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# Juvenile age estimation in physical anthropology: A critical review of existing methods and the application of two standardised methodological approaches

Louise Corron

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AIX-MARSEILLE UNIVERSITÉ

FACULTÉ DE MEDECINE DE MARSEILLE

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## THÈSE

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Par Louise CORRON

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**JUVENILE AGE ESTIMATION IN PHYSICAL ANTHROPOLOGY:  
A CRITICAL REVIEW OF EXISTING METHODS AND THE APPLICATION  
OF TWO STANDARDISED METHODOLOGICAL APPROACHES**

Pour obtenir le grade de DOCTORAT d'AIX-MARSEILLE UNIVERSITÉ

SPÉCIALITÉ : ANTHROPOLOGIE BIOLOGIQUE

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Mrs Eugenia Cunha (Professor, University of Coimbra),

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Mr Norbert Telmon (Professor, University Paul Sabatier, Toulouse – Hospital Practitioner, UMR 5288 AMIS)

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# Introduction

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The study of juvenile individuals in physical anthropology has seen considerable changes since the birth of the discipline at the end of the 19<sup>th</sup> Century. The numerous contexts of study of juveniles (medicine, physiology, sociology, psychology, ethics, law) have led to several denominations, each corresponding to subtle variations related to age and/or specific developmental characteristics: sub-adults or non-adults, immatures, juveniles, foetuses, new-borns, infants, children, adolescents, minors, etc. (Scheuer and Black 2000). In physical anthropology, these various terms can relate to a biological and/or social (or biocultural) status that characterises the individuals in question and as such, gives them a biological status and social and/or legal identity, depending on whether anthropologists are working in a bioarchaeological or forensic context, respectively.

Before becoming a particular field of interest for physical anthropologists, juvenile individuals were almost exclusively the subject of clinical studies alone. These were undertaken to control normal development (growth and maturation) and spot individuals presenting development-related pathologies to treat them. The notion of individual variability already peaks through these early works (Huxley 1924; Pryor 1907). The relationship between age and development then moved from clinical to scientific use, as development was no longer controlled but extensively studied and even predicted or mathematically modelled (Scammon and Calkins 1923). Most of the earliest anthropological studies involving juveniles were purely methodological and quickly made use of the development of medical imaging techniques to refine skeletal and dental analyses (Flecker 1932; Hess et al 1932): bone and dental developmental patterns led to direct application of the findings for juvenile age estimation, both on the living and the deceased. This particular and still very active field of interest, is therefore relatively ancient: the earliest occurrence found for the present study dates from 1894, with the work of L. Wacholz on bone development (Wacholz 1894). However, few studies were dedicated to using these methods for analysing and interpreting the role and status of these individuals in past populations before the 1960's (Johnston, *In* Brothwell 1968). It is now admitted that juveniles hold a central part in any osteoarchaeological study whenever they are involved.

The analysis of juveniles or juvenile remains in a forensic anthropological context has particular implications related to the potential legal status of the individuals, which is determined by the estimated age. Age is therefore a primary source of information for the study of juveniles in physical anthropology, regardless of the context and one of the key elements of an individual's biological profile.

The biological profile of an individual (age, sex, stature, individual characteristics) established from the study of human remains is a key concept in physical anthropology (Cunha et al 2009). In osteoarchaeology or bioarchaeology, the biological profile is constructed for each individual of the study to be analysed along with the other individuals at a global scale to participate in interpretations



of the archaeological, historical, ethnological and/or cultural context in terms of population studies. In a forensic context, the biological component takes on an even bigger role, as the context is set but the interpretation rests almost solely on the information provided by the biological profile. A juvenile's biological profile is quite succinct, because age and stature are the only parameters that can be reliably estimated from the juvenile skeleton (Saunders, *In* Katzenberg and Saunders, 2008).

Although the goals of juvenile age estimation differ according to context, the methods developed for that purpose follow similar principles. Today, anthropologists can choose from an array of methods to estimate age from juvenile remains (Cunha et al 2009; Wood, *In* Black and Ferguson 2011) such as dental and skeletal development indicators expressing growth and/or maturation changes that occur between early foetal life and early adulthood. Each growth and maturation event that can be related to age presents intrinsic and extrinsic variability, which needs to be considered to improve age estimation.

Because growth and developmental variability has different levels of expression (individual and population), and is caused by various factors (age, sex, genetic and epigenetic, secular trends, pathologies), it seems difficult to estimate age in juveniles following a standardised methodological approach. Indeed, current methods are often population-, age- or sex- dependent, which limits their practical application and can lead to biased estimates and interpretations. In addition to these methodological biases, the discrepancies observed between estimated (biological) and real (chronological, calendar or legal) age are also the result of the variable correlation between age and the biological indicator used to estimate it.

For several years now, the anthropological community has been advocating method uniformity to respect common statistical and methodological criteria and allow direct comparison between methods and results: reliability, standard error of estimation and accuracy should be known, sampling protocols should be standardised, etc. (Cattaneo 2007; Cunha et al 2009; Dirkmaat et al 2008; Ferembach et al 1979). By respecting these principles, methods could be objectively evaluated, bearing in mind the influence of methodological and population biases for estimating age in both osteoarchaeological and forensic contexts. The principles behind juvenile age estimation methods and the importance of context in both method construction and application are presented in Chapters 1 and 2.

The present study was undertaken to achieve two main goals:

- Present a critical methodological review of a large number of juvenile age estimation methods available to anthropologists today. This will lead to the elaboration of a methodological decisional tool, presented as a set of arborescent decisional trees. It is based

on objective criteria evaluating methodological construction protocols and method application. This practical tool can be used by anthropologists working in a forensic or archaeological context as a guide to the selection of methods according to the data available and highlights methods respecting valid methodological criteria (Chapter 2);

- Elaborate and apply standardised approaches of juvenile age estimation, respecting rigorous methodological protocols and criteria for data acquisition and analysis as well as statistical criteria, taking into account individual variability (Chapters 3 through 6).

The results obtained in answer to these two objectives will be discussed in Chapter 2 and conjointly in Chapter 7.

Juvenile age estimation in physical  
anthropology: concepts, contexts and  
methods

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## **Chapter 1. Age estimation of juvenile individuals in physical anthropology: definitions and contextualisation**

A sub-adult, non-adult or juvenile *sensus largo* is an individual who has not reached the biological (physiological, skeletal, dental), psychological, legal, or social status of an adult, *i.e.* biological, legal, or social maturity. This particular status is defined by the chronological (or calendar) age of an individual, *i.e.* the time (expressed in years, months, days, etc.) between his/her date of birth (or conception in some cases) and the present date. This simple notion of age as we understand it covers in fact a polyfactorial notion of immaturity that results from biological, cultural, psychological, social (Bogin 1997) and socio-legal parameters.

Age estimation is the principal component of the study of juvenile individuals in physical (or biological) anthropology. Indeed, physical anthropologists rely on indicators of biological immaturity (*i.e.* growth and development) to estimate chronological age, which is then used to interpret a social or legal status of immaturity. Estimated age is based on skeletal and/or dental remains or elements, so anthropologists work with skeletal and/or dental ages, inferred by maturation indicators or growth parameters (Wood and Cunningham, *In* Black and Ferguson 2011). Although biological growth and development are both highly correlated with chronological age, they are not perfectly equivalent to it. This correlation presents different levels of variability according to various factors. In addition to this, the polyfactorial definition of immaturity, its context of study, and the information available to anthropologists result in other inconsistencies, which we will present here.

### **1.1. Age differences: biological ages and chronological age**

To be able to compare the different definitions of immaturity, anchor them in a common time frame, and provide clear limitations for grading the different levels of immaturity, a neutral reference unit for numerically grading the level of immaturity and its temporal extent is needed. Developmental growth and maturation phases are strongly correlated with age (Adalian et al 2002; Cardoso 2008b; Fazekas and Kosa 1978; Scheuer and Black 2000; Ubelaker 1987), so age seems to be the best way to grade the appearance, extent and ending of developmental phases. This means that the different levels of biological immaturity are often the reflection of the variations in intensity of developmental activity (growth or maturation accelerations and decelerations, static periods, growth spurts, etc.) and can be anchored in time using age limits. These variations, particularly growth phases, are used to define sub-categories of immaturity. Indeed, two main growth phases are known (Bogin 1999): the prenatal phase (from conception to birth) and the postnatal phase (after birth).

Additional information on maturation and/or other particular growth events can also partake in refining these age categories.

### **1.1.1. Chronological age**

The only neutral unit is the “real” age, calendar age, or chronological age of the individual, as it is not subject to any confusions: chronological age is the mathematical difference between the date of birth and the date of death of the individual or between the date of birth and the date of an event of interest (*e.g.* the date of a crime) for a living individual. The definition of chronological age is a given and does not present any other possible value if both dates are known and considered accurate. The notions of “immaturity” and “age” are indeed inseparable but not equivalent. Along with context, they are the core elements of any anthropological study and interpretations of populations involving sub-adults.

Chronological age is usually expressed in years and/or months in official administrative documents, such as hospital or cemetery records and death certificates (Cardoso 2005; Cardoso and Severino 2010; Rissech et al 2013b; Saunders 1992; Scheuer and Black 2000). It can be established in two ways ([Insee](#)):

- The age reached during the year of the event, which is equal to the year of the event minus the year of birth. This age is expressed in full years (*e.g.* 19 years).
- The age in number of past years, which is the mathematical difference between the date of the event and the birth date. This age is more precise, and can be expressed in years, months, and days (*e.g.* 19 years 5 months 2 days). The exact age of an individual on date *d* is the difference between that particular date and his/her date of birth

Chronological age can be unknown for individuals of living populations, if birth and death dates are not registered, if the registers have been lost or destroyed or if the individuals refuse or cannot give their chronological age (Rösing et al 2007; Schmeling et al 2006b, 2007). Chronological age is almost never known for individuals of archaeological populations, unless the individuals possess authentic birth certificates, or the dates of birth and death are known. This is the case for the individuals buried in the cemeteries of St Bride’s Church (UK), Spitalfields (UK), and Lisbon (Portugal) where birth dates and death dates are registered or engraved on tombstones (Cardoso 2005; Molleson and Cox 1993; Rissech et al 2008, 2013a).

Chronological age is also the information used to determine the legal status of an individual. For juveniles, it is a decisive piece of information as it places an individual below or above a legal threshold. The position of an individual will determine the legal consequences concerning him/her

and the parties involved in the legal case. Chronological age also has social implications for an individual: reaching a particular age has important social and family meaning (Gélis 1986). This relates to the coming-of-age rituals or “rites of passage” that are still actively followed in some populations (van Gennep 1909). These rituals define particular statuses that anthropologists aim to interpret by studying the individuals.

### **1.1.2. Biological age**

In physical anthropology, the biological status of an individual is determined by his/her skeletal and/or dental remains, or any other physiological elements in living individuals. Biological parameters are often the only source of information available for determining a juvenile state and estimate a “biological age” that is compared to the “real” age of the individual, when the latter is available. Biological age is the only information of the biological profile/identity that can be established for juvenile individuals using osteological methods with sufficient reliability (Scheuer and Black 2000). Biological age can refer to several biological/physiological parameters or processes, and takes on as many adjectives, depending on the material used for estimation. This is why, depending on the material used, the terms skeletal age, dental age, physiological age, menstrual age, etc. can be found in literature. The biological status of juvenility is used to interpret a social and/or legal status of juvenility, by comparing the resulting biological age to chronological age groups defining these social or legal statuses (Buchet and Séguy 2008; Garcin 2009).

The concept of biological age was first used in a clinical context to assess the progression of an individual along the continuum of biological development, and check whether or not it was following its normal course (Gertych et al 2007; Lampl and Johnston 1996; Smith, *In* Kelley and Larsen 1991; Wood and Cunningham, *In* Black and Ferguson 2011). If development is normal, chronological age and biological age are relatively similar. If development is slow, or on the contrary accelerated, biological age will be respectively lower or higher than chronological age.

The age used by anthropologists is obtained from biological parameters mainly observed on bone and/or dental remains (Scheuer and Black 2000; White and Folkens 2005). Dental age is assessed by the degree of tooth development (dental emergence from the alveolar crypt, radicular closure, degree of enamel mineralisation). Skeletal age is assessed by the timing of appearance and fusion of ossification centres (primary and secondary), and by the changes of the size and shape of the bones with time (Scheuer and Black 2000). Skeletal age expresses the relation between a skeletal developmental state and the time that passed before this state was reached (Garcin 2009; Scheuer and Black 2000). It reflects one of the three phases of skeletal development: time of appearance of the ossification centre; time of appearance of a particular morphology and size of the ossification

centre; time of fusion between two ossification centres of a bone.

Dental or skeletal ages are often the only precise information on age that anthropologists can use to characterise juvenile individuals (Buikstra and Ubelaker 1994; Maples 1989; Scheuer and Black 2000; Quatrehomme 2015; Wood and Cunningham, *In* Black and Ferguson 2010). Because of the variability inherent to skeletal and dental development, it is important to note that biological age used by anthropologists is an *estimated* age. It is strongly and highly correlated with chronological age, and is more or less equal to it, depending on the reliability and precision of the estimation and where the individual is located on the normal range of developmental variability (Scheuer and Black 2000). Indeed, if an individual has a relatively slow skeletal developmental rate but is still in the normal range of variability for developmental rates, skeletal age will be lower than chronological age but will not be considered as significantly different. Indeed, his/her skeleton will seem to belong to a slightly or highly younger individual. Therefore, age will be more or less underestimated (Schmeling et al 2003a, 2003b; Schmidt et al 2008). Over- or under-estimation of age is also related to the method used for estimation. The composition of the study sample (age, sex ratio, age ratio, population characteristics) and the resulting method constructed with it can affect the estimated ages of different individuals (Coqueugniot et al 2010; Schillaci et al 2012; White and Folkens 2005).

The central question arising from this chapter is, when comparing chronological and biological ages, where (or rather, when) do we place the limit of normal variability that defines the level of acceptance of biological age as an estimator of chronological age? Anthropologists have provided an answer by proposing prediction intervals of the estimated age (biological age) and giving levels of accuracy for these estimates and intervals. Accuracy indicates the percentage of chronological ages found within the prediction intervals. However, it does not resolve another important question: the social and legal interpretation of biological. If the implications of being a juvenile, both in bioarchaeology and forensic anthropology, are known, related to age, relatively easy to understand and often context-dependent, the assessment of this particular status however, is not.

## **1.2. Juvenility: a polyfactorial, contextualised and pluridisciplinary concept**

### **1.2.1. Bioarchaeology: from bone to past populations, from biological individual to social status**

#### **a. General concepts**

Biological or physical anthropology is a pluridisciplinary field that aims to study and analyse human diversity and variability in past and present populations, by studying living humans and the remains of Humans and their ancestors (Dutour et al 2005; Johnston 1969; Jurmain et al 2008; Katzenberg and Saunders 2008).

Bioarchaeology focusses on a series of topics related to Human populations such as understanding funerary practices, palaeodemography, population movements and genetics, human activities, diets, and diseases through the study and analysis of human remains and the integration of data found in archaeological or historic contexts (Buikstra, *In* Buikstra and Beck 2008). This definition of bioarchaeology will be the one considered in our work and it will be used indifferently with osteoarchaeology. Bioarchaeological analyses include age estimation, sex determination, observation of pathologies and other particularities of skeletal and dental elements to characterise individuals of these past populations.

According to R. Hoppa and J. Vaupel (2002), palaeodemography is the “field of inquiry that attempts to identify demographic parameters from past populations (usually skeletal samples) derived from archaeological contexts, and then to make interpretations regarding the health and well-being of those populations”. Palaeodemographers use biostatistical approaches to estimate the age structure of past populations and understand their dynamics to construct and interpret demographic models of mortality by combining the reconstruction of the history of past populations from human remains and the information found in contemporary records, if available (Bocquet-Appel and Masset 1982; Lauwers, *In* Buchet et al 2006). The individuals of a past population are attributed different age groups to construct the demographic profile of the population in question (Bocquet and Masset 1977; Buchet and Séguy 2002, 2008; Milner et al, *In* Katzenberg and Saunders 2008).

The study of juvenile individuals in archaeological samples, be it for bioarchaeological analysis or palaeodemography, has long suffered from intrinsic, extrinsic and excavation-related sampling biases (Johnston, *In* Brothwell 1968; Saunders, *In* Katzenberg and Saunders 2008). However, they have now regained their rightful place as members of past populations in the same way as their adult peers (Garcin 2009) and they are often the main subject of anthropological studies.



## **b. The social juvenile: the social expression of biological development**

The social status of children (the generic social term used for juveniles) relies on both biological and cultural parameters (Hanawalt 2002). The place, image and role of the child are different according to populations, cultures, and historical periods (Halcrow and Tayles 2008). Considering the social place of juveniles brings additional information to the biological characteristics of juvenility and allows a global analysis of a population depending on its temporal and spatial contexts (Garcin 2009). In bioarchaeology, there are two complementary definitions for children: the biological juvenile, whose immature state is assessed by the skeletal remains of the individual, and the cultural child, represented by the artefacts associated with the grave or particular burial practices (Sofaer-Derevenski 2000). These definitions hide in fact several other concepts that participate in the definition of juveniles.

In Western societies, several “social phases” can be identified for juveniles:

- Infancy: infants or “babies” are juveniles for whom weaning is not complete. Weaning age is greatly socio-economically dependent, as it is directly linked to the type of food available and its relative abundance. Mean age of complete weaning is around 36 months (Dettwyler 1995).

- Childhood: B. Bogin (1997) gives a definition of childhood based on both biological and social factors: “[...] childhood is the period following infancy, when the youngster is weaned from nursing but still depends on older people for survival, feeding and protection. [...]”. This phase lasts from 3 to 7 years of age. The socio-cultural definition of childhood also englobes the biological juvenile stage (Pereira and Altmann, *In Watts* 1985), although B. Bogin differentiates them biologically. Juvenility lasts from 3 to 10-12 years (Bogin 1997). Generally, juveniles are socially considered as “older” children.

- Adolescence: adolescence is a relatively recent social concept. It designates a transitional period between childhood and adulthood when important developmental and psychological changes occur, before biological and social maturity is reached. It is marked by events of physical development leading to, and in some societies marking the entrance in, adulthood (puberty, pubertal growth spurt, development of secondary sexual characteristics). It is the period when sexual, social, economic and political behaviors start approaching those of adult individuals (Bogin 1997) and lead the way to accessing the rights and obligations related to adulthood (marriage, political and legal rights...). The age limits of this category depend on socio-cultural context.

For most periods in the past, the position of children in the family was at best equivocal. There was no concept of childhood; as soon as it was independent of the mother, the child was treated as a little adult (Molleson, *In Buchet* 1997). As seen above, the social progression of the juvenile

### ***Juveniles and age estimation: definitions and contextualisation***

approximately corresponds to developmental phases, but not all of them were used to mark this progression in past societies. In Roman and Medieval Western Europe, only three age groups defined juveniles: *infantia* (0-7 years), *puertitia* (7-14 years) and *adolescentia* (14-21 years) (Buchet and Séguy 2008). In medieval and modern societies, the status of newborns was linked to biological fragility and social exclusion (Séguy, *In* Buchet 1997) because infant and child mortality were a given until the beginning of the 19<sup>th</sup> Century in all pre-industrialised populations (Alduc-Le Bagousse, *In* Buchet et al 2006; Molleson, *In* Buchet 1997; Morel 2004; Schurr 1998). These individuals had no social status.

Indeed, until the late Middle-Ages, childhood had neither value nor specific place in society (Molleson, *In* Buchet 1997). Children belonged to the domestic sphere rather than the public sphere (Séguy, *In* Buchet 1997). During late medieval times, the growing influence of Christianity, mainly through baptism, greatly changed the social recognition of infants and children to finally abolish their social marginalisation and install a durable more positive attitude towards children (Treffort, *In* Buchet 1997). These changes can be seen in the appearance and progressive homogenisation of funerary treatments of new-borns throughout that period (Alduc-Le Bagousse, *In* Buchet et al 2006; Gourdon et al 2009; Séguy, *In* Buchet 1997; Treffort, *In* Buchet 1997). This positive attitude continued throughout the centuries, although the Church lost more and more of its influence after the Modern Period (18<sup>th</sup>-19<sup>th</sup> Centuries) as the social role of children became more and more characterised by its parent's and family circle affect.

In spite of this, child labour remained very common until the 20<sup>th</sup> Century. It began as soon as the individual was physically able to learn a trade to provide for the family (from 7-8 years onwards) (Orme 2003). Child labour has been regulated since the 19<sup>th</sup> Century, and progressively diminished with the instalment of free and mandatory education until the age of 16. These regulations are not always respected, particularly in developing countries where child labour is a real social problem ([International Labour Organization](#)). These cases can be the subject of legal proceedings requiring juvenile age estimation, to assess whether or not the child is old enough to be working.

The social child as we know it today results from gradual but major societal changes that have occurred since the 19<sup>th</sup> Century. These changes concern education, medical care, economics, culture, psychology, etc. that provoked the transition of the child as a collective body to a private, individual body (Gélis 1986). Based on the previous observations, it seems that all juveniles are defined as such by their biological characteristics and by the treatment and consideration they receive from their peers in the society to which they belong (Bogin 1997). As all these aspects are intrinsically variable, it is no surprise that the resulting definition of juveniles *sensus largo* would be too.

## **1.2.2. Forensic anthropology: from bone to individual, from biological individual to legal status**

### **a. General concepts**

Forensic Anthropology (FA) is the application and/or development of physical anthropology methods in a forensic context on a single individual. Its objective is twofold (Quatrehomme 2015). First, the identification of a partially or completely skeletonised individual from his/her skeletal and/or dental remains. This is done by constructing a biological profile that includes age, sex, stature, geographic origin (with some reservations, depending on authors), and other particularities (pathologies, asymmetries, other remarkable features) (Dirkmaat 2012; Lewis and Rutty 2003; Scheuer 2002; White and Folkens 2005). The second objective consists in finding the cause and circumstances of death or of the committed crime through trauma or lesion analysis, and assessing whether or not a second party could be involved. This aspect mainly implies analysing the context in which the remains or evidence were found. The most important element of the FA report is the construction of the biological profile of the individual from his/her skeletal remains, as it is the primary base of information used to guide the investigators in their search of a missing person, and ultimately formally identify the individual in question (Black and Ferguson 2011; Cunha et al 2009; Dirkmaat 2012; Franklin 2010; Quatrehomme 2015; Scheuer 2002; White and Folkens 2005).

The temporal frame of FA is defined by the statute of limitations from Penal law as inferior or equal to 10 years after the death of the concerned individual (article 213-5 of the French Code of Criminal Procedure (CCP), with a few exceptions (articles 7 and 8 of the CCP). For a minor, the number of years until majority is added to the time of the statute of limitation. In some cases, the legal time frame is overlooked and ethical principles take over to determine the forensic nature of the case (when dealing with identification of missing persons, or disaster victim identification for example). In practice, a forensic context rarely concerns periods of more than several decades after the date of death (Quatrehomme 2015).

### **b. Restrictions and regulations concerning the practice of forensic anthropology**

The practice, methods and results of a FA investigation and their interpretation have to rely on solid scientific arguments to be valid in front of a Court. In the United States, several institutions were created for such purposes: the American Academy of Forensic Sciences (AAFS) created a Physical Anthropology Section in 1972; the American Board of Forensic Anthropology (ABFA), founded in 1977, delivers certifications to FA experts.

In Northern America, the methods and protocols used by forensic experts must respect the Daubert criteria. This standard is used by a trial judge to make a preliminary assessment of whether an expert's scientific testimony is based on a reasoning or methodology that is scientifically valid and can properly be applied to the case (Rule 702: "Testimony by Expert witnesses", *Daubert v. Merrell Dow Pharmaceuticals* (92-102), 509 U.S. 579 (1993)).

Even if the FA community of Europe empirically follows the same precepts as the northern American regulations, no official legal restrictions and regulations exist for FA practice at a European level (Cattaneo 2007; Kranioti and Paine 2011). FA practitioners are mainly submitted to international and European recommendations for autopsy practice (e.g. recommendation n° R (99)-3 for harmonised practices, Rougé et al 2001), and regulations of the International Penal Court (IPC), installed in 1998 by the UN, but no regulations are explicitly dedicated to FA practice. However, the active role played by forensic anthropologists in the series of environmental or human international mass disaster events that occurred during the second half of the 20<sup>th</sup> Century (WWII, genocides, the Spanish and Balkan wars, etc.) and these past few years (the Tsunami in East Asia of 2002, plane crashes in Europe) has led to the official creation and optimisation of international cooperation in case of mass disasters (e.g. Belgium and UK Disaster Victim Identification (DVI) teams).

### **c. The legal juvenile: the minor**

If the term "juvenile" is the biological adjective for sub-adults, "minor" is its legal counterpart. Minority is defined *vis-à-vis* a specific threshold of chronological age: a minor is an individual younger than that threshold, and an individual older than the threshold has reached legal majority. This upper age limit determines the civil and criminal status of the individual following the corresponding articles in the Civil and Penal Codes. It refers to the notion of individual and penal responsibility in case of a crime.

The age of penal majority, *i.e.* the age from which an individual falls under common penal law is established at 18 in almost all European countries, except Denmark (15 years), Germany and Portugal (21 years). In the United States, it is either established at 18 or 21, depending on the state in question (International Juvenile Justice Observatory / [IJJO](#)). These differences can be problematic, especially in a world where international immigration (legal and illegal) is exploding (Focardi et al 2014). Indeed, the creation of the European Union and the free circulation of its inhabitants have blurred political and economic boundaries. More specifically where the European Union is concerned, based on the differentiating aspects and the common points that converge on the map of all European juvenile justice systems, the objective of the European Juvenile Justice Observatory (EJJO), founded on July 13<sup>th</sup> 2008 by the IJJO, is to create a European space for reflecting on and

developing initiatives, establishing codes and standards for good practices in serving the education and integration of minors in conflict with the law. This work in progress shows that the question of knowing and verifying legal minority is a national and international legal, social and ethical issue.

Another legal issue concerns minor individuals exclusively. Indeed, the civil and penal status of a minor change according to his/her age. Legislators adapt the legal consequences to the minority status of the concerned individual, be she/he a victim or a suspected culprit (Service of European Affairs 1999; Service of Legal Affairs 2007). The age of penal or criminal responsibility, *i.e.* the age after which minors are considered sufficiently old to be submitted to penal law, varies once again greatly between European countries and within the United States. It is an absolute notion in certain countries, where the minor who has not reached the age of penal responsibility cannot be held legally responsible for his/her actions. Age of penal responsibility ranges from a minimum of 10 years (Switzerland, England and Wales) to a maximum of 16 years (Portugal). In the United States, criminal responsibility ranges from 7 years (North Carolina) to 10 years (Wisconsin) (IJJO).

When a minor is sentenced, priority is given to educational measures. However, because individual responsibility is greatly variable from 0 to 18 years, several sub-age thresholds exist to distinguish specific levels of criminal or civil responsibility or legal representation of minors according to chronological age. Some exceptions to this principle can be found. For example, in Germany, an individual aged between 18 and 21 will be judged as minor or adult, depending on his/her mental and moral development state (Schmeling et al 2003a, 2007).

These sub-thresholds also determine the “underage” status of an individual. In France, minors are not judged by ordinary penal jurisdictions (criminal court, crown court), but they can be judged by a juvenile court judge, in a juvenile court for criminal cases, surveillance and education. If the minor individual is the victim of a crime, the culprit’s sentence will vary according to the position of the individual in regard to these thresholds. If there is a grave suspicion that a crime was committed by a minor, he/she can be taken into custody pending trial (Order n°45-174 of February 2<sup>nd</sup>, 1945, modified by law n°2011-392 of April 14<sup>th</sup> 2011).

In France, jurisprudence considers that children as young as 8 or 10 have developed enough discernment to be legally responsible for their actions. Minors younger than 10 years can receive an educational sanction if deemed necessary by the legal authorities. Individuals younger than 13 can only be the object of “protection, assistance, and can in no way be taken into custody”. Article 122-8 of the French Penal Code states that minors capable of discernment are considered legally responsible, but only minors aged between 13 and 18 can be concerned by criminal penalties, if the circumstances and personality apply. The French Civil Code adds the 16-year threshold as the maximum age for a minor to be legally represented by a parent or tutor (Article 17-3 of the French

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Civil Code). Minors aged between 13 and 16 years can be taken into custody for a maximum duration of 6 months, and custody can last up to one year for individuals aged between 16 and 18 years. However, the duration of the final sentence cannot be more than half the duration of the same sentence applied to an adult individual. Legal age thresholds for minors are only several years apart, which means that age needs to be precisely and reliably known, or precisely and reliably estimated by experts, in order to carry out a just and adapted sentence.

A special legal case concerns the status of children whose death occurs before the acquisition of a civil birth certificate. With the Circular of March 3rd 1993, a "still birth" certificate is drawn up only if it has not been established that the child was born alive and viable. Three situations are concerned by this circular:

- If the child was born alive, but was not viable, and died before the acquisition of a birth certificate. The civil status officer draws up the certificate upon production of a medical certificate regardless of the gestation period;
- If the child was born alive and viable, but died before the acquisition of a birth certificate, without a medical certificate stating it was born alive and viable;
- If the child was still-born. Since the 30<sup>th</sup> November 2001 circular, a still-life bulletin can be established if the gestation period was at least 22 gestational weeks (GW) or if the child had reached a weight of 500 grams (foetal viability criterion of the World Health Organization, WHO 2006b).

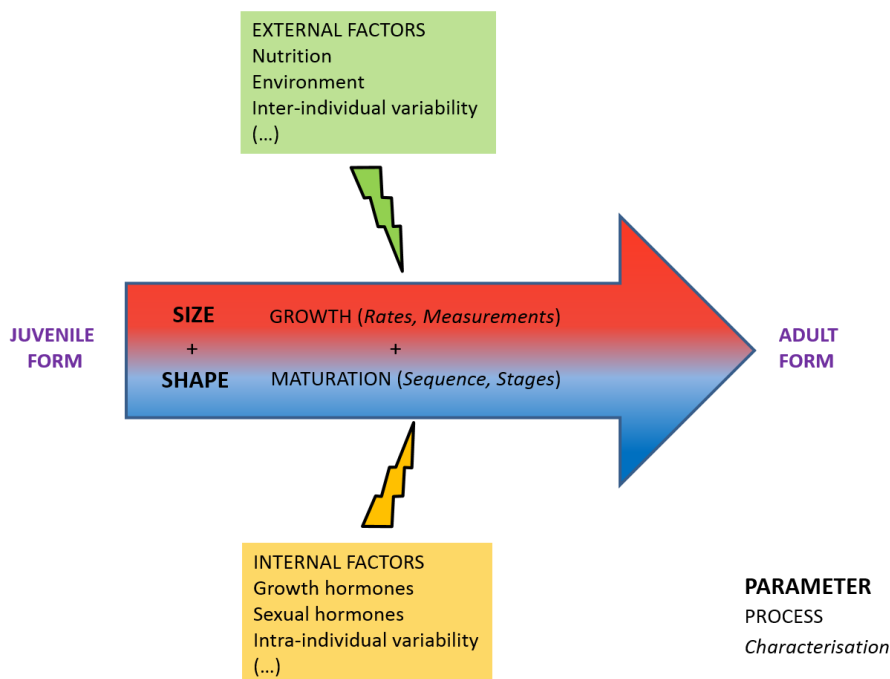
If these conditions are met, the child can be legally registered on a civil status certificate. This threshold applies for the acquisition of a birth certificate, a death certificate if the child was born alive but died within three days after birth, or for establishing a certificate of still-born birth (Circular n°2001/576 of November 30<sup>th</sup> 2001 and decree of July 19<sup>th</sup> 2002). A still-born child of less than 22 GW and weighing less than 500 grams is legally considered as an "anatomical specimen" and cannot be civically registered.

Almost all other countries use criteria of weight and gestation duration to establish foetal viability. However, the definition of a lifeless child varies between countries and does not always originate from a legal institution (for Germany, Switzerland, and Belgium). Since Decree n°2008-800 of August 20<sup>th</sup>, 2008 redefined the notion of juvenile deaths, the death certificate of a child has been established on the basis of a medical certificate obtained at childbirth. Gestation duration (22 gestational weeks), or a weight of 500 grams are no longer taken into account as viability criteria.

This overview of the notion of legal minority indicates how the legal and social status of juvenile individuals is partly defined (and almost entirely defined for foetuses and new-borns) by biological parameters, and mainly indicators of biological development that are accessible to anthropologists.

### 1.2.3. The biological juvenile: an expression of biological development

Development is the result of a series of mechanisms occurring in a living organism, from the time of fecundation to the attainment of its definitive morphology (Eveleth and Tanner 1990). According to S. Gould (1977), studying biological development aims to comprehend the evolution of human ontogeny. Development is the progressive change in the size and conformation of an element with time. Biological immaturity is therefore a state of ongoing development, with changes influenced by internal and external factors, but which, in the end, always result in the individual attaining complete maturity (Scheuer and Black 2000). Ontogenic changes can be fully perceived by studying the evolution of the form of an immature organism until it reaches its mature form. Form is the result of the combination between two components: size and shape. Shape is the result of geometric characteristics of an object, independently of its size, position and rotation (Kendall 1977). Size is a more variable concept, as its evaluation is always relative to a reference unit or object. Changes in the size and shape of an element are the result of two biological dynamic processes: growth and maturation respectively (**Figure 1.1**).



**Figure 1.1** Parameters, processes and their characterisations involved in the biological development of a juvenile or immature form to attain an adult or mature form

B. Bogin (1997) suggested characterising human biological development using five stages or phases that precede the final stage of adulthood or biological maturity. Each phase is characterised by a particular growth pattern or trajectory, or by specific developmental events. Human development can therefore be separated into six biological phases that correspond to variably precise age ranges:

- Intra-uterine life/pre- and perinatal phase: the phase during which primary ossification and dental

### ***Juveniles and age estimation: definitions and contextualisation***

mineralisation occurs, and the structure of the skeleton is formed (Boller 1964; Jeffery and Spoor 2004; Jit 1957; Morimoto et al 2008; Noback 1943; Ogata and Uhthoff 1990; Scheuer and Black 2000, 2004). It is characterised by a very high growth rate (Bareggi et al 1996; Bertino et al 1996; Deter and Harrist 1992; Fazekas and Kosa 1978; Ford 1956; Guihard-Costa 1991, 1993; Lampl and Jeanty 2003). It ranges from the 7<sup>th</sup> month *in utero* (age limit of foetal viability) to the 28<sup>th</sup> day after birth.

- Infancy: infancy is the phase when the deciduous dentition emerges and progressively appears in the buccal cavity, when the individual progressively masters voluntary movements and locomotion, implying significant changes in that regard in the pelvic girdle and the lower limb bones (Reynolds 1945). It is characterised by rapid growth in height (by extension of post-cranial bones, and long bones in particular) until the age of one, followed by progressive deceleration (Johnston 1962, 1996; Maresh and Deming 1939; Reynolds 1945). It ranges from the 2<sup>nd</sup> postnatal month to the 3<sup>rd</sup> year.

- Childhood: this period is characterised by moderate growth (Johnston 1962, 1996; Reynolds 1947; WHO multicentre Growth Reference Study Group 2006b), but a high need of energetic food: indeed, childhood is the phase when the brain develops the most and the most rapidly, and demands a high caloric intake to do so. Of course, the bony structures of the skull follow the growth in size and the development of the brain (Madeline and Elster 1995a). A small increase in general growth velocity called the mid-growth spurt (a sudden and rapid increase in growth rate, Bogin 1997) appears at the end (Tanner 1962). This mid-growth spurt marks the transition from childhood to the juvenile stage. It starts around three years and ends with the emergence of the first permanent molars and the upper permanent incisors (around 7 years, Bogin 1999). Children present characteristically mixed dentitions: both deciduous and permanent teeth are present in the maxillary and mandibular crypts and/or have erupted (Clements et al 1953; Hurme 1948, 1949; Nanda and Chawla 1966; Nolla 1960). Ossification centres continue to mature (Elgenmark 1946; Tupman 1962).

- Juvenile stage: the main biological events of this phase are the emergence of the first permanent molars and the end of brain growth. Growth continuously decelerates to reach its lowest rate around 10-12 years, when the next phase starts.

- Adolescence: this phase is characterised by an important growth spurt in stature, and by extension, of skeletal elements (Grave and Brown 1976; Smith and Buschang 2005; Tanner 1962, 1981), followed by the progressive cessation of growth. Once growth is over, an active phase of bone maturation takes over and results in the fusion of the majority of secondary ossification centres to form mature bone elements (Baughan et al 1980; Moss and Noback 1958; Tanner 1981).

As seen previously (**see section 1.2.1.**), childhood and adolescence are defined not only by biological events and developmental milestones, but also by several social parameters. In that way, these two



phases are specific to humans (Bogin 1997) and reflect the intertwining of social and biological components that define the status of juvenility.

- Adulthood: adulthood is characterised by the attainment of maximum stature, and the completion of dental and skeletal maturation. During early adulthood, development is still active for young adults, with the fusion of the last secondary ossification centres (sternal end of the clavicle, iliac crest and sphenoid-occipital synchondrosis). This phase is followed by a period of equilibrium/stasis, during which individuals are referred to as “adults” and then as “mature adults”. The process of senescence then gradually takes over: it is responsible for the aging of tissues, leading to a progressive loss of the perfect functioning of activities and regulatory processes (White and Folkens 2005). Individuals are then referred to as “elder adults”.

This chapter shows the strong intrication between the social, legal, biological definitions and markers of juvenility *sensus largo* and of its different sub-categories. To avoid confusion, **throughout this work, the terms “infancy”, “childhood”, “adolescence” and their derivatives will be used in their social sense. The terms “juvenile”, “sub-adult” or “non-adult” will be used indifferently in their larger biological sense, in relation to ongoing growth and development.** The term “juvenile” covers several sub-categories of juveniles, because demographics, most modern cultures and legal systems rely on specific age limitations where juveniles are concerned. All three statuses of juvenility (biological, legal or social) do not necessarily concern the same age ranges and do not always cover the same sub-categories of age. These inconsistencies are the first cause of difficulty in interpreting a social and/or legal status from biological parameters, independently from methodological limitations. The biological component of juvenility is indeed the main (and often only) available source of information for anthropologists to assess social or legal juvenility from an individual based on his/her body or his/her skeletal and dental remains.

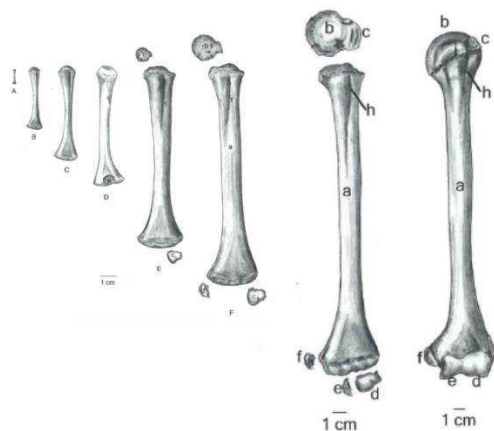
## **1.3. Skeletal development: growth and maturation**

### **1.3.1. Skeletal development**

Bones and teeth carry remarkable evidence of ongoing development expressed through growth and maturation processes, whose markers are used as age estimators for juveniles. The first studies on juvenile development date from the beginning of the 20<sup>th</sup> Century, and were based on research data from autopsies of the second half of the 19<sup>th</sup> Century (Scammon and Calkins 1929). The number of studies on biological development soared dramatically in the 1960's and between the 1980's and the 1990's, although the conclusions drawn then were limited by three major publications focusing

on the limits of developmental studies of past populations (Saunders et al, *In* Grauer 1995; Saunders and Hoppa 1993; Wood et al 1992).

Skeletal formation is the result of a complex sequence of events: induction of bone pattern, cell differentiation, and subsequent growth and maturation of the cells. Normal growth and maturation depend on the genetically-regulated balance between the specialised activities of osteoblasts (productive bone cells) and osteoclasts (destructive bone cells) (Encha-Razavi and Escudier 2008). Development is controlled by a complex interaction between regulation and structural proteins, growth factors, other interactive molecules (O'Connor et al 2010), and mechanical factors such as intermittent pressure on developing articulations, mechanical wear, loading on bone and articular zones influences remodelling (Zaleske, *In* Buckwalter et al 1998). This specificity is responsible for individual variation in shape, size, mineral density, and range of responses to external or internal stimuli (O'Connor et al 2010). Skeletal development can be assessed by studying the appearance of the bones, *i.e.* primary ossification, the changes that occur in the morphology of the bone elements as the individual ages, and the final developmental events (*i.e.* secondary ossification) that lead to the attainment of mature elements (Scheuer and Black 2000, 2004; White and Folkens 2005) (**Figure 1.2**).

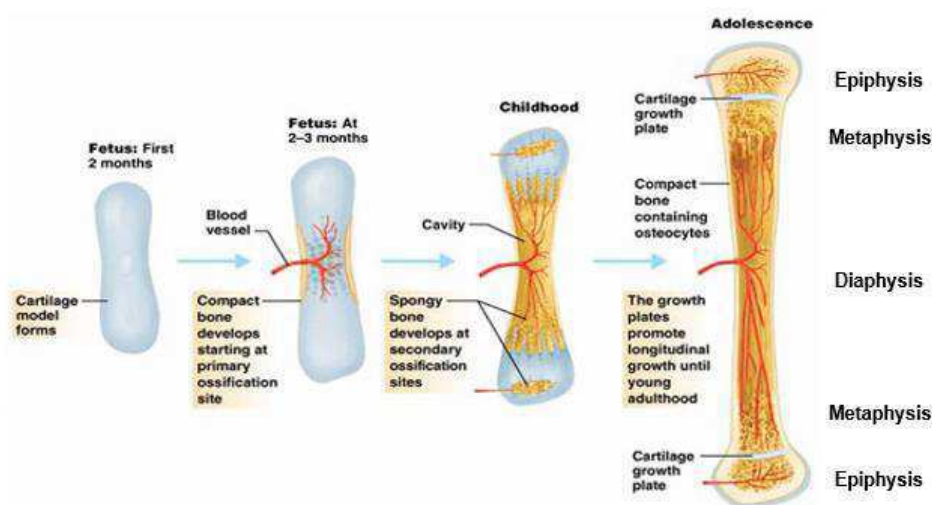


**Figure 1.2** Increase in size, appearance of ossification centres for the epiphyses and changes in conformation of the juvenile humerus with age. From left to right: (A) first foetal trimester, (B) second foetal trimester, (C) third foetal trimester, (D) perinate, (E) 1.5 years, (F) 5 years, 9 years and 15 years; a: diaphysis, b: head, c: greater tubercle, d: capitulum, e: trochlea, f: medial epicondyle, g: lateral epicondyle, h: inter-tubercular sulcus, i: lesser tubercle. Taken from Baker et al 2005, p.104-105

Primary ossification (the first step of bone formation) can be intramembranous or endochondral (Scheuer and Black 2000, 2004; Tuan, *In* Buckwalter et al 1998) depending on the future bone and its location in the skeleton. Primary ossification starts around the end of the embryonic period and the beginning of the foetal period (around the 7<sup>th</sup>-8<sup>th</sup> gestational weeks/GW). The clavicle is the first bone to start primary ossification (at 5-6 GW), followed by most cranial elements (between 6 and 16 GW). The other bones (flat and short postcranial bones) appear subsequently from 8 GW onwards.

Primary ossification centres of long bones appear during the 12<sup>th</sup> gestational week. From then on until birth, primary centres grow in size and mature in shape via the complementary modelling action of osteoblasts and osteoclasts, following specific patterns.

Almost all primary ossification centres appear during the embryonic or foetal periods, but some form much later (*e.g.* carpal bones that appear in pre-adolescent children). A bone presents one or several primary ossification centres that will progressively fuse with one another. After birth, bone diaphyses (the central part of long bones) are ossified. During the postnatal period, long bone cartilaginous secondary ossification centres (epiphyses) will progressively appear (**Figure 1.3**).



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**Figure 1.3** Endochondral ossification processes of a long bone occurring between the foetal period and adolescence

The epiphyses will then ossify via endochondral ossification (secondary ossification), but conjugate cartilage will persist between the diaphysis and the epiphyses until puberty, to ensure endochondral growth in length of the long bones (Scheuer and Black 2000, 2004). The end of fusion indicates that mature bone shape is reached.

All these steps occur sequentially and most importantly, chronologically. They are therefore correlated to age (Green 1961).

### **1.3.2. Skeletal maturation**

Maturation transforms an element's conformation by making it evolve through different morphological stages. It is simultaneously quantitative and qualitative and is often studied using qualitative parameters (Gili 1996). It can also be assessed at both macroscopic and microscopic levels by evaluating and grading changes, in the morphology or constitution of bony tissue (Chan et al 2007;

Glorieux et al 2000; Kunos et al 1999; Salle et al 2002; Schnitzler et al 2009; Smith 1963; Streeter 2010; Thomas et al 2000). Bone maturation is a two-fold process: it causes changes in bone conformation and fusion between two bone centres (between the diaphysis and the epiphyses for example) to enable the bony element to attain its next developmental stage.

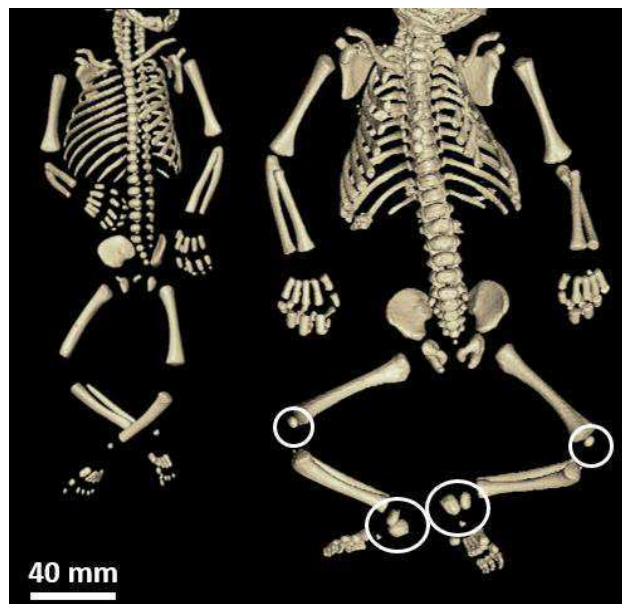
Three skeletal maturation phases can be identified (Scheuer and Black 2000, 2004):

- Appearance of ossification centres
- Morphological changes of ossification centres
- Fusion of ossification centres

These three phases grossly correspond to three periods of time (Bogin 1997; Scheuer and Black 2000): the prenatal period, from conception to birth (Bogin's intra-uterine life period), childhood (Bogin's periods of infancy from 0 to 1 year, childhood and juvenility from 1 year to adolescence) and adolescence (the same period for Bogin) (Wood and Cunnigham, *In* Black and Ferguson 2011).

#### **a. Appearance of ossification centres**

The majority of primary ossification centres (POCs) appear during intra-uterine life (at the transition between the embryonic and foetal periods). Prenatal POCs form the skull, vertebral column, sternum, ribs, long bones, girdles, and phalanges. The last to appear are the POCs of the ankle and knee (Figure 1.4).



**Figure 1.4** Post-cranial skeletal elements present in a foetus aged 22 GW (left) and in a foetus aged 41 GW (right). In the last 20 weeks of foetal life, bone development is particularly active, with an increase in bone size (long bones in particular), the appearance of the primary ossification centres of the distal femoral epiphyses (superior white circles) and maturation of the primary centres of the calcaneus and the talus (3D reconstruction using Avizo, Photo credit: L. Corron)

Until 1895, the appearance of POCs was dated using anatomical drawings of dissected embryos, fetuses and perinatals. Currently, several methods are used: histomorphology of bone trabeculae, bone tissue coloration using specific coloration of bone tissue, radiographic detection of radio-opaque calcified cartilage and bone, and other medical imaging techniques (ultrasonography, CT scan, MRI...). Dating the precise appearance of POCs is difficult: it is greatly dependent of the method used (Scheuer and Black 2000, 2004).

Postnatal primary ossification centres appear at the wrist, the ankle and part of the hand and foot. Secondary ossification centres (SOCs) or epiphyses appear some time during postnatal life, until maturity is reached (Lefebvre and Koifman 1956). At least one is present for each bone (except for the skull and the ossicles of the inner ear). Their appearance is assessed by longitudinal (multiple examinations of the same individuals at different but regular time-intervals) or cross-sectional (unique examination of several individuals at a given time) radiographic studies, from birth to maturity (Scheuer and Black 2000).

#### **b. Morphology and size of ossification centres**

Each ossification centre (OC) has a particular morphology, which facilitates its identification when it is isolated. Once the centre is identified, age can be correlated to its size and its morphology. Antenatal ossification centres can be recognised starting from the second half of foetal life. Morphological changes during development are often assessed by two-dimensional media (*e.g.* radiographies) that do not integrate the three-dimensional changes of the ossification centre. The shape of the various OCs remains similar until a critical stage of differentiation is reached for the elements. The timing and nature of this differentiation depends on the element itself (Cope 1920; Jit 1957; Noback 1954) but are always included in a given time frame.

During the second half of foetal life, most elements present are recognisable. Bony elements (epiphyses and OCs of the hand/wrist and ankle/foot) become sequentially recognisable throughout childhood and adolescence. The scapula and the iliac bone acquire their adult morphology during late adolescence. This particular phase is not as well documented as the others. It lacks morphological data obtained on dry bones (few adolescent individuals are entirely preserved in archaeological populations) or any other means of study (Scheuer and Black 2000).

#### **c. Fusion of ossification centres**

Fusion occurs in a bone region called a growth plate, constituted of conjugate cartilage that links the primary centre of the bone to the secondary one(s). This cartilage is gradually transformed into

bony tissue connecting the centres resulting in a mature bone shape (Anderson and Shapiro 2010). The timing and duration of fusion of the OCs are extremely variable. They are partially submitted to their association with soft tissues, and, more specifically, muscular tissues: for example, the early fusion of the vertebrae or the occipital bone during the beginning of childhood is the reflection of an early development of the central nervous system and ensures its physical protection (Scheuer and Black 2000). Generally speaking, all cranial elements fuse early (most often *in utero*) compared to post-cranial elements, to ensure the protection of the brain. During infancy and early childhood, occipital bone elements fuse with one another, and the vertebral body fuses with the corresponding neural arches.

Fusion of post-cranial bones always occurs between a POC and one or several SOC, once the elements and the enviroing soft tissues have finished growing. Long bone fusion is indeed greatly regulated by the growth rate of the associated muscle structures.

Adolescence is a very active period for bone fusion: OCs of long bones (with the exception of the sternal epiphysis of the clavicle), of the hands and feet, and all the POCs as well as some SOC of the iliac bone start and/or finish fusing. In the period between adolescence and adulthood, the last epiphyses finish fusing: the cranial jugular plate, the vertebral SOC (epiphyseal rings, other anatomical reliefs), the sacrum, the scapular SOC, the iliac crest, the ischial tuberosity. Bones acquire their adult morphology. The sternal extremity of the clavicle and the spheno-occipital synchondrosis are the last epiphyses to fuse, between 21 and 30 years (Baker et al 2005; Scheuer 2002; Scheuer and Black 2000, 2004; White and Folkens 2005). When bones have finished growing and all other epiphyses except the clavicle and the iliac crest are fused, the individual is no longer considered juvenile, but is categorised as a “young adult”.

Skeletal maturation presents a high number of milestones, from the first appearance of an ossification centre to the fusion of the last epiphysis. These milestones are a way of controlling normal development, but their sequential and chronologically-related appearance make them optimal candidates for age estimation.

### **1.3.3. Skeletal growth**

Growth is the progressive increase with time of the size of a biological unit or of a unit related to biological parameters, which occurs without any loss of individuality or interruption of functional activities (Mayrat et al 2015; Susanne 1991, 1993). A more intuitive definition of growth is the increase in size of an element (for example, the increase of length, width, volume of a bone). This increase is usually measured in relation with time, and therefore, growth of an element is generally

expressed in units/time<sup>1</sup>. Growth is not necessarily synchronised with maturation events (Scheuer and Black 2000) and it can therefore be studied independently. Growth can be evaluated by quantitative parameters (*e.g.* measurements, height, weight...) and assessed at a macroscopic or a microscopic level. It is a continuous developmental process. It has specific patterns, with phases of acceleration, deceleration and stasis, defining rhythms and phases that are regulated by internal (hormonal, genetic, metabolic) and external (nutrition, physical activity, environmental stress, socio-economic status) factors (Cameriere et al 2007b; O'Connor et al 2008; Prakash and Bala 1979).

### **a. Growth studies**

The scientific study of growth is auxology. The term was coined by P. Godin in 1910 but came into general use after 1977 (Spencer 1997). P. Godin defined auxology as “the study of growth by the method of following the same subjects during numerous successive six-monthly periods with a great number of measurements” (cited in Tanner 1981, p.226). The general definition of auxology is the analysis of quantitative and qualitative changes of an organism from conception to adult age, *i.e.* the study of the increase in body dimensions and the changes in physiological and biochemical body components (respectively) as the individual ages (Hauspie and Wachholder 1986). In physical anthropology, auxology is mainly studied using quantitative osteological data.

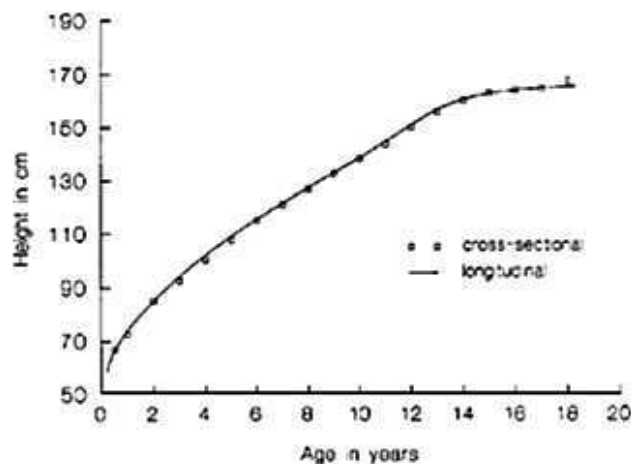
The definition of auxology implies the notion of time as a reference frame, of biometry, and implies the notions of “longitudinal” versus “cross-sectional” studies of growth. Indeed, according to P. Godin, to be accurately comprehended, growth has to be studied longitudinally, *i.e.* by successively measuring the same individuals or individual parameters for a given period of time. Cross-sectional studies measure the same individual parameters only once per individual, in individuals of different ages. This means that longitudinal studies follow individual growth and growth patterns (Coleman 1969) and their intra- and inter-individual variability, usually from the beginning to the end of the active growth period. Therefore, they are considered to be a more exact reflection of population growth variability by several authors (Godin 1910; Goldstein 1979, 1986; Lampl and Johnston 1996; Sempé and Pavia 1979). Seriated measurements allow modelisation of changes due to growth and the relation with other parameters, such as maturation (Smith and Buschang 2004). However, many authors consider that longitudinal studies do not provide a correct estimate of individual variability, as repeated measurements are highly intercorrelated. Therefore, total variance (the extent of inter-individual variability) is undervalued (Demirjian and Levesque 1980; Garn et al 1958; Haavikko 1970; Levesque et al 1981; Moorrees et al 1963a). Cross-sectional

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<sup>1</sup> I. Fazekas and F. Kosa (1978) have proposed diagrams to model growth of body size as a function of bone length

studies are more useful to study inter-individual growth variability, as they include a great number of different individuals at different ages, each with different growth patterns (Baroncelli et al 2006; Pinhasi et al 2005; Stull et al 2014a).

Studying growth in anthropology means studying growth of biometric variables. Biometry, or biological measurements, defines human growth and its spatial and temporal variability, resulting from genetic, environmental and social factors. Biometry is essential to model growth (Lalys, *In Chapuis-Lucciani et al 2010*). Growth studies began in a clinical context to monitor and characterise the normal progression of growth and estimate adult stature (WHO Multicentre Growth Reference Study Group 2006b; Kimura 1992; Lampl 1993; Tanner 1981). These extensive studies on bone growth resulted in several clinical (Adair 2007; Deter and Harrist 1992; Falkner et al 1972; Johnston 1996; Kedzia et al 2009a, 2009b; Lampl and Thompson 2007; Maresh 1943, 1955, 1970) and anthropological standards (Gindhart 1973; Jantz and Owsley 1984; Johnston 1962; Mays et al 2008; Miles and Bulman 1995; Saunders et al 1993b; Schillaci et al 2012; Smith and Buschang 2004, 2005) of several skeletal elements. Generally, growth standards are presented as curves, *i.e.* mathematical models of the relation between age and a biometric variable. There is a positive but complex correlation between age and individual growth (Pan and Goldstein 1998; Scheuer and Black 2000) that can be modelled using different mathematical expressions, depending on the bone, the age group considered, and growth variability. L. Humphrey (1998), H. Pan and H. Goldstein (1998) used sigmoid Gompertz curves (**Figure 1.5**) for growth modelisation.



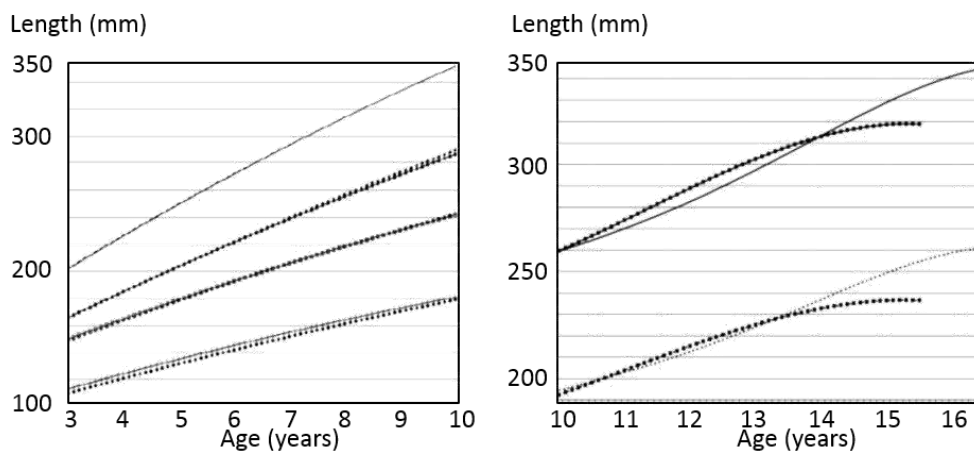
**Figure 1.5** Growth in stature (height) from birth to 20 years of age, modelled by a sigmoid Gompertz curve between age and height. The curve is fitted to longitudinal data. The dots correspond to cross-sectional means. Taken from Pan and Goldstein 1998

C. Rissech and collaborators used second, third, or fifth degree polynomials (Rissech and Malgosa 2005, 2007; Rissech et al 2003, 2008, 2013a). A double-S curve, or Pineau curve is customary to



model post-natal growth in height and long bone measurements from the beginning to the end of the process (Hunt and Hatch 1981; Marchal 1997, 2003; Pineau 1965). These particular curves perfectly illustrate the changes that occur in bone growth rates: the three parts of the characteristic “S” can be modelled separately using linear regressions (**Figure 1.6**). Each of them represents a sub-phase where growth occurs at a regular rate (Lopez-Costas et al 2012).

Foetal growth, and foetal long bone growth in particular, and growth during early childhood is usually modelled using linear regressions (Adalian et al 2002; Bagnall et al 1982; Brough et al 2013; Black and Scheuer 1996) but more complex models can also be found (Deter and Harrist 1992), such as the rule of Hääse (Fazekas and Kosa 1978), a rather complex exponential equation modelling the correlation between age and height (*i.e.* growth in height) for foetuses.



**Figure 1.6** Different models of long bone growth. Left: growth of diaphyseal long bone lengths of the Denver Growth Study children aged 3 to 10 years modelled by linear regression (from top to bottom: humerus, radius, femur, tibia). Right: Model curves of the total length (including the epiphyses) of the humerus and the radius for the Denver subjects aged 10 to 16.5 years. Curves for boys are modelled by dots and curves for girls are modelled by full lines. Taken from Smith and Buschang 2004, 2005

Normal growth curves of various biometric variables (height, weight, etc.) were constituted to serve as references to which individuals were compared to for assessing possible delays or advances in growth (Falkner et al 1972). These references can also be used in a more global comparative approach of growth parameters and growth models (Berkey 1982; Schillaci et al 2012; WHO multicentre Growth Reference Study Group 2006b).

The World Health Organisation (WHO) presented growth standards describing how normal and healthy children “should” grow (WHO 2006b). WHO standards are based on longitudinal and cross-sectional data obtained from healthy children of several countries across the world. These standards represent normal human growth under optimal conditions, independently of society or environment. They are used to assess and compare growth patterns of children from different populations as well

as clinically monitor normal growth (Schillaci et al 2012; WHO 2006a).

The Child Research Council of the University of Colorado conducted a series of studies on longitudinal growth between the 1930's and the 1960's. The most prominent work came from the physician M. Maresh, who constituted one of the only longitudinal radiographic databases on long bone growth available today (Maresh 1943, 1955, 1970) that is used as an abacus by anthropologists to estimate age. Other than this extensive study, there are fewer longitudinal than cross-sectional studies of long bone growth.

C. Ruff (2003) used part of the sample constituted by M. Maresh to study age-related changes in length and robusticity of the femur and the humerus (Ruff 2003). Comparing the growth of different limb bones aims to apprehend a particular level of variability: intra-individual variability, *i.e.* intra- and inter-member differences, provides information on bone growth coordination, its internal and external regulatory mechanisms, as well as the relation between bone size and its growth rate at a given age (Smith and Buschang 2004). Other than their use for studying variability, data obtained in auxology studies is often considered as a reference for the correlation between age and biometric variables and exploited for indirect age estimation by anthropologists.

## **b. Skeletal growth phases and patterns**

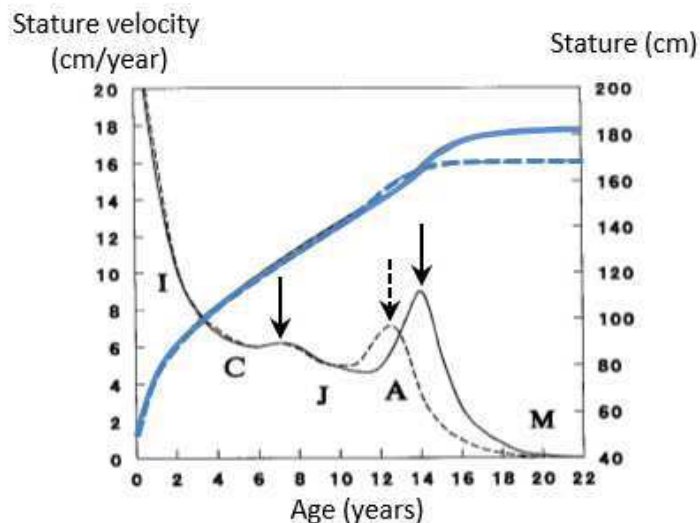
Bone growth starts *in utero* as soon as the first primary ossification centres appear, after the embryonic phase (7<sup>th</sup> gestational week). The *de novo* formation of bony tissue, following endochondral or intramembranous ossification, results in the increase in size of the bone: the ossification centres are separated from each other and from secondary ossification centres by a cartilaginous region between the ossified central part of the bone and the metaphyseal region, or growth plate (Zaleske, *In* Buckwalter et al 1998). This particular region is where additional bony tissue appears, leading to the increase of the size of the bone (Hunziker, *In* Buckwalter et al 1998; Scheuer and Black 2000; Trippel, *In* Buckwalter et al 1998). For long bones, growth occurs longitudinally at the proximal and distal ends. Growth of flat and short bones follows a more complex orientation, leading to the final shape of the bone (Marieb 1999). Bone growth ends during adolescence and bone fusion starts (Garn et al 1961; Parfitt et al 2000). Like maturation, bone growth is controlled and regulated by genetic and epigenetic (hormonal, physical, environmental) factors that ensure bone homeostasis and normal development (O'Connor et al 2010).

If bone maturation has three distinct phases, growth is characterised by a diversity of phases and particular events called spurts (a spurt is a sudden and short increase in growth). This is why growth is referred to as "saltatory" (Lampl 1993; Lampl and Thompson 2007), as it is a continuous, but non-

linear and irregular process (**Figure 1.7**). B. Bogin (1999) identified two principal growth phases: the prenatal phase (from conception to birth) and the postnatal phase (after birth).

Each of these phases can be subdivided following specific variations in growth rates that identify particular biological events. All authors agree that the prenatal (foetal) phase is a phase of extremely rapid and constant growth (Adalian 2001; Deter and Harrist 1992; Fazekas and Kosa 1978; Guihard-Costa 1991, 1993; Haj Salem et al 2010; Olivier 1962, 1974; Olivier and Pineau 1960; Scheuer and Black 2000; Sellier et al 1997).

The postnatal phase can be divided into several sub-phases according to changes in growth rates at different periods of life. Typically, the first year is characterised by high growth velocity followed by a continuous period when growth progressively slows until it reaches a plateau after puberty (Hoppa 1992; Miles and Bulman 1995; Okazaki 2004); between one and two years (or four years, depending on the variables measured) growth is fast and closely regulated by controlled secretions of growth hormones (Bogin 1999; Gowland and Chamberlain 2002; Guihard-Costa 1993; Guihard-Costa and Ramirez-Rozzi 2004); between the ages of 2 and 3 years, a change in the pattern of cranial and cerebral growth occurs (Guihard-Costa and Ramirez-Rozzi 2004).



**Figure 1.7** Theoretical mean velocity (black curves) and distance curves (blue curves) of postnatal growth in stature for girls (dashed lines) and boys (solid lines) showing phases and pattern of human growth. The arrows point to the mid-growth spurt (around 7 years) and the pubertal growth spurt (dashed arrow around 12 years for girls and full arrow 14 years for boys). The stages of postnatal are abbreviated as follows: I, infancy; C, childhood; J, juvenility; A, adolescence; M, mature adult. Taken from Bogin 1997, p.64

The next phase is characterised by remarkably moderate and stable bone growth (Maresh 1943, 1955) and even presents a slight deceleration in long bone growth (Smith and Buschang 2004) whereas brain growth is extremely active until it ends around 7 years (Bogin 1997) or 8 to 10 years of age (Coleman and Coleman 2002; Legge 2005; Mitani and Sato 1992). This last phase includes the pre-pubertal growth spurt, when juveniles reach 70 to 90% of their adult size (Humphrey 1998). This

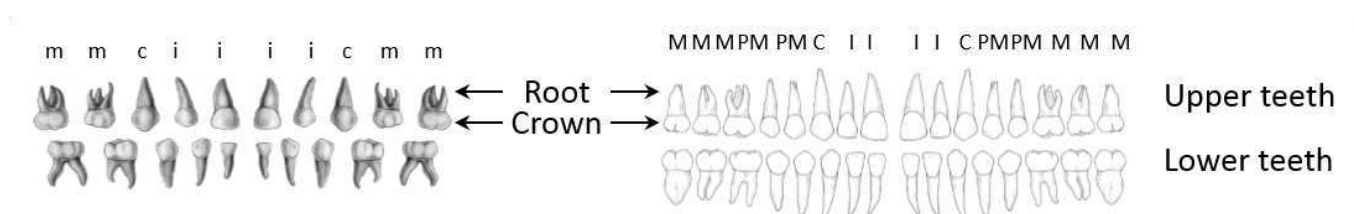
prolonged period is a unique characteristic of the human species (Bogin 1997).

After puberty, individuals enter the adolescent period which is characterised by the pubertal or prepubertal growth spurt, followed by a continuous and stable deceleration of growth (Anderson et al 1956; Bogin 1997; Smith and Buschang 2005; Tanner 1962). When bone growth ends, fusion of the secondary ossification centres begins (Coleman and Coleman 2002; Grave and Brown 1976; Tanner 1981). Although it is generally admitted that bone growth ends with SOC fusion, M. Schillaci and collaborators have observed that long bone robusticity increases throughout adulthood, via the increase of bone diameters (Schillaci et al 2012).

### 1.3.4. Dental development: growth and mineralisation

Because of their hardness a high preservation rate, teeth can be observed in living and deceased individuals in past and present populations (including fossils). They present an important range of morphological variability in both size and shape, and strong heritability (tooth development is closely monitored by genetic factors). Connective teeth are composed of a crown and a root and four types of tissues, from exterior to interior: enamel, cementum, dentine and pulp. The crown is covered by an acellular biological substance: enamel. It is secreted by ameloblasts and is rapidly mineralised to attain its mature state (Scott, *In* Katzenberg and Saunders 2008). Like most mammals, Humans present two types of dentitions (sets of teeth): the deciduous and the permanent dentition (**Figure 1.8**). The deciduous dentition appears first. It is then replaced by the permanent dentition that appears in the jaw as tooth germs, following a specific sequence (Garn et al 1957; van der Linden 1983).

In the same way as skeletal development can be subdivided into skeletal growth and maturation, dental development is characterised by dental growth and mineralisation. Mineralisation is a specific term to describe the progressive sequential apposition of layers of tooth enamel to form the crown of the tooth (Smith, *In* Kelley and Larsen 1991).



**Figure 1.8** Deciduous (left) and permanent (right) Human dentitions. Images taken from Schaefer et al 2009. M/m : molars; PM: premolars; C/c: canines; I/i: incisors

### ***Juveniles and age estimation: definitions and contextualisation***

As development is sequential and progressive for both deciduous and permanent teeth, both dentitions can overlap and be simultaneously emergent in the mouth (**Figure 1.9**): this is characterised as mixed dentition (Ubelaker 1989). Tooth formation starts during the embryonic period. The first evidence of deciduous tooth germs was found at 6 GW (AlQahtani et al 2010).

Tooth development concerns tooth formation (calcification/mineralisation, degree of development of dental crowns and roots) and tooth eruption (the process the tooth goes through to come out of the alveolar crypt to its occlusal level) and emergence (the next stage where the tooth has erupted and the alveolar bone is resorbed, and the alveolar ridge level is lower than the dental cusps) (Scheuer and Black 2000).



**Figure 1.9** Examples of mixed dentition. Left: orthopantomogram of the mixed dentition of a 9 year-old boy clearly showing the progressive emergence of the permanent premolars and canines, the emergence of the first permanent molars and the second permanent molar germs. The deciduous molars and canines are still erupted in the mouth. (Radiography courtesy of Dr B. Foti). Right: Right lateral view of the mixed dentition of a juvenile aged 6 +/- 2 years. Permanent teeth are in white, deciduous teeth are in green (taken from Ubelaker 1989)

From beginning to end, it lasts around 20 years (Smith, *In* Kelley and Larsen 1991). The ages of emergence of deciduous and permanent teeth were assessed by counting the number of emerged teeth on living patients from different populations (Hägg and Taranger 1985; Kaul et al 1992; Nyström et al 2000).

Tooth growth is characterised as appositional and incremental, increasing both in thickness and length by successive depositions of layers of enamel and dentine, to finish forming the crowns before the roots appear (Fitzgerald and Rose, *In* Katzenberg and Saunders 2008; Smith, *In* Kelley and Larsen 1991). Growth of deciduous and permanent teeth is constant from the embryonic period (emergence of deciduous tooth germs), throughout childhood (development of deciduous tooth roots and permanent crowns) to the end of adolescence (closure of the radicular apex of the third permanent molar when present) (Scheuer and Black 2000; Schmeling et al 2003a).

*In situ* tooth mineralisation was assessed pre-natally by dissecting the tissues surrounding the teeth (facial soft tissues, maxillary and mandibular bones) and post-natally using radiography (Smith,

*In* Kelley and Larsen 1991). Mineralisation sequences of deciduous and permanent teeth (Al-Qahtani et al 2010; Brauer and Bahador 1942; Demisch and Wartmann 1956; Fanning and Brown 1971; Gleiser and Hunt 1955; Gunst et al 2003; Kaul et al 1992; Kraus 1959; Kraus and Jordan 1965; Kronfeld 1935a, 1935b, 1935c; Lunt and Law 1974; Mincer et al 1993; Moorrees et al 1963a, 1963b; Nicodemo et al 1974; Nolla 1960; Orhan et al 2007; Smith, *In* Kelley and Larsen 1991; Sunderland et al 1987; Ubelaker 1978), their sequences of eruption (Schmeling et al 2003a; Smith, *In* Kelley and Larsen 1991) and their correlation with age have been extensively documented and are well-known today. They are routinely used for juvenile age estimation.

### **1.3.5. Variability, secular trends and developmental anomalies**

Variability can be found at all levels of observation and analysis; in samples (inter- and intra-population, inter- and intra-individual); in all biological factors (growth, maturation, aging, sexual dimorphism, response to pathological agents); at all ages (from the foetal period to older adults); in all anatomical regions (bones, teeth, soft tissues). It has different levels of expression and it comes from different sources (genetic, epigenetic and environmental). Its degree of expression itself is highly variable (Garcin 2009; Saunders 1992). Integrating and explaining different levels of expression for variability is an essential aspect to consider when analysing biological parameters in human populations and, of course, applies for the study of skeletal and dental growth and development.

#### **a. Skeletal growth and maturation**

Numerous references or standards on bone maturation were developed during the 20<sup>th</sup> Century (Brady 1924; Flecker 1932; Pryor 1925; Stevenson 1924; Stewart 1934). Some of them are still used today (Greulich and Pyle 1959; Sempé and Pavia 1979; Tanner et al 1962; 1975; 2001). Maturation presents inter-individual, sexual and population differences in the timing of epiphyseal fusion, one of the final and major skeletal maturation events (Ferembach et al 1979; O'Connor et al 2010; Scheuer and Black 2000; Zhang et al 2010). Even if the timing of development and the duration of morphological changes and fusion are variable (Scheuer and Black 2000), the order in which primary and secondary ossification centres appear is constant (McKern and Stewart 1957). This is why this order is qualified as the “skeletal maturation sequence” (Scheuer and Black 2000; Stevenson 1924). The sequence in which the ossification centres appear is presumably genetically determined, the same deviations from the most common sequence of ossification being manifest within the same families (Pryor 1907; Reynolds 1943; Reynolds and Schoen 1947). The hypothesis that the presence

of a maturation pacemaker could be present and active during early development comforts this statement (Gasser et al 2001). Maturation asymmetries can indeed reflect adjustments in proportional changes (Coleman 1969). This is partly why maturation is generally considered less variable than height or weight in healthy children and a good way to monitor physical development (Todd 1937). Delays or advances in skeletal maturation are assessed by comparing an individual's maturation state to a known "time-sequence" of ossification and by verifying if the individual is within the normal range of variation (Ingervall and Thilander 1972; Pryor 1925; Schaefer 2008). Timing also depends on the media used to assess it: fusion timing deduced from the study of dry bones is not the same as fusion timing deduced from bone radiographies or histological sections. In conclusion, it is admitted that maturation variability is multifactorial: it presents intra- and inter-individual, sexual, environmental and population specificities and secular trends, but is less important than growth variability (Banerjee and Agarwal 1998; Crowder and Austin 2005; Roche 1979; Schillaci et al 2012).

Growth is known to be extremely variable (Frelat 2007; Garcin 2009; Johnston 1969; Saunders 1992). Growth variability is the result of multiple influences in its expression and its levels of expression. In the same individual, body tissues do not present the same growth rates or ranges (intra-individual variability). Growth also varies according to individuals (inter-individual variability), sex, and populations. Variation is caused by genetic, environmental, epigenetic factors, with variable effects depending on their intensity, the individuals, their sex, and their age (pre-natal and post-natal effects are different) (Coleman 1969; Garcin 2009; Humphrey 1998; O'Connor et al 2010; Scheuer and Black 2000; Stull et al 2014a; Sundick 1978). Factors of variability presented here are age, sex, intra- and inter-individual patterns, environmental factors and secular trends.

During the whole growth period, there is a constant increase of inter-individual variability with age, as juveniles approach their adult format. It is well-known that inter-individual variability is important in adults, both in bone size and shape (Corsini et al 2005; Merritt 2015; White and Folkens 2005; White et al 2012). This results from the multiple factors that influenced growth and maturation during the juvenile state. Between the lower inter-individual variability observed at birth and the high inter-individual adult variability, variability increases as the individuals get older. This can be detected through differences in the onset of puberty according to sex, geographic origin and other life events that can have an impact on bone growth (Cardoso 2005, 2007; Garcin 2009; Heuzé and Cardoso 2008; Scheuer and Black 2000, 2004). This normal variation must be considered as a wide interval of possibilities for biological expression. This is why studies concerning growth must contain statistics and probabilities to model this variability (Garcin 2009).

In addition to changes in growth rates in respect of age, intra-individual variation is observed. This

level of variation is expressed by variations in growth according to anatomical regions: studies agree that growth occurs earlier in the cranial region (skull and mandible) compared to the postcranium during infancy and childhood (Scheuer and Black 2000). This can be directly correlated to the functional role of the corresponding anatomical structures: the sequence of development reflects the steps of construction of the bodily functions.

L. Humphrey identified five different groups of bone growth according to growth rates (from intermediate-very late to very fast), measured by the percentage of attainment of adult-sized bones. She found that variables presenting earliest and fastest growth were those measured on the bone structures of the cranial region. Comparatively, postcranial growth starts and ends later. The study done by L. Humphrey also clearly shows that, amongst the growth continuum, there are functional and spatial patterns of bone growth, reflecting the variation in growth patterns of the muscles associated to the bone structures to allow the corresponding functional properties. Growth patterns are therefore regionalised, to respect “energetic and functional distributions” (Humphrey 1998; Schillaci et al 2012). Growth regionalisation can also be seen between different anatomical regions of a single bone that contribute in various degrees to the general growth of the bone by following specific growth rates. This association of growth parameters between different segments is indirect proof of the general coordination of long bone growth (Cameron et al 1982; Tanner 1962).

The goal of growth studies is to show how much the extent of a particular developmental pattern is specific to modern populations (Garcin 2009; Humphrey, *in* Cox and Mays 2000). A great dispersion of values exists for older juveniles, illustrating inter-individual variability in growth rates of the body structures (Guihard-Costa and Ramirez-Rozzi 2004). P. Eveleth and J. Tanner conducted several studies on growth variation at a global scale (*e.g.* Eveleth and Tanner 1990; Tanner 1981). They found growth was more submitted to environmental factors than maturation. This postulate was also emitted by several other authors (Sundick 1978; Tupman 1962). L. Adair (2007) observed that size at birth influenced growth trajectories, adding to growth variability. Comparing variations of ontogenic trajectories gives information on control mechanisms. Differences are the biological proof of genetic variability and of the relative plasticity of biological development and the different impacts environmental factors have on bones (Meadows and Jantz 1995, 1999).

Sexual dimorphism (differences between male and female individuals) is present in both the timing of appearance and maturation of ossification centres. For example, it has been observed that females adapt more quickly to post-natal conditions than males, and therefore female development is more advanced than males of the same age. Skeletal sexual dimorphism does not appear uniformly and the levels of sexual dimorphism increase with age and are linked to growth parameters. The participation of a specific bone region to bone growth depends on the type of bone, the age and the



sex of the individual (Baughan et al 1980; Humphrey 1998; Pritchett 1991, 1992). In humans, it is expressed as differences in post-cranial elements in terms of relative contributions to the differences in growth rates and in duration of growth, for each sex. Males and females have comparable growth trajectories until adolescence, because their functional needs are comparable before sexual maturation starts (Humphrey 1998; Smith and Buschang 2005). After that, the influence of sexual hormones on biological development increases, leading to sexual dimorphism in growth and maturation patterns and timing (onsets, rates, duration) (Humphrey 1998).

Chronological and spatial evolution of individuals makes them subject to variations of temporal and environmental conditions (Rona 2000), as well as periodical socio-economic and sanitary states (Greulich and Pyle 1959). Sanitary and socio-economic conditions influence growth rates (**Figure 1.10**), so studying growth patterns can help interpret these conditions (Heuzé and Cardoso 2008).

Both growth and maturation are submitted to patternal changes due to secular trends (Cole 2003; Fishman 1982; Johnston 1996; Kalichman et al 2008; Langley-Shirley and Jantz 2010; Maresh 1972; Tanner 1981). Growth processes are neither stable, nor regular, in space and time (Buchet and Séguy 2008). Secular trends have a significant impact on growth and maturation (Scheuer and Black 2000) and can explain the differences of age corresponding to developmental milestones (menarche, adolescent growth spurt, etc.) when internal and external sources of variation change with time. Secular acceleration of ontogenic development is partly caused by societal modernisation, but its effects are limited (Herdeg 1992).

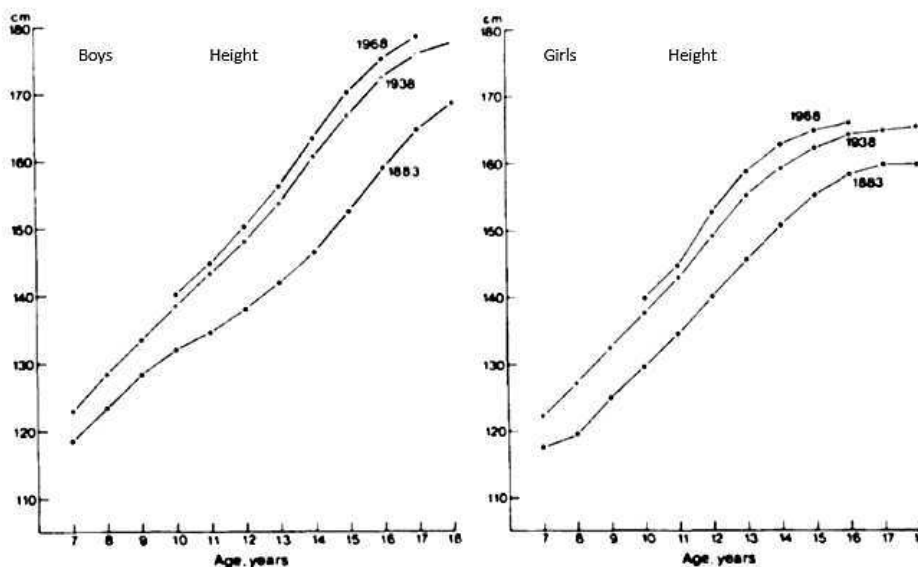


Figure 1.10 Secular trends in growth of height of Swedish boys and girls between 6 and 18 years measured in 1883 (bottom curve), 1938-1939 (middle curve) and 1965-1971 (top curve) showing that growth follows sensibly comparable patterns, but that the initial and final values increase with time. Taken from Tanner 1981

General developmental bone pathologies have been extensively studied by palaeopathologists

(Aufderheide and Rodriguez-Martin 1998; Barnes 2012; Ortner 2003; Sherwood et al 2000), radiologists, and clinicians (Risser 1958; Schmid and Moll 1960). As bone pathologies are not the object of this study, we will not expand on this subject. However, it is interesting to dedicate a few lines to anomalies in developmental rates, because they are characterised as advances or delays in development and are therefore related to time and, by extension, to age (Greulich and Pyle 1959). An anomaly can express itself as advances or delays in the appearance or fusion of ossification centres, or advances or delays in normal growth rates (Chapuis-Lucciani et al 2010; Scheuer and Black 2000). Indeed, an individual with an advance or delay in bone development presents ossification centres or entire bones outside the normal biometric or morphological range corresponding to his/her age.

The general health and nutritional status of a child influences normal development (Cardoso 2007b; Eckardt and Adair 2002; Greulich and Pyle 1959; Saxena and Saxena 1980). Developmental disorders are often expressed as delays in the appearance of ossification centres, rather than bone malformations. A delayed appearance cannot be used to assess the general maturity of an individual, until the constitutional deficiency is compensated. Evidence of delays in bone development, called skeletal stress markers, are visible on radiographies (*e.g.* Harris lines). In case of disruption of normal bone growth caused by important individual stress (malnutrition, pathologies, etc.), stress-markers can appear on the bones: instead of progressing, the growth plate remains in the same position it was in just before the stress occurred and stays there until the stress period is over (Byers 1991). Even though bone growth is momentarily slowed down or stopped, bone maturation still progresses. Therefore, the cartilage continues to ossify but because growth is interrupted, the result is an excess of bony tissue near the growth plate called “growth arrest lines” (Greulich and Pyle 1959; White et al 2012). Environmental stress also plays a part in bilateral asymmetry of long bones in particular (Albert and Greene 1999). Delays in bone growth are a bias for age estimation as they can lead to age underestimation (Franklin 2010; Rösing et al 2007; Schmeling et al 2000).

## **b. Dental development**

The dental mineralisation sequence is ontogenetically extremely stable (Franklin 2010; Scheuer and Black 2000, 2004; Smith, *in* Kelley and Larsen 1991) and less subject to environmental variability than skeletal development (Liversidge and Molleson 2004), although several factors can influence dental growth and eruption (Liversidge et al 2006; Liversidge et al, *in* Alt et al 1998). Dental developmental rate is also less sexually dimorphic than it is for skeletal development (Scheuer and Black 2000). The presence of sexual dimorphism in deciduous or permanent dental developmental

rates varies according to different studies (Cardoso 2007a; Gleiser and Hunt 1955; Saunders, *In* Katzenberg and Saunders 2008; Saunders et al 2007). Population variability however is generally admitted (Cardoso 2005; Liversidge et al 1999). It seems to be present in the time of eruption (for the canine, Smith, *In* Kelley and Larsen 1991) and in permanent teeth that emerge during late childhood or adolescence, when sexual dimorphism is also strong for skeletal development (Baughan et al 1980; Hernandez and Pena 2011).

Authors disagree on the association or independence of skeletal maturation and dental development (Lacey et al 1973). It is a fact that both processes are not controlled equally by the same factors (Franklin 2010) and have different levels of variability (Lewis and Garn 1960; Smith and Garn 1987). However, dental development is known to present high intra-individual dependency, meaning tooth development is globally homogenous for the same individual (Heuzé 2004) although some differences in relative dental development, *i.e.* different developmental rates per tooth, have been observed (Chaillet 2003).

If dental development is normal, it presents a remarkably constant regularity in the apposition of enamel layers (Lynnerup et al 2010). However, in case of biological stress, defects in enamel or in dentine apposition can appear as a reflection of the individual's status at that moment and can be microscopically and sometimes macroscopically observed. Enamel and dentine microstructures contain markers of growth, providing an "endogenous record of development" (Fitzgerald and Rose, *In* Katzenberg and Saunders 2008, p.237) that can be used for dating certain events of the individual's prenatal and postnatal life. An individual's teeth also carry traces of past individual stress, which can be observed during his/her entire lifetime. A few examples are enamel hypoplasia (external enamel defect), or the neonatal line (thought to relate the physiological stress of birth), etc. These stress markers can also be observed and studied *post-mortem*. This is done in palaeodemographic and palaeopathological studies of past populations (Lynnerup et al 2010). In conclusion, it is important to note that although dental development is less variable, its normal course can still be affected if socio-economic, sanitary status and other factors are unbalanced (Buchet et al 2002; Cardoso 2009a).

### **c. Growth and maturation in past populations**

Although several authors had already published work on growth in past populations without giving any name to the subject, A.-m. Tillier coined the term palaeoauxology to define this particular field of study in anthropology (Tillier 2000). More precisely, palaeoauxology is the study of growth rates, norms, rhythms, in ancient populations, and aims to construct skeletal growth profiles, compare skeletal growth in different past populations, and relate growth modalities to socio-

economic and sanitary statuses (Hoppa 2000; Humphrey, *In* Cox and Mays 2000; Saunders 1992, *In* Katzenberg and Saunders 2008; Tillier 2000). Palaeoanthropology can be studied following two approaches.

The first is to construct skeletal growth profiles of past populations using “classic growth curves”, *i.e.* by tracing biometric variables as a function of age of the juvenile individuals of the population. This was done by J. Martin-Gonzales and collaborators to compare the growth patterns of Neanderthal and Modern human juveniles (Martin-Gonzales et al 2012). The term “skeletal growth profiles” is preferred to skeletal growth curves because the data used to construct them is cross-sectional and not longitudinal (LampI and Thompson 2007). In archaeological populations, this means modelling the relation between skeletal measurements as a function of estimated dental or skeletal age or mean skeletal measurements as a function of annual age groups, because age is often unknown (Bolanos et al 2000; Franklin 2010; Voors and Metselaar 1958). The best model is selected, differences between growth profiles of different populations and the covariance between profiles and social, sanitary, environmental, geographical, temporal parameters is analysed and interpreted (Cole 2003; Pinhasi et al 2005; Stout and Lueck 1995; Susanne 1985; Susanne et al 2001). These skeletal growth profiles can also be compared to the growth curves of extant reference populations (Cardoso and Garcia 2009; Garcin 2009).

The second approach consists in using young adult (less than 25-30 years old) bone size as an approximal limit to skeletal growth by creating an asymptotic limit for the skeletal growth profile (Garcin 2009). Several authors have proposed the use of individual percentages of attainment of mean adult size to uniformise data (Humphrey, *In* Thompson et al 2003; Mays et al 2008).

“Growth is only constant in its variability” (Garcin 2009). This statement made by V. Garcin in her comparative study of several European past populations illustrates the extent of the limitations that come from studying growth in past and present populations and exploiting it to construct age estimation methods. Juveniles belonging to past populations are individuals who never reached adult variability (Saunders and Hoppa 1993), therefore they cannot collectively reflect the biological modalities of a normal juvenile state (Bennike et al 2005) with the exception of victims of natural disasters or sudden epidemics that do not discriminate their victims based on age, sex or sanitary state. This bias in representation needs to be considered when interpreting and comparing skeletal growth profiles.

The main problem in physical anthropology lies in applying methods constructed on contemporary populations to ancient populations whose growth and development are unknown or, at best, modelled (Garcin 2009; Saunders, *In* Katzenberg and Saunders 2008). Therefore, the relationship between age and growth or maturation variables is not constant, because, in addition to

the levels of variability previously evoked, the method used to estimate the individuals' age will also influence the outcome of the study (Garcin 2009). The exceptions to that rule are past reference populations, for which age and sex are known (through registers, civil records, engraved tombstones, etc.). Growth of skeletal and dental elements can be directly studied in these populations, with the reservations linked to working on cross-sectional data (Lampl and Johnston 1996) and their questionable position within the ranges of development normality. Considering these biases, age estimation methods could indeed be constructed directly on these past individuals.

Modelling the relationship between chronological age and biometrics provides growth norms for these populations, to do accurate and reliable comparisons with present populations, study secular trends in ancestral populations or simply compare global tendencies (Garcin 2009). Furthermore, age estimation methods constructed on referenced past populations are more likely to be applicable to other past populations whose growth and maturation could logically seem closer to past populations than living, even descending ones. Indeed, mortality rates in pre-Jennerian populations are specific to them and not applicable to other periods of time. Therefore, the specific demographic profile of these populations leads to specific skeletal profiles, and sometimes specific age estimation methods. These cases are a clear example of sample-dependency of the results, be they growth patterns or anthropological methods (Coqueugniot et al 2010; Garcin 2009; Gowland and Chamberlain 2002).

V. Garcin has exhaustively exposed the limitations in the use and interpretation of skeletal growth profiles and the study of growth in past populations in general (Garcin 2009). These limitations concern sampling (number of individuals, age and sex ratios) and methodological biases of juvenile age estimation, the informative relevancy in the use of mathematical models to interpret biological processes (Elston et al 2005; Huxley 1924; Needham 1932; Pollard 1973; Rashevsky 1954; Suppes 1960; Yoccoz 1991), and comparisons between populations. These limitations seem unsolvable, but several leads involving growth trajectories modelisation or the use of break-curves illustrating changes in growth patterns could be considered for past population studies (Garcin 2009).

The first two parts of this chapter showed that different contexts, be they bioarchaeological, forensic, or socio-economic, imply different definitions and age ranges for sub-categories of immaturity, leading to different consequences or interpretations regarding an individual, depending on his/her level of immaturity. This third part presented the additional difficulty for biological anthropologists in the form of diachronic and synchronic individual and population variability in skeletal and dental growth and development. These sources of variability in juveniles can be studied and understood when they are met, but cannot be exhaustively understood or controlled. However, anthropologists can level on their approach of juveniles by adopting methodological standardisation

for the study of juvenile individuals. It is indeed an essential aspect of juvenile age estimation.

## **1.4. Juvenile age estimation in physical anthropology: a case in point for the need of methodological standardisation**

### **1.4.1. Age as a biological and social indicator of juveniles in bioarchaeology**

Bioarchaeology aims to understand the structure and organisation of past populations through the study of the deceased. Independently of the period of study, a common problem encountered in bioarchaeology and palaeodemography is the representativity of an anthropological corpus compared to a population. Accurate representativity of a population can never be reached, as it would mean that a necropolis contained the whole population for a given site, that it was entirely uncovered by the dig and that every individual was represented. These successive sources of bias, appearing between the death of the individuals and the study of their remains mean that the living population is always under-represented. Other than preservation issues, sociocultural selectivity must also be taken into consideration when making inferences about archaeological or historical population structures (Hoppa and Vaupel 2002; Lauwers, *In* Buchet et al 2006). All these factors are biases for the interpretation of an archaeological sample, even before age estimation is attempted.

Age, sanitary state, archaeological analysis of the immediate environment (funerary structure in which the remains are found, the presence or absence of archaeological artefacts in the fossa alongside the individual, etc.) and the place of the individual amongst his/her peers, are the elements for interpreting an archaeological site and the funerary treatment that was given to the individual. Conclusions can then be interpreted at an individual and/or global scale. They allow the emission of hypotheses for the organisation of the past living population and the possible presence of age-related funerary practices that can be compared throughout chronological periods and/or geographic regions.

An interpretation of the place of juvenile individuals amongst the living can be attempted from their biological and cultural study. Biological age is the only information that can be both reliably and precisely estimated from juvenile remains (Wood and Cunningham, *In* Black and Ferguson 2011). Age estimation presents one central difficulty from which all others ensue: in past populations, developmental patterns are unknown or at best estimated through modelisation. Moreover, age cannot be considered as an equivalent to or an indicator of maturity. This is particularly true for Human fossils: the more ancient they are, the less certain their developmental patterns are. Although a juvenile state can be assessed using biological indicators of development (bone

epiphyses, deciduous teeth), the accuracy and reliability of individual age are uncertain.

Knowing the precise age of the juvenile individuals of a population helps detect and precisely interpret possible age-related funerary practices (Buchet and Séguy 2008; Garcin 2009). High mortality and, until relatively recent periods, the absence of any social or religious status can explain the exclusion of very young children from the collective funerary space, or the presence of a space dedicated to them. This postulate has to be moderated however, as there are regional, traditional, cultural and religious specificities to funerary practices for juveniles in general, and very young juveniles in particular (Alduc-Le Bagousse, *In* Buchet et al 2006). Studies of funerary practices from specific periods, the spatial organisation of necropolis and of mortality curves constructed using the age distribution of a population show that juveniles also held a particular place amongst the deceased (for example, the space dedicated to foetal individuals in the Gallo-Roman necropolis of Chantambre, Murail, *In* Buchet 1997).

The differences in funerary practices can also appear amongst the same age groups. In these cases, age is not the only discriminant factor for differentiating funerary practices. It is completed by archaeological or archivist data to help interpret the differences observed. In medieval Europe, juvenile funerary practices were dictated by religious authorities and differed if the child was baptised or not and if the child was still-born or not: unbaptised children did not have access to sanctified church grounds. They were mostly buried near non-religious structures (Treffort, *In* Buchet 1997). Confronting this historic information with archaeological and anthropological data can help understand the organisation of the necropolis and the society behind it (*e.g.* Gourdon et al 2009).

Quantitative age estimation of juveniles is necessary for palaeodemographic studies and for comparing different populations of similar or different chronological periods. This is possible because age expressed in years is less subjective than terms such as newborn, infants, children, etc. that cover several months or years (Buchet and Séguy 2008). Precision of age influences the reliability of a palaeodemographic study. Quinquenal palaeodemographic age groups regularly but arbitrarily subdivide the continuum that is a juvenile state. The first goal of these age groups is assessing mortality rates in past populations. When the estimated age covers several age groups, the postulate is to attribute the individual in the age group that includes the mean age. However, the probability of belonging to the adjacent age group(s) is not zero, as it is covered by the error risk. To remedy this, several solutions were suggested. Some authors have proposed to do a statistical subdivision of individuals in age groups (Bocquet and Masset 1977; Buchet and Séguy 2008). This allows the distribution of the individuals in the age group with the highest probability. It can be done for single individuals or groups of individuals using a common single indicator (Buchet and Séguy 2008).

In any case, age is indispensable for any bioarchaeological or palaeodemographic study implicating juveniles.

#### **1.4.2. Age as an individual identifier of juveniles in forensic anthropology**

Identifying a deceased individual is a prerequisite for officially declaring the individual's death. It is the basis of any criminal, collective catastrophe or war crime investigation (Schmeling et al 2007). A precise and reliable age estimate is indeed expected and required by legal authorities (Franklin 2010). Age-at-death is an essential component of the biological profile to restrain possibilities for identification. It is the only parameter that can be precisely estimated from juvenile remains, although precision greatly depends on the method and the element (Wood and Cunningham, *In Black and Ferguson 2011*). Juvenile age estimation methods in a forensic context must provide the necessary scientific arguments to address the demands of the Court, and, in the United States, they have to respect the Daubert criteria: methods must be constructed on referenced populations (osteological reference collections, or other equivalents), provide a reliability superior or equal to 95%, and give an estimate of age with known precision (ideally, less than a year) (Dirkmaat et al 2008; Wood and Cunningham, *In Black and Ferguson 2011*). Methods used for age-at-death estimation are those expressing the relationship between age and developmental states (**see section 1.3.**), while respecting these prerequisites set by the legal authorities in charge.

When dealing with age estimation of the living for example, experts are required to follow several recommendations and regulations to provide an age estimate (Wood and Cunningham, *In Black and Ferguson 2011*). Other than providing an indication of age, the estimate has to be associated with the probability for the person to have reached a legal threshold (Schmeling et al 2007). It must be precise and accurate and ideally consider the origins and socio-economic status of the individual, although this last condition remains debatable (**see section 1.3.5**).

An anthropologist is submitted to ethical principles concerning the handling, study, and curation of human remains in bioarchaeological and forensic contexts (Buikstra and Ubelaker 1994; White and Folkens 2005), with additional particularities when working in a forensic context (Garcin 2009; Quatrehomme 2015). In age estimation of the living, the main ethical issues concern the use of medical imaging for age estimation (Focardi et al 2014; Ramsthaler et al 2009). Many juvenile age estimation methods require the use of medical imaging without medical purposes, and most often, without the consent of the examined individual. Therefore, the use of such techniques has to be legally and ethically justified, and emitted by explicit demand by the legal authority concerned (Pruvost et al 2010; Schmeling et al 2003a). The French National Consulting Committee for Ethics



(CCNE) in Life Sciences emitted a notice on age estimation for legal purposes, precisely questioning the use of medical imaging outside a clinical context and the validity of a physical examination for pubertal assessment and suggesting the preferential use of non-ionising imaging techniques (25<sup>th</sup> November 2004 seizing of the CCNE by Mrs C. Brisset).

For the medical examiner conducting the expertise, the ethical principles of juvenile age estimation are extremely trying. Medical reasoning aims for reliable age estimation, whereas legal reasoning aims for precise age estimation. An interval covering several legal age ranges is problematic for legal interpretation; however, it is the only scientifically satisfying response (Bartoli 2006). Expertise is demanded in a context where the scope of scientific conclusions is limited by legal boundaries. Moreover, the data collected for medical and collective purposes are transformed into individual age estimations for legal purposes. The question is, what becomes of it after the report is sent and the case is closed? These ethical requirements are also valid for age estimation of the deceased.

Generally, juvenile age is estimated using the most easily accessible elements: in the living, this includes the growth of somatometric parameters, such as individual stature, or the analysis of pubertal changes using Tanner's method (Tanner 1962). These methods are imprecise because they use parameters known for presenting important individual and population disparities, and a strong influence of environmental factors (*e.g.* nutrition), hormonal factors and pathologies. This results in an extremely variable sex- and population-dependent timing of puberty, and therefore, these developmental milestones cannot correspond to absolute ages (Bartoli 2006).

The most common juvenile age estimation methods used for the living are the comparison of hand/wrist radiographies with the radiographic atlas standards of W. Greulich and S. Pyle (Greulich and Pyle 1959) constructed on American children of the 1930's-1940's, or of J. Tanner and R. Whitehouse (Tanner and Whitehouse 1959, Tanner et al 1961, 1962), constructed on British children of the 1950's. However, a study of these two methods by K. Chaumôitre and collaborators has proved that these standards do not present sufficient accuracy to be used in current cases because of secular trends that have modified developmental patterns during the past century (Chaumôitre et al 2006). Moreover, these atlases give statistical population-based standards, whose applicability for individual age assessment remains debatable.

Imprecision pertaining to bone development methods generally results in resolving to dental methods (orthopantomogram analysis) or pubertal stages to refine age estimation. However, these methods still present limitations due to individual and population variability. Juvenile age estimation remains a forensic procedure involving an important part of imprecision, whether it uses one method or a combination of methods to refine age estimates (Hill et al 1992; Huxley 1998; Reece et al 1989).

Of course, the possibilities of age estimation of the deceased depend on the state of decomposition of the body, the anatomical parts and state of the skeleton recovered (incomplete, fragmented bones and teeth), and the extent of taphonomic damage *i.e.* the quality and quantity of the material available for analysis (Bartoli 2006; Baud et al 1986; Bello et al 2002; Guy et al 1997; Schmeling et al 2007). Several age estimation methods can be used on the available bones. The estimate is often the result of the combination of skeletal and dental elements, to take into consideration the influence of internal and external factors on bone development, while also including less variable parameters, such as dental development (Conceicao and Cardoso 2011; Helm 1990; Lewis 1991).

Although these material considerations are an important because unavoidable argument for method selection, objectively and scientifically justifying the choice of a method can only be done by using parameters of the method itself. Several studies have addressed the concerns and conditions of age estimation in a forensic context (Schmeling et al 2003a, 2007, 2008) and have presented a way to objectively and effectively evaluate the quality of a method: methodological standardisation.

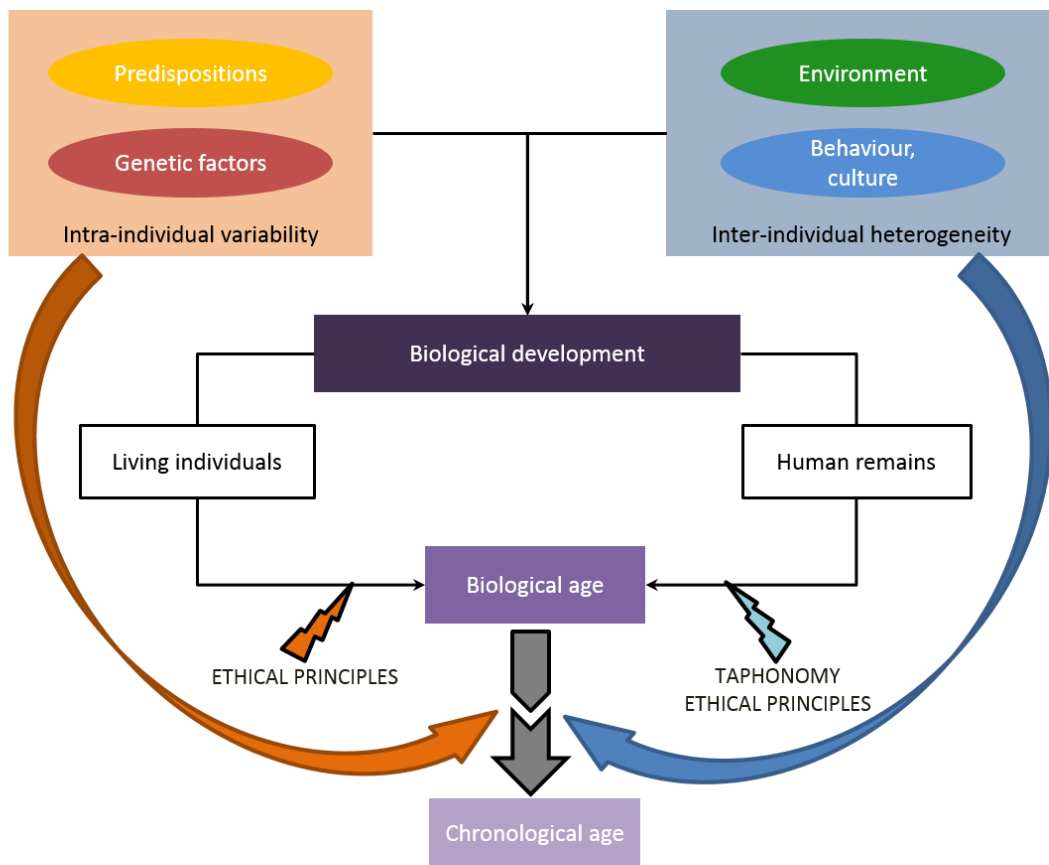
### **1.4.3. The importance of standardised juvenile age estimation protocols and methods**

As presented throughout this chapter, the heart of the problem for juvenile age estimation in anthropology lies in the absence of strict concordance between the social, legal and biological (physiological, dental and/or skeletal) notions of juvenility and the corresponding age ranges (Buchet and Séguy 2008; Garcin 2009). This multi-faceted definition of juvenility is why it is particularly difficult to estimate age in bioarchaeology or forensic anthropology, as most of the time only the biological part of this definition is attainable through the study of the individuals' remains. Remains are also subject to taphonomic damage, adding yet another bias to age estimation (**Figure 1.11**).

As well as the discordance between biological age and chronological age, a relative discordance also exists between the different types of biological ages. This is due to different levels of developmental variability of the elements used as estimates (teeth, or bones). Each presents developmental patterns that are more or less influenced by internal (genetic factors, biological predispositions) and external factors (environmental, cultural). The impact of geographic origin or ancestry on age estimation is still subject to debate as to whether or not it should be considered in age estimation (Cunha et al 2009; Schmeling et al 2007; Thevissen et al 2010). Some authors or legal instances suggest that socio-economic indicators should also be included in age estimation, to complete the biological age estimated using scientific criteria with information on social context

(CCNE; Heuzé and Cardoso 2008). The fact remains that biological age is variable, as it depends on the element used to estimate it (**Figure 1.11**).

Methods available for ae estimation have different potentials, limitations and biases both in construction and application. These need to be known prior to method application. Error risks are mainly due to intrinsic factors of the biological parameters exploited for method construction and to methodological defects. Heterogeneous and population-specific bone and tooth development (Cunha et al 2009; Franklin 2010; Schaefer and Black 2005).



Modified from Kemkes-Grottenhaler,  
(In Hoppa and Vaupel 2002)

**Figure 1.11 Summary of biological developmental factors, their characteristics, their use for biological age estimation and the limits implied by the lack of complete consistency between biological age and chronological age**

Moreover, methods often give results that are meant to be used for statistical rather than individual analysis, increasing the risk of outliers when applying the methods (Garcin 2009) to other samples or individuals.

The general incoherence in juvenile age estimation is also related to the “quality” of the method used to estimate biological age, as it will determine the reliability, accuracy and precision of the

estimated age, and therefore, the extent of the difference with chronological age. Most of the methods currently used for juvenile age estimation in a forensic context are sufficiently reliable, but require other methodological improvements (Schmeling et al 2007). It is a fact that a substantial number of methods do not respect the methodological requirements needed for their validation and determining error rates of combined estimates resulting from several methods remains problematic.

It is a given that different expert groups tend to use specific “methodological packages” for juvenile age estimation, so methodological divergence is found at national and international levels. Each group of experts uses its own protocol and procedures for method construction and evaluation (Schmeling et al 2007). This leads to important limitations in comparison, reproducibility, repeatability and verification of age estimations, adding another factor for incoherent results.

Developing reliable and useful osteological methodologies in physical anthropology are the backbone of any bioarchaeological or forensic study. Therefore, methodology remains an active and essential field of research for the discipline (Garcin 2009). The development of DNA-based methods has greatly enhanced the knowledge of past and present populations, and can allow very complete and precise biological profiling (sex determination in adults and juveniles, complete DNA-profiling, etc. (Dirkmaat 2012; Quatrehomme 2015; Scheuer 2002). It is in no way in competition with osteological methods. Both types should be used complementarily when possible and always as a means to compare and complete an individual biological profile or a population study. DNA-based methods applied to skeletal or dental material are destructive, time-consuming and are greatly limited by risks of contamination, and dependent on preservation rates of dentine and collagen, especially in archaeological populations (Bello et al 2002, 2006; Stojanowski et al 2002; Walker and Johnson 1988). This justifies the development and use of non-invasive osteological methods. Evidently, all osteological methods are also submitted to variable availability, and their use is conditioned by the preservation states of the elements for their application.

Until the past decade, few attempts had been made to standardise, calibrate, evaluate or re-evaluate past age estimation methods. In 2000, some German forensic pathologists and anthropologists founded the ArbeitsGemeinschaft für Forensische AltersDiagnostik (AGFAD) or Study Group on Forensic Age Diagnostics (Schmeling et al 2000). This group is dedicated to forensic age diagnostics of the living and methodological standardisation and evaluation. In 2003, A. Schmeling and his collaborators working with AGFAD published a series of recommendations on juvenile age estimation of the living and the deceased in a forensic context (Schmeling et al 2003a). Generally speaking, to be considered valid, anthropological methods must respect several construction and application criteria. These criteria comprise valid sampling parameters (samples of sufficient size, of known age and sex, and with homogenous age and sex ratios), resorting to statistical testing to

construct the methods, attaining sufficient statistical significance in the results (at least 95%), and, finally, validating the methods on independent test samples (Adalian et al 2002; Schmeling et al 2007). These requirements are the proof of the scientific quality of a method. Moreover, to ensure methodological quality, the methods used by AGFAD are revised every year: by conducting ring tests to check whether methods are still sufficiently reliable, precise and accurate to be used in a forensic context (Schmeling et al 2000, 2003a, 2003b, 2007). Standardisation of juvenile age estimation methods would benefit any study in all anthropological contexts, because it would reduce and ultimately eliminate the bias due to methodological discrepancies, which until now is one of the obstacles limiting valid comparisons between age estimates obtained by different methods.

Anthropologists agree on the respect of methodological quality (Cunha et al 2009; Dirkmaat et al 2008; Schmeling et al 2007), the standardisation of individual tasks, the compliance to uniformity of appropriate standards on all examined series, and the use of common statistical and methodological criteria that will facilitate method comparison. Rigorous protocols are a prerequisite for the validation of any anthropological method. However, it is a fact that methods do not always respect them and that methodological standardisation is still underway (Cunha et al 2009). The Forensic Anthropology Society of Europe (FASE), founded in 2003 as a subsection of the International Academy of Legal Medicine (IALM), aims amongst other things to promote the harmonisation and standardisation of FA practice and methodology at a European level. This includes harmonising anthropological methods used to construct biological profiles (Quatrehomme 2015).

**Therefore, the first step of our study on juvenile age estimation was to conduct a detailed critical meta-analysis of methodological and statistical criteria included in methods constructed and/or applied by anthropologists today.** Our goal was to illustrate and quantify the heterogeneity of the current situation in terms of respecting methodological and statistical criteria and highlight the best path to follow for standardised juvenile age estimation methods by constructing a classification of juvenile age estimation methods.

## **Chapter 2. A critical review of juvenile age estimation methods**

In consideration of the issues raised at the end of the previous chapter, it was decided to conduct a critical analysis of juvenile age estimation methods used in bioarchaeology and forensic anthropology. A corpus of 256 well-known or less known juvenile age estimation methods was collected. The methods in the corpus range from first studies done on bone development in the 19<sup>th</sup> Century and the beginning of the 20<sup>th</sup> Century to the ones constructed in respect of methodological principles promoted today. This chapter starts with a historic review of juvenile age estimation in biological anthropology, using data from literature and the methods in our corpus, detailing the evolution of its goals, methods, and perspectives of this field of research.

This is followed by a more detailed overview of the methods in the corpus, of their composition, construction and application by categorising them following the work done by B. Smith in 1991 (Smith, *In* Kelley and Larsen 1991). The resulting methodological typology highlights general tendencies followed by juvenile age estimation methods according to the type of estimate, the type of data used as age estimators, and the type of method used to estimate age (regression equations, abacuses, etc.). This typology also enabled us to highlight the main limitations existing in juvenile age estimation, principally due to a lack of standardised sampling and statistical protocols.

This is why it was decided to conduct a thorough review of the methods in our corpus, using a set of standardised sampling and statistical criteria characterising the methods, to assess and quantify existing methodological limitations. These criteria were then used to construct a classification of the methods in our corpus, highlighting the ones respecting all valid standardised sampling and statistical criteria as the “best” that should be privileged by anthropologists. Two types of classifications were constructed: an empirical one, where the criteria were ordered following a logic of method application and construction, and an automatic one, where the order of the criteria was determined by an algorithm. Both classifications were then analysed and confronted, to present the conclusions of our critical analysis as a decisional tool that can be used by anthropologists. Recaps on juvenile age estimation are presented in boxes (grey for particular sub-sections, brown for general conclusions).

## **2.1. Evolution of age estimation concepts and principles: from clinical or empirical studies to statistically robust methods**

### **2.1.1. First occurrences of juvenile age estimation (1940's and before): understanding biological development**

The beginning of the 20<sup>th</sup> Century was marked by a relatively important number of studies on juvenile bone growth (Borovansky and Hnevkovsky 1929; Krogman 1941) with a special focus on foetuses (Balthazard and Dervieux 1921; Flecker 1932; Ford 1956; Hesdorffer and Scammon 1928; Noback 1943; Scammon 1937; Scammon and Calkins 1923, 1929), maturity assessment of bones (Brauer and Bahador 1942; Davies and Parsons 1927; Galstaun 1930, 1937; Hrdlicka 1942; Paterson 1929; Reynolds 1945, 1947; Todd 1937) and teeth (Cattell 1928; Hurme 1948, 1949; Schour and Massler 1941). The primary aim of growth and development studies of children was for clinical applications, to check if skeletal and dental development were normal (Elgenmark 1946; Hurme 1949; Klein et al 1937). Age estimation from skeletal or dental development as a methodological goal came only later, with a few early exceptions (Balthazard and Dervieux 1921; Stevenson 1924). The notion of timing of ossification and dental mineralisation sequences, appearance of bone centres, of tooth germs, etc. was the first step towards direct age estimation (Bengston 1935; Brady 1924; Cope 1920; Pryor 1925; Sidhom and Derry 1931; Stewart 1934). Only a few age estimation methods were published as such (Klein et al 1937; Scammon 1937; Stevenson 1924), although both types of approaches are used in practice for age estimation.

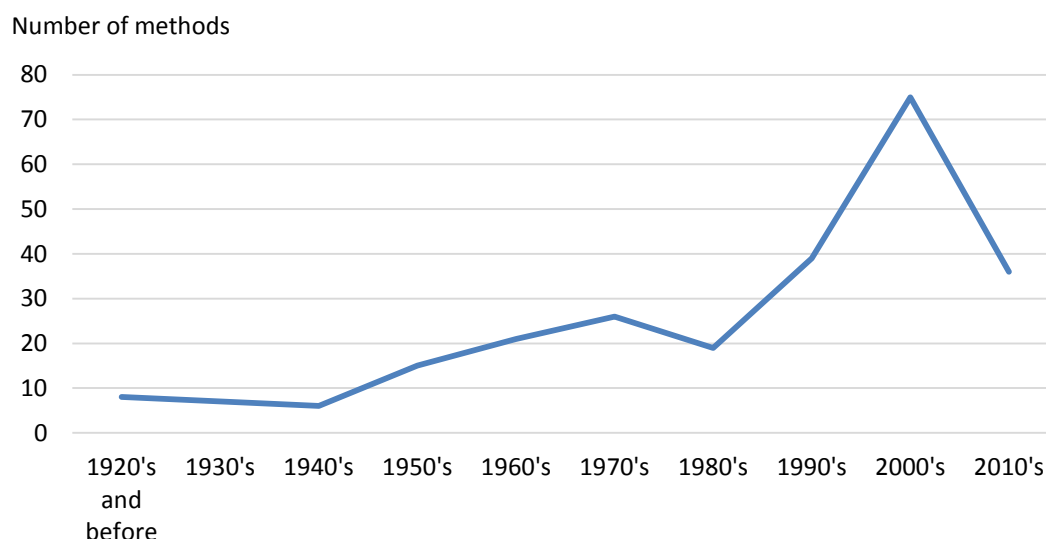
The standardisation of the use of radiography (roentgenography) as a mean to assess skeletal and dental development became more and more present (Davies and Parsons 1927; Flecker 1932; Hess et al 1932; Maresh and Deming 1939; Paterson 1929; Reynolds 1945; Sidhom and Derry 1931) and allowed the expansion of the knowledge of biological development by exploring the internal structures of bones and teeth on living individuals or without destroying them if they came from cadavers or skeletal remains. This led to the study of extensive samples of living individuals (Elgenmark 1946; Galstaun 1937; Paterson 1929; Pryor 1925) and of archaeological remains or osteological collections (*i.e.* dry bones) that were beginning to be gathered for anthropological research purposes, such as the Hamman-Todd collection and the Western Reserve osteological collection (Hrdlicka 1942; Krogman 1941; Stevenson 1924; Stewart 1934; Todd and d'Errico 1928; Vallois 1946). Other osteological collections began to be constituted in the USA and in Europe at that time (Buikstra, *In* Buikstra and Beck 2008). However, these ancient collections are often

characterised by the lack of consistency between recorded and true biological data (Owings-Webb and Suchey 1985) and are often biased in their composition (only male individuals, very few young individuals, etc.).

The growth and maturation studies from the pre-1950's are valuable by their exploratory approach that led to the knowledge and continued interest in human skeletal and dental growth and developmental patterns, which ultimately paved the way towards their exploitation for juvenile age estimation methods and their application in archaeological, forensic and legal contexts.

### **2.1.2. Juvenile age estimation (1950's-2000's): exploring, improving and testing**

Based on our corpus, the number of published juvenile age estimation methods increased from the end of the 1940's, with an important spurt occurring between 1980 and 2000 (**Figure 2.1**).



**Figure 2.1 Evolution of the number of published works on juvenile age estimation by decade (the data used for this plot comes from the corpus of 256 methods collected for this study)**

From the 1950's to the 1980's, the successive wars implicating the US overseas led to the extensive study of the remains or cadavers of young adults and older adolescents for identification purposes in forensic contexts. W. Krogman can be considered as the first renowned practitioner of collaborations with the police that became known as "forensic anthropology." Before W. Krogman's time, the history of the field had been written mostly by the contributions of diverse "anatomists-morphologists-anthropologists" (Kerley 1978, p.160), who conducted research on variation of the human skeleton aiming to answering questions that at times arose in forensic settings and concerned individual identification (Dirkmaat 2012). A particular need for age estimation methods for young



adults led to several methods focusing on the three main epiphyses showing active maturation during that final phase of the developmental period: the sternal end of the clavicle (Owings-Webb and Suchey 1985), the iliac crest (Katz and Suchey 1986; Owings-Webb and Suchey 1985), the speno-occipital synchondrosis (Ingervall and Thilander 1972; Konie 1964; Melsen 1969, 1972; Powell and Brodie 1963) and the mineralisation and/or emergence of the third permanent molar (Demisch and Wartmann 1956; Harris and Nortje 1984).

Parallel to this, clinical studies on growth and maturation were still actively pursued (Acheson 1957; Deter and Harrist 1992; Fishman 1982; Haavikko 1970; Maresh 1955, 1970). The approach of maturation and growth assessment became more and more significant, leading to specific research in the fields of medicine and physical anthropology, to be applied on the living and/or the deceased. In response to that need, this period of nearly 60 years saw a shift in the proportion of age estimation methods and the number of studies on growth and maturation, with the first becoming more important than the second. T. Stewart and M. Trotter each published guidelines on human identification from the skeleton, with an emphasis on age estimation (McKern and Stewart 1957; Stewart and Trotter 1954).

Their example was followed by the publication of a certain number of methodological recommendations for juvenile age estimation in both contexts: bioarchaeological (Ferembach et al 1979) and forensic (Ubelaker 1987, 1989). The media of study to obtain the variables for juvenile age estimation became more and more diversified (**Figure 2.2**).

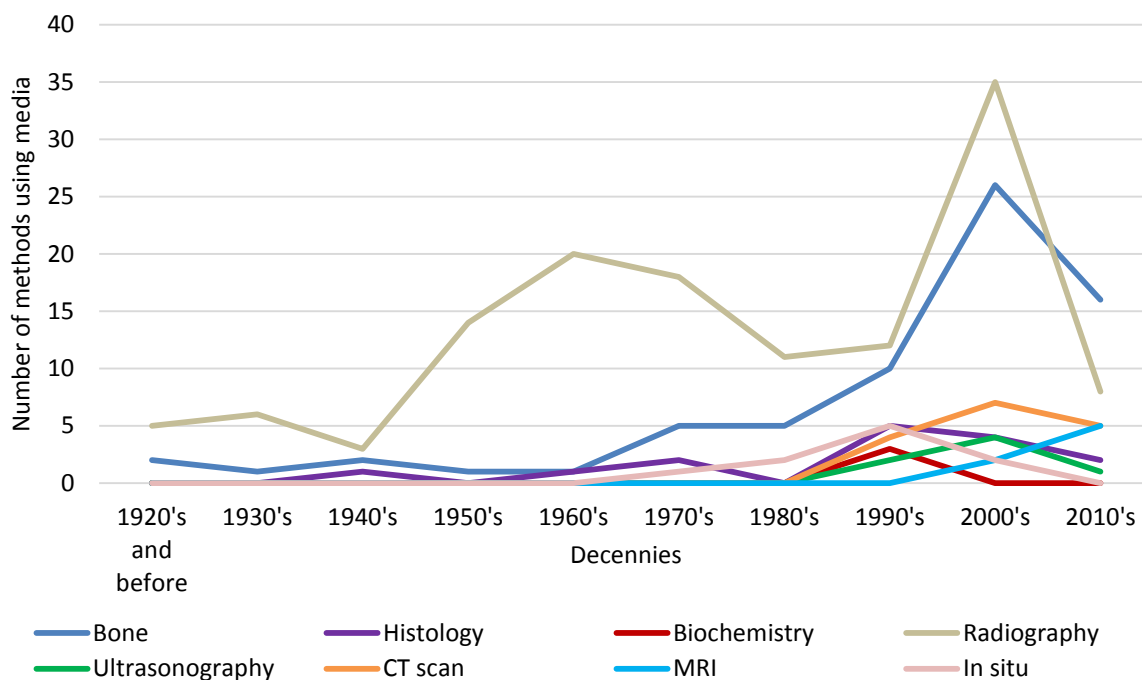
Radiography remained predominant until the mid-2000's, but with the increase in size and number of juvenile osteological reference collections<sup>2</sup> and the need for methods applicable to skeletal and dental remains, more and more methods were directly constructed on dry bones from individuals found in archaeological or modern (sometimes forensic) contexts (Black and Scheuer 1996; Johnston 1962; Liversidge et al, *In* Alt et al 1998; Liversidge et al 1999; Merchant and Ubelaker 1977; Redfield 1970; Scheuer and MacLaughlin-Black 1994; Stloukal and Hanakova 1978; Ubelaker 1978, 1989).

The invention of other medical imaging techniques in the 1950's (ultrasonography), and the 1970's (computed tomodensitometry/CT scan and Magnetic Resonance Imaging/MRI) found useful applications in physical anthropology. They allowed the exploration of internal structures of bones and teeth without destroying them, and provided durable osteological data acquisition.

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<sup>2</sup> The number of osteological reference collections with an important proportion of juveniles is still much lower than for adults. However, some reference collections are composed of an important number (or exclusively composed) of remains from juvenile individuals (Scheuer collection, Luis Lopes collection, Bologna collection, St Bride's Church, Spitalfields...) (Ardagna 2004; Cardoso 2006; Scheuer and Black 2000, 2004).

### *Juvenile age estimation: methodological issues and perspectives*



**Figure 2.2 Evolution of the media used for data acquisition in juvenile age estimation methods according to decade and medium type (the data used for this plot comes from the corpus of methods constituted for this study)**

The data was then used to construct age estimation methods that could be applied to dry bone material (Gustafson and Koch 1974; Maresh 1955, 1970; Moorrees et al 1963a and 1963b; Odita et al 1991; Young 1957). These methods were also a way to estimate age in the living by directly obtaining variables from the images (Castriota-Scanderberg and de Micheli 1995; Kreitner et al 1998; Madeline and Elster 1995a and 1995b). Histological and biochemical techniques were also explored for variable acquisition. However, they present limited applications for anthropologists as they are often destructive, time-consuming and require specialised practitioners (Calonius et al 1970; Glorieux et al 2000; Ohtani 1994; Pfeiffer and Thievens 1995; Pfeiffer et al 1995).

One major subject of publication during these years was the testing, evaluation and revision of previous methods (Aicardi et al 2000; Daito et al 1989; Nykänen et al 1998; Staaf et al 1991), sometimes as a way to assess the quality of new methods (Kullman 1995; Sauvegrain et al 1962; Stout et al 1996) or to create new methods based on older ones (Baumann et al 2009; Demish and Wartman 1956; Heuzé and Cardoso 2008; Millard and Gowland 2002; Mincer et al 1993; Wagner et al 1995). These studies provided disparate results (over- or under-estimation, inconsistent observer errors) (Tocheri and Molto 2002). Methodological heterogeneity, pointed out by a series of recommendations (Cunha et al 2009; Ritz-Timme et al 2000; Rösing et al 2007; Santoro et al 2009; Schmelting et al 2008; Schmidt et al 2008) led to a new research context in biological anthropology: method standardisation along with the improvement of accuracy and precision.

None of the age estimation systems rapidly presented above (age estimation methods, methods for growth or maturation assessment) seem ideally suited for age estimation but all can be used for that purpose. Dental and skeletal development may be such good estimators that the methods can work to some extent. “Misses” in literature are assimilated to dental variation or population differences, although some part of them might be due to methodological difficulties (Smith, *In* Kelley and Larsen 1991). Indeed, during the past decades, several studies found that age estimation methods did not always provide accurate age estimates, depending on populations (Chiang et al 2005; Haiter-Neito et al 2006; Mora et al 2001; Schnitzler et al 2009; Waldmann et al 1977). This is also true for past populations, for which developmental norms are unknown and the relationship between growth and maturation has not been extensively studied. Variation, or variability, is indeed the notion that emerged from the studies undertaken these past decades and its global apprehension is still pursued today.

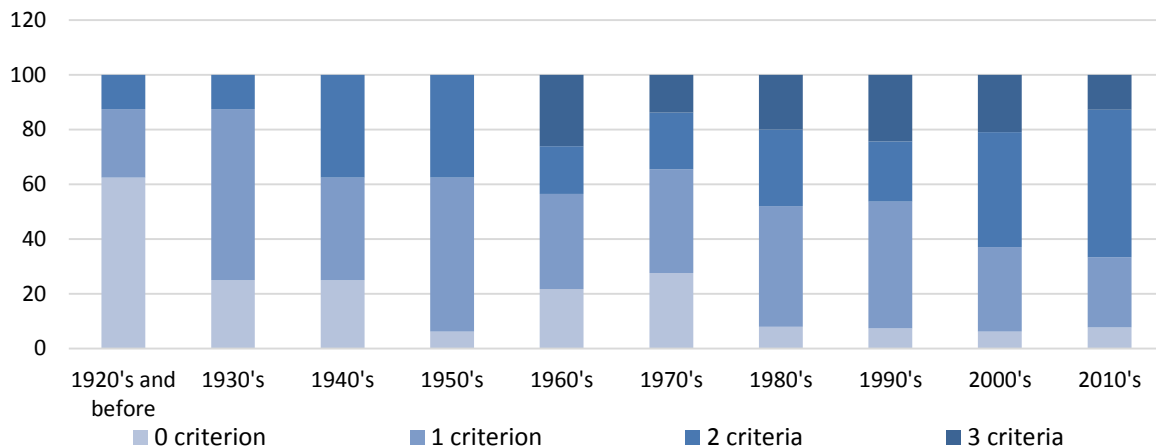
### **2.1.3. Present of juvenile age estimation (2000's-...): optimising, harmonising and standardising**

Methodological standardisation in physical anthropology could be seen with the progressive appearance and democratisation of statistical tests and parameters (Hens and Godde 2008) as a means for objective method evaluation and validation on independent reference samples (Crossner and Mansfeld 1983). This became a prerequisite for any new age estimation method constructed during that time although it was not always respected (Hassel and Farman 1995; Ogden and McCarthy 1983; San Roman et al 2002; Towlson and Peck 1990). It became clear that the objective way to compare different methods and their results, as recommended by several methodological reviews (Cattaneo 2007; Cunha et al 2009; Saunders, *In* Katzenberg and Saunders 2008; Schmeling et al 2007), was to do so using quantitative statistical and methodological criteria: mainly by providing and testing reliability, accuracy, and precision of the methods. Their inclusion in studies on development or for age estimation was progressive (**Figure 2.3**): not all criteria were included at once and, although the global tendency through time showed an increase of the number of methods including them and of the number of criteria considered, some irregularities were still present.

At this point, a differentiation needs to be made between statistical criteria, common to most methods used in physical anthropology for age estimation, stature estimation, sex determination, etc. and intrinsic methodological criteria related to method construction and application. Most of the methodological and statistical criteria identified by the Arbeitsgemeinschaft für Forensische Altersdiagnostik (AGFAD) for forensic age diagnostics of the living are also applicable for juvenile age

### *Juvenile age estimation: methodological issues and perspectives*

estimation methods constructed and/or used on skeletal remains (e.g. Baumann et al 2009; Schmeling et al 2004, 2007). Statistical criteria correspond to the level of significance given to the results and statistical tests compared to an error risk, whose threshold is formerly decided. Typically, the risk of error is set at 5%, but it can be lower (1%). It defines a level of significance of 95% for all the methodological parameters (regression equation coefficients, reliability, repeatability and reproducibility). This value of 5% is also chosen as the maximum percentage of individuals for whom age is incorrectly estimated using a given method.



**Figure 2.3 Evolution of the number of statistical criteria (reliability, accuracy and standard estimation error) in the past decades (the data used for this barplot comes from the corpus of methods constituted for this study)**

Several authors also pointed out methodological difficulties or artefacts. Most studies using similar methods give similar results, so these can represent sampling effects, giving partial, truncated distribution functions (Gowland and Chamberlain 2002; Smith, *In* Kelley and Larsen 1991). The age structure of the study sample affects the results and increases the error of estimates, in case of tailing off and truncation of samples at young ages. Another problem is the use of different scoring systems. Population differences cannot be inferred before controlling these sources of variation (Smith, *In* Kelley and Larsen 1991). Recommendations published by AGFAD and other authors all set several conditions for sample composition and method construction that need to be met for method validation and would allow objective and accurate method comparisons (Cattaneo 2007; Cunha et al 2009; Ferembach et al 1979; Schmeling et al 2000, 2003a, 2007, 2008):

- Chronological (calendar) age of the subjects must be known
- The number of individuals must be comparable for each age group to avoid systematic error, *i.e.* age ratio must be respected
- Sex must be known and sex ratio must be respected

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- The study samples must be of sufficient size for each age group and section of the population represented. The number of subjects for each sex must be at least ten times the number of observed criteria (variables, maturation stages, etc.)
- The geographical origin, the socio-economic and health status of the reference sample/population must be known
- Each sex must be analysed separately, because bone maturation and growth is different between sexes (girls mature before boys)
- The observed criteria must be clearly defined
- Examination techniques must be precisely described
- Intra- and inter-observer errors of the variables used must be known

The last few years have seen the creation of new juvenile age estimations methods, in respect of methodological recommendations. The principal characteristics that emerge from these articles are the importance of considering biological variation, and the use of objective statistical parameters that enable valid comparisons between methods. It seems that the harmonisation and standardisation objectives are now a work in progress. Recommendations still concern physical anthropology in general (Rissech et al 2013b), but a specific focus is put on forensic and legal contexts (age estimation of the living and the deceased) (Franklin 2010; Kranioti and Paine 2011; Lynnerup et al 2010; Wood and Cunnigham, *In Black and Ferguson* 2011).

All the possible biological factors, media of study, methodological criteria seem to be acquired today. Even if methodological criteria have become a prerogative for constructing reliable, accurate and precise methods, they remain strongly dependent on the objectives of the study: individual or population, forensic or bioarchaeological, living or deceased individuals (Garcin 2009).

When applying age estimation methods on juveniles of a given population, authors found that the problem was the relative population-dependency of the methods. Indeed, the methods can reflect the age and sex structures of the population used to construct them (Coqueugniot et al 2010; Gowland and Chamberlain 2002) and the developmental patterns of the population itself. This led to the recalibration of methods to provide population-specific references (Chen et al 2010b; O'Connor et al 2008; Singh and Chavali 2011; Wolff et al 2012).

When estimating age on a single individual, the difficulty lies in choosing the method that is the best suited. This means considering the population or sample composition used to construct it, as well as its statistical and methodological criteria.

Where age estimation is concerned, there are now two research paths that could still be followed. The first is to recalibrate past methods to best fit the biological reality and its variations (*i.e.* all levels of variability and secular trends). Several studies from countries in Asia, Africa and South America

have been dedicated to testing and recalibrating European and American age estimation methods of the 20<sup>th</sup> Century to make them applicable to a great diversity of populations. Although some studies had already started doing so in the past, their number has increased these past years (Büken et al 2007, 2009; Eid et al 2002; Garamendi et al 2005; Haiter-Neto et al 2006; Nyarady et al 2005; Sisman et al 2007; Tunc and Koyuturk 2008). Recalibration is also needed for applying current methods constructed on extant populations or on populations of the 20<sup>th</sup> Century to individuals from past populations whose biological variability was different (Boccone et al 2010; Cardoso 2009b; Garcin 2009; Merchant and Ubelaker 1977; Olivares et al 2014; Pinhasi et al 2005; Saunders, *In* Katzenberg and Saunders 2008). Standardised protocols regarding sampling procedures and statistical analyses are the only way to accurately and harmoniously recalibrate methods constructed on present populations to apply them to past populations and construct new age estimation methods using common objective criteria for method comparison.

The second research path is to keep trying to find user-friendly methods to obtain the most reliable, accurate and precise age estimates possible, without sacrificing the material of study, and their applicability to the greatest number of individuals.

This historical overview shows that juvenile age estimation methods in biological anthropology have followed a contextualised evolution, accompanied by an increase in scientific rigor and objectivity. In spite of methodological recommendations and the general scientific consensus concerning juvenile age estimation, there is still a possibility for improvement. In all contexts but in the forensic context in particular, juvenile age estimation still needs improvement in the results it can provide, from foetuses to late adolescents. These paths are trailed with the milestones left by past and present authors who have cleared the way by identifying the means, limits, and implications of juvenile age estimation that need to be used or surpassed to progress in this challenging field. To do this, a general apprehension and evaluation of existing methods needs to be done.

## **2.2. Methodological typology**

### **2.2.1. Method construction: objectives and exploited data**

The death of an individual stops the process of skeletal and dental development at the particular state of the individual at the moment of death. This fixed state is used as a predictor of age corresponding to that particular state. In the same way, in a living individual, the particular developmental state she/he is in at the time of examination will be used to estimate the corresponding age.

**a. Types of age estimation**

Summaries of different types of juvenile age estimation methods based on skeletal and dental growth and maturation parameters are proposed in **Tables 2-1, 2-2, 2-3 and 2-4**. The types of age estimation methods presented in these tables are inspired from the critical analysis done by B.H. Smith on dental age estimation methods for juveniles (Smith, *In* Kelley and Larsen 1991) and were adapted for this study to include skeletal age estimation methods. B.H. Smith conducted a review of several methods available to anthropologists based on tooth mineralisation and subdivided them into three main groups and eight subgroups depending on the form of the results, the type of study and the media used to obtain the predictive variables. The three main groups were:

*1. Age of attainment of developmental stages or element size.*

This type of method answers the question: at what age does a particular developmental state appear? This form of age assessment consists in constructing attainment schedules for each maturation or mineralisation stage, or for a certain value of a biometric variable (bone size). Age of attainment takes into account deviation from normality that is common in growth data. Age of attainment can be assessed on one or several elements. Methods can be presented as cumulative distribution functions, frequencies of attainment of a stage or value, the average of median age of attainment, abacuses, etc. The main limitation of these methods is the size of the study sample.

*2. Age prediction.*

This type of method answers the question: what is the age of the individual based on his/her stage of development, or size? Age is assessed by constructing methods that consider the continuity of development, by providing individual age estimates corresponding to the individuals' developmental state (maturation stage or size). Age prediction takes into account deviation from normality and often provides statistical parameters such as reliability, accuracy and precision of the prediction. Age can be predicted for one or several elements by independently estimating age for each element and calculating the mean of all ages to provide the final estimated age. Methods can provide direct estimates using mean age and standard deviation per stage or size, regression equations, calculating the age corresponding to a stage or value as the midpoint between age of appearance of that stage and the next, or providing posterior probabilities of age for a given stage or combination of stages. This last method cannot work for the final developmental stage (older individuals), because there is no further stage so no midpoint can be calculated. The limitations of such methods are data overfitting, small sample size, and sample composition (age and sex ratios).

*3. Maturity or growth assessment.*

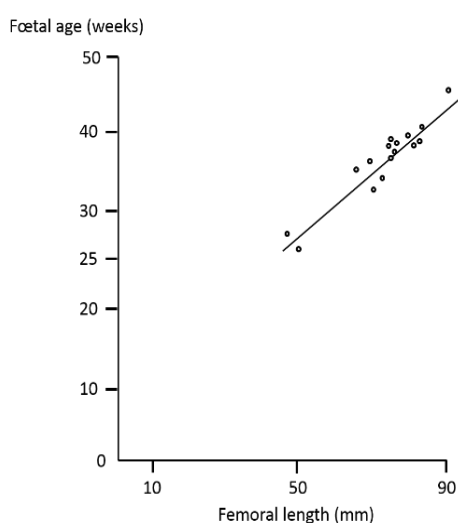
This type of method answers the question: what is the age associated with a particular maturity

state? This type of method was originally developed for the clinical evaluation of a child's overall maturity using maturity scales or for controlling growth normality using bone growth curves. It is the reverse relation of age prediction between age and the developmental variable. These maturity or growth scales can be atlases or diagrams showing the successive average maturity states and the corresponding ages or age ranges, growth curves presenting the percentiles of size per age or age range. The main limitations of these methods occur because their initial purpose was not age prediction, so their application to such matters can be questioned. They do not always provide the statistical parameters needed for method justification and as they are developmental scales, they should theoretically be constructed using longitudinal data (although this postulate is not fully accepted, **see section 1.3.3.**). Depending on the sample used, they can be too specific. They often do not allow for missing data, as maturation assessment is done as a whole (*e.g.* concurrent maturation of several epiphyses of the hand and wrist in the Greulich and Pyle atlas, Greulich and Pyle 1959) and are therefore not always applicable to human remains.

## **b. Measurements of ossification centres**

"Growth is an instrument whose biometric expression is a means to age estimation" (Adalian 2001). All age estimation methods using growth and growth parameters are based on the correlation between age and any biometric variable reflecting growth (*e.g.* stature, skeletal measurements...) (**Table 2-1**). This correlation can be expressed by two principal means:

- Growth and growth parameters can be mathematically expressed and modelled with equations and curves of the relation between a skeletal biometric variable (*e.g.* bone length, bone surface, bone



**Figure 2.4** Linear regression of age against femoral length for foetuses (Scheuer et al 1980)

volume...) as a function of time (*e.g.* age) (Twielsselmann 1969). The basis of age prediction is to mathematically express and model the relation between age and a biometric variable (**Figure 2.4**).

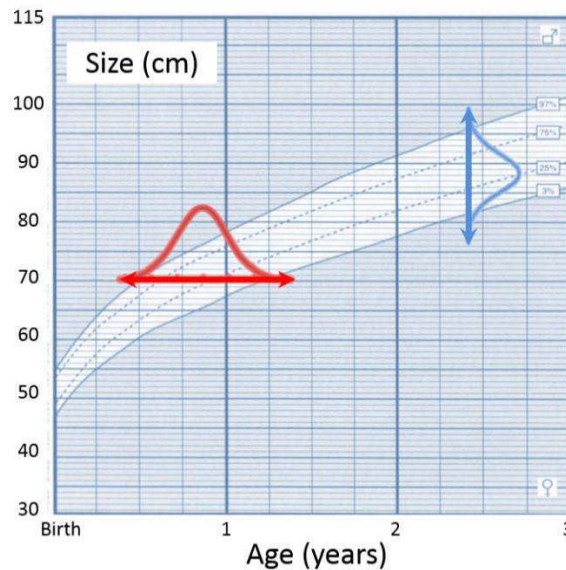
For example, L. Scheuer and collaborators modelled the relation between age and femoral length of prenatal individuals using the following linear equation (Scheuer et al 1980):

$$\text{Age (weeks)} = (0.3303 \times \text{femoral length}) + 13.5583 \pm 2.08$$

This example is the most frequent model. It uses least squares regression and inverse calibration, meaning age is considered as the dependent variable, estimated by long



bone length which is the independent variable (Cardoso et al 2014). Other functions can also be used to model the relation between age and a biometric variable (Konigsberg et al, *In Paine* 1994; Lucy and Pollard 1995). The approach aims to find the mathematical function that best expresses the degree of correlation between the two variables. The goal is to provide a biological estimate of real age that is the closest as possible to real age. The true difficulty of using a mathematical model is that it needs to express both the range of variation of the variable for a given age and the range of variation of age for a given value of the variable (**Figure 2.5**).



**Figure 2.5** Two-way normal variability of the relationship between age and size: variability of age (red normal distribution and range of values) for a given size and variability of size (blue normal distribution and range of values) for a given age

This variability is why individuals with the same chronological age can have different skeletal ages and vice versa (Olivier 1974; Scheuer and Black 2000).

Considering variability can be attempted by including a great number of individuals in the study sample, of all ages and both sexes. The complexity of the function is highly variable, depending on the complexity of the relation between age and the predictor variable.

- The correlation between age and biometric variables can also be presented as abacuses or tables. This type of method presents standard values of variables corresponding to a given age range. The range of values for the biometric variable is presented as a mean and standard error, percentiles, or directly by the minimum and maximum values found for a corresponding age or age range. For example, M. Maresh produced abacuses for the length of all limb bones of postnatal individuals (Maresh 1943, 1955, 1970). The type of age estimate, the type of method and the way it should be used (regression, equation, abacus, etc.) are mostly a matter of choice from the author. The choice is made depending on the scope of the method (population, individual), the context and the demands

of the study (is reliability to be privileged over precision, etc.) and is sometimes defined by the material used as the estimator(s). Because the relationship between growth and age is in itself complex and extremely variable on a global scale (Eveleth and Tanner 1990), it is no surprise that the inverse relation would present some level of complexity as well. Several authors argue that because of this, growth cannot be a reliable indicator of real age (Scheuer and Black 2000; Stull et al 2014a). In spite of its limitation, growth remains a much used marker of development and maturity.

The high and multiple levels of variation have led several authors to recommend the construction of population-specific age estimation methods (Cameriere et al 2007a; Liversidge et al, *In* Alt et al 1998; Liversidge et al 1999; Maber et al 2006).

**Juvenile age estimation: methodological issues and perspectives**

**Table 2-1: Methodological typology of several juvenile age estimation methods exploiting skeletal growth variables as age predictors**

Type of estimate	Type of age estimation method	Type of study	Medium of study	Goal of study	Examples
Age of attainment	Frequencies or percentages of individuals per value range and the corresponding age ranges: + Quantified distribution - Imprecision of the estimates, subject to sampling biases, not age estimation <i>stricto sensu</i>	Cross-sectional	Medical imaging, dry bone	Age estimation and growth study	Flory 1936; Hrdlicka 1942
Age prediction	Mean (and sd) age per biometric value ranges: Regression equations: + Provide mean estimate and sd with reliability and precision of the estimate known - Limits: risk of overfitting, sample size and composition	Cross-sectional	Medical imaging, dry bone, histology	Age estimation (and study)	Adalian et al 2002; Alhadlaq and Al-Maflehi 2013; Faruch Bilfeld et al 2008; Braga and Treil 2007; Cameriere et al 2006, 2007a; Cameriere et al 2008; Facchini and Veschi 2004; Fazekas and Kosa 1978; Franklin and Cardini 2007; Kunos et al 1999; Lalys et al 2006; Lopes-Costas et al 2012; Micheletti and Boccone 2004; Passalacqua 2011; Pinhasi et al 2005; Rissech and Black 2007; Rissech et al 2003, 2008, 2013a; Rissech and Malgosa 2005, 2007; Stout and Paine 1992; Stull et al 2014a
Growth assessment	Mean biometric value (and sd) per age or age group: Abacuses Tables + Give minimum and maximum biometric values per age; give age ranges - Limits: reliability not always known or sufficient	Cross-sectional and longitudinal	Medical imaging, dry bone, fresh bone	Age estimation and growth study	Anderson et al 1956, 1964; Baccetti et al 2002; Black and Scheuer 1996; Castriota-Scanderberg and de Micheli 1995; Eklof and Ringertz 1967; Facchini and Veschi 2004; Ghantus 1951; Gindhart 1973; Johnston 1962; Kimura 1992; Krogman 1941; Lang 1989; Maresh, 1943, 1955, 1970; Merchant and Ubelaker 1977; Odita et al 1991; Olivete and Rodrigues 2010; Plato et al 1981; Reynolds 1945, 1947; Saunders et al 1993a; Scheuer and MacLaughlin-Black 1994; Stloukal and Hanakova 1978; Sundick 1978; Trotter and Peterson 1970; Vallois 1946
	Growth curves: + Past and present populations - Longitudinal data is recommended for "correct" growth evaluation; population, sex, and individual specificities underestimated	Longitudinal and cross-sectional (past populations)	Medical imaging, dry bone	Growth study	Baroncelli et al 2006; Black and Scheuer 1996; Frelat and Mitteroecker 2011; Hunt and Hatch 1981; Merchant and Ubelaker 1977; Miles and Bulman 1995; Smith and Buschang 2004, 2005; Trotter and Peterson 1970

**Methods using measurements of ossification centres**

- Are useful in a clinical context to assess growth normality and predict adult height
- Can be used to estimate juvenile age in archaeological and forensic contexts, on a single individual or a more important series of individuals
- Are constructed on dry bones or medical images
- Provide individual age estimates using regression equations, abacuses, growth curves...
- Often provide statistical parameters: reliability, precision and accuracy (but not always)

Limitations:

- Sample composition
- Population and secular specificities (all ages)
- Extent of the apprehension of variability

**c. Bone maturation: morphology, maturation stages and sequences, fusion states**

Developmental changes are relatively stable and predictable: the appearance of primary and secondary ossification centres, changes in size and morphology of the centres and finally their fusion, all follow a specific order that can be dated in time and therefore, correspond to a given age or more likely, age range. This order is why we use the term “sequential maturation” to refer to the absolute and relative maturation events of the bones. A bone will follow a constant order of internal maturation events, and these events also follow a constant order in comparison with the maturation events of other bones. For example, the proximal epiphysis of the femur always starts fusing before the distal epiphysis (internal maturation sequence), and both epiphyses always finish fusing before the medial epiphysis of the clavicle starts fusing (Cardoso 2008a; Coqueugniot et al 2010; Scheuer and Black 2000). Some reserves need to be made on this postulate. If the prenatal phase of ossification centre appearance is relatively preserved from significant variations in timing, some authors have found that the dates of post-natal bone fusion are variable, and that variability increases with age (Scheuer and Black 2000).

Bone maturation is a reliable developmental indicator that has led to the elaboration of maturation sequences of all skeletal elements in relation to age. Age estimation using maturity indicators is based on the knowledge of the succession of the three types of maturation events occurring to the ossification centres (appearance, shape changes and fusion) (Wood and Cunnigham, *In* Black and Ferguson 2011) and their corresponding age ranges. A summary and typology of juvenile age estimation methods using skeletal maturation criteria can be found in **Table 2-2**.

**Juvenile age estimation: methodological issues and perspectives**

**Table 2-2 Methodological typology of several juvenile age estimation methods exploiting skeletal maturation variables as age predictors**

Type of estimate	Type of age estimation method	Type of study	Medium of study	Goal of study	Examples
Age of attainment	Cumulative distribution functions: Age of attainment of a stage for X% of individuals, until 100% is reached + Standard deviation provided + Chronology of bone formation - Limit: small sample size	Cross-sectional	Medical imaging, dry bone, <i>in situ</i>	Study and age estimation	Acheson 1957; Albert et al 2010, Albert and Maier 2013; Albert and Maples 1995; Baumann et al 2009; Cardoso et al 2013a; Elgenmark 1946; Garn et al 1967; Mackay 1952
	Average age of appearance or fusion for a maturation stage: Mean or median age and sd Frequency of individuals at a given stage/score or first appearance of a centre + Give minimum and maximum ages per state - Limits: long intervals between examinations; uneven distribution of subject ages; truncation of study groups - Less one-half interval between examinations to correct the postdatation of age of appearance				Banerjee and Agarwal 1998; Baumann et al 2009; Cardoso 2008a and 2008b; Cardoso et al 2013a; Cardoso and Severino 2010; Crowder and Austin 2005; Davies and Parsons 1927; Garn et al 1961; Humphrey and Scheuer 2006; Ingervall et al 1976; Ingervall and Thilander 1972; Jit and Kulkarni 1976; Jit and Singh 1971; Jopp et al 2010; Konie 1964; McKern and Stewart 1957; Moss and Noback 1958; Nemade et al 2010; Pryor 1925; Rios and Cardoso 2009; Sahni and Jit 1995; Todd and d'Errico 1928; Veschi and Facchini 2002; Owings-Webb and Suchey 1985; Webster and de Saram 1954
Age prediction	Mean age per developmental state: + Gives mean or median age and sd per state + Provides minimum and maximum ages per state - Limits: uneven distribution of subject ages; truncation of study groups	Cross-sectional or longitudinal	Medical imaging, dry bone	Study and age estimation	Chumlea et al 1989; Garn et al 1961; Kellinghaus et al 2010b; Roche et al 1988; Saint-Martin et al 2014; Schmeling et al 2004; Schmidt et al 2010, 2011, 2013; Whitaker et al 2002; Wittschieber et al 2013b
	Other methods: Posterior probabilities of age per stage, age-at-transition: - limited by sample composition, uneven age groups; Midpoint between mean ages at current and subsequent state Regression equations of stages or scores	Cross-sectional or longitudinal	Medical imaging, dry bone, histology, biochemistry	Study and age estimation	Bassed et al 2011; Cardoso et al 2013b; Coqueugniot et al 2010; di Gangi et al 2009; Ericksen 1991; Pfeiffer and Thievens 1995; Pfeiffer et al 1995; Ritz et al 1994; San Roman et al 2002; Stout and Stanley 1991; Thomas et al 2000
Maturity assessment	Mean developmental stage per age group: - Limits: results not comparable with other method types, importance of age interval and stage definition Probability of stage per age or age group	Cross-sectional or longitudinal	Medical imaging, dry bone, histology, biochemistry	Study and age estimation	Jit and Kaur 1989; Meijerman et al 2007
	Maturity scales/sequence /central tendency of stage for age or score calculations for age assessment: - Limits: no missing data allowed, deviation is minimised in the direction of stage/score not age	Longitudinal	Medical imaging, <i>in situ</i>	Clinical studies and age estimation	Baccetti et al 2002; Brodeur et al 1981; Noback et al 1960; Sempé 1987; Sempé and Pavia 1979; Tanner and Whitehouse 1959, Tanner et al 1962, 1975; Thiemann and Nitz 1991
	Pictorial charts and atlases: +Comparable to "age of attainment" methods - Limits: asymmetrical errors of assessment	Cross-sectional or longitudinal	Medical imaging, dry bone, histology, biochemistry	Study and age estimation	Bilgili et al 2003; Flory 1936; Gertych et al 2007; Gilsanz and Ratib 2005; Greulich and Pyle 1959; Todd 1937
	Miscellaneous	Cross-sectional or longitudinal	All media	Age estimation	Fishman 1982; Serinelli et al 2011

### ***Juvenile age estimation: methodological issues and perspectives***

Using the appearance of ossification centres as age indicators implies recording the exact date when the centre is observed. For primary ossification centres appearing during the prenatal period, this implies using medical imaging (radiography, ultrasonography) on living or recently deceased individuals (radiography, CT scan), dissecting recently deceased individuals, or directly observing dry bone remains. Studies of primary bone appearance were mainly conducted in a clinical context as a means to control the normal development of unborn children (Ogata and Uthoff 1990), therefore, most of them are not adapted to bioarchaeological contexts where the remains are dispersed and not always found. This is even more the case for primary centres at the beginning of development, when their size is inferior to a few millimetres. Particular cases in osteoarchaeology (mummies, completely preserved individuals such as bog bodies) or forensic contexts (partially decomposed individuals) can be x-rayed to assess the appearance of primary centres using the previously mentioned clinical standards.

Changes in skeletal conformation consist in analysing successive conformation stages. Changes in bone conformation have been studied using geometric morphometrics (Braga et al 2005; Chatzigianni and Halazonetis 2009; Jeffery and Spoor 2004; Morimoto et al 2008).

More frequently, studies assessing the changes in bone shape use qualitative descriptors to represent the successive bone states. The succession of states defines the maturation sequences of the bones. These sequences are obtained by identifying and coding maturation stages as a reflection of the successive phases of skeletal maturation of the primary and secondary ossification centres.

The sequence is the same for all individuals, but its rate and rhythm vary from one sample (or population) to another, according to the period concerned and the duration of the stages.

N. Cameron (Cameron 1997; Cameron, *In* Hauspie et al 2004) identified six conditions that need to be met for a maturity indicator to be useful and valid for age estimation:

- They must be universal and found for all children of both sexes
- They must appear sequentially and have the same sequence for all children
- They must easily discriminate juveniles and adults
- They must be reliable, and give similar results when repeated
- They must give a valid estimation of maturity
- They must show the complete path from the immature to the mature state

Studies involving bone maturation sequences are done by describing a particular epiphyseal bone state using a descriptive stage at the time *t* of observation.

This information is then used to give the minimum and maximum ages observed per stage, or the frequency distributions of stages per age, or to build more complex age estimation models (*e.g.* probabilistic models). The best maturation age indicators are those for which the period of fusion activity is the most limited in time (no more than a few months, or a year) as they give smaller age ranges for a given stage, *i.e.* more precise estimations of age (Scheuer and Black 2004). For example,

fusion of the elements of the occipital bone and the vertebral neural arches occur during infancy (over a period of less than 2 years).

The limits of this approach lie in the coding system of maturation stages: the use of qualitative criteria presents a certain level of subjectivity and, if it is complex, a certain level of experience may be required to use the system. The difficulty is finding the optimal number of stages and providing precise and user-friendly methods: too many stages lead to complicated and user-unfriendly methods, too few diminish precision (Saint-Martin 2014; Smith, *In* Kelley and Larsen 1991; Whitaker et al 2002; Wood and Cunningham, *In* Black and Ferguson 2011).

The chronological order of fusion is relatively constant, and is independent of age (Stevenson 1924; McKern and Stewart 1957). Variations are present in fusion timing during childhood for some bones (Cardoso 2008b; Cardoso and Severino 2010; Gertych et al 2007; Todd 1937), and become a given with the onset of adolescence (Baughan et al 1980; Moss and Noback 1958). This results in very variable age estimations depending on the methods used, because not all the ages are referenced and sex differences are not always mentioned for every method (Garn et al 1961; Humphrey 1998). As previously shown for primary ossification centres, the best maturity indicators (and age estimators) are the ones that change stages rapidly, and for which sex differences are either insignificant or greatly significant but always referenced.

The correlation between physical maturity and skeletal age is less marked when measuring the ossification ratio (the relation between the length and width of various bones), and least in the planimetry method. The reason estimation of skeletal age is less accurate when determined by the ossification ratio or by planimetry is presumably that these methods do not contain criteria for differentiation. These methods are least accurate during the years of and after puberty (Flory 1936).

S. Schmidt and collaborators (2008) separated the types of methods for assessing bone maturation into two categories:

- "Single bone" methods analyse the degree of maturation of the selected elements one-by-one and relate it to a corresponding age or age range;

- "Atlas" methods compare the maturation state of the studied individual(s) to standards obtained on study samples or populations. These standards are often sexed radiographic images of maturation states each corresponding to particular ages. Atlases can use two approaches for age estimation:

1. *Matching the general developmental state with a standard presented in an atlas.*

The age of the individual is estimated as the same age of the matching standard. For example, if the radiography of individual X bear the most resemblance to the radiography of a standard individual aged 12 years and 6 months, then that is his/her estimated age (or bone age). The difficulty of this approach is to find a suitable correspondence between the individual and a referenced developmental standard (Scheuer and Black 2000). For example, the Greulich and Pyle atlas (1959) presents standards of

hand/wrist development recorded longitudinally on living children of the 1940's to 1960's at different ages within a period of 1, 3 or 6 months.

2. *Cumulative system of maturation scores.*

Each skeletal element is observed and all the elements under study are given a stage according to their maturation state. A numerical score is then attributed to each developmental stage according to its level of development. The scores are then summed to provide a global maturity score that corresponds to an age. This approach was adopted by M. Sempé and C. Pavia (1979) and J. Tanner and R. Whitehouse (1959, revised as Tanner et al 1961, 1962) for their atlases on hand/wrist development. The main difficulty when using atlas methods or maturation diagrams is that they require the presence of all the epiphyseal elements presented in the charts to assess the general maturity of the individual. This is problematic when dealing with human remains, as elements are often missing and their position in the soil tends to be different from when the individual was alive. This is why these methods are mostly applied for age estimation of the living.

Another limitation is the "static" representation of the continuous process that is development (White and Folkens 2005). As each standard image or score value is a mean representation of a maturation state, individual variability can cause some individuals to be placed between two or more standards, therefore diminishing the precision of the estimation. This is particularly true for adolescents: because the onset of puberty is variable, the timing of the maturation sequence and the interval between the beginning and ending of fusion for each epiphysis is also variable (Lampl and Johnston 1996; Todd 1937).

The dates of fusion also depend on the method used to assess them (dry bone, radiography, histology) (Scheuer and Black 2004). Using dry bones presents the advantage of the absence of cartilage, but can lead to staging errors by mistakenly taking cartilage as bone and therefore staging the state at a higher level of maturity advancement than it really is. The general limits of age estimation methods using skeletal maturation variables are:

- Systematic and various errors of evaluation;
- Methodological objections to the constancy of the order and scheme of appearance of the centres, that are in fact individual-dependent;
- Referenced developmental stages can be separated by long periods of time;
- Distribution of the post-natal ossification centres does not follow a normal distribution, making the atlases are insufficiently reliable as variability is too high.



**Methods using the morphology and maturation of ossification centres**

- Are useful in a clinical context to assess maturity levels by studying morphological changes, maturation stages and scores with great caution (Example: ultrasounds to control foetal development)
- Can be used to indirectly estimate juvenile age in archaeological and forensic contexts from their maturation state using charts, atlases, tables
- Are constructed on dry bones, histological sections and medical images

Limitations:

- Statistical parameters (reliability, precision and accuracy) are not always known
- Static standards VS dynamic development
- Population- and sex-specificities
- Sample composition, and the extent of variability apprehension

**Methods using and providing the state of fusion of ossification centres**

- Are useful in a clinical context to detect premature fusion (pathological developmental anomalies)
- Can be used to estimate juvenile age in archaeological and forensic contexts from their fusion state using frequency or number tables, posterior probabilities
- Are less precise for age estimation from skeletal remains because of the high sexual dimorphism in adolescents and the difficulty to reliably and precisely determine sex for juveniles (age intervals are wider if sex is unknown). However, these methods are frequently used in both archaeological and forensic contexts by pooling the sexed methods

Limitations:

- Difficulty of distinguishing between stages, increase of intra- and inter-observer errors when the number of fusion stages increases, subjectivity of stage definition and identification
- Difficulty in associating observations made on dry bone and radiographic observations (the epiphyseal borders of dry bones are not included in the cartilaginous growth plates seen on radiographies)
- Population- and sex-specificity, extent of variability apprehension

**d. Dental growth: dental measurements**

Dental growth is less used than the other three developmental parameters (skeletal growth and maturation, dental mineralisation and eruption). However, a few methods were developed to predict age using tooth biometrics (**Table 2-3**).

**Table 2-3 Methodological typology of several juvenile age estimation methods exploiting dental growth variables as age predictors**

Type of estimate	Type of age estimation method	Type of study	Medium of study	Goal of study	Examples
Age of attainment	Age range for number of <i>striae</i> counted: Use of incremental growth rates of <i>striae</i>	Cross-sectional	Histology	Age estimation	Boyde 1963; Huda and Bowman 1995
Age prediction	Regression equations: + Give mean predicted age with associated prediction interval (precision) and reliability  - Limits: sample composition, goodness of fit	Cross-sectional	Dry bone and radiography	Age estimation	Aka et al 2009; Cameriere, et al 2006-2007a; Cameriere and Ferrante 2008; Deutsch et al 1985; Harris and Nortje 1984; Liversidge et al 1998,1999; Miles 1963; Mörnstad et al 1994; Olivares et al 2014
Growth assessment	Mean and sd of biometric values per age group	Cross-sectional or longitudinal	Dry bone and radiography	Growth study and age estimation	-

Their use and limitations are very similar to age estimation methods based on bone growth parameters (tables, abacuses, regression equations).

Two main quantitative variables are used for age estimation methods based on dental growth: histological criteria (number of incremental lines) and dental measurements (tooth length, root length, crown length, etc.). Quantitative data on dental development offer additional information about tooth development; they can provide more objective methods of age estimation and overcome some of the problems that come with the use of developmental stages (subjectivity in defining and identifying stages) (Cardoso 2007b).

Other methods use dental microstructures as juvenile age estimators by dating specific events recorded in tooth enamel or dentine that occurred during development, and/or by counting the number of layer appositions. Because their apposition is incremental and respects a given periodicity, it is possible to determine the amount of time that has passed between each layer and between two events. The quantification of enamel incremental lines provides the highest accuracy in age estimation from teeth because it is an absolute method without any reference to growth standards of a particular population. The disadvantages of this technique are that it is sometimes destructive, always requires laboratory facilities and experienced technicians and is both expensive and time consuming (Cardoso 2007b).

**Methods using dental measurements**

- Can be used to estimate juvenile age from human remains in archaeological and forensic contexts, on a single individual or a more important series of individuals
- Can use deciduous or permanent teeth
- Are constructed on dry bones, histological sections or medical images (radiographies)
- Provide individual age estimates using regression equations (mostly)
- Often provide statistical parameters: reliability, precision and accuracy (but not always)

Limitations:

- Sample composition
- Risk of overfitting the data
- Histological sections are destructive, time-consuming and user-unfriendly

**e. Dental maturation: mineralisation sequences and tooth eruption**














Dental age estimation exploits tooth formation (degree of completion of dental crowns and roots) and tooth eruption (the level of each tooth compared to the bone alveolar ridge).

Most dental methods and studies use maturation criteria, by identifying qualitative criteria to represent the progression of dental development (mineralisation and eruption). These criteria are often mineralisation stages or scores (**Figure 2.6**) or eruption stages.

Mineralisation is considered less variable, and it presents a higher genetic determinism than tooth eruption (Scheuer and Black 2000; Smith, *In* Kelley and Larsen 1991). The different types of dental age estimation methods for juveniles and their associated characteristics are summarised in **Table 2-4**.

This technique dates from the 1960's (Fitzgerald and Rose, *In* Katzenberg and Saunders 2008), but because it is based on histological criteria, it is destructive, or requires high-resolution medical imaging and is seldom used.

Sequences of Human dentition were established by several authors, starting in the early 1880's (Smith, *In* Kelley and Larsen 1991).

	<p>Cl: initial cusp formation</p>		
	<p>Cco: Coalescence of cusps</p>		<p>R 1/4: root length less than crown length with visible bifurcated area</p>
	<p>Coc: Cusp outline complete</p>		<p>R 1/2: root length equals crown length</p>
	<p>Cr 1/2: crown half completed with dentine formation</p>		<p>R 3/4: three quarters of root length developed with diverge ends</p>
	<p>Cr 3/4: crown three quarters completed</p>		<p>Rc: root length completed with parallel ends</p>
	<p>Crc: crown completed with defined pulp roof</p>		<p>A 1/2: apex closed (root ends converge) with wide PDL</p>
	<p>Ri: initial root formation with diverge edges</p>		<p>Ac: apex closed with normal PDL width</p>

**Figure 2.6 Mineralisation stages of permanent teeth, modified from Morrees et al 1963 (Taken from Al-Qahtani et al 2010)**

**Juvenile age estimation: methodological issues and perspectives**

**Table 2-4 Methodological typology of several juvenile age estimation methods exploiting dental maturation variables as age predictors**

Type of estimate	Type of age estimation method	Type of study	Medium of study	Goal of study	Examples
Age of attainment	Cumulative distribution functions: Age of attainment of a stage for 50% of individuals + Standard deviation provided + Chronology of tooth formation - Limit: small sample size	Cross-sectional or longitudinal	Medical imaging, dry bone	Age estimation	AlQahtani et al 2010; Brook and Barker 1972; Carr 1962; Demirjian and Levesque 1980; Fanning and Brown 1971; Frucht et al 2000; Garn et al 1958; Gates 1966; Demirjian and Levesque 1980; Haavikko 1970; Kumar and Sridhar 1990; Levesque et al 1981; Liversidge and Molleson 2004; Moorrees et al 1963a; Nyström et al 2000; Wolanski 1966
	Average age of appearance per stage or number of teeth: Less one half interval between examinations to correct the postdating of age of appearance - Limits: long intervals between examinations; uneven distribution of subject ages; truncation of study groups	Longitudinal	Medical imaging, <i>in situ</i>	Study and age estimation	Anderson et al 1956
Age prediction	Mean age per developmental state or number of erupted teeth: Gives mean or median age and sd per state + Can also provide minimum and maximum ages per state -Limits: uneven distribution of subject ages; truncation of study groups	Cross-sectional or longitudinal	Medical imaging, dry bone, living, histology	Study and age estimation	Demisch and Wartmann 1956; Fass 1969; Gleiser and Hunt 1955; Hägg and Taranger 1985; Harris and Nortje 1984; Kullman et al 1992; Olze et al 2007; Rozkocova et al 2012
	Other methods: Regression equations: limited by sample composition, goodness of fit evaluation Midpoint between mean ages at current and subsequent state Posterior probabilities of age per stage or number of teeth	Cross-sectional or longitudinal	Medical imaging, dry bone, histology, biochemistry	Study and age estimation	Braga et al 2005; Buchet et al 2006; Chaillet and Demirjian 2004; Foti et al 2003; Fu et al 1995; Griffin et al 2008; Harris and Nortje 1984; Heuzé 2004; Kaul and Pathak 1988; Kaul et al 1992; Leinonen et al 1972
Maturity assessment	Mean developmental stage or number of teeth per age group: - Results not comparable with other method types - Importance of age interval and stage definition	Cross-sectional or longitudinal	Medical imaging, dry bone, histology, biochemistry	Study and age estimation	Nolla 1960; Nanda and Chawla 1966; Nyström et al 1977
	Maturity scales/ central tendency of formation stage or eruption by age, score calculations: - No missing data allowed - Deviation is minimised in the direction of stage/score not age	Longitudinal	Medical imaging, <i>in situ</i>	Clinical studies and age estimation	Demirjian et al 1973; Demirjian and Goldstein 1976; Nyström et al 1986; Roberts et al 2008; Thomas et al 2000
	Pictorial charts and atlases of dental stages and/or eruption: + Comparable to “age of attainment” methods - Limits: asymmetrical errors of assessment	Cross-sectional or longitudinal	Medical imaging, dry bone, histology, biochemistry	Study and age estimation	AlQahtani et al 2010; Lunt and Law 1974; Schour and Massler 1941; Ubelaker 1978; van der Linden and Duterloo 1976
	Miscellaneous: mixed or unknown methodologies, observations of stages/maturation states at a certain age	Cross-sectional or longitudinal	All media	Age estimation	Calonius et al 1970; Gustafson and Koch 1974

R. Lunt and D. Law did a review of deciduous tooth formation to estimate age in fetuses and provided a precision around  $\pm 3$  GW when recalibrated for modern populations (Lunt and Law 1974). R. Kronfeld (1935), and I. Schour and M. Massler (1941) presented tables containing the ages corresponding to the first evidence of tooth calcification, the ages at which crowns and then roots are completed.

C. Moorrees and collaborators constructed two dental age estimation methods using mineralisation stages of the deciduous teeth (as well as radicular resorption stages) or the permanent teeth (Moorrees et al 1963a and 1963b). These methods are probit analyses, meaning that the age corresponding to a given mineralisation stage in the method is the age at which 50% of the individuals of the study sample presented that stage, with the standard deviation provided to include a larger percentage of individuals and increasing reliability of the estimate.

Mineralisation stages of the second permanent molar are used to estimate age until 16 years. The root finishes its development between 21 and 23 years, with a variation of 3 years (Schmeling et al 2003a, 2003b), which means that this method is sufficiently accurate for determining if an individual is older than 18, provided the apex of the root of M2 is completely closed.

Permanent dentition is mostly studied using radiographies of living children (Black and Ferguson 2011; Cunha et al 2009; Schmeling et al 2001), deciduous and permanent eruption is observed directly on the children *in situ* by tooth count (Hägg and Taranger 1985; Nyström et al 2000; Towlson and Peck 1990). The use of radiology to assess dental development and age of adolescents and young adults is common in a legal context (Cunha et al 2009; Schmeling et al 2003a; Wood and Cunningham, *in* Black and Ferguson 2011), although it is submitted to strict regulations and ethical principles (see section 1.4.2).

Dental age is a major juvenile indicator: teeth have very good preservation rates and are often recovered in archaeological and forensic contexts (Bello et al 2006; Scheuer and Black 2000), both deciduous and permanent teeth grow over very long periods of time, from the embryonic stage to adolescence; dental age is less variable than skeletal age, because deciduous and permanent tooth development is highly preserved during the pre-natal phase and less submitted to environmental fluctuations. Because of this and the high genetic determinism and stability of dental developmental factors, it is generally accepted that dental age is closer to chronological age than skeletal age. However, even if dental development is less influenced by environmental factors than skeletal development (Garcin 2009; Scheuer and Black 2000, 2004), dental age cannot be assimilated to real age (Bolanos et al 2000) because the methods used to estimate it are far from perfect. The composition of the study sample, the lower but still existent variability of tooth development that is not always fully considered (is it ever?), population-specificity of developmental rates, are but a few of the factors that explain the differences between dental age and real age. The main limitations of

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all these dental age estimation methods is the fact that because they were not constructed using the same criteria, result comparisons are not always accurate (Smith, *In* Kelley and Larsen 1991). This is problematic in forensic and bioarchaeological contexts alike, where several methods are often used and results are compared or combined to provide an estimate (Adalian et al 2006; Martrille et al 2007).

#### **Methods using dental eruption states**

- Are useful in a clinical context to assess dental maturity levels by counting the number of erupted teeth and comparing them to standards
- Can be used to indirectly estimate juvenile age in archaeological and forensic contexts (living and deceased) from their emergence state using charts, atlases, tables...
- Are constructed on medical images

#### Limitations:

- Unclear distinction between eruption and emergence, biasing estimates on dry bones
- Not suitable for single teeth
- Statistical parameters (reliability, precision and accuracy) are not always known
- Static standards VS dynamic development
- Population- and sex-specificities

#### **Methods using and providing dental mineralisation states**

- Are useful in a clinical context to detect premature fusion (pathological developmental anomalies)
- Can be used to estimate juvenile age in archaeological and forensic contexts from their mineralisation state using abacuses, regression equations, atlases, diagrams, charts...
- Can be used on isolated teeth but give better results with several teeth

#### Limitations:

- Difficulty of distinguishing between stages, increase of intra- and inter-observer errors when the number of mineralisation stages increases, subjectivity of stage definition and identification
- Difficulty to associate observations made on dry bone and radiographic observations (included teeth not always visible from the exterior) leading to the necessity of resorting to radiography
- Population- and sex-specificities
- Ethical principles controlling irradiation of living subjects

## **2.2.2. Method application: methods adapted to the subjects of study**

### **a. Skeletal age estimation**

#### *1. Skeletal age estimation of fetuses and newborns (Figure 2.7)*

Using the number and location of primary ossification centres compared to the developmental sequences of the Primary Ossification Centres (POCs) gives an estimate of the individual's age. Age is given in lunar months or gestational weeks (Bagnall et al 1982; Cunha et al 2009; Wood and Cunningham, *In* Black and Ferguson 2011).

The most precise methods use histological criteria by observing bone tissue sections (Calonius et al 1970; Piercecchi-Marti et al 2004; Scheuer and Black 2000; Wood and Cunningham, *In* Black and Ferguson 2011; Zhang et al 2011). Radiography is of limited use because most POCs appear before radiographic visualisation, as they are mainly composed of cartilage and are radiotranslucent.

On dry bones, the quality of age estimation depends on the age range of the individual and the bones examined. POCs are not often recovered because of their small size and their use is of limited interest before they reach their stage of morphological recognition (Lewis and Ruttly 2003).

Foetal bone measurements are highly correlated with age, especially long bone lengths, and are often used as perinatal age estimators using regression equations (Adalian 2001; Fazekas and Kosa 1978; Olivier and Pineau 1960; Olivier 1974; Scheuer et al 1980) or abacuses (Black and Scheuer 1996).

Cranial elements and basicranial elements in particular are the best age estimators for prenatal and perinatal individuals. Indeed, the brain is the anatomical structure that presents the highest stability in prenatal growth and maturation (Piercecchi-Marti et al 2004). The normal development of the brain requires the normal development of the structures protecting and surrounding it, *i.e.* the cranial bones, facial bones, teeth and first cervical vertebrae. These structures are the best possible reflection of normal growth and maturation if the brain is unavailable for study (as it is often the case when dealing with skeletal remains due to its rapid lysis *post-mortem*), because they are the most preserved structures of the skeleton and therefore provide reliable and accurate age estimates (Piercecchi-Marti et al 2004). They can be used as growth indicators (*e.g.* measurements of the *pars basilaris*, Fazekas and Kosa 1978; Scheuer and MacLaughlin-Black 1994) or maturation indicators (*e.g.* shifts in the morphology of the *pars basilaris*, Scheuer and MacLaughlin-Black 1994; POC fusion sequence, Humphrey and Scheuer 2006; Redfield 1970).

#### *2. Skeletal age estimation of infants, children and adolescents (Figure 2.7)*

Infants are characterised by active cranial growth and maturation: age estimation methods for that age group include the timing in fusion of the cranial fontanelles, the fusion of the cranial bones

and the general growth of postcranial bones and long bones in particular, which is constant throughout childhood. Age estimation methods for this period mostly use bone measurements as estimators (long bone diaphyseal length, widths, heights of girdle bones, etc.). Although the presence of sexual dimorphism for this period is not consensual (Marchal 1997, 2003; Rissech and Malgosa 2005, 2007; Rösing et al 2007; Veschi and Facchini 2002; Ubelaker 1987), the increasing number of studies on growth suggest that patterns may be population-specific and that age estimation methods should maybe follow the same trend. The objective is to find a simple and precise system reflecting growth and maturation parameters in a given population.

Although a geometric morphometrics approach is generally used for statistical analysis of shape, independently of size (Mitteroecker and Gunz 2009), a set of Cartesian landmark coordinates are used for statistical analysis in a mathematical metric space, to compare similarities or differences (taken as distances) of the length and direction of developmental and evolutionary trajectories. Thus it can also be used for juvenile age estimation as is the case in a few studies, to analyse the changes in shape of cranial or postcranial structures with age (Braga et al 2005; Chatziginani and Halazonetis 2009).

The use of epiphyseal fusion on dry bones is useful if their formation and fusion periods are relatively short. This process is used extensively for estimating the age of adolescents, as growth ends and maturation takes over (Parfitt et al 2000) for girls before it does for boys (Baughan et al 1980; Humphrey 1998; Lopez-Costas et al 2012; Rissech et al 2003, 2013a and 2013b). The advantage of using epiphyseal fusion is the very high number of studies on population-specific fusion patterns, so there is a high chance of finding a study of the same population as the one at hand. However, imprecision of estimation is added when working on skeletal remains, from which ethnicity cannot be reliably determined. Differences in attainment of maturation stages vary between sexes: girls mature before boys, and their Secondary Ossification Centres (SOCs) often start and finish fusing before boys' (Scheuer and Black 2000). This characteristic of skeletal maturation is often considered in maturation studies that provide sexed references for maturation states. In bioarchaeological and often in forensic contexts, because sex is unknown, the standards for girls and boys have to be used together to provide an age estimate for individuals of unknown sex. These estimates are less precise and cover larger age ranges (Black and Scheuer 1996; Cardoso 2006, 2008b; Moorrees et al 1963a).

They can be compared to maturation standards, or be converted to maturation stages used in posterior probability prediction models, or compared to previous developmental studies conducted on dry bones or radiographies. The main problems when using dry bones concern preservation rates of the bones and taphonomic actions that can damage the bones and prevent correct stage assessment (Franklin 2010; Garcin 2009; Wood and Cunningham, *in* Black and Ferguson 2011).



Because the fusion sequence can be relatively different if it is assessed on radiographies or on dry bones, the application of results obtained on radiographies to dry bone elements can lead to errors, particularly when using radiographic atlases (Cardoso and Severino 2010; Guihard-Costa and Droullé 1990; Scheuer and Black 2000; Ubelaker 1987; Veschi and Facchini 2002). Using scoring methods diminishes the risk of error, but a part of subjectivity remains when matching an observation to a static representation of the dynamic process that is maturation.

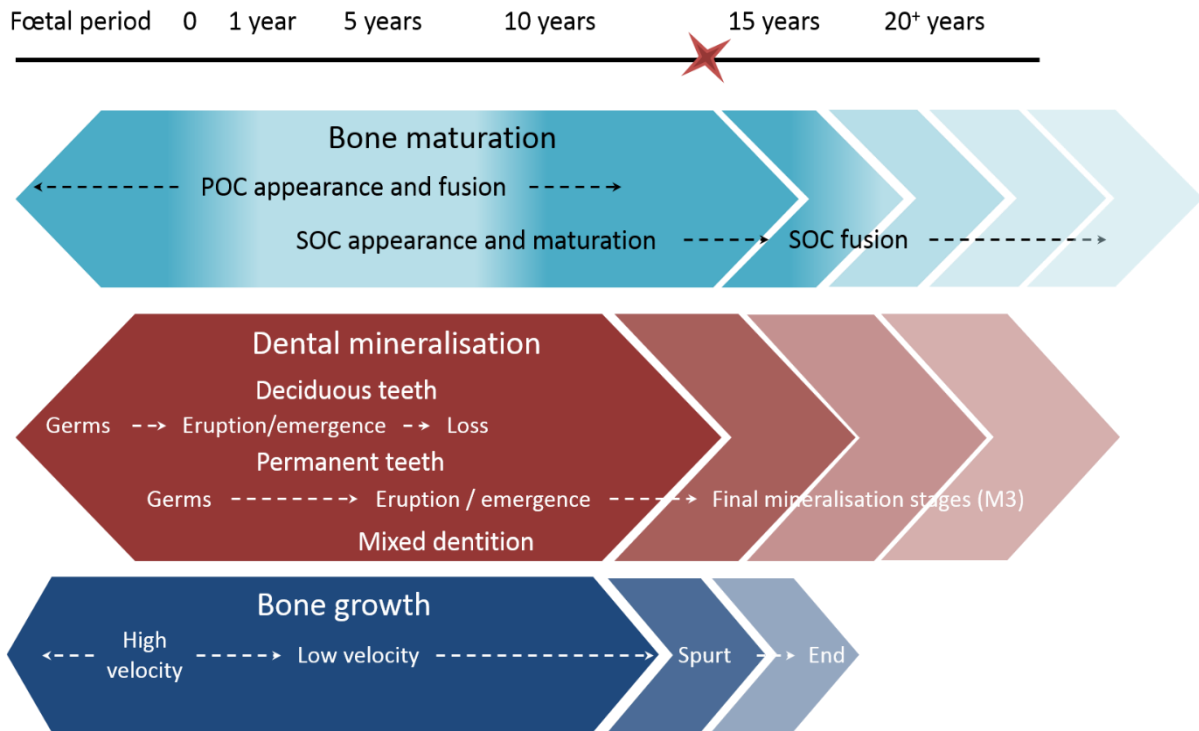
## **b. Dental age estimation**

### *1. Dental age estimation of fetuses and newborns (Figure 2.7)*

The neonatal line is present on more than 90% of all deciduous teeth and the first permanent molar. Because of the constant and incremental apposition of enamel and dentine and because these tissues record every biologically important life event of the individual (periods of stress in particular), it is relatively easy to calculate the time passed between the neonatal line and the last layer of tissue, therefore providing the age-of-death of the individual. During the foetal period, dental age can be estimated by the degree of formation and growth of the deciduous teeth and the germ of the first permanent molar, present at birth or appearing shortly after (Aka et al 2009; Boller 1964; Minier et al 2014; Schour and Massler 1941; Sema et al 2009; Ubelaker 1989).

### *2. Dental age estimation of infants, children and adolescents (Figure 2.7)*

Deciduous tooth formation can be used for age estimation of prenatal and postnatal subjects, until the deciduous teeth have all fallen from the alveolar crypts (around 10 years of age). The permanent counterparts are used for age estimation from the moment they start forming in the alveolar crypts (neonatal individuals) until the radicular apex of the third permanent molar is completely closed. This occurs between 16 and 23 years (de Salvia et al 2004; Demisch and Wartmann 1956; Harris and Nortje 1984; Kullman 1995; Kullman et al 1992; Lysell et al 1962; Mesotten et al 2003; Mincer et al 1993; Stewart 1934; Thevissen et al 2012). Both eruption and mineralisation can be used as stages (Demirjian et al 1973, Demirjian and Goldstein 1976; Gustafson 1950; Gustafson and Koch 1974; Hess et al 1932; Hunt and Gleiser 1955; Kronfeld 1935b and 1935c; Roberts et al 2008; Schour and Massler 1941) as well as tooth count (Filipsson 1975; Towlson and Peck 1990) to provide age indicators.



**Figure 2.7** Summary of the three main biological parameters used for juvenile age estimation and their developmental milestones or characteristics. The red star corresponds to the emergence of the second permanent molar, around 14 years of age; POC=Primary Ossification Centre, SOC=Secondary Ossification Centre

### 2.2.3. Limitations of juvenile age estimation

Difficulties of juvenile age estimation concern method construction and method application. Both are the source of great disparities in age estimates. Juvenile age estimation is difficult because it requires the arbitrary division of the continuous and variable process that is biological development. Dividing a continuum leads to imprecision, because an individual estimation always implies age intervals, to cover a certain percentage of the normal distribution of age. Continuity and variability imply that different developmental states can correspond to the same chronological age. Most often, age estimation comes with a degree of imprecision (White and Folkens 2005). The question is: how high is this degree?

For all the skeletal and dental elements used for age estimation, there are standards that exist, established at best on referenced populations composed of individuals of known age and sex (Cunha et al 2009; Scheuer and Black 2000). The hypothesis for applying these standards on different past and present populations is that different populations follow the same developmental periodicity. However, we have seen that this is not exactly the case, because of secular trends and other factors that had an unknown impact on these past individuals (Garcin 2009). Differences found between populations and individuals are often due to the use of different statistical methods and different

sample compositions (Smith, *In* Kelley and Larsen 1991). The problem is that results and method comparisons are difficult because the statistical criteria used for method construction are different. To address this problem, AGFAD aims to establish standards for age estimation in a forensic context, using discriminant and common statistics and exploring maturation dates in specific populations (Schmeling et al 2007). Moreover, the question of the legitimacy of comparing results obtained on skeletal or dental elements, using different methods (with specific methodological biases) raises the notion of methodological approximation for their interpretation (Garcin 2009).

Another bias in method construction results from their construction protocols. If we take the example of juvenile age estimation using long bone lengths, we find that there is a large variety of approaches used as methods: abacuses, regression equations, descriptive statistics, etc. (Johnston 1962; Hoffman 1979; Lopez-Costas et al 2012; Maresh 1955; Rissech et al 2003). The application of several of these approaches that were originally bone growth studies, not initially developed as age estimation methods, for the purpose of age estimation is questioned in the article of H. Cardoso and collaborators (2014): the standards often used for age estimation were developed to provide the range of values (minimum, mean, maximum) of bone length per given age. The samples used for these standards are either modern individuals of known age (Anderson et al 1964; Gindhart 1973; Maresh 1943, 1955, 1970) or archaeological material, for which age is often estimated (Hoppa 1992; Johnston 1962; Stloukal and Hanakova 1978; Sundick 1977). Moreover, juveniles present in archaeological series are often a biased representation of the past living population they belong to, because juvenile mortality is often the result of poor living conditions that could have affected normal development (Black and Scheuer 1996; Clark et al 1986; Garcin 2009; Johnston 1962; Saunders and Hoppa 1993; Saunders et al 1995). Therefore, age estimation methods constructed on such samples provide biased estimates (Bocquet-Appel and Masset 1982), with no means of verification.

Age prediction *sensus stricto* with age considered as the dependent variable was first attempted by T. Stewart (1934) and J. Hoffman (1969) using regression analysis. A large number of juvenile age estimation methods based on long bone growth use this approach (*e.g.* Adalian et al 2002, 2006; Facchini and Veschi 2004; Rissech et al 2008, 2013a; Scheuer et al 1980). The most frequent model used is inverse calibration, meaning age is estimated by long bone length (Cardoso et al 2014). The main bias of this approach is that the estimate errors are in the direction of long bone length and not age. Therefore, variability of age is underestimated (Cardoso et al 2014). Several authors have recommended using classical calibration, *i.e.* regressing long bone length on age, providing a model of pseudo-growth relationship, which is then inverted mathematically to obtain age (Konigsberg et al, *In* Paine 1994; Lucy and Pollard 1995). Although the estimate errors are in the direction of age,

they are more complicated to calculate, and therefore, the estimates are less efficient and variability is greater, even more so if the correlation between age and the indicator (bone length) is not sufficiently high (Aykroyd et al 1997). This approach is therefore adapted in archaeological contexts, where reliability is predominant but precision is less important as individuals need to be placed in quinquennial age groups. However, it is not applicable in forensic contexts, where both reliability and precision are essential (Cardoso et al 2014). The choice of the approach used to construct an age estimation method plays a significant role in the quality of the results and the types of bias inherent to it. However, no choices must disregard the necessity of providing objective statistical parameters (significance thresholds, standard estimation errors) that will enable method evaluation, comparison and justification.

The difficulties concerning method application reside in the fact that methods are used for particular purposes: collective age estimation in bioarchaeology or individual age estimation in forensic contexts. In the first case, the individuals are placed in quinquennial age groups. In the second case, the precision of the estimate should be less than a year, because the minimum number of years between two successive legal thresholds is often one or two (Wood and Cunningham, *In Black and Ferguson 2011*).

According to C. Lovejoy et al (1997), there are two sources of major errors in age estimation:

- Variation inherent to the development process;
- The ability of the expert to estimate an individual's age.

The first source of error relates to the different ranges of variability of growth or development. Bone growth is known to be more impacted by stress or pathologies than bone maturation, and it is in any case a more variable developmental parameter (Scheuer and Black 2000). Therefore, the age range corresponding to a given size is higher than the age range corresponding to a given maturation state. The opposite relation is also true: a given age covers a larger range of sizes than a range of maturation states. This means that age estimation methods based on growth have a higher risk of misestimation, by over- or under-estimating age if the individual is in an advanced or delayed growth state when the estimation is made. A verification of age estimation can be done by calibrating the estimates obtained with growth parameters by comparing them to the *a priori* more stable maturation state of the individual and verifying their concordance or discordance for a given age or age range. This was done for dental age estimation of adult individuals by D. Lucy and collaborators (2002), and proved to provide more accurate estimates in the extreme age ranges.

As a general rule, an age estimation method should not be applied to individuals whose growth or maturation state is not included in the range of values constituted by the study sample of the method in question: methods cannot be reliably extrapolated to lower or higher values than the ones

included in the study sample (Sellier et al 1997; Tardivo 2011). This postulate is directly linked to the fact we cannot model accurately the range of variability taken by these higher or lower values, and therefore, we cannot know the corresponding age ranges. However, when analysing methods and data taken from individuals covering large age ranges (Anderson et al 1964; Heuzé and Cardoso 2008; Gindhart 1973; Maresh 1955; Stull et al 2014a), authors found that inter-individual variability increases with age. This is why methods are often age-dependent, *i.e.* the quality of the estimates (reliability, precision and accuracy) is often better for particular age ranges (Chaillet 2003; Karki et al 2006; Reppien et al 2006). This leads to age estimation methods that are constructed on (and can only be applied to) a certain range of values, corresponding in fact to a certain age range (foetal period, adolescence), reflecting the duration of a particular growth phase or maturation sequence (Adalian et al 2002; Dedouit et al 2012; Pruvost et al 2010; Saint-Martin et al 2014; Schmeling et al 2004; Smith and Buschang 2004, 2005; Stull et al 2014a).

The second source of error can be moderated by user experience, even though it is highly dependent on the quality of the age estimation method itself. Seriation is necessary when studying a large sample of individuals before proceeding with individual age estimation. All unknown specimens must first be classified sequentially from the youngest to the oldest-looking individual. This provides a relative classification of the specimens, without any loss of reliability because the individuals are then distributed into age groups (White and Folkens 2005).

Individual age estimation does not require seriation, however the choice of the element and the method used for estimation is decisive in the quality of the results. In both contexts, a bias subsists in the subjectivity in the choice of the method used. This can be moderated by the selection of method(s) for which the statistical and methodological parameters are objectively (*i.e.* statistically) evaluated.

When working on dry bones or radiographed bones, referenced material is often used for comparison. The number of referenced osteological collections is limited so methods are often tested on living individuals (Al-Emran 2008; Chen et al 2010b; Hackman and Black 2012, 2013a, 2013b, 2013c; Kirzioglu and Ceyhan 2012; Sanders 2009), or on archaeological samples (Boccone et al 2010; Merchant and Ubelaker 1977; Olivares et al 2014; Saunders et al 1993b; Tocheri and Molto 2002). Moreover, these samples are not always documented, and they often correspond to specific populations and/or represent different periods in time, each characterised by developmental patterns more or less population-specific (Garcin 2009).

As previously evoked, the applicability of 20<sup>th</sup> Century methods to past populations, or even current populations is limited, because of secular changes (Cardoso et al 2013b). Reference atlases are often the result of studies conducted on middle-class individuals, from specific populations. The results obtained with radiographic methods are not comparable to the results obtained with dry

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bone methods: the ages of appearance and fusion of ossification centres vary according to the medium of study (Tables 2-5 and 2-6).

**Table 2-5 Conclusions drawn from the testing of four juvenile age estimation methods based on skeletal elements, as an illustration of limitations in methodological application and interpretation of the results**

Method type	Tested	Testers	Results
Skeletal growth	Hadlock et al 1982	Carneiro et al 2013 (Portugal)	Best accuracy for Adalian 2001, followed by Scheuer and Black 1994 (slight overestimation); underestimation by Fazekas and Kosa's method 1978. Other methods gave bad results: Hadlock et al 1982, Jeanty et al 1981, Olivier 1974
		Adalian et al 2002 (France)	Best accuracy for Adalian 2001, worst accuracy for Fazekas and Kosa 1978
	Fazekas and Kosa 1978	Tocheri and Molto 2002 (Archaeological, Egypt)	Inconsistencies between age estimations by all four methods (Fazekas and Kosa 1978; Redfield 1970; Scheuer and Black 1994; Ubelaker 1989)
Skeletal maturation	Greulich and Pyle 1959 (GP)	Aicardi et al 2000 (Italy), Büken et al 2007, 2009 (Turkey), Calfee et al 2010 (USA), Cantekin et al 2012 (Turkey), Carpenter and Lester 1993 (USA), Castriota-Scanderberg et al 1998 (Italy), Chaumoître et al 2006, 2007a and 2007b (France), Haavikko and Kilpinen 1973 (Finland), Hackman 2012, Hackman and Black 2012, 2013c (UK), Khan et al 2009 (USA), Kullman 1995 (Sweden), Lampl and Johnston 1996 (Mexico), Loder et al 1993 (USA), Maniar 1987 (India), Mora et al 2001 (USA), Moradi et al 2012 (Iran), Oh et al 2012 (Korea), Patil et al 2012 (India), Roche et al 1988 (USA), Serinelli et al 2011 (International)	Over- or underestimation depending on populations, sex, age and medium of study. New population standards recommended with an updating of the method. When compared with Tanner and Whitehouse's methods (TW1 1962, TW2 1975), GP is generally found less accurate
		Moradi et al 2012 (Iran), Murat-Aydin et al 2014 (Turkey), Paxton et al 2013 (Australia), Pechnikova et al 2011 (International), Santoro et al 2012 (Italy), Santos et al 2011 (Portugal), Sauvegrain et al 1962 (France), Sempé 1987 (France), van Rijn et al 2001 (Netherlands)	Sufficient accuracy, but not always sufficient for forensic studies
		Büken et al 2007 (Turkey), Bull et al 1999 (UK), Stiehl et al 2009 (Germany), Tisè et al 2011 (Italy), Wenzel et al 1984 (Austria)	Insufficient accuracy
		de Donno et al 2013 (Italy), Groell et al 1999 (Central Europe), Hackman and Black 2012 (UK), Johnson et al 1973 (USA)	High inter-observer errors and/or experience needed for scoring
		Schmelting et al 2004	Cameriere et al 2012c (Italy), Hillewig et al 2013 (Germany), Kellinghaus et al 2010a (Germany), Mühler et al 2006 (Germany), Tangmose et al 2014 (Denmark)
Baumann et al 2009 (Germany), Hillewig et al 2011 (Belgium), Schmidt et al 2008 (Germany), Tangmose et al 2013 (Denmark)	No observer errors, sufficient accuracy, applicability to MRI or CT scans		

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This is why authors sometimes find very different and even contradictory results when applying the same methods to different samples

**Table 2-6 Conclusions drawn from the testing of three juvenile age estimation methods based on dental elements, as an illustration of limitations in methodological application and interpretation of the results**

Method type	Tested	Testers	Results
Dental growth	Cameriere et al 2006 (Italy)	Cameriere et al 2012b (Italy), Cameriere et al 2007b (Peru), Rai et al 2010 (India)	Underestimation New references for the Indian population
		de Luca et al 2012 (Mexico), El-Bakary et al 2010 (Egypt), Galic et al 2010, 2011 (Bosnia-Herzegovia), Jeevan et al 2011 (India)	Overestimation, but sufficient accuracy
Dental mineralisation	Demirjian et al 1973, Demirjian and Goldstein 1976	Al-Emran 2008 (Saudi Arabia), Celikoglu et al 2011 (Turkey), Chen et al 2010b (China), Cruz-Landeira et al 2010 (Spain), Davis and Hägg 1994 (China), Eid et al 2002 (Brazil), Feijoo et al 2012 (Spain), Jayaraman et al 2012 (China), Kirzioglu and Ceyhan 2012 (Turkey), Koshy and Tandon 1998 (India), Liversidge et al 1999, 2003, 2006 (UK), Lynnerup et al 2008 (International), Nik-Hussein et al 2011 (Malaysia), Nyarady et al 2005 (Hungary), Qudeimat and Behbehani 2009 (Kuwait), Staaf et al 1991 (Scandinavia), Tao et al 2007 (China)	Overestimation, new references for populations needed, generally sufficient accuracy, absence/presence of observer errors
		Cruz-Landeira et al 2010 (Venezuela), Frucht et al 2000 (Germany), Heuzé and Cardoso 2008 (Lisbon reference collection), Lampl and Johnston 1996 (Mexico), Leurs et al 2005 (Netherlands), Mitchell et al 2009 (UK)	Underestimation, new references for populations, generally sufficient accuracy, absence/presence of observer errors
	Moorrees et al 1963a	Daito et al 1989 (Japan) Merchant and Ubelaker 1977 (Archaeological, Indian Knoll) Saunders et al 1992 (Archaeological, Canada)	Need for new population references Inconsistencies with Schour and Massler's method (1941) Sufficient accuracy, variation increases with age

Other than the few examples presented here, the inconsistencies between results of methods applied on the same sample or different samples illustrate the need for methodological harmonisation. This need is accentuated by the fact that some of the results are not even technically comparable because the methods were constructed using different approaches (Smith, *In* Kelley and Larsen 1991). This is particularly problematic when comparing two or more past populations, for which juvenile age estimation was done using different methods: as there is no way to verify the accuracy of the estimates, there is no way to evaluate them and directly compare the methods. Moreover, it seems rather far-fetched to compare ages estimated on past populations whose growth patterns were unknown or at best estimated, and who most likely expressed different levels of individual and population variability, much in the same way extant populations do. It would seem

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wise to compare estimates obtained on different populations using the same methods, if these methods provided the same results independently of factors such as sex, population origin, age, etc. but that is clearly not the case. This problem also appears when comparing different skeletal methods or dental methods amongst and between each other. In addition, it raises the question of dental VS skeletal age and the choice of the “preferable” age when it is possible, *i.e.* when both skeletal and dental remains/data are available for study. It seems difficult to occult one completely in favour of the other, as they both express different levels of individual and population reliability, providing no pathologies are present. However, calculating a “mean” age obtained from different methods AND different data seems quite imprudent (Smith, *In* Kelley and Larsen 1991) although some authors have suggested it as a way to calibrate age estimation (Bartoli 2006; Martrille et al 2007; Schmeling et al 2003a; Schulze et al 2006).

The following boxes serve as recaps of our analysis on juvenile age estimation methods: they highlight the type of data generally used for age estimation, the presentation of the results, the age groups concerned and the influence of context on the results and the methods themselves. They can serve as guidelines for method selection and provide arguments to justify the user’s choice.



**Skeletal age: exploitation of bone growth and maturation**

- Apparition of ossification centres: direct timing
  - Primary ossification centre apparition in prenatal and perinatal individuals
  - Secondary ossification centre apparition during childhood and early adolescence
- Morphological changes (size and shape): growth of the size of the centres, timing of maturation changes
  - Biometric variables used as age indicators or age predictors for prenatal individuals and prepubertal children
    - ⇒ Growth charts, abacuses, regression equations. Provide age ranges, mean age (with or w/out sd), and/or an age estimate with a corresponding prediction interval
  - Maturation stages of primary and secondary ossification centres for children and young adolescents
    - ⇒ Atlases, maturation scales, geometric morphometrics. Provide age ranges or mean age (with or w/out sd)
- Timing of fusion between two elements: direct or indirect timing
  - Fusion of primary ossification centres in prenatal individuals and young children (exception made of the fusion of the primary centres of the acetabular region during early adolescence)
  - Fusion of primary and secondary ossification centres during late childhood and adolescence. Provides age ranges for the duration of the fusion phase and for each identified fusion state:
    - ⇒ Direct timing: mean (with or w/out sd), minimum and maximum ages for a given stage, frequencies of stages per age range: atlases, maturation scales, tables
    - ⇒ Indirect timing: predicted age ranges using posterior probabilities of age, scores, and/or regression equations with stages as predictors

**Dental age: exploitation of tooth formation (growth and mineralisation) and eruption**

- Tooth growth: growth of deciduous tooth length (crown and root growth) during early childhood, growth of permanent tooth length during childhood and early adolescence
  - Biometric variables (tooth length) used as age indicators or age predictors
    - ⇒ Growth charts, regression equations
      - Provide age ranges, mean age (with or without sd), and/or an age estimate with a corresponding prediction interval
- Tooth mineralisation: apparition of tooth germs (deciduous teeth during the prenatal phase, permanent teeth during childhood), maturation of the deciduous and permanent teeth during the prenatal period (deciduous teeth and first permanent molar), childhood (deciduous and permanent teeth) and adolescence (permanent teeth and third permanent molar until early adulthood)
  - Mineralisation stages of crowns and roots formation (and root resorption for deciduous teeth) during infancy, childhood and adolescence
    - ⇒ Direct timing: mean age per stage, maturity scales, pictorial charts and atlases. Provides mean age (with or without sd), age ranges
    - ⇒ Indirect timing: average age of appearance and of each stage, sum of scores, cumulative distribution functions, posterior probabilities of age per stage or combination
      - Provide age ranges, or mean age (with or without sd)
  - Microstructural changes of enamel and/or dentine during the prenatal period, childhood, adolescence and adulthood
    - ⇒ Direct timing: microstructural standards. Provides age intervals, mean age (with or without sd)
    - ⇒ Indirect timing: Provides age estimates with associated prediction intervals
- Tooth eruption and emergence: timing of eruption and/or eruption stages of deciduous and /or permanent teeth during childhood (all teeth and both dentitions) and adolescence (second and third permanent molars)
  - ⇒ Direct timing: mean (with or w/out sd), minimum and maximum ages for a given stage, frequencies of stages per age range: atlases, maturation scales, tables

**Dental VS skeletal age**

- Bone growth is more variable than maturation: more subject to environmental stress, high level of sexual dimorphism and population variation, in rate, duration and resulting ranges of bone size
- Bone maturation follows a given sequence. Relatively high level of population variation, sexual dimorphism, in both rhythm and duration of maturation phases
- Dental development (mineralisation and eruption) is less subject to intra- and inter-population variation and sexual dimorphism although both are still present: variation in eruption times, variation of the presence of the third permanent molar
- Dental methods are more reliable and accurate (general consensus)

**Age estimation of the living (forensic or clinical context) or the deceased**

- Living: forensic or clinical context
  - *In situ* or medical imaging
  - Bone maturation: radiography of the hand/wrist, hip, knee and foot/ankle during childhood and adolescence, radiography of the sternal end of the clavicle during late adolescence/early adulthood
  - Tooth development: observation of the level of dental eruption *in situ*, orthopantomogram of the jaws, during childhood and adolescence
- Deceased (human remains): bioarchaeological or forensic context
  - Dry bones and teeth, medical imaging, skeletal or dental histological microstructures
  - Bone growth: measurements taken on dry bones, on medical images of bones, during the prenatal period, infancy and childhood
  - Bone maturation: presence/absence of centres (taphonomic bias), stages of formation and fusion, shape, assessed on dry bones or skeletal medical images, during the prenatal period, infancy and childhood
  - Tooth growth: measurements taken on isolated teeth, on medical images of teeth, during the prenatal period, infancy and childhood
  - Tooth emergence: observation of the level of dental emergence in the jaw bone, orthopantomogram of the jaws, during childhood and adolescence
  - Tooth mineralisation: presence/absence of deciduous and/or permanent teeth and tooth germs (taphonomic bias), stages of mineralisation, assessed on dry teeth or dental medical images, during the prenatal period, infancy and childhood

## **2.3. Critical review and classifications of juvenile age estimation methods**

### **2.3.1. Juvenile age estimation: method construction and method selection**

#### **a. Methodological deconstruction to understand method construction**

One question comes to mind when using an age estimation method: is it good?

Behind this question hides another question: does the estimated age come close enough to the real individual age? The term “close” reflects reliability (is the estimated age representative of real age in a sufficient number of cases?) and accuracy (is the difference between estimated age and real age small enough?). The answer to this question can be evaluated objectively using a set of statistical parameters and/or can be evaluated subjectively by deciding which parameter(s) should be privileged. This choice is mostly context-dependent. Authors agree that results must present a reliability of at least 95% to be valid. In a forensic context, the largest prediction interval must be considered to reach 95% reliability whereas in an archaeological context, the smallest prediction interval must be chosen to be the most accurate (Quatrehomme 2015; Wood and Cunningham, *In Black and Ferguson* 2011).

In reality, anthropologists are rarely confronted with the situation where one method is perfectly adapted to the goal of the study and the material at their disposal, respects all statistical and methodological criteria and gives the best results possible. Therefore, anthropologists often use several methods on the material they have at their disposal and compare the results. More or less explicitly, authors have agreed on several method construction criteria that need to be respected:

- Quantitative criteria are more objective than qualitative criteria and lead to more repeatable and reproducible methods. They need to be used to construct reliable, simple, fast and user-friendly methods.
- If possible, the results obtained on different bones can be combined to cover individual variability and obtain the most reliable or precise prediction interval (depending on the context of study). This approach considers allometric relations between bones and teeth: each bone has its own more or less specific growth and maturation patterns, and it is known that dental and skeletal development present slight discrepancies, leading to different dental and skeletal ages.

The question is now more specific: which method(s) is/are the best, *i.e.* which estimated age should be retained?

Like any anthropological method, juvenile age estimation methods are constructed following specific protocols. Protocols concern data acquisition (sampling), data analysis (statistical testing and

analysis) and validation of the results. To fulfil the requirements cited above, all these steps need to respect a strict methodological and statistical framework. To evaluate the framework used to build a method, it was decided to define methodological and statistical criteria, each referring to a particular condition of construction and/or application of the method concerned and use them as method evaluation parameters.

To do this, a large sample of referenced age estimation methods was collected. These references originate from several sources of information: original publications of age estimation methods (*e.g.* Black and Scheuer 1996; Cardoso and Rios 2011; Coqueugniot et al 2010), collations of referenced age estimation methods (*e.g.* Krogman 1941; Schaefer et al 2009), and studies on bone or dental growth and/or maturation that are used in practice for age estimation (*e.g.* Acheson 1957; Maresh 1955; Nolla 1960). These methods or studies were developed in anthropological (forensic or bioarchaeological) or clinical contexts.

The methods were selected according to the availability of the original publications, if they were cited in several reference textbooks on osteology and osteological methods (Baker et al 2005; Buikstra and Ubelaker 1994; Schaefer et al 2009; Scheuer and Black 2000, 2004; White and Folkens 2005), articles and books on juvenile age estimation in forensic anthropology (Cunha et al 2009; Gök et al 1985; Santoro et al 2009; Schmeling et al 2003a-2008; Ubelaker 2008; Wood and Cunningham, *In* Black and Ferguson 2011) or bioarchaeology (*e.g.* Ubelaker 1987).

Each method was identified by the names of the authors or the name of the first author followed by “et al” if there were three or more and the year of publication (*e.g.* Scheuer et al 1980). Criteria were either directly indicated in the published references, as was mostly the case, or they were found in other publications citing, testing, or using said reference. For example, in the 1996 publication of Black and Scheuer on the clavicle, all criteria were recorded as found and described in the original article (Black and Scheuer 1996). Criteria of Greulich and Pyle’s atlas of hand/wrist maturation (1959) were assembled by compiling the information of the original published atlas and of 60 different references citing or testing the method. If no information was available for the criteria, the modality “Unknown” was attributed. If less than half of the criteria were collected, the method was eliminated from the corpus to avoid the bias caused by too much missing information.

After selection, a total of 256 different references was obtained to conduct a meta-analysis of juvenile age estimation methods. Because foetal individuals are characterized by specific *in utero* growth and maturation patterns and are often the object of separate and specific studies (Adalian et al 2002; Schmeling et al 2007), age estimation methods specifically aimed at foetuses were not included in this study. Methods only concern post-natal juveniles, aged 0 or older. For each of these references, 21 descriptive qualitative parameters were defined (presented in bold font below),

characterising five different groups of criteria (presented in italic font below). The majority of our method evaluation criteria were selected following several methodological recommendations concerning anthropological samples and juvenile age estimation that were published in the past decades by various authors (Cunha et al 2009; Ferembach et al 1979; Schmeling et al 2000, 2003a, 2007, 2008) (**see section 1.4.3.**).

These criteria cover the element of study (bone, teeth), sampling parameters, statistical and methodological parameters of construction, application and validation, and several “transversal” parameters that can be placed in two or more groups of parameters. They can be taken as an ensemble or separately and are direct parameters for evaluating and comparing the qualities, weaknesses and biases of each method that will be used to draw conclusions on the state of juvenile age estimation in biological anthropology. The criteria are characterised by a specific number of modalities, and are the same for all references. All criteria were chosen to be as objective as possible and therefore can be considered as objective scientific arguments in case the choice of a method needs to be justified, in Court, in a report, or in any other context. 15 of the 21 criteria were ultimately included in the empirical classification, whereas only the five sampling and five statistical criteria were included in the automatic classification.

*1. Element identification criterion (1):*

This criterion is the **skeletal and/or dental element** from which the variables used for age estimation are obtained. It is either a single bone (*e.g.* humerus), several bones (*e.g.* limb bones: humerus, radius, ulna, femur, tibia and fibula), an anatomical zone (*e.g.* hand/wrist), specific states (*e.g.* deciduous teeth, mixed dentition, permanent teeth). The high number of modalities results from the variability of material used by the authors for their methods. No separation according to individual bones in the database was made for methods using several bones to avoid repetitions of references that would bias the statistical analysis by giving additional weight to methods using several different bones. However, such methods were indicated in the empirical classification for each bone concerned. For example, the method developed by K. Stull and collaborators using measurements of all six long bones (Stull et al 2014a) was indicated in the six classifications done for these bones. For the statistical classification, this criterion was combined with the reference of the method because the number of modalities was too high (*e.g.* Scheuer et al 1980 clavicle).

*2. Sample criteria (11):*

This group of ten criteria concerns the criteria related to sample composition and criteria related to the acquired variables. Criteria to characterise sample composition relate to the type of study, the number of individuals in the sample, and biological information of the individuals.

The **type of study** was either cross-sectional, semi-longitudinal or longitudinal. A cross-sectional study uses different and random individuals, who only contribute once to the sample, at a given point in time, by the developmental state they are in at that particular moment. One individual belongs to one age group. A longitudinal study follows the same individuals from a starting point to a finish point in time, and data is repeatedly collected at specific moments for each individual between these two dates (Lampl and Johnston 1996).

The **total number of individuals** in the study sample was arbitrarily subdivided into five categories from less than a hundred individuals to more than a thousand: [<100], [100-200], [200-500], [500-1000], [>1000]. The **geographical origin** of the individuals was also recorded (this mainly relates to the country of origin of the individuals, but sometimes covered “ethnic” groups) and resulted in 29 modalities.

The **age** and **sex** of the individuals used to construct the age estimation methods were also used as sampling criteria: for all methods, the fact that real age and/or sex of the individuals was known or unknown, and whether **age and sex ratios** are respected or not (comparable number of individuals of both sexes in each annual age group) is noted. The **precise age ranges** of the individuals from the study sample were also recorded for each method, resulting in 166 modalities.

Variable criteria are greatly redundant with one another: they concern the **type of factor** used for age estimation (growth, maturation, or both). This category is directly correlated to the second category, the **type of variable** used for method estimation (qualitative, quantitative or both), which is a generalisation of the third category, the specific **variable** used as an age estimator (*e.g.* bone measurement, dental mineralisation stage, etc.) for which 17 modalities were identified.

### *3. Methodological criteria (2):*

This group includes two criteria: the **form of the results** chosen by the authors (*e.g.* regression equations, frequencies, abacuses, etc.), leading to 16 modalities, and whether or not the **method** was **sexed**, *i.e.* if results are presented separately according to sex. This group also includes the **medium** used for data acquisition, resulting in 18 modalities including dry bone, radiography, computed tomodensitometry, biochemical analysis, etc.

### *4. Statistical criteria (5):*

This group includes four statistical criteria that need to be known to evaluate the predictive quality and validity of the age estimation method, independently of sampling and other parameters. These criteria are reliability, standard estimation error, accuracy and methodological validation. Two other criteria also belong to this group: whether or not intra- and inter-observer errors were tested, and whether or not the method was validated and if so, how it was done.

The **standard estimation error** (see) or standard deviation (sd) is the degree to which further calculations (or estimations) give similar results (or predicted ages) (Ferrante and Cameriere 2009). It is the standard deviation of the estimation errors. Standard error is estimated following the normal distribution of the estimation errors. The standard estimation error multiplied by 1.96 is the precision of the method associated to 95% reliability. It is often indicated under the mathematical expression +/- X years, with  $X = 1.96 * \text{see}$ .

**Reliability** is the degree of conformity between a real value and an estimated value. For an age estimation method, reliability is expressed as the confidence interval of the estimated age (White and Folkens 2005). It is typically set at 95%, and is associated with a standard estimation error. The size of a 95% confidence interval is equal to twice the standard error associated with the 95<sup>th</sup> percentile multiplied by 1.96.

Reliability and precision depend on the material available for study, sample composition, the methods used for estimation (White and Folkens 2005). The prioritisation of one or the other depends on the questions asked and the answers required, and are therefore mainly context-dependent.

**Accuracy** or validity is the degree of conformation of a measured or calculated value to its (actual) true value (Ferrante and Cameriere 2009). It can be presented as the percentage of successful correct estimations, *i.e.* the percentage of estimated values that do not differ significantly from the real values for a given error risk  $\alpha$ .

For age estimation, the notion of prediction interval (PI) is used: a 95% PI includes their real age for 95% of the individuals. A 5% estimation error is therefore associated to it. PIs are always equal to or bigger than confidence intervals.

Percentiles can also be indicated: they correspond to the percentages of individuals from the sample included in an interval of values (ages). For example, 80% of individuals are included between the 10<sup>th</sup> and 90<sup>th</sup> percentiles of a value. This value of 80% is wrongfully considered a prediction interval: it reflects the extent of the variability of variable values at a given age, and not the variability of ages for a given variable value. It is insufficiently reliable for age estimation. To increase estimation reliability, all the 80% prediction intervals associated with the confidence intervals of the measurements (percentiles) into which the individual falls need to be considered (Stull et al 2014a).

Accuracy can also be indicated by a statistical parameter that must not be mistaken for see, as it is presented under the mathematical expression +/- X years.

Testing **intra- and inter-observer errors** of the variables is a prerequisite for any methodology. Indeed, if variable acquisition is observer-dependent, the variables cannot be considered objective and there is a risk of error during acquisition, which can bias the results. Different types of tests for repeatability (intra-observer error) and reproducibility (inter-observer error) exist to test variable acquisition (t-tests, Wilcoxon tests, Cohen's kappa...). Seven modalities were identified for this



criterion: not tested, presence of intra- and inter-observer error, presence of inter-observer error, presence of intra-observer error, absence of intra-observer error, absence of inter-observer error, absence of intra- and inter-observer error. This criterion was assessed when the concerned method included it, or when it was tested in different publications.

**Methodological validation** is the last statistical criterion selected for method evaluation. Testing the method is a prerequisite for methodological validation. It aims to verify the applicability of the method on different samples, and normally, it is the way to calculate the accuracy of the method. Several validation techniques can be used by the authors: cross-validation is a way to test the method on a subset of the study sample that is not used to construct the method. It is also a way to construct the method by alternatively including and leaving-out several individuals to construct and test the method. Validation can also be done using an independent test sample by the authors themselves (Heuzé and Cardoso 2008; Lalys 2002; Saint-Martin et al 2014; Tardivo 2011) or by different researchers in an independent study either to validate (Cardoso 2009b; Dimeglio et al 2005; Saunders et al 1993a; van Lenthe et al 1998), invalidate it/point out inconsistencies (Sahin Salgam and Gazilerli 2002; Tocheri and Molto 2002), modify it (Millard and Gowland 2002; San Roman et al 2002; Schaefer and Black 2005; Stout et al 1996) or confront it with other methods (Boccone et al 2010; Dhanjal et al 2006; Lynnerup et al 2008). The question remains as to whether or not this can be considered as validation, but since several methods are in fact used because of repeated independent testing, it was considered as a legitimate way of assessing methodological validity. If no validation technique was found in literature (in the original article or any article mentioning it) but the method or study is mentioned, validation was indicated as “not done” in the database. If no reference was made of validation either in the original article or literature, validation was marked as “unknown”.

##### *5. Transversal criteria (2):*

This group includes the two criteria that could be included in at least two criteria groups.

The first transversal criterion is the **primary goal** of the methods included in the corpus: a publication can either be explicitly aimed to be an age estimation method, or it can be a study of the growth or maturation of a skeletal element that can be used as an age estimation method (*e.g.* a study on the maturation sequence of a bone, giving the minimum and maximum ages for maturation states). This criterion can either be considered a sample criterion, or a methodological criterion.

The second transversal criterion is the **age group** to which the methods are meant to be applied. This criterion can be considered as a sample criterion, as it provides information on the age of the individuals, but it is also the translation of the variables that are used in the method: Indeed, several

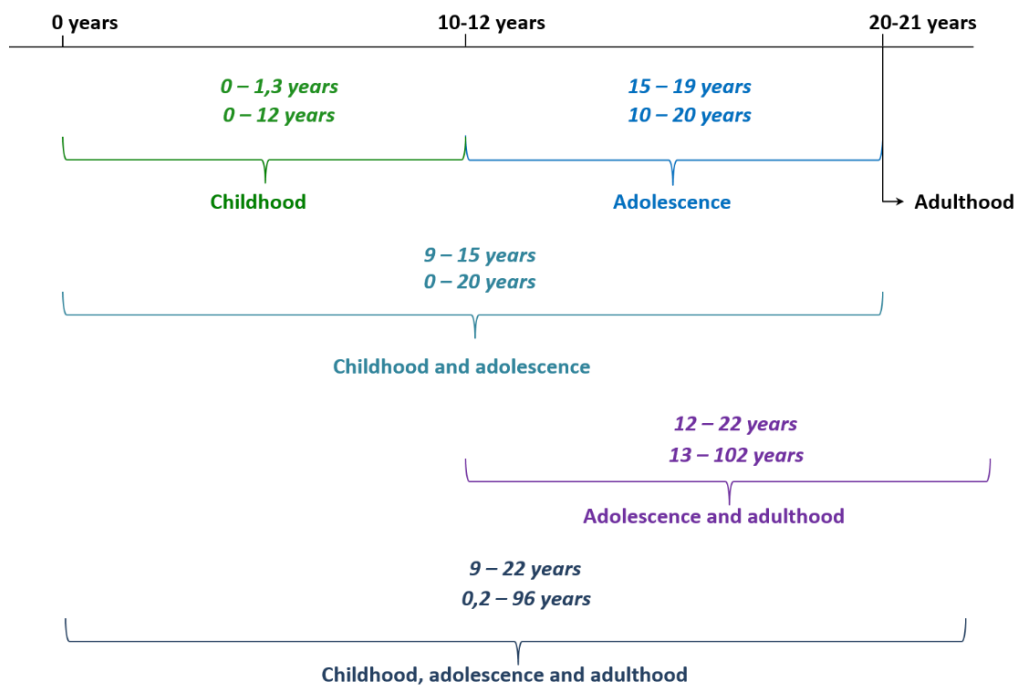
### *Juvenile age estimation: methodological issues and perspectives*

publications have established which variables were the best age estimators depending on the individuals' general age groups (foetal period, childhood, adolescence) (Cunha et al 2009; Wood and Cunningham, *In Black and Ferguson* 2011).

Three main age groups were defined:

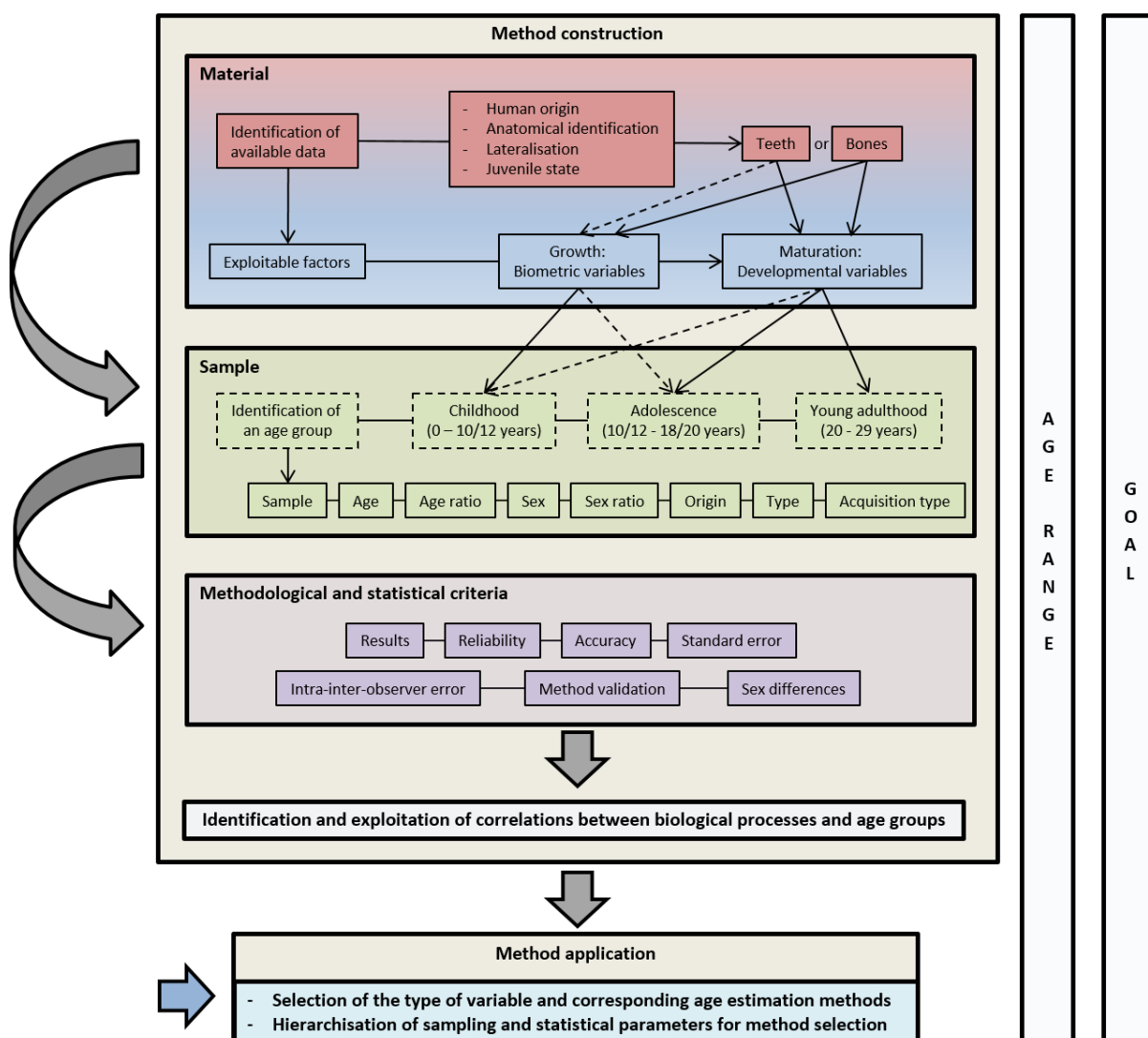
- Childhood: includes individuals aged 0 to 12 years (included). Age estimation methods for children relate to all methods constructed and aimed at individuals included in this age range exclusively. The methods can cover only part of the age group (for example, they can concern individuals aged between 1 and 6 years) or the entire age group (from 0 to 12 years);
- Adolescence: was defined as the age group including individuals aged between 10 or 12 and 20 years (included). In the same way, methods can cover only part of the adolescent phase or its entire duration;
- Adulthood: concerns individuals aged more than 20 years. As it is not a juvenile age group, it is only found in association with one or both previous age groups, when methods also include adult individuals in the study sample.

Because several methods covered two or all three of the age groups, we decided to create additional age groups for each possible overlap: childhood-adolescence, adolescence-adulthood, childhood-adolescence-adulthood. Once again, the overlapping methods can cover only part of one or two age groups, as illustrated in **Figure 2.8**.



**Figure 2.8** Different age groups corresponding to the age ranges covered by the methods of the corpus of study, with the minimum (upper age ranges) and maximum (lower age ranges) age ranges covered by each age group

All the criteria included in method construction and/or selection and the successive steps followed in this study are resumed in **Figure 2.9**.



**Figure 2.9** Interactions between the criteria and steps followed for constructing and selecting juvenile age estimation methods. The criteria were used for the critical analysis of the corpus of methods

**b. General structure of juvenile age estimation methods**

A simple calculation of frequencies by type of criterion, or of combinatory frequencies of two or three criteria characterising the methods of the total corpus provided a general overview of the tendencies followed by juvenile age estimation methods. Detailed results can be found in **Appendix A, Tables A1-A7**. Two general observations can be made from the descriptive analysis of the corpus:

1. *Maturation of post-cranial elements is the most exploited process in the corpus: the percentage of post-cranial elements used in the methods is nearly twice that of cranial elements (69.88% against*

35.59%), and 69.06% of the postcranial elements used are maturation indicators. Amongst postcranial elements, the most exploited anatomical region is the upper limb (17.13%), and the limb bones in particular (13.81%). However, the most exploited individual bones are the femur and tibia (11.05% each).

In the cranium, maturation is also more exploited than growth for both bones and teeth (around 63% and 85% respectively). The most exploited cranial elements come from the cranial base (78.79%), and the occipital bone in particular (84.85%). Mineralisation and eruption of permanent teeth stands out as the most exploited cranial developmental elements and factor (62.5%). Dental growth exclusively concerns deciduous teeth. These observations can be interpreted as the double consequence of a higher number of post-cranial elements for which maturation can be used for age estimation. Bone maturation is also a continuous and long process, with several marked periods of activity, which means that it can be used for age estimation at almost any age from conception to adulthood. Therefore, the number of age estimation methods using bone maturation is bound to be significant. The intrinsic qualities of dental mineralisation (less variability), make it a privileged factor for age estimation, thus explaining the high number of dental methods.

2. *Age groups determine variables and media of study*: this find is more or less intuitive when analysing the developmental patterns of juvenile individuals, so again, it confirms the biological reality of development. Most methods concern the childhood-adolescent period, from 0 to 20 years (34.9%), followed by methods covering the childhood-adolescent-adulthood periods (27.96%). This can partially be explained by the aim of authors to construct methods that can be applied similarly to a greater number of individuals, in order to avoid method heterogeneity. Also, these age groups cover most of the whole post-natal growth and maturation activity period for both skeletal and dental elements. Once again, maturation is the predominant factor, independently of age group. Maturation is most exploited in the adolescent-adult age group (91.88%), followed by the adolescent (85,71%), child-adolescent-adult age group (71.23%), child-adolescent (63.74%) and finally the child age group (50%) who is the only one with more than 40% of methods using growth variables (41.18%).

Several tendencies can also be highlighted on the choice of skeletal or dental element according to age group: methods destined for individuals between 0 and 12 years (childhood) mostly use cranial elements, teeth and limb bones as age indicators; methods for age estimation of adolescents use joint regions and limb bones; methods for both children and adolescents use teeth and joint regions; age is estimated for adolescents and adults using elements of the scapular girdle (mainly the sternal end of the clavicle), and methods destined to individuals from childhood to adulthood use

limb bones, the pelvic girdle and joint regions. Once again, no surprise, as these tendencies are the reflection of normal developmental patterns and particular phases of activity.

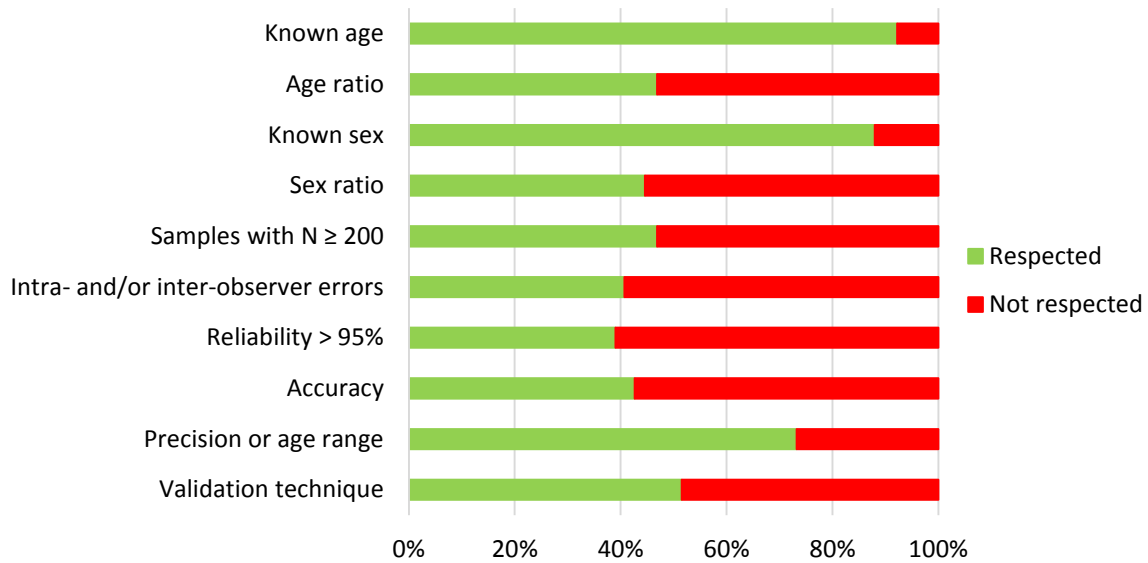
Of all 11 media of study, the most exploited is radiography (51.74%), followed by dry bones (27.79%). All the other media (histology, biochemistry, living, *in situ*, CT and MRI) fall under 10%. Maturation is the clear tendency, independently of the medium of study.

When compared to age groups, the type of medium used for age estimation is the reflection of the purpose of age estimation for each age group: methods that use *in situ* and variables taken from living individuals without resorting to medical imaging are found in relation with the age groups including childhood (33.33%, 50%). This is explained by the fact that these media are mostly used for clinical assessment of normal maturity in children. Invasive techniques such as histology and biochemistry are reserved for the group with the longest age range: childhood-adolescence-adulthood (42.85% and 66.67%). These methods also mostly concern cranial elements (bones and teeth). Medical imaging is predominantly used for individuals included in three of the four age groups that include adolescents. These media are also frequently used to assess age from the joint regions (31.34% for radiography and 42.86% for ultrasonography) and the scapular girdle (28.57% for MRI 35.29% for CT scans). Radiography is also often associated with teeth and limb bones (25.37%). Radiography used for age estimation of juveniles covers all age groups and all types of bones, as do dry bone studies. This is partly because these media provide methods that are maybe more likely to be applicable on human remains.

The analysis of the sampling and statistical parameters of the methods composing the corpus led us to observe that the majority of these parameters (6 out of 9) did not meet the methodological standards pointed out by the series of recommendations previously cited. Only two sampling criteria were respected for most methods (the number of known age and sex methods is higher than the unknown counterpart), and the two respected methodological criteria are precision (or standard error of estimation) with 73.4% and the use of validation techniques with 51.73%. However, most of the validation techniques were indirectly done by different authors in other studies testing the concerned method. So in reality, validation techniques done by the authors of the method themselves are only respected for 11.58% of the methods. The other four sampling criteria, the testing of intra- and inter-observer errors, a reliability superior or equal to 95% and provided accuracy were not respected for most methods (**Figure 2.10**). A more detailed summary of frequencies for all criteria can be found in **Appendix A, Table A7**.

Now that the main methodological limitations pointed out in literature (Schmelting et al 2000, 2004, 2007; Scheuer and Black 2000; Smith, *In* Kelley and Larsen 1991; Wood and Cunningham, *In* Black and Ferguson 2011) have been confirmed and quantified, the question remains: which juvenile

age estimation method(s) fulfil the necessary requirements and may be considered the best?



**Figure 2.10** Frequencies of respected and non-respected validity of the five sampling criteria and the five statistical criteria in the corpus of methods

To answer this question, two types of classifications of the methods in the corpus were constructed using the five sampling and five statistical criteria: an empirical classification and an automatic classification.

**c. Statistical analysis by Multiple Correspondence Analysis (MCA): from method to bone**

In order to describe the interaction between the criteria used to describe the methods without segregating them and find different groups of methods that share similar criteria modalities, Multiple Correspondence Analysis (MCA) of the criteria was undertaken on the dataset. MCA is the only multivariate analysis tool that can be used to describe the underlying structures of a dataset composed of more than two qualitative variables (the criteria), characterising a set of observations (the methods) (Heuzé 2004).

Multiple correspondence analysis is the counterpart of principal analysis done on categorical data. It is a simple correspondence analysis done on a dataset with a large number of variables. The idea behind correspondence analysis is to reduce the dimensionality of a data matrix and visualise it in a sub-space of low-dimensionality, often two- or three-dimensional (Nenadic and Greenacre 2007) *i.e.* to reproduce the distances between the observations represented as points in a lower-dimensioned Euclidian space. MCA transforms qualitative data into vector coordinates in a multidimensional

space, *i.e.* into quantitative data.

The total variance of the data is measured by inertia. The axes of the successive dimensions will explain part of the global inertia of the dataset. The important parameters are the distances between the points in the factorial space: proximity between points illustrates similar structure of the relative frequencies between the columns. Distances between points are weighted distances and are represented by spatial coordinates. In the two-dimensional spaces obtained with MCA, each point represents a categorical modality and possesses coordinates on the first and second dimension. The coordinates were then used for discriminant analysis of the variables. Discriminant analysis determines which variables are more discriminative and determine different groups of observations. Here, the ranking variables, *i.e.* the ones that we want to be discriminant, are the age group and the bone used for age estimation.

Discriminant analysis of the variables will determine the items implicated in discriminating methods for each age group. This procedure estimates the relation between variables. Different independent discriminant functions are calculated. Each successive function will contribute less and less to the global discriminant power. The maximum number of functions will always be inferior or equal to the total number of variables (criteria) or groups minus one. The databases were constructed using Microsoft Excel® 2013 Software (*Microsoft Inc.*). MCA and discriminant analyses were done using the R® Software (RStudio Interface, v0.98.1056, RCommander Interface v2.1-2) and the FactoMiner R® Package.

MCA can present several problems, depending on the approach adopted: in the classic approach, the data matrix is an indicator matrix (also called a complete disjunctive table) composed of lines of observations and several columns (more than two) of descriptive categorical variables. In this case, the matrix columns contain dummy variables (values of only 0 or 1) for each category (*i.e.* modality) of the set of categorical variables (*i.e.* criteria). This approach provides principal inertias and coordinates. It was not possible to use it here, as the categorical values would be restrained to 0 or 1, and that did not cover the whole range of modalities in our dataset.

A more preferable approach according to O. Nenadic and M. Greenacre (2007) is to perform an eigenvalue-eigenvector decomposition based on the Burt matrix. This matrix is equal to the cross-product of the indicator matrix, *i.e.* the matrix that concatenates all two-way cross-tabulations between pairs of variables. However, the Burt matrix presents submatrices on the main diagonal that are cross-tabulations of each variable with itself, and therefore overestimates total inertia.

To overcome this particular problem, there are two options: the adjustment of inertias or joint correspondence analysis (JCA). The adjustment approach rescales the coordinates of the solution to best fit the pairwise cross-tabulations off the main diagonal of the Burt matrix. JCA uses an iterative

algorithm to find the optimal weighted-least-squares fit to these off-diagonal tables. In both cases, the initial data frame is restrained to the columns containing the modalities for the analyses and is called a response pattern matrix. It is then converted into a Burt matrix. Adjustment of inertias is considered the best default option, since the optimal scaling properties of MCA are conserved while raising the percentages of inertia and squared correlations to obtain results very similar to JCA (Nenadic and Greenacre 2007). For these reasons, the adjusted approach was adopted to analyse the structure of our data.

Because MCA is an exploratory statistical tool, it is not possible to test the significance of the results. The primary objective of this tool is to provide a simplified representation of all the components of a categorical database. This will help us understand the structure of our database and observe the interactions existing between the categories in order to find which methods can be grouped, based on objective statistical and methodological criteria.

The results of the MCA were then used to build a classification of the methods with a Hierarchical Classification on Principal Components (HCPC). HCPC or hierarchical clustering is performed on the principal component coordinates of the observations (methods) to identify homogenous subgroups, or clusters (Husson et al 2010). Hierarchical trees constructed here use Ward's criterion. This criterion is based on the Huygens theorem which decomposes total inertia (total variance) in inter- and intra-group variance. HCPC consists in aggregating two clusters until the growth of intragroup - inertia is minimum (in other words, it minimises the reduction of inter-group inertia) at each step of the algorithm. Intra-group inertia characterises the homogeneity of a cluster. Hierarchy is represented by a dendrogram which is indexed by the gain of intra-group inertia. A cluster is defined by the most frequent modalities of the categories. Each cluster is characterised by the frequencies of one or several modalities of each category. The most discriminant criteria are identified by a Chi-squared test ( $p > 0.05$ ).

As the number of categories and modalities for each category was very high, some of them could not be used in MCA. Moreover, a higher number of modalities implies a small number of occurrences per modality. This is the case for four criteria that presented too many modalities to compose homogenous ensembles and for one criterion that was redundant with other information provided by the dataset. Because of this, it was not possible to conduct a multiple correspondence analysis with the categories "Elements", "Origin", "Variables", "Variable type", "Medium of study" and "Results". We decided to distribute the remaining 18 criteria into three sub-groups. The first group is composed of seven criteria related to sampling conditions and are used for MCA; the second group is composed of five criteria that define the statistical parameters of the age estimation methods; and



the third group is composed of six criteria which are additional information deemed too variable and not objective enough to be relevant for analysing the overall structure of the dataset. For this reason, no MCA was done using the criteria of the third group. In the first two groups, the first step was to detect rare modalities (represented by less than 10 individuals) and recode them to avoid biasing the results. Moreover, MCA cannot be done on individuals for whom there is missing data, so we had to conduct it by omitting missing values in the corpus, for sampling and statistical criteria. This only concerned one or two criteria in three different methods. The second step was deciding which criteria would be the active variables of the MCA and which ones would be the illustrative ones. As the methods are destined to estimate age, we decided to use “age group” as the illustrative variable and analyse the five sampling parameters and the developmental factors as of the methods “active variables” in each age group.

MCA followed by HCPC created clusters of methods based on the similarities or differences between their sampling protocols. They are referred to as “Sa” for “Sampling”, preceded by the cluster number. Then, for each of these groups, a second MCA followed by HCPC was done using the statistical parameters of the observations/methods. They are referred to as “St” for “St”, preceded by the cluster number. For each cluster, the frequencies of modalities were observed and compared to find out which modalities were clustered, and ultimately, which methods presented similar parameters.

MCA followed by HCPC was also done using the statistical parameters of the methods to see if the resulting statistical clusters were comparable to the sampling clusters. The sampling clusters were then each compared to the results of another clusterisation based on the statistical and methodological criteria of the methods. The cluster composed of the methods present in both the “best” sampling cluster and the “best” statistical cluster is the cluster that is objectively and statistically supposed to be composed of the best juvenile age estimation methods of our corpus.

#### **d. Empirical classifications: from bone to method**

##### **i. Approach adopted for constructing the classifications**

When confronted to practical application of age estimation methods, the main limitation is the material, *i.e.* the skeletal and/or dental elements. This already conditions the choice of the method(s) to be used to estimate the age of the individual whose remains are available for analysis.

Because of this, and because we could not use “Bone” as discriminant factor in the statistical classification, an empirical classification of the methods was done, using the same methodological

and statistical criteria as above (for MCA and hierarchical clustering) to evaluate the quality of the methods and to be able to compare the clusters of methods obtained with both approaches (statistical and empirical). The empirical tree follows the same progression as naturalist classifications (Janvier 2012): it starts at the root (first criterion), and progresses towards terminal leaves following a series of branches and knots. Each knot is a particular category from which a certain number of branches emerge to lead to the next knot. With each branch comes a modality of the precedent knot (criterion). In its structure, it is also similar to statistical decisional trees (Breiman et al 1984), but the branches are not associated to any probabilities of the events (modalities) occurring. The empirical classification aims to address methodological concerns relating to both method construction and method application:

*1. Constructing a juvenile age estimation method:*

Each step is directly dependent of the previous one and shows the progression of the constructor. The protocol results from the answers provided to a certain number of questions:

1. Is the element a useful material for practical age estimation? Does it have good preservation rates, can it be easily identified, can it be used on large age ranges, and can it provide a simple but “good” age estimation method?
2. The type of process to be exploited:
  - a. Growth: is it adapted to the element at our disposal? What are the characteristics of this process for the element? Is it punctuated by recognisable phases, patterns?
  - b. Development/maturation: is it adapted to the element at our disposal? What are the characteristics of this process for the element? Is it punctuated by recognisable phases, patterns?
3. The type of variable that best represents the relation between age and the biological process selected previously. If it is growth, the variables of choice are quantitative osteometrics (Lalys 2002). If it is maturation, it can be assessed by stages, descriptions, number, and other qualitative or quantitative variables. This step also concerns the choice for the medium of study, namely selecting between dry bone, radiography, other imaging techniques, and biochemistry as the primary source of variables acquisition. The choice depends on several factors: the context of study and more importantly, the context in which the method can be applied, the possibility of obtaining a substantial amount of data, ethical and legal requirements (where medical imaging and *in situ* variables are concerned), the advantages of using one medium in particular to obtain otherwise inaccessible information (*e.g.* visualising tooth germs by radiography), costs, user-friendliness, reliability of the variables, etc.

4. Statistical criteria used for method construction and validation: population (sex, age, number of individuals, type of variability covered by the sample...), level of statistical significance, aim of the study (clinical or anthropological study on growth and/or maturation, age estimation method...). These criteria evaluate the parameters of the samples used to construct age estimation methods for juveniles: it is admitted that the best samples for method construction originate from archaeological, forensic or modern reference populations, *i.e.* for which at least the age and sex of the individuals are known and verified and where age and sex ratios are respected. However, these populations are rare, and are composed of an insufficient number of individuals, or age and sex ratios are not respected.
5. Method presentation, *i.e.* how the method is presented to the scientific community and therefore, how it should be used by practitioners. For example, it can take on the form of a regression equation, an abacus, a maturation atlas... what are the statistical and interpretational advantages and limits caused by the author's choice of presentation?

2. *Choosing and using a juvenile age estimation method:*

To decide how to estimate the age of a juvenile individual from his/her skeletal or dental elements, practitioners analyse the elements at their disposal by asking themselves a series of questions:

1. Which element(s) of the individual can be used?
2. Considering the developmental state of the element, where is the individual on the developmental scale? Near the beginning (prenatal period), near the end (adolescence)? The answer to this question greatly depends on the type of element at the disposal of the practitioner, on personal experience, and on the practitioner's knowledge of juvenile osteology. Some elements are characteristic of a given period (*e.g.* an unfused pars basilaris is characteristic of the foetal period, fully developed deciduous teeth still present in the jaw characterise childhood, long bone epiphyses still fusing are characteristic of older children or young adolescents).

The answers to these two questions are material-dependent. Depending on the available elements and their preservation, the practitioner's choice of material can be very limited.

3. Which developmental process can be exploited (growth or maturation?) and which variable(s) can be used as an age estimator(s)? Measurements, maturation stages, etc.

This question leads to a various number of answers, and it also depends on the material and its preservation rate. However, the practitioner can also choose to exploit one developmental process over the other or even both processes if she/he thinks it is relevant. Moreover, the practitioner can identify one or several variables to be used as estimators.

4. We then sorted the different methods used for age estimation according to several criteria characterising method construction and method application. This final step is directly related to the fact that FA practitioners and bioarchaeologists need to justify their choice of age estimation methods when presenting their results in Court, or in an official report.

ii. Organisation of the empirical classification

The aim of the empirical classifications is to highlight the “best” methods available amongst our methodological corpus using objective criteria and see if they meet the methodological requirements demanded by osteoarchaeologists, anthropologists and Courts of Law (Black and Ferguson 2011; Cunha et al 2009; Schmeling et al 2007). It was decided to construct the empirical classifications to illustrate two levels of evaluation.

The first level is a general level, *i.e.* it gives an overview of the methods according to the skeletal or dental element and the biological process used to construct them. The first set of classification trees present all methods per element and per biological process (growth or maturation). It highlights the “best” methods, *i.e.* the ones that respect sampling and statistical criteria for each type of bone and variable exploited.

The second level aims to confront our findings with what is generally presented in literature as the best biological parameters identified for age estimation according to the age group of the individuals (Cattaneo 2007; Cunha et al 2009; Wood and Cunningham, *In* Black and Ferguson 2011). A second set of classification trees was constructed, separating the methods according to the element of study, the biological process used to construct the methods, and adding another discriminant factor: the age group on which the method was constructed.

Building the empirical trees was done by identifying several groups of criteria used as successive discriminant levels for separating the methods:

- Identification of the skeletal/dental element(s) used in the method (construction and application criteria): in practice, the first selection parameter is often the available data, as it conditions the analysis. When thinking of constructing an age estimation method, one of the first parameters is also the element to be used as an estimator;
- Sampling criteria: parameters describing the method for sample constitution, *i.e.* the biological characteristics of the individuals of the study sample and sample composition. These criteria are the same as the sample criteria used for the statistical classification;
- Statistical criteria: these parameters mainly concern the mathematical arguments of the methods that are chosen or result from their construction: the level for the alpha error, confidence intervals, intra- and inter-observer errors... These criteria are also the same as the

statistical criteria used for the statistical classification;

- The age group concerned: this step was difficult to take, as it implies an *a priori* knowledge or at least a vague idea of which age group the individual belongs to. Because age mirrors its related biological processes, it conditions method construction. An age estimation method for adolescents will use the most active biological parameter for that age group: it will almost never use growth parameters, and almost always use maturation variables. Therefore, the variables used as age estimators reflect the age group of the individuals whose ages they are meant to predict: the suspected age of the individual conditions the choice of the method. In practice, when faced with a single individual whose age needs to be estimated, as is often the case in a forensic context, the user will almost always have at least a vague idea of which general age group the individual belongs to. He/she will therefore select the age estimation method(s) accordingly.

When dealing with a larger number of individuals, as is the case when studying osteoarchaeological collections, the methods selected for age estimation can either be chosen because they cover several general age groups, or they can be chosen to be specifically applied to certain age groups.

Method selection can therefore be either dependent or independent of the concerned age groups. For this reason, it was decided to present the classification trees following two approaches: the first one considers all the methods independently of age, and only uses sampling and statistical parameters; the second one considers age as a decisive parameter for method selection, in the same way as sampling and statistical criteria.

The order of the sampling criteria was decided according to their importance *vis-à-vis* the object of the study: age estimation. For this reason, it seemed logical that the most discriminative criteria would be those related to age: the fact that the age estimation methods were built either on samples of known or unknown (or estimated) age and whether or not age ratios were respected. Indeed, skeletal or dental age estimated from inaccurate chronological ages seemed the core default of any method and needed to be identified first.

Study samples with heterogeneous age ratios (significantly different numbers of individuals in the age groups) have been found to bias the method constructed using them in favour of the age categories or ranges that are the most represented (Coqueugniot et al 2010; Gowland and Chamberlain 2002). This leads to overestimation of the individuals that are younger than the overrepresented age groups, and underestimation of the older individuals. For this reason, age ratio was chosen as the second most discriminative criterion.

Authors disagree on the significance of sexual dimorphism in juveniles (exception made of adolescents) and sex determination from juvenile remains has been proven insufficiently reliable.

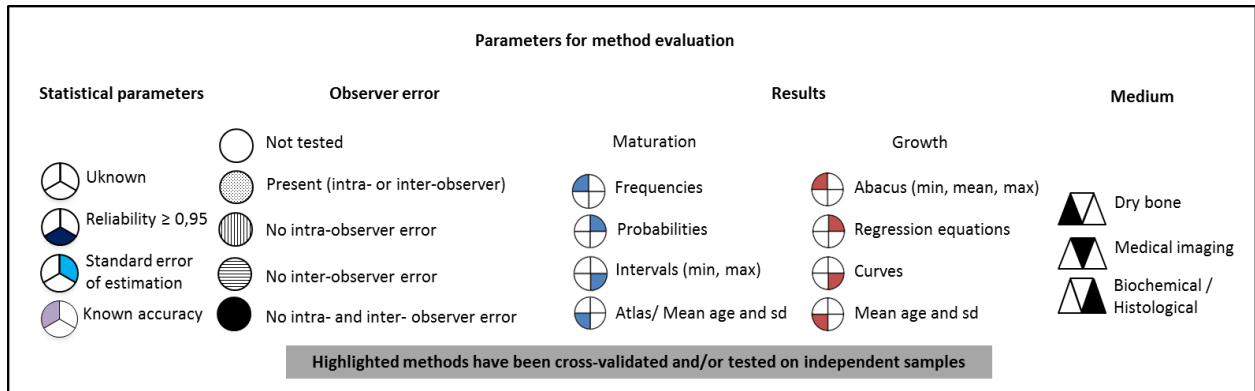
However, an important number of methods use sexual dimorphism to discriminate their results and provided “sexed” methods (Aka et al 2009; Banerjee and Agarwal 1998; Cameriere et al 2007a; Demirjian et al 1973; Greulich and Pyle 1959; Moorrees et al 1963a; Rios and Cardoso 2009; Roche et al 1988). It is a fact that sex plays a part in developmental patterns, and therefore, growth and maturation are different between boys and girls. Moreover, sexed methods provide inaccurate results if applied on individuals of the opposite sex (Cardoso 2008b). This is why it is best to know the sex of the individuals of the study sample, as it can influence the results. Because of sexual differences in development, and for the same methodological implications as age ratio, it is also best to have study samples with equal sex ratios per age group. This is our fourth level for discriminating sampling criteria.

The last discriminant level concerns sample size. It is a fact that juvenile reference collections of osteological data are hard to come by (Ardagna 2004; Scheuer and Black 2000), and that collecting extensive amounts of data is a long process. Nevertheless, results need to be obtained on samples of sufficient size to be considered significant (Coqueugniot et al 2010; Garcin 2009; Hoffman 1979; Schmeling et al 2007). The minimum number of individuals composing the study sample and considered as sufficient for statistical significance was chosen to be 200, after a general overview of the corpus of methods. This value did not exclude too many methods, and provided sufficient numbers of individuals for the age groups covered by the methods (Schmeling et al 2007).

In the same way as MCA, no statistical parameters to evaluate significance of the classification can be provided, as it is only illustrative. Unlike MCA, this classification presents a risk of subjectivity. Indeed, although the criteria are chosen to respect published recommendations, there is no guarantee that different observers would choose the same order of criteria if they were to build a classification. This is countered by the fact that the empirical classification can be used starting from the top (starting at the first sampling criteria: age of the individuals of the study sample) or the bottom of the trees (the statistical parameters, represented by different coloured circles), following the priority given to the criteria by the user.

Both levels of classification start with the element of study, followed by the factor used in the method (growth or maturation). The next discriminant steps are sampling criteria, progressing from age, to age ratio, sex, sex ratio and ending with sample size to arrive at the terminal leaf: the reference of the methods using that particular element. Each reference is associated with four colour-coded circles, each representing methodological or statistical parameters (**Figure 2.11**): the first one includes reliability, precision and accuracy; the second represents intra- and inter-observer testing; the third is the form taken by the method (regression equation, abacus, atlas, etc.); and the

fourth is the medium of study (dry bone, medical imaging...). Their order is not relevant.



**Figure 2.11** Legend of the modalities presented in the empirical classification of juvenile age estimation methods

### 2.3.2. Statistical and empirical classifications: a complementary approach for method evaluation

#### a. Statistical classification (MCA and clusterisation)

##### i. Sampling criteria

MCA conducted on the five sampling criteria (age, sex, age ratio, sex ratio, sample size) and the developmental factors exploited (growth and maturation) showed an important inertia gain between the first and the second dimension, followed by a regular but lower increase in inertia between dimensions 2 to 4, and another regular increase between dimensions 5 and 21 (**Figure 2.12**). This indicates that the principal processes separating the methods are along the first and second dimensions. Percentages along the two dimensions are quite low, attesting for low variance.

The individual plot shows a concentration of individuals between -1 and 1 in both dimensions (**Figure 2.13**). The scatterplot is quite homogeneous, and no particular group of individuals can be highlighted. The first dimension separates the methods according to sex, sex ratio, age, age ratio, sample size, factor, age group and type of study (from the most discriminant to the least discriminant variable).

Methods with unknown age, sex, age and sex ratios and with small samples or samples of unknown size are in the positive range of the first dimension, whereas methods with bigger sample size, respected age and sex ratios and for which age and sex is known are at the negative values of the first dimension. The first dimension also groups “childhood”, “childhood-adolescence” and “growth” in the positive values and “childhood-adolescence-adulthood”, “adolescence-adulthood”, “adolescence” and “maturation” in the negative values. The second dimension separates the methods according to sex ratio, age ratio, type of method (cross-sectional or longitudinal), sex, age, age groups, sample size and finally developmental factor, from most to least discriminant. Methods

with unrespected sex and age ratios, known and unknown sex and age, and small sample size are found in the positive values of the second dimension.

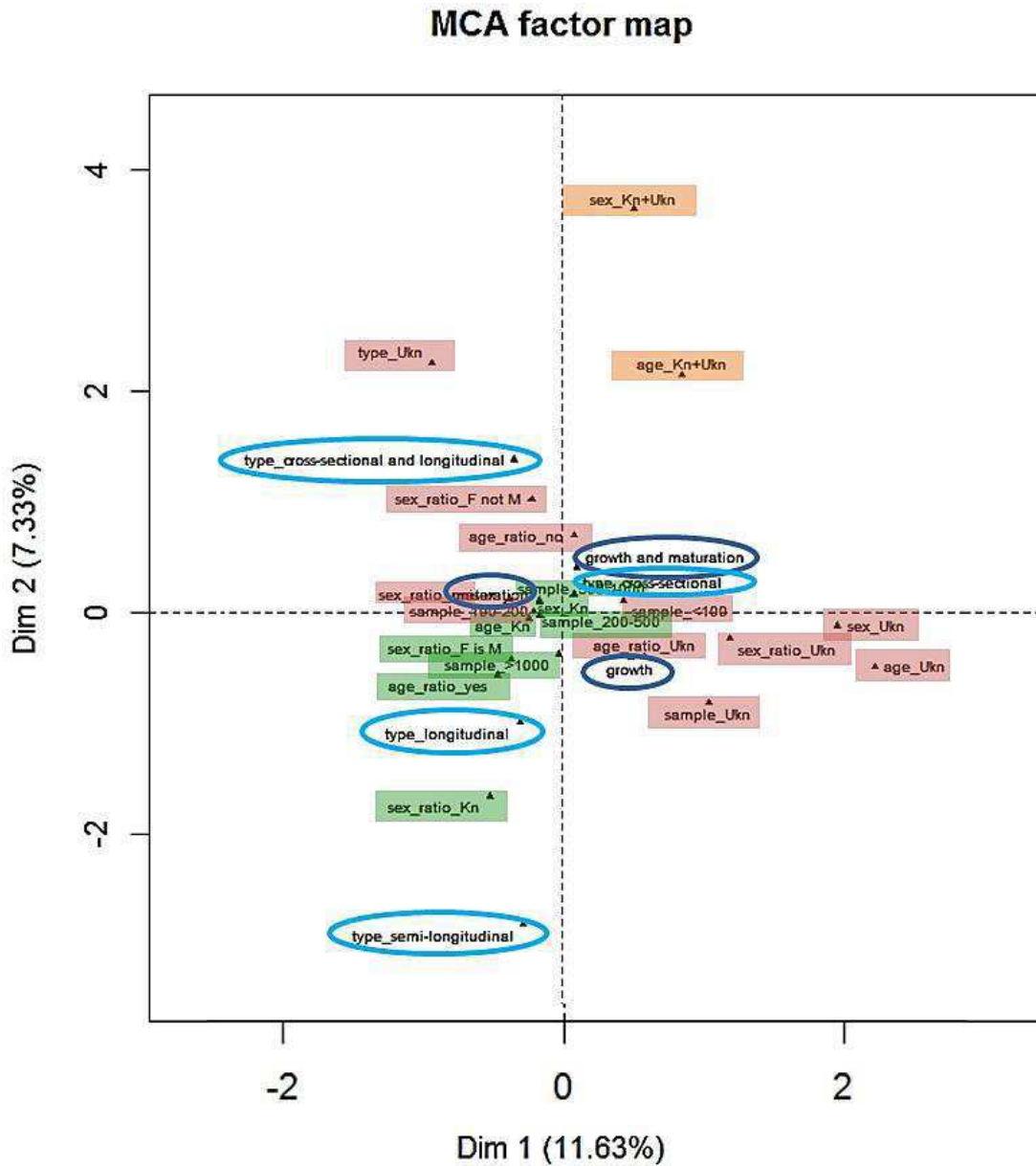
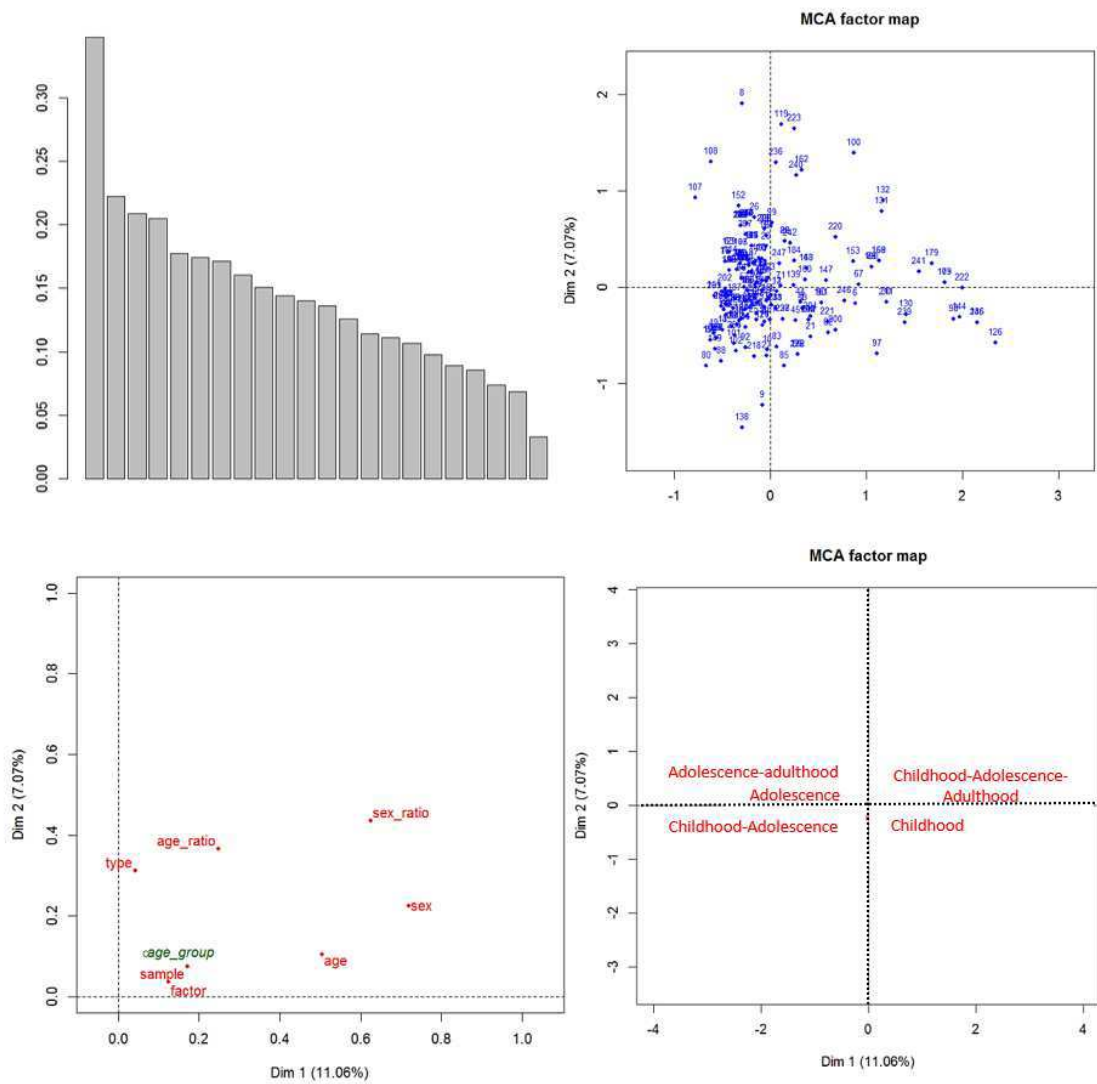


Figure 2.12 Multiple Correspondence Analysis of the five sampling criteria of our methodological corpus (age, sex, age ratio, sex ratio and sample size), factor (growth or maturation in dark blue circles), type of data (cross-sectional or longitudinal in light blue circles) and age group as the illustrative variable. Red modalities correspond to non-respected criteria; green modalities correspond to valid criteria; orange modalities correspond to “mixed” modalities

The negative values of the second dimension correspond to methods respecting age ratio, sex ratio, constructed on samples of known sex. The second dimension also separates cross-sectional (positive values) from longitudinal (negative values) methods and “adolescence-adulthood”, “adolescence” and “childhood-adolescence-adulthood” (positive values) from “childhood” and “childhood-adolescence” (negative values).





**Figure 2.13** Results of the MCA of sampling criteria conducted on the methods of our corpus. Top left: barplot of inertia gain; Top right: distribution of the methods in the factorial plane; Bottom left: distribution of the methods in the factorial plane (first and second dimensions); Bottom right: distribution of the age groups in the factorial plane

The ellipses traced around the modalities are the 95% confidence intervals of the distribution of the modalities around their barycentres (**Figure 2.14**). They allow the rapid visualisation of the degree of separation between two modalities in the factorial plane.

We can see that sex, age, age ratio, sex ratio, and type of study are significantly separated (their respective ellipses do not overlap). “Unisex” and “F is M” sex ratios overlap, but as they both indicate the respect of sex ratio (the difference being that the first modality illustrates unisex samples), their overlapping is normal. Sample sizes and developmental factor modalities all overlap, and are not well separated in the factorial plane. They are less discriminant than the other five variables.

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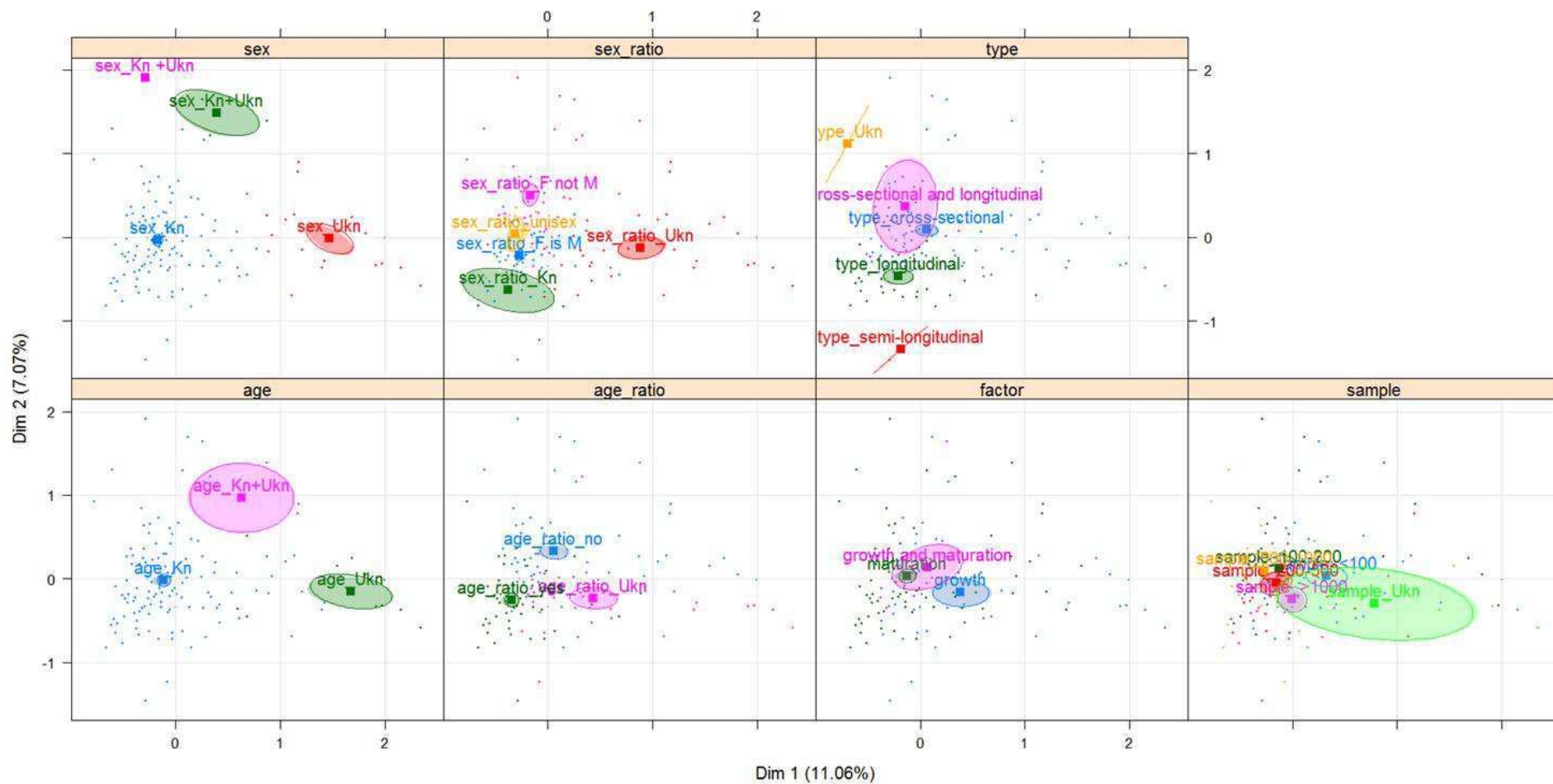


Figure 2.14 Results of the MCA of sampling criteria conducted on the methods of our corpus. Plot of the 95% confidence ellipses of modalities for sampling criteria

### ***Juvenile age estimation: methodological issues and perspectives***

The general description of the categories gives the list of the modalities for which the Chi-squared test is significant according to age group, *i.e.* the modalities that are the most discriminant for methods according to the age groups they cover. The v-test associated to the frequencies is the statistical test of a hypergeometric law and the value is the quantile of the normal distribution indicated by a sign. A positive sign indicates an overrepresentation of the variable in the age group. A negative sign indicates underrepresentation of the variable in the age group. It is a good method to pinpoint the weaknesses and strengths of the methods for each age group. The variables absent from the tables are neither over- or under-represented in the age groups. The tables highlight the modalities of the categories “sex ratio”, “factor”, “sample size” and “type of study” as the most or least represented in the different age groups and their frequencies can be compared to other general frequencies of these modalities in the whole corpus. The high percentage values (between 60 and 100%) indicate highly discriminant categories and the associated modalities characterising the cluster. Intermediate values (30-60%) are not particularly discriminant, and values inferior to 30% are not discriminant at all. For example, in the age group “childhood” (**Table 2-7**), we can see that 44.12% of the methods constructed on that age group have samples with less than 100 individuals. 52.94% of childhood methods are constructed using maturation data, and 38.24% using growth data. Compared to the frequencies found in the whole sample for these three variables (Global frequency), they are respectively higher for sample size and growth, and lower for maturation.

What transpires from the representation of the modalities according to age group, is that there is a general heterogeneity in method construction in terms of sampling parameters. The “worst” methods are those concerning the “adolescence-adulthood” age group (unrespected or unknown age and sex ratios) and “childhood” (unknown sex ratios and small sample size). Childhood-adolescence-adulthood is the only age group for which age and sex ratios are the most respected. Unisexed methods are predominantly found in the adolescent age group, which is a sign of the consideration of sexual dimorphism that becomes significant during that period. The “adolescence-adulthood” methods are mainly based on cross-sectional studies. This seems logical, as longitudinal studies are supposed to follow the same individuals for the whole duration of the growth or maturation process, and seem more appropriate for younger individuals. They are indeed more present in the “childhood-adolescence” age group, and accompanied by an overrepresentation of big sample sizes (>1000), characteristic of longitudinal growth studies.

Overall, MCA discriminates “bad” sampling modalities, distributed in the diagonal between the positive end of the first and second dimensions from “good” sampling modalities, distributed in the negative end of the first and second dimensions (**Figure 2.12**).

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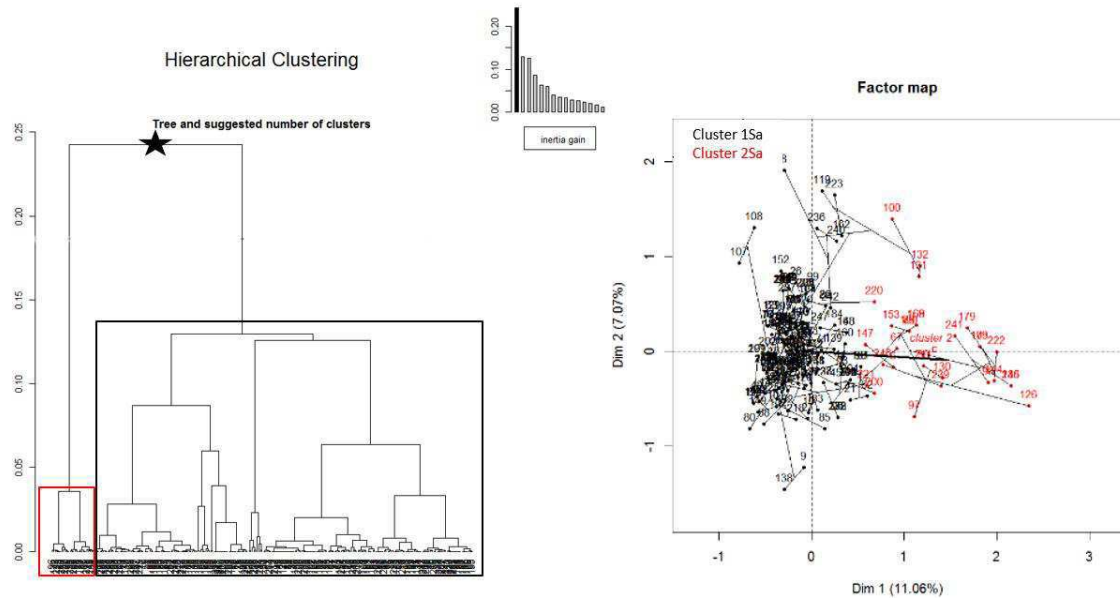
**Table 2-7: Frequencies (in percentages) of the sampling modalities representing the different age groups. Positive v-test values correspond to over-represented modalities and negative values correspond to under-represented modalities**

Age group	Modality	Frequency of the modality in the age group	Frequency of the age group for the modality	Global	p-value	v-test
Childhood	Unknown sex ratio	27.27	44.12	21.48	0.0015	3.17
	Sample size < 100	23.44	44.12	25	0.0094	2.60
	Growth	23.64	38.24	21.48	0.017	2.39
	Maturation	9.89	52.94	71.09	0.017	-2.388
Adolescence	Unisex sex ratio	33.33	41.67	5.86	0.00	3.69
	Known sex ratio	50.00	16.67	1.56	0.01	2.52
	Maturation	6.59	100	71.09	0.02	2.43
	Sample size 100-200	10.61	58.33	25.78	0.02	2.38
Childhood-adolescence	Longitudinal data	59.52	28.09	16.41	0.00	3.55
	Sample size >1000	60.53	25.84	14.84	0.00	3.48
	Growth	52.72	32.58	21.48	0.00	3.07
	Maturation	30.77	62.92	71.09	0.04	-2.07
	Sample size 100-200	24.24	17.98	25.78	0.04	-2.09
	Cross-sectional data	28.71	65.17	78.91	0.00	-3.81
Adolescence-adulthood	Unrespected sex ratio	35.82	48.98	26.17	1.34e-04	3.82
	Maturation	24.73	91.84	71.09	1.144e-04	3.80
	Cross-sectional data	23.27	95.92	78.91	3.83e-04	3.55
	Sample size 500-1000	35.14	26.53	14.45	1.30e-02	2.48
	Unrespected age ratio	25.93	57.14	42.19	2.05e-02	2.32
	Unknown data type	100.00	4.08	0.78	3.60e-02	2.10
	Unknown age	0.00	0.00	5.47	4.68e-02	-1.99
	Unknown age ratio	8.62	10.20	22.66	1.65e-02	-2.40
	Unknown sex ratio	7.27	8.16	21.48	8.11e-03	-2.65
	Growth	3.64	4.08	21.48	3.03e-04	-3.61
Childhood-adolescence-adulthood	Longitudinal data	0.00	0.00	16.41	5.38e-05	-4.04
	Respected age ratio	37.78	47.22	35.16	0.01	2.48
	Growth and maturation	52.63	13.89	7.42	0.02	2.99
	Respected sex ratio	34.78	55.56	44.92	0.03	2.12
	Sample size 500-1000	13.51	6.94	14.45	0.03	-2.19
	Unisex sex ratio	0.00	0.00	5.86	0.01	-2.75

This discrimination is also visible in the second dimension, along with the discrimination between cross-sectional and longitudinal data along the second dimension, and growth and maturation along the first dimension. The separation of the age groups along the two dimensions always tends to separate age estimation methods that concern children from methods destined for adolescents. Maturation is predominant in methods concerning adolescents and adolescents-young adults. Growth and maturation are both used quasi-equivalently for estimating age for children and adolescents. Cross-sectional and longitudinal studies seem to be mostly found for adolescents and children and adolescents respectively. MCA based on sampling criteria confirms the general consensus on the existence of heterogeneous sampling biases in juvenile age estimation, while highlighting specific methodological limitations and the heterogeneity of the qualities and defaults of age estimations methods according to the age group they are constructed on and destined to be used for.

Hierarchical clusterisation was then done according to sampling criteria. The resulting clusters are

identified as cluster number followed by “Sa” for “Sampling”. By default, clusterisation of the methods based on MCA results separates the methods into two groups. The dendrogram proposes a partition into two clusters, clearly separated as assessed by the inertia gain between one and two clusters (**Figure 2.15**).



**Figure 2.15** Results of the hierarchical clusterisation of methods according to sampling criteria. Left: dendrogram of the methods showing the cut-off point (Star) into two clusters (red and 1Sa in black) and the barplot of inertia gain according to the number of clusters. Right: map of distribution of the methods into 1Sa and 2Sa according to sampling criteria. Segments represent the “proximity” between two methods (represented by numbers) according to their sampling modalities

The associated Chi-squared test gives a list of the variables for which the modalities are significantly different in the two clusters (**Table 2-8**).

**Table 2-8:** List of the criteria significantly different between the sampling clusters and the associated p-values of the chi squared test, from the most to the least discriminant

Two-way clusterisation			Six-way clusterisation		
Criteria	p-value	df	Criteria	p-value	df
Sex	4.62e-44	3	Sample size	3.21e-85	25
Age	1.89e-25	2	Sex ratio	5.42e-72	20
Sex ratio	1.61e-21	4	Sex	7.94e-42	15
Sample size	3.12e-04	5	Age ratio	1.79e-21	10
Age ratio	5.70e-04	2	Age	2.07e-19	10
Factor	2.96e-03	2	Age group	5.05e-06	20
Age group	4.28e-02	4	Type of study	1.85e-05	20
			Factor	2.92e-04	10

The categories are ordered from most to least discriminant: sample size, sex ratio, sex, age ratio, age, age group, type and factor.

### *Juvenile age estimation: methodological issues and perspectives*

The first cluster is composed of 224 methods and mostly includes methods with samples of known age, sex, respected age and sex ratios. It also has fewer methods with unrespected age and sex ratios (all “positive” modalities are present in that cluster with frequencies higher than 90%). The type of study, sample size and the developmental factors are less discriminant because their modalities are represented with frequencies ranging from 75 to 97%, although maturation and longitudinal studies are more represented, as well as big sample size (>1000). This can be explained by the general predominance of maturation data in the methods composing the corpus, and we can see that longitudinal studies are once again linked to big samples, as was assessed with MCA. The predominant age group is childhood; it concerns 73.53% of the methods in that cluster.

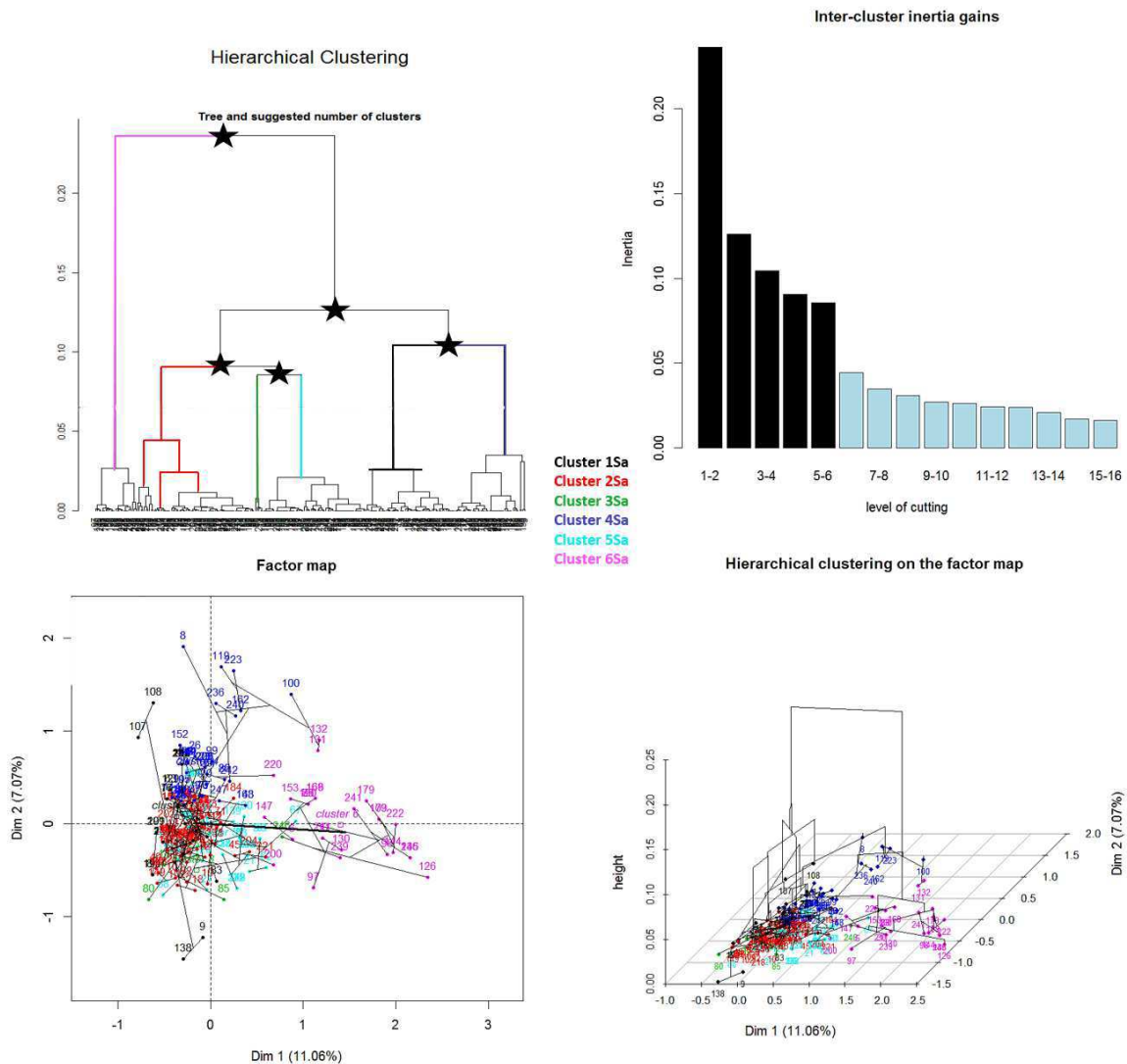
The second cluster is composed of 32 methods, all constructed on samples of unknown sex and age (frequencies of 100%), and mostly with unknown sex ratio, and small or unknown sample size. The type of study is mostly cross-sectional, the predominant factor is growth. Generally, the percentages are lower than they are in the first cluster (between 15.35 and 52.72%).

Although the default clusterisation discriminates the methods that generally respect (cluster 1Sa) from the methods that generally do not respect (cluster 2Sa) sampling criteria, it only allows a general description of the methods. Also, both clusters have very different sizes: cluster 1 is much larger than cluster 2Sa. This is a good thing, because it means that most methods generally respect sampling criteria, but it also limits the level of discrimination as it does not specifically highlight the “best” methods that respect all sampling criteria uniformly.

For this reason and to obtain additional levels of discrimination, we added a condition for clusterisation by imposing that the minimum number of clusters be equal to three or six. These numbers were chosen by observing the plot of inter-inertia gains, which seemed to present a high increase in inertia between two and three and five and six clusters, but less significant increases after that. Indeed, the dendrogram obtained for clusterisation separated the methods into six different clusters in both cases (**Figure 2.16**).

Cluster 6Sa corresponds to the major part of cluster 2Sa, so the other five clusters are refined classifications of cluster 1Sa, which means that several of them should be composed of samples of known age and sex. The order of the discriminative variables changes compared to the previous classification: sex, sample size, sex ratio, age, age ratio, type of study, developmental factor, and age group are the most to least discriminant. Sex-related variables and sample size are still part of the most discriminant parameters, and age group is still the least discriminant variable. This means that age group has a less discriminative power for objectively discriminating juvenile age estimation methods compared to biological criteria (age and sex) and sample construction (sample size, type of study, developmental factor used for the variables). Method evaluation seems to be relatively

independent of the age group concerned.



**Figure 2.16** Results of the hierarchical clusterisation of the methods according to sampling criteria. Top left: dendrogram of the methods showing the cut-off points (stars) into six clusters; Top right: barplot of inertia gain according to the number of clusters. Bottom left: map of distribution of the methods into six clusters; Bottom right: 3-dimensional distribution of the methods into six clusters. Segments represent the “proximity” between two methods (represented by numbers) according to their sampling modalities

Sample size is very discriminant for cluster 1Sa. The other variables are less discriminant: the type of sample seems to be variable (longitudinal or unknown). Cluster 2Sa is the cluster composed of the methods that respect the highest number of sampling criteria (four out of five). Cluster 3Sa is composed of less than ten methods of cluster 1Sa and one method of cluster 5Sa (**Table 2-9**).

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**Table 2-9: Sampling clusters of age estimation methods and their characteristics obtained by automatic clusterisation. Bold elements correspond to valid modalities**

Sampling cluster	Characteristics
1Sa (36 methods)	<b>Known sex (100%)</b> <b>Known age (100%)</b> <b>Sample size &gt; 500 (94.44%)</b> Unrespected sex ratio (47.22%) Adolescence-adulthood (36.11%) Unknown sample type (5.56%) Semi-longitudinal sample type (5.56%)
2Sa (97 methods)	<b>Known sex (100%)</b> <b>Known age (97.94%)</b> <b>Respected sex ratio (80.41%)</b> <b>Respected age ratio (63.92%)</b> Childhood-adolescence-adulthood (37.11%) Sample size > 100 (54.95%) Growth and maturation (12.37%)
3Sa (6 methods)	<b>Known sex ratio (66.67%)</b> Unknown sample size (50%) Adolescence (33.33%)
4Sa (50 methods)	Cross-sectional sample type (94%) Unrespected age ratio (88%) Unrespected sex ratio (62%) Sample size 100-200 (52%) <b>Unisex methods (16%)</b> Adolescence (12%) Known and unknown sex (8%)
5Sa (39 methods)	<b>Known age (100)</b> <b>Known sex (97.44%)</b> Maturation (94.87%) <b>Sample size &gt; 1000 (82.05%)</b> Childhood-adolescence (58.97%) Unknown sex ratio (56.41%) Unknown age ratio (51.28%) Longitudinal sample type (28.20%)
6Sa (28 methods)	Cross-sectional sample type (96.43%) Unknown sex ratio (92.86%) Unknown sex (89.29%) Sample size < 100 (57.14%) Unknown age (46.43%) Growth (46.43%) Unknown age ratio (39.29%) Childhood (32.14%)

Because of its small size and heterogeneous composition, it is doubtful that this cluster is significantly different from clusters 2Sa and 5Sa, as 3Sa is indeed very close to these two clusters (**Figure 2.16**).

Cluster 6Sa however, is clearly separated from all the others and respects no sampling parameters. Cluster 6Sa contains the “worst” sampling criteria as it is mostly composed of methods that respect neither of the five (known age and sex, respected age and sex ratios, sufficient sample



size) sampling parameters (**Table 2-9**).

The imposition of a higher number of clusters based on sampling criteria refines the first two clusters obtained by the default clusterisation by providing a gradual hierarchy of the methods. Imposing a higher number of clusters was attempted (between 7 and 16, the last level of inertia gain), but it did not provide any other significant pairings between modalities that were not already highlighted with six clusters. Indeed, the inertia gain is less important after 6 clusters. Clusterisation also highlights the heterogeneity of respected and unrespected sampling criteria (**Table 2-9**).

However, age- and sex-related criteria are always associated in all clusters: if sex is predominantly known, age is also predominantly known. This is also true for sex and age ratios, which are paired in all clusters, except for clusters 1Sa and 3Sa, which can be considered as “transitional” clusters, as the discriminative power of the other criteria is variable (lower percentages).

The type of study, age group and developmental factor are secondary parameters for method discrimination and their association is less obvious and more method-dependent. However, longitudinal studies are often associated with large samples, cross-sectional methods often have small samples, and methods concerning childhood are often discriminated from methods concerning adolescence. These four additional criteria (type of study, factor, and age group) can be considered for a more precise discrimination, but have less discriminant power for separating methods respecting standardised samples.

The methods respecting a standardised sampling protocol can be identified by five parameters: known age, known sex, respected age and sex ratios, and sample size superior to 100 individuals. Assessing these five parameters is sufficient for selecting methods constructed on standardised and valid sampling protocols.

ii. Statistical and methodological criteria

MCA done on the whole corpus using the five statistical and methodological criteria (reliability, accuracy, standard estimate error, observer error, and validation technique) showed an important inertia gain between the first and the second dimension, followed by a regular but lower increase in inertia between dimensions 2 to 4, and another regular increase between dimensions 5 and 24 (**Figure 2.17**). This indicates that the principal processes separating the methods are distributed on the first and second dimensions. The individual plot shows a concentration of individuals between -1 and 1 (**Figure 2.17**), although several methods are located within larger ranges in the positive and negative ranges of the second dimension. Three groups of individuals can be highlighted (central concentration, and two groups of dispersed methods located in the negative and positive ranges of the second dimension).

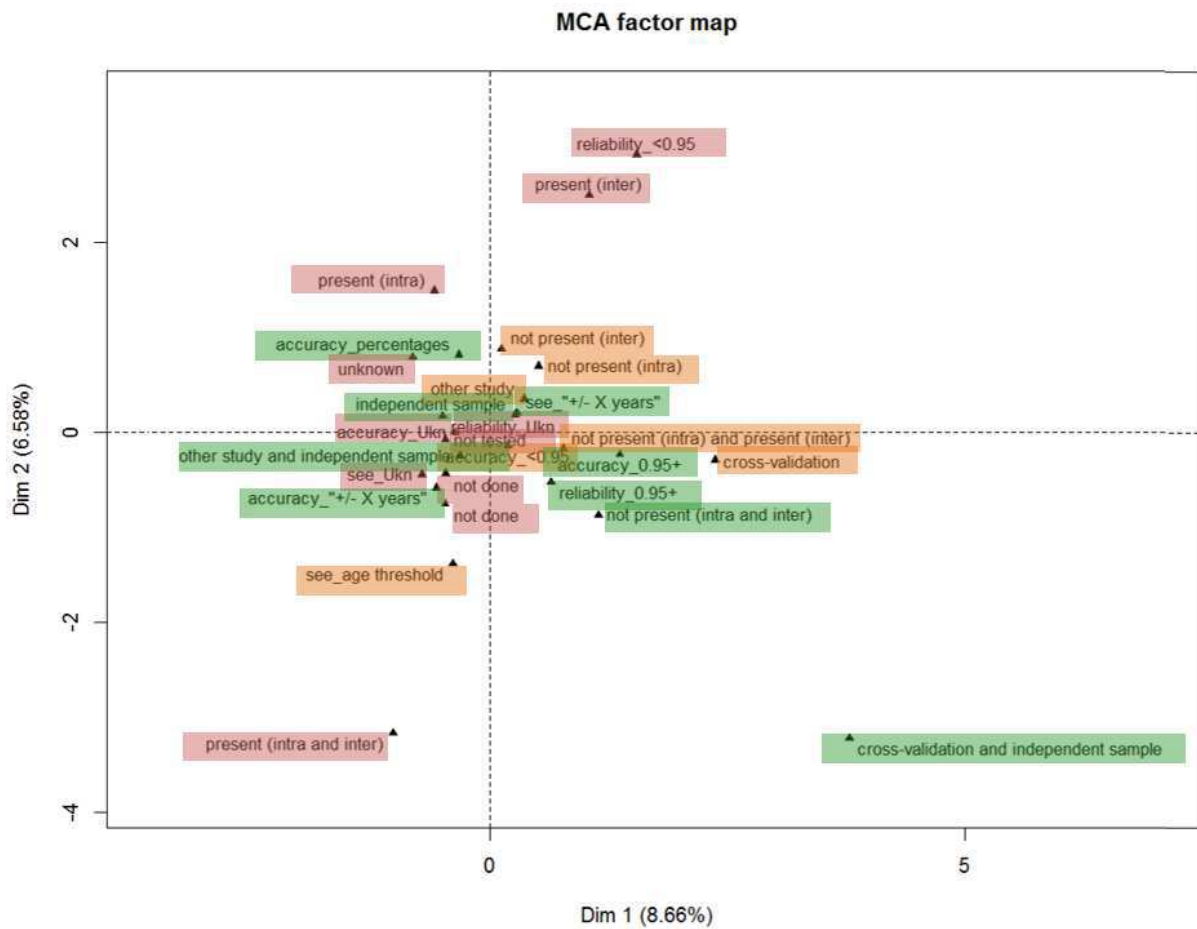


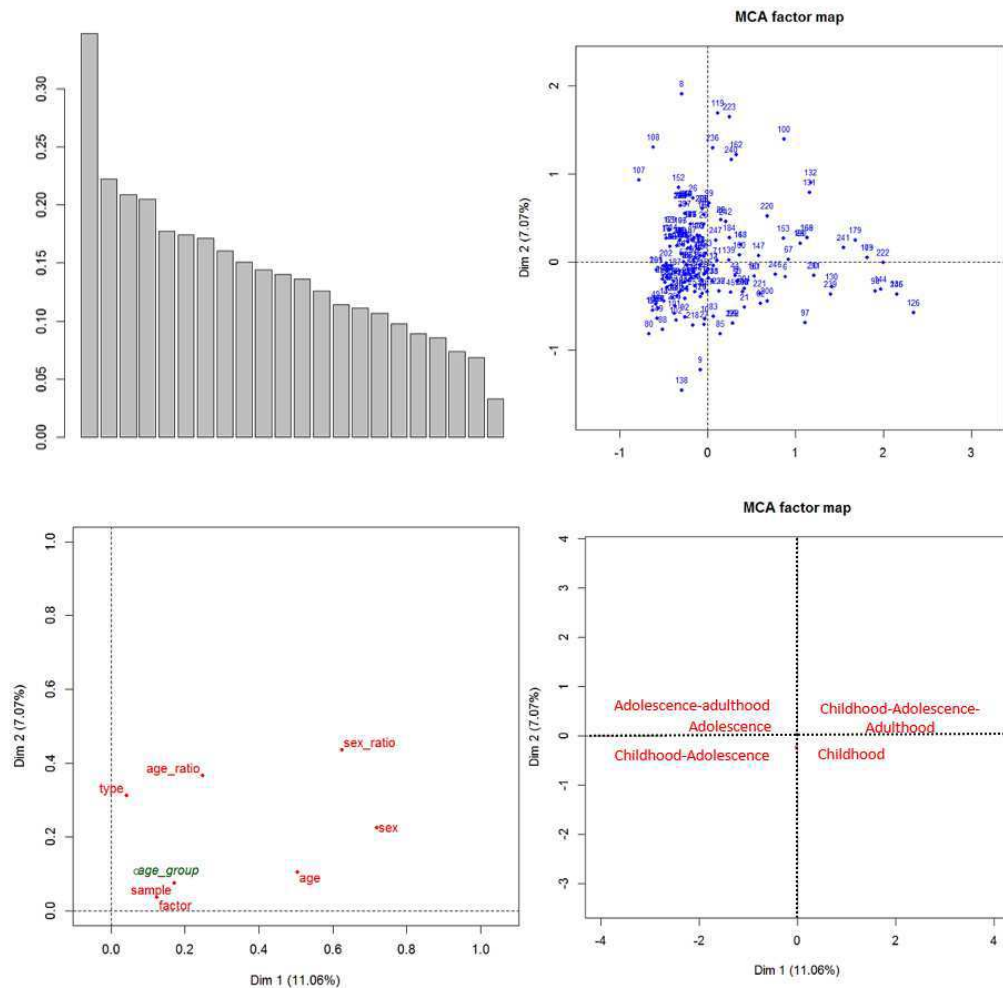
Figure 2.17 Multiple Correspondence Analysis of the five statistical criteria of our methodological corpus (reliability, accuracy, standard estimation error, repeatability/reproducibility and validation technique) and age group as the illustrative variable. Red modalities correspond to non-respected criteria; green modalities correspond to valid criteria; orange modalities correspond to “mixed” modalities

The first dimension separates the methods according to accuracy, observer error, reliability, validation technique and standard error of estimation (from the most discriminant to the least discriminant variable). Methods for which validation was done using cross-validation or an independent sample, with accuracy equal to 95%, for which intra- and inter-observer errors were tested and were not significant, for which standard error of estimate was given and reliability was known (95% or less) are in the positive range of the first dimension. The negative values of the first dimension correspond to methods for which validation was not done or was unknown, unknown accuracy, unknown standard estimation error, untested observer errors and unknown reliability. The “childhood-adolescence” age group is relatively central, as is the use of other studies for validation and 95% reliability. The distribution of the methods along the first dimension shows a dominant presence of “good” statistical parameters in the positive values and “bad” ones in the negative values.

The second dimension separates the methods according to observer error, reliability, validation technique, standard error of estimation and accuracy, from most to least discriminant. Methods for

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which observer errors are present or absent at intra- and inter-levels, absent at an inter-observer level, for which validation was done using independent samples and other studies, with a reliability inferior to 0.95, and accuracy expressed in years (“+/- X years”) or percentages are distributed in the positive values of the second dimension. The negative values of the second dimension correspond to methods for which observer error was not tested, absent or present. The second dimension also separates methods according to validation techniques (absence of validation, cross-validation and independent sample in the negative ranges, use of another studies and independent samples in the positive ranges). The separation of “good” and “bad” modalities is not as marked as it is for the first dimension. Although age groups are well separated along both axes, no modality pattern or grouping related to age ranges can be highlighted (**Figure 2.18**). The ellipses traced around the modalities show that modalities for reliability and standard error of estimation are significantly separated in the factorial plane (**Figure 2.19**).



**Figure 2.18** Results of the MCA of statistical criteria conducted on the methods of our corpus. Top left: barplot of inertia gain; Top right: distribution of the age groups in the factorial plane; Bottom left: distribution of the criteria centroids in the factorial plane; Bottom right: plot of the 95% confidence ellipses of criteria modalities

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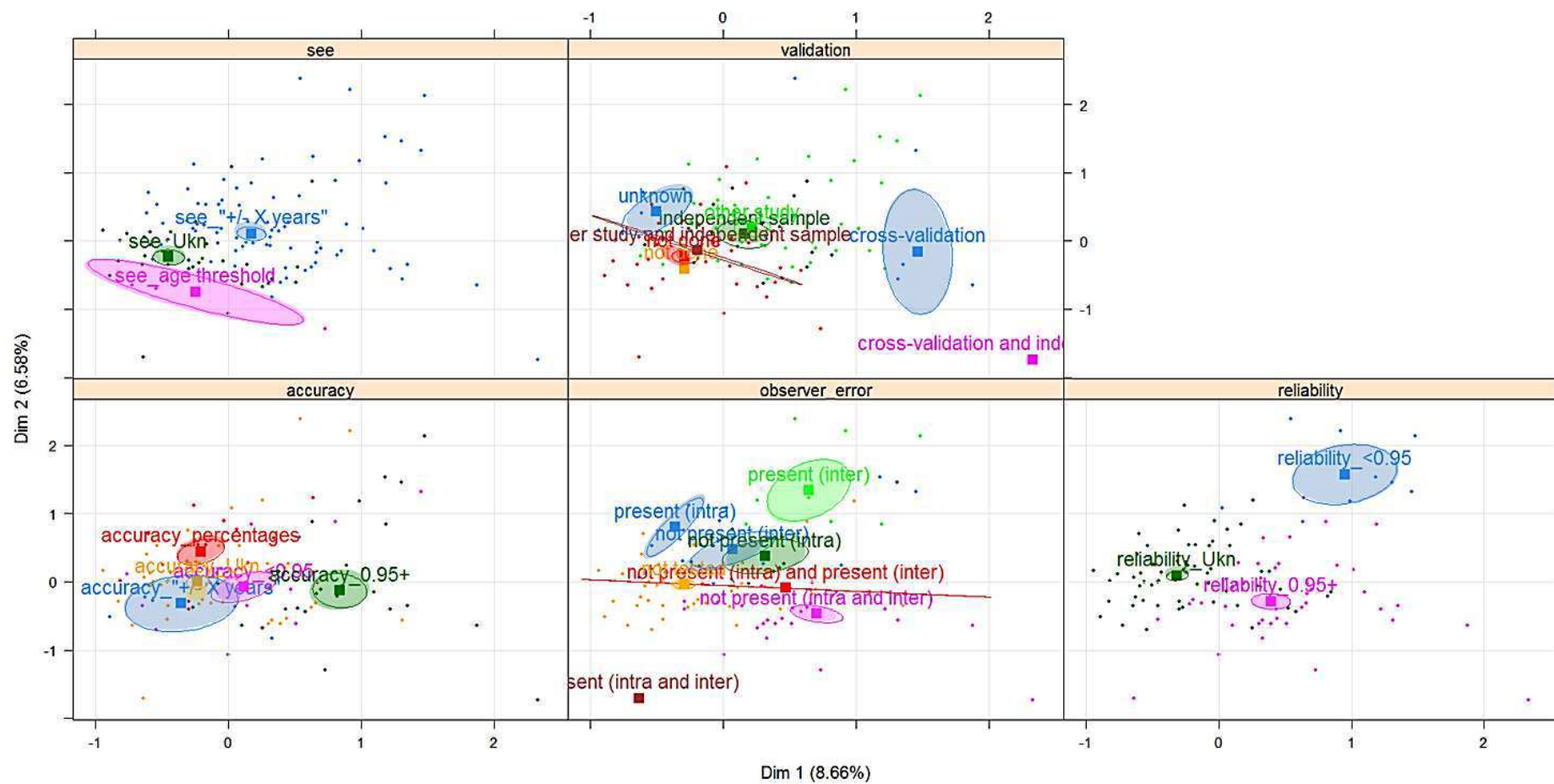


Figure 2.19 Results of the MCA of statistical criteria conducted on the methods of our corpus. Distribution of the criteria centroids in the factorial plane and plots of the 95% confidence ellipses of modalities for statistical criteria

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Three modalities for accuracy have overlapping confidence intervals (<0.95, “+/- X years” and unknown), but are well separated from the other two modalities (0.95+ and percentages). Cross-validation and cross-validation and independent sample are well separated from the other modalities: validation by other studies overlaps with “independent sample”, “other studies and independent samples” and “unknown” validation overlap with the absence of validation. The two modalities assessing the presence of observer errors (inter- and intra-) are well separated from the modalities assessing the absence of observer errors (intra-, inter-, intra- and inter-). The two modalities “not tested” and “absence of intra-error and presence of inter-error” overlap on one another, as do the absence of intra- and the absence of inter-observer errors.

The general description of the categories by the most discriminant modalities according to age group identify validation technique, observer error, reliability and accuracy as the most discriminant variables (**Table 2-10**).

**Table 2-10: Frequencies (in percentages) of the statistical modalities representing four age group (no tendencies were detected for the adolescence age group). Positive v-test values correspond to over-represented modalities and negative values correspond to under-represented modalities**

Age group	Modality	Frequency of the modality in the age group	Frequency of the age group for the modality	Global	p-value	v-test
Childhood	Unknown reliability	17.33	76.47	58.59	0.022	2.28
	Intra-observer error	66.67	5.88	1.17	0.05	1.97
	Accuracy ≥ 0.95	4.26	5.88	18.36	0.03	-2.11
Childhood-adolescence	Accuracy percentages	15.38	4.49	10.16	0.03	-2.23
	No inter-observer error	10.53	2.25	7.42	0.02	-2.38
Childhood-adolescence-adulthood	No validation	39.6	55.56	39.45	0.001	3.24
	Unknown validation	6.25	1.39	6.25	0.037	-2.09
	Validation by another study	20.95	30.06	41.02	0.03	-2.13
	Presence of inter-observer error	0.00	0.00	4.30	0.02	-2.25
Adolescence-adulthood	Reliability ≥ 0.95	25.77	51.02	37.89	0.04	2.06
	No intra-observer error	0.00	0.000	5.47	0.05	-1.99
	Unknown reliability	14.67	44.90	58.59	0.03	-2.13

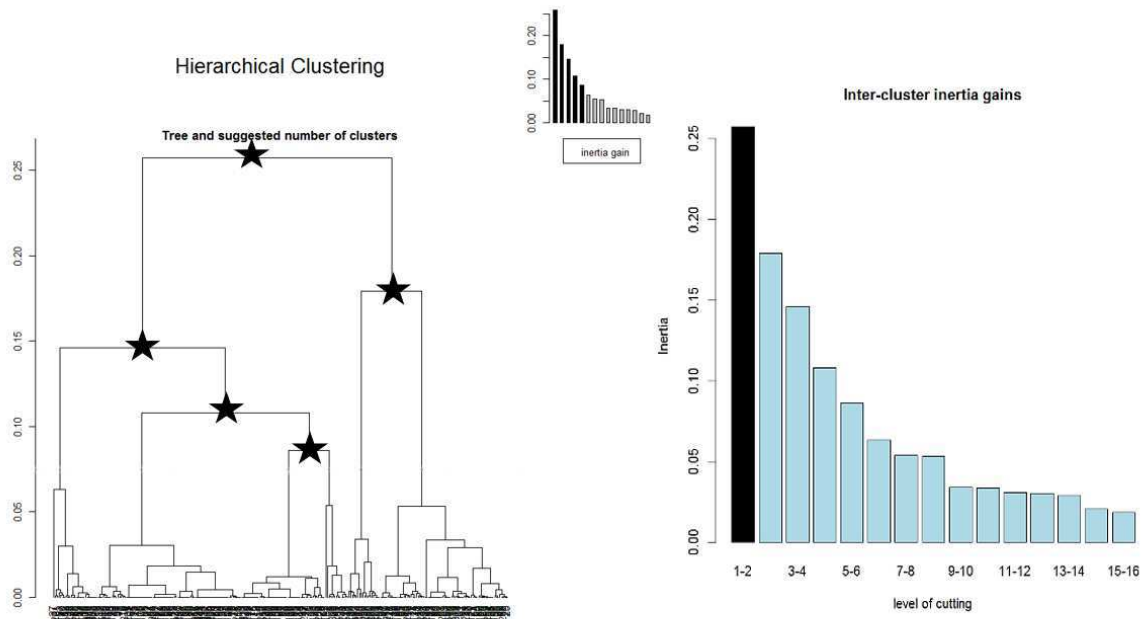
The age group adolescence-adulthood is characterised by a majority of 0.95+ reliability (51.02%), most methods for the childhood age group have unknown reliability (76.47%), and present intra-observer error. The “childhood-adolescence” age group is characterised by very few methods for which accuracy is expressed in percentages and no inter-observer error is present; the methods of the age group “childhood-adolescence-adulthood” mostly have not done any form of validation (55.56%), and other types of validation are underrepresented.

Overall, MCA of statistical criteria does not differentiate “bad” modalities or “good” modalities as well as the sampling parameters did and the range of variation for these criteria is larger (**Figure 2.19**). However, intermediate modalities seem to be found around the centre of the factorial plane,

“good” modalities are distributed along the first dimension and “bad” modalities are mostly present along the second dimension. MCA based on sampling criteria confirms the results found with sampling parameters and the general consensus on the heterogeneous use and respect of statistical parameters in juvenile age estimation, while highlighting specific methodological limitations and the heterogeneity of the qualities and defaults of age estimations methods according to the age group they are constructed on and destined to be used for.

Hierarchical clusterisation was then done according to statistical criteria. The resulting clusters are identified as cluster number followed by “St” for “Statistical”. The default clusterisation of the methods based on the five statistical criteria separated the corpus into two groups (**Figure 2.20**). The most discriminant criterion is observer error, followed by accuracy, reliability, validation, standard estimation error, and age group.

The first cluster is composed of 173 methods characterised by untested observer errors (80.92%), unknown reliability (73.99%), accuracy (72.25%), and standard estimation error (35.26%). All these modalities are highly represented in this first cluster. Several methods also present accuracy as percentages (13.29%).



**Figure 2.20** Results of the hierarchical clusterisation of the methods according to statistical criteria. Left: dendrogram of the methods showing the cut-off points (stars) into two, three, four, five and six clusters; Right: barplot of inertia gain according to the number of clusters showing the important inertia gains for two, three, four, five and six clusters

The second cluster contains the 83 methods for which accuracy and reliability are both equal to at least 0.95 (49.38% and 63.86%) or inferior to 0.95 (53.85% and 88.89%). Validation techniques include the use of cross-validation and other studies (100% and 48.57%). Neither intra- or inter-observer error were present for most methods (54.22%) but all methods with inter-observer errors

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are in this second cluster. the principal age group of this cluster is childhood-adolescence (49.40%). The unknown modalities of the criteria are all underrepresented (less than16%).

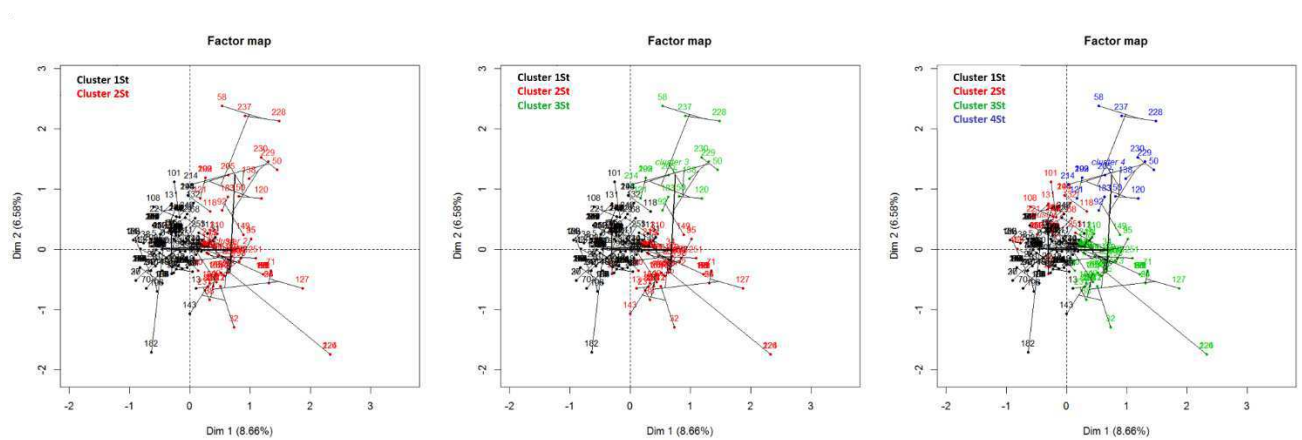
Default clusterisation identifies the methods that are mostly constructed with “bad” statistical parameters, which are the most numerous, whereas the second cluster which is mostly composed of methods respecting the statistical norm for methodological validity. This first rather general clusterisation confirms the general observations made in literature concerning the lack of standardised statistics and validation in juvenile age estimation methods.

To obtain a more refined view of the “best” methods, *i.e.* to highlight the smallest group of methods that respect all the standardised statistical criteria, additional clusterisation was conducted by setting the minimum number of clusters between 3 and 6, until the last significant inertia gain was obtained and the number of methods composing each cluster was not too different. This led to the identification of 6 clusters. The general hierarchy of discrimination for the criteria observed for the initial clusterisation (2 groups) remains the same whether 3, 4, 5 or 6 clusters are identified (**Table 2-11**).

**Table 2-11: List of the criteria significantly different between the sampling clusters and the associated p-values of the chi squared test, from the most to the least discriminant**

Two-way clusterisation			Six-way clusterisation		
Criteria	p-value	df	Criteria	p-value	df
Observer error	9.61e-27	7	Observer error	2.88e-86	35
Accuracy	1.29e-19	4	Accuracy	1.48e-54	20
Reliability	5.30e-13	2	Reliability	1.96e-29	10
Validation technique	1.42e-07	8	Standard estimation error	1.59e-26	10
Standard estimation error	7.02e-05	2	Validation technique	7.39e-12	35
Age group	1.14e-02	4	Age group	2.22e-02	20

Clusterisation into three groups subdivides cluster 2 into two clusters, with cluster 2/3 being the one composed of the most methods respecting statistical criteria (**Figure 2.21**).

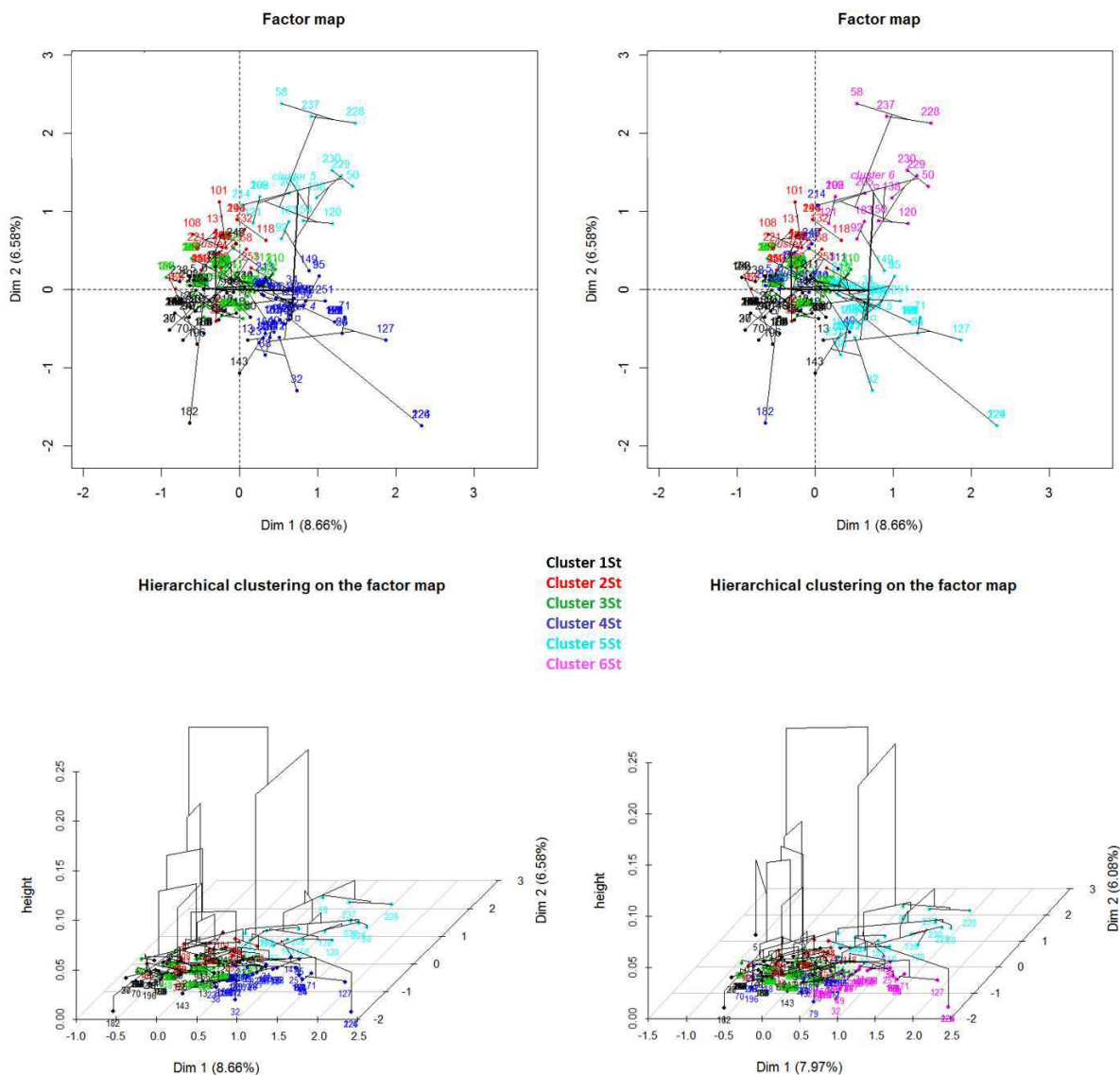


**Figure 2.21 Map of the distribution of the methods according to statistical criteria into two clusters (left), three clusters (middle) and four clusters (right). Segments represent the “proximity” between two methods (represented by numbers) according to their statistical modalities**

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Clusterisation into four groups subdivides the initial cluster 1 into two clusters (1/4 and 2/4), while clusters 3/4 and 4/4 remain globally unchanged compared to the previous clusterisation.

Clusterisation using five and six as minimum number of clusters continues to subdivide initial cluster 1, while the other two clusters remain globally constant (clusters 4/5 and 5/5 in the five-way clusterisation, clusters 5/6 and 6/6 in the six-way clusterisation). This means that the level of discrimination concerning the initial “best” group of methods is attained at the five-way clusterisation (**Figure 2.22**). However, as the inertia gain is higher between five and six clusters, the detailed analysis of the clusters is provided for the results obtained with the six-way clusterisation.



**Figure 2.22** Results of the hierarchical clusterisation of the methods according to statistical criteria. Top left: map of distribution of the methods into five clusters; Top right: map of distribution of the methods into six clusters; Bottom left: 3-dimensional distribution of the methods into five clusters; Bottom right: 3-dimensional distribution of the methods into six clusters. Segments represent the “proximity” between two methods (represented by numbers) according to their sampling modalities



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Age group is the least discriminative criterion, and is only present in three clusters (**Table 2-12**). The modalities of the inter-observer error criterion are the most discriminant and are found in all clusters. No significant associations are identified between criteria, but there are some between modalities: cluster 5St is the one with the most respected and “best” statistical criteria (five out of five), whereas clusters 1St and 3St are characterised by at least three “unknown” or “untested” modalities for validation, observer error, or accuracy.

The distribution of the criteria does not follow a clear pattern in clusters 2St, and 6St, and some modalities are contradictory (inter-observer error in cluster 2St). The presence of cluster 4St is somewhat surprising, as it is formed by only one discriminative statistical criterion, the absence of inter-observer error. However, because this subdivision groups all the methods using this modality, it is justified as a particular subgroup of methods.

**Table 2-12: Statistical clusters of age estimation methods and their characteristics obtained by automatic clusterisation. Bold elements correspond to valid modalities**

Statistical cluster	Characteristics
1St (57 methods)	Untested observer error (92.98%) Unknown standard estimation error (84.21%) See “threshold” (75%) Unknown reliability (74.44%) <b>Accuracy: “+/- X years” (10.53%)</b> Accuracy <0.95 (19.30%) No validation (61.40%)
2St (28 methods)	Unknown reliability (92.86%) <b>Accuracy: percentages (85.71%)</b> Absence of intra-observer error (17.86%) Presence of intra-observer error (10.71%)
3St (78 methods)	<b>Standard estimation error: “+/- X years” (97.47%)</b> Unknown accuracy (93.59%) Untested observer errors (92.31%) Unknown validation (12.82%)
4St (16 methods)	Absence of inter-observer error (81.25%) Adolescence-adulthood (43.75%) <b>Validation by another study and independent sample (18.75%)</b>
5St (61 methods)	<b>Standard estimation error: “+/- X years” (88.52%)</b> <b>Reliability: 0.95+ (73.77%)</b> <b>Absence of intra- and inter-observer error (70.97%)</b> <b>Validation by other studies (60.66%)</b> <b>Accuracy: 0.95+ (54.10%)</b> Childhood-adolescence (47.54%) <b>Validation by cross-validation (6.65%)</b>
6St (16 methods)	<b>Standard estimation error: “+/- X years” (93.75%)</b> <b>Validation by other studies (68.75%)</b> Presence of inter-observer error (68.75%) Childhood-adolescence (62.5%) Reliability < 0.95 (50%)

The six-way clusterisation using statistical criteria clearly highlights cluster 5St as the group of

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methods that mostly respect the all of the best statistical and methodological criteria.

The comparison between sampling clusters and statistical clusters led to the identification of the “best” methods, *i.e.* the ones contained in both clusters 2Sa and 5St. Twenty-one methods are therefore qualified as the methods mostly respecting sufficient sampling and statistical parameters defined by expert study groups and publications (**Table 2-13**).

**Table 2-13: Sampling and statistical modalities of the 21 “best” methods found by automatic clusterisation and their corresponding occurrences in the corpus. Bold characters represent the highest number of methods per criterion**

Criterion	Modality	Number of methods
Sample size	<100	7
	100-200	5
	<b>200-500</b>	<b>7</b>
	>1000	2
Sex ratio	Not respected	7
	<b>Respected</b>	<b>15</b>
Sex	<b>Known</b>	<b>21</b>
Age ratio	Not respected	6
	<b>Respected</b>	<b>11</b>
	Unknown	4
Age	<b>Known</b>	<b>21</b>
Type	<b>Cross-sectional</b>	<b>17</b>
	Longitudinal	4
Observer error	Not tested	5
	Not present (inter-observer)	1
	<b>Not present (intra- and inter-observer)</b>	<b>15</b>
Accuracy	Unknown	4
	<0.95	4
	Percentages	1
	<b>≥0.95</b>	<b>12</b>
Reliability	Unknown	3
	<b>≥0.95</b>	<b>18</b>
Validation	Not done	5
	<b>Other study</b>	<b>12</b>
	Cross-validation	2
	Independent sample	2
Standard estimation error	Unknown	6
	Threshold	1
	<b>"± X years"</b>	<b>14</b>
Age group	Childhood	4
	<b>Childhood-adolescence</b>	<b>8</b>
	Adolescence-adulthood	5
	Childhood-adolescence-adulthood	4
Factor	Growth	4
	Growth and maturation	5
	<b>Maturation</b>	<b>12</b>

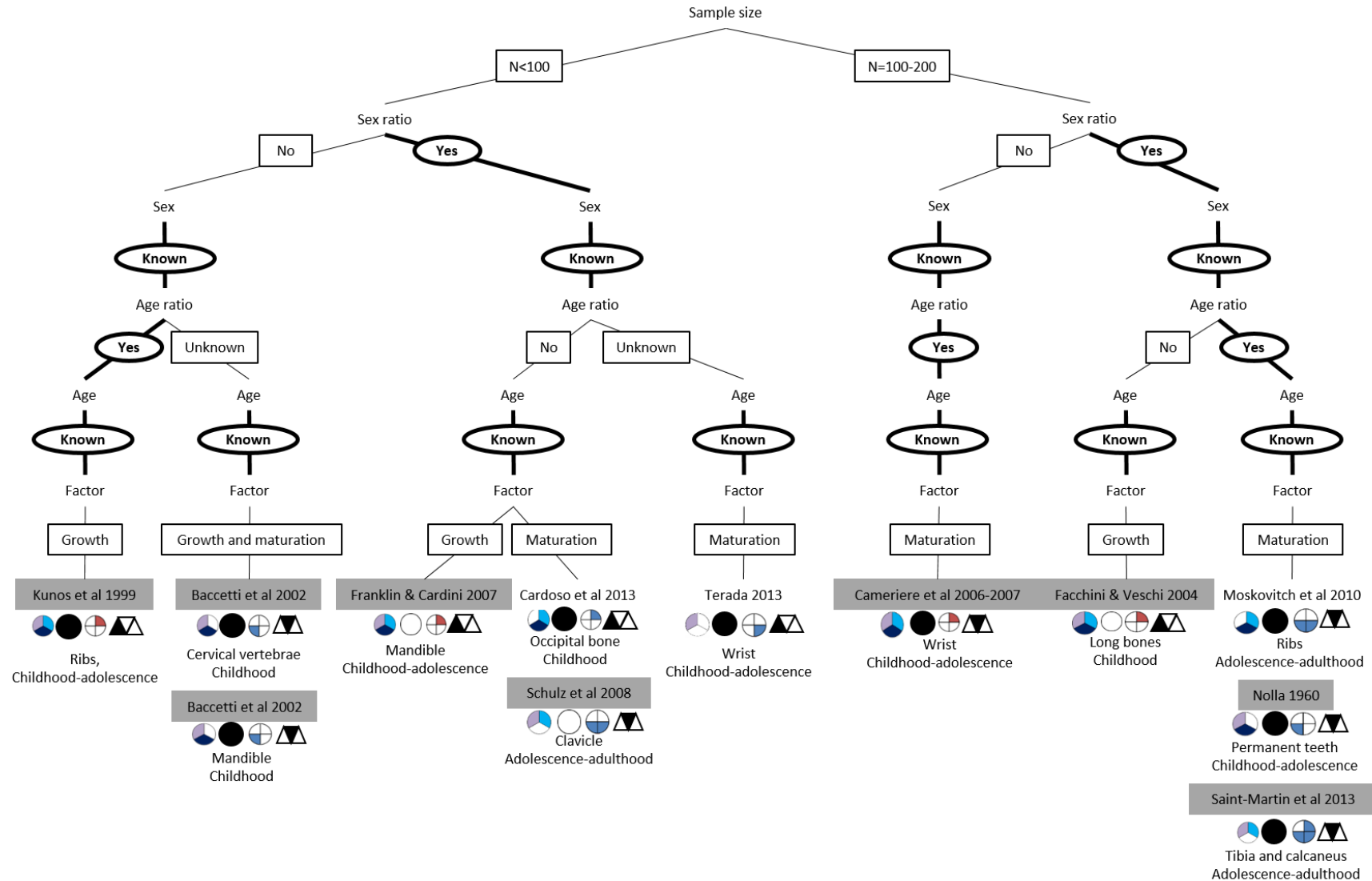
For direct comparison with the empirical classification, these final results are presented as trees (Figures 2.23 and 2.24).

Seventeen of these methods were published as age estimation methods. Although some heterogeneities are perceivable amongst the methods, the overall tendency is a majority of respected sampling and statistical criteria. All methods are of known age and sex, and a clear majority do not present any intra- or inter-observer errors, have a 95% or higher reliability, and provide standard estimation errors and accuracy (superior or inferior to 95%). Most methods respect sex ratio, but age ratio is only respected by one method. Validation of the methods is mainly done by their testing or use in other studies, and that is what is mostly found in literature, as a high number of methods are tested by different authors to evaluate their applicability on different samples or populations. However, the methodological guidelines advise the authors to test their methods themselves first on independent samples, in the original publication or in following work (Adalian et al 2002, 2006; Cunha et al 2009; Heuzé and Cardoso 2008; Vignolo et al 1992).

Although sample size was the most discriminant criteria for sampling clusterisation, the “best” methods resulting from the analysis have heterogeneous sample sizes (four different modalities). It appears that the size of the sample is not necessarily positively correlated to the quality of the method, as seven methods were constructed on less than 100 individuals and only two on more than 1000 individual samples. The most represented age group is childhood-adolescence, but as it is the most represented age group in the corpus, no conclusions can be drawn on the existence of a link between method quality and age group. The same thing can be said for cross-sectional data and maturation. The 21 methods cover all skeletal elements: five use dental elements, five use long bones (three are focused on the knee joint), three use the hand and wrist, three use the ribs, two the mandible, one the cervical vertebrae, one uses the occipital bone and one uses the foot/ankle.

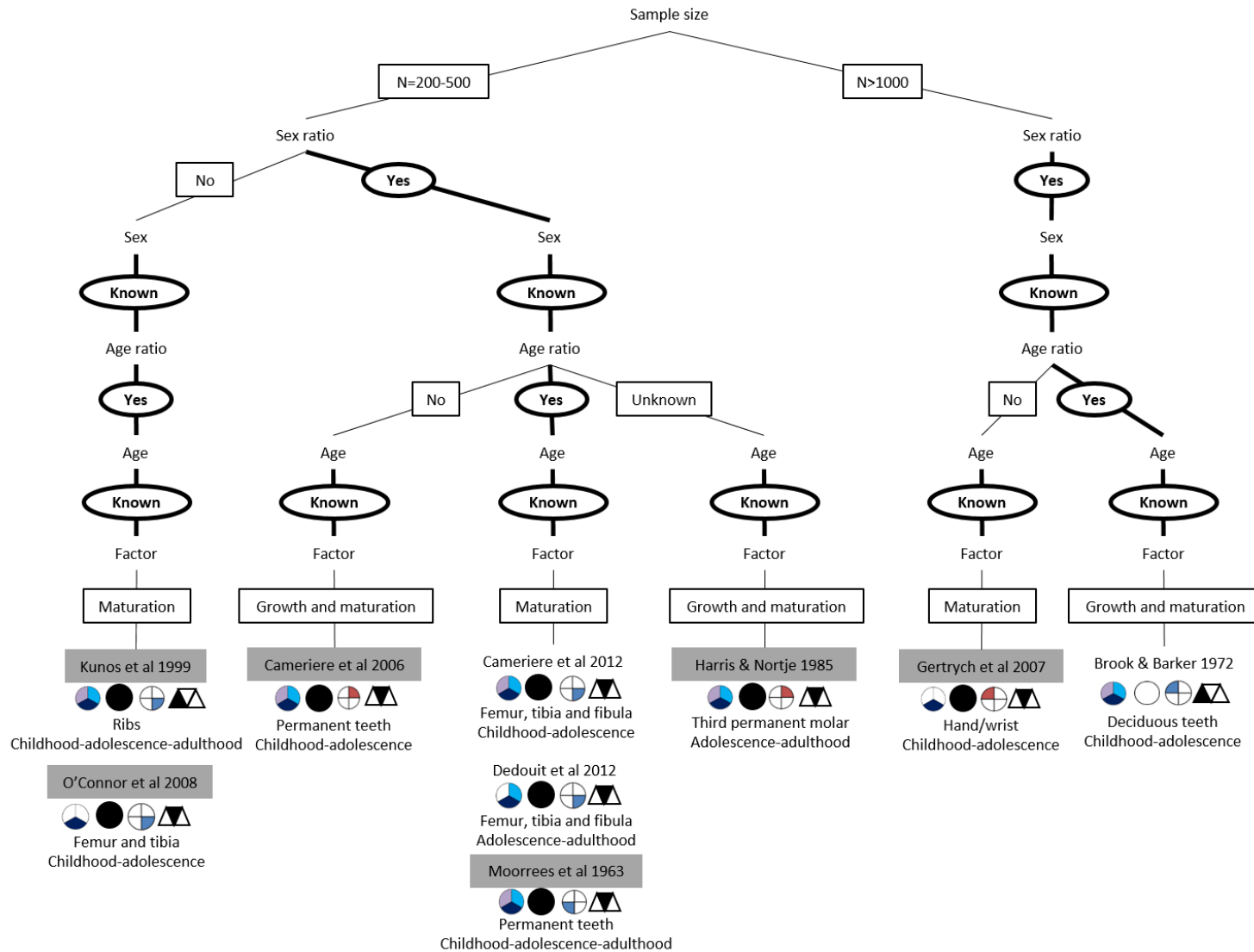
**Based on this “automatic” classification system, these twenty-one methods are the “best” post-natal juvenile age estimation methods. At least ten of them are applicable on dry bones, because they were constructed on dry bone material or tested for such purposes: Cameriere et al 2012a (femur, tibia and fibula), Cameriere et al 2006, 2007a (permanent teeth), Cardoso et al 2013b (occipital bone), Facchini and Veschi 2004 (all six long bones), Franklin and Cardini 2007 (mandible), Harris and Nortje 1984 (third permanent molar), Kunos et al 1999 (ribs), Moorrees et al 1963a (permanent teeth), Moskovitch et al 2010 (ribs).** The other methods were constructed on medical images (radiography, CT scans, MRIs, ultrasounds) or living individuals and were not developed or tested for application on dry bones.

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**Figure 2.23 Classification of the juvenile age estimation methods using samples of less than 200 individuals found in both the best sampling and statistical clusters obtained by automatic clusterisation**

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**Figure 2.24** Classification of the juvenile age estimation methods using samples of 200 or more individuals found in both the best sampling and statistical clusters obtained by automatic clusterisation

## **b. Empirical classifications**

All empirical classifications can be found in **Appendix A**. There is one classification per bone element and per developmental factor used to construct the method (growth or maturation for skeletal elements, growth or mineralisation or eruption for dental elements). The classification of the methods using the growth of the iliac bone contains 14 methods (**Figure 2.25**). Three of them respect all sampling criteria (Rissech et al 2003; Rissech and Malgosa 2005, 2007), one of them respects all three statistical criteria (Micheletti and Boccone 2004). The bold path leads to the methods that respect all sampling criteria, which are also indicated in bold characters.

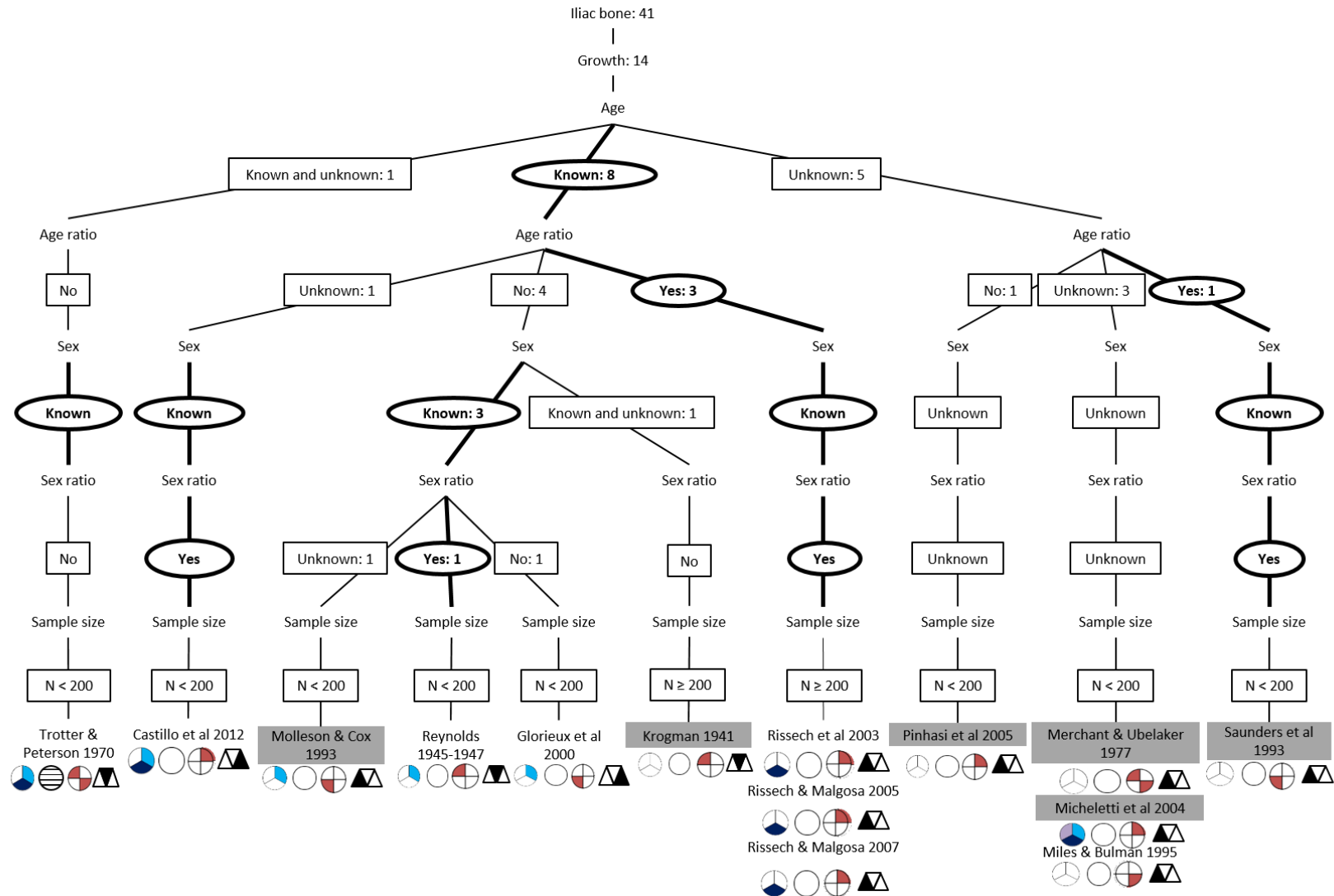
The classification of these fourteen methods was also done according to the age group targeted by the method. These classifications show that most of the juvenile age estimation methods using growth parameters of the iliac bone are constructed on samples covering childhood, adolescence and the beginning of adulthood (**Figure 2.26**) and are therefore aimed to be applied on series of large age ranges, *i.e.* osteoarchaeological series. The next age group most covered by this type of method is childhood-adolescence, followed by childhood (two methods) and finally adolescence (one method). The three methods respecting all five sampling criteria can be used to estimate the age of individuals from childhood to adulthood. The only method respecting all three statistical criteria is destined to children and adolescents.

The general results obtained for all empirical classifications are presented in **Table 2-14** for sampling criteria and **Table 2-15** for statistical criteria.

**Table 2-14** shows the 34 methods that respect all five sampling criteria (known age, known sex, homogenous age and sex ratios, sample size over 200 individuals). The general predominance of methods using maturation parameters is still present, but can be explained by the higher number of methods using them.

All bone regions are covered except for the cranial elements only represented by Konie's method using maturation of the spheno-occipital synchondrosis. All methods except one (Nanda and Chawla 1966) cover the age groups childhood-adolescence or childhood-adolescence-adulthood, meaning the methods are meant to be applied on postnatal juveniles of any age. This is particularly interesting for bioarchaeological studies, where methodological homogeneity is preferable. Indeed, using a limited number of methods diminishes the bias induced by the use of methods built using different criteria that limit result comparison (Smith, *in* Kelley and Larsen 1991).

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**Figure 2.25 Classification of the juvenile age estimation methods based on growth parameters of the iliac bone. The bold lines and words correspond to the methods respecting empirical sampling criteria**

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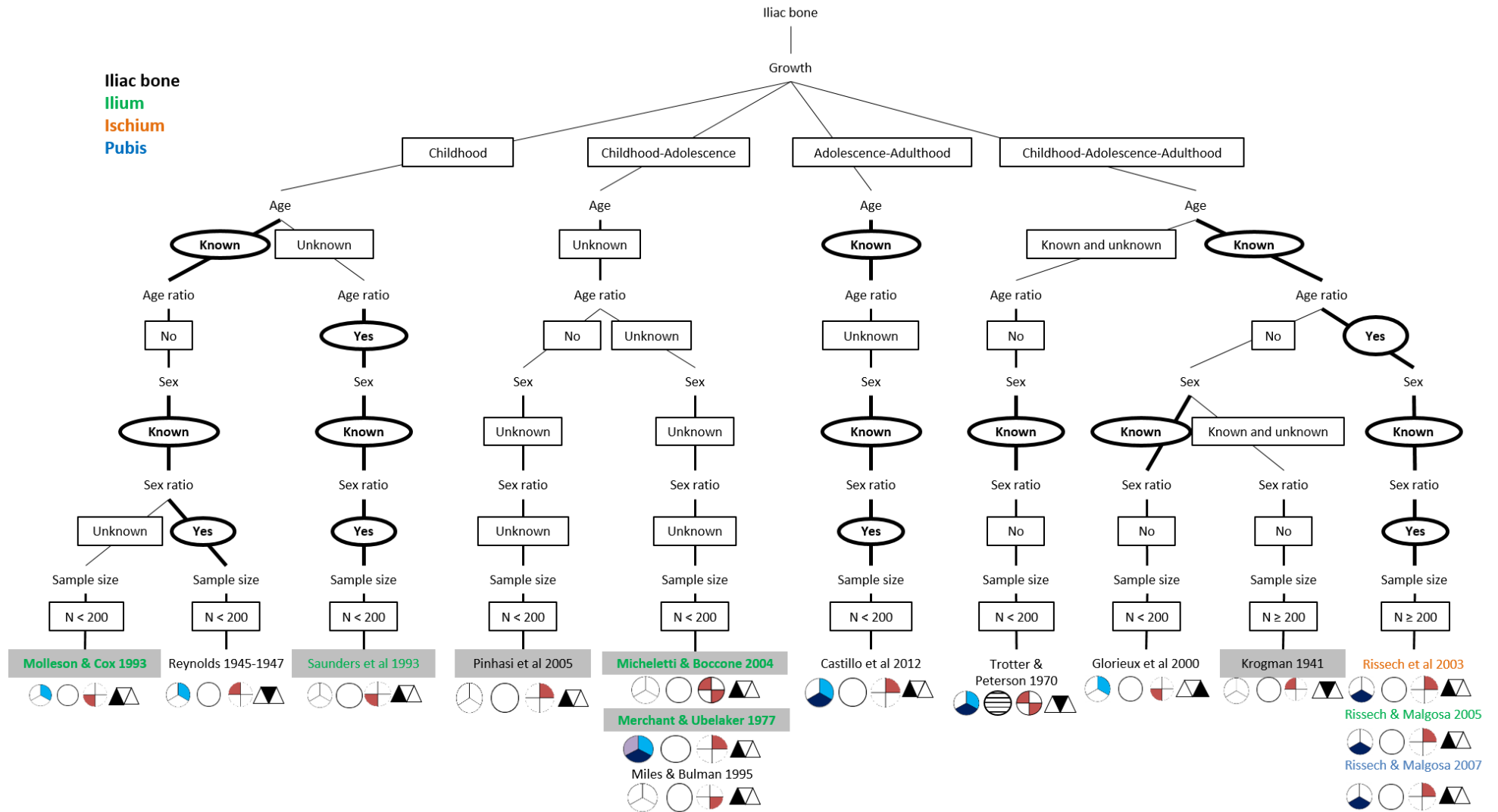


Figure 2.26 Classification of the juvenile age estimation methods based on growth parameters of the iliac bone according to age group



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**Table 2-14 Methods that respect the best “empirical” sampling criteria, with the factor and the element used for age estimation and the age range of the individual samples**

Process	Skeletal and/or dental elements	Methods	Age range	Applicable on dry bones/teeth**
Growth	Humerus, radius, ulna, femur, tibia, fibula	Maresh 1955, 1970*	0.125-18 years	Yes
	Hand/wrist	Baroncelli et al 2006	2-21 years	
		Cameriere et al 2008	5-17 years	
		Lalys et al 2006*	0-20 years	
		Olivete and Rodrigues 2010*	6-16 years	
	Iliac bone	Rissech et al 2003		Yes
		Rissech and Malgosa 2005	0-97 years	Yes
		Rissech and Malgosa 2007		Yes
	Femur	Castriota-Scanderberg and de Micheli 1995	1.9-14 years	
		Rissech et al 2008	0-25 years	Yes
Sternum	Bayarogullan et al 2014	0-20 years		
Mixed dentition	Nanda and Chawla 1966	6-12 years	Yes	
Maturation	Spheno-occipital synchondrosis	Konie 1964	7.5-24 years	Yes
	Humerus, radius, ulna, hand/wrist, foot/ankle	Garn et al 1967	0-18 years	
		Webster and de Saram 1954	9-17 years	Yes
	Hand/wrist	Baroncelli et al 2006	2-21 years	
		Chumlea et al 1989	0-18 years	
		Fishman 1982	0-25 years	
		Garn et al 1967	0-18 years	
		Gilsanz and Ratib 2005	0.67-18 years	
		Greulich and Pyle 1959*	0-20 years	
		Konie 1964	7.5-24 years	
		Roche et al 1988	1-18 years	
	Femur, tibia, fibula	Cameriere et al 2012a*	14-24 years	
		Dedouit et al 2012	10-30 years	Yes
		Garn et al 1967	0-18 years	Yes
		Roche et al 1975	1-18 years	
	Sternum	Bayarogullan et al 2014	0-25 years	Yes
Deciduous dentition	Brook and Barker 1972*	0-16 years		
Mixed dentition	Foti et al 2003*	6.1-21.1 years	Yes	
	Nanda and Chawla 1966	6-12 years	Yes	
Permanent dentition	Foti et al 2003*	6.1-21.1 years	Yes	
	Moorrees et al 1963a*	0-25 years	Yes	
	Rozkovcova et al 2012	5-21 years	Yes	

\*Methods respecting all five sampling criteria and the three main statistical criteria: reliability, accuracy and precision. Methods in bold characters do not present any intra- or inter-observer errors. \*\* These methods can be applied directly on dry bones/teeth, or medical images of dry bones/teeth, or histological sections of dry bones/teeth

Most of these methods are applicable to dry bone material, with the exception of methods using the hand/wrist (Baroncelli et al 2006; Cameriere et al 2008; Chumlea et al 1989; Fishman 1982; Garn et al 1967; Gilsanz and Ratib 2005; Greulich and Pyle 1959; Konie 1964; Lalys et al 2006; Olivete and Rodrigues 2010; Roche et al 1988).

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**Table 2-15 Methods that respect the best “empirical” statistical criteria, with the factor and the element used for age estimation and the age range of the individual samples**

Process	Skeletal and/or dental elements	Methods	Age range	Applicable on dry bones/teeth**
Growth	Mandible	Franklin and Cardini 2007	1-17 years	Yes
	Clavicle and 6th rib	Stout and Paine 1992	13-62 years	Yes
	Ribs	Kunos et al 1999	1-20 years	Yes
	Humerus, radius, ulna, femur, tibia, fibula	Facchini and Veschi 2004	0-12 years	Yes
		Maresh 1955, 1970*	0.125-18 years	Yes
		Stull et al 2014a	0-12 years	Yes
	Humerus, radius, ulna, ilium, femur, tibia, fibula	Micheletti and Boccone 2004	0-15 years	Yes
	Hand/wrist	Cameriere et al 2006-2007a	5-17 years	
		Lalys et al 2006*	0-20 years	
		Olivete and Rodrigues 2010*	6-16 years	
	Teeth and hand/wrist	Cameriere and Ferrante 2008	6-15 years	
	Femur	Castriota-Scanderberg and de Micheli 1995	1.9-14 years	
	Permanent teeth	Cameriere et al 2006-2007a	5-15 years	Yes
	Third permanent molar	Harris and Nortje 1984	15-21 years	Yes
Mixed dentition	Liversidge et al 1998, 1999	0-20 years	Yes	
	Mörnstad et al 1994	5.5-14.5 years	Yes	
Maturation	Clavicle	Kreitner et al 1998	0-29 years	
		Langley-Shirley and Jantz 2010	11-33 year	Yes
		Schmeling et al 2004	16-30 years	
	Clavicle and 6 <sup>th</sup> rib	Stout and Paine 1992	13-62 years	Yes
		Ribs	Kunos et al 1999	1-25 years
	Sternum and ribs	McCormick and Stewart 1988	15-60 years	
		Thouvenin et al 2009	10-97 years	
	Vertebrae	Cardoso and Rios 2011	9-30 years	Yes
	Hand/wrist	Dvorak et al 2007a, 2007b	14-19 years	
		Garn et al 1961	Unknown	
		Greulich and Pyle 1959*	0-20 years	
	Femur, tibia, fibula	Cameriere et al 2012a*	14-24 years	Yes
	Foot/ankle	Hoerr et al 1962	0.18 years	
		Whitaker et al 2002	0-20 years	
	Deciduous dentition	Brook and Barker 1972*	0-16 years	
		Moorrees et al 1963b	0-15 years	Yes
		Ohtani 1994	1-15 years	
	Mixed dentition	Buchet et al 2006	3-17 years	Yes
		Gustafson and Koch 1974	0-15 years	Yes
		Hägg and Taranger 1985	0-18 years	Yes
Kaul and Pathak 1988		6-14 years		
Permanent dentition	Cameriere et al 2006-2007a	5-15 years	Yes	
	Chaillet and Demirjian 2004	2-18 years	Yes	
	Demirjian et al 1973	3-16 years	Yes	
	Demirjian and Goldstein 1976	2.5-17 years	Yes	
	Foti et al 2003*	6.1-21.1 years	Yes	
	Gates 1966	6-15 years		
	Heuzé 2004	3.5-16 years	Yes	
	Moorrees et al 1963a*	0-25 years	Yes	
Third permanent molar	Roberts et al 2008	1.8-26.1 years	Yes	
	Wolanski 1966	5-21 years		
	Harris and Nortje 1984	15-21 years		
	Kullman et al 1992	15-25 years		

The methods presented here respect the three main statistical criteria: reliability, accuracy and precision. Methods with sufficient variable repeatability and reproducibility are indicated in bold characters. \*Methods respecting all five sampling criteria. \*\* These methods can be applied directly on dry bones/teeth, or medical images of dry bones/teeth, or histological sections of dry bones/teeth

Using the classifications, we can also highlight the 49 methods that respect all the statistical and methodological criteria (reliability, accuracy, precision, repeatability and reproducibility). The number of methods respecting statistical criteria is more important than the ones respecting sampling criteria (**Table 2-15**). This can be explained by the difficulty of obtaining referenced samples (with individuals of known age and sex) of consequent size, and homogenous age and sex ratios, especially in osteological collections, whereas obtaining sufficient statistical criteria relies on the constructor and the quality of the age indicator. It is therefore less material-dependent than sampling and less restrictive for method selection.

Most of these methods were developed between the 1960's and the 2000's, which confirms the observations previously made (**see section 2.1.**). The predominance of maturation remains, although the number of methods using growth parameters is higher than in the previous table. The methods also cover a larger diversity of skeletal and dental elements and can be used for age estimation in archaeological or forensic contexts (with the exception of a few methods applicable to living individuals via medical imaging). The main problem with a few of these methods is that caution is necessary before applying them to dry bone material, as they were constructed on medical images obtained from living individuals.

Seventeen methods also tested variable repeatability and reproducibility. Four methods respect the five sampling criteria (known age and sex, homogenous age and sex distribution, consequent sample size) and the three main statistical criteria important for method application (sufficient reliability, known precision and accuracy) and were successfully tested for repeatability and reproducibility.

**Based on this empirical classification system, these four methods are objectively the “best” post-natal juvenile age estimation methods. The references of these methods are: Cameriere et al (2012a), Greulich and Pyle (1959), Lalys et al (2006), Moorrees et al (1963a).**

**Two of them are applicable on dry bones. R. Cameriere and collaborators (2012a) use maturation scores to place an individual below or over the 18-year threshold, and C. Moorrees and collaborators (1963a) use mineralisation stages of deciduous or permanent teeth, applicable to juveniles of all ages.**

A brief parenthesis can be made at this point: our classification system highlights the methods that respect all sampling, statistical and methodological criteria, so, in theory, they should be the “best” methods. They provide sufficient scientific arguments to be included in an official report, have a reliability higher than 95%, are accurate and precise. What our classification cannot account for are methods that are frequently used by the anthropological community, and provide sufficiently good

(accurate and reliable) results, but are constructed on questionable or biased samples.

This is the case, for example, of the dental age estimation method of D. Ubelaker, constructed on an archaeological sample of Amerindians of unknown age and sex (Ubelaker 1978, 1989). This method provides accurate estimates of age, even if other methods are better (Moorrees et al 1963a and Roberts et al 2008, both of which respect all standardised criteria). This leads to several conclusions:

- The strength of the relation between age and biological development depends on too many factors to be constant in all bones, ages, populations, or both sexes. Indeed, it varies according to age, the variable expressing developmental changes, populations, sex, secular changes, sanitary state, environmental factors... Even if, theoretically, the best estimators of age can be inferred from developmental patterns, it is probable that a part of that variability will not be grasped, incorporated in the age estimation method and therefore, will not participate in the improvement of the estimate. This is why a method using an estimator with a stronger relation with age (correlation, covariation, etc.) can give better age predictions than another one that respects all methodological criteria but uses a less powerful estimator of age;
- The choice of the method depends on the objective of the user. The user can choose to privilege sample composition, statistical criteria, or simply the most accurate method, independently of its construction. This is why all the discriminant criteria appear independently in the empirical classification as indicators, and leave the possibility of choice to the user;
- It must be remembered that the criteria selected for building the classification and evaluating the methods were chosen for methodological standardisation and therefore, their primary goal is methodological harmonisation to enable method comparison and evaluation. There is always the possibility that the methodological frame could restrict the expression of certain biological realities (such as the increase in individual variability with age) and artificially bias them by hiding them or enhancing them.

### **c. Comparison of the results**

The discriminative power of the sampling criteria was different for clusterisation using MCA and HCPC than for the empirical classifications: where clusterisation was done according to sample size, followed by sex-related criteria, and age-related criteria, empirical classification was done using the developmental factor, followed by age-related criteria, sex-related criteria and lastly, sample size. It is interesting to see that the automatic clusterisation grouped the modalities that are empirically

considered the most important for sampling in cluster 2Sa: known age and sex, and mostly respected age and sex ratios. The modalities of these four criteria are often paired to follow similar trends (both age and sex are known or unknown, both age and sex ratios are respected or not). Lower discriminative power was awarded to age, age ratio and sex ratio in the automatic clusterisation, compared to the empirical classifications. Although these modalities seem less important than known age and sex, they can severely bias age estimation methods if the number of individuals in the different age groups is unbalanced, and if one sex is significantly more represented than the other. Although sex ratio becomes irrelevant for unisexed methods, the bias induced by unrespected sex ratios is particularly true for methods including adolescent individuals, for whom sexual dimorphism is marked in both growth and maturation (**see sections 1.3.2. and 1.3.3.**). Indeed, in most forensic or archaeological cases, sex is unknown and cannot be reliably determined for juveniles. Therefore, including general individual variability to construct an unsexed age estimation method also includes variability according to sex.

Sample size is an important parameter and is the most discriminant in automatic clusterisation. In sample 2Sa, it selected methods with 100-200 or 200-500 individuals. However, the final top-methods obtained by combining the sampling and statistical clusterisations showed heterogeneity in sample size, ranging from the smallest (<100) to the largest (>1000). This shows that our empirical threshold for sample size at 200 was possibly too restrictive. However, some reserves need to be made on the sufficient apprehension of developmental variability in small samples (**see section 1.3.5.**).

In the empirical and automatic classifications, the age group childhood-adolescence-adulthood was found to be associated more frequently with respected sampling criteria. No tendency of this kind was observed with automatic clusterisation or empirical classification based on the statistical criteria.

The total number of methods identified with automatic clusterization as respecting sampling and statistical parameters is higher (21) than it is in the empirical classification (4). Only two out of the four empirically “best” methods are common to the 21 statistically “best” methods (Cameriere et al 2012a and Moorrees et al 1963a). Eleven of the best clusterisation methods respect either the best empirical sampling or the best empirical statistical criteria. Three of them respect both criteria types (Brook and Barker 1972; Cameriere et al 2012a; Moorrees et al 1963a), one respects empirical sampling modalities (Dedouit et al 2012) and six respect empirical statistical modalities (Cameriere et al 2006, 2007a; Facchini and Veschi 2004; Franklin and Cardini 2007; Harris and Nortje 1984; Kunos et al 1999). The predominance of statistically acceptable methods is once again visible.

The automatic and empirical classifications highlight the same associations of statistical criteria: 95% reliability, known standard estimation error and known accuracy, and low intra- (and/or inter-) observer errors are opposed to unknown reliability, standard estimation error and accuracy associated with untested observer errors. Both classifications separate “good” and “bad” statistical modalities. Automatic clusterisation allowed the evaluation of validation techniques and found that any form of validation is acceptable for method evaluation. However, following the recommendations emitted by AGFAD, it is best to include methodological testing in the original publication of any new method, if only to provide a reference to compare future results to.

Although criteria were given different priorities, the final results are comparable: methods that respect sampling and/or statistical criteria are discriminated from methods that do not. The empirical classification is more precise and more restrictive, but it takes longer to build, as it considers each method independently and it is bound to be submitted to user and constructor subjectivity in choosing the hierarchy of the criteria. However, the statistical classification confirms the importance of particular sampling and statistical parameters for method validity that were empirically chosen and bibliographically identified in the series of recommendations. This critical analysis allowed to justify the choices made in criteria selection for method evaluation, therefore reaffirming their objectivity and validating their use for methodological evaluation and comparison. The ideal solution for an objective classification would be no imposed hierarchy for criteria selection, as it seems more objective to leave the prioritisation to the practitioner who will adapt his priorities to the goal behind the age estimation and choose the selective criteria accordingly. This would guarantee total freedom in criteria prioritisation and would generate methods adapted to personal preferences and goals. Complete objectivity and freedom of choice would require developing an automatic decisional tool under the form of an algorithm in a software, R package, or mobile application. This is our first perspective of research following the present study.

## **2.4. Conclusions on juvenile age estimation**

Variability can be found at all levels of biological development: intra-/ inter-individual, intra-/ inter-population; its sources are themselves variable: sex, age, socio-economic and sanitary status, secular trends... The variability inherent to biological development is why it is difficult to construct methods applicable to all juveniles, independent of age, sex, or population and also explains the limitations in terms of methodological precision of the estimated age, as variability increases.

### ***Juvenile age estimation: methodological issues and perspectives***

Theoretically, the best method(s) for estimating the age of an individual using bone or tooth elements would be the one(s) exploiting the most representative and correlated biological process(es) of the individual's age group. This implies knowing the phases of activity of skeletal and dental development and referring them to the five main juvenile age groups: prenatality, infancy, childhood, adolescence and early adulthood. In this lies the paradox of juvenile age estimation: the age group of an individual has to be presumed in order to know which methods are the most likely to provide reliable and precise age estimates. This is also why certain methods are more precise or reliable for certain age groups.

Several practical limitations rise to complicate the situation: depending on the preservation rates and the material, the most representative parameter of an age group is not necessarily available for age estimation. This is even more problematic when dealing with several juvenile individuals, as is the case in past population studies. Depending on the preservation of the elements, different methods are likely to be applied to the individuals. Therefore, age comparison is biased by the lack of methodological homogeneity; the most representative parameter of an age group is not necessarily the best estimator, because the corresponding methods are not always the "best" (most accurate, precise and reliable, built on solid statistical and sampling criteria); individual age estimation does not have the same implications as collective age estimation and must not be planned in the same way. Individual age estimation relies on the available material to provide the most reliable and precise estimate. Collective age estimation must be done serially, and include the highest number of homogenous estimates possible, at least according to the main age groups.

The problem of heterogeneous methods is visible in the absence of consistency between results obtained on archaeological and forensic collections. This is mainly due to the differences in methodological objectives (collective versus individual age estimation), but respecting homogenous methodological construction criteria would help standardise method application and legitimate result comparisons (Cunha et al 2009; Schmeling et al 2003b, 2007).

Most methods developed for age estimation of the living are not applicable to age estimation of the deceased: indeed, they often consider several elements anatomically connected by cartilage (*e.g.* hand/wrist, knee, and clavicle), which is absent in human remains. In addition to this, age estimation in the living often uses medical imaging techniques that can induce application biases or incompatibilities for human remains. Even though some methods can be used in both cases, authors treat these two contexts separately, as the implications and applications are very different.

Concerning the choice of age estimation methods according to age groups, the following guidelines can be proposed as a summary of what is already recommended in literature and of the conclusions of this study.

**Summary: Guidelines for selecting juvenile age estimation methods (according to literature and confirmed by the present study)**

- Prenatals and perinatals: privilege methods with growth parameters of the postcranium (long bone measurements) and maturation parameters of the cranium (basicranium, teeth) as estimators (more precise estimates)
- Infants: privilege methods using cranial maturation and dental mineralisation. Age can be estimated reliably and precisely without knowing sex
- Children: this age group (0-12 years) suffers from less discriminant methods for age estimation compared to the other age groups. Growth and maturation are both active in the cranium and postcranium, and can both be exploited by identifying and focusing on particular phases of pronounced activity. This means ossification sequences of the bones must be known to select the most appropriate one. Age can be estimated reliably and precisely without knowing sex
- Adolescents: this age group covers several legal thresholds (10 years, 14 years, 15 years and 16 years), so precise estimates are required. Sexual dimorphism becomes more and more significant, starting before the pubertal growth spurt, so age estimation is generally more precise and reliable when methods are sexed. Growth progressively stops after the pubertal spurt, and maturation is extremely active with the fusion of secondary ossification centres. Age estimation of adolescents should combine information from as many epiphyses as possible to provide the most accurate estimate of age (O'Connor et al 2008)
- Young adults: this period is marked by the final maturation processes of three bones (the iliac crest of the coxal bone, the sternal end of the clavicle and the spheno-occipital synchondrosis), and, with a relatively high variability, the end of formation of the third permanent molar. These parameters are extensively exploited for determining legal majority (under or over 18 years) in forensic contexts, and in the living in particular. In bioarcheology, the presence of one of the three bone epiphyses in an unfused state automatically places the individual in the young adult age group

**The methods should respect sampling and statistical criteria to meet the scientific standards of method construction and application, necessary in any context to justify the results and the conclusions drawn from the estimation**



**Summary: Juvenile age estimation in physical anthropology**

- Juvenility is a pluridisciplinary, plurifactorial and context-dependent concept
- Biologically, juvenile individuals are still undergoing growth and maturation (development)
- Age is the principal component of a juvenile's biological profile, used for individual identification or population studies
- Indicators of skeletal or dental development are used to estimate age of juveniles in physical anthropology
- The difference between real age and estimated age is due to various sources and levels of variability
- Age estimation methods must respect standardised criteria and protocols to be scientifically and biologically valid. A majority of methods do not respect these conditions

In conclusion, the discussion on juvenile age estimation still remains open, because of the heterogeneity of the methods, of the populations they were constructed on and the ones they are applied to. One partial solution is to standardise all methods, old and new, by respecting common construction criteria and common recalibration protocols, to at least be able to compare the results using a common basis. Concerning the debate on population- and sex-dependent methods versus population- and sex-independent methods, although there is reason to believe that specific methods have better accuracy (Cameriere et al 2008; Dvorak et al 2007a and 2007b; Ericksen 1991; Franklin 2010; Frucht et al 2000; Hunt and Hatch 1981; Olze et al 2007; Ontell et al 1996; Rissech et al 2013a; Schmeling et al 2007; Stull et al 2014a), forensic anthropologists and bioarchaeologists are still faced with the problem of dealing with human remains whose ancestry/geographic origin and sex cannot be reliably determined (Braga et al 2005; Pechnikova et al 2011; Saint-Martin et al 2014; Townsend and Hammel 1990).

## **2.5. Constructing a standardised juvenile age estimation method**

### **2.5.1. What are the needs in terms of juvenile age estimation from skeletal elements?**

The critical review and classification systems proved there is a lack in standardised methodological and statistical criteria for the common application and evaluation of juvenile age

estimation methods using skeletal or dental remains. This is particularly true for methods constructed and/or applied on dry bone material, which interest bioarchaeological studies, and the recovery of juvenile human remains in forensic contexts. Indeed, precise, reliable and accurate age estimates are essential for any study done on juvenile dry bones and teeth as they are the most discriminant and often sole information of the individuals' biological profile (Cunha et al 2009; Quatrehomme 2015; Scheuer and Black 2000).

The critical review also highlighted the particular lack of standardised age estimation methods for pre-pubertal juveniles that meet all the scientific validity parameters demanded by Courts (*e.g.* Daubert criteria, AGFAD recommendations).

It is difficult to construct methods incorporating "sufficient" developmental variability: because of insufficient sample sizes, the use of cross-sectional or longitudinal data, and other sampling biases, it is difficult to incorporate all levels of individual, sexual, population variability. This remark also leads to the controversial reflection on whether or not population-specific methods should be developed. Opinions diverge on that particular question.

How do we proceed to address all these points?

### **2.5.2. Methodological goals of our juvenile age estimation method**

The goal of the second part of our study is to address the need in terms of juvenile age estimation methods respecting the standardised sampling and methodological criteria of construction that would improve age estimation by providing a more accurate estimation of age. The method we would construct should be applicable on human remains found in a forensic or archaeological context. The material chosen for our study would be skeletal elements, because it seems that standardised methods using skeletal elements lack more than dental methods, and since variability is more important and less incorporated in skeletal methods, it seems research is needed in that area. The material of study should be dry bones or other medical imaging data obtained from bones, bearing in mind that the chosen variables should also be applicable to dry bones, as the method aims to be applicable in both archaeological and forensic contexts.

Methodological recommendations and the critical review presented previously provide the guidelines to construct juvenile age estimation methods that respect strict sampling, statistical and methodological criteria. By adopting this approach, the method can be objectively evaluated using statistical criteria, it can be compared to the statistical criteria of other methods, and it presents all the arguments for justifying method selection by practitioners.

# A standardised approach of juvenile age estimation

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## Chapter 3. Material

### 3.1. Study sample and variables

This study was done using different sources of osteological data:

- the medical imaging database of the Assistance Publique des Hôpitaux de Marseille (AP-HM) was used to collect CT scans of patients living in Marseilles to obtain three-dimensional reconstructed bone surfaces;
- biometric iliac data directly obtained from a sample of individuals from the Centre Hospitalier Universitaire (CHU) of Toulouse was used to test age prediction equations;
- a sample of juvenile individuals from the osteological Luis Lopes reference collection of the Museu de Historia Natural et da Ciencias in Lisbon, Portugal was studied. Biometric data of the clavicle, L5 and ilium and non-biometric data of the iliac bone were recorded for each individual and used to test age prediction models.

For each sample, all or part of biometric and/or non-biometric variables were recorded depending on available data (preservation state of the dry bones, integrity of the scanned bones, etc.). Data concerned one, two or all three of the different bones per individual and is presented thereafter (**see section 4.1.**).

The three bones each represent one type of bone as defined by osteologists (White and Folkens 2005; White et al 2012): long, short and flat bones (**Figure 3.1**). Long bones (*e.g.* radius) are composed of a central part, the diaphysis, and two extremities, the epiphyses. Flat bones have a distinctive flat part (*e.g.* scapula) or are entirely flat (*e.g.* cranial bones). Short bones are compact and rather massive, with no particular orientation other than anatomical orientation (*e.g.* patella).



**Figure 3.1** Three types of bones found in the Human skeleton: flat bone (iliac bone, left), long bone (clavicle, centre) and short/irregular bone (lumbar vertebra, right). (Source: eskeletons.org)

### **3.1.1. Growth and maturation patterns of the three bones studied**

If the starting date corresponds to the last menstruation cycle, age is called gestational age. The time until birth is typically equal to 280 days / 40 gestational weeks (GW) / 10 lunar months.

If age is calculated starting from the date of fertilisation, it is called fertilisation age, or embryonic/foetal age. Time until birth is typically equal to 266 days/38 weeks/9.5 lunar months.

The ages used hereafter are gestational ages.

#### **a. Flat bone: the iliac bone**

The iliac bone or coxal bone is a paired flat bone. It constitutes the pelvic girdle; along with its opposite counterpart, the sacrum and the coccyx. An iliac bone has three articular surfaces: it articulates medially via its auricular surface to the auricular surface of the sacrum; anteriorly, via the symphysis, with the symphysis of the opposite iliac bone; and laterally, via the acetabulum, with the femoral head, to form the hip joint.

The three elements of the pelvic bone (ilium, ischium and pubis) all appear during foetal life following an endochondral primary ossification process. At the fifth gestational week, mesenchymatous cells appear in the future pelvic region, and form the entire inferior limb (bones and soft tissues). The proximal cells form three bone processes, each corresponding to one of the three pelvic bone elements (Fazekas and Kosa 1978). Foetal and more generally juvenile iliac bones are therefore composed of three distinctive bony elements that will later progressively fuse with one another: the pubis (antero-inferior), the ischium (postero-inferior), and the ilium (supero-lateral).

The iliac primary ossification centre appears at the beginning of the third prenatal month, followed by the ischial centre around 4-5 months, and finally the pubic centre around 5-6 months (**Figure 3.2**). All centres are located close to the future acetabular cavity (Scheuer and Black 2004). Ossification of the ilium progresses in a characteristic radiating manner. At the beginning of the second trimester, the ilium is ossified and its length is greater than its width (Fazekas and Kosa 1978). After the third month, the iliac crest becomes more and more convex, and ilium width increases. The greater ischiatic notch and the postero-inferior iliac spine are formed at 4.5 months, and the antero-inferior iliac spine is visible at 5 months. Because of this, the ilium is recognisable as early as 4-5 foetal months.

The ischium's initial shape resembles an apple seed, and changes to resemble a comma around the fifth foetal month. The ischium acquires its identifiable morphology (curved antero-superior part

to form the obturator foramen and the premises of the acetabular surface externally) by the third trimester.

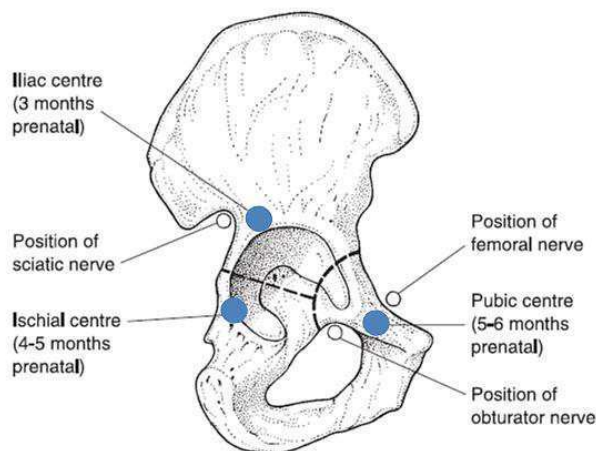


Figure 3.2 The three primary ossification centres of the iliac bone (blue dots) and their time of appearance. From *The Juvenile Skeleton*, Scheuer and Black, 2004 p.318

The pubis develops last from one or more primary centres that unite a few months after birth. The posterior part facing the ischium is pointy, and the antero-superior part (the future symphysis) is curved (Fazekas and Kosa 1978). The line of the pecten pubis is clearly visible on the lateral side of the bone (Scheuer and Black 2000). At birth, the three elements of the iliac bone are connected at the acetabulum by what is called a “Y-shaped” cartilage (Fazekas and Kosa 1978). Significant postnatal changes occur in the region of the acetabulum. The iliac articular part becomes less and less flat, and more and more concave and depressed (Baker et al 2005; White and Folkens 2005; White et al 2012).

The last step of primary ossification of the iliac bone is the fusion of the pubic and ischial rami, which generally occurs between 5 and 8 years (Figure 3.3). It sometimes provokes an enlargement of the ends of the rami, which disappears around 10 years of age.

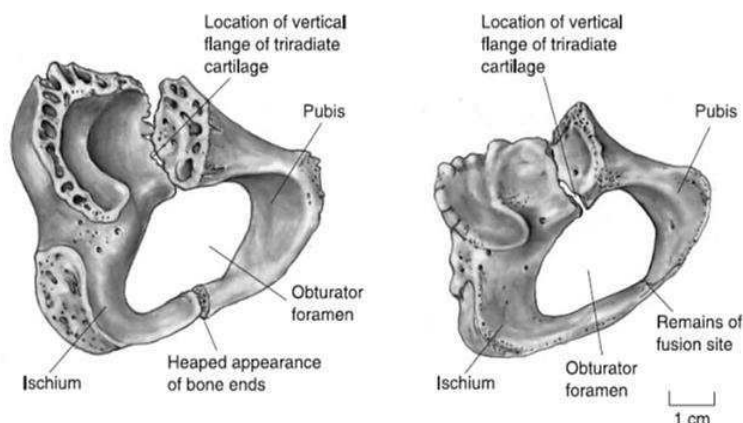
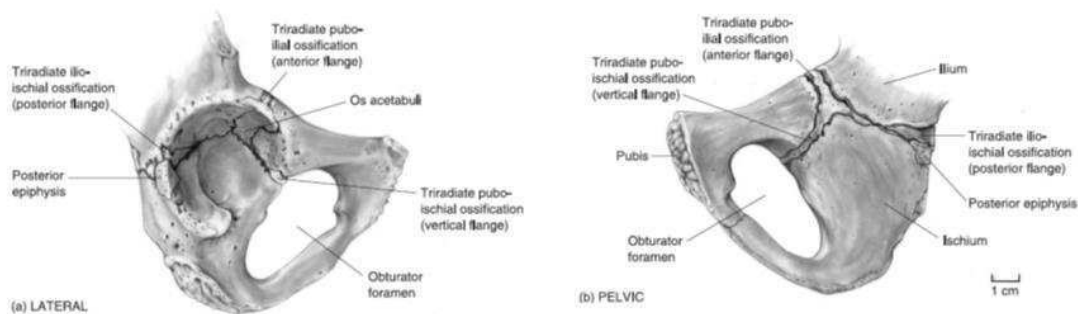


Figure 3.3 Incomplete (left) and complete (right) ischio-pubic fusion. From *The Juvenile Skeleton*, Scheuer and Black, 2004 p.324

At this point, the puberty growth spurt takes effect and has a dramatic effect on the iliac bone (White and Folkens 2005). A secondary centre of ossification for the acetabulum has appeared around 9-12 years. Other accessory ossification centres can also be present, but are rare and variable. Acetabular fusion is radiative and quite complex, which is why the acetabulum is also named “triradiate zone” (Scheuer and Black 2000). Indeed, three types of cartilage are present (**Figure 3.4**):



**Figure 3.4** External (left figure) and internal (right figure) view of the acetabular region showing the ossification of the triradiate complex and the acetabular epiphyses. Modified from *The Juvenile Skeleton*, Scheuer and Black, 2004 p.326

- The **growth/conjugation cartilage**, surrounding;
- The **epiphyseal cartilage (Figure 3.9)**. This cartilage is composed of three principle lateral elements or epiphyses: the **anterior epiphysis** or *os acetabuli* (the first epiphysis to fuse), between the pubis and the ilium, which forms around 9-10 years of age (**Figures 3.5 and 3.6**). Other accessory epiphyseal islets of bone can also be found in the epiphyseal cartilage; the **posterior epiphysis**, between the ischium and the ilium, which appears at 10-11 years; and the **superior epiphysis**, on the ilium, which forms around 12-14 years. These epiphyses “project” medially into three borders or “flanges”: one vertical, one anterior and one posterior. These flanges form the triradiate or “Y-shaped” cartilage;
- The articular cartilage, presenting a distinctive half-moon-like shape. It is surrounded by the epiphyseal cartilage.

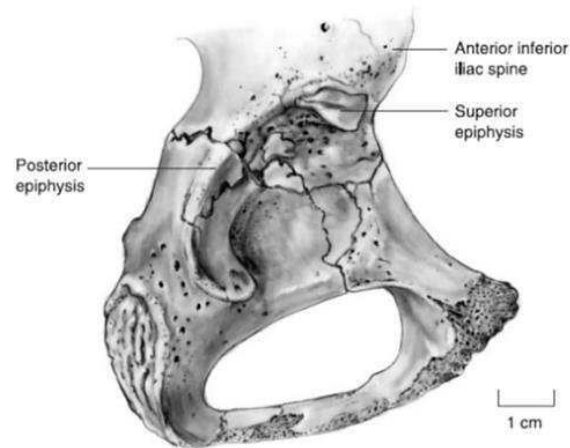


Figure 3.5 Posterior and superior acetabular epiphyses. From *The Juvenile Skeleton*, Scheuer and Black, 2004 p.328

The three main epiphyses composing the epiphyseal cartilage of the acetabular region appear and develop in the cartilaginous space between the ilium, ischium and pubis. They will extend to form the borders and the articular surface of the acetabulum.

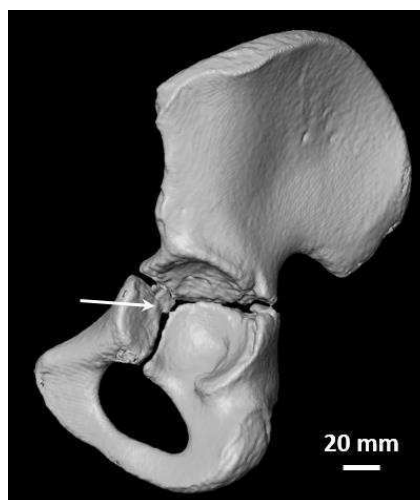


Figure 3.6 *Os acetabuli* (white arrow) observed on the reconstructed left coxal bone of a 9 year-old girl from Marseilles

The interstitial growth process of this zone allows the expansion of the acetabulum during childhood, following the enlargement of the femoral head (Harrison 1961). Fusion with the other triradiate epiphyses starts around 10-11 years, along with the fusion of the ischium and the pubis. All epiphyses are fused between 14-17 years of age, with the superior one fusing last (around 16-17 years) (**Figure 3.7**). Generally, fusion begins around 11 years for girls to end around 15 years and starts around 14 years for boys to end around 17 years (Cardoso 2008b; Coqueugniot and Weaver 2007; Scheuer and Black 2000, 2004).



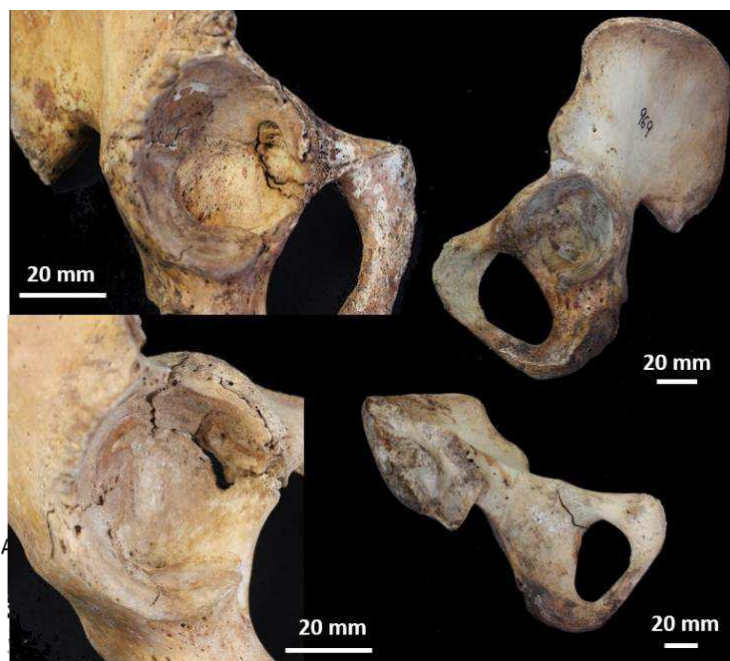
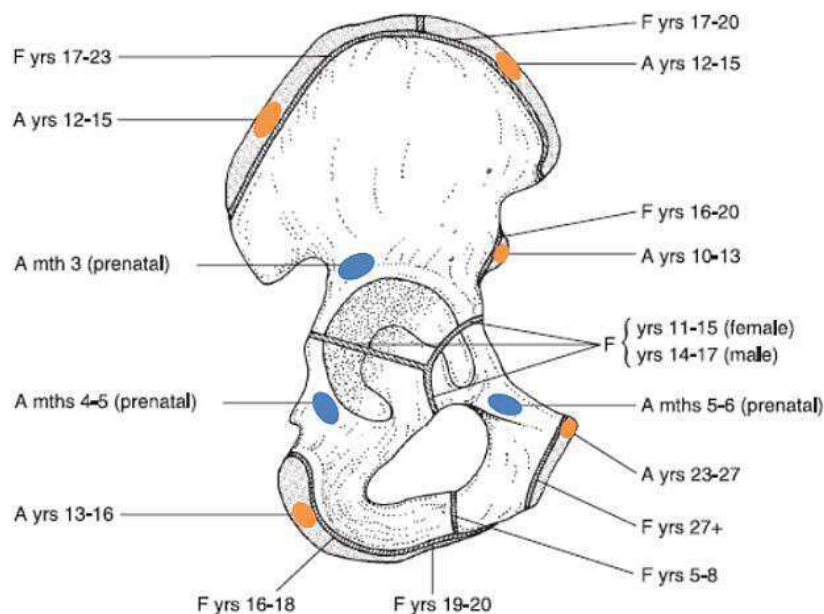


Figure 3.7 Acetabular regions of a 14-year old boy (upper left), a 15-year old boy (lower left), and a 14-year old girl (upper and lower right) from the Luis Lopes collection illustrating inter-sexual variations in timing and degree of fusion

Apart from the acetabular region, other secondary ossification centres are present on the iliac bone (**Figure 3.8**). Epiphyses of the four iliac spines, the pubic symphysis and the ischial tuberosity start and end fusion with the bony elements in a specific order, but at various ages. The maturation sequence of the iliac bone has been extensively studied (Cardoso 2008b; Cardoso et al 2013a; Flecker 1932; Owings-Webb and Suchey 1985; Schaefer 2008; Schaefer et al 2009; Schmidt et al 2007; Wittschieber et al 2013b), particularly on dry iliac bones (Cardoso 2008b, Cardoso et al 2013a; Owings-Webb and Suchey 1985; Schaefer et al 2009) for developmental studies and/or age estimation purposes (see **Chapter 1**).

D. Wittschieber and collaborators (Wittschieber et al 2013b) showed that evaluation of the ossification of the iliac bone is a good indicator to discriminate the 13- and 16-year thresholds. Risser's sign (Risser 1958) is used to detect iliac developmental anomalies and clinical risk of vertebral scoliosis because there is a very high correlation between ossification of the iliac apophysis/crest and vertebral growth. This examination is done by observing radiographies of the iliac crest, to assess its ossification from the fronto-lateral to the posterior part (the postero-superior iliac spine), until it joins with the iliac crest. For Californian girls of the 1930's-1940's, ossification of the crest occurs between 10 and 18 years, and between 13 and 20 years for boys. There are two classifications (for French and Americans) with 6 stages each that give similar age ranges.



**Figure 3.8** Ages and age ranges of appearance (A) and fusion (F) of the primary (blue) and secondary (orange) ossification centres of the iliac bone. From *The Juvenile Skeleton*, Scheuer and Black, 2004 p.338

Fusion of the iliac bone is complete when the iliac crest, which is one of the last epiphysis to finish fusion, is fused to the ilium. The starting and ending ages are variable, but complete fusion of the iliac bone always means that the individual is at least 18 years old (Cardoso 2008b; Schaefer et al 2009; Scheuer and Black 2000, 2004).

Iliac growth has been studied by several authors. I. Fazekas and F. Kosa (1978) have modelled intra-uterine ilium growth using linear regressions of ilium length and width against body length, and found that it followed the same rates as the clavicle: a mean growth rate of 1 mm/GW, except for an increased rate during the fourth lunar month (1.5 mm/GW). The ischium and the pubis show the same acceleration phase during the fourth lunar month in length and width, but with lower growth rates: 1.25 mm/GW and 0.75 mm/GW during the fourth lunar month, and 0.625 mm/GW and 0.375 mm/GW during the rest of the pregnancy respectively.

After birth, the morphology of all three iliac elements does not change much compared to the same foetal elements, but growth shows a rapid rate for the first three months, before progressively slowing down until 2-3 years of age, and getting even slower until puberty (Scheuer and Black 2000, 2004).

In L. Humphrey's study (1998) on postnatal bone growth patterns, the author found that growth patterns of the maximum and minimum iliac breadths followed Gompertz curves and were qualified as having intermediate-late growth rates: 90% of adult measurements of iliac breadth were attained at the ages of 17.2 and 17.9 years respectively. Sexual dimorphism of the minimum iliac breadth values seems to appear at 8.6 years old for 73% of the individuals.

A study by C. Rissech and A. Malgosa in 2005 showed that growth of ilium width and ilium length could be expressed by polynomial functions (degree 4 and 2 respectively). Sexual dimorphism was present for ilium length and ilium length growth rate in individuals aged 15 and more (growth is faster for males). In their study, a growth spurt was found only for ilium width, preceded by a stagnant growth phase. This growth spurt occurs at 11 years for females and 15 years for males. Sexual dimorphism in ilium dimensions seemed to appear when females stopped growing, around 15 years of age (Rissech and Malgosa 2005).

Growth of pubic length was studied on a large sample of juvenile bone remains from several osteological reference collections. It was found to follow a polynomial curve (Marchal 1997), with two growth spurts preceded by growth stasis: one at 10-14 years and the second at 15-19 years (Rissech and Malgosa 2007). After the first spurt, pubic growth accelerates for both sexes. Sexual dimorphism was also found: the first female pubic growth spurt begins before the males', but also ends first leading to a stabilisation in growth for females at age 12 as fusion of the acetabular region starts, whereas growth is continuous until 16 years for males.

Growth of the ischium was found to follow a polynomial (Marchal 1997) or linear (Rissech et al 2003) pattern. It slows down for females around age 9 and stops around age 12 when the acetabulum starts fusing, whereas it remains constant for males until 16 years of age. From then on, sexual dimorphism is significant for all ischiatic variables (Rissech et al 2003).

The acetabular zone shows increased growth rates in the vertical plane as opposed to the constancy of growth rates in the horizontal plane and can be expressed by first or second degree functions. They do not present any growth spurt because the acetabulum fuses before the first pubic growth spurt. The growth spurt of the ischio-pubic region occurs around 10 years for females and 14 years for males (Wittschieber et al 2013a, 2013b, 2013c).

It is generally admitted that skeletal elements do not present significant sexual dimorphism in size or conformation until puberty starts (Baker et al 2005; Scheuer and Black 2000, 2004; White and Folkens 2005). However, both the characteristic growth spurt (Castillo et al 2012; Krogman 1941; Trotter and Peterson 1970) and the beginning of fusion (Cardoso 2008b; Cardoso et al 2013a; Flecker 1932; Owings-Webb and Suchey 1985; Schaefer 2008; Schaefer et al 2009; Schmidt et al 2007) of the iliac bone in particular, but of other bones also, occur earlier in girls than they do in boys (Schaefer et al 2009; Scheuer and Black 2000, 2004). Acetabular maturation is a typical example of the expression of sexual differences in bone maturation for adolescents. Sexual dimorphism of the iliac bone starts in fact during ontogenesis, increases throughout childhood and leads to the constitutional differences of size and conformation of the adult iliac bone (Marchal 1997, 2003). A recent study by L. Wilson and collaborators (2015) using geometric morphometrics of the ilium has shown that iliac

ontogeny of size and shape is different for males and females: trajectory divergence provokes a sexually and ontogenic dissociated appearance of shape and size differences, with female ilia growing and maturing earlier and faster. Sexual dimorphism of the iliac bone will be even more flagrant after bone development is over (Bruzek 2002; Murail et al 2005; White et al 2012).

To conclude on the development of the iliac bone, it is safe to say that it is composed of a relatively long growth period, expanding from foetal life to puberty, and of maturation including both significant morphological changes as well as central and epiphyseal fusion. Most of the morphological changes occur between the end of primary ossification and the end of secondary ossification. A few years before and during puberty, development of the iliac bone shows sexual dimorphism in timing (starting and ending ages) of secondary ossification sequence, and as a result, in timing of iliac growth, as bone growth slows then stops when secondary ossification centres start fusing (Cardoso 2008b; Scheuer and Black 2000, 2004; White and Folkens 2005). Female growth and maturation of the iliac bone start and end earlier than they do for boys. This can be seen in biometric growth studies clearly showing two distinct growth spurts for boys and girls respectively (Marchal 2003; Wilson et al 2015) that lead to adult sexual dimorphism of both size and conformation. Studies of iliac maturation often result in sexed fusion sequences of the bone, or at least by mentioning the appearance of significant sexual dimorphism during puberty.

Identification of the iliac elements is fairly reliable, as the three elements present anatomical characteristics that can be distinguished from foetal life onwards (Scheuer and Black 2000, 2004; White and Folkens 2005; White et al 2012).

Of all the flat bones (cranial bones, scapula and iliac bone) of the skeleton, the iliac bone presents the best overall preservation rates in an archaeological context (Bello et al 2002, 2006), especially for juveniles. In adult iliac bones, the most fragile regions are the flat and very thin centre part of the bone (*i.e.* the iliac ala), the extremities: iliac spines, pubic symphysis, iliac crest contour; and the regions connecting the elements two by two, the ischio-pubic and ilio-pubic branches. The many reliefs of the bone make it highly susceptible to localised damage or breakage due to taphonomic processes.

In juveniles for which the three elements are not yet fused, there is a greater chance for the ischium and pubis of not being recovered, or being crushed, especially for very young individuals (foetuses and perinatals), because of their small size and their high composition in trabecular bone (White and Folkens 2005; White et al 2012). Rates of extremity alterations by taphonomic agents are comparable to those of adults (Bello et al 2006), because of the high composition in trabecular bone. In spite of extremity taphonomy which is still significant, there is a lower rate of overall breakage of

the ilium because of its smaller size, more compact shape and higher composition in compact bone compared to the other two elements.

The iliac bone is frequently present in medical CT examinations, at least in part, as it expands from the lower abdominal region to the pelvic region and the upper part of the lower limbs. Medical CT examinations of the pelvis and abdomen are relatively frequent as they contain the digestive and reproductive organs. These are elements mostly subject to infectious, inflammatory, tumoral diseases or surgical procedures and are often checked via medical imaging techniques, and CT in particular (Mettler Jr et al 2000). These examinations roughly encompass an anatomical region ranging from the beginning of the abdominal aorta to mid-thigh, so are likely to include the whole iliac bone.

Because of the higher probability of obtaining greater numbers of individuals, its good preservation rate, its long growth period and the many maturational changes occurring throughout its development, the choice was made on the iliac bone, with particular focus on the ilium, to be the flat bone of our study.

A development chart of the iliac bone illustrating both size increase and maturation changes observed for individuals aged 0 to 19 years from the Marseilles study sample is presented in **Figure 3.9** (male and female development). The individuals were chosen because they reflected the average state of development for their annual age group and sex.

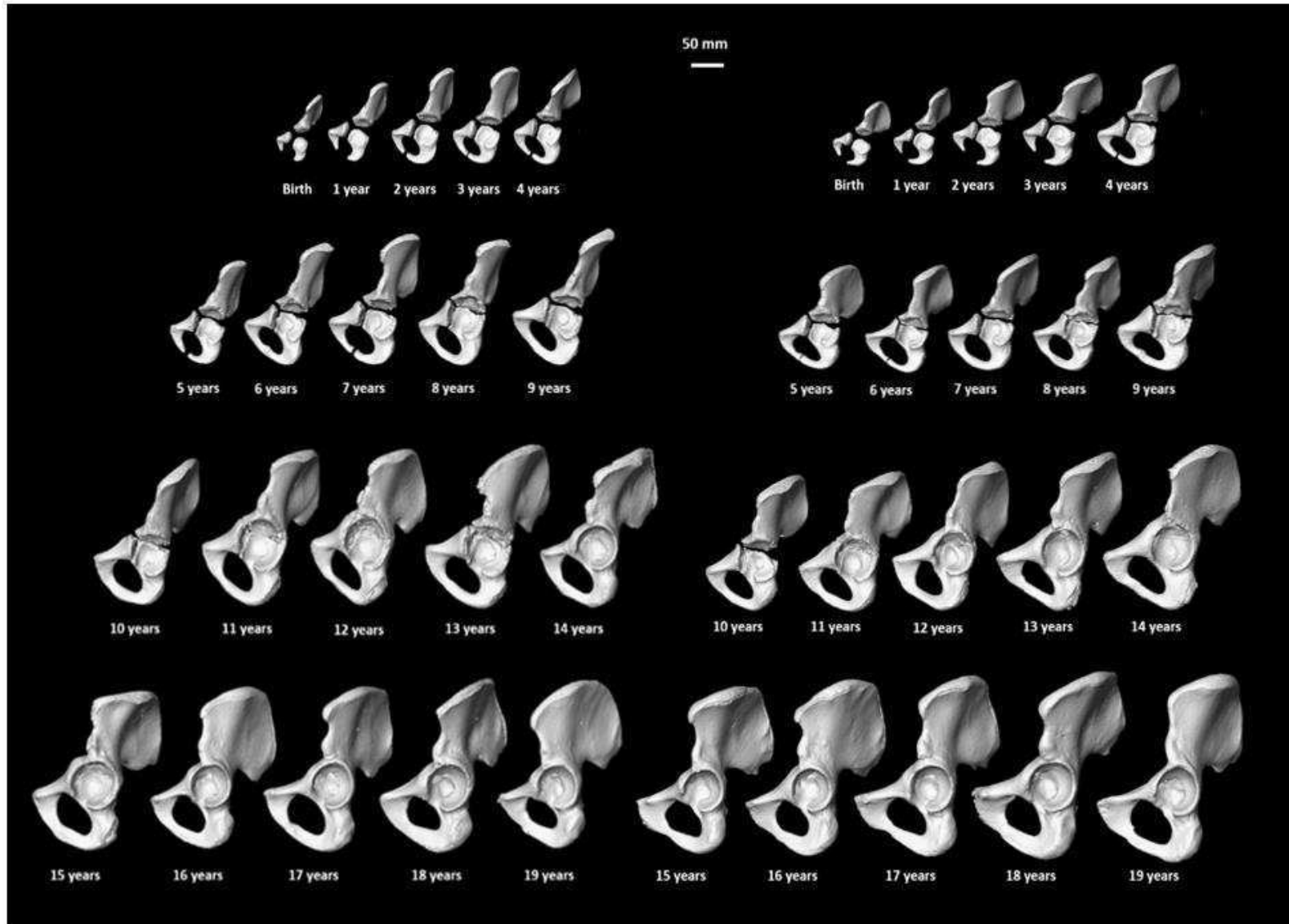


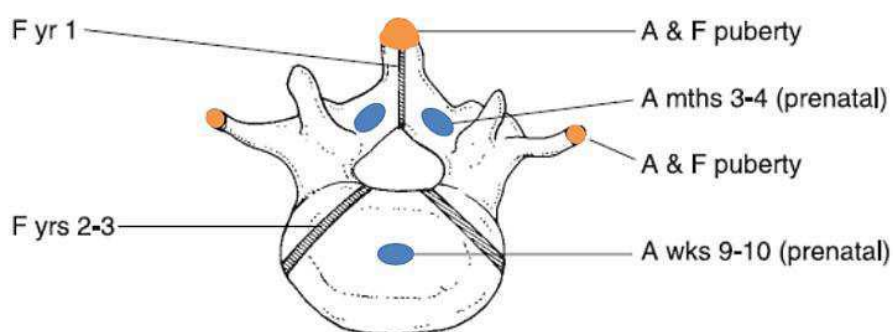
Figure 3.9 Developmental changes in size and shape of the juvenile iliac bone from birth to 19 years of age (left = male iliac bone, right = female iliac bone). The timing differences between the male and female ossification sequences of the primary and secondary ossification centres are clearly visible

## b. Short bone: the fifth lumbar vertebra

The fifth lumbar vertebra (subsequently referred to as L5) is the last of the mobile vertebrae of the vertebral column. It articulates superiorly with the fourth lumbar vertebra and inferiorly with the first sacral vertebra. Like all vertebrae (except for the atlas and the axis), an adult fifth lumbar vertebra has a central part, the centrum or vertebral body, and two lateral laminae fused posteriorly to form the neural arch and a horizontal spinous process. Lumbar vertebrae possess several characteristics: the centrum is wedge-shaped (anterior height is superior to posterior height), it is higher than the other vertebrae centra, its transverse diameter is bigger than its antero-posterior diameter; lumbar vertebrae have robust and quasi-horizontal pedicles, reduced superior articular facets, square blade-like shape and inferiorly concave laminae; and two lateral horizontal transverse processes. The inter-vertebral articulations are medially incurved, providing support and stability for the entire upper body (White et al 2012).

The whole chronology of L5 ossification (primary and secondary) is relatively long, and occurs between 12-16 GW and adolescence (Barnes 2012). Few morphological changes occur during that growth and maturation period, other than the sequential fusion of the elements (body, vertebral arches, transverse processes, epiphyseal rings of the body).

Primary ossification of the centrum/body involves between one and four primary centres, depending on authors (Baker et al 2005; Scheuer and Black 2000, 2004). It is entirely endochondral, and begins by the end of the third prenatal month (**Figure 3.10**). The first vertebral centres for the bodies appear in the thoraco-lumbar region. Ossification of the other vertebrae then spreads to both the cephalic and caudal regions (Bagnall et al 1977).



**Figure 3.10** Ages and age ranges of appearance (A) and fusion (F) of the primary (blue) and secondary (orange) ossification centres of a lumbar vertebra. Adapted from *The Juvenile Skeleton*, Scheuer and Black, 2004 p.214

The neural arches/posterior laminae appear during the fourth prenatal month. There is no clear order of appearance for primary ossification of the vertebral arches. However, it seems to occur in

the cervical/upper thoracic region first. The sequence then spreads down towards the thoracic region. A second group of centres appears in the lower thoracic/upper lumbar region, then moves cranially to meet the first group and caudally to the sacral region. The lower lumbar and sacral arches follow a slower ordered development, moving caudally: the fifth sacral arch centre is the last to appear (Bagnall et al 1977).

L5 is the lumbar vertebra that generally presents the largest body, the longest transverse processes and the lowest neural arch. These characteristics are already noticeable in juveniles (Scheuer and Black 2000, 2004). Indeed, lumbar vertebrae and L5 in particular are readily identifiable from the end of the 4th prenatal month.

At birth, each lumbar vertebra is represented by three bony masses connected by hyaline cartilage *in vivo*: an anterior centrum and paired posterior unfused neural arches (Barnes 2012; Scheuer and Black 2000). Fusion of the neural arches occurs in ascending order from the 1<sup>st</sup> to the 3<sup>rd</sup> year (Barnes 2012) or at 2-3 years of age (Scheuer and Black 2000), followed by fusion to the vertebral bodies in descending order between the 3<sup>rd</sup> and 8<sup>th</sup> years (Barnes 2012). Other authors have found that fusion of the posterior arches starts during the 4<sup>th</sup> year, but can also take place later on during life, or not at all (Scheuer and Black 2000, 2004), resulting in cases of *spina bifida* of L5.

A typical vertebra presents five epiphyses or secondary centres of ossification: one at each tip of the transverse and spinous processes (three in total) and two as annular rings that cover the periphery of the superior and inferior surfaces of the vertebral bodies. Most authors concur that the secondary centres appear during puberty (12-16 years) and finally fuse at the end of puberty (18+ years), and certainly by 24 years of age. Lumbar vertebrae have two additional secondary ossification centres, one at each mammillary process. These are the first to appear, followed by the transverse and spinous epiphysis, during puberty. I. Fazekas, F. Kosa (1978) and J. Ogden and M. McCarthy (1983) consider that the lumbar 'transverse process' is formed from the fusion of the true transverse process with the costal element. The former authors stated that the accessory and mammillary processes only develop around 6-8 years of age as muscle mass starts to increase. Whatever the actual origin of the lumbar transverse process, it does not start to develop and become visibly detectable until the end of the first and the beginning of the second year of life (Scheuer and Black 2000, 2004).

Fewer studies focusing on vertebral growth (Alhadlaq and Al-Maflehi 2013) were found compared to studies of long bones. Indeed, most studies of the vertebrae are maturation studies, and aim to give standards for the appearance, maturation and fusion of ossification centres, i.e. their maturation sequence (Albert and Maples 1995; Albert et al 2010, 2013; Baccetti et al 2002; Cardoso and Rios



2011; Chatzianni and Halazonetis 2009; Hassel and Farman 1995; Lamparski 1972; San Roman et al 2002; Veschi and Facchini 2002). K. Bagnall and collaborators (1977, 1982) studied growth and development of the spine during the foetal period and found that all five lumbar vertebrae present very similar polynomial growth patterns for the width of the vertebral arch before 15 GW. After this date, growth of L1 shows a slight deceleration, followed by L2, L3, and L4 around 22-23 GW, whereas L5 presents a quasi-continuous growth rate up to 28 GW. This was confirmed in a longitudinal ultrasound study of prenatal vertebral growth by R. Deter and R. Harrist (1992).

Vertebrae, and vertebral bodies in particular, are short bones that show relatively good preservation rates in archaeological samples. Of all vertebrae, lumbar ones show amongst the highest preservation rates in dry bone samples (Bello et al 2002, 2006). Identification of the centrum of L5 on dry bones can be done with sufficient certainty and reliability, even when it has not yet fused to the neural arch, and even in case of an incomplete vertebral column, as it adopts adult morphology early during foetal life.

The fifth lumbar vertebra is not as likely to be specifically scanned during a medical CT examination as the clavicle. However, it is located at the junction of the abdomen and the pelvic regions, both highly scanned areas of the body as they contain the digestive and reproductive organs. These are elements mostly subject to infectious, inflammatory, tumoral diseases or surgical procedures and are often checked or diagnosed via medical imaging techniques, and CT in particular (Mettler Jr et al 2000).

A development chart of the fifth lumbar vertebra illustrating both size increase and maturation changes observed for some individuals aged 0 to 19 years from the Marseilles study sample is presented in **Figure 3.11** (male and female development). The individuals were chosen because they reflected the average state of development of their annual age group and sex.

Once again, because of the higher probability of obtaining greater numbers of individuals, its inherent interesting physiological characteristics and its good preservation rate as a dry bone, the choice was made on the fifth lumbar vertebra as the short bone of our study.

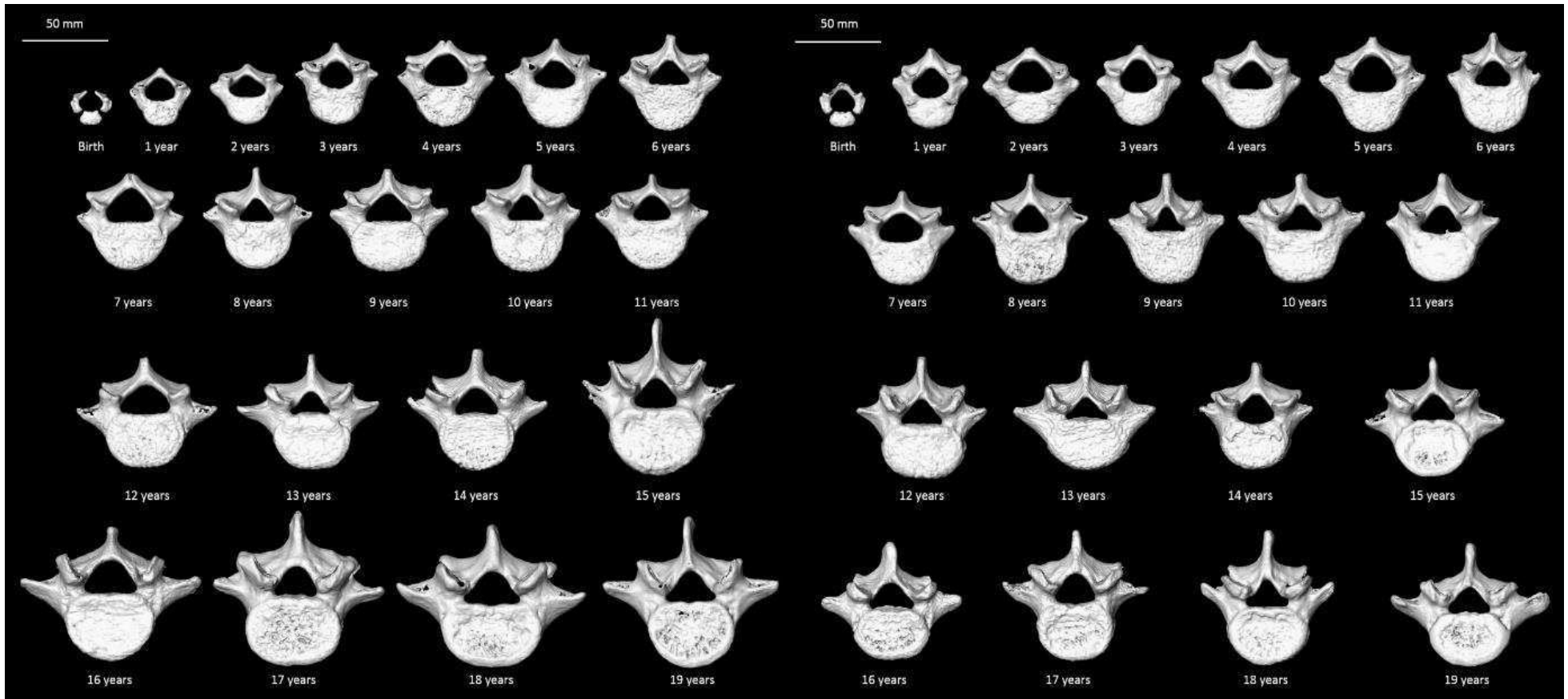
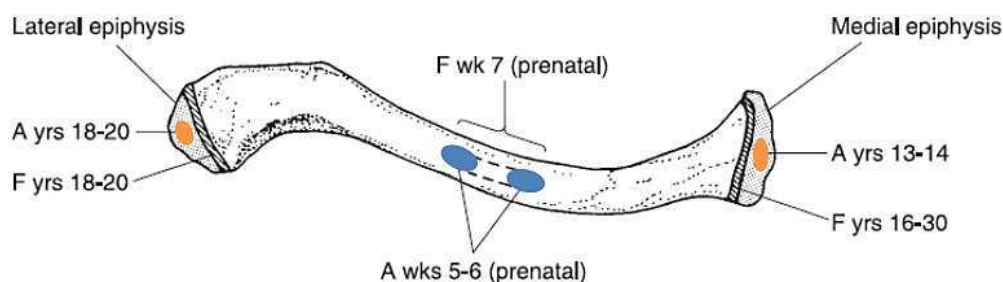


Figure 3.11 Developmental changes in size and shape of the juvenile fifth lumbar vertebra from birth to 19 years of age (left = male fifth lumbar vertebrae, right = female fifth lumbar vertebrae)

### c. Long bone: the clavicle

The clavicle is a bilateral long bone: it is composed of a main central part, the diaphysis, and two smaller secondary parts, the medial and lateral epiphyses. The medial or sternal epiphysis articulates with the manubrium and sometimes the first rib; the lateral or acromial epiphysis articulates with the acromion and sometimes the coracoid process of the scapula to form the upper part of the shoulder. Both articulations are synovial (White and Folkens 2005, White et al 2012).

The clavicle is the first bone of the skeleton to begin primary ossification *in utero*. Contrary to other long bones, primary ossification of the clavicle is endomembranous and not endochondral (Kreitner et al 1998). At around day 39, during the 6<sup>th</sup> week of intra-uterine life, ossification commences in the precartilaginous anlage. Although some authors consider there to be only a single primary ossification centre, most agree that there are probably two primary centres (**Figure 3.12**), one medial and one lateral (Scheuer and Black 2004). After the fusion of these centres, ossification becomes a mixture of endomembranous and endochondral processes. The lateral aspect of the clavicle may develop from a membranous tissue (as evidenced by its more flattened appearance), while the more medial aspect develops via true endochondral ossification (given its tubular appearance, the presence of an articular disc and a medial epiphysis) and contributes more to total bone length. This dual origin has also been used to explain the presence of paired centres of ossification (Baker et al 2005; Scheuer and Black 2000).



**Figure 3.12** Ages and age ranges of appearance (A) and fusion (F) of the primary (blue) and secondary (orange) ossification centres of the clavicle. Adapted from *The Juvenile Skeleton*, Scheuer and Black, 2004 p.248

Osteoformative cells are detected as early as the 6<sup>th</sup> gestational week (GW) and the clavicle rapidly reaches its distinct adult “S-shape”, around 11-12 GW (Fazekas and Kosa 1978; Scheuer and Black 2000). This illustrates the ontogenetic and phylogenetic stability of this bone. Indeed, during a relatively long period of time, beginning at the foetal period and ending around 15-17 years of age, clavicular morphology does not show any significant changes, only an increase in size, *i.e.* active growth (**see below**).

The epiphyses, or secondary ossification centres, are two thin bony « flakes » that fuse with the diaphysis (**Figure 3.12**). The first to fuse is the lateral epiphysis, around 18-20 years (Todd 1937; Todd and d'Errico 1928). The medial epiphysis is the last bony epiphysis of the skeleton to fuse completely. It starts to form between 12 and 14 years of age, but fusion with the diaphysis is not complete before 21 to 31 years and occurs earlier for female individuals (Cardoso 2006; Meijerman et al 2007; Owings-Webb and Suchey 1985; Scheuer and Black 2000, 2004; Schmidt et al 2007). Fusion of the sternal end of the clavicle is therefore useful for aging individuals from late adolescence to early adulthood and has been massively studied for that purpose, using different types of degree of fusion staging systems resulting in at least as many methods (*e.g.* Black and Scheuer 1996; Jit and Kulkarni 1976; Kreitner et al 1998; McKern and Stewart 1957; Meijerman et al 2007; Owings-Webb and Suchey 1985; Schmeling et al 2004, 2006a, 2006b; Schulz et al 2008; Stevenson 1924).

This slow ossification process is characteristic of the sternal extremity of the clavicle and is used to attribute individuals into several age groups, both in forensic and archaeological contexts. When all other epiphyses are fused except for the sternal end of the clavicle, the concerned individual is described as belonging to the “young adult” age group. Complete fusion of the clavicle occurs between 22 and 31 years (Black and Scheuer 1996; McKern and Stewart 1957; Owings-Webb and Suchey 1985; Schaefer 2008). It symbolises the end of bone maturation and the transition to the osteoarchaeological “adult” age group (Scheuer and Black 2000). The fusion state of the sternal end of the clavicle is therefore typically used in a forensic context to decide whether or not an individual has reached the legal threshold of 18 or 21 years of age (Jit and Kulkarni 1976; Schmeling et al 2006a, 2006b; Schulze et al 2006).

Growth of the clavicle, as for all long bones, is centrifugal and starts at both epiphyseal ends. The clavicle has the longest growth period of all bony elements of the skeleton: it starts at 5-6 GW and ends at the end of puberty (Black and Scheuer 1996). This characteristically long and continuous growth period, without any dramatic changes of bone shape, is why the clavicle is a particularly interesting bone to use for biometric growth studies or age estimation of foetuses to pre-adolescent individuals (before the fusion of the acromial and sternal epiphyses) (Black and Scheuer 1996; Fazekas and Kosa 1978; Schaefer 2008; Sherer et al 2006).

The clavicle is known for having an important growth rate for the whole duration of the process (Garcin 2009). Several phases have been identified through biometric studies of the clavicle and more specific growth studies of dry bones or ultrasound data. It has been found that *in utero* growth of the clavicle follows a linear pattern, with a rate of 1mm per foetal week (Black and Scheuer 1996). Growth of the clavicle during the foetal period is also presented in the work of I. Fazekas and F. Kosa (1978) as following a linear pattern with a mean growth rate of 6-7mm/lunar month during the third

and fourth lunar months, followed by a stable growth rate of 4mm/lunar month until the end of intra-uterine life.

Postnatal growth of the clavicle is slowed compared to intra-uterine growth, until the first postnatal growth spurt around 5-7 years. This spurt is followed by a second slowed growth phase until the pubertal growth spurt. Clavicular growth is incremental and does not seem to show differences between populations (Black and Scheuer 1996).

L. Humphrey (1998) conducted a cross-sectional study to compare postnatal growth patterns of skeletal elements and discuss the appearance of skeletal sexual dimorphism for these elements, using measurements taken on the St Bride collection of identified skeletons. Individuals were aged 0 to 20 years. L. Humphrey found that the growth of clavicle length, maximum and minimum diameters follow Gompertz curves of different parameters. 70% of adult clavicle length is reached at age 7.9 years, and 90% of total clavicular length is reached at 16.5 years. Maximum and minimum clavicular diameters reach 90% of their total values later on, respectively at 8.2 and 10.7 years. Based on these results, she qualified the growth of clavicular length and of its maximum diameter as intermediate-late, and growth of its minimum diameter as intermediate-very late. L. Humphrey also found that sexual dimorphism in growth rates of clavicular diameters was present at birth (for 60% and 74% of the individuals respectively), whereas it only appeared at 4.9 years for clavicular length but for 95% of the individuals and continued to increase until the end of the clavicular growth period (around 17 years of age).

Although the femur has been found to show the best preservation rates of all long bones, the clavicle is a long bone that generally shows relatively good preservation rates (Brough et al 2013) for juvenile dry bones in archaeological and forensic contexts because of its relative robusticity (Bello et al 2002, 2006) and a high composition in cortical bone (Black and Scheuer 1996). The main problem, apart from diaphyseal *post-mortem* fracturing, is taphonomic damage of bone extremities (sternal and acromial), because of the clavicle's higher composition in trabecular bone and thinner layer of cortical bone in these regions (White et al 2012). This problem was indeed observed for 59 clavicles/43 individuals of the Luis Lopes sample.

As previously exposed, the clavicle presents a long developmental period, characterised by very few morphological changes: the global shape of the clavicle stays the same from birth to late adolescence, and clavicular epiphyses start fusing around 16 years of age. In comparison, the other long bones show distinctive changes in morphology and several successive ossifications of primary and secondary epiphyses throughout their development. These characteristics may cause difficulties to obtain reliable and identical variables and landmark positions for the whole duration of bone growth. This is why the clavicle seems a better candidate for representing long bone growth.

However, the clavicle is a bone that presents high inter-individual variation in its morphology (Shirley 2009; Voisin and Balzeau 2004) which may be problematic for reliable landmark positioning. The clavicle also presents significant sexual dimorphism in its adult size and shape (Brough et al 2013; Humphrey 1998; Shirley 2009), which may require the construction of sexed age estimation methods.

The clavicle is also a bone that is most likely to be scanned during a medical CT examination. It is located between the head and the thorax, which are the two regions containing the most vital organs (the brain, the heart and the lungs) and are therefore systematically checked in case of accidents, traumas, or even post-mortem. It is also a bone that presents a relatively high prevalence for fractures in living individuals, especially male adolescents or young adults (Postacchini et al 2002; Robinson 1998), and calls for systematic checking using medical imaging in case of polytraumas. Because of this, choosing the clavicle would provide a higher probability of obtaining a greater number of individuals. In addition to this, its good preservation rates as a dry bone and its inherent interesting and “stable” developmental characteristics make the clavicle a good candidate for juvenile age estimation.

A development chart of the clavicle illustrating both size increase and maturation changes observed for some individuals aged 0 to 19 years from the Marseilles study sample is presented in **Figure 3.13** (male and female development). The individuals were chosen because they reflected the average state of development for their annual age group and sex.

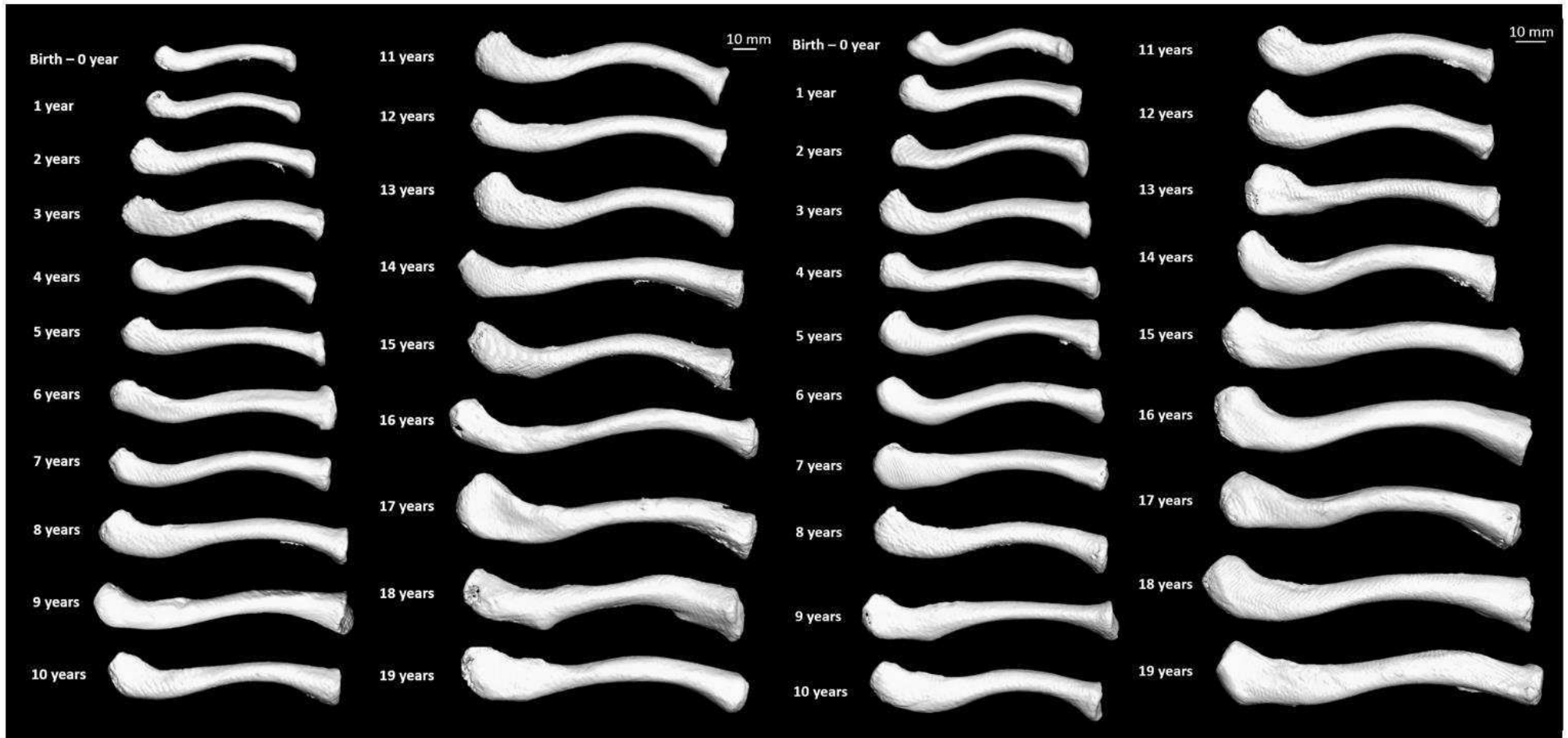


Figure 3.13 Developmental changes in size and shape of the juvenile clavicle from birth to 19 years of age (left = female clavicles, right = male clavicles). This diagram also illustrates the important inter-individual variability of clavicular morphology

**d. Summary of developmental events of the three bones studied**

All three bones follow particular ossification sequences and growth phases. All of them start during the prenatal period but end sequentially: the first is the fifth lumbar vertebra, the last is the clavicle. All significant maturation events of each bone are summarised in **Figure 3.14**. Growth of the three elements starts and ends roughly at comparable ages, whereas maturation sequences are more divergent. This will allow the age prediction models constructed on growth parameters of each of these three bones to apply to similar juvenile age groups.

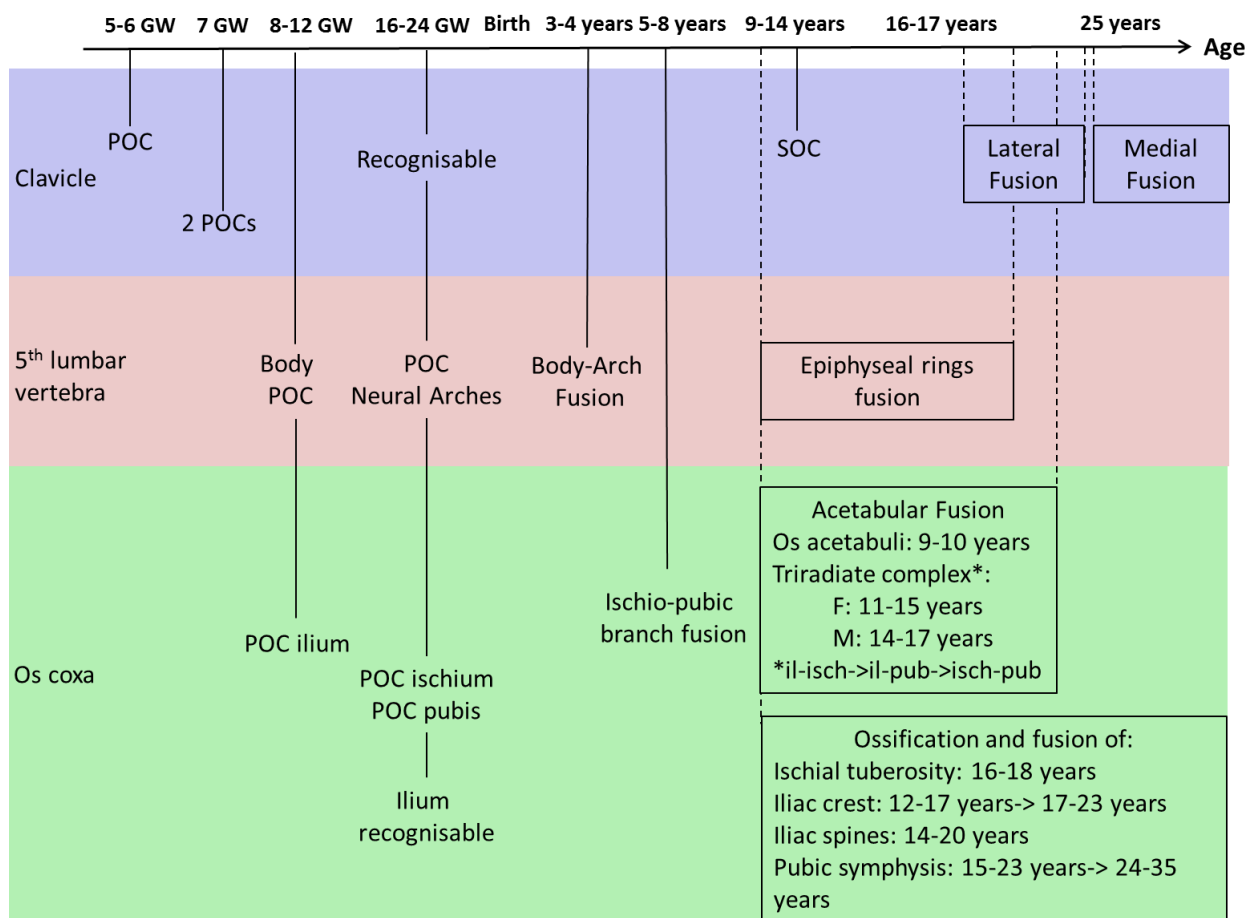


Figure 3.14 Summary of the developmental events occurring for the clavicle, fifth lumbar vertebra and the iliac bone (or coxal bone).GW: Gestational Weeks, POC: Primary Ossification Centre; SOC: Secondary Ossification Centre

**3.1.2. Study sample from Marseilles**

**a. Characteristics of reference samples**

Constructing new anthropological methods ideally requires using samples for which biological parameters, such as age and sex, amongst others, have to be known (Baroncelli et al 2006; Cameron, In Hauspie et al 2004; Sellier et al 1997). Such samples originate from referenced osteological collections or populations (White and Folkens 2005). These collections can be used as training



material for creating new methods but also as test material for verifying newly created methods or evaluating existing methods.

According to V. Garcin (2009), there are two types of reference collections:

- groups of data/individuals obtained in modern or forensic contexts (Schmelting et al 2003a), for which data is reliable but samples are not always homogeneous or representative of a given population, and complete skeletons are not a given. Such collections are, for juveniles, the reference collection of the forensic medicine department of the Strasbourg Faculty, in France (Coqueugniot and Hublin 2012), the reference osteological collection of Bologna, in Italy (Veschi and Facchini 2002);

- osteoarchaeological reference collections, composed of the skeletal remains of individuals from different archaeological contexts for whom at least age and sex are known with certainty. Such collections are for example Spitalfields (Molleson and Cox 1993) in England; the Scheuer collection (Scheuer and Black 2000, 2004) in Scotland; the Human Identified Osteological collection of Coimbra, Portugal (Coqueugniot and Weaver 2007); the Luis Lopes osteological collection (Cardoso 2005; 2006) in Lisbon, Portugal; the Belleville collection (Saunders et al 1993b), in Canada and other major reference collections of adult skeletons hosted in several countries worldwide (White and Folkens 2005). The main criterion for selecting an osteoarchaeological reference population is to favour a deceased population that is a perfect sample of the living population at the time (Garcin 2009). This is the case for a population whose individuals died of violent epidemics (*i.e.* the plague, cholera) or of another non-selective cause. The idea is to find a population with individuals of all ages and sexes whose mortality profile is the same as the demographic profile of the living population at the time.

With the application of medical imaging techniques to academic or non-academic research, the past decades have led to the idea of creating anthropological digital databases (Zollikofer and Ponce de Leon 2005) that could lead to new digital or virtual reference collections (Kistler et al 2013). Juvenile osteological reference collections are relatively rare. Moreover, they do not always include a sufficient number of individuals of all age groups, and of both sexes. This is problematic not only for studying population variability, but also questions the validity of the results and statistical parameters obtained through the study of these samples. Medical imaging has proven to be a good alternative to avoid these biases. If sampling is done correctly and in respect with the ethical and legal principles regulating the concerned institutions (mainly hospitals and clinics for data acquisition and research laboratories and universities for data storage and analysis), virtual databases of anonymised data containing only the biological information of the individuals (exact age, sex, eventually ethnicity) can be collected and used as reference collections for research purposes. Contrary to dry bone material, it allows the non-invasive collection of data from an important number of individuals, without the problem of material alteration due to multiple handling; it also

limits the problem of storage space; it allows visualisation of internal structures by non-invasive acquisition (White and Folkens 2005; Zollikofer and Ponce de Leon 2005). Several studies have shown that variables acquired or methods applied on dry skeletal or dental elements are comparable to the same variables or methods acquired on the same digitised elements (Brough et al 2013; Grabherr et al 2009). Indeed, a substantial number of studies directly use digital skeletal elements for age estimation of juveniles acquired via medical imaging (Cao et al 2000; Gertych et al 2007; Pietka et al 2003). Almost all age estimation methods of the living rely on medical imaging techniques for data acquisition (*e.g.* Kreitner et al 1998; Saint-Martin et al 2013, 2014; Schulze et al 2006). Although it cannot cover all the information contained in skeletal and dental material, medical imaging can be used to make osteoarchaeological material durable by digitising skeletal and dental elements (Zollikofer and Ponce de Leon 2005). In that sense, digital reference collections are a good way to complete documented osteological series (Franklin 2010).

To construct bone age estimation methods, the individuals included in the studies must therefore originate from a referenced osteological sample, collection or population. Ideally, a method should be constructed on a large reference study sample and evaluated on several large independent and referenced test samples.

According to A. Schmeling and collaborators (2007), a set of individuals must respect several criteria to qualify as “reference” sample/collection/population and therefore provide a reference study on growth, development, maturation, etc.

- The study sample or population must be of sufficient size for each age group and section of the population represented. The number of subjects for each sex must be at least ten times the number of observed criteria (variables, maturation stages, etc.);
- The number of individuals must be comparable for each age group, to avoid systematic error
- Data must be collected separately for each sex, because development is different between sexes;
- Date of examination must be recorded;
- Observed criteria must be clearly defined;
- Examination techniques must be precisely described;
- Information on geographic and genetic origins, and socio-economic and health status of the reference sample/population must be documented;
- Size of the sample, mean values and statistical deviation must be known for each observed criterion;
- Intra- and inter-observer errors must be known.

A sample/collection/population respecting all these criteria will be the best reflection of the normal range of inter- and intra-individual variability in that particular population. This raises the question of defining a population of study. Several definitions can be given to define a population (Larousse dictionary). In biology, a population is a group of individuals from the same species living in a defined spatial area. In statistics, a population is a group of individuals submitted to a statistical study. In genetics, a population is a group of individuals of the same species who can interact with one another during reproduction periods. The notion of population refers here to spatial, temporal, and genetic criteria. A population represents a genetic community, formed by all the genotypes of the individuals composing it. It is characterized by a collective genome, genetic patrimony or genetic pool. According to M. Lampl and F. Johnston (1996), methods constructed on reference populations produce better results, even though the universality of their application is not a given.

In all three definitions, the common criterion is variability, be it at a population or individual level (intra- and inter-individual). Variability can come from several sources: it can be genetic and/or epigenetic and expresses itself as the range of values taken by any measurable or descriptive characteristic in the population studied. It is one of the main characteristics that anthropologists aim to decipher and that they need to consider and discuss in their studies. Variability defines how to construct the study sample, and raises the question of the role of secular trends (variation with age, time and space of a biological variable) on possible growth and maturation acceleration/delay (Cole 2003; Heuzé and Cardoso 2008; Kalichman et al 2008; Roche 1979).

If the study sample is of sufficient size, it is customary to isolate a small subsample (often 30% of the total sample) that will be used to test the method constructed on the rest of the sample and evaluate validation criteria, such as the accuracy and standard estimation error of the method.

Cross-validation also enhances the reliability of the results obtained on a study sample. It consists in randomly picking a certain number of individuals in the whole sample that will compose successive different study samples and test samples. The method is constructed as a “mean” of the results obtained for each study sample and applied to the successive test samples. It is then customary to test the resulting method on one or several independent test samples to assess accuracy and standard estimation error.

If the total sample is too small to be subdivided into a study sample and an independent test sample, it is recommended to find one or several independent test sample(s) to test the new method.

One problem caused by reference population composition concerns method application. If a method is constructed on a given reference population, a bias may arise when the method is applied

to a population of a completely different structure. Because of its inherent characteristics and variability, the structure of a reference sample can influence the results of the study, particularly if the study concerns method construction, and even more so if it concerns age estimation. Indeed, if the age structure of the population used to construct an age estimation method is not uniform, it will influence the estimated ages in the validation/test population (Coqueugniot et al 2010). This is particularly true when the correlation between chronological age and the studied criteria is slow. This problem is known as demographic profile bias (Garcin 2009; Gowland and Chamberlain 2002).

To avoid this bias, it is recommended to use a standardised reference population to construct any method, so its structure does not influence the population on which the same method is applied (Buchet and Séguy 2002). L. Konigsberg and S. Frankenberg (1992) define three cases where the age structure of the reference sample does not influence the age distribution in the test sample:

- If the correlation between age and the indicator is perfect;
- If the reference sample and the test sample have the same age distribution;
- If the reference sample has a uniform age distribution.

For these reasons, methods constructed on reference populations must be tested on different populations and different taphonomic contexts to see if results are not influenced by structure (Cunha et al 2009).

A method must not be applied to external data without considering secular and regional trends of the reference collection used to construct it (Rissech et al 2013b). Indeed, bone development is known to be influenced by geographic origin and environmental conditions (Lewis 2002; 2007) as well as genetic parameters and secular changes or pathologies influencing growth or maturation, significantly more so than tooth development (Garcin 2009). Sampling the population adds other biases to the apprehension of bone development, through sample composition, intra- and inter-individual variability, which can lead to a correct or incorrect representation of the corresponding population (Garcin 2009). For this reason, several authors insist that although anthropological methods may be reliable, they remain population-dependent (Bocquet-Appel and Masset 1982; Cameriere et al 2007a; Cunha et al 2009; Liversidge et al 1998; Liversidge et al 1999; Maber et al 2006) and need to be applied cautiously on different populations (Garcin 2009; Rissech et al 2013b; Stull et al 2014a) or recalibrated/adjusted on specific populations before use (Cunha et al 2009; Lunt and Law 1974; Schmeling et al 2007).

As well as inter-population variability, intra-population variability has to be considered when constructing and applying age estimation methods, especially when several methods are used to estimate age for different individuals. Indeed, mainly because of preservation rates, we cannot

always estimate age with the same methods for the individuals of a given population. It is customary to privilege dental age over bone age, as it is considered less variable (Garcin 2009; Lampl and Johnston 1996; Scheuer 2002). However, method application is highly dependent on the available data. Depending on the individuals' preservation rates, it will be possible to estimate both dental and bone age, or bone age, or dental age. It is a fact that real age, dental age and bone age are not exactly the same for a given individual. This is the perfect illustration of intra-individual variability (Bolanos et al 2000; Garcin 2009) and questions the relevance of comparing individual ages of a same population estimated using different methods and different variables (teeth and/or bones). In these cases, there is the bias created by estimating and comparing different "types" (dental or bone) of age, reflecting and perhaps enhancing intra-individual and intra-population variability. Added to these biases, there is the influence of the composition of the original study samples used to construct the age estimation methods.

According to V. Garcin (2009), the influence of the reference population (Bocquet-Appel and Masset 1982) and the bias created by multiplying the number of age estimation methods in the same sample can be limited by constructing a secondary age estimation method using the individuals for whom dental age could be estimated as the reference sample. The goal is to use intra-population variability to construct the secondary age estimation method: primary age estimation is done on individuals to estimate their dental age. The secondary age estimation method is constructed on these primarily estimated individuals, who serve as a study sample, using other variables (*e.g.* bone measurements) that are well-preserved. This procedure allows the prediction of age for a maximum number of individuals, while limiting the biases implied by intra-population, intra-individual variability and preservation rates. It therefore provides reliable and precise information for population studies. A secondary age estimation method should be used specifically on other individuals of the same population as the study sample, but the principle should also be tested on other reference populations. The main limitation of this procedure is to find a study sample of sufficient size, in order for it to be representative of the whole population, to construct the secondary method.

Of course, this solution is restricted to bioarchaeological contexts and is inapplicable for isolated forensic cases. In a forensic context, the course of action would be to identify all available data, privilege variables known for their lower variability rates and use methods addressing legal and scientific requirements.

Authors have also questioned the validity of applying methods constructed on extant reference populations to unknown past population samples (Boccone et al 2010; Garcin 2009; Merchant and

Ubelaker 1977; Saunders et al 1992; Scheuer and Black 2000; Stout et al 1996). V. Garcin (2009) has pointed out the same problem when using growth standards or growth charts constructed on modern populations to evaluate growth in past populations, leading to the reevaluation of the principles of palaeoanthropology.

Conversely, it has been shown that methods constructed on reference data of the beginning of the 20<sup>th</sup> Century are probably no longer valid for present-day populations (Cao et al 2000; Chaumoitre et al 2006). Time, and everything that time implies for changes in human morphological features, is indeed a powerful and active agent of change in secular trends, genetic traits, environment, culture, and society. All these parameters show different but stable variations and actively partake in the evolution of growth and developmental patterns. Because of this, authors also recommend recalibrating methods before their use (Cunha et al 2009; Lunt and Law 1974; Schmeling et al 2007).

Another problem that can occur is when the method is inapplicable to other populations (if the subjects of the reference sample are pathological for example) (Chaillet 2003). The reference sample must always be constructed with a particular aim in mind. This is why it is important to indicate relevant information on the population from which the sample originates (**see criteria above**) to check whether or not the corresponding method can be applied to the test sample. Method-recalibration or the use of correction factors has also been suggested and done by several authors to apply dental or skeletal age estimation methods on populations different from the ones used for their construction (Haider-Neito et al 2006; Kurita et al 2007; Schnitzler et al 2009; Singh and Chavali 2011; Sisman et al 2007).

## **b. Inclusive criteria and sample composition**

To conduct this study, the following criteria for constructing the reference sample were applied: individuals had to be non-pathological, of known age and sex, be selected in a sufficient number and englobe as much variability as possible. This last point is submitted to the bias of randomised sample selection, and cannot be precisely monitored. In addition to this, the selection of acquired data had to be done to ensure that the constructed methods were applicable to osteological data taken on dry bones from archaeological populations (**see section 3.2.1.**). As well as the preservation state of each type of bone considered, precautions were taken to select bones that presented “known” periods of activity for growth and developmental patterns. This was evaluated by the extent of the age ranges proposed by several authors to describe developmental changes: growth had to be the longest

possible with short or inexistent periods of stasis before it ended, and maturation changes (mainly the states of fusion of iliac ossification centres) had to occur in a relatively short time frame (Cameron, *In* Hauspie et al 2004; McKern and Stewart 1957).

The individuals included in the study sample are patients of the hospital services of Marseilles, France, who underwent a CT examination for various medical reasons. Individuals suffering from pathologies that could affect or directly concern skeletal growth and/or development were excluded, as were individuals presenting fractures of the studied bones. Retained individuals often underwent medical examination for acute diseases (such as appendicitis), trauma (road accidents, accidents...), or for forensic purposes (virtual autopsies). For each retained individual, a set of CT scans was collected from the PACS (Picture Archiving and Communication System, McKesson Medical Imaging Group, Richmond, BC, Canada) of the hospital services of Marseilles, France (AP-HM). The CT scans were performed with a 64-row multidetector CT scan (Somatom Sensation 64, Siemens®, Erlangen, Germany). Scanning parameters were as follows: 120 KV, 50-150 mAs, thickness: 0.6 mm. Most scans were obtained after administration of an intravenous contrast media.

In accordance with the standards of the French National Consulting Committee for Ethics and the Helsinki Declaration of 1975, revised in 1983, concerning the principle of personal privacy, all personal patient information was anonymized. Precise genetic/geographic origin and socio-economic status of the individuals are unknown. Age was calculated as the difference between date of examination and date of birth and is expressed in weeks, months and years. Sex is known for all individuals.

The advantage of working on a living population is that there is no bias related to mortality rates, contrary to archaeological populations. The demographic profile of the sample can be chosen to be the same as in the living population it comes from, or, as is the case here, the number of individuals can be chosen to be consistently similar for all ages, in order to avoid under- and over-representation of certain age groups. This is particularly important for constructing age estimation methods, as it has been found that an over-representation of one or several age groups tends to skew the results in favour of said age group(s) (Coqueugniot et al 2010; Gowland and Chamberlain 2002). The sample aims to reflect the “normal” population variation of bone growth and maturation patterns present in that population. Other than respecting uniform age group and sex distribution, the individuals were chosen randomly and independently in the AP-HM database. The children live in environments where they experience normal levels of infectious diseases and are not protected from environmental insults, corresponding to the definition of a reference sample (Cameron, *In* Hauspie et al 2004).

For the same reasons as a homogeneous age distribution is necessary to avoid skewness, uniform sex distribution is also necessary. Indeed, in case of sexual dimorphism of bone growth and/or maturation, an over-representation of one sex or the other could skew the results in favour of the pattern followed by the over-represented sex (Schmeling et al 2007, 2008). This can be solved by conducting separate analyses for each sex if sexual dimorphism is detected beforehand, although authors disagree on that point. Firstly, sexual dimorphism in bone developmental variables is often suspected but never unanimously assessed with sufficient reliability for any bone in prepubertal juveniles (Humphrey 1998; Rösing et al 2007; Todd 1937; Ubelaker 1987). Secondly, because sex cannot be determined reliably from juvenile bones (Ferembach et al 1979; Rösing et al 2007; Scheuer and Black 2000), authors, and anthropologists in particular, have taken to constructing age estimation methods for pooled sexes as well as separate sexes. Sexed methods have proven to provide more precise age estimations (Cardoso 2006; Scheuer and Black 2000), but “unisixed” methods are applicable to greater numbers of sub-adult skeletal elements and seem more appropriate to study the juveniles of archaeological series, for whom sex can almost never be known.

The study is cross-sectional, which means that each selected individual data is included in the sample only once. In other words, an individual can only be included in the study once, at the age he/she was at the moment of the CT examination, with the corresponding osteological data at that same date. Their individual data (osteometric, biometric, etc.) is recorded at regular and identical time intervals. A cross-sectional study makes it possible to include a greater range of inter-individual variability, whereas a longitudinal study is more appropriate for studying intra-individual variability. For these reasons, cross-sectional studies are more suited for working on age estimation, whereas longitudinal studies are more adapted for studies on growth patterns (Stull et al 2014a).

The total sample consists in 525 individuals aged from 0 to 19 years of age, and covers the whole growth periods of the clavicle, fifth lumbar vertebra and iliac bone, as well as the maturation of the acetabulum (**Table 3-1**). The individuals were selected in order to obtain a study sample of respected age and sex ratio, but were chosen arbitrarily, provided they respected the conditions cited above. This leaves a good chance of collecting individuals representing a large range of biological and osteological variability.



Table 3-1 Total number of individuals from Marseilles, by age and sex

Age group (years)	Females	Males	Total
0	19	11	30
1	11	17	28
2	10	12	22
3	11	12	23
4	11	17	28
5	11	15	26
6	13	11	24
7	11	11	22
8	12	17	29
9	13	16	29
10	13	20	33
11	9	22	31
12	17	17	34
13	12	11	23
14	16	12	28
15	10	11	21
16	12	10	22
17	10	11	21
18	12	14	26
19	11	14	25
<b>Total</b>	<b>244</b>	<b>281</b>	<b>525</b>

## 3.2. Test samples

### 3.2.1. Test sample from Toulouse

This test sample is composed of 30 juveniles that underwent clinical Multislice Computed Tomography (MSCT) in the Medical Imaging Institutes of Toulouse (Services de Radiologie of the CHU Toulouse-Rangueil and the CHU Toulouse-Purpan), in the South-west of France. These MSCT examinations were mainly requested in clinical contexts of abdominal diseases and were undertaken between April 2005 and November 2011. Patients with known history of diseases affecting bones were excluded before sampling. The individuals are children currently living in this particular geographic region, and come from various ethnic backgrounds. In the same way as the sample from Marseilles, all data (images and osteometric data) was recorded anonymously. Only age and sex is known for each individual.

A total of 30 individuals aged 0 to 12 years (16 boys and 14 girls) are included in the sample. All age groups are represented by at least one individual of each sex (**Table 3-2**).

Table 3-2 Total number of individuals from Toulouse, by age and sex

Age group (years)	Females	Males	Total
0	2	3	5
1	1	1	2
2	1	1	2
3	1	1	2
4	1	2	3
5	1	1	2
6	1	1	2
7	1	1	2
8	1	1	2
9	1	1	2
10	1	1	2
11	1	1	2
12	1	1	2
<b>Total</b>	14	16	30

### 3.2.2. Test sample from the Luis Lopes osteological reference collection

The Luis Lopes identified skeletal collection is one of the few osteological reference collections with a considerable number of juvenile individuals (Cardoso 2005). It is housed at the Bocage museum (Museum of Natural History and Science) in Lisbon, Portugal. The individuals of the Luis Lopes collection are a 20<sup>th</sup> Century cemetery sample. Their remains were collected between the late 1980's and 1991. The collection is composed of Portuguese individuals who died in Lisbon between 1880 and 1975. The entire collection is composed of over 1700 individuals, amongst which 126 juveniles (aged less than 21 years old). They are all Portuguese-born or have at least one Portuguese-born parent and were buried in Lisbon between 1913 and 1972. Most of them represent the middle and low social class of the city (Cardoso 2005, 2007; Heuzé and Cardoso 2008), as the origin of the remains (temporary graves) and the reported male occupations can attest for (Cardoso 2007b) and were living during an extremely difficult social and economic context under Salazar's dictatorial regime (Cardoso 2005). Information on the individuals include the name, age at death, date of death, cause of death, address at the time of death, nativity, name of the parents, professional occupation (for individuals aged 14 or more), and other administrative data obtained from cemetery records (Cardoso 2007b).

The time frame of the juvenile sample taken from the Luis Lopes identified skeletal collection is 1900 to 1960. This 60-year period englobes the birth and death dates of all studied individuals. The overall socio-economic status at that time was that of the late 19<sup>th</sup> Century in other European countries. Until 1960, Portugal was isolated from the rest of Europe and its social, economic, and

medical progress. Socio-economic growth only began after 1968, when Salazar struck commercial deals with foreign states and brought Portugal out of its isolation. The main causes of death during the first half of the 20<sup>th</sup> Century were infectious or communicable diseases, particularly present in cities. Health conditions in Portugal were very bad before 1960, and amongst the lowest in Western Europe. Low life expectancy was paired with high infant mortality rates everywhere, especially in Lisbon where people lived in overcrowded and unsanitary environments (Cardoso 2007b). Tuberculosis remained a major health problem until the 1970's, as vaccination campaigns against this disease and other common infantile diseases only started in the 1960's (Cardoso 2005).

The living conditions had a relatively negative impact on the health and death rates of the Portuguese people living at that time (Cardoso 2005). Previous studies on the juvenile individuals of the Luis Lopes collection have shown that socioeconomic status had an impact on bone growth and dental development patterns (Cardoso 2005, 2007b). Indeed, individuals from lower socioeconomic groups showed pronounced delay in skeletal growth and in dental development for the lowest levels (Cardoso 2007b), assessed by significant differences found between physiological (dental and skeletal) ages and calendar age.

Several types of pathologies were identified as the cause of death for these young individuals (**Table 3-3**). The highest prevalence is observed for infectious diseases such as tuberculosis and pneumonia, two highly contagious respiratory pathologies well-known to be associated with insalubrious environments and overpopulated living conditions (World Health Organization factsheets).

The skeletal remains of each individual are contained in separate metallic drawers or trays, for a total of ten individuals per cabinet. Up to n°480, all individuals are contained in closed metal drawers; from n°481 to 1679, they are contained in open trays, except for the individual n°1126-A, whose remains are contained in a cardboard box in a separate room. Remains are sorted by anatomical region or type of bones. Long bones, iliac bones, sacrum and cranial bones are usually directly positioned at the bottom of the compartments, whereas the rest of the bones and fragmentary or loose elements (mainly teeth lost *post-mortem*) are placed in hermetic plastic bags bearing the individuals' number. For younger individuals, all elements are bagged.

At least two documents are present with the remains:

- a preservation sheet, documenting all the biological data of the individual: age at death, sex, identified skeletal elements, and other information (pathologies, accompanying objects...);
- a document reporting the social status of the deceased individual, name, date of death, as well as the cemetery where the remains were found and his/her number in the collection.

**Table 3-3 Nosocomial groups and pathologies recorded at the date of death of the juveniles from the Luis Lopes collection and their frequency in the total sample of 85 individuals**

Type of disease or pathology	Disease name	Frequency
Congenital anomaly or disease	Congenital debility	2/85
	Mitral malformation	1/85
	Cardiac insufficiency	1/85
Idiopathic disease	Aneurism of the arterial canal	1/85
	Dystrophy	1/85
	Epilepsy	1/85
	Right emphysema	1/85
Infectious disease	Tuberculosis (pulmonary, acute, intestinal, meningeal, military, primo-infection, tuberculous adenitis)	19/85
	Meningitis (tubercular, bacillary)	16/85
	Meningitis (tubercular, bacillary)	10/85
	Typhoid fever	3/85
	Septicaemia	2/85
	Osteomyelitis	1/85
	Poliomyelitis	1/85
	Toxaemia	1/85
	Acute liver atrophy	1/85
Diarrhoea	1/85	
Infectious disease/Systemic malfunction	Acute or toxic dyspepsia	2/85
Inflammatory disease	Acute peritonitis	1/85
	Poliserositis	1/85
	Rheumatism	1/85
	Chronic myocarditis	1/85
	Rheumatic endocarditis*	1/85
Trauma	Bone fracture	3/85
Unknown	Internal haemorrhage	1/85
	Meningeal haemorrhage	1/85
	Unknown	8/85

The number of the individual is written in black ink on every corresponding skeletal element. The total number of individuals studied included in the test sample is 82 (**Table 3-4**). Individuals are aged 0 to 19 years.

Table 3-4 Total number of individuals from the Luis Lopes collection, by age and sex

Age group (years)	Females	Males	Total
0	3	2	5
1	3	6	9
2	2	4	6
3	2	0	2
4	3	3	6
5	2	1	3
6	1	1	2
7	0	1	1
8	0	1	1
9	2	1	3
10	2	1	3
11	3	2	5
12	1	2	3
13	1	1	2
14	1	3	4
15	3	2	5
16	2	5	7
17	1	3	4
18	5	2	7
19	2	2	4
<b>Total</b>	39	43	82

### 3.3. Taphonomic alterations

The main downside of working on osteological samples is the presence of taphonomic alterations. Taphonomy, or “the laws of the grave” as defined by I. Efremov (1940), designates any biological, physical, or chemical agent, factor, or parameter present in the immediate environment of the bones that can modify its appearance or composition. These modifications can originate from the primary environment from which the bones were extracted (soil, grave, etc.), but repeated handling and preservation conditions can also partake in their creation by what is called “laboratory taphonomy” (Dastugue and Gervais 1992), resulting in breakage, extremity alterations, etc. of the bones.

Taphonomic alterations were found for several individuals in the Luis Lopes collection (**Table 3-5**), on one or both clavicles, on the lumbar vertebrae and the iliac bones. If the variables could not be obtained, these individuals were excluded from the study. These alterations were either partial and, in that case, often concerned bone extremities (the acromial extremity of the clavicles in particular), or they were total, and resulted in the absence of the whole bone.

**Table 3-5** Frequencies of taphonomic alterations observed on the clavicles, L5, iliac bones and iliae of individuals from the Luis Lopes sample and the total number of bones (and individuals) from that collection included in the final test sample

Bone	Frequency of taphonomic alterations preventing variable acquisition	Total number of bones (individuals) included in the final test sample
Clavicle	62/170 = 36.47%	108 (60)
L5	24/85=28.20%	66 (66)
Ilium	28/104 = 26.92%	76 (43)
Iliac bone	33/170 = 19.41%	137 (78)

Left and right iliae, iliac bones and clavicles are counted separately. Lumbar vertebrae: cases for which at least one of the variables could not be measured.

For a total number of 82 individuals, if all bones had been entirely preserved, there would have been 164 clavicles (82 left and 82 right), 82 fifth lumbar vertebrae, 164 (82 left and 82 right) iliac bones and 98 (49 left and 49 right) iliae. However, all 82 of the individuals did not provide all the variables of study, and therefore, age and sex ratios are not respected for all the variables.

**Summary: Characteristics of the material used in the study**

- The study samples from Marseilles used in this study qualify as reference samples: the individuals are non-pathological, of known age and sex; age and sex ratios are respected
- The three bones selected for this study (ilium/iliac bone, fifth lumbar vertebra, clavicle) have strong preservation rates, known growth and maturation phases and patterns
- The age ranges cover an important period of growth and/or maturation of the elements of interest: from 0 to 19 years for the fifth lumbar vertebra, the clavicle and the iliac bone; from 0 to 12 years for the ilium
- The test samples are composed of extant (Marseilles and Toulouse) or modern (Luis Lopes collection from Lisbon) individuals of known age and sex. They are used to assess the accuracy of the age estimation methods constructed on the study samples

## Chapter 4. Methods

### 4.1. Acquired data

#### 4.1.1. Biometric variables

The biometric variables measured on the clavicles and iliae and two lumbar variables (AVH and UVW) are well-known anthropological and/or osteological measurements defined by several authors (Martin 1957; Martin and Saller 1959, 1962; Olivier 1965) on adult bones. They are commonly used in biological anthropology. The matching measurements taken on juvenile and/or foetal bones were also defined and used in previous works by different authors (Black and Scheuer 1996; Buikstra and Ubelaker 1994; Fazekas and Kosa 1978). The following variable definitions are found in anatomical, anthropological and anthropometric manuals and articles aforementioned. They correspond to measurements taken on dry bones.

Six of the ten biometric variables measured on the fifth lumbar vertebra were used in a geometric medical and anthropometric study of CT scan slices of adult lumbar vertebrae done by S. Zhou and collaborators (2000) and a morphometric study of dissected juvenile lumbar vertebrae done by V. Mavrych and collaborators in 2014 (Mavrych et al 2014). These variables were adapted from previous anthropometric and morphometric studies of the lumbar spine (Berry et al 1987; Fang et al 1994; Jones and Thomson 1968; Panjabi et al 1992; Zhou et al 2000).

All variables are expressed in millimetres (mm) or squared millimetres (mm<sup>2</sup>).

#### a. Iliac variables

Two unidimensional and two bidimensional variables were measured on the iliae. The variables were obtained for the individuals of the Marseilles sample, the Toulouse sample and the Luis Lopes test sample (**Figure 4.1**):

- Ilium Length (**IL**) is the distance between the Anterior Superior Iliac Spine (ASIS) and the Posterior Superior Iliac Spine (PSIS) and is expressed in mm;

- Ilium Width (**IW**) is the maximum distance between the most cranial point of the iliac crest, the Iliac Crest Summit (ICS) and the most prominent internal point of the acetabular surface of the ilium, the Internal Acetabular Point (IAP). IAP corresponds to the most anterior part of the ilium, and the most superior part of the Y-cartilage that will fuse with the ischium and the pubis. IW is expressed in mm;

- Ilium Module (**IM**) is the product of IL and IW, and is expressed in mm<sup>2</sup>. IM corresponds to the surface of the quadrilateral whose diagonals are IL and IW. It is a rough geometric estimate of the internal surface of the ilium;

- Ilium Area (**IA**) is also expressed in mm<sup>2</sup>. IA is the measurement of the internal bone surface of the ilium as seen perpendicularly when the bone is projected on the iliac plane. The iliac plane is an anatomical plane defined by the three following points: ASIS, PSIS and IAP. IA is an exact measurement of the internal ilium surface projected in the iliac plane. To obtain IA, the observer must position the ilium in the iliac plane and face the internal bone surface perpendicularly. IA can be measured on scanned bone and dry bone (see sections 4.2.2.a. and 4.3.1.a.). The dry bone measurement of IA was used in the PhD theses of P. Adalian (2001) and F. Marchal (1997).

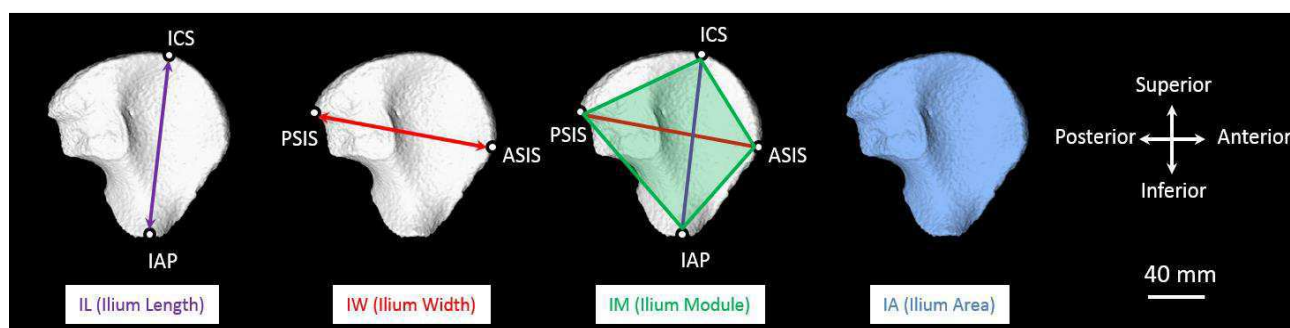


Figure 4.1 Variables taken on the juvenile iliae (dry bones and reconstructed bones): IL = Ilium Length, IW = Ilium Width, IM = Ilium Module = IL x IW, IA = Ilium Area. These variables are defined by four landmarks: ICS = Iliac Crest Summit, IAP = Inferior Acetabular Point, PSIS = Postero-Superior Iliac Spine, ASIS = Antero-Superior Iliac Spine

All four iliac variables were measured on the individuals of the Marseilles sample and the Luis lopes sample. IL and IW were obtained from landmark coordinates in the Toulouse sample (Figure 4.2) and IM was calculated as the product of IL and IW.

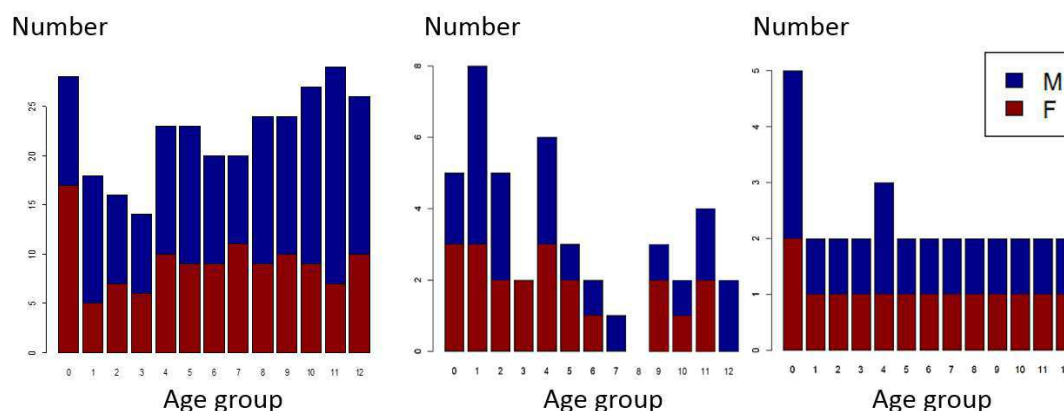
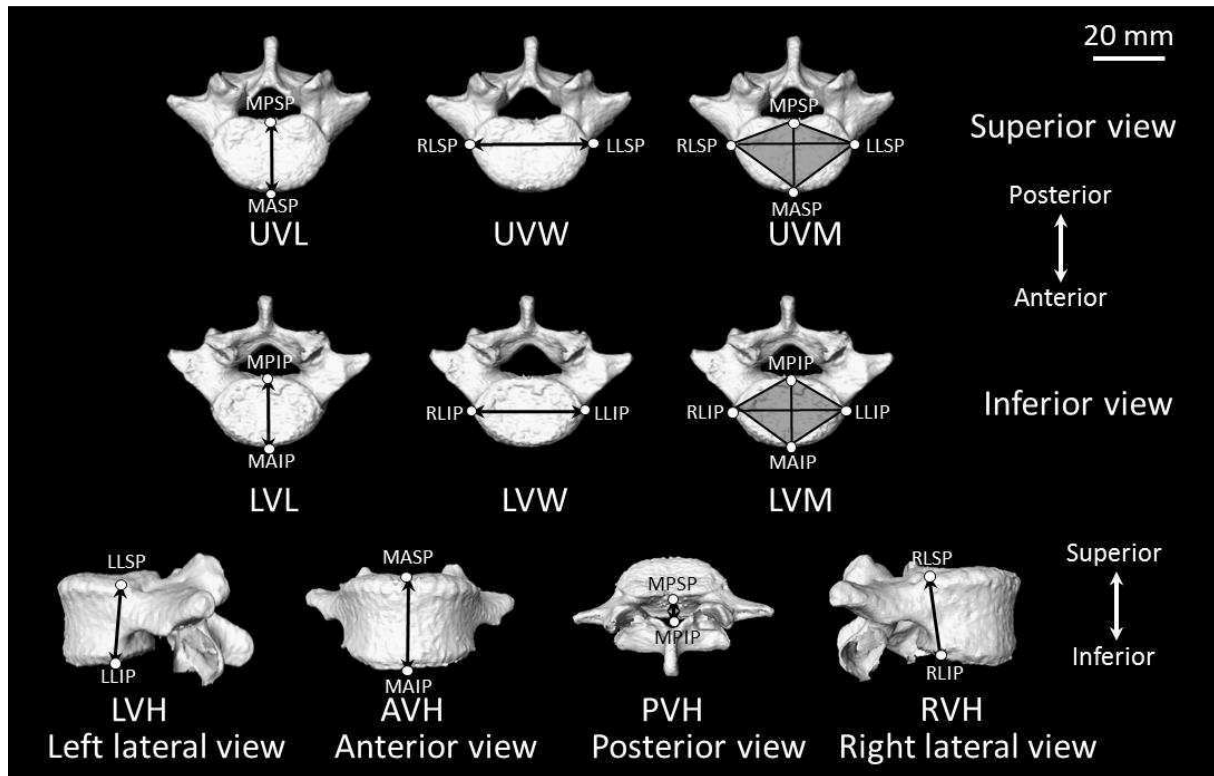


Figure 4.2 Histogram of distribution of the number of individuals from Marseilles (left), from the Luis Lopes collection (middle) and from Toulouse (right) in annual age groups and by sex (red = female, blue = male) for whom the iliac biometric variables were measured



## b. Lumbar variables

Eight unidimensional and two bidimensional variables were measured on the vertebral body of the fifth lumbar vertebra (**Figure 4.3**):



**Figure 4.3** Variables taken on the juvenile fifth lumbar vertebrae (dry bones and reconstructed bones): UVL = Upper Vertebral Length, UVW = Upper Vertebral Width, UVM = Upper Vertebral Module, LVL = Lower Vertebral Length, LVW = Lower Vertebral Width, LVM = Lower Vertebral Module, LVH = Left Vertebral Height, AVH = Anterior Vertebral Height, PVH = Posterior Vertebral Height, RVH = Right Vertebral Height

- Upper Vertebral Width (**UVW**): the greatest distance between the two lateral borders of the upper articular face of the vertebral body;
- Upper Vertebral Length (**UVL**): this variable is the distance between the anterior and posterior borders of the upper vertebral body taken in the mid-sagittal plane of the vertebral body;
- Lower Vertebral Width (**LVW**): the greatest distance between the two lateral borders of the lower articular face of the vertebral body;
- Lower Vertebral Length (**LVL**): this is the distance between the anterior and posterior borders of the lower vertebral body taken in the mid-sagittal plane of the vertebral body;
- Anterior Vertebral Height (**AVH**): this variable is the distance between the anterior border of the upper articular face and the anterior border of the lower articular face of the vertebra in the mid-sagittal plane. Both these points are the same as the anterior points used to measure UVL and LVL respectively;

- Posterior Vertebral Height (**PVH**): this variable is the distance between the posterior border of the upper articular face and the posterior border of the lower articular face of the vertebra in the mid-sagittal plane. Both these points are the same as the posterior points used to measure UVL and LVL respectively;

- Right Vertebral Height (**RVH**): the distance between the most lateral border point of the upper articular face and the most lateral border point of the lower articular face of the right side of the vertebral body. Both these points are the same as the right lateral points used to measure UVW and LVW respectively;

- Left Vertebral Height (**LVH**): the distance between the most lateral border point of the upper articular face and the most lateral border point of the lower articular face of the left side of the vertebral body. Both these points are the same as the left lateral points used to measure UVW and LVW respectively;

The first four variables were also used to calculate two unpublished bidimensional variables:

- Upper Vertebral Module (**UVM**): UVM is the product of UVL and UVW. UVM is a quadrilateral whose diagonals are equal to UVL and UVW; this variable was constructed to give a geometric approximation of the upper articular surface of the vertebral body. It is expressed in mm<sup>2</sup>;

- Lower Vertebral Module (**LVM**): LVM is the product of LVL and LVW. LVM is a quadrilateral whose diagonals are equal to LVL and LVW; this variable was constructed to give a geometric approximation of the lower articular surface of the vertebral body. It is expressed in mm<sup>2</sup>.

Lumbar variables were measured on the individuals of the Marseilles sample and the Luis Lopes collection (**Figure 4.4**).

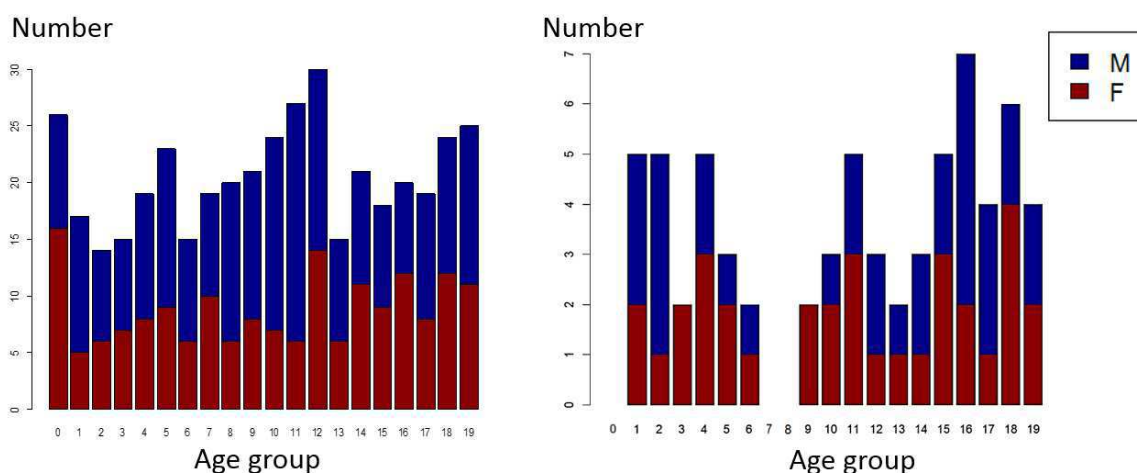


Figure 4.4 Histogram of distribution of the number of individuals from Marseilles (left) and from the Luis Lopes collection (right) in annual age groups and by sex (red = female, blue = male) for whom the lumbar variables were measured

### c. Clavicular variables

Five variables were measured on the clavicle (Figure 4.5):



Figure 4.5 Variables taken on the juvenile clavicles (dry bones and reconstructed bones). Upper left: Minimum diameter at half-length (**Min\_diam**); Upper right: Maximum diameter at half-length (**Max\_diam**); Middle: Supero-inferior diameter at half length (**SI\_diam**); Lower centre: Maximum length (**Ln**) and Antero-posterior diameter at half-length (**AP\_diam**)

- Maximum length (**Ln**): maximum distance between the medial/sternal extremity and the lateral/acromial extremity. This measurement does not include unfused clavicular epiphyses, but includes partially and completely fused ones;

- Antero-posterior diameter at half-maximum length (**AP\_diam**): the distance between the two landmarks marking the middle of the anterior side (Ant) and the middle of the posterior side (Post) at mid-length of the bone shaft;

- Supero-Inferior diameter at half-maximum length (**SI\_diam**): the distance between the two landmarks marking the middle of the superior side (Sup) and the middle of the inferior side (Inf) at mid-length of the bone shaft;

- Maximum diameter at half-maximum length (**Max\_diam**): a geometric variable, taken by finding the greatest distance between two points at mid-length of the total length of the clavicle, perpendicular to the principal axis of the bone shaft and passing through the centre of the ellipse formed by the mid-shaft section of the bone;

- Minimum diameter at half-maximum length (**Min\_diam**): a geometric variable, taken by finding the smallest distance between two points at mid-length of the total length of the clavicle, perpendicular to the principal axis of the bone and passing through the centre of the ellipse formed by the mid-shaft section of the bone.

Clavicular variables were taken on the individuals of the Marseilles sample and the Luis Lopes collection (Figure 4.6).

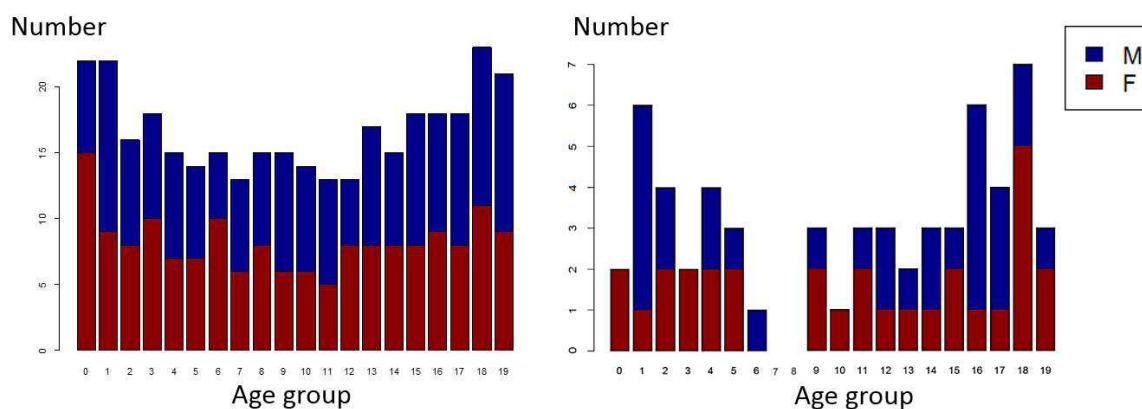
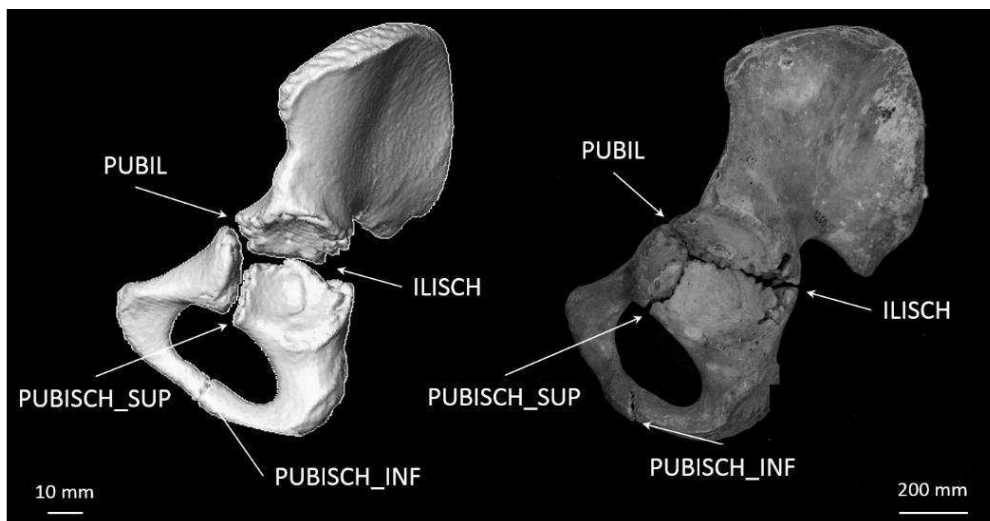


Figure 4.6 Histogram of distribution of the number of individuals from the Marseilles sample (left) and the Luis Lopes collection (right) in annual age groups and by sex (red = female, blue = male) for whom the clavicular variables were measured

#### 4.1.2. Non-biometric variables

Non-biometric variables were used to assess maturation of the acetabular region, by studying the ossification pattern of the three elements of the iliac bone. Two primary and three secondary epiphyses were observed. The three secondary epiphyses participate in the fusion of the acetabular region. One site corresponds to the junction of two epiphyses. Four ossification sites were identified and studied overall in both the Marseilles sample and the Luis Lopes sample (Figure 4.7):

- Inferior Pubic and Ischiatic epiphyses (**PUBISCH\_INF**): a primary ossification site, located between the inferior pubic and ischiatic epiphyses. After fusion, it will form the ischio-pubic branch;
- Iliac and Ischiatic epiphyses (**ILISCH**): this site englobes the posterior epiphysis, between the ischium and the ilium and the superior epiphysis, on the ilium;
- Superior Pubic and Ischiatic epiphyses (**PUBISCH\_SUP**): a secondary ossification site, located between the superior pubic and ischiatic epiphyses. After fusion, it will form the inferior part of the acetabulum and of the internal part of the innominate bone;
- Pubic and Iliac epiphyses (**PUBIL**): another secondary ossification site of the acetabulum, located between the pubic and iliac epiphyses. This site encloses the anterior epiphysis (*os acetabuli* and other bone islets) formed by the pubis and the ilium and the superior epiphysis, on the ilium.



**Figure 4.7** The four epiphyseal sites of the innominate bone studied on reconstructed bones from the Marseilles sample (left) and on dry bones from the Luis Lopes collection (right). PUBIL = Pubo-Iliac epiphysis, ILISCH = Ilio-Ischiatic epiphysis, PUBISCH\_SUP = Superior Pubo-Ischiatic epiphysis, PUBISCH\_INF = Inferior Pubo-Ischiatic epiphysis

The system adopted is similar to several methods of age estimation by maturation staging of dry bone epiphyses (Albert and Maples 1995; Iscan et al 1984, 1985; McKern and Stewart 1957; Owings-Webb and Suchey 1985). Qualitative data can be used to determine the degree of fusion, by describing the maturation state of the epiphyses. However, using qualitative descriptive data is bound to subjectivity of evaluation, intra- and inter-observer errors (Black and Scheuer 1996; Franklin 2010; Wood and Cunningham, *In* Black and Ferguson 2011). Moreover, it is difficult to undertake any statistical analyses using qualitative descriptive data other than descriptive statistics. Therefore, this approach is not appropriate to construct reliable age estimation methods using bone maturation.

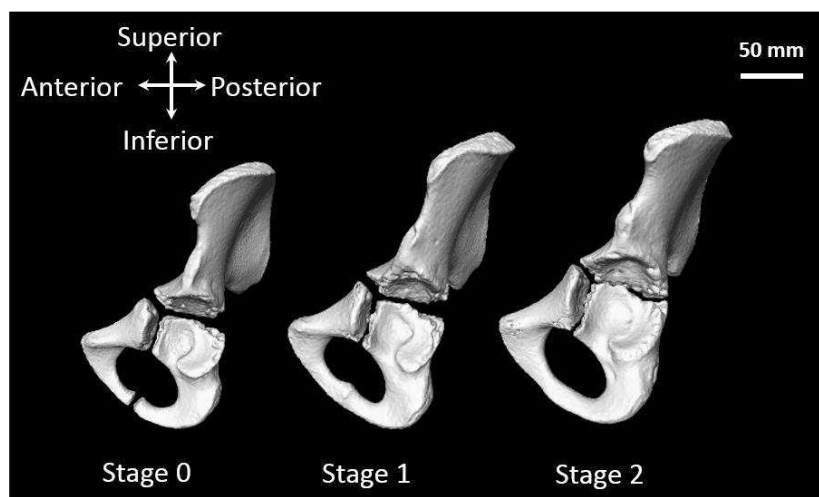
Bone maturation is often studied by staging the maturation status of the epiphyses using an n-level staging system. Stages are ordinal measures designated by whole integers (Smith, *In* Kelley and Larsen 1991). Each stage represents a particular epiphyseal state at the given time of observation. The principle of staging presents different variations in number of stages (from 2 to n), of associated descriptive characteristics (more or less detailed).

The main bias of maturation assessment by staging is subjectivity. Indeed, the goal is to develop a reliable staging system using objective stages with low intra- and inter-observer errors, *i.e.* high repeatability and reproducibility. Studies have shown that increasing the number of stages seems to be more precise, but also tends to increase intra- and inter-observer errors and takes longer to learn (Saint-Martin 2014). To reduce staging system error, the observer's implication in the evaluation of maturation needs to be reduced, *i.e.* the number of stages has to be restricted. Typically, the number of stages lies between 3 and 6 (Saint-Martin 2014). For this reason, we have chosen to use a three-stage maturation assessment system (**Figure 4.8**). Stages were defined as follows:

0 = no union/fusion

1 = partial union/fusion (or epiphyseal line still visible), including the presence of an *os acetabuli*.

2 = complete union/fusion (no visible line of fusion)



**Figure 4.** 8 Different maturation stages defined for the study of the fusion of the four epiphyses, illustrated by the fusion states of the PUBISCH\_INF site. Stage 0 = no fusion between the pubis and the ischium (*innominate bone* of a 5 year-old boy); Stage 1 = fusion still occurring between the pubic and ischiatic epiphyses with presence of a fusion line (*innominate bone* of a 6 year-old boy); Stage 2 = complete fusion between the pubis and the ischium, resulting in the ischio-pubic branch of the *innominate bone* (*innominate bone* of an 8 year-old boy)

Some methods for maturation assessment use several epiphyseal locations simultaneously. This can be represented as a score  $S$ , or a combination of stages (Smith, *In* Kelley and Larsen 1991):

- A score is the sum of the maturation stages at  $n$  epiphyseal sites:  $S = \sum_{i=1}^n M_i$ .
- A combination of stages at  $n$  epiphyseal locations can be represented as  $(M_1-M_2-...-M_n)$

Staging was done on the left and right innominate bones whenever possible for each individual, at the four fusion sites cited above: PUBISCH\_INF, PUBIL, PUBISCH\_SUP and ILISCH. This gives a minimum number of one and a maximum number of eight stages per individual. Staging was done on the left and right innominate bones whenever possible for each individual.

In addition to the stages, a four-digit combination is given for each iliac bone. Each digit corresponds to a stage at each epiphyseal site. The digits are always in the same order, following the order of the maturation sequence of the iliac bone: PUBISCH\_INF, ILISCH, PUBISCH\_SUP and PUBIL (Scheuer and Black 2000, 2004; White and Folkens 2005). Scoring was not attempted as it seemed too dependent on preservation rates of all four epiphyses, whereas it is possible to analyse combinations of two, three or four stages.

A maturation sequence also means that for a given element, the order of epiphyseal fusion is always the same, even though timing can vary (Scheuer and Black 2000, 2004). This means that all the epiphyses will fuse following a given order, but not all at the same time and they will always go from one stage (n) to the next (n+1). Therefore, once we consider these conditions and the sequential iliac maturation patterns, the real number of combinations is much lower.

The time lapses between stages are unequal, so standard mean and variance calculations are problematic (Smith, *In* Kelley and Larsen 1991) and were not done here. However, the minimum, maximum and mean ages per stage per site and per combination can be calculated.

Iliac non-biometric variables were taken on the individuals of the Marseilles sample and the Luis Lopes collection (Figure 4.9).

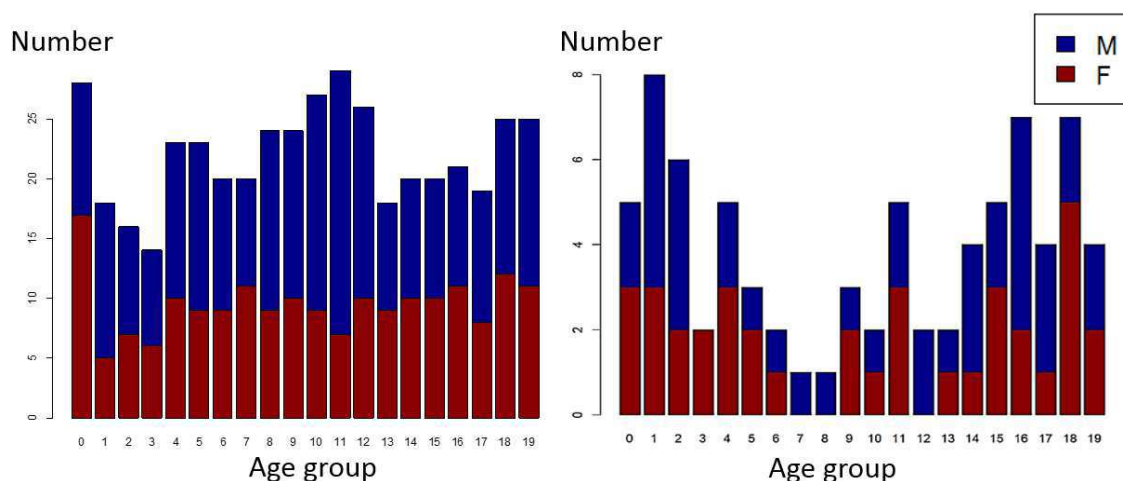


Figure 4.9 Histogram of distribution of the number of individuals from Marseilles (left) and from the Luis Lopes collection (right) in annual age groups and by sex (red = female, blue = male) for whom the non-biometric variables were recorded

## 4.2. Acquisition of variables on CT scan data

### 4.2.1. Segmentation and reconstruction techniques

After medical computed tomographic examination, the CT scans obtained for each individual are a stack of two-dimensional transversal greyscale images of their body (in Hounsfield units). Greyscale values are the values taken on by different elements of the CT scan and correspond to their respective radiodensity. The stack of images or CT slices can be compiled using software developed for medical image analysis, in order to obtain a virtual three-dimensional representation of the body and/or its anatomical components by virtual reconstruction.

The next step after acquiring the images was image data processing, to extract an object from a set of images (Zollikofer and Ponce de Leon 2005). Using these stacks of CT scans, we isolated and extracted the bone surfaces needed for our study from the other anatomical elements of the body, mainly the soft tissues surrounding the bones and other surrounding skeletal elements. This step is called segmentation and is done using Softwares such as Amira® (Mercury Computing Systems, Inc.) and Avizo® (Visualizing Sciences Group, SAS). For each image of a given stack, the bony elements and their corresponding greyscale level values have to be identified. CT scans were first converted to the digital standardized format Digital Imaging and Communication in Medicine (DICOM) format, in order to be treatable by all types of reconstruction softwares. The stack of CT scans was then loaded in the ImageJ® v1.48 Software (National Institute of Health, USA) as an image sequence, in order to open all the slices simultaneously and navigate through them, from the first to the last. For each bone studied (both clavicles, the fifth lumbar vertebra and the iliac bones), the greyscale level corresponding to the separation between bone tissue and its immediate environment (mainly soft tissue) was calculated independently using the Half-Maximum Height (HMH) method (Spoor et al 1993). HMH is an objective and systematic method to differentiate components/materials of a CT scan image and is easily reproducible. It is calculated on Image J® first by tracing a line at the interface between the bone (for example the ilium) and its environment. A histogram of the greyscale values (*i.e.* the corresponding anatomical elements) covered by this line is obtained. The minimum and maximum values of the histogram at the transition zone or threshold between bone and soft tissue are then used to calculate the mean greyscale value at that location. This is done on ten different CT slices including the same bone, to obtain ten mean threshold values (one per selected slice). Each mean is then added and divided by the number of means (ten) to obtain the HMH value (Fajardo et al 2002). HMH values were calculated for each left and right clavicle, each lumbar vertebra, and each left and right iliac bones of the individuals.

Several segmentation techniques have been developed to isolate objects of interest: manual segmentation, semi-automatic segmentation and automatic segmentation. The first two are mostly used for 3D bone reconstruction and give comparable and satisfactory results, with surface or volume estimation errors of less than 4% (Bondioli et al 2010; Puymerail 2011). However, as manual segmentation requires the segmentation of each region of interest, slice by slice, with several hundreds of slices per bone, it is much more time-consuming. Therefore, for practical and time-related reasons, semi-automatic segmentation was privileged over manual segmentation unless bone reconstructions were deemed of insufficient quality and required slice-by-slice segmentation. HMH values were used for semi-automatic segmentation of the bone surface and the transformation of two-dimensional objects into three-dimensional surfaces. This was done using the Image



Segmentation module of Avizo® v7.0.0 (Visualizing Sciences Group, SAS). The CT series were loaded into Avizo® and slices were visualised in all three planes as a binary Avizo® object. The modules “Isosurface” and “Extract Surface” were then used to transform the series of scans into bone surfaces. No specific smoothing process was applied as we wished to obtain reconstructed surfaces as similar as possible to the real bone surfaces and reliefs. The threshold value was set to the HMM calculated for each bone and the bone surfaces were thus reconstructed: they correspond to the interface between bones and surrounding soft tissues (Zollikofer and Ponce de Leon 2005).

As bones all present cortical and trabecular components, they are likely to have relatively comparable greyscale and threshold values, with variations depending on the ratios of both bone tissues. For this reason, most of the skeleton and sometimes medical equipment of comparable density and associated to the individual were also reconstructed along with the bone of interest (for example, the ilium). Moreover, as some skeletal elements are anatomically positioned to be very close to one another (*e.g.* the sacrum and the ilium at the sacro-iliac joint), segmentation could therefore prove difficult. In spite of this, in the end, no manual segmentation was required as the elements of interest were always sufficiently separated from the surrounding soft or hard tissues (tendons, ligaments, muscles, inter-vertebral discs).

To isolate the surfaces of the bone(s) of interest from the rest of the skeletal elements, the module “Surface Editor” and several tools (“Magic Wand” and “Draw Contour”) were used to select and isolate the element(s) of interest. The rest of the elements were then erased, leaving only the bones needed. The final step was to save the reconstructed bone surfaces used for analyses in a .surf format, specific to Avizo® or Amira®. The virtual bone surfaces were the basic objects for all the following steps of biometric and non-biometric variable acquisition.

All these steps were done for both clavicles, the fifth lumbar vertebra and both iliac bones for each individual of the Marseilles sample.

### **4.2.2. Biometric variables: uni- and bi-dimensional data**

The variables defined previously were taken on dry bone material. However, our study was conducted partly on dry bone material (individuals of the Luis Lopes collection) and partly on virtual three-dimensional bones reconstructed from computed tomographic scans (CT scans) of living individuals. Several publications have verified consistency between dry bone shape and three-dimensional reconstructed bone shape (Stephan and Guyomarc’h 2014) and between measurements taken on dry bone and two-dimensional bone images (Adalian et al 2002; Brough et al 2013) or three-dimensional bone reconstructions (Citardi et al 2001; Guyomarc’h et al 2012; Stull et al 2014b).

Adapting biometric variables taken on dry bones to three-dimensionally reconstructed bones was done by placing landmarks on the bone surfaces that correspond to the extremities of the dry bone measurements. A landmark is a reference point that corresponds to a specific structural limit on a given object. A landmark can represent a positive or negative anatomical relief, or bone extremity, and identifies a point of conversion between three or more anatomical units (Zollikofer and Ponce de Leon 2005). In that case, landmarks are virtual homologues to anatomical (skeletal) points, as defined by F. Bookstein (1991). In other words, a landmark has to represent the same anatomical structure for any individual. A landmark can also reflect a geometric property, such as a local maximum of curvature, or a projected point on a different plane, etc. Because of their various degrees of homology,

F. Bookstein established a classification of “landmark-types” (Bookstein 1991):

- Type 1 landmarks represent anatomical structures with a high degree of homology between individuals. For example, the antero-superior iliac spine is a type 1 landmark;
- Type 2 landmarks present inter-individual homology supported by surrounding geometric characteristics. For example, the summit of the iliac crest is a type 2 landmark;
- Type 3 landmarks are not defined by surrounding structures. Their position is defined as an extreme compared to another point. For example, the most lateral points of the upper surface of the vertebral body are type 3 landmarks.

Landmarks are used in biological anthropology, and more particularly in palaeoanthropology for visualising and analysing three-dimensional variations of bone conformation using geometric morphometrics (Bookstein 1991; Puymeraill 2011; Zollikofer and Ponce de Leon 2005). They are also used for taking measurements on virtually reconstructed objects, and bones in particular. In our study, landmarks were used as reference points to obtain the same variables on virtual bones as the ones defined on dry bones. Once the virtual bone surface is reconstructed after image segmentation and saved as a work object, landmarks can be placed on the bone surface by creating them as new data associated to the bone surface. Any number of landmarks can be created for a single object. Their position can be changed and they can be removed as easily using different options. A set of three-dimensional coordinates ( $x_i, y_i, z_i$ ) is associated to each landmark  $i$  created in the virtual plane of the object of study (the bone surface). The coordinates are saved as a file in the .ascii format. It corresponds to the three-dimensional coordinates of each landmark created, in the same order they were placed on the object.

### a. Iliac variables

After semi-automatic segmentation (Spoor et al 1993), the bones were isolated and bone surfaces of the iliae were reconstructed for the individuals from Marseilles.

#### i. Plane definition and landmark positioning

The landmarks defining the variables are type 1 or type 2, and correspond to their equivalent anatomical or geometrical points on dry iliae (**Figure 4.10**). Variables IL, IW and IM were obtained after positioning four landmarks in the following order:

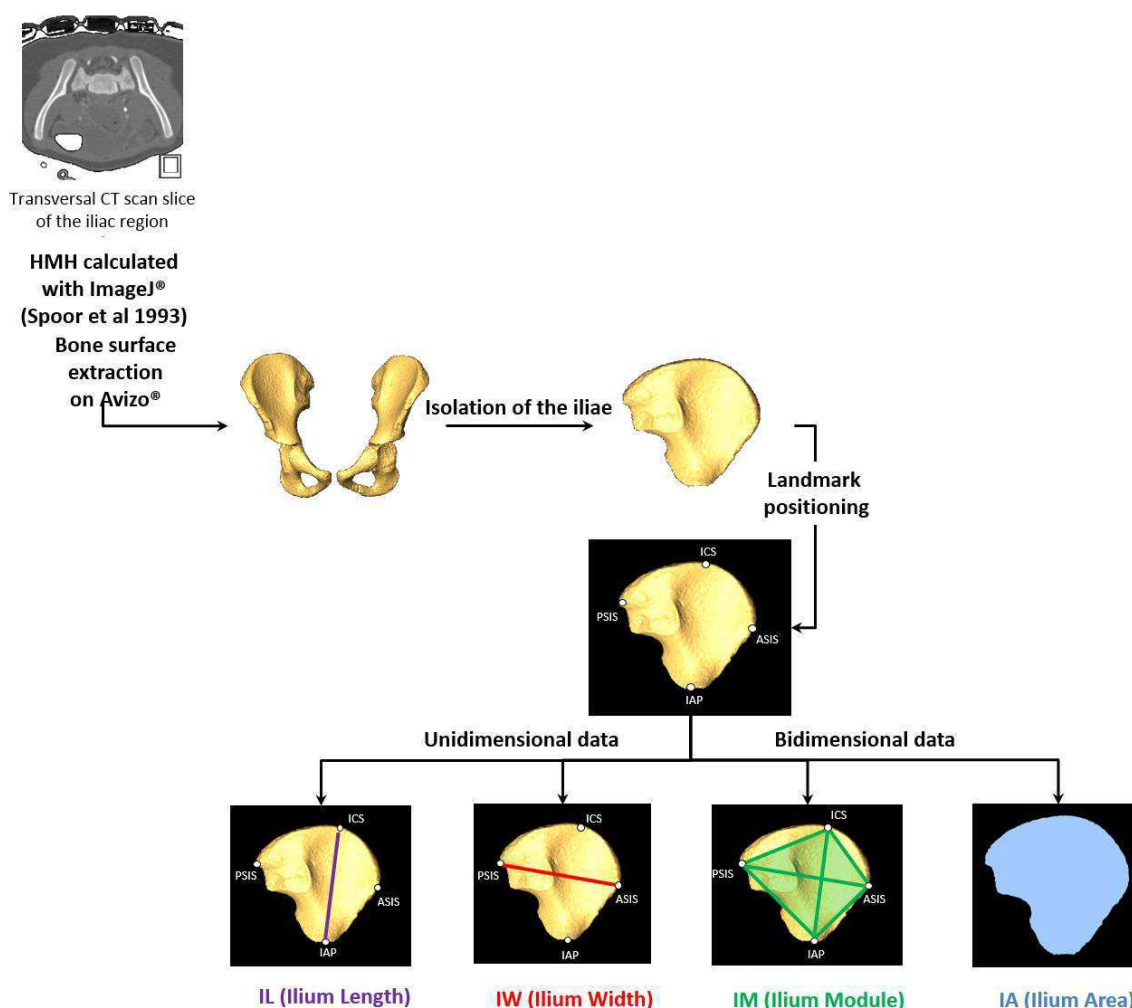


Figure 4.10 Protocol for iliae variable acquisition on iliae reconstructed from CT scans. Semi-automatic segmentation and iliae isolation are followed by landmark positioning on four anatomical points of the iliae: ICS = Iliac Crest Summit, IAP = Inferior Acetabular Point, PSIS = Postero-Superior Iliac Spine, ASIS = Antero-Superior Iliac Spine. Using the coordinates of these landmarks, two variables are obtained: IL = Ilium Length, IW = Ilium Width, IM = Ilium Module is the product of IL and IW, IA = Ilium Area is calculated using three of the landmarks to define a reference plane (the iliac plane) on which the internal iliac surface is projected

1. Antero-Superior Iliac Spine (**ASIS**): this landmark is positioned on the extremity of the antero-superior iliac spine of the ilium. It is a type 1 landmark. Its position in the virtual space is defined by the following coordinates: (xASIS, yASIS, zASIS);
2. Postero-Superior Iliac Spine (**PSIS**): this landmark is positioned on the extremity of the postero-superior iliac spine of the ilium. It is a type 1 landmark. Its position in the virtual space is defined by the following coordinates: (xPSIS, yPSIS, zPSIS);
3. Iliac Crest Summit (**ICS**): this landmark is positioned on the most prominent point of the iliac crest, at the maximum point of the curve made by the crest. It is a type 2 landmark. Its position in the virtual space is defined by the following coordinates: (xICS, yICS, zICS);
4. Internal Acetabular Point (**IAP**): this landmark is positioned on the most prominent point of the internal acetabular ridge of the ilium when the articular surface is seen from an infero-lateral view. It is a type 2 landmark. Its position in the virtual space is defined by the following coordinates: (xIAP, yIAP, zIAP).

ii. Variable acquisition protocols

IL and IW were measured as Euclidian distances by calculating the formula of vector length between each set of landmarks two by two as follows:

$$IL = \|\vec{IL}\| = \sqrt{((x \text{ ASIS} - x \text{ PSIS})^2 + (y \text{ ASIS} - y \text{ PSIS})^2 + (z \text{ ASIS} - z \text{ PSIS})^2)}$$

$$IW = \|\vec{IW}\| = \sqrt{((x \text{ ICS} - x \text{ IAP})^2 + (y \text{ ICS} - y \text{ IAP})^2 + (z \text{ ICS} - z \text{ IAP})^2)}$$

The third variable, Ilium Module (IM) was used by C. Rissech and collaborators (2003) in a previous study on dry bones (Rissech et al 2003). IM is the simple product of IL and IW, and is expressed in mm<sup>2</sup>. IM is the first bidimensional variable of our study.

$$IM = IL \times IW$$

The fourth variable is Ilium Area (IA), also expressed in mm<sup>2</sup>. The ilium was placed in a plane defined by three landmarks: ASIS, PSIS and IAP. This plane was then oriented to be parallel to the screen using the Slice ViewPoint tool of Avizo®. A scalebar of constant pixel size, but corresponding to various mm sizes, depending on the size of the ilium bone itself, was placed next to the bone and a screenshot was taken with the snapshot tool. The screenshot of the ilium oriented in the iliac plane was then exported in the ImageJ® Software. The projected area was converted into a binary black and white image, by adjusting the colour threshold of the picture. We obtained a black and white image of the ilium area: the ilium area is white and is called a “phantom”, the background is black. IA

was calculated on the “phantom” image using a correspondence in pixels with the length of the scalebar (Figure 4.11).

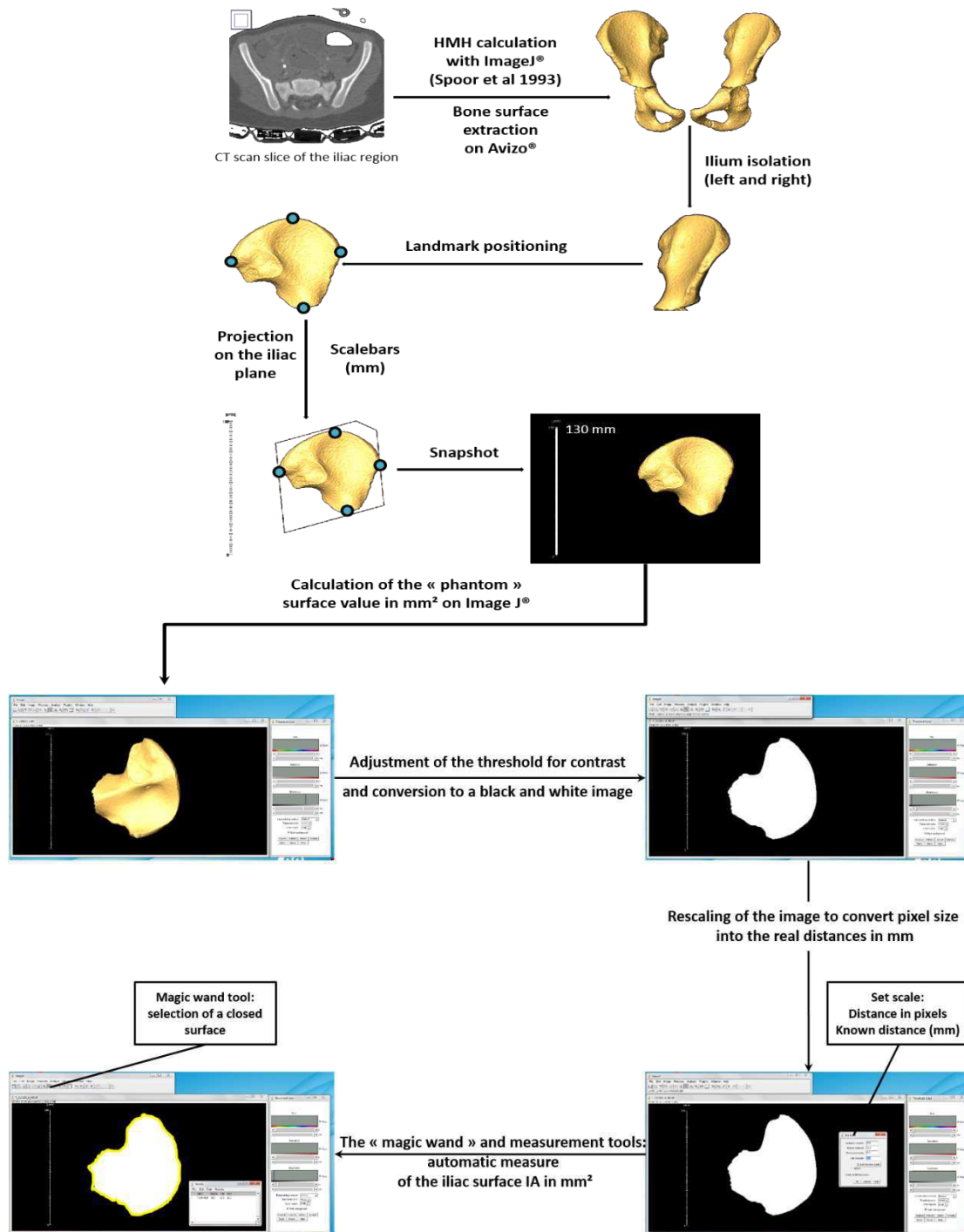


Figure 4.11 Detailed protocol for the acquisition of variable Ilium Area (IA): once the ilium is reconstructed and the four landmarks are positioned, three of them are used to define the iliac plane, a common reference plane for all the iliae studied. The ilium is then projected on the plane so the iliac surface is seen as if it were perpendicular to the observer’s vision axis. A scalebar of constant length is added and the iliac surface and the scalebar are photographed using the Avizo® snapshot tool and exported as a .tiff file in Image J®. The image is then binarised and the colour threshold is adjusted to obtain a “phantom” of the surface. The image is rescaled into pixel values using the “Scale” tool and the original scalebar. The surface is then selected using the magic wand tool and the area of the surface is directly calculated with Image J®’s measurement tool

Constant pixel size of the Avizo® scalebar avoids errors due to repeated measurements: the size in mm indicated next to the scalebar always corresponds to the same value in pixels, established with the first ilium image analysed. The scale of the image is fixed at the same value in pixels, but variable values in mm.

During the acquisition of the variables on virtual bone surfaces, we were forced to restrict the upper age limit of the sample to 12 years included. The reason for this is the beginning of acetabular fusion around 11-13 years of age that led to the fusion of all three bone elements in both the external and internal faces of the region of interest. This prevented us from accurately and reliably identifying and placing IAP, in which case the variables IW, IM and IA could not be measured accurately.

All four variables were measured on the left and right 3D-reconstructed iliae of 244 individuals from Marseilles, aged between 0 and 12 years included.

## **b. Lumbar variables**

Both vertebral laminae and vertebral body were reconstructed for all individuals even when they were not fused, because it facilitated both anatomical orientation of the bone in the three-dimensional virtual space and landmark positioning.

### **i. Plane definition and landmark positioning**

There was no need to proceed to reorientation or repositioning of the lumbar vertebrae: anatomical reliefs and faces of the bones were easily identifiable and landmark positioning was not difficult (**Figure 4.12**).

To obtain the same lumbar variables as the study done by S. Zhou and collaborators (2000), eight landmarks were defined and placed on the bone surfaces in the following order:

1. Median Postero-Superior Point (**MPSP**): the intersection between the posterior border of the upper vertebral surface and the mid-sagittal plane of the vertebral body;
2. Median Antero-Superior Point (**MASP**): the intersection between the anterior border of the upper vertebral surface and the mid-sagittal plane of the vertebral body;
3. Right Lateral Superior Point (**RLSP**): the most lateral point on the right side of the upper articular surface of the vertebral body;
4. Left Lateral Superior Point (**LLSP**): the most lateral point on the left side of the upper articular surface of the vertebral body;

5. Median Postero-Inferior Point (**MPIP**): the intersection between the posterior border of the lower vertebral surface and the mid-sagittal plane of the vertebral body;
6. Median Antero-Inferior Point (**MAIP**): the intersection between the anterior border of the lower vertebral surface and the mid-sagittal plane of the vertebral body;
7. Right Lateral Inferior Point (**RLIP**): the most lateral point on the right side of the lower articular surface of the vertebral body;
8. Left Lateral Inferior Point (**LLIP**): the most lateral point on the left side of the lower articular surface of the vertebral body;

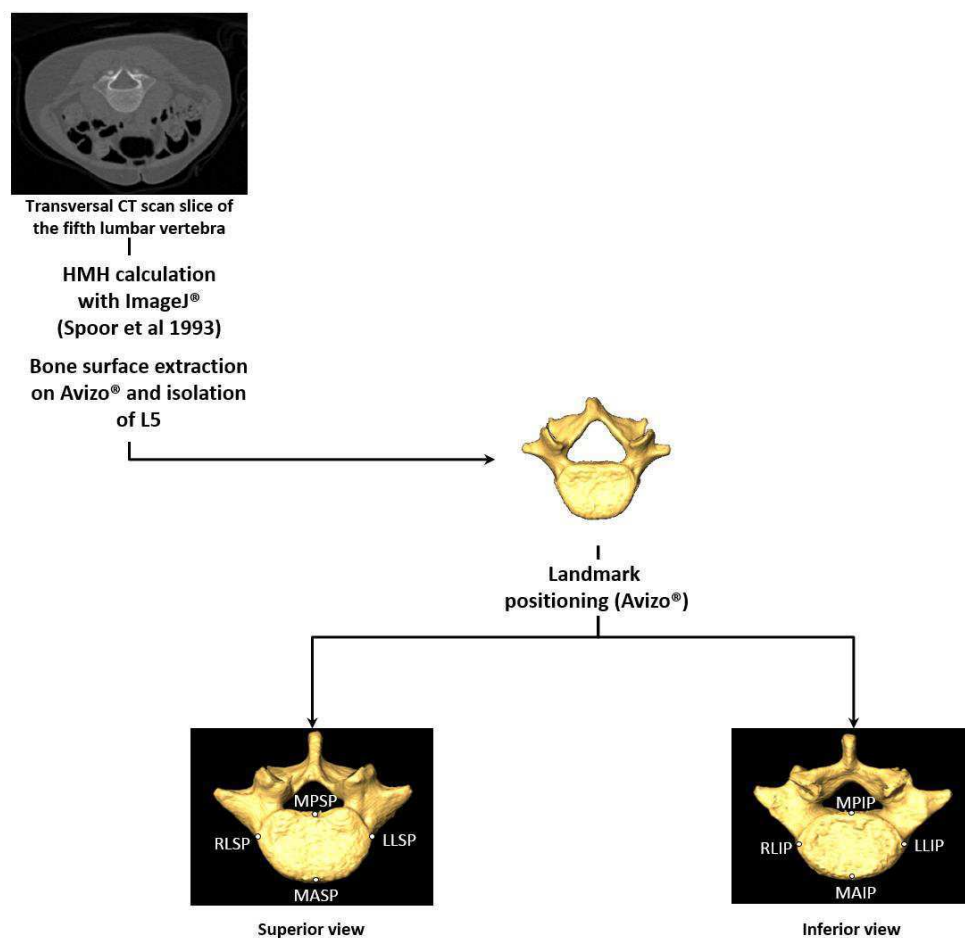


Figure 4.12 Main steps of the protocol for fifth lumbar surface extraction and reconstruction from CT scans and the positioning of eight landmarks defining eight of the lumbar variables; MPSP = Median Postero-Superior Point, MASP = Median Antero-Superior Point, RLSP = Right Lateral Superior Point, LLSP = Left Lateral Superior Point, MPIP = Median Postero-Inferior Point, MAIP = Median Antero-Inferior Point, RLIP = Right Lateral Inferior Point, LLIP = Left Lateral Inferior Point

ii. Variable acquisition protocol

All eight unidimensional variables were measured as Euclidian distances expressed in mm by calculating the formula of vector length between each set of landmarks two by two as follows:

$$UVL = \|\overrightarrow{UVL}\| = \sqrt{((x \text{ MPSP} - x \text{ MASP})^2 + (y \text{ MPSP} - y \text{ MASP})^2 + (z \text{ MPSP} - z \text{ MASP})^2)}$$

$$UVW = \|\overrightarrow{UVW}\| = \sqrt{((x \text{ RLSP} - x \text{ LLSP})^2 + (y \text{ RLSP} - y \text{ LLSP})^2 + (z \text{ RLSP} - z \text{ LLSP})^2)}$$

$$IVL = \|\overrightarrow{IVL}\| = \sqrt{((x \text{ MPIP} - x \text{ MAIP})^2 + (y \text{ MPIP} - y \text{ MAIP})^2 + (z \text{ MPIP} - z \text{ MAIP})^2)}$$

$$IVW = \|\overrightarrow{IVW}\| = \sqrt{((x \text{ RLIP} - x \text{ LLIP})^2 + (y \text{ RLIP} - y \text{ LLIP})^2 + (z \text{ RLIP} - z \text{ LLIP})^2)}$$

$$AVH = \|\overrightarrow{AVH}\| = \sqrt{((x \text{ MASP} - x \text{ MAIP})^2 + (y \text{ MASP} - y \text{ MAIP})^2 + (z \text{ MASP} - z \text{ MAIP})^2)}$$

$$PVH = \|\overrightarrow{PVH}\| = \sqrt{((x \text{ MPSP} - x \text{ MPIP})^2 + (y \text{ MPSP} - y \text{ MPIP})^2 + (z \text{ MPSP} - z \text{ MPIP})^2)}$$

$$RVH = \|\overrightarrow{RVH}\| = \sqrt{((x \text{ RLSP} - x \text{ RLIP})^2 + (y \text{ RLSP} - y \text{ RLIP})^2 + (z \text{ RLSP} - z \text{ RLIP})^2)}$$

$$LVH = \|\overrightarrow{LVH}\| = \sqrt{((x \text{ LLSP} - x \text{ LLIP})^2 + (y \text{ LLSP} - y \text{ LLIP})^2 + (z \text{ LLSP} - z \text{ LLIP})^2)}$$

The two bidimensional variables UVM and IVM were calculated as the product of UVL and UVW, and IVL and IVW respectively.

$$UVM = UVL \times UVW$$

$$IVM = IVL \times IVW$$

They are expressed in mm<sup>2</sup> and correspond to geometric approximations of the upper vertebral and lower vertebral body surfaces respectively (**Figure 4.13**).

Of the 540 individuals from Marseilles for whom CT scan sets were collected, 400 aged from 0 to 19 years included presented exploitable fifth lumbar vertebrae.



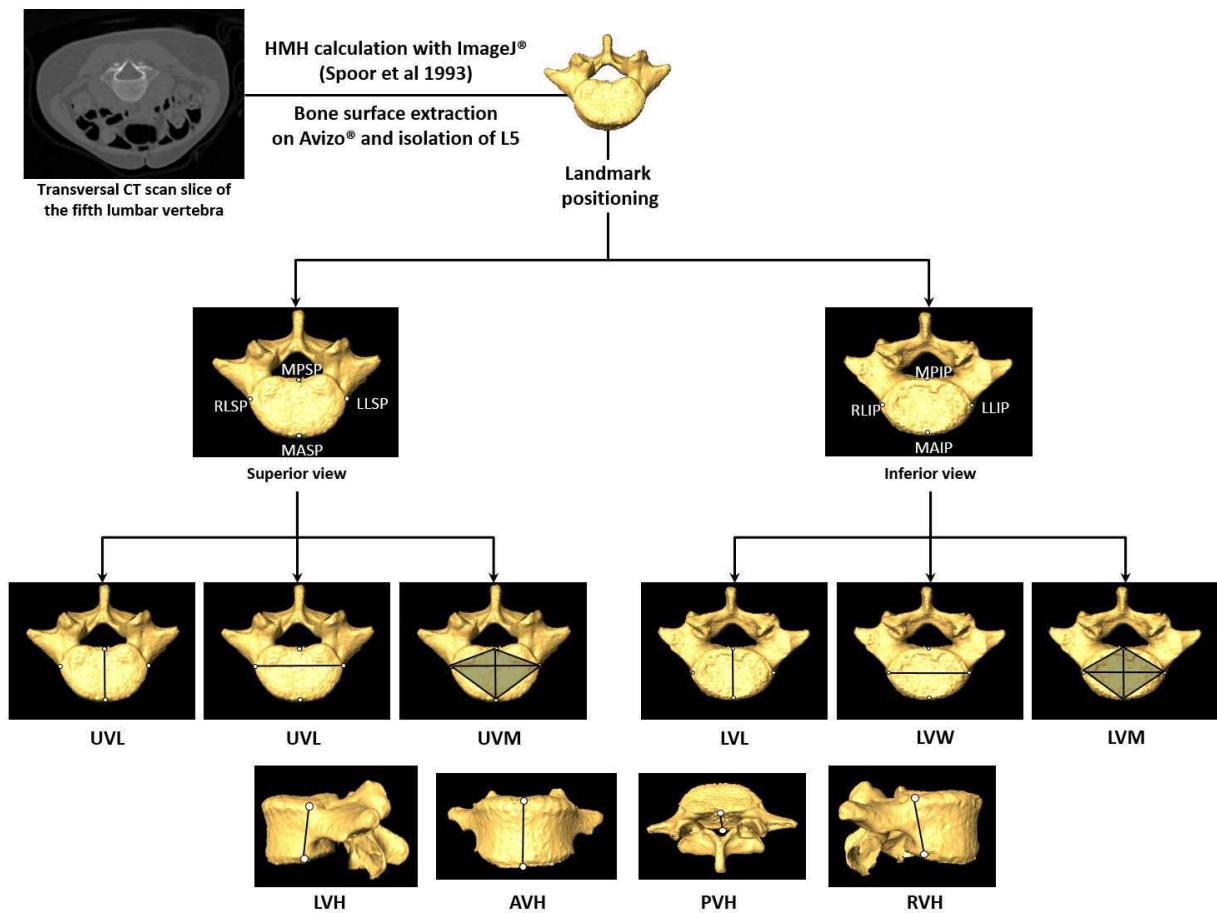


Figure 4.13 Eight landmarks placed on the reconstructed fifth lumbar bone surfaces and the variables obtained by their coordinates: UVL = Upper Vertebral Length, UVW = Upper Vertebral Width, UVM = Upper Vertebral Module, LVL = Lower Vertebral Length, LVW = Lower Vertebral Width, LVM = Lower Vertebral Module, LVH = Left Vertebral Height, AVH = Anterior Vertebral Height, PVH = Posterior Vertebral Height, RVH = Right Vertebral Height

### c. Clavicular variables

#### i. Plane definition and landmark positioning

The difficulty with using data taken from patient CT examinations is that the orientation of the bones (e.g. the clavicles) varies according to the position of the patient. Depending on the purpose of the CT examination, the patient will have his/her arms in different positions (mostly lifted over the head, but sometimes laid flat, or extended laterally). Their position can vary from patient to patient but also between the left and right bones of the same patient. We therefore had to verify that the position of the clavicles did not influence the values of the variables, to avoid errors due to differences in the positioning of the bones.

Several studies (Brough et al 2012, 2013) have shown that measurements taken on dry bones, on CT slices of the bones or on the three-dimensional surfaces of the same bones virtually reconstructed

from CT scans were consistent. However, dry and virtual bones were either placed in the same plane (flat surface) for comparison (Brough et al 2013; Stull et al 2014b), or no mention was made of the position of the bones, so the influence of the initial position and orientation of the bones on measurement error was not evaluated. In our study, only two landmarks were identifiable (most lateral and medial points of the acromial and sternal extremities respectively), but they did not respect the definition of a landmark as their position is purely geometrical. The extreme morphological variability of the sternal extremity of the clavicle (Cook et al 2013; Langley-Shirley and Jantz 2010) raises questions about placing a point there: should it be in the centre, on the most inferior point of the surface..? Moreover, a plane is defined by three points and no third point could be identified and qualified as reliable. The possibilities are numerous, and the risk of error is important. Indeed, no common reliable and reproducible plane has been identified to this day for virtually reconstructed clavicles (Voisin and Balzeau 2004).

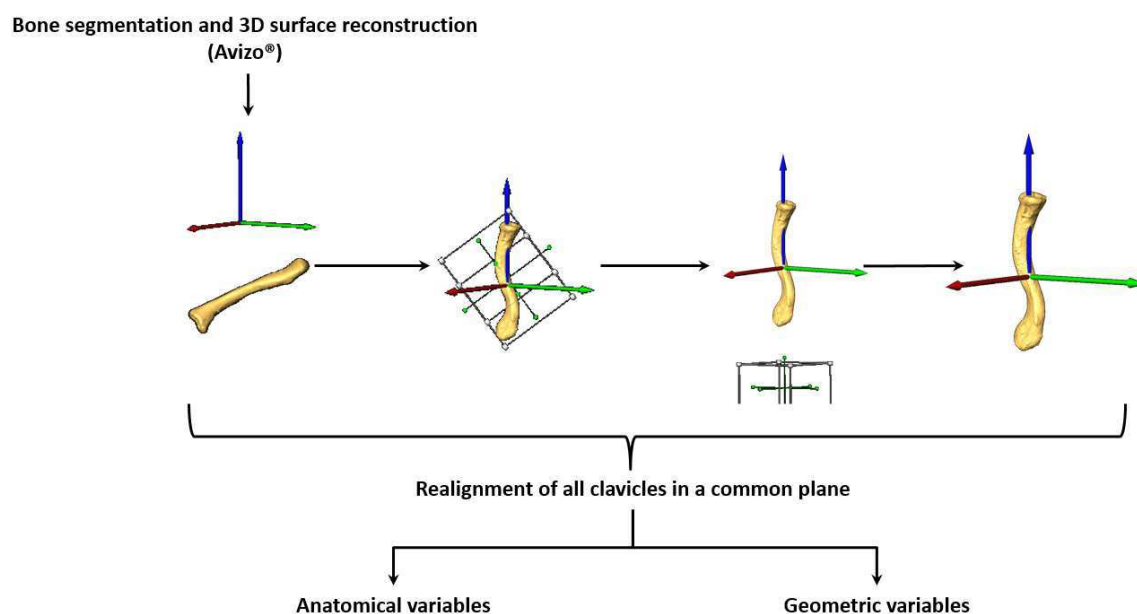
For these reasons, a protocol for obtaining the five clavicular variables from bones positioned in random planes was elaborated and tested. A pre-study was done to compare variables taken on 40 random dry bone clavicles and variables taken on the same 40 dry bone clavicles after virtual surface reconstruction. The clavicles belonged to 30 adults (13 right bones and 17 left bones) and 10 unknown juveniles (nine right bones and one left bone). 24 of the adult clavicles came from the "Cimetière des Trois Maisons" archaeological site (18th-19th Century) in Nancy, France (Dohr 2012). The other six adult clavicles and the ten juvenile ones belonged to individuals from unknown archaeological backgrounds that are used as reference material for osteology and anatomy courses in the UMR 7268 ADES. All bones are housed in the collections of the UMR 7268 ADES.

This pre-study evaluates both the error relative to the type of material on which variables are taken (dry bone VS virtually reconstructed bone) and the error due to differences in orientation, the dry bones being the anatomically oriented bones and the virtual reconstructed bones being the randomly oriented bones. The dry bone clavicles were measured using generic variable tools (sliding calliper and osteometric board) and served as the bones measured in the reference anatomical plane, with the anterior, posterior, superior and inferior faces clearly identified.

The clavicles were placed on a flat surface in their position of stability for acquisition by computed tomography (Brough et al 2013). A 64-row multidetector CT scan (Somatom Sensation 64, Siemens®, Erlangen, Germany) was used with the following scanning parameters: 120 KV, 315 mAs, thickness: 0.6 mm. The plane is the same, but the orientation of the bones is variable, as they are resting on the points defining their stable position. The stability points depend on the morphology of the bones. This is particularly true for the extremity points where bone reliefs, such as the conoid tubercle, can be more or less prominent. Therefore, the stable position cannot systematically be considered as the anatomical position.

After virtual reconstruction of the clavicles (using HMH for segmentation and the Avizo® Software), we observed that it was still relatively easy to distinguish the acromial plane (parallel to the acromial surface) on virtual bones. Because of this, and following what is generally done when taking variables on dry clavicles (Voisin and Balzeau 2004), we then considered that the acromial plane would be the superior plane; the inferior plane would be defined as being parallel to it and the anterior and posterior planes as perpendicular to the previous two. These planes then allowed landmark positions for the variables taken using the virtual variable tools of Avizo® Software (**see above**).

Because of their various positions of stability, the reconstructed clavicles had random orientations. To avoid errors due to different orientations, it was decided to construct a common plane for all the clavicles by realigning the clavicles in a common geometric plane directly in Avizo®, without relying on common landmarks or acquisition parameters (**Figure 4.14**).



**Figure 4.14** Clavicle realignment protocol in Avizo®: the clavicle is reconstructed by semi-automatic segmentation. The bone surface is then aligned on one of the three axes defining the Euclidian space (x, y, z) using the geometry transform tool. All clavicles are then aligned on the same axis, in a common geometrical plane and the variables can then be measured

Therefore, the orientation of each bone follows the same axes: the axis parallel to the maximum length of said bone (x) and the ones perpendicular to it (y and z). For the anatomical orientation of the bone, it was once again decided to consider the acromial surface as an equivalent to the superior surface of the bone (Voisin and Balzeau 2004). The other three anatomical planes were once again defined according to the acromial plane. To verify that this choice of orientation was acceptable, we then proceeded to evaluate the error of measurement between dry bones and re-positioned and re-

oriented scanned bones by comparing both sets of variables obtained by the same observer twice and by different observers (see section 4.4.1.).

ii. Variable acquisition protocols

In the same way as for the dry clavicle test study, we proceeded to realign all the patients' reconstructed clavicles on the same axis (x) and we considered the flat surface of the acromial plane as the superior surface of the clavicle. Once the position and the orientation of the bone were established, the protocol for acquiring the five biometric variables was applied (Figures 4.15 and 4.16).

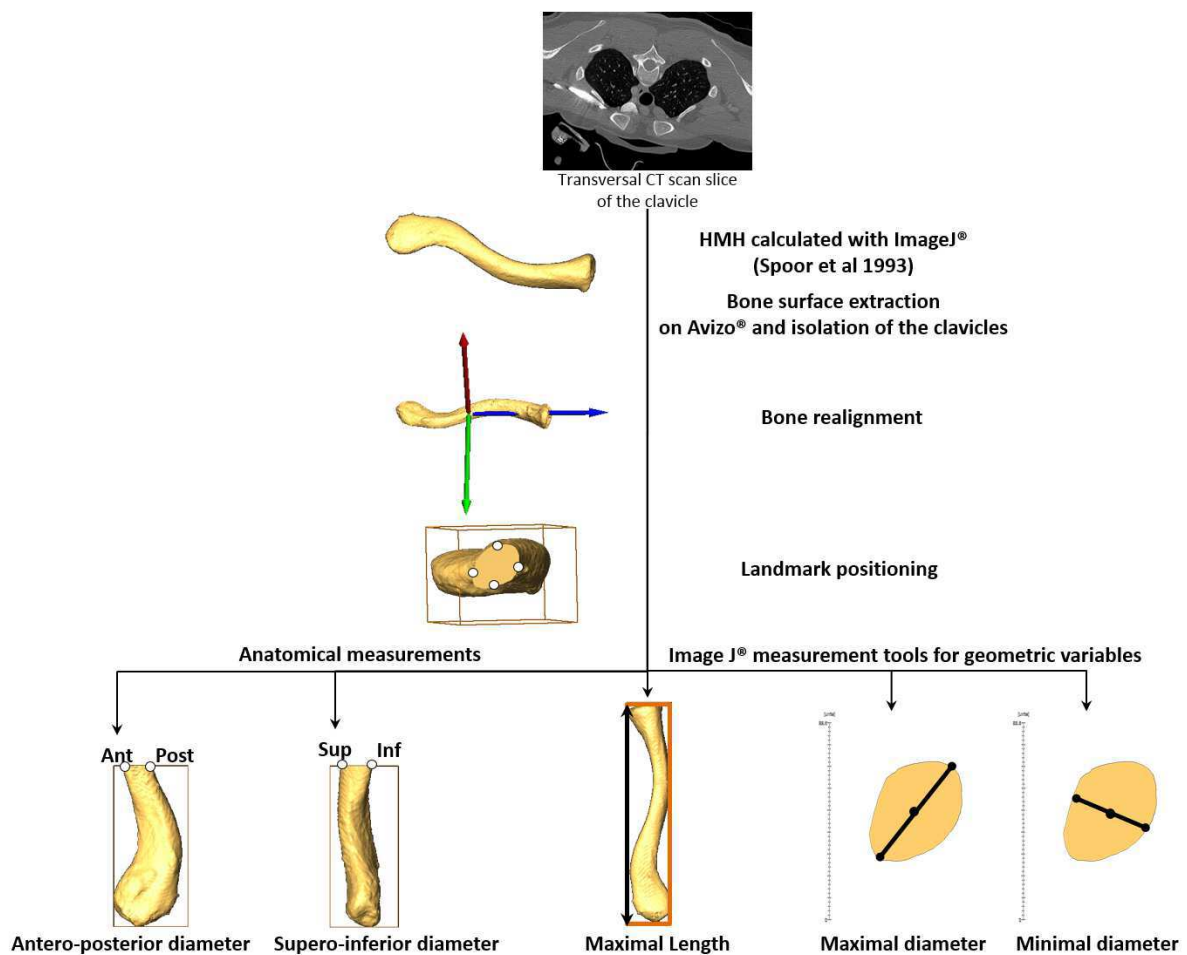


Figure 4.15 Successive steps for clavicular variable acquisition, from CT scan to three-dimensionally reconstructed bone surfaces on which all five variables are taken. Maximum length is directly measured in Avizo® using the measurement tool; Antero-posterior and supero-inferior diameters at mid-length are measured indirectly by the coordinates of four landmarks placed on the bone surfaces using Avizo® tools; Maximum and minimum diameters at mid-length are measured indirectly using measurement tools available in the Image J® Software

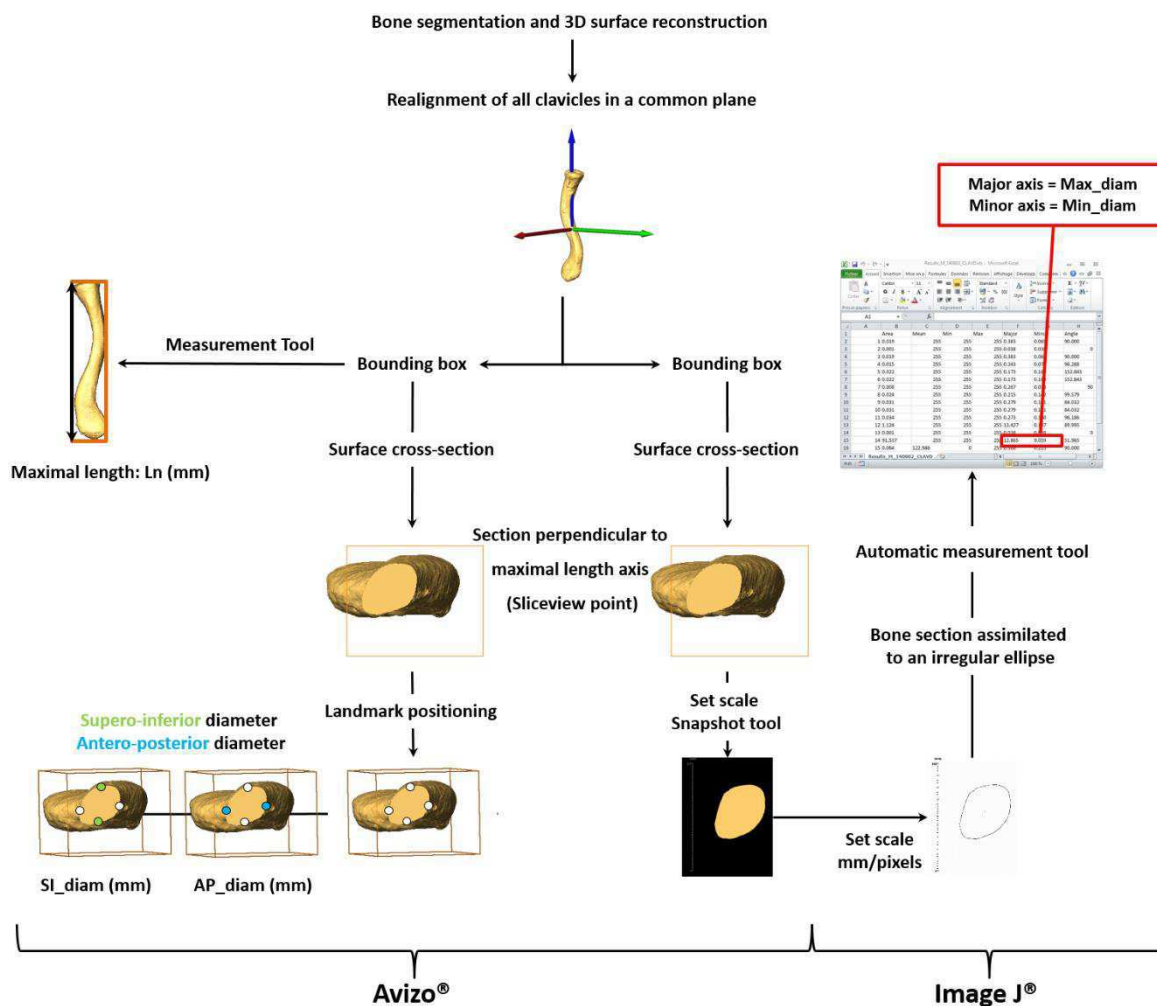


Figure 4.16 Protocol for clavicular realignment and osteometric data acquisition, showing the steps for anatomical variables (acquired using landmark coordinates or direct measurement tools in Avizo®) and geometric variables (acquired using measurement tools of the Avizo® and Image J® Softwares

We therefore have 4 landmarks each with three (x, y, z) coordinates at half-length of the clavicle using the Avizo® surface cross-section tool placed at 50% of total length on the superior (ldmk S, xS, yS, zS), inferior (ldmk I, xI, yI, zI), anterior (ldmk A, xA, yA, zA) and posterior (ldmk P, xP, yP, zP) sides of the section, in that order. The diameters are then calculated using the formula for vector length between landmarks taken two by two, giving the Euclidian distance between the two points:

$$SI\_diam = \|\vec{SI}\| = \sqrt{((xS - xI) * (xS - xI) + (yS - yI) * (yS - yI) + (zS - zI) * (zS - zI))}$$

$$AP\_diam = \|\vec{AP}\| = \sqrt{((xA - xP) * (xA - xP) + (yA - yP) * (yA - yP) + (zA - zP) * (zA - zP))}$$

The other two diameter variables (Min\_diam and Max\_diam) and maximum clavicular length are purely geometrical (as opposed to SI\_diam and AP\_diam that are anatomical variables) (Figure 4.16). Of the 540 individuals from Marseilles for whom CT scan sets were collected, 324 aged from 0 to 19 years included presented exploitable clavicles.

#### 4.2.3. Non-biometric variables: bone fusion assessment on virtual bones

The protocol for assessing acetabular maturation on 3D reconstructed bones was largely inspired by similar protocols developed to assess dry bones maturation (Cardoso and Rios 2011; Coqueugniot et al 2010). There are three main steps:

1. Reconstruct the entire iliac bone surfaces on Avizo® using semi-automatic or manual segmentation;
2. If possible, attribute maturity stages for each epiphyseal site (0, 1 or 2) and add the stages for each side (left and right) to give maturation scores;
3. In case of problematic staging, *i.e.* if choosing between two stages is difficult, it is possible to use the “Surface Cross Section” tool to construct virtual sections of the bone. It is also useful to visualize the CT scans directly, by using the “Ortho slice” tool and hiding the reconstructed bone surface (Figure 4.17). This is particularly useful, as fusion follows a centrifugal direction, and is not always clearly visible from the external part of the bone. Allowing the user to view sections of the bone parts in three different planes adds accuracy to the staging by seeing whether or not the epiphyses are fusing.

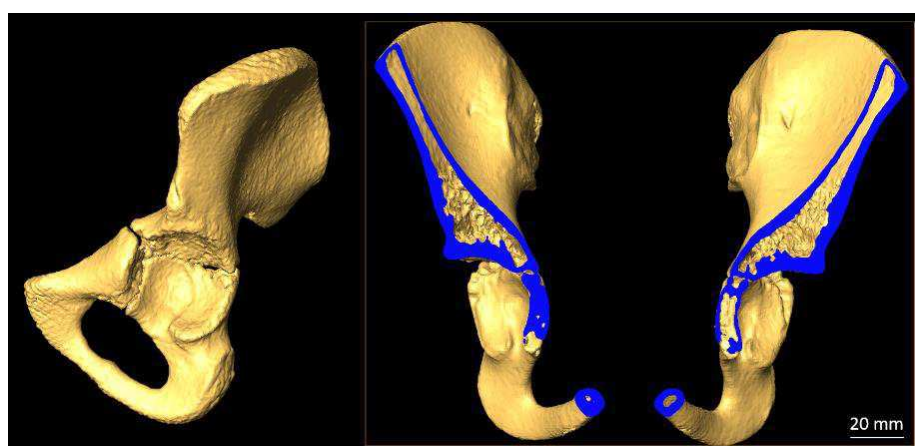


Figure 4.17 Staging assessment on a reconstructed iliac bone. The acetabular area could be either stage 0 (non-union between the epiphyses) or stage 1 (partial union between the epiphyses). Using the surface cross-section tool of Avizo®, it is possible to virtually section the bone and see if the elements are connected by bony tissue in regions that are not visible from an external view of the bone. Here, we can see that the left and right PUBIL epiphyses are connected by a small bony bridge, so the maturation stage for these epiphyses is 1

The iliac maturation study sample from Marseilles is composed of 221 of the 244 individuals included in the ilium growth study. For 21 individuals, the fusion site PUBISCH\_INF was not observable on either iliac bones, because the limits of the CT examinations did not extend to this anatomical region. For two individuals (F\_061000 and M\_180306), this same fusion site was not observable on the left innominate bones and one of these individuals (F\_061000) presented a fracture of the left ischio-pubic branch. Therefore, these 23 individuals were excluded from the maturation study.

As complete acetabular fusion was observed in literature at different ages ranging from 15 to 18 years (Baker et al 2005; Cardoso 2008b, Cardoso et al 2013a; Scheuer and Black 2000, 2004; Owings-Webb and Suchey 1985), it was decided to include individuals aged up to 19 years, to leave a one-year long margin of certainty for complete fusion. 148 older individuals aged from 13 to 19 years were therefore added to the sample in order to include the whole age range of acetabular fusion. Of these 148 individuals, maturity assessment was not possible at the left and right PUBISCH\_INF site for three of them, and at the left and right PUBIL sites for one of them. Moreover, in order to obtain a higher number of individuals and respect a homogenous age group distribution, additional children aged 0 to 12 years were included in the sample.

Over a total of 417 individuals collected, there were 392 for whom the four fusion sites were all observable. Age of these 392 individuals ranged from 0 to 19.95 years.

### **4.3. Acquisition of variables on dry bone**

Validating an anthropological method implies validating the entire protocol set up for constructing it: this includes validation of the results, but also validation of variable acquisition. Our methods are developed on virtual bone reconstructions, but aim to be used on all types of bone samples, mainly dry bone samples, which are the most common in osteoarchaeology and forensic anthropology. We are aiming for four levels of validation: validating the protocol for variable acquisition on 3D reconstructed bones, validating the application of the protocol on dry bone samples, validating the results of the models on a test sample of 3D reconstructed bones and validating the application and the results of the models and on a test sample of dry bones. For these reasons, we were careful to select standardised variables that had already been defined and/or used on both types of material in previous studies (Black and Scheuer 1996; Brough et al 2013, 2014). We also aimed to develop protocols for data acquisition with the least possible differences between virtual and dry bones, in order for them to be applicable in the largest number of cases.

### 4.3.1. Biometric variables

#### a. Iliac variables

Unidimensional variables IL and IW were measured using a digital sliding calliper, with a precision of 0.01 mm (**see above**). In the same way as its virtual counterpart, IM was calculated as the product of IL and IM.

The protocol for calculating IA on dry iliae has been developed in several previous studies (Adalian 2001; Daumas et al, *in press*; Marchal 1997) and is largely based on the work of P. Adalian (Adalian 2001). The left and right iliae of the individual were placed on a black cloth placed on a flat surface, perpendicularly to the objective of a digital camera (Canon®) that was maintained in the same vertical plane by an adjustable tripod. The camera was fitted with a macroscopic lens in order to obtain the most detailed photographs of the bone borders and epiphyseal sites. Lighting was arranged to limit shadowing. A ruler of constant size (50 mm) was placed next to the bones to serve as the reference scale in mm and photographed with every bone.

The digital photographs were then treated using Adobe Photoshop® Software to even out the background. The same protocol as the one used for obtaining IA from the image of the scanned iliae was then adopted to measure IA on these photographs of dry iliae (**Figure 4.11, steps following the “snapshot” step**).

In the Luis Lopes sample, left and/or right ilium variables were collected for 43 individuals aged 0 to 12 years. Because the sample is composed of dry bones, unavoidable taphonomic damages were observed for 14 of these 43 individuals on one or both of the iliae (**Tables 3-3 and 4-1**). For three of the fourteen individuals, only one of the four measurements was available for either the left or right bone.

3D coordinates ( $x, y, z$ ) of four landmarks placed on the ilium were directly available for the 30 individuals of Toulouse: the Anterior Superior Iliac Spine (ASIS), the Posterior Superior Iliac Spine (PSIS), the Iliac Crest Summit (ICS) and the Internal Acetabular Point (IAP). From these landmarks, the two variables IL and IW were calculated using the formula for vectorial norms (Euclidian distance between two points) and IM was then calculated as their product (**see section 4.1.1.a.**). IA was not available for these individuals.



**b. Lumbar variables**

All measurements were taken using a digital sliding calliper with a precision of 0.01 mm. the neural arch of the vertebrae was sometimes higher than PVH, which prevented us from measuring this variable with the calliper. In these cases, a supple metric ribbon was used to measure PVH, with a precision of 1mm.

**c. Clavicular variables**

Dry bone clavicular measurements used in this study were defined by R. Martin in 1957 and R. Martin and K. Saller in 1959 and 1962 and by G. Olivier in 1965. They have been used in several osteometric studies of the clavicle for age estimation (Black and Scheuer 1996; Miles and Bulman 1995; Pinhasi et al 2005).

- **Maximum length (Ln)**: this variable is taken using a sliding calliper (precision 0.01 mm) or with an osteometric board with a precision of 1mm for clavicles longer than 152 mm (maximum distance measured by the digital sliding calliper). The clavicle is placed on the board with the most lateral point of the acromial end blocked against the edge. A wooden rectangle is then placed perpendicularly to the board and used to find the extremity of the sternal end. The maximum length is read in millimetres on the graduated paper.

The other dry bone variables are taken with a digital sliding calliper (precision 0.01mm):

- **Antero-posterior diameter at half-maximum length (AP\_diam)**: the diameter between the anterior and posterior faces of the bone at half the total length of the bone. The calliper must be oriented in a way that the handle is parallel to the antero-posterior axis;

- **Supero-inferior diameter at half-maximum length (SI\_diam)**: the diameter between the superior and inferior faces of the bone at half the total length of the bone. The calliper must be oriented in a way that the handle is parallel to the supero-inferior axis;

- **Maximum diameter at half-maximum length (Max\_diam)**: the maximum diameter at half of total bone length. It is found by turning the calliper around the bone at half-length perpendicularly to the axis of total bone length until the highest value is obtained;

- **Minimum diameter at half-maximum length (Min\_diam)**: the minimum diameter at half of total bone length. It is found by turning the calliper around the bone at half-length perpendicularly to the axis of total bone length until the smallest value is obtained.

### 4.3.2. Non-biometric variables

The iliac maturation study sample from the Luis Lopes collection is composed of 85 individuals overall, aged 0 to 19 years. Taphonomic alterations were present on one, two or all three elements of the left and/or right innominate bones for 28 individuals. Overall, there are 57 individuals for whom all fusion sites were observable on both left and right iliac bones, 68 individuals for whom all fusion sites were observable on the left iliac bones and 67 individuals for whom all fusion sites were observable on the right iliac bones.

Staging was done by macroscopic observation of the five epiphyses of the three elements of the innominate bone when present: PUBISCH\_INF, ILISCH, PUBISCH\_SUP and PUBIL.

Staging, scores and combinations were obtained in the same way as for the virtual innominate bones.

### 4.3.3. Difficulties in data acquisition on dry bones

In the Luis Lopes collection, all measurements were taken directly on dry bones. In this sample, as in any archaeological or forensic osteological collection, we were confronted with relatively poor preservation rates and/or missing data because of field and laboratory taphonomy. For some individuals, the fifth lumbar vertebra was completely absent; for others, it was too altered for one or several of the measurements to be taken. This provokes a bias in the fact that some of the age groups are underrepresented in the test sample, and therefore the validity of age prediction models cannot be verified for these particular age groups. Of the 85 individuals from the Luis Lopes sample, all variables (clavicular, lumbar and iliac biometric variables and iliac non-biometric variables) were measured for only 64 of them.

**Table 4-1** Number of bones fully preserved (according to laterality) and number of missing or deteriorated bones (left and/or right) for the three bone types of the juveniles from the Luis Lopes collection. The preservation rates reflect the percentage of individuals from the collection for whom the variables (biometric and non-biometric) could be acquired

Bone	Left	Right	Left and right / Present	Number of bones missing	Preservation rates
Clavicle	14	10	36	74	0.565
L5	-	-	66	19	0.776
Ilium	6	4	33	28	0.731
Iliac bone	8	6	64	28	0.835

#### 4.4. Validation of data acquisition methods

All the following statistical analyses were done using the R Software® version 3.1.0 (2014-04-10) or with Microsoft® Excel® 2013 (version 15.0.4701.1001). Unless specified otherwise, all statistical tests were chosen following the work of biostatistician H. Motulsky (1995).

##### 4.4.1. Testing scanned bone/dry bone variable consistency

Six series of five variables (Ln, AP\_diam, SI\_diam, Max\_diam, Min\_diam) were measured on 40 clavicles: three were done directly on the dry bones and three on the virtual reconstructed bone surfaces of these dry bones. Variables were measured using the protocols detailed in **section 3.3.** by two observers (twice by a first observer and once by a second observer).

As all measurements were taken on the same bones, each set of variables obtained by one observer is paired with the other two sets of variables taken by the same observer and the second observer.

##### a. Consistency between variables taken on dry and scanned bone

Consistency between dry bone variables and scanned bone variables was assessed for each variable by comparing the dry bone variable to its scanned counterpart, two by two. All variables were tested for normality using a Shapiro-Wilk test ( $p > 0.05$ ). Variable consistency was then tested with a paired Student t-test if they follow a normal distribution, or with a signed-rank Wilcoxon test if they do not follow a normal distribution ( $p > 0.05$  for both tests).

##### b. Equality of geometric and anatomical variables taken on dry and scanned bone

When faced with the problem of bone orientation and position on a common plane, the idea came to mind to see whether geometrical measurements of the clavicular diameters were easier to measure than anatomical diameter measurements and could therefore be used instead of them in biometric studies of the clavicle. If this hypothesis were validated, it would be an advantage for measurement reliability on scanned bones as geometrical variables are obtained quasi-automatically and therefore user bias would be decreased dramatically. Moreover, and for the same reasons, variable acquisition on scanned bones would be greatly facilitated and accelerated. We therefore decided to test the equality between geometrical and anatomical variables two by two: AP\_diam was compared to Max\_diam and Min\_diam, and SI\_diam was compared to Max\_diam and Min\_diam

using paired Student t-tests if variables follow a normal distribution, or with a signed-rank Wilcoxon test if they do not follow a normal distribution ( $p > 0.05$  for both tests).

#### **4.4.2. Repeatability and reproducibility of the variables**

Tests for intra- and inter-observer errors were undertaken for both landmark positioning and variables. Indeed, as the landmarks are used to calculate the variables, their positioning needs to be reliable, repeatable and reproducible (Bookstein 1991; Richtsmeier et al 1995; Sholts et al 2011; Slice et al 2004; Utermohle and Zegura 1981). If landmark positioning shows sufficiently low intra- and inter-observer errors, it is safe to say that the variables also will. On the other hand, if landmark positioning does not show satisfactory repeatability or reproducibility, the same problem might not necessarily be found for the variables.

If one observer places two landmarks at a distance  $D$  from one another, a second observer can very well position the homologous landmarks at the same distance  $D$  from each other, but they can have different positions from the exact positions of the first set of landmarks placed by observer one.

A typical example of this is vector translation: the norm of a translated vector  $AB$  is the same as the norm of the original one  $AB$ , however the coordinates of the homologous points ( $A$  and  $A'$ ;  $B$  and  $B'$ ) are different.

Intra- and inter-observer error testing is not only important for validating measurement protocol (Jamison and Zegura 1974), it also determines whether or not forward regression models can be used for age prediction. Indeed, if the errors are significant, calibration or Bayesian approaches need to be used instead of forward regressions. Therefore, it is crucial to verify that the variables can be obtained with sufficient repeatability and reproducibility.

##### **a. Landmark positioning**

First, reliability of landmark positioning is assessed with intra- and inter-observer tests for repeatability and reproducibility. The distances between two repeated and reproduced homologous landmarks are calculated with the formula of vector length using landmark coordinates (**see above**). These distances between homologous landmarks are then tested to see if their distribution is normal using a Shapiro-Wilk test ( $p > 0.05$ ). Distances are then compared to fixed values of 0 mm (no difference between homologous landmark positions) and 1 mm (maximum distance allowed between homologous landmarks) with a paired Student t-test if they follow a normal distribution, or

with a signed-rank Wilcoxon test if they do not follow a normal distribution ( $p > 0.05$  for both tests) (McCrum-Gardner 2008).

To test repeatability and reproducibility of the four clavicular landmarks, two series of tests were undertaken:

- On the scanned dry clavicles, repeatability and reproducibility of the four landmarks were tested on all 40 bones twice by the same observer and once by an independent observer trained on Avizo®;
- On the clavicles collected from the hospitals, repeatability and reproducibility were tested on 40 different randomly picked individuals of the Marseilles sample, of both sexes and all age groups, twice by the same observer and once by an independent observer trained on Avizo®.

Repeatability and reproducibility of the eight lumbar landmarks were tested by placing all landmarks on the fifth lumbar vertebrae of 40 different randomly picked individuals of the Marseilles sample, of both sexes and all age groups, twice by the same observer and once by an independent observer trained on Avizo®.

Repeatability and reproducibility of the four iliac landmarks were tested by placing all landmarks on the iliae of 30 different randomly picked individuals of the Marseilles sample, of both sexes and all age groups, twice by the same observer and once by an independent observer trained on Avizo®.

## **b. Biometric variables: measurements**

All biometric variables present a latent error, resulting from the technical error of measurement, the error linked to the medium of measurement (calliper, osteometric board, digital measurement tool, etc). This latent error must be evaluated (Adalian 2001). For this reason, repeatability and reproducibility of the variables measured on the bones were tested. Repeatability and reproducibility of the variables were tested using intra-class correlation coefficients (Koch 1982), Bland-Altman plots (Bland and Altman 1986), and by evaluating the Technical Error of Measurement (TEM) (Goto and Nicholas Mascie-Taylor 2007; Stull et al 2014b; Ulijaszek and Kerr 1999). The mean and standard deviation of the differences between each set of variables were also calculated.

Intra-class correlation (ICC) is used to assess consistency or agreement of paired quantitative variables made by the same observer or by different observers respectively. It was first presented by R. Fisher (1925) as a statistical evaluation of rating reliability. The data (variables measured by the observers) are pooled to estimate the mean and the variance of each variable. ICC gives a composite

of intra-observer and inter-observer variability by providing agreement and consistency coefficients to see if the variable can be measured by any observer without significant error. However, finding a high correlation between paired variables does not automatically imply that there is good agreement between observers as a high correlation between similar variables is expected to be found.

Bland-Altman plots (Bland and Altman 1986) are a way to visualise observer errors by plotting the differences between the paired variables ( $d_i = x_i - x_j$ ) against the mean values of each pair of variables ( $\bar{x} = \frac{(x_i + x_j)}{2}$ ). M. Bland and D. Altman pointed out that that any two methods that are designed to measure the same parameter (or property) should have a good correlation when a set of samples are chosen such that the property to be determined varies considerably. A high correlation for any two methods designed to measure the same property could thus in itself just be a sign that one has chosen a widespread sample. However, a high correlation does not automatically imply that there is good agreement between the two methods. Bland-Altman plots are extensively used to evaluate the agreement among two different instruments or two measurements techniques. They allow the investigation of the existence of any systematic difference between the measurements (*i.e.* fixed bias) and to identify possible outliers. The limits of the acceptable error rate are represented by 95% confidence intervals, for a 5% error rate. If more than 90% of the points are included within the limits of the confidence interval, observer errors are accepted as sufficiently low for validation.

The Technical Error of Measurement (TEM) is a statistical parameter used to measure imprecision of variables (Mueller and Martorell 1988; Ulijaszek and Kerr 1999). It is the square root of measurement error variance. It is obtained by calculating the difference ( $d_i$ ) between  $N$  repeated measurements of the same variable taken by the same observer or by different observers, to calculate the value of the intra- and inter-observer errors respectively.

$$\text{TEM} = \sqrt{(\sum d_i^2)/2N}$$

The size of TEM has been found to be positively associated with the size of the measurement, meaning large values of variables are associated with high TEM and vice versa (Ross et al 1994). For this reason, and to be able to compare imprecision between different variables and different populations, Norton and Olds (1996) propose converting absolute TEM into relative TEM, or %TEM.

$$\% \text{TEM} = (\text{TEM} / \text{mean } (\bar{x})) \times 100$$

%TEM with values inferior to 5% are considered acceptable measurement repeatability and reproducibility.

The maximum difference tolerated for both landmark positioning and variable repeatability and reproducibility was one millimetre, i.e. a threshold confidence interval of [-1 ; +1] mm for the inter-landmark distances and the maximum difference between two series of variables.

**c. Non-biometric variables: stages and scores**

Maturation stages are considered ordinal variables, as only three values are possible: 0, 1 or 2. The same goes for scores, which can take any whole number between 0 and 8. Intra and inter-observer error for this type of variable were evaluated by Cohen’s Kappa coefficient (Cohen 1960). Cohen’s kappa coefficient measures inter-rater agreement rates for N qualitative variables classified into m exclusive modalities. It is more robust than “simple correlation coefficients” or percentages of agreement because it considers agreement due to chance, *i.e.* that observers could attribute random modalities to one or several observations.

J. Landis and G. Koch (1977) have established a table for the correspondence between the values of Cohen’s Kappa coefficient and the quality of observer agreement (**Table 4-2**).

**Table 4-2 Values of Cohen’s Kappa coefficient and their corresponding agreement rates, for assessing intra-observer consistency and inter-observer agreement between two sets of ordinal variables (e.g. stages)**

Cohen’s Kappa coefficient	Strength of agreement
< 0.00	Poor
0.00-0.20	Slight
0.21-0.40	Fair
0.41-0.6	Moderate
0.61-0.8	Substantial
0.81-1.00	Almost Perfect

Although the authors themselves have stated that the divisions were arbitrary, this table is referred to in a great number of studies that use qualitative variables. In this study, we will only consider an almost perfect strength of observer agreement as acceptable.

## 4.5. Descriptive statistics of the samples

### 4.5.1. Age and sex ratios of the different samples

Tests were undertaken to verify sample homogeneity for age and sex in all three samples, *i.e.* to test if age and sex ratios were respected or not in the different samples. This was done using a chi-squared test of independence on the number of individuals present in each annual age group, for sexes combined and for males and females separately. The chi-square test of independence is used to compare the proportions of one nominal variable to those of the other nominal variable (McDonald 2007). The null hypothesis was to consider that the number of individuals per age group was comparable, with both sexes pooled and between sexed age groups ( $p < 0.05$ ).

The Marseilles sample and the Luis Lopes sample comprised 13 age groups for the ilium (from 0 to 12 years), and 20 age groups for the clavicle, the fifth lumbar vertebra and the *innominate bone* (from 0 to 19 years).

Individuals from the Toulouse sample were categorised into 13 age groups for the ilium (from 0 to 12 years).

### 4.5.2. Non-biometric variables: iliac maturation stages

Descriptive statistics of the qualitative variables taken on the iliac bone consisted in calculating the mean age and standard deviation, and indicating the minimum, and maximum ages for each maturation stage (from 0 to 2). Frequencies of each stage per annual age group (from 0 to 19 years) were also calculated. These statistics were done for the Marseilles and Luis Lopes samples.

### 4.5.3. Biometric variables: iliac, lumbar and clavicular variables

Mean and variance were calculated for each biometric variable per annual age group, for combined and separate sexes. This was done for the variables measured for the Marseilles sample, the Luis Lopes sample and the Toulouse sample. Reference collections for which biological parameters and context are known, as is the case for these three samples, allow the comparison of the composition or developmental patterns of samples by testing the homogeneity and evaluating the differences between variables in these different samples (Pinhasi et al 2005; Rissech et al 2013a). This was also done in this study using analysis of covariance (ANCOVA) for all variables obtained for individuals of at least two or of all three samples. This method consists in evaluating the degree of



influence of several independent categorical factors (in our case, age and sample of origin) on a dependent variable (biometric data). The results indicate the part of variance of the variables explained by each independent factor and indicate whether samples are homogeneous or not.

## **4.6. Interactions between the variables, age, and biological factors**

### **4.6.1. Relationship between age and the variables**

Pearson and Spearman correlation coefficients were calculated between age and each of the quantitative variables of all three bones to evaluate the strength of the relation between them. High Pearson correlation coefficients imply a possible linear relation between age and the variables that could in theory be modelled using a linear regression (**see section 4.7.**) (McCrum-Gardner 2008; McDonald 2007).

Spearman's ranked correlation coefficient is a non-parametric measure of statistical dependence between two quantitative or qualitative variables without presuming any kind of relation between them. A high Spearman correlation coefficient indicates a monotonous relationship between the variables (McDonald 2007). Pearson and Spearman correlation coefficients were calculated between age and all biometric variables and Spearman correlation coefficients were calculated between age and the ordinal variables (maturation stages) of the iliac bone.

### **4.6.2. Influence of other biological factors: asymmetry, sex, variable collinearity**

#### **a. Bilateral asymmetry**

Bilateral asymmetry between left and right bones was tested on the clavicular and iliac biometric variables with a paired Welsh t-test ( $p < 0.05$ ), to establish whether or not the individuals presented asymmetry in growth and whether or not models could be built for left or right bones separately, or if they could be constructed similarly for variables taken on left and right bones (McCrum-Gardner 2008; McDonald 2007).

Bilateral asymmetry between the maturation stages of left and right iliac bones was tested using the non-parametric permutation test. This test is used to compare the distribution of ordinal variables (maturation stages) in paired samples, when the intervals between the variables are comparable. Here, the interval between one stage and the next is always one (0 to 1 to 2). The test

only considers the non-null differences between paired variables (*i.e.* the individuals for whom there are differences between right and left stages). The ranks of these differences are ordered according to their range. The pairs are then randomly flipped: the right maturation stage of random individuals is switched with the corresponding left one, to give a permutation distribution. The observed t-statistic is compared to the distribution of the t-statistic when the pairs are flipped at random. If the observed statistic is extreme relative to this permutation distribution, then the null hypothesis of equality between distributions is rejected. The null hypothesis is that there is no difference in stages depending on laterality, *i.e.* the two samples are identically distributed. The level of significance was fixed at  $p > 0.05$  (McCrum-Gardner 2008; McDonald 2007).

### **b. Sexual dimorphism**

All biometric variables were tested for sexual dimorphism to establish if any differences in growth could be related to the sex of the individual and if age prediction models could be built for sexes pooled, or needed to be specific to each sex. This was done using a two-sample t-test or, in case of non-normality of the data, a Wilcoxon test ( $p < 0.05$ ). The null hypothesis is that there is no significant difference between the variables according to sex. This test was done on the whole sample (all ages combined) and in each annual age group, as the presence of sexual dimorphism is related to age (McCrum-Gardner 2008; McDonald 2007).

Sexual dimorphism of maturation stages was tested using a non-parametric Mann-Whitney-Wilcoxon test for independent samples (McCrum-Gardner 2008; McDonald 2007). This test compares the ranks of the variables and can be used to compare two samples of different size. This is particularly interesting in anthropology as it is relatively rare to compare two samples of identical size (for example, when there is more males than females) and data is often missing (for example, one or several maturation sites could not be staged for all individuals). This test was also done on the whole sample and for each annual age group ( $p > 0.05$ ).

## 4.7. Modelling the relationship of biological variables with age

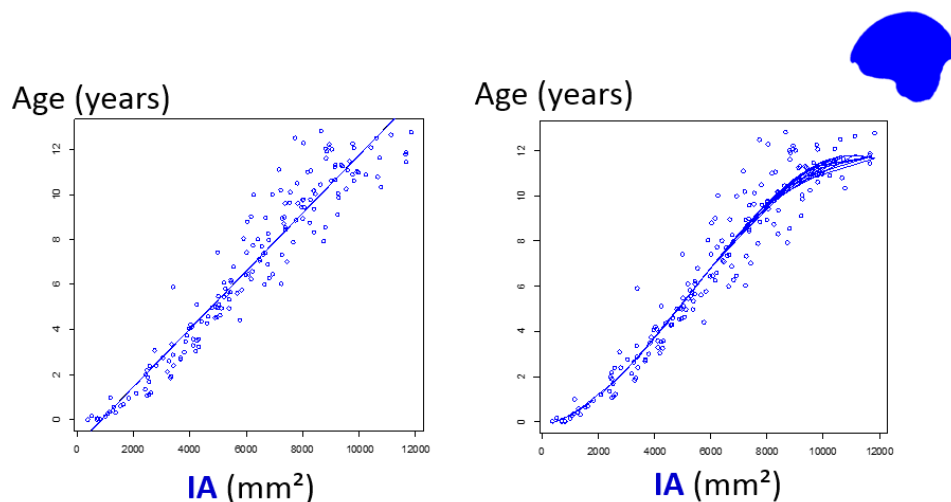
### 4.7.1. Parametric regression models

#### a. Univariate linear regression

A regression study aims to find the mathematical model that provides the best adjustment to the scatterplot representing a relationship between two variables, one dependent or predicted, and one independent or predictive (**Figure 4.18**). The regression has a double role: to describe the relationship between the two variables and to estimate the predicted variable using a predictor (Bland and Altman 1986). In case of a linear regression, the relationship is modelled by a linear function with a mathematical expression as follows:

$$y_i = a \cdot x_i + i + e_i,$$

Where  $y_i$  is the predicted variable,  $x_i$  is the predictor variable,  $a$  is the regression coefficient,  $i$  is the intercept and  $e_i$  is the error (McDonald 2007; Motulsky 1995).



**Figure 4.18** Examples of linear (left) and third degree polynomial (right) regression equations of age predicted by IA on the Marseilles sample

#### b. Polynomial regression

If the relationship between age and the variables seems to be curvilinear, a polynomial regression must be tested. The degree of the polynomial will depend on the number of inflection points detected on the curve  $n$ , such as  $\text{degree} = n + 1$ . If the relationship presents  $n$  inflection points, the model will be an  $n + 1$  degree polynomial with the mathematical expression as follows:

$$y_i = a \cdot x_i + b \cdot x_i^2 + c \cdot x_i^3 + \dots + n \cdot x_i^n + i + e_i,$$

Where  $y_i$  is the predicted variable,  $x_i$  is the predictor variable,  $a$  is the regression coefficient of the first degree term,  $b$  is the coefficient of the second degree term, etc.,  $i$  is the intercept and  $e_i$  is the error term of the equation (McDonald 2007; Motulsky 1995).

#### **4.7.2. Selective criteria for parametric regression models**

##### **a. Regression coefficients and tests**

The determination coefficient ( $R^2$ ) of the regression is the squared correlation coefficient between the predicted and predictor variables. It expresses the proportion of variation of the predicted variable explained by the regression model using the predictor variable. A strong linear relationship between variables provides a linear regression with a high determination coefficient.

For polynomial models that present several times the predictor variable with different degrees,  $R^2$  represents the correlation between the predicted variable and the predictor variable, and the different degrees of the predictor variable.  $R^2$  tends to overestimate the correlation between variables. This is why the adjusted  $R^2$  is used, as it considers the number of variables in the model and the size of the sample (McDonald 2007; Motulsky 1995).

To construct valid models, all regression parameters must be tested for statistical significance. This means that the regression coefficients (slope and intercept) are tested to check if they are significantly different from zero, using the F statistic for the slope coefficient and the t statistic for the intercept. Coefficients are significantly different from zero if the associated p-values are  $< 0.05$ . In that case, the significant coefficients are used in the regression that is considered as a valid model of the relationship between the variables involved (McDonald 2007; Motulsky 1995).

##### **b. Residuals: normality, homoscedasticity and independence**

The difference between a predicted value  $y$  and an observed value  $y'$  is called a residual. Residuals are estimates of true error. So in theory, a good model should have small residuals. This is why parametric regressions are constructed using “least squares” methods, *i.e.* they estimate coefficients and provide models with the smallest values of the sum of squared residuals.

The standard error of estimation, or Root Mean Square Error (RMSE), estimates residual dispersion around the regression. It is an indication of the standard deviation of the predicted variable. The smaller the RMSE, the better the predictive model (McDonald 2007; Motulsky 1995).

Coefficient estimation does not require residual normality. However, this condition is required to run verification tests and obtain confidence intervals for the coefficients. Therefore, residuals must respect three conditions (McDonald 2007; Motulsky 1995):

- a normal distribution, with a mean equal to zero;
- constant variance, *i.e.* homoscedasticity;
- independence, *i.e.* no auto-correlation.

Normality of the residuals can be verified graphically and statistically (**Figure 4.19**).

A graph showing a histogram of residual frequency superposed to the theoretical normal distribution of the residuals with a mean of zero and a standard deviation equal to one can be used to see if the two distributions are comparable.

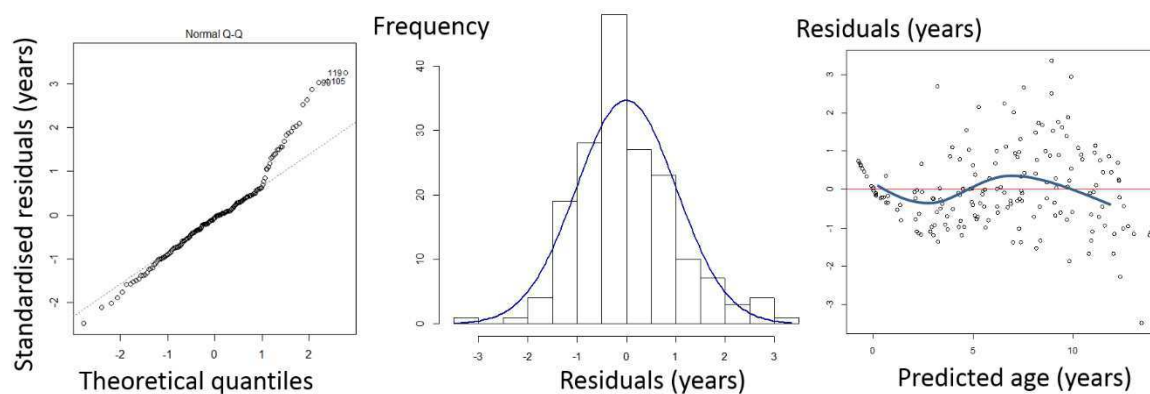
The distribution of the theoretical against observed cumulative quantiles of the residuals (QQplot) is used to see whether the two distributions are similar or not. Residuals are normal if the points follow the theoretical line.

It is also possible to test residual normality using the Shapiro-Wilk or Kolmogorov-Smirnov tests. Residuals are considered to follow a normal distribution if the statistic of the tests is high and the associated p-value is  $> 0.05$ .

Residual homoscedasticity is a crucial condition for prediction models, as it determines whether or not valid prediction intervals can be constructed for the model. Residual homoscedasticity can be assessed graphically and statistically (**Figure 4.19**). Heteroscedastic residuals mean that the standard errors associated to the estimates are incorrect; therefore statistical inference (*i.e.* constructing prediction intervals) is invalid.

Residuals (or standardised residuals, *i.e.* residuals divided by their estimated standard deviation) are plotted against the predicted variable  $y$ . If the model is valid, residuals will have a mean equal to zero and a standard error of one. 95% of them will be distributed between -1.96 and +1.96 on the graph. Moreover, their distribution will be random and homogenous, without following any kind of pattern. Homoscedasticity can be verified using the Breusch-Pagan test (Chatterjee et al 2006). The p-value associated to the BP statistic must be  $> 0.05$  for residuals to be homoscedastic.

Independence of the residuals is tested using the autocorrelation test of Durbin-Watson (Chatterjee et al 2006). The DW statistic always lies between 0 and 4. A DW value close to 2 indicates no autocorrelation ( $p > 0.05$ ).



**Figure 4.19** Graphical testing for the normal distribution of regression residuals (left and middle plots) and residual homoscedasticity (right plot). The residual quantiles do not follow the theoretical line (left plot), but follow their theoretical normal distribution represented by the blue curve (middle plot); however, they are heteroscedastic as they follow a horn-like distribution when plotted against predicted age: their variation is not constant and increases with age

When the Durbin-Watson test was not applicable (as was the case with one type of model), residual autocorrelation was diagnosed using the autocorrelation function (acf). Sample autocorrelation coefficients measure the correlation between observations at different times. The set of autocorrelation coefficients arranged as a function of separation in time (or lag) is the sample autocorrelation function, or the acf. Acf is a time series model constructed to capture the patterns in the data. Autocorrelation is signalled by significant spikes of the acf plot at different lag values. If no particular pattern is found and the residuals are within a 95% range of significance, there is no autocorrelation.

### c. 95% prediction intervals and precision

Prediction intervals (PIs) of estimated values are especially important for age estimation. The predicted value is the mean age corresponding to the value of the predictor, but a maximum and a minimum age are also possible for the same predictor value. PIs provide a range for the estimated values of age: a minimum, a maximum and a mean value, with a percentage of confidence that is decided beforehand. To construct valid and reliable age prediction models, a prediction interval must be indicated with the estimated age. Prediction intervals are typically fixed at 95%, which means that the estimated age is given with an associated range of possible ages covering 95% of the variability. The minimum and maximum ages are calculated by respectively subtracting or adding the Mean Absolute Error (MAE) obtained with the regression model to the mean estimated age multiplied by 1.96 (the statistic associated with an error of 0.05). This value,  $\pm 1.96 \cdot \text{MAE}$  is the precision of the estimation model. For example, the formula of P. Adalian (2001) for predicting age in fetuses using

the ilium area is age (in Gestational Weeks or GW) =  $0.0322 \times \text{Ilium area (mm}^2) + 18.244$  with a precision of  $\pm 7$  GW, so the prediction interval has a size of 14 GW.

### **4.7.3. Validation of regression models**

#### **a. Cross-validation**

The predicted values  $y$  returned by the models are those corresponding to the individual used to build the regressions. The accuracy of prediction, i.e. the proportion of individuals for whom  $y$  was correctly predicted is therefore overrated. To best reflect the correct accuracy of prediction, it is possible to construct the models using cross-validation. The principle of cross-validation is “leave-one-out”, i.e. excluding a random individual from the others, fitting a model on all the other individuals and testing it on that same individual (Adalian 2001; Milborrow 2014; Shirley 2009). This is done as many times as there are individuals, with each individual being successively left-out. The result is a “mean” predictive model, which gains in reliability, as it takes into account inter-individual variation.

#### **b. Separate independent samples**

Another method for model validation consists in constructing all the predictive models on a subset of the original sample, called the training sample, and testing it on the second independent subset, called the test sample. This allows the evaluation of the predictive power of a model obtained in one go on a “static” dataset. It is a good way to establish whether or not the model overfits the training sample, i.e. whether or not the model only provides good predictions for the sample on which it was built.

It is customary to select around 70% of the whole sample as the training sample, and the remaining 30% as the validation sample. Individuals can be selected randomly for both samples. However, the risk of this is that the age and sex ratios of the training sample will not be respected, and this could bias the resulting predictive model in favour of the overrepresented age groups, and could also provide a model too specific to the sample’s age and/or sex distribution (Gowland and Chamberlain 2002). For this reason, we were careful to check that the number of individuals of similar ages and of both sexes were comparable between and within age groups in the training sample, using a Chi-squared test ( $p > 0.05$ ).

#### 4.7.4. Collinearity and Multicollinearity

Two variables are collinear if there is a linear association between them. Multicollinearity refers to the same phenomenon for more than two variables. Because the bidimensional data used in this study is a product of two unidimensional variables, the presence of multicollinearity and collinearity seems unavoidable. Collinearity and multicollinearity of the variables measured for each bone need to be tested to evaluate whether or not multiple regression models could be constructed.

Multiple regression models are models using two or more variables as predictors. Multiple regression models are lines in a multidimensional plane and take on the following mathematical expression:

$$y = a*x + b*w + \dots + n*z + e,$$

Where a, b and n are the slopes or coefficients of the line according to the axes x, w and z respectively and e is the intercept.

Constructing multiple prediction models with linearly correlated predictor variables can affect the validity of the coefficient estimators of the model. They can become incoherent and variables which in theory are relatively important in their relationship with the predicted variable, can seem insignificant and therefore be wrongfully eliminated from the model. Results are very unstable, as a slight modification of the data can lead to an important modification of the estimated parameters (slopes and constant) of the model.

Although the model can be valid as a whole, the parameters of each predictor considered individually might not be. Student's t-test that is used to test the non-nullity of the parameters of the models gives biased p-values, so this cannot be verified with significant certainty.

Moreover, high collinearity between predictor variables tends to cause similarity between all multiple regression models adjusted to the same data, making it difficult to decide which predictor variable is better than other(s) (O'Brien 2007). The presence of Multicollinearity between all biometric variables was tested using the Variance Inflation Factor (VIF).

$$VIF = \frac{1}{\text{tolerance}}$$

With tolerance =  $1 - R^2_j$ , and where  $R^2_j$  is the coefficient of determination of a regression of variable  $j$  on all other variables.

A VIF higher than 10 indicates the presence of multicollinearity (O'Brien 2007).



#### 4.7.5. Heteroscedasticity

Heteroscedasticity is the opposite of homoscedasticity and means that sub-sets of random variables do not have the same variability. Heteroscedastic residuals do not have a constant variance, their distribution does not follow a random pattern and they are not all included in a 95% confidence distribution interval (**Figure 4.19**).

Residual heteroscedasticity is often a given when using parametric regressions as mathematical models of biological phenomena, and for age-related variables in particular. The variance of biometric variables for very young individuals is different and much lower than that for older individuals. Residual heteroscedasticity is direct mathematical proof of the increase of inter-individual variability as age increases. In terms of modelling and prediction, this phenomenon translates into a decrease in prediction accuracy as the predicted variable increases (Palm 1994), prevents the calculation of valid coefficients for the regression and their confidence intervals (slope and constant coefficients) and questions the results of all parametric statistical tests (t-test, etc.) applied to the regression parameters.

Furthermore, because of heteroscedasticity, the constant prediction intervals (PIs) of age that are usually constructed as “mean” prediction intervals with these models to serve as estimate range are invalid, as they do not encompass the totality of sample variability. This results in overestimation of smaller variables and underestimation of higher variables. Consequently, they are not applicable for either growth or age estimation models.

Several solutions are possible for dealing with heteroscedasticity:

- Variable **normalisation**: the predictive variable is normalised by subtracting the mean of the variable to all the values of the variable and dividing each resulting value by the variable standard

$$\text{error: } V' = \frac{(V - \mu)}{\sigma}$$

With  $\mu$  the mean and  $\sigma$  the standard error of variable  $V$ . The regression models are then constructed with  $V'$  as the predictor variable.

- Other variable **transformations**: this method is used to modify the variables so that their relationship can be modelled by a parametric regression and provide valid statistical parameters. Several transformations can be attempted (**Table 4-3**) on both the predicted variable and/or the predictors. A particular set of transformations can be attempted to “linearise” the relationship between age and the variables, so it can be modelled with a linear parametric regression.

Table 4-3 Possible variable transformations of the predicted (Y) and /or predictor (X) variables to reduce heteroscedasticity of the regression residuals. The last two transformation pairs aim to linearise the relationship between X and Y

Predicted variable Y transformation	Predictor variable X transformation
Y	Ln(X)
Log(Y)	1/X
Ln(Y)	X
$\sqrt{Y}$	X
1/Y	X

- **Weighted Least Squares (WLS)** method: classically, parametric regression models are constructed using the Ordinary Least Squares (OLS) method, which is a particular Generalised Least Squares model. OLS is used to estimate the parameters in a linear regression model, with the goal of minimising the errors (residuals). OLS is the maximum likelihood estimator of the relationship between the predicted and predictive variables. To be valid, OLS requires that all the conditions mentioned previously (see section 4.7.2) be met. If that is not the case, a Weighted Least Squares (WLS) approach can be attempted. WLS creates a regression model by providing a different weight to the variables following their variance. The goal of WLS is to homogenise variance to obtain homoscedastic residuals and be able to construct valid models and PIs (Palm 1994).

A way to provide weighted least squares is to directly transform the predictor variables by providing different weights to the predictor variables, according to their changes in variance. This can be done in three ways:

1. The first consists in observing the graph of the residuals of the parametric regression plotted against the predictor variable X and visually identifying changes of pattern in the scatterplot. Particular patterns corresponding to different subgroup of predictor variables with homogenous variances are identified. The variance of the corresponding groups of predicted variables is then calculated in each group and will be used as the weight of the variables in new weighted regressions.
2. The second consists in observing the plot of the predicted variable against the predictor variable and visually detecting changes in the relationship. These changes are chosen to be cut-points and define different homogenous sub-groups, for which the variance of the predicted variable will be calculated and used as the weight in new weighted regressions.
3. The third consists in identifying which variances calculated for each age group of the training sample have close values and can be considered as homogenous subgroups. The variances of the corresponding predicted variables of each subgroup are calculated and used to construct weighted regressions.

- **Matrix of variance-covariance robust to heteroscedasticity:** this method aims to correct the standard errors of the regression coefficients and make them robust against heteroscedasticity. With this correction, the standard errors, confidence intervals and the p-values associated with the regression coefficients are more accurate and valid. However, this correction only validates the coefficients and their confidence intervals and does not correct residual heteroscedasticity. It is therefore not useful for prediction purposes, as it does not allow the construction of valid PIs. The function HC3 of the R® Package “sandwich” is used on the parametric regressions and provides corrected valid coefficients for said regressions.

When all else fails, if residuals are still heteroscedastic, do not follow a normal distribution and/or are auto-correlated, the ultimate solution is to turn to non-parametric regressions, such as Multi-variate Adaptive Regression Splines (MARS) models.

#### 4.7.6. Multi-variate Adaptive Regression Splines (MARS) models

##### a. Principles of MARS models

The constraints associated with the presence of heteroscedasticity using parametric predictive models were encountered by K. Stull and collaborators (Stull et al 2014a) when modelling regressions of age against long bone lengths for age prediction of juveniles aged 0 to 12 years. The authors used Multi-variate Adaptive Regression Splines (MARS) of the R Software® Earth Package to bypass these constraints and obtain valid prediction intervals. These models were therefore tested on our data. MARS provides non-parametric regression models, without assuming a linear relationship between the variables. These models incorporate several types of functions (linear, but not exclusively) (Friedman 1991; Hastie et al 2009). MARS identifies a “mean” function modelling the relationship between  $y$  (age) and  $x$  (variable(s)) using piecewise linear basis functions. Construction of the models is based on a forward pass, returning a set of basis functions, for each predictor variable, called the basis matrix, followed by a pruning pass that finds the subset of terms (functions) of the basis matrix with the “best” model parameters (listed below). The resulting function is built around “hinge” points, *i.e.* variable values after which the slope of the regression changes (Friedman 1991; Hastie et al 2009).

MARS subdivides the predictor into several regions, limited by particular values of  $x$  called  $h_i$ , the knots of the function (**Figure 4.20**).

The knots represent changes in the slope of the transition between polynomials. The retained knots are the ones that provide the best fit. The coefficients of the final function are estimated by minimizing the Residual Sum of Squares (RSS). The resulting function takes the form of a constant and a hinge function or a combination of hinge functions, with an expression as follows:

$$F(x) = c + a_1*(x-h_1) + a_2*(h_2-x) + \dots + a_n*(x-h_n), \text{ with } \begin{cases} (x - h_i)_+ = x - h_i, & 0 \text{ if } x < h_i \text{ otherwise} \\ (h_i - x)_+ = h_i - x, & 0 \text{ if } x > h_i \text{ otherwise} \end{cases}$$

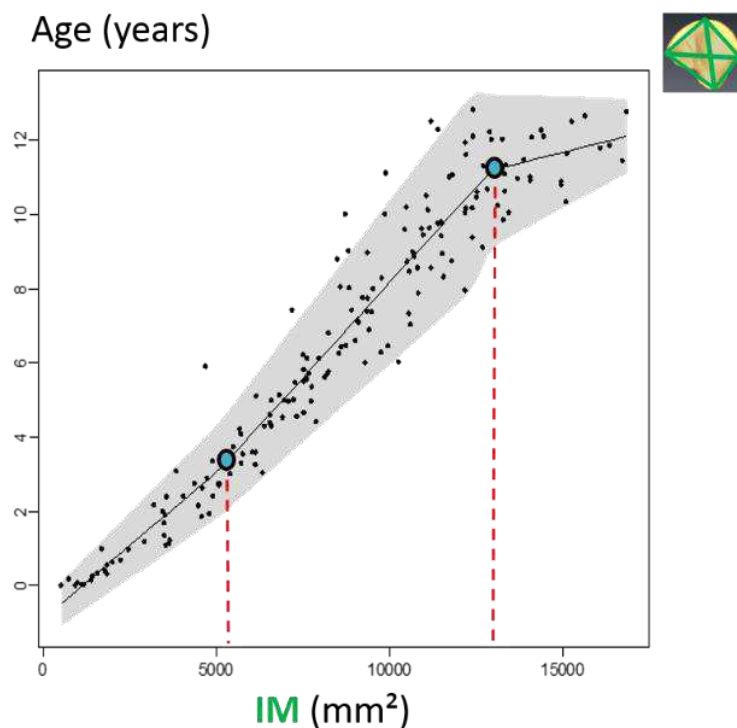


Figure 4.20 Example of a MARS model predicting age with the Ilium Module (IM). The function has detected two hinge points or knots (blue dots) where the slope of the curve changes, corresponding to two particular values of IM. This results in a hinged function with three functional sub-expressions: one before the first knot one between the two knots and one after the second knot

The contents of the parentheses cannot be negative.

For example, if we are looking to model the relationship between two variables y and x, and the algorithm detects two hinge points at x = 2 and x = 10, the resulting MARS function will have three different expressions depending on the value of x:

$$\begin{aligned} \text{If } x < 2, & \quad y = c + 0 + a_2*(10-x) \\ \text{If } 2 < x < 10, & \quad y = c + a_1*(x-2) + a_2*(10-x) \\ \text{If } x > 10, & \quad y = c + a_1*(x-2) + 0 \end{aligned}$$

## b. Selective criteria for MARS models

Prediction accuracy of MARS models is estimated using **Generalized Cross Validation (GCV)**, which estimates what the performance of the model would be on new independent data. The GCV is a penalized **Residual Sum-of-Squares (RSS)**, where the RSS is penalized by the complexity of the model. The GCV is used internally by the MARS algorithm to select the set of basis functions that perform best on new data without overfitting the training data (Milborrow 2014).

In spite of its name, no actual cross-validation is done to calculate the GCV. However, as an independent measure of prediction accuracy, actual **cross-validation** of the MARS models with the n-fold argument of the function was also done. The principle of cross-validation is “leave-one-out”, *i.e.* separate the sample into n-fold subsets with equal number of iterations, and fit a model on all but one of the subsets (the out-of-fold subset), therefore constructing different independent training sets. The n-fold value is usually equal to 5 or 10; n-fold=5 was chosen here, to have an out-of-fold subset of at least 35 individuals (approximately 20% of the total sample). This process is then repeated ten times (by setting the argument n-cross to 10 in the model), so the measure of prediction error is averaged across all out-of-fold predictions.

Using the n-fold and n-cross arguments, an average estimate of R-Squared (**Cross-Validated R-Squared, or CVRSq**) and of the variation across folds (average prediction error) using cross-validation was obtained, after averaging many models from the data (Milborrow 2014).

Finally, MARS models allow the calculation of valid prediction intervals with dynamic ranges, taking residual heteroscedasticity into account. Indeed, by considering that variance is not constant in the whole sample, but increases with age (as it is the case), MARS calculates individual prediction intervals for each occurrence with a size adapted to the corresponding variance.

### 4.7.7. Application of predictive models for age estimation

In matters of age prediction, meeting statistical requirements is essential and unquestionable for validating the predictive models.

Parametric linear, degree 2 and 3 polynomial regression models were constructed on 72% of the total Marseilles sample (176 individuals), which was therefore used a training sample, and 28% (68 individuals) was used as an independent test sample for the models. The age and sex ratios of both samples were tested to check if they were respected or not.

Each statistical parameter was checked for model validation. In case of non-respected parameters, we proceeded to transform the variables (predicted and/or predictors) to obtain valid models. If this was not sufficient, we then tried to construct weighted age prediction models.

When all else failed, non-parametric MARS models were tested for age prediction. Univariate/simple (using one variable as age predictor) and multivariate/multiple (using several or all variables as age predictors) models were built using cross-validation on the same training sample used for constructing the parametric regressions, composed of 176 individuals. To assess model performance, we compared the values of CVRSq, of the standard deviation across folds, of GRSq, of prediction accuracy, variable importance in multivariate models and the smallest and largest prediction intervals.

All the valid models were then tested on the independent test samples of 68 individuals from Marseilles, the individuals from the Luis Lopes collection for whom the variables were measured and the 30 individuals from Toulouse. We were careful to verify that the range of values for the variables of the test samples were not outside the range of values for the variables of the training sample. On the independent test samples, the RSq, and mean and standard deviation of residuals are analysed for comparison with parametric models. All intervals are set at a 95% level and the error risk is set at 0.05.

## **4.8. Age estimation using Bayesian probabilities on non-biometric ordinal data**

### **4.8.1. The Bayesian approach**

Bayesian probability is one interpretation of the concept of probability. A probability is a mathematical, statistical concept that quantifies the occurrence of random events. Probability theory allows us to estimate how frequently a particular event A is observed in a population, and is written  $P(A)$ . Bayes' theorem includes new evidence (*a priori* knowledge of an observed event B) to estimate the probability of an event A happening *a posteriori*. It is a different way of interpreting probability, by adding information.

Bayes' theorem is closely related to conditional probabilities. Bayes' theorem postulates that given two events A and B in a probability space  $\Omega$ , (with  $P(B) > 0$ ), the conditional probability of event A knowing that a different event B has occurred is written  $P(A|B)$  and is equal to:

$$P(A|B) = \frac{P(A \cap B)}{P(B)} = \frac{P(A) \times P(B|A)}{P(B)}$$

With  $P(A)$ : *a priori* probability of event A

$P(B)$ : *a priori* probability of event B

$P(B|A)$ : joint probability

$P(A|B)$ : *a posteriori* probability of A, conditional probability

$P(A \cap B)$ : probability of events A and B happening together

The generalised expression of Bayes' theorem is:

$$P(A_i|B) = \frac{P(A_i) \times P(B|A_i)}{\sum_{i=1}^n P(B|A_n) \times P(A_n)}$$

To calculate the conditional probability, we must know the *a priori* probability of the observed event B, the probability of event  $A_i$ , and the probability of B in the theoretical space  $\Omega$ .

The terms of this equation can also be referred to as priors ( $P(A)$  and  $P(B)$ ), posterior ( $P(A|B)$ ) and likelihood ( $P(B|A)$ ) (Lucy et al 2002), with the following proportional relation:

$$\text{Posterior} = \propto (\text{Prior} \times \text{Likelihood}).$$

The prior probability is the theoretical distribution of all events in  $\Omega$  without considering any additional information. For example, a prior probability can be the probability for an individual to belong to the particular age group  $A_3$ , knowing that the individual is included in the variability of the population. It is written  $P(A_3)$ .

Likelihood is the conditional probability of the observed event S, knowing  $A_i$  is true. Likelihood is  $P(M|A_3)$ . For example, likelihood is the probability of an individual to present a maturation stage M knowing she/he is in the age group  $A_3$ .

The posterior probability is the probability of observing event  $A_i$ , knowing the previous events M. It is written  $P(A_3|M)$ . In our example, it is the probability for an individual to be in the age group  $A_3$  knowing his/her maturation stage is M and the distributions of all age groups  $A_n$  and of the maturation stage M in the population (priors).

The final formula to calculate the posterior probability of being in the age group  $A_3$  knowing the maturation stage is M would be:

$$P(A_3|M) = \frac{P(A_3) \times P(M|A_3)}{\sum_{i=1}^n P(M|A_i) \times P(A_i)}$$

With  $P(A_3)$ : prior probability of being in age group  $A_3$

$P(M|A_3)$ : joint probability of being at a maturation stage  $M$  in the age group  $A_3$

$\sum_{i=1}^n P(M|A_i) \times P(A_i) = P(M|A_1) \times P(A_1) + \dots + P(M|A_n) \times P(A_n)$ : the sum for all age groups of the probability of being at maturation stage  $M$  in age group  $i$  multiplied by the prior probability of age group  $i$  for the  $n$  age groups.

The distribution of the posterior probabilities, which is the end result obtained, can be presented in many forms: we can either consider the maximum value of the posterior probability as the correct one, or calculate the mean of the distribution, or calculate the 95% confidence interval of one of these values (an interval covering 95% of the probability, as a sum of probabilities). It is the posterior probability that is used as a probabilistic estimate of age. More precisely, the result is the posterior probability for an individual of being in the age group  $A_i$ , knowing certain biological information (maturation status, biometric value, etc.).

#### 4.8.2. Choosing the *a priori* probabilities

The prior probability can be calculated using two approaches: non-uniform or uniform priors.

##### a. Non-uniform priors

Non-uniform priors consider the relative frequencies observed in the reference sample, *i.e.* the prior probability of an event  $A$ ,  $P(A)$  is equal to the number of observations of  $A$  divided by the total number of observations in the population. For example, the probability for an individual of being in age group  $A_3$ ,  $P(A_3)$ , is equal to the number of individuals in the age group  $A_3$  divided by the total number of individuals. The generalised formula for the non-uniform prior of event  $A_i$  is:

$$P(A_i) = \frac{N(A_i)}{\sum_{i=1}^n N(A_i)} \text{ and } \sum_{i=1}^n P(A_i) = 1.$$

Non-uniform priors imply that the resulting probabilities will depend on the distribution of the events in the reference sample (Heuzé 2004). For this reason, they present the risk of not being consistent with the distribution of similar events in an independent validation sample. Because of this, non-uniform priors tend to overestimate the precision of the same event in an independent sample from



the same population. In our example, this means that the prior probabilities, depend on the age distribution of the reference sample (Coqueugniot et al 2010; Cunha et al 2009). By extension, the posterior probability of age estimated for an unknown individual will depend on the age distribution of the reference sample of the method.

The non-uniform prior approach was adopted by a few authors for age estimation using Bayesian posterior probabilities (Chaillet 2003; Lucy et al 2002). This is problematic for method comparison, because the results of two probabilistic methods can only be compared if their reference samples present the same prior probability distributions. In our example, this means that the reference samples of the methods must have the same age distributions. In practice, this is rarely the case as reference samples are tribute to sampling criteria (availability of the individuals or variables, reliability and accuracy of biological information...).

However, in their study, H. Coqueugniot and collaborators (2010) show that if sample size is sufficient and all age groups are represented by a high number of individuals, the use of relative frequencies of events (in their case, maturation stages) in each age group as priors is acceptable. The extension of this postulate is to use the distribution of events in the population from which the sample originates as prior probabilities, therefore reflecting the occurrence of events in the natural population. In our example, this would amount to attributing the priors to the demographic profile of the population of study. Unfortunately, even in that case, the problem of result comparison still remains, unless the compared populations present the same demographic profile.

## **b. Uniform priors**

Uniform priors consider that the probability of each event  $A_i$  is the same in the entire sample or population, *i.e.* that  $P(A_1) = P(A_2) = \dots = P(A_n)$ .

In our example, this means that the probability of being in the age group  $A_i$  is the same for all age groups, and is equal to one divided by the number of age groups  $N$ . The generalised formula for the uniform prior of event  $A_i$  happening in a population where  $N$  events are possible is:

$$P(A_i) = \frac{1}{N} \text{ and } \sum_{i=1}^n P(A_i) = 1$$

No particular distribution of the events is presumed for the reference sample. In this case, it is possible to compare results obtained by different methods that use uniform prior probabilities, as

they are independent from the distribution of events in the reference samples used to obtain the posterior probabilities.

In these matters of age estimation, the prior probability is the probability of being in age group  $A_i$ . If the priors are uniform, the age distribution is the same, *i.e.* the prior probability is the same for all age groups and independent of the age structure of the reference sample. L. Konigsberg and S. Frankenberg (1992) state that using uniform priors as the age distribution in the reference sample will not affect the age distribution of the individuals in the test sample, or the estimation of age of a single individual for whom the population of origin (and its demographic structure) is unknown. Indeed, this approach is useful to evaluate the precision of the posterior probability (age estimate) of an isolated individual when population demographics are unknown (Coqueugniot et al 2010; Rissech et al 2013b).

The uniform priors approach was adopted by J. Braga and collaborators (2005), H. Coqueugniot and collaborators (2010) and H. Cardoso and L. Rios (2011) for age estimation using Bayesian posterior probabilities. All authors specify that this approach was adopted to avoid the bias caused by the age distribution of the reference sample. Therefore, the result obtained with these methods can be directly compared on independent samples, even if the number of age groups  $N$  is different.

However, R. Gowland and A. Chamberlain (2002) have questioned the use of uniform priors because they do not necessarily reflect natural population demographics. Their use can be considered artificial and unrealistic. Indeed, unless the study sample is constructed in a way that uniform priors reflect the real age distribution, *i.e.* that individuals are selected to respect this particular distribution, uniform priors are rarely met in demographics. Individuals of living populations are very rarely equally distributed in all age groups, or in all populations, as different factors (socio-economic status, secular trends, environment, cultural and religious beliefs, migratory events) play various roles in their demographic composition. This is also true for past populations of particular periods, where infant mortality was a given and old age was seldom reached (Garcin 2009; Morel 2004; Scheuer and Black 2000). R. Gowland and A. Chamberlain (2002) have suggested a sort of calibration of the priors, by using model life tables modelling prior probabilities that take into account natural population patterns, such as mortality risks per age group. In any case, authors agree that the use of uniform priors for constructing a probabilistic age estimation method is therefore problematic if the study is done in a population perspective (Bocquet-Appel and Masset 1982; Gowland and Chamberlain 2002; Heuzé 2004).

Nevertheless, this approach seems more adapted for individual age assessment than the non-uniform priors approach. **This is why a uniform prior approach was selected for our probabilistic age estimation method.**

### **4.8.3. Choosing the appropriate Bayesian approach for age estimation**

Several authors have already developed juvenile age estimation methods using posterior Bayesian probabilities of age and maturation indicators (Braga et al 2005; Cardoso et al 2013b; Cardoso and Rios 2011; Coqueugniot et al 2010; di Gangi et al 2009; Heuzé 2004; Jit and Kaur 1989; Kaul and Pathak 1988; Kaul et al 1992; Saint-Martin et al 2013).

**The principle of estimating age of an individual using posterior probabilities is to provide a range of probabilities for the individual of belonging to an age group  $A_i$  (expressed in years, months, or weeks), knowing that his/her maturation stage at an epiphyseal location  $i$  is  $M_i$ .**  $M_i$  can represent the maturation stage at a single epiphyseal site, or it can be the combination of several maturation stages at different epiphyseal sites (see section 4.1.2.).

The number of maturation stages is the range of values of  $M_i$  corresponding to the number of possible maturation stages. It is decided *a priori*, as is the order of the sites in case of combinations of maturation stages.

**In our case, the order is always PUBISCH\_INF-ILISCH-PUBISCH\_SUP-PUBIL for four-digit combinations, and the sites are explicitly indicated for two- or three-digit combinations.**

The precision of the estimation is also established *a priori*: it defines the number and the range of the age groups. For example, with a precision  $p$  of 6 months, the estimated age will be the posterior probability for an individual to belong to the age group  $A_i$ , where  $A_i$  is equal to  $p \cdot i$ . A training sample is used to estimate the age group to which the individuals belong. Each individual in the training sample is therefore defined by his/her precise age (real age), the age group to which she/he belongs, his/her sex and his/her maturation status (stage, score, or combination of stages). The resulting method is then applied on an independent test sample to obtain the distribution of the posterior probabilities of belonging to each age group defined for the training sample for an individual of maturation state  $M_i$ .

The group with the highest posterior probability of age is considered to be the estimated age group of an individual. However, posterior probabilities can be summed to obtain a total posterior

probability of X % of belonging to several age groups. This gives us the minimum and maximum ages of an individual with X % reliability. This possibility is one of the main advantages of the Bayesian approach, as reliability and precision of the estimated age can be chosen by the user.

It is possible to obtain a flattened distribution of the posterior probabilities: for a maturation state  $M_i$ , the posterior probabilities of belonging to the age groups can be similar. This is intuitively the case for the younger and older individuals, for whom the maturation states and combinations are similar in several age groups (0-0-0-0 for the younger children, 2-2-2-2 for the older children). If the number of individuals is the same in each of these age groups, and if the prior probabilities are uniform, the posterior probabilities will be very similar. For example, the maturation combination 0-0-0-0 for the iliac bone presents comparable probabilities in the age groups 0, 1, 2 and 3 years (0.1684 each) (Figure 4.21).

A flattened distribution of the posterior probabilities can also result from the age range selected to define the age groups. If the range is too small, for example if it is chosen at 6 months, individuals of the age group  $N + 1$  are 6 months older than the individuals from the age group  $N$ . In that case, and depending on the rate of the process and its action on the bone considered, there can be a bigger risk of obtaining similar posterior probabilities of age, because no significant maturation or growth changes occur between two or more consecutive age groups.

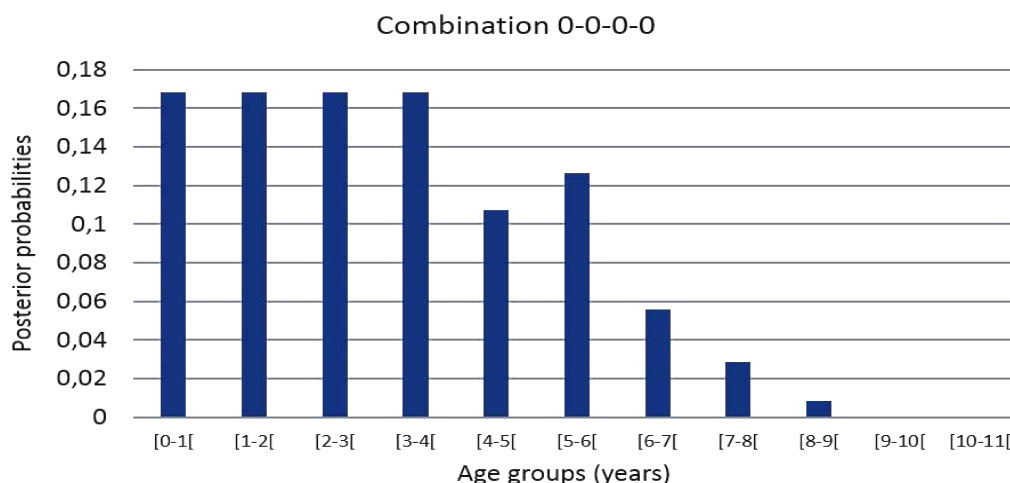


Figure 4.21 Flattened distribution of posterior probabilities of age for the combination of the four maturation sites of the iliac bone

In our case, age estimation using posterior probabilities can be based on the maturation stages of single epiphyseal sites or combinations of the maturation stages of two, three, or all sites. In the first case, the variables are independent. In the second case (combinations), they can be considered

dependent or independent. There are in fact two different approaches for Bayesian statistics using multiple variables as estimators: independent (IBM) and dependent (DBM) Bayesian methods.

### **a. Dependent Bayesian method (DBM)**

DBM considers the maturation stages of different sites as dependent attributes. This approach seems therefore more adapted to biological reality and particularly when analysing different epiphyseal sites of a same bone, such as we are doing with the iliac bone: since PUBISCH\_INF matures before the other three epiphyses, if it is at a stage 1, we know that the other three sites cannot be at a stage higher than 0 or 1. Y. Heuzé (2004) has also pointed out that the intensity of the relation between two or more maturation sites (in this case, teeth) varies according to the sites in question. When using the dependent Bayesian method, posterior probabilities are calculated for all sites combined, with variables presented as scores (the sum of the maturation stages at all four sites) or as combinations. Several authors have used this approach for juvenile age estimation (Braga et al 2005; Heuzé and Cardoso 2008).

Other than biologically incoherent combinations, another disadvantage of using DBM arises when we want to assess age of individuals for whom the maturation combination is not represented in the reference sample. Estimating the age of such individuals is not possible using Bayesian probabilities, as the probability of presenting these combinations is equal to zero.

For individuals with incomplete combinations or scores, the maturation score is calculated without one or more of the maturation stages, and the resulting score will automatically be inferior or equal to the real one and therefore, age will be underestimated. For the same reasons as the ones cited above, in case of incomplete scores or combinations, no age estimation can be done using dependent Bayesian probabilities. This is a problem when working on osteological series where fragmented and incomplete bones are often a given.

There is also a problem if there are too few occurrences of specific values of scores or of certain combinations in the reference sample: if there is a smaller number of individuals per combination than the number of individuals in the age groups, the resulting posterior probabilities will be false because the combinations are underrepresented in the reference sample. This was noticed by H. Coqueugniot and collaborators (2010) who stated that likelihood (the probability of observing a particular stage at a particular age) depends on sample size and sample aberrations (unrespected age and/or sex ratios) and therefore provides limited information on the distribution of stage probabilities in the reference sample. This issue was dealt with using kernel smoothing and non-

parametric regressions for calculating weighted likelihoods for age and stage. Smoothing can be applied when the size of the sample is insufficient and/or the age distribution is not uniform.

**b. Independent Bayesian method (IBM)**

This approach considers the maturation stages of different sites as independent variables. Posterior probabilities can be calculated for each site separately for the individuals. Several authors have used this approach for juvenile age estimation (Foti et al 2003; Gowland and Chamberlain 2002; Heuzé 2004; Heuzé and Cardoso 2008). IBM offers the possibility to generate age estimations for more individuals as calculations are done site by site because the maturation stages are considered as independent attributes. For this reason, IBM can assess age for each site separately but also for complete or incomplete combinations of stages.

For example, if an individual has an iliac bone with the maturity combination (2-1-1-0), the posterior probability of being in the age group  $A_i$  will be:

$$P(A_j | (2-1-1-0)) = \frac{P(A_j) \times P((2-1-1-0) | A_j)}{\sum_{i=1}^n P((2-1-1-0) | A_i) \times P(A_i)}$$

Because the maturation stages are independent,

$$P(A_j | (2-1-1-0)) = P(2 | A_j) \times P(1 | A_j) \times P(1 | A_j) \times P(0 | A_j)$$

And therefore,

$$P(A_j | (2-1-1-0)) = \frac{P(A_j) \times P(2 | A_j) \times P(1 | A_j) \times P(1 | A_j) \times P(0 | A_j)}{\sum_{i=1}^n P(2 | A_i) \times P(1 | A_i) \times P(1 | A_i) \times P(0 | A_i) \times P(A_i)}$$

In this case, the order of the sites used for the combination, PUBISCH\_INF, ILISCH PUBISCH\_SUP and PUBIL is unimportant because all probabilities are independent. This is called the conditional independence hypothesis. It relies on the fact that the correlation between maturation stages at different sites is related to age rather than genetics or development (Coqueugniot et al 2010).

The ability to assess age using IBM is greater than with DBM because the probability of finding the same site at the same stage in samples is always higher or equal to the probability of finding the same combination of maturation stages or score of different sites in the samples. Contrary to DBM, the stages of the test individuals can be represented by different individuals in the same age group of the reference sample. However, age cannot be assessed on an individual whose stages of

development are not represented in at least one age group of the reference sample. IBM can be associated with either uniform or non-uniform priors.

Several authors have argued that this approach is not appropriate for estimating age using several sites, because assuming that epiphyseal or dental maturation is independent does not reflect biological reality (Heuzé and Cardoso 2008). However, the main advantage of **IBM** is that it **allows the estimation of age with complete (all four sites) and incomplete (less than four sites) combinations**: because the probabilities of all individual sites are independent, they can be multiplied at will according to the variables available for study. For example, if only three stages out of four are observable, and provide the incomplete combination (0-0-1-), the posterior probability is:

$$P(A_j | (0-0-1-)) = \frac{P(A_j) \times P(0|A_j) \times P(0|A_j) \times P(1|A_j)}{\sum_{i=1}^n P(0|A_i) \times P(0|A_i) \times P(1|A_i) \times P(A_i)}$$

This is particularly interesting for cases with missing data (missing or fragmented bones or teeth), which often occur in anthropology, in both osteoarchaeological and forensic contexts.

Bearing that in mind, it would be interesting to find out if there is a minimum number of sites that could be sufficient to provide satisfactory age estimates. Indeed, if two or three maturation sites are sufficient to provide good age estimates instead of four, perhaps part of the bias due to the absence of data could be avoided. Moreover, as different combinations of sites would be tested, and knowing the maturation sequence of the iliac bone, perhaps certain combinations could be more adapted for certain age groups. For example, as we know that the PUBISCH\_INF epiphysis fuses between 5 and 8 years, it would be logical to think that a combination using this site would be useful for age estimation of individuals from this particular age range.

Combinations can include the maturation stages of two (A-B) to four (A-B-C-D) different sites. Theoretically, when using  $n$  epiphyseal sites with three possible maturation stages, there are  $3^n$  possible maturation combinations. In this study, there are four maturation sites, and three possibilities for staging (0, 1, and 2). Using three possible values, there are in theory  $3 \times 3 \times 3 \times 3 = 3^4$  possible four-digit combinations. For two-digit and three-digit combinations with three different possible numbers, there are in theory  $3^2$  and  $3^3$  possible combinations of stages respectively. These values have to be multiplied by the number of possible combinations of the two or three sites considered for the two-digit and three-digit combinations of stages. Because there are four sites, there are in theory four possibilities of three-site combinations and six possibilities of two-site combinations without repetition.

Therefore, there are in theory, **for a fixed order and without repetition** of sites:

- $3^4 = 81$  possible four-digit combinations of maturation stages
- $3^3 \times 4 = 108$  possible three-digit combinations of maturation stages
- $3^2 \times 6 = 54$  possible two-digit combinations of maturation stages

However, as maturation is sequential (Scheuer and Black 2000, 2004; Todd 1937), stages are always successive and unidirectional: stage 0 will always be followed by stage 1, which will in turn always be followed by stage 2. For example, a four-digit combination of 0-0-1-0 cannot be directly followed by the combination 0-2-1-2. For the same reasons and because the order of the epiphyses is fixed to avoid repetitions, the real numbers of three- and two-digit combinations are also inferior to the ones calculated above. The “real” numbers of combinations, *i.e.* the ones reflecting the biological phenomenon of maturation are therefore much lower.

In this study, 24 four-digit combinations, 88 three-digit combinations and 48 two-digit combinations were observed.

Adopting a dependent or independent Bayesian method for age estimation is in fact a matter of context, and objectives: the choice has to optimise the results considering the different biases exposed above. Although an **independent Bayesian approach** seems to be less adapted to reflect the biological reality of bone maturation, it presents several advantages compared to a dependent approach: it **allows age estimation using the maturation stages of each site separately and on the combination of the maturation stages even in case of missing data**. If we use the same approach on separate and combined stages, a direct comparison of the results can be done. Moreover, the possibility of estimating age even in case of missing data seems more useful for forensic anthropologists and osteoarchaeologists alike.

**In a perspective of optimising method application, aiming to apply the method to the highest number of individuals possible, on both scanned bone (living or deceased individuals) and dry bone (complete or incomplete bones of deceased individuals), an independent Bayesian approach is the best choice.**

Sexual dimorphism of bone maturation rates has been found by several authors (Coqueugniot and Weaver 2007; Scheuer and Black 2000, 2004; Todd 1937; White and Folkens 2005), and is particularly present for the iliac bone (Cardoso 2008b; Garn et al 1961; Marchal 1997, 2003; Rissech and Malgosa



2005, 2007; Rissech et al 2003). This is why posterior probabilities could be calculated for both sexes separately to obtain more specific and precise estimations (Cardoso 2008b; Heuzé 2004). However, because the number of male and female individuals was insufficient in each annual age group to provide reliable sexed results, no analyses were done for separate sexes.

Posterior probabilities were calculated on a reference sample composed of 285 individuals from the Marseilles sample. The probabilities were estimated for each epiphyseal site separately and for the combinations of maturation stages.

#### **4.8.4. Evaluation and validation of Bayesian posterior probabilities for age estimation**

Validation of probabilistic age estimates using ordinal data relies on similar criteria as the ones obtained for regression models using quantitative continuous data: reliability, precision, accuracy (see sections 4.7.2 and 4.7.3.). The posterior probabilities obtained on the training sample were tested on an independent sample of 132 individuals from Marseilles (58 girls and 74 boys) and between 76 and 80 individuals from the Luis Lopes collection (38/40 girls and 38/40 boys); all are aged between 0 and 19 years. These samples were used to evaluate the quality of the estimation through five statistical parameters (Heuzé 2004):

- Precision of the method (fixed at one year)
- Performance of the method (proportion of comparable bone age and real age)
- The size of the confidence interval with a 95% reliability
- The absence of asymmetry in the estimates (no significant over- or under-estimation of age)
- The absence of significant differences between the quality of the estimates for all age groups

Reliability is obtained by summing the posterior probabilities until a satisfactory value is obtained. Its level is left to the choice of the user. The initial precision is fixed before the calculations, and corresponds to the number of years between two successive age groups (1 year here).

Accuracy is obtained by comparing the real age group to the estimated age group (the age group with the highest probability) of the individuals. Age groups are all annual. The estimated age group is subtracted from the real age group and provides a ranking system of the group differences between the estimate and real age groups (Heuzé 2004; Heuzé and Cardoso 2008). A negative difference will

mean that (“real age group” – “estimated age group”) is negative, *i.e.* bone age is superior to real age.

When an individual presents the rank value 0, the bone age group and the real age group coincide, meaning that bone age is equal to real age with a maximum difference strictly inferior to 1 year (the size of the age group) and a confidence interval of +/- 0.5 years. When an individual presents the rank value +1, bone age is lower than real age with a maximum difference strictly inferior to two years (the sum of the sizes of the two age groups) and a confidence interval of +/- 1 year.

To evaluate the accuracy of the method not only on individual occurrences but on a sample, it is interesting to represent the distribution of the ranks for the estimates of the individuals of the whole test sample. This also gives the performance of the method, which is equal to the proportion of individuals with a rank value of 0, *i.e.* for whom bone age and real age groups are the same (Heuzé 2004; Heuzé and Cardoso 2008). Performance was also assessed for all variables used as age estimates (individual maturation sites and combinations) (Figure 4.22).

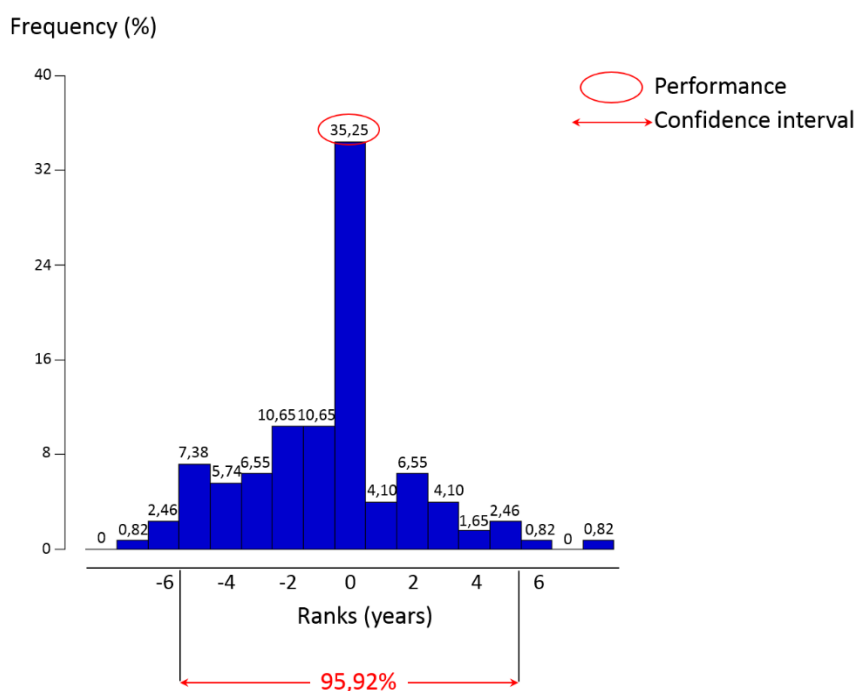


Figure 4.22 Rank distribution of the test sample from Marseilles using the combinations of the maturation stages from the four iliac sites. Each individual is ranked between -7 and +8

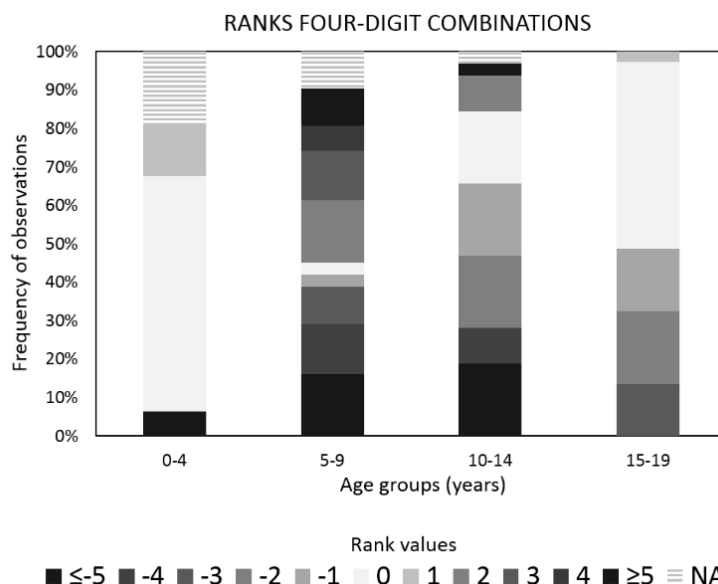
The size of the confidence interval of the estimation method associated with 95% reliability is determined by counting the minimum number of ranks containing at least 95% of the individuals from the test sample. The number of ranks  $N_{R\ 95\%}$  is then multiplied by the number of years covered by one rank (1 year), which gives us the size of the confidence interval in years.

The distribution of the ranks around 0 can also provide information on the quality of the method. Indeed, if there are more negative rank values, it means that estimated bone age is more frequently higher than real age, therefore that the method tends to overestimate age. A Kruskal-Wallis test is done to test the differences between the distributions of ranks in each age group ( $p > 0.05$ ).

The graphical representation of the ranks is used to calculate the kurtosis of the distribution. The higher the kurtosis value, the more pointed the distribution, and the more symmetrical and closer it is to 0.

A test was also done to check whether the accuracy of the estimations was comparable in all age groups (Heuzé 2004). To do this, we grouped the annual age groups five by five to obtain four groups of sufficient size: the first group is composed of individuals aged 0 to 4 years, the second group is composed of individuals aged 5 to 9 years, the third group includes individuals between 10 and 14 years of age, and the fourth group includes individuals aged 15 to 19 years (**Figure 4.23**). This way, the results can be directly interpreted according to the age groups representing the quinquennial age groups used in bioarchaeological studies.

The ratios of the number of individuals per rank value on the total number of individuals in the year groups are represented by cumulative histograms. All rank values are represented, as well as individuals for whom age was not estimated (NA). The distributions of ranks between the quinquennial age groups were compared using a multiple comparison Chi-squared test ( $p > 0.05$ ).



**Figure 4.23** Distribution of ranks obtained for the four-digit combination of maturation stages in the different quinquennial age groups of the Marseilles test sample

The evaluation and validation of the estimates were done by comparing the five parameters for all probabilistic methods constructed with pooled and separate sexes (using individual maturation sites,

or combinations) applied to the test samples from Marseilles (131 individuals) and the Luis Lopes collection (82 individuals).

**Summary: Key steps of the standardised methodological protocols**

- 19 biometric variables taken on the ilium (4), the fifth lumbar vertebra (10) and the clavicle (5) were measured on reconstructed bone surfaces; maturation stages were recorded for the epiphyses of the iliac bone
- Repeatability and reproducibility of the variables and the acquisition protocols were tested
- The protocol for data acquisition of the clavicular variables was tested on dry and scanned bone surfaces
- Variables were tested for sexual dimorphism and bilateral asymmetry
- Parametric and non-parametric age prediction models were constructed on study samples from Marseilles using the biometric variables as predictors
- Independent posterior probabilities of age were calculated using maturation stages of the iliac bones of individuals from a study sample from Marseilles
- The regression models and the posterior probabilities of age were applied on three different test samples from Marseilles, Toulouse and the Luis Lopes reference collection from Lisbon

# Results

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## Chapter 5. Results I: Protocol validation and sample descriptions

This chapter covers the statistical tests done on the data and the descriptive statistics of each variable obtained for the three samples (Marseilles study sample, Luis Lopes and Toulouse test samples). The first part concerns reliability, repeatability and reproducibility of landmark positioning and of biometric and non-biometric variable acquisition. The second part presents the statistical tests done for bilateral asymmetry of the paired variables of the ilium, clavicle and iliac bone) and for sexual dimorphism of all variables. All the tests used are presented in **Table 5-1**.

**Table 5-1 Statistical tests used for validation of data acquisition protocol and descriptive statistics of the variables.**  
\*symbols indicate parametric tests

Aim of the test	Test used	Hypotheses
Type of distribution	Shapiro-Wilk test for normality*	H0: The set of values follows a normal distribution H1: The set of values does not follow a normal distribution
Repeatability and reproducibility of landmarks and variables	Paired t-test* Paired Wilcoxon test	H0: the mean difference between two variables is equal to zero H1: the mean difference between two variables is different than zero
Repeatability and reproducibility of the variables	- Intra-Class Correlation (ICC)	Assess consistency or agreement of paired quantitative variables made by the same observer or by different observers. High coefficients imply high observer agreement
	- Bland-Altman plots	Evaluate the agreement among two different instruments or two measurements techniques. If more than 90% of the differences are within the 95% Confidence Interval of the values, agreement is reached.
	- Technical Error of Measurement (TEM) and percentage of TEM	TEM = Measurement of intra- and inter-observer variables (mm). %TEM = relative TEM. If %TEM ≤ 5%, variables are repeatable and reproducible.
Bilateral asymmetry -biometric variables	Paired t-test* Paired Wilcoxon signed rank test	H0: the mean difference between left and right variables is equal to zero H1: the mean difference between left and right variables is different than zero
-non-biometric variables	Permutation test  Exact binomial test	H0: the range of the differences between left and right epiphyseal sites is comparable to zero H1: the range of the differences between left and right epiphyseal sites is not comparable to zero  H0: the probability of similar stages on left and right epiphyseal sites of the same individual is ≥ 95% H1: the probability of similar stages on left and right epiphyseal sites of the same individual is not ≥ 95%
Sexual dimorphism -biometric variables	Independent Welch t-test* Independent Wilcoxon signed-rank test	H0: the mean difference between male and female variables is equal to zero H1: the mean difference between male and female variables is different than zero
-non-biometric variables	Independent Mann-Whitney Wilcoxon test	H0: the distributions of male and female variables are equal H1: the distributions of male and female variables are not equal

The detailed descriptive statistics of the three samples can be found in appendices B and C; the detailed results of the tests for sexual dimorphism per age group can be found in appendix D.

All tests are done with an error rate  $\alpha$  set at 0.05 and/or a maximum of more or less 2.5% of the mean value of differences between variables.

## 5.1. Validation of data acquisition protocols

### 5.1.1. Variable consistency on scanned bone surfaces and dry bones

We considered that clavicular variables taken on dry bones were sufficiently repeatable and reproducible, as they are referenced in several studies (*e.g.* Buikstra and Ubelaker 1994; Martin 1957; Martin and Saller 1959). However, we needed to test whether the consistency of the variables was sufficient when measurements were taken on dry bones (dry clavicles) and the virtually reconstructed surfaces of the same dry bones. The protocol was therefore tested on 40 dry bone clavicles, of unknown archaeological origin, curated in the laboratory UMR 7268 ADES that are used for anatomy and osteology courses. The choice was made for the clavicle, because it is the only bone of this study that did not have referenced “virtual” equivalents for all the osteometric variables used in this study. Moreover, contrary to the three iliac variables IL, IW and IM and the eight unidimensional vertebral variables, none of the five clavicular variables had been previously defined by landmarks on scanned bones, nor by specific reliable anatomical markers on dry bones. This means the risk for intra- and inter-observer errors needed to be assessed on dry and scanned bone.

#### a. Testing the consistency of variables taken on dry bone and scanned dry bone

The mean and standard deviation of all five variables were calculated on dry clavicles and scanned bone surfaces of the same dry clavicles from a series of measurements taken by observer 1 as  $v_{dry}$ - $v_{scanned}$  (Table 5-2).

Table 5-2 Mean and standard deviation of the clavicular variables taken on dry and reconstructed scanned dry bones

Variable	Dry bone		Scanned bone	
	Mean (mm)	Standard deviation (mm)	Mean (mm)	Standard deviation (mm)
Ln	124.670	40.788	124.914	40.746
Max_diam	11.792	3.866	11.885	3.927
Min_diam	8.149	3.009	8.295	2.956
AP_diam	11.068	3.618	11.150	3.648
SI_diam	8.841	3.338	8.403	3.067

The Shapiro-Wilk test of the normal distribution of the differences between the variables taken on

dry bone and scanned dry bone showed that the differences for Ln, Max\_diam and AP\_diam follow a normal distribution, but the differences between Min\_diam and SI\_diam do not (**Table 5-3**).

**Table 5-3 Results of the Shapiro-Wilk test for the distribution of the differences between clavicular variables taken on dry and scanned dry bones. The greyed p-values correspond to a distribution significantly different from normality**

Difference scanned-dry	W	p-value
Ln	0.973	0.436
Max_diam	0.956	0.120
Min_diam	0.941	0.038
AP_diam	0.986	0.905
SI_diam	0.869	<0.001

Paired t-tests for equality of measurements between dry and scanned dry bones were done for Ln, Max\_diam and AP\_diam and showed a significant difference for Ln (**Table 5-4**).

**Table 5-4 Results of the paired t-tests (upper table) and paired Wilcoxon tests (lower table) for variable reliability between dry and scanned dry clavicles. The greyed p-values correspond to significant differences between the variables; CI = Confidence Interval**

Variable	t	Df	CI 95% (mm)	Mean difference (mm)	p-value
Ln	2.881	39	[0.073 ; 0.415]	0.244	0.0064
Max_diam	1.280	39	[-0.054 ; 0.240]	0.093	0.208
AP_diam	0.91	39	[-0.101 ; 0.264]	0.082	0.370
Variable	V		CI 95% (mm)	Pseudo-median (mm)	p-value
Min_diam	649		[0.073 ; 0.261]	0.169	0.0013
SI_diam	213		[-0.658 ; -0.065]	-0.326	0.007

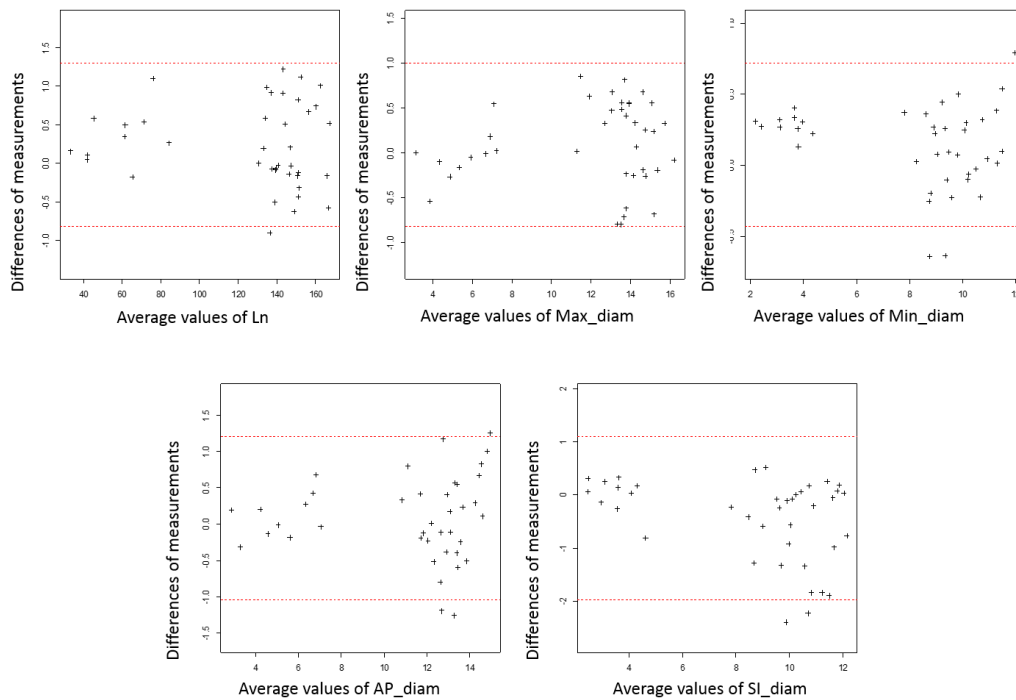
Non-parametric paired Wilcoxon rank-signed tests were done for Min\_diam and SI\_diam taken on dry and scanned dry bones and showed significant differences (**Table 5-51**).

All p-values are inferior to 0.05 except for Max\_diam and AP\_diam. Therefore, we have to reject the hypothesis of equality of measurements for Ln, Min\_diam and SI\_diam because the confidence intervals (CI) do not include zero. However, all the CIs, means and medians of the differences between measurements are included in the interval [-1 mm; +1mm] and the mean or pseudo-median differences are lower than 0.5 mm. We can therefore assess that the measurements between dry and scanned bone present an error of less than +/- 1mm, which is sufficient for their validation.

All measurement differences are within the 95% range of the Bland-Altman plots, with less than 10% of measurement differences outside said range (**Figure 5.1**). Min\_diam and AP\_diam have three, SI\_diam has two, Ln has one and Max\_diam has none.

We can conclude that in all five cases, there is an insignificant difference between measurements taken on dry bones and the same measurements taken on the scanned surfaces of the same dry bones.





**Figure 5.1 Bland-Altman plots of the differences between the five clavicular variables taken on dry and reconstructed scanned dry bones surfaces**

**b. Testing the equality of geometric and anatomical variables**

Both geometric (Max\_diam and Min\_diam) and anatomical (AP\_diam and SI\_diam) clavicular diameter types are likely to be studied (Buikstra and Ubelaker 1994; Martin and Saller 1959; Moore-Jansen et al 1994; Shirley 2009). However, because the two geometric variables are obtained using a semi-automatic protocol on scanned bone, they are much more reliable than the anatomical variables that require placing the clavicles on an anatomical plane, which can sometimes be tedious and/or unreliable on both dry and scanned bone, mostly because of the extreme variability of clavicular shape (Cook et al 2013; Mays et al 1999; Voisin and Balzeau 2004). For these reasons, and for the sake of developing fast, reliable and user-friendly protocols for data acquisition (particularly on scanned bone), equality between geometric and anatomical variables taken on the same bones was tested on both dry and scanned clavicles.

**i. Dry clavicles**

The Shapiro-Wilk test done on the differences between geometric and anatomical variables show that the differences between AP\_diam and Min\_diam and SI\_diam and Max\_diam follow a normal distribution, whereas the differences between AP\_diam and Max\_diam and SI\_diam and Min\_diam do not (Table 5-5).

**Table 5-5 Results of the Shapiro-Wilk test for the distribution of the differences between anatomical and geometric clavicular variables taken on dry bone. The greyed p-values correspond to a distribution significantly different from normality**

Variable difference	W	p-value
AP_diam - Max_diam	0.92	0.01
AP_diam - Min_diam	0.98	0.71
SI_diam - Max_diam	0.98	0.86
SI_diam - Min_diam	0.94	0.01

The paired t-test for equality of measurements between AP\_diam and Min\_diam and SI\_diam and Max\_diam showed that differences between these pairs of geometric and anatomical were significant (**Table 5-6**) with Confidence Intervals (CIs) larger than [-1 ; +1]. The non-parametric paired Wilcoxon rank-signed test for equality of measurements between AP\_diam and Max\_diam and SI\_diam and Min\_diam showed that the differences between these pairs of geometric and anatomical measurements were significant. However, the confidence intervals of the differences are inferior to [-1 ; +1] (**Table 5-6**).

**Table 5-6 Results of the paired t-tests (upper part of the table) and paired Wilcoxon tests (lower part of the table) for equality of anatomical and geometric variables taken on dry clavicles. The greyed p-values correspond to significant differences between variables; CI = Confidence Interval**

Variable difference	t	Df	CI 95% (mm)	Mean difference (mm)	p-value
AP_diam - Min_diam	-17.287	39	[-3.26 ; -2.58]	-2.92	<2.2e-16
SI_diam - Max_diam	16.9234	39	[2.60 ; 3.30]	2.95	<2.2e-16
Variable difference	V	CI 95% (mm)	Pseudo-median (mm)	p-value	
AP_diam - Max_diam	812	[0.46 ; 0.91]	0.66	<2.2e-16	
SI_diam - Min_diam	17	[-0.92 ; -0.44]	-0.68	<2.2e-16	

We must therefore reject the hypothesis of strict equality between the two geometric and the two anatomical variables taken on dry clavicles.

ii. Scanned dry bone surfaces

The Shapiro-Wilk test done on the differences between geometric and anatomical variables showed that the differences between AP\_diam and Min\_diam and SI\_diam and Max\_diam followed a normal distribution, whereas the differences between AP\_diam and Max\_diam and SI\_diam and Min\_diam did not (**Table 5-7**).

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**Table 5-7 Results of the Shapiro-Wilk test for the distribution of the differences between anatomical and geometric clavicular variables taken on scanned bones. The greyed p-values correspond to a distribution significantly different from normality**

Variable difference	W	p-value
AP_diam - Max_diam	0.91	0.005
AP_diam - Min_diam	0.97	0.45
SI_diam - Max_diam	0.98	0.65
SI_diam - Min_diam	0.93	0.02

The paired t-test for equality of measurements between AP\_diam and Min\_diam and SI\_diam and Max\_diam showed that differences between these pairs of geometric and anatomical were significant (**Table 5-8**), with CIs larger than [-1 ; +1].

The non-parametric paired Wilcoxon rank-signed test for equality of measurements between AP\_diam and Max\_diam and SI\_diam and Min\_diam showed that the differences between these pairs of geometric and anatomical variables was significant. However, the confidence intervals of the differences are smaller than [-1 ; +1] (**Table 5-8**).

**Table 5-8 Results of the paired t-tests (upper table) and paired Wilcoxon test (lower table) for equality of anatomical and geometric variables taken on scanned clavicles. The greyed p-values correspond to significant differences between variables; CI = Confidence Interval**

Variable difference	t	Df	CI 95% (mm)	Mean difference (mm)	p-value
AP_diam - Min_diam	-14.42	39	[-3.26 ; -2.45]	-2.86	<0.001
SI_diam - Max_diam	16.10	39	[3.04 ; 3.92]	3.48	<0.001
Variable difference	V		CI 95% (mm)	Pseudo-median (mm)	p-value
AP_diam - Max_diam	817		[0.47 ; 0.92]	0.70	<0.001
SI_diam - Min_diam	346		[-0.22 ; 0.079]	-0.07	0.39

The differences between geometric and anatomical variables are significant in all cases, except for SI\_diam and Min\_diam when taken on scanned bones. Moreover, differences between AP\_diam and Max\_diam are small (inferior to +/- 1mm) on both dry and scanned dry bones.

**Conclusions on variable acquisition on dry and scanned dry clavicles:**

- Landmark positioning is sufficiently repeatable but not sufficiently reproducible for all landmarks
- All five variables can be taken on dry and scanned dry clavicles with a reliability higher than 95%
- All five variables are sufficiently repeatable and reproducible on scanned bone with errors inferior to +/- 1mm and a reliability higher than 95%
- Geometric measurements (Max\_diam and Min\_diam) cannot be used as “substitutes” for anatomical measurements (AP\_diam and SI\_diam) on dry or scanned bone, even though differences between SI\_diam and Min\_diam and AP\_diam and Max\_diam are inferior to 1 mm

### **5.1.2. Repeatability and reproducibility**

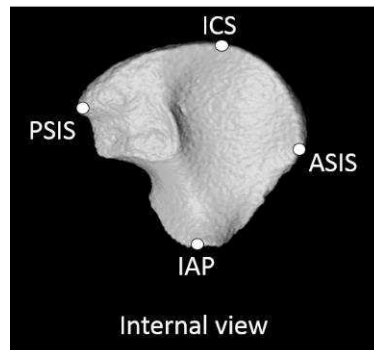
Variable repeatability and reproducibility are essential for protocol validation. A second set of tests for repeatability and reproducibility was done on the three bones studied (clavicle, lumbar vertebra, ilium and iliac bone) by randomly picking 30 individuals of both sexes (15 males and 15 females) and all age groups (from 0 to 12 years for the ilium, from 0 to 19 years for the other three bones) in the total sample from Marseilles. Because the bones belonged to living individuals, they were not on the same planes during acquisition, contrary to the dry clavicles that were scanned lying on a wooden board. Consequently, the reconstructed bone surfaces were not in the same plane either. Moreover, segmentation of scanned dry bones is different than reconstructing bone surfaces from entire living individuals, because of the absence of surrounding tissues and the changes in bone density that occur in time (Villa and Lynnerup 2012). The presence of surrounding tissues (muscles, cartilage, ligaments, etc.) can add difficulty to bone segmentation. This is why it is necessary to test repeatability and reproducibility of landmark positioning and variables on the individuals used in the study.

#### **a. Landmark positioning**

##### **i. Ilium**

Four landmarks were placed on the internal surface of the ilium (**Figure 5.2**) of 30 randomly

selected individuals to test the repeatability and reproducibility of landmark positioning.



**Figure 5.2** The four landmarks placed on the internal face of the iliae. PSIS = Postero-Superior Iliac Spine; ICS = Iliac Crest Summit; ASIS = Antero-Superior Iliac Spine; IAP = Internal Acetabular Point

*Repeatability*

The distance between two sets of homologous landmarks was calculated using the formula for vector norm (see section 4.2.2.) between the two landmarks. The mean, median, standard deviation, minimum and maximum values of the distances were calculated for each set of homologous landmarks (Table 5-9). The means were all inferior to 1 mm, as were the standard deviations.

The Shapiro-Wilk test showed that only the distance between the ICS homologous landmarks followed a normal distribution (Table 5-10).

**Table 5-9** Mean, median, standard deviation, minimum and maximum values of the distances between homologous iliac landmarks placed by the same observer twice

Intra-observer landmark distance	N	Distribution of the distances between repeated landmark positioning				
		Mean (mm)	Median (mm)	Standard deviation (mm)	Max (mm)	Min (mm)
ASIS-ASIS	30	0.585	0.510	0.375	1.493	0.074
PSIS-PSIS	30	0.857	0.539	0.914	4.311	0.149
ICS-ICS	30	2.688	2.434	1.592	6.668	0.245
IAP-IAP	30	1.124	0.913	0.818	3.336	0.115

**Table 5-10** Results of the Shapiro-Wilk test for the distribution of the differences between iliac landmarks placed by the same observer twice. The greyed p-values correspond to a distribution significantly different from normality

Intra-observer landmark distance	W	p-value
ASIS-ASIS	0.922	0.030
PSIS-PSIS	0.695	<0.001
ICS-ICS	0.967	0.469
IAP-IAP	0.902	0.009

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All distances are significantly different from zero (**Table 5-11**). The mean difference between two ICS landmarks is high (2.688 mm) and the corresponding Confidence Intervals (CI) are much higher than the threshold CI of [-1 ; +1]. The mean distances between the ASIS and PSIS landmarks and their respective CIs are included in the threshold CI. The mean and CI for IAP however, are not (mean=1.08 mm and CI=[0.815 ; 1.405]).

**Table 5-11 Results of the paired t-test (upper part of the table) and paired Wilcoxon tests (lower part of the table) for landmark repeatability on scanned iliae. The greyed p-values correspond to significant differences between repeated landmarks; CI = Confidence Interval**

Intra-observer landmark distance	t	Df	CI 95% (mm)	Mean difference (mm)	p-value
ICS-ICS	9.25	29	[2.094 ; 3.283]	2.688	<0.001
Intra-observer landmark distance	V	CI 95% (mm)	Pseudo-median (mm)	p-value	
ASIS-ASIS	465	[0.427 ; 0.697]	0.547	<0.001	
PSIS-PSIS	465	[0.465 ; 0.902]	0.623	<0.001	
IAP-IAP	465	[0.815 ; 1.405]	1.08	<0.001	

*Reproducibility*

The same four landmarks were placed by an independent observer on the same 30 individuals. The distance between two sets of homologous landmarks was calculated using the formula for vector norm (see section 4.2.2.) between the two landmarks. The mean, median, standard deviation, minimum and maximum values of the distances were calculated for each set of homologous landmarks (**Table 5-12**). The means were all inferior to 1 mm, as were the standard deviations.

**Table 5-12 Mean, median, standard deviation, minimum and maximum values of the distances between homologous iliac landmarks placed by different observers**

Inter-observer landmark distance	N	Distribution of the distances between repeated landmark positioning				
		Mean (mm)	Median (mm)	Standard deviation (mm)	Max (mm)	Min (mm)
ASIS-ASIS	30	0.551	0.410	0.374	1.681	0.111
PSIS-PSIS	30	0.718	0.645	0.414	1.794	0.134
ICS-ICS	30	2.323	1.762	1.662	6.027	0.445
IAP-IAP	30	1.739	1.573	0.863	3.419	0.128

The Shapiro-Wilk test showed that only the distance between the IAP homologous landmarks followed a normal distribution (**Table 5-13**).

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**Table 5-13 Results of the Shapiro-Wilk test for the distribution of the differences between iliac landmarks placed by two observers. The greyed p-values correspond to a distribution significantly different from normality**

Inter-observer landmark distance	W	p-value
ASIS-ASIS	0.877	0.002
PSIS-PSIS	0.920	0.027
ICS-ICS	0.870	0.002
IAP-IAP	0.967	0.453

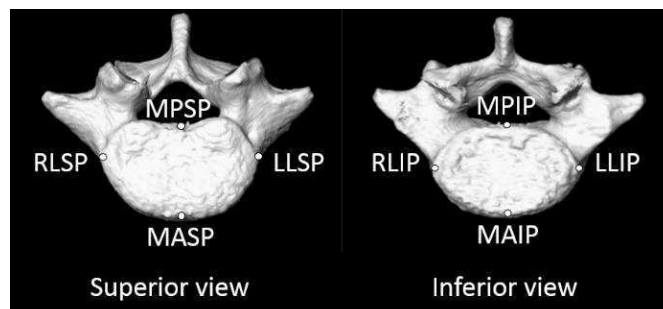
All distances are significantly different from zero (**Table 5-14**). The mean difference between two ICS landmarks is high (2.082 mm) and the corresponding Confidence Intervals (CI) are much higher than the threshold CI of [-1 ; +1]. The mean distances between the ASIS and PSIS landmarks and their respective CIs are included in the threshold CI. The mean and CI for IAP however, are not (mean=1.739 mm and CI=[1.417 ; 2.061]).

**Table 5-14 Results of the paired t-test (upper part of the table) and paired Wilcoxon tests (lower part of the table) for landmark reproducibility on scanned ilia. The greyed p-values correspond to significant differences between reproduced landmarks; CI = Confidence Interval**

Inter-observer landmark distance	t	Df	CI 95% (mm)	Mean difference (mm)	p-value
IAP-IAP	11.043	29	[1.417 ; 2.061]	1.739	<0.001
Inter-observer landmark distance	V	CI 95% (mm)	Pseudo-median (mm)	p-value	
ASIS-ASIS	465	[0.358 ; 0.661]	0.523	<0.001	
PSIS-PSIS	465	[0.534 ; 0.844]	0.664	<0.001	
ICS-ICS	465	[1.495 ; 2.969]	2.082	<0.001	

ii. Fifth lumbar vertebra

Eight landmarks were placed on the body of the fifth lumbar vertebrae (**Figure 5.3**).



**Figure 5.3 The eight landmarks placed on the bodies of the fifth lumbar vertebrae. RLSP = Right Lateral Superior Point; MPSP = Median Postero-Superior Point; LLSP = Left Lateral Superior Point; MASP = Median Antero-Superior Point; RLIP = Right Lateral Inferior Point; MPIP = Median Postero-Inferior Point; LLIP = Left Lateral Inferior Point; MAIP = Median Antero-Inferior Point**

Thirty individuals randomly selected for testing the repeatability and reproducibility of landmark positioning and variables.

*Repeatability*

The distance between two sets of homologous landmarks was calculated using the formula for vector norm (see section 4.2.2.) between the two landmarks. The mean, median, standard deviation, minimum and maximum values of the distances were calculated for each set of homologous landmarks (Table 5-15). The means were all inferior to 1 mm, as were the standard deviations.

**Table 5-15 Mean, median, standard deviation, minimum and maximum values of the distances between homologous lumbar landmarks placed by the same observer twice**

Intra-observer landmark distance	Distribution of the distances between repeated landmark positioning					
	N	Mean (mm)	Median (mm)	Standard deviation (mm)	Max (mm)	Min (mm)
MPSP-MPSP	30	0.838	0.815	0.519	2.288	0.081
MASP-MASP	30	0.61	0.581	0.318	1.327	0.084
RLSP-RLSP	30	0.805	0.967	0.464	2.059	0.145
LLSP-LLSP	30	0.839	0.733	0.443	1.955	0.239
MPIP-MPIP	30	0.602	0.529	0.394	1.506	0.304
MAIP-MAIP	30	0.643	0.576	0.428	2.326	0.141
RLIP-RLIP	30	0.739	0.702	0.486	1.829	0.082
LLIP-LLIP	30	0.807	0.772	0.409	1.693	0.115

The Shapiro-Wilk test for normality of the distances between landmarks assessed that distances followed a normal distribution for MPSP, MASP, RLSP and LLIP (Table 5-16).

**Table 5-16 Results of the Shapiro-Wilk test for the distribution of the differences between lumbar landmarks placed by the same observer twice. The greyed p-values correspond to a distribution significantly different from normality**

Intra-observer landmark distance	W	p-value
MPSP-MPSP	0.96	0.241
MASP-MASP	0.97	0.477
RLSP-RLSP	0.93	0.055
LLSP-LLSP	0.93	0.039
MPIP-MPIP	0.92	0.029
MAIP-MAIP	0.81	<0.001
RLIP-RLIP	0.93	0.048
LLIP-LLIP	0.964	0.384

All distances were significantly different than zero (p-values < 0.001). However, the means and



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Confidence Intervals (CI) of inter-landmark distances were all included in the threshold CI of [-1 ; +1], except for the CI for MPSP [0.644 ; 1.032] (**Table 5-17**).

**Table 5-17 Results of the paired t-tests (upper part of the table) and paired Wilcoxon tests (lower part of the table) for landmark repeatability on scanned fifth lumbar vertebrae. The greyed p-values correspond to significant differences between repeated landmarks; CI = Confidence Interval**

Intra-observer landmark distance	t	Df	CI 95% (mm)	Mean difference (mm)	p-value
MPSP-MPSP	8.841	29	[0.644 ; 1.032]	0.838	<0.001
MASP-MASP	10.512	29	[0.491 ; 0.729]	0.610	<0.001
RLSP-RLSP	9.507	29	[0.632 ; 0.978]	0.805	<0.001
LLIP-LLIP	10.803	29	[0.654 ; 0.959]	0.807	<0.001
Intra-observer landmark distance	V	CI 95% (mm)	Pseudo-median (mm)	p-value	
LLSP-LLSP	465	[0.630 ; 0.999]	0.799	<0.001	
MPIP-MPIP	465	[0.419 ; 0.743]	0.550	<0.001	
MAIP-MAIP	465	[0.479 ; 0.715]	0.585	<0.001	
RLIP-RLIP	465	[0.529 ; 0.915]	0.694	<0.001	

*Reproducibility*

The same eight lumbar landmarks were placed by an independent observer on the same 30 individuals. The distance between two sets of homologous landmarks was calculated using the formula for vector norm (**see section 4.2.2.**) between the two landmarks. The mean, median, standard deviation, minimum and maximum values of the distances were calculated for each set of homologous landmarks (**Table 5-18**).

**Table 5-18 Mean, median, standard deviation, minimum and maximum values of the distances between homologous lumbar landmarks placed by different observers**

Inter-observer landmark distance	Distribution of the distances between reproduced landmark positioning					
	N	Mean (mm)	Median (mm)	Standard deviation (mm)	Max (mm)	Min (mm)
MPSP-MPSP	30	0.796	0.779	0.475	1.996	0.120
MASP-MASP	30	0.815	0.614	0.531	2.075	0.094
RLSP-RLSP	30	1.440	1.351	0.844	4.167	0.180
LLSP-LLSP	30	1.413	1.472	0.850	3.92	0.241
MPIP-MPIP	30	0.955	0.904	0.667	3.602	0.130
MAIP-MAIP	30	0.604	0.591	0.317	1.448	0.063
RLIP-RLIP	30	0.955	0.792	0.693	2.705	0.082
LLIP-LLIP	30	0.950	0.880	0.605	2.585	0.198

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The means were all inferior to 1 mm, as were the standard deviations. The normality of the inter-landmark distances tested using a Shapiro-Wilk test showed that neither values were normally distributed (**Table 5-19**).

**Table 5-19 Results of the Shapiro-Wilk test for the distribution of the differences between lumbar landmarks placed by two observers. The greyed p-values correspond to a distribution significantly different from normality**

Inter-observer landmark distance	W	p-value
MPSP-MPSP	0.904	0.011
MASP-MASP	0.900	0.008
RLSP-RLSP	0.936	0.069
LLSP-LLSP	0.933	0.060
MPIP-MPIP	0.807	<0.001
MAIP-MAIP	0.974	0.646
RLIP-RLIP	0.924	0.035
LLIP-LLIP	0.925	0.036

All distances were significantly different than zero (p-values < 0.001). The means and Confidence Intervals (CI) of inter-landmark distances of MAIP, MPSP and LLIP are included in the threshold CI of [-1 ; +1]. The pseudo-medians of MASP, MPIP and RLIP are inferior to 1 mm, but the upper limits of their 95% CI are higher than 1 mm. RLSP and LLSP cannot be considered as reproducible as both their mean values and 95% CIs are higher than 1mm (**Table 5-20**).

**Table 5-20 Results of the paired t-tests (upper part of the table) and paired Wilcoxon tests (lower part of the table) for landmark reproducibility on scanned fifth lumbar vertebrae. The greyed p-values correspond to significant differences between reproduced landmarks; CI = Confidence Interval**

Inter-observer landmark distance	t	Df	CI 95% (mm)	Mean difference (mm)	p-value
RLSP-RLSP	9.345	29	[1.125 ; 1.755]	1.440	<0.001
LLSP-LLSP	9.104	29	[1.0956 ; 1.730]	1.413	<0.001
MAIP-MAIP	10.422	29	[0.485 ; 0.723]	0.604	<0.001
Inter-observer landmark distance	V	CI 95% (mm)	Pseudo-median (mm)	p-value	
MPSP-MPSP	465	[0.588 ; 0.92]	0.756	<0.001	
MASP-MASP	465	[0.552 ; 1.011]	0.729	<0.001	
MPIP-MPIP	465	[0.699 ; 1.078]	0.882	<0.001	
RLIP-RLIP	465	[0.654 ; 1.189]	0.896	<0.001	
LLIP-LLIP	465	[0.625 ; 0.971]	0.806	<0.001	

iii. Clavicle

Four landmarks were placed on the reconstructed clavicles of 30 randomly selected individuals (Figure 5.4).

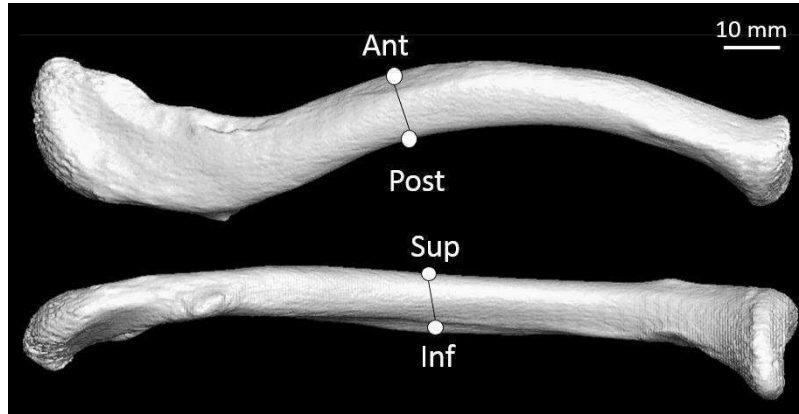


Figure 5.4 The four clavicular landmarks defining the antero-posterior (AP\_diam) and supero-inferior (SI\_diam) diameters at mid-length. Ant = point located at the intersection of the anterior plane and the sagittal plane at half-length of the clavicle; Post = point located at the intersection of the posterior plane and the sagittal plane at half-length of the clavicle; Sup = point located at the intersection of the superior plane and the sagittal plane at half-length of the clavicle; Inf = point located at the intersection of the inferior plane and the sagittal plane at half-length of the clavicle

Repeatability

These four landmarks were placed twice by the same observer on two separate occasions. The distance between two sets of homologous landmarks were calculated using the formula for vector norm (see section 4.2.2.) between the two landmarks. The mean, median, standard deviation, minimum and maximum values of the distances were calculated for each set of homologous landmarks (Table 5-21). The means were all inferior to 1 mm, as were the standard deviations. The maximum distances were relatively important, especially between the posterior landmarks (5.07 mm) and were all higher than 1 mm.

Table 5-21 Mean, median, standard deviation, minimum and maximum values of the distances between homologous clavicular landmarks placed by the same observer

Intra-observer landmark distance	N	Distribution of the distances between repeated landmark positioning				
		Mean (mm)	Median (mm)	Standard deviation (mm)	Max (mm)	Min (mm)
Sup-Sup	30	0.760	0.533	0.722	2.908	0.041
Inf-Inf	30	0.636	0.434	0.542	2.125	0.061
Ant-Ant	30	0.753	0.471	0.803	3.921	0.087
Post-Post	30	0.769	0.527	0.880	5.068	0.074

The normality of the inter-landmark distances tested using a Shapiro-Wilk test showed that

neither values followed a normal distribution (**Table 5-22**). Because none of the distances follow a normal distribution, non-parametric paired Wilcoxon tests were used to test repeatability of landmark positioning.

**Table 5-22 Results of the Shapiro-Wilk test for the distribution of the differences between clavicular landmarks placed by the same observer twice. The greyed p-values correspond to a distribution significantly different from normality**

Intra-observer landmark distance	W	p-value
Sup-Sup	0.858	<0.001
Inf-Inf	0.850	<0.001
Ant-Ant	0.747	<0.001
Post-Post	0.664	<0.001

The non-parametric paired Wilcoxon rank-signed test showed that the distances between two homologous landmarks placed twice by the same observer were significantly different from zero. However, the confidence intervals of the differences were included in the threshold 95% interval [-1 ; +1] (**Table 5-23**).

**Table 5-23 Results of the paired Wilcoxon tests for landmark repeatability on scanned clavicles. The greyed p-values correspond to significant differences between repeated landmarks; CI = Confidence Interval**

Intra-observer landmark distance	V	CI 95% (mm)	Pseudo-median (mm)	p-value
Sup-Sup	820	[0.407 ; 0.916]	0.693	<0.001
Inf-Inf	820	[0.379 ; 0.769]	0.540	<0.001
Ant-Ant	820	[0.412 ; 0.815]	0.566	<0.001
Post-Post	820	[0.428 ; 0.822]	0.642	<0.001

*Reproducibility*

The same four landmarks were placed by an independent observer on the same 30 individuals. As before, the mean, median, standard deviation, minimum and maximum values of the distances between homologous landmarks were calculated for each set (**Table 5-24**). Once again, the maximum distances were relatively considerable, especially between the inferior landmarks (5.161 mm) and were all over 1 mm.

The normality of the inter-landmark distances tested using a Shapiro-Wilk test showed that neither values were normally distributed (**Table 5-25**).

**Table 5-24 Mean, median, standard deviation, minimum and maximum values of the distances between homologous landmarks placed by different observers**

Inter-observer landmark distance	Distribution of the distances between reproduced landmark positioning					
	N	Mean (mm)	Median (mm)	Standard deviation (mm)	Max (mm)	Min (mm)
Sup-Sup	30	0.669	0.616	0.512	2.065	0.033
Inf-Inf	30	1.196	0.942	1.186	5.161	0.079
Ant-Ant	30	0.792	0.510	0.868	4.196	0.036
Post-Post	30	0.821	0.657	0.702	2.927	0.093

**Table 5-25 Results of the Shapiro-Wilk test for the distribution of the differences between clavicular landmarks placed by two observers. The greyed p-values correspond to a distribution significantly different from normality**

Inter-observer landmark distance	W	p-value
Sup-Sup	0.936	0.025
Inf-Inf	0.825	<0.001
Ant-Ant	0.747	<0.001
Post-Post	0.842	<0.001

Because none of the distances followed a normal distribution, non-parametric paired Wilcoxon tests were done to test the reproducibility of landmark positioning (**Table 5-26**). All distances were significantly different than zero (p-values < 0.001), so we have to conclude that the positioning of the inferior landmark is not reproducible, as the corresponding CI includes the value of 1 mm and the pseudo-median of the distance between two inferior landmarks is slightly higher than 1 mm (1.002 mm). However, the other CIs of inter-landmark distances are all included in the threshold CI of [-1 ; +1].

**Table 5-26 Results of the paired Wilcoxon tests for landmark reproducibility on scanned clavicles. The greyed p-values correspond to significant differences between reproduced landmarks; CI = Confidence Interval**

Inter-observer landmark distance	V	CI 95% (mm)	Pseudo-median (mm)	p-value
Sup-Sup	820	[0.480 ; 0.819]	0.646	<0.001
Inf-Inf	820	[0.698 ; 1.436]	1.002	<0.001
Ant-Ant	820	[0.437 ; 0.867]	0.594	<0.001
Post-Post	820	[0.527 ; 0.940]	0.684	<0.001

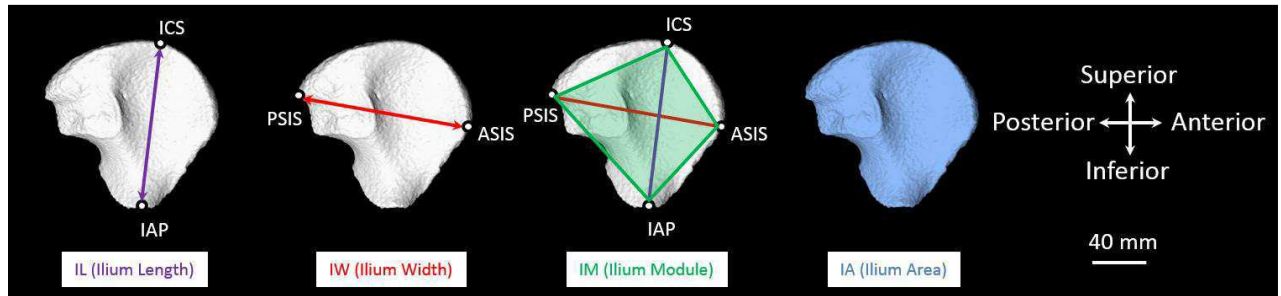
**b. Biometric variables**

i. Ilium

The four biometric variables measured on the reconstructed iliae of 30 randomly selected

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individuals were tested for repeatability and reproducibility. These variables include two unidimensional ones (IL and IW) and two bidimensional ones (IM and IA) (Figure 5.5).



**Figure 5.5** Four variables taken on the ilium. IL: Ilium Length; IW: Ilium Width; IM: Ilium Module; IA: Ilium Area

**Repeatability**

The mean and standard deviations of the variables were calculated for the series of homologous variables taken twice by the first observer (Table 5-27).

**Table 5-27** Means and standard deviations of the variables taken on iliae by the same observer twice (Observer 1 and Observer 1bis). \*Refers to bidimensional variables, expressed in mm<sup>2</sup>

Variable	Observer 1		Observer 1 bis	
	Mean (mm)	Standard deviation (mm)	Mean (mm)	Standard deviation (mm)
IL	90.080	22.773	90.003	22.874
IW	82.823	21.132	83.109	21.013
IM*	7916.592	3890.749	7941.737	3907.350
IA*	5561.896	2719.871	5566.882	2713.227

The Shapiro-Wilk test for assessing normality of the differences between the series of measurements was used and showed that no variables followed a normal distribution (Table 5-28). The differences are calculated as  $v_1 - v_{1bis}$ .

**Table 5-28** Results of the Shapiro-Wilk test for the distribution of the differences between iliac variables taken by the same observer twice. The greyed p-values correspond to a distribution significantly different from normality

Variable	W	p-value
IL	0.793	<0.001
IW	0.977	<0.001
IM	0.924	0.034
IA	0.888	0.004

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The differences between the two sets of the variables IM and IA are comparable to zero. Although the p-values associated to the tests done on IL and IW are inferior to 0.05, the corresponding CIs and pseudo-median values are all included in the threshold interval [-1 ; +1] (**Table 5-29**).

**Table 5-29 Results of the paired Wilcoxon tests for variable repeatability on scanned iliae. The greyed p-values correspond to significant differences between repeated variables. \*Refers to bidimensional variables, expressed in mm<sup>2</sup>; CI = Confidence Interval**

Variable	V	CI 95% (mm)	Pseudo-median (mm)	p-value
IL	331	[0.003 ; 0.263]	0.146	0.043
IW	119	[-0.512 ; -0.049]	-0.271	0.019
IM*	143	[-62.538 ; 1.599]	-31.193	0.067
IA*	196	[-34.903 ; 13.518]	-9.193	0.465

The intra-class correlation coefficients (ICC) indicate significant consistency rates (>0.95) between all sets of homologous variables measured twice by the same observer (**Table 5-30**).

**Table 5-30 Intra-Class Correlation (ICC) coefficient values and the associated 95% Confidence Interval (CI) for the consistency of the same observer on iliac variables**

Variable	F	CI 95%	ICC (consistency)	p-value
IL	11655	[0.999 ; 1]	0.9998	<0.001
IW	4087	[0.999 ; 1]	0.9996	<0.001
IM	6491	[0.999 ; 1]	0.9997	<0.001
IA	3782	[0.999 ; 1]	0.9995	<0.001

Because the p-values associated to the tests were significant, Bland-Altman plots were done on the differences between dry and scanned bone variables. Here we consider that two variables are comparable if less than 10% of the differences are found outside a 95% distribution range on a Bland-Altman plot. Therefore, there has to be fewer than four cases outside that range (10% of 30), materialised by the red dotted lines to assess variable equality.

All differences between homologous variables measured on the iliae are within the 95% range of the Bland-Altman plots, with less than 10% of differences outside said range (**Figure 5.6**). IW and IA have one outsider; IL and IM have none.

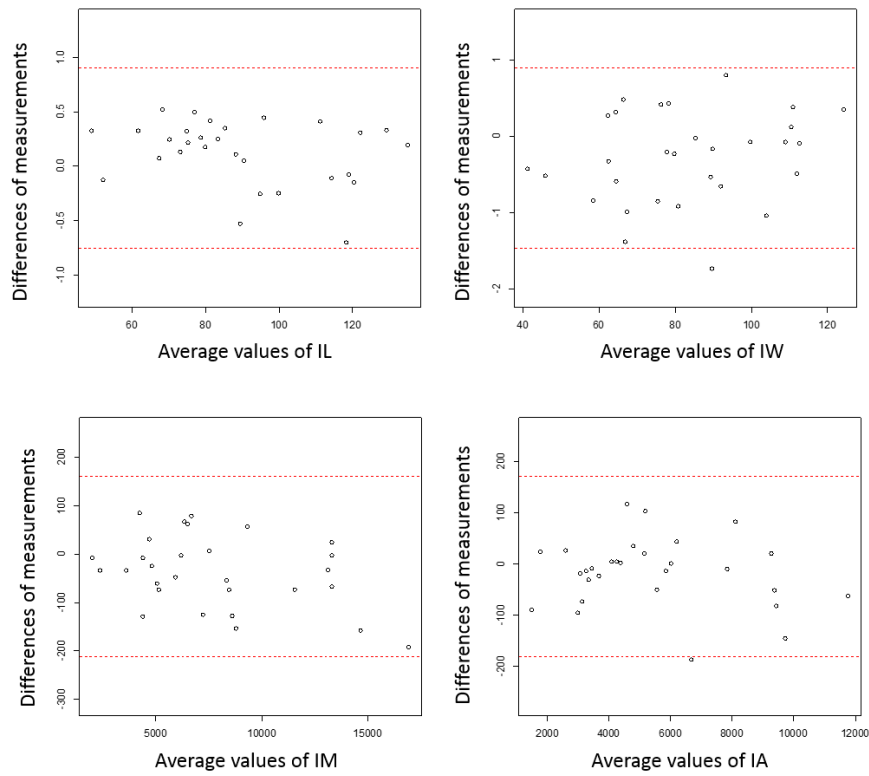


Figure 5.6 Bland-Altman plots of the differences between the four iliac variables taken on scanned bones by the same observer twice

We can conclude that in all four cases, there is no significant difference between the two sets of iliac biometric variables taken by the same observer.

*Reproducibility*

The mean and standard deviations of the iliac biometric variables were calculated for the series of homologous variables taken by two observers (Table 5-31).

Table 5-31 Means and standard deviations of the variables taken on iliae by a first observer (Observer 1) and by a second observer (Observer 2). \*Refers to bidimensional variables, expressed in mm<sup>2</sup>

Variable	Observer 1		Observer 2	
	Mean (mm)	Standard deviation (mm)	Mean (mm)	Standard deviation (mm)
IL	90.080	22.773	90.301	22.799
IW	82.823	21.132	83.461	20.957
IM*	7916.592	3890.749	7992.299	3890.623
IA*	5561.896	2719.871	5574.784	2740.761



The Shapiro-Wilk test for assessing normality of the differences between the series of measurements was used and showed that IL and IW followed a normal distribution (**Table 5-32**). The differences are calculated as  $v_1-v_2$ .

**Table 5-32 Results of the Shapiro-Wilk test for the distribution of the differences between iliac variables taken by two observers. The greyed p-values correspond to a distribution significantly different from normality**

Variable	W	p-value
IL	0.933	0.057
IW	0.965	0.403
IM	0.920	0.027
IA	0.864	0.001

The differences between the two sets of IA are comparable to zero. Although the p-values associated to the tests done on IL and IW are inferior to 0.05, the corresponding CIs and pseudo-median values are all included in the threshold interval [-1 ; +1] (**Table 5-33**). The confidence interval for IM does not include zero and the variable has a mean difference equal to -68.450 mm<sup>2</sup>.

**Table 5-33 Results of the paired t-test (upper part of the table) and paired Wilcoxon tests (lower part of the table) for variable reproducibility on scanned iliae. \*Refers to bidimensional variables, expressed in mm<sup>2</sup>; the greyed p-values correspond to significant differences between reproduced variables; CI = Confidence Interval**

Variable	t	Df	CI 95% (mm)	Mean difference (mm)	p-value
IW	-5.034	29	[-0.898 ; -0.379]	-0.638	<0.001
Variable	V		CI 95% (mm)	Pseudo-median (mm)	p-value
IL	100		[-0.342 ; -0.057]	-0.173	0.006
IM*	20		[-104.029 ; -41.998]	-68.450	<0.001
IA*	224		[-36.777 ; 34.443]	-3.482	0.871

The minimum value of IM in the test sample is  $IM_{min} = 2003.991 \text{ mm}^2$ . The minimum 5% error for this variable is therefore equal to  $\epsilon_{IM} = +/- 0.025 * IM_{min} = +/- 50.100 \text{ mm}^2$ .

The mean difference between reproduced measurements of IM is higher than  $\epsilon_{IM}$  and the corresponding 95% CI is larger than the one defined by  $\epsilon_{IM}$ .

However, the intra-class correlation coefficients (ICC) indicate significant agreement rates (>0.95) between all sets of homologous variables measured by both observers (**Table 5-34**).

Table 5-34 Intra-Class Correlation (ICC) coefficient values and the associated 95% Confidence Interval (CI) for the agreement of two observers on iliac variables

Variable	F	CI 95%	ICC (agreement)	p-value
IL	10875	[0.999 ; 1]	0.9998	<0.001
IW	2026	[0.998 ; 1]	0.9990	<0.001
IM	5419	[0.999 ; 1]	0.9996	<0.001
IA	2009	[0.998 ; 1]	0.9990	<0.001

All differences between homologous biometric iliac variables measured by the two observers are within the 95% range of the Bland-Altman plots, with less than 10% of differences outside said range (Figure 5.7). IL and IM have three outsiders; IW has one; IA has none.

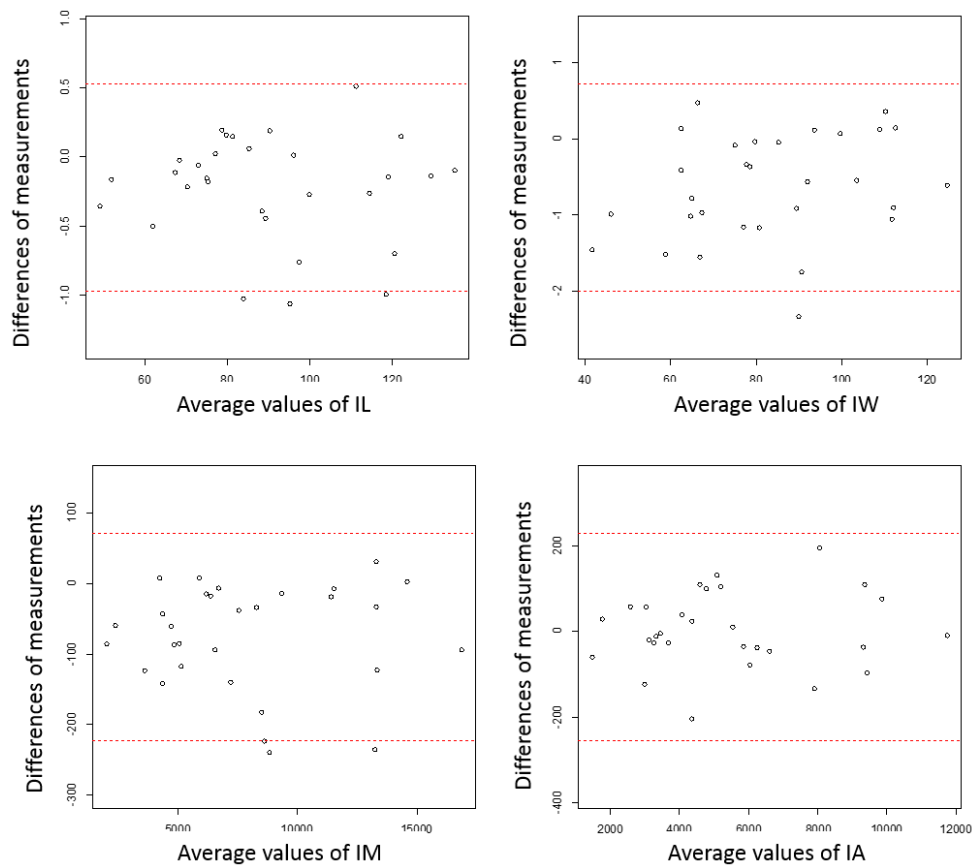


Figure 5.7 Bland-Altman plots of the differences between the four iliac variables taken on scanned bones by two observers

We can conclude that in all four cases, there is no significant difference between measurements taken on scanned iliae by two observers. All iliac variables present a technical measurement error of less than 5% when taken by one or two observers (Table 5-35). Reliability is high for all and always superior to 0.95.

Table 5-35 Intra- and inter-observer technical errors of measurement (TEM), percentages of TEM (%TEM) and reliability obtained for the iliac variables. \*Refers to bidimensional variables, expressed in mm<sup>2</sup>

Variable	Intra-observer		Inter-observer		Intra-observer	Inter-observer
	TEM (mm)	% TEM	TEM (mm)	% TEM	reliability	reliability
IL	0.299	0.332	0.309	0.343	0.999	0.999
IW	0.466	0.562	0.661	0.795	0.999	0.999
IM*	68.438	0.863	74.739	0.940	0.999	0.999
IA*	62.460	1.122	86.118	1.547	0.999	0.999

ii. Fifth lumbar vertebra

The ten biometric variables (**Figure 5.8**) measured on the reconstructed lumbar vertebrae of 30 randomly selected individuals were tested for repeatability and reproducibility. These include the eight unidimensional (UVL, UVW, LVL, LVW, PVH, AVH, RVH and LVH) and the two bidimensional (UVM and LVM) variables.

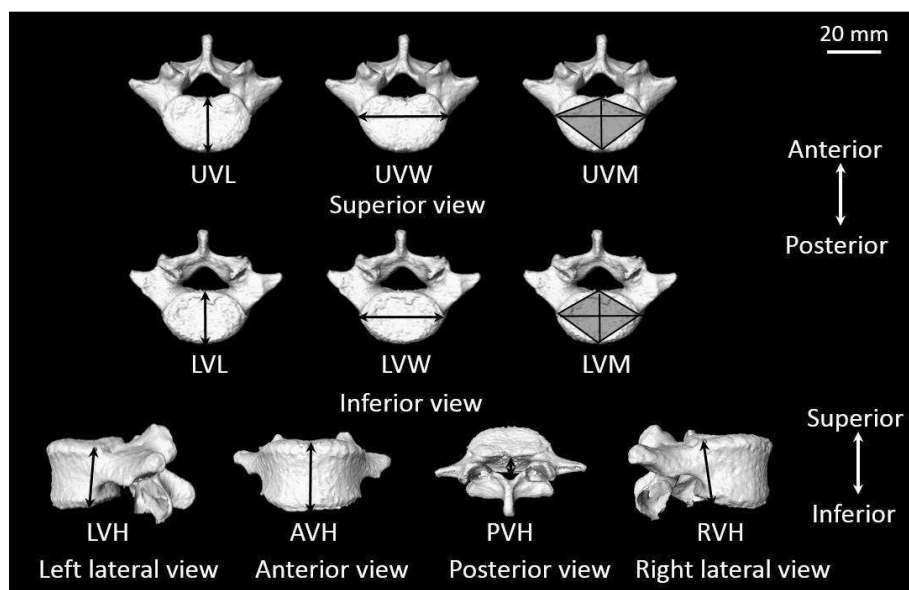


Figure 5.8 The ten variables measured on the fifth lumbar vertebrae. UVL: Upper Vertebral Length; UVW: Upper Vertebral Width; UVM: Upper Vertebral Module; LVH: Lower Vertebral Length; LVW: Lower Vertebral Width; LVM: Lower Vertebral Module; LVH: Left Vertebral Height; AVH: Anterior Vertebral Height; PVH: Posterior Vertebral Height; RVH: Right Vertebral Height

Repeatability

The mean and standard deviations of the variables were calculated for the series of homologous variables taken twice by the first observer (**Table 5-36**).

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**Table 5-36 Means and standard deviations of the variables taken on fifth lumbar vertebrae twice by the same observer (Observer 1 and Observer 1bis). \*Refers to bidimensional variables, expressed in mm<sup>2</sup>**

Variable	Observer 1		Observer 1 bis	
	Mean (mm)	Standard deviation (mm)	Mean (mm)	Standard deviation (mm)
UVL	22.002	6.736	22.096	6.699
UVW	36.298	9.287	36.283	9.339
UVM*	856.777	439.273	860.259	437.426
LVL	21.859	6.835	21.922	6.792
LVW	35.653	9.456	35.829	9.375
LVM*	839.128	431.749	843.734	430.297
PVH	13.976	5.741	13.761	5.681
AVH	16.331	5.650	16.392	5.637
RVH	13.278	6.142	13.113	5.957
LVH	12.893	5.937	13.019	6.201

The Shapiro-Wilk test for assessing normality of the differences between the series of measurements was used and showed that six variables followed a normal distribution: UVL, UVW, UVM, LVW, AVH and RVH (**Table 5-37**). The differences are calculated as  $v_1 - v_{1bis}$ . The differences between the two sets of the variables UVL, UVW, UVM, LVL, AVH, RVH and LVH are comparable to zero.

**Table 5-37 Results of the Shapiro-Wilk test for the distribution of the differences between lumbar variables taken twice by the same observer. The greyed p-values correspond to a distribution significantly different from normality**

Variable	W	p-value
UVL	0.949	0.163
UVW	0.949	0.158
UVM	0.976	0.713
LVL	0.845	<0.001
LVW	0.955	0.229
LVM	0.897	0.007
PVH	0.925	0.035
AVH	0.944	0.119
RVH	0.952	0.195
LVH	0.921	0.028

Although the p-values associated with the tests done on LVW and PVH are inferior to 0.05, the corresponding CIs and pseudo-median values are all included in the threshold interval [-1 ; +1] (**Table 5-38**). The confidence intervals for UVM and LVM do not include zero and have mean differences equal to -2.461 mm<sup>2</sup> and -6.210 mm<sup>2</sup> respectively.

The minimum value of UVM in the test sample is  $UVM_{min} = 172.300 \text{ mm}^2$  and the minimum value of

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IVM is  $IVM_{\min} = 155.123 \text{ mm}^2$ . The minimum 5% errors for these two variables are therefore equal to  $\epsilon_{UVM} = +/- 0.025 * UVM_{\min}$  and  $\epsilon_{IVM} = +/- 0.025 * IVM_{\min}$  respectively.

$\epsilon_{UVM} = +/- 4.308 \text{ mm}^2$  and  $\epsilon_{IVM} = +/- 3.878 \text{ mm}^2$ .

The mean difference between repeated measurements of UVM is inferior to  $\epsilon_{UVM}$ , but the mean of LVM is not inferior to  $\epsilon_{IVM}$  and both 95% CIs are larger than the ones defined by  $\epsilon_{UVM}$  and  $\epsilon_{IVM}$ .

**Table 5-38 Results of the paired t-tests (upper table) and paired Wilcoxon tests (lower table) for variable repeatability on scanned fifth lumbar vertebrae. \*Refers to bidimensional variables, expressed in mm<sup>2</sup>; the greyed p-values correspond to significant differences between repeated variables; CI = Confidence Interval**

Variable	t	Df	CI 95% (mm)	Mean difference (mm)	p-value
UVL	-0.870	29	[-0.316 ; 0.127]	-0.094	0.391
UVW	0.524	29	[-0.192 ; 0.324]	0.066	0.604
UVM*	-0.45	29	[-13.645 ; 8.724]	-2.461	0.651
LVW	-2.252	29	[-0.335 ; -0.016]	-0.175	0.032
AVH	-0.587	29	[-0.274 ; 0.152]	-0.061	0.562
RVH	1.462	29	[-0.066 ; 0.396 ]	0.165	0.016
Variable	V		CI 95% (mm)	Pseudo-median (mm)	p-value
LVL	154		[-0.238 ; 0.023]	-0.125	0.109
LVM*	126		[-10.870 ; -1.052]	-6.210	0.028
PVH	344		[0.056 ; 0.459]	0.280	0.021
LVH	218		[-0.359 ; 0.251]	-0.077	0.777

All differences between homologous lumbar variables are within the 95% range of the Bland-Altman plots, with less than 10% of differences outside said range (**Figure 5.9**). UVW has three outsiders; UVM, LVW, LVM, AVH and LVH have two, UVL, LVL and RVH have one, PVH has none.

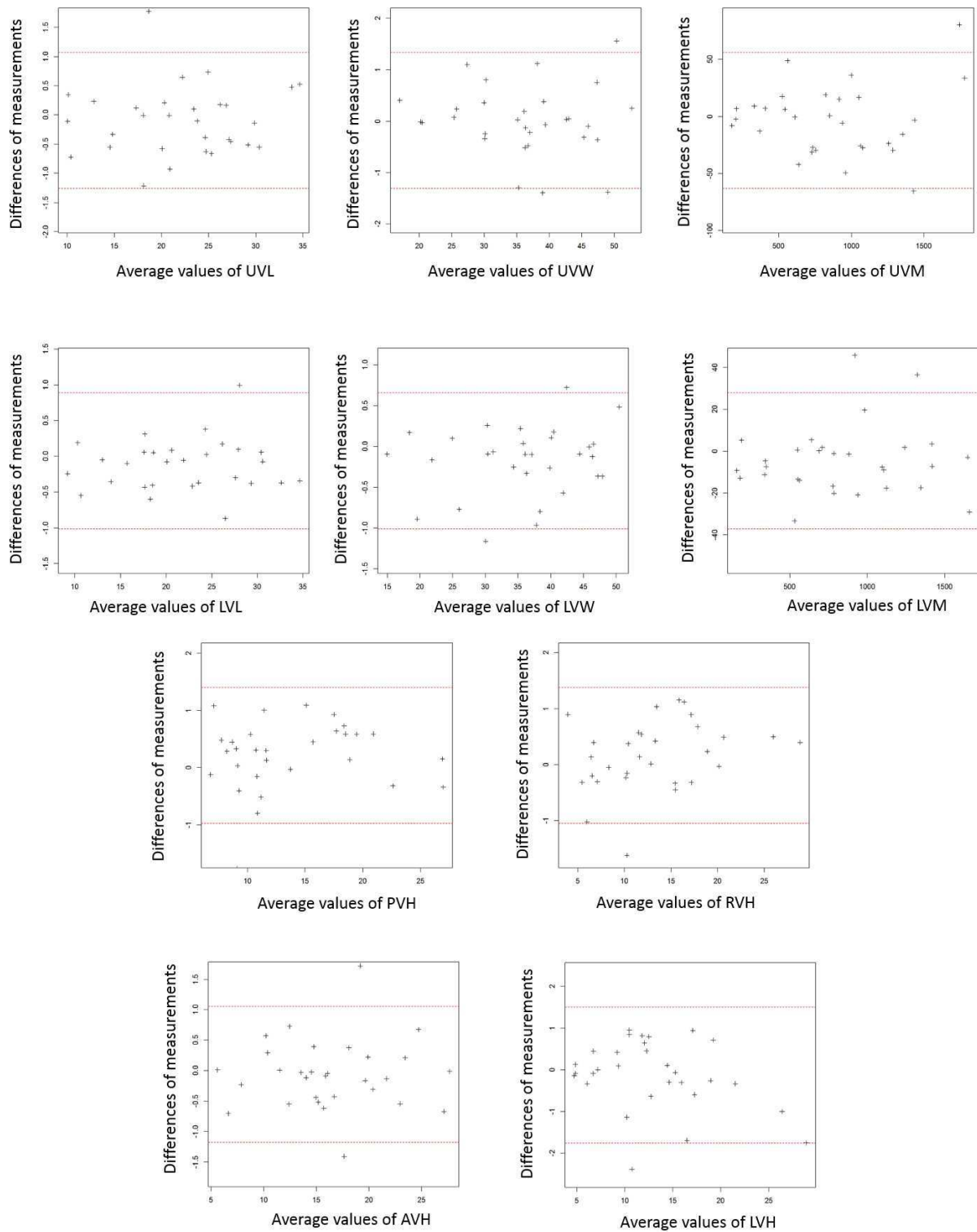


Figure 5.9 Bland-Altman plots of the differences between the ten lumbar variables taken on scanned bones by the same observer twice

However, the intra-class correlation coefficients (ICC) indicate significant consistency rates (>0.95) between all sets of homologous variables measured twice by the same observer (Table 5-39).

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**Table 5-39 Intra-Class Correlation (ICC) coefficient values and the associated 95% Confidence Interval (CI) for the consistency of the same observer on fifth lumbar vertebrae variables**

Variable	F	CI 95%	ICC (consistency)	p-value
UVL	514	[0.992 ; 0.998]	0.996	<0.001
UVW	789	[0.995 ; 0.999]	0.997	<0.001
UVM	847	[0.995 ; 0.999]	0.998	<0.001
LVL	798	[0.995 ; 0.999]	0.997	<0.001
LVW	1713	[0.998 ; 0.999]	0.999	<0.001
LVM	2570	[0.998 ; 1]	0.999	<0.001
PVH	325	[0.987 ; 0.997]	0.994	<0.001
AVH	401	[0.990 ; 0.998]	0.995	<0.001
RVH	367	[0.989 ; 0.997]	0.995	<0.001
LVH	215	[0.981 ; 0.996]	0.991	<0.001

We can conclude that in all ten cases, there is no significant difference between the two sets of lumbar variables taken by the same observer.

*Reproducibility*

The mean and standard deviations of the lumbar variables were calculated for the series of homologous variables taken by two observers (**Table 5-40**).

**Table 5-40 Means and standard deviations of the variables taken on fifth lumbar vertebrae by a first observer (Observer 1) and by a second observer (Observer 2). \*Refers to bidimensional variables, expressed in mm<sup>2</sup>**

Variable	Observer 1		Observer 2	
	Mean (mm)	Standard deviation (mm)	Mean (mm)	Standard deviation (mm)
UVL	22.002	6.736	22.222	6.840
UVW	36.349	9.289	36.392	9.369
UVM*	857.798	438.960	868.387	448.908
LVL	21.859	6.835	22.332	6.952
LVW	35.653	9.456	35.903	9.457
LVM*	839.128	431.749	862.996	444.496
PVH	13.976	5.741	13.534	5.383
AVH	16.331	5.650	16.135	5.695
RVH	13.278	6.142	12.510	5.798
LVH	12.893	5.937	12.753	5.587

The Shapiro-Wilk test for assessing normality of the differences between the series of measurements was used and showed that seven variables followed a normal distribution: UVL, UVW, UVM, PVH,

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AVH, RVH and LVH (Table 5-41). The differences are calculated as  $v_1-v_2$ .

**Table 5-41 Results of the Shapiro-Wilk test for the distribution of the differences between lumbar variables taken by two observers. The greyed p-values correspond to a distribution significantly different from normality**

Variable	W	p-value
UVL	0.980	0.837
UVW	0.969	0.509
UVM	0.975	0.677
LVL	0.861	0.001
LVW	0.869	0.002
LVM	0.887	0.004
PVH	0.965	0.413
AVH	0.971	0.573
RVH	0.967	0.451
LVH	0.971	0.576

The differences between the two sets of the variables UVW, AVH and LVH are comparable to zero. Although the p-values associated with the tests done on UVL, LVL, LVW and PVH are inferior to 0.05, the corresponding CIs and pseudo-median values are all included in the threshold interval [-1 ; +1] (Table 5-42).

**Table 5-42 Results of the paired t-test (upper table) and paired Wilcoxon tests (lower table) for variable reproducibility on scanned fifth lumbar vertebrae. \*Refers to bidimensional variables, expressed in mm<sup>2</sup>; the greyed p-values correspond to significant differences between reproduced variables; CI = Confidence Interval**

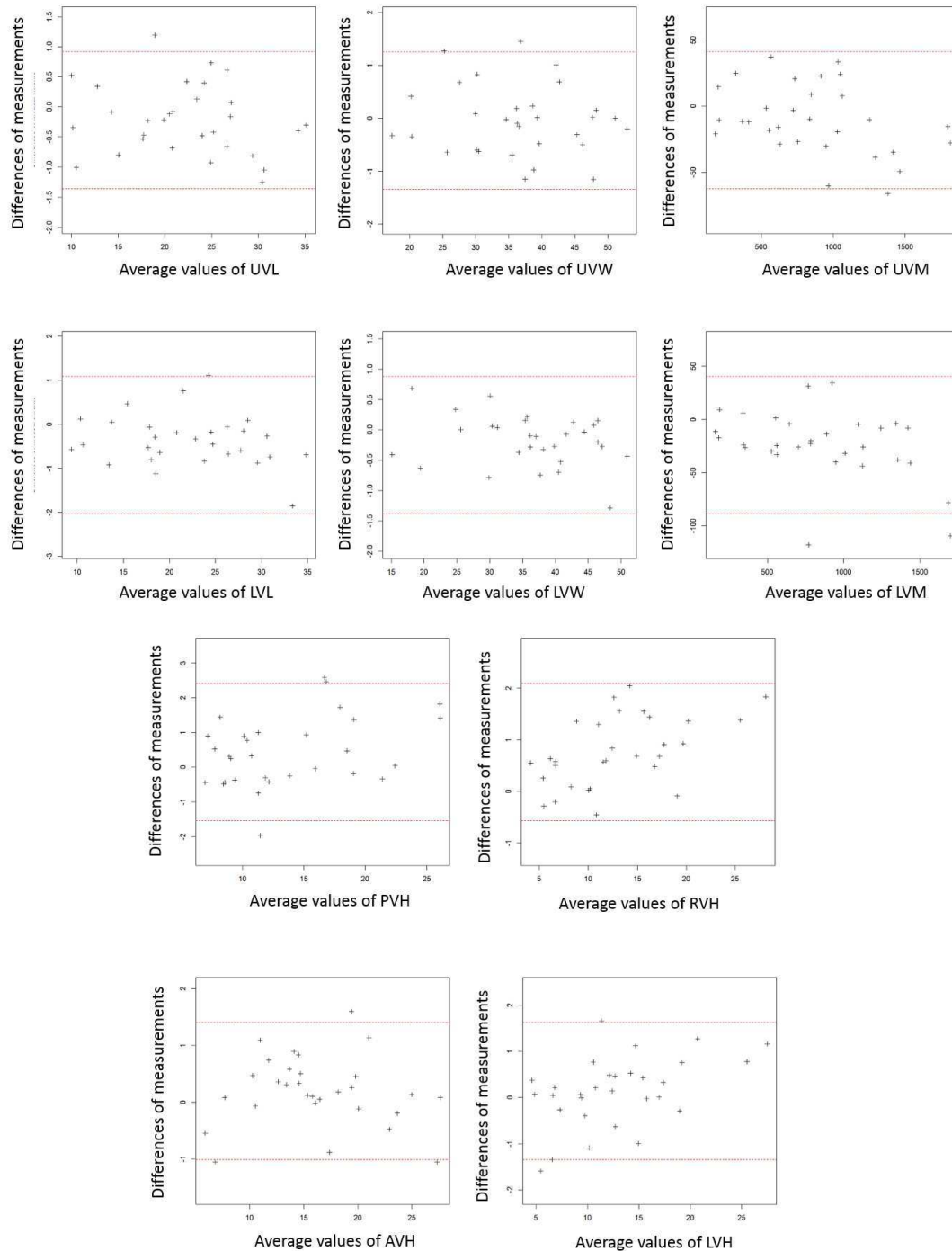
Variable	t	Df	CI 95% (mm)	Mean difference (mm)	p-value
UVL	-2.078	29	[-0.438 ; -0.003]	-0.221	0.047
UVW	-0.355	29	[-0.290 ; 0.204]	-0.043	0.726
UVM*	-2.189	29	[-20.484 ; -0.694]	-10.589	0.037
PVH	2.396	29	[0.065 ; 0.819]	0.442	0.023
AVH	1.737	29	[-0.035 ; 0.426]	0.196	0.093
RVH	6.184	29	[0.514 ; 1.021]	0.767	<0.001
LVH	1.014	29	[-0.142 ; 0.422]	0.140	0.319
Variables	V	CI 95% (mm)	Pseudo-median (mm)	p-value	
LVL	73	[-0.634 ; -0.213]	-0.426	<0.001	
LVW	123	[-0.361 ; -0.028]	-0.185	0.023	
LVM*	58	[-30.325 ; -12.122]	-21.093	<0.001	

The confidence intervals for UVM and LVM do not include zero. The mean differences for these two variables are equal to -10.589 mm<sup>2</sup> and -21.093 mm<sup>2</sup> respectively and are much higher than  $\epsilon_{UVM}$  and  $\epsilon_{LVM}$ . We cannot assess sufficient reproducibility for these two variables using statistical tests.



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All differences between homologous lumbar variables measured by the two observers are within the 95% range of the Bland-Altman plots, with less than 10% of differences outside said range (**Figure 5.10**).



**Figure 5.10 Bland-Altman plots of the differences between the ten lumbar variables taken on scanned bones by two observers**

PVH, AVH and LVH have three outsiders; UVW and LVM have two, UVL; UVM and LVL have one; LVW

and RVH have none.

All measurement differences are in the 95% range with less than 10% of measurement differences outside said range.

However, the intra-class correlation coefficients (ICC) indicate significant agreement rates (>0.95) between all sets of homologous variables measured by both observers (**Table 5-43**).

**Table 5-43 Intra-Class Correlation (ICC) coefficient values and the associated 95% Confidence Interval (CI) for the agreement of two observers for fifth lumbar vertebra variables**

Variable	F	CI 95%	ICC (agreement)	p-value
UVL	490	[0.992 ; 0.998]	0.996	<0.001
UVW	817	[0.995 ; 0.999]	0.998	<0.001
UVM	996	[0.996 ; 0.999]	0.998	<0.001
LVL	226	[0.982 ; 0.996]	0.991	<0.001
LVW	930	[0.996 ; 0.999]	0.998	<0.001
LVM	473	[0.991 ; 0.998]	0.996	<0.001
PVH	104	[0.961 ; 0.991]	0.981	<0.001
AVH	316	[0.987 ; 0.997]	0.994	<0.001
RVH	137	[0.970 ; 0.993]	0.986	<0.001
LVH	231	[0.982 ; 0.996]	0.991	<0.001

Six unidimensional lumbar variables taken by one or two observers present a technical measurement error of less than 1 mm and a %TEM of less than 5% (**Table 5-44**).

**Table 5-44 Intra- and inter-observer technical errors of measurement (TEM), percentages of TEM (%TEM) and reliability obtained for the fifth lumbar variables. \*Refers to bidimensional variables, expressed in mm<sup>2</sup>; the greyed values correspond to %TEM higher than the error threshold fixed at 5%**

Variable	Intra-observer		Inter-observer		Intra-observer reliability	Inter-observer reliability
	TEM (mm)	% TEM	TEM (mm)	% TEM		
UVL	0.418	1.898	0.433	1.960	0.996	0.996
UVW	0.469	1.291	0.461	1.268	0.997	0.998
UVM*	21.283	2.479	19.887	2.304	0.998	0.998
LVL	0.341	1.557	0.647	2.928	0.997	0.991
LVW	0.322	0.900	0.438	1.225	0.999	0.998
LVM*	12.021	1.429	28.462	3.344	0.999	0.996
PVH	0.448	3.228	0.769	5.587	0.994	0.981
AVH	0.398	2.434	0.450	2.774	0.995	0.994
RVH	0.446	3.379	0.720	5.581	0.994	0.985
LVH	0.584	4.505	0.535	4.173	0.991	0.991

The two variables PVH and RVH present a TEM lower than 1 mm, but a %TEM higher than 5%. The two bidimensional variables UVM and IVM have %TEM lower than 5%, but relatively high values of TEM. Intra- and inter-observer reliabilities are high for all variables and always superior to 0.95.

We can conclude that in all ten cases, there is no significant difference between measurements taken on scanned fifth lumbar vertebrae by two observers.

iii. Clavicle

The five biometric variables (**Figure 5.11**) measured on reconstructed clavicles of 30 randomly selected individuals of both sexes (15 males and 15 females) and all age groups (two per age group) were tested for repeatability and reproducibility.



**Figure 5.11** Variables measured on the clavicles. Min\_Diam: minimum diameter of the clavicle at mid-length; Max\_diam: maximum diameter of the clavicle at mid-length; SI\_diam: Supero-Inferior diameter of the clavicle at mid-length; AP\_diam: Antero-Posterior diameter of the clavicle at mid-length; Ln: Maximum length of the clavicle

*Repeatability*

The mean and standard deviations of the variables were calculated for the series of homologous variables measured twice by the first observer (**Table 5-45**).

The Shapiro-Wilk test for assessing normality of the differences between the series of measurements was used and showed that no variables followed a normal distribution (**Table 5-46**). The differences are calculated as  $V_1 - V_{1bis}$ .

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**Table 5-45 Mean and standard deviation of the clavicular variables measured twice by the same observer (Observer 1 and Observer 1bis)**

Variable	Observer 1		Observer 1 bis	
	Mean (mm)	Standard deviation (mm)	Mean (mm)	Standard deviation (mm)
Ln	110.396	29.013	110.434	28.936
Max_diam	10.125	2.682	10.094	2.676
Min_diam	8.191	2.340	8.163	2.336
AP_diam	9.465	2.480	9.376	2.565
SI_diam	8.404	2.316	8.613	2.296

**Table 5-46 Results of the Shapiro-Wilk test for the distribution of the differences between clavicular variables take twice by the same observer. The greyed p-values correspond to a distribution significantly different from normality**

Variable	W	p-value
Ln	0.870	<0.001
Max_diam	0.926	0.012
Min_diam	0.810	<0.001
AP_diam	0.888	<0.001
SI_diam	0.651	<0.001

The differences between the two sets of Ln are comparable to zero. Although the p-values associated with the tests done on the other four variables are inferior to 0.05, the corresponding CIs and pseudo-median values are all included in the threshold interval [-1 ; +1] (**Table 5-47**).

**Table 5-47 Results of the paired Wilcoxon tests for variable repeatability on scanned clavicles. The greyed p-values correspond to significant differences between repeated variables; CI = Confidence Interval**

Variable	V	CI 95% (mm)	Pseudo-median (mm)	p-value
Ln	289	[-0.110 ; 0.020]	-0.035	0.240
Max_diam	818	[0.023 ; 0.038]	0.030	<0.001
Min_diam	817	[0.017 ; 0.030]	0.023	<0.001
AP_diam	564	[0.005 ; 0.179]	0.093	0.038
SI_diam	187	[-0.216 ; -0.041]	-0.116	0.002

Moreover, the intra-class correlation coefficients (ICC) indicate a significant consistency higher than 0.95 between all sets of homologous variables, with three cases of perfect consistency (Ln, Max\_diam and Min\_diam) (**Table 5-48**).

Table 5-48 Intra-Class Correlation (ICC) coefficient values and the associated 95% Confidence Interval (CI) for the consistency of the same observer on clavicular variables

Variable	F	CI 95%	ICC (consistency)	p-value
Ln	48765	[1 ; 1]	1	<0.001
Max_diam	18799	[1 ; 1]	1	<0.001
Min_diam	15290	[1 ; 1]	1	<0.001
AP_diam	136	[0.973 ; 0.992]	0.985	<0.001
SI_diam	76.2	[0.952 ; 0.986]	0.974	<0.001

Bland-Altman plots show that less than 10% of the individuals are outside the 95% range of acceptable values for differences between variables (**Figure 5.12**).

Two individuals are outside the range for AP\_diam and Max\_diam, and only one is outside for Ln, SI\_diam and Min\_diam. We can conclude that in all five cases, there are no significant differences between variables measured twice on scanned clavicles by the same observer.

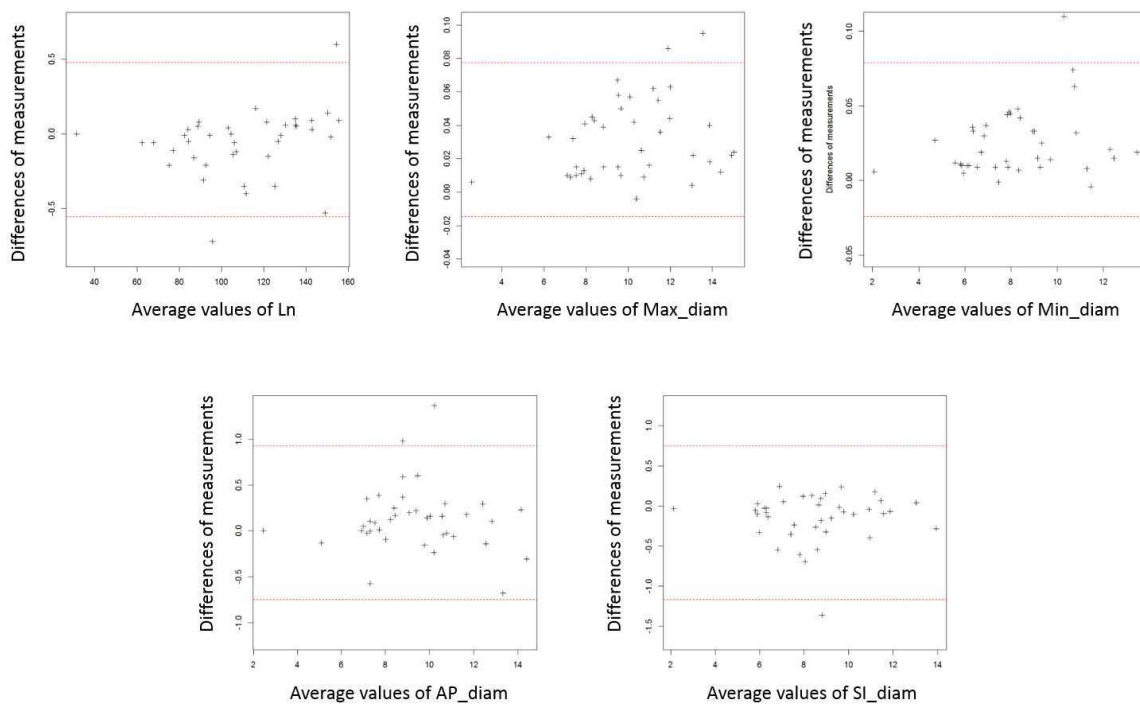


Figure 5.12 Bland-Altman plots of the differences between the five clavicular variables taken on reconstructed scanned bones by the same observer twice

*Reproducibility*

The mean and standard deviations of the variables were calculated for the series of homologous variables taken by two observers (**Table 5-49**).

**Table 5-49 Means and standard deviations of the clavicular variables taken by a first observer (Observer 1) and a second observer (Observer 1bis)**

Variable	Observer 1		Observer 2	
	Mean (mm)	Standard deviation (mm)	Mean (mm)	Standard deviation (mm)
Ln	110.396	29.013	110.433	28.940
Max_diam	10.125	2.682	10.147	2.698
Min_diam	8.191	2.340	8.213	2.364
AP_diam	9.465	2.480	9.642	2.575
SI_diam	8.404	2.316	8.664	2.373

The Shapiro-Wilk test for assessing normality of the differences between the series of measurements was used and showed that only Ln followed a normal distribution (**Table 5-50**). The differences are calculated as  $v_1-v_2$ .

**Table 5-50 Results of the Shapiro-Wilk test for the distribution of the differences between clavicular variables taken by two observers.** The greyed p-values correspond to a distribution significantly different from normality

Variable	W	p-value
Ln	0.982	0.767
Max_diam	0.217	<0.001
Min_diam	0.219	<0.001
AP_diam	0.899	0.002
SI_diam	0.845	<0.001

The differences between the two sets of Ln are comparable to zero. Although the p-values associated to the tests done on the other four variables are inferior to 0.05, the corresponding CIs and pseudo-median values are all included in the threshold interval [-1 ; +1] (**Table 5-51**).

**Table 5-51 Results of the paired t-test (upper table) and paired Wilcoxon tests (lower table) for variable reproducibility on scanned clavicles.** The greyed p-values correspond to significant differences between reproduced variables; CI = Confidence Interval

Variable	t	Df	CI 95% (mm)	Mean difference (mm)	p-value
Ln	-1.098	39	[-0.105 ; 0.031]	-0.037	0.279
Variable	V	CI 95% (mm)	Pseudo-median (mm)	p-value	
Max_diam	720.5	[0.0125 ; 0.029]	0.020	<0.001	
Min_diam	676.5	[0.0075 ; 0.022]	0.015	<0.001	
AP_diam	214	[-0.248 ; -0.032]	-0.123	0.008	
SI_diam	194	[-0.342 ; -0.047]	-0.166	0.003	

Moreover, the intra-class correlation coefficients (ICC) indicate significant agreement rates (>0.95) between all sets of homologous variables measured by two observers, with one case of perfect

agreement (Ln) (Table 5-52).

Table 5-52 Intra-Class Correlation (ICC) coefficient values and the associated 95% Confidence Interval (CI) for the agreement of two observers on clavicular variables

Variable	F	IC 95%	ICC (agreement)	p-value
Ln	73579	[1 ; 1]	1	<0.001
Max_diam	391	[0.990 ; 0.997]	0.995	<0.001
Min_diam	391	[0.990 ; 0.997]	0.995	<0.001
AP_diam	167	[0.978 ; 0.994]	0.988	<0.001
SI_diam	71	[0.948 ; 0.985]	0.972	<0.001

All differences between homologous clavicular variables are within the 95% range of the Bland-Altman plots, with less than 10% of differences outside said range (Figure 5.13). AP\_diam and Ln have two outsiders; SI\_diam has one; Max\_diam and Min\_diam have none.

All clavicular variables taken by one or two observers present a technical measurement error of less than 0.5 mm and a %TEM of less than 5% (Table 5-53). Intra- and inter-observer reliability is high for all and always superior to 0.95.

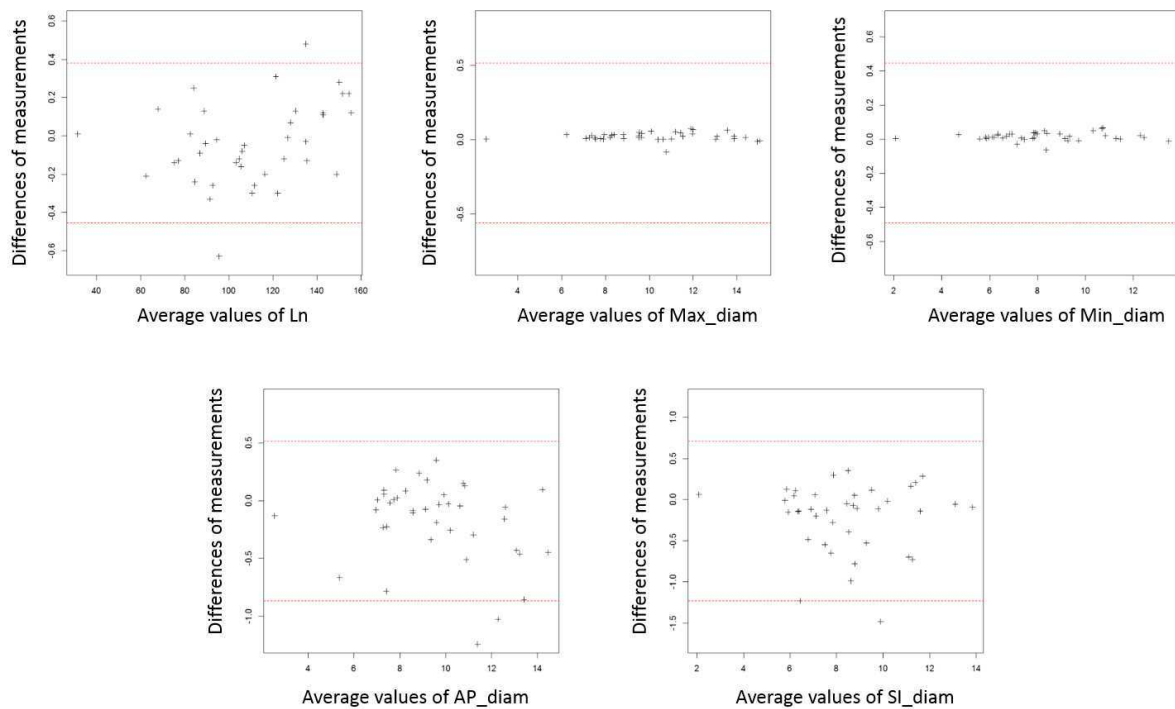


Figure 5.13 Bland-Altman plots of the differences between the five clavicular variables taken on reconstructed scanned bones by two observers

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**Table 5-53** Intra- and inter-observer technical errors of measurement (TEM), percentages of TEM (%TEM) and reliability obtained for the clavicular variables

Variable	Intra-observer		Inter-observer		Intra-observer	Inter-observer
	TEM (mm)	% TEM	TEM (mm)	% TEM	reliability	reliability
Ln	0.178	0.156	0.165	0.145	0.999	0.999
Max_diam	0.031	0.361	0.222	2.110	0.999	0.993
Min_diam	0.031	0.297	0.194	2.294	0.999	0.993
AP_diam	0.322	3.267	0.315	3.153	0.983	0.983
SI_diam	0.415	4.758	0.399	4.553	0.965	0.969

We can conclude that in all five cases, there is no significant difference between variables taken on scanned clavicles by different observers.

Overall, at least three out of four statistical measures give significant results for variable repeatability and reproducibility for all the biometric variables used in this study, except for reproducibility of PVH and RVH, which only gave two (**Table 5-54**).

**Table 5-54** Summary of the results from the statistical tests done to assess repeatability and reproducibility of the biometric variables. ICC = Intra-Class Correlation coefficient; TEM = Technical Error of Measurement

Bone	Variable	Repeatability				Reproducibility			
		Wilcoxon/t-test	Bland-Altman	ICC	TEM - % TEM	Wilcoxon/t-test	Bland-Altman	ICC	TEM / % TEM
Ilium	IL	✗	✓	✓	✓	✗	✓	✓	✓
	IW	✗	✓	✓	✓	✗	✓	✓	✓
	IM	✓	✓	✓	✓	✗	✓	✓	✓
	IA	✓	✓	✓	✓	✓	✓	✓	✓
Fifth lumbar vertebra	UVL	✓	✓	✓	✓	✗	✓	✓	✓
	UVW	✓	✓	✓	✓	✓	✓	✓	✓
	UVM	✓	✓	✓	✓	✗	✓	✓	✓
	LVL	✓	✓	✓	✓	✗	✓	✓	✓
	LVW	✗	✓	✓	✓	✗	✓	✓	✓
	LVM	✗	✓	✓	✓	✗	✓	✓	✓
	PVH	✗	✓	✓	✓	✗	✓	✓	✗
	AVH	✓	✓	✓	✓	✓	✓	✓	✓
	RVH	✗	✓	✓	✓	✗	✓	✓	✗
	LVH	✗	✓	✓	✓	✓	✓	✓	✓
Clavicle	Ln	✓	✓	✓	✓	✓	✓	✓	✓
	Max_diam	✗	✓	✓	✓	✗	✓	✓	✓
	Min_diam	✗	✓	✓	✓	✗	✓	✓	✓
	AP_diam	✗	✓	✓	✓	✗	✓	✓	✓
	SI_diam	✗	✓	✓	✓	✗	✓	✓	✓



**c. Non-biometric variables**

Repeatability and reproducibility of the three-level staging system (0-1-2) was tested on 99 individuals of both sexes (48 females and 51 males) and all twenty age groups (0 to 19 years) from the Marseilles study sample (Figure 5.14).

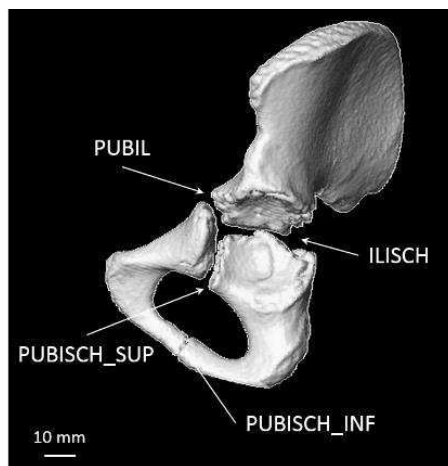


Figure 5.14 Four epiphyseal sites of the iliac bone for which maturation was assessed. PUBISCH\_INF: Inferior ischio-pubic epiphysis; PUBISCH\_SUP: Superior ischio-pubic epiphysis; PUBIL: Pubo-iliac epiphysis; ILISCH: Ilio-ischiatic epiphysis

Repeatability and reproducibility of maturation staging at the four epiphyseal sites were assessed using Cohen’s Kappa for intra-observer consistency and inter-observer agreement. All four epiphyseal sites were assessed twice by the same observer and once by an independent observer on the left iliac bones. Repeatability and reproducibility of staging was sufficient for all four epiphyseal sites (Table 5-55) and corresponded to almost perfect agreement (or consistency) for every site.

Table 5-55 Cohen’s kappa values of the maturation stages for the four epiphyseal sites of the iliac bone and their corresponding agreement ratings for repeatability and reproducibility of staging

Epiphyseal site	Repeatability		Reproducibility	
	Cohen’s kappa	Strength of agreement	Cohen’s kappa	Strength of agreement
PUBISCH_INF	0.881	Almost Perfect	0.882	Almost Perfect
PUBISCH_SUP	0.949	Almost Perfect	0.916	Almost Perfect
PUBIL	0.930	Almost Perfect	0.831	Almost Perfect
ILISCH	0.952	Almost Perfect	0.952	Almost Perfect

Therefore, the three-stage maturation rating adopted for this study is reliable, repeatable and reproducible.

**Conclusions on landmark positioning and variables obtained on scanned bone surfaces:**

Landmark positioning:

- Three out of four clavicular landmarks (Ant, Post and Sup) are sufficiently repeatable and reproducible. The Inf landmark is not sufficiently reproducible
- Seven out of eight lumbar vertebra landmarks are sufficiently repeatable. MPSP is neither sufficiently repeatable nor reproducible. Six lumbar landmarks are sufficiently reproducible. The two superior lateral landmarks (RLSP and LLSP) are not
- Two ilium landmarks, ASIS and PSIS, are sufficiently repeatable and reproducible. The other two ilium landmarks, ICS and IAP, are not

Variables:

- The five clavicular variables Ln, AP\_diam, SI\_diam, Min\_diam and Max\_diam are sufficiently repeatable and reproducible with a reliability > 95%
- The ten lumbar vertebra variables are sufficiently repeatable and reproducible with a reliability > 95%, although PVH and LVH have higher errors ( $p < 0.05$ )
- The four biometric iliac variables are all sufficiently repeatable and reproducible with a reliability > 95%
- The three-stage maturation assessment system of the iliac bone is sufficiently repeatable and reproducible with “almost perfect” agreement rates

## 5.2. Descriptive statistics of the sample from Marseilles

Complete descriptive statistics of all bones studied in the Marseilles sample, the Luis Lopes and Toulouse test samples (clavicle, fifth lumbar vertebra, ilium and iliac bone) can be found in tables in **appendices B to C**. These tables present the mean, standard deviation, median, minimum and maximum values of each variable used in the study per annual age group and can be used as abacuses.

### 5.2.1. Age and sex ratios

The homogeneity of the distribution by age and sex in the samples was assessed using Chi-Squared tests ( $p > 0.05$ ). The tests were done on the total number of individuals in the Marseilles sample and for each subsample according to the four bones studied (clavicle, fifth lumbar vertebra, ilium and coxal bone). All bone samples have homogeneous sex (**Table 5-56**) and age (**Table 5-57**) ratios ( $p > 0.05$ ).

**Table 5-56 Results of the Chi-squared test to assess the homogeneity of age ratio in the total samples from Marseilles**

Samples	Chi-squared	Df	p-value
<b>Marseilles total sample</b>	11.571	19	0.903
Marseilles clavicle sample	11.556	19	0.904
Marseilles L5 sample	13.700	19	0.801
Marseilles coxal bone sample	14.182	19	0.773
Marseilles ilium sample	7.424	12	0.828

Because homogeneous age and sex ratios are necessary to avoid over- or under-representation of age groups or sex that could bias the age estimations (**see section 3.1.2.b.**), chi-squared tests were also done on the four study samples from Marseilles for each bone studied

**Table 5-57 Results of the Chi-squared test to assess the homogeneity of sex ratio in the total samples from Marseilles**

Samples	Chi-squared	Df	p-value
<b>Marseilles total sample</b>	12.682	19	0.854
Marseilles clavicle sample	8.132	19	0.985
Marseilles L5 sample	16.316	19	0.636
Marseilles coxal bone sample	14.576	19	0.749
Marseilles ilium sample	10.930	12	0.535

Age (**Table 5-58**) and sex (**Table 5-59**) ratios are respected in all four study samples.

**Table 5-58 Results of the Chi-squared test to assess the homogeneity of age ratios in the different study samples from Marseilles**

Samples	Chi-squared	Df	p-value
Clavicle study sample	7.189	19	0.993
L5 study sample	5.881	19	0.998
Coxal bone study sample	11.632	19	0.901
Ilium study sample	4.523	12	0.972

**Table 5-59 Results of the Chi-squared test to assess the homogeneity of sex ratios in the different study samples from Marseilles**

Samples	Chi-squared	Df	p-value
Clavicle study sample	7.762	19	0.989
L5 study sample	7.266	19	0.993
Coxal bone study sample	12.242	19	0.875
Ilium study sample	6.521	12	0.888

## **5.2.2. Iliac variables**

### **a. Biometric variables of the ilium**

The boxplots of the biometric iliac variables show high variation of all four variable between birth and the age of one, followed by stable variation increase for IW and IL until the ages of 9 and 11 respectively (**Figure 5.15**).

The number of outliers is relatively low for all variables. The corresponding values of the variables lie within the ranges of the corresponding variables for neighbouring age groups one or two years older or younger. Therefore, these individuals were not excluded from the sample, as there was no reason to believe they did not represent extreme normal variability. Moreover, an individual can be an outlier for one variable but not the others (*e.g.* the 2-year old outlier for IW), once again illustrating variability. The bidimensional variables IM and IA show an important increase in variation with age from the age of five that is particularly strong at 11 and 12 years (**see Appendix B**).

The relationship between age and IL, and age and IW changes at the age of one year and remains stable from then on, illustrating continued linear growth, meaning the curves modelling these relationships should have one inflexion point at the age of one.

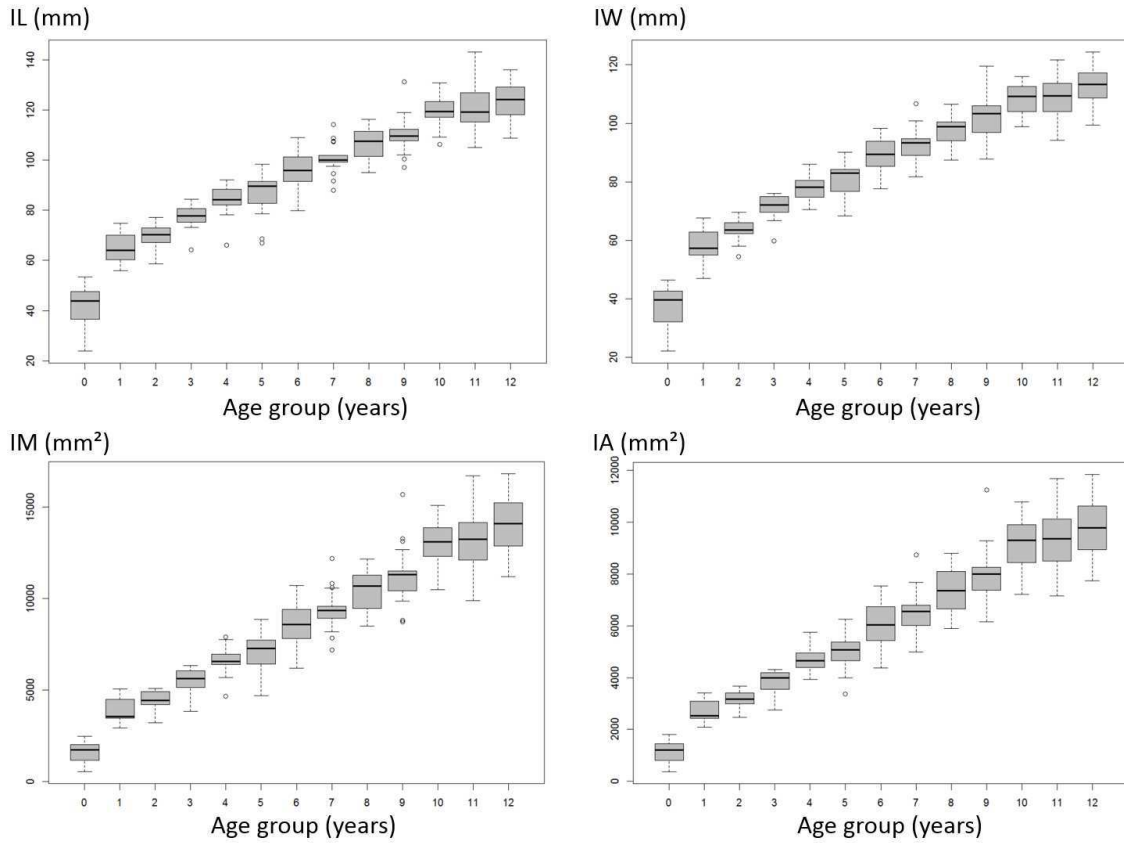


Figure 5.15 Boxplots of the four iliac variables per annual age group. Each boxplot presents the mean (bold black line), 25<sup>th</sup> to 75<sup>th</sup> percentiles (grey box), 10<sup>th</sup> to 90<sup>th</sup> percentiles (dotted line) and outliers (circles) of the variables for each age

IM and IA seem to have a stable continuous relationship with age as no inflexion point can be observed. This is confirmed by the high value of the Pearson coefficients showing a strong linearity of the relationship between age and each of these two variables (Table 5-60). For IL and IW on the other hand, Spearman’s coefficients are higher, meaning their relationship with age could be modelled using second degree polynomial functions.

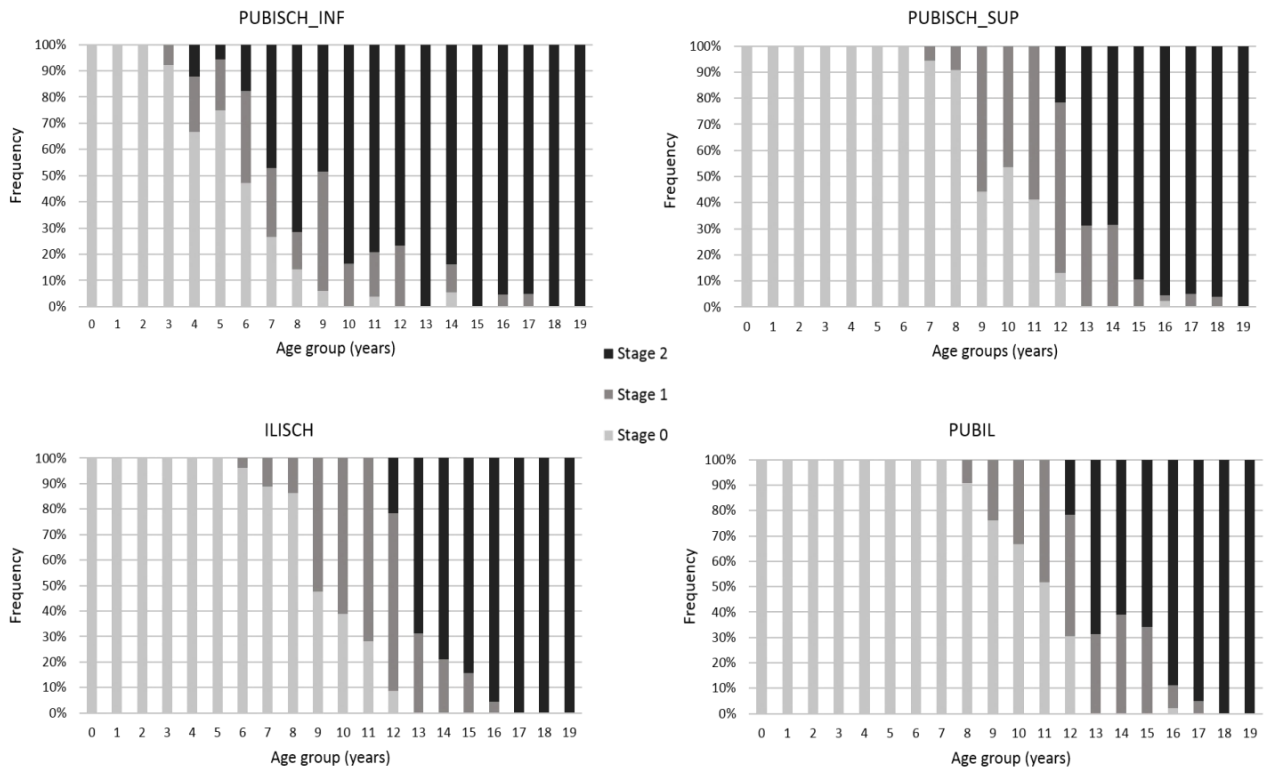
Table 5-60 Values of the Pearson and Spearman correlation coefficients between age and the four iliac variables

Variables	Pearson	Spearman
IL	0.941	0.959
IW	0.944	0.961
IM	0.966	0.962
IA	0.965	0.961

Bidimensional iliac variables IM and IA could possibly be modelled against age using linear regression, whereas unidimensional ones IL and IW seem to have a more complex relationship with age that could be modelled using polynomial functions, provided variable dispersion does not imply mathematical limitations.

**b. Maturation stages of the iliac bone**

The distribution of the three stages used to assess maturation of the four epiphyseal sites of the iliac bone according to the annual age groups illustrate the sequence of events that occur between an unfused iliac bone to a completely fused iliac bone (Figure 5.16).

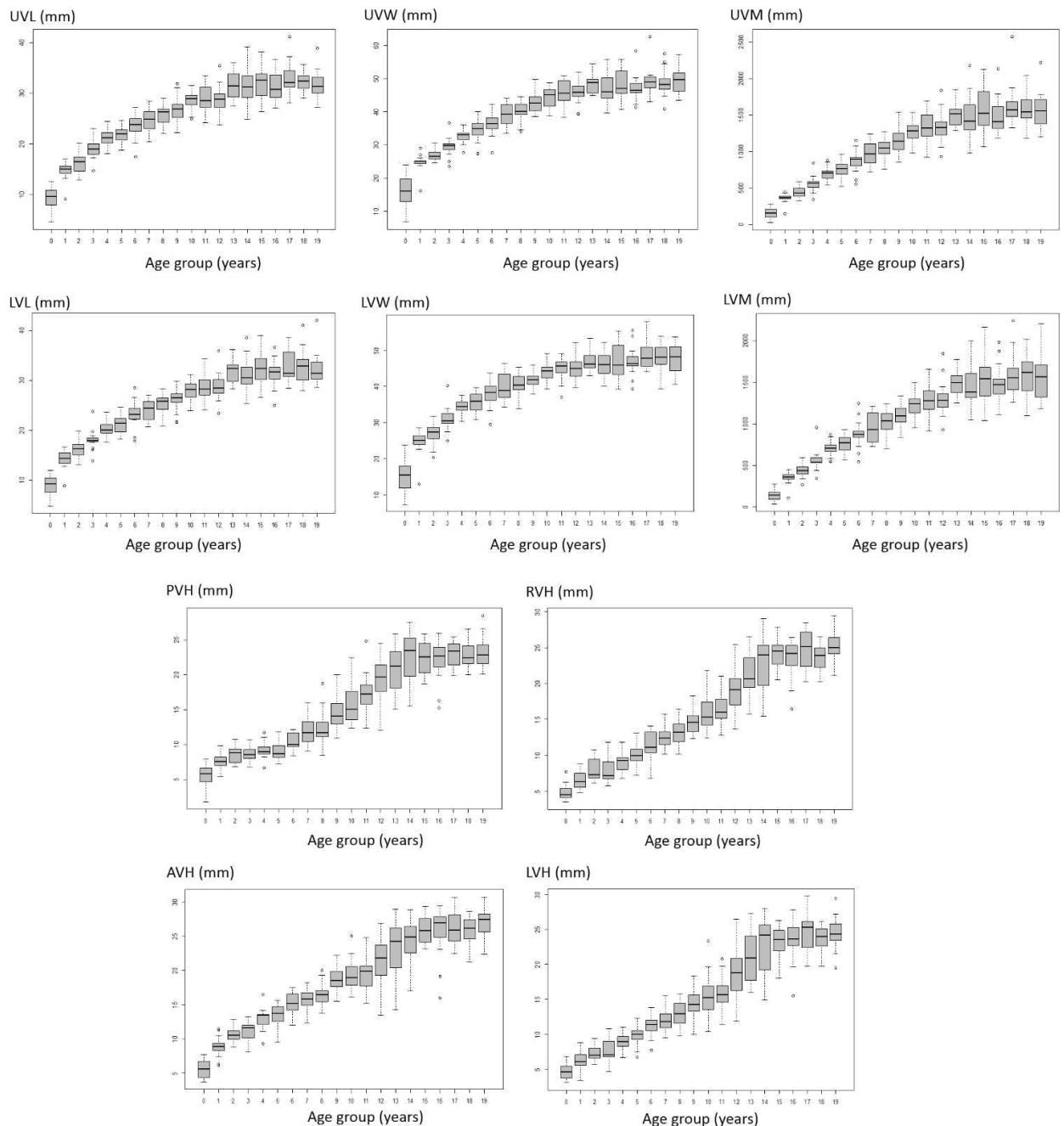


**Figure 5.16** Distribution of the three maturation stages of the four epiphyseal sites of the iliac bone in the Marseilles sample according to age

The first epiphysis to start fusion is PUBISCH\_INF, as early as three years. Maturation states are then very varied for this epiphysis from three to eight years, with the number of unfused states (stage 0) continuously decreasing and the number of fused states (stage 2) rapidly increasing until they are dominant, around ten years of age. The transitional stage (stage 1) is relatively long, from 4 to 12 years approximately, where all three stages are represented with a dominance of stage 2. Some partially fused states (stage 1) remain from 12 to 17 years, but they are a minority. The three Y-cartilage epiphyses, PUBISCH\_SUP, ILISCH and PUBIL, start fusing later, around 7-8 years, and follow comparable fusion sequences. Fusion is more progressive than for PUBISCH\_INF, but seems to occur slightly faster for ILISCH as the number of partially fused epiphyses continuously decreases after 13 years. PUBIL and PUBISCH\_SUP follow very similar patterns, although PUBIL seems to finish fusing slightly earlier than PUBISCH\_SUP (around 18 and 19 years respectively). The mean, standard error, minimum and maximum ages per stage for each epiphysis can be found in **Appendix B**.

### 5.2.3. Lumbar variables

The boxplots of each lumbar variable per age group (**Figure 5.17**) show that the variables seem to follow different patterns: individual variation is highest between the ages of 8-9 and 14-15 years for the variables AVH, PVH, RVH and LVH, at the ages 0 for PVH, UVL, UVW, LVL and LVW, and after 8-9 years for UVM and LVM (see Appendix B).



**Figure 5.17** Boxplots of four fifth lumbar vertebra variables per annual age group. Each boxplot presents the mean (bold black line), 25<sup>th</sup> to 75<sup>th</sup> percentiles (grey box), 10<sup>th</sup> to 90<sup>th</sup> percentiles (dotted line) and outliers (circles) of the variables for each age group

### **Results I: Protocol validation – sample descriptions**

The plots of RVH, LVH, UVM and LVM against age seem to present one inflexion point between 12 and 14 years, with a steady growth before that age is reached. Variables AVH, UVL, UVW, LVL and LVW present two inflexion points at around 1 and 14 years, with a decrease of the slope modelling the relationship between age and the variables after each inflexion point. Growth of PVH however seems to present three inflexion points at the ages of 1, 6 and 14 years.

The number of outliers is relatively constant for all the variables. They are equally distributed in the lower and upper ranges of values. Also, their values do not excessively exceed the ranges of the neighbouring age groups, with the exception of two individuals in the 17- and 18-year age groups for UVM and LVL and two 16-year old individuals for AVH. As the individuals cannot all be considered as outliers for all ten lumbar variables, they can very well be considered as representing extreme normal variability and were kept for the study.

Age explains a great part of the variance of the lumbar variables, as assessed by the high values (all > 0.85) of Pearson and Spearman coefficients (**Table 5-61**).

**Table 5-61 Values of the Pearson and Spearman correlation coefficients between age and the ten fifth lumbar vertebra variables**

<b>Variables</b>	<b>Pearson</b>	<b>Spearman</b>
UVL	0.888	0.897
UVW	0.877	0.879
UVM	0.901	0.901
LVL	0.899	0.909
LVW	0.858	0.867
LVM	0.906	0.905
AVH	0.937	0.932
PVH	0.921	0.919
RVH	0.945	0.944
LVH	0.937	0.936

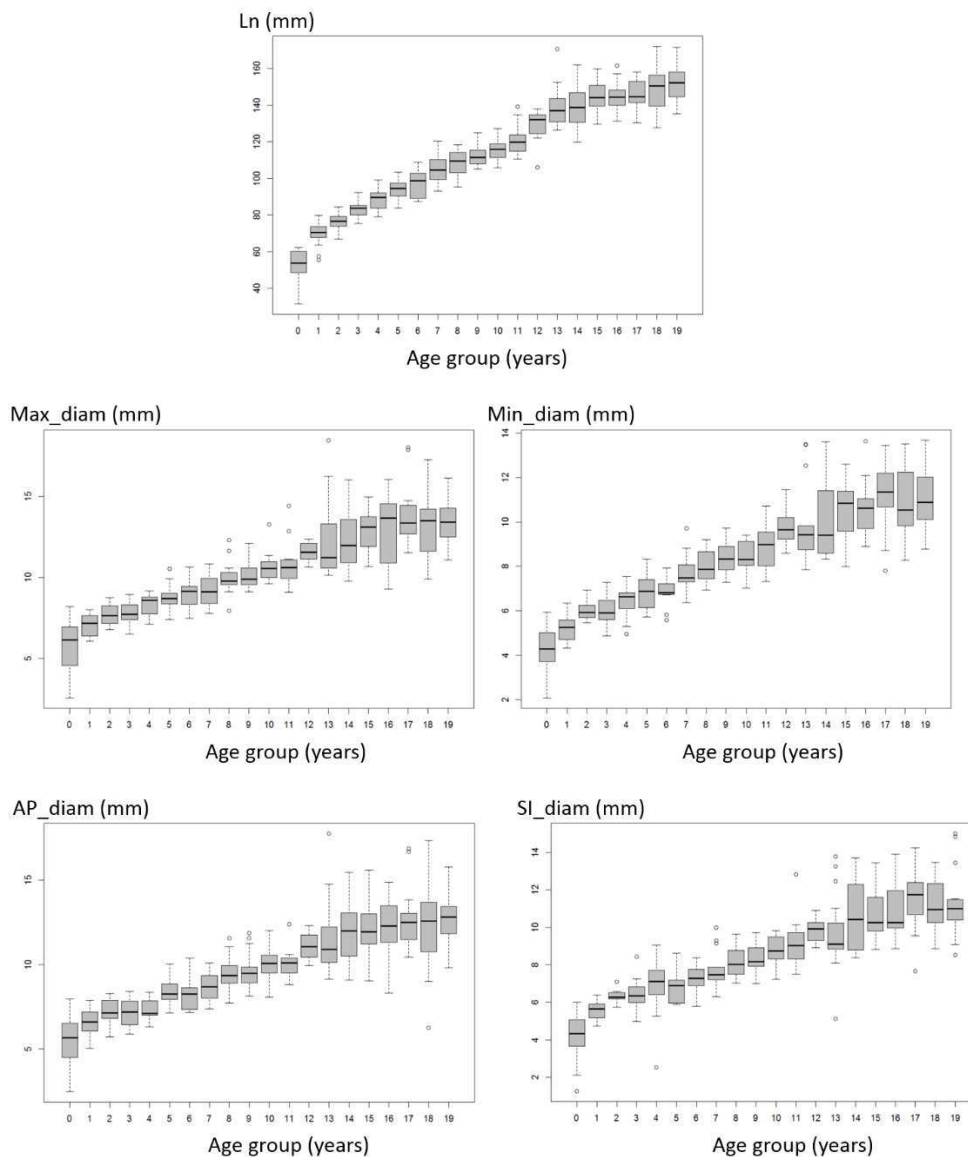
The variables with the strongest hypothetically linear relationship with age are RVH, AVH, LVH, and PVH, as their Pearson coefficients are higher than their Spearman's. Both bidimensional variables UVM and IVM have higher Pearson coefficients than the other four unidimensional ones, but have comparable Spearman coefficient values, meaning the relationship between age and the variables could be modelled by a linear or polynomial function, considering the existence of inflexion points as noted previously. Considering the dispersion of the variables as shown in the boxplots and the coefficient values of each lumbar variable, their relationship with age could be modelled using parametric linear or polynomial functions.



Once again, the notable increase in variation with age starting at 8-9 years may limit the application of parametric models.

### 5.2.4. Clavicular variables

The boxplots of each clavicular variable per annual age group (**Figure 5.18**) modelling growth show that all variables seem to have two main growth phases, separated by an inflexion point (a moment when the relationship between two variables seems to change).



**Figure 5.18** Boxplots of the five clavicular variables per annual age group. Each boxplot presents the mean (bold black line), 25<sup>th</sup> to 75<sup>th</sup> percentiles (grey box), 10<sup>th</sup> to 90<sup>th</sup> percentiles (dotted line) and outliers (circles) of the variables for each age

The inflexion point is between the ages of 0 and 1 year: rapid growth between the ages 0 and 1 year is followed by a steady but slower increase in values until the age of 18-19 years. Variation is

high for all variables between birth and the age of one and generally lower between one and 12 years for maximum clavicular length (Ln) before increasing until 19 years. The four diameter variables have overall high variances that increase with age (see Appendix B), with several individuals outside the 90% percentile range of values (materialised by the open circles).

The number of potential outliers varies depending on the variable, but is higher for the diameter variables. Only one 13-year old individual presents values outside the normal ranges for all variables and was therefore excluded from the study.

Age explains a great part of the variance of the clavicular variables, as assessed by the high values (all > 0.85) of Pearson and Spearman coefficients (Table 5-62).

The equal values of both coefficients for Ln suggests that the relationship between Ln and age could possibly be modelled using a linear function ( $a \cdot x + b$ ), whereas the Spearman coefficient of the other four variables is higher than Pearson's, meaning the relationship between age and these variables may not be linear, but polynomial, as suggested by the inflexion points in the boxplots.

**Table 5-62 Values of the Pearson and Spearman correlation coefficients between age and the five clavicular variables**

Variables	Pearson	Spearman
Ln	0.957	0.957
Max_diam	0.868	0.903
Min_diam	0.886	0.908
AP_diam	0.854	0.882
SI_diam	0.867	0.896

Considering the relationship between age and the variables as shown in the boxplots and the coefficient values, we could hypothetically be modelled by a parametric linear regression for Ln, and parametric polynomial functions for Max\_diam, Min\_diam, AP\_diam and SI\_diam. However, some difficulty may arise from the increase of variation with age, especially after 14 years and limit the use of parametric regressions (see section 4.7.2.).

### **5.3. Bilateral asymmetry of the variables in the Marseilles sample**

#### **5.3.1. Ilium**

The tests show that there is significant bilateral asymmetry for the variables IL, IW, and IM (Table

5-63). However, the pseudo-median values of the differences between left and right IL and IW are inferior to 1 mm and the confidence intervals are both included in the [-1 ; +1] threshold interval. Therefore, we can conclude that bilateral asymmetry is absent for IL and IW with a maximum error of +/- 1 mm. IM however, presents significant bilateral asymmetry.

**Table 5-63 Results of the Wilcoxon tests done for assessing bilateral asymmetry of the iliac biometric variables. The greyed p-values indicate significant differences between left and right variables**

Variables	V	CI 95% (mm)	Pseudo-median (mm)	p-value
IL	12415	[-0.382 ; -0.028]	-0.199	0.028
IW	11825	[-0.486 ; -0.077]	-0.282	0.006
IM	11451	[-64.009 ; -13.844]	-38.205	0.002
IA	16438	[-3.215 ; 21.767]	9.499	0.141

### 5.3.2. Fifth lumbar vertebra

There is significant bilateral asymmetry between RVH and LVH (Table 5-64). However, the pseudo-median value of the differences between the two variables is inferior to 1 mm and the confidence interval is included in the [-1 ; +1] threshold interval. Therefore, we can conclude that bilateral asymmetry is absent with a maximum error of +/- 1 mm.

**Table 5-64 Results of the t-test done for assessing bilateral asymmetry of the left and right fifth lumbar variables. The greyed p-value indicates significant differences between left and right variables**

Variables	V	CI 95% (mm)	Pseudo-median (mm)	p-value
RVH-LVH	48992	[0.120 ; 0.359]	0.241	0.0001

### 5.3.3. Clavicle

Bilateral asymmetry was tested with a paired t-test for the normally distributed variable SI\_diam and with non-parametric paired Wilcoxon tests for the other four variables (Table 5-65). There is no significant bilateral asymmetry for Min\_diam and AP\_diam.

Bilateral asymmetry is present for Ln, SI\_diam and Max\_diam. However, the pseudo-median values of the differences between left and right bone variables are inferior to 1 mm and the confidence intervals are included in the [-1 ; +1] threshold interval with the exception of Ln. Considering the range of these three variables (see Appendix B), we can consider that the differences between left and right variables Max\_diam and SI\_diam are significantly different than zero, but remain inferior to 1mm. Therefore, we can conclude that bilateral asymmetry is present with a maximum error of +/- 1

mm for diameter variables and for maximum clavicular length (Ln).

**Table 5-65 Results of the t-test (upper part of the table) and the Wilcoxon tests (lower part of the table) done for assessing bilateral asymmetry of the clavicular variables. The greyed p-values indicate significant differences between left and right variables**

Variable	t	df	CI 95% (mm)	Pseudo-median (mm)	p-value
SI_diam	-3.623	277	[-0.347 ; -0.101]	-0.222	<0.001
Variables	V		CI 95% (mm)	Pseudo-median (mm)	p-value
Ln	25828		[0.465 ; 1.060]	0.760	<0.001
Max_diam	9449		[-0.255 ; -0.109]	-0.180	<0.001
Min_diam	14437		[-0.064 ; 0.055]	-0.004	0.8954
AP_diam	21606		[-0.014 ; 0.164]	0.076	0.099

### 5.3.4. Iliac bone

Bilateral asymmetry of maturation stages of the iliac bone was tested using a binomial test on the differences of stages between paired left and right bones. A difference between left and right bone equal to zero is considered as a “success”. The test computes the mean probability of success and its confidence intervals, *i.e.* the probability of having no difference between left and right stages. The permutation test estimates whether the range of the differences between paired left and right stages is comparable to zero or not.

There is no significant bilateral asymmetry for epiphyses PUBISCH\_INF, ILISCH and PUBIL (**Table 5-66**). The probability of similarly staging the left and right sites are all higher than 0.95 and the permutation test show that the differences between paired left and right stages are comparable to zero.

**Table 5-66 Results of the binomial test and the permutation test done for assessing bilateral asymmetry of the non-biometric iliac variables. The greyed p-values indicate significant differences between left and right variables**

Variables	Differences between stages of right and left bones					CI 95%	Probability of similar stage	Binomial test p-value	Permutation test p-value
	-2	-1	0	1	2				
	PUBISCH_INF	0	3	272	0				
PUBISCH_SUP	1	5	238	6	0	[0.918 ; 0.975]	0.952	1	0.0455
ILISCH	0	3	271	1	0	[0.963 ; 0.996]	0.985	0.003	0.655
PUBIL	0	3	267	4	0	[0.948 ; 0.990]	0.974	0.070	1

The permutation test p-value for PUBISCH\_SUP is very close to 0.05 and the mean probability of success is higher than 0.95. Because the associated confidence interval includes values lower than 0.95, we can say that there is no significant differences between left and right stages for

PUBISCH\_SUP with an error risk higher than 5%.

**Conclusions on bilateral asymmetry of the variables:**

- Of the four iliac variables, IL and IW show slight bilateral asymmetry. IM shows significant bilateral asymmetry, and IA does not show any bilateral asymmetry
- The right (RVH) and left (LVH) lumbar heights show slight bilateral asymmetry
- Maximum clavicular length (Ln) shows significant bilateral asymmetry. Min\_diam and SI\_diam show slight bilateral asymmetry, contrary to Max\_diam and AP\_diam
- Maturation stages of the iliac bone show bilateral asymmetry for the PUBISCH\_SUP epiphysis

## 5.4. Sexual dimorphism of the variables in the Marseilles sample

### 5.4.1. Ilium

The presence of sexual dimorphism of the iliac variables was tested on the whole sample with independent Wilcoxon tests. They show that no significant sexual dimorphism is present for any iliac variable (Table 5-67).

**Table 5-67 Results of the Wilcoxon tests done for assessing sexual dimorphism of the iliac variables in the whole sample.**  
\*Refers to bidimensional variables, expressed in mm<sup>2</sup>

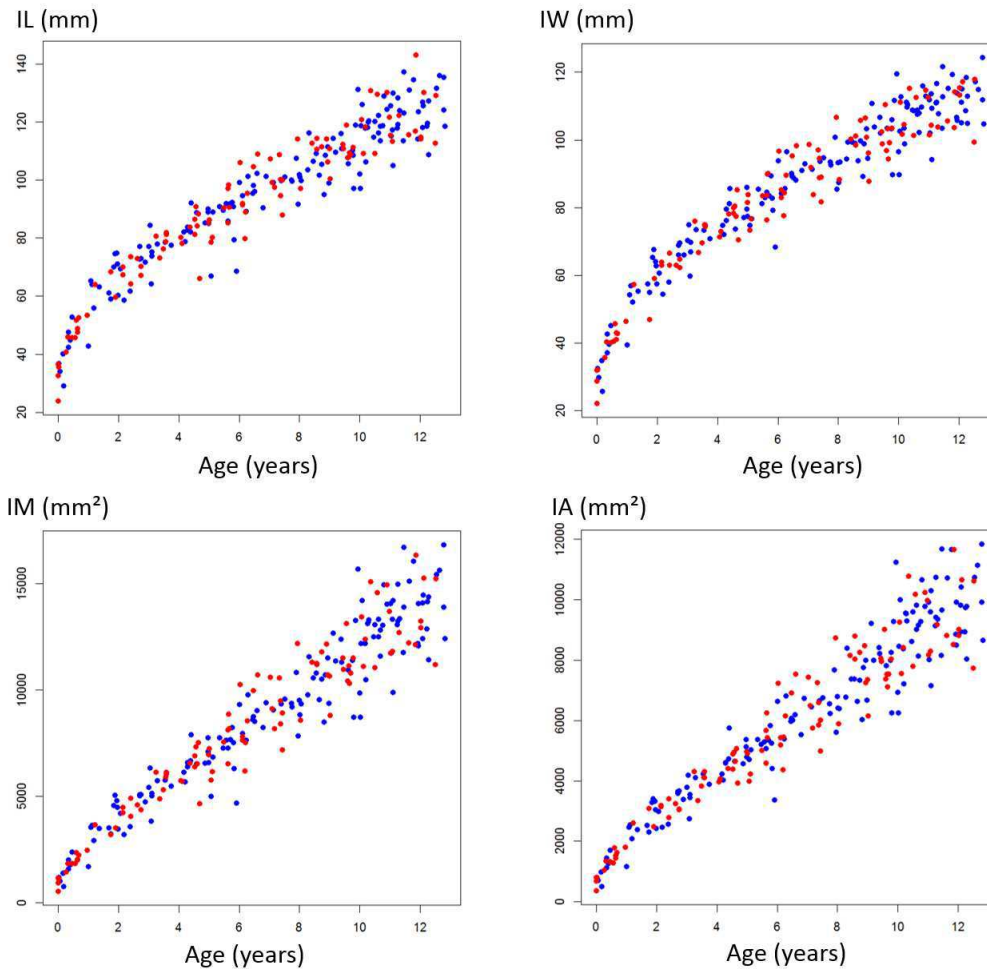
Variables	W	CI 95% (mm)	Difference in location (mm)	p-value
IL	6346	[-10.362 ; 4.362]	-3.527	0.362
IW	6295	[-9.680 ; 3.133]	-3.538	0.313
IM*	6286	[-1817.672 ; 649.283]	-610.746	0.305
IA*	6257	[-1331.978 ; 405.871]	-478.265	0.279

This is corroborated by the fact that boys and girls seem to follow the same growth patterns and the same variation range at all ages between 0 and 12 years (Figure 5.19). Nevertheless, because the confidence intervals of the differences between male and female variables were relatively important (Table 5-68/ Figure 5.20), and to detect the possible punctual presence of sexual dimorphism, we tested it in each annual age group using the same two methods exposed previously. The details of the test results are presented in Appendix D.

**Results I: Protocol validation – sample descriptions**

**Table 5-68 Iliac variables and the corresponding age groups for which sexual dimorphism is present, when using Wilcoxon tests on the male and female variable means of each annual age group**

Variables	Sexual dimorphism	Age groups
IL	Yes	[8-9 years]
IW	Yes	[8-9 years]
IM	Yes	[8-9 years]
IA	Yes	[8-9 years]



**Figure 5.19 Plots of the iliac variables against age, with male (blue) separated from female (red) individuals**

Sexual dimorphism is present for all four iliac variables at the ages of 8 and 9 years (**Table 5-68**). When using moving averages, sexual dimorphism was only significant for iliac width (IW), between the ages of 9 and 11 years (**Figure 5.20**).

Sexual dimorphism of ilium growth seems limited to a particular period of time, between the ages of 8 and 9 years for all four variables and is less significant overall than it is for the clavicular and lumbar variables. IW has the most significant sexual dimorphism.

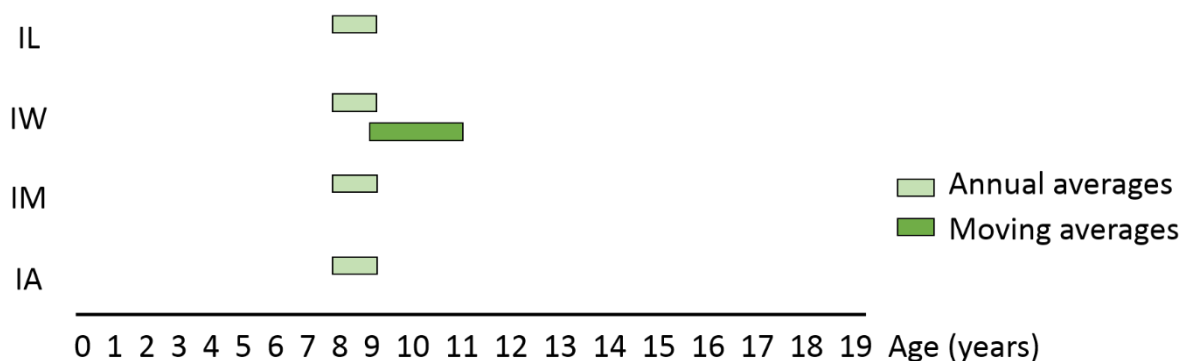


Figure 5.20 Ages or age ranges of significant sexual dimorphism for iliac variables assessed using Wilcoxon tests on annual averages (light green) of the variables, calculated for the individuals of each annual age group N, or moving averages (dark green) of the variables, calculated for the individuals of age groups (N-1), N and (N+1)

### 5.4.2. Fifth lumbar vertebra

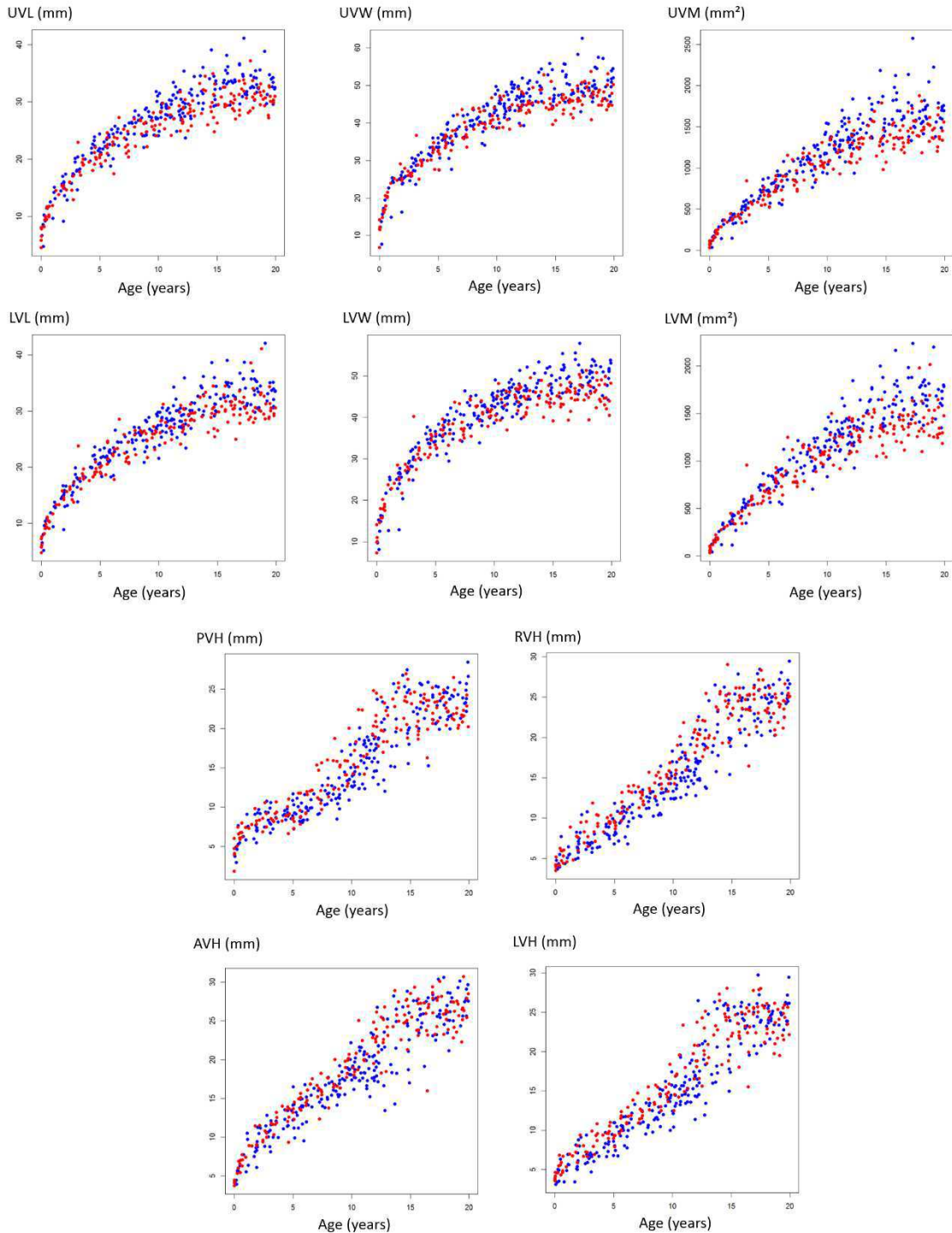
The presence of sexual dimorphism was tested on the whole sample with independent Wilcoxon tests. They show that sexual dimorphism is present for all lumbar variables, except PVH, AVH and LVH (Table 5-69).

Table 5-69 Results of the Wilcoxon tests done for assessing sexual dimorphism of the fifth lumbar vertebra variables in the whole sample. \*Refers to bidimensional variables, expressed in mm<sup>2</sup>; the greyed p-values indicate significant differences between male and female variables

Variables	W	CI 95% (mm)	Difference in location (mm)	p-value
UVL	16533	[-2.899 ; -0.488]	-1.701	0.006
UVW	16886	[-3.536 ; -0.397]	-1.954	0.015
UVM*	16518	[-219.415 ; -35.880]	-128.628	0.006
LVL	17419	[-2.604 ; -0.010]	-1.288	0.048
LVW	16437	[-3.581 ; -0.651]	-2.101	0.005
LVM*	16903	[-206.402 ; -22.850]	-114.213	0.015
PVH	21222	[-0.393 ; 2.255]	0.853	0.181
AVH	21524	[-0.249 ; 2.621]	1.152	0.11
RVH	22007	[0.031 ; 3.021]	1.458	0.043
LVH	21738	[-0.121 ; 2.775]	1.271	0.08

When looking at the graphs showing the growth of each variable according to sex, we can see that once again differences between boys (blue dots) and girls (red dots) seem to appear at a certain period of time, between ten and fifteen years and seems particularly important for UVL, UVVW, UVM, LVL, LVW and LVM (Figure 5.21).

**Results I: Protocol validation – sample descriptions**



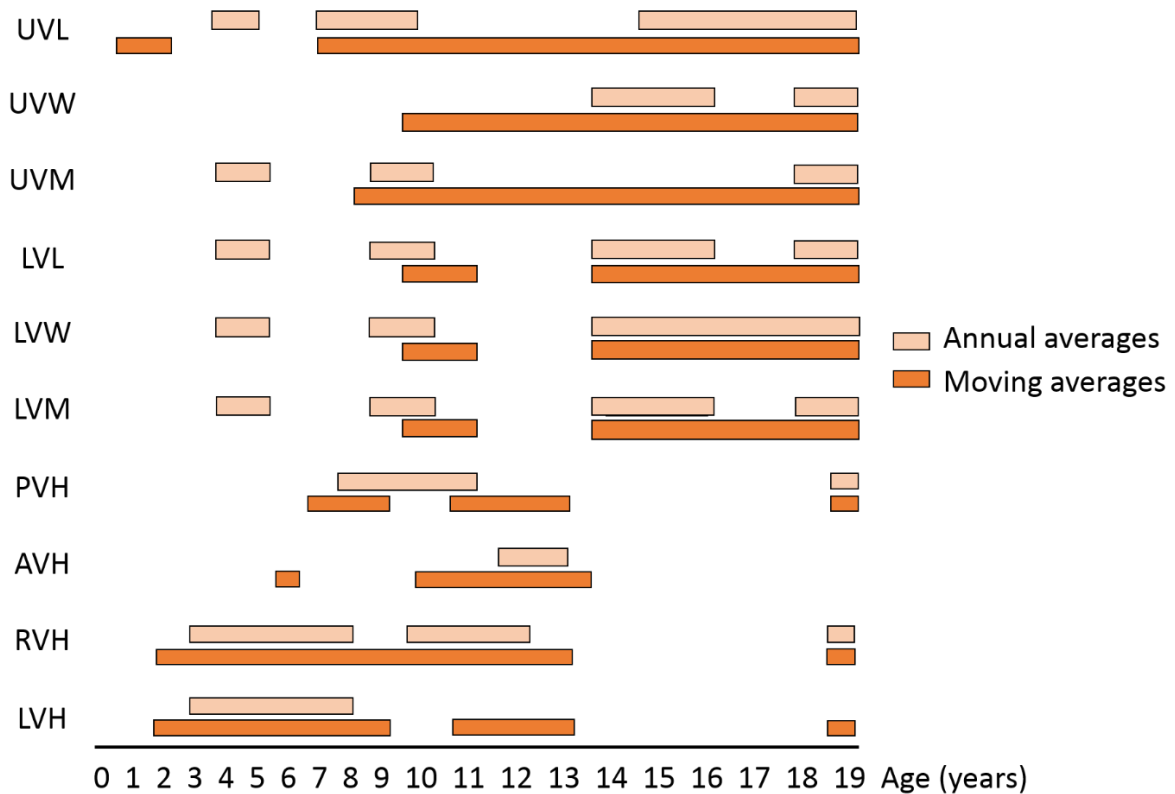
**Figure 5.21** Plots of the fifth lumbar vertebra variables against age, showing the differences between male (blue) and female (red) growth patterns for UVL, UVW, UVM, LVL, LVW and LVM

It also seems to be present for PVH, AVH, RVH and LVH at younger ages, between 1 and 10 years. In the same way as the clavicular variables, we tested the presence of sexual dimorphism for the ten lumbar variables in each annual age group using the same two methods exposed previously. The details of the test results are presented in **Appendix D**.



**Results I: Protocol validation – sample descriptions**

Using the Wilcoxon test on each annual age group, it was found that sexual dimorphism is present during early childhood (between 4 and 5 years) for UVL, UVM, LVL, LVW, and LVM and continues until 8 years for RVH and LVH (**Figure 5.22**). It reappears during late childhood for UVL, UVM, LVL, LVW, LVM, and RVH and appears at that same time for PVH. It is present during early adolescence for AVH and reappears at the age of fourteen or fifteen to remain until early adulthood for UVL, UVM, LVL, LVW and LVM. It is no longer present at 19 years for AVH and LVH, but it is for all the other variables.



**Figure 5.22** Ages or age ranges of significant sexual dimorphism for fifth lumbar variables assessed using Wilcoxon tests on annual averages (light orange) of the variables, calculated for the individuals of each annual age group N, or moving averages (dark orange) of the variables, calculated for the individuals of age groups (N-1), N and (N+1)

Using Wilcoxon tests on the moving averages (**Figure 5.22**), we can highlight six periods where sexual dimorphism is present: during early childhood for UVL, RVH and LVH; during childhood for AVH, RVH and LVH; during late childhood for UVL, UVM, PVH, RVH and LVH; during the prepubertal phase for all ten variables; during the whole adolescent period for UVL, UVW, UVM, LVL, LVW, LVM; at 19 years of age, it is still present for all variables except AVH.

Sexual dimorphism of the lumbar variables seems less period-specific than it was for the clavicular variables. However, it is present for all during the prepubertal phase (9 to 13 years) and for the upper

(UVL and UVW), lower (LVL and LVW) and bidimensional (UVM and LVM) variables during adolescence (14-19 years).

### 5.4.3. Clavicle

The presence of sexual dimorphism was tested on the whole sample with independent Wilcoxon tests. They show that sexual dimorphism is present for all five clavicular variables (**Table 5-70**).

**Table 5-70 Results of the Wilcoxon tests done for assessing sexual dimorphism of the clavicular variables in the whole sample. The greyed p-values indicate significant differences between male and female variables**

Variables	W	CI 95% (mm)	Difference in location (mm)	p-value
Ln	37247.5	[-14.770 ; -5.0999]	-10.060	<0.001
Max_diam	29521	[-1.524 ; -0.658]	-1.097	<0.001
Min_diam	27699	[-2.009 ; -1.028]	-1.515	<0.001
AP_diam	33977	[-1.737 ; -0.812]	-1.278	<0.001
SI_diam	34620	[-1.500 ; -0.657]	-1.071	<0.001

When looking at the graphs showing the growth of each variable according to sex, we cannot help but notice that differences between boys (blue dots) and girls (red dots) seem to appear at a certain period of time, between ten and fifteen years (**Figure 5.23**).

Because of this, we tested the presence of sexual dimorphism for the five clavicular variables in each annual age group using two methods:

- the first is an independent Wilcoxon test done on the subsample composed of girls and boys of age group n, to assess the presence of sexual dimorphism in that age group n. The tests are done on the means of the individuals of the age group n;
- the second is an independent Wilcoxon test done on the girls and boys of a subsample composed of individuals from age groups n-1 to n+1, to assess the presence of sexual dimorphism in the group n. This method uses moving averages: the tests are done on the means calculated with the individuals from age groups n-1, n and n+1. This method is used to smooth out punctual fluctuations and highlight longer-term trends (Yaffee and McGee 2000). For the inferior and superior age groups (0 and 19 years), we used 0-1 years and 18-19 years for mobile means, respectively.

The details of the test results are presented in **Appendix D**.

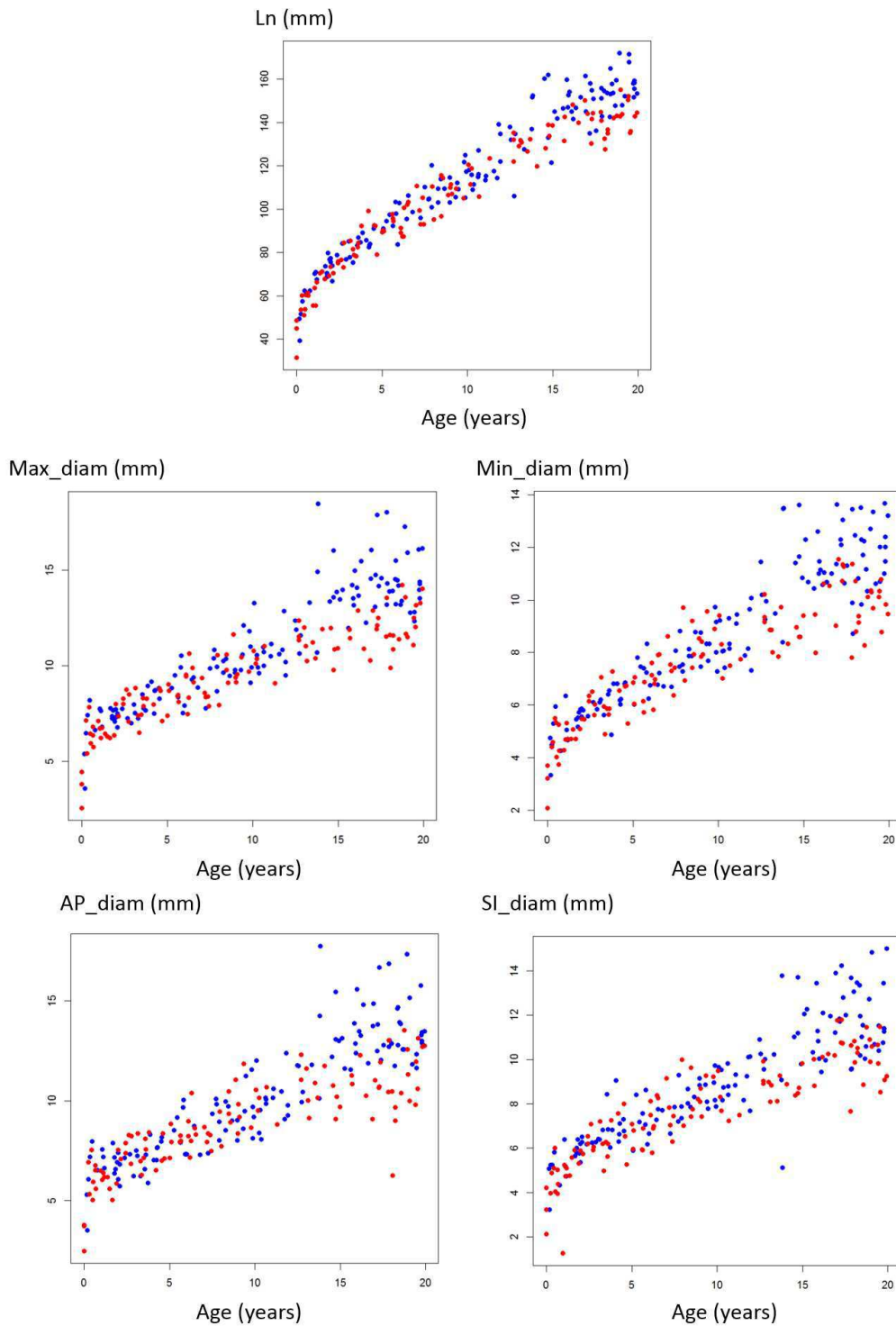


Figure 5.23 Plots of the clavicular variables against age, showing the differences between male (blue) and female (red) growth patterns

Using the Wilcoxon test applied on each annual age group (**Figure 5.24**), we can see that sexual dimorphism is present for all variables around 1 to 2 years and between 13 and 19 years. Max\_diam and Min\_diam also show sexual dimorphism at the age of 5 years, and Max\_diam and SI\_diam show it at the ages of 11 and 10 respectively.

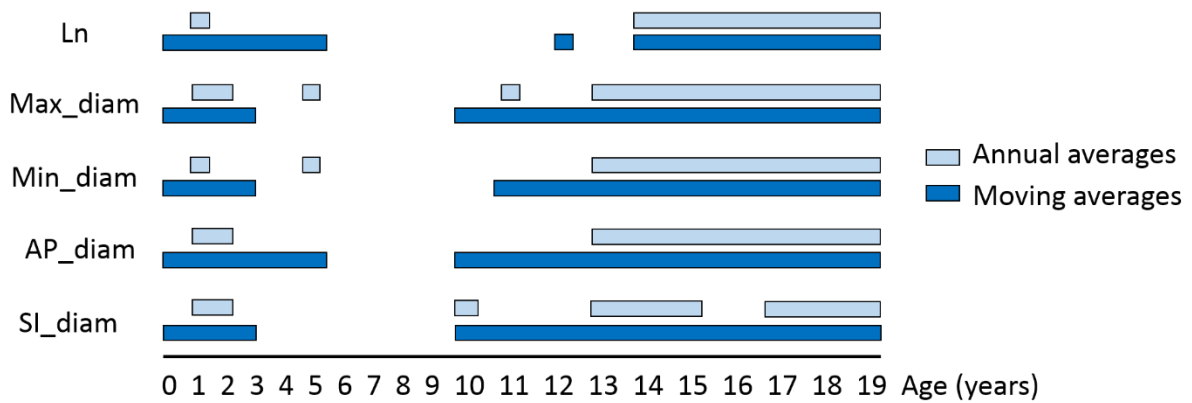


Figure 5.24 Ages or age ranges of significant sexual dimorphism for clavicular variables assessed using Wilcoxon tests on annual averages (light blue) of the variables, calculated for the individuals of each annual age group N, or moving averages (dark blue) of the variables, calculated for the individuals of age groups (N-1), N and (N+1)

Using the moving averages method (Figure 5.24), we can see that sexual dimorphism is present for all variables during two main periods: between the ages of 0 and 3 years and up to 5 years for Ln and AP\_diam, and after 10 years for all variables, except Ln. Ln shows sexual dimorphism at the age of 12 years, coinciding with the sexually dimorphic onset of the prepubertal growth spurt, and then again between 14 and 19 years of age, during adolescence.

Overall, sexual dimorphism is present during early childhood and adolescence for all five clavicular variables, which corroborates sexual bimaturism of long bone growth (Humphrey 1998).

#### 5.4.4. Iliac bone

Sexual dimorphism was tested on the whole sample with independent Wilcoxon tests for each epiphyseal site. They show that sexual dimorphism is significant for the maturation of the three epiphyses of the acetabular region: PUBISCH\_SUP, ILISCH and PUBIL (Table 5-76).

In this study, we wanted to refine the acknowledgment of sexual dimorphism of the iliac bone by precisely detecting the moment when it becomes significant in the bone’s acetabular maturation pattern. To do this, we conducted independent Wilcoxon tests on each annual age group using annual means and moving averages.

The results (Figure 5.25) show that sexual dimorphism of maturation states appears earlier for ILISCH and PUBIL (8-9 years) than it does for PUBISCH\_SUP (10 years). However, it persists longer for ILISCH and PUBIL (until 15 years) than it does for PUBISCH\_SUP (until 12 years).

Table 5-71 Results of the Wilcoxon tests done for assessing sexual dimorphism of the non-biometric iliac variables in the whole sample. The greyed p-values indicate significant differences between male and female variables

Variables	W	CI 95%	Difference in location	p-value
PUBISCH_INF	19711	[-2.731e-5 ; 5.900e-5]	4.838e-5	0.438
PUBISCH_SUP	24383	[7.110e-5 ; 9.257e-6]	5.808e-5	0.012
ILISCH	24062	[1.714e-5 ; 3.954e-5]	6.435e-5	0.027
PUBIL	24623.5	[1.582e-5 ; 2.320e-5]	2.724e-5	0.004

Tests done on the moving averages show that the differences in maturation patterns first appear at the PUBIL epiphysis (8 years), followed by PUBISCH\_SUP (9 years) and ILISCH (11 years) and last until 14 years, when sexual dimorphism of acetabular maturation is not significant anymore (Table 5-78 / Figure 5.28).

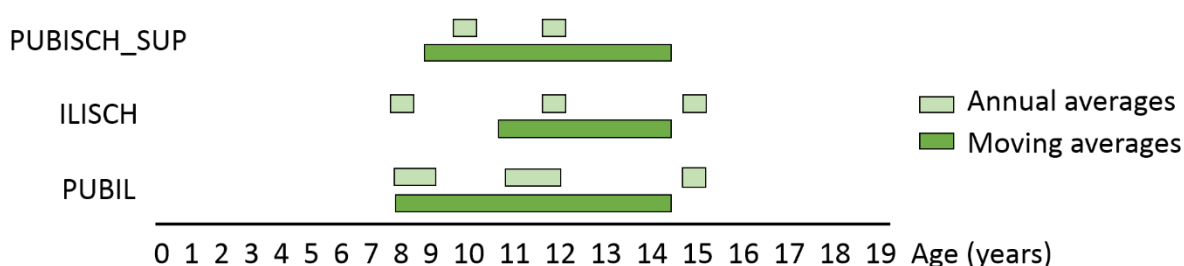


Figure 5.25 Ages or age ranges of significant sexual dimorphism for maturation of the coxal bone assessed using Wilcoxon tests on annual averages (light green) of the variables, calculated for the individuals of each annual age group N, or moving averages (dark green) of the variables, calculated for the individuals of age groups (N-1), N and (N+1)

Sexual dimorphism of the maturation pattern of the acetabular region is present from the age when the epiphyses start fusing until the age when they are for the most part completely fused. This period extends from 8 to 15 years and covers the entire maturation period of the acetabular region, starting with PUBIL, followed by ILISCH and PUBISCH\_SUP.

**Conclusions on sexual dimorphism of the variables:**

- All iliac variables (IW in particular) present sexual dimorphism between the ages of 8 and 9
- All lumbar variables present sexual dimorphism throughout the developmental period
- All clavicular variables present sexual dimorphism during early childhood and from the pre-pubertal period onwards
- The three acetabular epiphyseal sites (ILISCH, PUBISCH\_SUP, PUBIL) present significant sexual dimorphism in maturation stages

## 5.5. Homogeneity of the variables from the different samples

All results of the analyses of covariance for testing homogeneity between the samples can be found in **Appendix D**. Inhomogeneity was found for the values of all iliac variables between the three samples from Marseilles, Toulouse and Lisbon ( $p < 0.001$ ). Inhomogeneity was also found for the four vertebral heights (PVH, AVH, RVH and LVH) between the samples from Marseilles and Lisbon.

No significant differences of covariance were found for any of the three iliac variables IL, IW and IM measured in samples from Marseilles and Toulouse (**Table 5.72**), nor for the other lumbar variables, the five clavicular variables and one iliac variable (IA), between the Marseilles and Lisbon samples.

**Table 5-72 Results of the covariance test for homogeneity of biometric iliac variables between the samples for Marseilles and Toulouse. Greyed values indicate significant factors of inhomogeneity of the variables**

Variable	Factor of influence	Df	Sum of squares	Mean Square value	F value	p-value
IL	Age	1	159879	159879	2489.0054	<0.001
	Sample	1	2480	1240	17.631	0.007
IW	Age	1	130572	130572	2608.558	<0.001
	Sample	1	531	531	10.618	0.001
IM	Age	1	4068611768	4068611768	3160.80	<0.001
	Sample	1	373337	373337	0.29	0.591

**Summary: Principal results for protocol validation and sample description**

- The acquisition protocol for clavicular variables is applicable on dry and scanned dry bones
- Landmark positioning is sufficiently repeatable, but not sufficiently reproducible for all landmarks
- Biometric and non-biometric variables are sufficiently reliable, repeatable and reproducible. Some have higher error rates, such as PVH or bidimensional variables of the lumbar vertebrae
- Some of the variables present significant bilateral asymmetry (Ln, IM).

For this reason, the growth and age prediction models that were constructed on the left variables cannot be applied to right variables without caution and verification of the significance of bilateral asymmetry. Age prediction models were constructed separately for left and right maximum clavicular length (Ln) and Ilium Module (IM)

- Sexual dimorphism is present for all clavicular variables during early childhood and adolescence
- Sexual dimorphism is variably present for all lumbar variables at different periods of time, during childhood and adolescence
- Sexual dimorphism is less distinctly present for ilium growth (prepubertal phase), but is present throughout the entire maturation of the acetabular region of the iliac bone (from 8 to 15 years)
- The iliac variables IL, IW and IM of the samples from Marseilles and Toulouse were homogeneous.

The four vertebral heights (PVH, AVH, RVH and LVH) and three iliac variables (IL, IW and IM) were significantly different in the samples from Lisbon and Marseilles. The five clavicular variables, the other six lumbar variables and the iliac variable IA were homogeneous

## **Chapter 6. Results II: Age estimation**

This chapter presents the mathematical regressions constructed on the Marseilles sample to estimate individual age from the clavicle, the fifth lumbar vertebra and the ilium. Bayesian posterior probabilities of age were calculated using maturation stages of the iliac bone.

The relationship between age and the biometric variables was mathematically modelled using regression equations with age as the predicted variable ( $y$ ) and the biometric variables as predictors ( $x$ ), to provide new juvenile age estimation methods based on biometric variables of the three bones. The regression models were considered valid if they complied with standard validation requirements concerning residuals (independence, normality, and homoscedasticity).

The resulting valid methods were then applied on three independent samples from Marseilles (France), Toulouse (France) and Lisbon (Portugal). The model using iliac biometric variables was also tested in a forensic context on a single individual, whose identity was later confirmed *via* DNA analysis, providing the true chronological age for verification.

Complete validation of the models was awarded provided they complied with the standard 95%-reliability and accuracy thresholds. The valid models were then ranked using the precision of the estimations.

Maturation of the iliac bone was studied using a three-level staging system defining fusion states (0 = unfused, 1 = partially fused, 2 = completely fused) on four epiphyseal sites (one for the ischio-pubic ramus and three for the acetabulum) considered individually or as two-, three-, or four-digit combinations. These non-biometric variables were then used for age estimation using a Bayesian probabilistic approach.

The Bayesian probabilities were then tested on an independent sample from Marseilles and on the individuals of the Luis Lopes collection to provide mathematical validation parameters. The results were then compared using these parameters.

All statistical tests, statistical parameters, regression models and graphical iconography were obtained using the R© Software and the RStudio© interface (R Core Team 2014, <http://www.R-project.org/>).



## 6.1. Age estimation using regression models and biometric variables

### 6.1.1. Parametric models

Because the objective of this study is to provide valid and user-friendly age estimation models, and considering the relatively high values of Pearson correlation coefficients for a majority of the variables, linear regressions were first tested to model the relationship between age and the variables with age as the predicted factor (**Table 6-1**). However, the variables also showed high Spearman correlation coefficients. For this reason, and considering the general aspect of the plots of age against the variables (**see section 5.2.**), second- and third-degree polynomial regressions were also constructed. As no bilateral asymmetry was found for variable growth, all models were constructed on left-sided variables when applicable. The quality of the models was assessed using the value of the regression coefficient ( $R^2$ ) and residual standard error (RSE).

**Table 6-1** Parameters of linear and polynomial growth models of maximum clavicular length (Ln) and their associated statistical parameters. Estimate = regression coefficient value; Standard error = standard error of the regression coefficient; p-value associated with the regression coefficient;  $R^2$  = correlation coefficient of the regression; Residual standard error (RSE): difference between the real and estimated values of Ln. Residual test results: DW = Durbin-Watson statistic (upper) and associated p-value (lower); BP = Breusch-Pagan statistic (upper) and associated p-value (lower); Shapiro-Wilk statistic (upper) and associated p-value (lower); Bold italic terms correspond to non-significant regression coefficients ( $p < 0.05$ ); greyed p-values correspond to non-significant test results ( $p$ -value  $< 0.05$ )

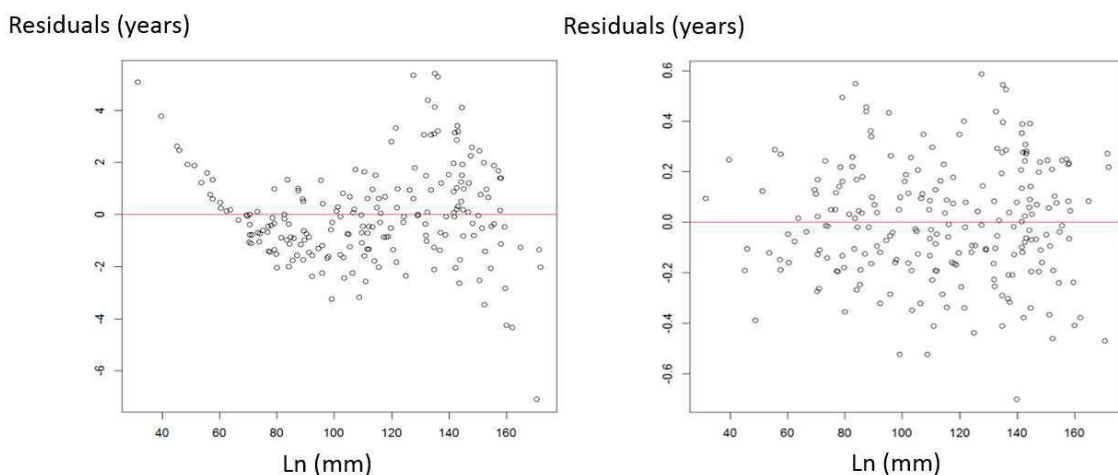
Linear regression model					Residual test results			
Coefficient variables	Estimate	Standard error	p-value	$R^2$	Residual standard error (years)	DW	BP	Shapiro-Wilk
Intercept	-10.911	0.464	<2e-16	0.913	1.813	1.108	8.760	0.968
Ln	0.185	0.004	<2e-16			<0.001	0.003	<0.001
Second degree polynomial regression model					Residual test results			
Coefficient variables	Estimate	Standard error	p-value	$R^2$	Residual standard error (years)	DW	BP	Shapiro-Wilk
Intercept	-8.361	1.372	<0.001	0.912	1.800	1.244	30.180	0.964
Ln	0.134	0.026	<0.001			<0.001	<0.001	<0.001
<b><i>Ln<sup>2</sup></i></b>	<b><i>0.00024</i></b>	<b><i>0.0001</i></b>	<b><i>0.05</i></b>					
Third degree polynomial regression model					Residual test results			
Coefficient variables	Estimate	Standard error	p-value	$R^2$	Residual standard error (years)	DW	BP	Shapiro-Wilk
Intercept	14.406	2.790	<0.001	0.938	1.535	1.133	29.128	0.985
Ln	-0.608	0.0867	<0.001					
Ln <sup>2</sup>	0.00776	0.000857	<2 e-16			<0.001	<0.001	0.0288
Ln <sup>3</sup>	-2.384e-05	2.694e-06	<0.001					

A p-value is associated to each of these parameters, providing the level of significance of its difference with zero. A non-significant coefficient means that the associated term of the predictor variable ( $x$ ,  $x^2$ ,  $x^3...x^n$ ) does not significantly contribute to the model. This is particularly interesting when comparing linear and polynomial models, and second and third degree polynomial models.

As previously exposed in **section 4.7.2.**, residuals are crucial parameters for predictive model validation: their independence, homoscedasticity and normal distribution have to be verified in order to construct valid prediction intervals.

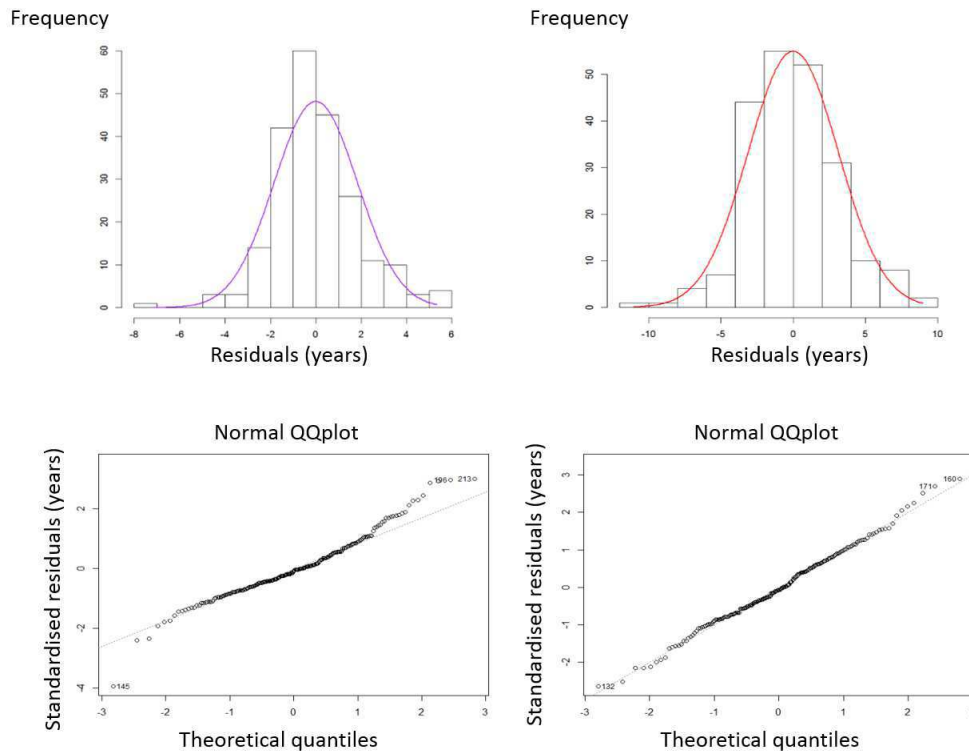
Independence of the residuals is tested using the Durbin-Watson test for autocorrelation, or by plotting the Autocorrelation function (acf). The Durbin-Watson test for samples greater than 100 individuals using one regression variable ( $x$ ) provides upper (dU) and lower (dL) limits for the associated DW value with a risk of error equal to 0.05:  $dU=1.653$  and  $dL=1.611$ . We can conclude to no significant positive (respectively negative) autocorrelation of the residuals if DW is superior to dU (respectively if  $4 - DW$  is inferior to dL) Therefore, DW has to be included in the interval  $[1.653 ; 2.389]$  to conclude to no residual autocorrelation. If  $dL < DW < dU$ , the test is inconclusive. Acf indicates autocorrelation if the residual lags follow a particular pattern and/or are not within the 95% confidence interval of significance levels.

Homoscedasticity of the residuals was tested using the Breusch-Pagan test with an error risk set at 0.05 and confronted to the plot of the residuals against predicted age and/or the predictor variable (**Figure 6.1**). Homoscedasticity of the residuals was declared when both the test and the plot were in agreement.



**Figure 6.1** Plots of heteroscedastic (left) and homoscedastic (right) residuals against the predictor variable (Ln). Heteroscedastic residuals present a specific pattern, with a tail at lower values of Ln, whereas homoscedastic residuals are homogeneously dispersed between -1.96 and +1.96 without following any particular pattern

The normal distribution of the residuals was tested using the Shapiro-Wilk test ( $p$ -value < 0.05), by observing the QQplot of the standardised residuals against their theoretical quantiles, and the histogram of the residuals (**Figure 6.2**).



**Figure 6.2** Non-normal versus normal residual distribution. Upper figures show the histograms of the distribution of residuals against their theoretical normal distribution curve (mean = 0 and standard error = 1) in purple and red. Left: the residuals do not follow the theoretical distribution (purple); Right: the residuals follow the theoretical distribution (red). Lower figures are the plots of the standardised residuals against their theoretical quantiles. A non-normal distribution is characterised by residuals that do not follow their theoretical quantiles (solid line) and often present “tails” at the extremities that diverge from the normal distribution. Left: Normal residuals do not follow their theoretical quantiles; Right: Normal residuals globally follow their theoretical quantiles

A normal distribution of the residuals was declared when both the test and the plots were in agreement (mean  $\sim$  0, sd  $\sim$  1, 95% of the residuals distributed in the [-1.96 ; 1.96] interval).

The respect of these three statistical requirements, added to high regression coefficients and low residual standard errors determine the selection of the regression model to be tested on an independent sample.

Because of the important number of models constructed and tested, it was decided not to present all the tests and regression formulae. **Tables 6-2 to 6-7** resume the conclusions drawn from the interpretation of the regression parameters and the tests done on the models and the residuals to select those considered as valid age estimation methods. **Figures 6.3 to 6.12** are the results obtained for the iliac variables and are representative of the general tendency followed by similar variables (uni- and bidimensional) of all three bone types. All the curves and mathematical

expressions of the parametric untransformed and transformed clavicular and lumbar variables can be found in **Appendix E**.

**a. Ordinary Least Squares (OLS) regressions**

i. Iliac variables

*Linear and polynomial regressions of the iliac variables against age*

Models were constructed on a training sample of 176 individuals (72 females and 104 males) aged 0 to 12 years, presenting uniform age and sex distributions (see **Chapter 5**). Because no significant sexual dimorphism was found for iliac variables, age estimation models were built for combined sexes. The highest regression coefficients ( $R^2$ ) are found in the models using bidimensional variables as predictors. There is no important increase ( $\geq 0.05$ ) in the values of  $R^2$  between the linear and polynomial models (**Table 6-2**).

**Table 6-2** Conclusions drawn from the three tests done on the residuals of the linear and polynomial regressions of age against the iliac variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase ( $\geq 0.05$ ) in the value of  $R^2$  between the linear and polynomial regression. The greyed areas correspond to valid residual parameters ( $p\text{-value} > 0.05$ )

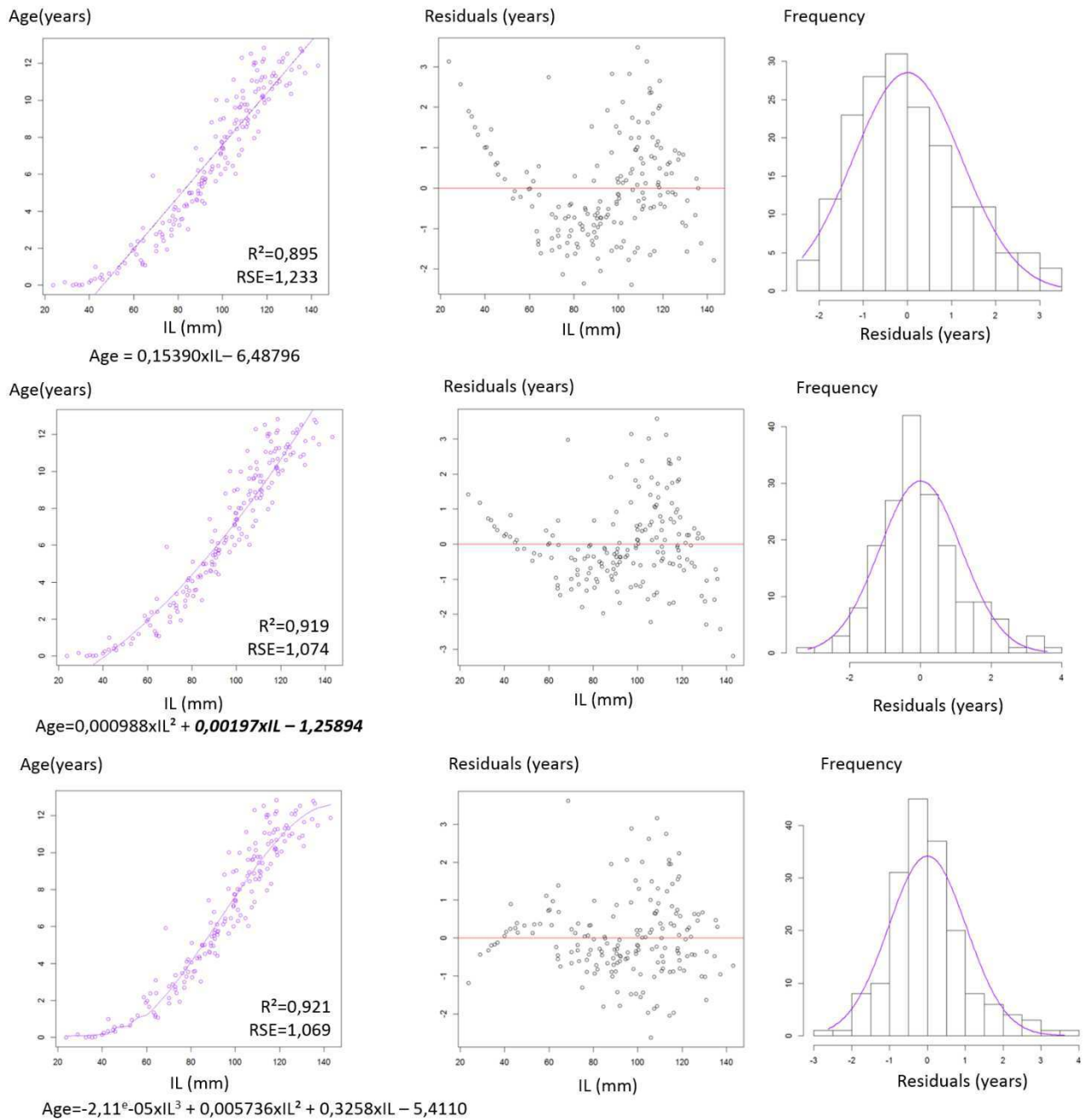
Variable	Linear regression				Second degree polynomial				Third degree polynomial			
	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.
IL	0.894	Neg.	No	No	0.907	Neg.	No	No	0.926	No	No	No
IW	0.895	Neg.	No	No	0.919	No	No	No	0.921	No	No	No
IM	0.921	Neg.	No	No	0.923	Neg.	No	No	0.935	No	No	No
IA	0.916	Neg.	No	No	0.921	No	No	No	0.934	No	No	No

All regression coefficients are significant at the 0.05 level, except the intercept and linear coefficients of the second degree polynomial regression with IW and of the third degree polynomial regression with IM. The third degree polynomial regressions of IL and IW are the only ones that includes the younger individuals in the model (**Figures 6.3 and 6.4**).

The linear and polynomial regressions seem equally well-fitted to bidimensional data. This confirms the more linear relationship of bidimensional variables with age, and the more polynomial relationship between age and unidimensional variables, although both model types have significant fits.

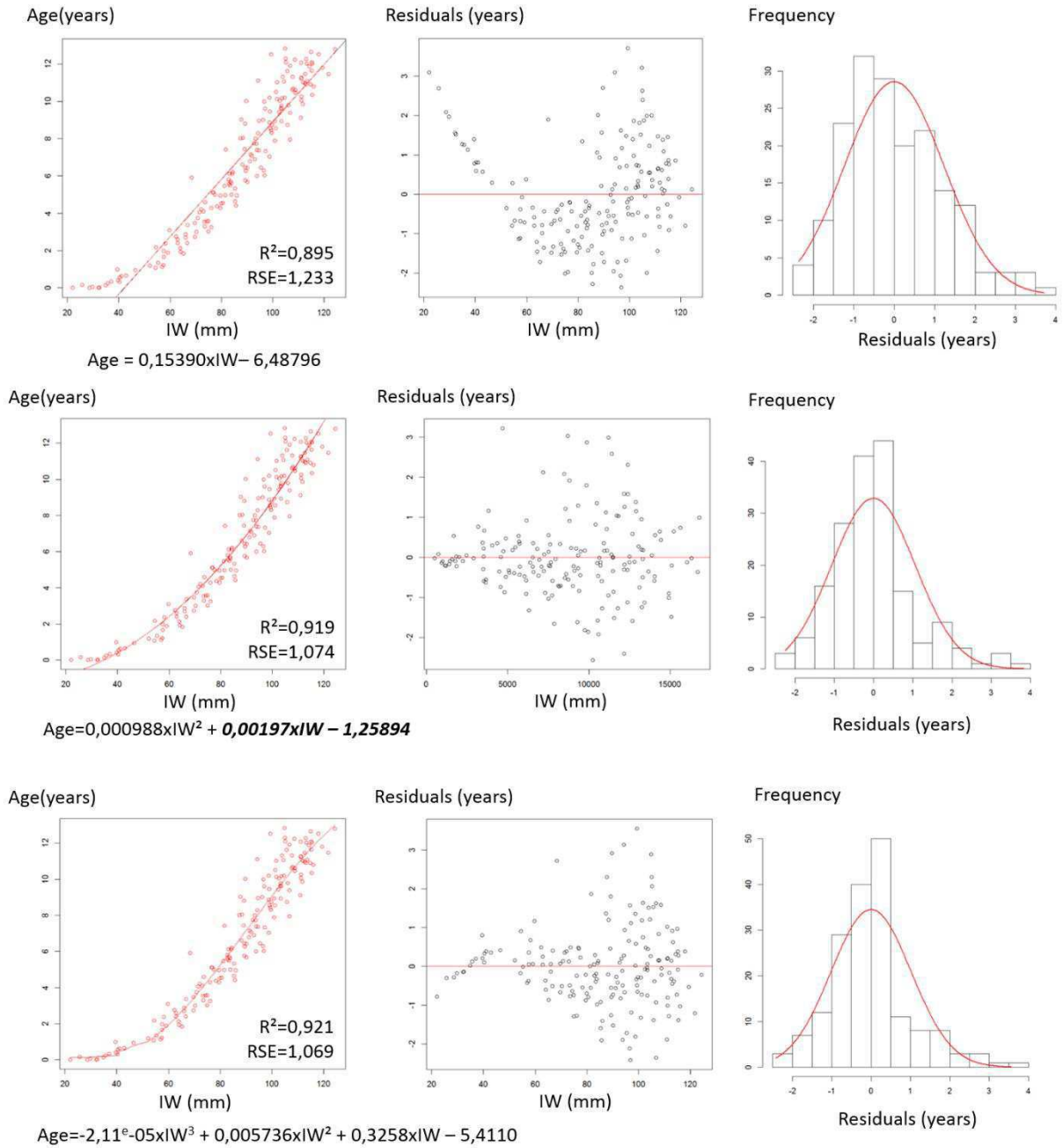
The residuals are only independent for all third degree polynomial regressions and the second degree polynomial with IW. Although they seem more dispersed for the third degree polynomial

regression, none of them are homoscedastic for any model used, nor normally distributed. They follow a triangular dispersion, with the presence of a tail for the lower values (**Figures 6.3 to 6.6**).



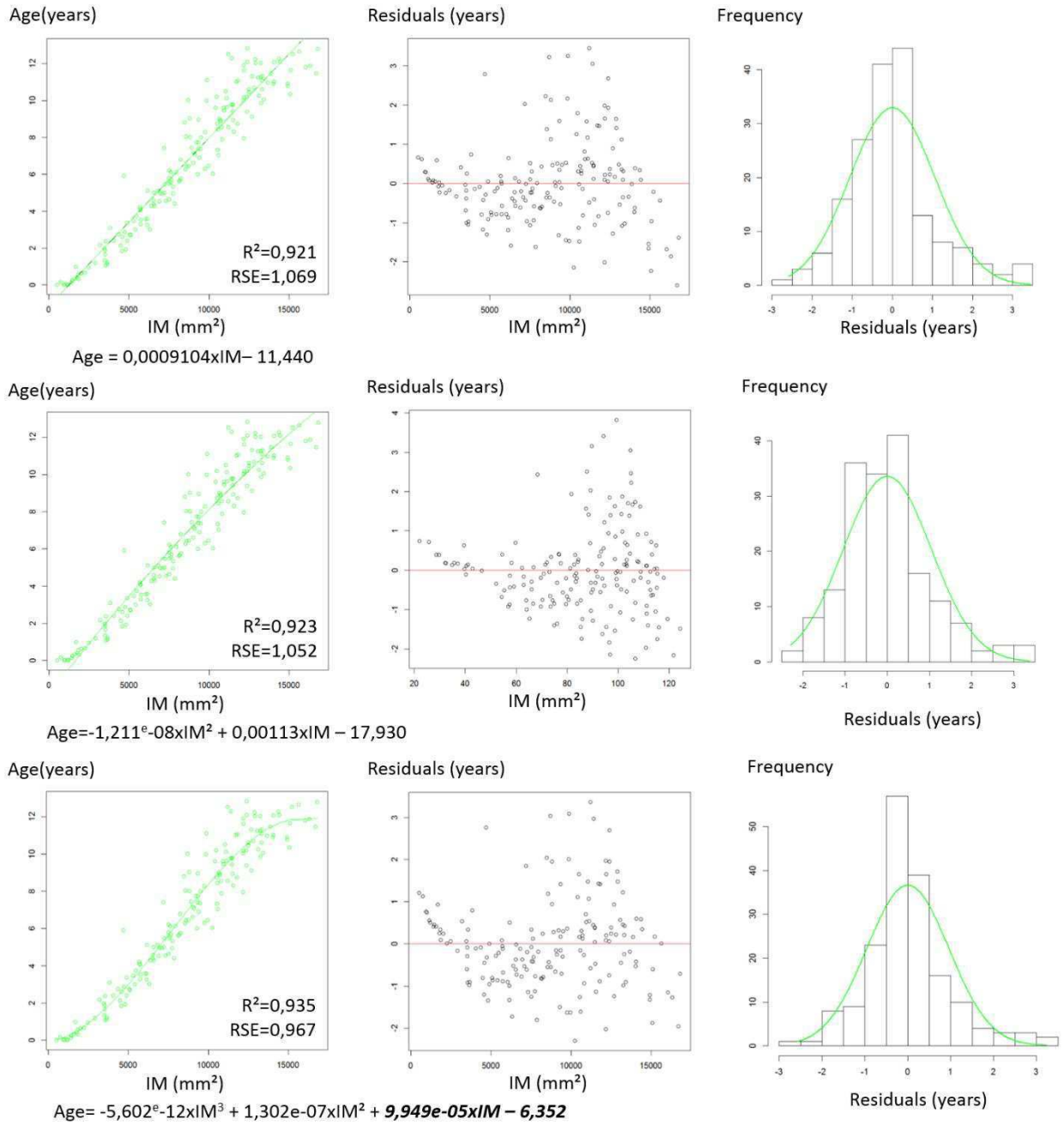
**Figure 6.3** Age prediction using linear, second and third degree polynomial regressions of IW with the mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against the predictor variable. ***Bold italic terms correspond to non-significant regression coefficients***

**Results II: Age estimation**



**Figure 6.4** Age prediction using linear, second and third degree polynomial regressions of IW with the mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against the predictor variable. Bold italic terms correspond to non-significant regression coefficients

**Results II: Age estimation**



**Figure 6.5** Age prediction using linear, second and third degree polynomial regressions of IM with the mathematical expressions, associated R<sup>2</sup> and RSE values and the plot of the residuals against the predictor variable. Bold italic terms correspond to non-significant regression coefficients

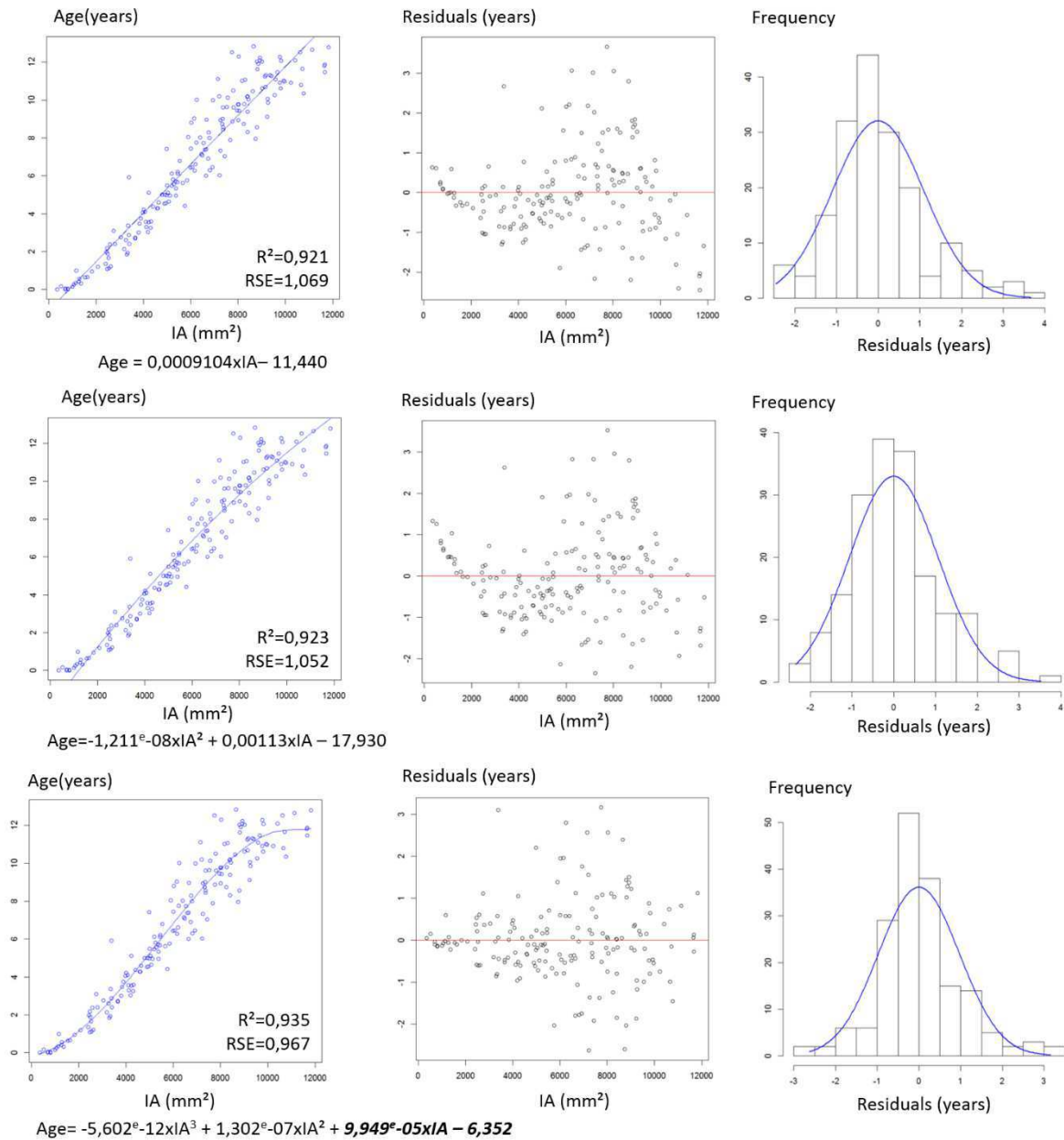


Figure 6.6 Age prediction using linear, second and third degree polynomial regressions of IA with the mathematical expressions, associated R<sup>2</sup> and RSE values and the plot of the residuals against the predictor variable. Bold italic terms correspond to non-significant regression coefficients

Because of this, new linear, second, and third polynomial regression models were constructed on transformed variables to try and obtain independent, homoscedastic, normally distributed residuals. These transformations concern the predicted variable (age) and/or the predictor variables.



Variable transformations

Transformation of age into  $\ln(\text{age})$  did not improve the previous regressions, nor did it significantly improve the residuals.  $R^2$  values are lower or equivalent to models with untransformed variables and only two models have independent residuals: second and third degree polynomials using unidimensional variables as predictors (**Table 6-3**).

The polynomial models improve the  $R^2$  values dramatically, although RSE values remain comparable for all three model-types. All models present residual heteroscedasticity that is not improved by the polynomial models (**Figures 6.3 to 6.6**) and no residual values follow a normal distribution.

**Table 6-3** Conclusions drawn from the three tests done on the residuals of the linear and polynomial regressions of natural logarithm of age against the iliac variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase ( $\geq 0.05$ ) in the value of  $R^2$  between the linear and polynomial regression; \*\*Indicates a significant increase ( $\geq 0.05$ ) in the value of  $R^2$  between the second and third degree polynomial regressions. The greyed areas correspond to valid residual parameters ( $p\text{-value} > 0.05$ )

Variable	ln(age) = f (variable)											
	Linear regression				Second degree polynomial				Third degree polynomial			
	$R^2$	Corr.	Hom.	Norm.	$R^2$	Corr.	Hom.	Norm.	$R^2$	Corr.	Hom.	Norm.
IL	0.731	Neg.	No	No	0.894*	No	No	No	0.918*	No	No	No
IW	0.752	Neg.	No	No	0.897*	No	No	No	0.921*	No	No	No
IM	0.615	Neg.	No	No	0.817*	Neg.	No	No	0.877**	Neg.	No	No
IA	0.610	Neg.	No	No	0.820*	Neg.	No	No	0.881**	Neg.	No	No

Polynomial models present a better fit to the data for all four variables (**Table 6-4**), with a second significant increase in  $R^2$  and decrease in RSE values using third degree models and bidimensional data instead of second degree polynomial models.

Residuals of linear regressions have a convex pattern, whereas residuals of second degree polynomial regressions present a concave pattern and third degree polynomial residuals show a horizontal “S-curve”. Residual plots show higher dispersion of the lower values (**Figure 6.7**).

However, due to quasi non-existent valid residual parameters, these models are not selectable for age estimation.

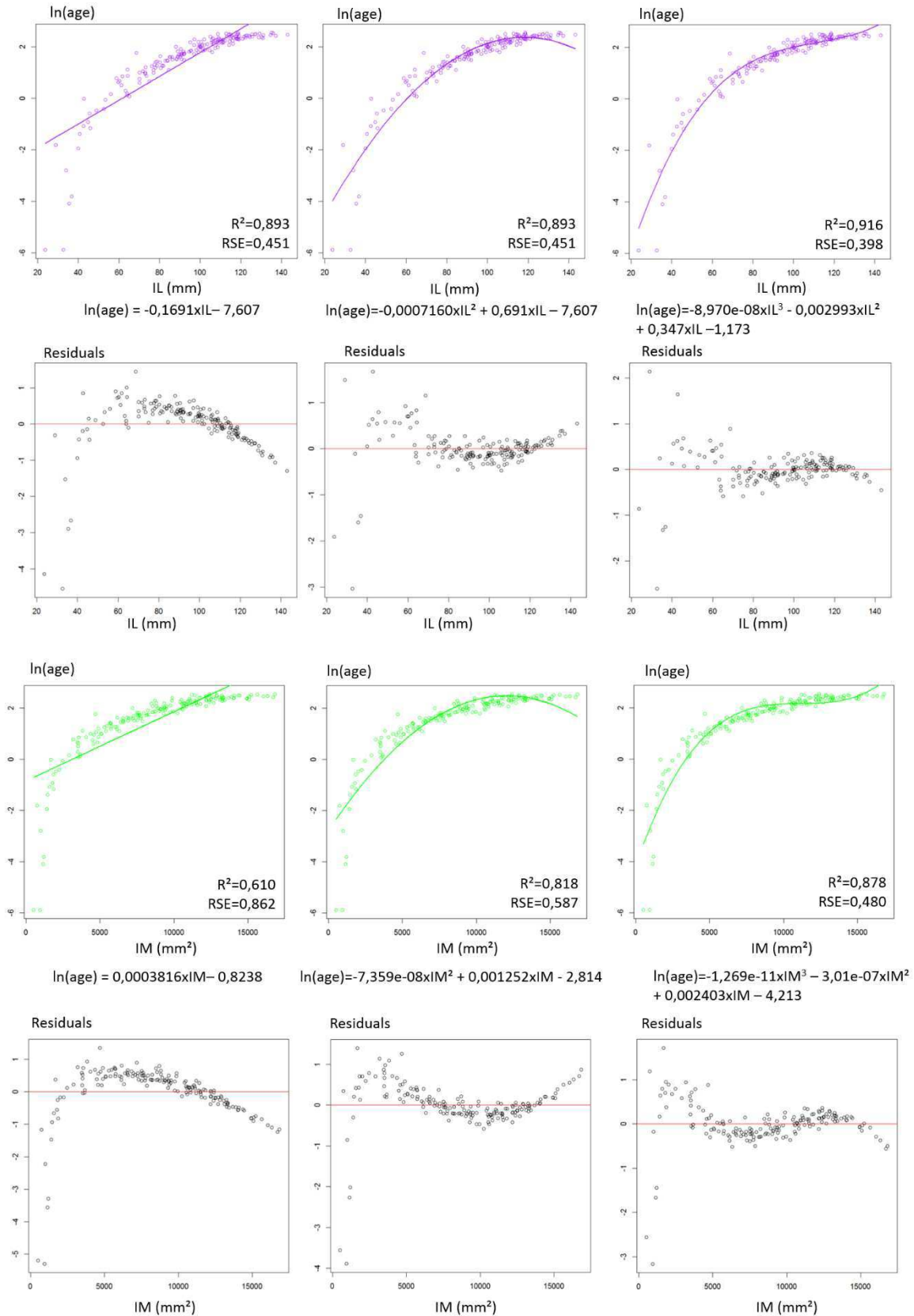


Figure 6.7 Examples of the linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against IL (purple) and IM (green), with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (second and fourth line of plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

Transformation of age into its square root (root(age)) improved the results compared to the “untransformed variables” regressions, but did not always significantly improve the residuals. The increase in R<sup>2</sup> between linear and polynomial regressions is important for the bidimensional variables (Table 6-4). R<sup>2</sup> values are higher or equivalent to the “untransformed” regressions and the residual standard errors are lower. Third degree polynomial models are the best fit for unidimensional data (Figure 6.8), whereas the best fit for bidimensional data could be linear or a second degree polynomial model (Figure 6.9).

Table 6-4 Conclusions drawn from the three tests done on the residuals of the linear and polynomial regressions of the square root of age against the iliac variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase (≥ 0.05) in the value of R<sup>2</sup> between the linear and polynomial regression. The greyed areas correspond to valid residual parameters (p-value >0.05)

Variable	root(age) = f (variable)											
	Linear regression				Second degree polynomial				Third degree polynomial			
	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.
IL	0.935	Neg.	No	Yes	0.943	No	No	No	0.950	No	Yes	No
IW	0.949	No	No	No	0.951	No	No	No	0.954	No	Yes	No
IM	0.899	Neg.	No	Yes	0.956*	No	No	No	0.956*	No	Yes	No
IA	0.893	Neg.	No	No	0.956*	No	No	No	0.956*	No	Yes	No

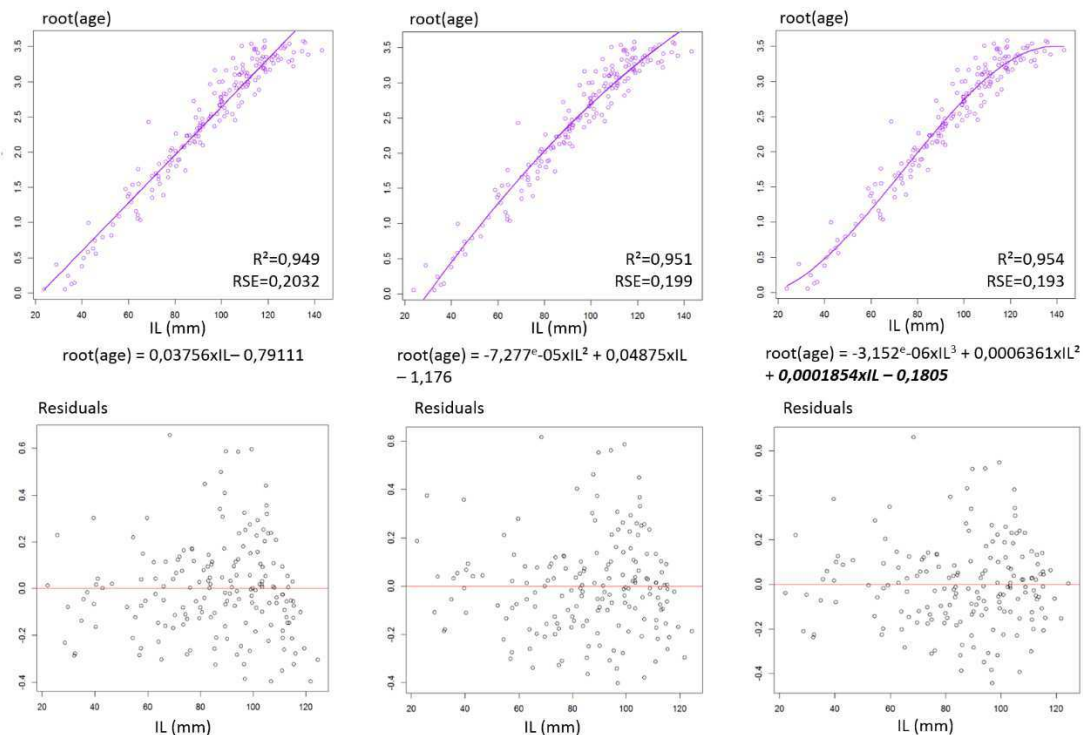


Figure 6.8 Example of the linear, second and third degree polynomial models of root(age) against IL (purple), with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plot of the residuals against predictor variables (lower plots). Third degree polynomial residuals of the regressions are homoscedastic. Bold italic terms correspond to non-significant regression coefficients

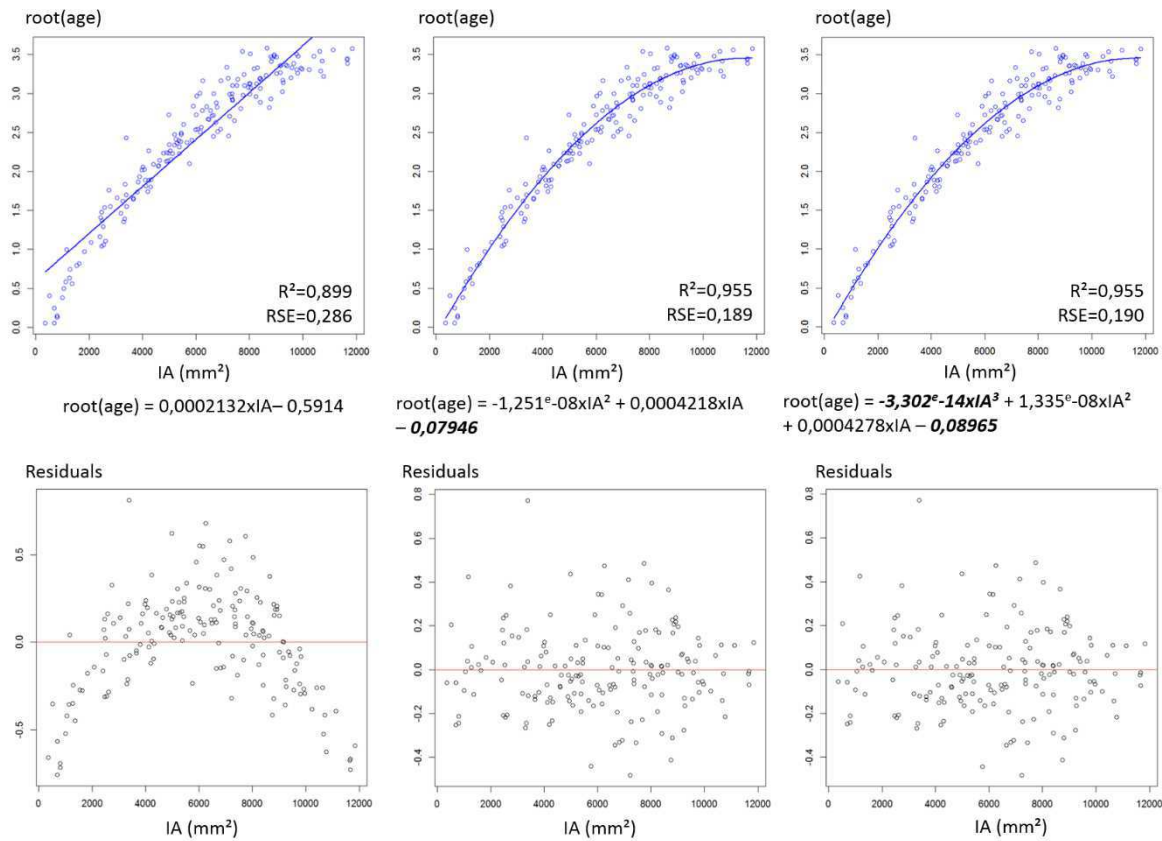


Figure 6.9 Example of the linear, second and third degree polynomial models of root(age) against IA (blue), with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (lower plots). Third degree polynomial regression residuals are homoscedastic. Bold italic terms correspond to non-significant regression coefficients

Only two linear models have normal residuals. The residuals of linear regressions using bidimensional variables follow an arch-like pattern. The third degree polynomials all have independent and homoscedastic residuals (Table 6-4, Figures 6.8 and 6.9) but they do not follow a normal distribution. No model fulfils all the validation requirements.

Transformation of age into its inverse value did not improve the “untransformed variables” regressions. No attempts were made to use polynomial regressions. Dispersion of the data (Figure 6.10), seemed to point out to an exponential function to model the relationship of the inverse of age with each variable. The function seems well-suited (Figure 6.10) although  $R^2$  values are not particularly high (Table 6-5). The residuals follow a normal distribution (Table 6-5). The presence of autocorrelation or heteroscedasticity could not be assessed using parametric tests as it was done for the other models, without resulting to neperian logarithm transformation of the exponential function to obtain a linear regression.

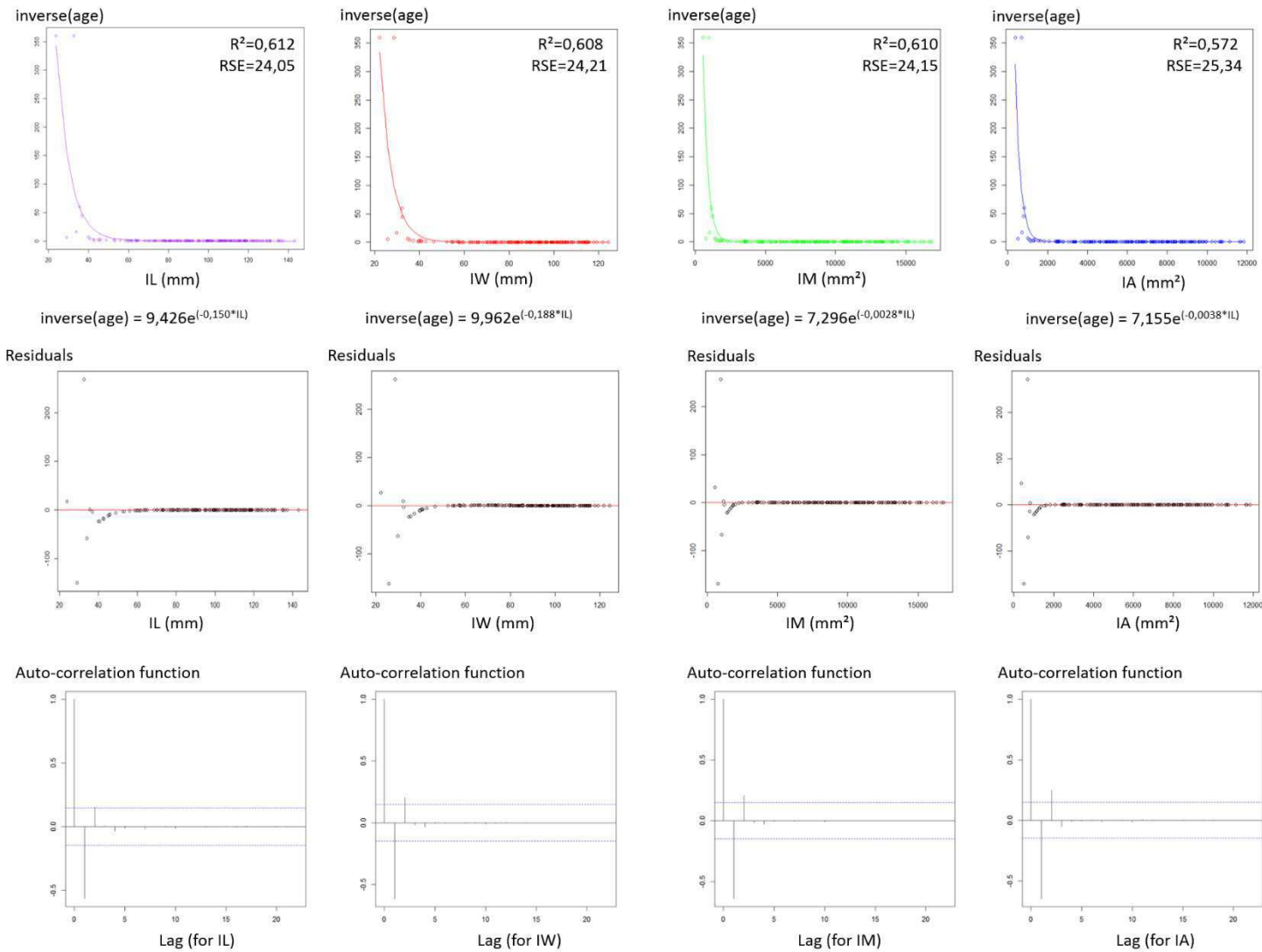


Figure 6.10 Exponential regressions of the inverse of age against IL (purple), IW (red), IM (green) and IA (blue) with their mathematical expressions, associated  $R^2$  and RSE values (upper plots), plot of the residuals against the predictor variables (middle plots) and the autocorrelation functions of the residuals (lower plots)

Table 6-5 Conclusions drawn from the three tests done on the residuals of the exponential regression of the inverse of age against the iliac variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. The greyed areas correspond to valid residual parameters

Variable	inverse(age) = f (variable)			
	Exponential regression			
	R <sup>2</sup>	Corr.	Hom.	Norm.
IL	0.612	No	No	Yes
IW	0.608	No	No	Yes
IM	0.610	No	No	Yes
IA	0.572	No	No	Yes

Graphical diagnostics were used instead. Auto-correlation function estimation (acf) showed that residuals were not significantly correlated as they were distributed within the 95% significance range (except the first two) and showed progressive decrease as lags progress (Figure 6.10). When plotted against predictor variables, residuals were distributed around 0, again with the exception of the smaller individuals. This specific pattern indicates heteroscedasticity. (Figure 6.10). Because of heteroscedastic residuals, and because this transformation and the further steps to obtain a linear model or come back to “normal” age seemed to denature the core relationship between age and the variables, it does not seem suited to model the relationship between age and ilium variables.

Transformation of the variables into their natural logarithmic values did not significantly improve the “untransformed variables” regressions, not did it significantly improve the residuals: R<sup>2</sup> values are equivalent or lower. Residual parameters are invalid for all linear regressions. Polynomial regressions provide independent residuals in half the cases for second degree polynomials and all but one case (IL) for third degree polynomials (Table 6-6).

Table 6-6 Conclusions drawn from the three tests done on the residuals of the linear and polynomial regressions of age against the natural logarithm of the iliac variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase (≥ 0.05) in the value of R<sup>2</sup> between the linear and polynomial regression. The greyed areas correspond to valid residual parameters (p>0.05)

Variable	Age = f (ln(variable))											
	Linear regression				Second degree polynomial				Third degree polynomial			
	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.
IL	0.800	Neg.	No	No	0.915*	Neg.	No	No	0.915*	Neg.	Yes	No
IW	0.791	Neg.	No	No	0.923*	No	No	No	0.925*	No	No	No
IM	0.798	Neg.	No	No	0.925*	Neg.	No	No	0.926*	No	No	No
IA	0.798	Neg.	No	No	0.924*	No	No	No	0.924*	No	No	No

Switching from linear to polynomial models does not improve the value of R<sup>2</sup> as much as the other transformations: R<sup>2</sup> are significantly increased between linear and second degree polynomial

models for all variables but not between second and third degree polynomial regressions. (Table 6-6 and Figure 6.11).

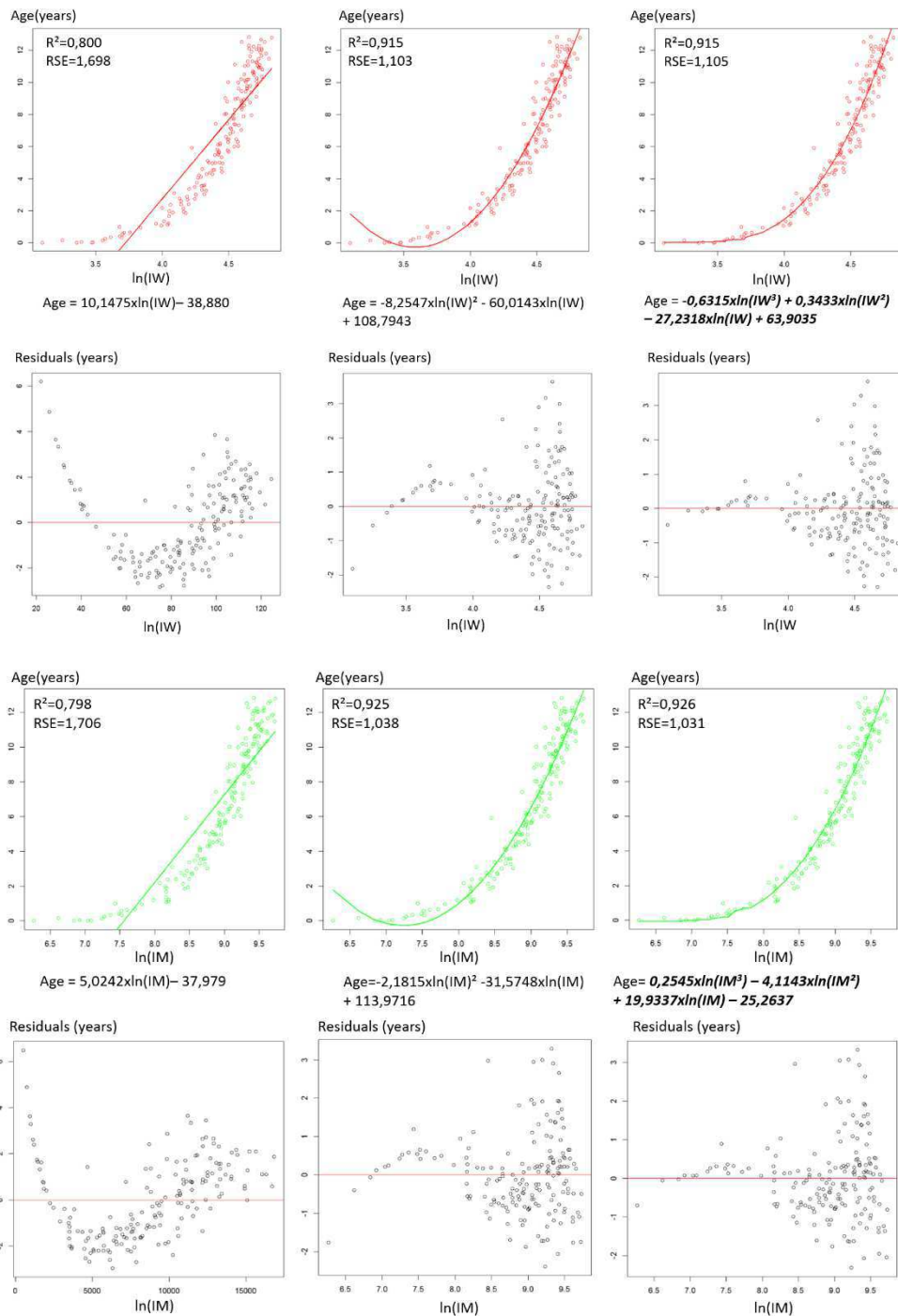


Figure 6.11 Linear, second and third degree polynomial models of age against  $\ln(IW)$  (red) and  $\ln(IM)$  (green) with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (second and third rows). Bold italic terms correspond to non-significant regression coefficients

The relationship between age and the variables is therefore clearly polynomial. Third degree models do not provide significant coefficients for the third degree polynomial terms of unidimensional or

bidimensional variables (**Figure 6.11**). Moreover, all residuals are heteroscedastic (except for the third degree polynomial with IL as a predictor). They present a tail with linear regression models and a funnel-like shape with polynomial models.

No model using the natural logarithm of the variables fulfils all the residual validation requirements.

Transformation of the age into  $\log(\text{age})$  and of the predictor variables into their inverse values did not improve the “untransformed variables” regressions, nor did it significantly improve the residuals:  $R^2$  values are lower or equivalent for all variables, and only the two models with unidimensional predictor variables have independent residuals (**Table 6-7**). All other residual parameters are invalid. The model seems to fit well with the data and have a better fit with unidimensional variables (**Figure 6.12 and Table 6-7**). However, there is a very high concentration of values in the lower ranges of  $\text{inverse}(\text{IW})$  and  $\text{inverse}(\text{IA})$  and a dispersion of the higher ranges of values, which are not included in the mean model (**Figure 6.12**).

**Table 6-7** Conclusions drawn from the three tests done on the residuals of the linear regressions of the logarithm of age against the inverse values of the iliac variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. The greyed areas correspond to valid residual parameters ( $p > 0.05$ )

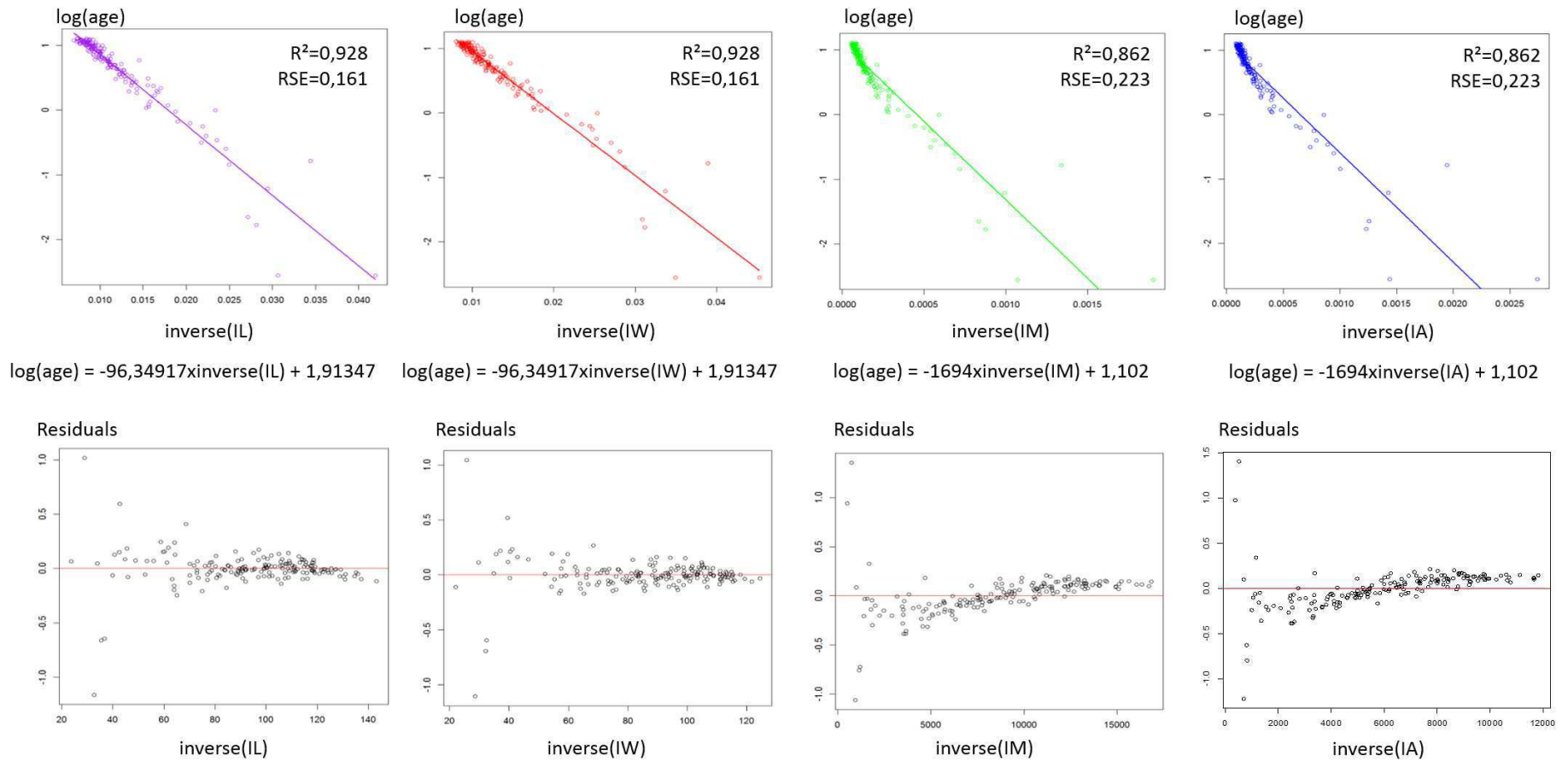
Variable	$\log(\text{age}) = f(\text{inverse}(\text{variable}))$			
	Linear regression			
	$R^2$	Corr.	Hom.	Norm.
IL	0.923	No	No	No
IW	0.928	No	No	No
IM	0.873	Neg.	No	No
IA	0.862	Neg.	No	No

The residuals follow a narrow horizontal dispersion with unidimensional predictor variables, although dispersion is greater for the smaller predictor values, and are concentrated near zero with sporadic and more dispersed points for higher predictor values of bidimensional predictor variables (**Figure 6.12**).

These models are therefore invalid for age estimation.



**Results II: Age estimation**



**Figure 6.12** Examples of the linear regression of the decimal logarithm of age against the inverse of IL (purple), IW (red), IM (green) and IA (blue) with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (lower right and left)

## ii. Lumbar variables

*Linear and polynomial regressions of the lumbar variables against age*

Models were constructed on a training sample of 268 individuals (121 females and 147 males) aged 0 to 19 years, presenting uniform age and sex distributions (see Chapter 5). The bidimensional variables (UVM and LVM) and vertebral body height variables (PVH, AVH, RVH and LVH) have the highest  $R^2$  values with both linear and polynomial regressions. There is an important increase ( $\geq 0.05$ ) in the values of  $R^2$  between the linear and polynomial models for UVW and LVW (Table 6-8, Appendix E).

**Table 6-8** Conclusions drawn from the three tests done on the residuals of the linear and polynomial regressions of age against the lumbar variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase ( $\geq 0.05$ ) in the value of  $R^2$  between the linear and polynomial regression. The greyed areas correspond to valid residual parameters ( $p\text{-value} > 0.05$ )

Variable	Linear regression				Second degree polynomial				Third degree polynomial			
	$R^2$	Corr.	Hom.	Norm.	$R^2$	Corr.	Hom.	Norm.	$R^2$	Corr.	Hom.	Norm.
UVL	0.803	Neg.	No	No	0.812	Neg.	No	No	0.834	Neg.	No	No
UVW	0.770	Neg.	No	No	0.785	Neg.	No	No	0.805*	Neg.	No	No
UVM	0.819	Neg.	No	No	0.824	Neg.	No	No	0.846	Neg.	No	No
LVL	0.813	Neg.	No	No	0.816	Neg.	No	No	0.844	Neg.	No	No
LVW	0.738	Neg.	No	No	0.772	Neg.	No	No	0.782*	Neg.	No	No
LVM	0.820	Neg.	No	No	0.824	Neg.	No	No	0.844	Neg.	No	No
PVH	0.853	Neg.	No	Yes	0.868	Neg.	No	Yes	0.873	Neg.	No	Yes
AVH	0.871	Neg.	No	No	0.872	Neg.	No	No	0.883	Neg.	No	No
RVH	0.885	Neg.	No	Yes	0.896	Neg.	No	Yes	0.901	Neg.	No	Yes
LVH	0.871	Neg.	No	Yes	0.889	Neg.	No	Yes	0.898	Neg.	No	Yes

All regression coefficients are significant at the 0.05 level for the polynomial terms, except in the second degree regression of AVH and the intercept and linear coefficients of the third degree polynomial regression with UVM. However, linear and polynomial regressions seem equally well-fitted for the modelling the relationship between age and the variables, as assessed by the comparable values of  $R^2$  and RSEs. None of the residuals are homoscedastic. They follow a triangular shape, with the presence of a tail for the lower values of residuals from models using upper and lower vertebral variables, and they are not independent. They are normally distributed for only two or three variables out of ten depending on the model used (Table 6-8).

Because of this, new linear, second, and third polynomial regression models were constructed on transformed variables to try to obtain independent, homoscedastic, normally distributed residuals.

These transformations concern the predicted variable (age) and/or the predictor variables. Details and curves of all the transformations can be found in **Appendix E**.

*Variable transformations*

Transformation of age into  $\ln(\text{age})$  did not improve the previous regressions, nor did it significantly improve the residuals.  $R^2$  values are lower or equivalent to models with “untransformed” variables and only two models have independent residuals: second and third degree polynomials using unidimensional variables UVW and LVW and UVW, LVL and LVW, respectively (**Table 6-9**).

**Table 6-9** Conclusions drawn from the three tests done on the residuals of the linear and polynomial regressions of the logarithm of age against the lumbar variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase ( $\geq 0.05$ ) in the value of  $R^2$  between the linear and polynomial regression. The greyed areas correspond to valid residual parameters

Variable	In(age) = f (variable)											
	Linear regression				Second degree polynomial				Third degree polynomial			
	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.
UVL	0.733	Neg.	No	No	0.878*	Neg.	No	No	0.903**	No	No	No
UVW	0.750	Neg.	No	No	0.893*	No	No	No	0.908*	No	No	No
UVM	0.628	Neg.	No	No	0.804*	Neg.	No	No	0.860**	Neg.	No	No
LVL	0.712	Neg.	No	No	0.874*	Neg.	No	No	0.904*	No	No	No
LVW	0.770	Neg.	No	No	0.873*	No	No	No	0.883*	No	No	No
LVM	0.628	Neg.	No	No	0.803*	Neg.	No	No	0.852**	Neg.	No	No
PVH	0.528	Neg.	No	No	0.728*	Neg.	No	No	0.813**	Neg.	No	No
AVH	0.621	Neg.	No	No	0.826*	Neg.	No	No	0.878**	No	No	No
RVH	0.539	Neg.	No	No	0.714*	Neg.	No	No	0.766**	Neg.	No	No
LVH	0.531	Neg.	No	No	0.717*	Neg.	No	No	0.761*	Neg.	No	No

The polynomial models improve the  $R^2$  values dramatically, and RSE values decrease for all three model-types. Only two second degree and three third degree models provide independent residuals. All models present residual heteroscedasticity that is not improved by the polynomial models. No residual values follow a normal distribution.

This transformation does not provide valid age estimation models.

The transformation of age into its square root ( $\text{root}(\text{age})$ ) did not significantly improve the results of the “untransformed variables” regressions (**Table 6-10**), but did provide normally distributed residuals for all third degree polynomial models. The increase in  $R^2$  between linear and polynomial regressions is important for five variables: UVM, LVM, PVH, RVH and LVH (**Table 6-10**).

Third degree polynomial models are the best fit for upper and lower unidimensional data, whereas the best fit for bidimensional data and vertebral height variables could be linear or a second degree polynomial model.

**Table 6-10 Conclusions drawn from the three tests done on the residuals of the linear and polynomial regressions of the square root of age against the lumbar variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase ( $\geq 0.05$ ) in the value of  $R^2$  between the linear and polynomial regression. The greyed areas correspond to valid residual parameters ( $p\text{-value}>0.05$ )**

Variable	root(age) = f (variable)											
	Linear regression				Second degree polynomial				Third degree polynomial			
	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.
UVL	0.883	Neg.	No	Yes	0.888	Neg.	No	Yes	0.898	Neg.	No	Yes
UVW	0.866	Neg.	No	Yes	0.868	Neg.	No	Yes	0.881	Neg.	No	Yes
UVM	0.852	Neg.	No	Yes	0.902*	Neg.	No	Yes	0.903*	Neg.	No	Yes
LVL	0.878	Neg.	No	No	0.893	Neg.	No	Yes	0.902	Neg.	No	Yes
LVW	0.852	Neg.	No	Yes	0.852	Neg.	No	Yes	0.862	Neg.	No	Yes
LVM	0.852	Neg.	No	Yes	0.900	Neg.	No	Yes	0.901*	Neg.	No	Yes
PVH	0.813	Neg.	No	No	0.883*	Neg.	No	Yes	0.884*	Neg.	No	Yes
AVH	0.872	Neg.	No	No	0.913	Neg.	No	No	0.914	Neg.	Yes	Yes
RVH	0.842	Neg.	No	No	0.910*	Neg.	No	Yes	0.911*	Neg.	No	Yes
LVH	0.829	Neg.	No	No	0.909*	Neg.	No	Yes	0.909*	Neg.	No	Yes

Polynomial models improve residual normality but are not sufficient to obtain independent or homoscedastic residuals (**Table 6-10**). Only the third degree regression with AVH has homoscedastic residuals, presenting a homogenous and randomly dispersed pattern. Therefore, we can conclude that no model fulfils all the validation requirements.

Transformation of age into its inverse value did not improve the “untransformed variables” regressions, nor did it significantly improve the residuals (**Table 6-11**). This transformation is not suited to model the relationship between age and lumbar variables.

Table 6-11 Conclusions drawn from the three tests done on the residuals of the exponential regressions of the inverse values of age against the lumbar variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. The greyed areas correspond to valid residual parameters

Variable	inverse(age) = f (variable)			
	Exponential regression			
	R <sup>2</sup>	Corr.	Hom.	Norm.
UVL	0.582	No	No	Yes
UVW	0.580	No	No	Yes
UVM	0.583	No	No	Yes
LVL	0.630	No	No	Yes
LVW	0.508	No	No	Yes
LVM	0.552	No	No	Yes
PVH	0.515	No	No	Yes
AVH	0.559	No	No	Yes
RVH	0.475	No	No	Yes
LVH	0.196	No	No	Yes

Transformation of the variables into their natural logarithmic values did not significantly improve the “untransformed variables” regressions, not did it significantly improve the residuals: R<sup>2</sup> values are equivalent or lower. Residual parameters are generally invalid for all linear regressions. Polynomial regressions provide a few models with normally distributed residuals for half the cases (Table 6-12). Switching from linear to polynomial models improved the values of R<sup>2</sup> for seven variables (all upper and lower variables and only one vertebral height variable, AVH).

Table 6-12 Conclusions drawn from the three tests done on the residuals of the linear and polynomial regressions of age against the natural logarithm of the lumbar variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase (≥ 0.05) in the value of R<sup>2</sup> between the linear and polynomial regression. The greyed areas correspond to valid residual parameters (p-value>0.05)

Variable	Age = f (ln(variable))											
	Linear regression				Second degree polynomial				Third degree polynomial			
	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.
UVL	0.707	Neg.	No	No	0.819*	Neg.	No	No	0.821*	Neg.	No	Yes
UVW	0.670	Neg.	No	No	0.790*	Neg.	No	No	0.794*	Neg.	No	No
UVM	0.698	Neg.	No	No	0.822*	Neg.	No	Yes	0.826*	Neg.	No	Yes
LVL	0.727	Neg.	No	No	0.828*	Neg.	No	No	0.828*	Neg.	No	No
LVW	0.624	Neg.	No	No	0.769*	Neg.	No	Yes	0.778*	Neg.	No	No
LVM	0.689	Neg.	No	No	0.823*	Neg.	No	No	0.828*	Neg.	No	No
PVH	0.840	Neg.	No	No	0.866	Neg.	No	Yes	0.870	Neg.	No	Yes
AVH	0.813	Neg.	No	No	0.876*	Neg.	No	No	0.877*	Neg.	No	No
RVH	0.870	Neg.	No	Yes	0.895	Neg.	No	Yes	0.898	Neg.	No	Yes
LVH	0.862	Neg.	No	Yes	0.886	Neg.	No	Yes	0.892	Neg.	No	Yes

The relationship between age and the natural logarithm of the variables is therefore clearly polynomial (**Table 6-12**).

However, no model fulfils all the validation requirements to be an age estimation method.

Transformation of age into log(age) and of the predictor variables into their inverse values did not improve the “untransformed variables” regressions. R<sup>2</sup> values are equivalent to the original models for all variables. The three models with the highest R<sup>2</sup> values also provided independent residuals (**Table 6-13**). All other residual parameters are invalid.

**Table 6-13** Conclusions drawn from the three tests done on the residuals of the linear regressions of the logarithm of age against the inverse of the lumbar variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase ( $\geq 0.05$ ) in the value of R<sup>2</sup> between the linear and polynomial regression. The greyed areas correspond to valid residual parameters (p-value>0.05)

Variable	log(age) = f (inverse(variable))			
	Linear regression			
	R <sup>2</sup>	Corr.	Hom.	Norm.
UVL	0.906	No	No	No
UVW	0.877	Neg.	No	No
UVM	0.697	Neg.	No	No
LVL	0.923	No	No	No
LVW	0.862	Neg.	No	No
LVM	0.751	Neg.	No	No
PVH	0.791	Neg.	No	No
AVH	0.914	No	No	No
RVH	0.802	Neg.	No	No
LVH	0.784	Neg.	No	No

The models seem to fit well with the data and have a better fit with unidimensional variables (**Table 6-13**). However, because of invalid residual parameters, these models are invalid for age estimation.

iii. Clavicular variables

*Linear and polynomial regressions of the clavicular variables against age*

Models were constructed on a training sample of 209 individuals (101 females and 108 males) using Ln, AP\_diam and SI\_diam as predictors, and on 191 individuals (90 females and 101 males) using Max\_diam and Min\_Diam as predictors because of missing values. Samples present uniform age and sex distributions (see **Chapter 5**).

The highest regression coefficients ( $R^2$ ) are found in the models using Ln as a predictor. There is an important increase ( $\geq 0.05$ ) in the values of  $R^2$  between the linear and third degree polynomial models for all variables except Ln (**Table 6-14**). All regression coefficients are significant at the 0.05 level, except the intercept of the third degree polynomial regression of AP\_diam. This confirms the third degree polynomial relationship between age and the diameter variables.

**Table 6-14 Conclusions drawn from the three tests done on the residuals of the linear and polynomial regressions of age against the clavicular variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase ( $\geq 0.05$ ) in the value of  $R^2$  between the linear and polynomial regression. The greyed areas correspond to valid residual parameters ( $p > 0.05$ )**

Variable	Linear regression				Second degree polynomial				Third degree polynomial			
	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.
Ln	0.913	Neg.	No	No	0.914	Neg.	No	No	0.938	Neg.	No	Yes
Max_diam	0.763	Neg.	No	Yes	0.784	Neg.	No	No	0.842*	Neg.	No	Yes
Min_diam	0.793	Neg.	No	No	0.803	Neg.	No	No	0.841*	Neg.	No	No
AP_diam	0.722	Neg.	No	No	0.745	Neg.	No	No	0.787*	Neg.	No	No
SI_diam	0.736	Neg.	No	No	0.741	Neg.	No	No	0.791*	Neg.	No	No

Third degree polynomial models seem to best explain the variance of age according to the four clavicular diameters, whereas age predicted by maximum clavicular length could be modelled using linear regression. The residuals all present significant negative autocorrelation.

Because of this, new linear, second, and third polynomial regression models were constructed on transformed variables to obtain independent, homoscedastic, normally distributed residuals. These transformations concern the predicted variable (age) and/or the predictor variables. Details and curves of all the transformations can be found in **Appendix E**.

*Variable transformations*

Transformation of age into  $\ln(\text{age})$  did not improve the previous regressions, nor did it significantly improve the residuals:  $R^2$  values are lower or equivalent, and only two models have independent residuals: third degree polynomials using Max\_diam and Min\_diam as predictors (**Table 6-15**).

The polynomial models improve the  $R^2$  values dramatically, although RSE values remain comparable. All models present residual heteroscedasticity that is not improved by the polynomial models.

Table 6-15 Conclusions drawn from the three tests done on the residuals of the linear and polynomial regressions of the natural logarithm of age against the clavicular variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase ( $\geq 0.05$ ) in the value of  $R^2$  between the second and third degree polynomial regressions; the greyed areas correspond to valid residual parameters ( $p > 0.05$ )

Variable	ln(age) = f (variable)											
	Linear regression				Second degree polynomial				Third degree polynomial			
	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.
Ln	0.702	Neg.	No	No	0.890*	Neg.	No	No	0.918*	Neg.	No	No
Max_diam	0.570	Neg.	No	No	0.824*	Neg.	No	No	0.850*	No	No	No
Min_diam	0.587	Neg.	No	No	0.823*	Neg.	No	No	0.859*	No	No	No
AP_diam	0.534	Neg.	No	No	0.777*	Neg.	No	No	0.826**	Neg.	No	No
SI_diam	0.576	Neg.	No	No	0.743*	Neg.	No	No	0.739*	Neg.	No	No

Transformation of age into its square root (root(age)) improved the results compared to the “untransformed variables” regressions, but did not always significantly improve the residuals. The increase in  $R^2$  between linear and polynomial regressions is important for all variables except for Ln.  $R^2$  values are higher or equivalent to the “untransformed” regressions and the residual standard errors are lower (Table 6-16).

Third degree polynomial models are the best fit for the diameter variables, whereas the best fit for Ln could be linear or polynomial. If only two linear models have normal residuals, the third degree polynomials using Ln, Max\_diam and Min\_diam as predictors all have normal residuals. Four third degree models have homoscedastic residuals (those using Ln, Max\_diam, AP\_diam and SI\_diam) and two have independent residuals (those using Min\_diam and SI\_diam). However, once again, no model fulfils all the validation requirements.

Table 6-16 Conclusions drawn from the three tests done on the residuals of the linear and polynomial regressions of the square root of age against the clavicular variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase ( $\geq 0.05$ ) in the value of  $R^2$  between the linear and polynomial regression. The greyed areas correspond to valid residual parameter ( $p > 0.05$ )

Variable	root(age) = f (variable)											
	Linear regression				Second degree polynomial				Third degree polynomial			
	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.
Ln	0.931	Neg.	No	Yes	0.952	Neg.	No	Yes	0.959	Neg.	Yes	Yes
Max_diam	0.751	Neg.	No	Yes	0.841*	Neg.	No	Yes	0.859*	Neg.	Yes	Yes
Min_diam	0.791	Neg.	No	Yes	0.864*	Neg.	No	Yes	0.875*	No	No	Yes
AP_diam	0.705	Neg.	No	Yes	0.799*	Neg.	No	No	0.802*	Neg.	Yes	No
SI_diam	0.754	Neg.	No	Yes	0.796	Neg.	No	No	0.825*	No	Yes	No



Transformation of age into its inverse value did not at all improve the “untransformed variables” regressions, nor did it significantly improve the residuals (Table 6-17).

**Table 6-17** Conclusions drawn from the three tests done on the residuals of the exponential regressions of the inverse of age against the clavicular variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. The greyed areas correspond to valid residual parameters

Variable	inverse(age) = f (variable)			
	Exponential regression			
	R <sup>2</sup>	Corr.	Hom.	Norm.
Ln	0.535	No	No	Yes
Max_diam	0.573	No	No	Yes
Min_diam	0.570	No	No	Yes
AP_diam	0.686	No	No	Yes
SI_diam	0.152	No	No	Yes

Transformation of the variables into their natural logarithmic values did not significantly improve the “untransformed variables” regressions, not did it significantly improve the residuals: R<sup>2</sup> values are equivalent or lower, and residual parameters are invalid, with one exception: polynomial regressions of Max\_diam result in normally distributed residuals (Table 6-18).

Switching from linear to polynomial models does not improve the value of R<sup>2</sup> as much as the other transformations: R<sup>2</sup> are significantly increased between linear and third degree polynomial models for the diameter variables, and between the linear and second degree polynomial regressions for Ln. The relationship between age and the variables is clearly polynomial (Table 6-18).

**Table 6-18** Conclusions drawn from the three tests done on the residuals of the linear and polynomial regressions of age against the natural logarithm of the clavicular variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. \*Indicates a significant increase (≥ 0.05) in the value of R<sup>2</sup> between the linear and polynomial regression. The greyed area corresponds to a valid residual parameter (p>0.05)

Variable	Age = f (ln(variable))											
	Linear regression				Second degree polynomial				Third degree polynomial			
	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.	R <sup>2</sup>	Corr.	Hom.	Norm.
Ln	0.853	Neg.	No	No	0.921*	Neg.	No	No	0.923*	Neg.	No	No
Max_diam	0.737	Neg.	No	No	0.762	Neg.	No	Yes	0.807*	Neg.	No	Yes
Min_diam	0.773	Neg.	No	No	0.804	Neg.	No	No	0.819*	Neg.	No	Yes
AP_diam	0.704	Neg.	No	No	0.723	Neg.	No	No	0.762*	Neg.	No	No
SI_diam	0.677	Neg.	No	No	0.752*	Neg.	No	No	0.759	Neg.	No	No

Third degree models clearly seem to be best suited for age estimation using clavicular diameter variables, whereas maximum clavicular length (Ln) seems a better predictor with a second or third degree polynomial model. However, all residuals are correlated and heteroscedastic. No model fulfils all the validation requirements.

Transformation of age into log(age) and of the predictor variables into their inverse values did not improve the “untransformed variables” regressions, nor did it significantly improve the residuals: R<sup>2</sup> values are lower or equivalent for all variables, and only one model has independent residuals (with Min\_diam as the predictor) (**Table 6-19**). All other residual parameters are invalid.

**Table 6-19** Conclusions drawn from the three tests done on the residuals of the linear regressions of the logarithm of age against the inverse of the clavicular variables. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality. The greyed area corresponds to a valid residual parameter (p>0.05)

Variable	log(age) = f (inverse(variable))			
	Linear regression			
	R <sup>2</sup>	Corr.	Hom.	Norm.
Ln	0.913	Neg.	No	No
Max_diam	0.818	Neg.	No	No
Min_diam	0.846	No	No	No
AP_diam	0.798	Neg.	No	No
SI_diam	0.531	Neg.	No	No

iv. Conclusions on OLS models

The previous analyses found that third degree polynomial regressions seem the best fit for age prediction using unidimensional iliac variables, and that age could be predicted using linear regressions when using iliac bidimensional variables. This is confirmed by the results of the F-test done on the linear and polynomial regressions (**Table 6-20**).

**Table 6-20** Results of the Fisher test for differences between linear and polynomial age prediction models using iliac variables. F-ratio = ratio between the variances of the models; 95% CI = 95% confidence interval for the F-ratio; Greyed p-values indicate significant differences between the models

Variable	F-ratio	95% CI	p-value	Best model
IL	1.416	[1.050 ; 1.910]	0.023	Third degree
IW	1.413	[1.048 ; 1.905]	0.024	Third degree
IM	1.063	[0.788 ; 1.433]	0.689	No difference
IA	1.121	[0.831 ; 1.512]	0.452	No difference

Therefore, third degree polynomial models were considered to be the best compromise between parsimony and best fit for age prediction using unidimensional iliac variables, and linear models were considered to be the best compromise between parsimony and best fit for age prediction using bidimensional iliac variables. The problem lies in obtaining models with valid residual parameters: homoscedasticity, normal distribution, and independence.

No significant differences in variance were found between linear and polynomial regressions of age against the ten lumbar variables (**Table 6-21**). However, age prediction models using third degree polynomial regression of the unidimensional variables (UVL, UVW, LVL, and LVW), provided higher values of  $R^2$ , lower values of RSE and presented the advantage of including the younger individuals. Polynomial and linear regression models of the bidimensional variables (UVM and LVM) and linear regressions of the vertebral height variables PVH, AVH, RVH and LVH were found to be comparable for their values of  $R^2$  and RSE. Therefore, linear and third degree polynomial models were considered to be the best compromise between parsimony and best fit for age prediction using lumbar variables.

**Table 6-21 Results of the Fisher test for differences between linear and polynomial age prediction models using lumbar variables. F-ratio = ratio between the variances of the models; 95% CI = 95% confidence interval for the F-ratio; Greyed p-values indicate significant differences between the models. *Italic terms indicate low significance of the results***

Variable	F-ratio	95% CI	p-value	Best model
UVL	1.187	[0.933 ; 1.511]	0.163	No difference
UVW	1.169	[0.918 ; 1.488]	0.205	No difference
UVM	1.176	[0.924 ; 1.497]	0.187	No difference
LVL	1.190	[0.935 ; 1.515]	0.158	No difference
LVW	1.191	[0.936 ; 1.516]	0.156	No difference
LVM	1.148	[0.902 ; 1.461]	0.263	No difference
PVH	1.170	[0.919 ; 1.489]	0.203	No difference
AVH	1.099	[0.863 ; 1.399]	0.443	No difference
RVH	1.171	[0.920 ; 1.490]	0.200	No difference
LVH	1.248	[0.980 ; 1.589]	<i>0.072</i>	<i>No difference</i>

Age prediction using maximum clavicular length Ln, Max\_diam and SI\_diam should be attempted using third degree polynomial models, because they presented a significantly better fit than linear models. It is interesting to note that the linear regression model using Ln also provided good results ( $R^2$  and RSE values are comparable to those of polynomial models) and has the advantage of simplicity in its application. Although F-tests found no clear difference between linear and polynomial regressions with Min\_diam and AP\_diam as predictors (**Table 6-22**), the associated p-

values are low and third degree polynomials showed rather important increases in R<sup>2</sup> values and decreases in RSE values (**Table 6-14 and Appendix E**). All third degree models are more significant than second degree models for all variables. Linear and third degree polynomial models could therefore be selected for age prediction using Ln, Min\_diam and AP\_diam; polynomial models could be chosen for age prediction using Max\_diam and SI\_diam.

**Table 6-22 Results of the Fisher test for differences between linear and polynomial age prediction models using clavicular variables. F-ratio = ratio between the variances of the models; 95% CI = 95% confidence interval for the F-ratio; Greyed p-values indicate significant differences between the models. Italic terms indicate low significance of the results**

Variable	F-ratio	95% CI	p-value	Best model
Ln	1.395	[1.060 ; 1.834]	0.017	Third degree
Max_diam	1.508	[1.132 ; 2.009]	0.005	Third degree
Min_diam	1.305	[0.980 ; 1.739]	0.069	<i>No difference (Third degree)</i>
AP_diam	1.314	[0.999 ; 1.728]	0.051	<i>No difference (Third degree)</i>
SI_diam	1.272	[0.967 ; 1.672]	0.023	Polynomial (second or third degree)

Thus, linear and third degree polynomial models were considered to be the best compromise between parsimony and best fit for age prediction using clavicular variables. The problem lies in obtaining models with valid residual parameters: homoscedasticity, normal distribution, and independence.

Simple linear and/or third degree polynomial regression models on the untransformed variables provided high determination coefficients (between 0.704 and 0.935) and RSE values comparable to those obtained with transformed variables. Variable and/or age transformations did not significantly increase determination coefficients, nor did they decrease RSE values, even for the best fitted models. Moreover, no model provided valid residual parameters. This means that no valid prediction intervals could be constructed and that regression coefficients could be biased. Therefore, some of the “untransformed” models could be suitable for age estimation using clavicular, lumbar and iliac variables, if all validation parameters concerning residuals were met. The following paragraph presents the models that would be hypothetically retained if they provided valid parameters. These conclusions will be used in the next step of the analysis: constructing models robust to residual heteroscedasticity.

**b. Heteroscedasticity- and autocorrelation-robust variance-covariance matrices (vcovHAC) - Weighted Least Squares (WLS) regressions**

- i. Dealing with heteroscedasticity I: variance-covariance matrices robust to residual heteroscedasticity and autocorrelation

Although the previous results give an idea of the general tendency of the relationship between age and the predictor variables, because of residual heteroscedasticity, dependence and autocorrelation, the standard errors of the estimated coefficients are likely to be biased. Consequently, the significance of the coefficients in the model could be biased, which could invalidate the regression model altogether, questioning the nature of the relationship between the predictor and predicted variables. For this reason, and because the OLS models provided relatively good or very good results, the significance of the coefficients was tested by constructing their variance-covariance matrices robust to residual heteroscedasticity and autocorrelation. These matrices provide unbiased standard errors and associated statistics for the regression coefficients that can then be compared to the original OLS parameters to check if they are concurring or not (Table 6-23).

This was done for all linear and third degree polynomial regression models of untransformed variables. The results proved that the coefficient estimates obtained for all models were significant and that the type of models used to model age against each predictor variable was valid. However, because of persistent heteroscedasticity, no valid prediction intervals can be constructed and models cannot be used for age prediction.

**Table 6-23 Coefficient estimates with their associated statistical parameters obtained for the OLS linear and polynomial regression equations of age using Ln as a predictor and their heteroscedastically robust parameters**

Model	Coefficient	OLS model				Heteroscedasticity and autocorrelation-robust parameters		
		Estimate	Standard error	t	p-value	Standard error	t	p-value
Linear	Intercept	-11.097	0.465	-23.87	<0.001	0.643	-16.982	<0.001
	Ln	0.187	0.004	47.34	<0.001	0.006	32.833	<0.001
Third degree polynomial	Intercept	14.450	2.790	5.122	<0.001	2.896	4.854	<0.001
	Ln	-0.6216	0.0876	-7.114	<0.001	0.0946	-6.424	<0.001
	Ln <sup>2</sup>	0.00791	0.00086	9.180	<0.001	0.00097	7.970	<0.001
	Ln <sup>3</sup>	-0.0000238	2.694e-06	-8.964	<0.001	3.158e-06	-7.550	<0.001

ii. Dealing with heteroscedasticity II: age prediction using WLS models

Resolving residual heteroscedasticity was attempted by constructing age prediction models using Weighted Least Squares (WLS). WLS models use the cause for heteroscedasticity, *i.e.* the changes in the variance of age in the whole sample, to construct valid models with homoscedastic residuals. The principle lies in identifying weights for the regression coefficients that will homogenise the residuals. The plots of age against the predictor variables and of the residuals versus predictor variables obtained for each Ordinary Least Squares (OLS) regression of age against the “untransformed” variables were used to identify intervals of predictor values for which the residuals seem homoscedastic (*i.e.* the residuals follow a homogenous and random dispersion around zero). Each interval is limited by an upper and a lower value of the predictor variable and corresponds to a specific age range (**Figure 6.13**). The inverse of the variance of each age range covered by the interval are then used as weights to construct WLS regressions.

Weighted Least Squares models were constructed for linear and third degree polynomial models for age estimation using the clavicular, lumbar and iliac variables. Because all results provided models that still provided heteroscedastic and non-normally distributed residuals, the entire procedure to obtain WLS models will be detailed only once with a single example of linear and polynomial WLS models.

The first step for constructing WLS models was to identify sub-groups or intervals of a predictor variable for which the associated residuals obtained via OLS seem to present a homogeneous pattern when plotted against said predictor variable (**Figure 6.13**).

The intervals of predictor variables were then used to calculate the mean and variance of the age ranges covered by each interval. The number of individuals in each interval was also noted. This was done for both linear and polynomial models (**Tables 6-24 and 6-25**).

**Table 6-24 Characteristics of the intervals of values of Ln used to calculate the weights of the linear WLS regression model. n = number of individuals in the interval; Mean = mean age in the interval; Variance = variance of age in the interval**

Linear regression model			
Interval of values	n	Mean	Variance
[31.48 ; 66.667[	16	0.488	0.169
[66.667 ; 128.333[	119	6.770	13.651
[128.333 – [	87	16.457	5.058

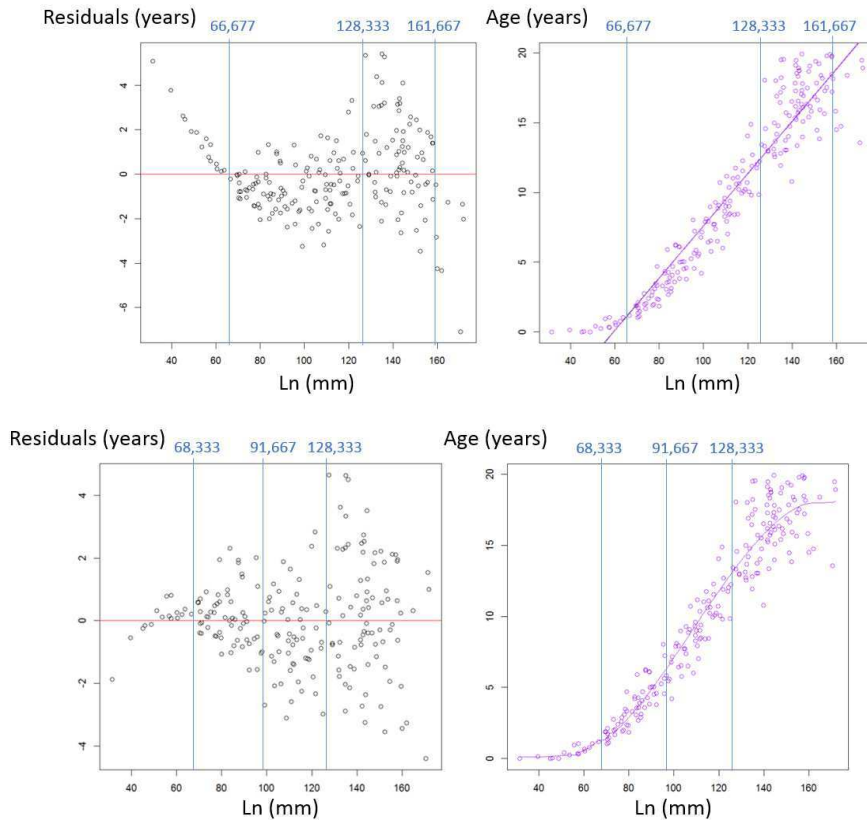


Figure 6.13 Example of the four intervals of Ln values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by Ln. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (Ln) (upper and lower right plots)

It is obvious that variance of age is extremely heterogeneous, particularly for the linear OLS model: the variances per age range are very different and the pattern of the residuals is very different in each interval (Figure 6.13).

Table 6-25 Characteristics of the intervals of values of Ln used to calculate the weights of the third degree polynomial WLS regression model. n = number of individuals in the interval; Mean = mean age in the interval; Variance = variance of age in the interval

Polynomial regression model			
Interval of values	n	Mean	Variance
[31.18 ; 68.333[	16	0.488	0.169
[68.333 ; 91.667[	49	3.345	2.089
[91.667 ; 128.333[	70	9.168	7.728
[128.333 – [	87	16.457	5.058

The inverse values of variance were then used as weights for each corresponding interval of the predictor variable. The results obtained by WLS were then compared to those previously obtained by OLS (Tables 6-26 and 6-27).

Table 6-26 OLS and WLS regression coefficients and their associated statistical parameters obtained for the linear and third degree polynomial models for age prediction using Ln. Estimate = estimated value of the coefficient; Standard error of the estimate; t statistic of the t-test for significance conducted on the estimates and the associated p-value

Model	Coefficient	OLS model				WLS model			
		Estimate	Standard error	t	p-value	Estimate	Standard error	t	p-value
Linear	Intercept	-10.911	0.464	-23.53	<0.001	-8.113	0.227	-35.670	<0.001
	Ln	0.185	0.004	46.71	<0.001	0.162	0.003	55.150	<0.001
Polynomial	Intercept	14.406	2.790	5.038	<0.001	8.455	0.983	8.604	<0.001
	Ln	-0.608	0.0867	-7.006	<0.001	-0.413	0.037	-11.204	<0.001
	Ln <sup>2</sup>	0.00776	0.00077	9.063	<0.001	0.0058	0.00043	13.454	<0.001
	Ln <sup>3</sup>	-0.000024	2.384e-05	-8.850	<0.001	-0.000018	1.538e-06	-11.457	<0.001

We can see that the intercept estimates differ significantly between the OLS and WLS models (Table 6-27, Figures 6.14 and 6.15).

However, the other coefficients have comparable estimates for both model types and all of them are significant. The R<sup>2</sup> values are higher and the RSE values are lower for both WLS models compared to OLS models, meaning they provide better age estimates (Figures 6.14 and 6.15).

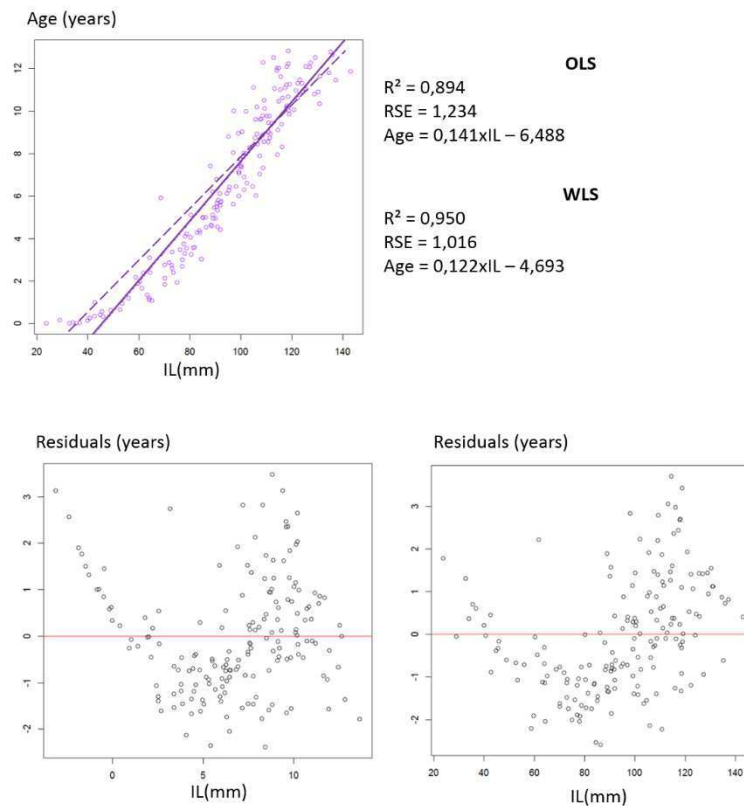


Figure 6.14 Plot of age against IL, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics (R<sup>2</sup> and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against IL; Right plot: plot of residuals of the WLS linear regression against IL



WLS improves the models significantly for all variables, especially the ten lumbar variables (Table 6-27). WLS models tend to flatten and even out the distribution of the residuals, by attenuating variability at the extreme values. However, residuals still present heteroscedasticity (Figures 6.14 and 6.15) and do not follow a normal distribution, excluding a few exceptions (Table 6-27).

Autocorrelation of the residuals cannot be verified statistically for WLS models.

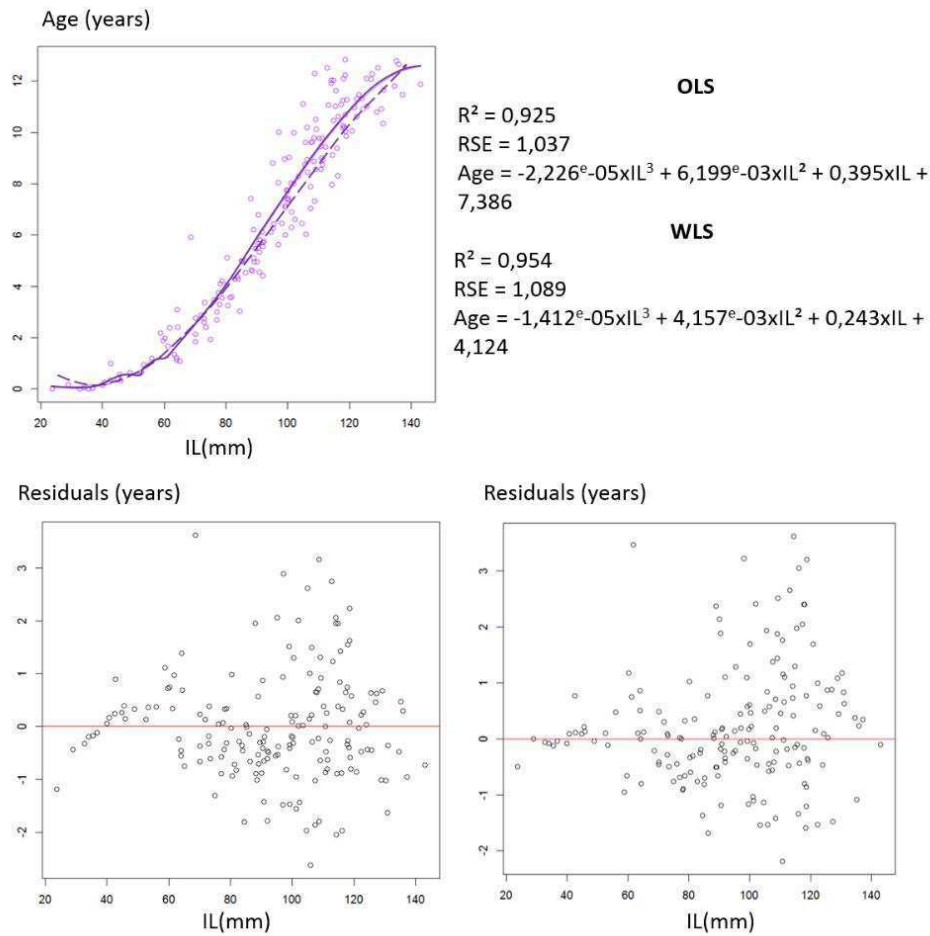


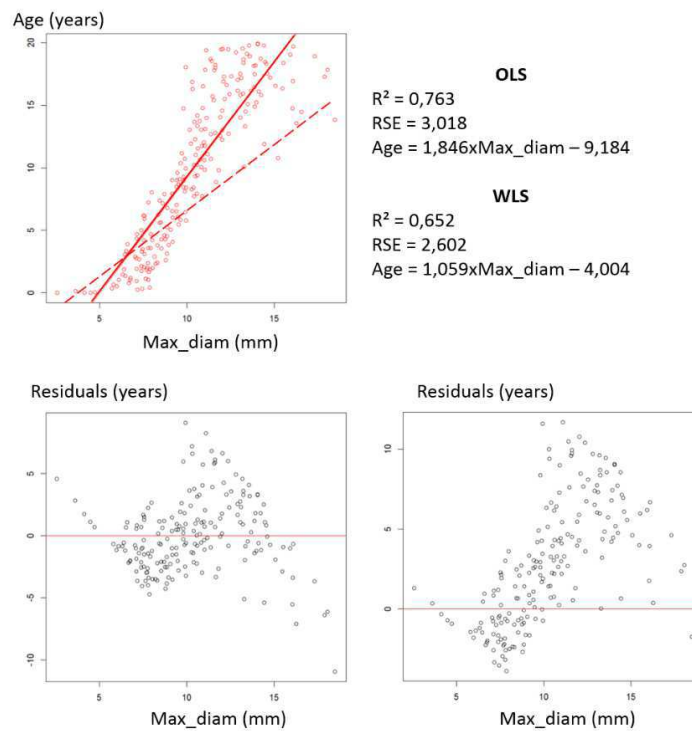
Figure 6.15 Plot of age against IL, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against IL; Right plot: plot of residuals of the WLS polynomial regression against IL

Table 6-27 Comparison of the results of linear and third degree polynomial OLS and WLS regression models of age against all the variables in the study. \*Values of R<sup>2</sup> and RSE significantly improved compared to their equivalents with OLS models. Greyed areas correspond to valid parameters. Residual parameters: Corr. = Autocorrelation; Hom. = Homoscedasticity; Norm. = Normality

Variable	OLS linear regression					OLS third degree polynomial					WLS linear regression				WLS third degree polynomial			
	R <sup>2</sup>	RSE	Corr.	Hom.	Norm.	R <sup>2</sup>	RSE	Corr.	Hom.	Norm.	R <sup>2</sup>	RSE	Hom.	Norm.	R <sup>2</sup>	RSE	Hom.	Norm.
Ln	0.913	1.813	Neg.	No	No	0.938	1.535	Neg.	No	Yes	0.936*	1.098*	No	No	0.974*	0.731*	No	No
Max_diam	0.763	3.018	Neg.	No	Yes	0.842	2.558	Neg.	No	Yes	0.652	2.602*	No	No	0.944*	1.027*	No	No
Min_diam	0.793	2.822	Neg.	No	No	0.841	2.470	Neg.	No	No	0.889*	1.416*	No	No	0.955*	0.776*	No	No
AP_diam	0.722	3.248	Neg.	No	No	0.787	2.834	Neg.	No	No	0.695	2.468*	No	No	0.940*	1.028*	No	No
SI_diam	0.735	3.165	Neg.	No	No	0.791	2.807	Neg.	No	No	0.846*	1.128*	No	No	0.922*	0.804*	No	No
Variable	OLS linear regression					OLS third degree polynomial					WLS linear regression				WLS third degree polynomial			
	R <sup>2</sup>	RSE	Corr.	Hom.	Norm.	R <sup>2</sup>	RSE	Corr.	Hom.	Norm.	R <sup>2</sup>	RSE	Hom.	Norm.	R <sup>2</sup>	RSE	Hom.	Norm.
UVL	0.803	2.572	Neg.	No	No	0.834	2.361	Neg.	No	No	0.889*	0.954*	No	No	0.938*	0.788*	No	No
UVW	0.770	2.779	Neg.	No	No	0.805	2.570	Neg.	No	No	0.850*	1.258*	No	No	0.933*	0.826*	No	No
UVM	0.819	2.456	Neg.	No	No	0.846	2.273	Neg.	No	No	0.938*	0.817*	No	No	0.935*	0.736*	No	No
LVL	0.813	2.506	Neg.	No	No	0.844	2.297	Neg.	No	No	0.904*	0.982*	No	No	0.945*	0.750*	No	No
LVW	0.738	2.966	Neg.	No	No	0.782	2.718	Neg.	No	No	0.845*	1.026*	No	No	0.906*	0.805*	No	No
LVM	0.820	2.462	Neg.	No	No	0.844	2.298	Neg.	No	No	0.940*	0.795*	No	No	0.939*	0.787*	No	No
PVH	0.853	2.227	Neg.	No	Yes	0.873	2.059	Neg.	No	Yes	0.917*	1.369*	No	Yes	0.944*	0.891*	No	Yes
AVH	0.871	2.085	Neg.	No	No	0.883	1.989	Neg.	No	No	0.940*	0.981*	No	No	0.947*	0.810*	No	No
RVH	0.885	1.967	Neg.	No	Yes	0.901	1.818	Neg.	No	Yes	0.953*	0.809*	No	Yes	0.962*	0.674*	No	Yes
LVH	0.871	2.082	Neg.	No	Yes	0.898	1.863	Neg.	No	Yes	0.901*	0.885*	No	Yes	0.920*	0.808*	No	Yes
Variable	OLS linear regression					OLS third degree polynomial					WLS linear regression				WLS third degree polynomial			
	R <sup>2</sup>	RSE	Corr.	Hom.	Norm.	R <sup>2</sup>	RSE	Corr.	Hom.	Norm.	R <sup>2</sup>	RSE	Hom.	Norm.	R <sup>2</sup>	RSE	Hom.	Norm.
IL	0.894	1.234	Neg.	No	No	0.926	1.037	No	No	No	0.950*	1.016*	No	No	0.954*	1.089	No	No
IW	0.895	1.233	Neg.	No	No	0.921	1.069	No	No	No	0.929*	0.945*	No	No	0.982*	0.623*	No	No
IM	0.921	1.069	Neg.	No	No	0.935	0.967	No	No	No	0.968*	0.765*	No	No	0.981*	0.703*	No	No
IA	0.916	1.098	Neg.	No	No	0.934	0.980	No	No	No	0.966*	0.897*	No	No	0.982*	0.617*	No	No

Moreover, although WLS models tend to increase  $R^2$  and decrease RSE values, thus providing overall better fits and lower residual dispersion, there are a few cases for which WLS models provide worse results than OLS (**Figure 6.16 and Table 6-27**). The linear regression of age against Max\_diam shows a much lower value of  $R^2$ , a lower value of RSE but a much higher dispersion of the residuals in the positive values for the WLS model compared to the OLS model. The fitted WLS line does not follow the direction of the majority of the individuals and seems to be based on the extreme values of Max\_diam (the younger and older individuals).

The best results in terms of  $R^2$  and RSE were obtained for the third degree WLS polynomial model of age against ilium area (IA). However, residuals are still heteroscedastic and do not follow a normal distribution (**Table 6-27**).



**Figure 6.16** Plot of age against Max\_diam, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against Max\_diam; Right plot: plot of residuals of the WLS linear regression against Max\_diam

WLS models could have been an improved solution for heteroscedastic OLS models, because they provide better age estimates. They also confirm that third degree polynomial models present a significantly better fit than linear models, excluding bidimensional variables of the fifth lumbar vertebra and the ilium. However, heteroscedasticity of the residuals is still present, and almost none of them follow a normal distribution.

Therefore, the choice was made to turn to non-parametric Multi-variate Adaptive Regression Splines (MARS) models as a heteroscedasticity-resistant alternative to parametric models for age prediction.

### **6.1.2. Non-parametric Multi-variate Adaptive Regression Splines (MARS) models**

Univariate/simple (using one variable as age predictor) and multivariate/multiple (using several or all variables of the same bone as age predictors) models were built on the same training samples used to construct the parametric regressions. The values of Cross-Validation R-Squared (CVRSq), standard deviation across folds, General R-Squared (GRSq), accuracy, variable importance in multivariate models and the smallest and largest prediction intervals were compared to assess model performance. The models were then tested on independent test samples from Marseilles. On these test samples, the RSq, the mean and the standard deviation of residuals were analysed for comparison with parametric models. The confidence intervals of the regression parameters and the prediction intervals were set at a 95% level.

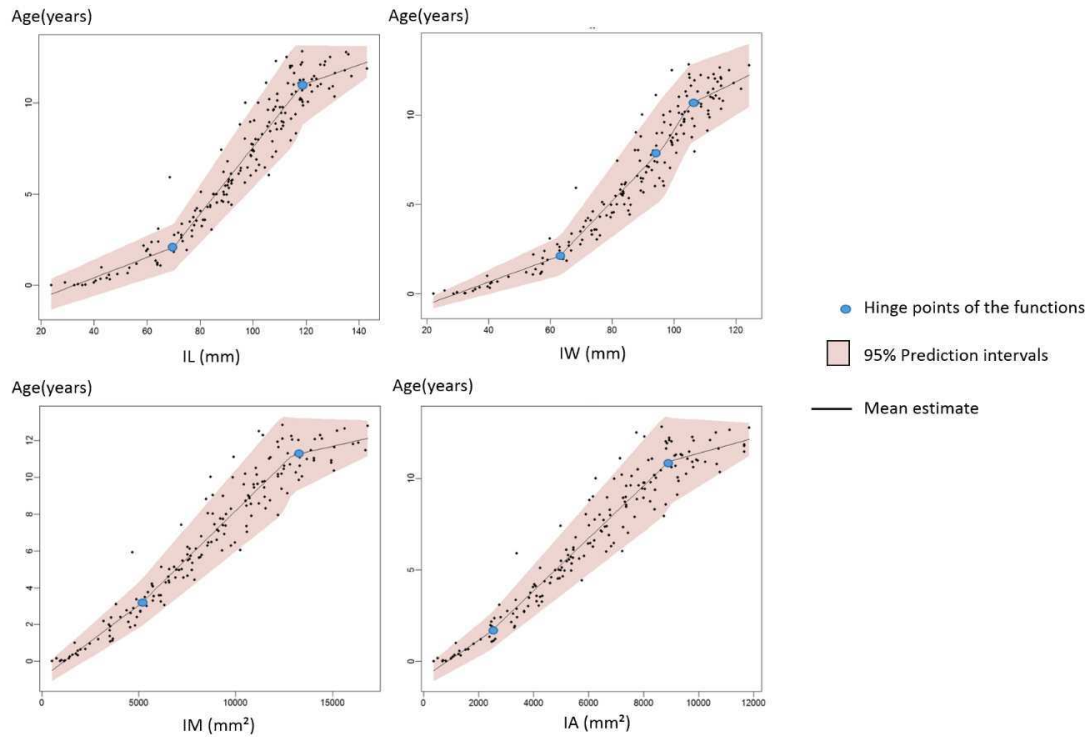
For a practical application of MARS models for age estimation, two options are possible. First, the measurement obtained for the variable can be reported on the plot of age against said variable to find the mean estimate, the maximum and the minimum ages predicted by the model. Second, the mathematical formula of the MARS function can be used for age estimation in the same way as a regression equation. The 95% prediction intervals associated with the best models selected after testing are calculated with the MARS function and can be found in **Appendix F** as an abacus with the corresponding values of predictor variables. The abacus also highlights the hinge points of the functions and therefore the corresponding ages, when the relationship between age and the variables changes.

#### **a. Iliac variables**

Models were constructed on a training sample of 176 individuals (72 females and 104 males) aged 0 to 12 years and presenting a uniform distribution of age and sex. They were tested on an independent sample of 68 individuals (28 females and 40 males). Both samples have uniform age and sex distributions (**see Chapter 5**).

MARS functions detected two hinge points for the univariate models using IL, IM and IA and three for the function modelling age against IW, identifying two or three occurrences for non-linearity of the

relation between age and the variables (**Figure 6.17**), and providing three or four sub-functions modelling this relationship. Bivariate and multivariate models present between one and two hinge points per variable, depending on the model (**Figure 6.18**).



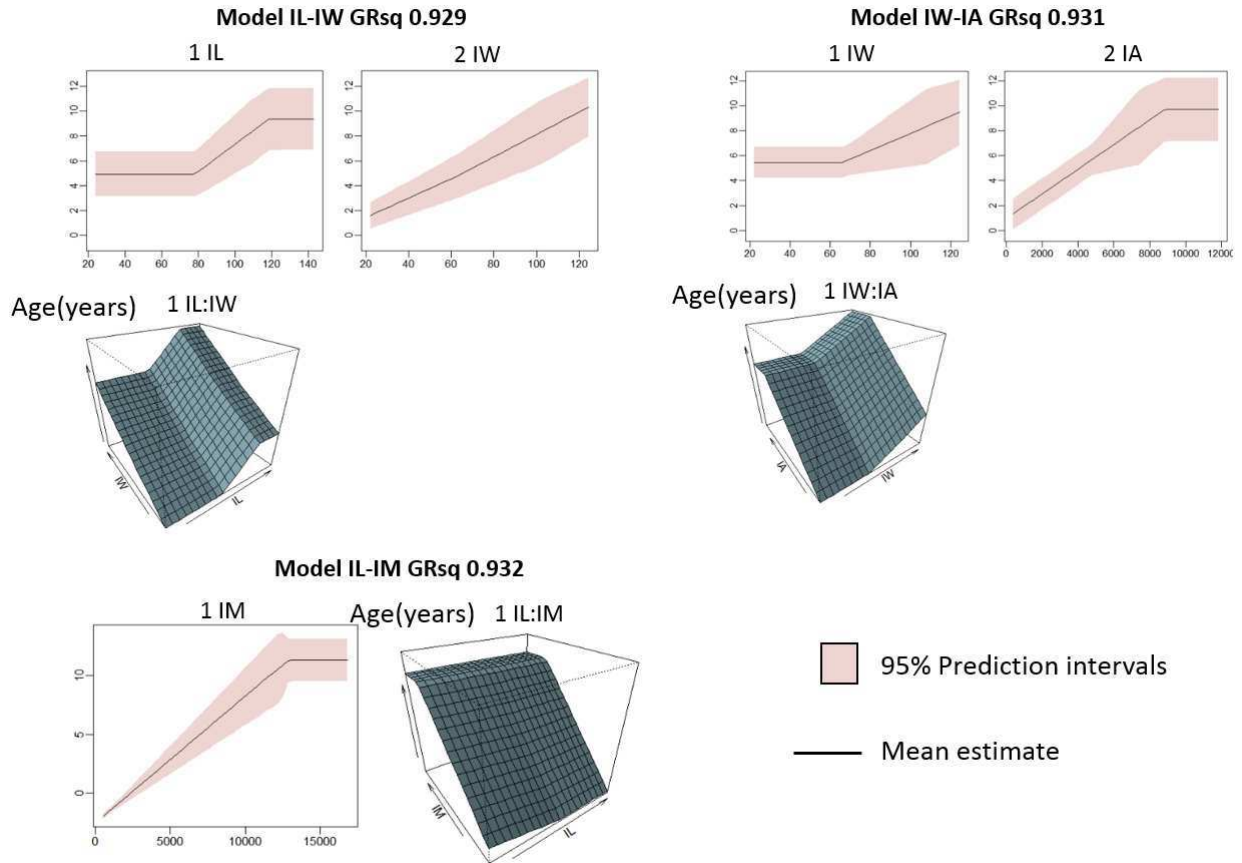
**Figure 6.17 Univariate MARS models for age prediction using iliac variables**

Overall, PI size is comparable for all univariate models. In comparison, smallest PI sizes are lower but largest PI sizes are higher for bivariate and multivariate models (**Tables 6-28 and 6-29**).

**Table 6-28 Regression parameters of the univariate MARS models for age prediction using iliac variables. RSq = R-Squared regression coefficient; CVRSq = Cross-Validated R-Squared; GRSq = Generalised R-Squared; GCV = Generalised Cross-Validation; PI=Prediction Interval; sd= standard deviation**

Univariate MARS models				
Predictor	IL	IW	IM	IA
CVRSq	0.923	0.919	0.929	0.928
GRSq	0.923	0.923	0.932	0.930
GCV	1.103	1.113	0.981	1.011
Standard deviation of CVRSq across folds	0.026	0.027	0.024	0.024
Smallest PI size (years)	1.670	1.168	1.198	1.160
Largest PI size (years)	5.468	4.912	5.364	5.464
Accuracy	95%	>94%	95%	>94%
RSq on the test sample	0.911	0.933	0.923	0.930
Residual mean (years)	0.171	0.102	0.116	0.061
Residual sd (years)	1.408	0.838	0.958	0.505

**Results II: Age estimation**



**Figure 6.18 Contribution of the iliac variables to the bivariate MARS models**

Accuracy is higher than 94% for all models, and GRSq are higher than 0.923. CVRSq, standard deviation across folds and GCV values are comparable between univariate and bi- or multi-variate models. However, the multivariate model using all four variables as predictors has a very low CVRSq and a very high value of standard deviation across folds (**Table 6-29**).

The 95% PIs range from a minimum of 0.867 years for the multivariate model IL+IW+IA to a maximum of 14.369 years for the multivariate model IL+IW++IM+IA (**Table 6-30**). The latter model is therefore invalid for age prediction.

Models with bidimensional variables IM or IA provide better results (higher GRSq, CVRSq, lower standard errors) than models using IL and IW. These variables always contribute more significantly to the model in bivariate and multivariate models (**Figures 6.19 and 6.20**). IW generally contributes more than IL.

However, bivariate and multivariate models do not improve univariate models significantly, except for some of the smaller PI sizes, GRSq and CVRSq values.

**Table 6-29 Regression parameters of the bivariate MARS models for age prediction using iliac variables. RSq = R-Squared regression coefficient; CVRSq = Cross-Validated R-Squared; GRSq = Generalised R-Squared; GCV = Generalised Cross-Validation; PI=Prediction Interval; sd= standard deviation**

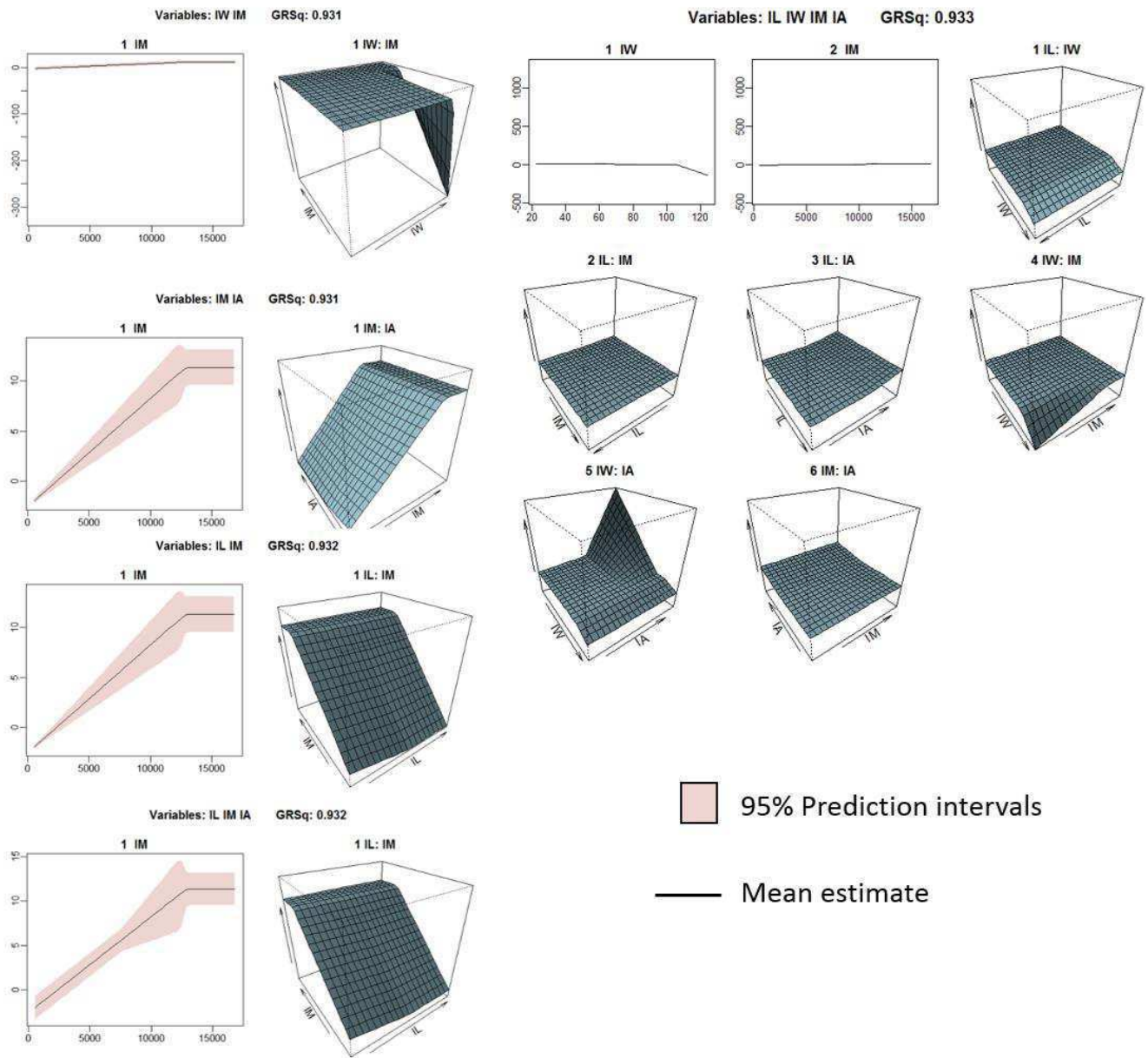
<b>Bivariate MARS models</b>						
Predictors	IL+IW	IL+IM	IL+IA	IW+IM	IW+IA	IM+IA
CVRSq	0.923	0.922	0.911	0.923	0.904	0.924
GRSq	0.929	0.932	0.932	0.931	0.931	0.931
GCV	1.028	0.986	0.978	0.996	0.992	0.993
Standard deviation of CVRSq across folds	0.029	0.057	0.11	0.027	0.25	0.026
Smallest PI size (years)	1.745	0.992	0.903	1.284	1.090	1.255
Largest PI size (years)	5.012	6.135	6.324	5.845	6.208	5.931
Accuracy	>94%	97%	96%	97%	96%	96%
Variable importance	IW>IL	IM>IL	IA>IL	IM>IW	IA>IW	IM >IA
RSq on the test sample	0.925	0.924	0.927	0.923	0.932	0.920
Residual mean (years)	0.117	0.133	0.098	0.103	0.092	0.141
Residual sd (years)	0.968	1.099	0.812	0.853	0.762	1.167

**Table 6-30 Regression parameters of the multivariate MARS models for age prediction using iliac variables. RSq = R-Squared regression coefficient; CVRSq = Cross-Validated R-Squared; GRSq = Generalised R-Squared; GCV = Generalised Cross-Validation; PI=Prediction Interval; sd= standard deviation. Greyed values correspond to invalid parameters**

<b>Multivariate MARS models</b>					
Predictors	IL+IW+IM	IL+IW+IA	IL+IM+IA	IW+IM+IA	IL+IW+IM+IA
CVRSq	0.913	0.884	0.918	0.920	0.295
GRSq	0.931	0.931	0.932	0.931	0.933
GCV	1.001	0.988	0.985	0.993	0.961
Standard deviation of CVRSq across folds	0.13	0.29	0.055	0.031	7.4
Smallest PI size (years)	1.138	0.867	1.153	0.997	1.147
Largest PI size (years)	6.319	6.388	6.371	6.315	14.369
Accuracy	97%	97%	97%	97%	96%
Variable importance	IM> IW >IL	IW>IL>IA	IM>IL IA unused	IM>IW IA unused	IM>IL>IW>IA
RSq on the test sample	0.925	0.868	0.924	0.923	0.923
Residual mean (years)	0.106	-0.057	0.135	0.102	0.102
Residual sd (years)	0.871	0.472	1.114	0.840	0.840

The parameters obtained on the test sample follow the same pattern: values are comparable between univariate, bivariate and multivariate models. Therefore, four multivariate models, all six bivariate models and all four univariate models are valid for age prediction.

**Results II: Age estimation**



**Figure 6.19 Contribution of the iliac variables to the multivariate MARS models**

These fourteen models all provide good results when applied to an independent test sample from the same population: all  $RSq$  values are high, mean and standard deviation of the residuals are low (Tables 6-28 to 6-30).

Maximum residual values are inferior to 2.10 for 95% of the individuals of the training sample in all univariate models. Heteroscedasticity and non-normality of the residuals are still present for all models, as assessed by the increasing dispersion of the residuals (Figure 6.20).

The parameters obtained on the test sample give  $RSq$  values between 0.90 and 0.94 and comparable or smaller residual means and standard errors for the univariate models compared to



OLS and WLS parametric models (**Table 6-31**). Overall, PI size is much smaller for MARS models than it would be for linear and polynomial models if they were valid, particularly for younger individuals.

**Table 6-31 Comparison of RSq, mean and standard deviation values between the OLS and WLS parametric models and the univariate MARS models using iliac variables. Greyed values correspond to invalid parameters**

Variable	OLS third degree polynomial			WLS third degree polynomial			MARS models		
	RSq	RSE	RM	RSq	RSE	RM	RSq	RSE	RM
IL	0.926	1.037	0.000	0.954	1.089	0.213	0.923	1.408	0.171
IW	0.921	1.069	0.000	0.982	0.623	0.022	0.919	0.838	0.102
IM	0.935	0.967	0.000	0.981	0.703	0.016	0.929	0.958	0.116
IA	0.934	0.980	0.000	0.982	0.617	0.083	0.928	0.505	0.061

Because bivariate and multivariate models give comparable results on the independent test sample to the univariate models, they do not provide any significant improvement in age prediction. For user-friendly purposes, univariate models were selected to be used for age prediction. Their application can be done with the following formulae:

- *IL model:*

For  $IL < 70.0635$  mm,

$$\text{age (years)} = 2.09985 - 0.05596 \cdot (70.0635 - IL)$$

For  $70.0635 \text{ mm} < IL < 118.491$  mm,

$$\text{age (years)} = 2.09985 + 0.18330 \cdot (IL - 70.0635)$$

For  $IL > 118.491$  mm,

$$\text{age (years)} = 2.09985 + 0.18330 \cdot (IL - 70.0635) - 0.13113 \cdot (IL - 118.491)$$

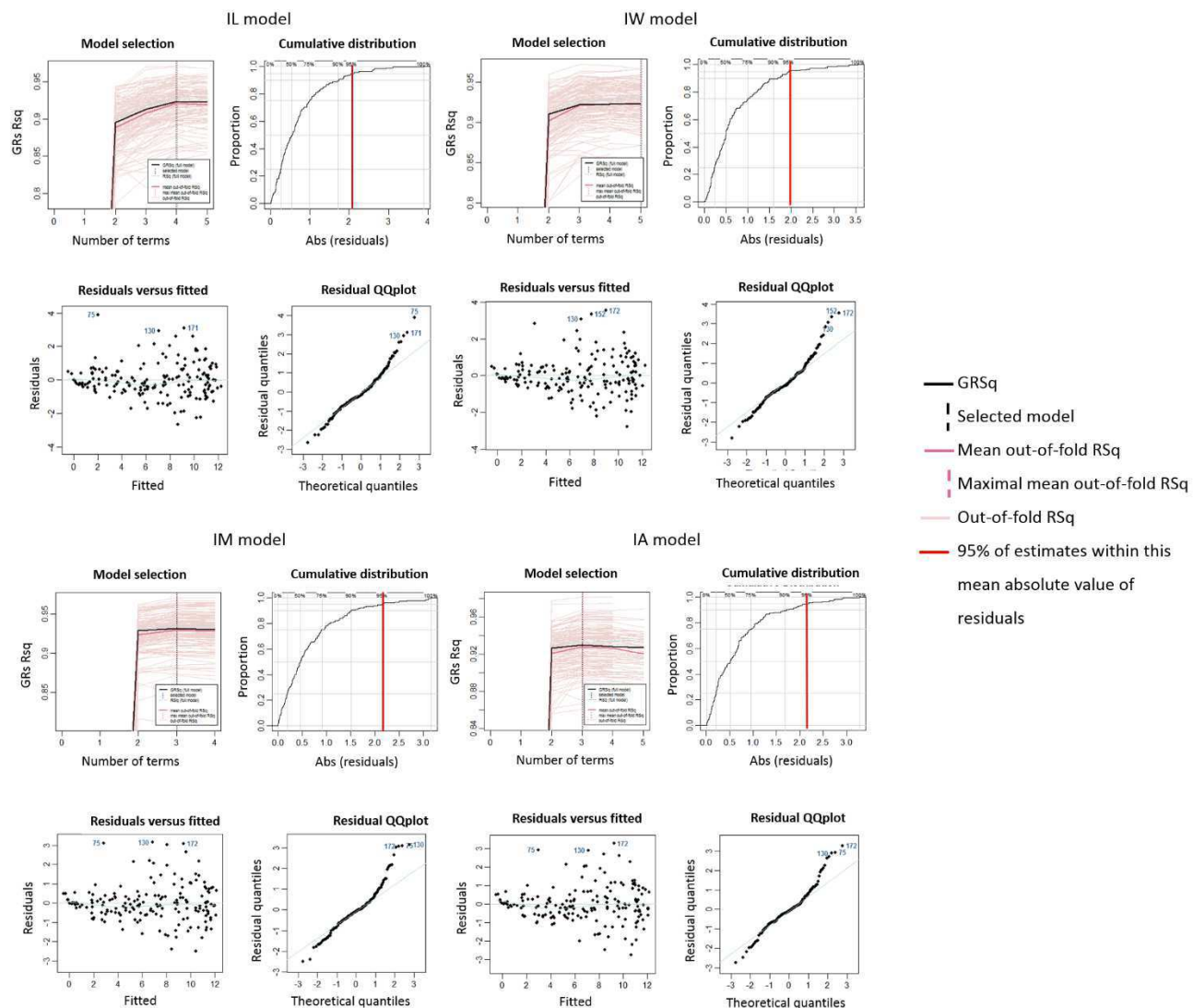


Figure 6.20 Parameters of MARS models using IL, IW, IM and IA as predictors: Mean CVRSq (pink line) and GRSq (black line) values; Mean absolute residual value of the 95% cumulative distribution (red line); number of terms of the regression equation (black dotted line); Plots of the residuals versus fitted values illustrating heteroscedasticity and outliers (numbered individuals); QQplot of residuals illustrating non-normality of the residuals

*IW model:*

For  $IW < 62.9477$  mm,

$$\text{age (years)} = 2.10555 - 0.06317*(62.9477 - IW)$$

For  $62.9477 \text{ mm} < IW < 96.5859$  mm,

$$\text{age (years)} = 2.09985 + 0.18104*(IW - 62.9477)$$

For  $96.5859 \text{ mm} < IW < 105.162$  mm,

$$\text{age (years)} = 2.09985 + 0.18104*(IW - 62.9477) + 0.09988*(IW - 96.5859)$$

For  $IW > 105.162$  mm,

$$\text{age (years)} = 2.09985 + 0.18104*(IW - 62.9477) + 0.09988*(IW - 96.5859) - 0.19634*(IW - 105.162)$$

*-IM model:*

For  $IM < 5080.840$  mm<sup>2</sup>,

$$\text{age (years)} = 9.33184 + 0.00024*(IM - 5080.840)$$

For  $5080.840 \text{ mm}^2 < IM < 12921.300$  mm<sup>2</sup>,

$$\text{age (years)} = 9.33184 + 0.00024*(IM - 5080.840) - 0.00079*(12921.300 - IM)$$

For  $IM > 12921.300$  mm<sup>2</sup>,

$$\text{age (years)} = 9.33184 - 0.00079*(12921.300 - IM)$$

*-IA model:*

For  $IA < 2399.240$  mm<sup>2</sup>,

$$\text{age (years)} = 8.28593 + 0.00041*(IA - 2399.240)$$

For  $2399.240 \text{ mm}^2 < IA < 8932.350$  mm<sup>2</sup>,

$$\text{age (years)} = 8.28593 - 0.00103*(8932.350 - IA) + 0.00041*(IA - 2399.240)$$

For  $IA > 8932.350$  mm<sup>2</sup>,

$$\text{age (years)} = 8.28593 - 0.00103*(8932.350 - IA)$$

The associated 95% prediction intervals are calculated with the MARS model and can be found in **Appendix F** as an abacus with the corresponding values of predictor variables. The abacus also indicates the ages associated with the hinge points of the functions.

**b. Lumbar variables**

Models were constructed on a training sample of 268 individuals (121 females and 147 males) aged 0 to 19 years. The test sample is composed of 132 individuals (54 females and 78 males). Age and sex distributions are uniform in both samples (see **Chapter 5**).

MARS functions detected between one (model using RVH) and four (model using PVH) hinge points for the univariate models, identifying them as occurrences for non-linearity of the relation between age and the variables (**Figures 6.21 and 6.22**). The bivariate and multivariate models detected between one and four hinge points per variable, depending on the model and the variable.

The model using PVH cannot be considered valid after the third hinge point, at PVH=21.652 mm, as it modelled a decrease in age between the third and fourth hinge point, which is biologically impossible. This artefact corresponds to lower ages for higher values of PVH (**Figure 6.21**).

Smallest PI sizes are lowest for models using UVL and RVH, and univariate models in particular; largest PI sizes are lowest for the four height variables (**Tables 6-32 and 6-33**).

**Table 6-32 Regression parameters of the univariate MARS models for age prediction using upper and lower lumbar variables. RSq = R-Squared regression coefficient; CVRSq = Cross-Validated R-Squared; GRSq = Generalised R-Squared; GCV = Generalised Cross-Validation; PI=Prediction Interval; sd= standard deviation**

Univariate MARS models						
Predictor	UVL	UVW	UVM	LVL	LVW	LVM
CVRSq	0.835	0.800	0.836	0.845	0.770	0.829
GRSq	0.839	0.805	0.844	0.848	0.777	0.838
GCV	5.410	6.560	5.256	5.112	7.511	5.439
Standard deviation of CVRSq across folds	0.038	0.047	0.039	0.041	0.053	0.044
Smallest PI size (years)	1.193	4.746	3.961	3.722	4.579	3.600
Largest PI size (years)	12.337	14.827	13.109	13.368	15.199	13.886
Accuracy	96%	96%	97%	97%	96%	97%
RSq on the test sample	0.799	0.808	0.827	0.841	0.792	0.840
Residual mean (years)	-0.193	-0.134	-0.091	-0.218	-0.097	-0.129
Residual sd (years)	2.221	1.545	1.047	2.506	1.118	1.478

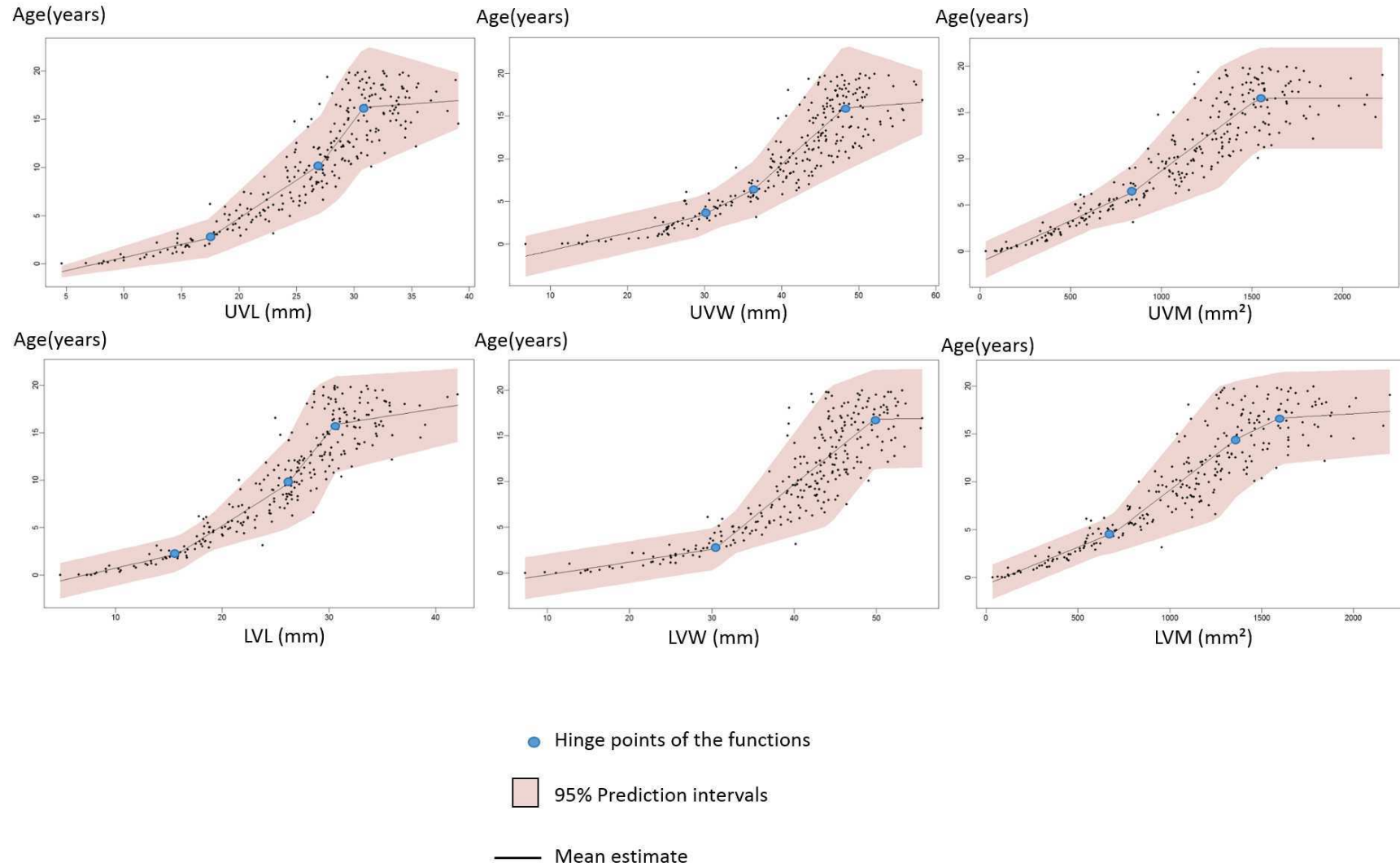


Figure 6.21 Univariate MARS models for age prediction using upper and lower lumbar variables

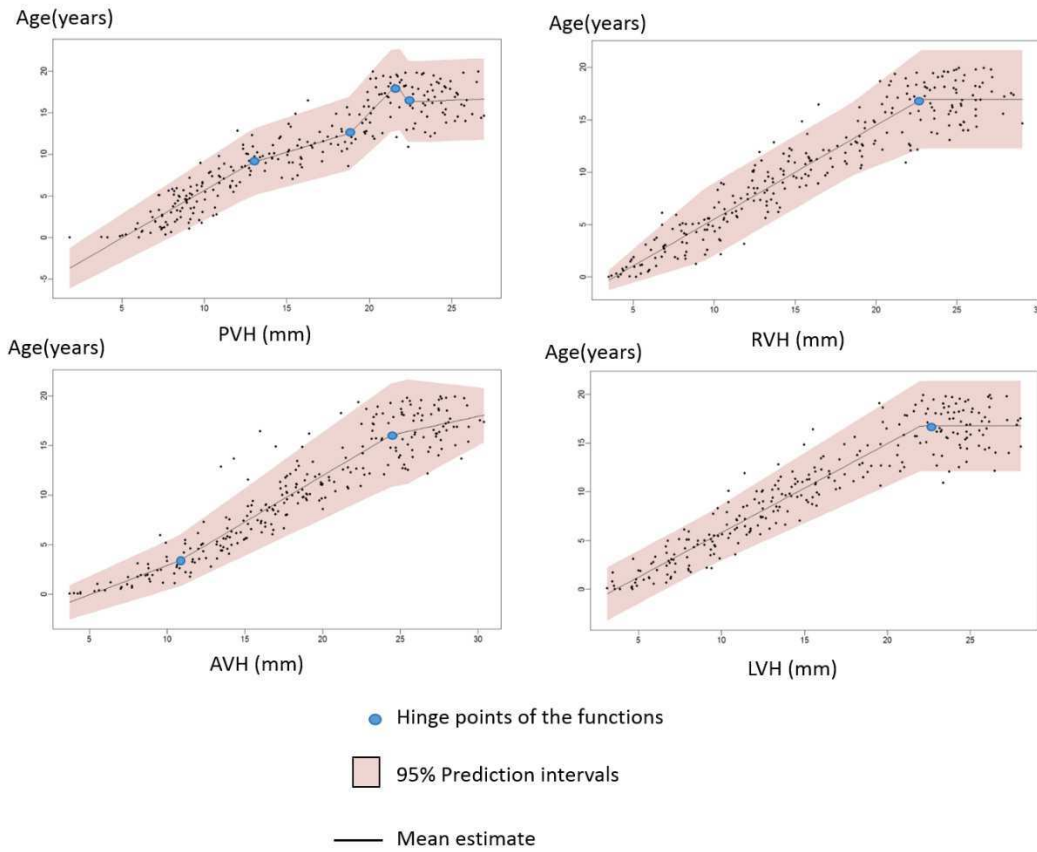


Figure 6.22 Univariate MARS models for age prediction using lumbar height variable

Table 6-33 Regression parameters of the univariate MARS models for age prediction using lumbar height variables. RSq = R-Squared regression coefficient; CVRSq = Cross-Validated R-Squared; GRSq = Generalised R-Squared; GCV = Generalised Cross-Validation; PI=Prediction Interval; sd= standard deviation

Univariate MARS models				
Predictor	PVH	AVH	RVH	LVH
CVRSq	0.869	0.875	0.896	0.892
GRSq	0.876	0.879	0.901	0.896
GCV	4.165	4.066	3.341	3.499
Standard deviation of CVRSq across folds	0.026	0.030	0.019	0.022
Smallest PI size (years)	4.886	3.445	1.919	5.465
Largest PI size (years)	9.793	10.604	9.379	9.292
Accuracy	97%	96%	97%	96%
RSq on the test sample	0.858	0.891	0.909	0.899
Residual mean (years)	-0.022	-0.352	-0.174	-0.217
Residual sd (years)	0.257	4.045	1.994	2.497

Accuracy is higher than 95% for all models, and GRSq are comprised between 0.777 (univariate model using LVW as predictor) and 0.938 (model with all variables as predictors). Regression parameters are comparable between univariate and bi- or multi-variate models, with two exceptions:

the bivariate model using UVL and UVW as predictors and the multivariate model using all ten variables. These two models also present the lowest CVRSq values, high standard deviation across folds and extremely large PI sizes (**Tables 6-33 and 6-34**). These two models can therefore be excluded from being valid age estimation methods.

Standard deviation values of CVRSq across folds are low and comparable for all other models. PI sizes are comparable for univariate, bivariate and multivariate models (**Tables 6-31 to 6-33**), especially the largest PI sizes. Smallest PI sizes are found for models using UVL, RVH, UVL+LVL, and upper and lower variables.

Length variables UVL and LVL and upper variables are more important for the models when used with other variables. Bidimensional variables UVM and LVM are always more important in bivariate and multivariate models using them (**Tables 6-33 and 6-34**), but the bivariate model using them both does not provide significantly better results than other more “simple” models.

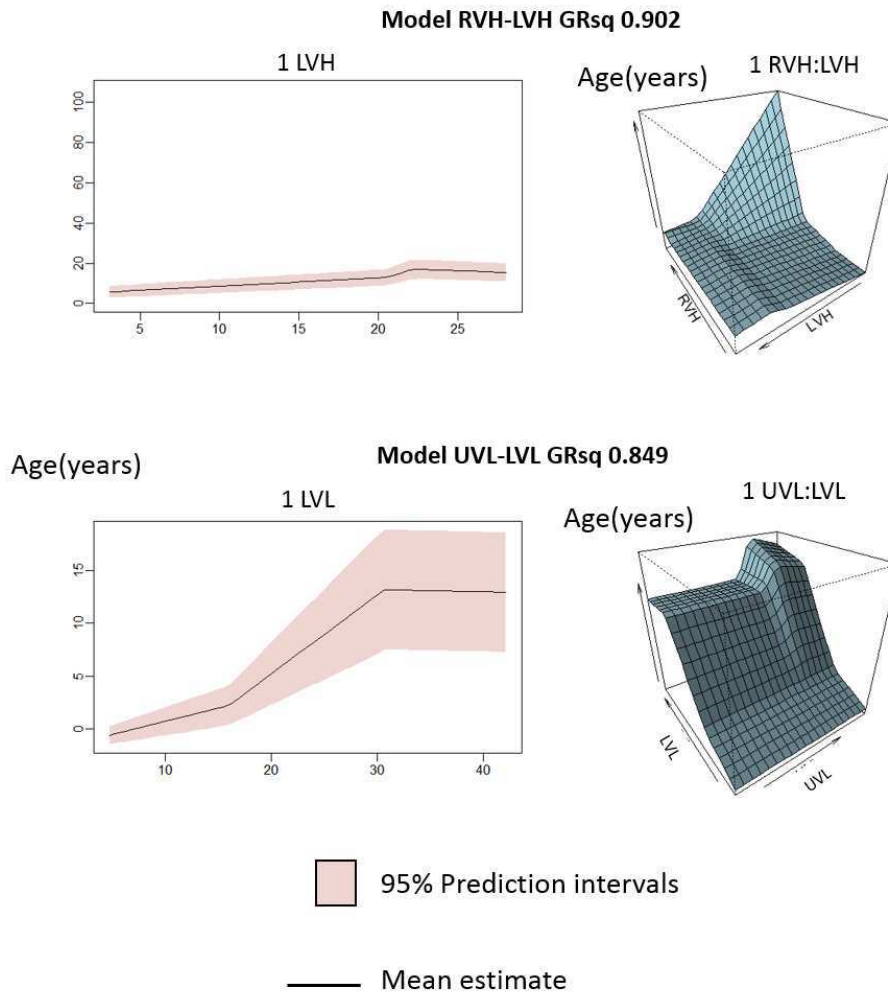
**Table 6-34 Regression parameters of the bivariate MARS models for age prediction using lumbar variables. RSq = R-Squared regression coefficient; CVRSq = Cross-Validated R-Squared; GRSq = Generalised R-Squared; GCV = Generalised Cross-Validation; PI=Prediction Interval; sd= standard deviation. Greyed values correspond to invalid parameters**

Bivariate MARS models							
Predictors	PVH+AVH	RVH+LVH	LVL+LVW	UVL+UVW	UVL+LVL	UVW+LVW	UVM+LVM
CVRSq	0.885	0.895	0.840	0.276	0.840	0.800	0.837
GRSq	0.901	0.902	0.845	0.846	0.849	0.815	0.848
GCV	3.310	3.292	5.202	5.176	5.078	6.231	5.107
Standard deviation of CVRSq across folds	0.089	0.021	0.043	6.800	0.041	0.049	0.043
Smallest PI size (years)	5.752	4.043	4.015	1.322	1.711	4.283	3.559
Largest PI size (years)	12.444	10.383	13.534	363.263	11.298	14.970	14.788
Accuracy	97%	96%	96%	97%	96%	97%	97%
Variable importance	AVH>PVH	RVH>LVH	LVL LVW unused	UVL>UVW	LVL>LVW	UVW>LVW	UVM>LVM
RSq on the test sample	0.903	0.892	0.838	0.807	0.831	0.818	0.816
Residual mean (years)	-0.213	-0.161	-0.226	-0.206	-0.186	-0.150	-0.265
Residual sd (years)	2.446	1.847	2.595	2.372	1.722	1.722	3.046

When applying the models on the test sample from Marseilles, comparable values were obtained for RSq, residual means and standard deviations than for the training sample (**Tables 6-31 to 6-33**). Considering this and PI sizes, the “best” models are therefore the univariate models using UVL, UVM, LVM, AVH, RVH and LVH; the bivariate models RVH+LVH, UVL+LVL; and the multivariate models Upper+Lower and Height. Contributions of the variables to the models can also be assessed: LVH

contributes more to the LVH+RVH model in lower ranges (younger individuals), LVL contributes more to the UVL+LVL model in higher ranges (older individuals) (**Figure 6.23**).

The lowest residual means and standard deviations obtained on the test sample correspond to the univariate models using UVM, LVM and RVH; the bivariate models RVH+LVH and UVL+LVL. These models can therefore be considered as valid for age estimation, even though the largest PI sizes can seem too important (**Tables 6-33 and 6-34, Figures 6.21 to 6.23**).



**Figure 6.23** Contribution of the variables to the bivariate age prediction models using RVH and LVH (upper) and UVL and LVL (lower) as predictors

The RSq values of univariate MARS models comparable to the ones obtained with the polynomial OLS models (**Table 6-35**).

The WLS polynomial models provided much higher RSq results and lower residual mean values, but residual standard deviations are comparable or lower (**Table 6-36**). This validates the MARS approach for age estimation, as no significant decrease in model performance can be found.



**Table 6-35 Regression parameters of the multivariate MARS models for age prediction using clavicular variables. RSq = R-Squared regression coefficient; CVRSq = Cross-Validated R-Squared; GRSq = Generalised R-Squared; GCV = Generalised Cross-Validation; PI=Prediction Interval; sd= standard deviation. Greyed values correspond to invalid parameters**

Multivariate MARS models						
Predictors	All	Upper+Lower	Height	UVL+UVW+LVL+LVW	Upper	Lower
CVRSq	0.349	0.830	0.830	0.830	0.841	0.827
GRSq	0.938	0.855	0.910	0.850	0.850	0.849
GCV	2.077	4.862	3.015	5.049	5.055	5.059
Standard deviation of CVRSq across folds	5.500	0.053	0.400	0.045	0.040	0.080
Smallest PI size (years)	4.031	1.741	2.322	3.832	3.444	3.081
Largest PI size (years)	25.721	12.160	12.148	13.571	13.214	14.781
Accuracy	96%	97%	99%	97%	96%	96%
Variable importance	RVH>UVM>LVH					
	>PVH>LVW>UV	UVM>LVL>UVW>	RVH>AVH>	LVL>UVL>LVW	UVM>UVL	LVM>
	L>AVH>LVL	LVW>LVM>UVL	PVH>LVH	UVW unused	UVW	LVL>
	UVW and LVM unused				unused	LVW
RSq on the test sample	0.874	0.800	0.893	0.828	0.824	0.844
Residual mean (years)	-0.050	-0.251	-0.183	-0.171	-0.206	-0.157
Residual sd (years)	0.574	2.881	2.102	1.960	2.362	1.798

All=UVL+UVW+UVM+LVL+LVW+LVM+PVH+AVH+RVH+LVH; Upper=UVL+UVW+UVM; Lower=LVL+LVW+LVM; Height=PVH+AVH+RVH+LVH

**Table 6-36 Comparison of RSq, mean and standard deviation values between the OLS and WLS parametric models and the univariate MARS models using lumbar variables. Greyed values correspond to invalid parameters**

Variable	OLS third degree polynomial			WLS third degree polynomial			MARS model		
	RSq	RSE	RM	RSq	RSE	RM	RSq	RSE	RM
UVL	0.834	2.361	0.000	0.938	0.788	0.309	0.835	2.221	-0.193
UVW	0.805	2.570	0.000	0.933	0.826	0.437	0.800	1.545	-0.134
UVM	0.846	2.273	0.000	0.935	0.736	0.088	0.836	1.047	-0.091
LVL	0.844	2.297	0.000	0.945	0.750	0.162	0.845	2.506	-0.218
LVW	0.782	2.718	0.000	0.906	0.805	0.213	0.770	1.118	-0.097
LVM	0.844	2.298	0.000	0.939	0.787	0.013	0.829	1.478	-0.129
PVH	0.873	2.059	0.000	0.944	0.891	0.252	0.869	0.257	-0.022
AVH	0.883	1.989	0.000	0.947	0.810	-0.038	0.875	4.045	-0.352
RVH	0.901	1.818	0.000	0.962	0.674	0.068	0.896	1.994	-0.174
LVH	0.898	1.863	0.000	0.920	0.808	-0.009	0.892	2.497	-0.217

Cross-validation enabled the calculation of mean RSq and CVRSq values, as well as the best fitted model with a specific number of terms in the regression equation. The five best age prediction models identified previously are valid despite the lack of residual homoscedasticity and of normally distributed residuals (**Figure 6.24**).

The residual distribution seems more uniform, closer to zero and to normality for models using RVH and RVH+LVH, and shows improvement compared to parametric models.

The choice of the “best” models was done by discarding models one by one based on regression parameters to ultimately privilege smaller PI sizes: the number of terms identified for each model provides simple models with only two terms when using RVH as a predictor, and more complex models using six or eight terms for the bivariate models. The formulae for these models to be used for age prediction are as follows:

*-UVM model:*

For  $UVM < 843.450 \text{ mm}^2$ ,

$$\text{age (years)} = 12.79522 - 0.00897*(1556.770 - UVM)$$

For  $843.450 \text{ mm}^2 < UVM < 1496.490 \text{ mm}^2$ ,

$$\text{age (years)} = 12.79522 + 0.00573*(UVM - 843.450) - 0.00897*(1556.770 - UVM)$$

For  $1496.490 \text{ mm}^2 < UVM < 1556.770 \text{ mm}^2$ ,

$$\text{age (years)} = 12.79522 + 0.00573*(UVM - 843.450) - 0.00570*(UVM - 1496.490) - 0.00897*(1556.770 - UVM)$$

For  $UVM > 1556.770 \text{ mm}^2$ ,

$$\text{age (years)} = 12.79522 + 0.00573*(UVM - 843.450) - 0.00570*(UVM - 1496.490)$$

*-LVM model:*

For  $LVM < 700.756 \text{ mm}^2$ ,

$$\text{age (years)} = 11.71588 - 0.00778*(1600.960 - LVM)$$

For  $700.756 \text{ mm}^2 < LVM < 1367.250 \text{ mm}^2$ ,

$$\text{age (years)} = 11.71588 + 0.00573*(LVM - 700.756) - 0.00778*(1600.960 - LVM)$$

For  $1367.250 \text{ mm}^2 < LVM < 1600.960 \text{ mm}^2$ ,

$$\text{age (years)} = 11.71588 + 0.00573*(LVM - 700.756) - 0.00579*(LVM - 1367.250) - 0.00778*(1600.960 - LVM)$$

For  $LVM > 1600.960 \text{ mm}^2$ ,

$$\text{age (years)} = 11.71588 + 0.00573*(LVM - 700.756) - 0.00579*(LVM - 1367.250)$$

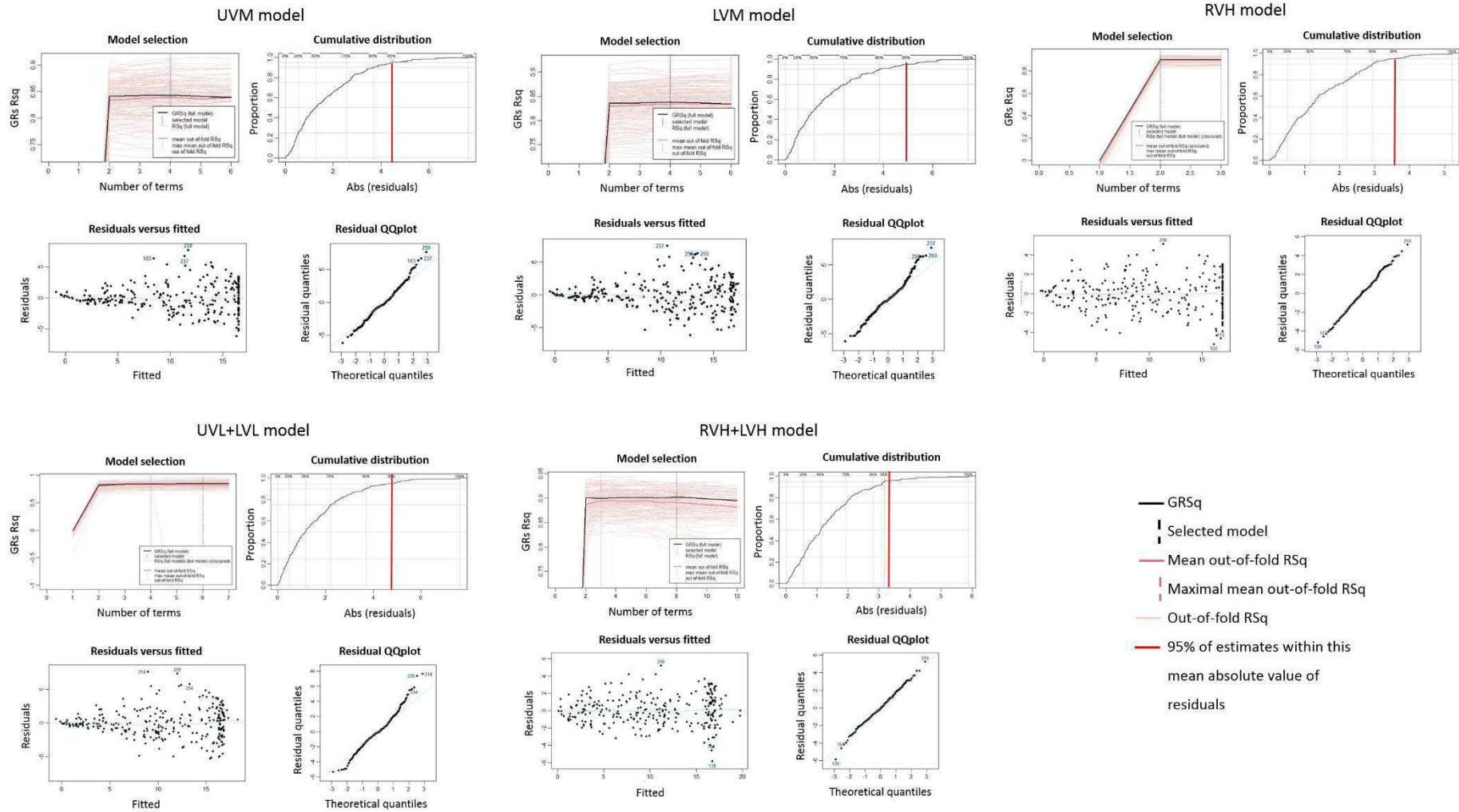


Figure 6.24 Parameters of MARS models using UVM, RVH, RVH+LVH and UVL+LVL as predictors: Mean CVRSq (pink line) and GRSq (black line) values; Mean absolute residual value of the 95% cumulative distribution (red line); number of terms of the regression equation (black dotted line); Plots of the residuals versus fitted values illustrating heteroscedasticity and outliers (numbered individuals); QQplot of residuals illustrating non-normality of the residuals

*-AVH model:*

For AVH < 10.808 mm,

$$\text{age (years)} = 11.45776 + 0.33745*(\text{AVH} - 10.8082)$$

For 10.808 mm < AVH < 24.379 mm,

$$\text{age (years)} = 11.45776 + 0.33745*(\text{AVH} - 10.8082) - 0.59582*(24.379 - \text{AVH})$$

For AVH > 10.808 mm,

$$\text{age (years)} = 11.45776 + 0.33745*(\text{AVH} - 10.8082)$$

*-RVH model:*

$$\text{age (years)} = 16.93605 - 0.89330*(22.7436 - \text{RVH})$$

*-LVH model:*

$$\text{age (years)} = 16.77597 - 0.91416*(21.981 - \text{LVH})$$

*-RVH+LVH model:*

For LVH < 20.608 mm and RVH < 9.643 mm,

$$\text{age (years)} = 13.59606 - 0.70762*(22.151 - \text{LVH})$$

For LVH < 20.608 mm and RVH > 9.643 mm,

$$\text{age (years)} = 13.59606 - 0.70762*(22.151 - \text{LVH}) + 0.05123*(\text{RVH} - 9.643)*(22.151 - \text{LVH})$$

For LVH < 20.608 mm and 19.138 mm < RVH < 25.693 mm,

$$\text{age (years)} = 13.59606 - 0.70762*(22.151 - \text{LVH}) + 0.46423*(\text{RVH} - 19.138)*(22.151 - \text{LVH})$$

For 20.608 mm < LVH < 22.151 mm and 19.138 mm < RVH < 25.693 mm,

$$\text{age (years)} = 13.59606 + 2.2864598*(\text{LVH} - 20.608) - 0.70762*(22.151 - \text{LVH}) + 0.46423*(\text{RVH} - 19.138)*(22.151 - \text{LVH})$$

For LVH > 22.151 mm and 19.138 mm < RVH < 25.693 mm,

$$\text{age (years)} = 13.59606 - 2.57743*(\text{LVH} - 22.151)$$

For LVH > 22.151 mm and 25.693 mm < RVH < 27.101 mm,

$$\text{age (years)} = 13.59606 - 2.57743*(\text{LVH} - 22.151) + 0.57960*(\text{RVH} - 25.693)*(22.151 - \text{LVH})$$

For LVH > 22.151 mm and RVH > 27.101 mm,

$$\text{age (years)} = 13.59606 - 2.57743*(\text{LVH} - 22.151) - 1.0352*(\text{RVH} - 27.101)*(22.151 - \text{LVH})$$

*-UVL+LVL model:*

For LVL < 16.058 mm and UVL < 27.311 mm,

$$\text{age (years)} = 2.27218 - 0.25237*(16.058 - \text{LVL})$$

For 16.058 mm < LVL < 30.649 mm and UVL < 27.311 mm,

$$\text{age (years)} = 2.27218 + 0.74839 * (\text{LVL} - 16.058)$$

For 16.058 mm < LVL < 30.649 mm and 27.311 mm < UVL < 30.676 mm,

$$\text{age (years)} = 2.27218 - 0.76975 * (\text{LVL} - 16.058) + 0.06373 * (\text{UVL} - 27.3114) * (\text{LVL} - 16.058)$$

For 16.058 mm < LVL < 30.649 mm and UVL > 30.676 mm,

$$\text{age (years)} = 2.27218 - 0.76975 * (\text{LVL} - 16.058) - 0.07021 * (\text{UVL} - 30.676) * (\text{LVL} - 16.058)$$

For LVL > 30.649 mm and 27.311 mm < UVL < 30.676 mm,

$$\text{age (years)} = 2.27218 - 0.76975 * (\text{LVL} - 30.6493) - 0.06373 * (\text{UVL} - 27.3114) * (\text{LVL} - 16.0583)$$

For LVL > 30.649 mm and UVL > 30.676 mm,

$$\text{age (years)} = 2.27218 - 0.76975 * (\text{LVL} - 30.6493) - 0.07021 * (\text{UVL} - 30.676) * (\text{LVL} - 16.058)$$

### **c. Clavicular variables**

Models were constructed on two training samples of individuals aged 0 to 19 years: 209 individuals (101 females and 108 males) using Ln, AP\_diam and SI\_diam as predictors, and 191 individuals (90 females and 101 males) using Max\_diam and Min\_Diam as predictors, because of missing values. The multivariate models were constructed on the training sample of 191 individuals. The test sample is composed of 97 individuals (46 females and 51 males). Age and sex distributions are uniform in all samples (**see Chapter 5**).

MARS functions detected three hinge points for the univariate models, identifying three occurrences for non-linearity of the relation between age and the variables (**Figure 6.25**), and providing four sub-functions modelling this relationship

The model using SI\_diam cannot be considered valid after the second hinge point, at SI\_diam=11.454 mm, as it modelled a decrease of age between the second and third hinge point, which is biologically impossible. This artefact corresponds to lower ages for higher values of SI\_diam. The bivariate and multivariate models detected between one and three hinge points per variable, depending on the model and the variable.

Overall, PI size is much higher for models using clavicular diameters as predictors. Accuracy is higher than 95% for all models, and GRSq are higher than 0.786. Regression parameters are comparable between univariate and bi- or multi-variate models. Eleven models present high CVRSq values, and low standard deviation across folds (lower than one): the five univariate models, five out of six bivariate models and one multivariate model (**Tables 6-37 and 6-38**).

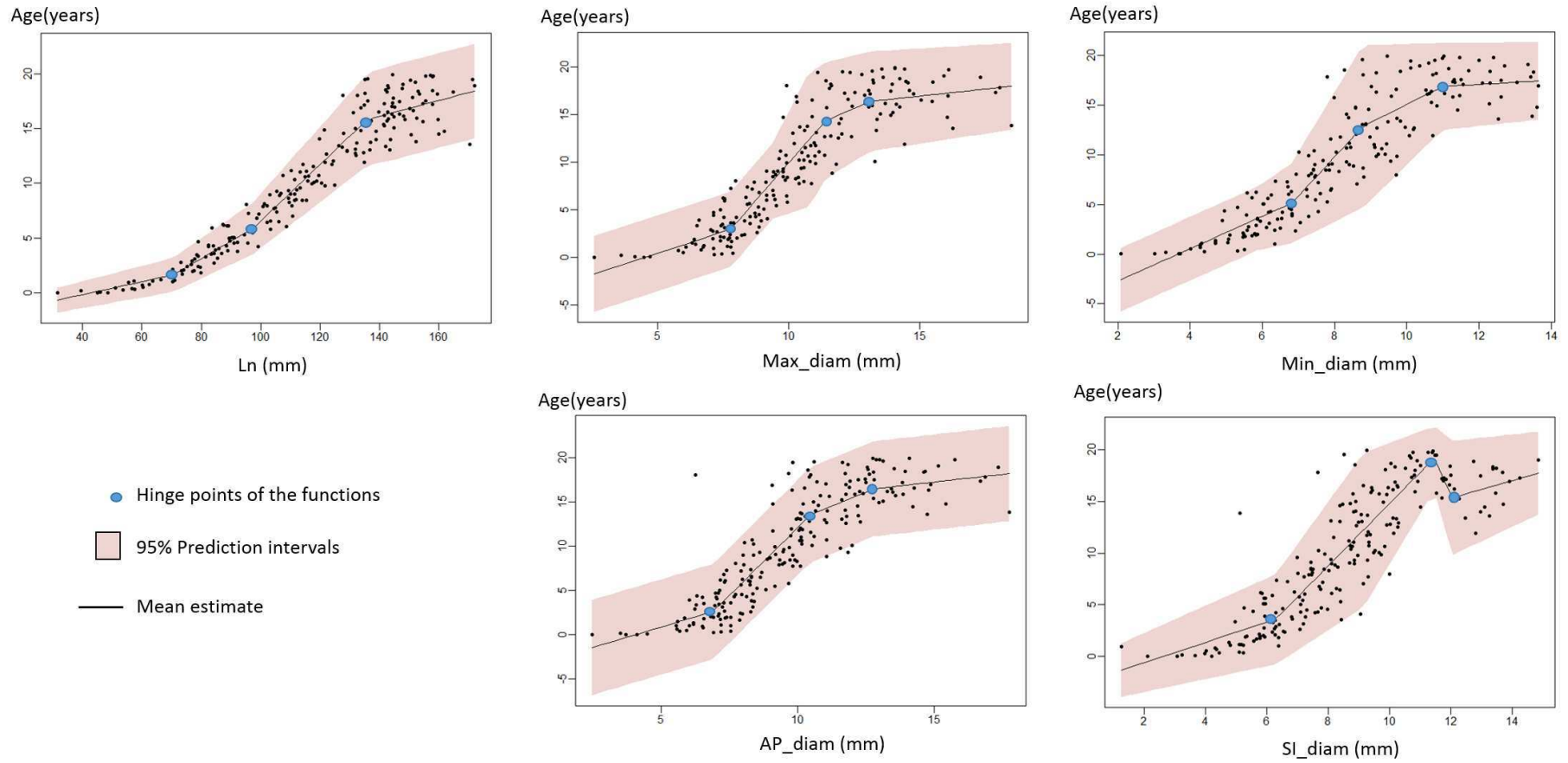


Figure 6.25 Univariate MARS models for age prediction using clavicular variables

Table 6-37 Regression parameters of the univariate MARS models for age prediction using clavicular variables. RSq = R-Squared regression coefficient; CVRSq = Cross-Validated R-Squared; GRSq = Generalised R-Squared; GCV = Generalised Cross-Validation; PI=Prediction Interval; sd= standard deviation. Greyed values correspond to invalid parameters

Univariate MARS models					
Predictor	Ln	Max_diam	Min_diam	AP_diam	SI_diam
CVRSq	0.930	0.832	0.819	0.780	0.790
GRSq	0.935	0.841	0.832	0.786	0.792
GCV	2.464	6.116	6.451	8.107	7.899
Standard deviation of CVRSq across folds	0.018	0.047	0.047	0.081	0.065
Smallest PI size (years)	2.254	7.979	6.400	10.742	5.173
Largest PI size (years)	8.653	14.056	16.329	10.742	14.959
Accuracy	97%	96%	98%	95%	95%
RSq on the test sample	0.928	0.832	0.361	0.784	0.847
Residual mean (years)	0.142	0.145	4.003	0.174	-0.001
Residual sd (years)	1.365	1.364	37.549	1.718	0.011

The bivariate model using AP\_diam and SI\_diam and three multivariate models have very low CVRSqs.

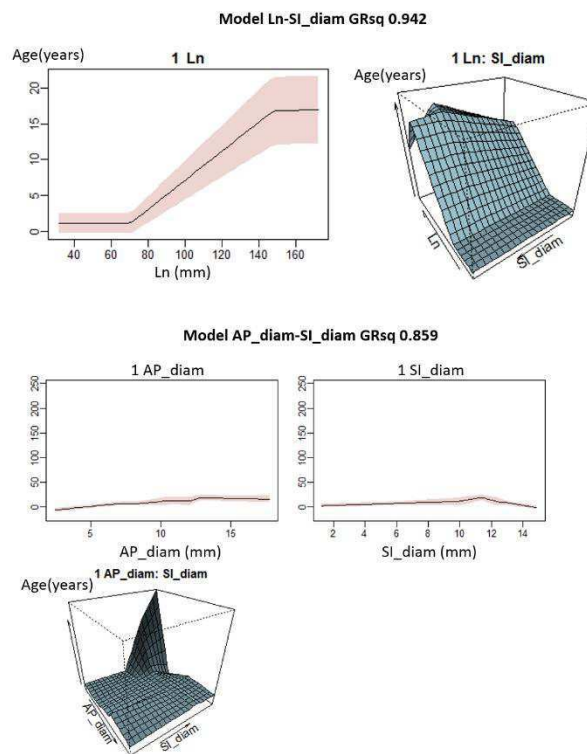


Figure 6.26 Bivariate age prediction models using Ln and SI\_diam (upper) and AP\_diam and SI\_diam (lower) as predictors

Compared to the bivariate model using Ln and SI\_diam, the combination of AP\_diam and SI\_diam leads to a flattened model, with a stronger contribution to the model at higher values of SI\_diam. The same thing was observed for the three multivariate models. The combination of Ln and SI\_diam

however, shows a regular increase in PI size and a relatively harmonious co-prediction of age (Figure 6.26). These models also have high standard deviation values, which invalidate them for age prediction.

PI size is relatively high for all ages when using clavicular diameters as predictors and seem too large for younger individuals. Indeed, smallest and/or largest prediction interval sizes are too large to provide precise age estimates for the univariate models using clavicular diameters (Table 6-38 and Figure 6.27), three of the five bivariate models and for the retained multivariate model (Tables 6-38).

**Table 6-38 Regression parameters of the bivariate and multivariate MARS models for age prediction using clavicular variables. RSq = R-Squared regression coefficient; CVRSq = Cross-Validated R-Squared; GRSq = Generalised R-Squared; GCV = Generalised Cross-Validation; PI=Prediction Interval; sd= standard deviation. Greyed values correspond to invalid parameters**

Bivariate MARS models						
Predictors	Max+Min	AP+SI	Ln+AP	Ln+SI	Ln+Max	Ln+Min
CVRSq	0.856	0.654	0.908	0.913	0.919	0.932
GRSq	0.868	0.859	0.942	0.942	0.942	0.940
GCV	5.090	5.440	2.249	2.216	2.231	2.309
Standard deviation of CVRSq across folds	0.043	0.750	0.057	0.070	0.089	0.020
Smallest PI size (years)	3.702	7.432	2.819	2.856	2.753	3.006
Largest PI size (years)	13.519	16.376	53.036	9.478	42.962	8.835
Accuracy	97%	97%	97%	97%	97%	97%
Variable importance	Min_diam>Max_diam	SI_diam>AP_diam	Ln>AP_diam	Ln>SI_diam	Ln>Max_diam	Ln>Min_diam
RSq on the test sample	0.843	0.842	0.927	0.937	0.936	0.931
Residual mean (years)	0.127	0.183	0.091	0.043	0.061	0.058
Residual sd (years)	1.188	1.718	0.854	0.406	0.570	0.545
Multivariate MARS models						
Predictors	All	Diameters	Ln+AP+SI	Ln+Max+Min		
CVRSq	-313.248	0.325	-0.565	0.904		
GRSq	0.950	0.875	0.944	0.941		
GCV	1.937	4.815	2.141	2.268		
Standard deviation of CVRSq across folds	3846	3.700	17	0.098		
Smallest PI size (years)	47.418	8.842	10.134	2.736		
Largest PI size (years)	47.418	20.825	10.134	52.158		
Accuracy	99%	98%	97%	98%		
Variable importance	Ln>SI >AP >Min>Max	Min>Max>SI>AP	Ln>SI>AP	Ln>Max Min unused		
RSq on the test sample	0.932	0.854	0.936	0.935		
Residual mean (years)	0.057	0.346	0.105	0.040		
Residual sd (years)	0.539	3.325	0.988	0.380		

All=Ln+AP\_diam+SI\_diam+Max\_diam+Min\_diam; Diameters=AP\_diam+SI\_diam+Max\_diam+Min\_diam; AP=AP\_diam; SI=SI\_diam, Max=Max\_diam; Min=Min\_diam



The six valid models left after elimination have CVRSq values ranging from 0.790 (SI\_diam) to 0.932 (Ln and Min\_diam), very low standard deviations of CVRSq across folds (between 0.018 and 0.081) and comparable residual means. Residual standard deviations are generally higher for univariate models, but remain sufficiently low for model validation. PI sizes are more variable, although the smallest are found for the univariate model using Ln, and the largest are found for the univariate models using SI\_diam, Max\_diam and Min\_diam as predictors.

The RSq, residual means and standard deviations of univariate MARS models are better or equivalent to the ones obtained with the polynomial OLS models (**Table 6-39**). The WLS polynomial models provided higher RSq results, but residual means and standard deviations are comparable.

Cross-validation enabled the calculation of mean RSQ and CVRSq values, as well as the best fitted model with a specific number of terms in the regression equation. In this case, the best models according to regression parameters are the univariate and two bivariate models including Ln as a predictor: Ln, Ln+SI and Ln+Min (**Tables 6-38 and 6-39, Figures 6.25 and 6.26**).

**Table 6-39 Comparison of RSq, mean and standard deviation values between the OLS and WLS parametric models and the univariate MARS models using clavicular variables. Greyed values correspond to invalid parameters**

Variable	OLS third degree polynomial			WLS third degree polynomial			MARS model		
	RSq	RSE	RM	RSq	RSE	RM	RSq	RSE	RM
Ln	0.938	1.535	0.000	0.974	0.731	-0.091	0.930	1.365	0.142
Max_diam	0.842	2.558	0.000	0.944	1.027	0.165	0.832	1.364	0.145
Min_diam	0.841	2.470	0.000	0.955	0.776	0.098	0.819	37.549	4.003
AP_diam	0.787	2.834	0.000	0.940	1.028	0.255	0.780	1.718	0.174
SI_diam	0.791	2.807	0.000	0.922	0.804	0.212	0.790	0.011	-0.001

These models are valid despite residual heteroscedasticity and un-normally distributed residuals (**Figure 6.27**).

The formulae for the retained clavicular models to be used for age prediction are as follows:

The choice of the “best” model would reside in privileging smaller PI sizes: this means the best models are the univariate MARS model using Ln as a predictor and the bivariate models Ln+SI and Ln+Min. There is no significant amelioration of the estimates by adding other predictor variables to the models. Maximum clavicular length is therefore the best age predictor of the five clavicular variables.

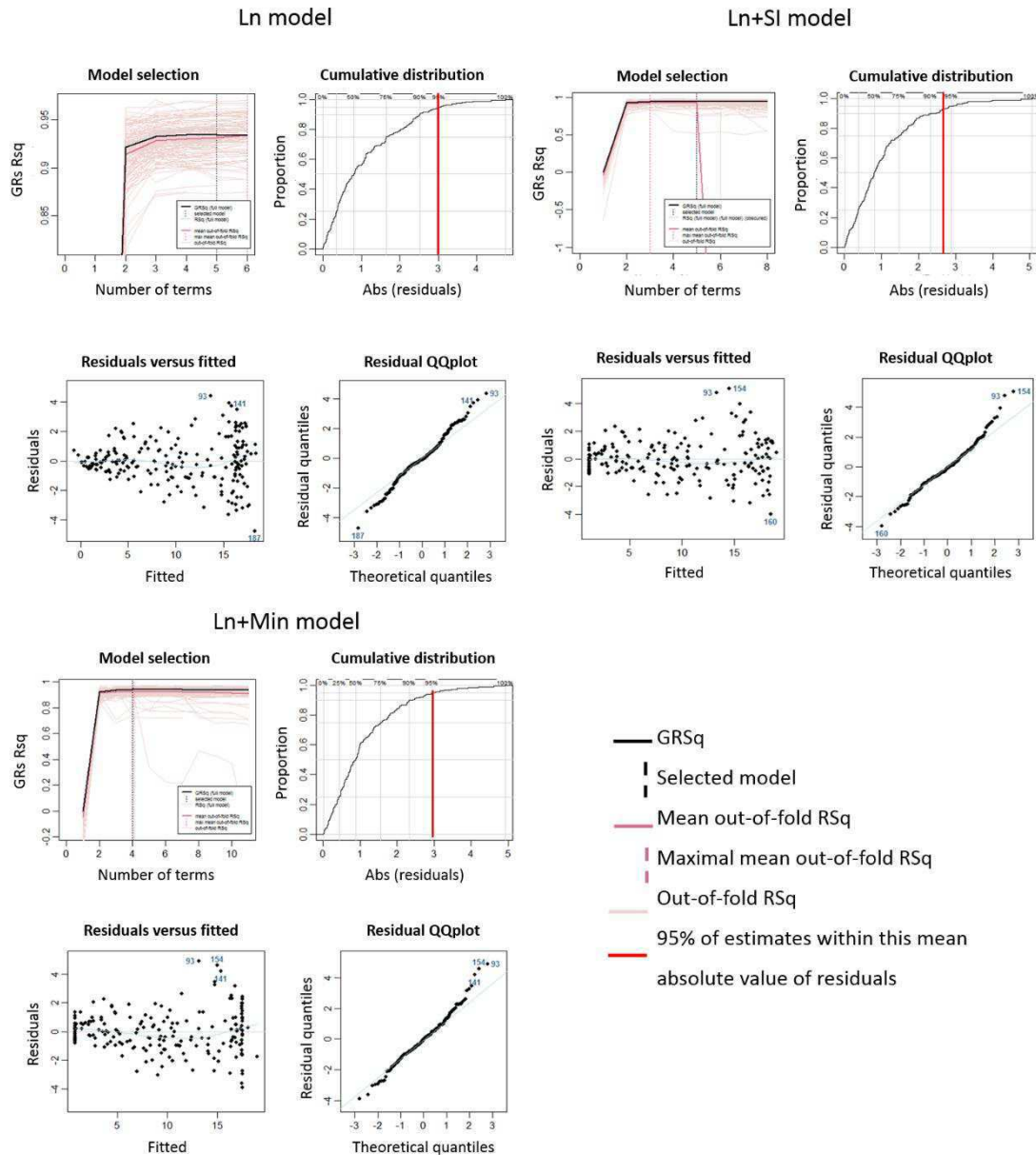


Figure 6.27 Parameters of MARS models using Ln, Ln+SI and Ln+Min\_diam as predictors: Mean CVRSq (pink line) and GRSq (black line) values; Mean absolute residual value of the 95% cumulative distribution (red line); number of terms of the regression equation (black dotted line); Plots of the residuals versus fitted values illustrating heteroscedasticity and outliers (numbered individuals); QQplot of residuals illustrating non-normality of the residuals

The three best MARS models predicting age with clavicular variables have the following mathematical expressions:

-Ln model:

For  $Ln < 70.320$  mm,

$$\text{age (years)} = 1.61406 - 0.05934 \cdot (70.320 - Ln)$$

For  $70.320 \text{ mm} < Ln < 97.390$  mm,

$$\text{age (years)} = 1.61406 + 0.15732 * (\text{Ln} - 70.320)$$

For  $97.390 \text{ mm} < \text{Ln} < 136.060 \text{ mm}$ ,

$$\text{age (years)} = 1.61406 + 0.15732 * (\text{Ln} - 70.320) + 0.10102 * (\text{Ln} - 97.390)$$

For  $\text{Ln} > 136.060 \text{ mm}$ ,

$$\text{age (years)} = 1.61406 + 0.15732 * (\text{Ln} - 70.320) + 0.10102 * (\text{Ln} - 97.390) - 0.18714 * (\text{Ln} - 136.060)$$

- *Ln+SI model:*

For  $\text{Ln} < 70.460 \text{ mm}$ ,

$$\text{age (years)} = 1.10344$$

For  $70.460 \text{ mm} < \text{Ln} < 147.620 \text{ mm}$  and  $\text{SI\_diam} < 11.3948 \text{ mm}$ ,

$$\text{age (years)} = 1.10344 + 0.22384 * (\text{Ln} - 70.460) - 0.00679 * (\text{Ln} - 70.46) * (11.3948 - \text{SI\_diam})$$

For  $70.460 \text{ mm} < \text{Ln} < 147.620 \text{ mm}$  and  $11.3948 \text{ mm} < \text{SI\_diam} < 11.518 \text{ mm}$ ,

$$\text{age (years)} = 1.10344 + 0.22384 * (\text{Ln} - 70.460)$$

For  $70.460 \text{ mm} < \text{Ln} < 147.620 \text{ mm}$  and  $\text{SI\_diam} > 11.518 \text{ mm}$ ,

$$\text{age (years)} = 1.10344 + 0.22384 * (\text{Ln} - 70.460)$$

For  $\text{Ln} > 147.620 \text{ mm}$  and  $\text{SI\_diam} > 11.518 \text{ mm}$ ,

$$\text{age (years)} = 1.10344 - 0.19596 * (\text{Ln} - 147.620) - 0.09854 * (\text{Ln} - 147.620) * (\text{SI\_diam} - 11.518)$$

This model estimates age as a constant for  $\text{Ln} < 70.460 \text{ mm}$ . It is therefore not adapted for very young individuals.

- *Ln+Min model:*

For  $\text{Ln} < 70.460 \text{ mm}$ ,

$$\text{age (years)} = 0.80570$$

For  $70.460 \text{ mm} < \text{Ln} < 145.020 \text{ mm}$  and  $\text{Min\_diam} < 10.530 \text{ mm}$ ,

$$\text{age (years)} = 0.80570 + 0.21573 * (\text{Ln} - 70.460)$$

For  $70.460 \text{ mm} < \text{Ln} < 145.020 \text{ mm}$  and  $\text{Min\_diam} > 10.530 \text{ mm}$ ,

$$\text{age (years)} = 0.80570 + 0.21573 * (\text{Ln} - 70.460) + 0.23675 * (145.020 - \text{Ln}) * (\text{Min\_diam} - 10.530)$$

For  $145.020 \text{ mm} < \text{Ln} < 147.620 \text{ mm}$  and  $\text{Min\_diam} > 10.530 \text{ mm}$ ,

$$\text{age (years)} = 0.80570 + 0.21573 * (\text{Ln} - 70.460)$$

For  $\text{Ln} > 147.620 \text{ mm}$  and  $\text{SI\_diam} > 10.530 \text{ mm}$ ,

$$\text{age (years)} = 0.80570 - 0.21375 * (\text{Ln} - 147.620)$$

This model estimates age as a constant for  $\text{Ln} < 70.460 \text{ mm}$ . It seems therefore not adapted for very young individuals.

### **6.1.3. Testing the models**

Regression models present risks of overfitting and population-dependency (Gowland and Chamberlain 2002). For this reason, the retained models were tested on two additional independent samples.

#### **a. Independent test sample from Toulouse**

A test sample composed of 30 living individuals (14 females and 16 males) from Toulouse (France) aged 0 to 12 years was used to test the MARS models based on iliac variables IL, IW and IM.

The univariate MARS models provided comparable values of RSq and residual means to the ones obtained on the Marseilles test sample, however residual standard errors were higher, especially for the model using IW as a predictor (**Table 6-40**). Once again, the bidimensional variable IM provides the best results.

This is even more perceptible when looking at the plots of the functions (**Figure 6.28**). The prediction intervals obtained when using IM as a predictor include all the individuals from the sample.

However, only a few individuals lie outside the range of the predictions intervals constructed with the functions using IL and IW as predictors.

Table 6-40 Validation parameters of the univariate MARS models for age prediction using iliac variables of individuals from Toulouse. RSq=R-Squared regression coefficient; sd=standard deviation. Greyed values correspond to invalid parameters, or insufficient for age estimation purposes (<0.95)

Univariate MARS models			
Predictors	IL	IW	IM
RSq	0.948	0.883	0.960
Residual mean (years)	-0.515	0.733	0.107
Residual sd (years)	2.821	4.013	0.586
Accuracy (%)	96.7	90.0	100

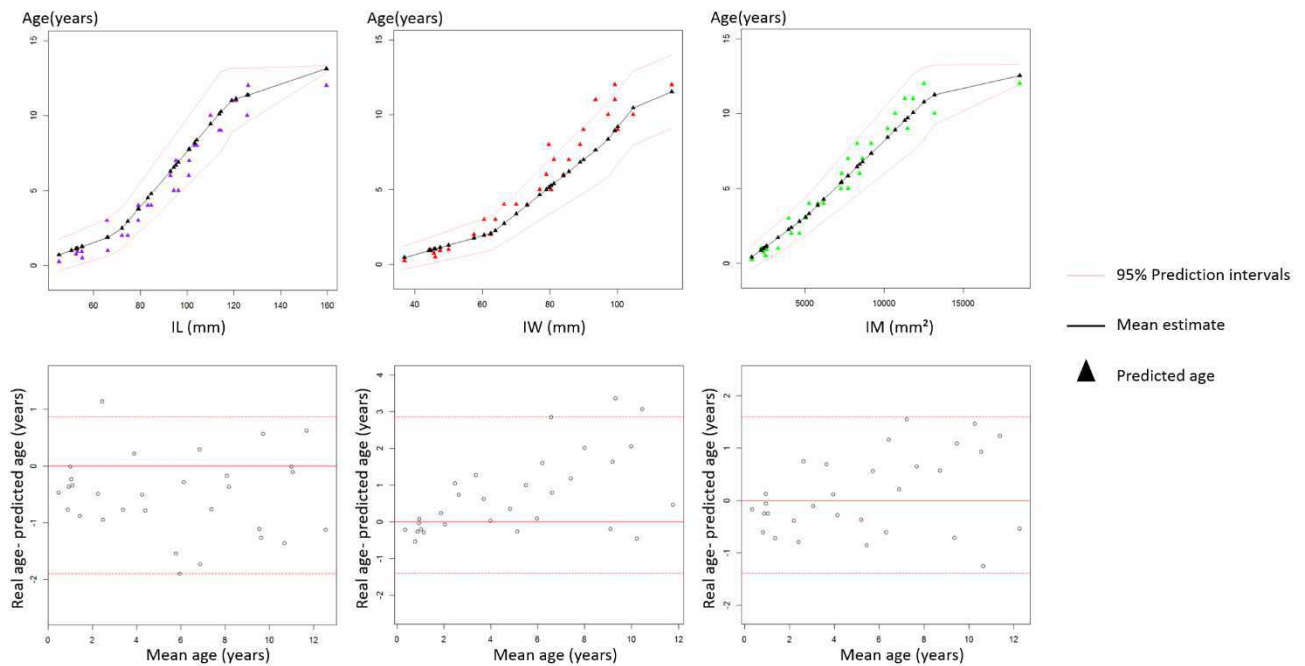


Figure 6.28 Univariate iliac MARS models applied to the Toulouse sample. Top: plots of age against the predictor variables, mean estimated age and prediction intervals; Bottom: Residuals against mean values of age

Age tends to be overestimated when using IL and underestimated when using IW. The residuals seem to be uniformly dispersed around zero with IM as a predictor, with a slight tendency to overestimate the age of younger individuals. Only one or two individuals lie outside the 95% range of residual dispersion, which is comprised between -1.96 and +1.96.

Bivariate and multivariate models provide very similar results to the test sample from Marseille when applied to the individuals from Toulouse. They also provide similar results to each other: the test parameters have comparable values, high RSq and low mean and standard deviations of the residuals (Table 6-41). The results of the bivariate and multivariate models are very close to the ones provided by the univariate model using IM as a predictor.

Table 6-41 Validation parameters of the bivariate and multivariate MARS models for age prediction using iliac variables of individuals from Toulouse. RSq=R-Squared regression coefficient; sd=standard deviation

Bivariate and multivariate MARS models				
Predictors	IL+IW	IL+IM	IW+IM	IL+IW+IM
RSq	0.963	0.963	0.962	0.964
Residual mean (years)	0.133	0.174	0.087	0.144
Residual sd (years)	0.731	0.951	0.475	0.787
Accuracy (%)	100	100	100	100

The residual distribution against mean values of age show comparable patterns, with a tendency to overestimate the age of younger individuals and underestimate the age of older individuals (**Figure 6.29**). Only one or no individuals lie outside the 95% range of residual dispersion, which is comprised between -1.96 and +1.96.

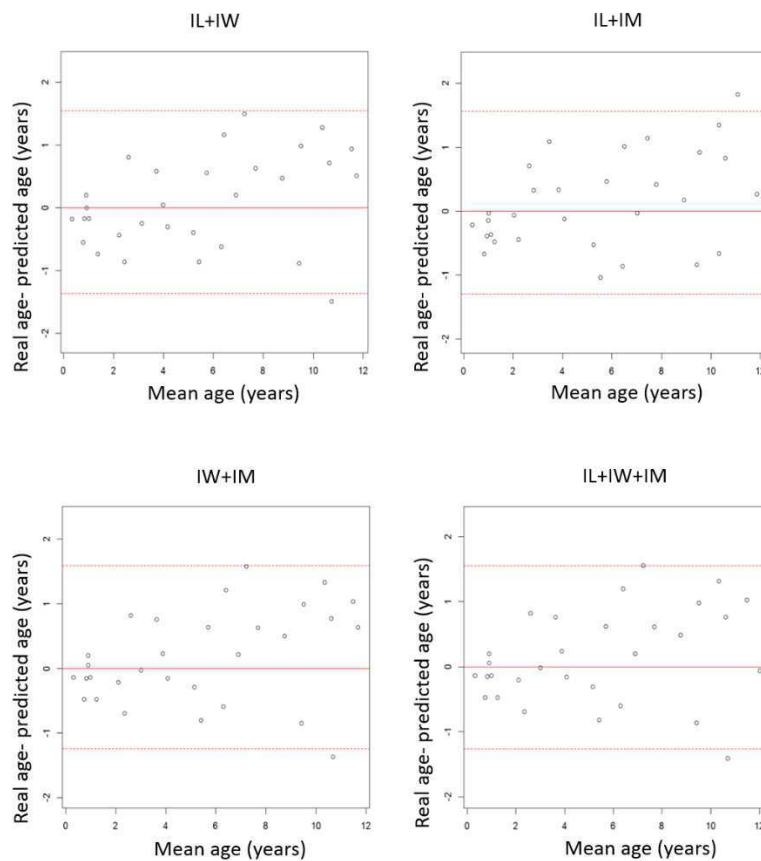


Figure 6.29 Residuals of the bivariate and multivariate iliac MARS models against mean values of age

All the models using IL, IW and IM are therefore validated on the test sample from Toulouse. These results also confirm the fact that IM seems a better age predictor than IL or IW.

### b. Independent test sample from Lisbon (Luis Lopes collection)

A test sample of 85 individuals (40 females and 45 males) from a Modern (first half of the 20<sup>th</sup> Century) population from Lisbon (Portugal) was used to test the MARS models constructed on the clavicle, fifth lumbar vertebra and iliac variables.

#### i. Iliac variables

All four iliac variables were obtained on 45 individuals (21 females and 24 males) from the Luis Lopes collection. The univariate MARS models provided lower values of RSq, comparable residual means and higher residual standard deviation values to the ones obtained on the Marseilles and Toulouse test samples (**Table 6-42**). Once again, the bidimensional variable IA provides the best results for the residual mean and standard deviation. IW provides the highest RSq value.

**Table 6-42 Validation parameters of the univariate MARS models for age prediction using iliac variables of individuals from the Luis Lopes collection. RSq=R-Squared regression coefficient; sd=standard deviation. The greyed values correspond to invalid parameters, or insufficient for age estimation purposes. Greyed values correspond to invalid parameters, or insufficient for age estimation purposes (<0.95)**

Predictors	Univariate MARS models			
	IL	IW	IM	IA
RSq	0.808	0.843	0.811	0.824
Residual mean (years)	0.921	0.663	0.799	0.335
Residual sd (years)	5.210	3.864	4.450	1.709
Accuracy (%)	69.2	80.8	73.1	78.5

The plots of the individuals and the corresponding mean estimated ages and prediction intervals show that age is generally underestimated (**Figure 6.30**). Four (IA) to eight (IL) individuals lie outside the range of the predictions intervals, which means the models have an accuracy between 69.2% and 78.5% for this sample.

The residual dispersion plots confirm the tendency to underestimate age as the 95% ranges of residual dispersion cover a larger part of positive values. They also show that age is slightly overestimated for younger individuals for all variables and intervals are larger than the ones of the Toulouse sample.

Bivariate and multivariate models provide similar if not slightly lower parameters compared to the univariate models: relatively high RSq, low means and high standard deviations of the residuals (**Table 6-43**). The bivariate and multivariate models using IA as a predictor provide the best results. Parameters show a lower performance than for the Toulouse sample.

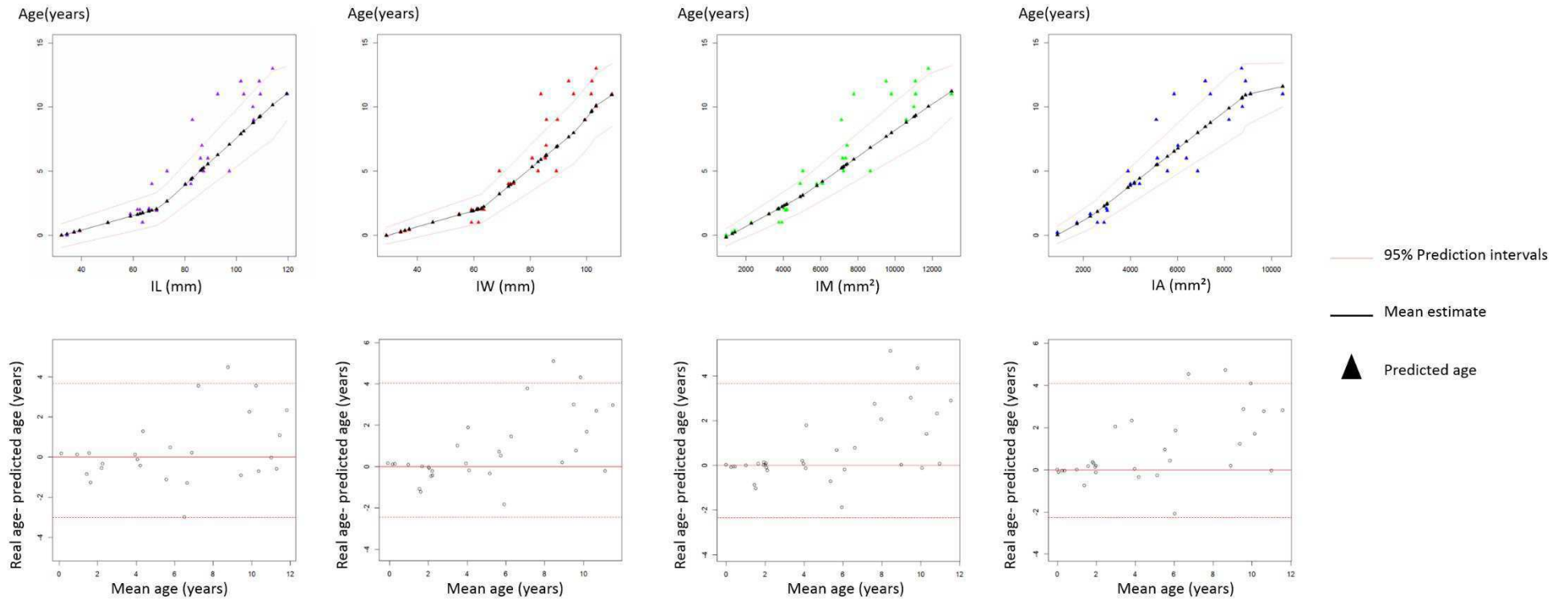


Figure 6.30 Univariate iliac MARS models applied to the individuals from the Luis Lopes collection. Top: plots of age against each variable, mean estimated age and prediction intervals; Bottom: Residuals against mean values of age



Table 6-43 Validation parameters of the bivariate and multivariate MARS models for age prediction using iliac variables of individuals from the Luis Lopes collection. RSq=R-Squared regression coefficient; sd=standard deviation. The greyed values correspond to invalid parameters, or insufficient for age estimation purposes. Greyed values correspond to invalid parameters, or insufficient for age estimation purposes (<0.95)

Bivariate MARS models						
Predictors	IL+IW	IL+IM	IL+IA	IW+IM	IW+IA	IM+IA
RSq	0.766	0.768	0.813	0.767	0.822	0.765
Residual mean (years)	0.932	0.953	0.545	0.954	0.504	0.966
Residual sd (years)	4.751	4.859	2.777	4.867	2.570	4.924
Accuracy (%)	65.4	65.4	73.1	73.1	80.8	80.8
Multivariate MARS models						
Predictors	IL+IW+IM	IL+IW+IA	IL+IM+IA	IW+IM+IA	IL+IW+IM+IA	
RSq	0.769	0.685	0.768	0.768	0.832	
Residual mean (years)	0.946	0.767	0.954	0.950	0.551	
Residual sd (years)	4.824	3.910	4.863	4.846	2.808	
Accuracy (%)	76.9	73.1	76.9	76.9	80.8	

The residual distribution against mean values of age show comparable patterns for bivariate and multivariate models, with the same tendency to overestimate the age of younger individuals and underestimate the age of older individuals as the univariate models. The range of dispersion of the residuals is higher than it was for the Toulouse sample (Figure 6.31).

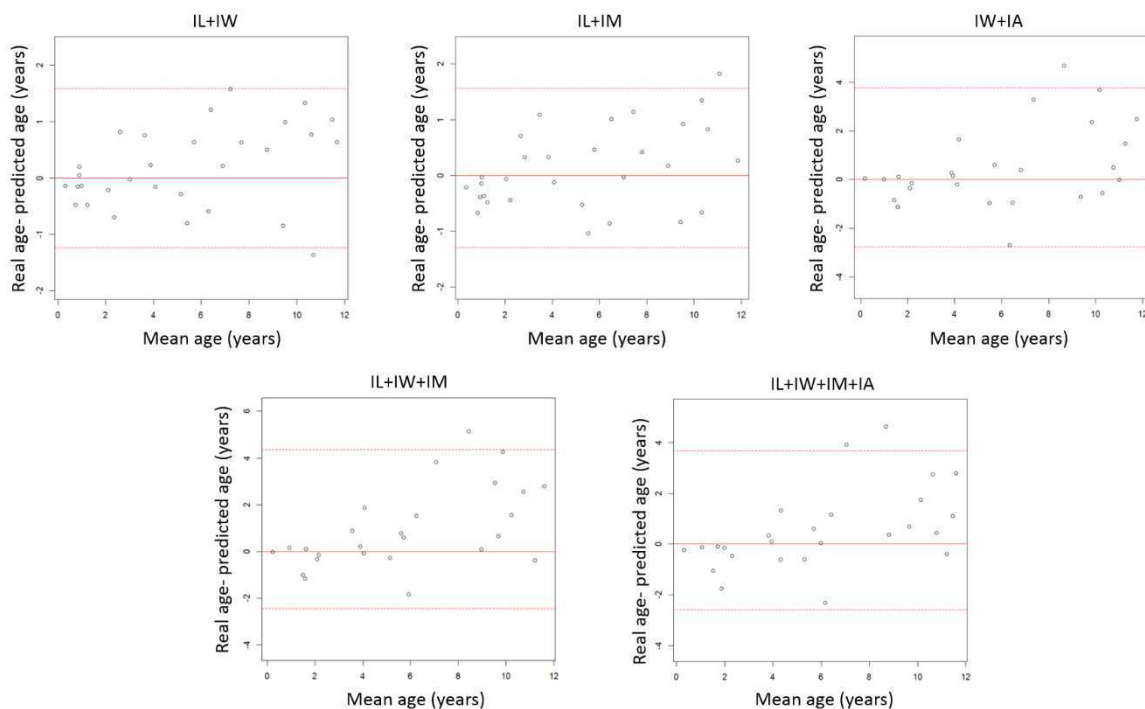


Figure 6. 31 Residuals of bivariate and multivariate iliac MARS models against mean values of age obtained for the individuals from the Luis Lopes collection

The univariate models using IW and IA, the bivariate IW+IA and IL+IA and the multivariate model using all four variables give the best results on the test sample from the Luis Lopes collection. These results confirm that a bidimensional variable is a better predictor of age than a unidimensional one. However, as the performance of age prediction is only slightly improved by bivariate and multivariate models, it seems simpler to use univariate models with IA and IW as predictors.

ii. Lumbar variables

All ten lumbar variables were obtained on 64 individuals (33 females and 31 males) from the Luis Lopes collection. The univariate MARS models provided lower values of RSq, comparable residual means and much higher residual standard deviation values to the ones obtained on the Marseilles test sample (**Table 6-44**). Lower variables (LVL, LVW and LVM) provide better results than upper variables (UVL, UVW and UVM).

**Table 6-44 Validation parameters of the univariate MARS models for age prediction using upper and lower lumbar variables of individuals from the Luis Lopes collection. RSq=R-Squared regression coefficient; sd=standard deviation. Greyed values correspond to invalid parameters, or insufficient for age estimation purposes (<0.95)**

Univariate MARS models						
Predictor	UVL	UVW	UVM	LVL	LVW	LVM
RSq	0.651	0.583	0.636	0.668	0.662	0.693
Residual mean (years)	2.258	2.259	2.299	2.119	2.022	2.040
Residual sd (years)	18.068	18.069	18.395	16.949	16.173	16.323
Accuracy (%)	79.7	79.7	79.7	79.7	84.4	84.4

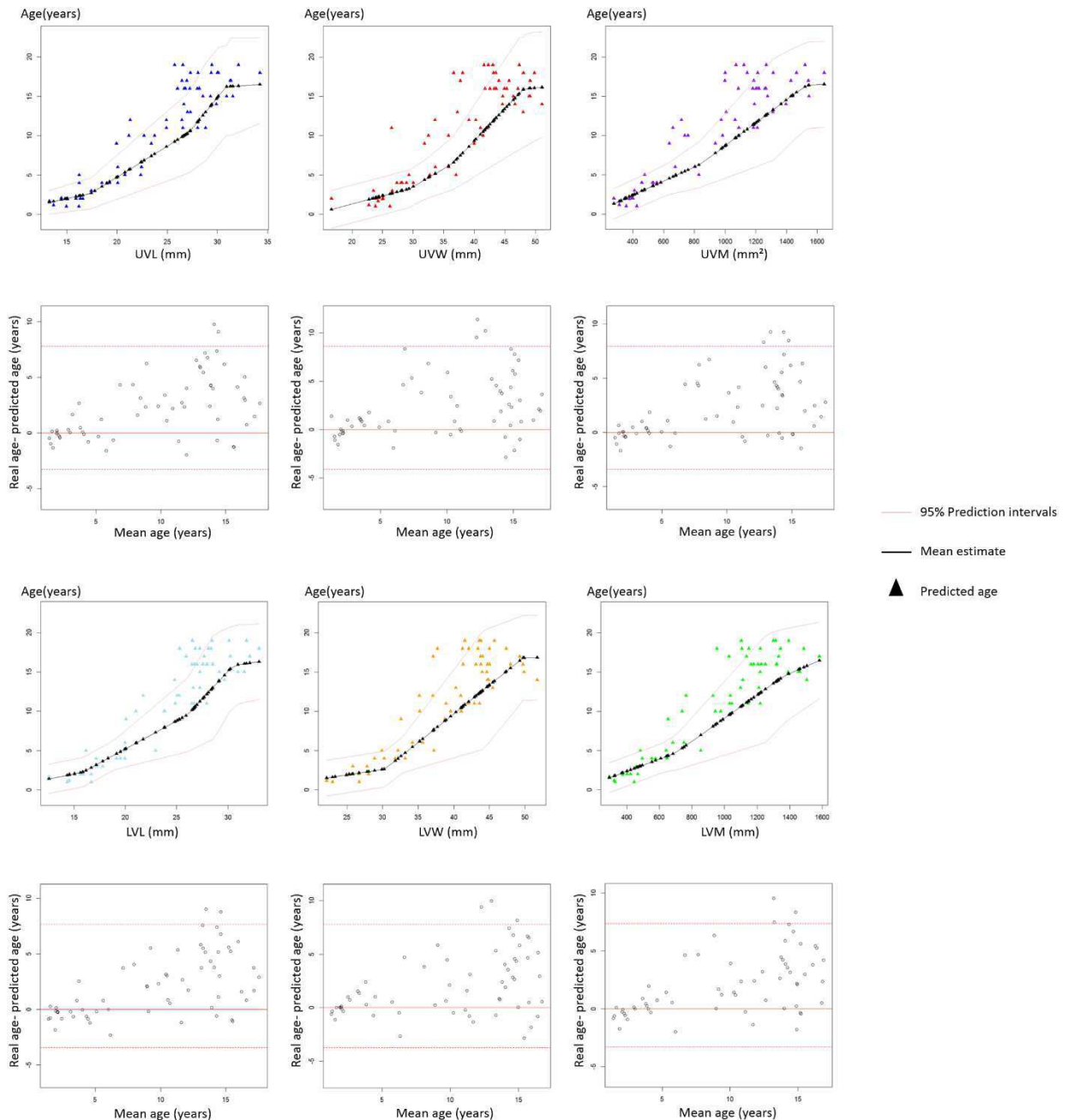
However, performances of the models using height variables are much better, especially for variables AVH, RVH and LVH (**Table 6-45**). The parameters are comparable to or better than those obtained with the Marseilles test sample: residual means are comparable and residual standard deviations are lower, particularly with PVH and AVH as predictors.

**Table 6- 45 Validation parameters of the univariate MARS models for age prediction using the lumbar height variables of individuals from the Luis Lopes collection. RSq=R-Squared regression coefficient; sd=standard deviation. Greyed values correspond to invalid parameters, or insufficient for age estimation purposes (<0.95)**

Univariate MARS models				
Predictor	PVH	AVH	RVH	LVH
RSq	0.797	0.884	0.879	0.885
Residual mean (years)	-0.073	0.081	0.455	0.164
Residual sd (years)	0.586	0.650	3.643	1.309
Accuracy (%)	87.5	95.3	89.1	89.1

The plots of the individuals and the corresponding mean estimated ages and prediction intervals confirm these observations. Plots of age against upper and lower variables with the associated prediction intervals show that age is generally underestimated (**Figures 6.32 and 6.33**).

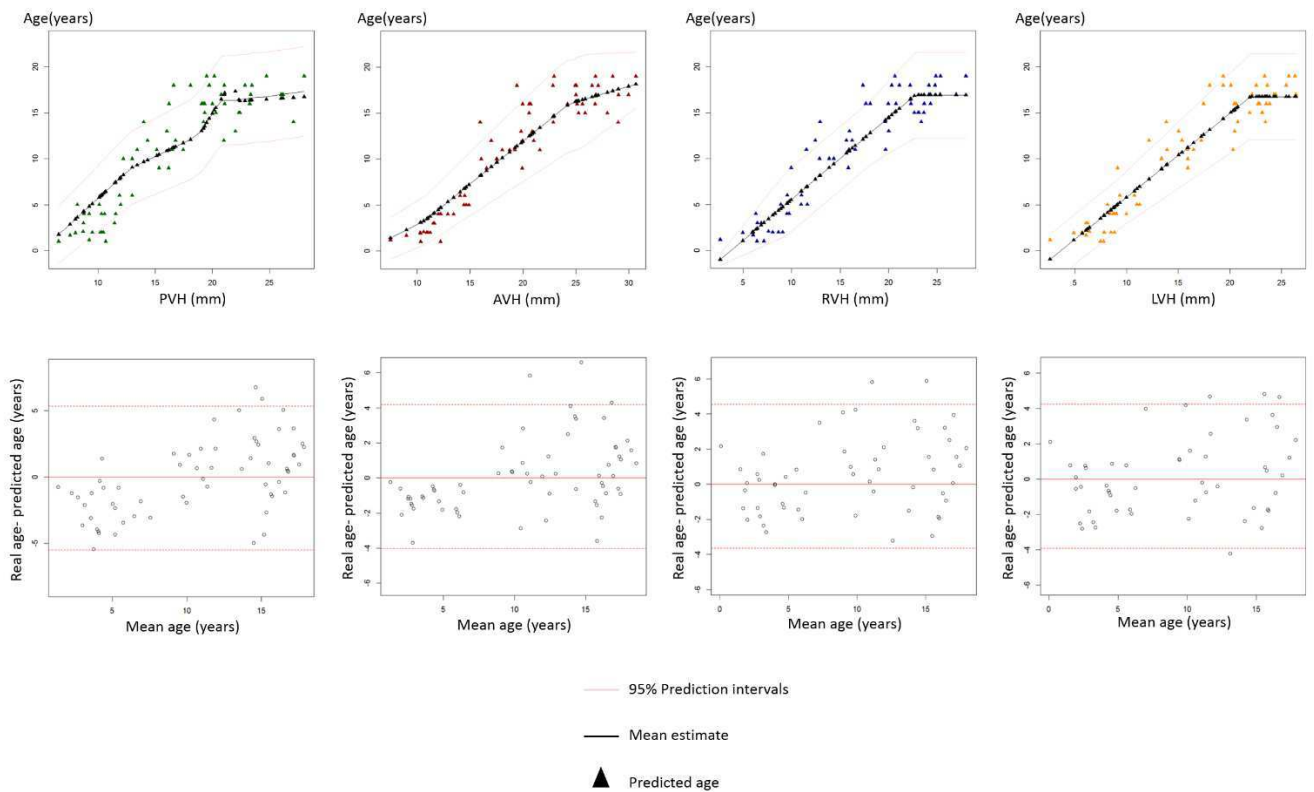
These six models leave between ten (LVW and LVM) and thirteen (UVL) individuals outside the range of the prediction intervals, which means they have an accuracy between 79.7% and 84.4%.



**Figure 6.32** Univariate MARS models using the lower lumbar variables as predictors applied to the individuals from the Luis Lopes collection. Top: plots of age against each variable, mean estimated age and prediction intervals; Bottom: Residuals against mean values of age

The residual dispersion plots confirm the tendency to underestimate age, as the 95% ranges of residual dispersion cover a larger part of positive values. They also show that age is either correctly or slightly overestimated for younger individuals for all variables. The ranges are much larger than they were for the Marseilles test sample.

Plots of age against height variables with the associated mean estimates and prediction intervals show a better fit to the data, although age seems slightly overestimated for younger individuals when using PVH or AVH as predictors (**Figure 6.33**). These four models leave between three (AVH) and eight (PVH) individuals outside the ranges of the prediction intervals, which means they have an accuracy between 87.5% and 95.3%.



**Figure 6.33** Univariate MARS models using the lumbar height variables as predictors applied to the individuals from the Luis Lopes collection. Top: plots of age against each variable, mean estimated age and prediction intervals; Bottom: Residuals against mean values of age

The residual dispersion plots confirm the tendency to overestimate age of younger individuals and underestimate age of older individuals (**Figures 6.32 and 6.33**). Because of this, the 95% ranges of residual dispersion cover equal parts of negative and positive values respectively. The ranges are much larger than they were for the Marseilles test sample at [-5 ; +5] years for PVH and [-4 ; +4] years for AVH. These plots also show the uniform dispersion of the residuals of the functions using

RVH and LVH as predictors; indeed, age is neither preferably over- or underestimated by these models. However, the ranges of dispersion remain high [-4 ; + 4] years.

Bivariate and multivariate models provide similar if not slightly lower parameters compared to the univariate models using the same variables: relatively average values of RSq for models using upper and lower variables, very high residual standard deviations and residual means around 2 years. The three models using height variables stand out with much higher RSq values, lower residual means and standard deviations (**Table 6-46**).

**Table 6-46 Validation parameters of the bivariate and multivariate MARS models for age prediction using lumbar variables of individuals from the Luis Lopes collection. RSq=R-Squared regression coefficient; sd=standard deviation. Greyed values correspond to invalid parameters, or insufficient for age estimation purposes (<0.95)**

Bivariate MARS models							
Predictors	PVH+AVH	RVH+LVH	LVL+LVW	UVL+UVW	UVL+LVL	UVW+LVW	UVM+LVM
RSq	0.860	0.893	0.667	0.644	0.661	0.604	0.616
Residual mean (years)	-0.009	0.331	2.120	2.303	2.168	2.174	2.362
Residual sd (years)	0.076	2.649	16.963	18.424	17.343	17.390	56.686
Accuracy (%)	87.5	100	96.9	79.7	75.0	81.2	73.4
Multivariate MARS models							
Predictors	All	Upper+Lower	Height	UVL+UVW+LVL+LVW	Upper	Lower	
RSq	0.792	0.655	0.882	0.658	0.636	0.656	
Residual mean (years)	1.118	2.137	0.167	2.263	2.363	2.233	
Residual sd (years)	8.941	17.098	4.004	18.105	18.902	17.863	
Accuracy (%)	84.4	95.3	93.8	75.0	78.1	79.7	

All=UVL+UVW+UVM+LVL+LVW+LVM+PVH+AVH+RVH+LVH; Upper=UVL+UVW+UVM; Lower=LVL+LVW+LVM; Height=PVH+AVH+RVH+LVH

The distribution of residuals against mean values of age show comparable patterns for bivariate and multivariate models than they did for univariate models. Models with upper and lower variables show the same tendency to slightly overestimate the age of younger individuals and predominantly underestimate the age of older individuals (**Figure 6.34**). The range of dispersion of the residuals is comparable to the one found with the univariate models.

The bivariate and multivariate models using height variables still show a more uniform dispersion of the residuals, although age tends to be overestimated for the younger individuals with the “height” model. The RVH+LVH model gives the best results overall.

The univariate, bivariate and multivariate models using lumbar height variables give the best results on the test sample from the Luis Lopes collection. These results confirm that these lumbar variable are better predictors of age than the other lumbar variables. However, as the performance of age prediction is comparable or only slightly improved by bivariate and multivariate models, it seems

simpler to use univariate models with AVH, RVH and LVH as predictors. However, the bivariate models PVH+AVH and RVH+LVH provide the best results.

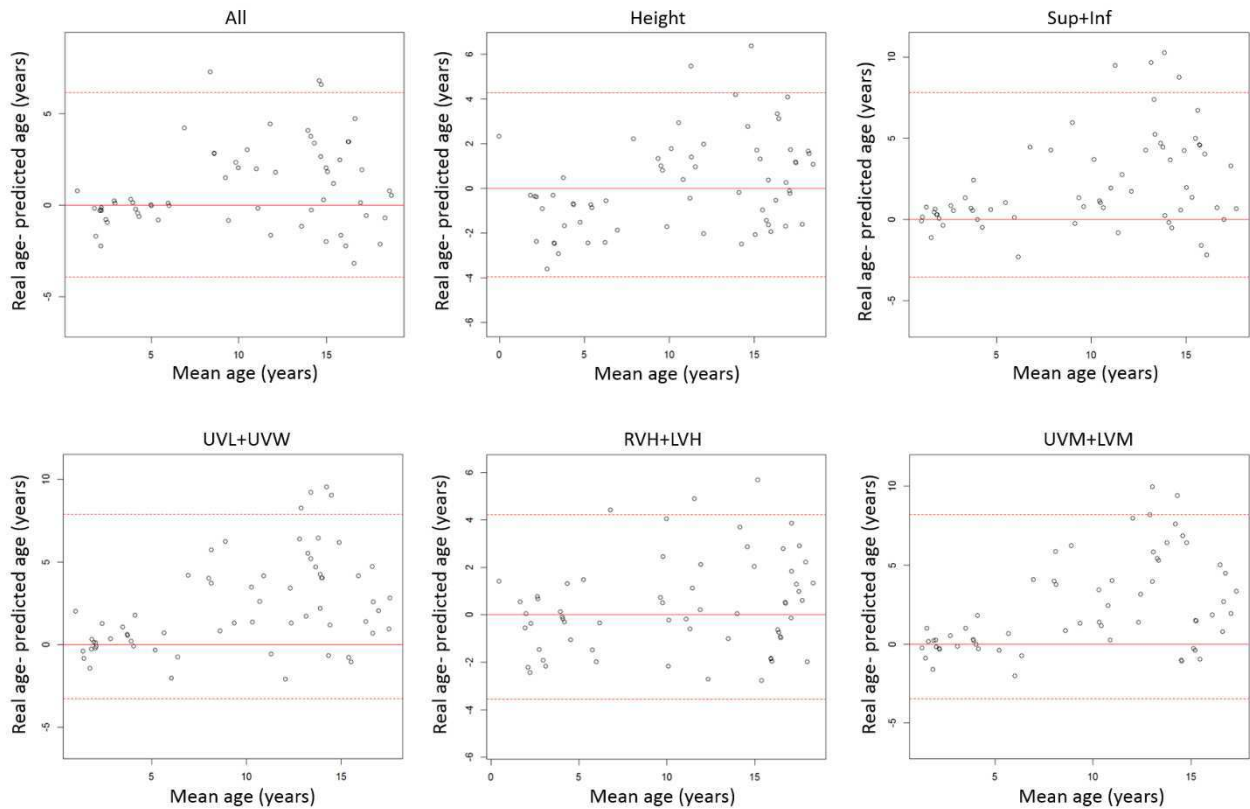


Figure 6.34 Residuals of the bivariate and multivariate lumbar MARS models against mean values of age obtained for the individuals from the Luis Lopes collection

iii. Clavicular variables

All five clavicular variables were obtained on 49 individuals (24 females and 25 males) from the Luis Lopes collection. The univariate MARS models provided much lower values of RSq, higher residual means and much higher residual standard deviation values to the ones obtained on the Marseilles test sample. The model using Ln as a predictor was the only univariate model to have a high RSq and a low residual mean. Residual standard deviation however is very high (Table 6-47).

The plots of the individuals and the corresponding mean estimated ages and prediction intervals confirm these observations. Plots of age against upper and lower variables with the associated prediction intervals show that age is generally underestimated for all variables (Figures 6.35).

**Table 6-47 Validation parameters of the univariate MARS models for age prediction using clavicular variables of individuals from the Luis Lopes collection. RSq=R-Squared regression coefficient; sd=standard deviation. Greyed values correspond to invalid parameters, or insufficient for age estimation purposes (<0.95)**

Univariate MARS models					
Predictors	Ln	Max_diam	Min_diam	AP_diam	SI_diam
RSq on the test sample	0.826	0.477	0.047	0.335	0.205
Residual mean (years)	1.751	3.601	5.181	4.037	4.170
Residual sd (years)	12.257	25.206	36.269	28.262	29.189
Accuracy (%)	79.6	73.5	20.4	71.4	65.3

Ln is the only predictor to include a relatively high number of individuals within the range of the 95% prediction interval: 10 individuals lie outside said range, which gives the model a 79.6% accuracy rate. The residual dispersion plot confirms underestimation of age, as the 95% range of residual dispersion covers a larger part of positive values. The range is larger than it was for the Marseilles test sample (**Figure 6.35**).

These four univariate models using diameters give very poor results: age is underestimated for all individuals older than five years old and the majority of the individuals lie outside the range of the prediction intervals. This is particularly noticeable for the model using Min\_diam as a predictor: only 10 out of 49 individuals are within the 95% prediction range, which gives the model a 20.4% accuracy rate. The other three model accuracies are between 65.3% (SI\_diam) and 73.5% (Max\_diam).

Residual dispersion confirms the importance of age underestimation by the four diameter variables. It presents a “bridge-shape”: age underestimation starts around five years, increases until 10 years and stabilises before decreasing around 15 years. Because of this, the 95% ranges of residual dispersion are almost entirely within positive values. The ranges are asymmetrical compared to zero and much larger than they were for the Marseilles test sample, including residual values higher than 10.

Bivariate and multivariate models using Ln as one of the predictors provide better results than the univariate models using diameter variables: the values of RSq have greatly increased, residual means have greatly decreased, but residual standard deviations are very high. However, the parameters are not better than the ones obtained with the univariate “Ln” model. Residual standard deviations are still very high.

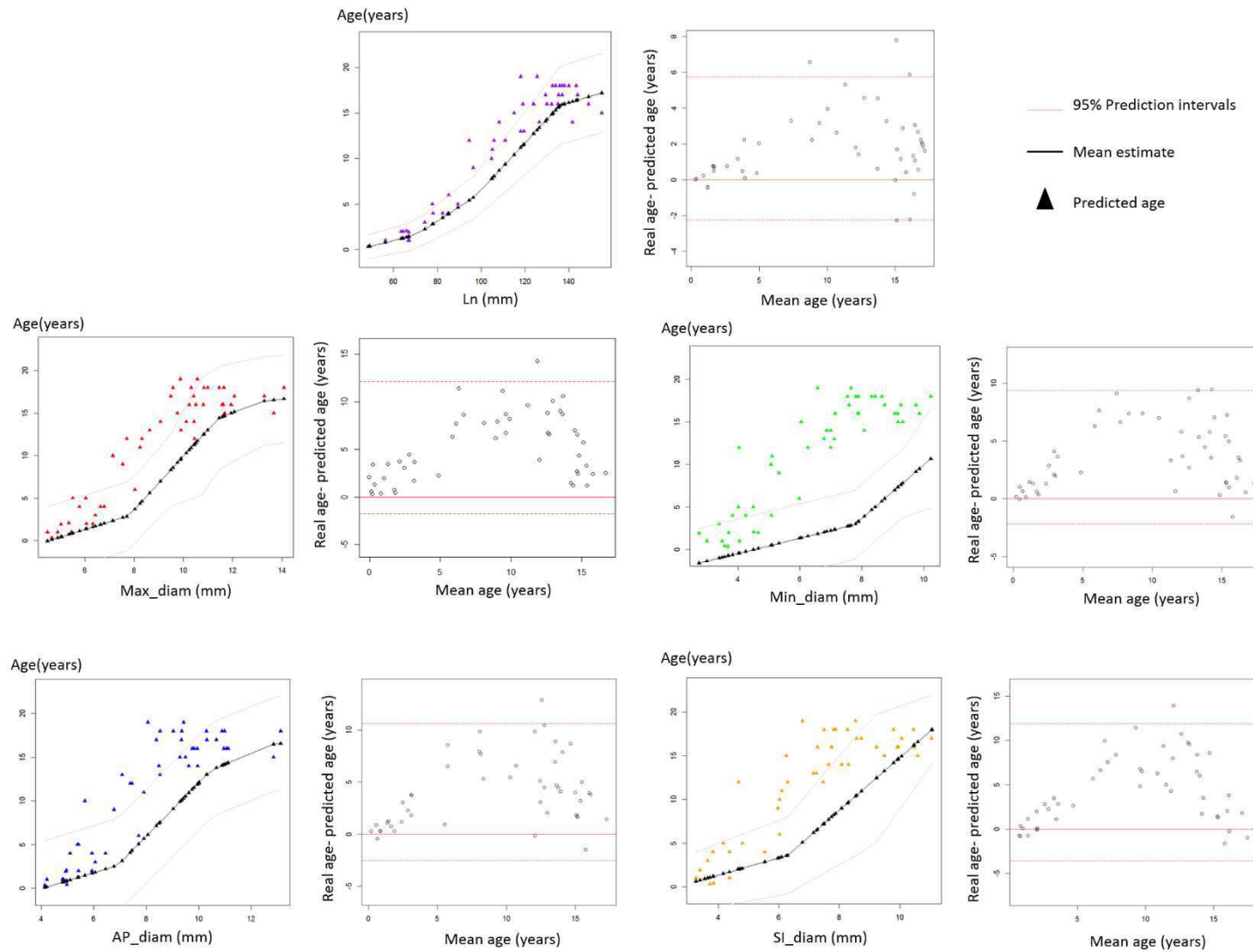


Figure 6.35 Univariate clavicular MARS model using Ln as a predictor applied to the individuals from the Lisbon sample. Left: plot of age against Ln, mean estimated age and prediction intervals; Right: Residuals against mean values of age



The two bivariate models using diameter variables stand out with much lower RSq values, higher residual means and standard deviations (**Table 6-48**).

The distribution of residuals against mean values of age show comparable patterns for bivariate and multivariate models. Models with Ln as a predictor provide a triangular distribution of the residuals, with differences between real and estimated age increasing in absolute value with age. Residuals of the models using diameter variables (*e.g.* Max+Min) show the same tendency noted previously to slightly overestimate the age of individuals younger than five years.

**Table 6-48 Validation parameters of the bivariate and multivariate MARS models for age prediction using clavicular variables of individuals from the Luis Lopes collection. RSq=R-Squared regression coefficient; sd=standard deviation. Greyed values correspond to invalid parameters, or insufficient for age estimation purposes (<0.95)**

<b>Bivariate MARS models</b>						
Predictors	Max+Min	AP+SI	Ln+AP	Ln+SI	Ln+Max	Ln+Min
RSq on the test sample	0.295	0.274	0.794	0.764	0.792	0.806
Residual mean (years)	4.364	4.313	1.988	2.145	2.022	1.972
Residual sd (years)	30.549	30.189	13.917	15.014	14.152	13.803
Accuracy (%)	49.0	61.2	77.6	77.6	73.5	81.6
<b>Multivariate MARS models</b>						
Predictors	All	Diameters	Ln+AP+SI	Ln+Max+Min		
RSq on the test sample	0.753	0.087	0.744	0.782		
Residual mean (years)	2.020	4.602	2.316	2.062		
Residual sd (years)	14.141	32.217	16.213	14.434		
Accuracy (%)	71.4	57.1	87.8	75.5		

The age of older individuals is either over- or underestimated by the models using Ln as one of the predictors and the range of the residuals is smaller: [-5 ; +5] years with diameter variables and around [-3 ; +3] years with models using Ln (**Figure 6.36**).

The univariate, bivariate and multivariate models using maximum clavicular length (Ln) give the best results on the test sample from the Luis Lopes collection, although they cannot be considered completely valid: accuracy is insufficient and residual standard deviations are too high. Nevertheless, these results confirm that Ln is a better predictors of age than the other clavicular variables.

However, as the performance of the MARS models using Ln is insufficient when applied on the individuals from the Luis Lopes collection, the use of these functions for age prediction is questionable.

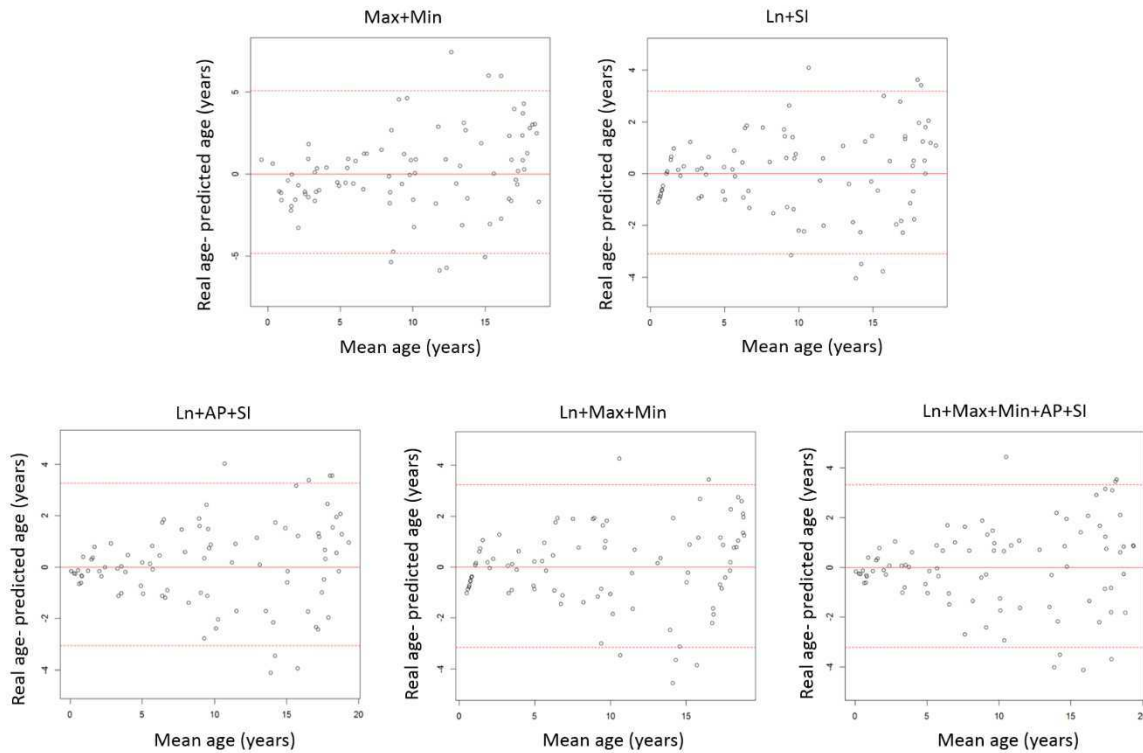


Figure 6.36 Residuals of bivariate and multivariate clavicular MARS models against mean values of age obtained for the individuals from the Luis Lopes collection

**Conclusions on the age estimation models using biometric predictor variables**

- Testing the models on various samples has either confirmed or infirmed their performance for age estimation
  - Iliac bidimensional data (IM and/or IA) generally gave better results on all three test samples. In multivariate models, bidimensional variables stood out as the most important in terms of contribution to the models.
- These models are valid on all three test samples, although their predictive power is lower on the individuals from the Luis Lopes collection.
- Both the Marseilles and Luis Lopes test samples showed better results with vertebral height variables (PVH, AVH, RVH and LVH) in univariate, bivariate or multivariate models. The three univariate lumbar models “AVH”, “RVH” and “LVH” are the best of all models constructed on data from any of the three types of bones from the individuals of the Luis Lopes collection
  - Maximum clavicular length (Ln) is the best clavicular age predictor when used in univariate or bivariate models on both test samples (Marseilles and the Luis Lopes collection). However, the results are only valid on the individuals from the Marseilles sample

## **6.2. Age estimation using Independent Bayesian probabilities and maturation stages of the iliac bone**

Age was also estimated by calculating posterior probabilities of age using non-biometric maturation stages of the four iliac epiphyses. All posterior probabilities calculated using Independent Bayesian Probabilities obtained from the maturation stages for the 285 individuals of the Marseilles training sample (uni-site and combinations) can be found in **Appendix G**. They can be used for age estimation by referring to the maturation indicator (stage or combination) and its corresponding posterior probabilities of age by summing the probabilities to obtain a satisfactory confidence interval.

### **6.2.1. Uni-site probabilities**

Flat distributions were found for the maturation stage values of 0 and 2 for all four sites of the iliac bone (**Figure 6.37**).

A high number of individuals present these two extreme stages. All sites remain unfused until at least 8 years, extending to 12 years for PUBISCH\_SUP, PUBIL and ILISCH, and they can be completely fused as early as 12 years. Therefore, fusion of the iliac sites considered separately is not relevant for age estimation before 8 years of age. The highest number of individuals with partially fused sites are 8 years old for PUBISCH\_INF, 11 years for ILISCH and 12 years for PUBISCH\_SUP and PUBIL.

All four sites can remain partially fused until a maximum of 17 years of age, although this intermediate state is shorter for PUBISCH\_INF, as most individuals at stage 1 at this site are aged between 8 and 13 years. The other three sites present intermediate states from 8 to 15 years of age.

In the following results, frequencies of rank 0 indicate the percentage of individuals placed in the correct annual age group. This is considered as the performance of the estimation and constitutes the “bone age group” of the individual. Positive ranks indicate an underestimation of age (the correct age group is superior to the bone age group) and negative ranks indicate an overestimation of age.

#### **a. Independent test sample from Marseilles**

When applied to an independent test sample from Marseilles composed of 132 individuals aged 0 to 19 years, the posterior probabilities provided age estimates with 95% or higher confidence intervals of +/- 6 years for PUBISCH\_INF, +/- 5 years for ILISCH and PUBISCH\_SUP and of +/- 4 years for PUBIL (**Figure 6.37**).

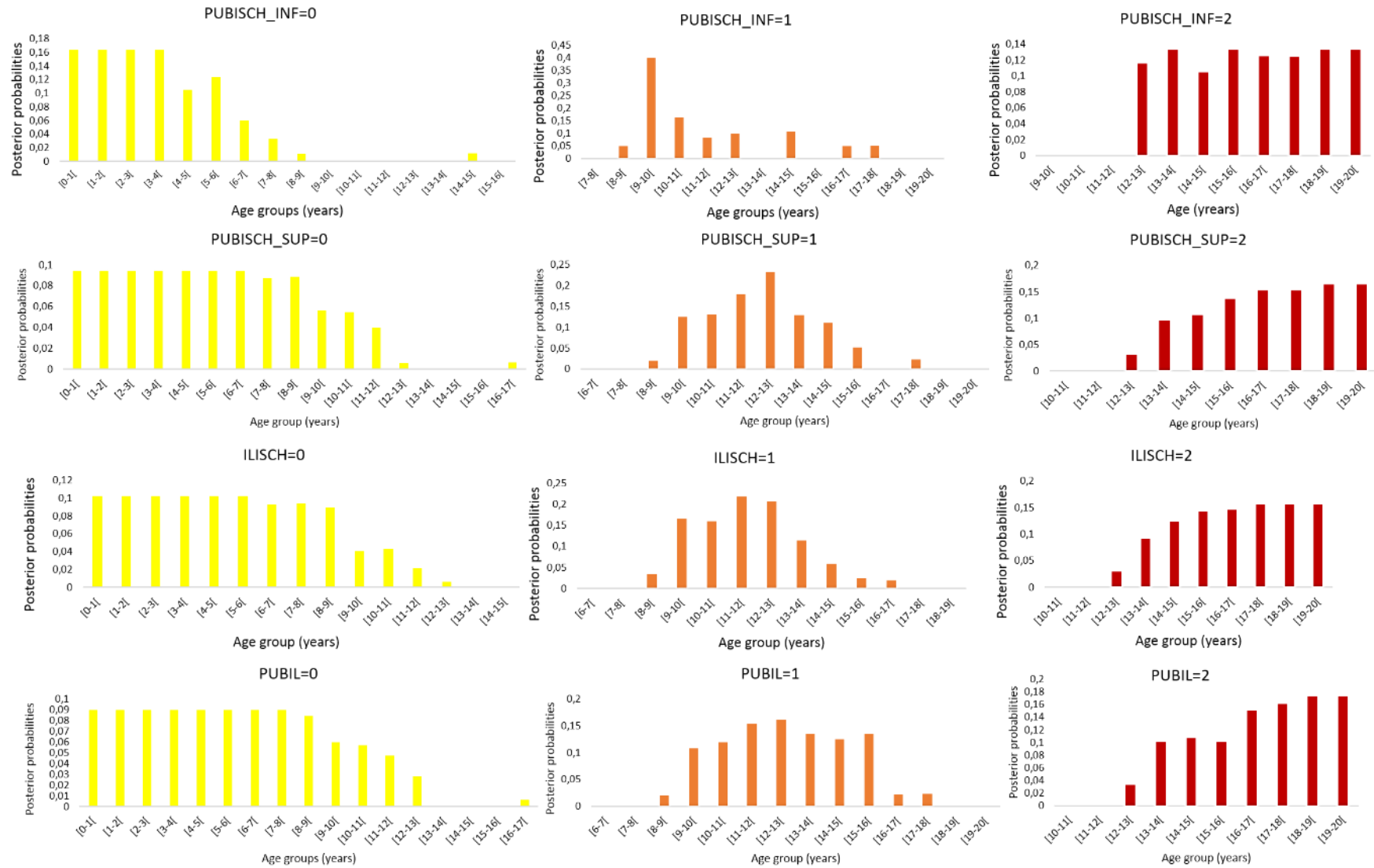


Figure 6.37 Posterior probabilities of age associated to the maturation stages of each of the four iliac sites

Reliability lower than 95% results in better precision. However, the best precision associated to a reliability of 91.66% can only be obtained using PUBIL, with a +/- 3 years precision.

The performance (the frequency associated with rank 0) is higher than 50% for all four sites. The highest performance is obtained with PUBIL (59.54%), and the lowest is obtained with ILISCH (50.02%).

The distributions of ranks are “peaked”, with much lower frequencies for the ranks other than rank 0. This is confirmed by the kurtosis values which are higher than 1 for all four sites (**Table 6-49**).

**Table 6-49 Results of the kurtosis, skewness and Kruskal-Wallis tests done on the distributions of the ranks obtained for the four iliac sites on the Marseilles test sample. Greyed values correspond to significant differences of rank distributions between age groups**

Test	PUBISCH_INF		PUBISCH_SUP		PUBIL		ILISCH	
Kurtosis	1.590		1.754		1.464		1.362	
Skewness	-0.164		0.152		-0.808		0.588	
Kruskal-Wallis	H	p-value	H	p-value	H	p-value	H	p-value
	3.128	0.372	20.307	<0.001	13.980	0.003	19.559	<0.001

Moreover, frequencies do not continuously decrease when adding (or subtracting) a positive (or negative) rank. Skewness is not significant for any of the distributions, although skewness is slightly negative for PUBISCH\_INF and PUBIL, meaning age is slightly more overestimated than underestimated. It is the opposite for PUBISCH\_SUP and ILISCH.

The Kruskal-Wallis test done on the rank distribution in the four quinquennial age groups for each site showed that the distributions were significantly different between the age groups for PUBISCH\_SUP, PUBIL and ILISCH (**Figure 6.38**). Rank 0 is clearly dominant for the age group 0-4 for all four sites, and for the age group 15-19 for PUBISCH\_INF. It is also dominant in age group 15-19 for PUBISCH\_INF.

The proportions of negative and positive ranks are comparable for age groups 5-9 and 10-14 for PUBISCH\_INF. Positive ranks are clearly dominant in age group 5-9 for PUBIL, PUBISCH\_SUP and ILISCH, whereas negative ranks are dominant for the age group 15-19 for the same sites.

Independent Bayesian probabilities using PUBISCH\_SUP, PUBIL or ILISCH have the tendency to underestimate age for individuals aged 0 to 14 years and overestimate it for individuals in the 15-19 age group. PUBISCH\_INF does not show any tendency for over- or underestimation of age.

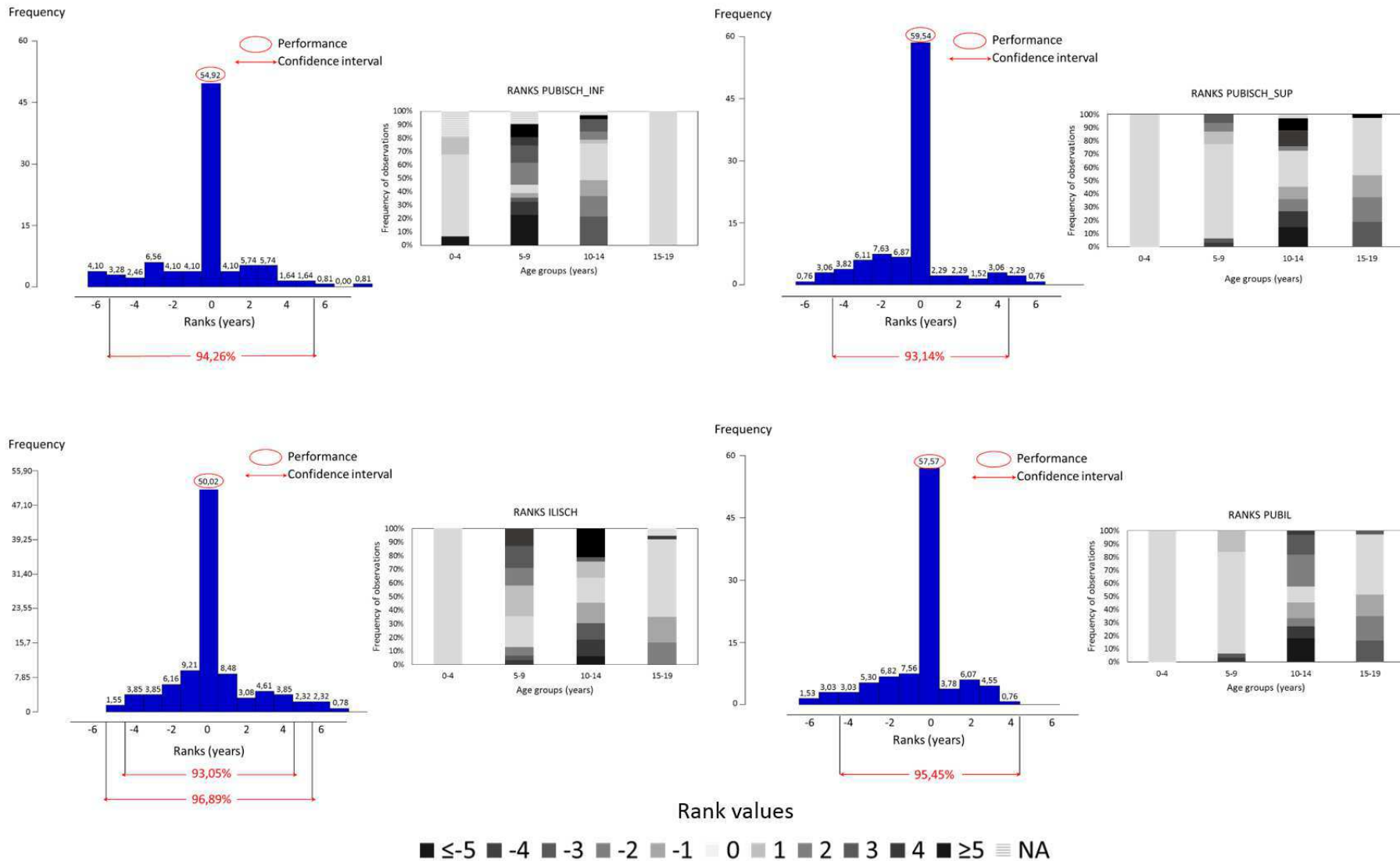


Figure 6.38 Validation ranks of age estimation using uni-site posterior probabilities and their distribution according to quinquennial age groups in the Marseilles sample

### b. Independent test sample from Lisbon (Luis Lopes collection)

When applied to the test sample from the Luis Lopes collection composed of 79 individuals aged 0 to 19 years, the posterior probabilities provided age estimates with 95% or higher confidence intervals of +/- 8 years for PUBISCH\_SUP, +/- 7 years for ILISCH and PUBISCH\_INF and +/- 4 years for PUBIL (**Figure 6.39**). Reliability lower than 95% results in better precision. However, the best precision associated to a reliability of at least 90% can only be obtained using PUBIL, with a +/- 3 years precision.

The performance (the frequency associated with rank 0) is higher than 50% for all four sites. The highest performance is obtained with PUBISCH\_INF (67.51%), and the lowest is obtained with PUBISCH\_SUP (52.50 %).

The distributions of ranks are “peaked” at rank 0, with much lower frequencies for the other ranks. This is confirmed by the kurtosis values which are higher than 1 for three sites, especially for PUBISCH\_INF (**Table 6-50**).

**Table 6-50 Results of the kurtosis, skewness and Kruskal-Wallis tests done on the distributions of the ranks obtained for the four iliac sites on the Luis Lopes test sample. Greyed values correspond to significant differences of rank distributions between age groups**

Test	PUBISCH_INF		PUBISCH_SUP		PUBIL		ILISCH	
Kurtosis	5.025		1.101		1.791		0.620	
Skewness	0.474		1.350		1.054		1.324	
Kruskal-Wallis	H	p-value	H	p-value	H	p-value	H	p-value
	6.703	0.082	30.963	<0.001	34.225	<0.001	52.549	<0.001

ILISCH however, does not present a very high kurtosis value. Moreover, frequencies do not continuously decrease when adding (or subtracting) a positive (or negative) rank. Skewness is not significant for the rank distribution of PUBISCH\_INF, although it is highly significant for the other three sites: it is positive for PUBISCH\_SUP, PUBIL and ILISCH, meaning age is mostly underestimated by the maturation stages of these sites. This can clearly be seen in **Figure 6.39**, with the right “tails” in the distributions of ranks for these three sites.

The Kruskal-Wallis test done on the rank distribution of the four quinquennial age groups for each site showed that the distributions were significantly different between the age groups for PUBISCH\_SUP, PUBIL and ILISCH. Because the p-value associated to the test for PUBISCH\_INF is only slightly superior to 0.05, it is also considered significant. This is visible in **Figure 6.39**. Rank 0 is clearly dominant for age group 0-4 for all four sites, for age group 5-9 for PUBISCH\_SUP and PUBIL and for age group 15-19 for PUBISCH\_INF.

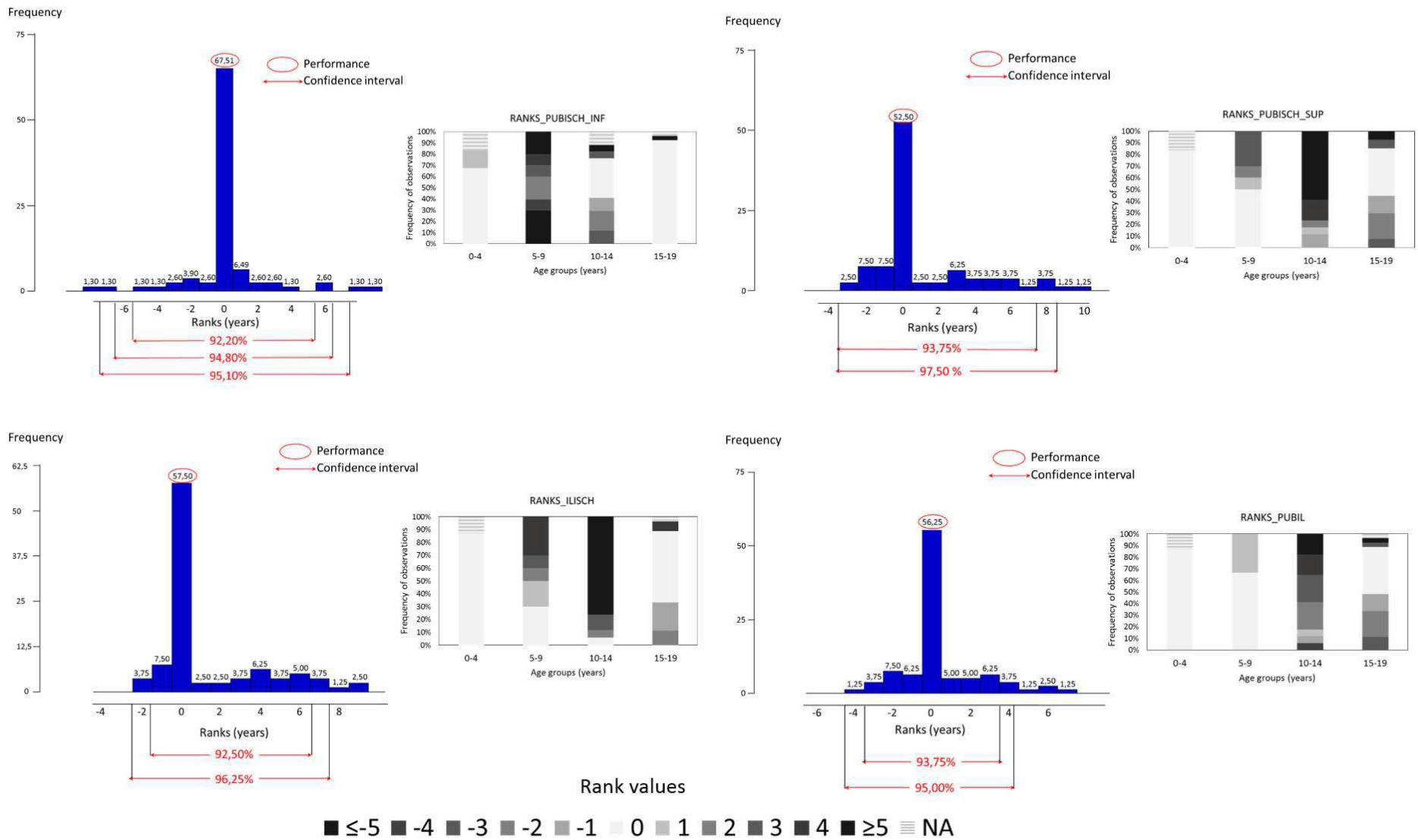


Figure 6.39 Validation ranks of age estimation using uni-site posterior probabilities and their distribution according to quinquennial age groups in the Luis Lopes sample

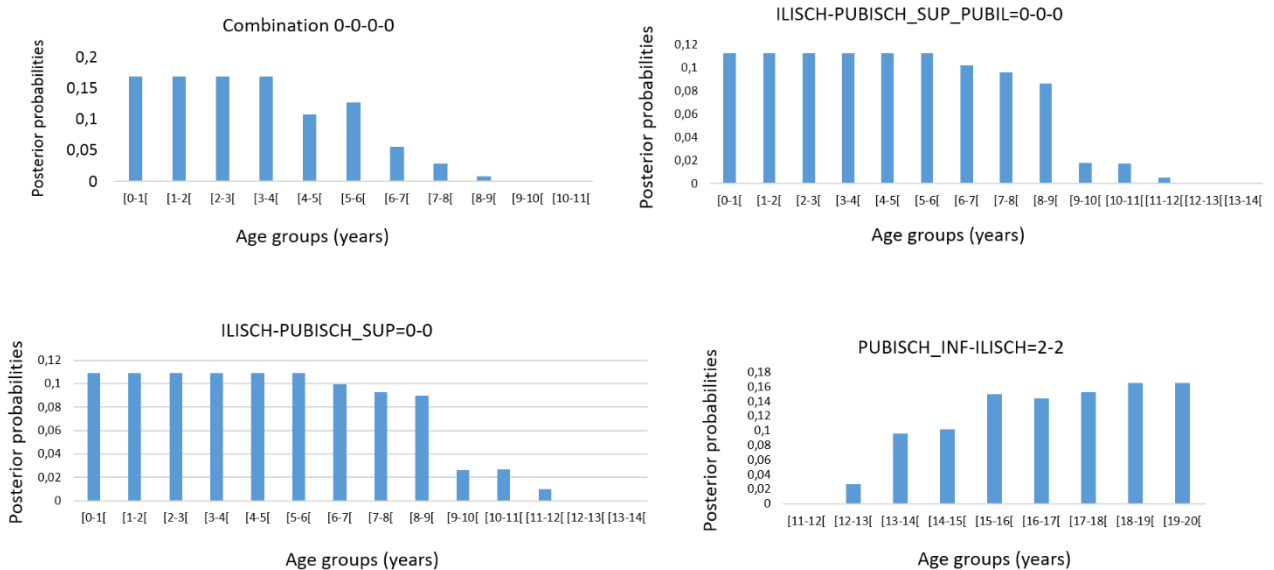


The proportions of negative and positive ranks are comparable for age groups 5-9 and 10-14 for PUBISCH\_INF. Positive ranks are clearly dominant in age groups 5-9 and 10-14 for PUBIL, PUBISCH\_SUP and ILISCH. Negative ranks are dominant for the age group 15-19 for the same sites.

Independent Bayesian probabilities using PUBISCH\_SUP, PUBIL or ILISCH have the tendency to underestimate age for individuals aged 0 to 14 years and overestimate it for individuals in the 15-19 age group.

### 6.2.2. Combinatory probabilities

Flat distributions were found for the two-, three- and four-digit combinations composed exclusively of values of 0 or 2 (Figure 6.40).



**Figure 6.40** Examples of flat distributions of posterior probabilities of age using combinations of maturation stages

A high number of individuals present these two extreme combinations of stages. Between two and six consecutive age groups present the same probabilities of age according to their combination. This means these combinations are not very discriminant for these age groups: combinations using only “0” as maturation stages do not discriminate well younger individuals, and combinations using only “2” as maturation stages do not discriminate well older individuals (see Appendix G for all posterior probabilities of age).

The results are presented following a decreasing number of digits in the combinations (from four to two). This choice was made because we wished to find the minimum number of digits required for sufficient reliability and precision of the estimates.

**a. Independent test sample from Marseilles**

i. Four-digit combination

When applied to the independent test sample from Marseilles, the posterior probabilities associated with the four-digit combinations of maturation stages provided age estimates with 95% or higher confidence intervals of +/- 5 years (Figure 6.41).

Because of the high values of the frequencies, no better precision could be obtained with a reliability between 90 and 95%: a precision of +/- 4 years would be associated with an 85.24% reliability.

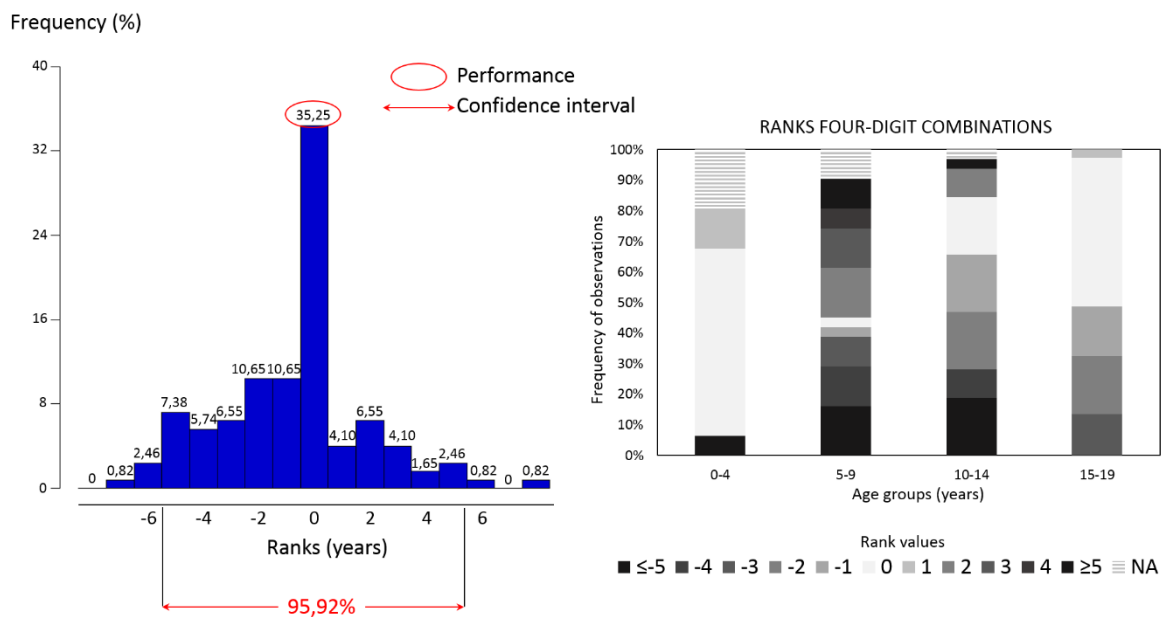


Figure 6.41 Validation ranks of age estimation using the four-digit combination of maturation stages and their distribution according to quinquennial age groups in the Marseilles sample

The performance is equal to 35.25%, which is lower than for the uni-site ranks. The distributions of ranks are “peaked” at rank 0, with much lower frequencies for the other ranks. This is confirmed by the kurtosis value which is higher than 1 (Table 6-51).

As previously, frequencies do not continuously decrease when adding (or subtracting) a positive (or negative) rank and their values are much higher in the negative ranges of ranks. Skewness is not

significant for the rank distribution meaning age is neither predominantly underestimated nor overestimated by the four-digit combination approach.

**Table 6-51 Results of the kurtosis, skewness and Kruskal-Wallis tests done on the distributions of the ranks obtained for the four-digit combination ranks on the Marseilles test sample**

Test	4-digit combination	
Kurtosis	5.025	
Skewness	0.474	
Kruskal-Wallis	H	p-value
	7.769	0.051

The Kruskal-Wallis test of the rank distribution between the four quinquennial age groups showed that the distributions were slightly different between the age groups. Because the p-value associated to the test is equal to 0.05 it is considered slightly significant. Rank 0 is clearly dominant for age groups 0-4 and 15-19, and underrepresented in age group 5-9. High negative ranks are also present in the 0-4 age group (**Figure 6.41**).

Negative ranks are dominant for age groups 10-14 and 15-19. No clear dominance of positive or negative ranks is visible in age group 5-9.

Independent Bayesian probabilities using the four-digit combination has the tendency to overestimate age for individuals aged 10 to 19 years, but shows no such tendencies for younger individuals. The application for age estimation could lead to important errors (and low precision) in the 5-9 and 10-14 age groups.

ii. Three-digit combinations

Posterior probabilities using three-digit combinations of maturation stages provided age estimates with 95% or higher confidence intervals and a precision between +/- 5 years and +/- 6 years (**Figures 6.42 and 6.43**). A reliability of 90.02% would provide a precision of +/- 4 years using ILISCH-PUBISCH\_SUP-PUBIL. The highest performance is obtained with ILISCH-PUBISCH\_SUP-PUBIL (46.16%), and the lowest is obtained with PUBISCH\_INF-ILISCH-PUBIL (34.09 %).

The distributions of ranks are “peaked” at rank 0, with much lower frequencies for the other ranks. This is confirmed by the kurtosis value higher than 1 for the three-digit combination ILISCH-PUBISCH\_SUP-PUBIL, and higher than 0.5 for the PUBISCH\_INF-ILISCH-PUBISCH\_SUP and PUBISCH\_INF-PUBIL-ILISCH (**Table 6-52**).

PUBISCH\_INF-PUBISCH\_SUP-PUBIL however, does not present a very high kurtosis value. Indeed, the ranks different than zero have higher values than for the other three-digit combinations. As before, frequencies do not continuously decrease when adding (or subtracting) a positive (or negative) rank, even if the general tendency is a decrease in frequency as the absolute value of ranks increases.

**Table 6-52 Results of the kurtosis, skewness and Kruskal-Wallis tests done on the distributions of the ranks obtained for the three-digit combination ranks on the Marseilles test sample. Greyed values correspond to significant differences of rank distributions between age groups**

Test	PUBISCH_INF-PUBISCH_SUP-PUBIL		PUBISCH_INF-ILISCH-PUBISCH_SUP		PUBISCH_INF-ILISCH-PUBIL		ILISCH-PUBISCH_SUP-PUBIL	
	H	p-value	H	p-value	H	p-value	H	p-value
Kurtosis	0.134		0.611		0.843		1.148	
Skewness	0.103		0.235		0.404		0.293	
Kruskal-Wallis	10.462	0.015	58.580	0.035	11.519	0.009	24.376	<0.001

Skewness is not significant for the rank distribution of all four three-digit combinations. Indeed, other than higher frequencies of negative ranks, the global distribution is symmetric around rank zero. The Kruskal-Wallis test done on the rank distribution of the four quinquennial age groups for each three-digit combination showed that the distributions were significantly different between the age groups for all combinations. This is visible in **Figures 6.42 and 6.43**.

Rank 0 is clearly dominant for age groups 0-4 and 15-19 for all four three-digit combinations. The highest proportion of absolute values of ranks superior or equal to 5 is found in the 10-14 age group for all combinations.

The proportions of negative and positive ranks are comparable for the four combinations: positive ranks are predominant in the 5-9 age group, while negative ranks are predominant in the 10-14 age group.

Independent Bayesian probabilities using three-digit combinations have the tendency to underestimate age of individuals aged 0 to 4 years, and overestimate age for individuals aged 10 to 19 years.

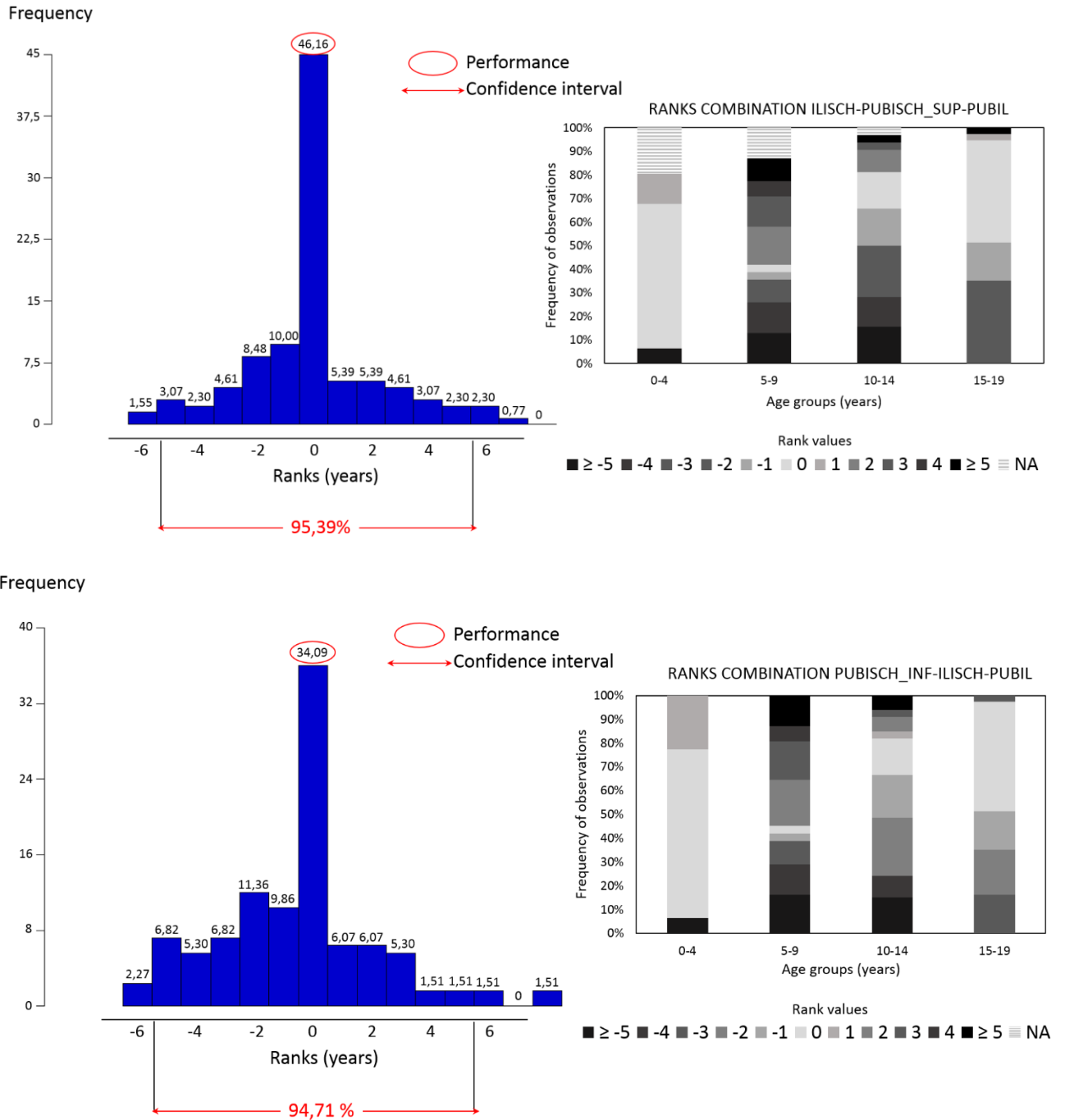


Figure 6.42 Validation ranks of age estimation using the three-digit combinations ILISCH-PUBISCH\_SUP-PUBIL and PUBISCH\_INF-ILISCH-PUBIL and their distribution according to quinquennial age groups in the Marseilles sample

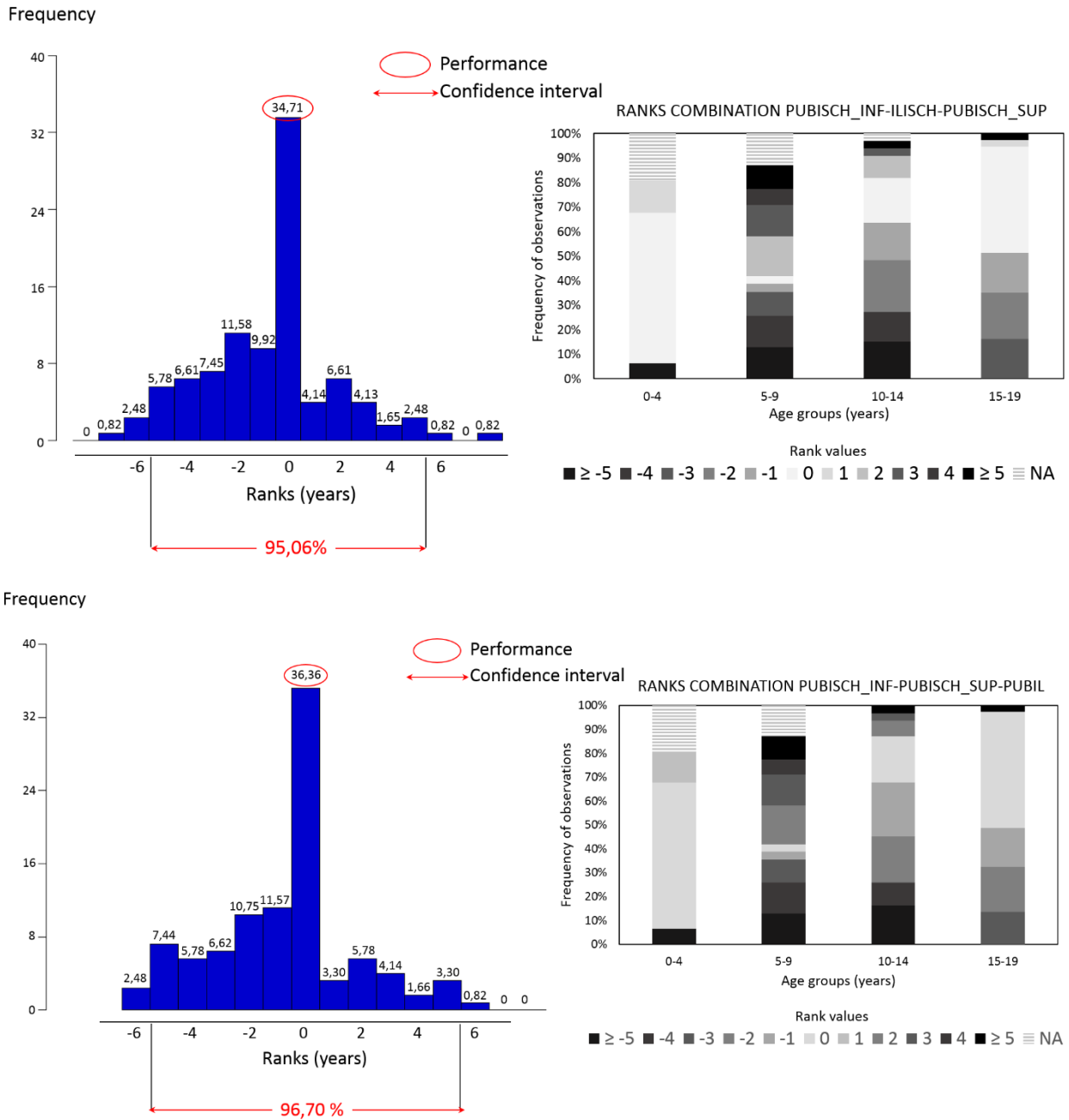


Figure 6.43 Validation ranks of age estimation using the three-digit combinations PUBISCH\_INF-ILISCH-PUBISCH\_SUP and PUBISCH\_INF-PUBISCH\_SUP-PUBIL and their distribution according to quinquennial age groups in the Marseilles sample

iii. Two-digit combinations

Posterior probabilities provided age estimates with 95% or higher confidence intervals of +/- 5 years for PUBISCH\_INF-PUBIL, PUBISCH\_INF-ILISCH and PUBISCH\_SUP-PUBIL, +/- 6 years for ILISCH-PUBISCH\_SUP and ILISCH-PUBIL and +/- 8 years for PUBISCH\_INF-PUBISCH\_SUP (Figures 6.44 and 6.45). No better precision associated to a reliability of at least 90% can be obtained using any of the two-digit combinations.

The highest performance is obtained with PUBISCH\_SUP-PUBIL (46.16%), and the lowest is obtained with PUBISCH\_INF-ILISCH (34.17 %).

The distributions of ranks are “peaked” at rank 0, with much lower frequencies for the other ranks. This is verified by the positive values of kurtosis, meaning the distributions have sharper peaks and heavier tails than a normal distribution (**Table 6-53**).

**Table 6-53 Results of the kurtosis, skewness and Kruskal-Wallis tests done on the distributions of the ranks obtained for the two-digit combination ranks on the Marseilles test sample. Greyed values correspond to significant differences of rank distributions between age groups**

Test	PUBISCH_INF-PUBIL		PUBISCH_INF-PUBISCH_SUP		PUBISCH_INF-ILISCH		PUBISCH_SUP-PUBIL		ILISCH-PUBISCH_SUP		ILISCH-PUBIL	
	H	p-value	H	p-value	H	p-value	H	p-value	H	p-value	H	p-value
Kurtosis		0.606		0.550		0.546		0.761		0.557		0.727
Skewness		0.341		-0.316		0.304		0.123		0.267		0.258
Kruskal-Wallis	10.668	0.014	11.199	0.011	9.917	0.019	30.455	<0.001	34.829	<0.001	33.625	<0.001

In this regard, it can be observed that frequencies do not continuously decrease when adding (or subtracting) a positive (or negative) rank and that they remain significant even at the extreme values.

Skewness is negative for PUBISCH\_INF-PUBISCH\_SUP, meaning the left tail is longer than the right in the rank distribution: the PUBISCH\_INF-PUBISCH\_SUP combination produces more negative rank values, meaning age is predominantly overestimated by this combination (**Figure 6.44**). Skewness is positive for the other five two-digit combinations, meaning they mostly underestimate age. This can clearly be seen in **Figures 6.44** and **6.45**, with the longer right “tails” in the distributions of ranks.

The Kruskal-Wallis test done on the rank distribution of the four quinquennial age groups for each site showed that the distributions were significantly different between the age groups for all two-digit combinations (**Table 6-53**). This is visible in **Figure 6.45**. Rank 0 is clearly dominant for age groups 0-4 for all six combinations, and for age group 15-19 with the PUBISCH\_INF-PUBIL, PUBISCH\_INF-PUBISCH\_SUP and PUBISCH\_INF-ILISCH combinations.

Negative ranks are more frequent in the 10-14 and 15-19 age groups, whereas positive ranks are predominant in the 5-9 age group for all combinations. High rank values are more often found in the 10-14 age group for all combinations, and in the 5-9 age group of the combinations using PUBISCH\_INF as one of the maturation sites.

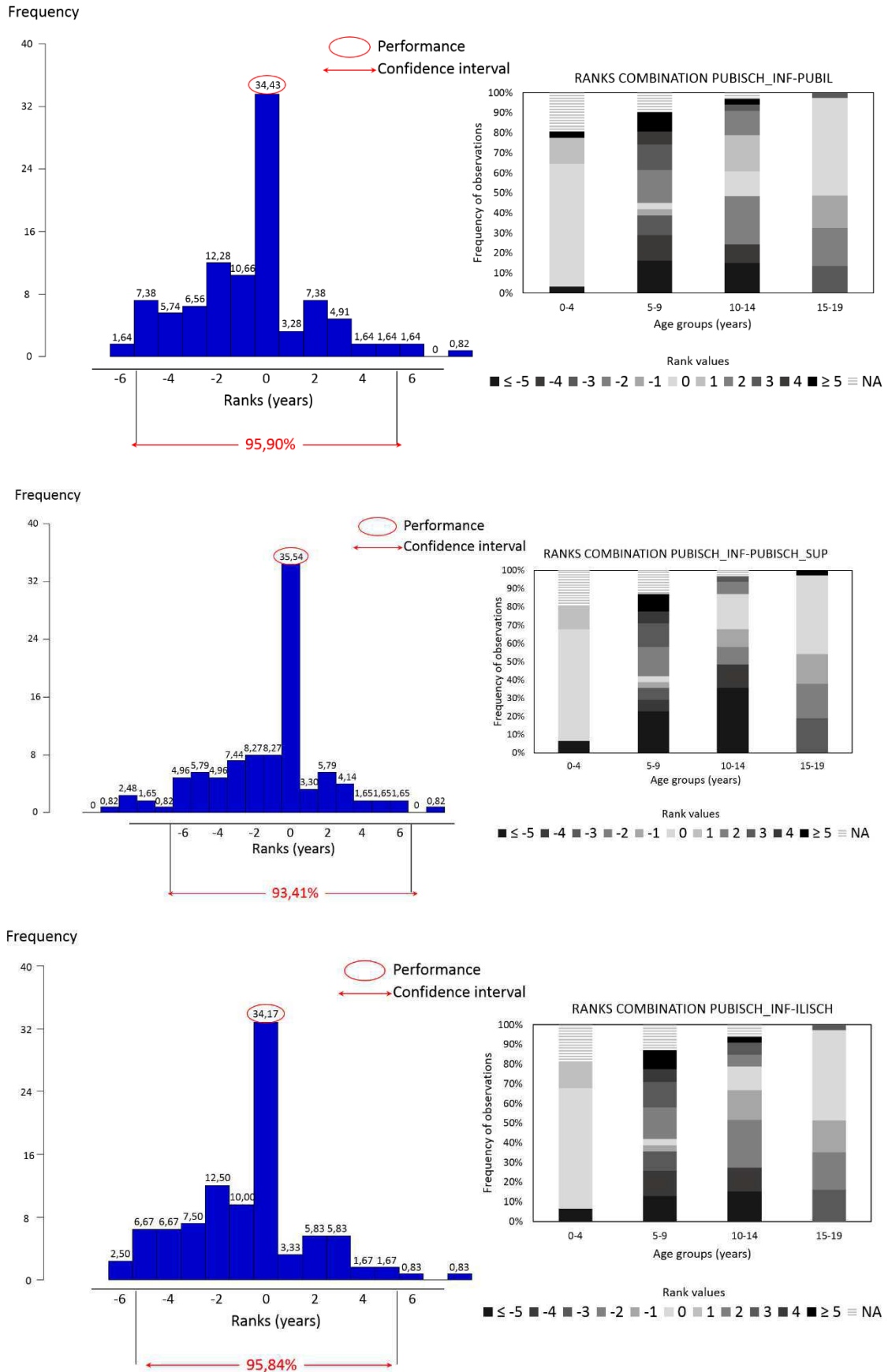


Figure 6.44 Validation ranks of age estimation using the two-digit combinations PUBISCH\_INF-PUBIL, PUBISCH\_INF-PUBISCH\_SUP and PUBISCH\_INF-ILISCH and their distribution according to quinquennial age groups in the Marseilles sample



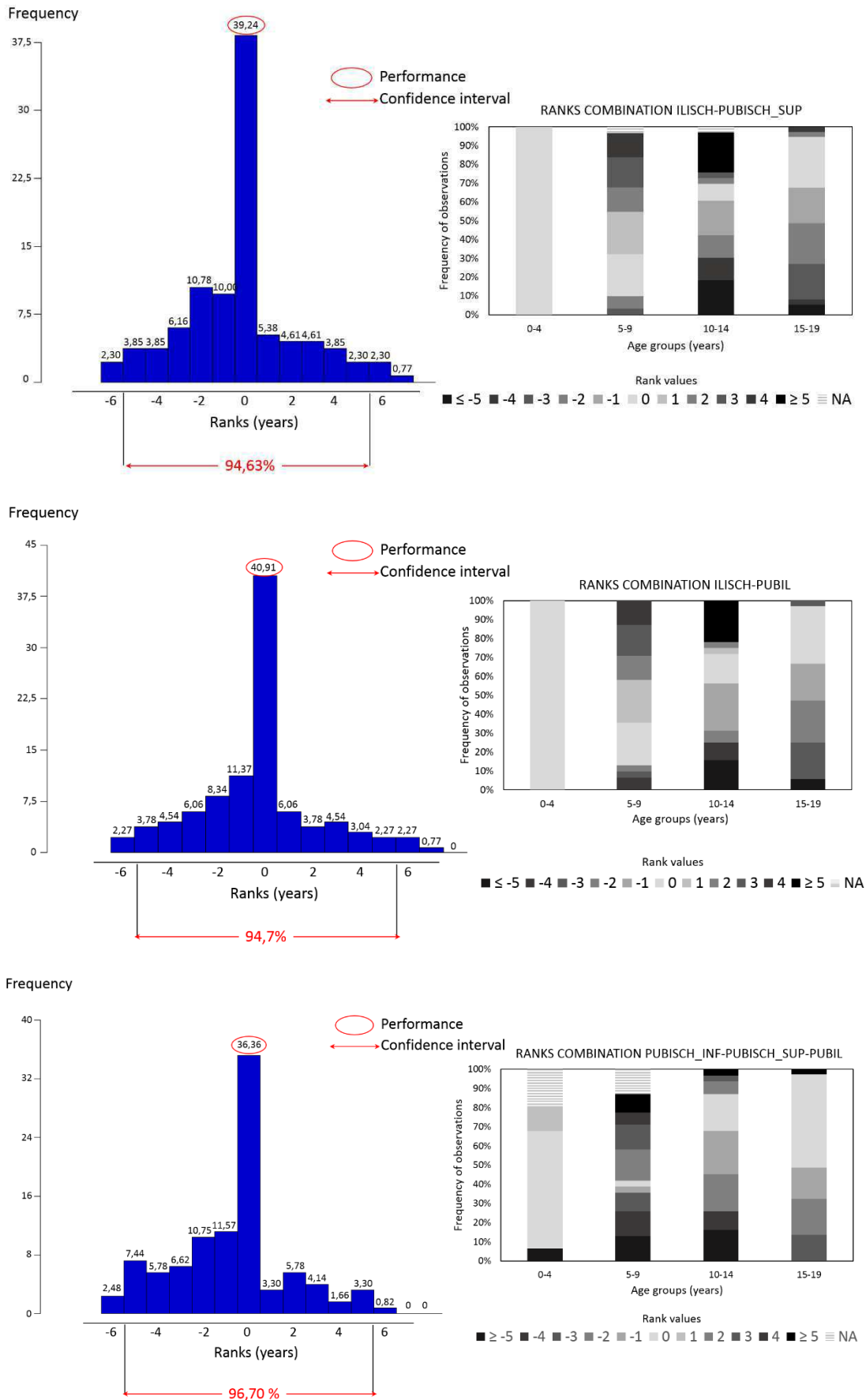


Figure 6.45 Validation ranks of age estimation using the two-digit combinations ILISCH-PUBISCH\_SUP, ILISCH-PUBIL and PUBISCH\_SUP-PUBIL and their distribution according to quinquennial age groups in the Marseilles sample

Independent Bayesian probabilities using two-digit combinations have the tendency to underestimate age for individuals aged 5 to 9 years and overestimate it for individuals between 10 and 19 years of age. The proportions of high ranks are too important in the age group 10-14 for all two-digit combinations, which means they cannot be applied to individuals from this age group to provide precise estimates. The PUBISCH\_INF-PUBISCH\_SUP, PUBISCH\_INF-ILISCH and PUBISCH\_INF-PUBIL combinations also tend to provide high rank values for the 5-9 age group. Their application for age estimation implies a risk for low precision of the estimates.

**b. Independent test sample from Lisbon (Luis Lopes collection)**

i. Four-digit combination

When applied on the independent test sample from the Luis Lopes collection, the posterior probabilities calculated on the Marseilles training sample provided age estimates with a 96.11% confidence interval of +/- 6 years with the four-digit combination (Figure 6.46).

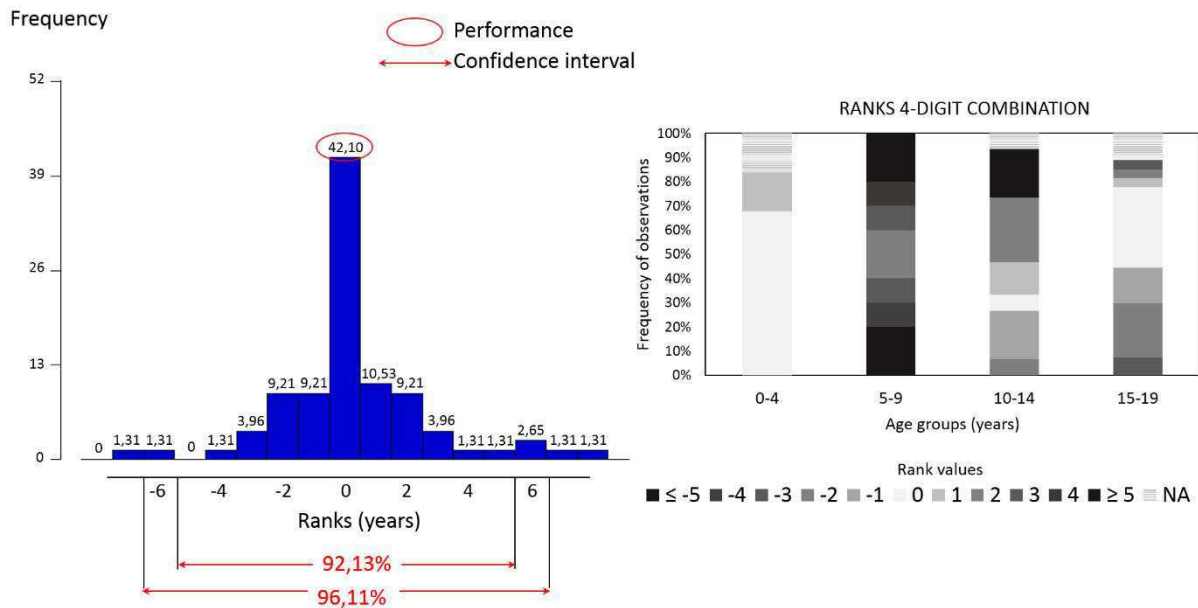


Figure 6.46 Validation ranks of age estimation using the four-digit combination of maturation stages and their distribution according to quinquennial age groups in the Luis Lopes sample

Reliability lower than 95% leads to better precision. The best precision associated to a reliability of 90.82% can be obtained, with a +/- 4 years precision.

The predictive power of the method is 42.10%. The distributions of ranks are “peaked” at rank 0, with much lower frequencies for the other ranks. This is confirmed by the high kurtosis value (**Table 6-54**), illustrating the higher peak and larger tail values of the rank distribution.

Skewness is slightly significant for the rank distribution: it is positive, meaning age in the Luis Lopes sample is mostly underestimated by the four-digit combination. Indeed, the right tail of the rank distribution is slightly longer than the left (**Figure 6.46**).

**Table 6-54 Results of the kurtosis, skewness and Kruskal-Wallis tests done on the distributions of the ranks obtained for the four-digit combination ranks on the Luis Lopes test sample. Greyed values correspond to significant differences of rank distributions between age groups**

Test	4-digit combination	
Kurtosis	2.307	
Skewness	0.462	
Kruskal-Wallis	H	p-value
	11.683	0.009

The Kruskal-Wallis test done on the rank distribution of the four quinquennial age groups showed that the distributions were significantly different between the age groups. This is also visible in **Figure 6.46**. Rank 0 is clearly dominant for age group 0-4, whereas positive ranks are predominant in age groups 5-9 and 10-14, and age group 15-19 mainly presents negative rank values. Age group 5-9 has the highest proportion of high rank values ( $\geq |5|$ ), so age is less precisely estimated in this age group using the four-digit combination.

Independent Bayesian probabilities using the four-digit combination tend to underestimate age for individuals aged 5 to 14 years and overestimate it for individuals in the 15-19 age group. This method is inappropriate for young individuals (5-9 age group), as the proportion of highly over- or underestimated ages is too important. It can be applied to the other three age groups (0.4, 10-14 and 15-19), keeping in mind that precision is at best +/- 4 years.

ii. Three-digit combinations

Posterior probabilities using three-digit combinations provided age estimates with 95% or higher confidence intervals of +/- 6 years for PUBISCH\_INF-ILISCH-PUBIL, PUBISCH\_INF-ILISCH-PUBISCH\_SUP and PUBISCH\_INF-PUBISCH\_SUP-PUBIL and +/- 7 years for ILISCH-PUBISCH\_SUP-PUBIL (**Figures 6.47 and 6.48**). The best precision associated to a reliability of at least 90% can only be obtained using the three-digit combinations PUBISCH\_INF-ILISCH-PUBISCH\_SUP, PUBISCH\_INF-PUBISCH\_SUP-PUBIL and PUBISCH\_INF-ILISCH-PUBIL, with a +/- 4 years precision and a 92.20% reliability.

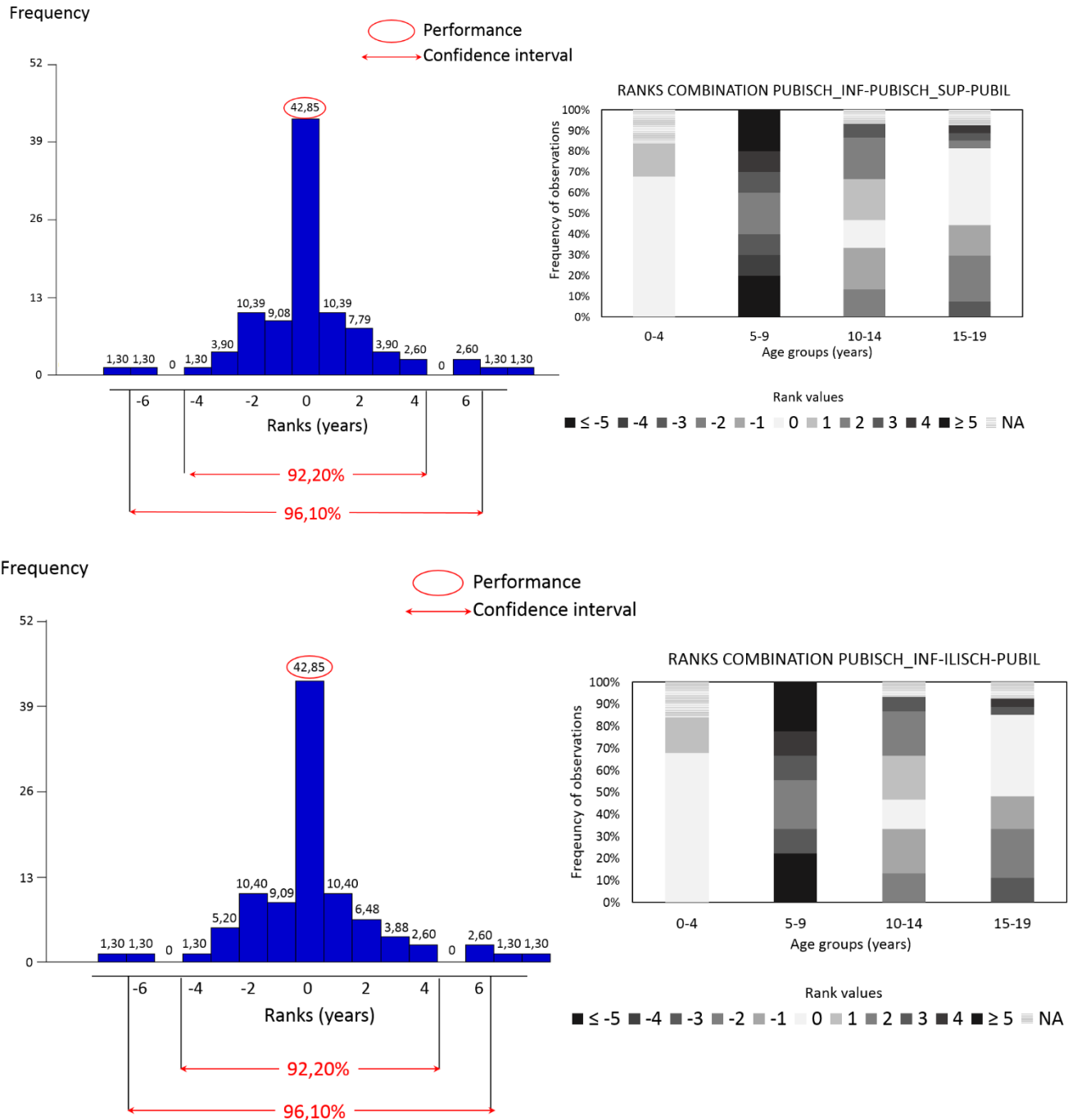


Figure 6.47 Validation ranks of age estimation using the three-digit combinations ILISCH-PUBISCH\_SUP-PUBIL and PUBISCH\_INF-ILISCH-PUBIL and their distribution according to quinquennial age groups in the Luis Lopes sample

The performance is between 42.85% (PUBISCH\_INF-ILISCH-PUBIL and PUBISCH\_INF-PUBISCH\_SUP-PUBIL) and 50% (ILISCH-PUBISCH\_SUP-PUBIL) for all four combinations.

The distributions of ranks are “peaked” at rank 0, with much lower frequencies for the other ranks that extend to high absolute rank values (+/- 6 to +/- 9).

This is confirmed by the kurtosis values that are higher than 2 for three combinations (Table 6-55).

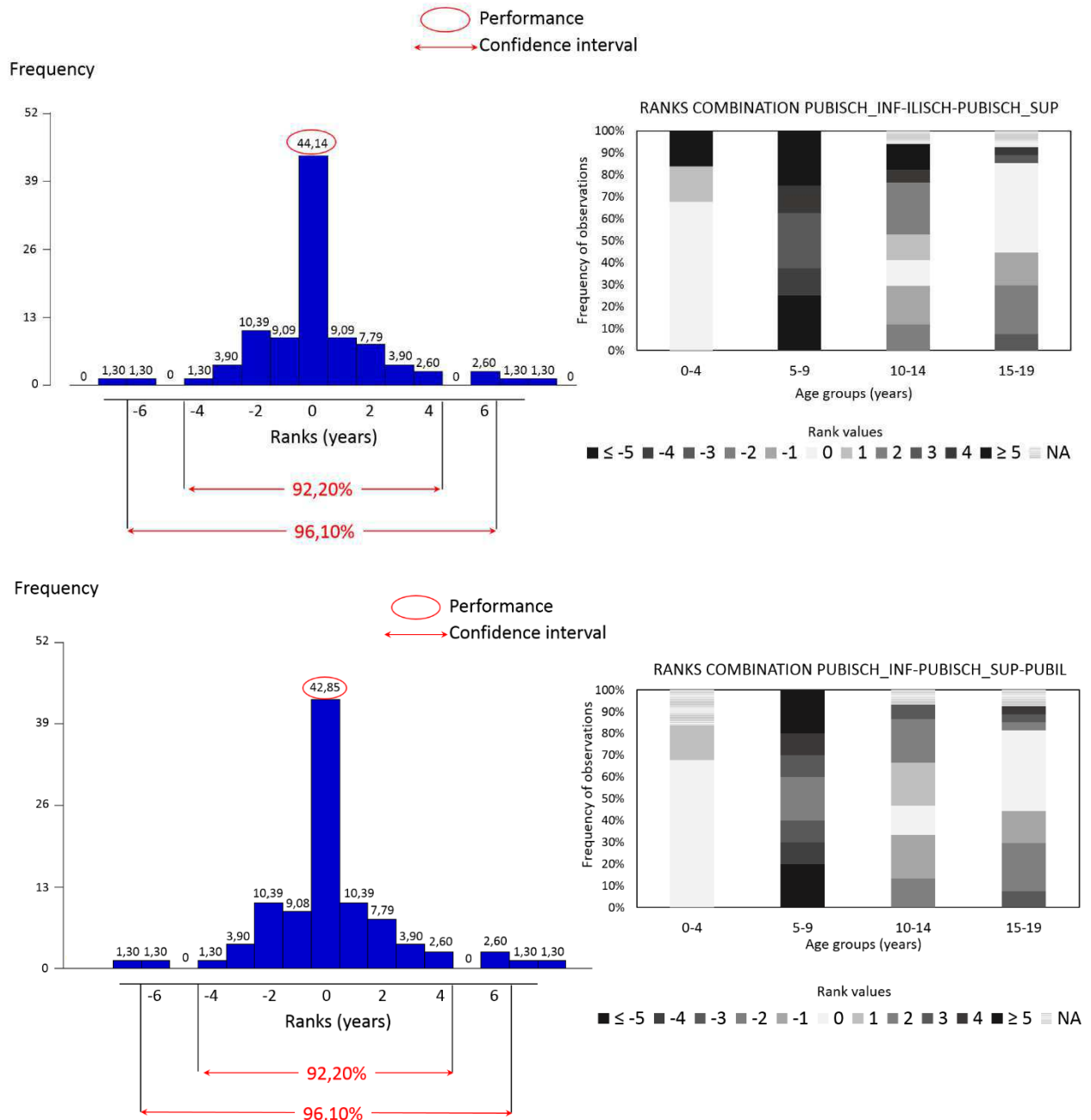


Figure 6.48 Validation ranks of age estimation using the three-digit combinations PUBISCH\_INF-ILISCH-PUBISCH\_SUP and PUBISCH\_INF-PUBISCH\_SUP-PUBIL and their distribution according to quinquennial age groups in the Luis Lopes sample

ILISCH-PUBISCH\_SUP-PUBIL however, does not present a very high kurtosis value.

Skewness is slightly significant for the rank distributions of PUBISCH\_INF-PUBISCH\_SUP-PUBIL, PUBISCH\_INF-ILISCH-PUBISCH\_SUP, and PUBISCH\_INF-ILISCH-PUBIL. It is more significant for the ILISCH-PUBISCH\_SUP-PUBIL combination.

All skewness values are positive, meaning the right tails are longer than the left (Figures 6.47 and 6.48) and age is mostly underestimated by the three-digit combinations.

**Table 6-55 Results of the kurtosis, skewness and Kruskal-Wallis tests done on the distributions of the ranks obtained for the three-digit combination ranks on the Luis Lopes test sample. Greyed values correspond to significant differences of rank distributions between age groups**

Test	PUBISCH_INF- PUBISCH_SUP-PUBIL		PUBISCH_INF-ILISCH - PUBISCH_SUP		PUBISCH_INF- ILISCH- PUBIL		ILISCH- PUBISCH_SUP- PUBIL	
	H	p-value	H	p-value	H	p-value	H	p-value
Kurtosis		2.476		2.503		2.404		0.476
Skewness		0.494		0.510		0.528		1.171
Kruskal- Wallis	8.027	0.045	9.559	0.023	10.620	0.014	42.348	<0.001

The Kruskal-Wallis test done on the rank distribution for each combination showed that distributions were significantly different between the quinquennial age groups for all combinations (**Table 6-55**).

This is visible in **Figures 6.47 and 6.48**. Rank 0 is clearly dominant for age group 0-4 and for age group 15-19 for all four combinations.

Positive ranks are clearly dominant in age groups 5-9 and 10-14 for ILISCH-PUBISCH\_SUP-PUBIL. Negative ranks are more frequent than positive ranks in the age group 15-19 for all combinations. ILISCH-PUBISCH\_SUP-PUBIL shows a very high proportion of ranks with values higher than 5 in the 10-14 age group (**Figure 6.47**). PUBISCH\_INF-ILISCH-PUBIL, PUBISCH\_INF-ILISCH-PUBISCH\_SUP and PUBISCH\_INF-PUBISCH\_SUP-PUBIL present high proportions of ranks with absolute values superior to 5 in age group 5-9, meaning the estimates are either higher or lower by at least five years than real age. This is also the case for the 10-14 age group when using the ILISCH-PUBISCH\_SUP-PUBIL combination.

Independent Bayesian probabilities using three-digit combinations tend to overestimate age for individuals in the 15-19 age group and underestimate age in the 10-14 group. The ILISCH-PUBISCH\_SUP-PUBIL combination is not suited for age estimation in the 10-14 age group; PUBISCH\_INF-ILISCH-PUBISCH\_SUP and PUBISCH\_INF-PUBISCH\_SUP-PUBIL are not suited for estimating the age of individuals aged 5 to 9 years. The precision is much lower when using these combinations for estimating age of individuals from these age groups.

iii. Two-digit combinations

The posterior probabilities calculated with two-digit combinations provided age estimates with 95% or higher confidence intervals of +/- 8 years for PUBISCH\_INF-PUBISCH\_SUP, +/- 7 years for ILISCH-PUBISCH\_SUP, ILISCH-PUBIL and PUBISCH\_SUP-PUBIL, and +/- 6 years for PUBISCH\_INF-PUBIL and PUBISCH\_INF-ILISCH (**Figures 6.49 and 6.50**).

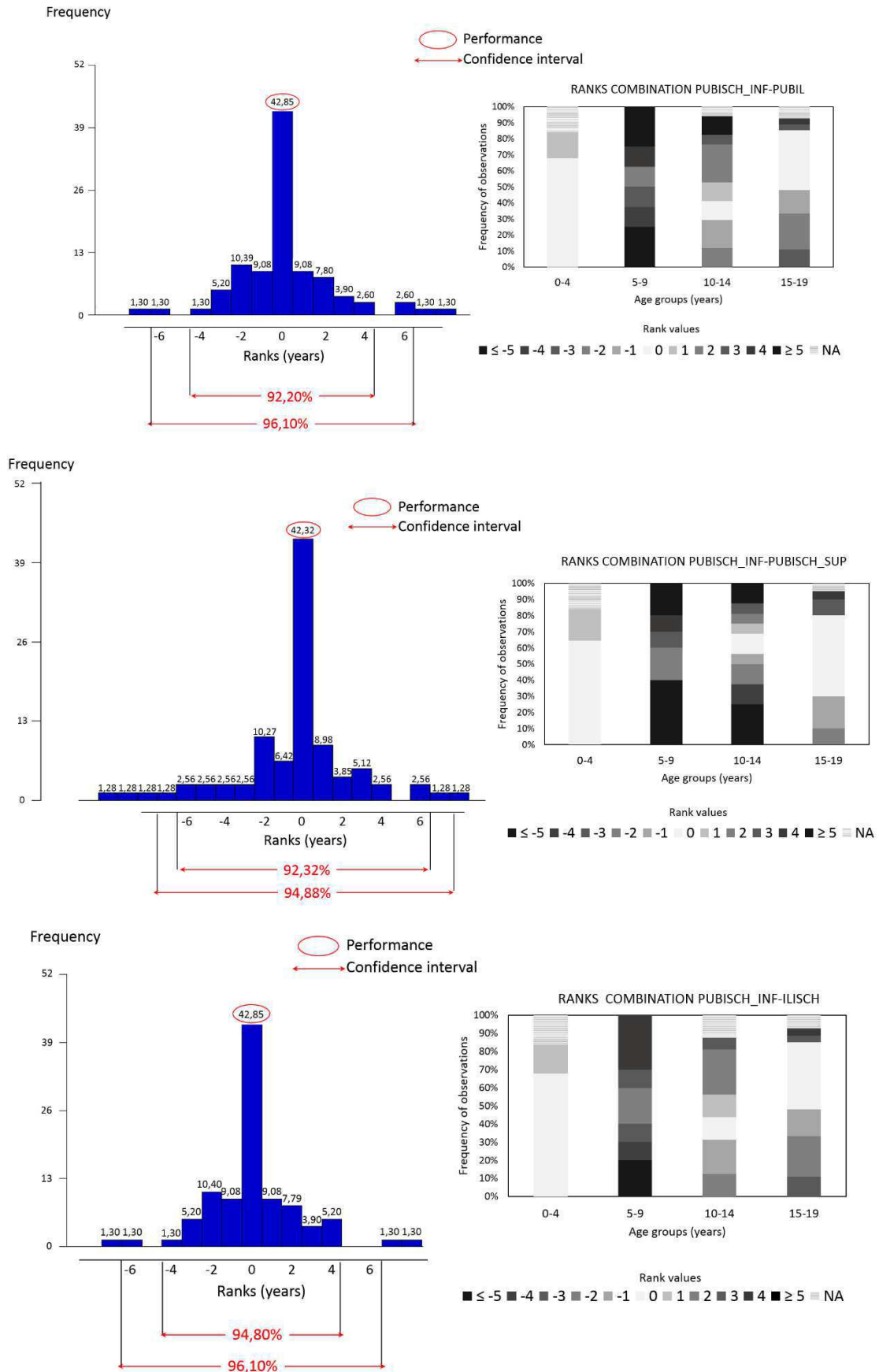


Figure 6.49 Validation ranks of age estimation using the two-digit combinations PUBISCH\_INF-PUBIL, PUBISCH\_INF-PUBISCH\_SUP and PUBISCH\_INF-ILISCH and their distribution according to quinquennial age groups in the Luis Lopes sample

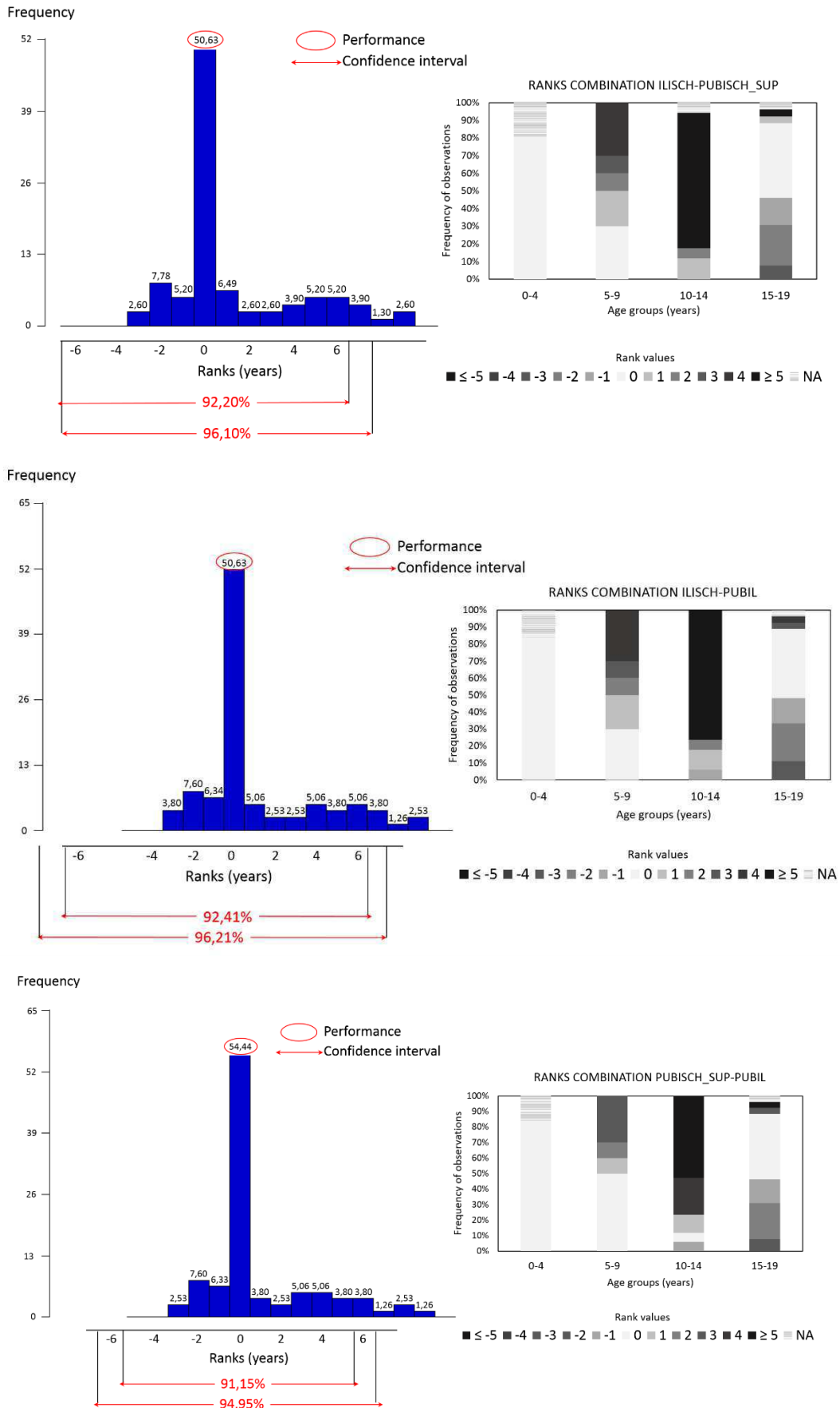


Figure 6.50 Validation ranks of age estimation using the two-digit combinations ILISCH -PUBISCH\_SUP, ILISCH-PUBIL and PUBISCH\_SUP-PUBIL and their distribution according to quinquennial age groups in the Luis Lopes sample



Reliability lower than 95% leads to better precision. Better precision associated to a reliability between 90 and 95% can be obtained using the combinations PUBISCH\_INF-PUBIL (92.20% reliability and +/- 4 years precision), PUBISCH\_INF-ILISCH (94.80% reliability and +/- 4 years precision). The other four combinations provide a reliability lower than 90% and/or lower precision.

The highest performance is obtained with PUBISCH\_SUP-PUBIL (54.44%), and the lowest is obtained with PUBISCH\_INF-PUBISCH\_SUP (42.32 %).

The distributions of ranks are all “peaked” at rank 0, with much lower frequencies for the other ranks. This is confirmed by the kurtosis values that are higher than 1 for four combinations, especially for PUBISCH\_INF-PUBIL and PUBISCH\_INF-ILISCH (**Table 6-56**).

ILISCH-PUBISCH\_SUP and ILISCH-PUBIL however, do not present very high kurtosis values. All kurtosis values are positive, meaning the peak is significantly higher and the tails are significantly larger than they would be for a normal distribution.

**Table 6-56 Results of the kurtosis, skewness and Kruskal-Wallis tests done on the distributions of the ranks obtained for the two-digit combination ranks on the Luis Lopes test sample. Greyed values correspond to significant differences of rank distributions between age groups**

Test	PUBISCH_INF-PUBIL		PUBISCH_INF-PUBISCH_SUP		PUBISCH_INF-ILISCH		PUBISCH_SUP-PUBIL		ILISCH-PUBISCH_SUP		ILISCH-PUBIL	
	H	p-value	H	p-value	H	p-value	H	p-value	H	p-value	H	p-value
Kurtosis	2.332		1.733		2.550		1.180		0.407		0.520	
Skewness	0.512		-0.440		0.351		1.326		1.157		1.172	
Kruskal-Wallis	10.685	0.013	5.832	0.120	10.618	0.014	38.432	<0.001	50.467	<0.001	48.280	<0.001

Skewness is positive and significant for the rank distributions of PUBISCH\_SUP-PUBIL, ILISCH-PUBISCH\_SUP and ILISCH-PUBIL: the right tails are longer than the left, and the rank distribution is asymmetric. It is less significant for the other three combinations: it is positive for PUBISCH\_INF-PUBIL and PUBISCH\_INF-ILISCH, but it is negative for PUBISCH\_INF-PUBISCH\_SUP. This means age is mostly underestimated by the PUBISCH\_SUP-PUBIL, ILISCH-PUBISCH\_SUP, ILISCH-PUBIL, PUBISCH\_INF-PUBIL and PUBISCH\_INF-ILISCH combinations, whereas it is mostly overestimated by the PUBISCH\_INF-PUBISCH\_SUP combination. This can clearly be observed in **Figures 6.49 and 6.50**.

The Kruskal-Wallis test done on the rank distribution of the four quinquennial age groups for each two-digit combination showed that the distributions were significantly different between the age groups for PUBISCH\_INF-PUBIL and PUBISCH\_INF-ILISCH, PUBISCH\_SUP-PUBIL, ILISCH-PUBISCH\_SUP

and ILISCH-PUBIL. However, they are not significantly different for the PUBISCH\_INF-PUBISCH\_SUP combination. This is also visible in **Figures 6.49 and 6.50**.

Rank 0 is clearly dominant for age group [0-4] for all six combinations. Rank 0 is also the most represented rank in age group [15-19] for all combinations.

The three combinations using PUBISCH\_INF as one of the two sites lead to high absolute values of ranks for age groups [5-9]. Negative ranks are predominant in the [15-19] age group; positive ranks are more frequently obtained for the [10-14] age group using PUBISCH\_INF-ILISCH and PUBISCH\_INF-PUBIL combinations.

High positive rank values are also obtained in the [10-14] age group with the PUBISCH\_SUP-PUBIL, ILISCH-PUBISCH\_SUP and ILISCH-PUBIL combinations. These three combinations also present a majority of positive ranks for the [5-9] age group and a majority of negative rank values for the [15-19] age group.

Independent Bayesian probabilities using two-digit combinations tend to highly underestimate age for individuals aged 0 to 14 years when PUBISCH\_INF is not one of the sites included in the combination and overestimate it for individuals in the [15-19] age group. The three combinations including PUBISCH\_INF follow the same tendency for age groups [0-4] and [15-19] as the other three combinations. However, high ranks are more frequent in the [5-9] age group and age is neither predominantly over- or under-estimated. PUBISCH\_INF-PUBIL and PUBISCH\_INF-ILISCH have a tendency to underestimate age in the [10-14] age group, but PUBISCH\_INF-PUBISCH\_SUP shows a higher rate of overestimation for that age group.

Overall, two-digit combinations of maturation stages can be useful for age estimation of young (0 to 4 years) and older (15 to 19 years) juveniles. High errors are more frequent in the [5-9] and [10-14] age groups and precision may be insufficient for younger individuals.

### **6.2.3. Influence of sex on age estimation using posterior probabilities**

Because sexual dimorphism had been observed on maturation stages, the influence of sex was tested on the ranks obtained for both test samples with a Kruskal-Wallis test.

#### **a. Independent test sample from Marseilles**

The Kruskal-Wallis tests done on uni-site and four-digit combination rank distributions of the Marseilles test sample show significant differences for the ranks obtained with ILISCH and PUBIL as

age predictors according to sex. Differences are particularly significant for PUBIL, and particularly insignificant for PUBISCH\_INF (**Table 6.57**).

**Table 6-57 Results of the Kruskal-Wallis tests done on the distributions of ranks on the Marseilles test sample using uni-site and four-digit combination of maturation stages. Greyed values correspond to significant differences of rank distribution between sexes**

Site/combination	Kruskal-Wallis test	
	H	p-value
PUBISCH_INF	0.011	0.915
PUBISCH_SUP	2.316	0.128
ILISCH	4.166	0.041
PUBIL	6.782	0.009
4-digit combination	0.453	0.501

Female individuals are more frequently located in the negative ranks, meaning age is mostly overestimated for female individuals using these uni-site posterior probabilities (**Figure 6.51**).

Differences of rank distributions between sexes were also found for the ILISCH-PUBISCH\_SUP-PUBIL three-digit combination and the PUBISCH\_SUP-PUBIL and PUBISCH\_SUP-ILISCH two-digit combinations (**Table 6.58**).

**Table 6-58 Results of the Kruskal-Wallis tests done on the distributions of ranks on the Marseilles test sample using two- and three-digit combination of maturation stages. Greyed values correspond to significant differences of rank distribution between sexes**

Combination	Kruskal-Wallis test	
	H	p-value
PUBISCH_INF-PUBISCH_SUP-PUBIL	0.871	0.351
PUBISCH_INF-PUBISCH_SUP-ILISCH	0.297	0.585
PUBISCH_INF-PUBIL-ILISCH	1.497	0.221
ILISCH-PUBISCH_SUP-PUBIL	7.757	0.005
PUBISCH_INF-PUBIL	1.114	0.291
PUBISCH_INF-PUBISCH_SUP	0.043	0.836
PUBISCH_INF-ILISCH	1.271	0.260
PUBISCH_SUP-PUBIL	8.735	0.003
ILISCH -PUBISCH_SUP	6.816	0.009
ILISCH-PUBIL	7.035	0.008

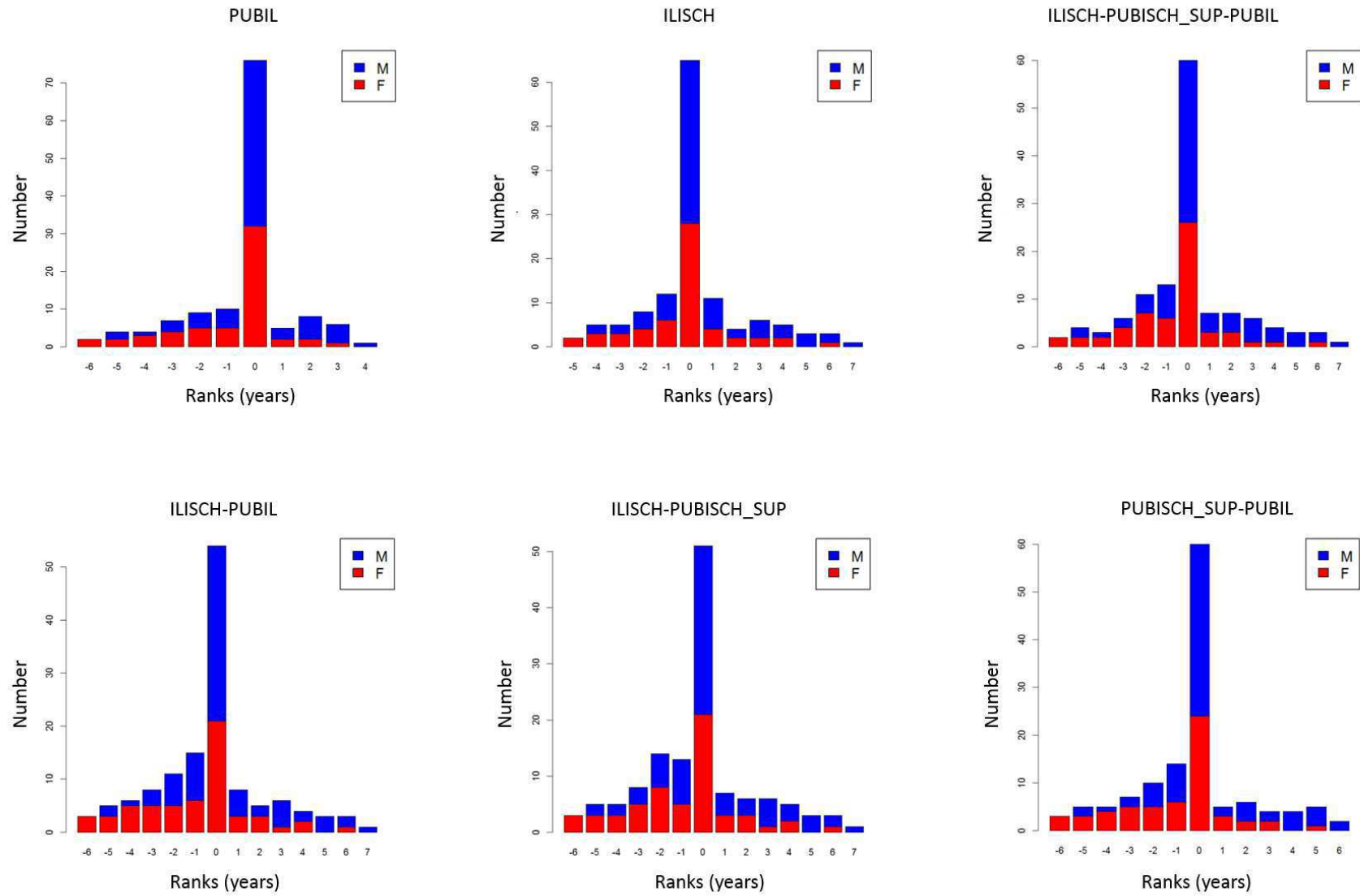


Figure 6.51 Rank distributions of sites and combinations showing significant differences according to sex in the sample from Marseilles

This is visible on the sexed rank distributions obtained for these three combinations (**Figure 6.70**). As with the other three sites/combinations, age estimates of female individuals are more often located in the negative ranks of the distributions. As previously, age is mostly overestimated by these three combinations for females, and mostly underestimated for male individuals. Only males are present in the most extreme positive rank values, and only females are present in the most extreme negative rank values.

Sexual dimorphism of iliac maturation in the Marseilles sample is expressed in these six age estimation approaches by a tendency for overestimation of female age and underestimation of male age.

**b. Independent test sample from Lisbon (Luis Lopes collection)**

The Kruskal-Wallis tests done on uni-site and four-digit combination rank distributions of the Marseilles test sample showed no significant differences in rank distribution according to sex (**Tables 6-59 and 6-60**).

**Table 6-59 Results of the Kruskal-Wallis tests done on the distributions of ranks on the Luis Lopes test sample using uni-site and four-digit combination of maturation stages**

Site/combination	Kruskal-Wallis test	
	H	p-value
PUBISCH_INF	0.430	0.512
PUBISCH_SUP	1.739	0.187
ILISCH	0.316	0.574
PUBIL	0.271	0.603
4-digit combination	0.023	0.880

**Table 6-60 Results of the Kruskal-Wallis tests done on the distributions of ranks on the Luis Lopes test sample using two- and three-digit combination of maturation stages**

Combination	Kruskal-Wallis test	
	H	p-value
PUBISCH_INF-PUBISCH_SUP-PUBIL	0.250	0.617
PUBISCH_INF-ILISCH-PUBISCH_SUP	0.474	0.491
PUBISCH_INF-PUBIL-ILISCH	0.645	0.422
ILISCH-PUBISCH_SUP-PUBIL	0.469	0.493
PUBISCH_INF-PUBIL	0.706	0.401
PUBISCH_INF-PUBISCH_SUP	1.640	0.200
PUBISCH_INF-ILISCH	0.688	0.407
PUBISCH_SUP-PUBIL	1.091	0.296
ILISCH-PUBISCH_SUP	0.586	0.444
ILISCH-PUBIL	0.234	0.628

**Conclusions on the age estimation method using non-biometric predictor variables**

- Testing the probabilistic method on two independent samples has either confirmed or infirmed its performance for age estimation
- Age estimation using posterior probabilities of age calculated from combinations of maturation stages of the iliac bone provided reliable results (higher than 90%) and prediction intervals of +/- 4 years at best on both samples from Marseilles and the Luis Lopes collection
- Depending on the number of stages used in the combinations, the method tends to overestimate or underestimate age in different age groups. High errors are more frequent in the [5-9] and [10-14] age groups
- The method tends to overestimate age for female individuals and underestimate it for male individuals in the Marseilles sample

### **6.3. Comparison with other age estimation methods**

#### **6.3.1. Age estimation using iliac biometric data: comparison with Rissech and Malgosa's method**

The univariate MARS models using IL and IW as predictor variables were compared to the age estimation method of C. Rissech and A. Malgosa using the same two variables (Rissech and Malgosa 2005). Their method was constructed on 327 individuals aged 0 to 97 years from four documented skeletal collections: 80 from the St Bride's collection (United Kingdom), 112 from the Luis Lopes collection, 99 from the collection of *Esqueletos Identificados* in Coimbra (Portugal) and 24 from the UAB collection (Spain). IL and IW were used as age predictors in parametric regression equations for the 89 juvenile individuals aged 0 to 19 years (47 females and 48 males). Unisex and sexed models were constructed. Regression equations were third degree polynomials for both IL and IW with the unisex and male regressions. The female regression equation using IL was a third degree polynomial, whereas it was a linear regression when using IW as a predictor.

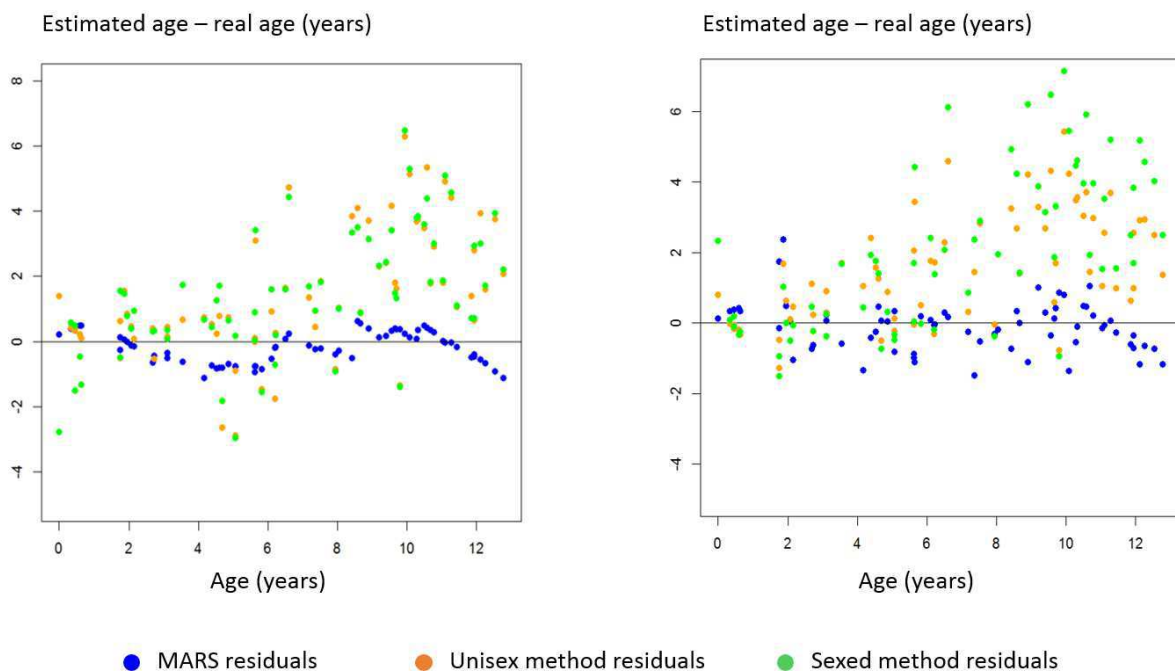
The unisex and sexed models were applied on the individuals of the Marseilles test sample and from the Luis Lopes collection. The differences between the three mean estimates (MARS, sexed and unisex) and the real age of the individuals were plotted against real age (**Figures 6.52 to 6.54**).

### a. Independent test sample from Marseilles

Estimates using IL or IW as predictors showed better results with the MARS models on the Marseilles sample: all residuals are within the  $\pm 2$  year range (**Figure 6.52**).

Residuals of the IL MARS model follow a sinusoidal pattern: age is alternatively over- and underestimated between 0-2, 2-8, 8-10.5 and 10.5-12 years. The MARS model using IW shows even better results, as the residuals are more evenly dispersed around zero and still within the  $\pm 2$  year range.

The sexed and unisex regression equations developed by C. Rissech and A. Malgosa overestimate age from 0 to 12 years. Residuals are higher for the sexed equations, and their dispersion and value increase with age to form a fan-like shape.



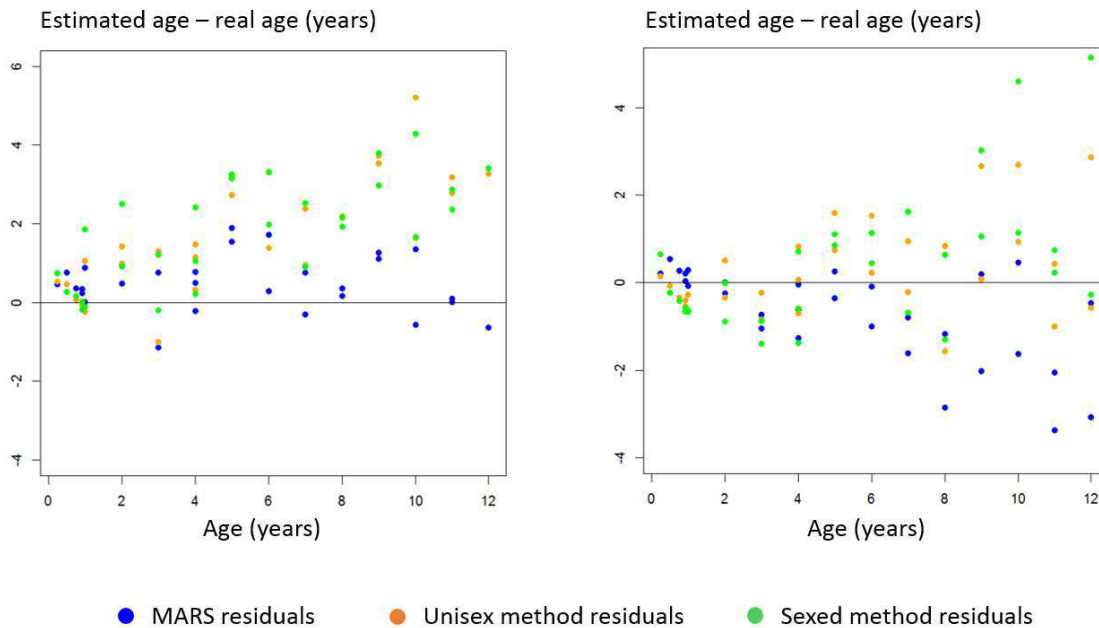
**Figure 6.52** Plots of differences between ages estimated using IL (left) and IW (right) as predictors with the MARS model and the regression equations of C. Rissech and A. Malgosa (2005) and real ages of the individuals from the Marseilles test sample

### b. Independent test sample from Toulouse

MARS models also showed better results when using IL or IW as predictors on the Toulouse sample. Residuals using IL are within the  $\pm 2$  year range, with a slight tendency to overestimate age (majority of positive residuals). Residuals using IW are distributed around 0 between 0 and 2 years and become clearly negative around 4-5 years of age, meaning the MARS model globally underestimates age between 5 and 12 years. They also have higher absolute values than the ones

with the model using IL as a predictor (**Figure 6.53**). The MARS model using IL shows better results, as the residuals are more evenly dispersed around zero and still within the +/-2 year range, even though they are mostly in the positive half of the plot.

The sexed and unisex regression equations developed by C. Rissech and A. Malgosa overestimate age from 0 to 12 years using IL and from 4 to 12 years using IW. Residuals are higher than for the MARS model, and their dispersion and value increase with age to form a fan-like shape.



**Figure 6.53** Plots of differences between ages estimated using IL (left) and IW (right) as predictors with the MARS model and the regression equations of C. Rissech and A. Malgosa (2005) and real ages of the individuals from the Toulouse test sample

**c. Independent test sample from Lisbon (Luis Lopes collection)**

Estimates using IL or IW as predictors showed comparable results with the MARS models and the equations of C. Rissech and A. Malgosa on the Luis Lopes sample: all residuals are within the +/-6 year range, although age is mostly underestimated using the MARS model and overestimated using the parametric regression equations (**Figure 6.54**).

Underestimation is particularly marked for the older individuals (between 9 and 12 years) for both MARS models but is also present for younger individuals aged 3 to 8. The parametric regressions overestimate the age of younger individuals aged between 0 and 6 years for IL, and of all individuals from 0 to 12 years for IW. The sexed and unisex regression equations developed by C. Rissech and A. Malgosa give comparable estimates using IL or IW. All three approaches show an increase of residual dispersion with age.



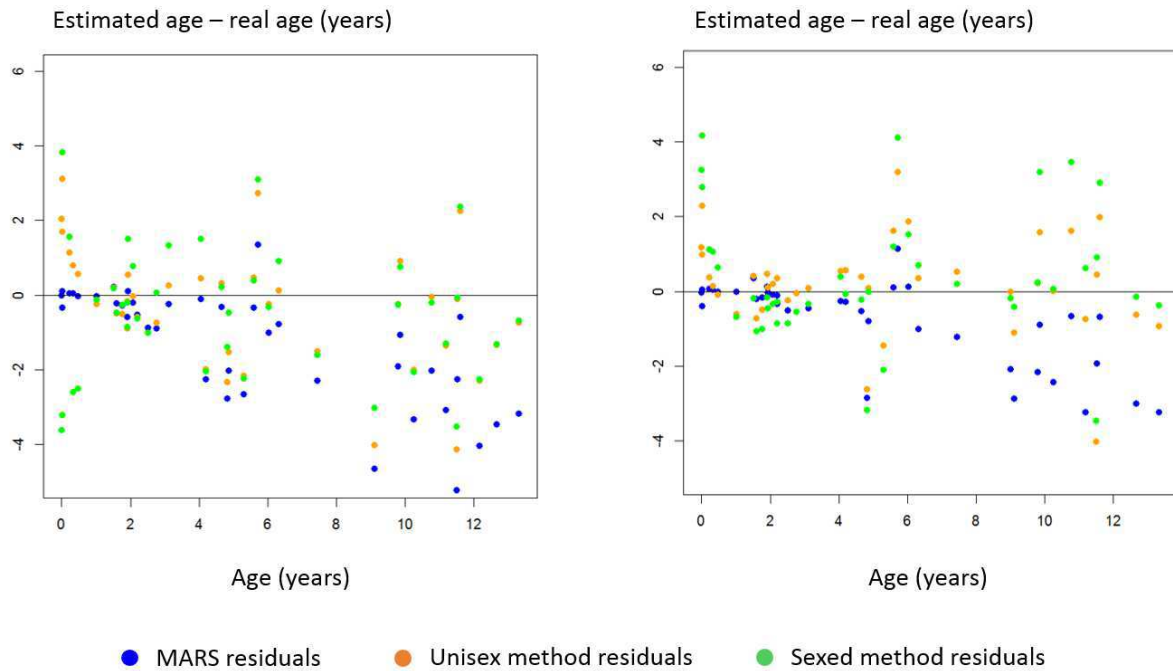


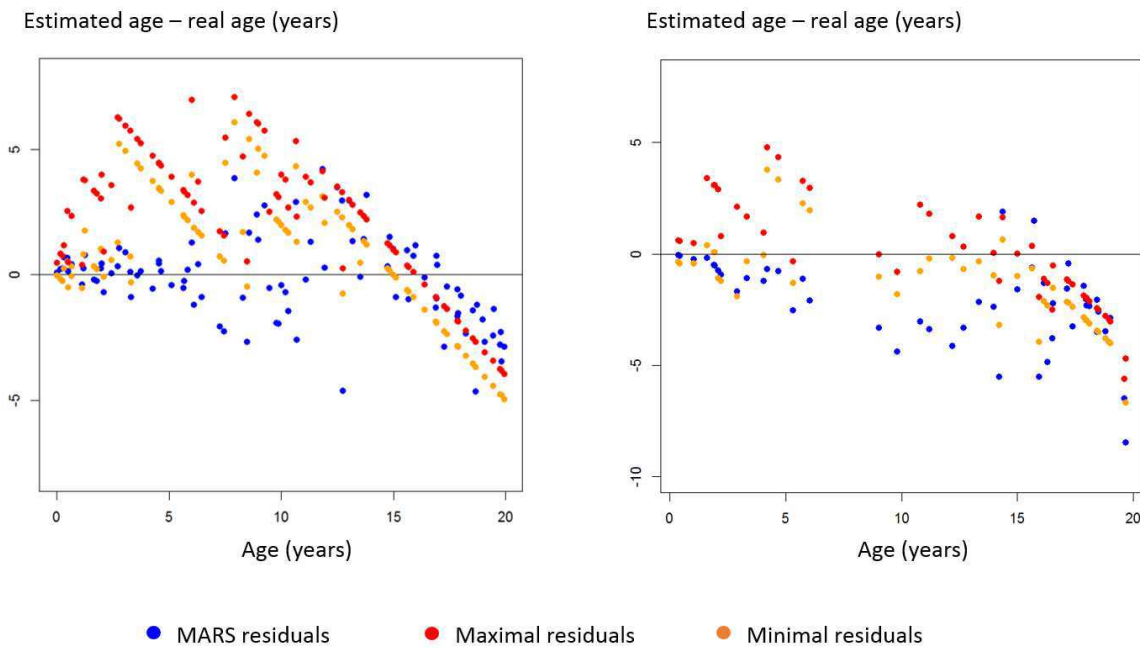
Figure 6.54 Plots of differences between ages estimated using IL (left) and IW (right) as predictors with the MARS model and the regression equations of C. Rissech and A. Malgosa (2005) and real ages of the individuals from the Luis Lopes sample

### 6.3.2. Age estimation using maximum clavicular length: comparison with Black and Scheuer's method

The results obtained with the MARS model using maximum clavicular length as a predictor of age were confronted with the ages obtained using the abacus of S. Black and L. Scheuer (Black and Scheuer 1996). This abacus was developed on 143 individual aged 0 to 30 years. Individuals originated from four documented skeletal collections: Spitalfields, St Bride's Church and St Barnabas in the United Kingdom and the Luis Lopes collection. Amongst these individuals, 103 were aged 0 to 20 years. Maximum diaphyseal length of the clavicle was measured on 87 individuals aged 0 to 16 years. The measurements are presented with mean, maximum and minimum values corresponding to semi-annual (up to 2 years) and annual age groups.

These were used for comparison with the mean MARS estimates obtained on the individuals aged 0 to 19 years in the test sample from Marseilles and the Luis Lopes sample. This was possible because the range of values in the abacus included the values of maximum clavicular length in both samples. Minimum and maximum ages were obtained for each individual in both samples using the Black and Scheuer abacus. The differences between the three estimates and the real age of the individuals were plotted against real age (Figure 6.55).

The results show that residual patterns are comparable in both samples and using both methods: age is overestimated for individuals younger than 15 years of age using the Black and Scheuer abacus in both the Marseilles and Luis Lopes samples. Differences between real and estimated ages are quite high (up to +6 and – 5 years' difference) and are the highest for the youngest and oldest individuals in both samples, especially the Marseilles sample.



**Figure 6.55** Plots of differences between ages estimated using maximum clavicular length with the MARS model and the abacus of Black and Scheuer (1996) and real ages of the individuals from the Marseilles test sample (left) and the Luis Lopes collection (right)

The MARS model tends to underestimate age for individuals aged 5 to 10 years and 16 to 19 years. Underestimation is stronger for the older individuals, especially in the Luis Lopes sample. Age is underestimated between 10 and 15 years for individuals of the Marseilles sample. Residuals have lower absolute values for MARS models and for the individuals in both samples. MARS model residuals follow the same pattern as residuals obtained with the Black and Scheuer approach, except for individuals aged 10 to 15 years. MARS model residuals have lower values, meaning age estimation is more precise.

### **6.3.3. Age estimation using iliac maturation stages: comparison with Coqueugniot and collaborators' method**

Eleven publications were found that used iliac bone maturation stages to estimate age for individuals belonging to the same age ranges as the specimens of the Marseilles and Lisbon samples.

Eight of these methods were abacuses, presenting mean, minimum and maximum ages per stage per maturation site. They were developed on dry bone collections (Cardoso 2008b; Cardoso et al 2013a; Coqueugniot and Weaver 2007; Stewart 1934) or radiographies of the iliac bone (Acheson 1957; Borovansky and Hnevkovsky 1929; Davies and Parsons 1927; Flecker 1932). These methods could be used to compare the mean, minimum and maximum ages per stage site by site, as they do not consider combinations of stages from several sites. Because of their descriptive quality, they cannot be compared to the posterior probabilities of age obtained for each site of our training sample. Two other methods cannot be used for comparison, because the stages used were defined on histological slices (Schnitzler et al 2009) or ultrasonographic images of the iliac bone (Wagner et al 1995).

The only method that uses posterior probabilities calculated from maturation stages of the iliac bone is that of H. Coqueugniot, T. Weaver and F. Houët (Coqueugniot et al 2010). It was developed on 137 individuals aged 7 to 29 years from the osteological reference collection *Esqueletos Identificados* of Coimbra. Stages of maturation were defined as follows: a=unfused, b=partially fused and c=completely fused. They are comparable to the three stages (0, 1, 2) defined for our approach. This method only allows age estimation using combinations of maturation stages observed on two to fourteen sites of the postcranial skeleton. In the same way as for our method (see section 4.8.), the posterior probabilities of age calculated using H. Coqueugniot and collaborators' method for two-, three- and four-digit combinations of iliac maturation stages were used to identify the "real annual age group" of each individual from the Marseilles test sample and the Luis Lopes sample. These real annual age groups were then used to calculate the rank of the estimation for each individual. The distribution of the ranks obtained with H. Coqueugniot and collaborators' method were then compared to the distribution of ranks obtained with our approach on both samples.

#### **a. Independent test sample from Marseilles**

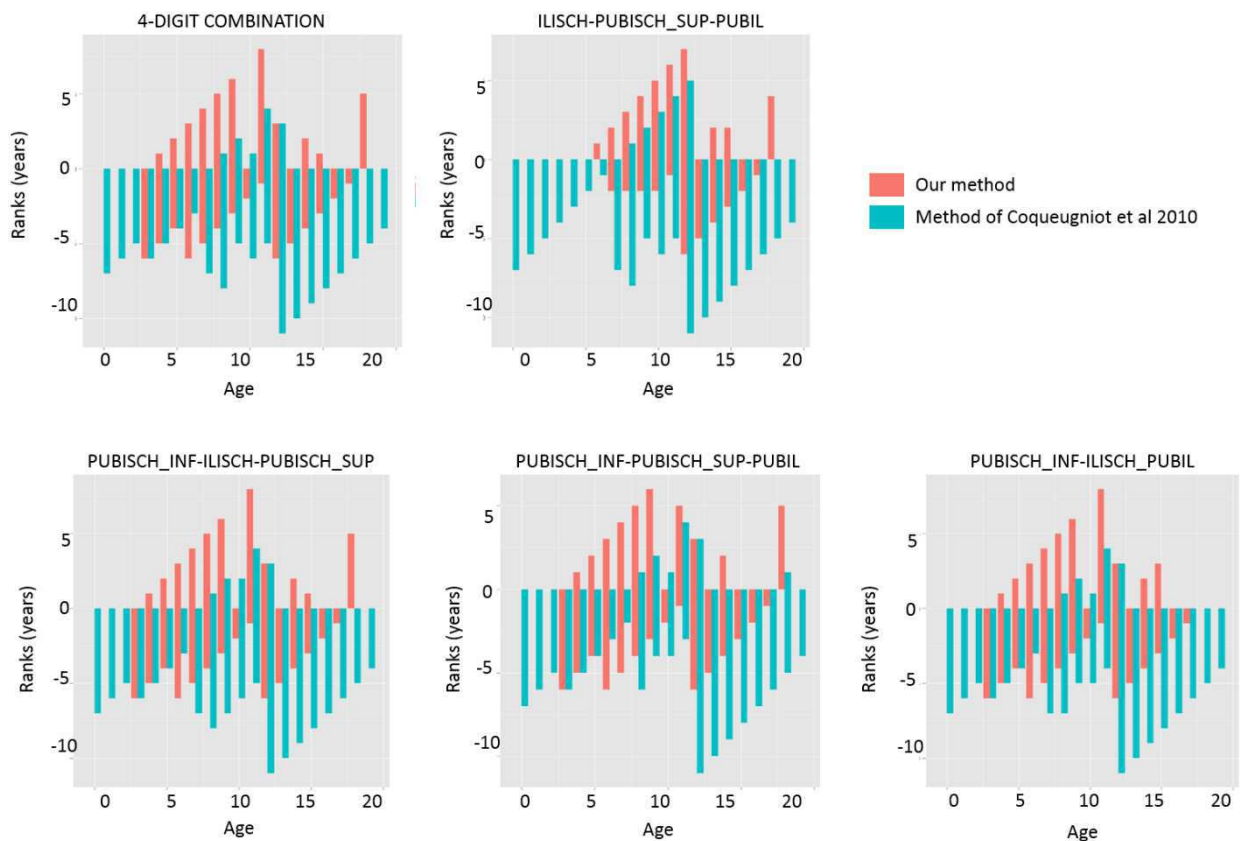
The ranges of rank values are comparable for both approaches, although the ranks obtained with H. Coqueugniot and collaborators' approach gives higher absolute rank values (up to -11) (**Figures 6.56 and 6.57**).

All four- and three-digit combinations produce ranks with similar dispersion patterns, specific to the approach used. Our approach generally estimates age correctly between 0 and 3 years. It equally overestimates and underestimates age between 3 and 11 years (except with the ILISCH-PUBISCH\_SUP-PUBIL combination for which age is mostly underestimated in that age range) and mostly overestimates age between 11 and 19 years (majority of positive rank values) (**Figure 6.56**).

Peaks of underestimation are seen around 10-11 years for all five combinations. Overestimation of age peaks at 2, 5 and/or 11 years, depending on the combination used. Overestimation is more important for combinations using PUBISCH\_INF as one of the maturation sites.

The method of H. Coqueugniot and collaborators tends to overestimate age from 0 to 19 years (majority of negative rank values) with peaks of overestimation between 0 and 1 years, 8-9 years and 11 years.

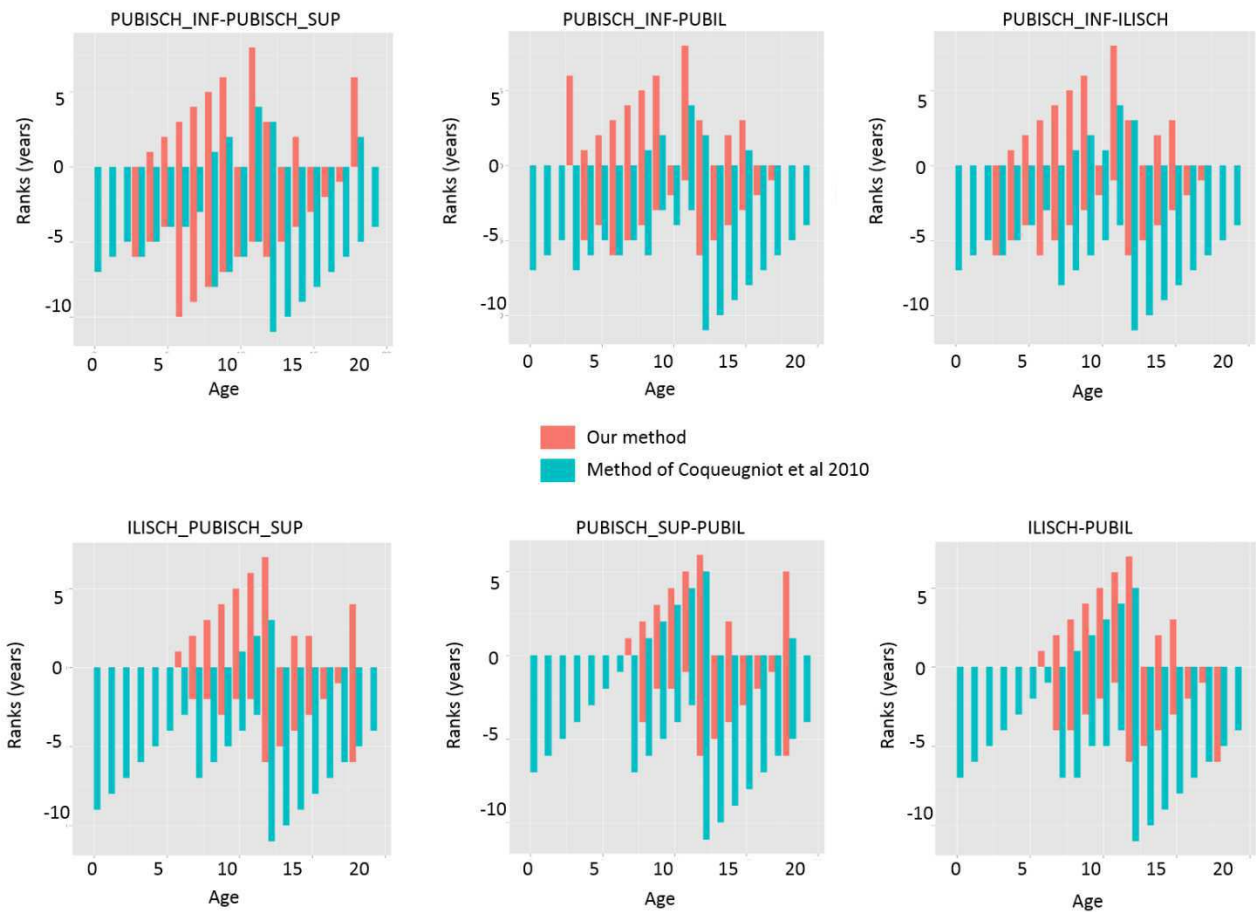
An equal rate of over- and under-estimation is observed between 5 and 11 years for the ILISCH-PUBISCH\_SUP-PUBIL combination. After the peaks, rank values decrease regularly with a rate of one year per added year of age. Overestimation is particularly important at the younger ages (0 to 7 years) and between 11 and 16 years.



**Figure 6.56** Comparison of the rank distributions of the individuals from the Marseilles sample using our approach and the method of H. Coqueugniot and collaborators on four- and three-digit combinations of iliac maturation stages

The two-digit combination ranks also follow different patterns depending on the approach used (**Figure 6.57**). The two-digit combinations using PUBISCH\_INF as one of the sites present a relatively similar pattern: age is equally over- and under-estimated for individuals aged less than 9 to 10 years, with one or two peaks between 0 and 5 years, whereas age is mostly overestimated after 11-12

years. The peak corresponds to an age of 12 years and ranks decrease until 19 years, except for a positive peak at 18 years for the PUBISCH\_INF-PUBISCH\_SUP combination.



**Figure 6.57 Comparison of the rank distributions of the individuals from the Marseilles sample using our approach and the method of H. Coqueugniot and collaborators on two-digit combinations of iliac maturation stages**

The combinations not using PUBISCH\_INF as one of the predictors also show a similar pattern: age is mostly underestimated between 5 and 11 years and mostly overestimated between 12 and 19 years, excepting an even distribution of ranks at 18 years with the ILISCH-PUBISCH\_SUP and PUBISCH\_SUP-PUBIL combinations and two positive peaks at 13 and 14 years with the ILISCH-PUBISCH\_SUP and ILISCH\_PUBIL combinations. Once again, the approach of H. Coqueugniot and collaborators shows a comparable pattern for all two-digit combinations. This approach mostly overestimates age from 0 to 19 years, with peaks of negative rank values around 0 and 12 years for combinations using PUBISCH\_INF as one of the maturation sites, and an additional peak around 7 years for the other three that do not. PUBISCH\_SUP-PUBIL and ILISCH-PUBIL also present a positive peak around 11 years, followed by the 12-year negative peak. After each peak, the absolute value of ranks decreases until the following peak.

Underestimation is always more important and extended to a larger number of age groups with our approach, whereas overestimation is inferior to underestimation by the approach of H. Coqueugniot and collaborators and concerns less age groups.

**b. Independent test sample from Lisbon (Luis Lopes collection)**

The Luis Lopes sample showed more heterogeneous patterns of rank distributions for the four- and three-digit combinations when our approach was used (Figure 6.58).

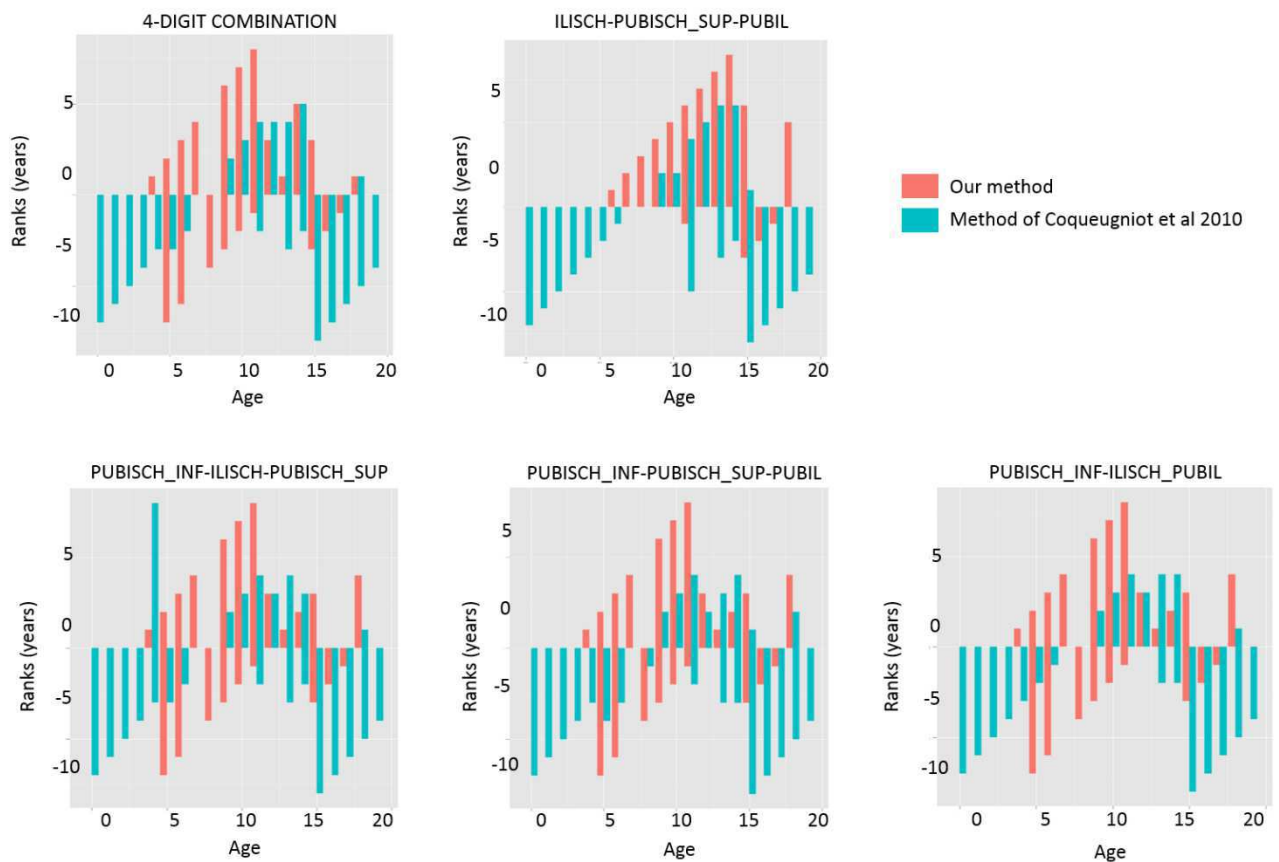


Figure 6.58 Comparison of the rank distributions of the individuals from the Luis Lopes sample using our approach and the method of H. Coqueugniot and collaborators on four- and three-digit combinations of iliac maturation stages

All combinations have a mean rank of zero for individuals aged 0 to 3 years and 19 years. The four-digit combination tends to underestimate age between 3 and 12 years with a peak value at 10 years, although important overestimation is also observed at 4 and 5 years. It is less important between 6 and 12 years and even less important between 15 and 17 years.

The three-digit combination ILISCH-PUBISCH\_SUP-PUBIL increasingly underestimates age between 5 and 14 years, and rank values show a positive peak at 18 years. The other three-digit combinations

using PUBISCH\_INF as one of their sites show almost exactly the same pattern: age is highly overestimated between 5 and 7 years as assessed by two important negative peaks, and slightly overestimated between 7 and 10 years and 13 to 15 years, whereas it is mostly and increasingly underestimated from 4 to 11 years and again at 18 years.

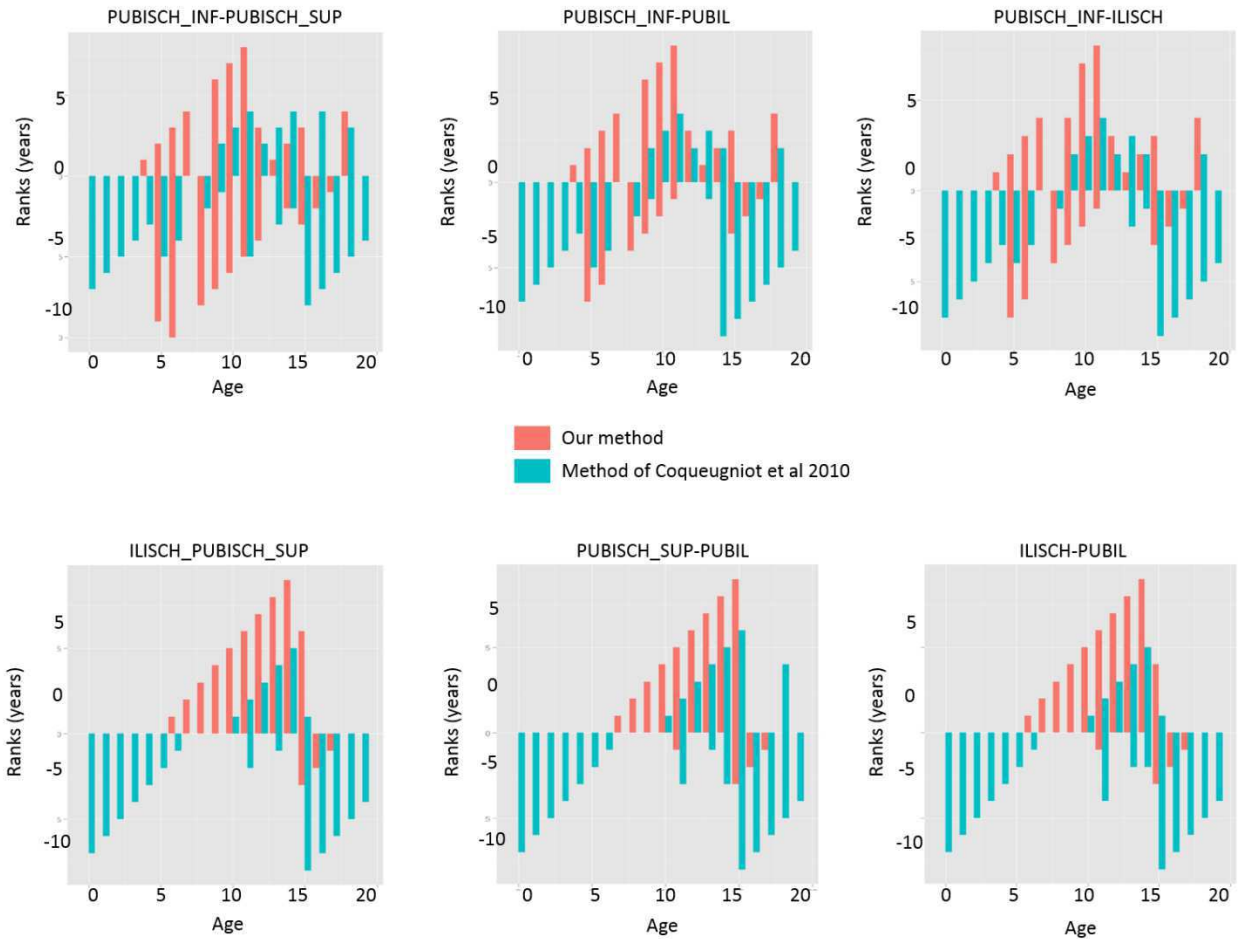
The approach of H. Coqueugniot and collaborators shows higher absolute rank values than our approach and quasi-similar patterns for ranks obtained using all five combinations: age is decreasingly overestimated from 0 to 7 years and from 15 to 19 years, correctly estimated between 7 and 8-9 years, whereas it is constantly of increasingly underestimated for individuals aged 9-10 to 14 years. Rank values are lower for that age group. The common peaks correspond to individuals aged 0 and 15 years. ILISCH-PUBISCH\_SUP-PUBIL also presents a negative peak at age 11, and PUBISCH\_INF-ILISCH-PUBISCH\_SUP presents a positive one at age 4. Underestimation is always more important and extended to a larger number of age groups with our approach, whereas overestimation is inferior or equal to the approach of H. Coqueugniot and collaborators (2010) and concerns less age groups.

The two-digit combination ranks also follow different patterns depending on the approach and/or the sites used (**Figure 6.59**). The two-digit combinations not using PUBISCH\_INF as one of the sites present a very similar pattern: age is correctly estimated from 0 to 5 years, mostly underestimated for individuals aged 6 to 14 years, and slightly overestimated between 15 and 17 years: the positive ranks increase until reaching a peak of underestimation at 14 years.

The combinations using PUBISCH\_INF as one of the predictors show a more erratic pattern: age is mostly overestimated between 5 and 8 years and mostly underestimated between 9 and 18 years, with peaks at 11 years or 12 years. However, important overestimation is also observed for individuals aged 4 to 10 years with the PUBISCH\_INF-PUBISCH\_SUP, and less importantly between 5 and 7 years with the PUBISCH\_INF-PUBIL and PUBISCH\_INF-ILISCH combinations. Once again, the approach of H. Coqueugniot and collaborators shows a comparable pattern for all two-digit combinations, and between the combinations using PUBISCH\_INF as one of the sites in particular. This approach mostly overestimates age from 0 to 6 years and from 14-15 to 19 years, with peaks of negative rank values around 0 and 15 years, followed by a decrease in rank values. Between 6 and 10 years, age is correctly estimated (majority of null rank values) and it is constantly or increasingly underestimated between 10 and 15 years of age.

Underestimation is more important and erratically present in a large number of age groups (4 to 17 years) with our approach used with two-digit combinations implying PUBISCH\_INF, whereas it is restrained to a smaller age range (6 to 14 years) when PUBISCH\_INF is not part of the combination. Overestimation is inferior to underestimation when using the approach of H. Coqueugniot and

collaborators and concerns the two extreme age groups: 0 to 6 and 15 to 19. Underestimation is dominant in the 9 to 14 age group.



**Figure 6.59 Comparison of the rank distributions of the individuals from the Luis Lopes sample using our approach and the method of H. Coqueugniot and collaborators on two-digit combinations of iliac maturation stages**

Overall, the method of H. Coqueugniot and collaborators (2010) tends to overestimate age for the younger and older individuals independently of the number of sites or the nature of the sample, and over- or underestimate age for intermediate ages, depending on the sites and the number of combinations.

Our approach provides less constant results: they are particularly erratic when posterior probabilities are applied to the Luis Lopes sample. However, the pattern that emerges is a general tendency for underestimating age of individuals between 5 and 10-12 years, and sometimes overestimating it depending on the number and nature of the sites used in the combination. Our approach overestimates age for older individuals between 15 and 18 years of age, independently of the combination used.



**Summary: Conclusions on our standardised approaches for juvenile age estimation**

- All biometric variables showed residual heteroscedasticity when used as age predictors with parametric ordinary or weighted least squares regression equations. As a result, non-parametric Multi-variate Adaptive Regression Splines (MARS) models were used to construct age estimation models

- MARS models using bidimensional iliac variables (IM or IA) provided the best results on two independent test samples, followed by the lumbar variables RVH and LVH, and lastly, by maximum clavicular length (Ln). These models have a 95% reliability, an accuracy of at least 89% and a precision that decreases with age. The estimates are comparable to or better than those obtained with the age estimation methods of S. Black and L. Scheuer (1996) and C. Rissech and A. Malgosa (2005)

- Age estimation using posterior probabilities of age calculated from maturation stages of the iliac bone provided reliable results (higher than 90%) but prediction intervals are too large for a precise estimation of age (+/- 4 years at best). Estimates are comparable to those obtained with the method of H. Coqueugniot and collaborators (2010)

All the valid, most reliable, accurate and precise predictive methods are available for practical use in Appendices F and G

# Discussion

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## **Chapter 7. Juvenile age estimation in physical anthropology: standardised methods to express and exploit developmental variability**

This study had two main goals: first, conduct an extensive and critical analysis of juvenile age estimation methods and implications and second, present a standardised and valid approach to juvenile age estimation. The first goal highlighted the importance of adopting standardised sampling and statistical criteria to obtain scientifically valid methodological protocols that are mandatory for correct method application and objective evaluation. This helped us elaborate a standardised and valid methodological protocol for our second goal: a standardised approach to juvenile age estimation, using biometric (skeletal measurements) and non-biometric (maturation stages) variables. In light of the results obtained in our study, the present chapter discusses all the implications of juvenile age estimation methods, from their elaboration to their application. This covers sample characteristics, sampling and statistical protocols, biological interpretations of our results, the role of context and goals in method application and the importance of individual variability.

### **7.1. Standardised sampling of population variability**

#### **7.1.1. Adopting a standardised and adapted sampling protocol**

We considered a sample to be a group of individuals included in a statistical study. Sampling parameters must conflate the theoretical background (goals, context of study) and the biological reality (range and factors of variability) behind an anthropological study. For example, a valid growth study would most likely require the collection of longitudinal data on a large number of individuals, so that it includes intra-individual variability (Lampl and Johnston 1996; Sempé and Pavia 1979). Although longitudinal data provides information on intra-individual variability, the successive values measured in a longitudinal dataset are highly correlated and under-estimate inter-individual variability by over-representing each individual in the sample (Stull et al 2014a). Cross-sectional data are more appropriate for constructing age estimation methods, because it enables to i a larger range of inter-population variability. Using Multi-variate Adaptive Regression Splines (MARS) models, this inter-individual variability is mathematically modelled by the dynamic prediction intervals of age against the variables measured in the sample. Moreover, it is rather difficult and time-consuming to

obtain longitudinal data on individuals other than in a clinical context. Even then, the risk of irradiating the patients (Rahmstahler et al 2009) greatly limits the amount of longitudinal data and restricts it mostly to dental radiographies (Heuzé 2004) or radiographies of the hand/wrist region (Lalys 2002) that are routinely and massively done to control normal postnatal development of these structures. It is much easier and faster to obtain cross-sectional data from osteological collections or medical imaging databases.

Sampling individual variability in a cross-sectional study requires obtaining data from a large number of individuals. Sample size is an important parameter for evaluating the validity of anthropological studies. Several authors have recommended that a study where age and sex are potential factors of influence, a minimum number of ten individuals per sex and per age group (the range of which is set prior to the study) must be respected (Humphrey 1998; Schmeling et al 2007). Unfortunately, because of the irradiative nature of tomodensitometric examinations, fewer examinations of this kind were found in the hospital databases for younger juveniles. This is why data were less available for individuals in certain age groups (1 to 3 years for the ilium, 0 to 11 years for the lumbar vertebra, 2, 5, 6, 7, and 10 to 12 years for the clavicle) although there were never less than 12 individuals and never more than 24 in a given age group for both sexes combined (**see Appendix B**). Uniformity also means that sample composition had to be relatively consistent between the groups of factors to avoid factor-related biases. The Marseilles study samples were therefore collected to respect uniform age and sex ratios for all bones and variables. This does not necessarily reflect normal population composition, but it avoids over- or under-representations of age groups and any bias of value ranges for the variables (Gowland and Chamberlain 2002; Schmeling et al 2007).

A uniform distribution and a sufficient number of individuals are also advised for test samples, because they are mainly used to obtain methodological accuracy and standard error that also depend on individual variability. These prerequisites partake in methodological standardisation and minimise the influence of sample structure on the quality and applicability of the method (Gowland and Chamberlain 2002). This is particularly important, because anthropological methods are predominantly applied to isolated individuals or samples for which structure is unknown. A standardised and non-biased method seems less likely to give biased results on an independent sample of unknown structure. The test samples from Marseilles and Toulouse respected uniform age and sex distributions. The test sample from the Luis Lopes collection did not respect uniform age and sex distributions, so results should be interpreted with caution and consider the general characteristics (sanitary state, socio-economic status) of that particular test sample.

### 7.1.2. Sample characteristics

The Marseilles sample can be considered as a standardised reference sample, composed of individuals with known age and sex and uniform age and sex ratios. However, the unknown but potentially diverse geographical origins of the individuals means it cannot be referred to as ancestrally, culturally and socio-economically homogeneous (Gastaut 2009; Insee). Because the Marseilles sample is composed of hospital patients, we cannot rely on any other factor but chance to state that the collected sample is a true reflection of variability of the juvenile population living in Marseilles. Therefore, the age estimation method built on this sample is not qualified as “population-specific” in a non-statistical (*i.e.* a geographical or genetic) sense: the sample from Marseilles is a group of individuals presenting a particular inter-individual variability and living in certain socio-economic and environmental conditions that can potentially influence the outcome of the age estimation method. The same thing could be said of the Toulouse sample as the individuals also originate from a hospital environment.

The main possible bias when working on clinical samples remains the risk of selecting pathological individuals. By definition, a hospital patient is unlikely to be completely healthy. Even though asserted pathological cases were excluded from the sample, the general chance of selecting individuals outside the normal ranges of developmental variation cannot be excluded. Indeed, all variables presented outliers in several age groups. However, an outlier for one variable was always within the range of normality for other variables taken on the same bone or on one or the other two bones (iliac bone, fifth lumbar vertebra and clavicle). Moreover, outliers belonging to an annual age group N were never outside the range of values of individuals from age groups N-1 or N+1, which means they only presented slightly advanced or delayed growth. This argument does not justify their exclusion from the sample and these individuals could not be considered biologically “abnormal”.

This issue is also true when working on individuals picked randomly in the general population (in a non-statistical sense) for whom medical or socio-economic history is unknown or incomplete. Working with medical imaging leads to a collaborative approach between anthropologists, pathologists, and radiologists. Hospital and autopsy samples present the advantage of being composed of high numbers of individuals, of various socio-economic, genetic backgrounds and for whom all the information of the biological profile is known (Hartnett 2010; Kistler et al 2013; Schmeling et al 2006b). This makes them ideal for methodological studies in anthropology, as they are likely to encompass an important portion of individual variability. These samples are also the best way to define the ranges of skeletal normality, as they are likely to include a certain number of outliers who can give an indication on where to set these limits (Laor and Jaramillo 2009) and also, perhaps, a more accurate vision of the population who very probably presents a certain proportion

of outliers. The question behind these observations remains the same as in any anthropological study: what is the definition of biological normality and where is the cut-point between normality and pathology (Stevenson 1924)? Studies need to qualify and/or quantify factors that could influence skeletal development of the individuals in the samples and characterise their intrinsic variability to identify factors that could bias the results. These remarks were addressed in the protocol for the selection of the individuals.

### **7.1.3. Past and present individuals: recalibrating variability**

The term "referenced sample or collection" is often used to characterise a group of individuals from a given population and for whom at least age and sex are known. It would perhaps be useful to specify the population (in a non-statistical sense) characteristics that could influence results of the construction or the application of anthropological methods. The Luis Lopes sample originates from a cemetery population. It is considered as an osteological reference collection, although its characteristics are rather specific and may be the cause of biased results. Compared to the other two test samples from Marseilles and Toulouse, it is composed of deceased individuals from a homogenous geographic, cultural and environmental background (Cardoso 2005, 2007b). Cemetery populations are biased by nature, because several biases (selection, preservation, etc.) cause discrepancies between the demographic structure of the deceased population and the past living population (Garcin 2009; Gowland and Chamberlain 2002).

In spite of those differences in population representation, similarities of juvenile skeletal formats between these past populations and extant ones are more frequently found than differences (Garcin 2009). This is why they can be considered as referenced populations, when age (at least) is known. It is useful to test new methods on such reference collections in view of being applied on archaeological populations.

Early death during childhood is often due to bad living conditions that probably had an effect on normal biological development. Such is the case for the Luis Lopes collection: all individuals suffered from various pathologies at their time of death. Moreover, the influence of socio-economic status, sanitary state, environment, and nutrition has been extensively studied for these individuals (Cardoso 2005, 2007b; Conceiao and Cardoso 2011) and has proved to be significant. This influence varies according to the developmental stage of the individuals: for example, these factors have a stronger influence on young children than on adolescents (Cardoso 2008b).

Pathologies are known to have an effect on the timing of maturation and on the quality of growth (Banerjee and Agarwal 1998; Crowder and Austin 2005; Giuca et al 2012). Age estimation of

pathologic individuals has been tested in a few studies and gives various results (Cundy et al 1988; de Moraes et al 2013; dos Santos et al 2013; Pludowski et al 2005; Sansilbano-Collilieux 1993). The problem of method application is the large discrepancies between chronologic, dental and/or skeletal ages caused by the various pathologies that alter the level of the correlation between chronological and biological age. This leads to various and inconstant discrepancies between the two types of age and often, invalid estimates. As no information is available concerning the duration of the sickness of the individuals from the Luis Lopes collection (with a few exceptions of sudden deaths), we cannot measure the impact the pathologies had on skeletal growth and maturation, but we cannot assume it was inexistent.

The Luis Lopes collection also raises the question of secular trends (Heuzé and Cardoso 2008; Himes 1984; Roche 1979) and their influence on age estimates obtained on past individuals with methods built on extant populations. Normal developmental patterns cannot be reliably verified for unreferenced past populations, but are likely to be different than those of extant populations because of the factors stated above. However, biological proximity between populations was found to be a stronger factor than chronological distance (Rissech et al 2013b), making population structure a more discriminating factor than chronology.

Inhomogeneity between the variables obtained for the three samples from Marseilles, Toulouse and Lisbon was found for the values of all variables used in this study ( $p < 0.001$ ), except for one iliac variable (IA) and the four vertebral height variables (PVH, AVH, RVH and LVH). The Marseilles and Toulouse samples presented no significant differences for the variability of iliac variables IL, IW and IM ( $p > 0.001$ ) (**see section 5.5.**). The individuals from the Luis Lopes collection tend to have lower values for the variables than the individuals from Marseilles and Toulouse at similar ages. These results could be interpreted in terms of media specificity (scanned bone versus dry bone), and/or population specificities such as chronological period, geographic origin, socio-economic and sanitary status (Clarck et al 1986; Garn et al 1973). Our pre-study on the reliability of biometric variables measured on dry bones VS reconstructed bone surfaces of the clavicle seems to rule out, or at least, lessen, the influence of the medium of acquisition (**see section 5.1.1.**), identifying the main source of variation between the extant French individuals and the early 20<sup>th</sup> Century Portuguese individuals as population specificities, such as socio-economic and sanitary status, and less importantly, secular trends. These hypotheses need to be verified.

## 7.2. Standardised data and data acquisition protocols

### 7.2.1. Protocols for data acquisition

#### a. Biometric variables

Segmentation of bone tissue from CT scans of living individuals or corpses for whom soft tissues are still present can be difficult: its quality depends on acquisition parameters (*e.g.* slice thickness), the state and density of environing tissues (Villa and Lynnerup 2012) and the method of segmentation. For instance, it can be difficult to separate cartilage from bone, and this was the case in the acetabular region. Cartilage ossifies simultaneously in several different locations, making it difficult to identify all the bony components of that region. The variability and progression of maturation patterns of the acetabulum can therefore be difficult to visualise on reconstructed bone surfaces of the iliac bone, which sometimes adds difficulty in attributing a stage to a particular maturation state.

Reconstructing bone surfaces from CT scan slices induces a smoothing bias, which modifies the reliefs of the “real” bone surface. An anatomical landmark corresponding to a particular bone relief can be smoothed to modify its appearance, or modified to the point that it no longer appears on the virtual bone surface (Weber and Bookstein 2011; Zollikofer and Ponce de Leon 2005).

The variables used for this study were defined by or adapted from previous works on dry bones (Martin 1957; Martin and Saller 1959, 1962) and/or on two-dimensional data, such as bone radiographs or CT slices (Brough et al 2012; Zhou et al 2000). They have been used on three-dimensional bone reconstructions and tested for consistency with dry bone measurements (Brough et al 2013; Citardi et al 2001; Hildebolt et al 1990; Lopes et al 2008; Lou et al 2007; Richtsmeier et al 1995; Stull et al 2014a; Waitzman et al 1992). However, these studies do not deal with the influence of bone orientation of the 2D or 3D virtual bone images on measurements. This can be a source of error, particularly when handling data obtained from living individuals, whose position is not always standardised during image acquisition. Indeed, the errors due to measurement techniques (Goto and Nicholas Mascie-Taylor 2007) or media biases such as examination settings or reconstruction protocols (Stout and Gehlert 1982) must be insignificant compared to normal and random biometric variations. However, they are not systematically tested in anthropometric studies (Harris and Smith 2009). Therefore, consistency tests were done to verify the equality of measurements taken on dry clavicles and on the reconstructed bone surfaces of the same dry clavicles after CT scan in random orientations. This allowed us to construct a simple protocol for geometric bone reorientation. Anatomic orientation seems to rely on anatomical landmarks that could not be precisely determined for the clavicle, but could be found and tested for other bones.



Variable reliability required testing repeatability and reproducibility of the landmarks defining them on the virtual bone surfaces (Richtsmeier et al 1995). Sufficient repeatability and reproducibility rates were not met for all landmarks (**see section 5.1.2.a**). This suggests that a certain level of experience with reconstructive softwares such as Avizo® is required and that perhaps the protocol should be revised to add more details on landmark positioning. However, all clavicular, lumbar and iliac variables were found to be sufficiently repeatable and reproducible and measurement errors were quantified as required by methodological recommendations (Shirley and Ramirez-Montes 2015).

The clavicle presents asymmetry in length and orientation (Bassed et al 2012; Mays et al 1999) as well as high variability of its anatomy and shape (Cook et al 2013). Because of this and contrary to the iliac and lumbar variables, the five clavicular variables seemed the most difficult to reproduce and repeat: they do not depend on anatomical landmarks or identifiable zones of dry bones. This is why the validity of variable acquisition protocol on both scanned and dry bone was verified by an independent study of 40 dry clavicles for which the surface was also reconstructed from CT scans. Biometric variables obtained on virtual bones are more precise: landmarks and geometric analytic tools provide variables with a precision higher than 0.0001 mm, compared to measurements done using a sliding calliper on dry bones. All clavicular variables were found to be repeatable and reproducible on dry and reconstructed bones and therefore, our protocol was deemed satisfactory in terms of methodological requirements for application on both dry bones and reconstructed bone surfaces.

Equality between geometric and anatomical variables was tested to see whether or not one type could replace the other: indeed, anatomical variables are easier to measure on dry bones and geometric variables are more reliable and easier to measure on reconstructed bone surfaces, where anatomical features are less visible. Because of this, measuring the anatomical variables of the clavicle on reconstructed bone surfaces can be particularly difficult and time-consuming. The antero-posterior and supero-inferior anatomical orientations are approximated from the lateral end as reference to measure the sagittal (AP\_diam) and vertical (SI\_diam) diameters on reconstructed clavicular surfaces (Shirley 2009). However, correct and reliable orientation of the bone is difficult.

Even if our methodological study of 40 clavicles could not cover the full range of clavicular morphological variability nor lead to conclusions on the influence of age, activity, or origin on morphology, the sample still provided very different clavicular morphologies and sizes that sometimes rendered anatomical orientation difficult. This could be bypassed by using geometric variables that are quasi-automatically measured by tools in Avizo® and Image J® softwares. Unfortunately, anatomical and geometric clavicular variables could not be considered equivalent, therefore both types of variables were used.

**b. Maturation stages**

Timing and sequence of fusion of the primary ossification centres of the iliac bone has been extensively studied using several types of media and different maturation indicators to present iliac developmental patterns (Acheson 1957; Cardoso 2008b; Cardoso et al 2013a; Coqueugniot and Weaver 2007; Flecker 1932; Schmidt et al 2011; Stewart 1934; Wagner et al 1995; Owings-Webb and Suchey 1985). These studies use between two and nine stages to characterise fusion patterns. Too many stages can lead to intra- and/or inter-observer errors (Gonsior et al 2013; Scheuer and Black 2000; Todd 1937; Veschi and Facchini 2002). Their qualitative character leads to higher risks of subjective staging (Danforth et al 1993; Garcin 2009). This is why a simple three-stage maturity rating system applicable on reconstructed bone surfaces and dry bones was adopted here: it does not necessitate experienced observers such as radiologists, who attribute maturation stages on CT scan slices that are sometimes difficult to read (Brodeur et al 1983; Castriota-Scanderberg et al 1996; Kaplowitz et al 2011; King et al 1994; Medicus et al 1971); it limits the risk of intra- and inter-observer errors and is the best compromise between accuracy and reliability for an application on dry bones and reconstructed bone surfaces (Cardoso and Severino 2010; Krogman and Iscan 1986). Moreover, the restricted number of stages (only three) and sites (only four) limits the number of combinations, compared to other studies with much higher numbers of stages and sites (Heuzé 2004). Extensive staging is more precise but often less reliable than a more restrictive number of stages (Heuzé 2004). It was decided to privilege reliability of staging rather than precision in the present study. Therefore, our system is less precise than other staging protocols used on bones or teeth (Demirjian et al 1973, Demirjian and Goldstein 1976; Kreitner et al 1998), because one stage is likely to cover a larger age range. This is the case for the extreme stages (0 and 2) and it is partly due to the age range selected for the study. However, reliability, repeatability and reproducibility of our staging system are quantified and sufficient.

The bias when obtaining and comparing qualitative data (*e.g.* maturation stages) on reconstructed bone surfaces compared to dry bones is due to the quality of the CT scan and the correct segmentation of the bone tissues from the surrounding elements (cartilage and ligaments). A default in either of these two steps can lead to unobservable maturation stages or incorrect staging.

Our staging system is not immune to the presence of persistent fusion lines or of unfused states at an advanced age. These two occurrences can underestimate the age of an individual and increase the age ranges corresponding to the unfused (0) and partially fused (1) stages, sometimes dramatically. This bias is well-known and recurrent in bone maturation studies (Cardoso et al 2013a; Weiss et al 2012). Fusion lines can be difficult to visualise because of surface smoothing. Reconstructed bone surfaces do not have the visualisation and superimposition biases of

radiographic images: the advantage of using reconstructed bones is the possibility to access the internal structures of the bone by using the cross-section tool of Avizo® and assess the maturation stage more accurately and reliably. In the end, staging maturation on reconstructed bone surfaces is no more different than it is on dry bones, provided segmentation is done correctly.

## **7.2.2. Predictor variables**

### **a. Biometric variables**

The variables chosen as predictors of age had to be reliable, repeatable and reproducible, measurable on dry bones and reconstructed bone surfaces and have a strong correlation with age.

The juvenile ilium is easily recognisable and relatively well-preserved in archaeological samples; iliac variables are well-defined and have been used for several age estimation methods, therefore methodological comparison is possible. Unidimensional biometric variables of the ilium (IL and IW) are well-known, repeatable and reproducible, and have been used for juvenile sex determination (Adalian et al 2001; Majo et al 1993; Rissech and Malgosa 2005) and/or age estimation (Rissech and Malgosa 2005) for both juvenile and adult individuals and have provided good results. The two bidimensional variables IM and IA are less used for age estimation or sex determination, but also provide good results (Adalian 2001; Daumas et al, *in press*). Several studies on growth trajectories of the ilium have also been done (Faruch Bilfeld et al 2013; Miles and Bulman 1995; Wilson et al 2015), so its developmental pattern is also well-known. The external acetabular point was not used as one of the defining points of ilium width as it was in C. Rissech and collaborators' study (Rissech et al 2001), because it was not observable on reconstructed scanned bone surfaces. The most caudal point of the internal side of the iliac acetabular surface was used instead as it was much more reliable.

All four iliac variables are highly correlated with age. The highest correlation was observed for the two bidimensional variables: the proxy of the internal ilium surface (IM) and the projected internal iliac area (IA). These two bidimensional iliac variables were selected for this study to provide a more complete apprehension of growth of this particular short bone than "classic" unidimensional variables, by covering possible spurts or patterns related to directionality. This could explain the lower number of outliers for IM and IA compared to IL (see Chapter 5). Using bidimensional data could be another way of considering variability, and therefore improve juvenile age estimation. The validity of this hypothesis is confirmed by our results with MARS models (see Chapter 6).

The vertebral body is an element often relatively well-preserved for adults and juveniles (Bello et al 2006). Moreover, the fifth lumbar vertebra is one of the most recognisable vertebrae, whether it is found in anatomical position or not (Baker et al 2005; Scheuer and Black 2000; White et al 2012). These two characteristics were decisive in the selection of the fifth lumbar vertebra as the representative of short bones.

No exhaustive studies on growth or age estimation using biometric variables of juvenile fifth lumbar vertebrae were found prior to this work. Six of the variables used here were adapted from the study of CT scan slices of adult lumbar vertebrae (Zhou et al 2000) and the growth study on dissected juvenile lumbar vertebrae (Mavrych et al 2014). The study of V. Mavrych and collaborators did not analyse the growth curves obtained, but provided mean growth rates for each variable for individuals aged 0 to 12 years. Our study presents four original lumbar variables as well as original results in the form of a new juvenile age estimation method using fifth lumbar biometric variables, applicable to individuals aged 0 to 19 years using dry bones or reconstructed bone surfaces.

All lumbar variables were sufficiently repeatable and reproducible and had a strong correlation with age. Indeed, they provided good results for age estimation (**see Chapter 6**). In the same way as the bidimensional variables of the ilium, the two proxies of the upper or lower vertebral surfaces (respectively UVM and LVM) provide a more complete apprehension of growth of this particular short bone than “classic” unidimensional variables, by covering possible spurts or patterns related to directionality. However, bidimensional and unidimensional lumbar variables present comparable numbers of outliers (10 to 15) and therefore, growth patterns and growth variability of the vertebra could be different than that of the ilium: perhaps growth directionality has a lower influence than other factors such as sexual dimorphism, or posture (Taylor 1975).

The clavicle was chosen as the representative of long bones because of its long growth, its relatively good preservation rate and the possibility to compare our age estimation method with other standards using the same predictor variable (maximum clavicular length).

In the present study, clavicular diameters were measured primarily to see whether they could be good estimators of age and to test whether or not anatomical measurements were comparable to geometric measurements to eventually facilitate analyses on dry and scanned bone. Geometric and anatomical clavicular measurements were not found to be equivalent. However all four are reliable, repeatable and reproducible and can be measured on dry and scanned bones. A previous study (Brough et al 2013) tested reliability between measurements taken on scanned clavicles and the same dry clavicles. However, the measurements taken in that previous study were taken on CT slices and not three-dimensionally reconstructed bones, and it did not deal with difficulties related to position and orientation. Both difficulties were considered when building our protocol.

Three of the clavicular variables used in this study are geometrical (maximum length, maximum and minimum diameters), two of them are anatomical (antero-posterior and supero-inferior diameters). Maximum clavicular length is known to be a good estimator of age. It has been used in several juvenile age estimation methods and clavicular growth studies (Black and Scheuer 1996; Krogman 1941; Miles and Bulman 1995; Pinhasi et al 2005; Stout and Paine 1992). Correlation with age was highest for maximum clavicular length, which confirms its high potential for age estimation for the whole duration of clavicular growth. This variable also presents the lowest number of outliers (six). Correlation was lower for the four diameter variables. It was still sufficiently high for them to be used as predictors of age, although our results show that they cannot be used for reliable or precise age estimation (see Chapter 6).

Sexual dimorphism is generally observed for both growth and maturation patterns at different developmental phases (Bogin 1997, 1999; Humphrey 1998; Sundick 1977; Weaver 1980). In this study, IL did not present sexual dimorphism, which corroborates the observations of C. Rissech and A. Malgosa (Rissech and Malgosa 2005) on the growth patterns of that variable. Neither of the two bidimensional variables IM and IA present significant sexual dimorphism, which means the punctual sexual dimorphism detected by the first series of tests for IL, IM and IA is either an artefact or is relatively insignificant compared to the global growth pattern of these variables. Our observations concur with a study done by M. Faruch Bilfeld and collaborators (2013) on growth patterns of the ilium between the ages of 1 and 18 on a sample of 188 individuals from Toulouse, France. Indeed, using geometric morphometrics, the authors showed that the ilium presented no significant sexual dimorphism of its size, but presented significant sexual dimorphism of iliac shape and developmental patterns from 11 years onward, as found in a previous study (Marchal 2003). Sexual dimorphism does not seem to influence the size of the ilium, but rather its shape, development and growth trajectories.

Sexual dimorphism was found for all biometric clavicular and lumbar variables from an early age onwards and for the maturation sequence of the iliac bone during adolescence. Other studies have found sexual dimorphism of skeletal developmental patterns for all bones, with different levels for different elements of the skeleton. This results in divergent developmental patterns between males and females, also called sexual bimaturism (Humphrey 1998) because of differential growth rates or of the combined result of differential growth rates and growth duration (Coleman 1969; Rissech and Malgosa 2005, 2007).

Significant bilateral asymmetry was found for maximum clavicular length (Ln) and diameter variables (SI\_diam and Max\_diam), left (LVH) and right (RVH) vertebral heights and ilium module

(IM). Although bilateral asymmetry is known for adult clavicles (Auerbach and Raxter 2008; Auerbach and Ruff 2006; White et al 2012), this study confirmed its presence during skeletal growth. This corroborates previous observations stating that long bone growth presents bilateral asymmetry, reflecting readjustments and changes in proportions (Byers 1991; Todd 1937). Bilateral asymmetry of IM, LVH and RVH could also be correlated with these same growth changes and readjustments, along with additional factors that could influence posture and skeletal development of the spine (Taylor 1975) or the pelvic girdle.

### **b. Maturation of the ischio-pubic ramus and the acetabular region**

Fusion of skeletal elements follows a similar sequence in all populations as assessed by several studies (Stevenson 1924; Stewart 1934). However, if the sequence is the same, timing is not: ages at which skeletal epiphyses start fusing or are completely fused vary according to populations and medium of study (dry bones, skeletal radiographies or ultrasonographic images) (Cardoso 2008b; Moss and Noback 1958; Thaler et al 2008).

In the Marseilles sample, the first epiphyses to fuse are the ones forming the ischiopubic ramus (PUBISCH\_INF<sup>‡</sup> site). The ischio-pubic epiphyses remain unfused for all individuals under the age of 3, up to 7 years for females and 8 years for males. However, unfused epiphyses were also observed on one male individual aged 14 years, even though the three acetabular epiphyses were fusing normally. Considering the important age difference between the 8-year olds and the 14-year old, this individual is clearly out of the normal range for ischiopubic ramus fusion. The ages of fusion observed in other studies (Acheson 1957; Coqueugniot and Weaver 2007; Cardoso et al 2013a) are in accordance with our findings, even in terms of outliers (older unfused individuals), although the ages of fusion for these particular individuals vary between our sample and these other studies. It seems more plausible to conclude that all three studies have out-of-range individuals, than to find such different maximum ages for an unfused ischiopubic ramus.

The elements start fusing at 3 years and partial fusion can be observed until 14 years included. Comparable results were computed by logistic regression on the Luis Lopes collection (Cardoso et al 2013a) and on a sample of English children from the late 1940's using the Oxford method on radiographies of the iliac bone (Acheson 1957). Partial fusion of the ischiopubic ramus starts much later but ends sooner for the individuals from Coimbra: the first occurrence is at 7 years, the last is at 10 years (Coqueugniot and Weaver 2007). This is biased by the small number of individuals presenting that particular stage.

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<sup>‡</sup> PUBISCH\_INF= Inferior Pubo-Ischiatic epiphyses, PUBIL=Pubo-Iliac epiphyses, ILISCH=Ilio-Ischiatic epiphyses, PUBISCH\_SUP=Superior Pubo-Ischiatic epiphyses

Complete fusion of the ischiopubic ramus is first observed at age 4 for males and age 5 for females from Marseilles. It occurs later for males but at a similar age for females than that observed in the Luis Lopes sample (Cardoso et al 2013a). Fusion is complete for all individuals from 15 years onwards with the exception of two male individuals of 16 and 17 years. The epiphyses of these two individuals were staged as partially fused, because an epiphyseal scar was still slightly visible at the meeting point of the two bone parts. The 17-year old individual also presented an epiphyseal scar at the ilio-pubic fusion site. Partial fusion of the ischiopubic ramus was found on the right and left iliac bones for both individuals. They can be considered as the upper age extremity for partial fusion of the ischiopubic ramus in the Marseilles sample. Other studies have reported fusion timing to be much later, with the line still visible at 6 years, but complete fusion occurring between 5 and 9 years (Birkner 1978; Coqueugniot and Weaver 2007; Davies and Parsons 1927; Flecker 1932; Frazer 1948; Galstaun 1937; Scheuer and Black 2000). It is difficult to interpret some of these studies, as no mention is made on whether fusion is completed during that period or whether it is first observed at that age (Cardoso et al 2013a).

The acetabular maturation sequence in the Marseilles sample is not clearly defined, because fusion of the elements seems to be strongly intricate for the three sites concerned (PUBIL, ILISCH and PUBISCH\_SUP). The sequence of fusion of the acetabular epiphyses that seems to emerge in this study is the following (see **Chapter 5**): ILISCH starts and finishes fusing first, followed by PUBISCH\_SUP and PUBIL (Stevenson 1924; Stewart 1934). Partial fusion is found between 6 and 18 years, which is comparable to that observed by R. Acheson (Acheson 1957). The earliest occurrence of complete acetabular fusion in the Marseilles sample is 12 years and the acetabuli of individuals aged 18 or more are all completely fused. These observations are comparable to other maximum age ranges generally reported in literature for acetabular fusion (Acheson 1957; Davies and Parsons 1927; Scheuer and Black 2000; Stevenson 1924). H. Cardoso did an extensive study of fusion patterns of the iliac bone in the Luis Lopes collection (Cardoso 2008b). He also found a comparable maturation sequence to the other studies and to the one reported for the Marseilles sample.

Sexual dimorphism of fusion timing, or bimaturation, of the ischiopubic ramus is not consensual and is known to depend on several factors (Cardoso et al 2013a; Galstaun 1937; Scheuer and Black 2000). The differences of stages according to sex were not significant for PUBISCH\_INF in the Marseilles sample, but differences were found in another study of individuals from Coimbra (Coqueugniot and Weaver 2007). It is known that bone maturation occurs significantly earlier for girls than it does for boys (Cardoso 2006; Scheuer and Black 2000, 2004; Todd 1937). Moreover, the adult iliac bone is the skeletal element that presents the highest level of morphological sexual dimorphism,

which is studied using measurements and/or morphoscopic observations (Bruzek 2002; Marchal 1997, 2003; Murail et al 2005). Sexual dimorphism for ilium variables is absent for the individuals of the Marseilles sample. This shows that sexual dimorphism of iliac biometry does not start to express itself significantly before at least 13 years (Faruch Bilfeld et al 2013). However, 8-year old individuals already present sexually dimorphic maturation patterns of the acetabulum. Sexual dimorphism of iliac maturation stages was indeed significant for individuals between 8 and 15 years of age. This was also observed in other studies (Reynolds 1947; Rissech and Malgosa 2005, 2007). Fusion starts at 6 for females and 7 for males, which is earlier than other reported ages (Acheson 1957). Complete fusion of the acetabulum was observed at age 12 for females, and at age 14 for males in the Marseilles sample. All female individuals aged 17 or more have completely fused acetabuli, whereas complete fusion was not observed before the age of 18 for males. Fusion of the acetabulum is reported to begin at 11 years and end at 15 years for females. For males, fusion is known to start at 14 and end at 17 years (Acheson 1957; Scheuer and Black 2000). The age ranges of acetabular fusion found in the Marseilles sample are slightly extended compared to what is presented in literature, but still comparable.

Acetabular fusion occurs before the pubertal growth spurt. It happens for females first, therefore maturation occurs earlier for females (at 12 years) than it does for males (14 years) (Rissech and Malgosa 2005, 2007; Rissech et al 2003, 2008). Most studies find that fusion generally occurred first for females (Acheson 1957; Rissech and Malgosa 2005, 2007; Rissech et al 2003, 2008; Scheuer and Black 2000), but other studies found that there were no differences between sexes for timing of acetabular fusion (Galstaun 1937), with females only having one or two years advance on males (Cardoso et al 2013a), exception made of one or two sites: PUBIL (Cardoso 2008b) or PUBISCH\_SUP (Coqueugniot and Weaver 2007). H. Coqueugniot and T. Weaver's study presented similar ages for the start of fusion of the three sites for females (12 years), but much later ages for males (17 years, as opposed to 14 years in the Marseilles sample). The considerable overlap of sexes for acetabular stages in their study can be explained by small sample size, and an under-estimation of individual variability.

No significant bilateral asymmetry was found between left and right iliac bone stages in the Marseilles sample, or in the Luis Lopes collection. (Cardoso 2008b). It was found in one study for the PUBIL and ILISCH sites (Coqueugniot and Weaver 2007), which also seemed to present delays in other maturation stages, particularly for male individuals.

Growth of the iliac bone ends around 21-22 years, when it has attained its adult size and morphology (Cardoso 2008b). Fusion scars can persist for a long time and lead to staging errors (overestimation of partial fusion). However, studies show comparable maturation sequences.



Differences are due to truncated samples (Coqueugniot and Weaver 2007; McKern and Stewart 1957; Schaefer and Black 2005; Veschi and Facchini 2002), small sample size or uneven age and sex ratios *i.e.* low inter-individual variability (Cardoso 2008b; Coqueugniot and Weaver 2007), the media used and their intrinsic defects (restored bones, superimposed radiographed structures) and socio-economic factors, geographic origin or secular trends (Cardoso et al 2013b, Lalys et al 2012).

A “poor” socio-economic status can lead to delays in maturation (Hennenberg and Harrison 2001; Heuzé 2004). Socio-economic status, secular trends and developmental levels in populations influence maturation timing and duration (Banerjee and Agarwal 1998, Cardoso 2005, 2008; Crowder and Austin 2005). This could explain the small differences found between the Marseilles sample and other referenced data on acetabular fusion.

### **7.3. Standardised statistical approaches**

#### **7.3.1. Regression models and age estimation: choosing the best approach**

##### **a. A prerequisite: methodological standardisation**

The aim of this study was to build a juvenile age estimation method with a standardised protocol to address the methodological issues pointed out in several publications (Cattaneo 2007; Cunha et al 2009; Schmeling et al 2007). These issues mainly concern the importance of the statistical validity of sampling protocols and methodology: sufficient sample size and adapted structure, sufficient repeatability and reproducibility of the variables, standard levels of reliability and accuracy, calculation and explicit presentation of prediction intervals and/or standard errors of estimation. It is recommended that authors explicitly present the limitations of methodological construction and application (comparison of the results obtained on independent samples, validity of the results, etc.).

All methods are based on intra-population variability and depend on the factors that this variability implies: sex, socio-economic status, sanitary state, nutritional state, etc. No consensus has been reached regarding the preferential use of sexed or unisexed age estimation methods. Sexual dimorphism implies different variability for males and females. Sexed methods should logically reflect the biological reality of skeletal development and should provide more accurate estimates of age (Hunt and Gleiser 1955; Rissech et al 2003; Scheuer and Black 2000; Ubelaker 1987). Dental development is less influenced by sex, although some studies found differences according to sex (Demirjian and Levesque 1980; Levesque et al 1981; Saunders et al 2007). However, sex cannot be reliably determined for juvenile individuals (Rissech et al 2008; Scheuer and Black 2000; White and

Folkens 2005) and is most likely unknown for individuals in archaeological samples or found in a forensic context if only skeletal remains are recovered. Moreover, if the clavicular and fifth lumbar vertebral variables used here were significantly different for males and females, male and female variability ranges mostly overlapped. Sexed age estimation methods would probably provide more precise estimates and they have proven to do so (Moorrees et al 1963a; Rissech and Malgosa 2005; 2007). However, priority was given here to reliability because age estimation is considered at an individual level and not a population-level, and in view of optimising methodological use, it seemed more relevant to construct a unisex method with a higher applicability rate.

Most importantly for anthropologists, standardised methods have the advantage of being directly comparable to one another, even if they were constructed on different referenced populations. Standardisation excludes or at least limits discrepancies in statistical biases and the variability of influence on the results. Using standardised methods allows for other sources of discrepancies, such as population variability, socio-economic status, etc., to be evoked and account for sample or population differences. By getting rid of sampling and statistical biases, methodological standardisation allows the expression of individual or population variability and its interpretation

#### **b. MARS models: an approach fitting individual variability**

The critical analysis (see Chapter 2) showed that age estimation using biometric variables is generally studied using a forward approach, *i.e.* regressing age against a biometric variable. The regression models obtained by Ordinary Least Squares (OLS), or other parametric approaches such as Weighted Least Squares (WLS) allow the prediction of age directly by using a mathematical function modelling the relationship of age with the variable. This approach is only valid if residuals of the regressions respect normality, homoscedasticity and independence. None of these prerequisites were met for any of the variables used in the present study.

Heteroscedasticity of the residuals (*i.e.* changes in residual variance) was observed in all the OLS and the WLS predictive regressions constructed in this study. It could not be considered and dealt with for age estimation purposes using WLS models or by transforming the variables. It could be a mathematical expression of individual variability. Indeed, heteroscedasticity expresses changes in the range of inter-individual biometric variability as age progresses. The plots of heteroscedastic residuals seem relatively similar and independent of the type of bone or the biometric variable. This particular residual pattern could reflect the irregular changes of the onset of growth phases in addition to inconstant ranges of normal individual variability. Understanding biometric (*i.e.* growth)

variability could be done by identifying the onset and duration of phases of changes in residual variance.

It is interesting to note that independently of the relative ranges of values of the variables, all of them lead to residual heteroscedasticity (inhomogeneous variances), auto-correlation and un-normal distributions. The increase of variance with age for biometric variables is a known phenomenon and is explicitly represented by the progressive increase of the distance between percentile curves modelling statural growth, especially in samples covering large age ranges (Garcin 2009; Humphrey 1998). Therefore, to be correct and make biological sense, the prediction intervals (PIs) of any age estimation method covering the concerned range of values (and the corresponding age ranges) should follow the same pattern, *i.e.* their size should increase as variability increases, as it does with MARS models.

The relationship between age and the variables used in this study is clearly polynomial (except for the bidimensional ilium variables Ilium Module IM and Ilium Area IA). The approach used to construct a simple polynomial model is “piecewise polynomials” (de Boor 1993; Milborrow 2014). The polynomial form is preserved without increasing the polynomial degree as predictor values increase. A function is constructed as a succession of low-degree polynomials regularly connected by knots. This allows non-derivability and a low convergence rate of linear interpolation functions at interpolation points. These are called spline functions. MARS models are based on spline polynomial functions and provide a single model composed of several sub-models with particular mathematical expressions depending on the predictor values. MARS models directly highlight the values for which the relationship between age and the variables changes via the hinge points (or knots): these points correspond to predictor values (and corresponding age ranges) before and after which variability patterns are different. They are the most efficient for modelling existing non-linearities in the data and taking residual heteroscedasticity into consideration. Therefore, MARS models could model transition stages of growth and allow a direct comparison of these stages between reference samples. From a biological perspective, this approach is more interesting than constructing parametric regressions on different truncated subsamples of data presenting homoscedastic residuals: it gives the true perspective of individual variability and uses it to provide biologically realistic estimates of age (Stull et al 2014a).

High multicollinearity can cause all MARS univariate and multivariate models to seem similar (one variable seems as good as another). This makes it difficult to decide which variable is the “best” predictor, as they somehow seem redundant. Indeed, CVRSq values, standard deviations of CVRSq and PI sizes are roughly similar. Collinearity can also lead to large standard errors and the risk of overfitting models to the data. If the test sample has a different pattern of multicollinearity than the

study sample, such extrapolation may introduce large errors in the predictions (Chatterjee et al 2006). These difficulties are compensated by cross-validating the MARS models on independent subsets of data from the same sample, which gives average standard errors per model. The models are therefore statistically robust and more reliable. MARS models are therefore the best suited models in case of variable collinearity, especially when constructing multivariate models.

Another approach for age estimation is the use of calibration models (*e.g.* inverse or fractional regression models) followed by Bayesian predictions (Boldsen et al, *In* Hoppa and Vaupel 2002; Braga et al 2005; Konigsberg et al, *In* Paine 1994; Prince and Konigsberg 2008). This Bayesian approach consists in constructing a growth model of the biometric variable and calculating the inverse function to obtain the mathematical expression of age by the variable. The next step is to use Bayesian probabilities to estimate the probability of age according to the inverse regression model. The calibration approach is necessary if there are significant measurement errors. It is also useful to solve the common phenomenon observed with forward regressions: the underestimation of age for older individuals and overestimation of age for younger individuals (Aykroyd et al 1997).

This approach would be valid if no heteroscedasticity were present, if it could be repressed by variable transformations or by using Weighted Least Squares (WLS), or by a smoothing method to bypass residual heteroscedasticity. Indeed, fractional regression can only be used if variables are normal or normalised, and is only valid if residuals are homoscedastic, normal and independent. None of these conditions were met here: measurement errors were insignificant for all variables and transforming or weighing the predictors or predicted variable did not solve the limitations previously evoked. Moreover, the use of forward regressions done here is not confronted with the bias that is the influence of the age composition of the sample, often identified in osteoarchaeological samples (Boldsen et al, *In* Hoppa and Vaupel 2002). Ensuring uniform age and sex distributions in our study samples makes it possible to use forward regressions of age against predictor variables. Inverse regressions are the method of choice when the distribution of the sample from which the individual originates is known *a priori* (Lopez-Costas et al 2012). If this prerequisite is obvious when constructing the method, it almost never applies when estimating the age of a single individual or several individuals, as is most often the case in both forensic anthropology and bioarchaeology. MARS models seemed the most adapted approach to model the normal increase in inter-individual variability with age on these particular study samples and with these predictor variables. These models are not used to conflate regression with causation, but simply present a way of regressing age on biometric variables that takes care of non-linearity and heteroscedasticity in a sound and practically useful way. All the usual caveats for regression apply, such as regression towards the mean (Aykroyd et al 1997), or heteroscedasticity. However, MARS (or similar techniques) are

definitely applicable because low degree polynomials or transforming age into a logarithmic variable cannot linearise the age curve and they provide a certain flexibility to model the non-linear relation between age and the variables (Stull et al 2014a).

Future analyses could test fractional polynomials and compare the results obtained with MARS models, provided application conditions are met. No consensus has been reached on which statistical approach is the best for age estimation (Schmeling et al 2007).

### **7.3.2. Age estimation using maturation stages and Bayesian probabilities**

Bayesian probabilities are a robust and appropriate method to use ordinal variables for age estimation. They present age estimates as probabilities of age, which leaves a certain liberty in the choice of precision and reliability levels according to personal objectives and context of study. A Bayesian approach is necessary when the objective is to construct a juvenile age estimation method using qualitative data and applicable on different populations (Lopez-Costas et al 2012). Ideally, it should also be constructed on a large sample composed of individuals from several populations to encompass a greater range of variability (Heuzé 2004). This last condition applies to any anthropological method.

Choosing the prior probabilities to be uniform (equal priors for all age groups) or non-uniform is the first step when using Bayesian probabilities. This step depends on sample structure. The prior probability is the probability of being of age X at the time of the examination/death (Gowland and Chamberlain 2002). If the frequencies of each age group are used as priors, a sample with heterogeneous age ratios will provide non-uniform priors.

Non-uniform priors are more appropriate for developmental population studies, because they mirror the natural frequencies of age groups in the population of study and therefore provide a more accurate representation of developmental patterns. Uniform priors are more appropriate for methodological studies (*e.g.* constructing an age estimation method) than for population studies because population structure (sample size, age and sex ratios, covered age range) has in fact more influence on the results than the geographical origin of the individuals (Heuzé 2004). Choosing uniform priors allows comparisons amongst methods that also use uniform age distributions in their study sample (Konigsberg and Frankenberg 1992).

Choosing between dependent and independent Bayesian probabilities is also a matter of context and objectives. In this study, an independent Bayesian approach was adopted. This implies that the four maturation sites of the iliac bone are independent from one another. This is biologically difficult

to admit, because maturation of the acetabular epiphyses cannot be considered as four independent maturation processes that end up merging into the acetabulum. Other than their coordination with one another, they are dependent on the maturation of the femoral head and the surrounding soft tissues that compose the hip articulation (Humphrey 1998). All three epiphyses fuse with one another following a specific sequence but have particular maturation rates that are subject to the factors previously cited (Greulich and Pyle 1959). However, the genetic component has a stronger influence and the sequence remains generally constant (O'Connor et al 2010; Pryor 1907; Reynolds 1943; Schmeling et al 2003a), despite allowing a certain developmental plasticity (Meadows and Jantz 1995, 1999). Although an independent Bayesian approach is less biologically compatible than a dependent one, it enables the calculation of posterior probabilities of age of any type of combination, be it observed in the study sample or not. This is particularly interesting in biological anthropology, because it is difficult to globally incorporate individual variability in study samples that are often of insufficient size (less than several hundreds of individuals). Independent probabilities allow the calculation of age using any combination of stages, even if it was not observed in the initial study sample, provided the stages and sites have been assigned individual probabilities. This means that age can be inferred even in case of missing data *i.e.* in case of bad preservation rates, an extremely likely scenario in osteoarchaeological series and forensic cases. Moreover, uniform priors associated with an independent approach give best results for past populations and are more suited for practical case studies in both osteoarchaeological and forensic contexts (Heuzé 2004).

Transition analysis, initially used for age estimation of adults with qualitative skeletal age indicators (Bethard 2005; Boldsen et al, *In* Hoppa and Vaupel 2002; di Gangi et al 2009; Kimmerle et al 2008; Milner and Boldsen 2012), is another approach that could be adapted for juvenile age estimation using qualitative data. However, it is much more sensitive to upper and lower extreme ages (*i.e.* extreme indicators) and sample truncations than posterior probabilities. As such cases were extremely frequent in our study sample, this approach was not attempted. It would require a much greater number of individuals between the two extreme stages to obtain substantial numbers of intermediate combinations (*i.e.* other than 0-0-0-0 or 2-2-2-2).

In the same way as for regression models, we considered constructing sexed and/or unisexed posterior probabilities of age. Sexual bimaturism is known to be present for maturation sequences, and it was observed between the ages of 8 and 15 in the Marseilles sample. Maturation sequences and rates present important sexual bimaturism: it is the first source of variability for dental maturation, followed by geographic origin (Heuzé 2004). Skeletal maturation also presents important sexual dimorphism and the iliac bone in particular (Cardoso 2008b; Coqueugniot and Weaver 2007). Sexual dimorphism of iliac bone maturation was observed in the samples from Marseilles and Lisbon,

with males presenting a delay of maturation stages compared to females of the same age. However, the number of individuals per sex per annual age group in the Marseilles study sample was too limited for the probabilities of stages per annual age group to be considered representative of population variability in maturation states. Because of this, probabilities of age are less precise than they would be if they were sexed. Sex cannot be reliably determined for prepubertal individuals (Rösing et al 2007) which represent the largest part of the study sample used here. Growth has not yet ended for males or females of that age range, so sexual dimorphism of biometric variables is not yet significant enough to be used for reliable sex determination. Moreover, although some differences in iliac shape have been identified as sexually-related (Faruch Bilfeld et al 2013), iliac bone conformation has not yet reached its highly sexually dimorphic adult shape, so morphoscopic methods such as the one by J. Bruzek (2002) cannot be used reliably either. Male and female variability of maturation stages is not significant enough either to be individually discriminant, even if it is observed at a population level. This is why posterior probabilities of age using maturation stages of the primary ossification centres of the iliac bone were calculated on males and females combined, using uniform priors and independent Bayesian probabilities.

## **7.4. Juvenile age estimation using two standardised approaches**

### **7.4.1. MARS models**

#### **a. Biological interpretations of statistical results**

Because parametric age prediction models did not provide valid residual parameters, even after variable transformations or weighing, MARS models seemed to be the best fit for effectively exploiting the biometric variables used in this study for age estimation. By providing prediction intervals whose size increase with age, they take into account the increase of growth variability with age (Garcin 2009). Considering multicollinearity and the values of the model parameters CVRSq and RSq, the “best” models were found to be the univariate MARS models using bidimensional ilium variables IM and IA, the univariate models using IW, Ln, AVH and RVH, and the bivariate models RVH-LVH, Ln-SI\_diam and Ln-Min\_diam.

Models using bidimensional iliac data provided higher accuracy, RSq values and lower residual means and standard deviations on all three test samples. The increase in predictive power was not extremely significant between univariate and multivariate models, so univariate models can be privileged for user-friendly reasons. These models provided the best predictions on the Marseilles

and Toulouse test samples, with extremely high RSq values and accuracy. Performance was good, but accuracy was lower than 81% for the Luis Lopes sample for all iliac models.

Both the Marseilles and Luis Lopes test samples showed better results with vertebral height variables (PVH, AVH, RVH and LVH) in univariate, bivariate or multivariate models. If RVH and LVH were the best predictors in the Marseilles sample, AVH also stands out as one of the best predictors in the Luis Lopes sample. The models using upper and lower vertebral variables performed very poorly on the Luis Lopes sample and cannot be considered as an age estimation method.

The “AVH” model, the models combining all four height variables and the “RVH+LVH” model present the highest accuracy. However, PI sizes of the “RVH+LVH” model are very important and often stretch from negative values of age for the lower estimate limit to very high positive values of age for the lower estimate limit, which means that age estimation using this model has a very limited value for precise age estimation. PI size is much more reasonable with the “Height”, “AVH”, “RVH” and “LVH” models and all three present relatively high accuracy, although it is lower than 95% for the models using RVH and LVH.

The clavicular models using Ln as a predictor are invalid for precise age estimation on the Luis Lopes sample: the formulae of the bivariate models are constants for younger individuals, and the residual standard deviations calculated on the Luis Lopes sample are too high for all models. However, they were valid on the sample from Marseilles and should be tested on other samples before deciding whether they are valid or not.

Standard error values are inconstant with MARS models. However, the mean standard errors are still much lower than they were for linear and polynomial regressions so there is no considerable loss of precision for the estimates and the individual PIs associated to the mean estimates are valid. The increase in inter-individual variability is particularly marked after 10 years of age for the ilium, and during puberty for the lumbar and clavicular variables (Humphrey 1998; Lewis 2007; Saunders *In* Katzenberg and Saunders 2008). This could be correlated to the variability of the age at which puberty is reached and the pubertal growth spurt occurs, a phenomenon observed by several authors (Greulich and Pyle 1959; Coleman and Coleman 2002). It explains why inter-individual variability is so important during that particular period and the increase in residual variance. It is interesting to note that inter-individual variability of the clavicular variables increases quite dramatically after the first two years, a phenomenon previously observed (Garzin 2009). This was indeed detected by an increase in PI size of the MARS models after that phase.

For long bones like the clavicle, maximum diaphyseal length seems to be the best predictor of age until growth stops, between 17 and 20 years of age (Maresh 1943, 1955). Maximum clavicular length is known to be a very good predictor of age for juveniles (Scheuer and Black 2000). This was verified



in the present study on individuals from the Marseilles and Lisbon samples. Diameter variables do not significantly improve the age estimation models when associated with maximum clavicular length. The four clavicular diameters measured for this study are known to be more influenced by sex and activity than maximum clavicular length. Clavicular breadths are also known to present greater directional bilateral asymmetry and this was verified in the present study. This adds to the range of variation for the four diameter variables (Auerbach and Raxter 2008; Auerbach and Ruff 2006). All these factors induce very important variability, independently of the age factor. Moreover, intra- and inter-variability seem too important for these variables to be sufficiently precise age predictors.

For the fifth lumbar vertebra, the body height variables gave the best estimates of age, with higher  $R^2$  values and smaller prediction intervals. The best predictions were obtained using AVH, RVH and LVH on both test samples from Marseilles and Lisbon. Previous studies had observed higher growth rates in vertebral height compared to sagittal length (UVL) of thoracic and/or lumbar vertebrae (Brandner 1970; Zhang et al 2010). J. Taylor found that growth of sagittal variables (UVL and LVL) was more likely to be affected by activity and weight-bearing due to the erect posture (Taylor 1975). This would statistically translate into higher variability of these variables compared to body height variables (AVH, PVH, RVH, and LVH). This is indeed observed in this study, particularly for adolescent individuals. He also found that middle body height was less subject to variation than peripheral body height whose growth can also be altered by pathologies (Taylor 1975). Precaution must therefore be taken when using LVH and/or RVH as predictors, and systematic checking of bilateral asymmetry must be done. If the middle of the upper and lower body surfaces could be reliably assessed on scanned and dry bones, this variable could be of interest for age estimation, as it could be the least influenced by external factors.

The initial hypothesis for this study was that bidimensional variables taken on a flat bone like the ilium covered more growth variability and followed a more linear relationship with age, therefore making them easily exploitable as age predictors. Bidimensional iliac data had a stronger linear relationship with age, although age prediction models were not linear. MARS models using bidimensional variables such as IM and IA and the unidimensional variable IW gave the most precise and accurate age estimates for prepubertal individuals from Marseilles, Toulouse and Lisbon, even more so than maximum clavicular length. Another study found that ilium variables were better age predictors for juveniles than any long bone lengths (Boccone et al 2010). Ilium Width (IW) is indeed a very good predictor of age in all three samples. A possible explanation for this is the fact that shape changes of the ilium during childhood are mostly located in the postero-superior and superior parts

of the ilium (Faruch Bilfeld et al 2013), so IW translates the more important age-related size and shape changes that occur in this region and therefore is a better age estimate than IL.

All MARS models present hinge points during the second half of adolescence (from 15 to 19 years) independently of the predictor variable. This could be interpreted as the progressive slowing and stopping of growth, until adult size is reached and growth stops (Kalifa and Merzoug 1999). The last interval includes hinge points of the ilium MARS models (between 10 and 12.3 years) is artificially truncated due to measurement limitations and does not correspond to an identified growth phase for the ilium. However, it is included in the age range corresponding to the normal onset of puberty for French males and females (8 to 14 years) (Tauber 2002) that corresponds to known changes in skeletal development (Humphrey 1998; Scheuer and Black 2000).

Although several hinge points seem to be clustered in age ranges corresponding to ages of specific changes in growth rates such as infancy, puberty, adolescence, as presented in B. Bogin's work (Bogin 1997), they are not sufficiently close to be systematically interpreted as such. At best, they can be considered as an illustration of the individual variability in the age of the onset of different growth phases. At worst, they can represent the change in the growth pattern of one or a few individuals outside the normal range of variability and deform the normal signal. This raises once again the question of outliers and of their weight in regression models, and the question of normal range of individual variation. The first issue can be tackled by eliminating out-of-range individuals and/or by using cross-validation, which attenuates the effect of such individuals (Milborrow 2014; Stull 2013). The choice of including or excluding seemingly out-of-range individuals is delicate. When dealing with "small" samples (*i.e.* composed of only a few hundred individuals) and/or cross-sectional data, it is difficult to exclude an individual for being "too small" or "too big" for his/her age: exclusion implies the risk of underestimating normal variability, which is not the objective of an age estimation method based on that particular principle.

As no significant sexual dimorphism was found, ilium models were built for combined sexes. This confirms that pre-pubertal girls and boys follow the same growth pattern (Faruch Bilfeld et al 2013; Coleman 1969). Ilium growth indicators are valid parameters for unisex age estimation at least until the onset of puberty, even though variability increases after 9 years. Growth is not significantly different between girls and boys before approximately 13 years, but differences were found for maturation: bone fusion starts earlier for girls, particularly for the iliac bone (Rissech and Malgosa 2005, 2007; Rissech et al 2003) and sexual dimorphism was observed in ilium shape and developmental patterns in general as early as 1 year (Faruch Bilfeld et al 2013). This was verified in the sample from Marseilles. Sexual dimorphism of ilium shape and ilium shape changes are

significant throughout development, particularly from 11 years onwards, even though growth patterns remain comparable (Faruch Bilfeld et al 2013). Because our sample was truncated, this could not be verified. Although sexual dimorphism was found for the variables, uni-sexed MARS models were built for age estimation with the clavicular and lumbar variables, because the number of individuals per sex and annual age group was insufficient to be considered as representative of the whole male and female population and provide statistically significant results. In addition to mirroring the normal increase of variability with age, this could explain why precision of MARS models is lower for older individuals.

#### **b. Comparison of MARS models with other age estimation methods**

The comparison of MARS models with other age estimation methods (Black and Scheuer 1996; Rissech and Malgosa 2005) provided interesting results concerning the limitations of age estimation methods related to population specificities and the influence of sample structure. MARS models gave more accurate age estimates on individuals from the Marseilles and Toulouse test samples than on the individuals from the Luis Lopes collection. The two French samples are composed of extant living individuals originating from presumably comparable geographic, socio-economic and sanitary backgrounds to the individuals of the training sample from Marseilles. The sample from the Luis Lopes collection is composed of juveniles that were born and lived during the first half of the 20<sup>th</sup> Century, when sanitary and socio-economic conditions were relatively bad. This certainly had an impact on normal skeletal growth and maturation (Cardoso 2005, 2007; Susanne 1985), along with the influence of secular trends on growth ranges and patterns (Amselem et al 2007; Cole 2003; Hauspie et al 1997; Helm 1969; Heuzé and Cardoso 2008; Lalys et al 2012; Maresch 1972). Both factors resulted in higher ranges of values and an increase in growth rates for the more recent populations (Roche 1979; Susanne et al 2001) These results seem consistent with the general consensus on the strong influence of socio-economic status and secular trends on growth and maturation patterns (Amselem et al 2007), which lead to divergences in age estimates between populations from different periods and socio-economic status (Cardoso 2005, 2007; Conceicao and Cardoso 2011; Heuzé and Cardoso 2008).

The better results obtained on the Luis Lopes sample using the abacus on maximum clavicular length of S. Black and L. Scheuer (Black and Scheuer 1996) and the regression equations of ilium length and width from C. Rissech and A. Malgosa (Rissech and Malgosa 2005) can be moderated. Results were only significantly better than MARS models for individuals older than 10 years using the abacus and for individuals older than 8 years using the regression equations. MARS models are still useful to predict age of prepubertal juveniles, an age range for which standardised age estimation methods

are scarce (Cunha et al 2009). Most importantly, the methods of S. Black and L. Scheuer (1996) and of C. Rissech and A. Malgosa (2005) were built on a sample composed of juveniles from several osteological reference collections, including the Luis Lopes individuals. It seems sensible to presume that testing a method on part of the sample used to construct it would undoubtedly provide better results, due to similar sample structures and the possibility of overfitting models to the data (Gowland and Chamberlain 2002; Millard and Gowland 2002). Unfortunately, no other referenced methods that used the same predictor variables as the MARS models were found to overcome the sampling bias.

Accuracy of MARS models is comparable to or better than other juvenile age estimation methods applicable to individuals of the same age range and respecting the same sampling and methodological parameters as our study. For instance, real age was not included in the 95% PIs for only 5 out of 68 individuals when using the MARS model with Ilium Module (IM) as a predictor. This provides a predictive model with a 93%-level of accuracy. The MARS models validated on the three test samples have accuracy rates ranging from 82 to 97%.

The prediction errors obtained with the MARS models were lowest when using IM as a predictor: “ $\pm 0.3$  years” to “ $\pm 3.4$  years”, with a mean prediction error of  $\pm 0.80$  years for the Marseilles sample. The highest prediction errors found for valid and applicable MARS models were obtained on the Luis Lopes test sample using maximum clavicular length as a predictor (mean prediction error of  $\pm 1.75$  years). The study of K. Stull and collaborators on modern South-African children gave prediction errors of “ $\pm 0.084$  years” to “ $\pm 5$  years” for their MARS models constructed on long bone measurements for individuals between 0 and 12 years of age (Stull et al 2014a). Their models were not tested on independent samples from different populations presenting different variability; L. Lalys and collaborators’ method based on radiographic hand/wrist measurements have a prediction error of “ $\pm 2.80$  years” (Lalys 2002; Lalys et al 2006) on an independent test sample from the same population as the training sample; R. Cameriere and collaborators’ method gives a constant prediction error of “ $\pm 0.96$  years” (Cameriere et al 2008) on an independent Italian sample, but mean prediction errors ranged from  $\pm 0.26$  years to  $\pm 2.68$  years when it was tested on different populations (Cameriere et al 2009; de Luca et al 2012; El-Bakary et al 2010; Galic et al 2011). Overall, our results are comparable and, most importantly, adapted to age-related changes in variability.

#### **7.4.2. Bayesian posterior probabilities for age estimation: strengths and weaknesses**

Several studies using dental or skeletal maturation and posterior probabilities of age based on maturation stages give varied but overall good results (Braga et al 2005; Coqueugniot et al 2010; Gowland and Chamberlain 2002; Heuzé 2004; Heuzé and Cardoso 2008; Langley-Shirley 2010; Millard and Gowland 2002; Thevissen et al 2010). In the present study, using combinations of stages from more than two maturation sites was not necessary to provide significantly higher confidence intervals and precision: 95% or higher confidence intervals and precision of +/- 4 years were obtained for the PUBIL site and the two-digit combination PUBISCH\_INF-ILISCH for both the Marseilles and Luis Lopes samples.

Precision could not be increased without compromising reliability; it is for the user to decide which parameter will be privileged (Kimmerle et al 2008), although a minimum reliability of 95% is considered the threshold for method validation. This is one of the advantages of using posterior probabilities for age estimation. Because confidence intervals are sufficiently high but the associated precision is at best equal to +/- 4 years, this approach should be used to confirm the attribution of an individual to a quinquennial age group, rather than provide individual age estimation. It is insufficient for age estimation in a forensic context; however, the results are comparable to other age estimation methods (Cardoso 2008b; Coqueugniot et al 2010) and can be used in an osteoarchaeological context to attribute, confirm or infirm a quinquennial age group to which an individual belongs, in association with other age estimation methods using different or similar age indicators.

Caution must be taken however when comparing several methods with one another, as differences exist between age estimates obtained from different regions of the skeleton depending on the methods used (Aicardi et al 2000). The biological limitations of age estimation need to be considered by evaluating the methods constructed with the same variables obtained from the same bones. Differences observed in estimates are often caused by the combination of factors such as differences between the methods used, between the observers' experience and because of biological variability of development. Validity of the methods should be verified and their biases and limitations should be clearly identified beforehand.

By combining the results obtained through iliac biometric variables with maturation indicators of the whole iliac bone, the posterior probabilities developed in this study could also be used to calibrate and refine the estimates obtained with MARS models for post-pubertal individuals. This would require finding a way to take reliable measurements of IL, IW and IM (IA could not be reliably measured because of acetabular fusion) after 12 years, which we could not do in this study. Biometric and non-biometric variables could be used as age predictors in regression models, by

attributing different weights to maturation stages, or both estimates could be combined after being obtained separately. The probability distribution of the MARS PIs and of the posterior probabilities of age would provide a combination of the two estimates that could help verify normal development of the iliac bone and could eventually indicate the age group presenting the higher probability of age if prediction intervals overlapped two or more age groups. As such, this study seems more adapted for population- or sample-scale analyses rather than individual age estimation, because of its lower precision (Garcin 2009).

The large size of the 95% confidence intervals (CIs) is due to the limited number of maturation stages, the long duration of each stage (several years) and the heterogeneous number of occurrences of the different combinations that are not equally represented in the study sample. The study done by Y. Heuzé in 2004 was based on a very large sample of individuals, used a higher number of stages so that each had a much shorter duration (Heuzé 2004; Heuzé and Cardoso 2008). This method estimated dental age with a precision of +/- 0.5-1 year for a 95% level of reliability.

In our sample, the most represented stages and combinations were the extreme values: "0" and "2" for single sites, and combinations using either only "0" or "2". In this case, it is not the age structure of the sample that is a source of bias in age distribution (Gowland and Chamberlain 2002); it is the frequency of distribution of the indicators used to estimate age and the fact that, because of individual variability, age-informative indicators do not present the same discriminative power from one individual and one population to another (Boldsen et al, *In* Hoppa and Vaupel 2002).

One of the most unfortunate defaults of this study is the important proportion of high ranking errors for the [10-14] age group. This age group corresponds to a period where acetabular fusion is particularly active, but its timing is also especially variable due to sexual differences in particular, and other less ostentatious factors (Cardoso 2008b). The two- or three-digit combinations correspond to much larger age ranges than expected for the higher number of combinations represented in that age group. One way to perhaps refine the posterior probability distributions of age corresponding to specific combinations would be to use transition analysis (Boldsen et al, *In* Hoppa and Vaupel 2002; Konigsberg et al 1998; Milner and Boldsen 2012): knowing the maturation stages of each epiphyseal site, the sequence of maturation of the entire iliac bone and because the sequence of maturation stages is ordered (Konigsberg et al 2008), calculating the age-of-transition for the different sites would perhaps enable one to refine the posterior probabilities of age (calculated using posterior density functions) and the associated confidence intervals (Kimmerle et al 2008). A comparison between the two approaches would help address this question.

Males show more positive rank values than females for all combinations, meaning age is mostly underestimated for males and overestimated for females. This could be due to the mutual influence of one sex on the other at different periods when maturation is significantly asynchronous between males and females (Tanner 1981). It is recognised that males have a slower maturation and will globally present lower stages of maturation than females at the same age, especially during and after puberty (Calfee et al 2010; Heuzé 2004; Kalifa and Merzoug 1999; Scheuer and Black 2000; Tanner 1981; Todd 1937). Precision of the estimates would certainly be increased by separating males and females to calculate the posterior probabilities of age, but such results would only be obtainable with much larger samples.

## **7.5. Limitations and perspectives of juvenile age estimation**

### **7.5.1. Practical use of MARS models and perspectives for improvement**

To appeal to the broadest crowd and facilitate their use, anthropological methods should respect the KISS principle: “Keep It Smart and Simple”. Although the process behind the building of a method can be complex, it should be user-friendly, provide fast results and not compromise statistical parameters such as reliability, accuracy or precision. The principle behind MARS models and the results they produce are simple enough to understand. Their practical use requires selecting the correct mathematical expression corresponding to the range of the predictor value and doing several calculations to obtain the mean estimate and the associated 95% dynamic prediction interval, or, more simply, referring to an abacus indicating the mean, maximum and minimum estimates corresponding to a predictor value (**see Appendix F**).

With the development of free and accessible softwares and/or applications on mobile devices, it seems relevant to turn to such resources for user-friendly, globalised and easily accessible interfaces to propose a simplified and fast use of a complex method. There has indeed been a tendency these past years to construct and use automatized forms of age estimation methods in the form of specific softwares (Bouchard and Sempé 2001; Cao et al 2000; Kbaier et al 2005; Pietka et al 2003). To facilitate the use of the MARS models in the future, we aim to create an R® function and/or an application for mobile devices implementing MARS models for individual or sample age prediction. By entering the value of the predictor variable, the algorithm would automatically detect the correct mathematical expression of the MARS model needed for age prediction and would provide the mean estimate and associated 95% prediction interval for each individual as the output.

Presenting this method as an R<sup>®</sup> function and mobile application will enable us to associate maximum likelihood age estimates to ease interpretation of the results. This is already done for age estimation of adult individuals using descriptive qualitative variables (maturation stages) and transition analysis as a means to bypass the influence of age distribution in the samples (Boldsen et al, *In* Hoppa and Vaupel 2002; Konigsberg et al 1998; Milner and Boldsen 2012). This approach could be adapted to complete our study, by fitting a probabilistic density function for random continuous variables of the estimates across each individual prediction interval, using a more complex approach (Buck et al 1996). Because the size of individual prediction intervals obtained with MARS models is not constant, associating a distribution function of the estimated age range could, for example, help orient the interpretation of the result. This should be particularly useful in a forensic context where a probability of age is easier to interpret than a mean age and the associated prediction interval and make it easier to place an individual below or above a particular age limit. It could also be used in bioarchaeology when the estimated prediction interval overlaps two quinquennial age groups: knowing the probability distribution of the estimates could help choose the demographic age group associated with the highest probability of age.

Moreover, adding Bayesian probability distributions to the prediction intervals obtained with biometric variables could allow their combination with the posterior probabilities of age obtained with iliac maturation stages and maturation combinations. This could be a first step for constructing an age estimation method combining maturation and growth indicators, which could perhaps improve the estimates.

### **7.5.2. The influence of context**

Although exploring new data and innovative techniques for juvenile age estimation are an undisputable component of scientific progress and ethical improvement (Focardi et al 2014; Hillewig et al 2011; Pruvost et al 2010), it seems they can only be considered as such if they rely on robust and valid protocols (Cunha et al 2009). This study has confirmed and quantified methodological heterogeneity, the lack of a standardised methodological approach, and the difficulty to compare and complement different juvenile age estimation methods. Method comparisons (Aicardi et al 2000; Büken et al 2009; El-Bakary et al 2010; Haiter-Neto et al 2006; Martrille et al 2007) and meta-analyses (Serinelli et al 2011) require the statistical integration of several methods that are often based on unknown or different parameters (Cunha et al 2009). Therefore, the validity of comparisons and of mean age estimates obtained as a combination of several methods should be systematically



questioned and verified, as it is the only viable argument for method justification, independently of the context of study.

### **a. Age estimation in bioarchaeology and palaeodemography**

In archaeological samples, biases in interpretation are mainly due to methodological and population parameters (Bocquet-Appel and Masset 1982). Using a standardised approach to construct an age estimation method promotes homogenisation of methodological biases and allows valid method comparisons. Once methodological biases are identified and quantified, they can be put aside to study the evolution and influence of population and biological characteristics, such as secular trends, socio-economic status and other factors, that could explain divergent results. For example, systematic underestimation of age for individuals of past populations could be due to bad living conditions and/or be a result of secular trends in growth patterns (Cardoso 2005, 2007). This can only be assessed with certainty if methodological sources of bias are excluded. That way, conclusions can be interpreted in light of the archaeological context without worrying about unknown or unclear methodological limitations.

Archaeological context has a more important role than biological characteristics for differentiating populations and can often help explain these differences (Garcin 2009). However, as biological components are the basis of any osteoarchaeological study, a solid and rigorous methodology is indispensable to obtain these components. Here, we decided to develop a method from an individual perspective, although it can also be used in a population-level study. In both these perspectives, MARS models seem well-suited to address these demands: the protocol respects standardised sampling and statistical criteria to avoid methodological biases, and therefore, the individual approach classically developed for age estimation in a forensic context can be transposed to the archaeological context. MARS models can reliably predict age across numerous samples of variable sets drawn from the same statistical population (Stull et al 2014a), in our case the test sample from Marseilles and for individuals from a sample of different age and sex distributions, such as the Luis Lopes collection. The use of cross-validation contributes to obtaining age estimates specific to each study of juvenile human remains, independently of the population they originate from. This study highlights the more important influence of the statistical structure of training samples compared to population characteristics (Gowland and Chamberlain 2002) for the construction of age estimation methods.

## **b. Age estimation in forensic anthropology**

Juvenile age estimation in forensic anthropology is mainly done to assess two levels of individual minority: first, to assess if the individual is indeed a minor and second, to what particular legal age range he/she belongs to. In France, minors have to be “placed” in age groups defined by legal thresholds. This is extremely important as it determines whether the procedure will be held in front of a juvenile court or a criminal court, depending on the age of the minor, and defines the rights, protections and conditions under which they can be tried and possibly held in custody (French National Consulting Committee for Ethics), or if they can be considered criminally responsible for their actions (Kreitner et al 1998). The “minority” status also has an impact on the other individuals implicated in the case at hand: for example, in France, if a minor is the victim of a criminal offence and is younger than 15, the criminal offender will face graver penal consequences (Bartoli 2006; Saint-Martin et al 2013) than if the individual were older.

Other than criminal implications, the minority status also determines the civil modalities of acquisition and loss of nationality (*e.g.* Articles 17-1, 18-1, 19, 21, 26, 32), acts of marriage and divorce (*e.g.* Articles 63, 75) with the legal threshold established at 16, as stated in the French Civil Code. Minority assessment and knowing the exact age of children and adolescents are also essential to define the rights and protection of isolated foreign juveniles staying in the country in question, and the conditions and possibility of conducting a trial if they are charged with a felony. These procedures have become relatively frequent in several European countries, as the number of illegal or undocumented immigrants has increased throughout the years (Focardi et al 2014; Schmeling et al 2003b, 2007). Indeed, the law forbids the deportation of an illegal immigrant from the country he/she is in if he/she is minor and sometimes, he/she can benefit from immigration regularisation (Law n°2002-305 of March 4<sup>th</sup> 2002 relative to parental authority, modified by articles 17 and 35 of Order n°45-2658 from November 1945 relative to the conditions of entry and residence of foreigners in France).

In case of loss of identity papers or other official documents that could provide that piece of information - if the individual refuses or is incapable of revealing his/her age, if the individual is represented by undocumented skeletal remains - the chronological age (or real age) of an individual cannot be known directly and has to be estimated. Because of the legal implications conditioned by the thresholds evoked previously, the estimated age has to be the most objective and precise possible to “place” the individual in the same age range as her/his chronological age (Bartoli 2006). The age of an individual is the temporal marker of biological development. Therefore, biological development (growth and maturation) is the process that is exploited to estimate chronological age by providing the biological age of the individual. This is when the medical examiner and/or the

forensic anthropologist enter, as experts appointed by the Court to proceed with the direct examination of the individual, or the indirect examination of relevant medical records, in order to provide the necessary information. This procedure raises a set of ethical problems, in relation with the fact of physically and/or medically examining living individuals or the handling of the skeletal remains of the individual (Knight 1985; Rahmstahler et al 2009). For example, the use of medical imaging and/or invasive analyses have significant ethical limitations for the construction and application of age estimation methods, because they are done in a non-clinical context. This is why exploring imaging techniques, such as MRI or ultrasound, with limited or inexistent irradiation risks (Dedouit et al 2012; Hillewig et al 2013; Saint-Martin et al 2013, 2015; Schmidt et al 2007, 2013) is more and more frequent.

The dual position of the expert, divided between her/his status of medical/anatomical examiner and her/his status of legal expert: indeed, his/her scientific expertise will be used as a legal argument (Bartoli 2006). Because the conclusions provided by the expert determine the status of the individual and the consequences that ensue, “scientific” age estimation must respect sufficient reliability, precision and accuracy to be valid and accepted in Court (Kreitner et al 1998; Schmeling et al 2003b, 2007). The method constructed in this study aims to respect these conditions of scientific validity. Moreover, from a methodological point of view, the anthropologist constructing a method that aims to be used in a forensic context must assess and explain the imprecisions and uncertainty of the method (Larsen et al 2015). Indeed, in a forensic context, methods for age estimation should consider technically unacceptable errors or false negatives (*e.g.* the proportion of majors estimated as minors) and ethically unacceptable errors or false positives (*e.g.* the proportion of minors estimated as majors) (Garamendi et al 2005) that need to be avoided.

Juvenile age estimation in a forensic context calls for reliable and precise estimates, to come as closely as possible to real age and give accurate and reliable information to help identify an individual. In this regard, the first step towards a better and accurate understanding and interpretation of age estimation has been pointed out these past decades: promoting standardised and explicit protocols to enable harmonised results, valid comparative studies and an objective (statistical) evaluation of each method (Cattaneo 2007; Cunha et al 2009). This study was conducted in respect of these particular requisites. In bioarchaeology, if the goals of age estimation studies are different, the same methodological requirements apply to approach the real demographic profile of a past population and propose interpretations using valid biological information. The scale of an anthropological study has more impact on the interpretation of the age estimates than on the

protocol for constructing the methods, although some methods are specifically designed to be used at a population (Masset 1982) or individual level.

**c. Juvenile age estimation: an example of the limitations of biological modelisation?**

Even if our approaches using MARS models and posterior probabilities of age respect standardised sampling and statistical criteria, the well-known interpretive limitation of threshold- or age group-overlapping remains. It translates the difficulty regarding correspondence between biological, anthropological, legal and social levels of juvenility. There are two main reasons for this.

The first one is that the biological processes of growth and maturation are not categorical, but follow a continuous trajectory from beginning to end. The demographic age groups classically used in osteoarchaeology do not follow biological growth or maturation phases of skeletal or dental elements, especially during childhood *sensus largo* (1-10 years) and cut right through the middle of the adolescent period (age groups [10-14 years] and [15-19 years]). They were even qualified as biologically nonsensical (Garcin 2009). For this reason, several authors (Buchet and Séguy 2008; Garcin 2009; Scheuer and Black 2000) have proposed redefining these different anthropological age groups following recognised developmental phases (respectively the long bone growth phases defined by B. Bogin in 1999, the new dental mineralisation stages defined by L. Buchet and I. Séguy in 2002, and general dental development, particularly the formation of the two sets of dentition). Age ranges of prepubertal long bone growth phases are defined following changes in growth rates of long bones by B. Bogin (1999): (0-2 years), (3-5 years), (6-8 years), (9-12 years), (13+ years). Growth rates increase once again after puberty starts but only for a short period of time. This is visually perceived by the pre-pubertal growth spurt that occurs between the beginning of this phase and the previous one. The onset of puberty is not clearly identified, as it varies according to sex, populations, and is subject to other external influences (environment, socio-economic status, etc.) (Coleman and Coleman 2002; Legge 2005; Mitani and Sato 1992). L. Buchet and I. Séguy pushed the notion of redefining age groups according to specific time-frames even further (Buchet and Séguy 2008). They propose different age groups for populations of Roman and Medieval France, defined by social, cultural, religious, legal living conditions, developmental phases and mortality risks specific to these age groups: less than 2 years, 3-4 years, 5-7 years, 8-12 years, 13-15 years, and 16-17 years (Buchet and Séguy 2008). However, this last subdivision seems too specific and inapplicable to different time frames and population structures. In any case, there is a clear lack of agreement on how and if age groups should be established and used in anthropology.

The second reason is directly linked to the first: the range of individual variability increases with age. For age estimation models, this translates mathematically and methodologically into increasing PI sizes as age advances for the MARS models and by large confidence Intervals (CI) for posterior probabilities of age. Because precision decreases with age (Stull et al 2014a), there are limitations to the use of biometric variables and MARS models for age prediction. The importance of these limitations depends on the context and the goal of the study. One might think that the prediction intervals of the MARS models obtained for older individuals would be too large to allow easy and useful interpretation, particularly in case of threshold overlapping, which is especially problematic when it concerns the 18-year threshold, as the individuals' status change completely, or if the prediction interval is included in two consecutive palaeodemographic age groups. The question is "if reliability and accuracy are sufficient (as set by the methods), is precision high enough to provide age estimates that lead to satisfactory biological, legal or cultural interpretations?"

In a forensic context, where reliability and precision are key parameters for interpretation, our MARS models provide insufficient precision to discriminate the 18-year threshold: all prediction intervals increase in size from 15 years onward and cover a range of at least 4 to 5 years. Therefore, they cannot reliably and accurately discriminate minor from major individuals. This seems understandable, as skeletal growth has reached its stopping point at the end of adolescence and therefore, biometric variables are no longer relevant enough for juvenile age estimation (Cunha et al 2009). Maturation indicators such as fusion stages of the sternal end of the clavicle (Kellinghaus et al 2010a and 2010b; Schmeling et al 2004) or of the secondary ossification centres of the iliac bone and/or lumbar vertebrae (Albert and Maples 1995), and/or identifying age-related stages for the general shape of the bones seem interesting paths to follow in order to identify relevant biological age indicators to refine age estimation for these sensitive and challenging cases.

The use of posterior probabilities for age estimation highlights even more clearly the problem caused by demographic age groups (Garcin 2009): almost every probability distribution of age associated with the combinations significantly overlaps two adjacent quinquennial age groups. This makes it difficult to choose one age group rather than the other. The probabilities cannot be used to determine whether or not an individual is below or above one of the legal thresholds. In this regard, the distribution of the ranks can help orient the selection of the age group: if the combination tends to overestimate age and the confidence interval slightly overlaps the upper age group, it can be concluded that the lower age group is most likely to be the right one.

Because it is due to biological reality, interval or threshold overlapping cannot be bypassed without resolving to artificial transformations of the variables. This can lead to the loss of biological

meaning of the primal relationship between age and the predictor variables and may not even provide valid results. It also points back to the main obstacle of this study and the complexity of the questions behind the definition and exploitation of juvenility, which is the incoherence between the statistical result that is an estimate of age, the biological reality it aims to encapture and the interpretation of the estimate. Statistics are the only known asserted, objective and scientific tool for evaluating any method. However, this study shows that a rigid statistical frame of work could possibly be a source of limitation for a “satisfactory” social or legal interpretation of age that would also entirely and accurately integrate biological variability. The reductive, simplifying and somewhat rigid nature of a mathematical model compared to the ever-changing biological reality that is development is an obstacle for the anthropologist who cannot confidently admit that a model is entirely (or at least sufficiently) adapted or not to translate this reality into an interpretable biological fact (Artigue et al 2009). This can also explain why some methods built with somewhat imperfect protocols and/or invalid statistical (*e.g.* Ubelaker 1978) can give seemingly comparable or sometimes better results from the user’s point of view than scientifically and statistically valid methods (*e.g.* our approach using posterior probabilities). This phenomenon can be explained by the different strength of the correlation existing between a biological indicator used as a predictor and age, which covers in fact the extent of its variability and of all the factors that are responsible for it. In this lies the contradictory contribution of statistics to juvenile age estimation methods: the rigor behind statistised results is the best approach for objective method application and evaluation but they do not suffice for biological interpretation of these results, *i.e.*, in our case, interpreting skeletal developmental patterns and their relationship with age. When conducting a growth or maturation study, verifying the quality of the consistency between biology and statistics is therefore crucial in order to get as close to biological reality as possible. This also means collecting enough data to represent that reality and finding the best possible modelisation of that data; one that takes into account and statistically translates all its biological particularities (at best). Our study took up the challenge of finding a more suitable model for mathematically representing the relationship between age and biometric variables, without transforming or including too many intermediates between age and the biological parameters used to estimate it. MARS models seemed best suited for that purpose, but studies with larger samples would be necessary to confirm this and provide more realistic biological interpretations of the results.

Knowing the estimating bias of a method is not a sufficiently valid argument to explain the choice of the age group attributed to an individual, and several cases can arise when no satisfactory choice can be made. This is partly why methods in forensic anthropology are often constructed with the

specific goal of determining the position of an individual in relation to a particular age threshold: these are age-specific methods.

### 7.5.3. Age-specific versus non age-specific methods

Samples covering large age ranges present an important increase in biological variability, which was expressed through residual heteroscedasticity of the age prediction models in this study. This leads to the following question: do methods need to be constructed for each specific range of variability *i.e.* for each range of similar growth patterns, comparable ranges of variability and therefore belonging to a certain age range, or can methods cover individuals from the beginning to the end of the active phases of growth and maturation, knowing this implies changes in the ranges of variability? Age-specific methods are common for foetal individuals (Adalian et al 2002, 2006; Aka et al 2009; Butt and Lim 2014; Minier et al 2012; Olivier 1974; Wozniack et al 2009) and for older individuals from specific periods but rather inconsistent age limits, like adolescence (Albert and Maples 1995; Cardoso 2008b; Hunt and Gleiser 1955). This approach is already adopted in forensic anthropology: one of the most active research topics in age estimation of the living is finding methods that reliably and precisely determine the position of an individual compared to the 18-year threshold (or 21-year threshold, in some countries) (Dedouit 2009; Dedouit et al 2012; Quirmbach et al 2009; Saint-Martin et al 2013, 2014; Schmeling et al 2007, 2008). The privileged developmental parameter is bone maturation, the privileged medium of study is medical imaging, and the privileged areas used for these “older juveniles” are the sternal extremity of the clavicle (Garamendi et al 2011; Jit and Kulkarni 1976; Kreitner et al 1998; Schmeling et al 2004; Schulz et al 2005; Shirley 2009), the knee (Cameriere et al 2012a; Dedouit et al 2012; Hackman and Black 2013a; O’Connor et al 2008; Pyle and Hoerr 1955), the foot and ankle (Hackman and Black 2013b; Krämer et al 2014; Saint-Martin et al 2014; Whitaker et al 2002), and the hip (Owings-Webb and Suchey 1985; Schmidt et al 2011; Wittschieber et al 2013a, 2013b).

Using age-specific methods seems to point out that method selection requires a prior idea of the age group of the individual whose age needs to be estimated, in order to select the most appropriate method. This seems somewhat confusing and contradictory, but it can in fact be explained by the age-related patterns of biological activity exploited for age estimation. It seems logical that the most active processes during a particular developmental phase will evidently be preferred as estimators to construct an age estimation method specifically designed for the corresponding age range. Method application and the estimate resulting from this reflects the combination of data availability (type of skeletal/dental element used), type of data (expression of a developmental process), method

construction and user-experience. The first two elements are where method construction and application meet, and are the reason for constructing and applying age-specific methods.

A comparable number of age estimation methods cover longer chronological periods, from birth to early adulthood for instance (Black and Scheuer 1996; Cardoso et al 2013b; Hess et al 1932), but they sometimes use different predictor variables as age progresses (Black and Scheuer 1996) or obtain much larger prediction intervals and/or lower confidence intervals (Cardoso et al 2013a, 2013b). MARS models are an example proving that such non-specific methods can remain sufficiently accurate and reliable from the youngest to the oldest individuals, although precision fatally decreases because of the increase in inter-individual variability. The hinge values of the predictor variables limit the ranges of variables for which the relationship with age is constant. Adopting an age-specific or rather a variability-specific approach would mean building a method for each age range or for each age threshold, which does not seem feasible, unless the method is presented as a software and the hinge points are proven to be independent from sample structure (which is not completely the case in this study).

Although targeted or age-specific methods seem to be a solution for differentiating individuals older or younger than 18, this precept becomes difficult to apply when using biometric variables as predictors. Would age-specific methods take care of, for instance, heteroscedasticity? Would they allow sufficiently precise estimates and no overlapping? It is far from certain, as the prediction intervals constructed with the MARS models are dynamic and continuously increase with most of the variable ranges. Moreover, how would the individuals corresponding to the values of the hinge points be integrated in targeted models? These questions point right back to the initial problem of more or less arbitrarily subdividing a continuous process such as biometric growth to obtain a single age estimate (White and Folkens 2005). This is another argument in favour of the development of “calibrated age estimation methods”, combining growth and maturation (Minier et al 2014). Authors have proposed comparing biometric age with maturational age using one or several skeletal elements (Cameriere and Ferrante 2008; Lacey et al 1973; Prah-Andersen and Roede, *In Prah-Andersen et al 1979*), sometimes without resorting to the concept of age (Acheson 1956; Garcin 2009). However, as exact age still remains the information that is most demanded in both archaeological and forensic contexts (with the exception of the minor or major status), threshold-methods have a more limited interest to our field of study.

This study shows that the same approach (MARS models using biometric data) can apply to different ranges of data, depending on said data: iliac variables cover a specific developmental period (growth of the ilium prior to acetabular fusion), and by extension, a specific age range (0 to 12 years); clavicular and lumbar variables cover the entire post-natal growth phase of the bones, from 0



to 19 years. No clear conclusion can be made concerning the age-specific or non-age specific methods debate: although they would be more satisfactory when it comes to not breaking the developmental continuum, biological reality does not seem to point to the relevance of non-age specific methods, at least not for all biological parameters.

#### **7.5.4. Populations of study**

One central question that arose from this work concerned the relevance of age estimation methods that aim to be applied on individuals from the same specific population the methods were constructed on.

In respect of international regulations, and with the increasing internationalisation of travelling, immigrations and the blurring of borders, forensic experts are more and more likely to handle cases concerning individuals from almost any country in the world. This postulate has separated researchers into supporters of population-specific methods, many of whom are forensic anthropologists or pathologists (Chaillet et al 2005; Loder et al 1993; Mora et al 2001; Stull et al 2014a) and supporters of multipopulation methods, applicable to any individuals regardless of their origin and ancestry (Braga et al 2005; Cardoso 2007b). A high number of original age estimation methods or standards are population specific, or were adapted by researchers to be used on individuals from their respective countries (Bokariya et al 2010; Brown 1978; Chandrakanth et al 2012; Dahlberg and Menegaz-Bock 1958; de Caldas et al 2007; Galic et al 2013; Gillett 1997; Haataja 1965; Karadayi et al 2012; Karkhanis et al 2015; Kashyap and Koteswara-Rao 1990; Kimura 1977; Maia et al 2010; Moze and Roberts 2012; Owsley and Jantz 1983; Patond et al 2012; Pavon et al 2010; Sahni et al 1998; Shi et al 2009; Trodden 1982; Vignolo et al 1990; Willems et al 2001).

The argument against population-specific methods is the difficulty in answering the questions “what can be defined as a population” and “how can population variability be included in global models?” There are differences between populations related to geographic origin, genetic factors and socio-economic status (Pinhasi et al 2005) that have various influences on dental and skeletal development. Anthropologists should consider individual biological variability of maturation rates and the systematic effects of environment (Lampl and Johnston 1996), sex, secular trends (Chaillet et al 2005) on growth and maturation to predict age for juveniles. Living conditions and socio-economic status have a significant influence on the extent of the differences between bone age and chronological age. Added to genetic factors, these factors influence skeletal and dental maturation rates. In a healthy population, they contribute to individual variability and are particularly visible in specific conditions.

Age estimation is complicated by the contradictory conclusions found on the effects of socio-economic status and ethnicity on age estimation: no general tendency can be identified (Garamendi et al 2005); effects are population- and method-dependent (Ontell et al 1996; Schmeling et al 2001, 2003a). This is why these effects are difficult to quantify with sufficient precision and accuracy. Moreover, ancestry and sex are mostly unknown in both forensic and archaeological contexts. The current genetic, social and cultural mixing of individuals, the lack of reliability for ancestry determination (Albanese and Saunders, *In* Schmitt et al 2006; Edgar and Hunley 2009) leads to difficulties in finding a consensual and precise definition of a population in the biological sense. Population-specific (in the biological sense) methods have limited interest for past populations other than being applicable to other populations with similar age and sex distributions (Bello et al 2006; Garcin 2009; Gowland and Chamberlain 2002) and for the analyses of human remains of a single individual. Since forensic cases often imply no *a priori* knowledge of the population of origin of the studied individual, it seems practically unsatisfying to use population-specific methods for age estimation. In a forensic context, the use of such methods would have to be ethically and scientifically justified. This is why we considered the term “population” in a statistical sense throughout this study, *i.e.* by its age and sex distributions and by the range of variability covered by the data. This definition can seem somewhat artificial, but it is the only one that allows objective comparison of methods applied on “groups of individuals presenting similar or different structures”. If the statistical structure of populations is known and comparable, the impact of biological and social factors can then be considered as possible sources of variation.

The combination of these sources of variability and the addition of secular trends and pathologies (**see section 7.1.3.**) can result in misestimation of age in Past populations, and in extreme cases, they can cause misinterpretations of an archaeological sample or population (Gowland and Chamberlain 2002). The application of our age estimation methods on the individuals from the Luis lopes collection has raised such concerns without completely confirming them. Our methods could therefore be tested on archaeological samples of juvenile individuals for whom age (through parish or civil registers), living conditions and sanitary status are known. Only then will we be able to identify the causes for biased estimations, quantify their impact and shed light on other samples with similar structures.

### 7.5.5. Understanding developmental patterns and variability to improve age estimation

Growth studies are done to characterise and verify normal individual growth, compare population growth patterns or re-evaluate and eventually update existing growth standards (Adair 2007; Anderson et al 1956; Berkey 1982; Cameron et al 1982; Eckardt and Adair 2002; Molleson, *In* Buchet 1997; Scherdel et al 2015; Tanner 1981). For example, the Maresh reference dataset of bone radiographies of children aged 0 to 18 years (Maresh 1955) was compared to the World Health Organisation (WHO) standards for normal growth (WHO 2006a) on a population sample from India to verify if they could both be used for comparing growth pattern between populations and/or for inferring advances/delays in normal growth (Schillaci et al 2012). Biological growth patterns of juvenile skeletal elements are known to present the following characteristics: an increase in variability with age, and a strong homogeneity and low variability for prenatal individuals and individuals aged less than one year (Bogin 1997; Garcin 2009). This is partly verified for all the biometric variables used in this study: dispersion of the scatterplots increases dramatically with age, especially after 10 years.

There is no reliable or accurate method for verifying normal growth of an individual of unknown age and sex with sufficient certainty (Beauthier 2011). Inter-individual variability of the age at which growth spurts occur is well-known (Björk and Helm 1967; Gindhart 1973; Grave and Brown 1976; Özer et al 2006). A growth spurt is not systematically detected during childhood (Smith and Buschang 2004; Tanner and Cameron 1980) and it is possible for growth models not to show any spurts at adolescence, because individual growth spurts do not occur simultaneously and do not emerge from cross-sectional data (Gindhart 1973; Humphrey 1998). If international growth curves (using percentiles) were constructed for young children (WHO Multicenter Growth Reference Study Group 2006), reference growth curves during late childhood and adolescence are known to be sex- and population-dependent (Amselem et al 2007; Kuczmarski et al 2002; Rogol et al 2000), so results have varied interpretations depending on the reference curve used to control normal growth. This leads to the concept of allometry (Bareggi et al 1996; Jungers and German 1981) as a means to verify the absence of abnormality of development by combining information obtained on several elements and reliably affirm a delay or advance in development (Scheuer and Black 2000). This would only reliably exclude abnormal development, but would not prevent the under- or over-estimation of age if the individual presented a delay or advance in growth at the time of death/ time of the examination that could have been/could be resolved in the future.

The mathematical definition of allometry is the quantification of the relationship between the variations of one factor (the independent variable) as a function of variation of several other factors

(dependent variables) (Froman and Gourdon 2003; Teissier 1948). Regarding skeletal elements, allometry is the study of the covariation between bone size and bone shape (Hallgrímsson et al, *In Katzenberg and Saunders 2008; Marchal 1997*). Allometric studies help comprehend specific developmental patterns of a particular bone (*i.e.* how size and shape evolve compared to one another), and also compare and interpret allometric similarities or differences between different bones (Cameron et al 1982). For example, P. Buschang interpreted allometric differences between upper limb bones and lower limb bones as a result of developmental modifications due to bipedalism (Buschang 1982). Allometric studies also enable the detection of growth phases that would otherwise be overlooked because the range of variability covered by the sample does not exactly reflect true variability (Bogin 1999; Lewis 2007; Scheuer and Black 2000; Ulijaszek and Kerr 1999). Indeed, intra-individual variability can also be studied in allometric studies. An allometric study of the three skeletal elements chosen in this work could help to analyse these different levels of variability more specifically and understand the global development of these bones, in view of improving age estimation.

MARS models have an intrinsic component of classifying variables according to their predictive power. Therefore, constructing allometric MARS models, combining variables measured on the three bones, would enable us to statistically detect which biometric variables are the best predictors of age. This approach could be used to rank the predictive power of all possible age prediction variables according to statistical parameters (*e.g.* degree of correlation with age) and other parameters (*e.g.* preservation rate, sexual dimorphism). The same thing could be done with maturation indicators. Because of the age-dependent nature of individual variability and successive phases of growth and maturation, this approach could be tested on the entire developmental period of the elements or on samples covering a specific age group to highlight which estimator would be best suited for a given period. In this lies the main difficulty of juvenile age estimation: the best predictor of age is often dependent on age or an age group itself, as previously evoked in the paragraph concerning age-specific or non-specific methods. Instead of being a limitation of age estimation, it could be used as an asset.

Sexual dimorphism of adult biometric variables is mainly caused by differences in growth rates or by the combined differences of growth rates and duration. This explains the difference between male and female biometric variables, because maturation occurs earlier for females and leads to growth ending (Humphrey 1998; Rissech et al 2008). This is why growth is said to be prolonged for males. Variables showed systematic sexual dimorphism according to sex for all clavicular and lumbar variables between the ages of 0 and 19 years. There is in fact a significant relationship between biometric growth patterns and skeletal sexual dimorphism: sexual dimorphism results from sexual

bimaturism of skeletal elements, in terms of rates and duration (Humphrey 1998), especially from puberty onwards, accompanied by high inter-individual variability (Coleman 1969).

Skeletal growth can be influenced by diverse factors, on different levels (Micklesfield et al 2011; Olze et al 2004), depending on their origin (genetic factors), immediate environment and living conditions (Clarck et al 1986; Garn et al 1973), mechanical factors (Carter et al 1996; Chen et al 2010a), tissue interactions and intrinsic regulations (Colnot and Alliston 2010; Roche et al 1996) and epigenetic factors, with different levels of importance according to skeletal region, bone type, population specificities, age, sex, sanitary state, chronological period (Cardoso 2005; Eveleth and Tanner 1990; Gindhart 1973; Humphrey 1998). These factors can therefore lead to misestimation of age.

Socio-economic and environmental factors have a relative influence on growth. Studies comparing growth in size and weight of children showed that under-privileged children (malnourished, with limited access to medical treatment) negatively affected normal growth and maturation, resulting in developmental retardation and possibly stunting (Pathmanathan and Raghavan 2006; Prakash and Bala 1979; Prakash and Cameron 1981; Tanner et al 1975). It is interesting to note that socio-economic impact is reduced for prenatal and early postnatal development. This has been confirmed by allometric and conformation studies of skeletal elements (Wang et al 2009).

The impact of external factors on normal development is still not consensual. Studies have shown no particular difference in growth patterns between populations that presented different living conditions (urban/rural environment) that could impact normal development (Lewis 2002; Mays et al 2008). The impact of such factors is clearly more complex than it appears and cannot be generalised. It depends on other population specificities (ontogenetic, epigenetic, cultural factors), that cannot always be reliably assessed (Frelat and Mittroecker 2011; Garcin 2009; Mays et al 2008; Pathmanathan and Raghavan 2006). Indeed, a number of studies have found similar or differential growth patterns of several skeletal elements assimilated to comparable or distinct inter-population variability of absolute dimensions in relation to age and variation in growth velocities (Pinhasi et al 2005; Miles and Bulman 1994, 1995) without any specific factor associations emerging from the analyses. Because of the biases inherent to the samples (estimated ages, unreferenced archaeological samples, unbalanced age and/or sex ratios), these results cannot be reliably compared. This leads back to the importance of standardised population structures for any referenced growth-related study.

A geometric morphometrics study of the shape of the ilium (Faruch Bilfeld et al 2013; Wilson et al 2015) and of the fifth lumbar vertebra could be done to comprehend the complete developmental patterns of these bones. These analyses could identify particular maturational changes to refine age

estimation for older individuals, for whom prediction intervals are too large to provide sufficiently precise age estimates using biometric growth: skeletal maturation is indeed more active than skeletal growth during adolescence and takes over as the best indicator of age (Cunha et al 2009; Scheuer and Black 2000; White and Folkens 2005). Size variability is more important than shape variability. The idea presented by several authors would be to calibrate the age estimated with biometric growth with maturation “checkpoints” to control the normality of development. Calibration of age estimation methods based on growth parameters by controlling the results using maturation standards is one way of ensuring that the estimated age is consistent with the general developmental state of the individual (Bhat and Kamath 2007; Hadlock et al 1987; Thevissen et al 2012; Thomas et al 2000; Tocheri and Molto 2002). This seems more reliable than simply checking the position of an individual within the percentile curves of biometric growth, as they are also population- and sex-dependent. An individual outside the normal percentile ranges, of abnormal size for his/her age, can be considered pathological if maturation is also abnormal, but could also simply follow a normal development that is not included in the normal range of variability.

All the factors influencing skeletal development and related to sample composition are likely to lead to differences in age estimation. The differences observed between developmental patterns of the individuals from the Luis Lopes sample and the Marseilles sample could be quantified in a future study to recalibrate methods to correct this particular bias. This can only be done if sampling protocols and methods are standardised, as they were here. Recalibrating would increase accuracy, although some part of error will always be unknown when applying methods constructed on different populations, because the source of their variability cannot be entirely explained nor quantified (Cunha et al 2009; Rissech et al 2013a).

All these characteristics of growth and maturation were reflected in the MARS models built for juvenile age estimation, the maturation sequence of the iliac bone and the different ranges of posterior probabilities of age based on iliac maturation patterns. Completely understanding and characterising the complexity of biological development (and biometric growth in particular) seems to be the key to constructing juvenile age estimation methods that make biological sense, even though the results can sometimes be difficult to interpret (overlap of adjacent age groups, overlap of a legal threshold). A follow-up to this work would be to construct an exhaustive growth and maturation study using very important samples of the French population, and, possibly, longitudinal data. A more important number of individuals would provide a higher number of different combinations and enable the calculation of reliable sexed posterior probabilities. Other bones could also be considered to study intra-individual variability of skeletal maturation.

# Conclusion

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A particular issue of juvenile age estimation is choosing and applying the most appropriate method. This choice is partly based on objective statistical and methodological parameters, partly based on the material available and its preservation, and partly based on subjective or contextual criteria prioritisation. Because of the inherently variable levels of factors influencing skeletal development, and of methodological heterogeneity, conclusions drawn from method comparison and/or evaluation are inconsistent from one study to another. They depend on the method used, the population of study (both in a statistical and biological sense), sampling protocols, etc. Bearing in mind that population differences do exist, but that in practice, they are likely to be unknown, the anthropologist must take her/his decision for both method construction and application as a compromise between scientific rigor (methodological protocols) and biological reality (known/unknown biological background, developmental patterns).

This study was done in that perspective. It is very difficult to affirm that a general tendency should be followed for juvenile age estimation in physical anthropology. This is why the first step of our work was to identify objective and valid standardised protocols for data acquisition and analyses explicitly presenting statistical parameters seemed the best solution to provide methods whose quality can be objectively evaluated. The critical review of 256 juvenile age estimation methods done in this work showed that only a small proportion of methods respect valid sampling, statistical and methodological criteria identified as “gold standards” for method construction (Cunha et al 2009; Schmeling et al 2007). These standardised criteria seem the best guidelines for method construction and the best arguments to put forward for objective method evaluation. This first step enabled us to construct a methodological decisional tree for objective method selection that can be used in practice by anthropologists to objectively justify their choice of method in bioarchaeological or forensic reports.

We aim to transpose the methodological decisional trees into an improved and interactive decisional tool (software or mobile application) as a guide for the selection of juvenile age estimation methods. The user would be able to select the descriptive criteria of the methods following her/his personal preference, to provide the method(s) best suited to her/his particular case. All the sampling, methodological and statistical criteria would be provided with the chosen method along with full reference of the method(s)'original publication, to give the user the necessary arguments of justification, needed in forensic reports or population studies.

Once the criteria for a standardised and valid methodological protocol had been identified, the second step of our study was to apply this protocol to construct two standardised and valid new age estimation methods. As the scientific rigor entirely depends on the authors of the methods and



biological reality does not, choosing a standardised, harmonised and valid approach seems the best solution to uniformly integrate biological developmental variability, in order to improve juvenile age estimation.

The two approaches adopted to construct the age estimation methods in this study respect the standardised criteria of validity identified in several critical reviews of anthropological methods. Both MARS models and posterior Bayesian probabilities are applicable for age estimation using dry skeletal remains of the clavicle, fifth lumbar vertebra, ilium (for MARS models) and iliac bone (for posterior probabilities) and on reconstructed bone surfaces. They were constructed on referenced samples collected from the hospital databases of Marseilles and tested on three independent samples for validation: two samples of individuals currently living in the Marseilles and Toulouse areas, and one sample from the Luis Lopes osteological collection composed of individuals who lived in Lisbon during the first half of the 20<sup>th</sup> Century. The best results were obtained using ilium width, ilium module, ilium area, anterior and lateral vertebral heights, and maximum clavicular length as predictors. They provide sufficient reliability and accuracy, and 95% prediction intervals along with the mean estimate of age.

These methods also pushed us to integrate normal developmental variability and patterns of skeletal growth and maturation in our methods: non-parametric MARS models were initially chosen because parametric prediction models invariably produced heteroscedastic residuals, but in the end, they seem to be a better fit to model and integrate the changes in growth variability and the relationship between age and biometric data by producing dynamic prediction intervals. Independent Bayesian posterior probabilities of age seemed the best approach to construct a standardised age estimation method based on maturation stages of the iliac bone, and the acetabular epiphyses in particular, considering the size of the sample collected for the study and the statistical prerequisites in terms of standardisation and validity for an application on juvenile skeletal remains. If MARS models can be used in both a forensic and bioarchaeological context, because their reliability, accuracy and precision are high, posterior probabilities of age are not sufficiently precise for forensic application. However, they can be used in bioarchaeological or palaeodemographic studies to help decide which quinquennial age group an individual belongs to.

To improve the user-friendliness of our two approaches, MARS models will be implemented as a simple script or application for automatic calculation of mean estimates and prediction intervals, at an individual or sample level. A probabilistic distribution of age will also be associated to the prediction intervals to facilitate its interpretation. Allometric regressions combining biometric variables taken on the three types of bones will also be tested.

Our approach using posterior probabilities of age according to maturation stages of the iliac bone can be improved by including a higher number of individuals, and/or a higher number of stages to provide sexed and/or more precise results.

Both approaches will be tested on referenced archaeological samples to tackle the question of method recalibration to provide more accurate estimates of age and study the influence of known factors of variability responsible for biased estimates, such as secular trends, or other population factors. Studies combining and comparing biometric and non-biometric variables, *i.e.* using indicators of growth and maturation, will also be attempted to refine age estimates and verify the consistency between skeletal and/or dental growth and maturation patterns.

The main prospect drawn from this study is the necessity to correctly and globally comprehend developmental variability and the sources of this variability, in order to propose valid juvenile age estimation methods. This will require working on very large referenced samples of different origins and of known characteristics as using different analytical tools (regression analyses, allometric analyses, Bayesian probabilities and geometric morphometrics) in order to study and account for the different factors influencing skeletal development. In the same way as juvenile age estimation, the validity of developmental studies will require the application of standardised and adapted sampling protocols and statistical analyses.

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## French summary

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**Estimation de l'âge des individus immatures en anthropologie biologique :**  
**Analyse critique de méthodes existantes**  
**et application de deux approches méthodologiques standardisées**

***Caractéristiques des individus immatures et contexte d'étude en anthropologie biologique***

Les individus immatures ou non-adultes ont un statut particulier quel que soit leur contexte d'étude anthropologique. La place de l'enfant dans une société, une population, une culture, un cercle familial, ou le statut légal de mineurs sont des concepts qui sont souvent des transpositions sociales et légales de l'âge réel ou chronologique d'un individu immature. C'est pourquoi connaître l'âge des immatures en archéanthropologie ou en anthropologie médico-légale permet d'interpréter leur statut social ou légal, respectivement. Il faut donc estimer leur âge lorsque celui-ci est inconnu, ce qui est majoritairement le cas, afin d'appréhender le plus justement possible ce statut particulier.

L'étude des immatures en anthropologie biologique se fait par l'analyse qualitative ou quantitative des éléments du squelette, qui portent des indicateurs du développement osseux et dentaire. Ces indicateurs sont souvent la seule source d'information sur le statut d'immaturité d'un individu mais ne représentent qu'une seule des composantes du statut d'immaturité, la composante biologique. L'expression de la forte corrélation qui existe entre les indicateurs de développement (croissance et maturation) et l'âge chronologique des individus est l'âge biologique (osseux ou dentaire) va permettre d'affiner le statut d'immaturité de l'individu en question par l'estimation de son âge biologique.

Pour estimer l'âge des immatures, les anthropobiologistes se basent sur des indicateurs de croissance et de maturation osseux et/ou dentaires. La croissance est l'augmentation de la taille d'un élément osseux ou dentaire avec l'âge jusqu'à obtention de la taille adulte. Elle se mesure par des indicateurs biométriques (variables). La croissance est incrémentielle et continue. Elle présente plusieurs phases, des accélérations et des ralentissements alternatifs et des pics caractéristiques qui peuvent se rapporter à des tranches d'âge particulières.

La maturation englobe tous les changements de conformation et la fusion des éléments osseux, la minéralisation de l'émail et l'éruption dentaires qui ont lieu jusqu'à atteindre la forme adulte (de pair avec la croissance). Elle s'appréhende par des indicateurs non-biométriques (stades, descripteurs). La maturation est séquentielle et périodique. Elle présente elle aussi des phases caractéristiques dont la succession permet d'en contrôler le déroulement normal.

Ces deux processus sont soumis à des variations chronologiques (tendances séculaires), populationnelles, environnementales, socio-économiques, sanitaires, qui peuvent provoquer des différences développementales, et ainsi mener à des mésestimations de l'âge. Ces sources de variations sont bien connues aujourd'hui. Cependant, l'importance, l'étendue et la variabilité de leur impact sur le développement ne sont pas totalement appréhendées, donc le biais qu'elles constituent dans l'estimation de l'âge n'est pas quantifié.

L'anthropologue va donc principalement baser l'interprétation du statut de l'individu sur une estimation de son âge (l'âge biologique) et non sur son âge exact (l'âge chronologique). Ceci a d'autant plus d'importance que l'âge est l'un des deux paramètres du profil biologique (qui comprend l'âge, le sexe, la stature et d'autres particularités anatomiques et/ou pathologiques) que l'on puisse estimer de manière suffisamment fiable, juste et précise pour caractériser un individu immature à partir de ses restes osseux. Ceci vaut pour une estimation de l'âge faite sur un individu vivant ou à partir de restes osseux, même si les conditions éthiques et légales de l'examen et les conséquences varient dans les deux cas.

En ostéoarchéologie, les informations biologiques (le profil biologique) sont confrontées et complétées par celles fournies par l'environnement immédiat (i.e. la structure funéraire) et l'ensemble est analysé à l'échelle populationnelle pour en déduire le statut social de l'individu, ou déterminer et interpréter la structure démographique de ladite population.

En anthropologie médico-légale, l'âge d'un immature va indiquer s'il est mineur ou majeur et sa position par rapport aux différents seuils d'âge de minorités légales va définir son statut légal précis. Ce statut va déterminer les conditions pénales pour l'individu et/ou les personnes impliquées dans la même affaire.

Les méthodes utilisées pour estimer l'âge doivent donc être suffisamment précises pour que la différence entre l'âge estimé (biologique, osseux ou dentaire) et l'âge réel (calendaire, chronologique) soit la plus faible possible, suffisamment justes pour que les âges estimés appartiennent aux intervalles des âges possibles et suffisamment fiables pour que le degré de conformité entre les âges estimés et les âges réels soit suffisamment élevé ( $\geq 95\%$  par exemple) pour que leur statut soit correctement interprété. Quel que soit le contexte d'étude, les méthodes se doivent d'être standardisées pour être valides et surtout comparables entre elles. En effet, le choix et l'utilisation des méthodes est sujette à des biais matériels, à la volonté d'apprécier les variations intra-individuelles du développement, au fait de considérer ou non les spécificités populationnelles et/ou contextuelles. Plusieurs publications indiquent que les méthodes d'estimation de l'âge des immatures présentent des biais variés, tant au niveau des critères d'échantillonnage que statistiques. Les différentes méthodes publiées, qu'elles soient basées sur des indicateurs biométriques de

croissance, sur la maturation osseuse ou la minéralisation dentaire, respectent de manière inégale des critères d'échantillonnage, de fiabilité et de précision suffisamment élevés pour être scientifiquement et biologiquement valides. Elles demeurent donc relativement critiquables. À notre connaissance, ces biais méthodologiques n'ont jamais été quantifiés ni analysés de manière objective.

Le principal objectif de cette thèse est donc de construire une nouvelle méthode d'estimation de l'âge des individus immatures qui respecterait un protocole et des paramètres standardisés, identifiés par une étude approfondie de la littérature.

### ***Etat des lieux et analyse critique des méthodes d'estimation de l'âge des immatures***

Les approches adoptées pour obtenir l'âge des immatures peuvent être classées dans deux groupes : les méthodes d'estimation ou prédiction de l'âge « directes », telles que les modèles de régressions, les fonctions discriminantes, les probabilités d'âge, etc. et les méthodes d'estimation de l'âge « indirectes », telles que les atlas ou les tables de références (abaques) qui servent à établir l'âge d'atteinte d'un état développemental particulier ou attester d'un état développemental qui est ensuite rapporté à un âge ou un intervalle d'âge correspondant. Les méthodes d'estimation de l'âge sont adaptées à leurs sujets d'étude et au contexte : les processus développementaux les plus actifs sur la durée  $t$  (donc ayant une meilleure corrélation avec l'âge) sont utilisés comme estimateurs de l'âge préférentiels pour les individus dont l'âge est inclus dans cette durée et le matériel est étudié de manière à respecter et accorder au mieux les prérequis éthiques et scientifiques.

Plusieurs publications ont mis en avant la nécessité d'une standardisation méthodologique en anthropologie. Celle-ci concernait d'abord l'utilisation de critères statistiques, puis la présentation explicite des paramètres d'échantillonnage et statistiques d'une méthode. En contexte médico-légal, la présentation des paramètres méthodologiques est nécessaire pour faire une expertise valide devant la Cour, évaluer et justifier scientifiquement l'utilisation des méthodes. En contexte archéologique, la présentation des paramètres méthodologiques permet la quantification des biais d'estimation et de comparer plusieurs méthodes grâce aux critères statistiques, afin de limiter les erreurs d'interprétation.

Cette standardisation vaut pour la révision d'anciennes méthodes encore utilisées aujourd'hui et pour la construction de nouvelles méthodes. Il est reconnu que seul un nombre restreint de méthodes respectent suffisamment de ces critères pour être considérées comme standardisés, mais aucune étude n'a quantifié à grande échelle les biais statistiques de ces méthodes.



La première étape de ce travail a donc consisté en une analyse critique d'un ensemble de méthodes d'estimation de l'âge des immatures afin d'identifier des critères méthodologiques standardisés et valides. Les avantages et les limites méthodologiques et statistiques de méthodes utilisées en pratique sont mis en avant explicitement, et permettent une évaluation objective de ces méthodes. Un corpus de 256 méthodes d'estimation de l'âge des immatures a été collecté : il s'agit de méthodes référencées (publiées dans des revues scientifiques), et/ou trouvées dans des ouvrages de référence d'anthropologie biologique ou d'ostéologie. Chaque méthode référencée dans notre corpus s'est vue attribuer 21 critères descriptifs couvrant l'élément anatomique (osseux ou dentaire) utilisé (1), les paramètres d'échantillonnage (11), les paramètres statistiques (5), les paramètres méthodologiques (2), des paramètres transversaux (2). Chaque critère est caractérisé par des modalités descriptives particulières, qui traduisent le respect ou le non-respect des standards méthodologiques présentés dans des publications de référence (recommandations de l'Arbeitsgemeinschaft für Forensische Altersdiagnostik ou AGFAD, gold standards de la Forensic Anthropology Society of Europe ou FASE, critères Daubert). L'objectif était d'évaluer les fréquences des différentes modalités, notamment de celles jugées valides, pour les cinq critères descriptifs d'échantillonnage les plus objectifs (taille de l'échantillon, âge, sexe des individus, âge ratio et sexe ratio) et des cinq paramètres statistiques (fiabilité, précision, justesse, validation de la méthode, test des erreurs intra- et inter-observateurs des variables de prédiction), afin de proposer une analyse critique des méthodes à la disposition des anthropologues. Une classification empirique des méthodes du corpus a été construite sous la forme d'arbres décisionnels basés sur ces modalités et mettant en évidence les méthodes respectant les modalités d'échantillonnage et statistiques considérées comme valides : âge et sexe connus, âge et sexe ratio respectés et une taille d'échantillon respectable (plus de 200 individus) pour les paramètres d'échantillonnage ; fiabilité suffisante, précision et justesse indiquées, validation de la méthode sur un échantillon indépendant, erreurs intra- et inter-observateurs non significatives pour les paramètres statistiques. Seules 4 méthodes sur un total de 256 respectent ces dix critères de validité. Une seconde approche, statistique, a été adoptée en utilisant des Analyses en Composantes Multiples (ACM) suivies d'une classification ascendante hiérarchique (logiciel statistique R®), toujours à partir de ces mêmes dix modalités descriptives. Par cette approche, nous avons identifié 21 méthodes comme étant valides. Ces deux approches fournissent des résultats comparables, même si l'approche statistique est moins restrictive. Une minorité des méthodes d'estimation de l'âge des immatures respectent des critères standards de validité méthodologique. Les arbres décisionnels constituent un outil décisionnel utilisable en pratique pour sélectionner les méthodes d'estimation de l'âge respectant des critères d'échantillonnage et statistiques valides et des protocoles standardisés. La classification met en évidence les méthodes qui respectent des protocoles d'échantillonnages et des paramètres

statistiques standardisés, mais laisse le choix de la méthode à l'utilisateur. Cette analyse permettra à terme de proposer un outil décisionnel automatisé de sélection méthodologique adaptée selon les contextes d'étude.

### ***Application de protocoles méthodologiques standardisés***

#### *Matériel et méthodes*

Les conclusions tirées de l'analyse critique ont servi à élaborer le protocole méthodologique standardisé d'une nouvelle méthode d'estimation de l'âge osseux des immatures qui respecte tous les critères requis pour sa validation scientifique et biologique. Le matériel des échantillons d'apprentissage est constitué de séries de coupes tomodensitométriques obtenues au service d'imagerie de l'hôpital Nord (APHM – CHU Marseille). Les échantillons d'apprentissage sur lesquels la méthode est construite sont qualifiés d'échantillons de référence : l'âge et le sexe sont connus, les ratios d'âge et de sexe sont uniformes, les individus ne présentent aucune pathologie pouvant affecter le développement osseux normal. Les surfaces osseuses des os iliaques, de la cinquième vertèbre lombaire (L5) et des clavicules ont été virtuellement reconstruites à partir de ces coupes grâce au logiciel AVIZO®. Les caractéristiques développementales de ces trois os sont connues et leur conservation dans les séries ostéoarchéologiques est généralement bonne. Les individus sélectionnés ont des âges couvrant tout ou partie de leur développement : de 0 à 19 ans pour l'os iliaque (390 individus, dont 244 individus âgés de 0 à 12 ans pour lesquels des variables biométriques et non-biométriques ont été enregistrées), la cinquième vertèbre lombaire (402 individus) et la clavicule (321 individus).

Des variables biométriques uni- (longueurs, largeurs, hauteurs, diamètres) et bidimensionnelles (surfaces projetées, modules), définies par des landmarks ou des paramètres géométriques, ont été calculées à partir de ces reconstructions : quatre variables (deux uni- et deux bidimensionnelles) ont été mesurées sur les iliums; dix variables (huit uni- et deux bidimensionnelles) ont été mesurées sur les corps vertébraux des L5 et cinq variables uni-dimensionnelles ont été mesurées sur les clavicules.

Des variables non-biométriques ont été définies pour coter le degré de maturation des trois épiphyses de la zone acétabulaire et celles de la branche ischio-pubienne de l'os iliaque. La cotation a été faite sur les os iliaques droit et gauches pour les individus âgés de 0 à 19 ans. Trois stades ont été choisis pour définir trois états de maturation successifs des sites épiphysaires: 0 (absence de fusion épiphysaire), 1 (fusion épiphysaire en cours) et 2 (fusion complète). Des probabilités a priori d'appartenir à une classe d'âge ont été considérées comme uniformes et calculées pour les 20

classes d'âge annuelles. La probabilité d'observer un stade ou une combinaison de stades allant de deux à quatre par os iliaque pour chaque classe d'âge annuelle ont également été calculées. Les probabilités ont été calculées pour chaque stade de maturation à chaque site et pour plusieurs sites de l'os iliaque par les combinaisons de stades.

### *Résultats*

Le dimorphisme sexuel et l'asymétrie bilatérale ont été testés pour toutes les variables dans l'échantillon marseillais total. Aucune asymétrie bilatérale n'a été détectée pour les variables de l'ilium ; en revanche, elle est présente pour toutes les variables claviculaires. Les variables claviculaires présentent toutes un dimorphisme sexuel significatif avant 5 ans et après 11 ans. Les variables lombaires présentent un dimorphisme sexuel à des âges différents selon les variables, tout au long de la croissance. Les stades de maturation des trois épiphyses de l'acétabulum présentent un dimorphisme sexuel significatif entre 8 et 15 ans, alors que la fusion de la branche ischio-pubienne se fait de manière identique chez les filles comme chez les garçons, à partir de 4.5-5 ans.

La répétabilité et la reproductibilité des variables biométriques et non-biométriques sont suffisantes. La concordance entre mesures prises sur os sec et sur surfaces osseuses virtuelles a été vérifiée. Nous avons également proposé un protocole simple pour la réorientation d'éléments osseux dans un même plan afin de limiter au mieux les sources d'erreurs de mesures. Il y a un haut degré de colinéarité pour les variables biométriques obtenues sur le même os, ce qui peut rendre l'interprétation de régressions multivariées difficile.

Un ensemble d'équations de régression classiques modélisant la relation des variables avec l'âge comme variable prédite a été obtenu pour les individus des trois échantillons d'apprentissage grâce au logiciel statistique R®. À cause de l'hétéroscedasticité des résidus résultant des régressions classiques paramétriques par la méthode des Moindres Carrés Ordinaires (MCO) ou par celle des Moindres Carrés Pondérés (MCP), une nouvelle approche non-paramétrique a été testée. Les équations ont été construites avec des modèles en Multi Adaptive Regression Splines (MARS), qui présentent deux avantages importants : ils permettent la construction d'intervalles de prédiction robustes à l'hétéroscedasticité et dont la taille varie suivant la variabilité biométrique normale des individus (intervalles dits dynamiques). Les meilleures prédictions de l'âge ont été obtenues avec les variables bidimensionnelles iliaques, la longueur maximale de la clavicule et les hauteurs droite et gauche de la cinquième vertèbre lombaire. Ces équations ont été testées sur trois échantillons indépendants pour déterminer la justesse des prédictions: le premier est composé d'individus issus

du même service d'imagerie des hôpitaux de Marseille ; le deuxième consiste en des individus actuels provenant des services hospitaliers de Toulouse pour lesquels seuls le sexe, l'âge et les jeux de landmarks placés sur les iliums étaient disponibles ; le troisième est composé d'individus de la collection ostéologique de référence d'immatures de Lisbonne (collection Luis Lopes), datant de la première moitié du 20<sup>ème</sup> siècle. Ces tests ont montré qu'il n'existait pas de différence significative entre les âges réels et estimés par les modèles de régression MARS. Ces modèles ont été comparés à deux méthodes d'estimation de l'âge des immatures qui utilisaient les mêmes variables de prédiction et couvraient des tranches d'âge similaires : celle de S. Black et L. Scheuer (1996) utilisant la longueur maximale de la clavicule (Ln) et celle de C. Rissech et A. Malgosa (2005) utilisant les variables biométriques de l'ilium IL et IW, sur les trois échantillons tests. Nos modèles ont donné des résultats meilleurs ou comparables à ceux obtenus avec ces deux méthodes.

Les variables non-biométriques ont été utilisées pour calculer des probabilités d'âge *a posteriori* en fonction du stade de maturation osseuse des épiphyses de l'os iliaque. Un système de cotation en trois stades a été choisi comme compromis entre fiabilité et précision. Ces probabilités ont été calculées pour les sites épiphysaires un à un et pour les combinaisons de sites. Les résultats obtenus donnent des estimations de l'âge suffisamment fiables. Les combinaisons à deux stades donnent une fiabilité et une précision similaires à celles utilisant trois ou quatre stades. Notre approche donne des résultats comparables à ceux obtenus avec la méthode de H. Coqueugniot et collaborateurs (2010) appliquée aux échantillons tests de Marseille et de Lisbonne, avec une justesse d'au moins 90% pour une précision maximum de +/- 4 ans.

### **Discussion**

Ce travail a été mené sur un échantillon ostéologique inédit, suivant un protocole standardisé d'acquisition et de traitement statistique des données. Échantillonner la variabilité populationnelle nécessite l'adoption d'un protocole standardisé et adapté aux objectifs de l'étude. Ceci vaut pour la taille de l'échantillon, sa composition (âge, sexe, âge et sexe ratios) et ses caractéristiques (statuts socio-économique et sanitaire, origine géographique, période chronologique, etc.). Cette standardisation vise à minimiser l'influence de la structure de l'échantillon sur la qualité et l'applicabilité de la méthode construite à partir de ces individus. L'homogénéité de la répartition par âge et par sexe évite la sur- ou sous-représentation d'une ou plusieurs classes d'âge et ainsi ne biaise pas les estimations en faveur d'un groupe d'âge ou d'un sexe particulier. Cet échantillon d'étude homogène n'est pas fidèle à la répartition démographique normale de la population marseillaise,

mais il peut être considéré comme une population de référence ostéologique au sens statistique du terme et servir à d'autres études anthropologiques. La notion de population de référence soulève également des questions relatives à la variabilité « normale » et à son appréhension lors de la phase d'échantillonnage. L'échantillon est-il représentatif de la population dont il est issu ? Où se trouve la limite entre la variabilité extrême (valeurs extrêmes) de la normalité biologique qu'il faut inclure pour s'approcher au plus près de cette variabilité et la non-normalité (valeurs aberrantes ou outliers), traduisant des individus pathologiques, qu'il faudrait donc exclure ? Ces questionnements sont d'autant plus légitimes lorsque l'échantillon est constitué de patients de milieux hospitaliers, comme dans cette étude. C'est pourquoi les facteurs pouvant influencer le développement osseux des individus de l'échantillon et illustrer leur variabilité intrinsèque ont été qualifiés et/ou quantifiés afin d'identifier des facteurs pouvant potentiellement biaiser les résultats. De manière générale, il serait sans doute nécessaire de préciser les caractéristiques populationnelles, au sens non-statistique, qui pourraient influencer les résultats lors de la construction ou de l'application de méthodes anthropologiques. Ceci vaut particulièrement pour les populations du passé (populations de cimetière), telles que l'échantillon de la collection Luis Lopes, qui sont par définition des représentations biaisées des mêmes populations vivantes. Lorsque l'on travaille sur des populations du passé, les tendances séculaires sont une source supplémentaire de variabilité du développement qu'il faut considérer car elles peuvent participer aux biais des estimations de l'âge. Cependant, il a été montré que la structure des populations était une source de biais plus importante que les différences chronologiques dans les schémas développementaux, d'où l'importance de construire et tester des méthodes sur des échantillons dont la structure est connue avant de les appliquer sur des échantillons inconnus.

Les variables biométriques et non-biométriques utilisées dans cette étude sont pour la plupart identifiées comme de bons estimateurs de l'âge chez les individus immatures. Leurs comportements biologique (dimorphisme sexuel biométrique, bimaturisme, asymétrie bilatérale, corrélation avec l'âge) et statistique (répétabilité, reproductibilité, concordance os sec/os scanné) ont été quantifiés ou qualifiés et sont en accord avec ce qui est décrit dans la littérature. Cependant, nous n'avons trouvé que très peu de références mentionnant la présence d'hétérogénéité des variances des variables entre les classes d'âge. Dans notre étude, la présence systématique d'hétéroscédasticité résiduelle (variance résiduelle hétérogène) pour chacune des régressions paramétriques de l'âge en fonction de chaque variable questionne les résultats trouvés préalablement, qui demanderaient donc peut-être vérification, notamment pour ceux obtenus avec les mêmes variables.

La persistance de l'hétéroscédasticité résiduelle avec les équations paramétriques, et ce malgré les nombreuses transformations de variables, invalidait toute approche paramétrique pour la

prédiction de l'âge, qu'elle soit classique ou inversée. Cette hétéroscedasticité pourrait être une expression indirecte des changements de la variabilité biologique avec l'âge et ainsi seraient peut-être le reflet de l'hétérogénéité des changements des phases de croissance. Les modèles non-paramétriques MARS permettent d'obtenir des régressions simples ou multiples valides, même avec des variables présentant une hétéroscedasticité et une autocorrélation résiduelles persistantes, un fort degré de colinéarité et des non-linéarités ponctuelles dans leur relation avec l'âge. Ces modèles sont donc les plus adaptés à notre jeu de données par rapport à d'autres approches telles que les régressions inverses et les modèles bayésiens, qui nécessitent une homoscedasticité résiduelle pour être valides. De plus, ils permettent de modéliser directement les changements liés à l'âge de la variabilité développementale individuelle par les intervalles de prédiction fluctuants.

Les meilleures estimations de l'âge sont obtenues avec les deux variables bidimensionnelles iliaques (module et surface) et la largeur de l'ilium, la longueur maximale de la clavicule et les hauteurs latérales du corps vertébral de la cinquième vertèbre lombaire. La qualité prédictive des variables claviculaires et iliaques ont été démontrées dans d'autres études, mais celle des variables lombaires est inédite. Le choix de construire des modèles non-sexés s'inscrivait dans une logique d'optimisation d'application, car le sexe des individus immatures est souvent inconnu et ne peut être estimé à partir du squelette avec une fiabilité suffisante. Cependant, puisque les variables claviculaires et lombaires présentaient un dimorphisme sexuel significatif, des modèles de prédiction sexés pourraient être construits sur un plus grand échantillon et comparés aux modèles non-sexés afin de vérifier si la précision pourrait être améliorée. Même si les prédictions faites avec les variables biométriques respectent une fiabilité de 95%, la précision des estimations baisse à partir de l'adolescence (12-13 ans). Ceci questionne tout d'abord l'intérêt général de l'utilisation des variables biométriques pour prédire l'âge à partir de la deuxième moitié de cette période particulière, où la croissance certes continue, mais sa vitesse est réduite, et où la variabilité augmente d'avantage. Cette augmentation traduit certainement la variabilité de l'âge auquel le pic de croissance pubertaire se déclenche et la variabilité de la durée de la croissance pré-pubertaire. L'adolescence est caractérisée par un dimorphisme sexuel biométrique et auxologique, accentuant l'étendue de la variabilité. Ceci est visible pour les modèles de prédiction utilisant les variables claviculaires et lombaires qui couvrent toute l'adolescence. La largeur des intervalles de prédiction est la traduction statistique de l'augmentation de la variabilité biologique avec l'âge. Peut-on dire que l'hétéroscedasticité résiduelle est le reflet statistique de la variabilité de croissance inter-individuelle ? Cette variabilité augmente avec l'âge, mais elle est irrégulière et ne semble pas suivre de schéma particulier. Même si c'était le cas, rien ne dit que la variabilité serait la même dans d'autres échantillons, et elle n'est probablement pas totalement appréhendée dans l'échantillon marseillais.

L'utilisation pratique des modèles MARS présente des limites intrinsèques : même en utilisant la validation croisée et des échantillons-tests indépendants, les modèles sont susceptibles de fournir des résultats biologiquement incompatibles avec la réalité de croissance et ne peuvent s'affranchir totalement de l'influence de la composition de l'échantillon, notamment des outliers. Il serait intéressant d'une part d'augmenter significativement la taille de l'échantillon marseillais afin de tenter d'appréhender au mieux cette variabilité, masquer les effets d'éventuels outliers et d'autre part de confronter plusieurs échantillons issus de zones géographiques éloignées afin de voir si les variabilités et les schémas d'hétéroscédasticité résiduelle sont comparables.

La comparaison de nos modèles avec les méthodes référencées de S. Black et L. Scheuer (1996) et C. Rissech et A. Malgosa (2005) valide notre approche et son applicabilité sur os sec et surfaces osseuses virtuelles. Les résultats meilleurs ou comparables obtenus sur l'échantillon de Lisbonne avec ces deux méthodes de comparaison peuvent être modérés par le fait qu'elles ont été construites en partie sur ce même échantillon et par les mauvaises conditions socio-économiques et sanitaires de cette population qui ont sans doute influencé le déroulement normal du développement osseux et induit des biais d'estimation de l'âge.

Les probabilités d'âge *a posteriori* ont été construites en choisissant des probabilités a priori indépendantes et uniformes, respectivement plus adaptées au contexte anthropologique (données manquantes) et à l'estimation de l'âge (indépendance de la structure de l'échantillon et possibilité de comparaison avec d'autres méthodes similaires). Elles associent une probabilité à un âge, ce qui fournit un argument scientifique pour la validité statistique de l'estimation et facilite l'interprétation du résultat. De plus, la précision est modulable selon la volonté de l'utilisateur et le contexte d'étude. La grande fréquence d'erreurs d'estimation dans la classe des 10-14 ans peut s'expliquer par la forte activité de maturation acétabulaire caractérisant les individus à ce moment de leur développement et de l'apparition d'un bimaturation sexuelle significatif de l'os iliaque: en effet, l'âge des filles a tendance à être sous-estimé, alors que c'est l'inverse pour les garçons. Les erreurs pourraient sans doute être réduites et la précision pourrait être améliorée en sexant les probabilités, et en travaillant sur un échantillon beaucoup plus grand. Des analyses transitionnelles pourraient également être utilisées pour calculer l'âge de transition d'un stade au suivant, afin d'affiner la précision de l'estimation.

Les deux méthodes d'estimation de l'âge proposées ici (modèles MARS et probabilités d'âge *a posteriori*) suivent le principe KISS : Keep It Smart and Simple : elles sont simples et rapides à utiliser, méthodologiquement et statistiquement standardisées et valides. Elles se présentent sous la forme d'abaques avec les intervalles de prédiction associés pour une lecture directe des résultats. La

création d'une application mobile ou d'un script implémentant ces méthodes automatiserait d'avantage l'obtention des résultats. Ceci permettrait d'associer une probabilité d'âge à l'intervalle de prédiction fourni par les modèles MARS pour faciliter l'interprétation des résultats.

Indépendamment du contexte d'étude, la confirmation qualifiée et quantifiée de l'hétérogénéité et le manque de standardisation méthodologique rendent difficile la comparaison et la combinaison de méthodes d'estimation de l'âge. La standardisation méthodologique diminue les biais méthodologiques, améliore la validité des estimations, des profils biologiques et de ce fait, des interprétations archéoanthropologiques et paléodémographiques basées sur ces données biologiques. En anthropologie médico-légale, les experts mandatés par la Cour donnent un âge biologique obtenu à partir des marqueurs du développement biologique. Plus que les limitations éthiques associées à cette démarche (utilisation d'imagerie médicale en dehors d'un contexte de soin), l'expert doit faire preuve de la plus grande objectivité scientifique dans son estimation. Ceci n'est possible qu'en appliquant des méthodes standardisées. Les modèles MARS et les probabilités *a posteriori* développées ici répondent à toutes ces contraintes.

Cependant, malgré leur fiabilité et leur justesse suffisantes, ces méthodes sont limitées par le risque de chevauchement des classes d'âges quinquennales utilisées en archéoanthropologie et des seuils d'âge légaux par les intervalles de prédiction. Ce chevauchement illustre une possible discordance entre la réalité biologique et l'interprétation socio-culturelle ou légale de l'âge. En effet, les processus biologiques sont continus par opposition à des classes ou seuils d'âge catégoriels et présentent tous une augmentation de la variabilité individuelle avec l'âge, qui augmente le risque de chevauchement. Il s'agit d'un bon exemple du non-sens biologique que représentent les classes d'âge quinquennales et des limites de l'association entre le résultat statistique et la réalité biologique à l'interprétation bio-socio-culturelle ou légale. Comment affirmer qu'un modèle est adapté ou inadapté pour traduire la réalité du changement biologique perpétuel en fait biologique interprétable ? Et si les modèles semblent adaptés, comme c'est le cas dans cette étude, la rigueur statistique qui permet l'application et l'évaluation objective de méthodes ne contourne pas toujours la limite interprétative d'une estimation de l'âge.

Toutes ces remarques renvoient également aux choix stratégiques de construire des méthodes d'estimation de l'âge des immatures ciblées ou non. La construction de méthodes destinées à une catégorie d'âge particulière, ou la discrimination d'un individu par rapport à un seuil d'âge particulier (*e.g.* 18 ans) sont des pratiques d'estimation de l'âge courantes. Il semble logique que le processus biologique le plus actif durant une phase de développement particulière soit pris comme estimateur de l'âge pour la tranche d'âge correspondante. L'application de méthodes et les âges estimés sont le reflet de la combinaison entre les données disponibles (type d'élément osseux ou dentaire), le type



de données (expression du processus biologique), le protocole méthodologique adopté et l'expérience de l'utilisateur. Les deux premières composantes se situent à l'interface de la construction et de l'application de méthodes et expliquent l'existence et l'application de méthodes d'estimation de l'âge ciblées. Ces méthodes « ciblées » présentent la difficulté supplémentaire du choix plus ou moins artificiel des limites entre lesquelles la méthode peut s'appliquer et de la sous-estimation de la variabilité que cela implique.

Les méthodes non-ciblées peuvent rester suffisamment fiables et justes sur toute la durée du processus développemental exploité, mais la précision décroît fatalement à cause de l'augmentation de la variabilité individuelle avec l'âge et l'influence variable d'autres facteurs, tel que le sexe.

Aucune conclusion claire ne peut être tirée sur le débat entre méthodes d'estimation de l'âge ciblées ou non-ciblées : bien que les méthodes non-ciblées seraient plus satisfaisantes car elles ne coupent pas le continuum du développement et donnent des méthodes valides, la réalité biologique ne semble pas pointer vers la pertinence de ces méthodes, du moins pas pour tous les paramètres biologiques utilisables comme estimateurs de l'âge.

La combinaison de ces sources de variabilité ajoutées aux tendances séculaires et aux pathologies peut entraîner des estimations biaisées des individus dans les populations du passé, voire des biais d'interprétation des sites archéologiques. L'application de nos méthodes d'estimation de l'âge aux individus de la collection Luis Lopes soulève ces questions sans les confirmer. Nos méthodes pourraient donc être testées sur des échantillons archéologiques d'âge connu par des archives civiles ou paroissiales et dont les conditions sanitaires et socio-économiques étaient également connues. Ceci permettrait d'identifier et quantifier les sources de biais, ce qui pourrait servir à interpréter d'autres échantillons présentant les mêmes caractéristiques.

Une notion essentielle se dégage de cette étude : celle de variabilité développementale et de tout ce qu'elle implique en termes d'estimation de l'âge. En effet, il semble que l'appréhension des schémas du développement et de leur variabilité permettrait d'améliorer les méthodes d'estimation de l'âge. Il y a une augmentation normale de la variabilité avec l'âge, il existe une variabilité individuelle des rythmes et des pics de croissance. La difficulté pour l'estimation de l'âge est la mesure de leurs conséquences aux échelles individuelle ou populationnelle.

La croissance osseuse peut être influencée par différents facteurs, à différents niveaux, selon leur origine (génétique/épigénétique), l'environnement immédiat, les conditions de vie, les facteurs mécaniques, les régulations intrinsèques et extrinsèques, avec des différences selon la région anatomique, le type d'os, l'âge, le sexe, l'état sanitaire, la période chronologique, etc. Tous ces facteurs peuvent potentiellement biaiser des estimations de l'âge. Calibrer les méthodes

d'estimation de l'âge basées sur des paramètres de croissance par des paramètres de maturation est une des solutions pour vérifier que l'âge estimé et l'état de développement d'un individu sont cohérents. Ce serait en effet un moyen de vérifier l'absence de trouble du développement, mais ne peut pas résoudre la sous- ou sur-estimation de l'âge si l'individu présente un trouble du développement à sa mort / pendant l'examen. La recalibration peut s'appliquer à des populations récentes et passées, afin d'augmenter la justesse d'estimation. Cependant, une part d'erreur sera toujours présente lors de l'application de méthodes construites sur des populations différentes, car la source de leur variabilité ne peut pas être entièrement expliquée ou quantifiée.

Malgré ces limitations insolubles et les difficultés d'interprétation posées par le chevauchement des classes d'âge ou des seuils d'âge légaux, comprendre et appréhender la complexité du développement biologique, semble être la clé pour construire des méthodes d'estimation de l'âge biologiquement cohérentes.

### **Conclusion**

L'évaluation critique d'un corpus de méthodes d'estimation de l'âge des immatures utilisées en anthropologie a permis de qualifier et quantifier les biais et les limites de construction et d'application de ces méthodes, ainsi qu'identifier les critères de validité à respecter pour notre protocole d'étude. Nous envisageons de transformer la classification empirique en logiciel/application mobile afin de l'automatiser et la rendre plus didactique. Par un jeu de questions (les critères méthodologiques)/réponses (les modalités associées), l'utilisateur sera libre de choisir la ou les méthodes en fonction des critères méthodologiques qu'il souhaite privilégier. La validité des méthodes ainsi que l'ensemble de leurs critères descriptifs seront systématiquement indiqués. Nous pourrions également intégrer d'autres paramètres d'évaluation statistiques et pratiques (*e.g.* l'état de conservation des éléments).

Nos nouvelles méthodes d'estimations de l'âge des immatures se présentent sous la forme d'un abaque et/ou d'équations de régressions relativement simples d'application. Ces méthodes sont statistiquement valides et utilisables en contexte médico-légal et/ou archéanthropologique. Les perspectives faisant directement suite à ce travail sont de mieux appréhender la variabilité du développement, notamment à l'aide de plus grands échantillons, plus diversifiés ; de construire des régressions allométriques combinant des variables de croissance (biométriques) et/ou de développement (stades de maturation), sur les mêmes os utilisés ici ou sur d'autres. Ceci permettrait

de hiérarchiser le pouvoir de prédiction des variables en fonction de l'âge et à terme d'affiner l'estimation.

Une perspective plus générale faisant suite à ce travail serait de mener une étude exhaustive sur la croissance et la maturation osseuse basée sur de très grands échantillons de la population française. Cette étude mêlerait données biométriques, relations allométriques et analyses de morphométrie géométrique afin de comprendre et quantifier l'étendue de cette variabilité.

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**Estimation de l'âge des individus immatures en anthropologie biologique :**  
**Analyse critique de méthodes existantes et application de deux approches méthodologiques standardisées**

L'âge est l'un des paramètres du profil biologique que l'on puisse estimer de manière suffisamment fiable et précise pour caractériser un individu immature à partir de ses éléments osseux ou dentaires. Les nombreuses méthodes utilisées respectent de manière inégale des critères méthodologiques scientifiquement et biologiquement valides. Elles demeurent donc relativement critiquables. Ce travail présente une analyse critique d'un corpus de 256 méthodes d'estimation de l'âge des immatures utilisées en anthropologie biologique. Cette première analyse a permis de qualifier et quantifier les biais et les limites de construction et d'application des méthodes et propose une classification objective des méthodes utilisable en pratique, mettant en évidence celles qui respectent des critères statistiques et méthodologiques valides. Les conclusions tirées de cette analyse critique ont servi à élaborer le protocole méthodologique d'une nouvelle méthode standardisée d'estimation de l'âge osseux d'individus immatures. Les surfaces osseuses de l'os iliaque, de la cinquième vertèbre lombaire et de la clavicule ont été virtuellement reconstruites à partir de coupes tomodensitométriques d'individus marseillais grâce au logiciel AVIZO®. Des variables de croissance biométriques et des variables maturationnelles non-biométriques ont été obtenues sur ces trois os. Un ensemble d'équations de régression non-paramétriques de type Multi-variate Adaptive Regression Splines (MARS) modélisant la relation des variables biométriques avec l'âge a été calculé à partir d'échantillons d'apprentissage grâce au logiciel statistique R®. Les meilleures prédictions de l'âge ont été obtenues avec les variables iliaques, les hauteurs latérales de la cinquième vertèbre lombaire et la longueur maximale de la clavicule. Ces équations ont été validées sur trois échantillons de validation de Marseille, Toulouse et de la collection ostéologique de référence d'immatures Luis Lopes. Ces modèles donnaient des résultats meilleurs ou comparables à d'autres méthodes « classiques » d'estimation de l'âge. Des probabilités d'âge *a posteriori* en fonction des indicateurs de maturation de l'os iliaque ont été calculées. Les résultats étaient suffisamment fiables, mais la précision était trop faible pour être valide. Notre nouvelle méthode d'estimation de l'âge des immatures se présente sous la forme d'un abaque et d'équations avec 95% de fiabilité et une justesse supérieure à 90%. Elle est standardisée, statistiquement valide, prend en compte les changements de la variabilité normale de croissance et utilisable en contexte médico-légal et archéologique.

**Mots-clés :** anthropologie biologique ; immatures ; estimation de l'âge ; standardisation ; méthodologie ; variabilité

**Juvenile age estimation in physical anthropology:**  
**A critical review of existing methods and the application of two standardised methodological approaches**

Age is one of the parameters of the biological profile of a juvenile individual that can be estimated from bones or teeth with sufficient reliability and precision. Many juvenile age estimation methods currently available to anthropologists do not follow scientifically nor biologically valid methodological criteria. This work starts with the critical analysis of a corpus of 256 juvenile age estimation methods used in physical anthropology. This analysis qualified and quantified the biases and limitations of method construction and application. It also presents an objective and practical classification of methods that highlights methods respecting valid sampling and methodological protocols and statistical criteria. The conclusions of this first study enabled the elaboration of a valid and standardised methodological protocol to construct a new standardised juvenile age estimation method. The surfaces of the iliac bone, fifth lumbar vertebra and the clavicle were virtually reconstructed from CT scans of individuals from Marseilles using the AVIZO® software. Biometric growth variables and non-biometric maturation indicators were taken on the three bone types. Several non-parametric Multi-variate Adaptive Regression Splines (MARS) models were calculated to model the relationship between age and the variables using the R® software. The best age prediction models were obtained with the variables taken on the ilium, left and right vertebral heights and maximum clavicular length. These models were validated on three test samples from Marseilles, Toulouse and the Luis Lopes referenced osteological collection and gave comparable or better results than other referenced juvenile age estimation methods. Posterior probabilities of age were calculated using the maturation stages of four iliac bone epiphyses. Results were reliable, but precision was too low for validation. Our new juvenile age estimation method can be used as an abacus or as regression equations to predict individual age with 95% reliability and at least 90% accuracy. It is standardised, statistically valid, integrates changes in normal growth variability and is applicable in both a forensic or bioarchaeological context.

**Key-words:** physical anthropology; juveniles; age estimation; standardisation; methodology; variability



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## THÈSE

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**JUVENILE AGE ESTIMATION IN PHYSICAL ANTHROPOLOGY:  
A CRITICAL REVIEW OF EXISTING METHODS AND THE APPLICATION  
OF TWO STANDARDISED METHODOLOGICAL APPROACHES**

- **APPENDICES** -

Pour obtenir le grade de DOCTORAT d'AIX-MARSEILLE UNIVERSITÉ

SPECIALITÉ : ANTHROPOLOGIE BIOLOGIQUE

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## APPENDIX A

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Critical analysis of juvenile age  
estimation methods: Frequency tables  
and empirical classifications

## Frequency tables of methodological criteria

Table A-1 Frequencies in percentages of methods using developmental factors of dental elements. The bold italic frequencies are the highest

	Cranium		
<b>Total</b>	33,590		
<b>Material</b>	Teeth		
	<b>64,37</b>		
<b>Process</b>	Growth	Mineralisation and/or eruption	Growth and mineralisation
	10,53	<b>83,93</b>	5,26
<b>Type of teeth</b>	Deciduous teeth		Total frequency per bone/region
	0	16,67	14,04
	Permanent teeth		Total
	0	<b>61,70</b>	<b>56,14</b>
	Deciduous and permanent teeth		Total
	<b>100</b>	25	31,58

Table A-2 Frequencies in percentages of methods using developmental factors of cranial elements. The bold italic frequencies are the highest

	Cranium		
<b>Total</b>	33,59		
<b>Material</b>	Bones		
	37,93		
<b>Process</b>	Growth	Maturation	Growth and maturation
	27,27	<b>63,634</b>	9,09
<b>Anatomical region</b>	Face		Total frequency per bone/region
	50	50	54,55
	Vault		Total
	33,33	33,33	36,36
	Base		Total
	50	<b>83,33</b>	<b>78,79</b>
	Frontal bone		Total
	33,33	37,5	39,39
	Parietal bone		Total
	25	33,33	33,33
<b>Bone and frequency</b>	Temporal bone		Total
	25	45,83	42,42
	Occipital bone		Total
	50	<b>91,67</b>	<b>84,85</b>
	Facial bones		Total
41,67	33,33	39,39	
Sphenoid		Total	
25	66,67	57,57	
Mandible		Total	
41,67	45,83	48,48	

Table A-3 Frequencies in percentages of methods using growth parameters taken on postcranial skeletal elements. The bold italic frequencies are the highest

		Postcranium				
<b>Total</b>		<b>69,88</b>				
<b>Process</b>		Growth				
		24,30939227				
<b>Anatomical region</b>	Upper Limb bones	Lower limb bones	Pelvic Girdle	Scapular Girdle	Vertebral Column	Thorax
	<b>17,13</b>	13,26	7,73	6,08	2,76	3,31
	Long bones*		Ilium	Clavicle	Cervical vertebrae	Ribs
	<b>13,81</b>		6,63	3,87	2,76	3,31
<b>Bone and frequency</b>	Humerus	Femur	Ischium	Scapula	Thoracic vertebrae	Sternum / Manubrium
	9,94	<b>11,05</b>	4,42	4,97	1,66	2,21
	Radius	Tibia	Pubis		Lumbar vertebrae	Hyoid bone
	9,94	<b>11,05</b>	4,42		1,66	1,66
	Ulna	Fibula	Sacrum			
	8,29	8,84	1,66			
	Elbow	Knee				
	8,29	7,73				
	Hand/Wrist bones	Foot/ankle				
	6,63	3,31				

Table A-4 Frequencies in percentages of methods using maturation parameters taken on postcranial skeletal elements. The bold italic frequencies are the highest

	Postcranium					
<b>Total</b>	<b>69,88</b>					
<b>Process</b>	Maturation					
	<b>69,06</b>					
<b>Anatomical region</b>	Upper Limb bones	Lower Limb bones	Pelvic Girdle	Scapular Girdle	Vertebral Column	Thorax
	<b>33,70</b>	21,55	14,36	17,68	9,94	13,26
	Long bones		Ilium	Clavicle	Cervical vertebrae	Ribs
	<b>26,52</b>		13,81	17,68	8,84	11,05
	Humerus	Femur	Ischium	Scapula	Thoracic vertebrae	Sternum/Manubrium
	16,02	17,12	13,81	7,73	6,08	6,08
	Radius	Tibia	Pubis		Lumbar vertebrae	Hyoid bone
	16,57	17,68	13,81		6,08	3,31
	Ulna	Fibula	Sacrum			
	16,02	16,57	6,08			
	Elbow	Knee				
	13,81	13,81				
	Hand/Wrist	Foot/Ankle				
	<b>23,76</b>	9,9				

Table A-5 Frequencies in percentages of developmental factors, skeletal and dental elements per age group. The bold italic frequencies are the highest

	Childhood	Adolescence	Childhood/Adolescence	Adolescence/Adulthood	Childhood/Adolescence/Adulthood
<b>Total number</b>	34	14	<b>91</b>	49	73
<b>Growth</b>	41,18	0	31,87	2,04	16,44
<b>Maturation</b>	<b>50</b>	<b>85,71</b>	<b>62,64</b>	<b>93,88</b>	<b>71,23</b>
<b>Growth and maturation</b>	8,82	14,28	4,39	4,08	12,33
<b>Cranial bones</b>	23,53	14,28	7,69	10,20	17,81
<b>Teeth</b>	<b>35,29</b>	0	<b>28,57</b>	12,24	16,44
<b>Limb bones</b>	<b>26,47</b>	28,57	27,47	28,57	<b>24,66</b>
<b>Hand</b>	8,82	28,57	<b>29,67</b>	2,04	17,81
<b>Foot</b>	2,94	7,14	8,79	0	13,69
<b>Iliac bone</b>	11,76	0	10,99	18,37	19,18
<b>Scapular girdle</b>	5,88	0	7,69	<b>40,82</b>	16,44
<b>Vertebral column</b>	8,82	7,14	7,69	10,20	8,22
<b>Thorax</b>	2,94	0	4,39	20,41	17,81
<b>Joint regions</b>	11,76	<b>42,86</b>	<b>29,67</b>	4,08	19,18

Table A-6 Frequencies in percentages of developmental factors, age group, and skeletal/dental elements of the methods according to the medium used for data acquisition. The bold italic frequencies are the highest

	Material type	Dry bones	Fresh bones	In situ	Radiographic images	CT scans	MRI scans	Ultrasounds	Histologic slices	Biochemical variables	Living	Unknown
	All methods	27,79	1,16	1,16	<b>51,74</b>	6,56	2,70	2,70	5,41	1,16	3,09	0,77
Factor	Growth variables	31,94	33,33	0	19,40	17,65	0	14,28	0	0	12,5	<b>50</b>
	Maturation variables	<b>55,56</b>	<b>66,67</b>	<b>100</b>	<b>76,87</b>	<b>82,35</b>	<b>100</b>	<b>71,43</b>	<b>78,57</b>	<b>100</b>	<b>87,5</b>	<b>50</b>
	Growth and maturation variables	12,5	0	0	3,73	5,88	0	14,28	14,28	0	0	0
Age group	Childhood	20,83	0	<b>33,33</b>	10,45	0	0	14,28	14,28	0	<b>50</b>	0
	Adolescence	1,39	0	0	6,72	5,88	<b>28,57</b>	0	7,14	0	0	0
	Childhood/Adolescence	16,67	0	<b>33,33</b>	<b>48,51</b>	29,41	14,28	<b>42,86</b>	7,14	0	<b>50</b>	<b>50</b>
	Adolescence/Adulthood	23,61	33,33	0	11,19	<b>47,06</b>	<b>28,57</b>	28,57	28,57	33,33	0	0
	Childhood/Adolescence/Adulthood	<b>37,5</b>	<b>66,67</b>	<b>33,33</b>	22,39	17,65	<b>28,57</b>	14,28	<b>42,85</b>	<b>66,67</b>	0	<b>50</b>
Cranium	Cranial bones	20,83	<b>33,33</b>	0	8,21	29,41	0	0	14,28	<b>33,33</b>	12,5	<b>50</b>
	Teeth	9,72	0	<b>100</b>	24,63	5,88	0	0	<b>35,71</b>	<b>33,33</b>	<b>87,5</b>	<b>50</b>
Post-cranium	Limb bones	<b>31,94</b>	<b>66,67</b>	0	25,37	17,65	<b>28,57</b>	14,28	21,43	0	0	0
	Iliac bone	29,17	<b>33,33</b>	0	7,46	11,76	0	28,57	14,28	0	0	0
	Scapular girdle	26,39	33,33	0	7,46	<b>35,29</b>	<b>28,57</b>	14,28	7,14	0	0	0
	Vertebral column	9,72	<b>33,33</b>	0	7,46	17,65	0	0	0	0	0	0
	Thorax	13,89	<b>66,67</b>	0	5,97	35,29	0	0	14,29	<b>33,33</b>	0	0
	Joint regions	2,78	33,33	0	<b>31,34</b>	17,64	0	<b>42,86</b>	7,14	0	0	0

Table A-7 Frequencies in percentages of the different sampling, statistical and methodological criteria recorded for the corpus of methods. The bold italic frequencies are the highest

Sampling criteria												
Age		Age ratio		Sex		Sex ratio		Sexed methods				
Unknown	Known	Unknown	Known	Unknown	Known	Unknown	Known	Unknown	Known			
7,72	<b>92,27</b>	22,39	<b>72,20</b>	11,97	<b>88,03</b>	20,077	<b>79,92</b>					<b>96,91</b>
			No	Yes			No	Yes	3,089	No	Yes and no	Yes
			<b>58,28</b>	49,19			32,37	<b>56,04</b>		33,86	5,58	<b>60,56</b>
Sample size												
Unknown		≤100	100-200		200-500		500-1000		≥1000			
2,32		24,71	<b>25,48</b>		17,76		14,29		14,67			
Type of study												
Cross-sectional			Longitudinal			Mixed			Unknown			
<b>78,38</b>			17,37			3,09			0,77			
Statistical and methodological criteria												
Intra- and inter- observer errors												
Not tested												
Tested												
40,54												
<b>59,07</b>	Presence of intra- and inter-observer error		Presence of intra-error observer error		Presence of inter-error observer error		Absence of intra-observer error		Absence of inter-observer error		Absence of intra- and inter-observer error	
	10,47		2,86		6,67		13,33		15,24		<b>51,43</b>	
Reliability			Standard prediction error			Accuracy / Percentiles						
Unknown	Known		Unknown	Known		Unknown	Known					
<b>57,14</b>	42,47		26,64	<b>73,97</b>		<b>57,14</b>	42,86					
Validation techniques												
Cross-validation		Independent sample		Other study		Not done		Unknown		Accuracy		Percentiles
2,32		10,04		41,31		<b>42,08</b>		6,18		9,09		<b>90,91</b>
Purpose of study												
Age estimation			Growth/maturation study			Age estimation and growth/maturation study						
<b>50,58</b>			39,38			9,65						



## Empirical classification trees for method evaluation and selection

The 256 juvenile age estimation methods analysed in our study are presented in empirical classification trees. This system is organised as follows:

- There is one classification tree per page. Each classification tree regroups the methods using variables obtained from the same anatomical region and reflecting the same developmental process: growth and/or maturation. The anatomical region and the developmental process represent the base of the tree. The branches range from the base of the tree (the anatomical element of study) to the leaves (the age estimation methods);

- Each branch represents a modality of one of the five sampling criteria of the methods. The user progresses through the tree by successively following the branches until the corresponding method is reached. Each method is characterised by the modalities the user followed to attain it.

The order of the sampling criteria is always the same: age, age ratio, sex, sex ratio, and finally sample size. Modalities circled in bold lines are considered valid, whereas framed modalities are not;

- Once the method is reached, it can be evaluated by its statistical characteristics using pictograms representing four statistical parameters: reliability, accuracy, precision and the testing of observer errors.

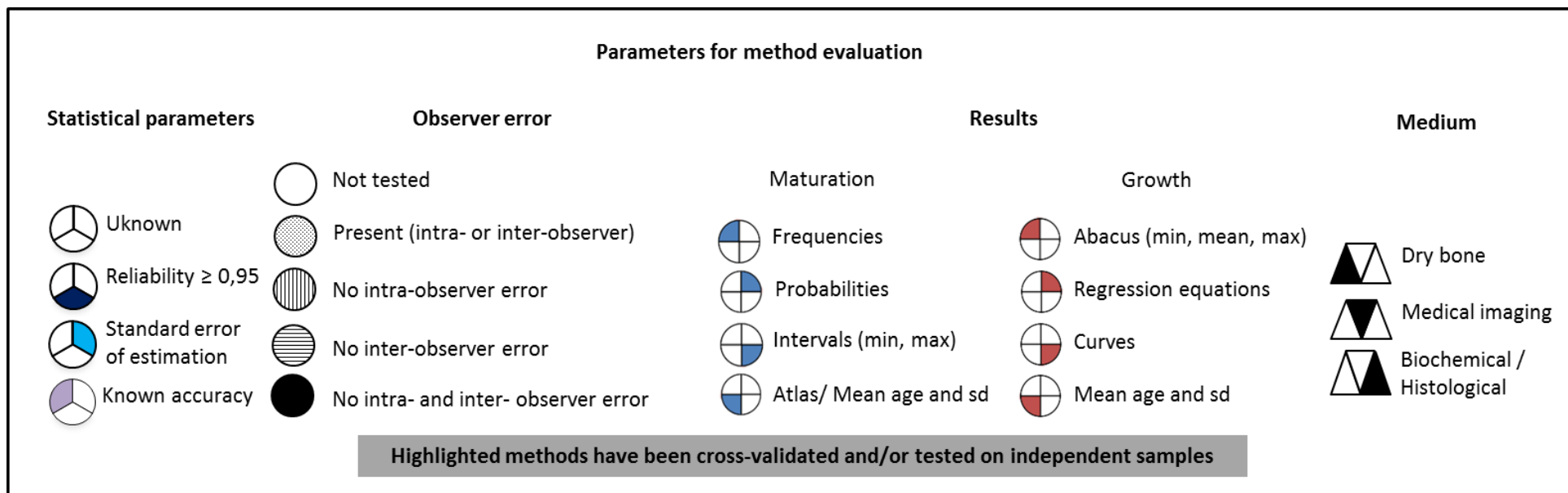
Additional indications on the method are also represented: the media of study of the anatomical element of interest (*e.g.* X-ray, dry bone) and the presentation of the method (*e.g.* curves, abacuses, frequencies).

The methods highlighted in grey have been tested by the authors themselves or in other studies for validation.

The references of the methods presented in the trees can be found in the “References” section at the end of Appendix A.

The classification and the references can be used in practice as a decisional tool to help with the selection, justification and citation of juvenile age estimation methods in forensic or bioarchaeological contexts.

The legend of the pictograms (below) can be cut out to serve as a reminder and bookmark when using the classification for method selection.



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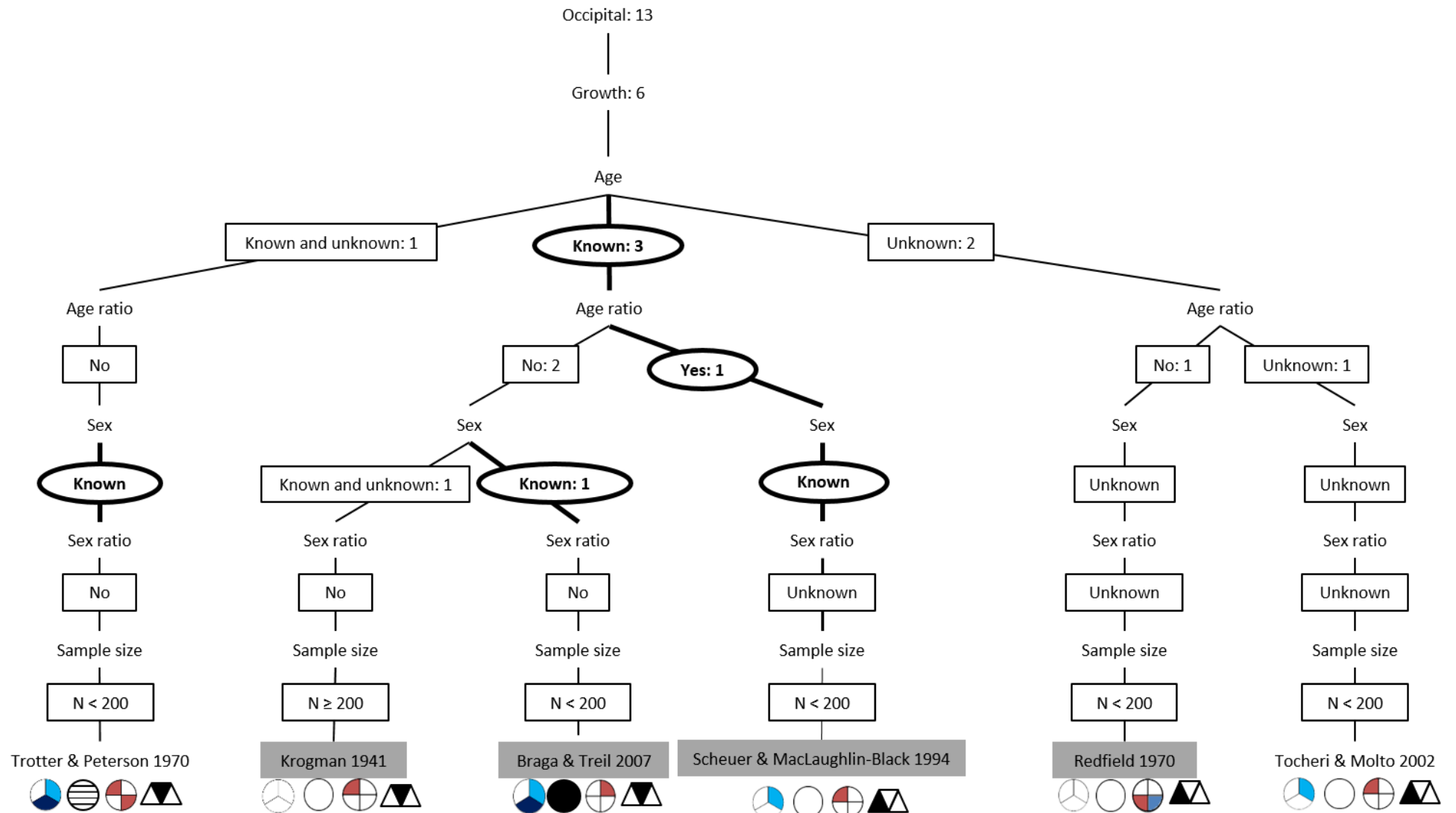


Figure A.1 Classification tree of the juvenile age estimation methods based on the growth of the occipital bone

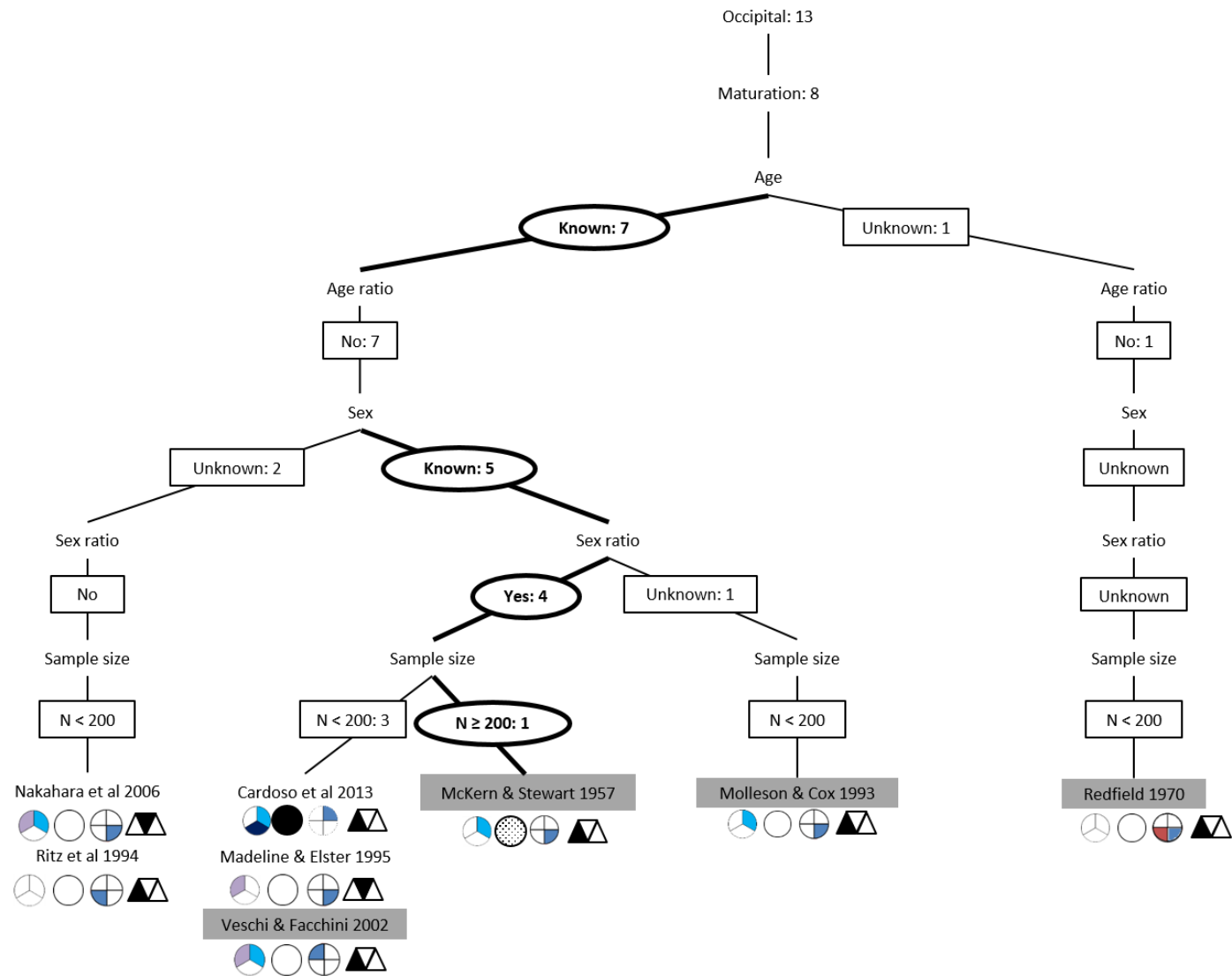


Figure A.2 Classification tree of the juvenile age estimation methods based on the maturation of the occipital bone

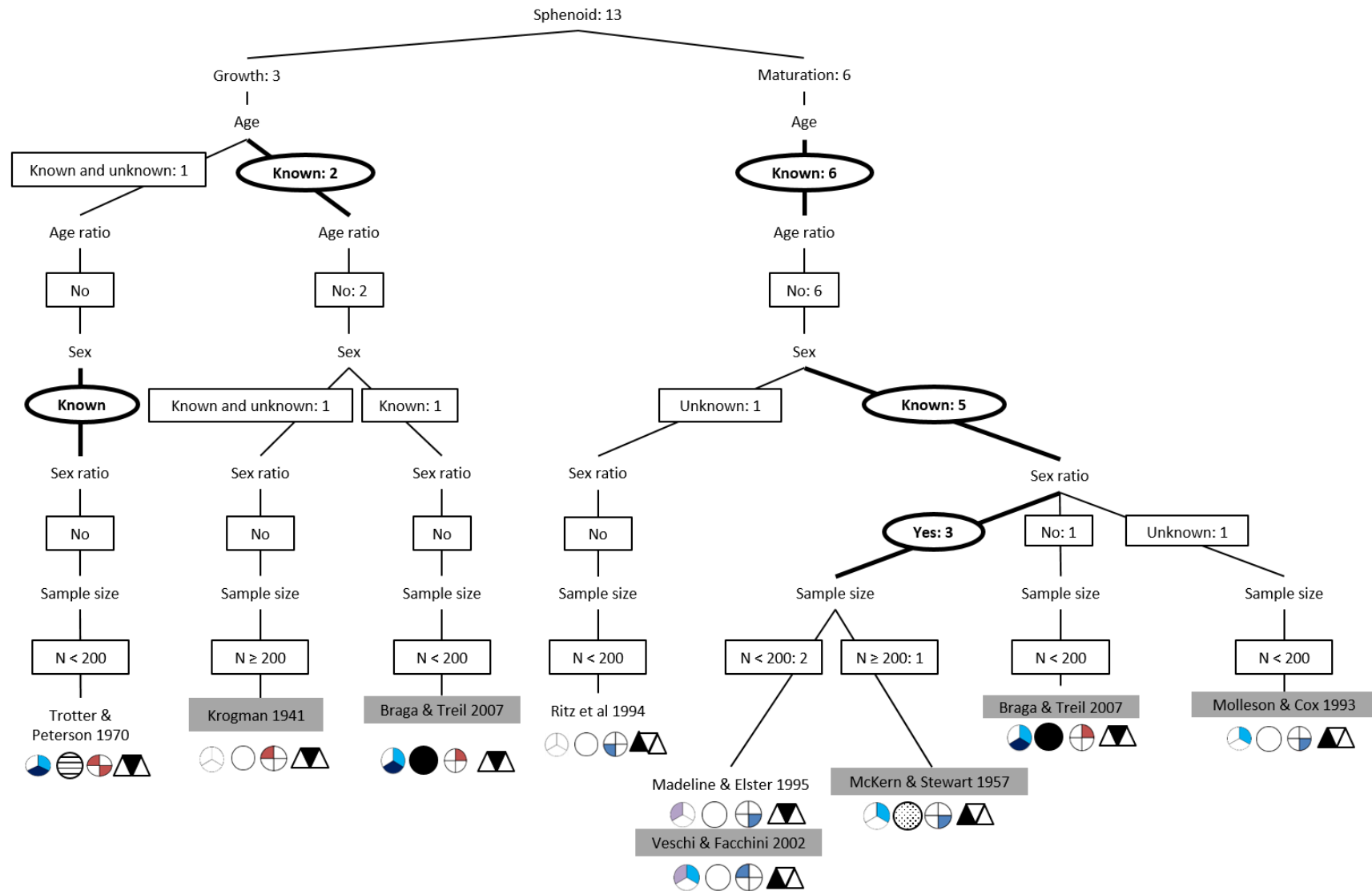


Figure A.3 Classification tree of the juvenile age estimation methods based on the growth or the maturation of the sphenoid bone





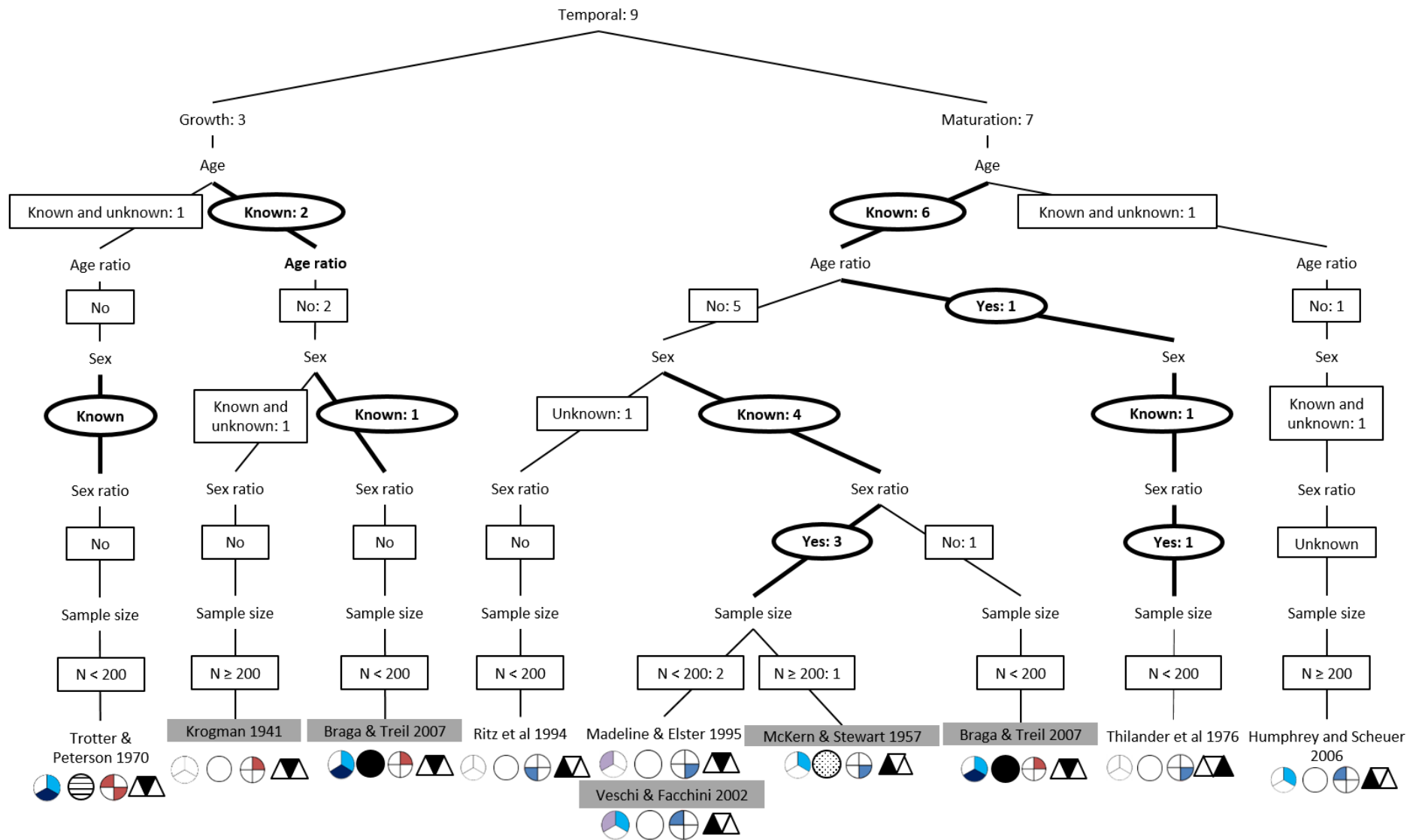


Figure A.5 Classification tree of the juvenile age estimation methods based on the growth or the maturation of the temporal bone

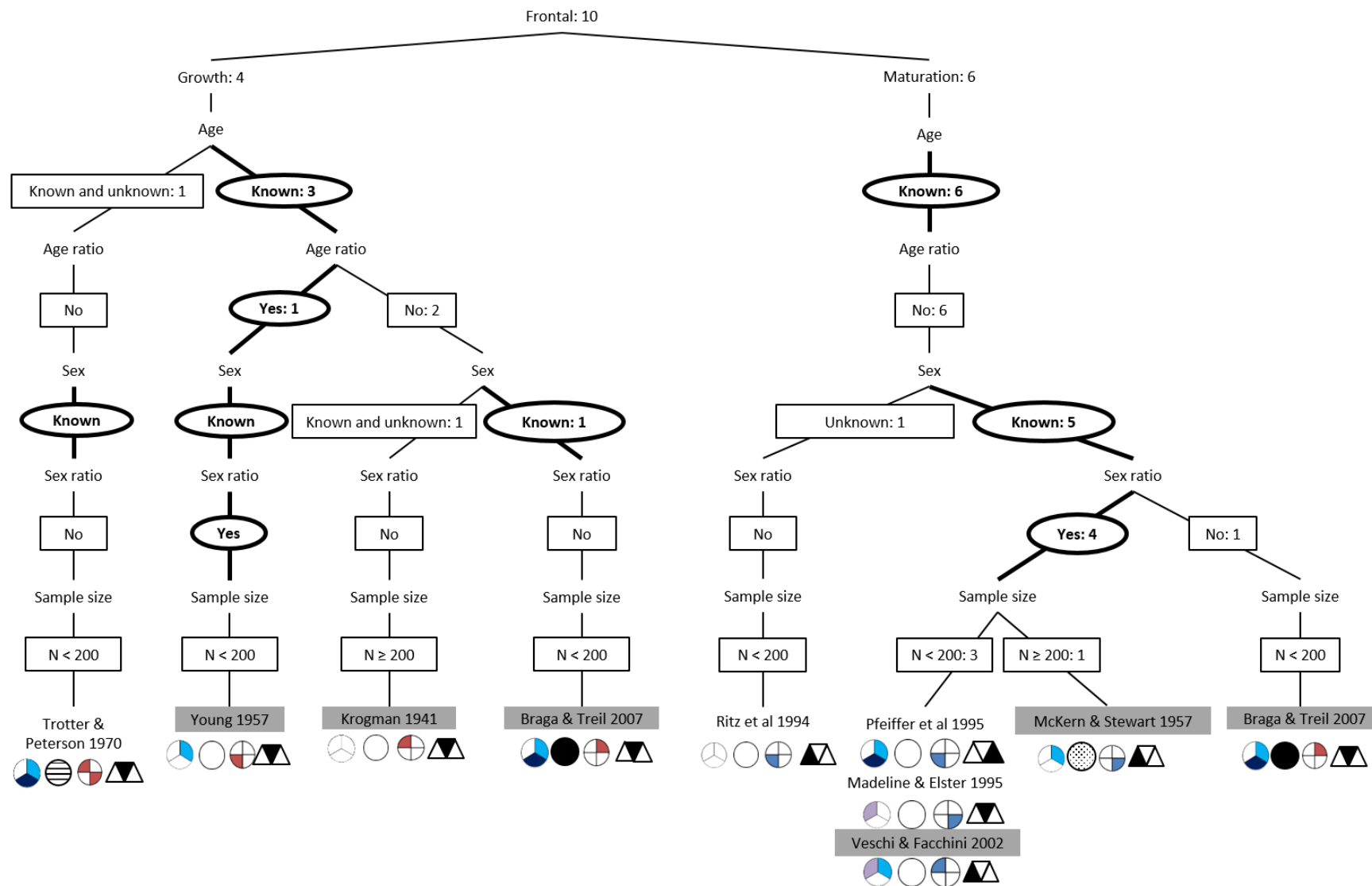


Figure A.6 Classification tree of the juvenile age estimation methods based on the growth or the maturation of the frontal bone

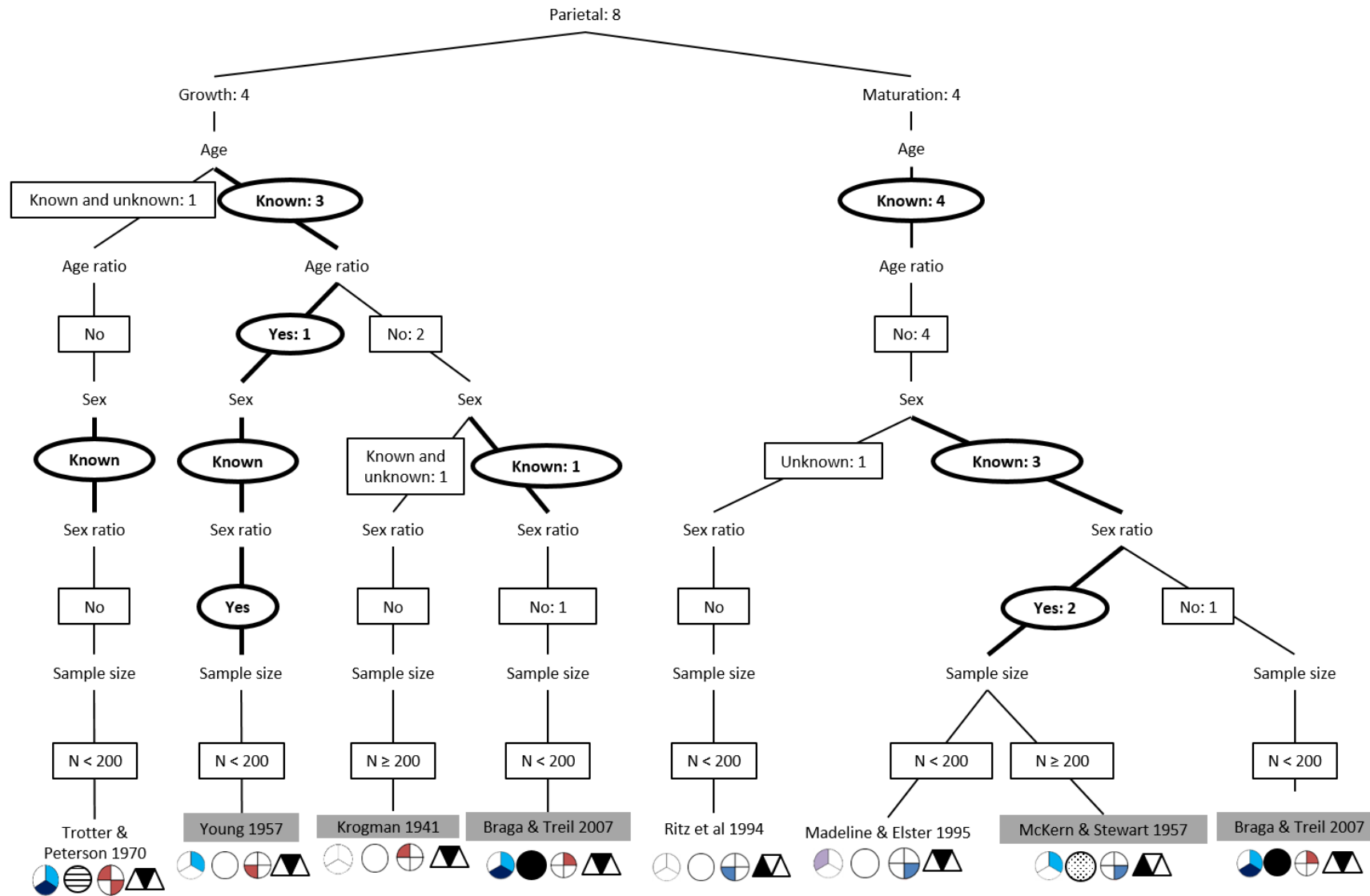


Figure A.7 Classification tree of the juvenile age estimation methods based on the growth or the maturation of the parietal bone

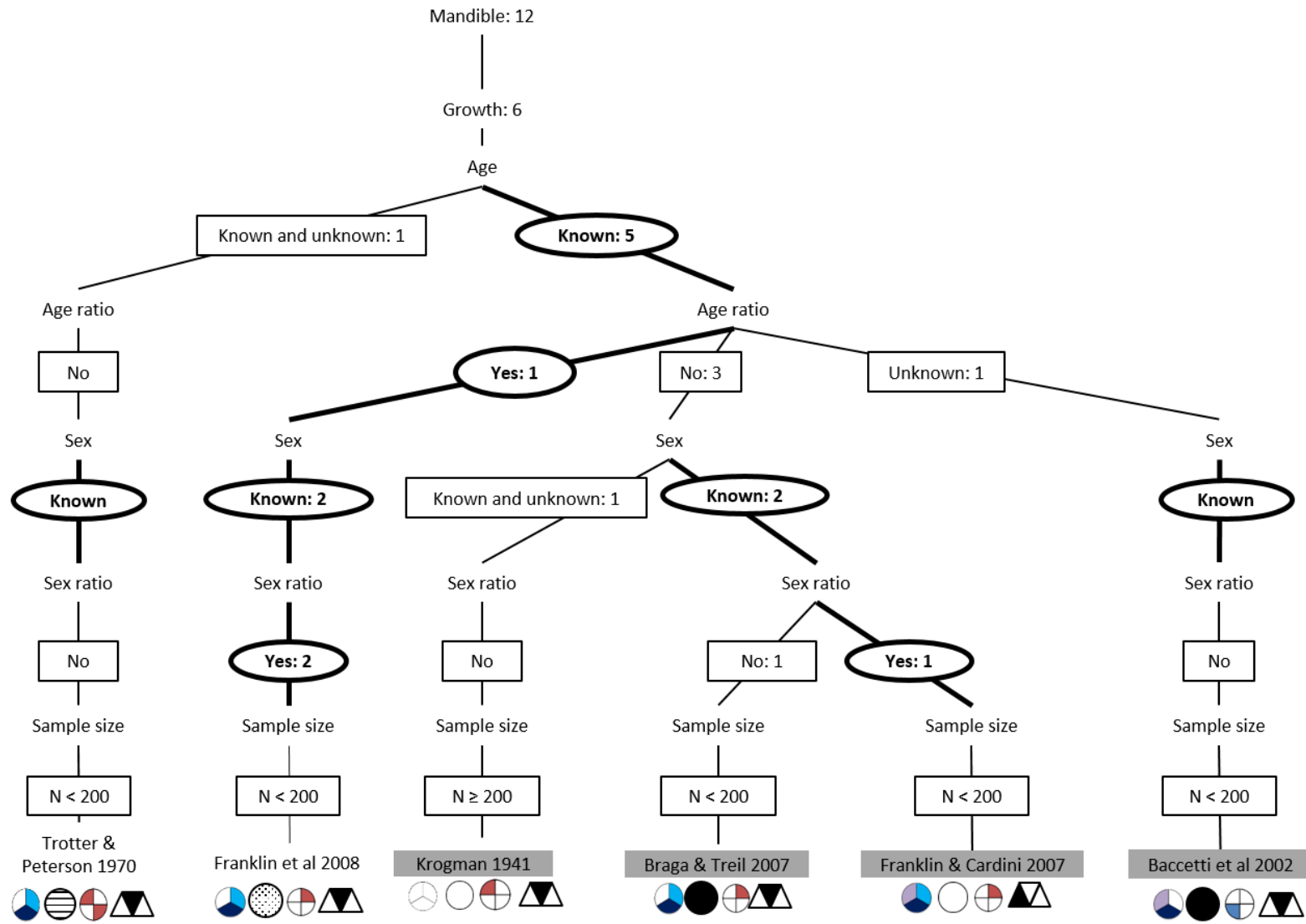


Figure A.8 Classification tree of the juvenile age estimation methods based on the growth of the mandible

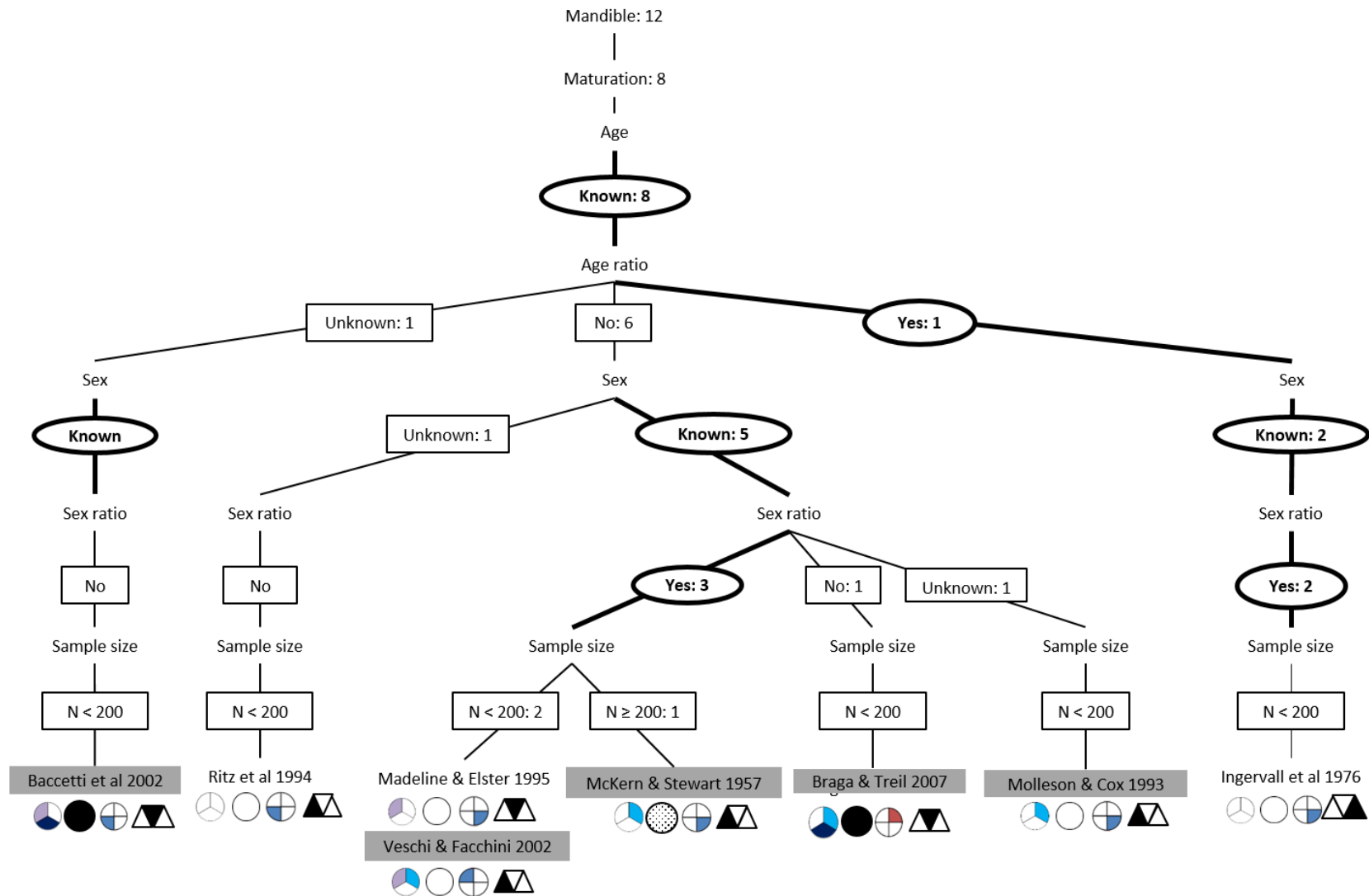


Figure A.9 Classification tree of the juvenile age estimation methods based on the maturation of the mandible

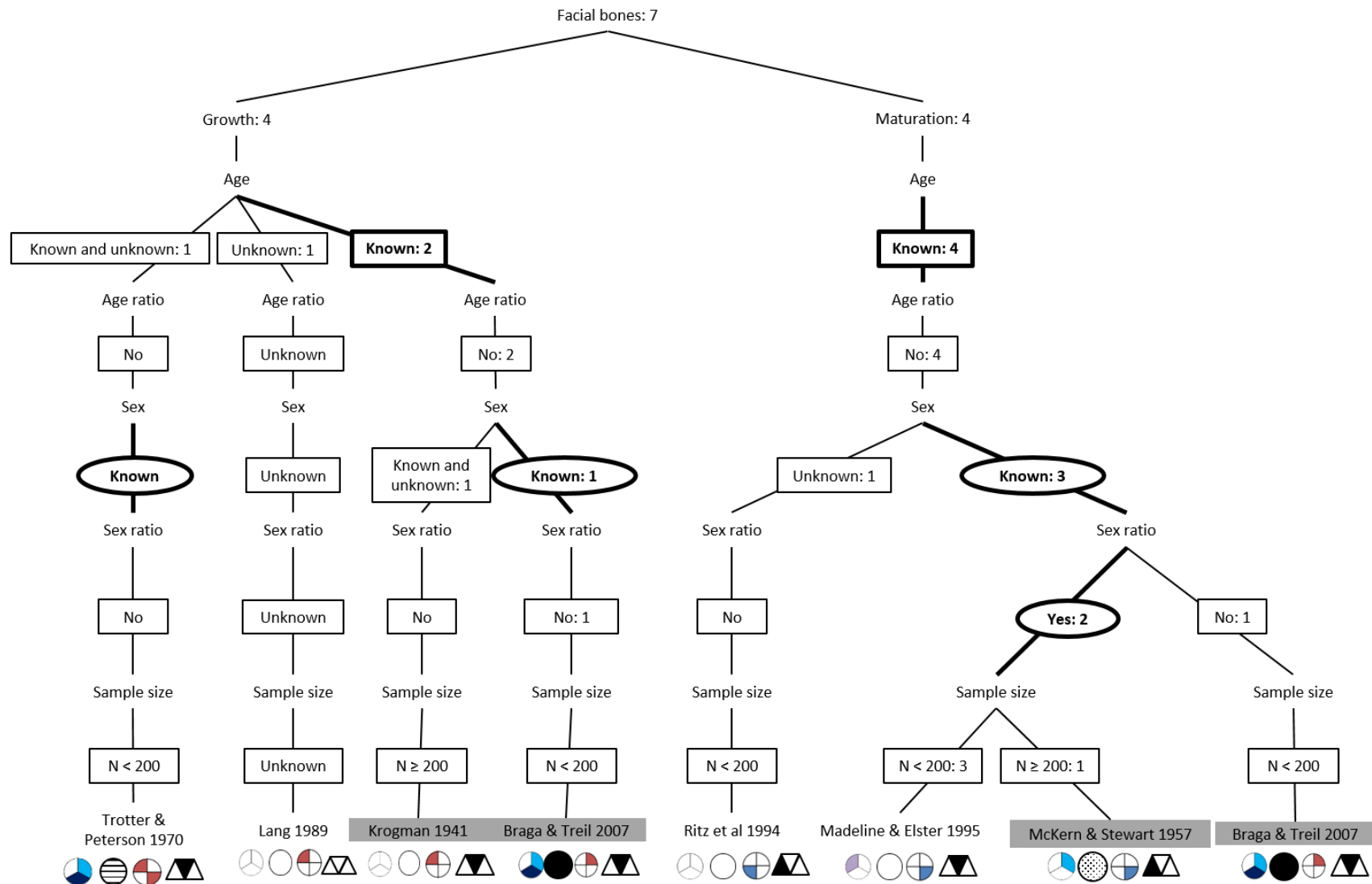


Figure A.10 Classification tree of the juvenile age estimation methods based on the growth or the maturation of the facial bones

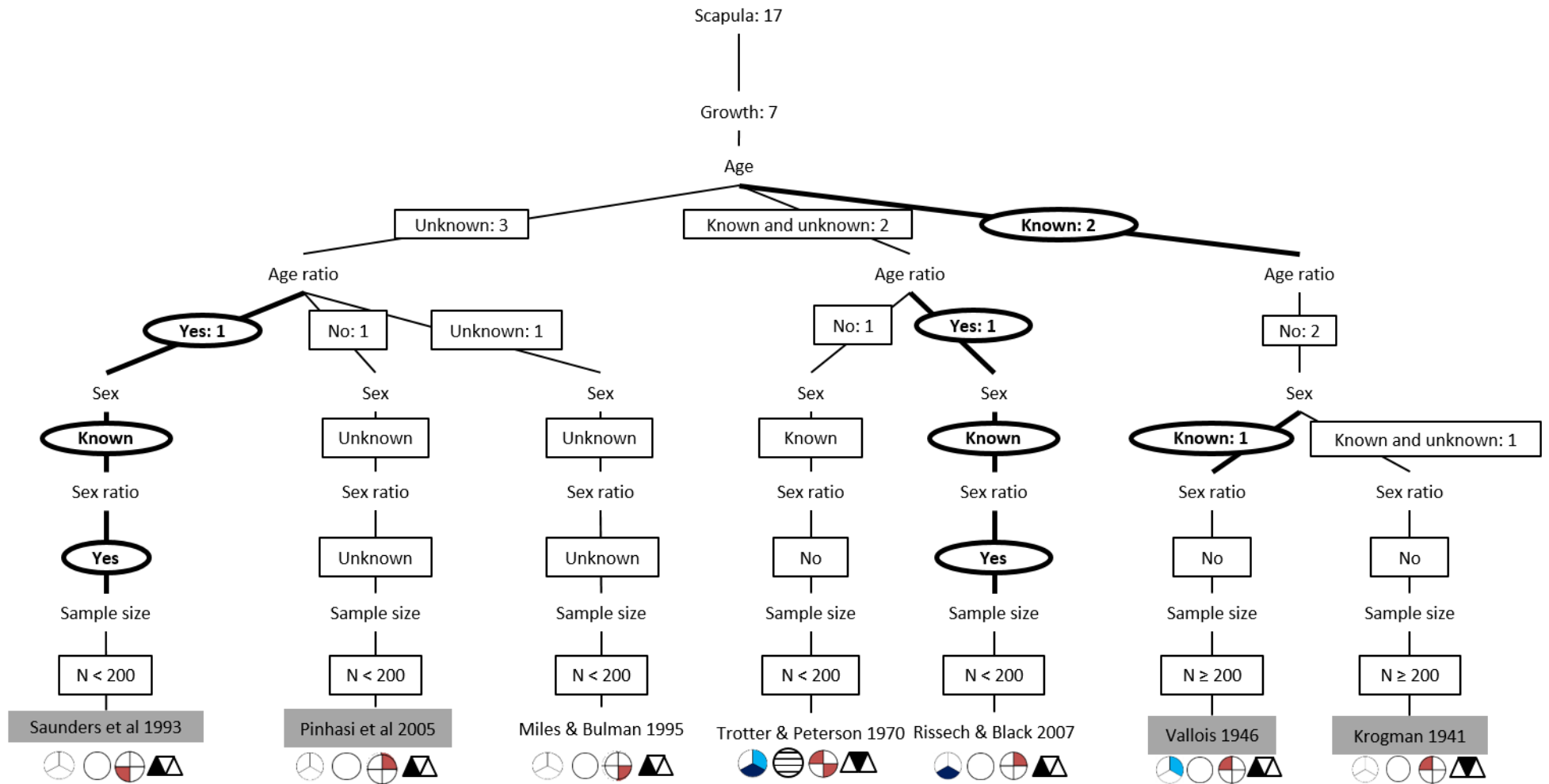


Figure A.11 Classification tree of the juvenile age estimation methods based on the growth of the scapula



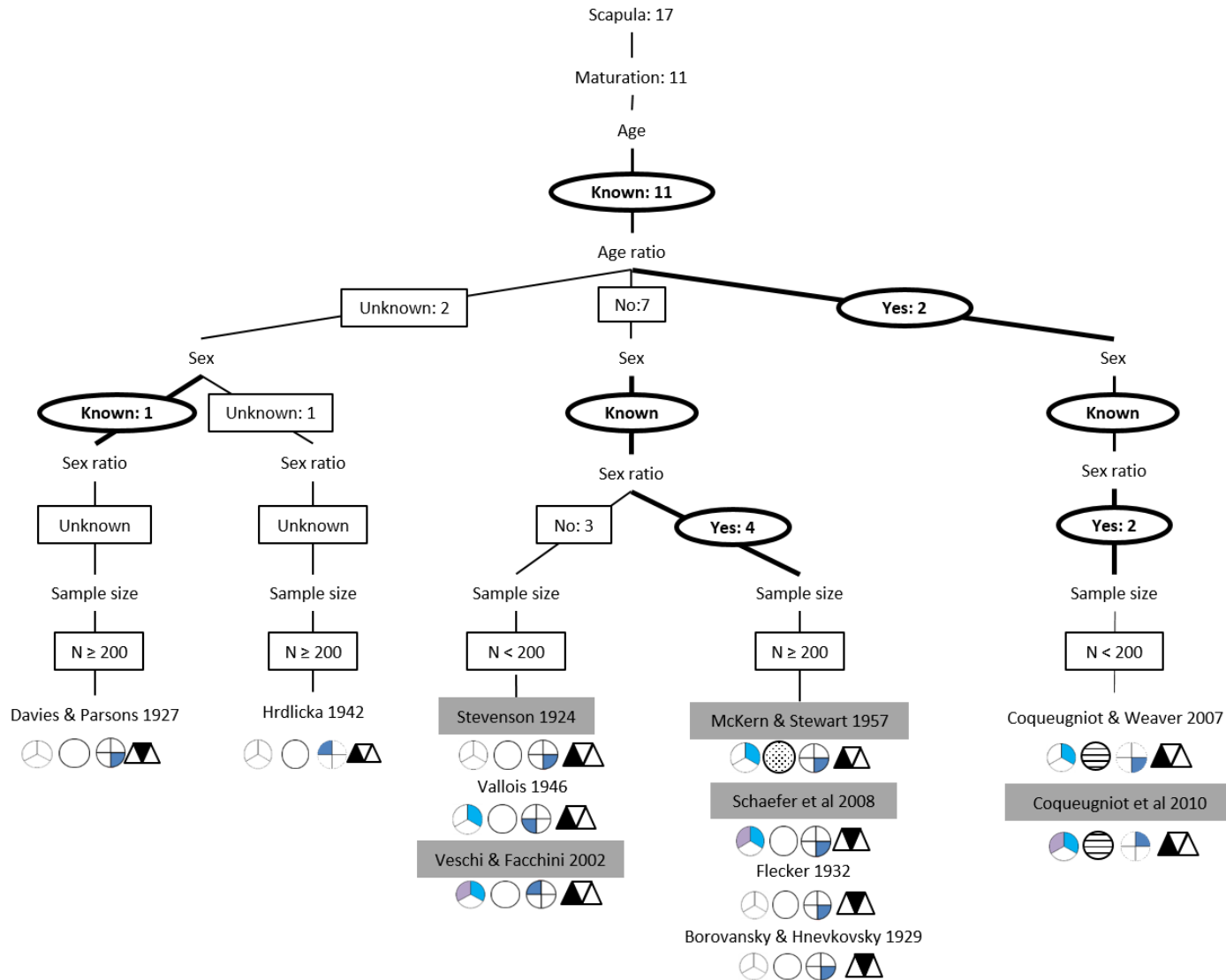


Figure A.12 Classification tree of the juvenile age estimation methods based on the maturation of the scapula

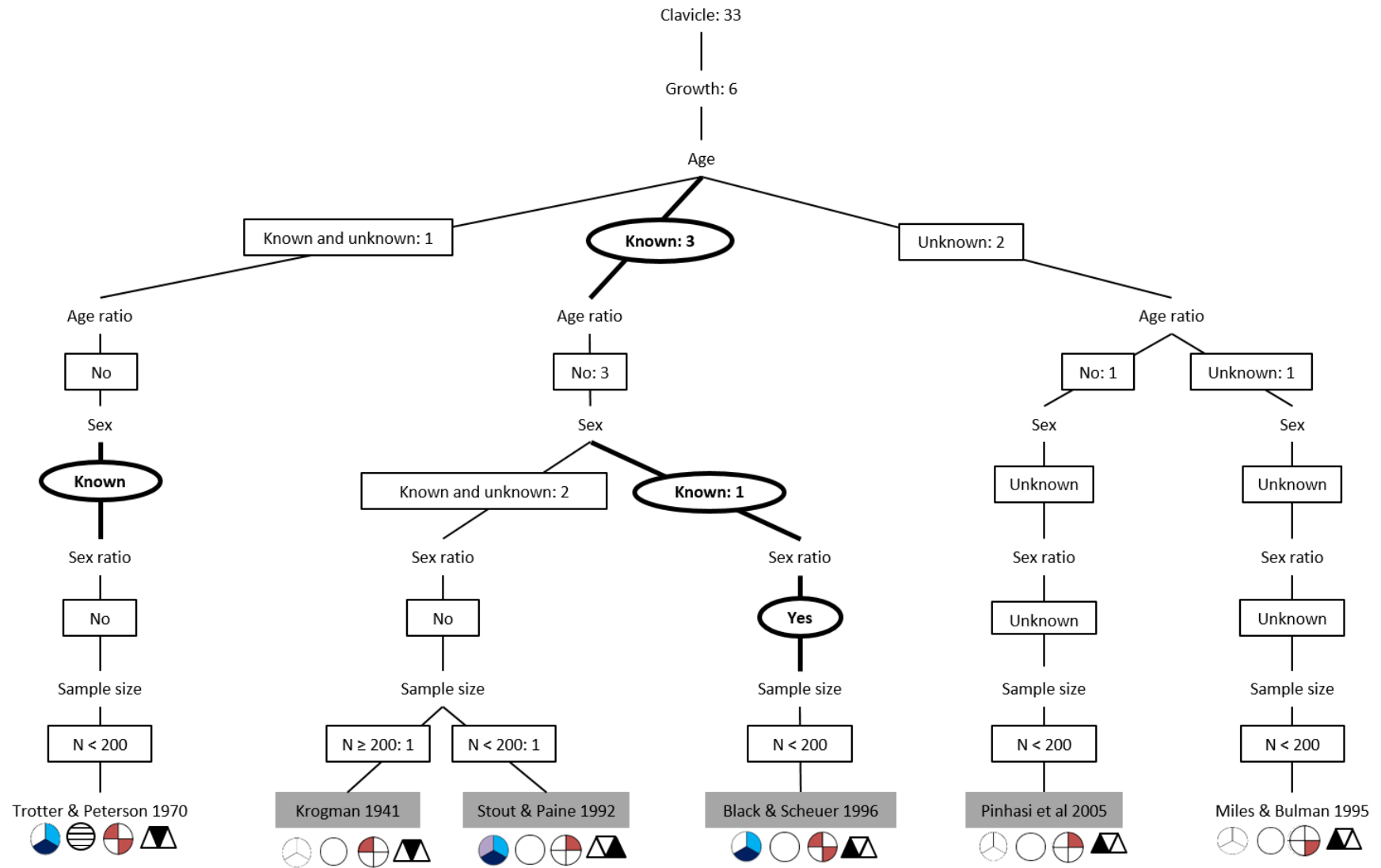


Figure A.13 Classification tree of the juvenile age estimation methods based on the growth of the clavicle

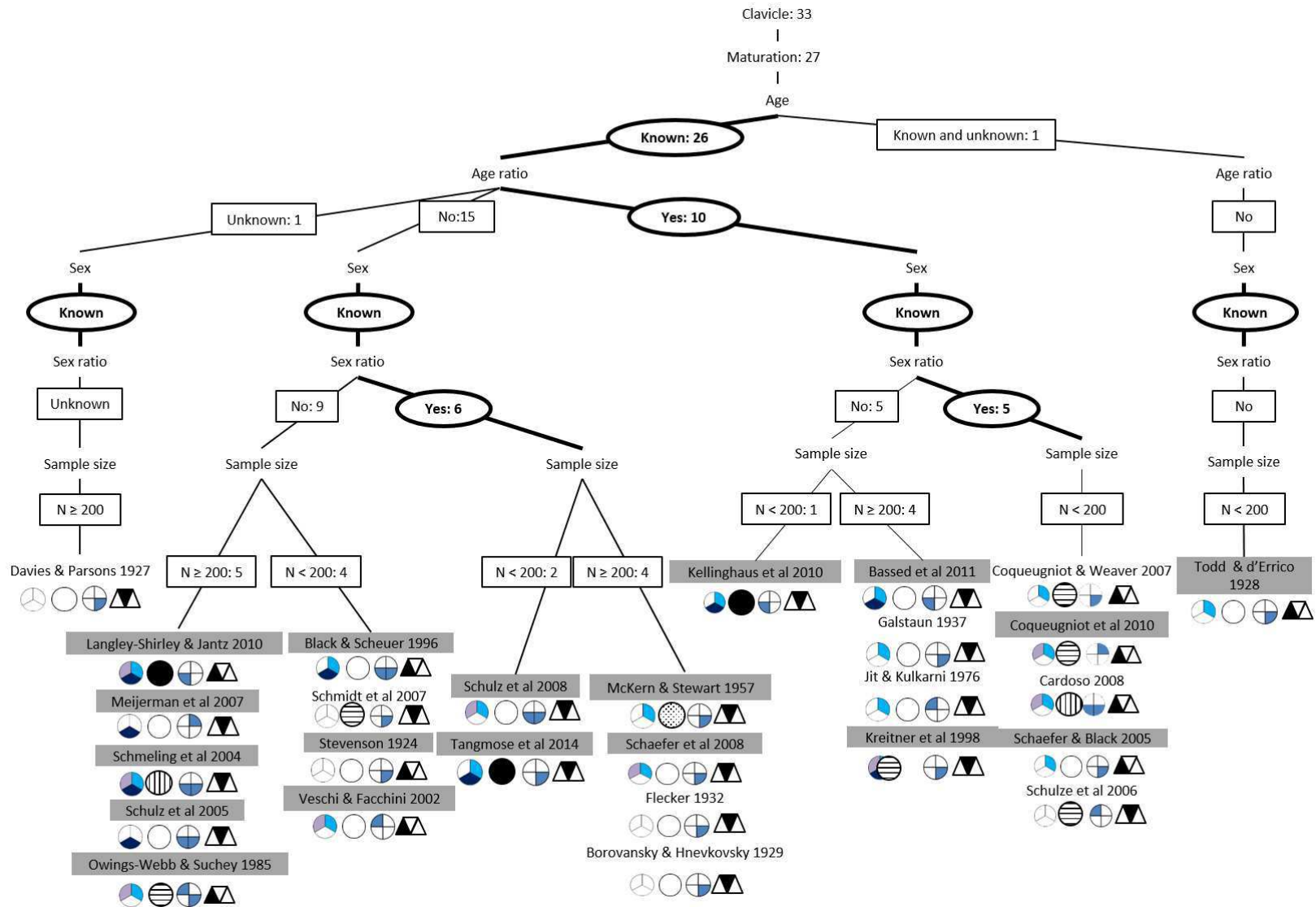


Figure A.14 Classification tree of the juvenile age estimation methods based on the maturation of the clavicle

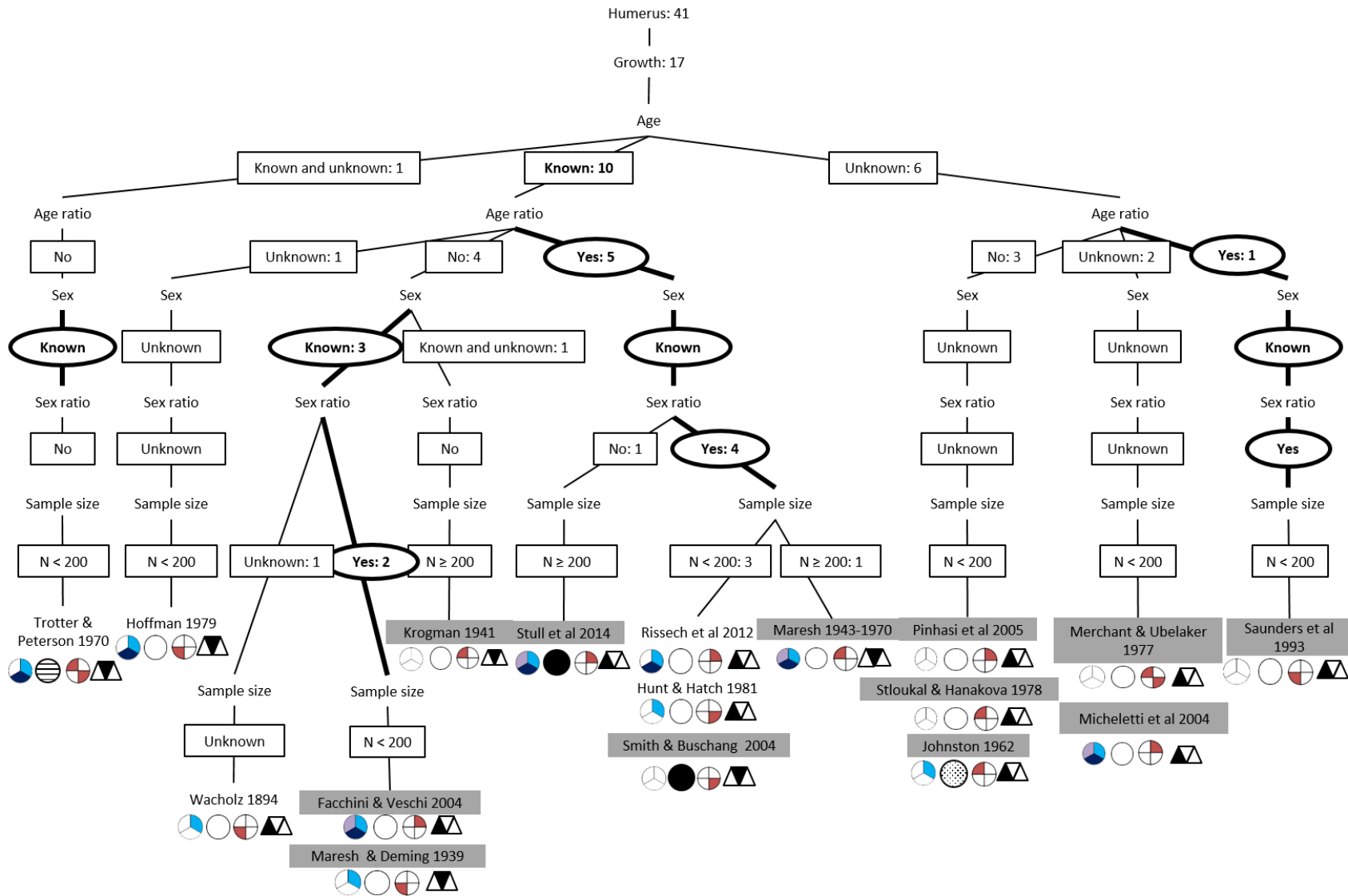


Figure A.15 Classification tree of the juvenile age estimation methods based on the growth of the humerus

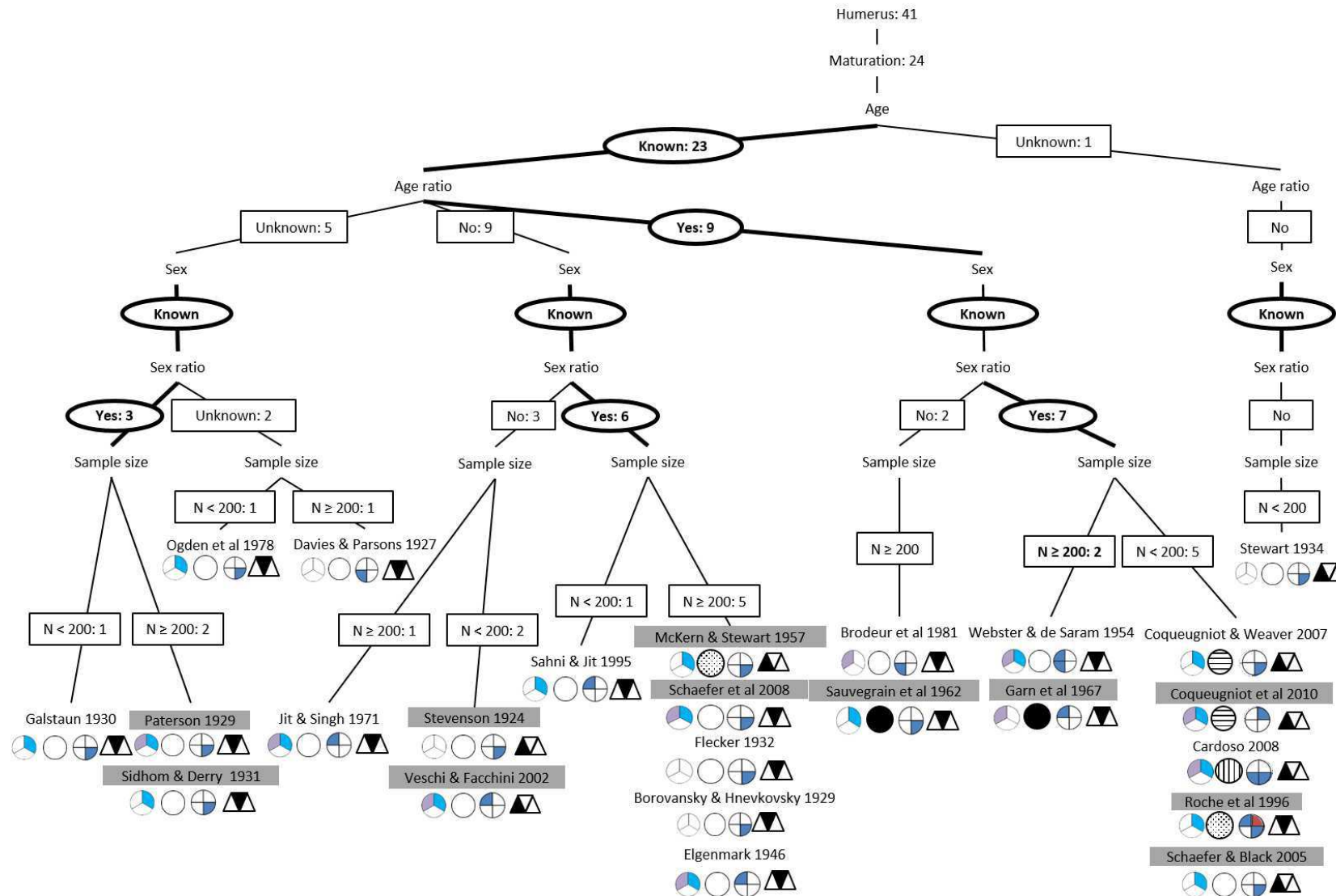


Figure A.16 Classification tree of the juvenile age estimation methods based on the maturation of the humerus

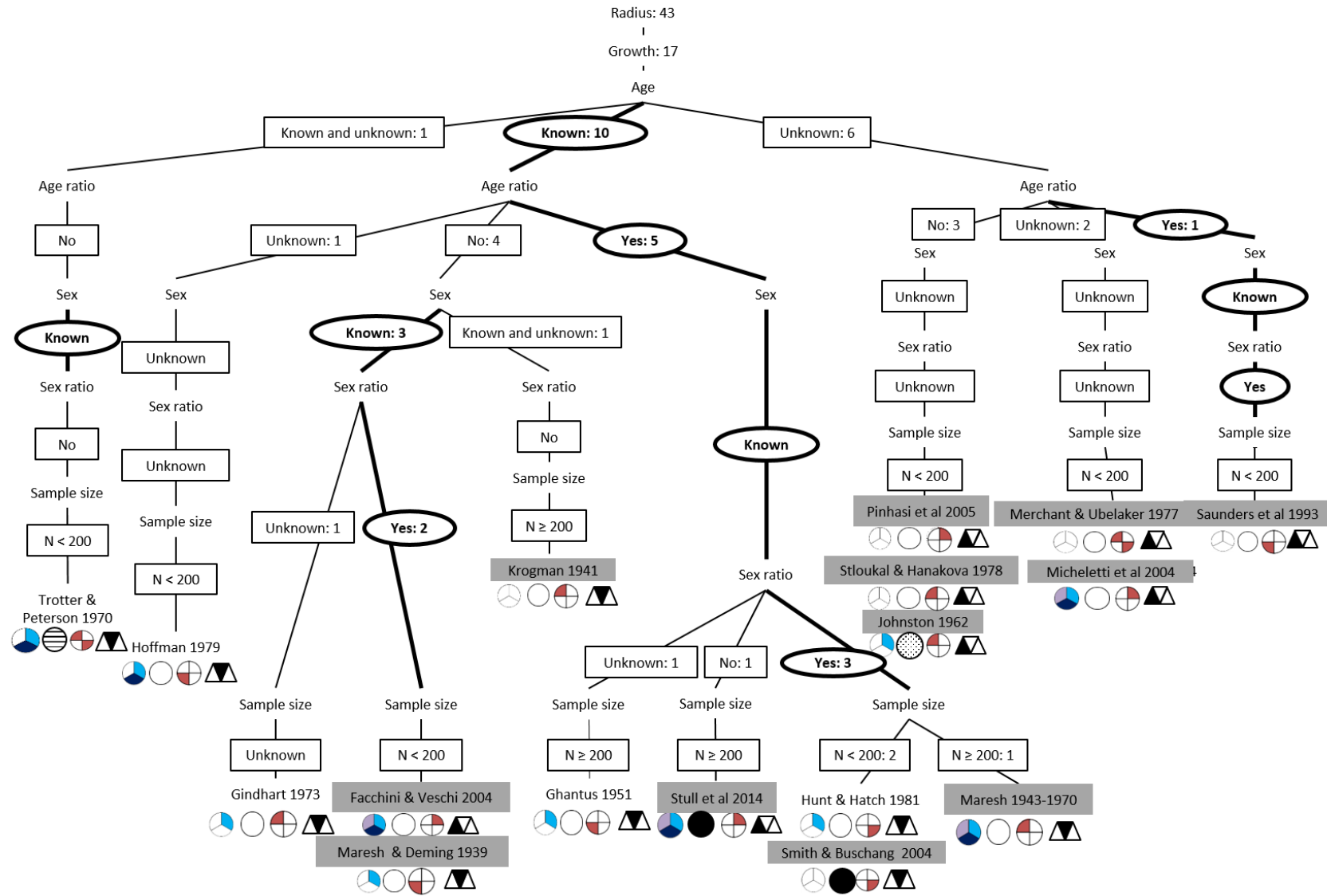


Figure A.17 Classification tree of the juvenile age estimation methods based on the growth of the radius

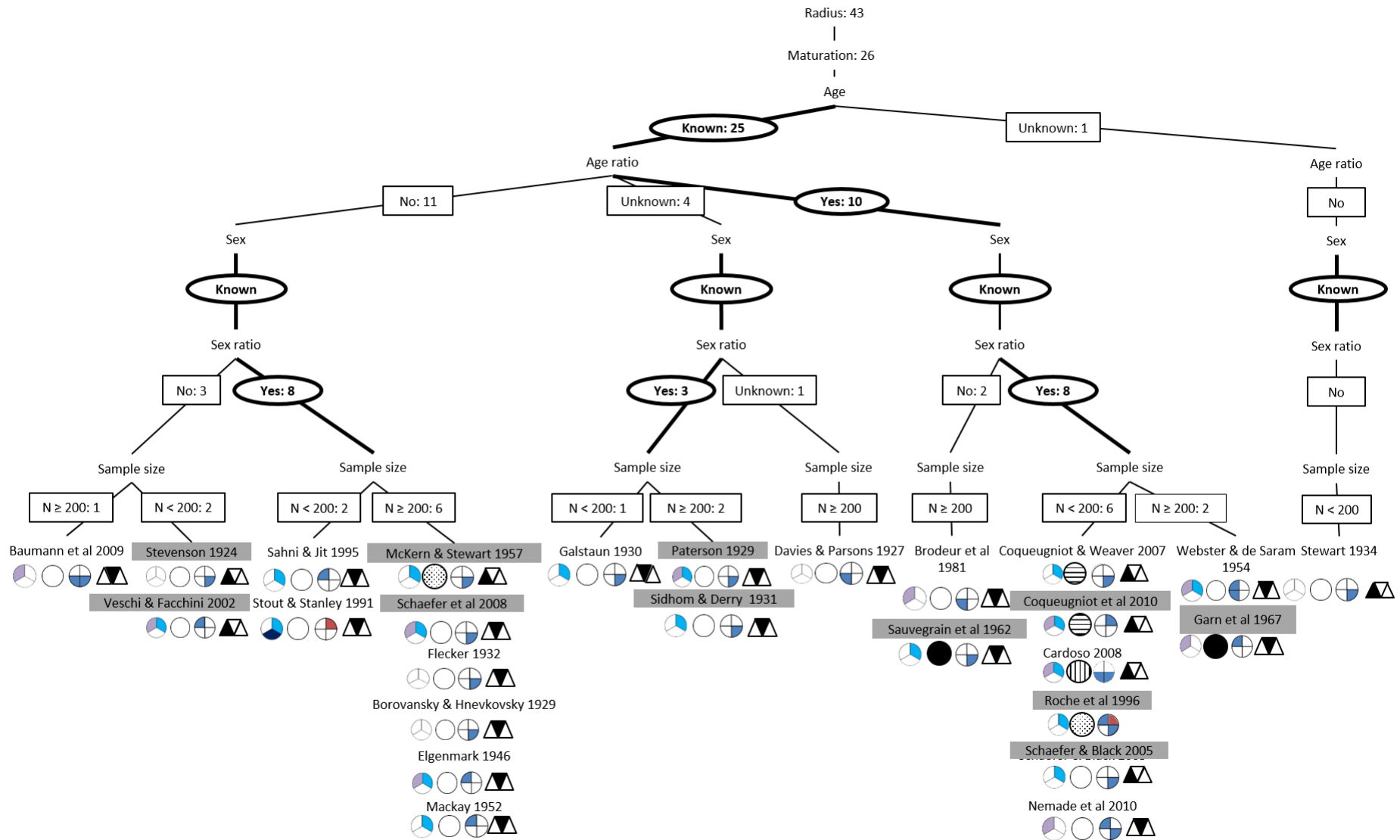


Figure A.18 Classification tree of the juvenile age estimation methods based on the maturation of the radius

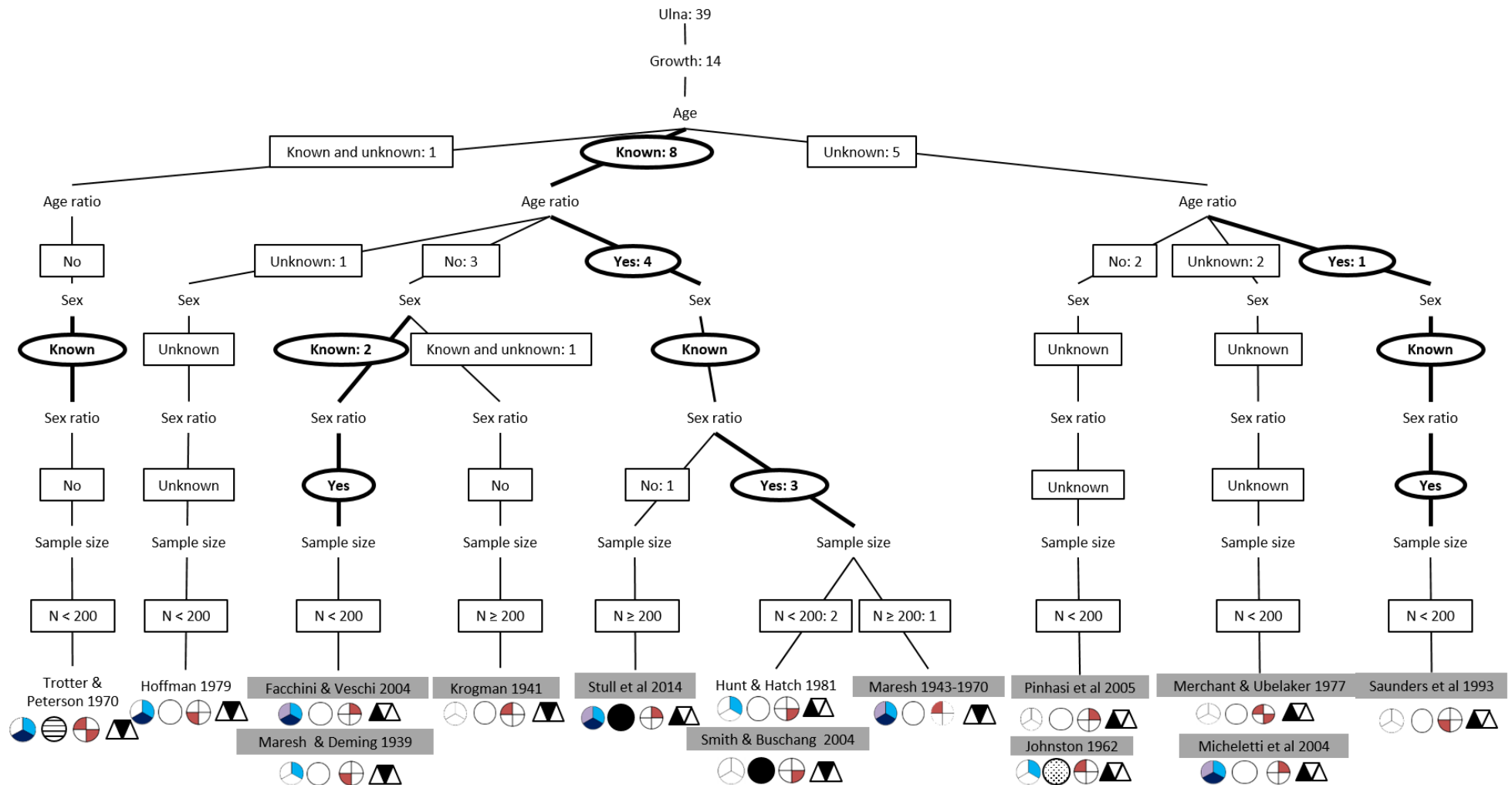


Figure A.19 Classification tree of the juvenile age estimation methods based on the growth of the ulna



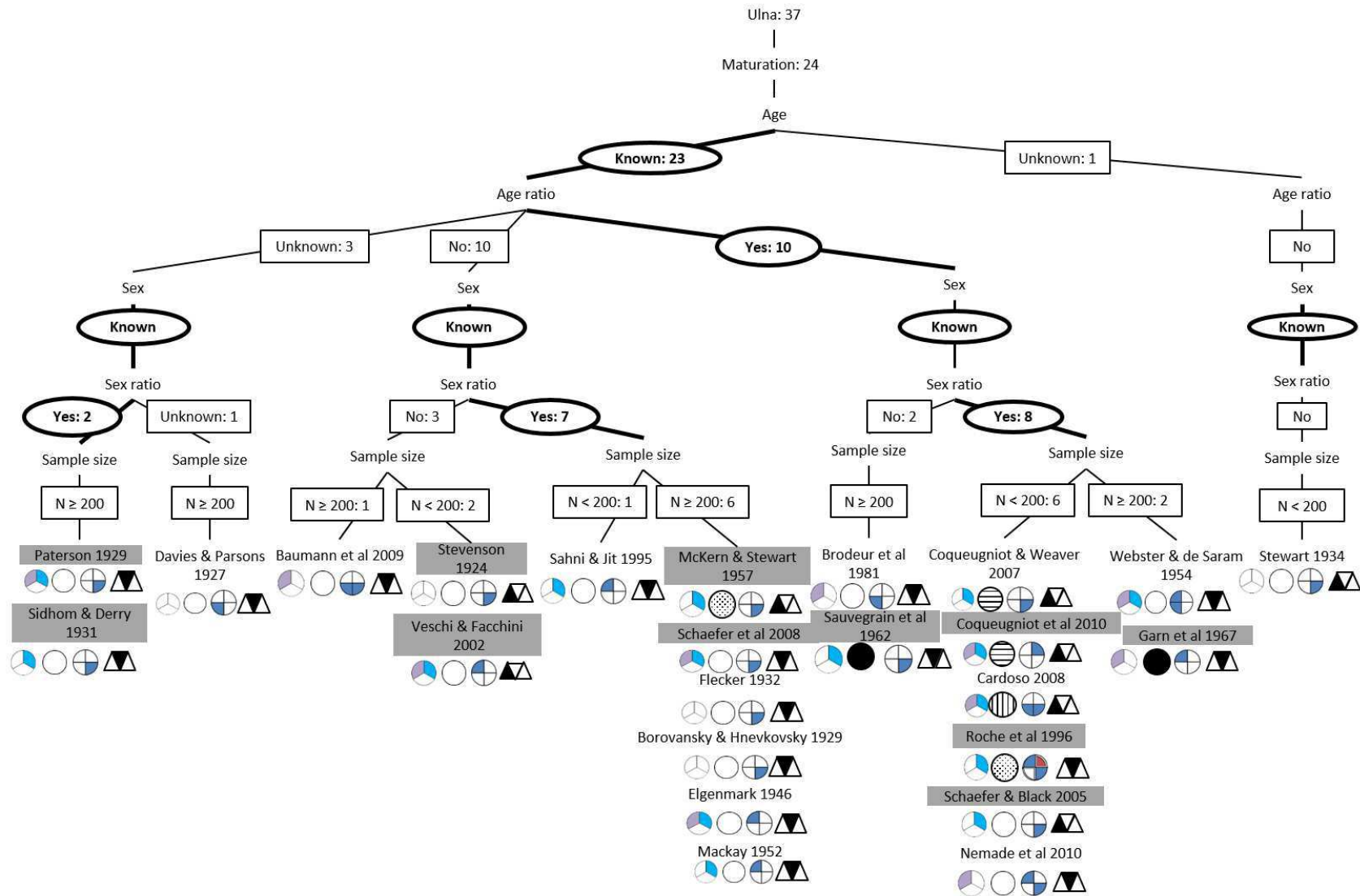


Figure A.20 Classification tree of the juvenile age estimation methods based on the maturation of the ulna

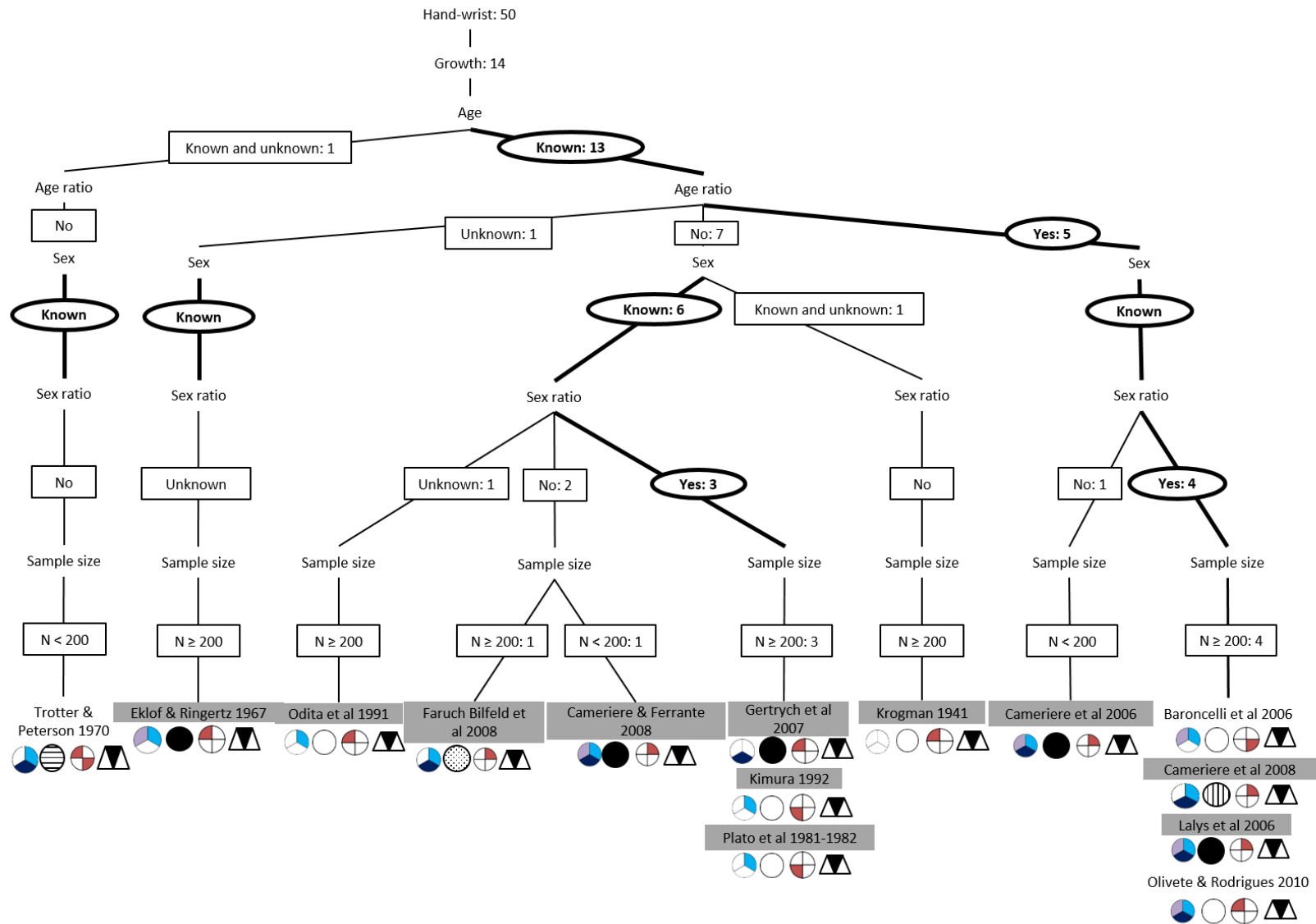


Figure A.21 Classification tree of the juvenile age estimation methods based on the growth of the hand and wrist bones

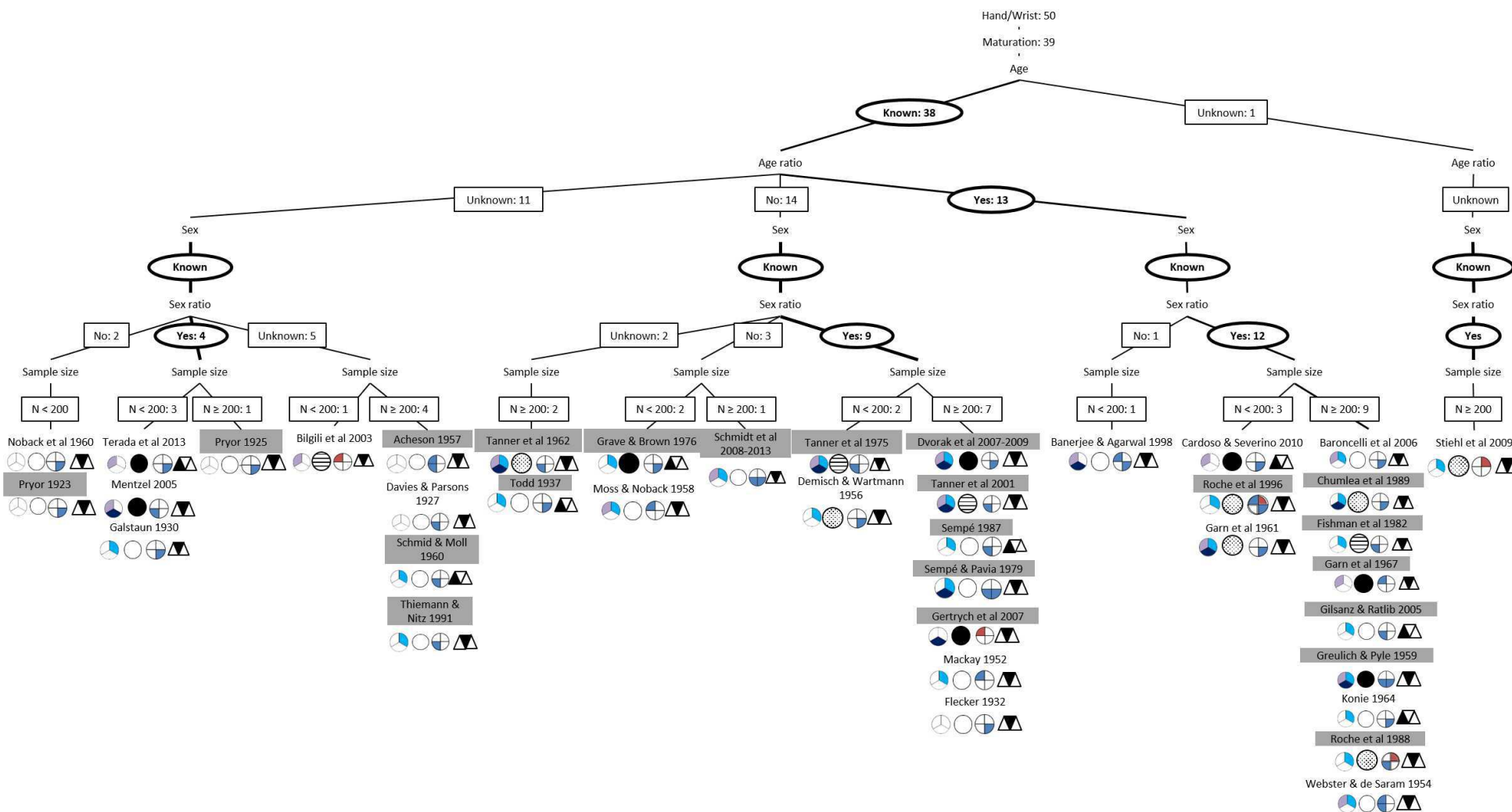


Figure A.22 Classification tree of the juvenile age estimation methods based on maturation of the hand and wrist bones

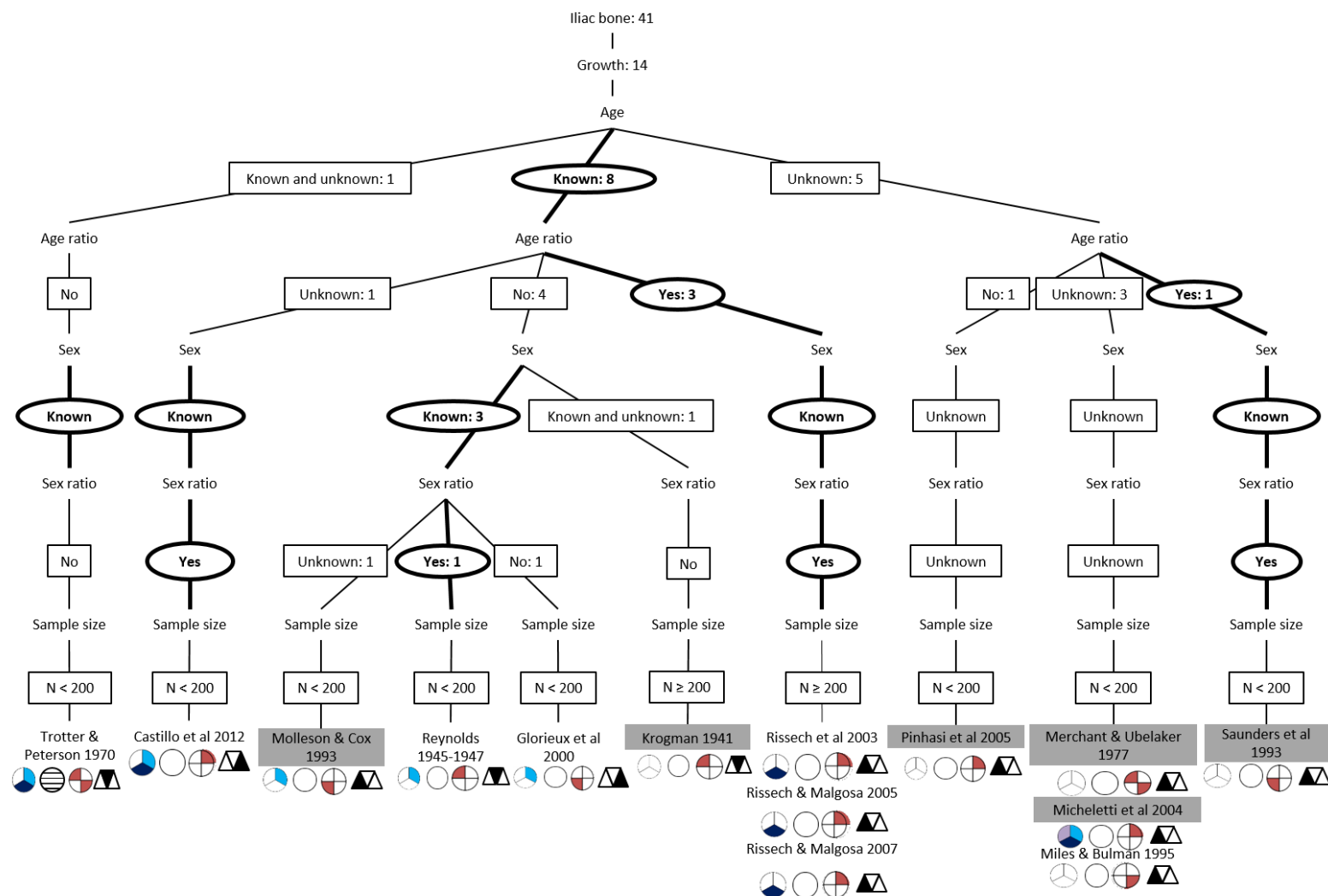


Figure A.23 Classification tree of the juvenile age estimation methods based on growth of the iliac bone

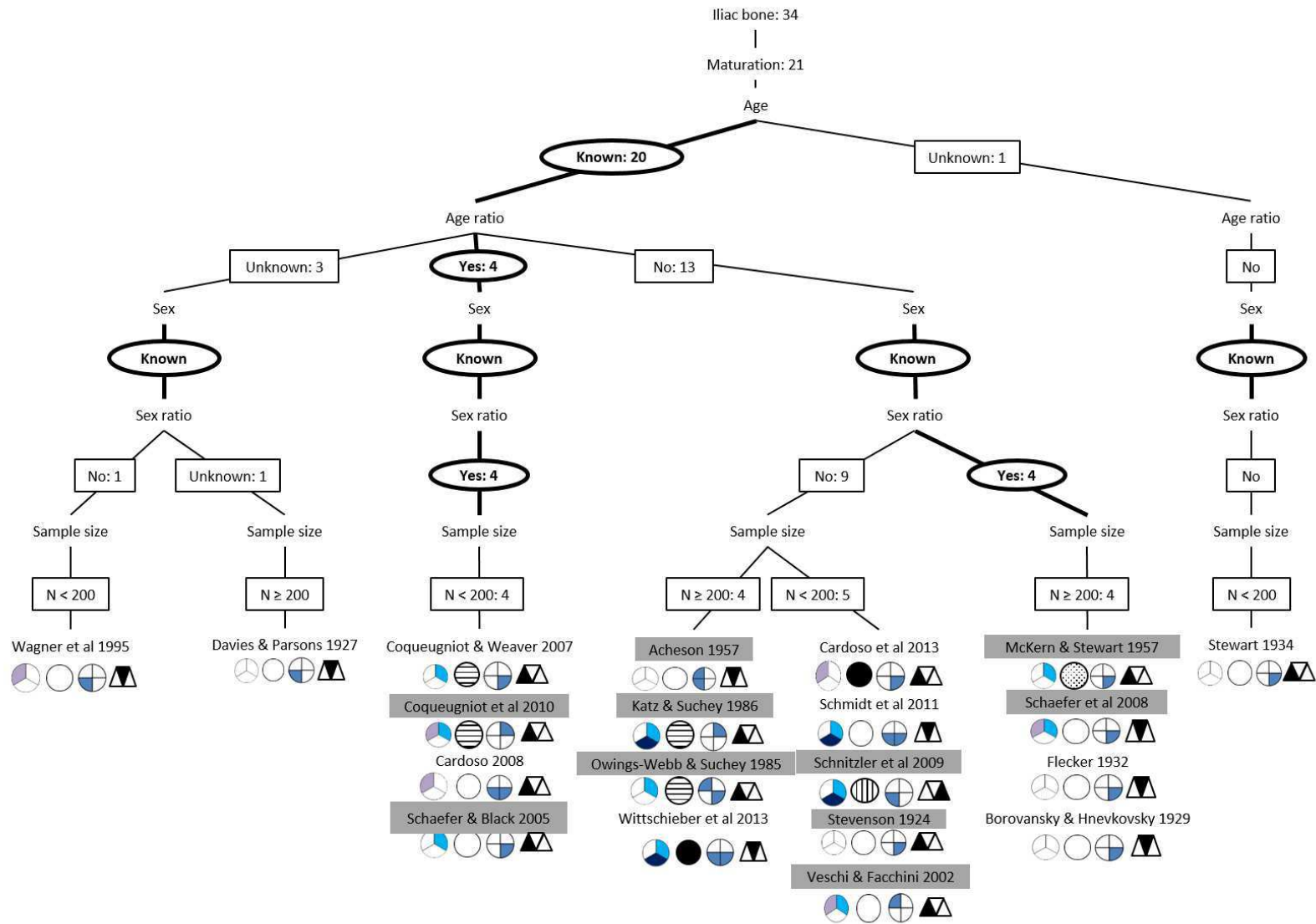


Figure A.24 Classification tree of the juvenile age estimation methods based on the maturation of the iliac bone

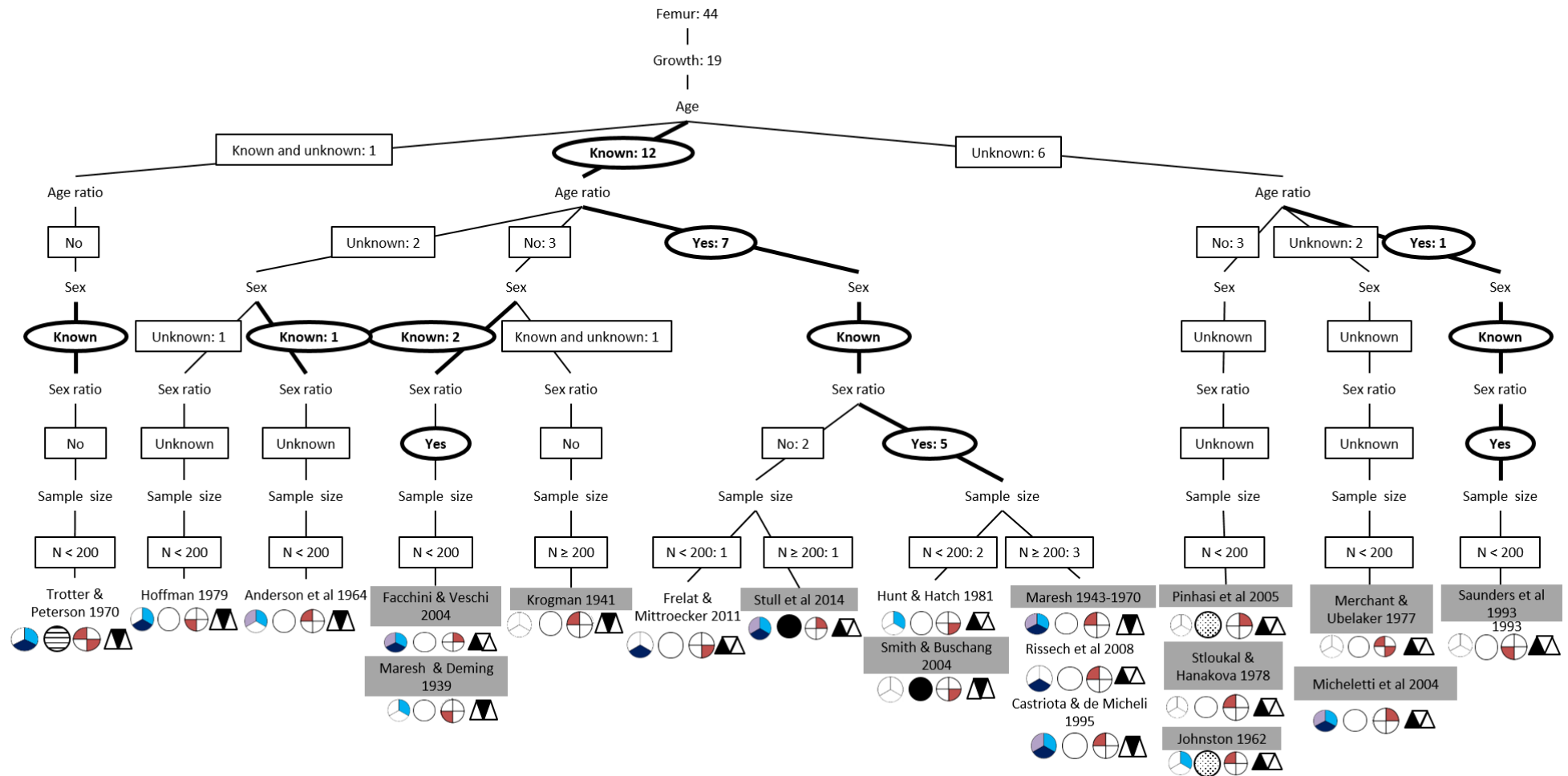


Figure A.25 Classification tree of the juvenile age estimation methods based on the growth of the femur

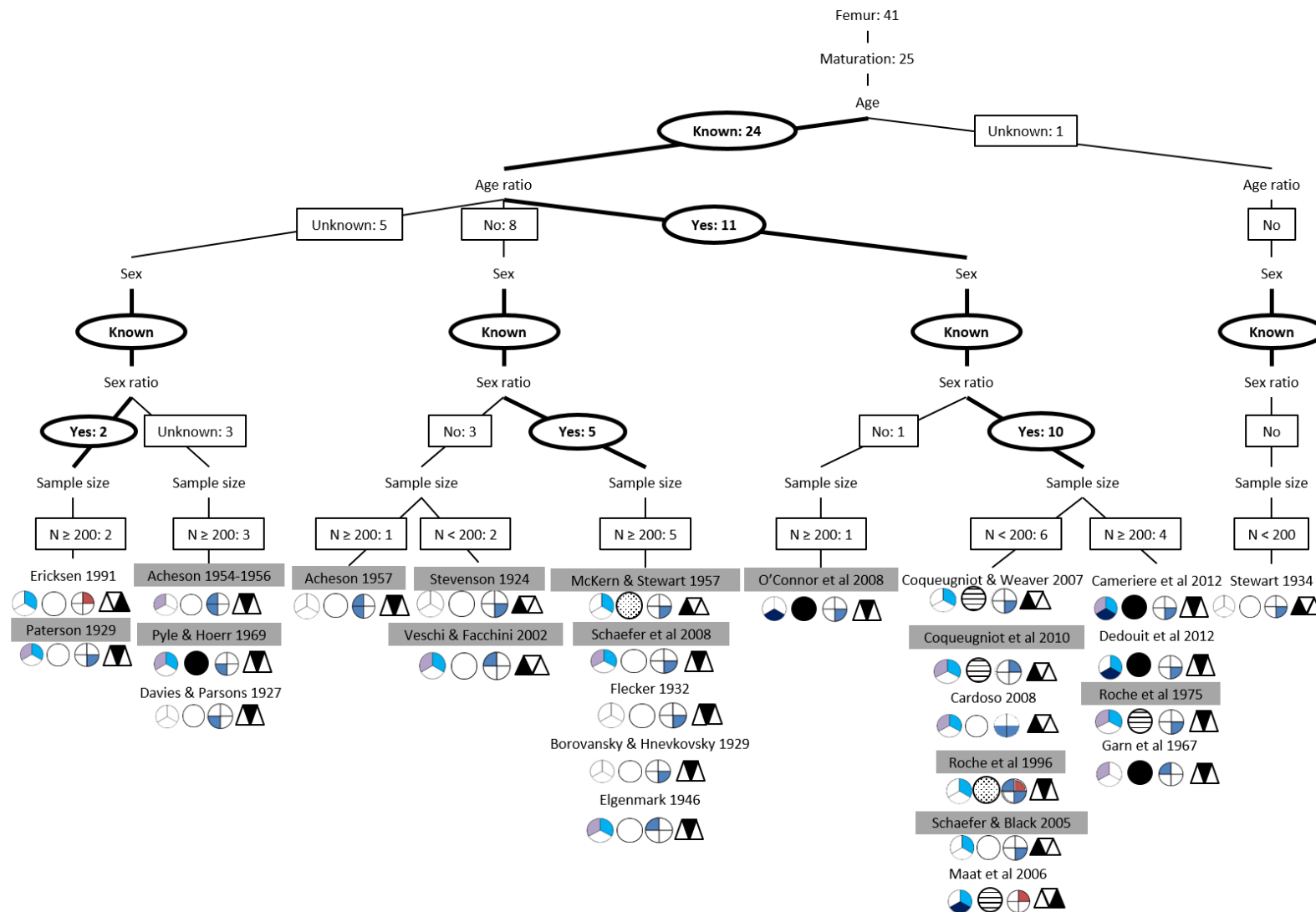


Figure A.26 Classification tree of the juvenile age estimation methods based on the maturation of the femur

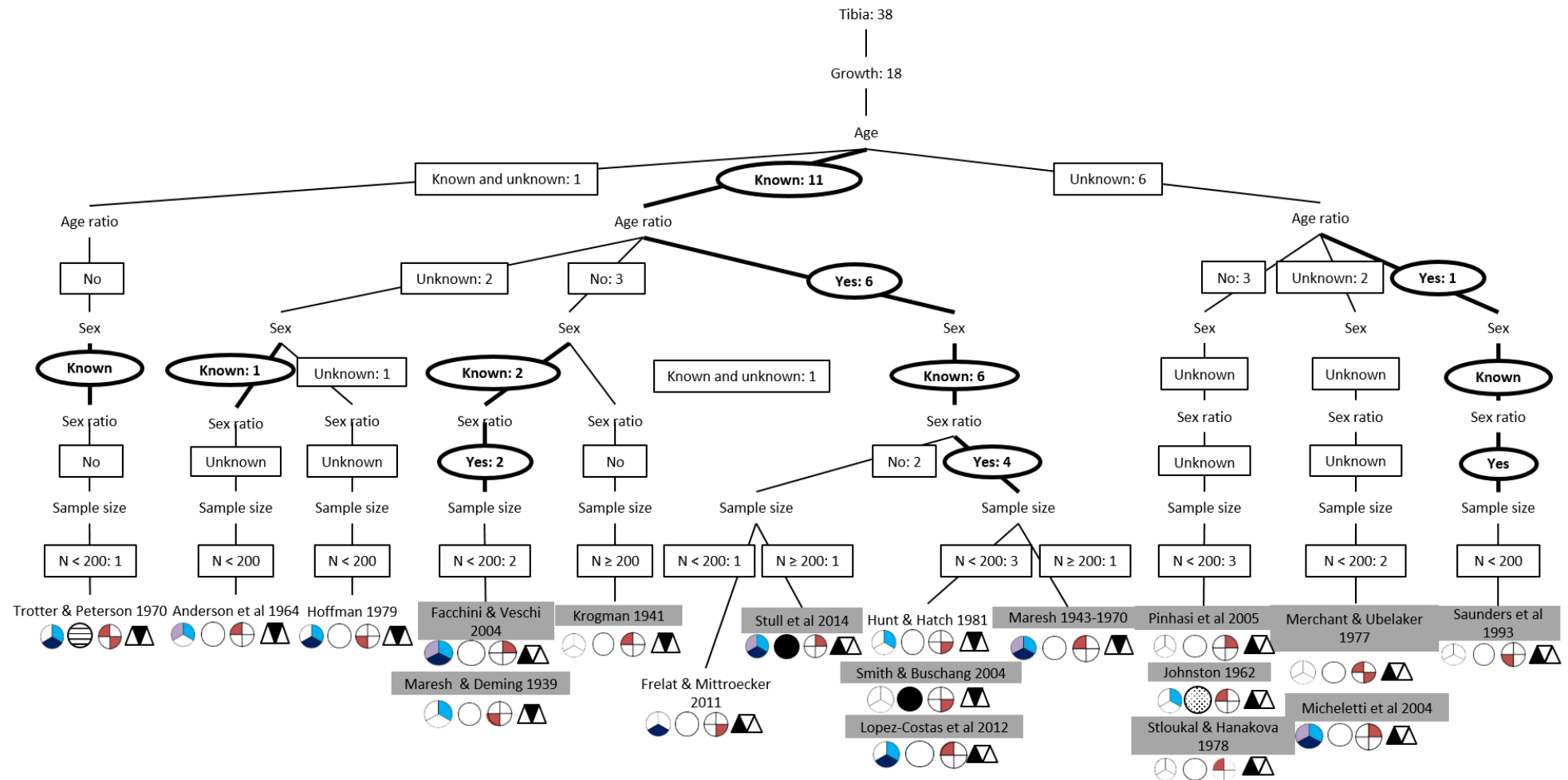


Figure A.27 Classification tree of the juvenile age estimation methods based on the growth of the tibia



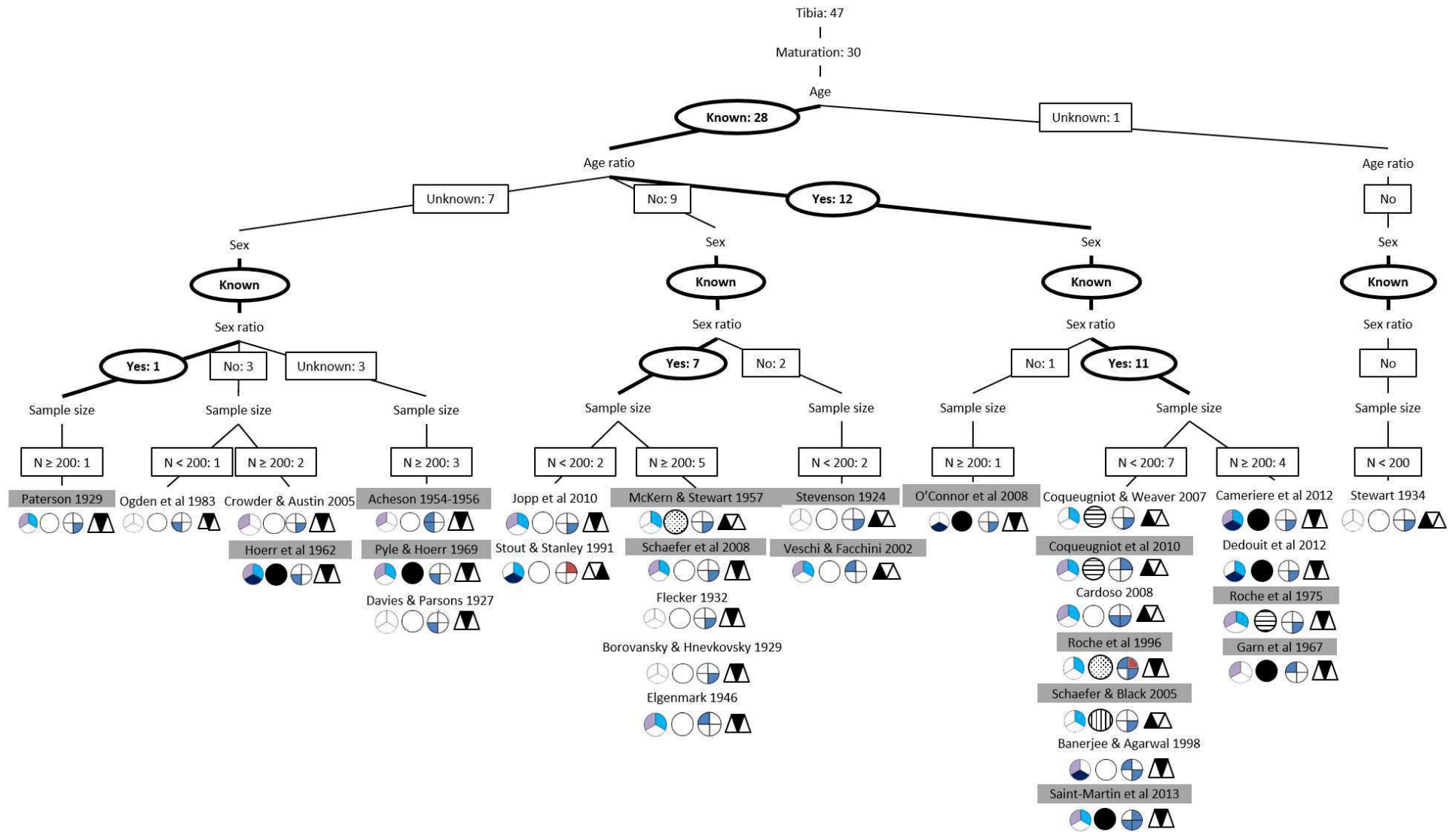


Figure A.28 Classification tree of the juvenile age estimation methods based on the maturation of the tibia

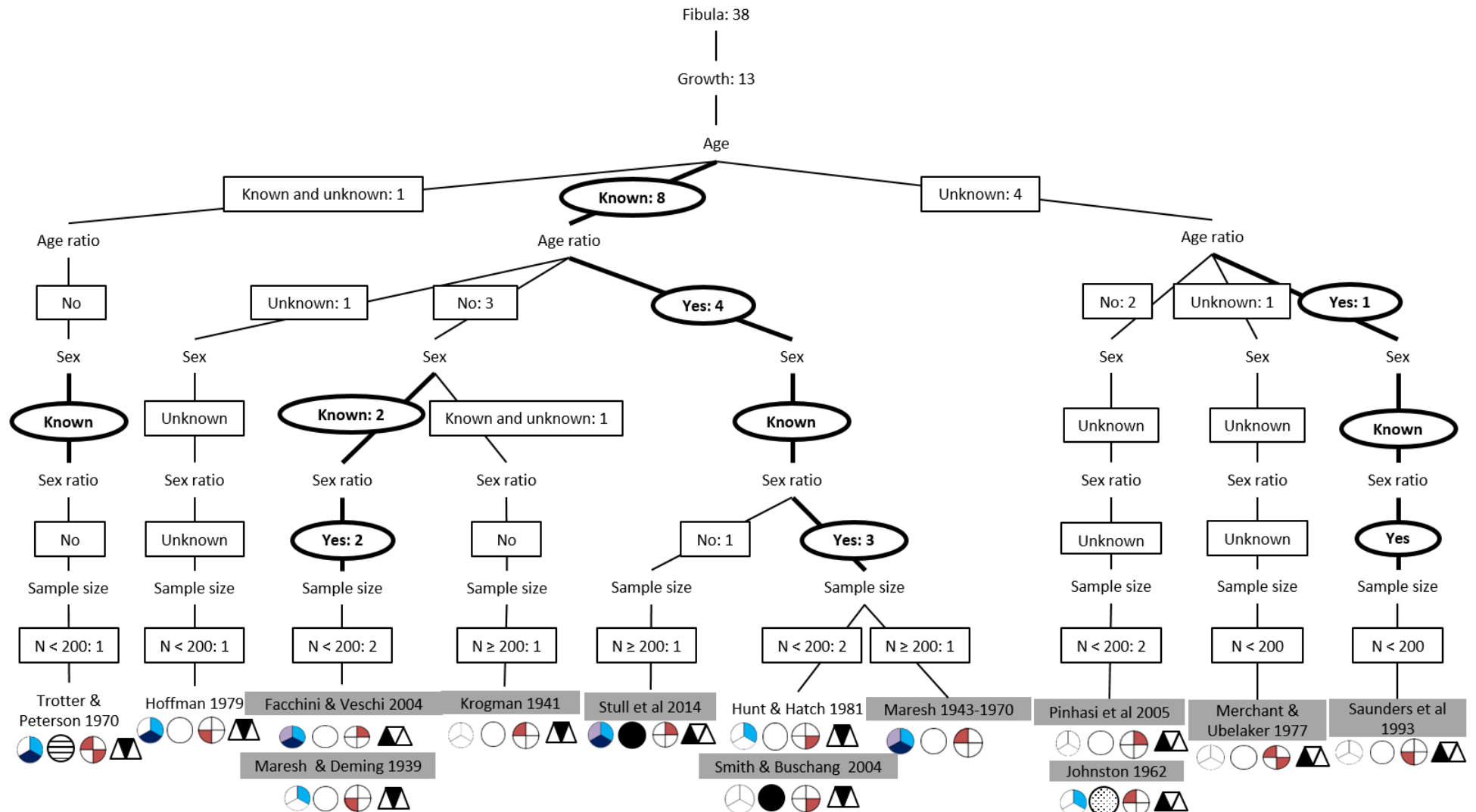


Figure A.29 Classification tree of the juvenile age estimation methods based on the growth of the fibula

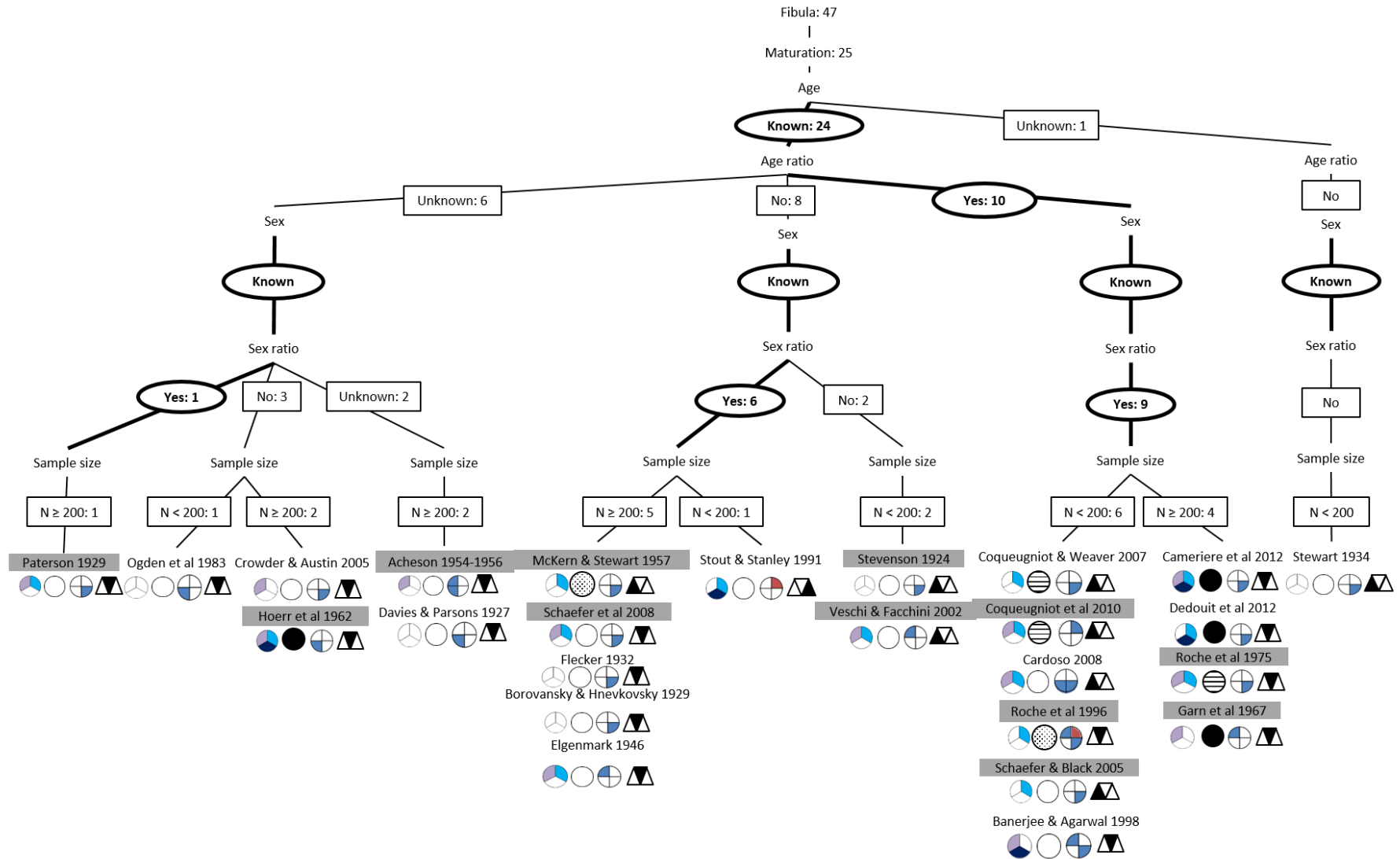


Figure A.30 Classification tree of the juvenile age estimation methods based on the maturation of the fibula

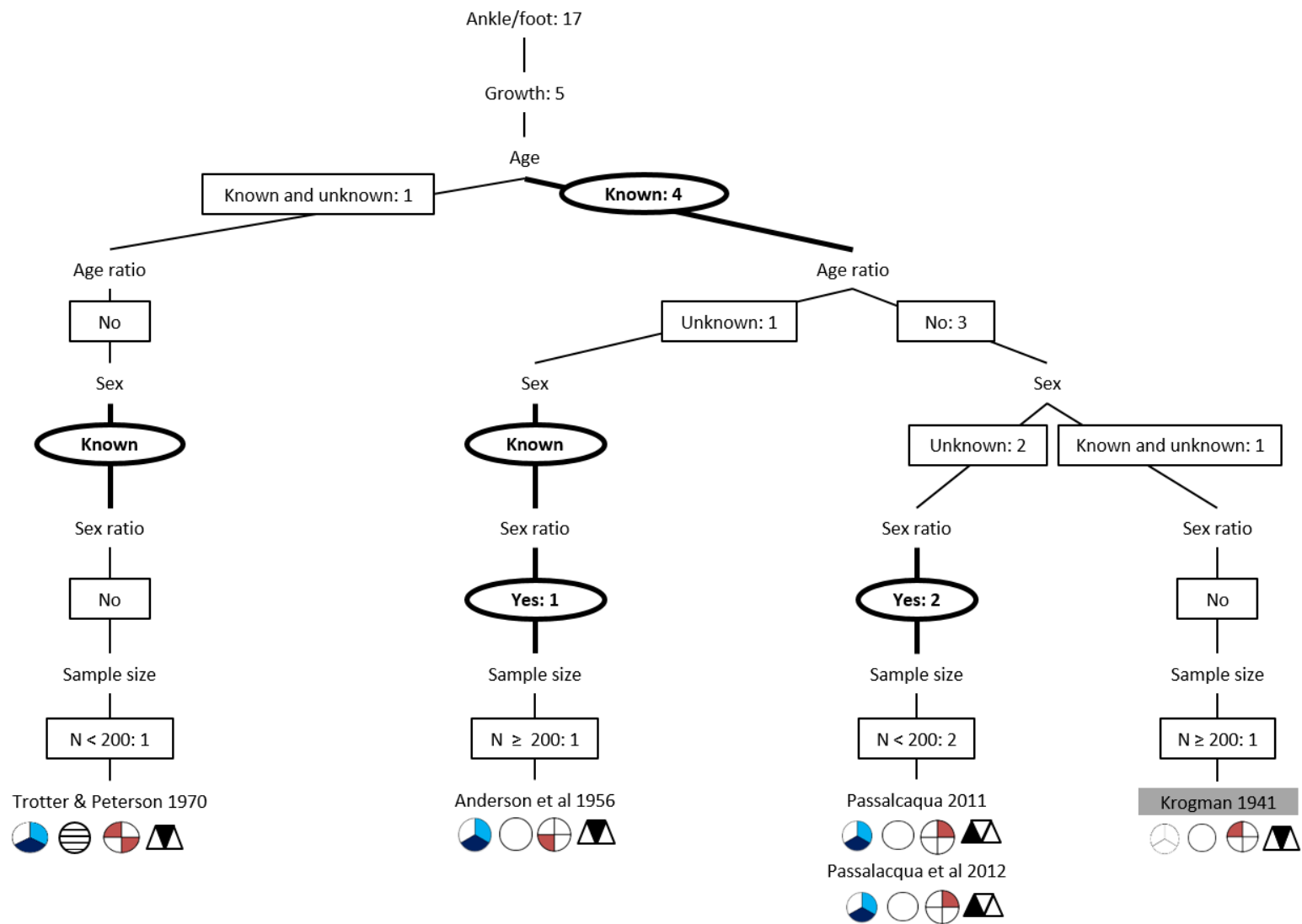


Figure A.31 Classification tree of the juvenile age estimation methods based on the growth of the ankle and foot bones

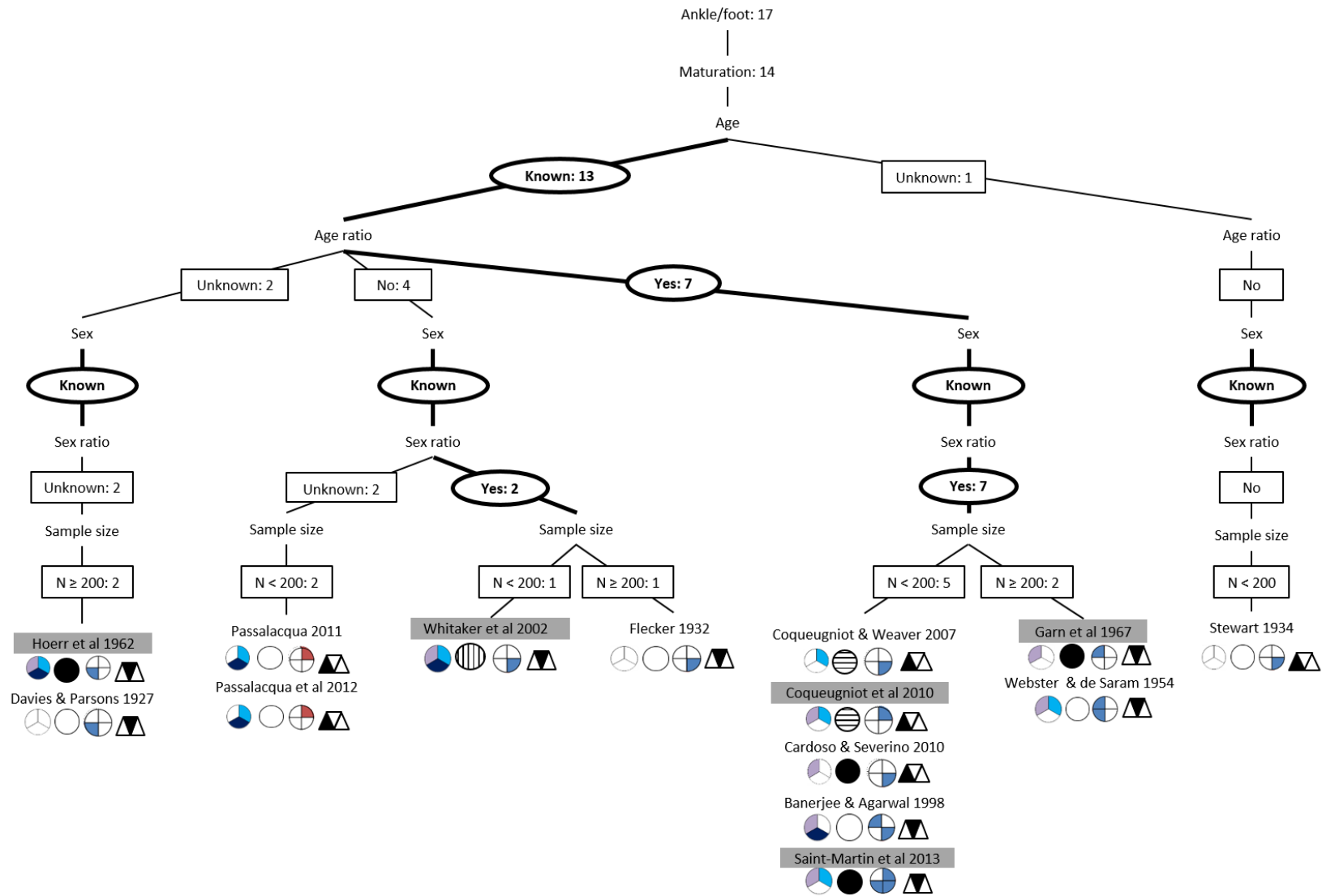


Figure A.32 Classification tree of the juvenile age estimation methods based on the maturation of the ankle and foot bones

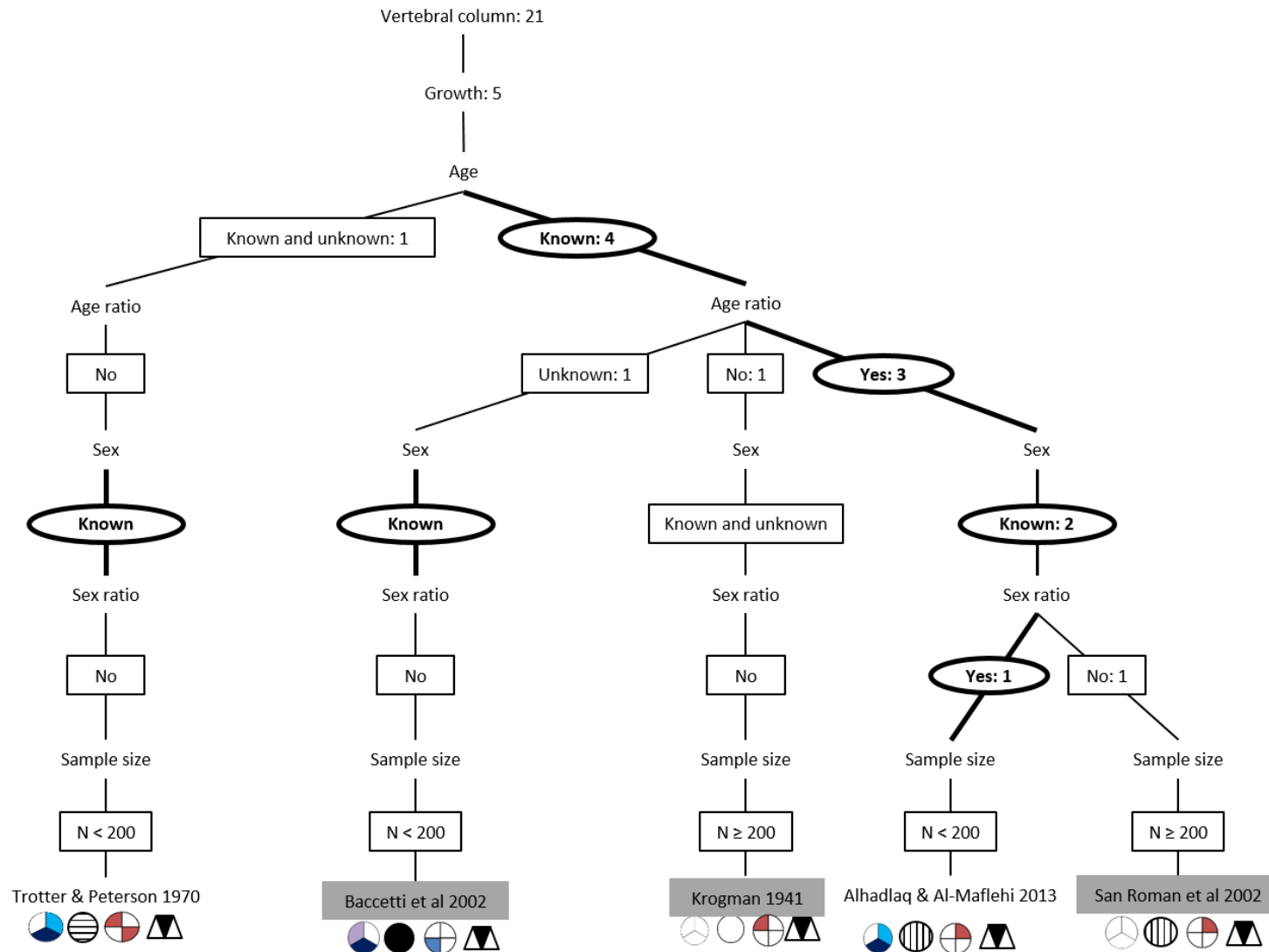


Figure A.33 Classification tree of the juvenile age estimation methods based on the growth of the vertebral column

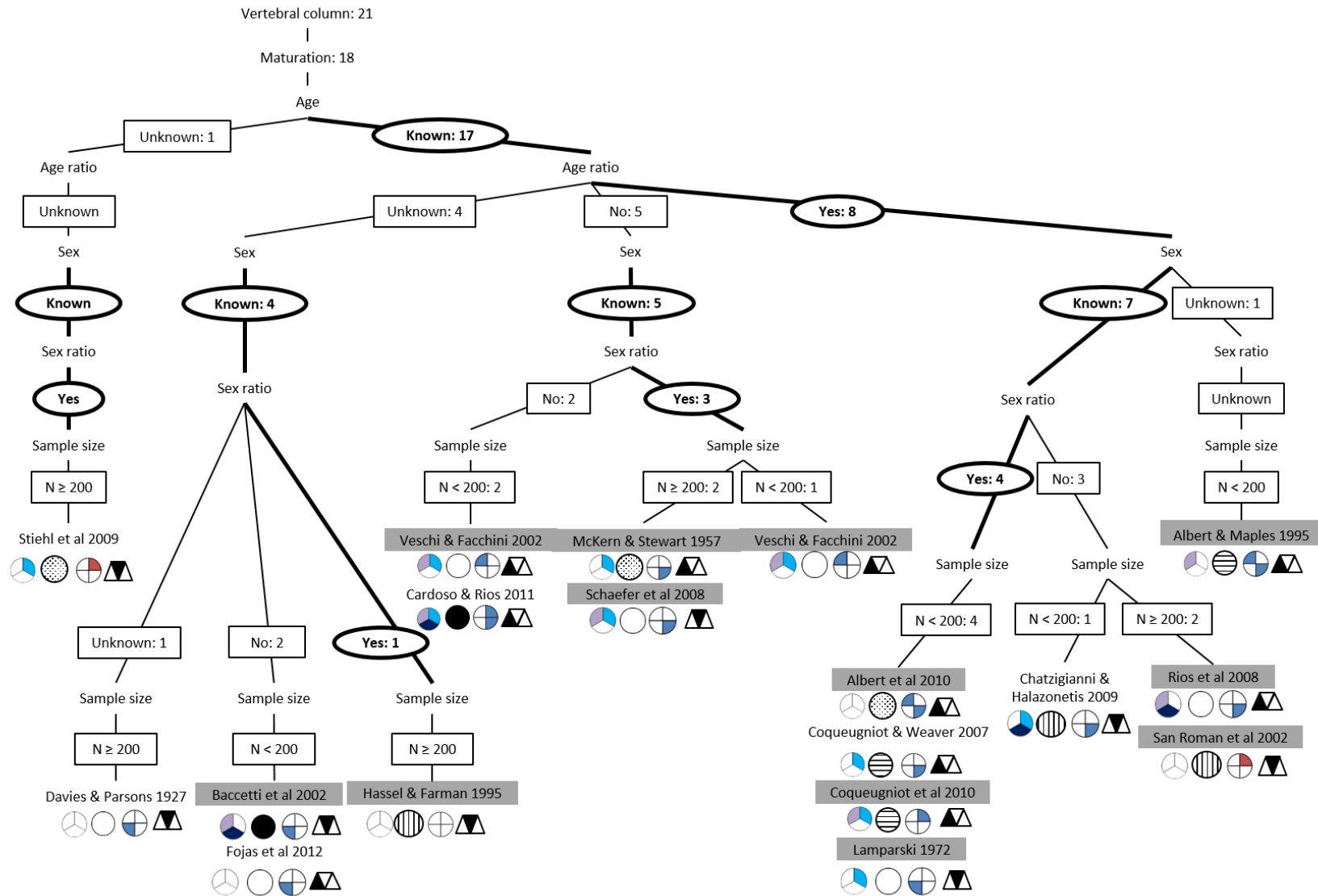


Figure A.34 Classification tree of the juvenile age estimation methods based on the maturation of the vertebral column

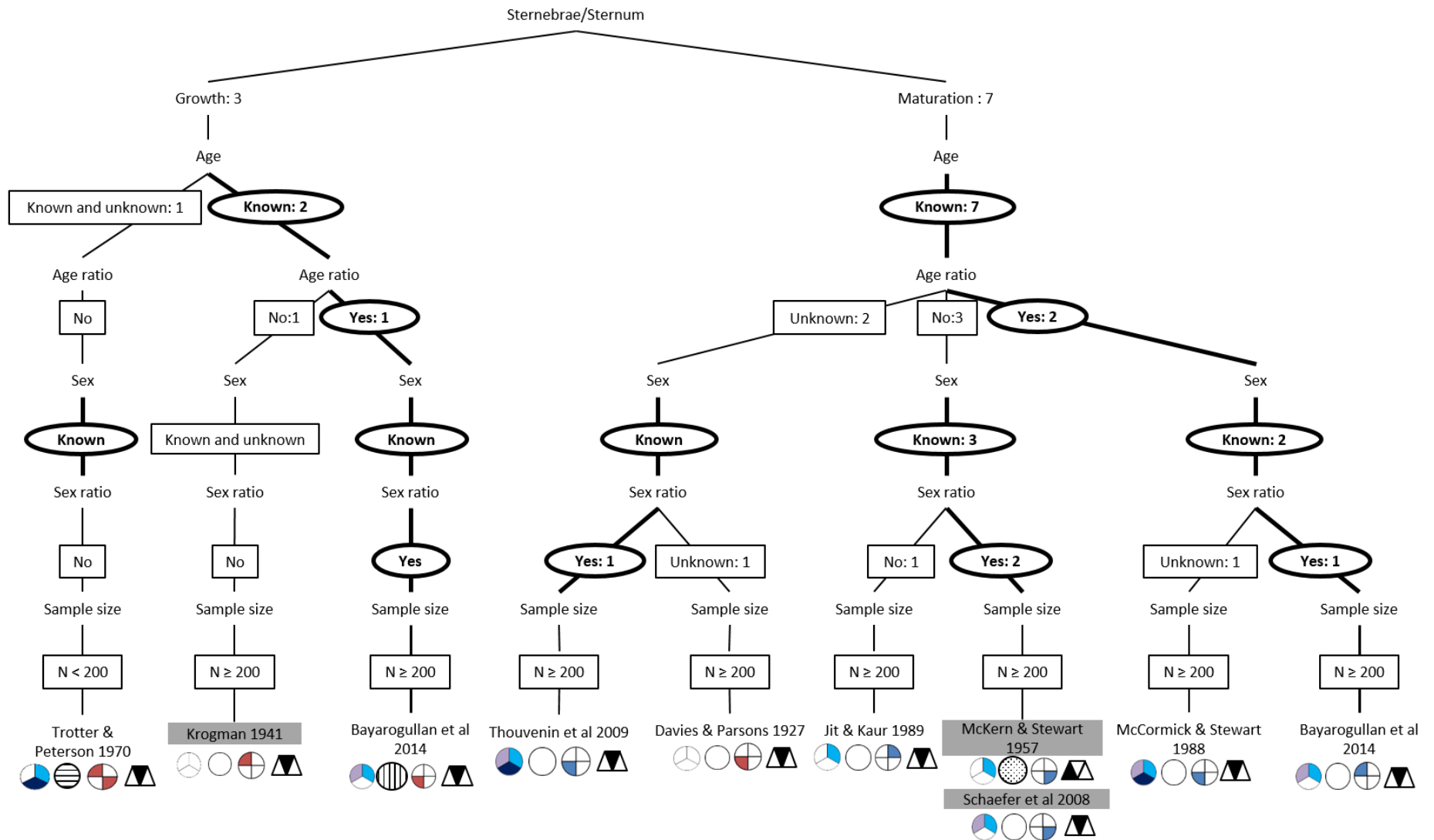


Figure A.35 Classification tree of the juvenile age estimation methods based on the growth or the maturation of the sternbrae or sternum



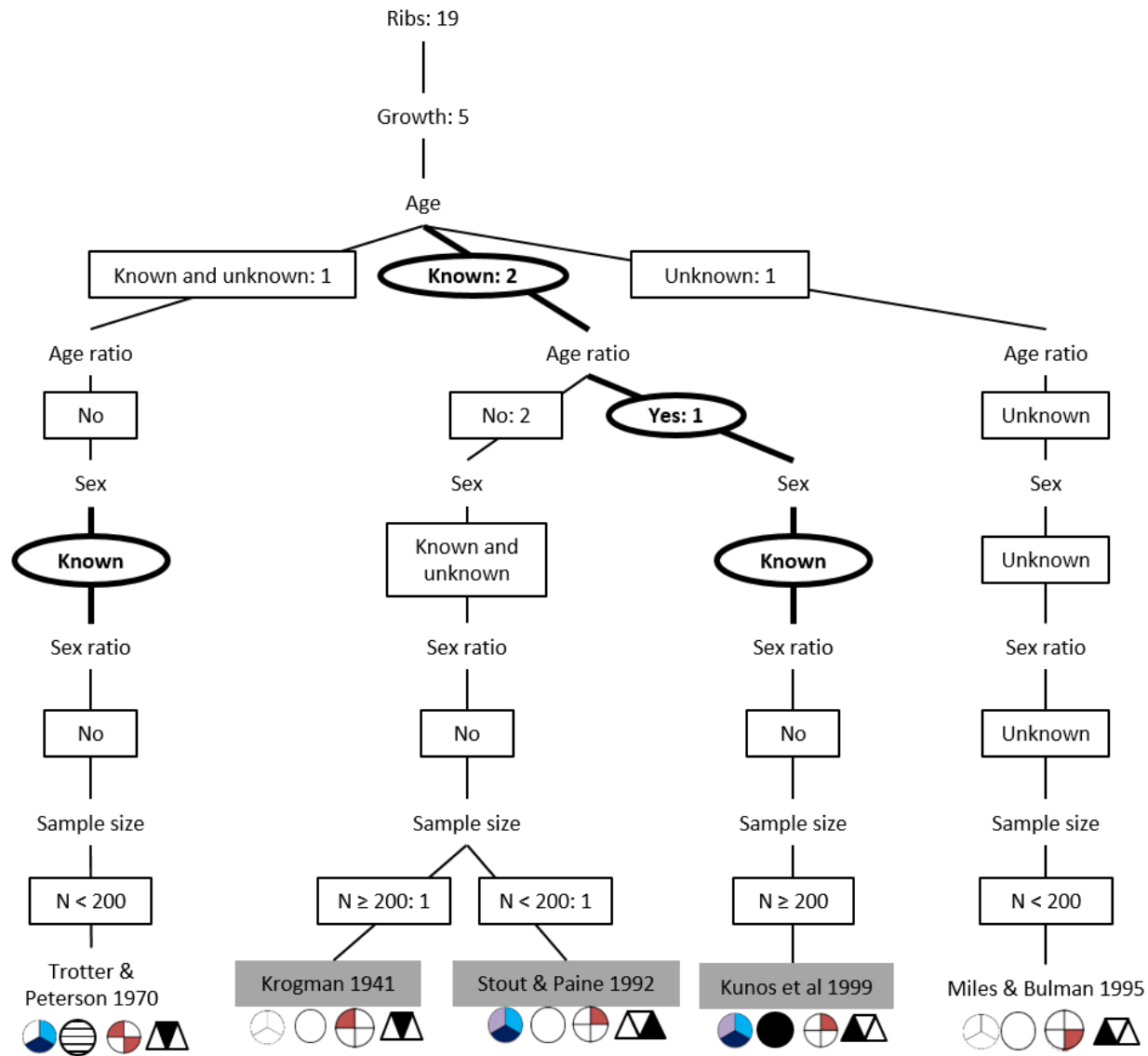


Figure A.36 Classification tree of the juvenile age estimation methods based on the growth of the ribs

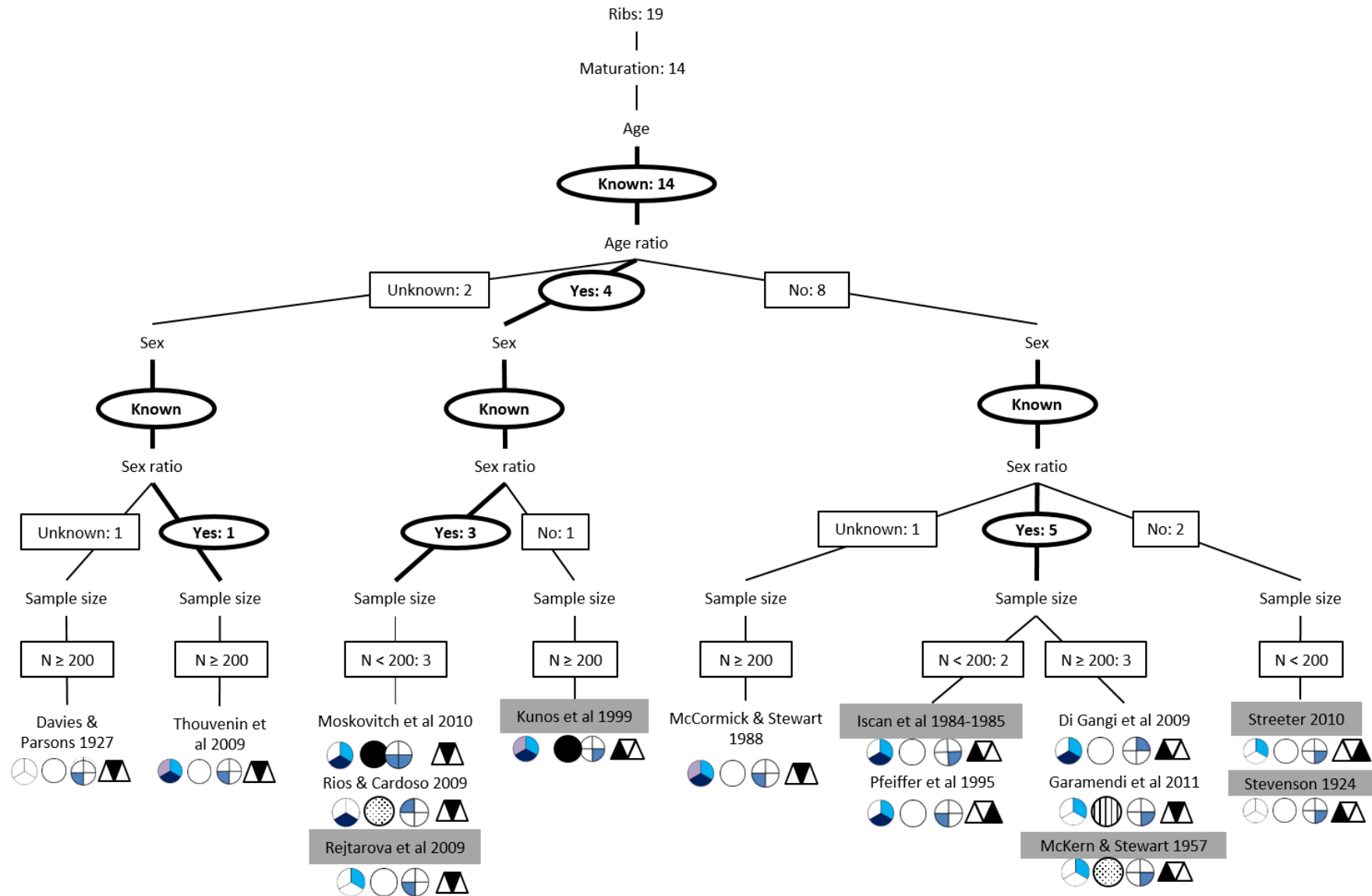


Figure A.37 Classification tree of the juvenile age estimation methods based on the maturation of the ribs

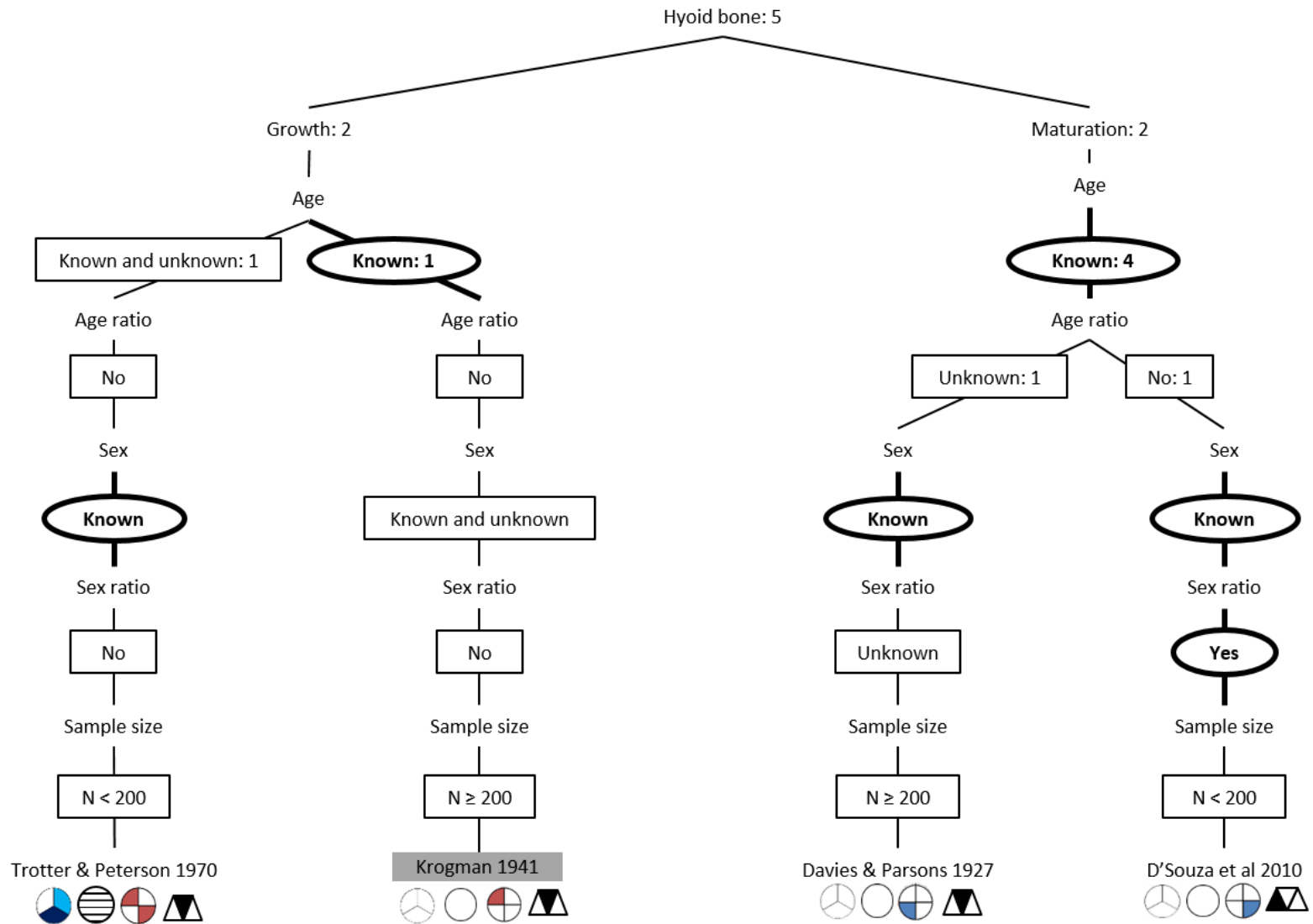


Figure A.38 Classification tree of the juvenile age estimation methods based on the growth or the maturation of the hyoid bone

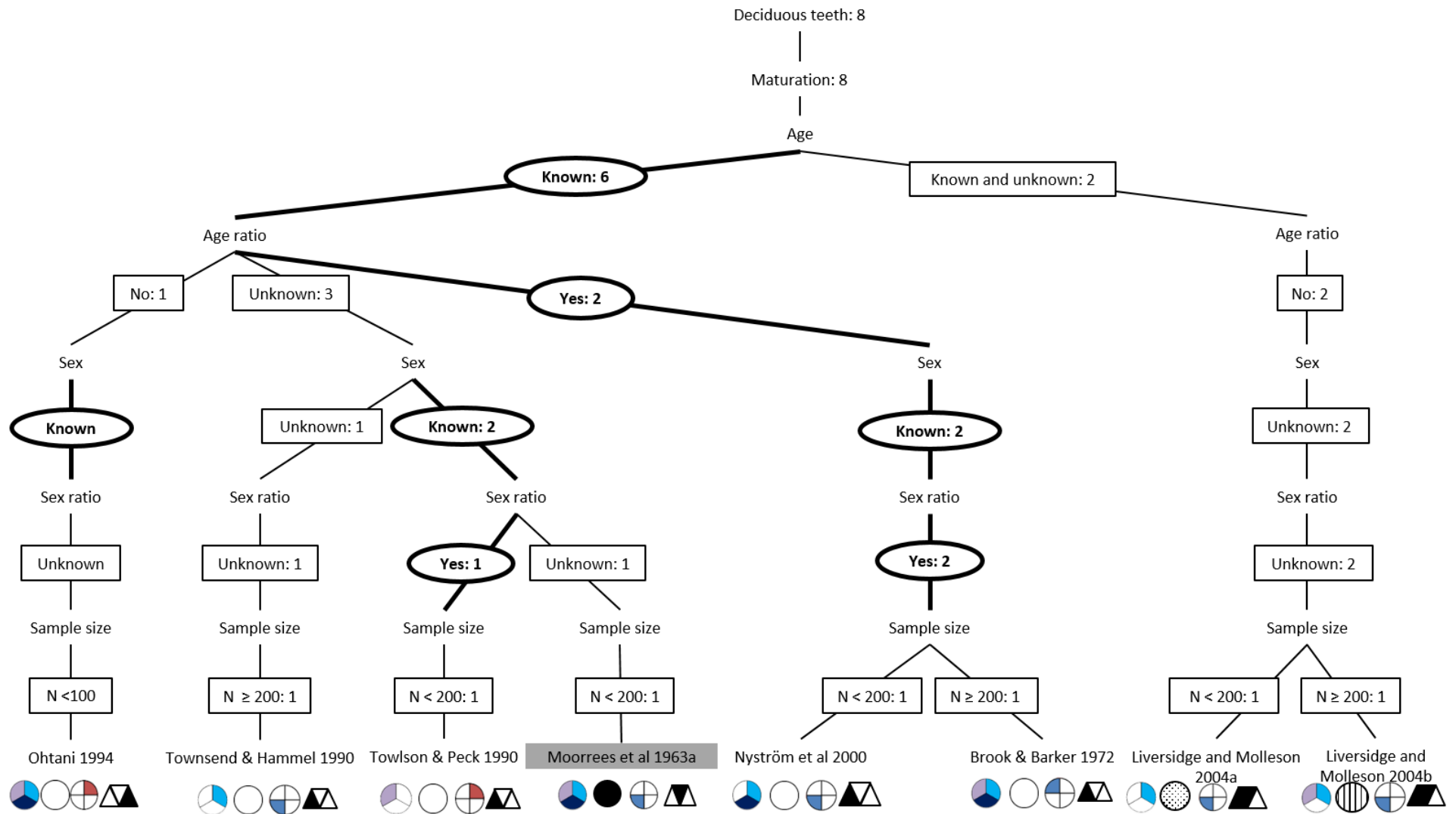


Figure A.39 Classification tree of the juvenile age estimation methods based on the maturation of deciduous teeth

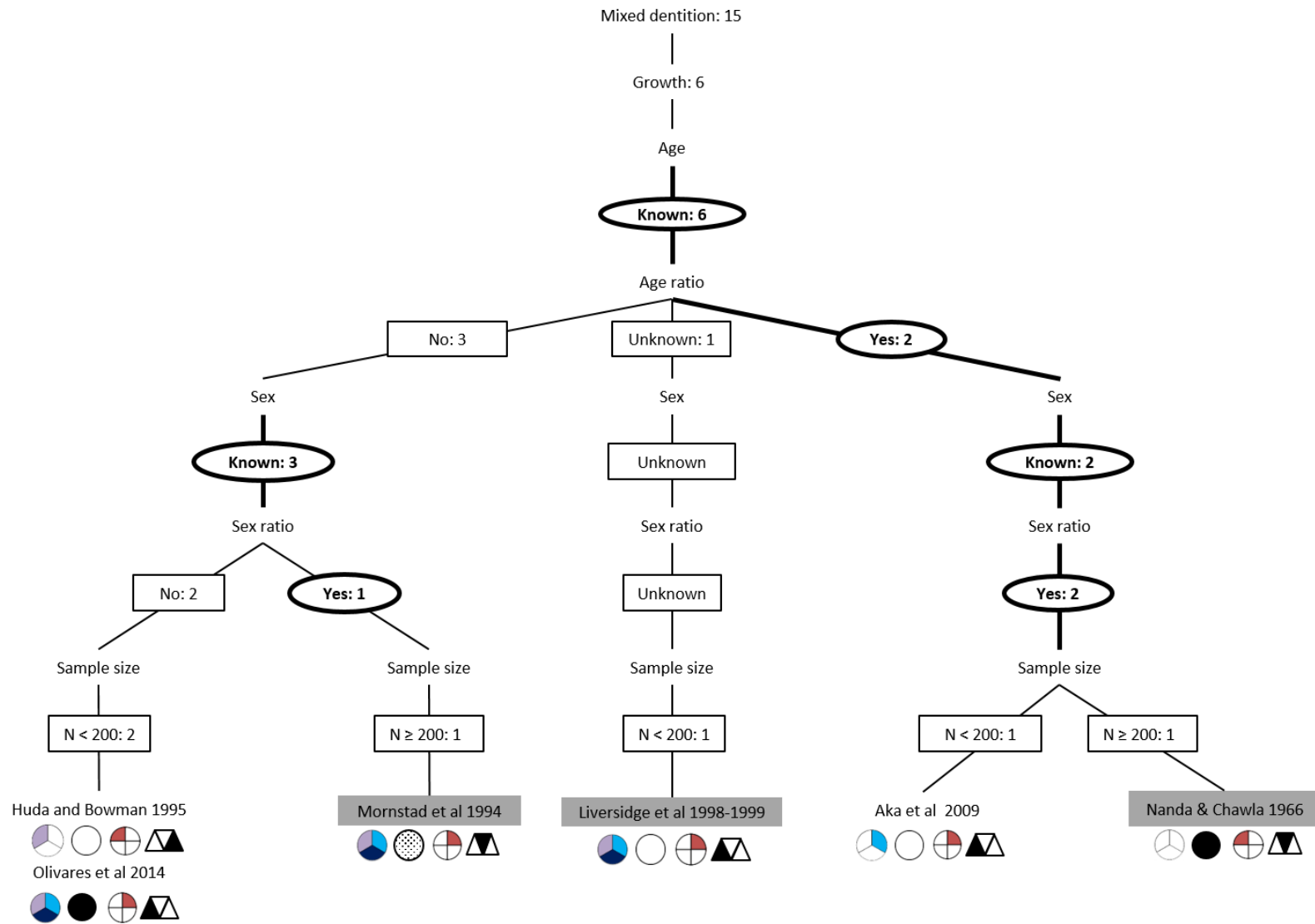


Figure A.40 Classification tree of the juvenile age estimation methods based on the growth of mixed dentition

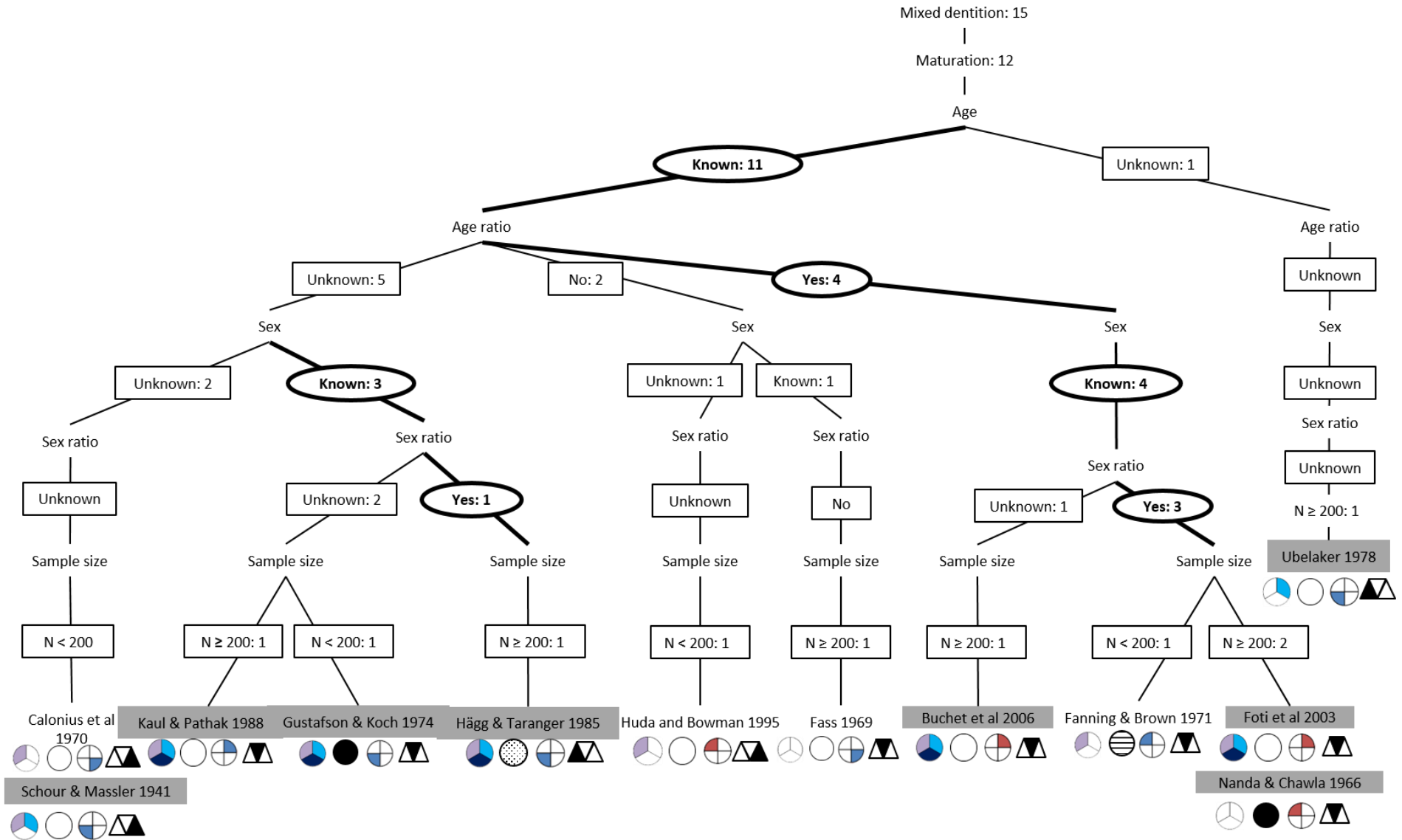


Figure A.41 Classification tree of the juvenile age estimation methods based on the maturation of mixed dentition

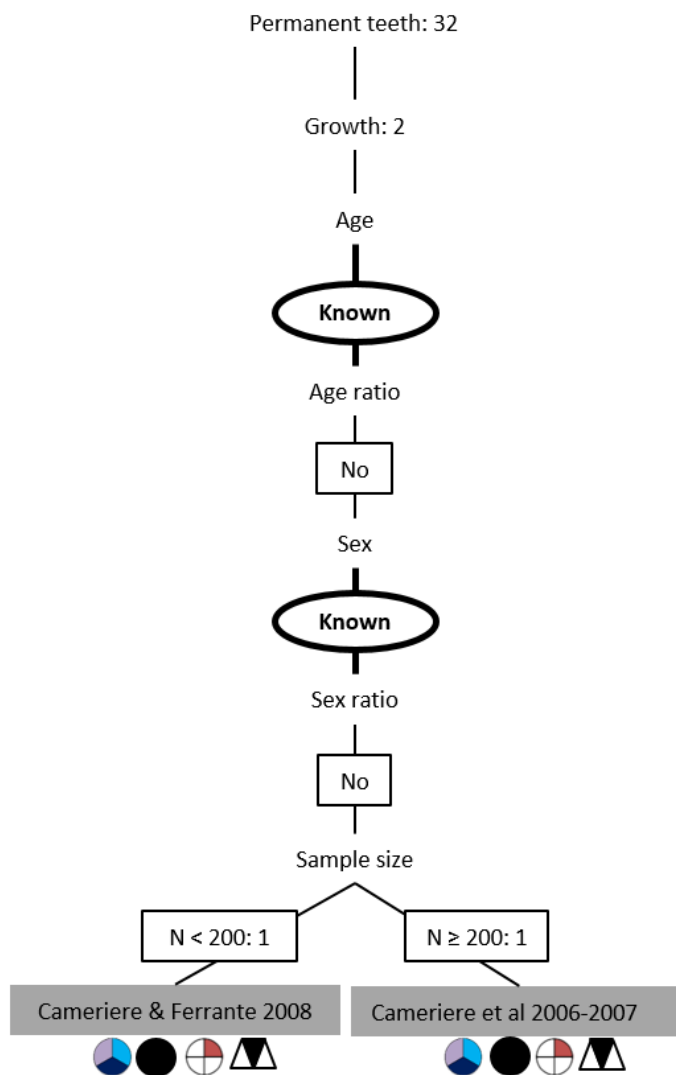


Figure A.42 Classification tree of the juvenile age estimation methods based on the growth of permanent teeth

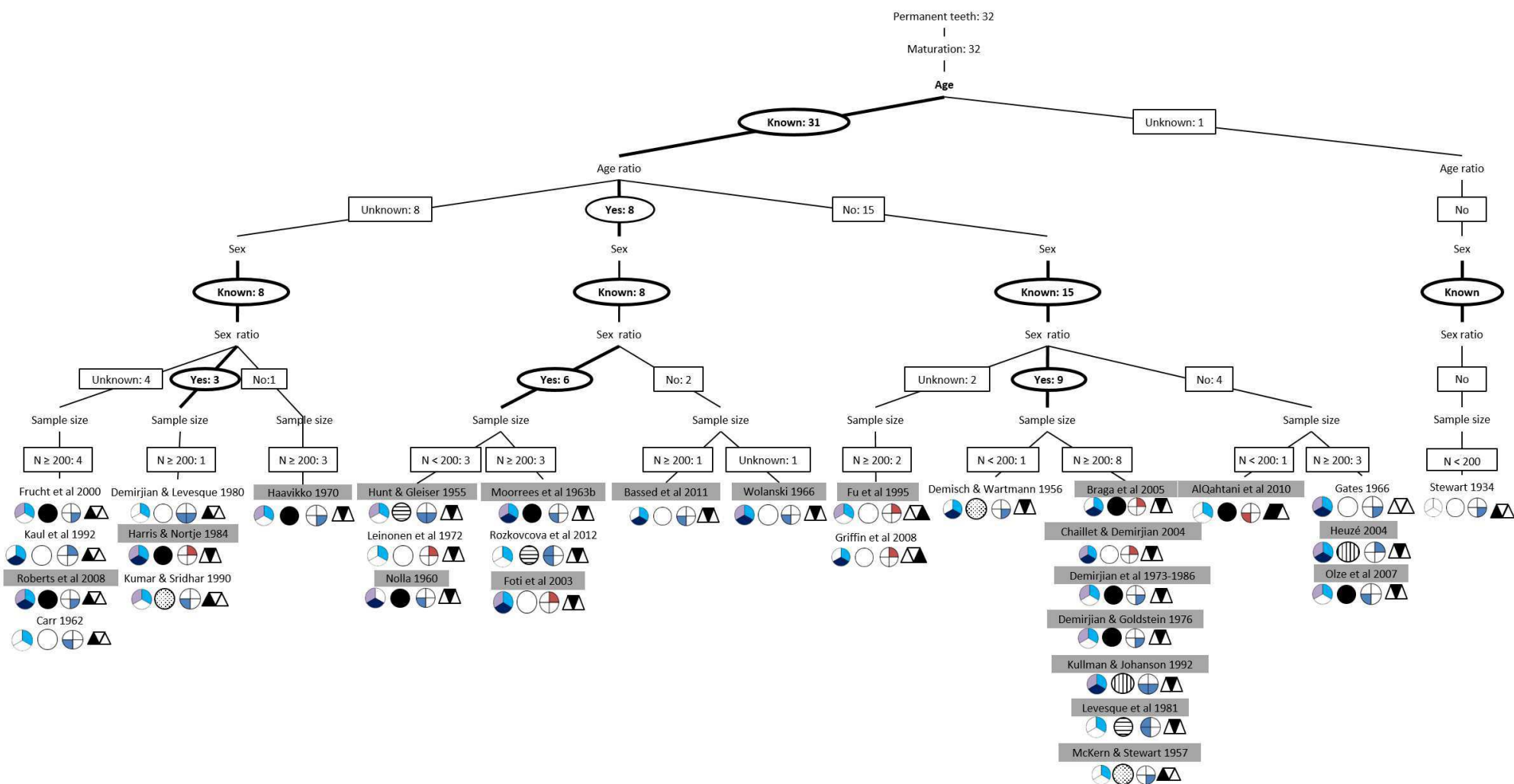


Figure A.43 Classification tree of the juvenile age estimation methods based on the maturation of permanent teeth



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## APPENDIX B

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### Descriptive statistics of the Marseilles sample



The following tables present the descriptive statistics of all the biometric and non-biometric variables taken on the ilium, the fifth lumbar vertebra, the clavicle and the iliac bone for the individuals from Marseilles.

These tables include the number of males and females, and the mean, standard deviation, median, minimal and maximal value of each variable per annual age group.

They can also be used as abacuses for age estimation, but the prediction interval and standard error of estimation have to be calculated individually.

The variables used in this study are represented in the figures for each section.

## Iliac variables

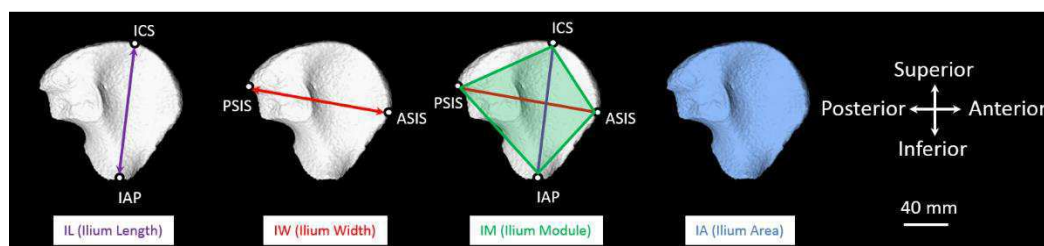


Figure B.1 Four biometric variables taken on the ilium

Table B-1 Descriptive statistics of the variable Ilium Length (IL)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	9	13	42.260	7.970	43.320	23.860	54.440
1	11	3	64.840	5.854	63.80	56.000	74.920
2	7	7	69.640	5.134	70.270	57.520	78.030
3	8	6	77.390	4.858	77.930	63.940	84.550
4	11	10	84.510	4.877	84.300	66.120	93.450
5	11	8	87.190	7.545	89.670	66.960	98.600
6	10	8	96.290	7.036	95.750	79.780	109.640
7	8	9	400.660	6.665	99.860	87.990	117.110
8	12	8	107.090	6.363	108.270	95.040	117.590
9	12	9	111.900	6.558	110.300	100.500	132.200
10	16	8	118.800	7.639	119.800	97.100	131.800
11	17	6	122.000	8.807	120.800	104.900	143.100
12	12	5	123.000	7.998	124.300	108.600	136.800

Table B-2 Descriptive statistics of the variable Ilium Width (IW)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	9	13	37.190	6.751	39.590	22.100	47.130
1	11	3	58.920	4.606	57.360	51.770	67.680
2	7	7	64.100	3.923	64.540	54.460	69.990
3	8	6	71.300	4.405	72.770	59.760	76.930
4	11	10	78.360	4.377	78.380	70.540	85.940
5	11	8	81.650	5.605	82.760	68.170	91.060
6	10	8	89.49	5.630	90.000	76.700	98.250
7	8	9	93.170	6.113	93.250	80.880	106.690
8	12	8	97.700	5.295	98.900	85.480	106.670
9	12	9	103.390	6.712	103.310	87.740	119.550
10	16	8	107.690	5.976	108.840	89.680	116.980
11	17	6	108.250	9.380	108.970	62.040	121.950
12	12	5	112.870	6.481	113.330	99.350	125.230

Table B-3 Descriptive statistics of the variable Ilium Module (IM)

Age group (years)	Number		Mean (mm <sup>2</sup> )	Standard deviation (mm <sup>2</sup> )	Median (mm <sup>2</sup> )	Minimal (mm <sup>2</sup> )	Maximal (mm <sup>2</sup> )
	Females	Males					
0	9	13	1623.315	553.635	1729.328	527.284	2495.700
1	11	3	3843.470	637.275	3544.814	2907.292	5050.304
2	7	7	4479.929	560.483	4470.446	3196.113	5257.656
3	8	6	5537.271	650.921	5696.840	3836.215	6504.482
4	11	10	6637.976	698.836	6585.207	4663.922	8023.632
5	11	8	7152.453	1019.934	7292.645	4686.343	8850.435
6	10	8	8650.103	1113.378	8695.339	6119.375	10694.980
7	8	9	9414.654	1222.668	9351.752	7131.021	12304.470
8	12	8	10490.250	1121.964	10713.870	8363.619	12236.660
9	12	9	11558.200	1417.861	11345.990	8815.682	15691.730
10	16	8	12830.650	1548.393	13007.310	8712.828	15084.070
11	17	6	13252.200	1880.204	13330.810	7125.997	16699.450
12	12	5	13922.680	1595.246	14039.100	11187.710	17136.210

Table B-4 Descriptive statistics of the variable Ilium Area (IA)

Age group (years)	Number		Mean (mm <sup>2</sup> )	Standard deviation (mm <sup>2</sup> )	Median (mm <sup>2</sup> )	Minimal (mm <sup>2</sup> )	Maximal (mm <sup>2</sup> )
	Females	Males					
0	9	13	1161.318	409.028	1204.856	365.102	1818.360
1	11	3	2711.292	416.216	2534.296	2084.070	3406.549
2	7	7	3170.364	370.672	3152.801	2463.473	3700.455
3	8	6	3870.368	446.202	4026.776	2722.856	4391.291
4	11	10	4643.637	459.122	4646.174	3912.472	5759.668
5	11	8	4994.122	664.202	5109.537	3379.753	6253.885
6	10	8	6032.691	798.397	6019.267	4294.335	7540.539
7	8	9	6579.162	875.202	6513.534	4983.055	8799.315
8	12	8	7383.634	858.288	7365.276	5898.726	8892.781
9	12	9	8124.960	1056.638	7990.760	6154.478	11320.660
10	16	8	9027.960	1082.454	9239.956	6127.868	10775.740
11	17	6	9452.253	1237.064	9368.779	7148.777	11944.530
12	12	5	9704.530	1130.032	9789.456	7605.842	12096.48

## Lumbar variables

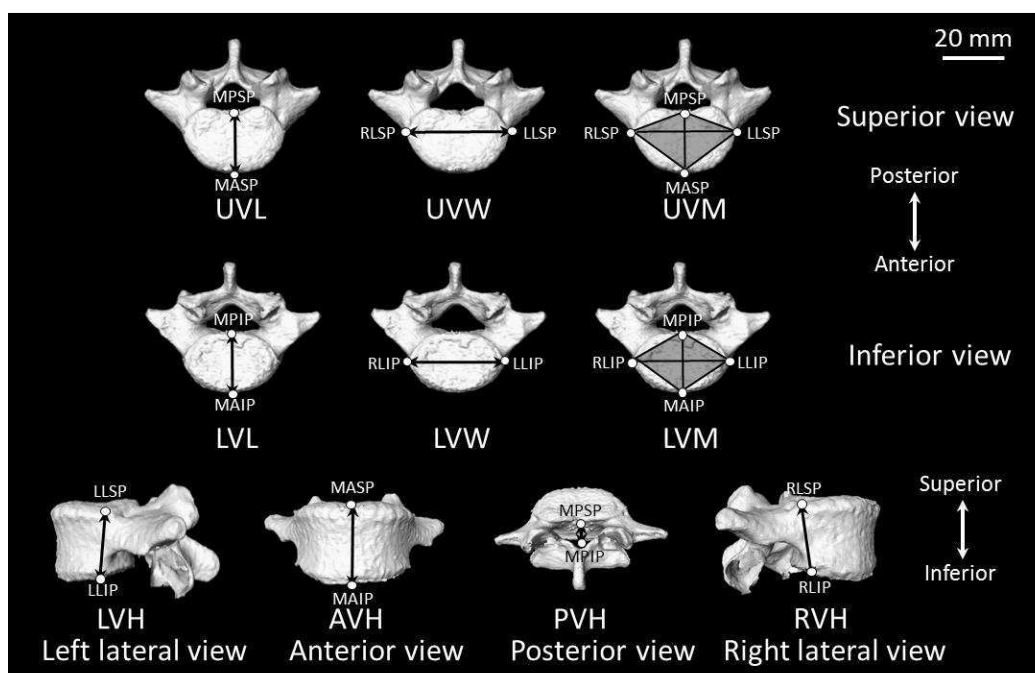


Figure B.2 Ten biometric variables taken on the fifth lumbar vertebra

Table B-5 Descriptive statistics of the variable Upper Vertebral Length (UVL)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	15	9	9.201	2.248	9.598	4.592	12.576
1	5	12	14.658	1.711	14.989	9.178	17.051
2	6	8	16.354	2.134	16.540	12.860	20.130
3	7	8	18.910	1.881	19.010	14.680	22.980
4	8	11	21.270	1.657	21.180	18.060	24.440
5	9	14	21.710	1.766	22.010	18.760	24.610
6	6	9	23.520	2.397	23.820	17.500	27.300
7	10	9	24.580	2.272	24.880	20.440	28.410
8	7	13	25.790	1.887	26.340	22.070	28.980
9	8	12	26.780	2.397	26.910	22.220	31.830
10	7	16	28.500	1.584	28.940	24.900	31.470
11	6	20	28.900	2.499	28.540	24.170	33.390
12	13	14	28.710	2.384	28.800	23.740	35.390
13	6	9	31.440	2.697	31.430	27.530	36.050
14	10	10	31.180	3.360	31.200	24.830	39.070
15	9	9	32.240	2.986	32.570	26.310	38.130
16	12	8	31.240	2.779	30.720	27.01	36.67
17	8	10	32.780	3.041	32.090	28.090	41.130
18	12	12	32.270	1.747	32.410	28.960	35.590
19	11	12	31.740	2.567	31.320	27.240	38.820

Table B-6 Descriptive statistics of the variable Upper Vertebral Width (UVW)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	15	9	16.059	4.402	16.217	6.799	23.952
1	5	12	24.730	2.520	25.130	16.230	29.060
2	6	8	29.960	1.642	26.610	24.670	30.650
3	7	8	29.470	2.998	29.900	23.610	36.700
4	8	11	32.710	2.075	32.960	27.640	36.020
5	9	14	34.530	3.322	34.890	27.410	40.10
6	6	9	36.530	3.587	36.470	27.650	42.240
7	10	9	39.520	3.141	39.330	33.560	44.020
8	7	13	40.210	2.766	40.070	34.100	44.420
9	8	12	42.740	3.108	42.580	38.540	49.700
10	7	16	44.200	3.049	45.150	38.690	48.750
11	6	20	45.800	3.426	45.500	38.330	50.890
12	13	14	45.910	3.065	45.770	39.320	51.940
13	6	9	48.390	3.011	48.810	44.800	54.420
14	10	10	47.050	4.258	45.980	39.580	55.840
15	9	9	48.070	4.456	47.050	40.630	55.780
16	12	8	47.210	3.469	46.460	41.380	58.270
17	8	10	49.250	3.981	49.010	42.990	62.590
18	12	12	48.740	3.665	48.260	40.860	57.480
19	11	12	49.150	3.645	49.680	43.510	57.220

Table B-7 Descriptive statistics of the variable Upper Vertebral Module (UVM)

Age group (years)	Number		Mean (mm <sup>2</sup> )	Standard deviation (mm <sup>2</sup> )	Median (mm <sup>2</sup> )	Minimal (mm <sup>2</sup> )	Maximal (mm <sup>2</sup> )
	Females	Males					
0	15	9	156.686	72.179	159.350	31.221	286.321
1	5	12	365.900	66.618	370.100	149.003	446.300
2	6	8	442.400	74.766	437.500	331.900	590.300
3	7	8	561.900	109.539	568.400	346.500	843.500
4	8	11	697.800	91.037	710.000	544.400	880.300
5	9	14	753.800	123.043	767.900	525.900	964.400
6	6	9	864.400	154.484	899.700	555.800	1153.400
7	10	9	976.600	155.622	968.000	718.300	1236.800
8	7	13	1040.600	133.796	1047.300	762.500	1275.700
9	8	12	1150.100	178.015	1140.900	856.500	1544.500
10	7	16	1262.400	138.172	1281.700	984.100	1534.300
11	6	20	1328.900	194.797	1323.600	926.500	1699.000
12	13	14	1324.000	190.067	1331.400	934.700	1838.300
13	6	9	1521.000	163.983	1516.000	1291.000	1848.000
14	10	10	1477.700	280.283	1418.900	982.600	2181.600
15	9	9	1561.000	282.623	1527.000	1069.000	2122.000
16	12	8	1481.000	229.462	1415.000	1186.000	2137.000
17	8	10	1622.000	276.363	1570.000	1330.000	2574.000
18	12	12	1577.000	184.411	1548.000	1183.000	2046.000
19	11	12	1566.000	224.364	1556.000	1205.000	2222

Table B-8 Descriptive statistics of the variable Lower Vertebral Length (LVL)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	15	9	8.862	2.028	9.203	4.802	11.927
1	5	12	14.170	1.798	14.4310	8.887	16.699
2	6	8	16.200	1.844	16.300	13.180	19.900
3	7	8	18.080	2.083	18.040	13.880	23.790
4	8	11	20.380	1.561	20.040	17.620	23.580
5	9	14	21.198	1.875	21.460	18.250	24.660
6	6	9	23.110	2.671	23.230	17.810	28.560
7	10	9	23.970	2.100	24.450	20.660	27.090
8	7	13	25.260	1.905	25.850	20.850	28.330
9	8	12	26.270	2.319	26.490	21.570	29.760
10	7	16	27.950	1.945	28.190	23.930	31.190
11	6	20	28.400	2.310	28.280	24.130	34.280
12	13	14	28.800	2.301	28.540	23.380	35.910
13	6	9	31.780	2.298	32.450	28.300	36.180
14	10	10	30.940	3.229	30.560	25.440	38.610
15	9	9	32.360	2.927	32.450	26.550	39.040
16	12	8	31.410	2.520	31.680	24.980	36.720
17	8	10	32.610	3.082	31.420	28.470	38.660
18	12	12	32.650	3.049	32.900	27.940	41.120
19	11	12	32.100	2.994	31.370	28.620	42.080

Table B-9 Descriptive statistics of the variable Lower Vertebral Width (LVW)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	15	9	15.054	4.266	15.530	7.322	23.757
1	5	12	24.520	3.411	25.070	12.930	28.500
2	6	8	26.890	2.992	27.380	20.380	31.810
3	7	8	31.040	3.447	30.430	24.870	40.210
4	8	11	34.340	2.003	34.530	30.350	37.660
5	9	14	35.800	2.674	35.990	30.860	39.610
6	6	9	37.760	3.676	38.350	29.500	43.700
7	10	9	39.820	3.936	38.840	34.200	46.400
8	7	13	40.450	2.851	40.370	33.860	45.300
9	8	12	41.960	2.093	41.870	37.880	45.880
10	7	16	43.810	2.667	44.370	39.380	49.050
11	6	20	45.060	2.755	45.710	37.010	49.170
12	13	14	45.100	3.105	45.080	39.710	52.170
13	6	9	47.030	2.714	46.220	43.050	53.400
14	10	10	46.450	3.287	46.120	40.060	52.260
15	9	9	47.070	4.318	45.870	39.190	55.390
16	12	8	46.960	3.614	46.390	39.360	55.580
17	8	10	48.670	3.461	47.850	44.170	57.880
18	12	12	48.240	3.532	48.110	39.400	53.960
19	11	12	47.780	3.840	48.270	40.520	53.770

Table B-10 Descriptive statistics of the variable Lower Vertebral Module (LVM)

Age group (years)	Number		Mean (mm <sup>2</sup> )	Standard deviation (mm <sup>2</sup> )	Median (mm <sup>2</sup> )	Minimal (mm <sup>2</sup> )	Maximal (mm <sup>2</sup> )
	Females	Males					
0	15	9	140.754	64.479	147.890	35.159	278.535
1	5	12	352.349	77.577	360.600	114.900	450.800
2	6	8	438.889	87.867	438.300	286.600	596.000
3	7	8	566.936	129.261	543.200	345.300	956.800
4	8	11	701.531	83.924	709.300	549.300	869.900
5	9	14	761.542	107.993	689.400	565.500	930.700
6	6	9	878.883	170.665	875.700	546.000	1248.200
7	10	9	960.700	170.447	930.900	731.900	1213.300
8	7	13	1025.708	136.389	1039.200	706.200	1238.600
9	8	12	1105.381	142.536	1097.300	833.500	1338.800
10	7	16	1227.598	142.746	1241.100	956.500	1500.700
11	6	20	1284.155	169.156	1281.700	921.100	1660.100
12	13	14	1303.028	175.838	1285.300	928.500	1844.500
13	6	9	1496.140	156.228	1495.000	1248.000	1771.000
14	10	10	1445.572	243.982	1388.000	1051.000	2001.000
15	9	9	1533.472	269.383	1544.000	1041.000	2162.000
16	12	8	1479.669	207.866	1469.000	1115.000	1983.000
17	8	10	1594.000	247.573	1552.000	1257.000	2238.000
18	12	12	1580.000	220.051	1617.000	1101.000	2014.000
19	11	12	1541.000	244.233	1564.000	1183.000	2199.000

Table B-11 Descriptive statistics of the variable Posterior Vertebral Height (PVH)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	15	9	5.560	1.526	5.859	1.830	7.988
1	5	12	7.703	1.145	7.571	5.446	9.836
2	6	8	8.753	1.183	8.863	6.873	10.756
3	7	8	8.658	1.117	8.549	6.737	10.663
4	8	11	9.222	1.135	8.949	6.635	11.710
5	9	14	9.105	1.302	8.741	7.225	11.784
6	6	9	10.533	1.233	10.031	8.384	12.171
7	10	9	12.055	2.169	11.703	9.108	16.104
8	7	13	12.420	2.404	11.701	8.473	18.738
9	8	12	14.560	2.214	14.120	10.900	20.060
10	7	16	15.760	3.016	15.090	12.330	22.440
11	6	20	17.220	2.462	17.220	12.350	24.820
12	13	14	19.220	3.407	19.700	12.020	24.500
13	6	9	20.820	3.497	21.210	15.110	25.830
14	10	10	22.590	3.440	23.460	15.520	27.490
15	9	9	22.490	2.307	22.570	18.670	25.760
16	12	8	22.170	2.687	22.650	15.270	25.910
17	8	10	23.050	1.691	23.380	19.900	25.370
18	12	12	22.830	1.666	22.410	19.980	26.520
19	11	12	23.100	2.193	22.880	20.070	28.470

Table B-12 Descriptive statistics of the variable Anterior Vertebral Height (AVH)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	15	9	5.660	1.283	5.643	3.724	7.754
1	5	12	8.877	1.438	8.902	6.108	11.470
2	6	8	10.562	1.008	10.559	8.814	12.822
3	7	8	11.100	1.414	11.640	8.100	13.210
4	8	11	12.907	1.505	13.434	9.333	16.492
5	9	14	13.380	1.687	13.778	9.547	15.648
6	6	9	15.230	1.499	15.180	11.980	17.510
7	10	9	15.750	1.522	15.830	12.360	18.260
8	7	13	16.500	1.492	16.520	13.770	20.030
9	8	12	18.640	1.548	18.560	15.510	22.210
10	7	16	19.340	20.029	18.950	16.190	25.05
11	6	20	19.600	2.325	19.880	15.210	24.810
12	13	14	21.620	3.339	21.810	13.460	26.830
13	6	9	23.360	4.206	24.250	14.300	28.920
14	10	10	24.380	3.048	24.910	17.000	28.860
15	9	9	25.960	1.879	25.780	23.160	29.370
16	12	8	25.800	3.332	26.950	15.990	29.390
17	8	10	26.290	2.568	25.870	22.500	30.610
18	12	12	25.780	1.909	26.150	21.180	28.670
19	11	12	27.160	1.989	27.470	22.300	30.730

Table B-13 Descriptive statistics of the variable Right Vertebral Height (RVH)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	15	9	4.812	0.997	4.480	3.475	7.682
1	5	12	6.484	1.212	6.363	4.815	8.877
2	6	8	7.942	1.538	7.335	6.101	10.761
3	7	8	7.993	1.818	7.124	5.756	11.855
4	8	11	8.996	1.184	9.257	6.784	11.802
5	9	14	9.976	1.458	9.943	7.175	13.169
6	6	9	11.316	2.028	11.114	6.793	14.060
7	10	9	12.380	1.337	12.440	10.200	15.680
8	7	13	13.130	1.835	13.190	10.230	16.420
9	8	12	14.640	1.585	14.590	12.350	18.310
10	7	16	15.820	2.589	15.370	12.360	21.830
11	6	20	16.490	2.153	16.040	12.800	20.990
12	13	14	18.920	2.914	19.140	13.680	25.470
13	6	9	21.320	3.076	20.660	15.750	26.510
14	10	10	22.850	3.363	23.990	15.420	29.050
15	9	9	24.200	1.912	24.540	20.580	27.860
16	12	8	23.500	2.605	24.230	16.460	26.450
17	8	10	24.730	2.665	25.100	20.270	28.480
18	12	12	23.620	1.902	23.930	20.250	26.570
19	11	12	25.110	1.887	24.970	21.170	29.460



Table B-14 Descriptive statistics of the variable Left Vertebral Height (LVH)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	15	9	4.722	1.045	4.640	3.098	6.832
1	5	12	6.354	1.331	6.123	3.444	8.783
2	6	8	7.391	1.221	7.039	5.766	9.406
3	7	8	7.689	1.658	7.102	4.671	10.841
4	8	11	8.960	1.262	8.979	6.654	11.005
5	9	14	9.819	1.450	10.004	6.733	12.339
6	6	9	11.266	1.576	11.430	7.749	13.845
7	10	9	12.099	1.560	11.870	9.507	15.508
8	7	13	12.976	1.677	12.994	9.764	15.731
9	8	12	14.831	1.925	14.332	9.982	18.404
10	7	16	15.510	2.906	15.240	10.420	23.360
11	6	20	15.990	2.123	15.710	11.400	20.820
12	13	14	18.890	3.657	18.810	11.940	26.470
13	6	9	21.170	3.783	20.890	15.990	27.310
14	10	10	22.690	3.727	24.180	14.980	28.050
15	9	9	23.320	2.086	23.590	18.040	26.270
16	12	8	23.400	2.777	23.620	15.510	27.790
17	8	10	24.690	2.784	25.300	19.730	29.750
18	12	12	23.790	1.684	23.980	19.730	26.140
19	11	12	24.410	2.101	24.370	19.520	29.460

## Clavicular variables

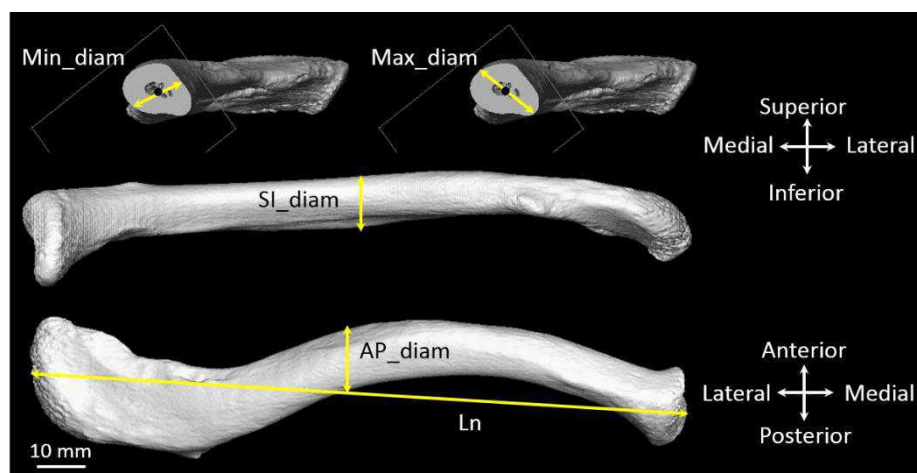


Figure B.3 Five biometric variables taken on the clavicle

Table B-15 Descriptive statistics of the variable Maximal length (Ln)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	14	7	53.24	8.186	54.88	31.48	62.71
1	10	12	69.83	5.840	69.9	55.22	79.88
2	7	7	76.84	4.693	76.79	66.81	85.13
3	10	8	83.23	4.222	83.55	74.48	92.28
4	7	8	87.79	5.318	88.02	78.99	99.12
5	7	7	93.48	5.216	94.47	82.85	104.85
6	9	5	96.92	7.403	97.88	84.51	108.76
7	6	7	103.49	7.620	102.69	92.59	120.19
8	8	7	108.3	6.724	110.31	94.83	118.43
9	6	9	111.40	6.758	109.50	102.30	125.80
10	6	8	116.10	7.480	115.80	105.70	139.90
11	4	8	121.00	8.314	118.30	110.40	139.20
12	7	5	127.60	9.510	130.80	105.10	141.00
13	8	9	138.90	13.129	136.80	123.10	172.40
14	8	6	137.90	12.395	137.80	118.60	161.90
15	8	10	143.80	8.656	144.20	128.60	159.70
16	9	8	143.80	8.128	144.30	131.20	161.40
17	7	10	144.50	8.444	144.20	130.30	158.00
18	10	12	147.80	11.964	148.60	124.60	172.10
19	9	11	150.90	8.789	151.70	135.10	171.40

Table B-16 Descriptive statistics of the variable Maximal diameter (Max\_diam)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	14	7	5.890	1.469	6.153	2.563	8.750
1	10	12	7.152	0.671	7.310	6.074	8.519
2	7	7	7.825	0.652	7.837	6.446	8.916
3	10	8	7.802	0.689	7.721	6.420	9.024
4	7	8	8.228	0.647	8.344	6.556	9.177
5	7	7	8.865	0.883	8.590	7.386	10.937
6	9	5	9.086	0.933	9.294	7.491	10.659
7	6	7	9.252	1.066	9.131	7.726	10.939
8	8	7	9.967	0.884	9.855	7.962	12.304
9	6	9	10.427	1.138	10.329	8.713	12.488
10	6	8	10.856	1.305	10.345	9.611	15.209
11	4	8	10.890	1.295	10.695	9.086	14.414
12	7	5	11.760	0.723	11.630	10.450	13.250
13	8	9	12.640	2.609	11.750	10.140	20.560
14	8	6	12.559	2.086	11.959	9.779	16.855
15	8	10	13.160	1.541	13.210	10.660	16.910
16	9	8	13.155	1.936	13.833	9.277	16.122
17	7	10	13.700	2.091	13.370	10.560	18.340
18	10	12	13.316	2.026	13.495	9.368	17.854
19	9	11	13.560	1.424	13.540	11.070	16.140

Table B-17 Descriptive statistics of the variable Minimal diameter (Min\_diam)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	14	7	4.335	0.957	4.278	2.080	6.013
1	10	12	5.247	0.537	5.200	4.326	6.359
2	7	7	5.980	0.476	5.966	5.214	6.927
3	10	8	6.105	0.819	6.065	4.545	8.615
4	7	8	6.355	0.673	6.399	4.956	7.662
5	7	7	6.823	0.723	6.873	5.627	8.334
6	9	5	6.956	0.662	6.981	5.587	8.168
7	6	7	7.788	0.948	7.541	6.366	10.253
8	8	7	7.957	0.750	7.864	6.562	9.204
9	6	9	8.420	0.858	8.340	7.163	10.595
10	6	8	8.349	0.780	8.325	7.009	9.593
11	4	8	8.775	0.993	8.809	7.320	10.721
12	7	5	9.658	1.094	9.732	7.620	12.090
13	8	9	9.943	2.030	9.355	7.345	15.719
14	8	6	10.318	1.830	9.877	8.333	13.892
15	8	10	10.449	1.330	10.607	7.997	13.323
16	9	8	10.695	1.171	10.573	8.896	13.638
17	7	10	11.119	1.527	11.304	7.805	13.692
18	10	12	10.812	1.479	10.518	8.268	14.273
19	9	11	11.095	1.382	10.807	8.776	13.674

Table B-18 Descriptive statistics of the variable Antero-posterior diameter (AP\_diam)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	14	7	5.520	1.384	5.629	2.468	8.203
1	10	12	6.504	0.825	6.512	4.692	8.048
2	7	7	7.148	0.765	7.184	5.716	8.517
3	10	8	7.169	0.781	7.186	5.893	8.420
4	7	8	7.216	0.713	7.070	5.725	8.403
5	7	7	8.170	0.969	7.957	6.399	10.033
6	9	5	8.680	0.922	8.196	6.973	10.386
7	6	7	8.685	0.902	8.693	7.224	10.104
8	8	7	9.444	0.931	9.319	7.727	11.554
9	6	9	9.494	1.196	9.183	7.694	12.090
10	6	8	9.914	1.189	9.848	7.672	12.831
11	4	8	9.962	0.906	10.092	8.244	12.395
12	7	5	11.113	0.923	11.013	9.119	13.085
13	8	9	11.526	1.916	10.895	9.145	17.746
14	8	6	11.886	2.113	11.730	8.851	16.851
15	8	10	11.981	1.482	12.048	9.017	15.576
16	9	8	12.301	1.820	12.649	8.316	15.398
17	7	10	12.478	1.921	12.486	8.261	16.868
18	10	12	12.150	2.203	12.545	6.268	17.338
19	9	11	12.698	1.669	12.749	9.813	16.211

Table B-19 Descriptive statistics of the variable Supero-inferior diameter (SI\_diam)

Age group (years)	Number		Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
	Females	Males					
0	14	7	4.393	1.092	4.501	1.255	6.010
1	10	12	5.600	0.566	5.659	4.000	7.076
2	7	7	6.337	0.432	6.279	5.727	7.278
3	10	8	6.439	0.874	6.349	4.973	8.952
4	7	8	6.883	1.250	7.232	2.526	9.065
5	7	7	7.117	0.925	7.020	5.880	9.065
6	9	5	7.410	0.866	7.504	5.793	9.293
7	6	7	7.934	1.088	7.827	6.308	10.459
8	8	7	8.219	0.801	8.138	6.929	9.655
9	6	9	8.584	0.856	8.374	6.983	10.531
10	6	8	8.853	0.904	8.880	7.031	10.935
11	4	8	9.397	1.646	9.110	7.506	14.338
12	7	5	9.890	0.817	9.669	8.925	12.122
13	8	9	9.956	2.083	9.273	5.134	15.665
14	8	6	10.550	1.803	10.441	8.155	14.100
15	8	10	10.715	1.703	10.225	8.011	16.555
16	9	8	10.974	1.438	10.496	8.860	13.910
17	7	10	11.436	1.630	11.347	7.665	14.858
18	10	12	11.271	1.472	10.960	8.625	14.815
19	9	11	11.320	1.624	11.375	8.521	15.014

## Iliac non-metric variables

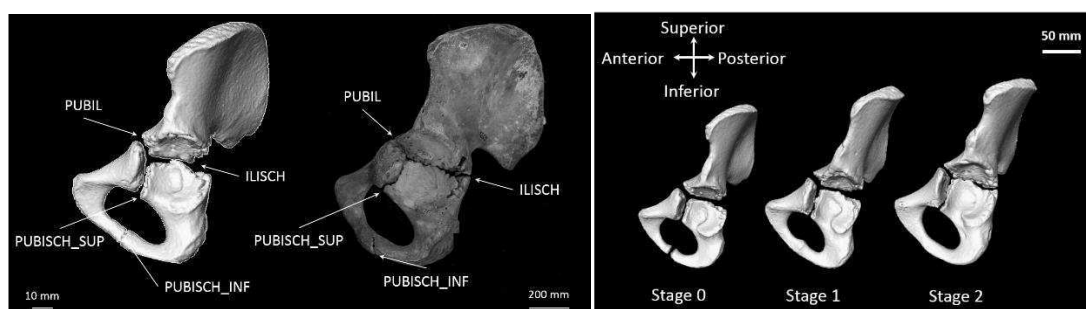


Figure B.4 The four epiphyseal sites and the three maturation stages of the iliac bone

Table B-20 Number of individuals per sex and age group for whom the iliac bone was studied

Age group (years)	Number	
	Females	Males
0	16	9
1	5	10
2	7	6
3	6	7
4	9	7
5	8	10
6	8	9
7	9	8
8	9	12
9	8	9
10	9	16
11	7	20
12	10	12
13	8	7
14	9	10
15	10	8
16	12	10
17	9	11
18	12	13
19	15	11

Table B-21 Descriptive statistics of the maturation stages at the Inferior pubo-ischiatic epiphysis (PUBISCH\_INF)

Stage	Number		Mean age (years)	Standard deviation (years)	Median (years)	Minimal age (years)	Maximal age (years)
	Females	Males					
0	52	56	3.50	2.77	3.039	0.003	14.84
1	16	32	9.22	3.28	9.322	3.11	17.22
2	126	118	14.13	3.87	14.638	4.54	19.95

Table B-22 Descriptive statistics of the maturation stages at the Inferior pubo-ischiatic epiphysis (PUBISCH\_INF) per sex

Stage	Females		Males	
	Minimal age (years)	Maximal age (years)	Minimal age (years)	Maximal age (years)
0	0.003	9.63	0.02	14.84
1	4.11	14.77	3.11	17.22
2	5.49	19.93	4.54	19.95

Table B-23 Descriptive statistics of the maturation stages at the Superior pubo-ischiatic epiphysis (PUBISCH\_SUP)

Stage	Number		Mean age (years)	Standard deviation (years)	Median (years)	Minimal age (years)	Maximal age (years)
	Females	Males					
0	86	125	5.74	3.56	5.642	0.003	16.44
1	27	41	11.89	1.98	11.794	7.94	18.19
2	76	61	16.91	2.09	17.107	12.07	19.95

Table B-24 Descriptive statistics of the maturation stages at the Superior pubo-ischiatic epiphysis (PUBISCH\_SUP) per sex

Stage	Females		Males	
	Minimal age (years)	Maximal age (years)	Minimal age (years)	Maximal age (years)
0	0.003	11.08	0.02	12.78
1	7.94	16.44	9.62	18.19
2	12.07	19.93	13.58	19.95

Table B-25 Descriptive statistics of the maturation stages at the Ilio-ischiatic epiphysis (ILISCH)

Stage	Number		Mean age (years)	Standard deviation (years)	Median (years)	Minimal age (years)	Maximal age (years)
	Females	Males					
0	81	113	5.29	3.32	5.114	0.003	12.49
1	32	51	11.47	1.82	11.458	6.61	16.44
2	77	63	16.90	2.09	17.141	12.07	19.95

Table B-26 Descriptive statistics of the maturation stages at the Ilio-ischiatic epiphysis (ILISCH) per sex

Stage	Females		Males	
	Minimal age (years)	Maximal age (years)	Minimal age (years)	Maximal age (years)
0	0.003	11.08	0.02	12.49
1	6.61	16.44	7.99	15.61
2	12.07	19.93	13.32	19.95

Table B-27 Descriptive statistics of the maturation stages at the Pubo-iliac epiphysis (PUBIL)

Stage	Number		Mean age (years)	Standard deviation (years)	Median (years)	Minimal age (years)	Maximal age (years)
	Females	Males					
0	89	136	6.03	3.64	6.028	0.003	16.44
1	27	33	12.47	2.14	12.036	8.57	17.22
2	73	58	17.01	2.11	17.295	12.07	19.95

Table B-28 Descriptive statistics of the maturation stages at the Ilio-ischiatic epiphysis (ILISCH)

Stage	Females		Males	
	Minimal age (years)	Maximal age (years)	Minimal age (years)	Maximal age (years)
0	0.003	11.08	0.02	12.84
1	8.57	16.92	9.93	17.22
2	12.07	19.93	13.58	19.95

## APPENDIX C

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Descriptive statistics of the Lisbon (Luis Lopes collection) and Toulouse samples



The following tables present the descriptive statistics of the variables taken on the iliae, fifth lumbar vertebrae, the clavicles and the iliac bones of the individuals from the Luis Lopes collection in Lisbon and on the iliae of the individuals from Toulouse.

The grey highlighted lines correspond to single individuals.

## Lisbon (Luis Lopes collection) sample

### Iliac variables

Table C-1 Descriptive statistics of the variable Ilium Length (IL)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	35.490	3.961	36.110	26.820	40.490
1	59.920	6.007	58.720	49.510	69.070
2	64.260	1.691	64.010	61.610	66.130
3	74.37	-	-	-	-
4	75.700	6.332	76.670	67.270	83.230
5	86.070	10.859	87.970	73.090	97.200
6	87.180	1.563	87.060	85.650	88.950
7	87.060	0.566	87.060	86.660	87.460
8	-	-	-	-	-
9	95.02	11.091	92.950	82.940	106.630
10	103.930	6.681	106.430	96.360	109.000
11	97.160	10.450	105.680	92.800	120.170
12	107.600	5.958	108.900	100.800	115.600

Table C-2 Descriptive statistics of the variable Ilium Width (IW)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	32.260	4.324	33.530	23.410	37.070
1	55.140	6.179	54.800	44.410	62.330
2	61.030	1.876	61.150	57.300	63.600
3	66.040	-	-	-	-
4	71.180	4.308	72.170	60.760	74.170
5	80.010	8.571	83.000	69.080	89.250
6	83.390	2.573	83.540	80.710	85.750
7	84.830	1.223	84.830	83.960	85.690
8	-	-	-	-	-
9	91.330	6.030	89.670	85.370	99.360
10	100.650	5.330	103.400	94.510	104.050
11	97.160	9.846	97.330	83.560	109.370
12	98.800	5.128	101.000	91.210	103.380

Table C-3 Descriptive statistics of the variable Ilium Module (IM)

Age group (years)	Mean (mm <sup>2</sup> )	Standard deviation (mm <sup>2</sup> )	Median (mm <sup>2</sup> )	Minimal (mm <sup>2</sup> )	Maximal (mm <sup>2</sup> )
0	1143.193	272.748	1209.190	627.856	1500.964
1	3354.047	684.866	3229.364	2198.739	4247.130
2	3902.006	202.423	3864.587	3652.302	4194.420
3	4911.395	-	-	-	-
4	5398.540	644.616	5569.746	4194.870	6107.158
5	6962.594	1567.208	7301.211	5050.439	8675.100
6	7266.300	96.299	7265.692	7179.154	7354.662
7	7384.519	58.516	7384.692	7343.142	7425.895
8	-	-	-	-	-
9	8769.599	1659.523	8340.404	7117.081	10594.760
10	10484.430	1204.718	11004.860	9106.984	11341.450
11	10393.410	2051.021	10272.420	7778.496	13142.990
12	10729.560	1133.155	11010.430	9189.407	11860.430

Table C-4 Descriptive statistics of the variable Ilium Area (IA)

Age group (years)	Mean (mm <sup>2</sup> )	Standard deviation (mm <sup>2</sup> )	Median (mm <sup>2</sup> )	Minimal (mm <sup>2</sup> )	Maximal (mm <sup>2</sup> )
0	848.367	223.288	902.312	460.295	1060.516
1	2480.874	503.356	2440.162	1671.422	3093.495
2	2862.663	289.092	2978.428	2423.204	3147.800
3	3437.072	-	-	-	-
4	4043.950	491.176	4184.558	2884.827	4385.354
5	5219.841	1205.446	5466.989	3736.389	6857.520
6	5607.224	558.885	5449.402	5144.262	6385.831
7	5980.483	56.570	5980.483	5940.482	6020.484
8	-	-	-	-	-
9	6732.522	1812.524	6713.696	5099.591	8403.106
10	8332.069	714.953	8726.927	7506.786	8763.111
11	8223.676	1756.783	8254.927	5850.373	10474.580
12	8406.453	1078.332	8788.837	7016.570	9761.308

## Lumbar variables

Table C-5 Descriptive statistics of the variable Upper Vertebral Length (UVL)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	9.870	-	-	-	-
1	14.630	1.174	14.950	13.270	16.200
2	15.630	0.943	15.920	14.450	16.590
3	17.600	0.240	17.600	17.430	17.770
4	18.610	1.448	18.990	16.230	20.080
5	19.790	3.176	20.770	16.240	22.360
6	21.260	1.669	21.260	20.080	22.440
7	-	-	-	-	-
8	-	-	-	-	-
9	21.680	2.440	21.680	19.950	23.400
10	22.540	1.277	22.690	21.190	23.730
11	27.070	1.718	27.280	24.900	28.800
12	24.220	2.637	24.900	21.310	26.450
13	27.160	0.205	27.160	27.020	27.310
14	27.730	1.859	26.660	26.660	29.880
15	29.350	2.030	29.300	26.420	31.540
16	27.280	0.817	27.250	26.010	28.180
17	28.990	2.661	29.110	26.440	31.320
18	29.840	2.417	29.680	27.300	34.230
19	28.450	2.902	27.990	25.720	32.090

Table C-6 Descriptive statistics of the variable Upper Vertebral Width (UVW)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	-	-	-	-	-
1	24.410	1.275	24.250	22.780	26.190
2	22.680	3.431	23.970	16.650	25.060
3	25.070	2.185	25.070	23.520	26.610
4	28.500	1.009	28.220	27.360	30.020
5	33.040	3.864	32.740	29.340	37.050
6	34.730	1.549	34.730	33.630	35.820
7	-	-	-	-	-
8	-	-	-	-	-
9	35.940	5.742	35.940	31.880	40.000
10	36.590	4.526	35.810	32.510	41.460
11	37.800	6.638	41.020	26.480	42.250
12	37.670	3.569	39.120	33.600	40.280
13	42.630	7.601	42.630	37.260	48.010
14	47.490	3.318	46.690	44.640	51.130
15	46.070	3.339	46.430	41.120	49.150
16	44.110	1.847	43.600	41.760	47.190
17	44.170	4.798	44.880	37.730	49.190
18	43.240	5.235	43.380	36.640	49.860
19	43.590	2.600	42.670	41.620	47.390

Table C-7 Descriptive statistics of the variable Upper Vertebral Module (UVM)

Age group (years)	Mean (mm <sup>2</sup> )	Standard deviation (mm <sup>2</sup> )	Median (mm <sup>2</sup> )	Minimal (mm <sup>2</sup> )	Maximal (mm <sup>2</sup> )
0	-	-	-	-	-
1	358.027	45.289	356.558	311.403	424.278
2	353.070	48.935	356.558	276.224	398.955
3	441.407	44.481	441.407	409.954	472.860
4	530.454	45.694	532.100	458.011	580.111
5	661.643	176.696	680.010	476.482	828.438
6	739.546	90.871	739.546	675.290	803.801
7	-	-	-	-	-
8	-	-	-	-	-
9	786.003	212.128	786.003	636.006	936.000
10	826.771	136.442	758.814	737.652	983.846
11	1035.364	255.962	1136.252	659.352	1209.600
12	918.503	181.206	974.088	716.016	1065.406
13	1158.959	215.235	1158.959	1006.765	1311.153
14	1316.108	110.289	1363.126	1190.102	1395.097
15	1356.515	179.152	1435.616	1086.390	1545.145
16	1203.195	59.923	121.0658	1086.178	1265.636
17	1288.496	243.749	1307.886	997.581	1540.631
18	1295.974	231.630	1261.835	1000.272	1645.094
19	1245.310	201.534	1195.013	1070.466	1520.745

Table C-8 Descriptive statistics of the variable Lower Vertebral Length (LVL)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	8.430	-	-	-	-
1	14.620	1.476	14.530	12.550	16.660
2	15.690	0.757	15.790	14.540	16.650
3	17.470	0.438	17.470	17.160	17.780
4	18.810	1.108	19.140	17.150	19.960
5	19.670	3.403	19.940	16.140	22.930
6	20.520	0.778	20.520	19.970	21.070
7	-	-	-	-	-
8	-	-	-	-	-
9	22.790	3.875	22.790	20.050	25.530
10	22.870	1.606	23.760	21.020	23.840
11	25.710	2.040	26.480	22.630	27.920
12	24.010	1.973	25.060	21.730	25.230
13	26.940	0.453	26.940	26.620	27.260
14	27.850	2.656	29.100	24.800	29.650
15	28.960	2.436	29.140	25.830	31.600
16	27.450	0.742	27.550	26.490	28.520
17	28.980	2.746	28.950	25.910	32.130
18	28.140	2.639	27.680	25.270	33.040
19	29.230	2.263	29.280	26.540	31.820

Table C-9 Descriptive statistics of the variable Lower Vertebral Width (LVW)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	15.880	-	-	-	-
1	24.030	1.807	23.360	22.170	26.720
2	26.220	1.155	25.700	25.290	28.040
3	26.820	1.407	26.820	25.830	27.820
4	30.600	1.877	30.230	28.890	33.190
5	33.110	3.737	32.180	29.920	37.220
6	34.910	1.004	34.910	34.200	35.620
7	-	-	-	-	-
8	-	-	-	-	-
9	35.660	4.335	35.660	32.600	38.730
10	38.600	3.043	39.550	35.200	41.060
11	40.830	2.943	42.060	36.660	43.640
12	37.740	2.985	37.050	35.160	41.010
13	42.880	3.677	42.880	40.280	45.480
14	47.110	4.001	44.860	44.740	51.730
15	45.140	3.716	44.660	39.960	49.880
16	45.100	2.880	44.740	41.330	49.740
17	43.540	4.979	43.900	37.100	49.250
18	42.800	3.318	42.740	37.710	47.380
19	43.670	1.697	43.700	41.560	45.710

Table C-10 Descriptive statistics of the variable Lower Vertebral Module (LVM)

Age group (years)	Mean (mm <sup>2</sup> )	Standard deviation (mm <sup>2</sup> )	Median (mm <sup>2</sup> )	Minimal (mm <sup>2</sup> )	Maximal (mm <sup>2</sup> )
0	133.868	-	-	-	-
1	352.786	59.306	328.930	293.168	445.155
2	412.036	36.512	401.434	367.717	466.866
3	468.324	12.823	468.324	459.257	477.391
4	576.330	57.229	576.644	495.635	647.205
5	659.344	185.904	641.669	482.909	853.455
6	716.744	47.758	716.744	682.974	750.513
7	-	-	-	-	-
8	-	-	-	-	-
9	821.203	236.985	821.203	653.630	988.777
10	886.121	127.679	942.872	739.904	975.586
11	1053.575	146.728	1045.191	829.616	1218.429
12	909.061	136.368	928.473	764.027	1034.682
13	1154.355	79.652	1154.355	1098.033	1210.678
14	1314.804	196.670	1326.541	1112.528	1505.343
15	1311.364	184.486	1390.084	1032.167	1463.185
16	1237.078	63.139	1223.930	1163.378	1321.573
17	1268.528	244.375	1232.575	1026.557	1582.402
18	1209.227	187.279	1183.452	952.932	1487.130
19	1277.570	123.646	1306.462	1103.002	1394.352

Table C-11 Descriptive statistics of the variable Posterior Vertebral Height (PVH)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	5.560	-	-	-	-
1	8.422	1.584	8.050	6.580	9.420
2	9.852	0.747	10.230	8.700	10.470
3	10.100	1.923	10.100	8.740	11.460
4	10.100	1.098	10.310	8.790	11.550
5	10.110	1.885	10.130	8.210	11.980
6	12.450	0.799	12.450	11.880	13.010
7	12.8	-	-	-	-
8	-	-	-	-	-
9	15.760	0.580	15.760	15.350	16.170
10	12.490	0.442	12.240	12.230	13.000
11	15.090	1.937	15.140	12.930	17.450
12	17.170	3.374	16.780	14.330	21.000
13	19.020	4.221	19.020	16.030	22.000
14	20.110	6.588	19.230	14.000	27.090
15	21.510	1.622	22.330	19.320	23.160
16	19.730	2.151	19.320	16.210	23.270
17	24.070	2.556	24.680	20.760	26.160
18	20.290	2.669	20.380	16.620	23.450
19	23.110	4.019	22.450	19.500	28.030

Table C-12 Descriptive statistics of the variable Anterior Vertebral Height (AVH)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	6.510	-	-	-	-
1	9.876	1.763	10.340	7.490	12.220
2	10.810	0.359	10.910	10.300	11.220
3	11.570	0.064	11.570	11.530	11.620
4	12.550	0.599	12.250	11.990	13.430
5	14.660	0.230	14.650	14.440	14.900
6	14.270	0.304	14.270	14.050	14.480
7	12.720	-	-	-	-
8	-	-	-	-	-
9	18.230	2.376	18.230	16.550	19.910
10	17.030	0.858	17.500	16.040	17.550
11	19.400	1.533	18.980	18.050	21.580
12	19.330	2.076	19.950	17.010	21.020
13	20.340	0.750	20.340	19.810	20.870
14	22.610	6.519	22.870	15.960	28.990
15	24.920	3.113	25.270	20.590	27.970
16	23.280	2.798	24.190	19.950	26.820
17	27.710	2.219	27.950	24.990	29.950
18	24.300	2.820	24.980	19.410	27.090
19	27.230	3.258	27.660	22.940	30.660

Table C-13 Descriptive statistics of the variable Right Vertebral Height (RVH)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	-	-	-	-	-
1	5.452	1.752	6.040	2.660	7.160
2	7.872	1.208	8.070	5.950	9.070
3	6.675	0.262	6.675	6.490	6.860
4	8.428	1.355	8.310	6.330	9.740
5	10.500	1.418	11.000	8.900	11.600
6	10.295	1.039	10.295	9.560	11.030
7	12.620	-	-	-	-
8	-	-	-	-	-
9	12.890	4.179	12.890	9.940	15.850
10	13.700	0.737	13.880	12.890	14.330
11	15.460	3.086	15.910	11.520	19.680
12	14.780	2.043	15.650	12.450	16.250
13	18.000	2.864	18.000	15.970	20.020
14	18.650	5.289	19.640	12.940	23.380
15	23.110	0.348	23.080	22.640	23.620
16	20.960	2.590	20.760	17.650	24.300
17	25.180	1.281	24.800	24.160	26.950
18	21.650	2.727	21.650	17.340	24.800
19	24.700	3.038	25.100	20.630	27.970

Table C-14 Descriptive statistics of the variable Left Vertebral Height (LVH)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	4.650	-	-	-	-
1	5.756	2.117	6.060	2.590	7.790
2	7.872	1.351	8.070	5.950	9.070
3	6.675	0.099	6.675	6.490	6.860
4	8.428	0.592	8.310	6.330	9.740
5	10.500	1.725	11.000	8.900	11.600
6	10.035	0.997	10.035	9.330	10.740
7	12.620	-	-	-	-
8	-	-	-	-	-
9	12.530	4.815	12.530	9.120	15.930
10	14.210	1.467	13.380	13.340	15.900
11	15.460	2.670	15.910	11.520	19.680
12	14.780	2.563	15.650	12.450	16.250
13	17.740	3.818	17.740	15.040	20.440
14	19.330	4.948	20.740	13.830	23.420
15	23.110	0.530	23.080	22.640	23.620
16	21.630	2.287	22.720	17.430	23.850
17	24.300	1.737	24.340	22.130	26.380
18	21.510	2.809	21.320	18.040	25.420
19	24.530	3.160	24.530	19.320	26.280



## Clavicular variables

Table C-15 Descriptive statistics of the variable Maximal length (Ln)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	48.810	0.361	48.810	48.560	49.070
1	64.220	4.429	66.680	56.290	67.800
2	65.210	1.274	65.340	63.530	66.760
3	73.570	3.333	74.320	69.930	76.470
4	79.440	7.211	80.890	66.060	85.870
5	84.630	6.286	86.270	77.720	91.390
6	85.190	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	98.710	6.337	96.340	93.900	105.890
10	104.700	-	-	-	-
11	112.000	8.491	111.100	104.500	121.100
12	101.770	7.924	100.800	94.570	110.930
13	119.200	1.041	119.500	118.000	120.000
14	122.100	13.568	124.000	108.200	141.700
15	134.000	18.779	131.800	113.500	157.000
16	130.200	9.876	130.000	115.000	149.000
17	135.200	6.241	136.100	127.700	144.000
18	135.700	4.282	136.900	128.000	143.300
19	125.500	13.107	125.500	115.500	148.000

Table C-16 Descriptive statistics of the variable Maximal diameter (Max\_diam)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	4.850	0.283	4.850	4.650	5.050
1	5.380	0.749	5.230	4.470	6.840
2	5.998	0.408	6.060	5.330	6.340
3	6.190	0.822	6.410	5.280	6.800
4	6.482	0.556	6.695	5.770	7.020
5	6.262	1.000	6.030	5.500	7.970
6	8.020	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	8.280	0.740	8.300	7.530	9.010
10	7.130	-	-	-	-
11	8.775	1.212	8.220	8.070	10.590
12	8.790	1.179	8.515	7.690	10.440
13	9.517	0.778	9.910	8.620	10.020
14	9.988	0.871	10.120	9.060	11.140
15	11.490	1.571	11.800	9.570	13.670
16	11.290	0.632	11.490	10.230	12.060
17	11.600	1.499	11.480	9.480	13.410
18	11.570	1.739	10.980	9.570	15.910
19	11.070	1.591	10.570	9.850	13.690

Table C-17 Descriptive statistics of the variable Minimal diameter (Min\_diam)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	3.615	0.078	3.615	3.560	3.670
1	3.546	0.512	3.490	2.740	4.150
2	4.270	0.401	4.490	3.690	4.650
3	3.803	0.392	3.850	3.690	4.170
4	4.412	0.477	4.445	3.820	5.080
5	4.524	0.489	4.490	4.010	5.120
6	5.980	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	5.517	0.686	5.320	4.950	6.280
10	5.060	-	-	-	-
11	5.798	0.687	5.815	5.100	6.460
12	5.845	1.270	6.180	4.030	6.990
13	6.827	0.263	6.780	6.590	7.110
14	7.362	0.543	7.080	6.860	8.080
15	8.138	1.710	8.525	6.040	10.170
16	8.596	1.105	8.770	6.680	10.250
17	8.746	1.109	9.000	6.890	9.960
18	8.463	0.932	8.430	7.150	10.300
19	7.596	0.895	7.650	6.570	8.920

Table C-18 Descriptive statistics of the variable Antero-posterior diameter (AP\_diam)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	4.550	0.594	4.550	4.130	4.970
1	5.043	0.768	4.845	4.210	6.800
2	5.642	0.484	5.720	4.950	6.210
3	6.167	0.960	6.050	5.270	7.180
4	6.002	0.588	6.140	5.090	6.630
5	5.848	0.821	5.420	5.300	7.260
6	7.710	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	7.823	1.035	7.860	6.770	8.840
10	5.650	-	-	-	-
11	8.070	0.948	7.960	7.030	9.330
12	8.345	1.229	7.985	7.390	10.020
13	8.233	1.040	8.520	7.080	9.100
14	9.368	0.829	9.620	8.480	10.270
15	10.527	1.538	9.885	9.260	12.860
16	10.568	0.638	10.815	9.750	11.420
17	9.947	1.150	10.145	8.390	11.680
18	10.610	1.881	10.300	8.520	15.290
19	10.230	1.930	10.230	8.060	12.970

Table C-19 Descriptive statistics of the variable Supero-inferior diameter (SI\_diam)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	3.780	0.085	3.780	3.720	3.840
1	3.928	0.607	3.675	3.260	4.820
2	4.534	0.246	4.650	4.110	4.720
3	4.080	0.590	3.850	3.640	4.750
4	4.618	0.622	4.605	3.820	5.530
5	4.832	0.475	4.790	4.160	5.370
6	8.02	-	-	-	-
7	-	-	-	-	-
8	-	-	-	-	-
9	5.947	0.400	5.960	5.540	6.340
10	6.02	-	-	-	-
11	6.700	1.151	6.820	5.380	7.780
12	6.360	1.227	6.650	4.670	7.470
13	7.137	0.111	7.150	7.020	7.240
14	8.266	0.420	8.310	7.650	8.680
15	8.575	1.966	8.850	6.240	10.600
16	9.237	1.264	9.620	7.280	10.810
17	9.621	1.460	9.395	7.750	11.640
18	9.157	1.450	8.850	7.500	11.640
19	8.146	0.879	8.440	6.780	9.100

## Iliac non-metric variables

Table C-20 Descriptive statistics of the maturation stages at the Inferior pubo-ischiatic epiphysis (PUBISCH\_INF)

Age group (years)	Mean age (years)	Standard deviation (years)	Median (years)	Minimal age (years)	Maximal age (years)
0	3.185	3.022	2.000	0.000	11.000
1	14.250	2.435	13.500	12.000	18.000
2	14.526	3.715	16.000	5.000	19.000

Table C-21 Descriptive statistics of the maturation stages at the Inferior pubo-ischiatic epiphysis (PUBISCH\_INF)

Stage	Girls		Boys	
	Minimal age (years)	Maximal age (years)	Minimal age (years)	Maximal age (years)
0	0.000	11.000	0.000	7.000
1	-	18.000	12.000	14.000
2	5.000	19.000	8.000	19.000

Table C-22 Descriptive statistics of the maturation stages at the Superior pubo-ischiatic epiphysis (PUBISCH\_SUP)

Age group (years)	Mean age (years)	Standard deviation (years)	Median (years)	Minimal age (years)	Maximal age (years)
0	6.400	4.951	5.000	0.000	16.000
1	13.667	2.535	13.500	11.000	18.000
2	17.182	1.281	17.000	15.000	19.000

Table C-23 Descriptive statistics of the maturation stages at the Superior pubo-ischiatic epiphysis (PUBISCH\_SUP)

Stage	Girls		Boys	
	Minimal age (years)	Maximal age (years)	Minimal age (years)	Maximal age (years)
0	0.000	12.000	0.000	16.000
1	11.000	14.000	15.000	18.000
2	15.000	19.000	16.000	19.000

Table C-24 Descriptive statistics of the maturation stages at the Ilio-ischiatic epiphysis (ILISCH)

Age group (years)	Mean age (years)	Standard deviation (years)	Median (years)	Minimal age (years)	Maximal age (years)
0	5.511	4.548	4.000	0.000	14.000
1	13.667	1.435	14.000	11.000	15.000
2	17.125	1.315	17.000	15.000	19.000

Table C-25 Descriptive statistics of the maturation stages at the Ilio-ischiatic epiphysis (ILISCH)

Stage	Girls		Boys	
	Minimal age (years)	Maximal age (years)	Minimal age (years)	Maximal age (years)
0	0.000	12.000	0.000	14.000
1	11.000	15.000	14.000	15.000
2	15.000	19.000	15.000	19.000

Table C-26 Descriptive statistics of the maturation stages at the Pubo-iliac epiphysis (PUBIL)

Age group (years)	Mean age (years)	Standard deviation (years)	Median (years)	Minimal age (years)	Maximal age (years)
0	5.820	4.726	5.000	0.000	15.000
1	13.625	2.264	13.500	11.000	18.000
2	16.958	1.443	17.000	14.000	19.000

Table C-27 Descriptive statistics of the maturation stages at the Pubo-iliac epiphysis (PUBIL) site

Stage	Girls		Boys	
	Minimal age (years)	Maximal age (years)	Minimal age (years)	Maximal age (years)
0	0.000	12.000	0.000	15.000
1	11.000	15.000	14.000	18.000
2	14.000	19.000	15.000	19.000

## Toulouse sample

### Iliac variables

Table C-28 Descriptive statistics of the variable Ilium Length (IL)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	52.230	4.051	53.110	45.300	55.200
1	58.390	10.962	58.39	50.640	66.140
2	73.440	1.744	73.440	72.200	74.670
3	72.480	9.490	72.480	65.770	79.190
4	82.370	2.820	83.190	79.230	84.700
5	95.280	1.345	95.280	94.320	96.230
6	96.830	5.560	96.830	92.900	100.760
7	98.080	4.078	98.080	95.190	100.960
8	103.700	0.733	103.700	103.200	104.200
9	114.200	0.582	114.200	113.800	114.600
10	117.900	11.13	117.900	110.100	125.800
11	120.100	1.282	120.100	119.200	121.000
12	142.900	23.722	142.900	126.100	159.600

Table C-29 Descriptive statistics of the variable Ilium Width (IW)

Age group (years)	Mean (mm)	Standard deviation (mm)	Median (mm)	Minimal (mm)	Maximal (mm)
0	44.160	4.158	45.680	36.940	47.460
1	47.130	4.088	47.130	44.240	50.020
2	59.960	3.495	59.960	57.490	62.430
3	62.180	2.340	62.180	60.530	63.830
4	69.870	3.410	69.990	66.410	73.220
5	78.680	2.370	78.680	77.010	80.360
6	81.440	3.578	81.440	78.910	83.970
7	83.350	3.161	83.350	81.120	85.590
8	84.360	6.557	84.360	79.720	88.990
9	95.030	7.234	95.030	89.910	100.140
10	100.910	5.254	100.910	97.200	104.630
11	96.370	4.075	96.370	93.480	99.250
12	107.650	11.973	107.650	99.180	116.120

Table C-30 Descriptive statistics of the variable Ilium Module (IM)

Age group (years)	Mean (mm <sup>2</sup> )	Standard deviation (mm <sup>2</sup> )	Median (mm <sup>2</sup> )	Minimal (mm <sup>2</sup> )	Maximal (mm <sup>2</sup> )
0	2319.711	374.731	2398.098	1673.346	2612.499
1	2774.730	755.404	2774.730	2240.579	3308.882
2	4406.043	361.174	4406.043	4150.655	4661.432
3	4517.683	759.689	4517.683	3980.501	5054.864
4	5761.814	472.939	5822.080	5261.631	6201.732
5	7498.222	331.600	7498.222	7263.746	7732.699
6	7896.096	799.326	7896.096	7330.887	8461.305
7	8181.415	649.971	8181.415	7721.816	8641.014
8	8747.138	618.244	8747.138	8309.974	9184.303
9	10852.130	881.188	10852.130	10229.040	11475.230
10	11931.080	1742.923	11931.080	10698.650	13163.510
11	11566.990	365.684	11566.990	11308.410	11825.560
12	15521.340	4264.227	15521.340	12506.080	18536.600

## APPENDIX D

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Sexual dimorphism of the variables in  
the Marseilles sample - Homogeneity  
between the samples from Marseilles,  
Toulouse and Lisbon



## Sexual dimorphism in the Marseilles sample

The following tables present the results of the tests done to assess the presence of sexual dimorphism of all the variables for each annual age group using annual means (calculated for individuals in each age group N) and mobile means (calculated for individuals in age groups N-1, N and N+1) .

### Iliac variables

**Table D-1 Results of the Wilcoxon tests for sexual dimorphism of Ilium Length (IL). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange**

Age group	W	95% confidence interval	Difference in location	p-value
0	71	[-5.848 ; 9.841]	3.132	0.431
1	14	[-10.950 ; 7.974]	-1.210	0.769
2	13	[-6.843 ; 11.285]	-2.085	0.792
3	22	[-3.857 ; 8.205]	1.988	0.530
4	26	[-7.767 ; 2.081]	-3.012	0.237
5	40	[-6.626 ; 11.702]	3.140	0.669
6	23	[-13.326 ; 9.958]	-0.405	0.859
7	34	[-5.434 ; 8.576]	0.247	0.837
8	50	[1.165 ; 12.825]	7.206	0.037
9	44	[-7.772 ; 5.093]	-0.827	0.710
10	80	[-5.673 ; 10.693]	2.827	0.350
11	41	[-11.314 ; 8.163]	-1.848	0.516
12	21	[-13.130 ; 6.073]	-4.905	0.383

**Table D-2 Results of the Wilcoxon tests for sexual dimorphism of Ilium Length (IL). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange**

Age group	W	95% confidence interval	Difference in location	p-value
0	319	[-10.884 ; 14.845]	2.490	0.780
1	214	[-14.900 ; 3.427]	-5.724	0.213
2	180	[-3.857 ; 7.706]	2.367	0.460
3	186	[-6.709 ; 4.306]	-1.512	0.594
4	261	[-4.851 ; 5.036]	-0.408	0.856
5	245	[-7.055 ; 3.042]	-2.577	0.283
6	306	[-3.646 ; 7.916]	1.307	0.651
7	316	[-2.602 ; 7.748]	2.711	0.403
8	390	[-2.168 ; 6.182]	1.499	0.428
9	468	[-3.697 ; 6.182]	1.409	0.566
10	420	[-6.955 ; 2.191]	-2.587	0.261
11	381	[-5.474 ; 3.386]	-1.659	0.502
12	118	[-9.841 ; 3.059]	-3.272	0.218

Table D-3 Results of the Wilcoxon tests for sexual dimorphism of Ilium Width (IW). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	71	[-4.742 ; 7.913]	1.465	0.431
1	12	[-15.959 ; 4.170]	-5.203	0.555
2	10	[-6.623 ; 8.468]	-2.997	0.429
3	23	[-3.648 ; 8.112]	1.682	0.432
4	26	[-7.767 ; 2.081]	-3.012	0.237
5	40	[-6.211 ; 8.483]	0.777	0.669
6	25	[-10.623 ; 7.212]	-0.120	1
7	29	[-8.957 ; 5.932]	-1.614	0.837
8	52	[1.164 ; 11.989]	6.462	0.019
9	26	[-11.923 ; 0.326]	-4.610	0.080
10	74	[-4.819 ; 5.661]	2.164	0.569
11	26	[-10.298 ; 0.572]	-5.841	0.087
12	35	[-7.192 ; 8.496]	1.727	0.646

Table D-4 Results of the Wilcoxon tests for sexual dimorphism of Ilium Width (IW). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	314	[-11.146 ; 10.537]	0.745	0.855
1	208	[-14.946 ; 2.382]	-6.654	0.169
2	175	[-3.952 ; 7.337]	1.819	0.561
3	215	[-4.914 ; 5.227]	0.450	0.846
4	290	[-3.126 ; 4.682]	0.751	0.678
5	276	[-5.807 ; 3.464]	-1.161	0.645
6	294	[-4.240 ; 5.299]	0.589	0.837
7	304	[-3.665 ; 5.900]	1.372	0.559
8	332	[-4.838 ; 3.972]	-0.293	0.824
9	429	[-4.340 ; 3.964]	-0.002	1
10	351	[-7.384 ; -0.130]	-4.224	0.041
11	389	[-5.073 ; 2.522]	-1.020	0.580
12	127	[-8.028 ; 2.253]	-2.412	0.338

Table D-5 Results of the Wilcoxon tests for sexual dimorphism of Ilium Module (IM). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	70	[-406.477 ; 699.461]	187.048	0.471
1	13	[-1386.252 ; 289.258]	-257.527	0.659
2	10	[-733.136 ; 1159.971]	-452.668	0.429
3	22	[-459.957 ; 1106.641]	369.635	0.530
4	32	[-1181.998 ; 419.491]	-136.446	0.515
5	42	[-1090.844 ; 1579.950]	521.309	0.536
6	24	[-2041.139 ; 1729.294]	-87.494	0.953
7	29	[-1178.410 ; 1363.067]	-148.674	0.837
8	52	[266.413 ; 2434.631]	1369.260	0.019
9	35	[-2108.721 ; 469.108]	-603.213	0.295
10	78	[-1089.590 ; 1823.304]	550.280	0.417
11	36	[-1738.810 ; 800.960]	-707.294	0.319
12	26	[-2488.111 ; 1359.166]	-381.513	0.721

Table D-6 Results of the Wilcoxon tests for sexual dimorphism of Ilium Module (IM). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	316	[-941.502 ; 814.682]	78.883	0.824
1	207	[-1509.561 ; 226.737]	-621.970	0.163
2	173	[-496.958 ; 992.868]	190.292	0.604
3	191	[-820.240 ; 762.090]	-139.911	0.687
4	272	[-626.833 ; 765.046]	12.457	0.975
5	256	[-1077.062 ; 513.921]	-293.118	0.393
6	299	[-724.079 ; 1212.707]	219.704	0.758
7	309	[-601.872 ; 1355.093]	426.191	0.490
8	349	[-699.691 ; 1000.746]	48.425	0.950
9	439	[-866.120 ; 1071.728]	109.354	0.888
10	384	[-1575.426 ; 147.153]	-719.042	0.109
11	382	[-1148.143 ; 660.679]	-358.555	0.512
12	120	[-1930.091 ; 702.903]	-755.484	0.241

Table D-7 Results of the Wilcoxon tests for sexual dimorphism of Ilium Area (IA). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	73	[-299.748 ; 558.953]	178.927	0.358
1	20	[-794.194 ; 661.752]	70.027	0.659
2	10	[-590.608 ; 677.790]	-336.266	0.429
3	23	[-351.952 ; 857.615]	186.390	0.432
4	31	[-720.151 ; 325.676]	-208.765	0.460
5	35	[-832.289 ; 958.640]	8.733	1
6	28	[-1295.424 ; 1381.244]	94.324	0.768
7	29	[-932.656 ; 976.983]	-172.943	0.837
8	51	[150.894 ; 1750.295]	880.551	0.027
9	29	[-1696.000 ; 178.419]	-475.460	0.131
10	72	[-887.229 ; 1118.596]	265.829	0.653
11	36	[-1738.810 ; 800.960]	-707.294	0.319
12	24	[-2004.620 ; 881.427]	-486.936	0.574

Table D-8 Results of the Wilcoxon tests for sexual dimorphism of Ilium Area (IA). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	327	[-611.221 ; 629.807]	-6.654	0.169
1	219	[-1026.676 ; 261.426]	-386.382	0.255
2	183	[-282.954 ; 703.212]	206.631	0.404
3	198	[-535.551 ; 488.012]	-118.673	0.825
4	251	[-487.265 ; 390.144]	-94.255	0.693
5	243	[-783.100 ; 246.130]	-314.441	0.266
6	295	[-566.988 ; 724.880]	68.972	0.821
7	308	[-448.286 ; 885.940]	254.997	0.504
8	346	[-629.080 ; 667.312]	14.572	0.993
9	428	[-706.344 ; 713.172]	-5.913	0.994
10	367	[-1252.843 ; 30.010]	-617.347	0.067
11	367	[-957.841 ; 384.258]	-342.189	0.381
12	116	[-1930.091 ; 702.903]	-755.484	0.241

## Lumbar variables

Table D-9 Results of the Wilcoxon tests for sexual dimorphism of Upper Vertebral Length (UVL). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	64	[-2.107 ; 1.786]	-0.097	0.861
1	27	[-1.282 ; 1.266]	-0.202	0.799
2	10	[-4.431 ; 0.078]	-2.423	0.081
3	30	[-1.187 ; 2.931]	0.810	0.867
4	12	[-3.511 ; -0.685]	-1.977	0.007
5	60	[-1.951 ; 1.676]	-0.147	0.878
6	20	[-2.792 ; 2.086]	-1.232	0.456
7	17	[-4.732 ; -0.608]	-2.484	0.022
8	38	[-2.379 ; 1.723]	-0.346	0.588
9	19	[-4.803 ; -0.328]	-2.005	0.025
10	33	[-2.832 ; 0.197]	-1.214	0.135
11	38	[-4.361 ; 0.609]	-2.070	0.196
12	58	[-3.113 ; 0.486]	-1.246	0.116
13	19	[-3.939 ; 2.334]	-1.460	0.388
14	25	[-5.918 ; 0.158]	-2.912	0.063
15	2	[-6.635 ; -2.780]	-4.239	<0.001
16	17	[-5.891 ; -1.052]	-3.550	0.016
17	31	[-3.941 ; 1.647]	-1.048	0.460
18	27	[-3.341 ; -0.632]	-1.883	0.008
19	23	[-4.425 ; -1.213]	-2.678	0.007

Table D-10 Results of the Wilcoxon tests for sexual dimorphism of Upper Vertebral Length (UVL). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	145	[-4.191 ; 0.304]	-1.798	0.093
1	256	[-4.191 ; -0.067]	-2.0306	0.042
2	246	[-1.481 ; 1.652]	-0.067	0.903
3	210	[-2.811 ; 0.452]	-1.149	0.130
4	309	[-1.973 ; 0.305]	-0.887	0.163
5	276	[-2.273 ; 0.037]	-1.214	0.062
6	316	[-2.229 ; 0.411]	-0.942	0.181
7	241	[-2.639 ; -0.035]	-1.450	0.044
8	223	[-2.987 ; -0.655]	-1.851	0.002
9	266	[-2.655 ; -0.509]	-1.606	0.007
10	260	[-3.172 ; -0.888]	-2.083	0.001
11	142	[-2.906 ; -0.103]	-1.519	0.037
12	207	[-2.802 ; -0.157]	-1.464	0.031
13	137	[-3.190 ; -0.074]	-1.657	0.040
14	91	[-4.418 ; -0.066]	-2.251	0.044
15	54	[-5.354 ; -1.916]	-3.805	0.0001
16	32	[-5.466 ; -2.782]	-4.115	2.893e-06
17	82	[-4.222 ; -0.933]	-2.711	0.0035
18	120	[-2.912 ; -0.456]	-1.605	0.064
19	99	[-3.285 ; -1.353]	-2.290	8.838e-05

Table D-11 Results of the Wilcoxon tests for sexual dimorphism of Upper Vertebral Width (UVW). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	74	[-3.053 ; 4.658]	0.984	0.726
1	36	[-0.611 ; 3.826]	0.376	0.574
2	25	[-2.750 ; 1.400]	0.062	0.950
3	24	[-2.683 ; 5.189]	-0.267	0.694
4	25	[-3.616 ; 0.603]	-1.400	0.129
5	44	[-3.963 ; 1.189]	-1.592	0.250
6	29	[-3.307 ; 5.103]	0.441	0.864
7	34	[-4.811 ; 2.062]	-1.885	0.400
8	52	[-1.457 ; 3.851]	0.941	0.643
9	26	[-4.782 ; 0.859]	-2.595	0.098
10	39	[-5.801 ; 1.691]	-1.752	0.278
11	37	[-5.636 ; 1.139]	-2.226	0.176
12	32	[-4.803 ; -1.044]	-2.838	0.003
13	16	[-5.278 ; 1.015]	-1.290	0.224
14	23	[-7.770 ; -0.033]	-2.611	0.043
15	6	[-9.867 ; -2.670]	-6.762	0.001
16	20	[-5.231 ; -0.291]	-2.180	0.031
17	41	[-3.374 ; 2.165]	0.067	0.965
18	34	[-6.162 ; -0.434]	-2.419	0.028
19	27	[-6.691 ; -0.844]	-3.713	0.016

Table D-12 Results of the Wilcoxon tests for sexual dimorphism of Upper Vertebral Width (UVW). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	165	[-5.632 ; 1.286]	-1.635	0.249
1	319	[-4.773 ; 1.103]	-1.178	0.335
2	294	[-0.646 ; 2.510]	0.749	0.354
3	243	[-3.190 ; 1.285]	-1.080	0.409
4	312	[-3.278 ; 0.606]	-1.301	0.179
5	315	[-2.928 ; 0.696]	-1.082	0.221
6	358	[-2.709 ; 1.548]	-0.611	0.508
7	357	[-2.058 ; 2.025]	0.019	1
8	359	[-2.683 ; 0.810]	-0.741	0.318
9	340	[-3.141 ; 0.499]	-1.312	0.112
10	298	[-4.458 ; -0.657]	-2.650	0.007
11	152	[-4.703 ; 0.162]	-2.247	0.064
12	169	[-4.271 ; -0.891]	-2.455	0.004
13	97	[-4.525 ; -0.864]	-2.419	0.002
14	82	[-5.187 ; -0.352]	-2.210	0.020
15	63	[-7.794 ; -1.903]	-4.393	0.0004
16	48	[-6.867 ; -1.970]	-4.276	5.066e-05
17	119	[-3.635 ; 0.112]	-1.844	0.077
18	146	[-3.551 ; 0.112]	-1.726	0.064
19	124	[-5.217 ; -1.375]	-3.230	0.001

**Table D-13 Results of the Wilcoxon tests for sexual dimorphism of Upper Vertebral Module (UVM). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange**

Age group	W	95% confidence interval	Difference in location	p-value
0	71	[-62.569 ; 78.470]	7.373	0.861
1	33	[-34.490 ; 69.567]	5.323	0.799
2	12	[-144.112 ; 18.275]	-69.023	0.142
3	28	[-88.873 ; 212.369]	2.415	1
4	14	[-178.581 ; -22.443]	-109.193	0.012
5	48	[-143.689 ; 87.286]	-48.797	0.369
6	21	[-188.574 ; 195.063]	-19.922	0.529
7	26	[-280.950 ; 26.047]	-138.424	0.133
8	48	[-115.782 ; 141.404]	17.714	0.877
9	21	[-325.151 ; -62.800]	-141.794	0.039
10	31	[-254.411 ; 28.861]	-104.937	0.103
11	33	[-356.140 ; 37.922]	-136.056	0.108
12	45	[-285.797 ; -17.641]	-146.027	0.025
13	14	[-301.334 ; 47.239]	-135.127	0.145
14	21	[-482.549 ; -18.826]	-206.906	0.029
15	3	[-617.479 ; -215.398]	-441.250	0.0003
16	13	[-428.152 ; -75.036]	-266.738	0.005
17	27	[-238.624 ; 120.136]	-96.662	0.274
18	22	[-302.588 ; -68.160]	-194.052	0.003
19	17	[-389.744 ; -100.635]	-246.647	0.002

**Table D-14 Results of the Wilcoxon tests for sexual dimorphism of Upper Vertebral Module (UVM). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange**

Age group	W	95% confidence interval	Difference in location	p-value
0	158	[-156.478 ; 18.850]	-57.695	0.181
1	286	[-156.478 ; 12.873]	-62.165	0.128
2	270	[-50.948 ; 71.069]	12.128	0.697
3	222	[-146.841 ; 35.966]	-53.152	0.207
4	303	[-135.068 ; 11.328]	-53.031	0.136
5	277	[-144.177 ; 2.480]	-64.013	0.065
6	324	[-138.067 ; 40.903]	-56.047	0.227
7	294	[-145.166 ; 46.127]	-55.122	0.281
8	283	[-182.294 ; -11.952]	-92.603	0.029
9	294	[-194.789 ; -17.004]	-106.882	0.023
10	249	[-256.591 ; -69.376]	-164.154	0.0007
11	131	[-254.187 ; -14.238]	-132.203	0.019
12	179	[-239.667 ; -31.868]	-136.200	0.007
13	114	[-275.045 ; -33.970]	-150.358	0.008
14	73	[-334.884 ; -29.484]	-178.381	0.008
15	48	[-492.791 ; -163.926]	-309.885	4.225e-05
16	28	[-466.940 ; -22.670]	-328.286	1.248e-06
17	80	[-301.167 ; -69.145]	-190.156	0.003
18	108	[-232.984 ; -43.188]	-140.538	0.004
19	84	[-302.483 ; -120.389]	-220.136	1.679e-05

Table D-15 Results of the Wilcoxon tests for sexual dimorphism of Lower Vertebral Length (LVL). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	61	[-2.028 ; 1.387]	-0.136	0.726
1	29	[-1.759 ; 1.679]	-0.074	0.959
2	16	[-3.178 ; 1.023]	-1.052	0.345
3	41	[-0.240 ; 4.542]	0.917	0.152
4	8	[-3.305 ; -0.773]	-1.910	0.002
5	53	[-2.477 ; 1.835]	-0.515	0.557
6	21	[-3.333 ; 3.894]	-0.721	0.529
7	23	[-4.010 ; 0.394]	-1.993	0.079
8	51	[-1.589 ; 2.334]	0.353	0.699
9	21	[-3.623 ; -0.203]	-1.536	0.039
10	44	[-2.496 ; 1.510]	-0.617	0.452
11	39	[-3.723 ; 1.318]	-1.231	0.219
12	91	[-1.748 ; 1.662]	-0.007	1
13	16	[-4.328 ; 1.307]	-1.311	0.224
14	19	[-5.847 ; -0.420]	-3.122	0.019
15	9	[-5.964 ; -1.445]	-3.513	0.004
16	15	[-4.586 ; -0.659]	-2.404	0.010
17	18	[-5.328 ; 0.093]	-2.487	0.055
18	36	[-4.585 ; -0.221]	-2.493	0.039
19	15	[-4.751 ; -1.627]	-3.143	0.001

Table D-16 Results of the Wilcoxon tests for sexual dimorphism of Lower Vertebral Length (LVL). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	144	[-4.139 ; 0.248]	-1.878	0.088
1	273	[-4.081 ; 0.232]	-1.771	0.081
2	281	[-0.986 ; 1.933]	0.455	0.525
3	239	[-2.217 ; 0.866]	-0.723	0.364
4	328	[-2.075 ; 0.545]	-0.819	0.278
5	278	[-2.438 ; 0.113]	-1.238	0.067
6	326	[-2.068 ; 0.559]	-0.750	0.239
7	292	[-2.168 ; 0.602]	-0.725	0.265
8	300	[-2.498 ; 0.028]	-1.032	0.056
9	340	[-2.136 ; 0.237]	-0.894	0.112
10	309	[-2.624 ; -0.323]	-1.524	0.010
11	170	[-2.466 ; 0.572]	-1.040	0.152
12	299	[-1.545 ; 1.038]	-0.209	0.666
13	180	[-2.416 ; 0.893]	-0.767	0.341
14	74	[-4.359 ; -0.523]	-2.381	0.009
15	61	[-5.102 ; -1.677]	-3.355	0.0003
16	42	[-4.544 ; -1.677]	-2.953	1.877e-05
17	70	[-4.294 ; -0.977]	-2.457	0.0009
18	106	[-4.212 ; -0.838]	-2.480	0.004
19	103	[-4.212 ; -1.337]	-2.907	0.0001



Table D-17 Results of the Wilcoxon tests for sexual dimorphism of Lower Vertebral Width (LVW). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	83	[-2.505 ; 5.319]	1.921	0.379
1	29	[-2.284 ; 2.905]	-0.053	0.959
2	19	[-4.476 ; 1.957]	-0.844	0.573
3	31	[-2.404 ; 6.262]	0.535	0.779
4	17	[-4.177 ; -0.154]	-2.324	0.026
5	68	[-2.242 ; 3.128]	0.199	0.781
6	24	[-4.513 ; 3.757]	-0.963	0.776
7	29	[-6.360 ; 1.515]	-2.386	0.211
8	56	[-1.849 ; 3.588]	1.687	0.438
9	20	[-3.966 ; -0.502]	-2.018	0.031
10	38	[-4.558 ; 1.722]	-1.543	0.249
11	53	[-2.583 ; 2.016]	-0.251	0.700
12	63	[-4.569 ; 0.514]	-1.922	0.185
13	19	[-4.433 ; 1.175]	-1.457	0.388
14	14	[-6.791 ; -1.218]	-3.785	0.005
15	5	[-9.441 ; -3.075]	-6.484	0.0008
16	16	[-7.859 ; -1.108]	-3.313	0.012
17	27	[-5.480 ; 1.821]	-1.382	0.274
18	17	[-6.512 ; -1.787]	-4.425	0.0009
19	15	[-7.886 ; -2.009]	-5.008	0.001

Table D-18 Results of the Wilcoxon tests for sexual dimorphism of Lower Vertebral Width (LVW). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	170	[-6.565 ; 2.081]	-2.240	0.306
1	304	[-5.993 ; 1.433]	-2.049	0.224
2	281	[-1.507 ; 3.062]	0.778	0.525
3	245	[-3.423 ; 1.616]	-1.013	0.433
4	358	[-2.456 ; 1.290]	-0.649	0.547
5	338	[-2.516 ; 0.933]	-0.813	0.396
6	368	[-2.475 ; 1.515]	-0.511	0.615
7	314	[-2.962 ; 1.672]	-0.878	0.466
8	332	[-2.905 ; 0.561]	-1.301	0.157
9	348	[-2.610 ; 0.351]	-1.086	0.140
10	331	[-3.529 ; -0.293]	-1.916	0.024
11	189	[-3.038 ; 0.979]	-0.856	0.318
12	249	[-2.726 ; 0.385]	-1.072	0.174
13	143	[-3.871 ; 0.027]	-1.813	0.057
14	64	[-4.778 ; -1.068]	-2.761	0.003
15	34	[-7.123 ; -3.075]	-4.978	3.550e-06
16	40	[-7.171 ; -2.937]	-5.129	1.321e-05
17	87	[-5.235 ; -0.916]	-2.856	0.006
18	96	[-5.209 ; -1.255]	-3.139	0.001
19	69	[-6.438 ; -2.975]	-4.671	2.525e-06

Table D-19 Results of the Wilcoxon tests for sexual dimorphism of Lower Vertebral Module (LVM). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	76	[-57.134 ; 67.256]	17.163	0.640
1	29	[-66.370 ; 79.454]	-2.965	0.953
2	15	[-146.209 ; 53.513]	-58.397	0.282
3	39	[-35.395 ; 242.602]	49.344	0.232
4	9	[-179.161 ; -40.686]	-118.160	0.0025
5	59	[-116.194 ; 94.325]	-11.189	0.829
6	20	[-223.887 ; 235.087]	-39.416	0.456
7	26	[-314.812 ; 26.852]	-142.695	0.133
8	55	[-107.050 ; 186.898]	67.582	0.485
9	16	[-259.438 ; -34.595]	-141.806	0.012
10	40	[-214.220 ; 82.753]	-72.088	0.308
11	44	[-263.249 ; 94.719]	-55.221	0.355
12	73	[-164.431 ; 74.982]	-53.503	0.402
13	14	[-294.503 ; 76.107]	-120.473	0.145
14	13	[-478.292 ; -65.211]	-271.751	0.004
15	5	[-550.606 ; -185.019]	-369.949	0.0008
16	10	[-444.718 ; -70327]	-245.440	0.002
17	20	[-373.232 ; 32.836]	-179.253	0.083
18	26	[-395.492 ; -108.841]	-261.255	0.007
19	10	[-474.541 ; -149.480]	-327.256	0.0002

Table D-20 Results of the Wilcoxon tests for sexual dimorphism of Lower Vertebral Module (LVM). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	160	[-161.195 ; 25.055]	-59.684	0.199
1	292	[-161.195 ; 21.5322]	-63.971	0.155
2	282	[-53.805 ; 99.684]	21.951	0.511
3	243	[-131.152 ; 51.007]	-44.216	0.621
4	337	[-114.634 ; 44.977]	-36.135	0.347
5	284	[-132.785 ; 8.359]	-60.151	0.083
6	331	[-120.969 ; 38.322]	-45.249	0.273
7	291	[-143.860 ; 52.663]	-58.369	0.258
8	301	[-160.611 ; 2.019]	-81.027	0.058
9	330	[-154.295 ; 13.262]	-77.789	0.082
10	290	[-207.771 ; -37.681]	-131.663	0.005
11	171	[-184.710 ; 39.287]	-65.870	0.159
12	260	[-139.298 ; 36.258]	-47.745	0.249
13	154	[-209.989 ; 21.765]	-87.723	0.106
14	61	[-335.416 ; -65.359]	-194.563	0.002
15	39	[-459.244 ; -177.876]	-307.280	90135e-06
16	27	[-431.835 ; -189.780]	-306.016	1.002e-06
17	58	[-347.111 ; -109.171]	-218.613	0.0002
18	93	[-337.522 ; -109.245]	-224.117	0.001
19	73	[-390.299 ; -172.047]	-294.323	4.294e-06

Table D-21 Results of the Wilcoxon tests for sexual dimorphism of Posterior Vertebral Height (PVH). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	88	[-0.753 ; 2.005]	0.779	0.238
1	44	[-0.829 ; 2.265]	0.702	0.16
2	19	[-2.192 ; 1.120]	-0.572	0.573
3	40	[-0.717 ; 1.844]	0.643	0.189
4	33	[-1.758 ; 0.514]	-0.391	0.395
5	63	[-1.285 ; 1.296]	0.0072	1
6	37	[-0.677 ; 2.213]	0.948	0.272
7	67	[-0.608 ; 3.953]	1.804	0.079
8	79	[0.683 ; 5.020]	3.366	0.006
9	60	[-1.251 ; 2.911]	0.819	0.384
10	90	[0.297 ; 5.890]	2.668	0.022
11	80	[-1.493 ; 4.672]	1.358	0.243
12	130	[-0.111 ; 5.485]	2.666	0.061
13	38	[-1.983 ; 6.242]	2.268	0.224
14	54	[-3.306 ; 4.624]	0.507	0.796
15	24	[-4.541 ; 0.884]	-1.872	0.162
16	45	[-2.341 ; 2.041]	-0.384	0.851
17	48	[-1.207 ; 2.756]	0.497	0.515
18	60	[-2.246 ; 0.959]	-0.801	0.514
19	14	[-4.199 ; -1.455]	-2.814	0.0007

Table D-22 Results of the Wilcoxon tests for sexual dimorphism of Posterior Vertebral Height (PVH). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	211	[-1.165 ; 0.952]	0.014	0.990
1	348	[-1.338 ; 0.764]	-0.226	0.364
2	306	[-0.316 ; 1.176]	0.460	0.231
3	259	[-0.795 ; 0.518]	-0.167	0.621
4	395	[-0.654 ; 0.553]	-0.008	0.994
5	390	[-0.777 ; 0.747]	-0.008	0.994
6	512	[-0.143 ; 2.131]	1.002	0.073
7	529	[0.568 ; 3.001]	1.683	0.002
8	605	[0.571 ; 3.157]	1.924	0.005
9	656	[0.797 ; 3.477]	2.149	0.003
10	630	[-0.325 ; 2.769]	1.334	0.102
11	324	[0.068 ; 4.137]	2.017	0.042
12	490	[1.054 ; 4.568]	2.715	0.002
13	303	[0.137 ; 4.642]	2.380	0.033
14	188	[-1.195 ; 4.208]	1.404	0.243
15	157	[-2.375 ; 1.347]	-0.746	0.506
16	136	[-2.433 ; 0.661]	-1.100	0.220
17	187	[-1.174 ; 1.504]	0.077	0.851
18	213	[-1.212 ; 1.140]	-0.175	0.871
19	141	[-2.759 ; -0.761]	-1.796	0.004

**Table D-23 Results of the Wilcoxon tests for sexual dimorphism of Anterior Vertebral Height (AVH). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange**

Age group	W	95% confidence interval	Difference in location	p-value
0	75	[-1.085 ; 1.450]	0.347	0.682
1	41	[-0.659 ; 2.705]	0.572	0.279
2	18	[-1.570 ; 0.798]	-0.553	0.491
3	44	[-0.242 ; 3.110]	1.568	0.072
4	49	[-1.550 ; 1.512]	0.140	0.717
5	94	[-0.088 ; 2.831]	1.147	0.053
6	34	[-1.104 ; 2.473]	0.564	0.456
7	64	[-0.601 ; 2.494]	1.139	0.133
8	54	[-0.748 ; 2.201]	0.765	0.536
9	63	[-1.007 ; 2.133]	0.877	0.270
10	73	[-0.745 ; 3.452]	1.036	0.278
11	89	[-0.269 ; 4.385]	2.239	0.083
12	134	[0.349 ; 5.081]	2.622	0.038
13	39	[-0.993 ; 8.850]	3.896	0.181
14	63	[-1.295 ; 4.325]	1.038	0.353
15	47	[-1.918 ; 2.867]	0.746	0.605
16	47	[-2.028 ; 2.625]	-0.054	0.970
17	43	[-2.522 ; 2.764]	0.113	0.829
18	63	[-2.260 ; 1.263]	-0.672	0.630
19	44	[-2.758 ; 0.761]	-1.211	0.190

**Table D-24 Results of the Wilcoxon tests for sexual dimorphism of Anterior Vertebral Height (AVH). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange**

Age group	W	95% confidence interval	Difference in location	p-value
0	175	[-1.983 ; 0.766]	-0.661	0.372
1	319	[-2.046 ; 0.664]	-0.769	0.335
2	335	[-0.092 ; 1.821]	0.825	0.063
3	312	[-0.732 ; 1.275]	0.288	0.564
4	495	[-0.230 ; 1.708]	0.735	0.112
5	477	[-0.258 ; 1.640]	0.636	0.166
6	544	[0.187 ; 2.081]	1.113	0.020
7	438	[-0.230 ; 1.435]	0.661	0.158
8	495	[-0.517 ; 1.545]	0.658	0.289
9	545	[-0.397 ; 1.794]	0.821	0.179
10	673	[0.152 ; 2.160]	1.146	0.027
11	334	[0.263 ; 3.113]	1.631	0.023
12	495	[1.222 ; 4.500]	2.819	0.001
13	316	[0.653 ; 5.090]	2.934	0.131
14	202	[-0.248 ; 5.228]	2.174	0.102
15	215	[-0.876 ; 2.541]	0.792	0.325
16	200	[-0.930 ; 1.870]	0.363	0.542
17	192	[-1.750 ; 1.809]	0.189	0.740
18	212	[-1.765 ; 1.294]	-0.198	0.852
19	206	[-2.081 ; 0.340]	-1.032	0.140

Table D-25 Results of the Wilcoxon tests for sexual dimorphism of Right Vertebral Height (RVH). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	74	[-0.702 ; 0.928]	0.162	0.726
1	39	[-0.810 ; 2.455]	0.880	0.383
2	29	[-1.706 ; 2.572]	0.353	0.573
3	55	[1.323 ; 4.081]	2.706	0.0006
4	71	[0.131 ; 1.974]	1.222	0.026
5	78	[-0.729 ; 2.297]	0.726	0.369
6	47	[0.468 ; 4.316]	2.522	0.018
7	72	[0.059 ; 2.505]	1.208	0.028
8	76	[0.256 ; 3.640]	2.068	0.014
9	62	[-0.983 ; 1.961]	0.644	0.305
10	88	[0.357 ; 5.133]	2.652	0.033
11	102	[0.756 ; 4.746]	2.495	0.009
12	141	[1.044 ; 5.151]	3.169	0.014
13	34	[-2.739 ; 4.787]	1.758	0.456
14	61	[-0.843 ; 5.479]	1.905	0.436
15	42	[-1.837 ; 2.291]	0.076	0.931
16	60	[-1.358 ; 3.215]	0.938	0.384
17	48	[-1.958 ; 3.634]	0.966	0.515
18	54	[-2.596 ; 0.691]	-1.083	0.319
19	29	[-3.135 ; -0.087]	-1.663	0.023

Table D-26 Results of the Wilcoxon tests for sexual dimorphism of Right Vertebral Height (RVH). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	178	[-1.214 ; 0.476]	-0.356	0.415
1	340	[-1.246 ; 0.709]	-0.314	0.541
2	391	[0.710 ; 2.540]	1.508	0.001
3	433	[0.483 ; 2.428]	1.447	0.002
4	572	[0.421 ; 2.260]	1.369	0.004
5	532	[0.154 ; 2.159]	1.225	0.021
6	594	[0.648 ; 2.547]	1.674	0.002
7	563	[0.835 ; 2.646]	1.733	0.0002
8	592	[0.205 ; 2.088]	1.152	0.010
9	642	[0.332 ; 2.560]	1.438	0.005
10	689	[0.298 ; 2.700]	1.488	0.015
11	371	[1.032 ; 4.148]	2.608	0.001
12	545	[2.081 ; 4.746]	3.387	1.423e-05
13	307	[0.600 ; 4.408]	2.502	0.025
14	197	[-0.541 ; 4.503]	1.644	0.142
15	206	[-0.843 ; 2.901]	0.655	0.470
16	197	[-1.116 ; 1.736]	0.412	0.601
17	209	[-1.208 ; 2.447]	0.639	0.409
18	211	[-1.876 ; 1.187]	-0.132	0.832
19	172	[-2.424 ; -0.123]	-1.299	0.027

Table D-27 Results of the Wilcoxon tests for sexual dimorphism of Left Vertebral Height (LVH). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	90	[-0.377 ; 1.529]	0.625	0.194
1	44	[-0.362 ; 2.122]	1.073	0.160
2	30	[-1.363 ; 1.465]	0.312	0.491
3	53	[1.050 ; 3.857]	2.612	0.002
4	73	[0.376 ; 2.450]	1.296	0.016
5	71	[-0.842 ; 1.903]	0.510	0.643
6	39	[-0.440 ; 2.918]	1.065	0.181
7	69	[-0.089 ; 2.887]	1.372	0.053
8	74	[0.109 ; 3.236]	1.657	0.024
9	61	[-0.947 ; 2.564]	0.731	0.343
10	74	[-1.135 ; 4.321]	1.460	0.249
11	89	[-0.290 ; 4.368]	1.813	0.083
12	128	[-0.213 ; 5.393]	2.822	0.076
13	38	[-2.671 ; 6.192]	2.907	0.224
14	63	[-1.475 ; 6.571]	1.130	0.353
15	23	[-1.837 ; 2.291]	0.076	0.931
16	58	[-1.205 ; 2.658]	0.986	0.473
17	49	[-1.880 ; 4.253]	1.262	0.459
18	58	[-2.333 ; 0.899]	-0.792	0.443
19	40	[-3.511 ; 0.484]	-1.356	0.118

Table D-28 Results of the Wilcoxon tests for sexual dimorphism of Left Vertebral Height (LVH). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	221	[-0.836 ; 1.087]	0.170	0.787
1	393	[-0.954 ; 1.045]	0.118	0.796
2	393	[0.492 ; 2.243]	1.269	0.001
3	431	[0.465 ; 2.429]	1.391	0.002
4	554	[0.314 ; 2.257]	1.331	0.010
5	516	[0.038 ; 1.878]	1.001	0.042
6	561	[0.351 ; 2.016]	1.243	0.009
7	519	[0.400 ; 2.224]	1.282	0.004
8	569	[0.110 ; 2.125]	1.146	0.027
9	592	[0.054 ; 2.402]	1.160	0.042
10	640	[-0.187 ; 2.423]	1.049	0.077
11	323	[0.059 ; 3.559]	1.637	0.044
12	500	[1.405 ; 4.653]	3.137	0.0007
13	302	[0.281 ; 4.867]	2.599	0.035
14	201	[-0.793 ; 5.410]	2.147	0.109
15	174	[-1.904 ; 1.854]	-0.256	0.863
16	156	[-1.726 ; 1.219]	-0.441	0.523
17	210	[-0.845 ; 2.365]	1.024	0.393
18	224	[-1.586 ; 1.450]	0.063	0.931
19	198	[-2.165 ; 0.141]	-1.065	0.099

## Clavicular variables

Table D-29 Results of the Wilcoxon tests for sexual dimorphism of Maximal Length (Ln). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	174	[-4.590 ; 6.490]	-0.090	0.977
1	79.5	[-9.670 ; -2.680]	-5.720	0.0005
2	67	[-4.660 ; 3.120]	-1.56	0.388
3	109	[-3.780 ; 3.550]	-1.29	0.502
4	126.5	[-1.420 ; 7.630]	3.780	0.189
5	77	[-5.580 ; 3.400]	-1.172	0.512
6	74	[-8.730 ; 4.470]	-1.660	0.604
7	63	[-9.390 ; 5.330]	-3.335	0.437
8	101	[-5.620 ; 5.760]	0.370	0.892
9	65	[-7.900 ; 1.170]	-2.690	0.191
10	60	[-8.430 ; 3.600]	-2.755	0.428
11	41	[-11.640 ; 3.560]	-2.210	0.641
12	41	[-9.640 ; 9.990]	-2.450	0.552
13	79	[-17.47 ; 1.590]	-6.525	0.1102
14	43	[-22.580 ; -1.140]	-13.235	0.036
15	47	[-15.620 ; -4.650]	-9.540	0.001
16	44	[-13.140 ; -4.070]	-8.805	<0.001
17	62	[-13.460 ; -1.130]	-8.470	0.028
18	39	[-22.730 ; -12.380]	-17.170	<0.001
19	38	[-16.190 ; -7.190]	-11.730	<0.001

Table D-30 Results of the Wilcoxon tests for sexual dimorphism of Maximal Length (Ln). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	477.5	[-12.310 ; -3.230]	-8.180	0.001
1	384.5	[-6.360 ; -0.500]	-3.405	0.019
2	1087.5	[-4.590 ; 1.520]	-1.600	0.266
3	898	[-2.770 ; 3.110]	-0.310	0.826
4	974.5	[-2.320 ; 3.860]	0.387	0.812
5	920	[-2.070 ; 4.590]	1.210	0.452
6	649.5	[-6.080 ; 1.360]	-2.318	0.212
7	680	[-6.490 ; 1.900]	-2.360	0.283
8	711.5	[-5.810 ; 1.590]	-2.071	0.320
9	662	[-5.470 ; 1.060]	-2.430	0.162
10	465.5	[-7.320 ; -0.030]	-3.866	0.045
11	536.5	[-5.420 ; 4.320]	-0.045	0.969
12	247	[-12.870 ; 0.960]	-5.420	0.085
13	226	[-9.920 ; 0.340]	-5.030	0.063
14	247	[-16.770 ; -2.470]	-8.925	0.011
15	165.5	[-16.610 ; -6.730]	-11.647	4.530e-05
16	182	[-12.950 ; -5.620]	-9.230	1.017e-06
17	224	[-12.160 ; -4.010]	-8.030	6.868e-05
18	195	[-16.560 ; -9.500]	-13.390	5.230e-08
19	153	[-17.720 ; -11.200]	-14.795	2.652e-11

Table D-31 Results of the Wilcoxon tests for sexual dimorphism of Maximal diameter (Max\_diam). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	141	[-1.434 ; 0.617]	-0.381	0.418
1	53	[-1.238 ; -0.617]	-1.011	<0.001
2	133	[0.319 ; 1.217]	0.759	0.002
3	100	[-0.716 ; 0.339]	-0.234	0.313
4	37.5	[-1.146 ; 0.018]	-0.481	0.061
5	49.5	[-1.383 ; -0.017]	-0.774	0.047
6	95	[-0.246 ; 1.301]	0.382	0.207
7	49	[-1.801 ; 0.302]	-0.798	0.123
8	87	[-0.628 ; 0.542]	0.082	0.918
9	52	[-1.763 ; 0.110]	-0.753	0.080
10	62	[-1.472 ; 0.253]	-0.232	0.495
11	14	[-2.211 ; -0.003]	-1.174	0.042
12	23	[-1.112 ; 0.384]	-0.315	0.236
13	29	[-4.334 ; -0.638]	-2.248	0.007
14	0	[-4.718 ; -1.978]	-3.204	<0.001
15	15	[-3.065 ; -1.011]	-2.085	<0.001
16	4	[-4.171 ; -1.839]	-3.183	<0.001
17	5	[-3.556 ; -1.676]	-2.467	<0.001
18	28	[-3.616 ; -2.113]	-2.840	<0.001
19	66	[-2.414 ; -0.683]	-1.539	0.002

Table D-32 Results of the Wilcoxon tests for sexual dimorphism of Maximal diameter (Max\_diam). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	364	[-1.366 ; -0.543]	-0.985	9.698e-05
1	450	[-0.778 ; 0.310]	-0.268	0.423
2	1035	[-0.525 ; 0.214]	-0.177	0.395
3	781.5	[-0.385 ; 0.286]	-0.054	0.720
4	575	[-0.834 ; -0.104]	-0.432	0.010
5	630	[-0.605 ; 0.205]	-0.174	0.344
6	570.5	[-0.858 ; 0.073]	-0.406	0.088
7	638.5	[-0.725 ; 0.290]	-0.194	0.413
8	540	[-1.096 ; -0.051]	-0.539	0.031
9	600	[-0.815 ; 0.088]	-0.321	0.137
10	358	[-1.255 ; -0.227]	-0.731	0.005
11	326	[-1.145 ; 0.042]	-0.521	0.075
12	143	[-1.973 ; -0.045]	-0.962	0.039
13	102	[-2.059 ; -0.377]	-1.093	0.002
14	55	[-3.923 ; -1.887]	-2.744	4.188e-07
15	26	[-3.251 ; -1.887]	-2.565	8.558e-10
16	44	[-3.278 ; -1.695]	-2.566	1.098e-08
17	23	[-3.532 ; -2.059]	-2.767	2.647e-12
18	64	[-3.269 ; -2.117]	-2.678	1.904e-13
19	185	[-2.829 ; -1.597]	-2.247	2.195e-08



Table D-33 Results of the Wilcoxon tests for sexual dimorphism of Minimal diameter (Min\_diam). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	136	[-1.014 ; 0.279]	-0.284	0.33
1	44	[-1.006 ; -0.407]	-0.686	<0.001
2	135	[0.242 ; 0.906]	0.565	0.001
3	114	[-0.685 ; 0.485]	-0.200	0.628
4	53	[-0.944 ; 0.338]	-0.326	0.341
5	46	[-1.241 ; -0.051]	-0.605	0.029
6	103	[-0.088 ; 1.085]	0.398	0.084
7	74	[-0.699 ; 0.930]	-0.077	0.852
8	63	[-0.957 ; 0.360]	-0.311	0.287
9	115	[-0.189 ; 1.267]	0.488	0.195
10	63	[-1.009 ; 0.381]	-0.197	0.542
11	23	[-1.735 ; 0.522]	-0.652	0.230
12	24	[-1.967 ; 0.406]	-0.648	0.277
13	36	[-3.680 ; -0.374]	-1.622	0.022
14	6	[-4.385 ; -2.231]	-2.825	<0.001
15	23	[-2.614 ; -0.679]	-1.508	0.003
16	10	[-2.141 ; -0.566]	-1.440	<0.001
17	68	[-2.035 ; 0.033]	-0.876	0.051
18	30	[-2.767 ; -1.299]	-2.026	<0.001
19	19	[-2.838 ; -1.302]	-2.089	<0.001

Table D-34 Results of the Wilcoxon tests for sexual dimorphism of Minimal diameter (Min\_diam). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	393	[-1.061 ; -0.346]	-0.708	0.0002
1	441	[-0.560 ; 0.233]	-0.214	0.357
2	1061.5	[-0.458 ; 0.218]	-0.111	0.512
3	854	[-0.260 ; 0.333]	0.045	0.753
4	642	[-0.713 ; -0.002]	-0.365	0.047
5	669	[-0.484 ; 0.231]	-0.091	0.593
6	655	[-0.525 ; 0.169]	-0.173	0.402
7	664	[-0.521 ; 0.270]	-0.132	0.583
8	764	[-0.386 ; 0.465]	0.025	0.940
9	707	[-0.465 ; 0.351]	-0.068	0.683
10	563.5	[-0.540 ; 0.381]	-0.090	0.690
11	361.5	[-0.894 ; 0.193]	-0.418	0.208
12	154	[-1.645 ; 0.068]	-0.717	0.072
13	114	[-2.292 ; -0.367]	-1.177	0.007
14	72	[-3.397 ; -1.431]	-2.601	1.783e-05
15	59	[-2.733 ; -1.629]	-2.210	2.775e-06
16	74	[-1.970 ; -0.853]	-1.424	1.002e-06
17	182	[-1.890 ; -0.469]	-1.15	0.0006
18	224	[-2.271 ; -0.987]	-1.634	1.549e-06
19	96	[-2.586 ; -1.566]	-2.082	2.303e-13

Table D-35 Results of the Wilcoxon tests for sexual dimorphism of Antero-Posterior diameter (AP\_diam). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	157	[-1.406 ; 0.596]	-0.357	0.608
1	61	[-1.408 ; -0.550]	-1.010	<0.001
2	152	[0.559 ; 1.520]	1.037	<0.001
3	158	[-0.271 ; 0.929]	0.249	0.261
4	58	[-1.151 ; 0.060]	-0.552	0.072
5	51	[-1.681 ; 0.001]	-0.845	0.054
6	113	[-0.166 ; 1.230]	0.653	0.170
7	52	[-1.464 ; 0.246]	-0.646	0.168
8	125	[-0.185 ; 1.315]	0.381	0.217
9	85	[-1.109 ; 0.602]	-0.274	0.711
10	75	[-1.074 ; 0.994]	0.018	1
11	24	[-1.495 ; 0.148]	-0.563	0.083
12	42	[-1.126 ; 0.599]	-0.231	0.603
13	53	[-3.193 ; -0.793]	-2.101	0.007
14	0	[-4.291 ; -2.256]	-3.241	<0.001
15	54	[-2.426 ; -0.521]	-1.449	0.003
16	17	[-3.610 ; -1.628]	-2.525	<0.001
17	10	[-3.245 ; -1.556]	-2.156	<0.001
18	39	[-3.699 ; -1.822]	-2.888	<0.001
19	35	[-3.078 ; -1.236]	-2.108	<0.001

Table D-36 Results of the Wilcoxon tests for sexual dimorphism of Antero-posterior diameter (AP\_diam). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	428	[-1.364 ; -0.473]	-0.931	0.0001
1	515	[-0.680 ; 0.320]	-0.186	0.460
2	1280	[-0.331 ; 0.403]	0.035	0.836
3	1103	[-0.080 ; 0.597]	0.249	0.125
4	806	[-0.691 ; 0.134]	-0.262	0.238
5	760	[-0.621 ; 0.278]	-0.154	0.471
6	655	[-0.812 ; 0.209]	-0.272	0.234
7	818	[-0.437 ; 0.578]	0.065	0.806
8	759	[-0.610 ; 0.338]	-0.136	0.588
9	855	[-0.361 ; 0.605]	0.117	0.674
10	559	[-0.829 ; 0.238]	-0.290	0.331
11	526	[-0.691 ; 0.501]	-0.045	0.864
12	264	[-1.474 ; 0.176]	-0.665	0.153
13	177	[-1.964 ; -0.452]	-1.183	0.005
14	88	[-3.311 ; -1.940]	-2.602	3.881e-08
15	76	[-3.011 ; -1.571]	-2.287	2.163e-09
16	141	[-2.648 ; -1.351]	-2.007	5.732e-08
17	64	[-3.094 ; -1.847]	-2.423	2.765e-11
18	92	[-3.314 ; -1.930]	-2.604	2.196e-12
19	150	[-3.192 ; -1.857]	-2.512	1.994e-11

Table D-37 Results of the Wilcoxon tests for sexual dimorphism of Supero-Inferior diameter (SI\_diam). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	121	[-1.246 ; 0.152]	-0.601	0.120
1	100	[-0.801 ; -0.145]	-0.511	0.002
2	90	[-0.368 ; 0.392]	0.022	0.801
3	86	[-1.203 ; 0.125]	-0.594	0.123
4	69	[-1.692 ; 0.340]	-0.707	0.201
5	62	[-1.268 ; 0.205]	-0.533	0.169
6	112	[-0.288 ; 1.263]	0.430	0.187
7	75	[-0.845 ; 0.935]	-0.024	0.894
8	61	[-1.169 ; 0.225]	-0.582	0.098
9	110	[-0.455 ; 0.977]	0.315	0.458
10	30	[-1.652 ; -0.169]	-0.938	0.012
11	25	[-2.293 ; 0.123]	-0.910	0.098
12	40	[-0.967 ; 0.408]	-0.268	0.503
13	36	[-3.179 ; -0.698]	-1.851	<0.001
14	22	[-3.105 ; -1.194]	-2.408	<0.001
15	69	[-2.127 ; -0.178]	-1.214	0.016
16	89	[-1.866 ; 0.294]	-0.828	0.153
17	56	[-2.590 ; -0.320]	-1.467	0.014
18	59	[-2.637 ; -1.022]	-1.881	<0.001
19	44	[-2.786 ; -0.939]	-1.888	<0.001

Table D-38 Results of the Wilcoxon tests for sexual dimorphism of Supero-Inferior diameter (SI\_diam). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	438	[-1.101 ; -0.336]	-0.704	0.0002
1	439	[-0.556 ; 0.027]	-0.283	0.094
2	925	[-0.612 ; -0.038]	-0.324	0.025
3	644	[-0.771 ; -0.084]	-0.413	0.015
4	602	[-1.020 ; -0.213]	-0.612	0.003
5	753	[-0.644 ; 0.274]	-0.195	0.432
6	756	[-0.528 ; 0.404]	-0.041	0.841
7	747	[-0.548 ; 0.353]	-0.095	0.669
8	746	[-0.574 ; 0.325]	-0.143	0.507
9	601	[-0.855 ; -0.004]	-0.435	0.047
10	464	[-0.950 ; -0.028]	-0.499	0.042
11	371	[-1.061 ; -0.059]	-0.565	0.031
12	229	[-1.563 ; -0.031]	-0.667	0.041
13	146	[-1.901 ; -0.457]	-1.059	0.0005
14	115	[-2.797 ; -1.283]	-2.183	8.052e-07
15	170	[-2.367 ; -1.061]	-1.610	3.033e-05
16	328	[-1.721 ; -0.266]	-0.997	0.008
17	286	[-1.926 ; -0.358]	-1.190	0.004
18	241	[-2.328 ; -1.034]	-1.685	1.407e-06
19	197	[-2.422 ; -1.280]	-1.841	1.219e-09

## Iliac non-metric variables

Table D-39 Results of the Wilcoxon tests for sexual dimorphism for the maturation stages of the Inferior pubo-ischiatic epiphysis (PUBISCH\_INF). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	72	-	-	-
1	-	-	-	-
2	21	-	-	-
3	18	-	-	0.44
4	30	-	-	0.897
5	43	-	-	0.760
6	34.5	-	-	0.917
7	43.5	-	-	0.468
8	76.5	[0.000-1.000]	7.351e-05	0.036
9	35.5	[-0.999 ; 0.999]	-4.254e-05	1.000
10	90	[0.000 ; 7.874e-05]	0.000	0.119
11	62.5	[-3.596e-05 ; 1.935e-06]	1.7935e-05	0.859
12	74	[-8.693e-05 ; 0.999]	8.292e-05	0.221
13	-	-	-	-
14	49.5	[-4.053e-06 ; 1.952e-05]	2.742e-05	0.607
15	-	-	-	-
16	66	[0.000 ; 7.0363e-05]	0.000	0.315
17	54	[0.000 ; 7.174e-05]	0.000	0.421
18	-	-	-	-
19	-	-	-	-

Table D-40 Results of the Wilcoxon tests for sexual dimorphism for the maturation stages of the Inferior pubo-ischiatic epiphysis (PUBISCH\_INF). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	72	-	-	-
1	-	-	-	-
2	198	-	-	0.403
3	310.5	-	-	-
4	384	-	-	-
5	316	-	-	0.850
6	377.5	-	-	0.428
7	467.5	[-4.758e-05 ; 0.999]	7.891e-05	0.099
8	457.5	[-4.323e-05 ; 0.999]	3.025e-05	0.127
9	588	[-6.433e-05 ; 1.090e-05]	7.227e-05	0.06
10	550.5	[-2.842e-05 ; 4.646e-05]	3.972e-05	0.583
11	703	[-5.215e-05 ; 2.862e-05]	7.096e-05	0.081
12	526	[-1.922e-05 ; 7.799e-06]	5.576e-05	0.199
13	444.5	[-2.898e-05 ; 3.336e-05]	4.322e-05	0.156
14	352.5	[-2.033e-05 ; 9.561e-05]	1.225e-05	0.511
15	467	[-7.289e-05 ; 2.746e-05]	7.182e-05	0.258
16	480.5	[0.000 ; 0.000]	0.000	0.147
17	594	[0.000 ; 0.000]	0.000	0.167
18	648	[0.000 ; 0.000]	0.000	0.324
19	-	-	-	-

Table D-41 Results of the Wilcoxon tests for sexual dimorphism for the maturation stages of the Superior pubo-ischiatic epiphysis (PUBISCH\_SUP). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	72	-	-	-
1	30	-	-	-
2	24.5	-	-	-
3	24	-	-	-
4	60	-	-	-
5	48	-	-	-
6	40	-	-	-
7	44	-	-	0.434
8	71.5	[0.000 ; 6.878e-05]	0.000	0.094
9	57	[-1.273e-05 ; 0.999]	9.334e-07	0.900
10	121.5	[4.336e-05 ; 1.000]	0.999	0.017
11	101	[-2.358e-05 ; 1.000]	1.403e-05	0.089
12	105	[0.000 ; 1.000]	0.999	0.004
13	44	[-5.104e-06 ; 1.000]	1.080e-05	0.133
14	53	[-4.288e-05 ; 0.999]	4.171e-09	0.448
15	55	[0.000 ; 7.866e-05]	0.000	0.145
16	55	[-6.836e-05 ; 0.000]	0.000	0.411
17	54	[0.000 ; 7.174e-05]	0.000	0.421
18	84	[0.000 ; 6.456e-05]	0.000	0.379
19	-	-	-	-

Table D-42 Results of the Wilcoxon tests for sexual dimorphism for the maturation stages of the Superior pubo-ischiatic epiphysis (PUBISCH\_SUP). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange

Age group	W	95% confidence interval	Difference in location	p-value
0	72	-	-	-
1	392	-	-	-
2	243	-	-	-
3	310.5	-	-	-
4	384	-	-	-
5	442	-	-	-
6	405	-	-	0.299
7	465	[0.000 ; 5.428e-05]	0.000	0.062
8	518	[-4.241e-05 ; 5.055e-05]	3.235e-05	0.246
9	735	[-3.357e-05 ; 5.691e-05]	8.372e-05	0.029
10	828	[2.211e-06 ; 0.999]	3.095e-05	0.025
11	1011.5	[8.365e-05 ; 0.999]	0.999	5.521e-05
12	799	[5.362e-05 ; 1.000]	0.999	9.04e-05
13	590	[6.977e-05 ; 0.999]	0.999	0.003
14	459	[-4344e-05 ; 3.437e-05]	4.598e-05	0.028
15	496	[-3.254e-05 ; 1.690e-05]	4.976e-05	0.272
16	495	[-3.688e-05 ; 4.669e-05]	2.313e-05	0.321
17	576	[-1.800e-05 ; 8.118e-05]	5.165e-05	0.612
18	684	[0.000 ; 0.000]	0.000	0.160
19	351	[0.000 ; 0.000]	0.000	0.317

**Table D-43 Results of the Wilcoxon tests for sexual dimorphism for the maturation stages of the Ilio-ischiatic epiphysis (ILISCH). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange**

Age group	W	95% confidence interval	Difference in location	p-value
0	72	-	-	-
1	30	-	-	-
2	24.5	-	-	-
3	24	-	-	-
4	60	-	-	-
5	48	-	-	-
6	45	-	-	0.314
7	39	-	-	0.935
8	78	[0.000 ; 0.999]	5.729e-05	0.033
9	52.5	[-0.999 ; 6.750e-07]	-7.714e-05	0.871
10	117	[-7.135e-05 ; 1.000]	8.985e-05	0.032
11	84	[-5.300e-05 ; 2.382e-05]	5.454e-05	0.480
12	102.5	[0.000 ; 1.000]	0.999	0.005
13	44	[-5.104e-06 ; 1.000]	1.080e-05	0.133
14	53.5	[-3.730e-05 ; 0.999]	4.335e-05	0.356
15	60	[0.000 ; 0.999]	5.597e-05	0.061
16	55	[-6.836e-05 ; 0.000]	0.000	0.411
17	-	-	-	-
18	-	-	-	-
19	-	-	-	-

**Table D-44 Results of the Wilcoxon tests for sexual dimorphism for the maturation stages of the Ilio-ischiatic epiphysis (ILISCH). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange**

Age group	W	95% confidence interval	Difference in location	p-value
0	72	-	-	-
1	392	-	-	-
2	243	-	-	-
3	310.5	-	-	-
4	384	-	-	-
5	459	-	-	0.267
6	407	-	-	0.487
7	482.5	[-9.287e-05 ; 2.554e-05]	3.388e-05	0.061
8	506.5	[-1.299e05 ; 8.856e-06]	6.870e-06	0.426
9	728	[-1.526e-05 ; 0.999]	7.519e-05	0.051
10	748	[-6.372e-05 ; 5.825e-05]	9.409e-07	0.201
11	943	[6.434e-05 ; 0.999]	7.724e-06	0.0006
12	760	[3.282e-05 ; 0.999]	0.999	0.0004
13	587	[8.074e-06 ; 0.999]	2.969e-05	0.003
14	472.5	[5.488e-05 ; 4.664e-05]	3.617e-06	0.01
15	513.5	[-2.970e-05 ; 7.706e-05]	7.471e-05	0.111
16	496	[-1.581e-05 ; 3.350e-05]	1.259e-05	0.297
17	544	[0.000 ; 0.000]	0.000	0.325
18	-	-	-	-
19	-	-	-	-

**Table D-45 Results of the Wilcoxon tests for sexual dimorphism for the maturation stages of the Pubo-iliac epiphysis (PUBIL). Tests were done on annual averages calculated for the individuals of each annual age group N. p-values corresponding to significant sexual dimorphism are highlighted in orange**

Age group	W	95% confidence interval	Difference in location	p-value
0	72	-	-	-
1	30	-	-	-
2	24.5	-	-	-
3	24	-	-	-
4	60	-	-	-
5	48	-	-	-
6	40	-	-	-
7	40	-	-	-
8	71.5	[0.000 ; 6.878e-05]	0.000	0.094
9	82.5	[0.000 ; 1.000]	0.500	0.010
10	108	[-3.344e-05 ; 1.000]	5.738e-05	0.095
11	98	[-1.045e-05 ; 1.000]	6.494e-06	0.141
12	115	[0.999 ; 1.999]	1.000	0.0009
13	44	[-5.104e-06 ; 1.000]	1.080e-05	0.133
14	50	[-4.71e-06 ; 1.000]	1.121e-05	0.318
15	65.5	[-4.404e-05 ; 1.000]	0.227	0.043
16	50	[-7.381e-05 ; 0.000]	0.000	0.209
17	54	[0.000 ; 7.174e-05]	0.000	0.421
18	-	-	-	-
19	-	-	-	-

**Table D-46 Results of the Wilcoxon tests for sexual dimorphism for the maturation stages of the pubo-iliac epiphysis (PUBIL). Tests were done on moving averages calculated for the individuals of age groups (N-1), N and (N+1). p-values corresponding to significant sexual dimorphism are highlighted in orange**

Age group	W	95% confidence interval	Difference in location	p-value
0	72	-	-	-
1	392	-	-	-
2	243	-	-	-
3	310.5	-	-	-
4	384	-	-	-
5	442	-	-	-
6	390	-	-	-
7	449.5	[0.000 ; 0.000]	0.000	0.133
8	576	[0.000 ; 6.600e-05]	0.000	0.004
9	784	[3.879e-05 ; 0.999]	6.210e-05	0.001
10	869	[7.340e-05 ; 0.999]	2.254e-05	0.004
11	1007	[4.618e-05 ; 0.999]	0.999	8.041e-05
12	819	[0.999 ; 1.000]	0.999	4.520e-05
13	593	[1.447e-06 ; 1.000]	0.999	0.0008
14	479	[3.691e-05 ; 0.999]	3.090e-06	0.006
15	507.5	[-1.353e-05 ; 3.268e-05]	5.347e-06	0.149
16	510	[-5.879e-05 ; 1.560e-05]	5.553e-05	0.297
17	543	[-3.302e-05 ; 3.091e-05]	-4.282e-06	0.540
18	666	[0.000 ; 0.000]	0.000	0.331
19	-	-	-	-

## Homogeneity between the samples

The following tables present the results of the tests for homogeneity of the variables between the three samples from Marseilles, Toulouse and Lisbon (Luis Lopes collection).

### Iliac variables

Table D-47 Test of homogeneity of the biometric iliac variables obtained on the individuals from Marseilles and Toulouse

Variable	Factor of influence	Df	Sum of squares	Mean Square value	F value	p-value
IL	Age	1	159879	159879	2489.0054	<0.001
	Sample	1	2480	1240	17.631	0.007
IW	Age	1	130572	130572	2608.558	<0.001
	Sample	1	531	531	10.618	0.001
IM	Age	1	4068611768	4068611768	3160.80	<0.001
	Sample	1	373337	373337	0.29	0.591

Table D-48 Test of homogeneity of the iliac variables obtained on the individuals from Marseilles and Lisbon. Greyed values indicate inhomogeneity of the variables between the samples

Variable	Factor of influence	Df	Sum of squares	Mean Square value	F value	p-value
IL	Age	1	188056	188056	2675.340	<0.001
	Sample	2	2480	1240	17.631	<0.001
IW	Age	1	153932	153932	2758.899	<0.001
	Sample	2	1326	663	11.879	<0.001
IM	Age	1	4635478935	4635478935	3311.671	<0.001
	Sample	2	34201819	17100909	12.217	<0.001
IA	Age	1	2041000506	2041000506	2787.7094	<0.001
	Sample	1	201339189	732143	5.8338	0.016



## Lumbar variables

**Table D-49** Test of homogeneity of the lumbar variables obtained on the individuals from Marseilles and Lisbon. Greyed values indicate inhomogeneity of the variables between the samples

Variable	Factor of influence	Df	Sum of squares	Mean Square value	F value	p-value
UVL	Age	1	16961.50	16961.5	1737.80	<0.001
	Sample	1	346.3	346.3	35.48	<0.001
UVW	Age	1	34108	34108	1497.952	<0.001
	Sample	1	826	826	36.263	<0.001
UVM	Age	1	80890628	80890628	1921.020	<0.001
	Sample	1	2437903	2437903	57.896	<0.001
LVL	Age	1	18219	18219	1906.769	<0.001
	Sample	1	331.4	331.4	34.682	<0.001
LVW	Age	1	30861.5	30861.5	1307.691	<0.001
	Sample	1	481.3	481.3	20.395	<0.001
LVM	Age	1	7878944	78978944	2033.444	<0.001
	Sample	1	1985279	1985279	51.114	<0.001
PVH	Age	1	15693.8	15693.8	2412.9919	<0.001
	Sample	1	0.0	0.0	0.0002	0.989
AVH	Age	1	18246	18546	3211.3567	<0.001
	Sample	1	0.4	0.4	0.0627	0.802
RVH	Age	1	19881.2	19881.2	3731.5436	<0.001
	Sample	1	28.7	28.7	5.3988	0.021
LVH	Age	1	19768		3322.9125	<0.001
	Sample	1	12.7	12.7	2.1376	0.144

## Clavicular variables

**Table D-50** Test of homogeneity of the clavicular variables obtained on the individuals from Marseilles and Lisbon. Greyed values indicate inhomogeneity of the variables between the samples

Variable	Factor of influence	Df	Sum of squares	Mean Square value	F value	p-value
Ln	Age	1	318858	318858	3668.555	<0.001
	Sample	1	4645	4645	53.439	<0.001
Max_diam	Age	1	1938.44	1938.44	1100.794	<0.001
	Sample	1	159.73	159.73	90.709	<0.001
Min_diam	Age	1	1437.45	1437.45	1200.05	<0.001
	Sample	1	212.77	212.77	177.63	<0.001
AP_diam	Age	1	1726.39	1726.39	899.47	<0.001
	Sample	1	428.17	428.17	223.08	<0.001
SI_diam	Age	1	1656.27	1656.27	1043.281	<0.001
	Sample	1	28.76	28.76	18.113	<0.001

## APPENDIX E

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### Variable transformations and Weighted Least Squares (WLS) models for age estimation

This appendix regroups all the Ordinary Least Squares models resulting from variable transformations (predicted and/or predictor variables) of the lumbar and clavicular variables and the Weighted Least Squares models built on the iliac, lumbar and clavicular variables attempted to obtain valid age prediction models.

The results of the transformations of the iliac variables and/or age can be found in chapter 6, section 6.1.1.a.

All models resulted in heteroscedastic, autocorrelated and/or non-normally distributed residuals.

## Variable transformations

### Lumbar variables

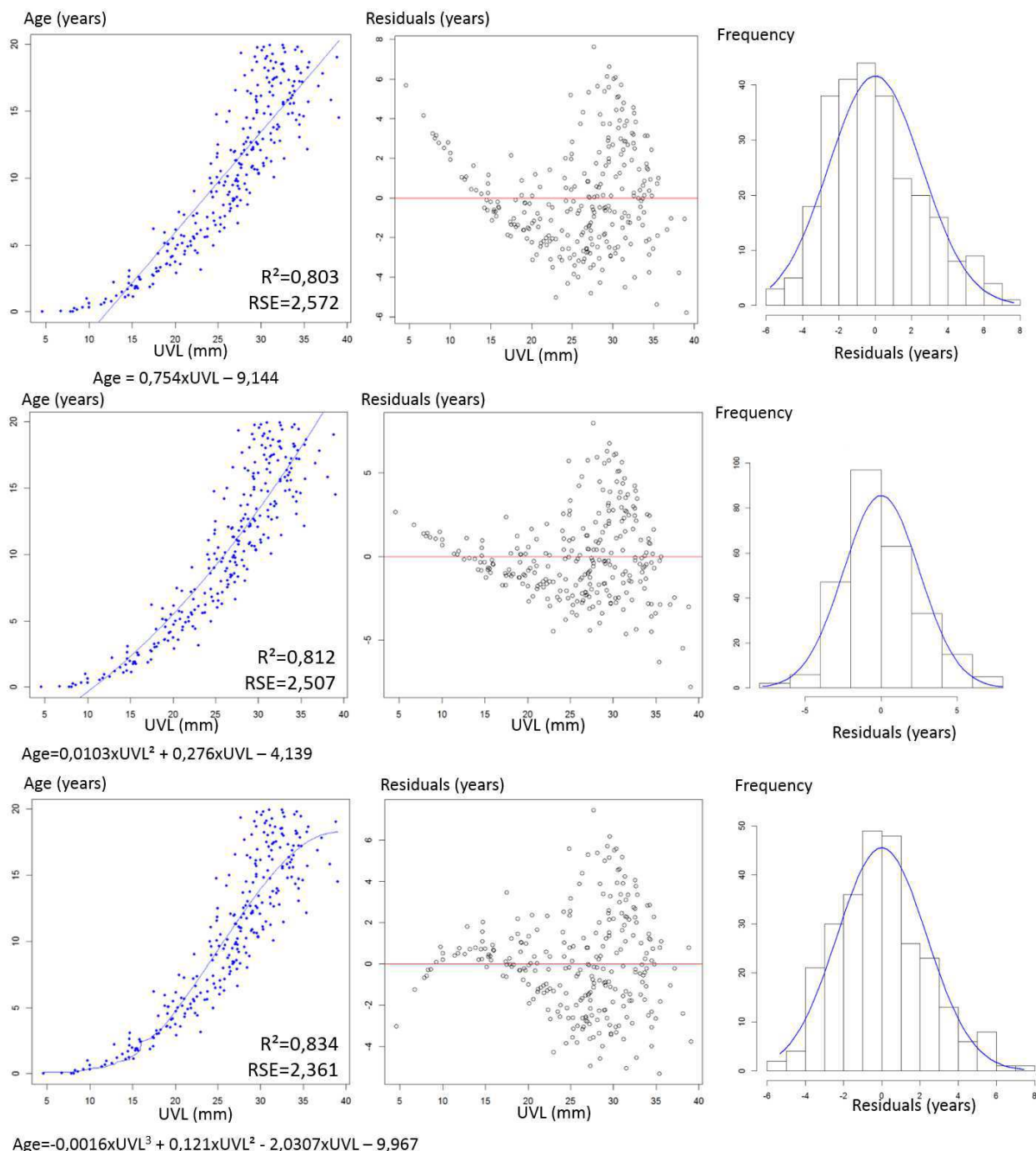


Figure E.1 Age prediction using linear, second and third degree polynomial regressions of UVL with the mathematical expressions, associated  $R^2$  and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients

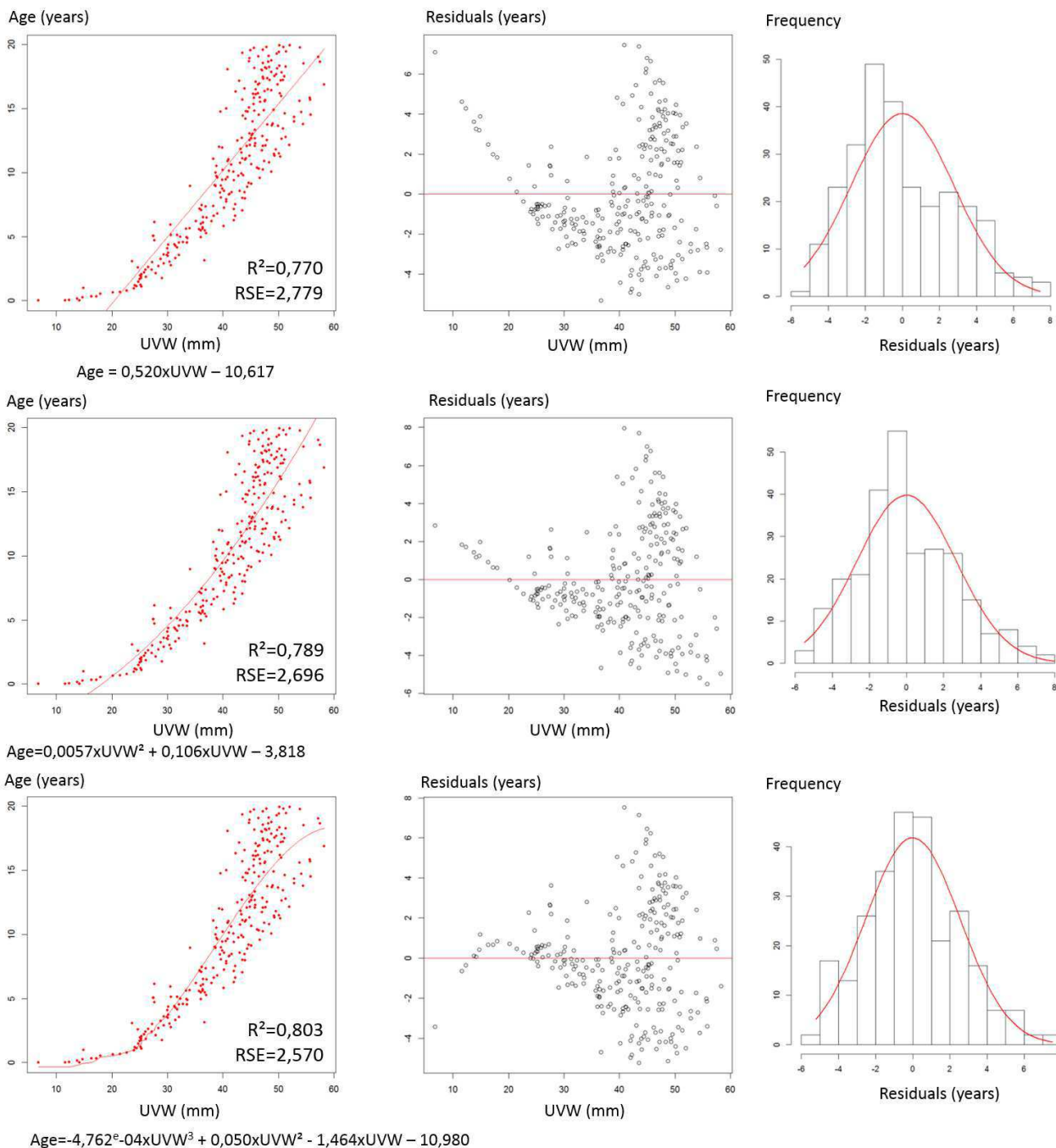


Figure E.2 Age prediction using linear, second and third degree polynomial regressions of UVW with the mathematical expressions, associated  $R^2$  and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients

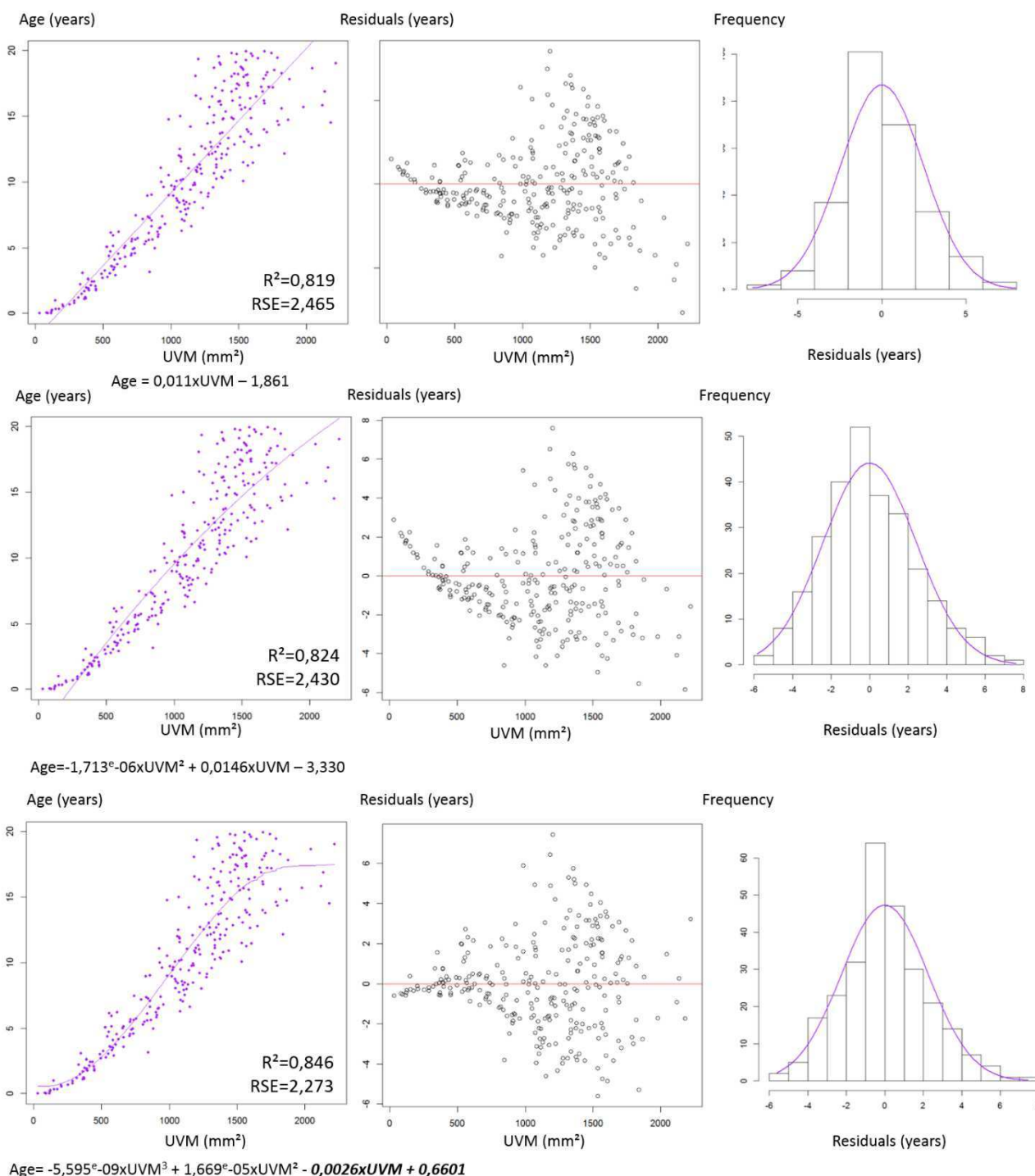


Figure E.3 Age prediction using linear, second and third degree polynomial regressions of UVM with the mathematical expressions, associated R<sup>2</sup> and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients

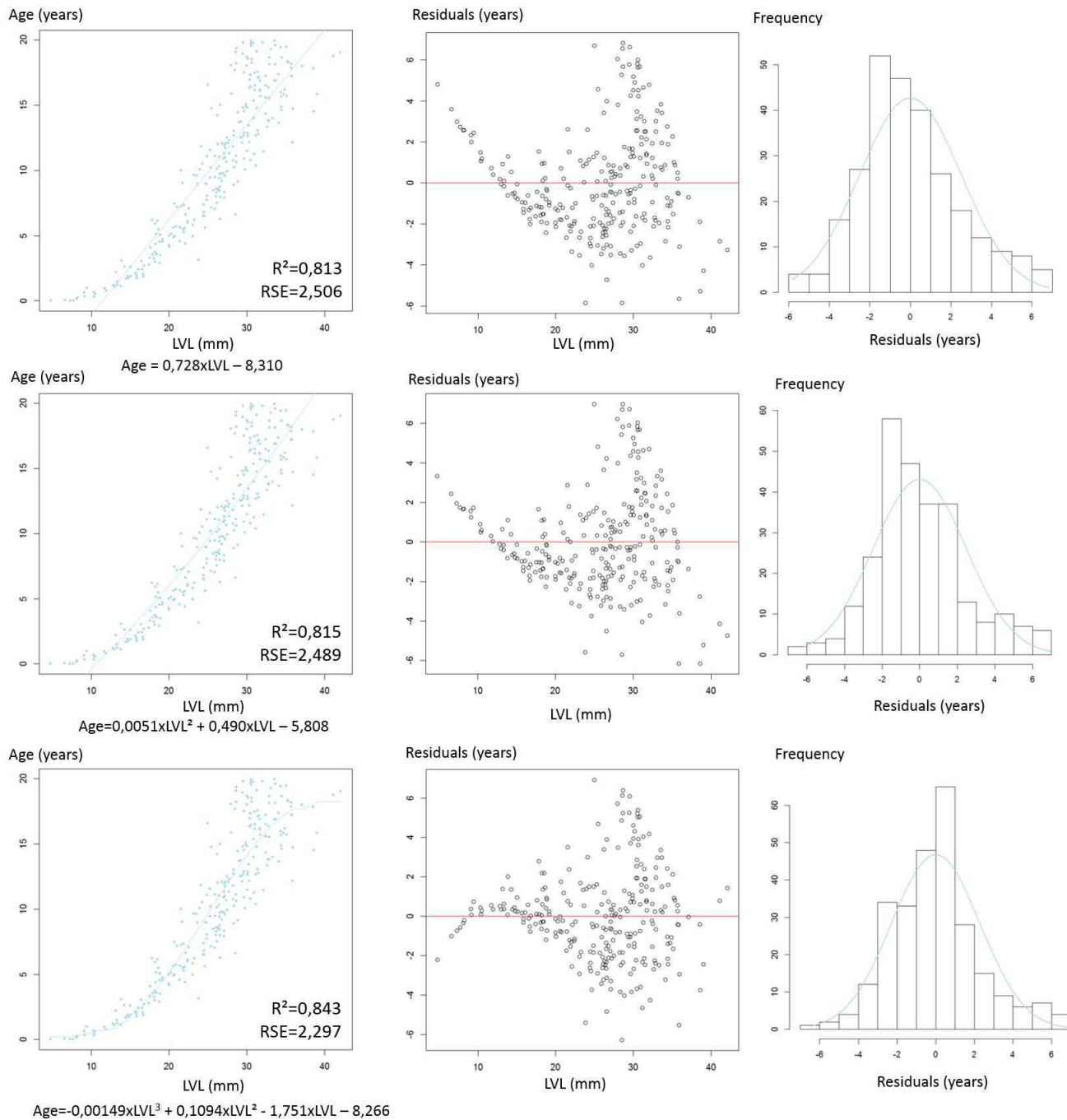


Figure E.4 Age prediction using linear, second and third degree polynomial regressions of LVL with the mathematical expressions, associated  $R^2$  and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients

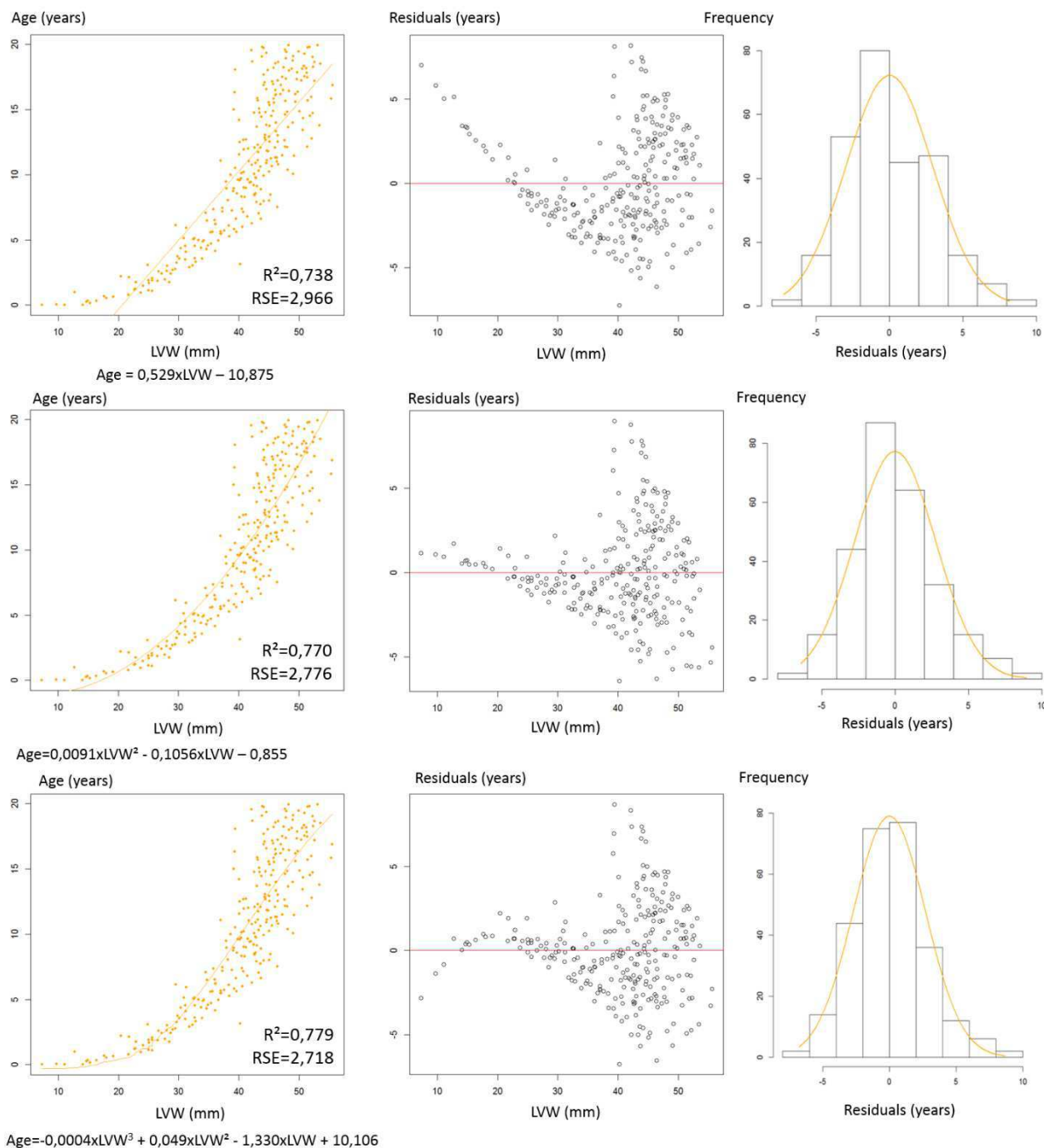


Figure E.5 Age prediction using linear, second and third degree polynomial regressions of LVW with the mathematical expressions, associated  $R^2$  and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients



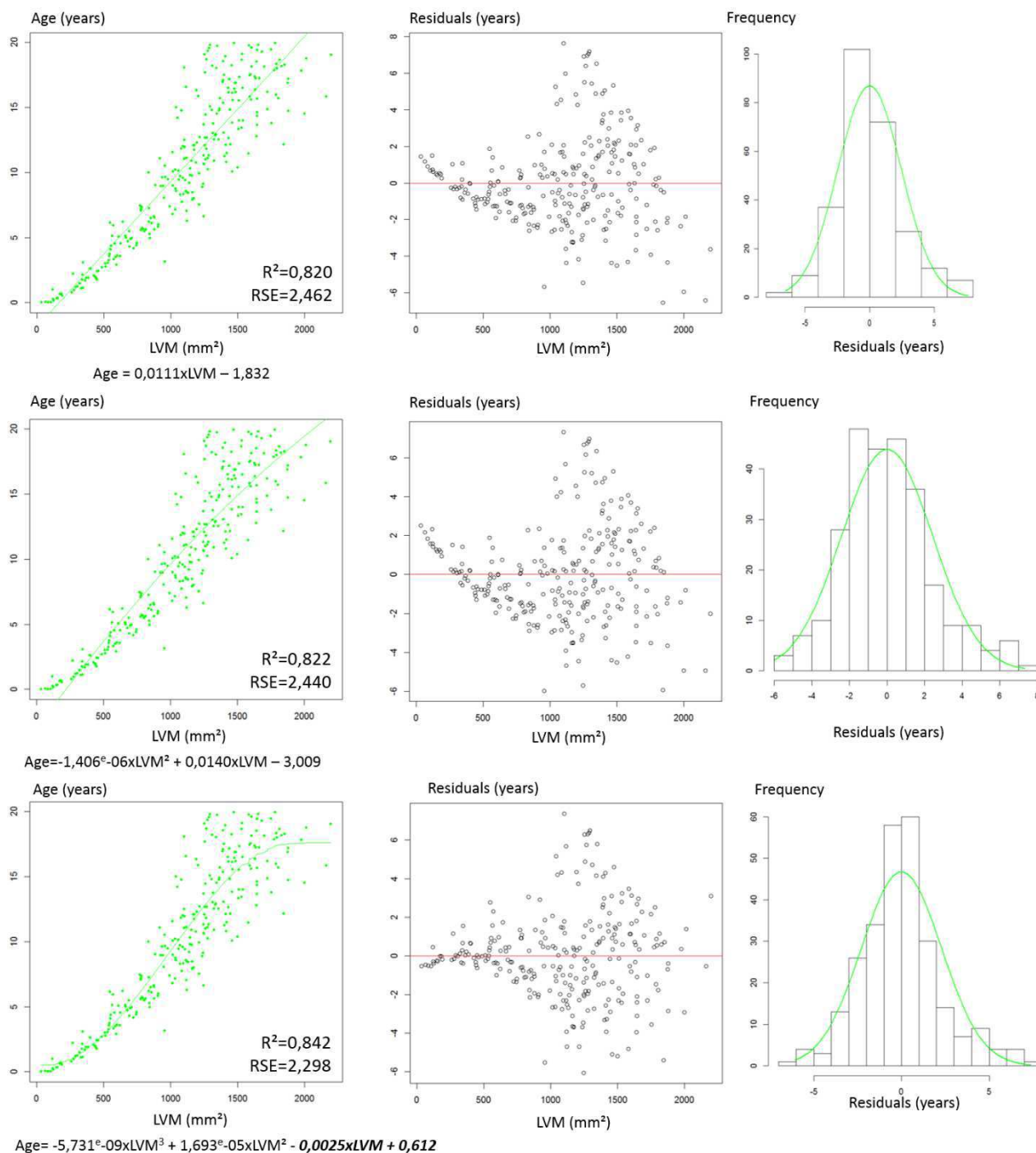


Figure E.6 Age prediction using linear, second and third degree polynomial regressions of LVM with the mathematical expressions, associated  $R^2$  and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients

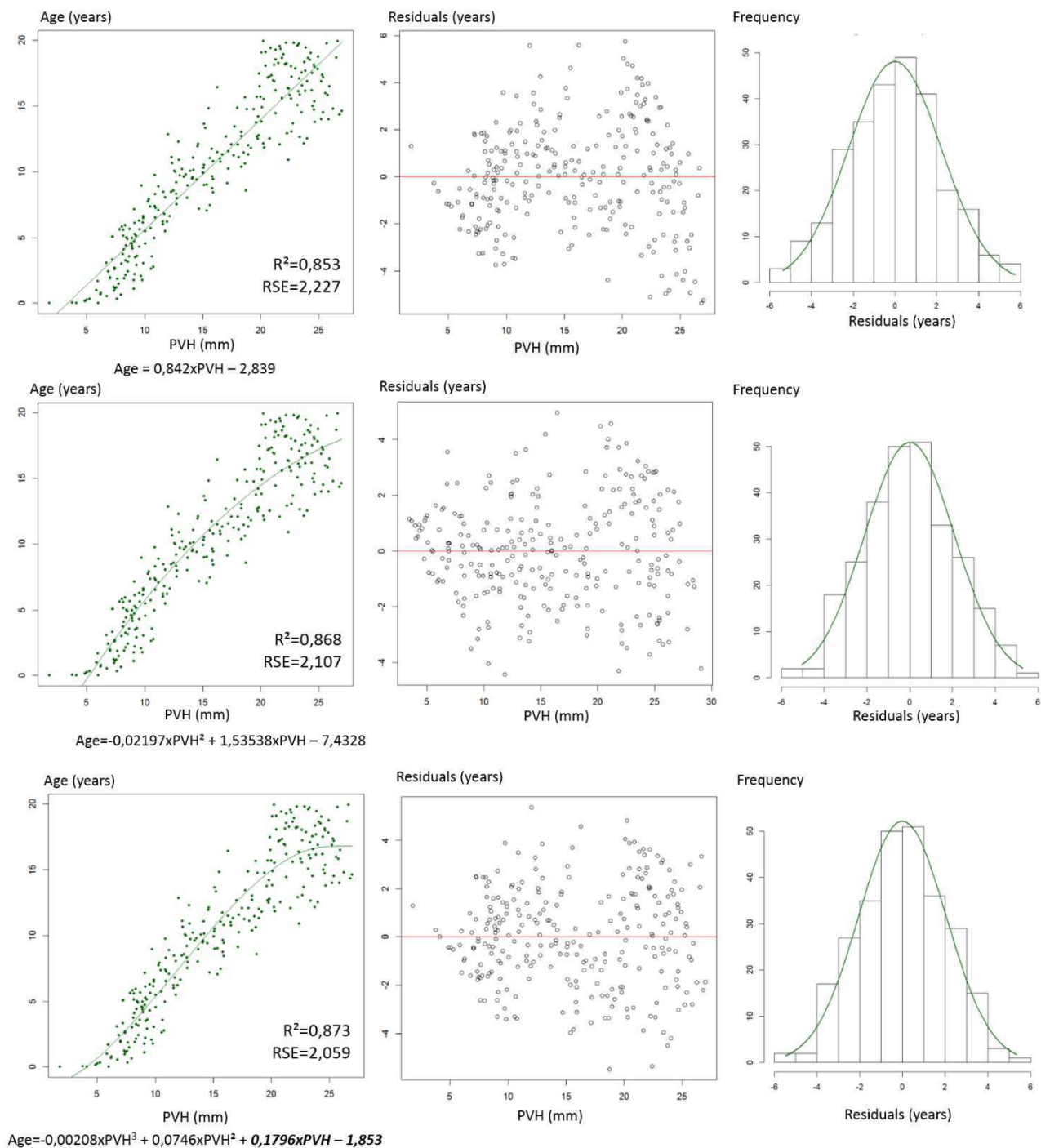


Figure E.7 Age prediction using linear, second and third degree polynomial regressions of PVH with the mathematical expressions, associated  $R^2$  and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients

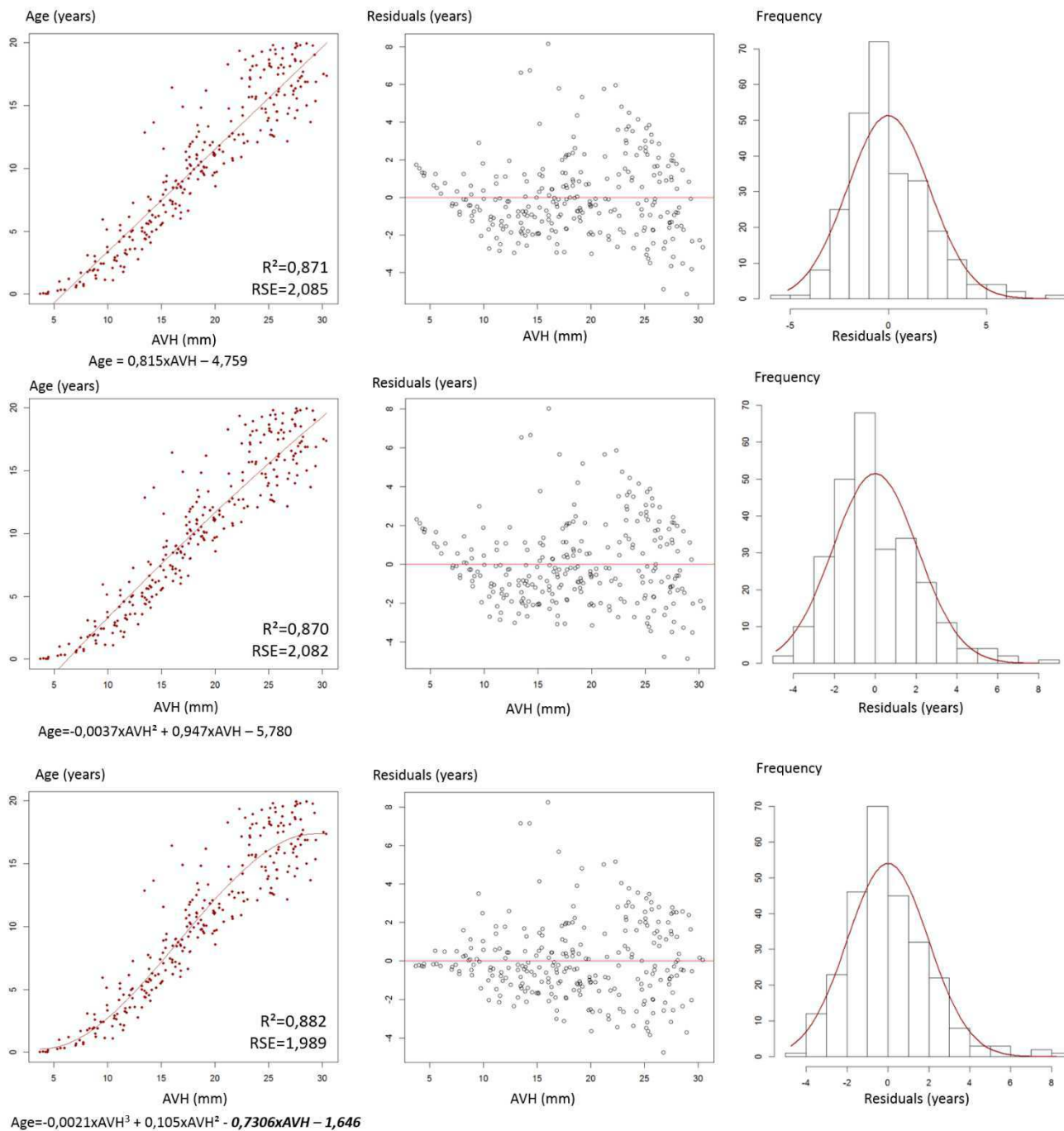


Figure E.8 Age prediction using linear, second and third degree polynomial regressions of AVH with the mathematical expressions, associated R<sup>2</sup> and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients

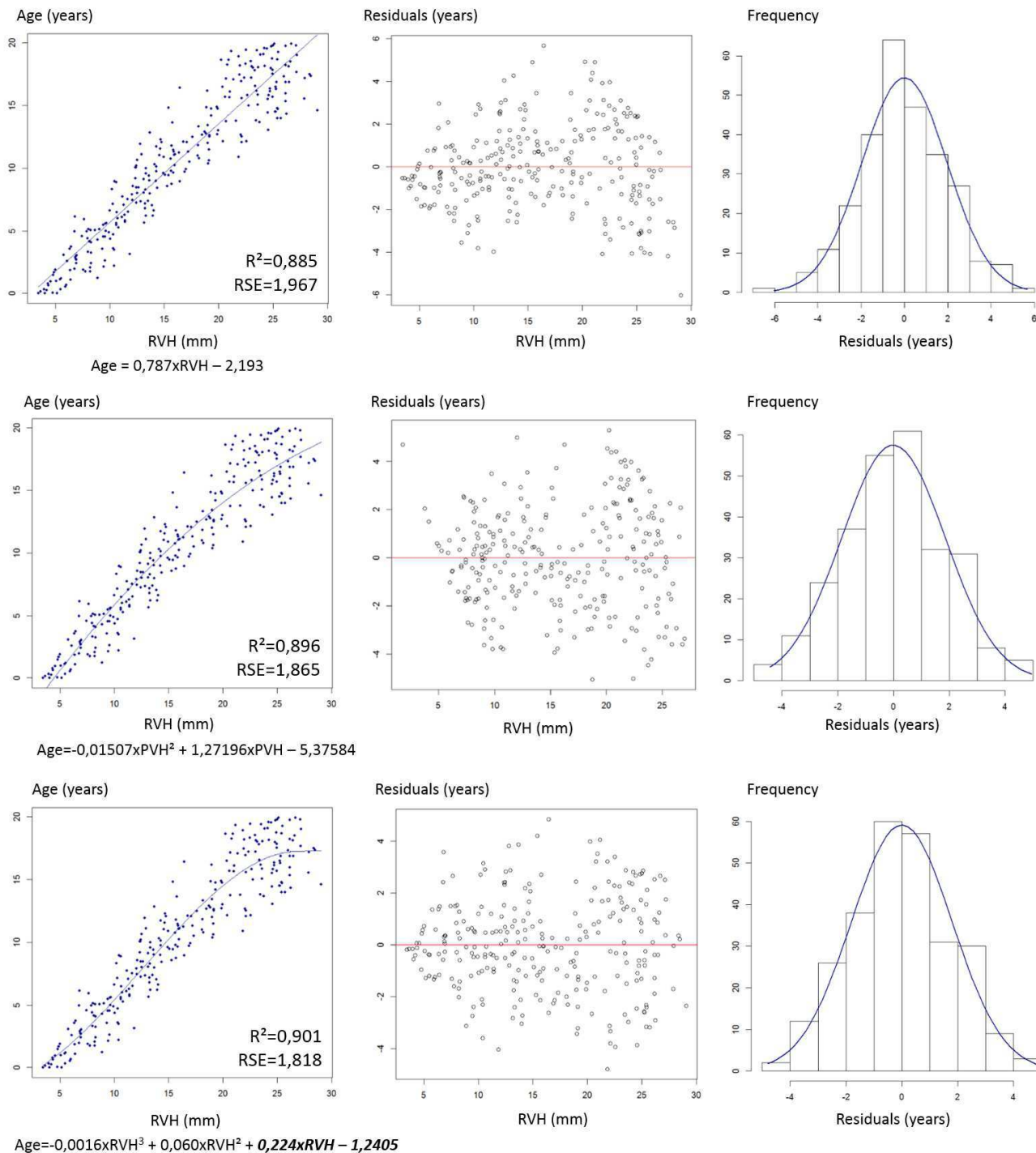


Figure E.9 Age prediction using linear, second and third degree polynomial regressions of RVH with the mathematical expressions, associated  $R^2$  and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients

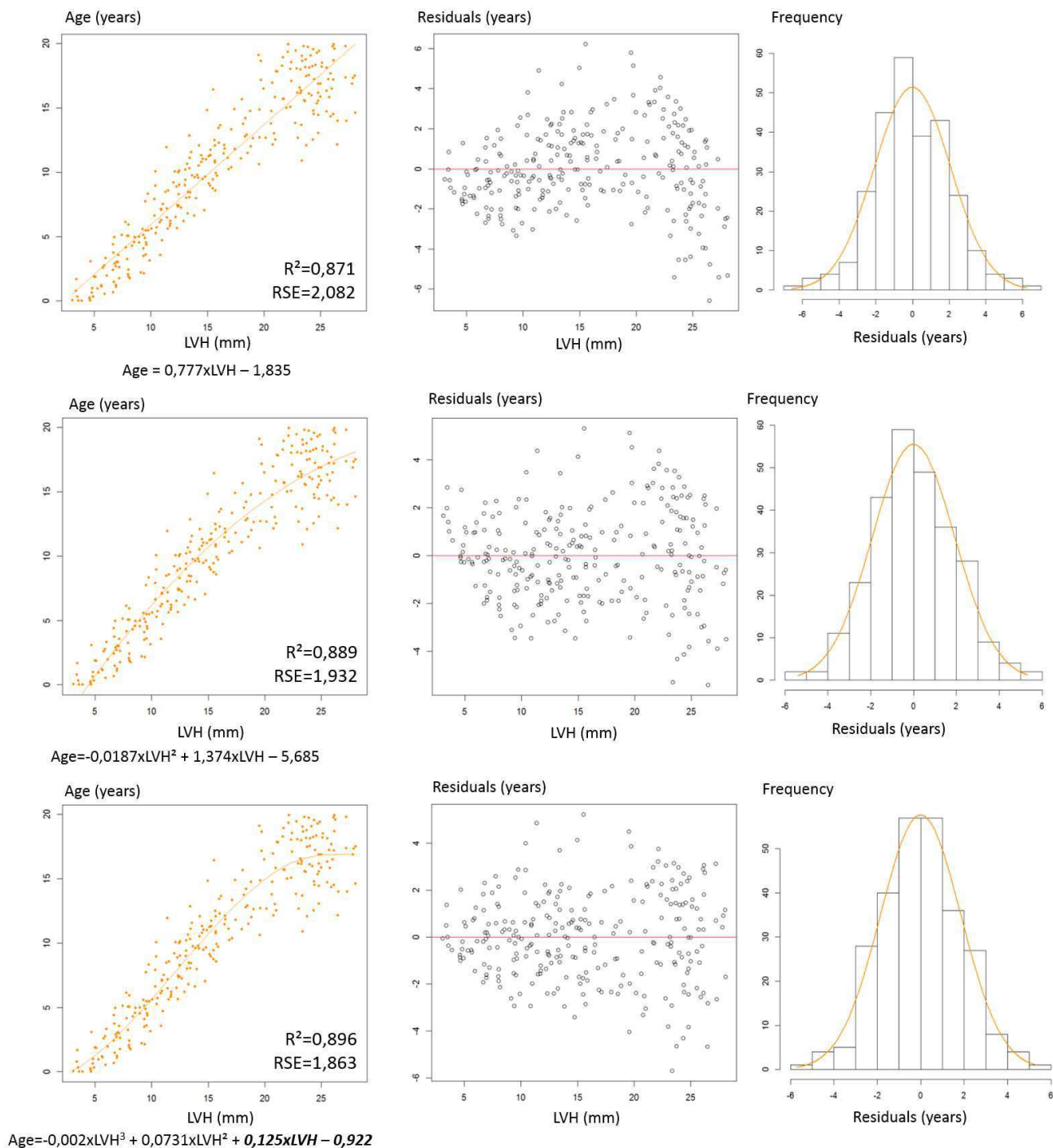


Figure E.10 Age prediction using linear, second and third degree polynomial regressions of LVH with the mathematical expressions, associated  $R^2$  and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients

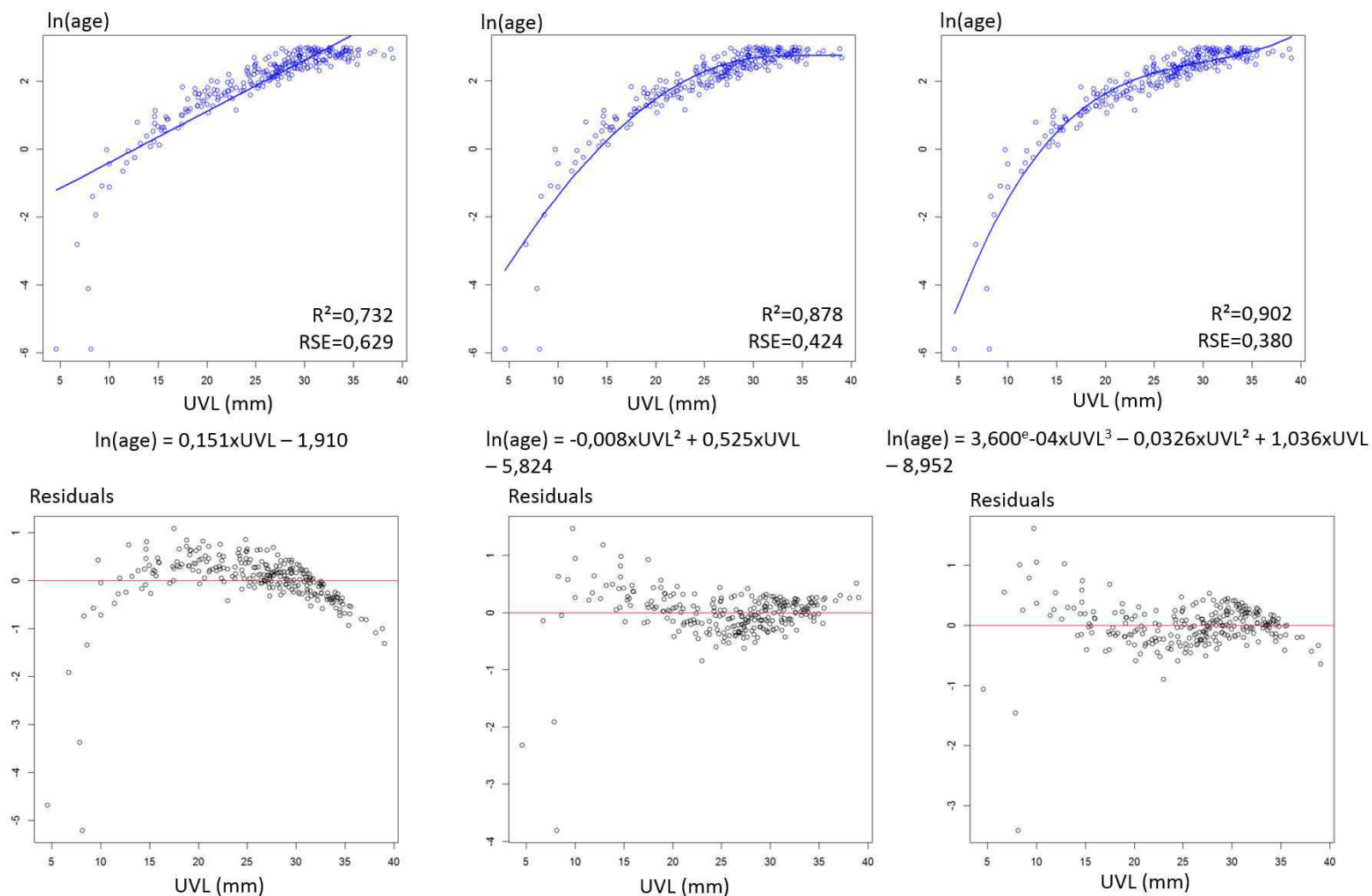


Figure E.11 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against UVL, with the mathematical expressions, associated  $R^2$  and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

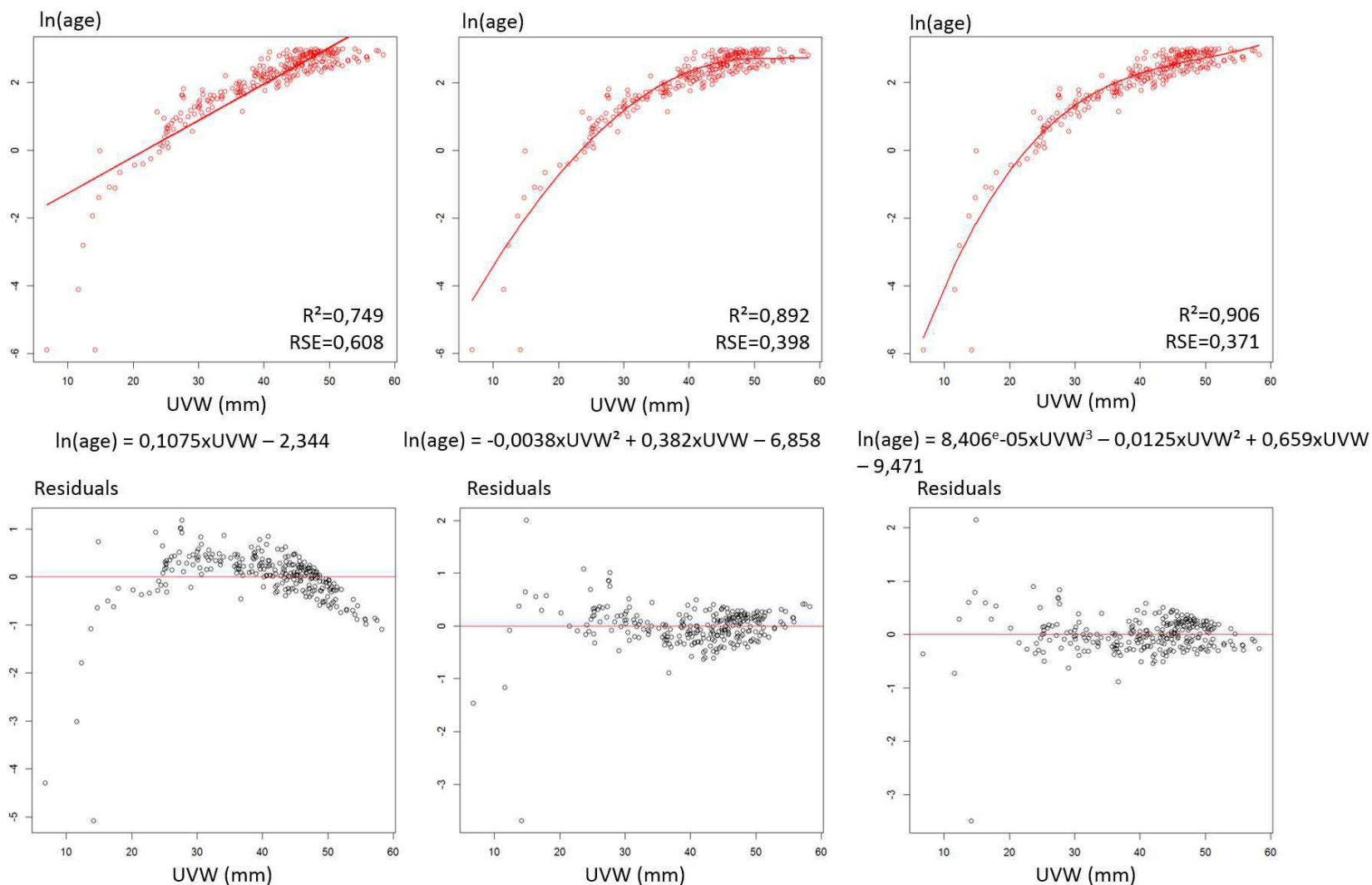


Figure E.12 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against  $\text{UVW}$ , with the mathematical expressions, associated  $R^2$  and  $\text{RSE}$  values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

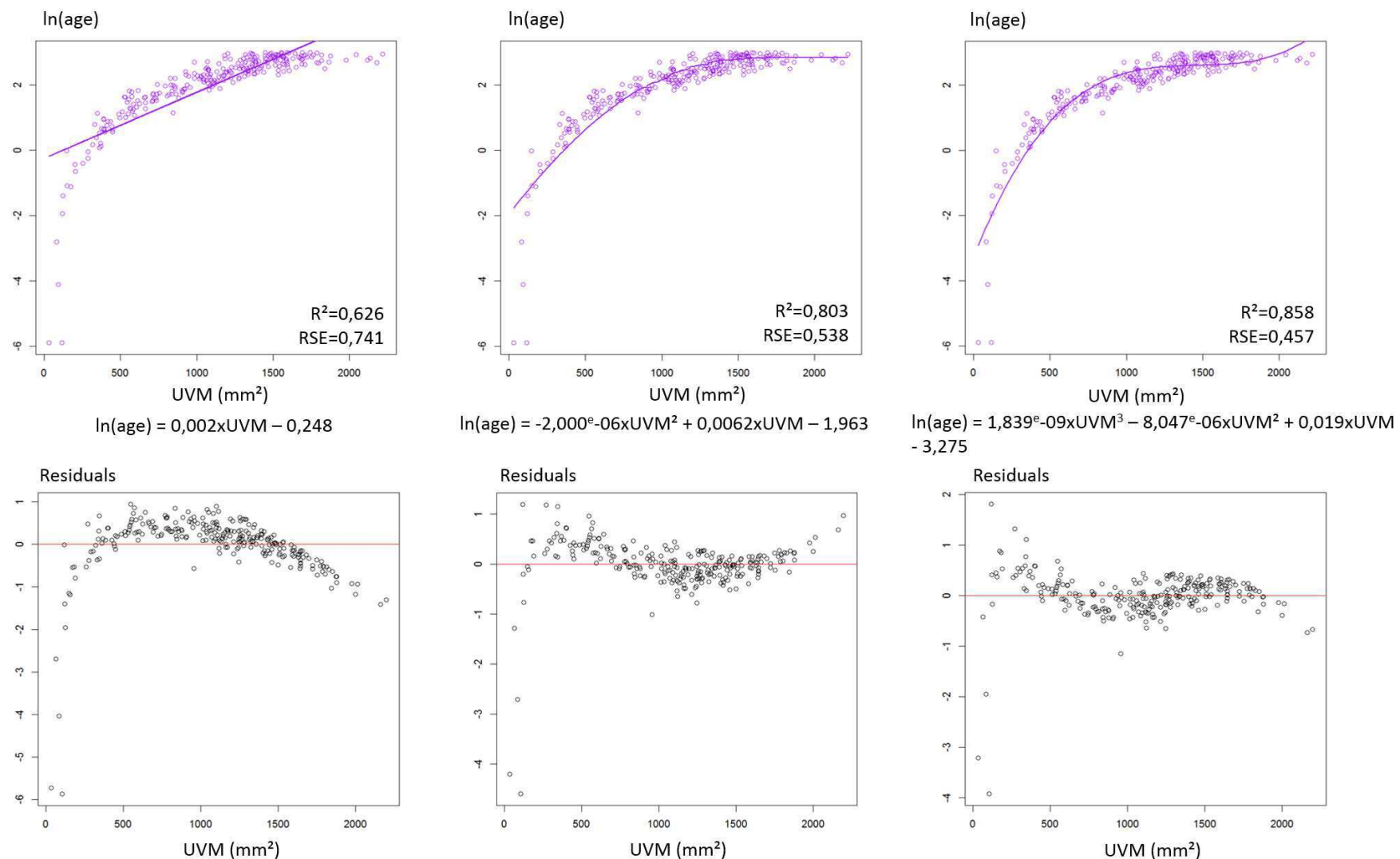


Figure E.13 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against  $\text{UVM}$ , with the mathematical expressions, associated  $R^2$  and  $RSE$  values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients



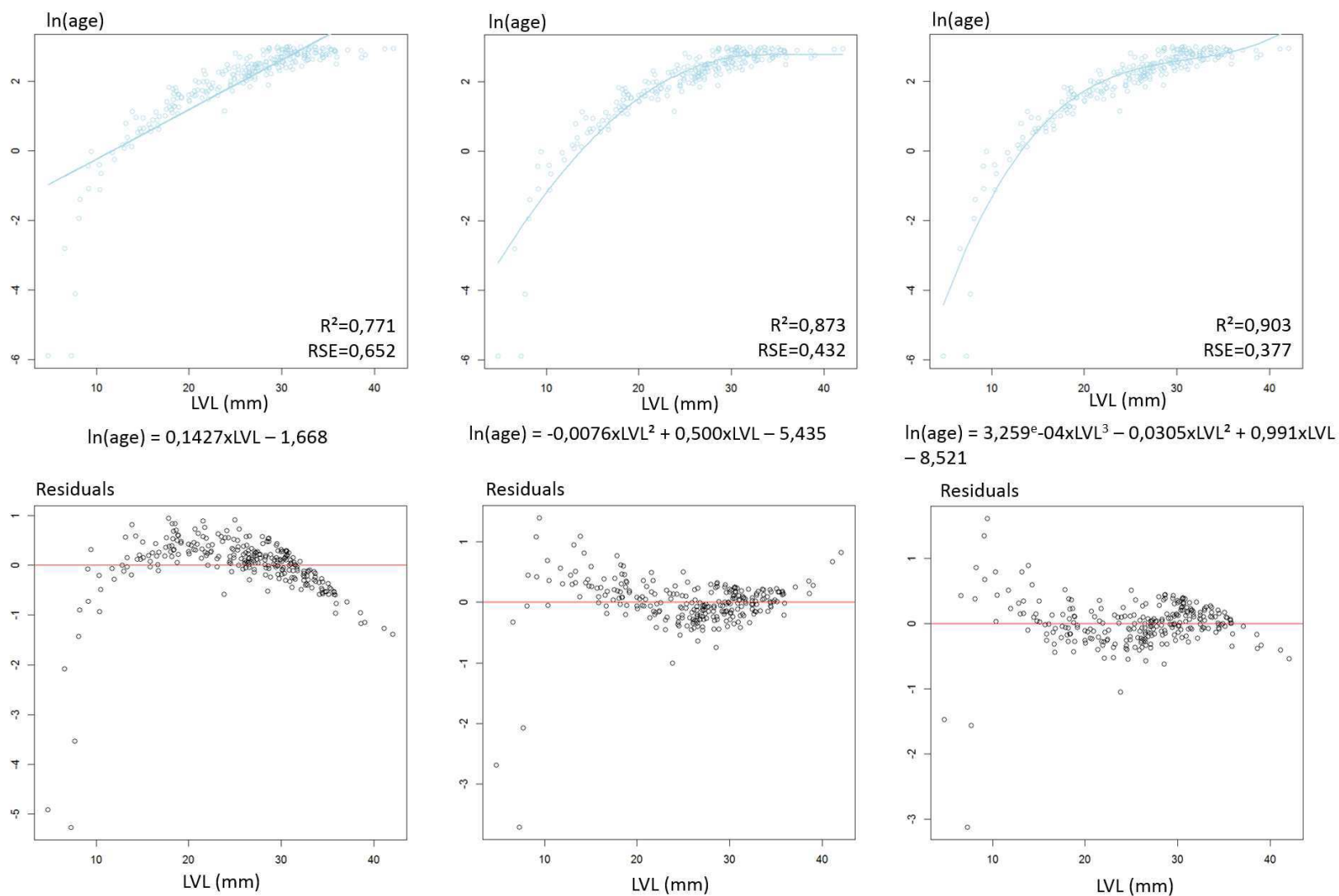


Figure E.14 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against LVL, with the mathematical expressions, associated  $R^2$  and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

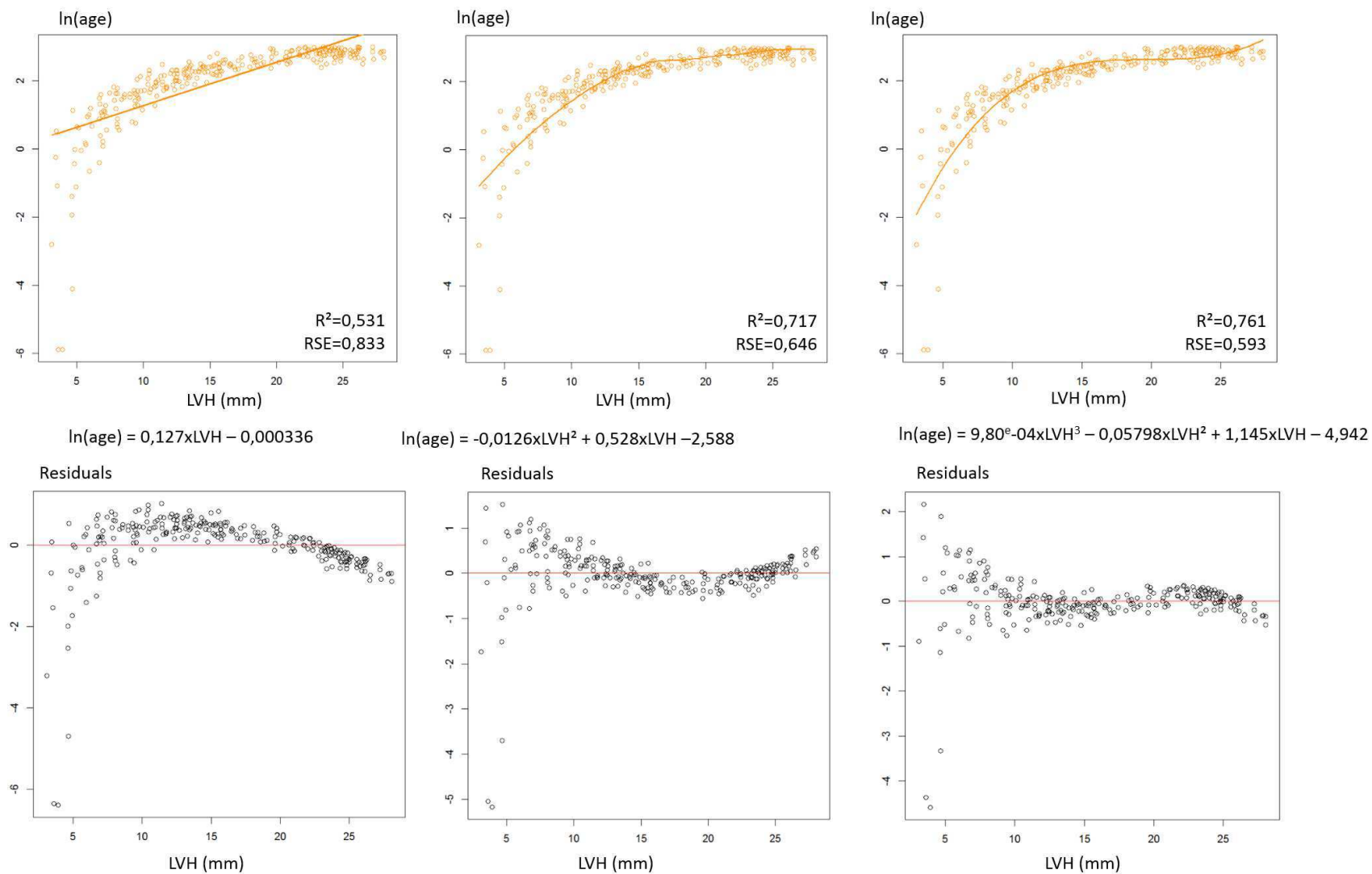


Figure E.15 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against LVW, with the mathematical expressions, associated  $R^2$  and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

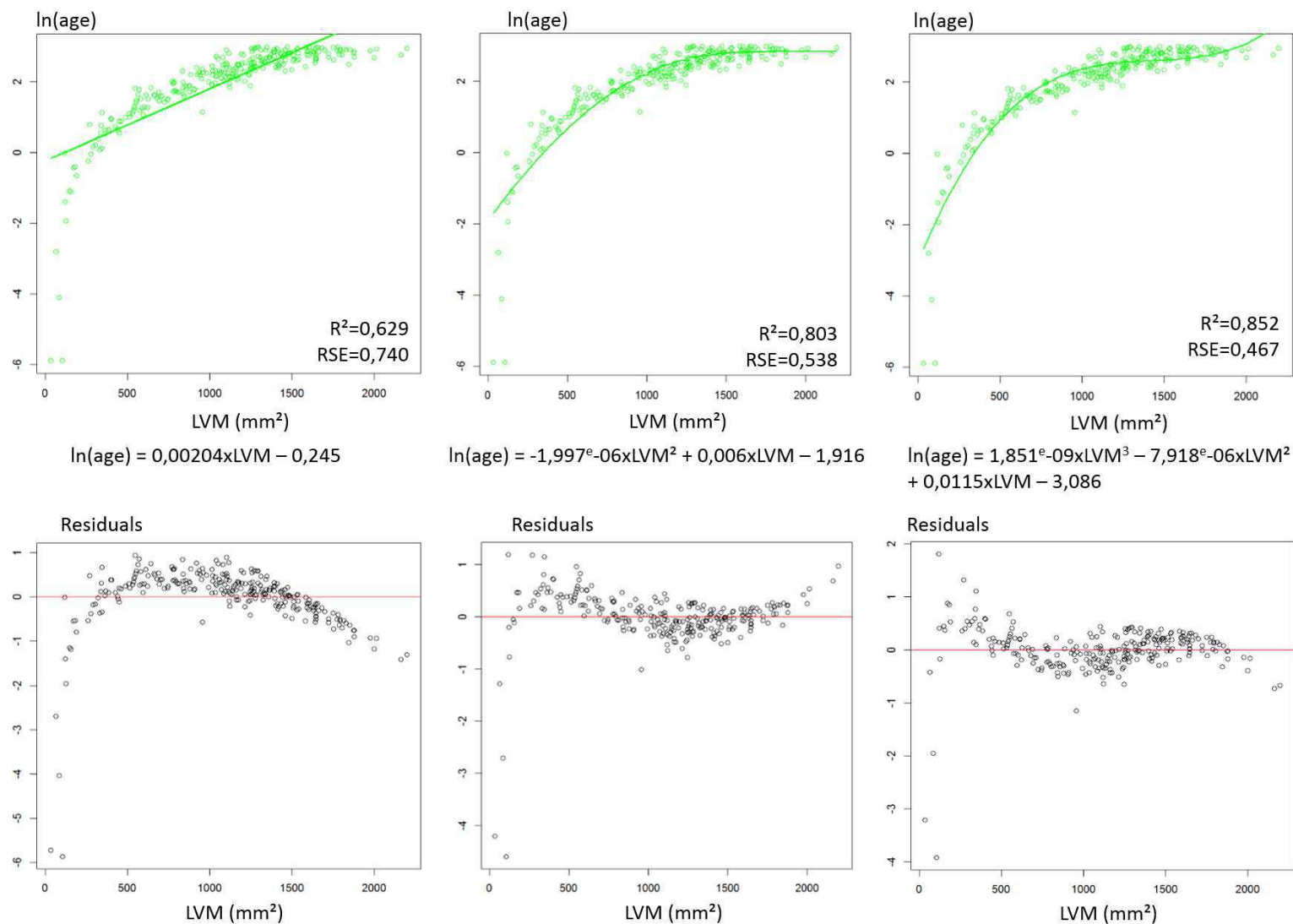


Figure E.16 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against  $\text{LVM}$ , with the mathematical expressions, associated  $R^2$  and  $RSE$  values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

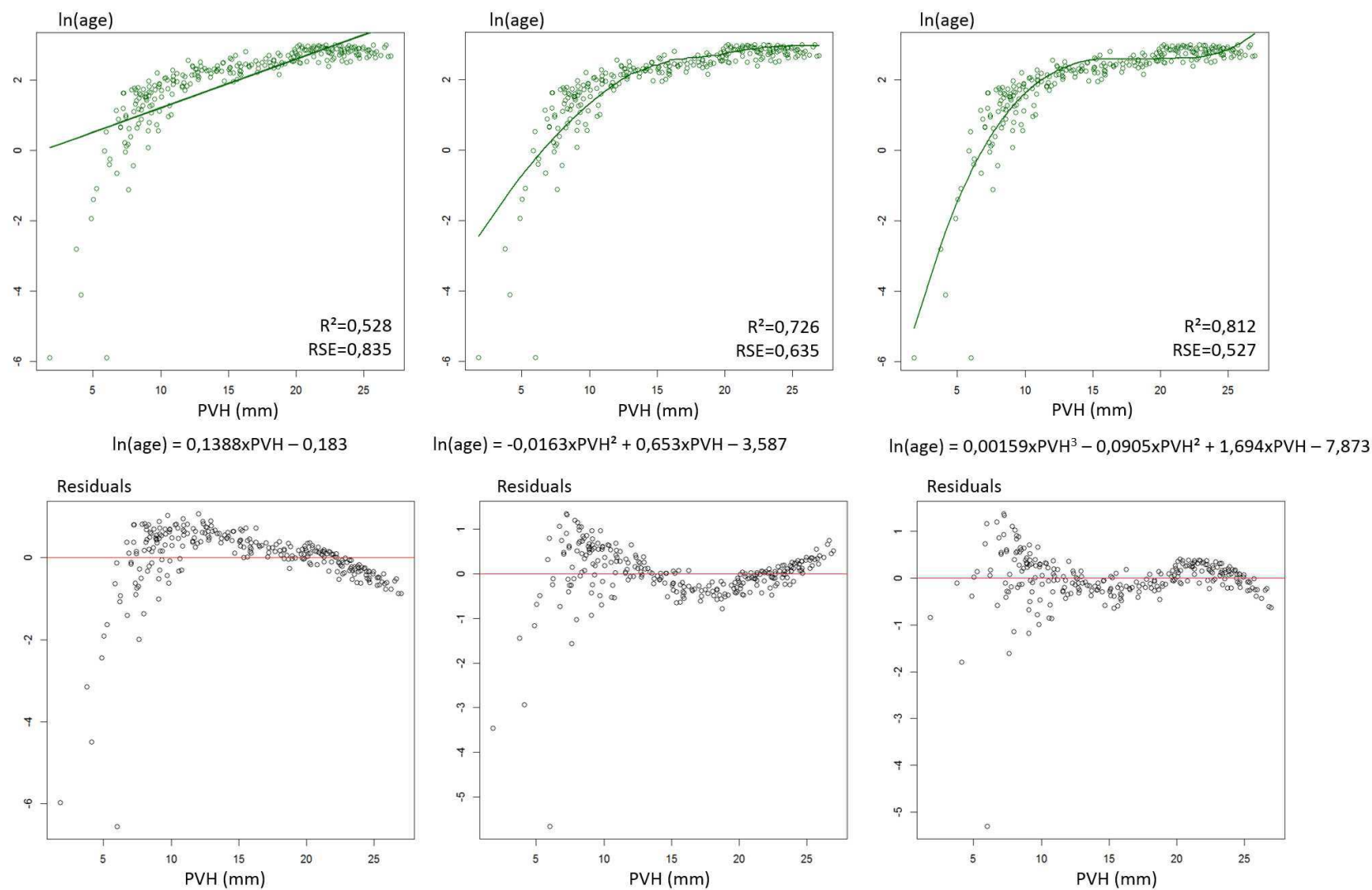


Figure E.17 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against  $\text{PVH}$ , with the mathematical expressions, associated  $R^2$  and  $RSE$  values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

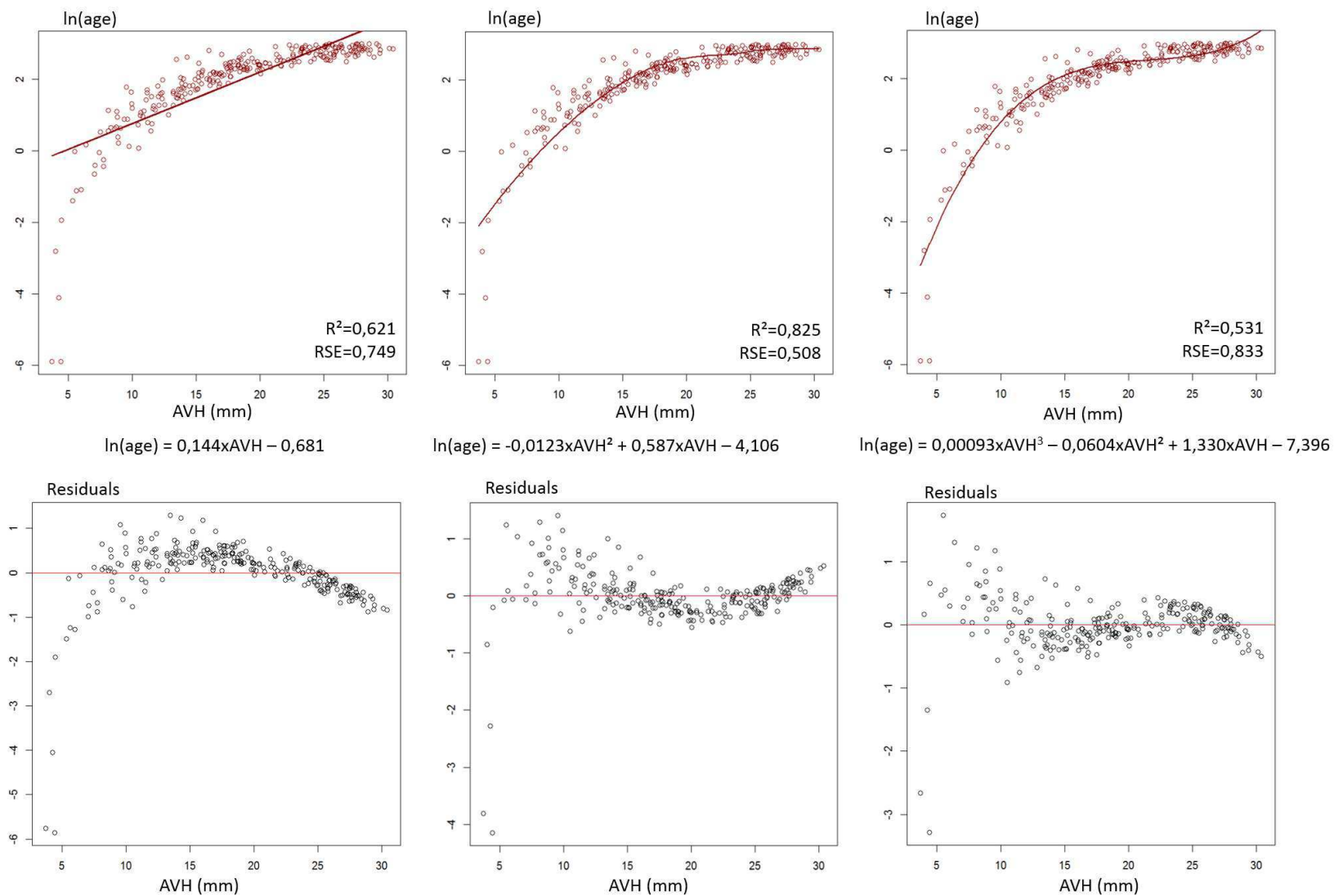


Figure E.18 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against AVH, with the mathematical expressions, associated  $R^2$  and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

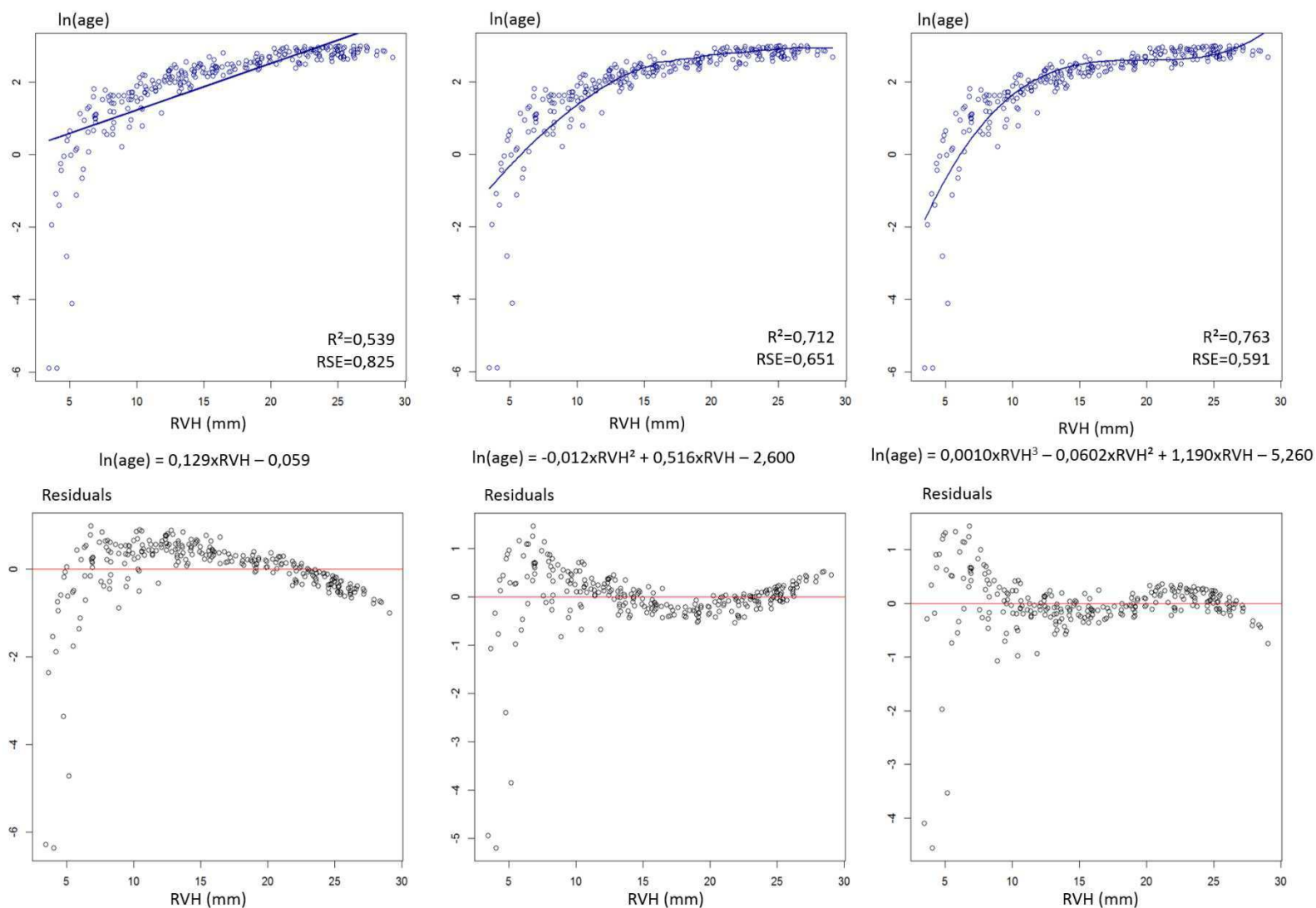


Figure E.19 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against  $\text{RVH}$ , with the mathematical expressions, associated  $R^2$  and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

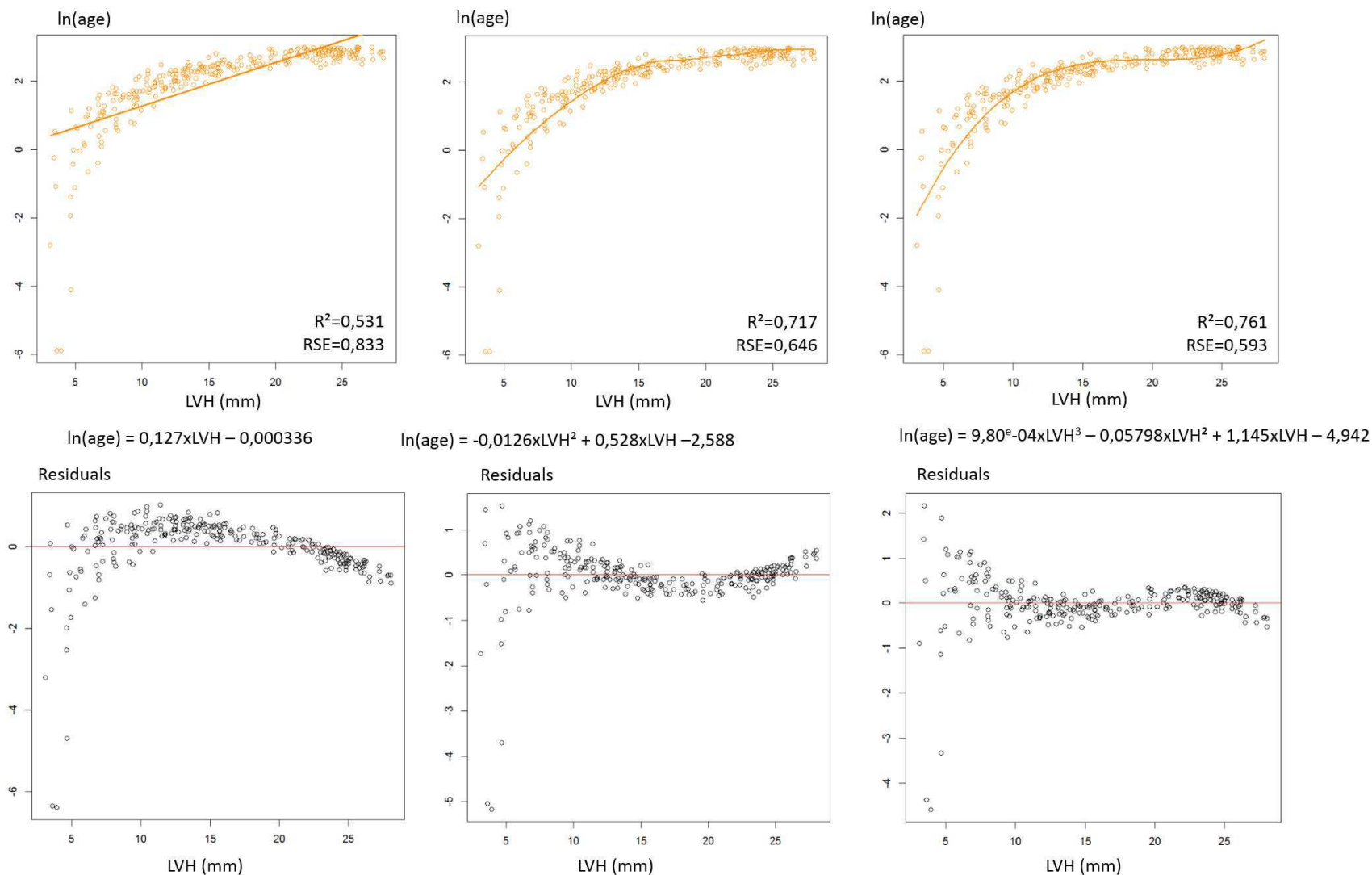


Figure E.20 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against LVH, with the mathematical expressions, associated  $R^2$  and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

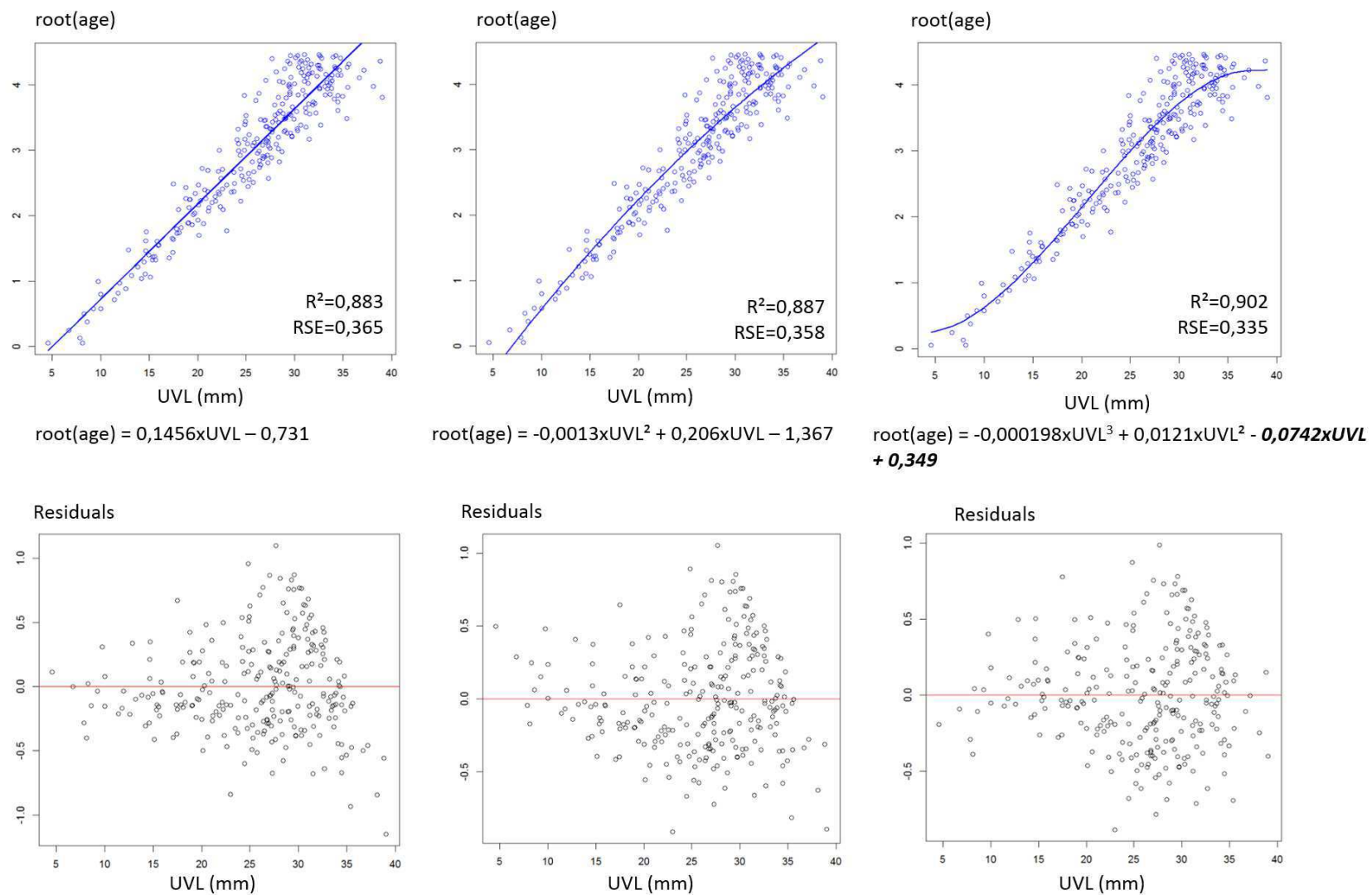


Figure E.21 Age prediction using linear, second and third degree polynomial models of root(age) against UVL, with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plot of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients



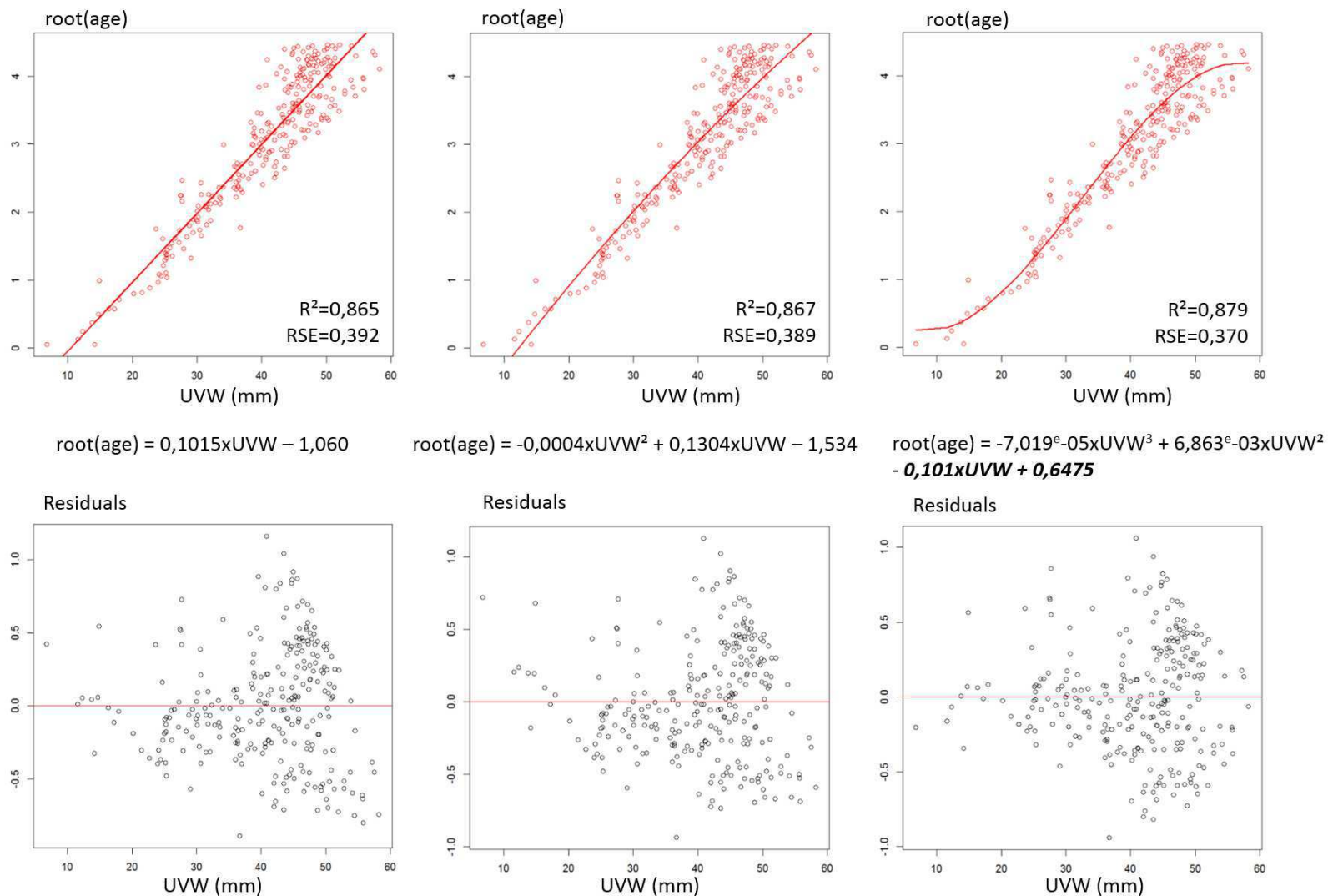


Figure E.22 Age prediction using linear, second and third degree polynomial models of root(age) against UVW, with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plot of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

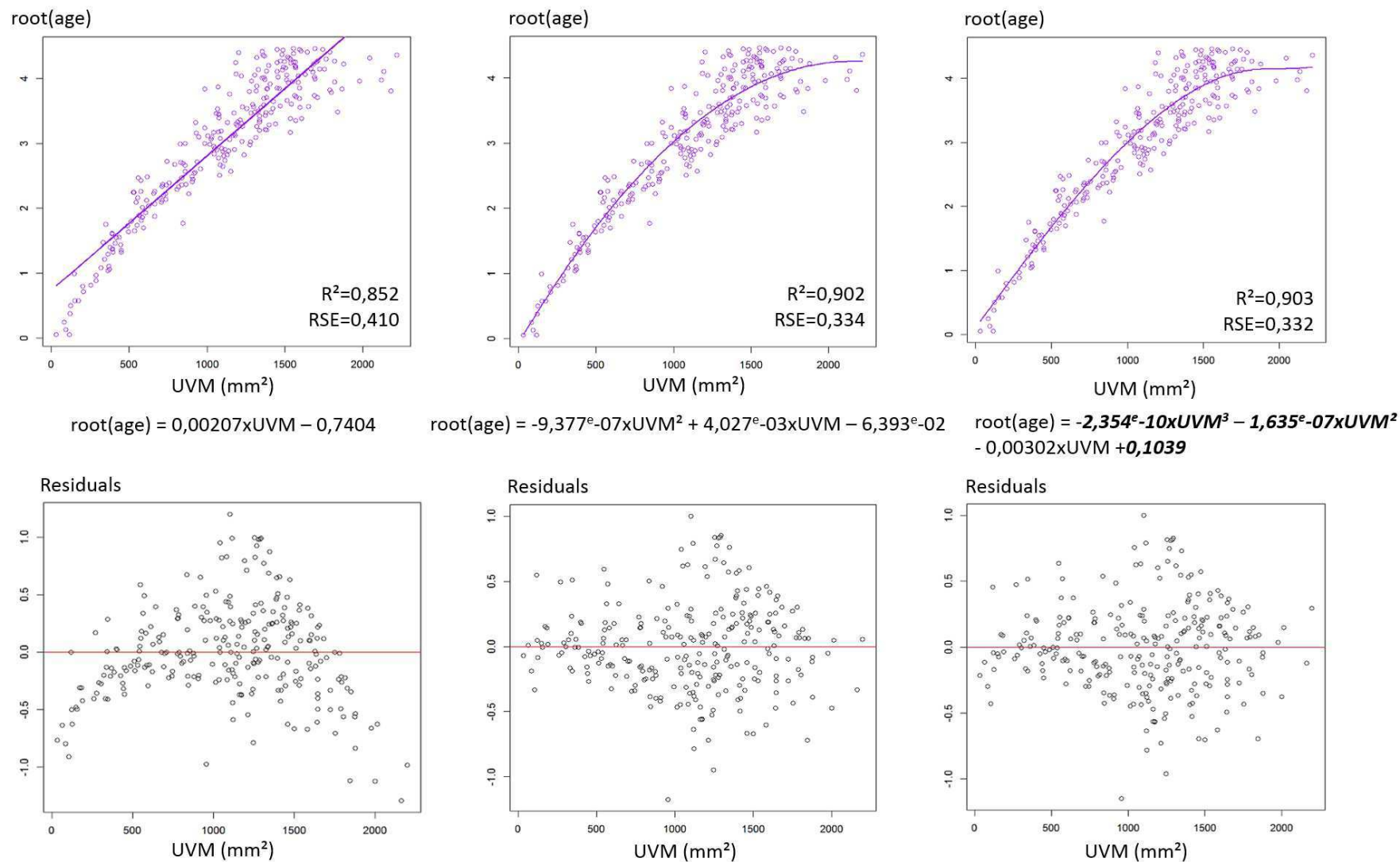


Figure E.23 Age prediction using linear, second and third degree polynomial models of root(age) against UVM, with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

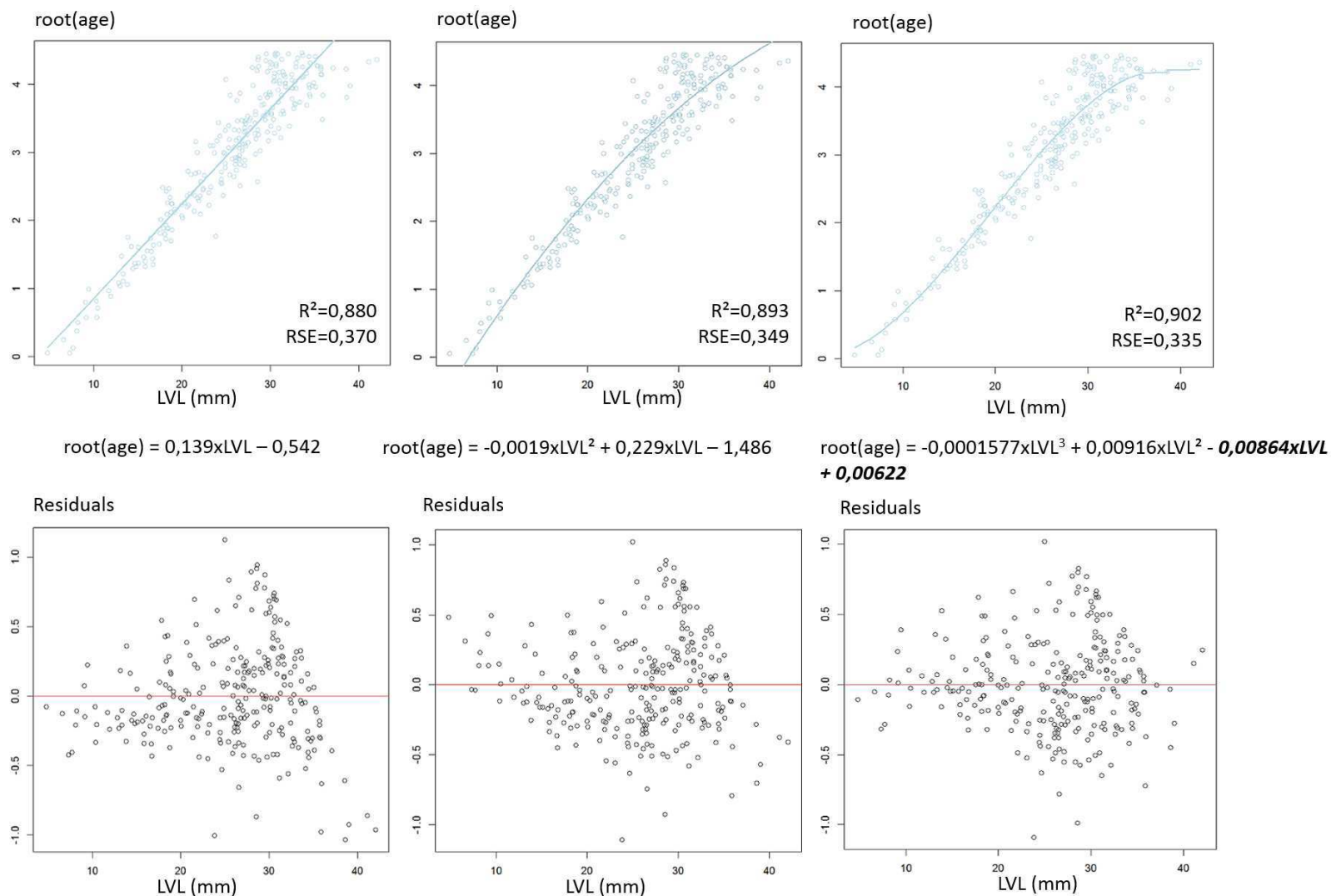


Figure E.24 Age prediction using linear, second and third degree polynomial models of root(age) against LVL, with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plot of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

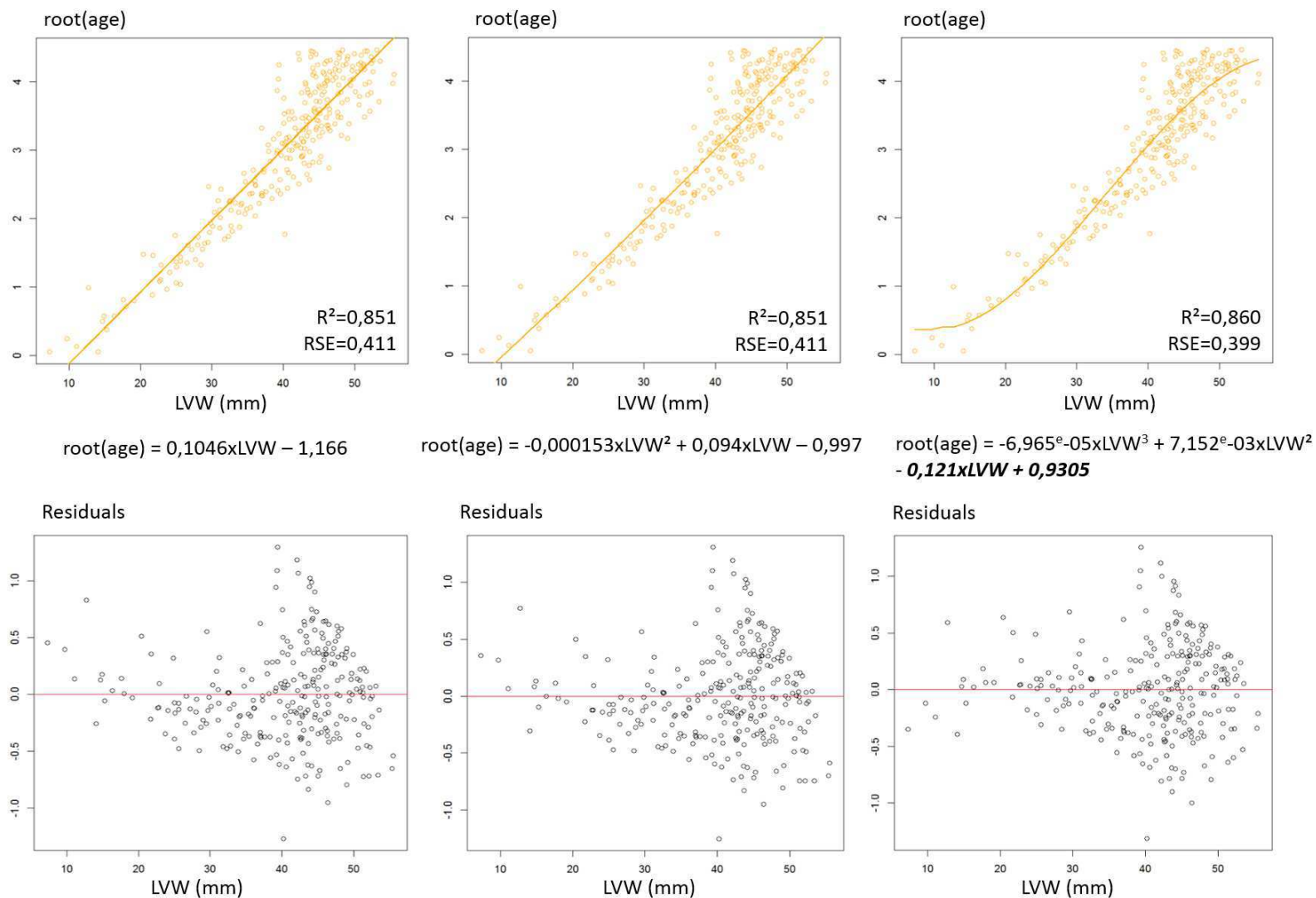


Figure E.25 Age prediction using linear, second and third degree polynomial models of root(age) against LVW, with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

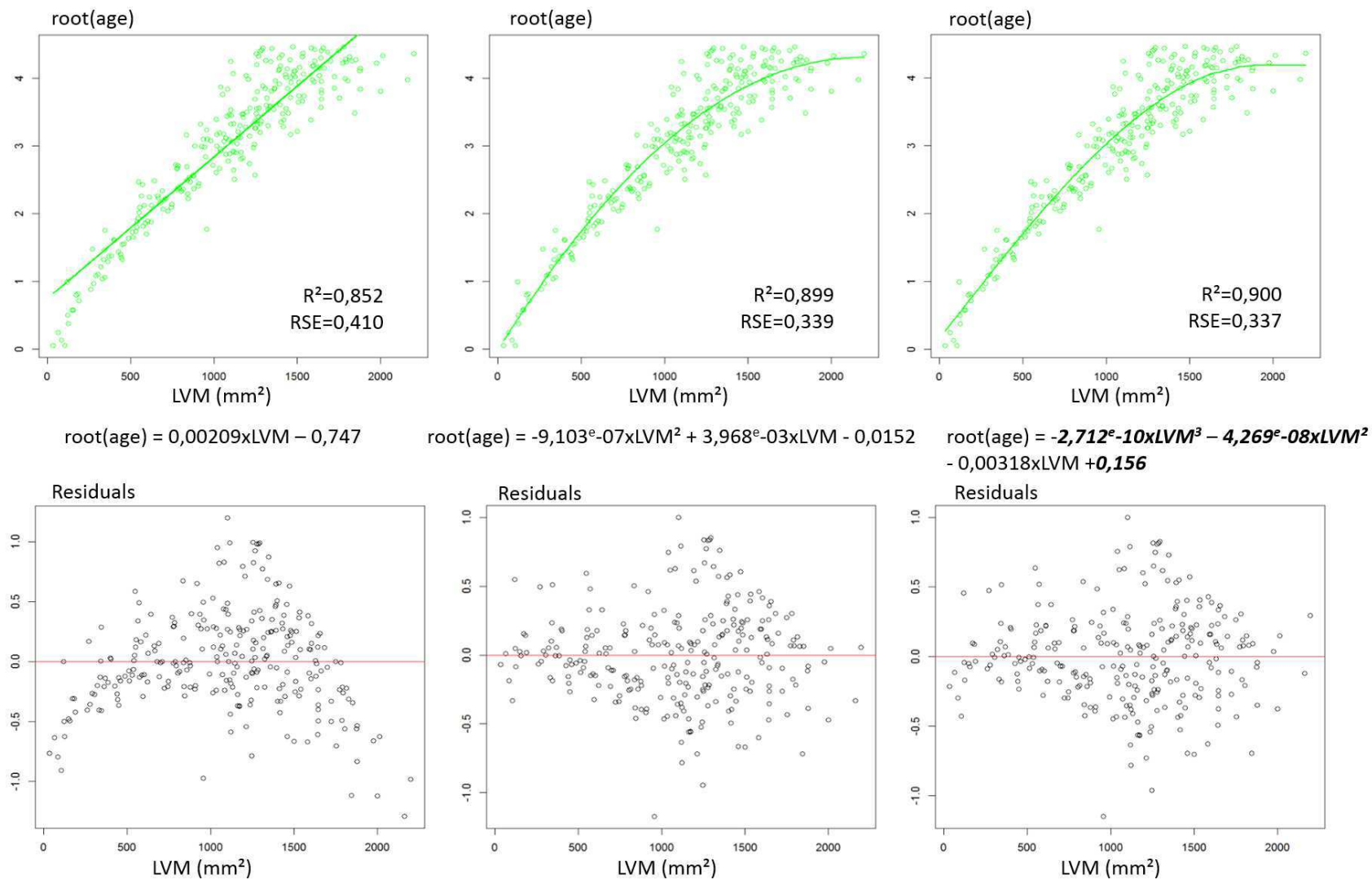


Figure E.26 Age prediction using linear, second and third degree polynomial models of root(age) against LVM, with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plot of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

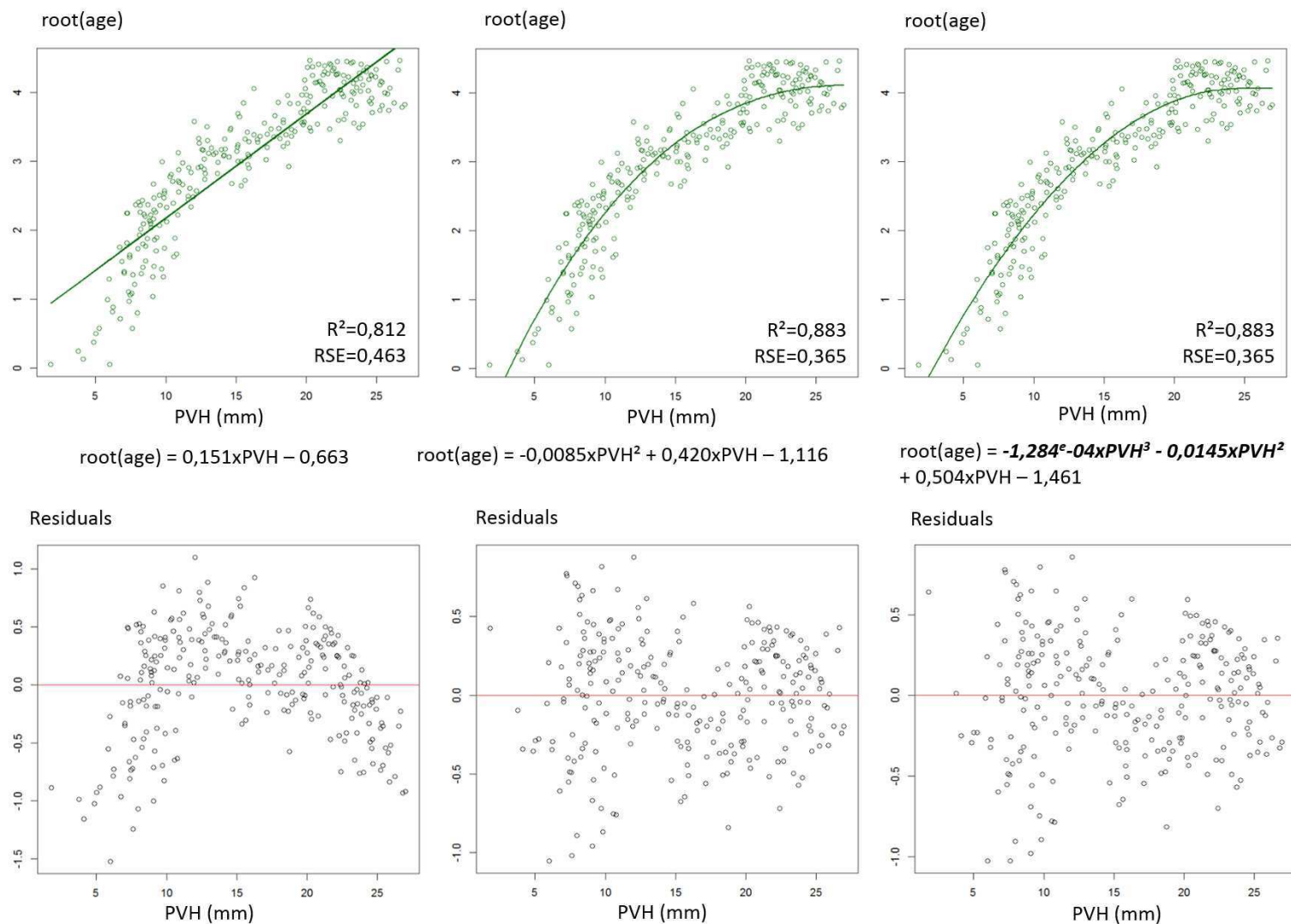


Figure E.27 Age prediction using linear, second and third degree polynomial models of root(age) against PVH, with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plot of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

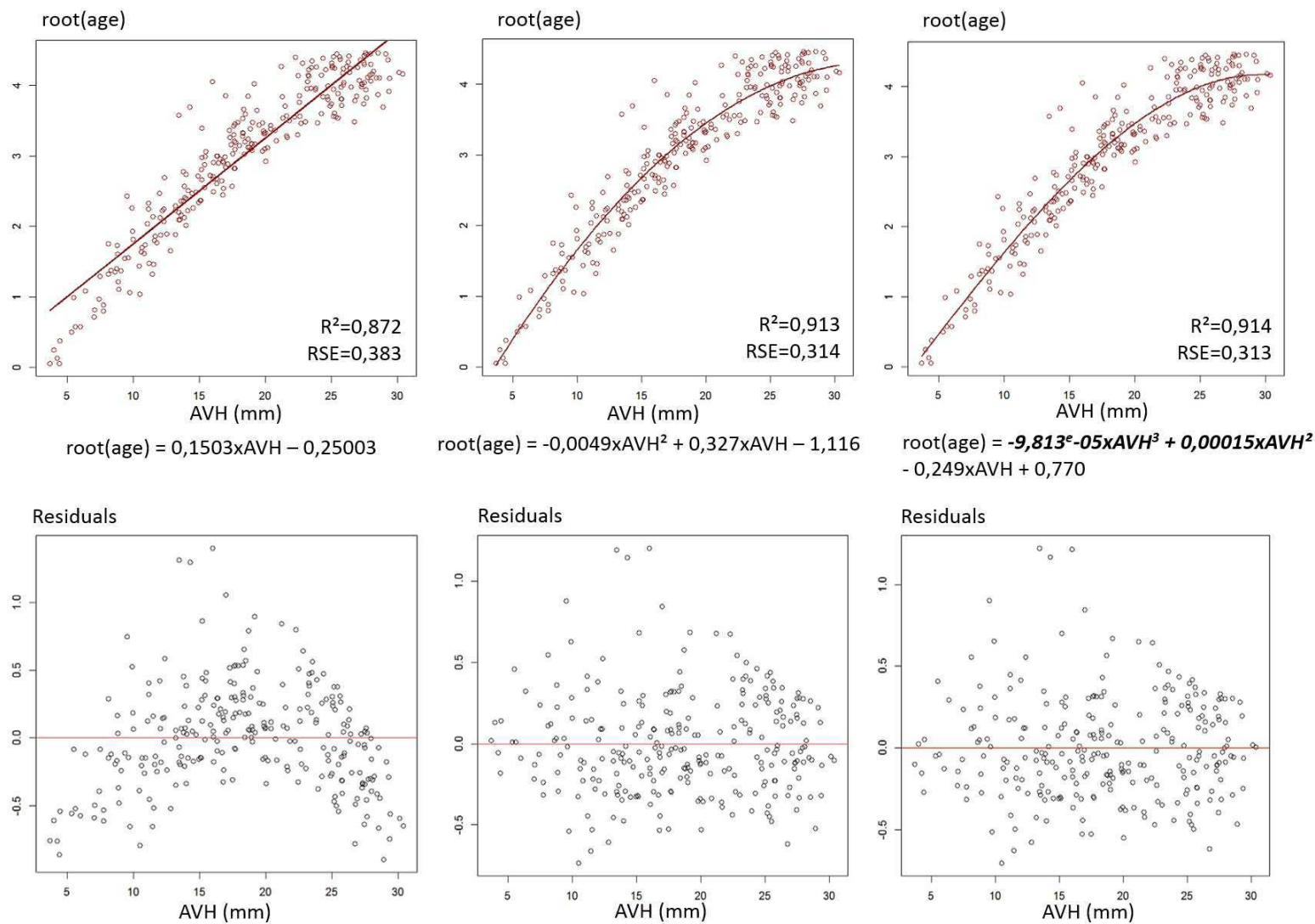


Figure E.28 Age prediction using linear, second and third degree polynomial models of root(age) against AVH, with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

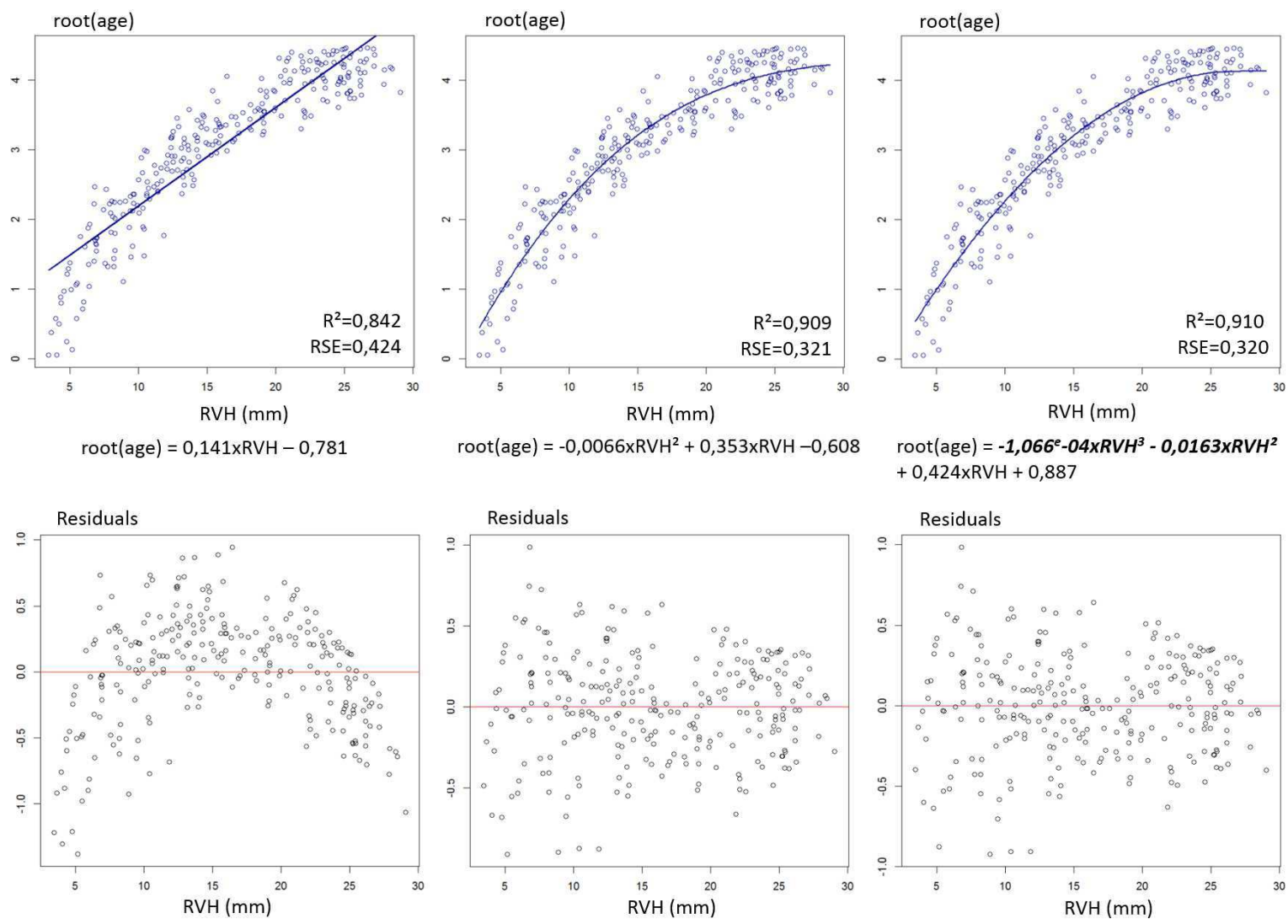


Figure E.29 Age prediction using linear, second and third degree polynomial models of root(age) against RVH, with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients



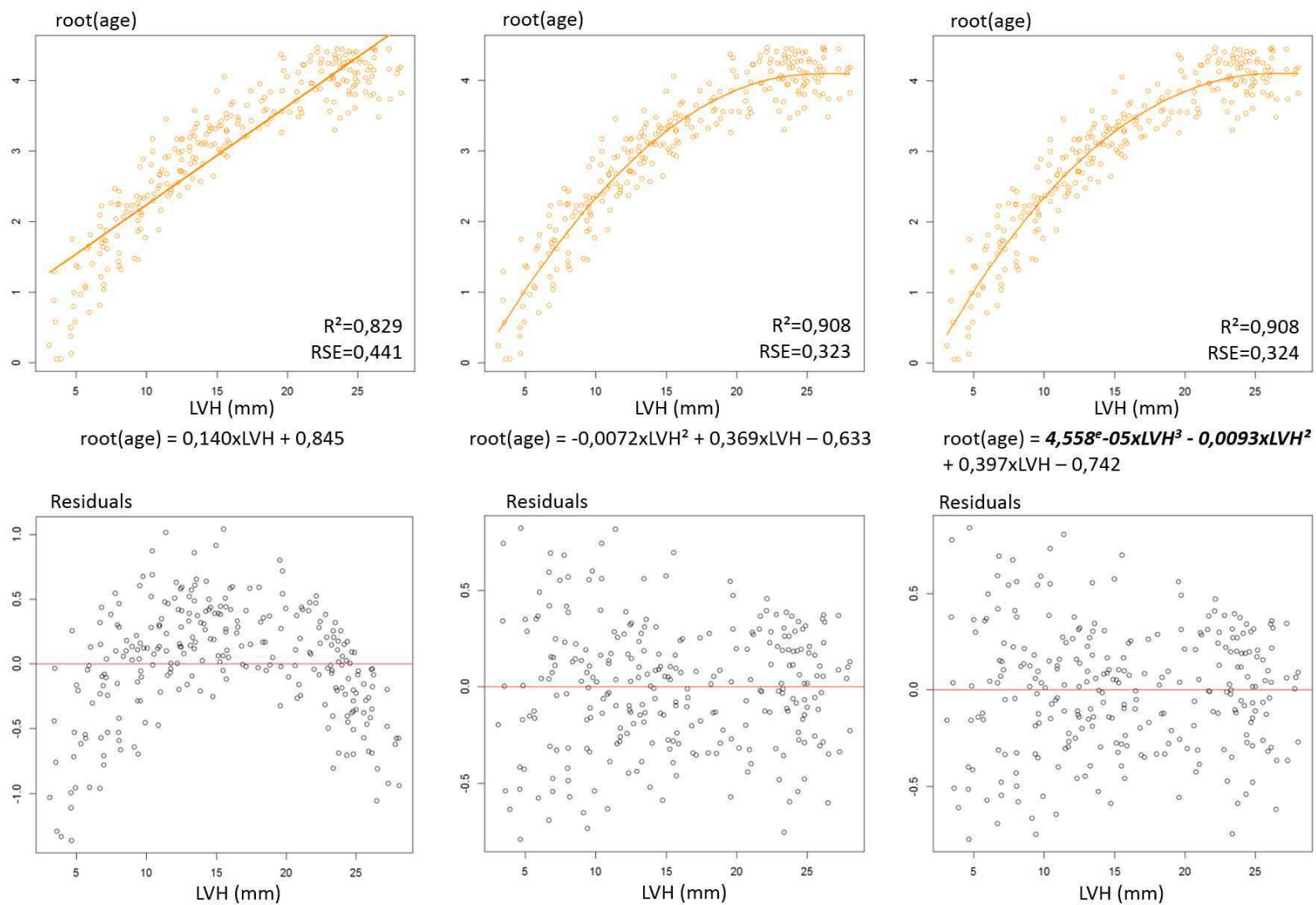


Figure E.30 Age prediction using linear, second and third degree polynomial models of root(age) against LVH, with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

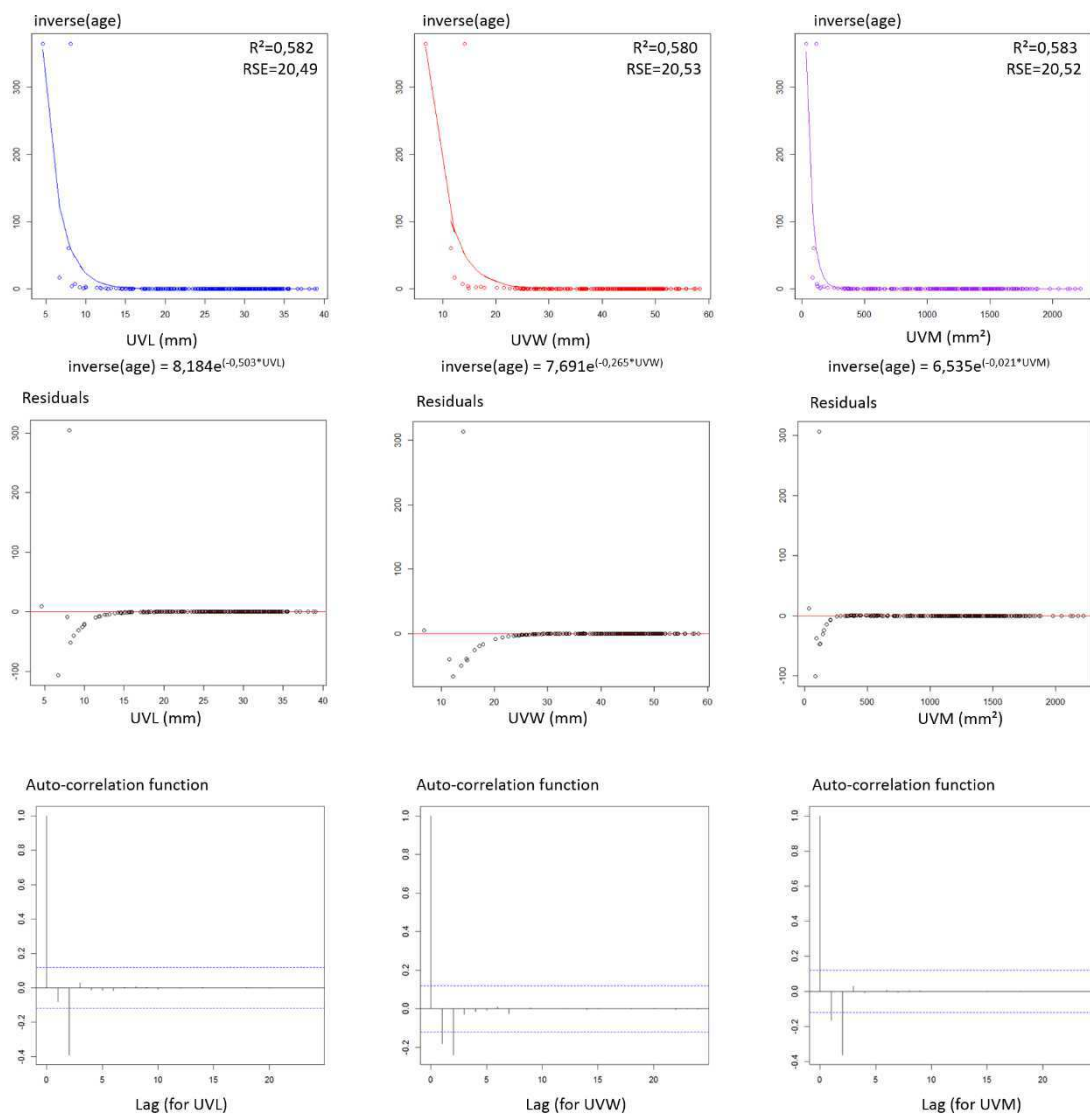


Figure E.31 Exponential regressions of the inverse of age against UVL (blue), UVW (red) and UVM (purple) with their mathematical expressions, associated R<sup>2</sup> and RSE values (upper plots), plot of the residuals against the predictor variables (middle plots) and the autocorrelation functions of the residuals (lower plots)

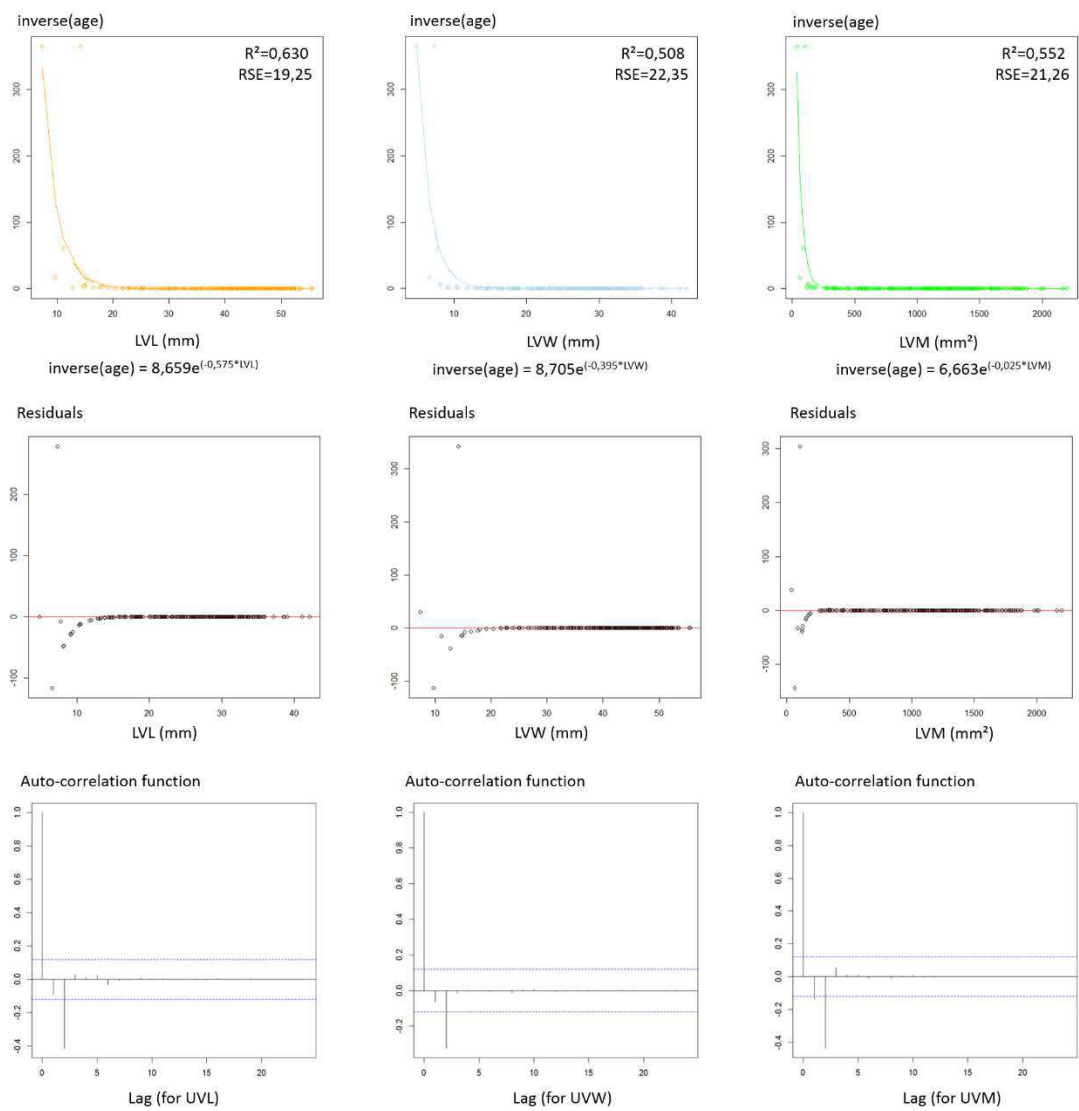


Figure E.32 Exponential regressions of the inverse of age against LVL (yellow), LVW (light blue) and LVM (green) with their mathematical expressions, associated R<sup>2</sup> and RSE values (upper plots), plot of the residuals against the predictor variables (middle plots) and the autocorrelation functions of the residuals (lower plots)

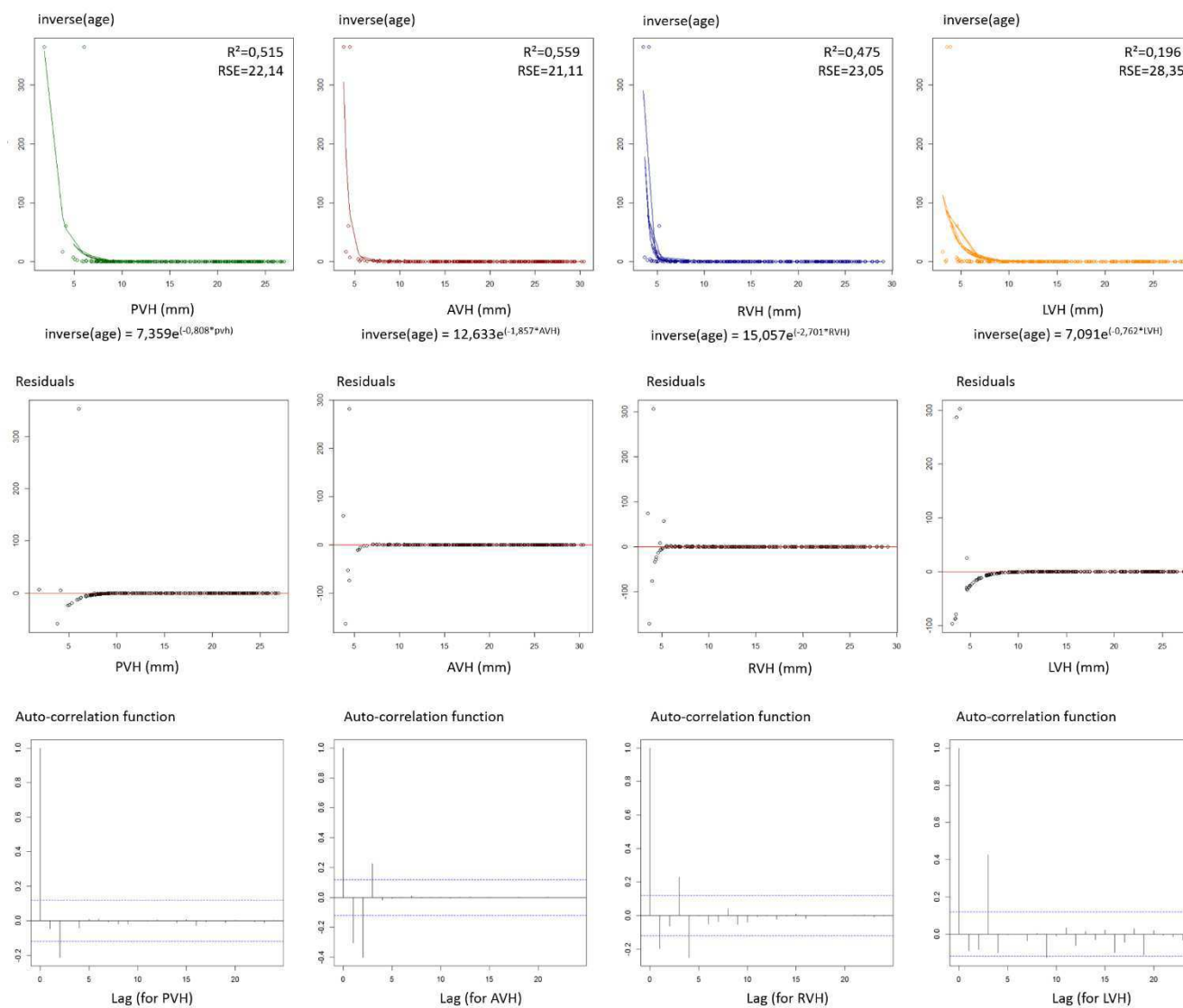


Figure E.33 Exponential regressions of the inverse of age against PVH (green), AVH (red), RVH (blue) and LVH (yellow) with their mathematical expressions, associated  $R^2$  and RSE values (upper plots), plot of the residuals against the predictor variables (middle plots) and the autocorrelation functions of the residuals (lower plots)

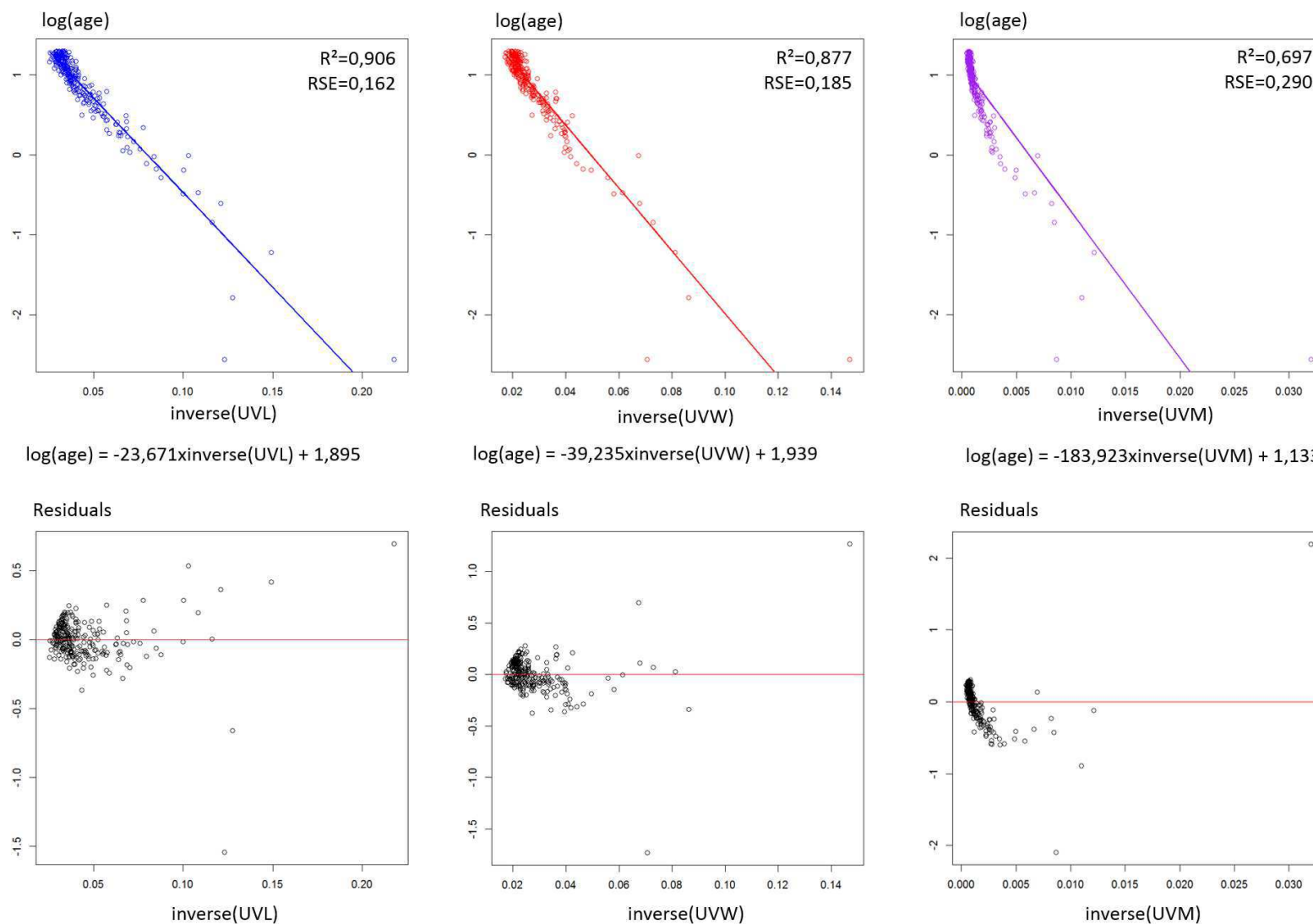


Figure E. 34 Age prediction using linear regression of the decimal logarithm of age against the inverse of UVL (blue), UVW (red) and UVM (purple) with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (lower plots)

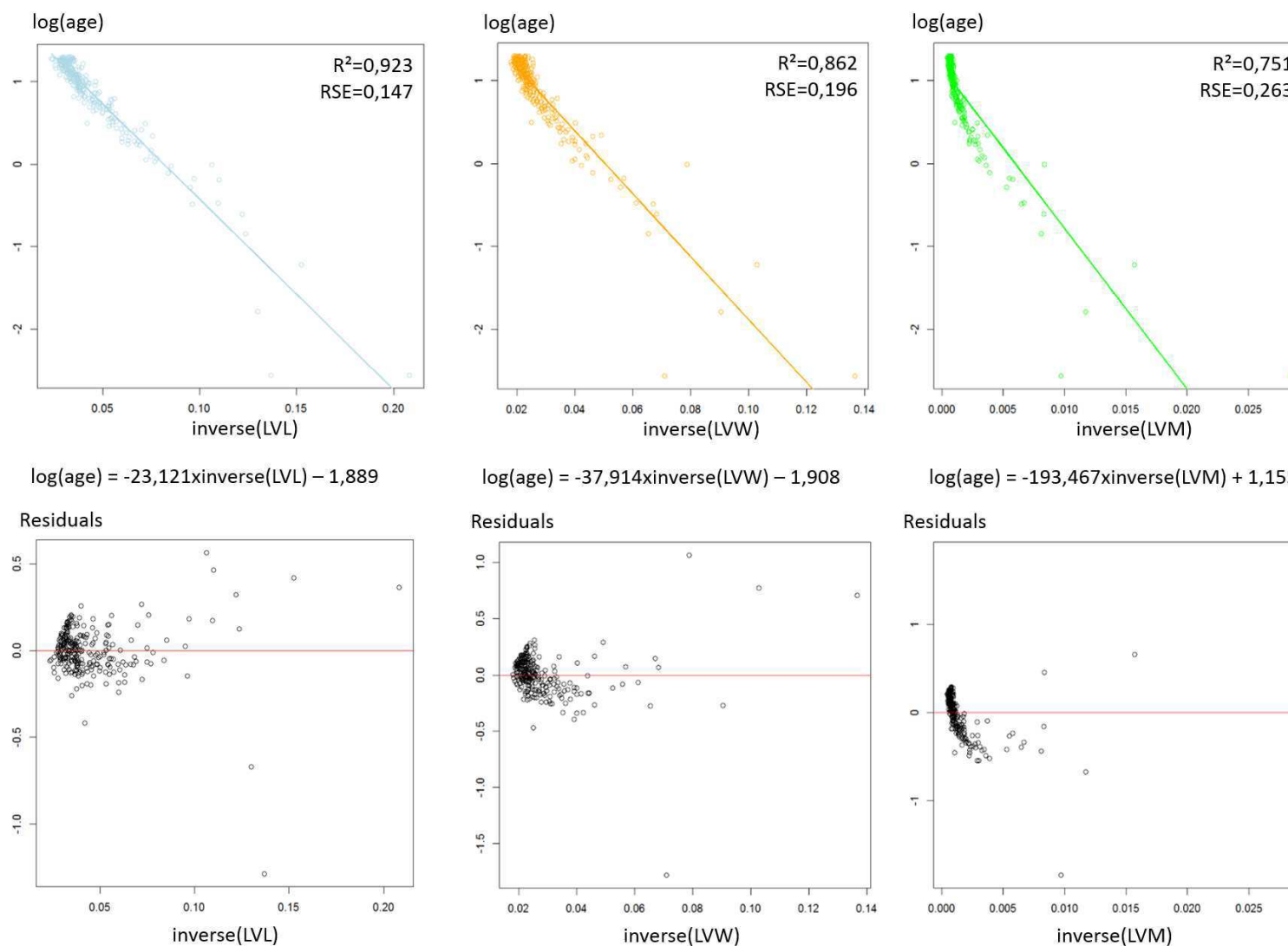


Figure E.35 Age prediction using linear regression of the decimal logarithm of age against the inverse of LVL (blue), LVW (yellow) and LVM (green) with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (lower plots)

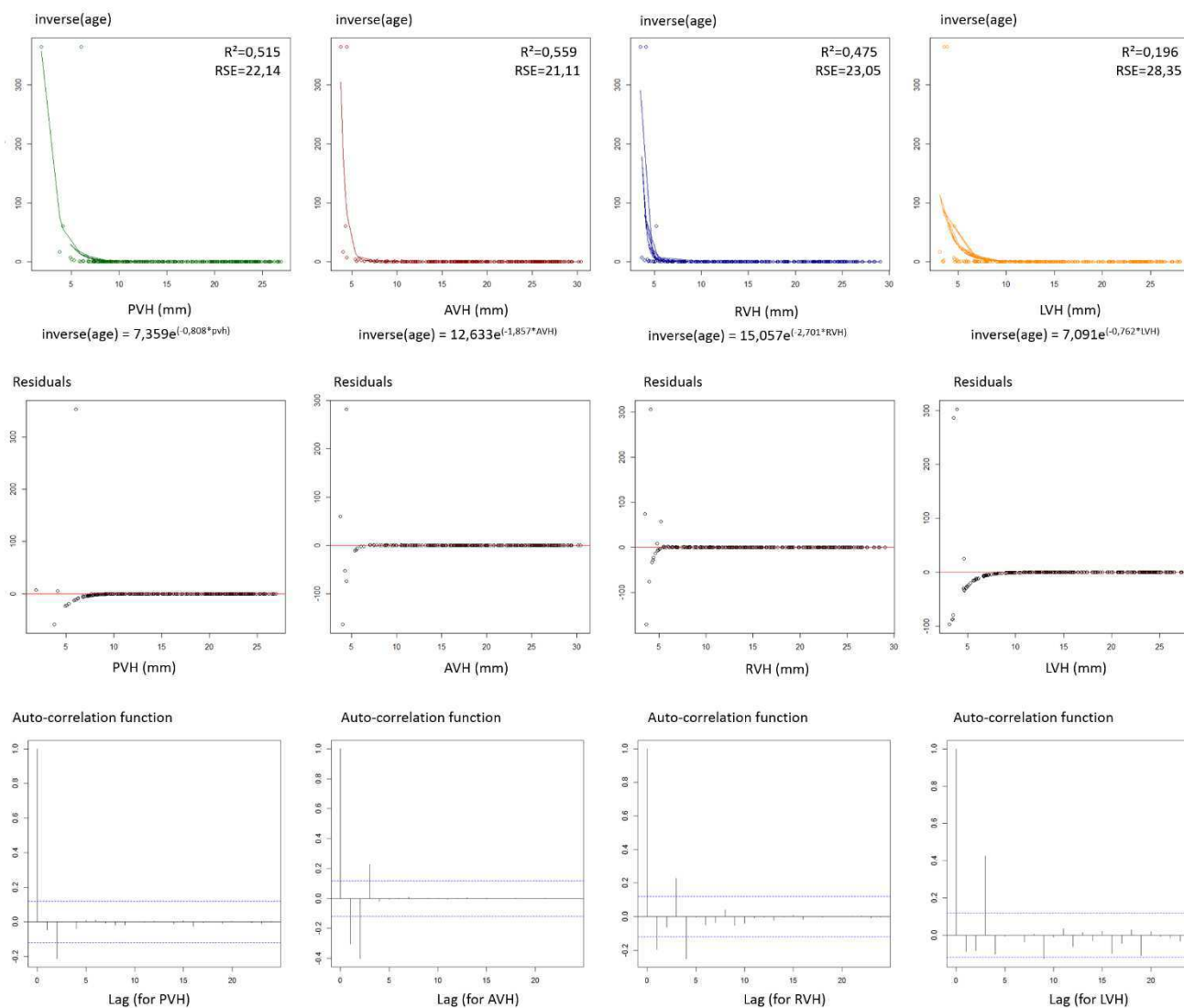


Figure E.36 Age prediction using linear regression of the decimal logarithm of age against the inverse of PVH (green), AVH (red), RVH (blue) and LVH (yellow) with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (lower plots)

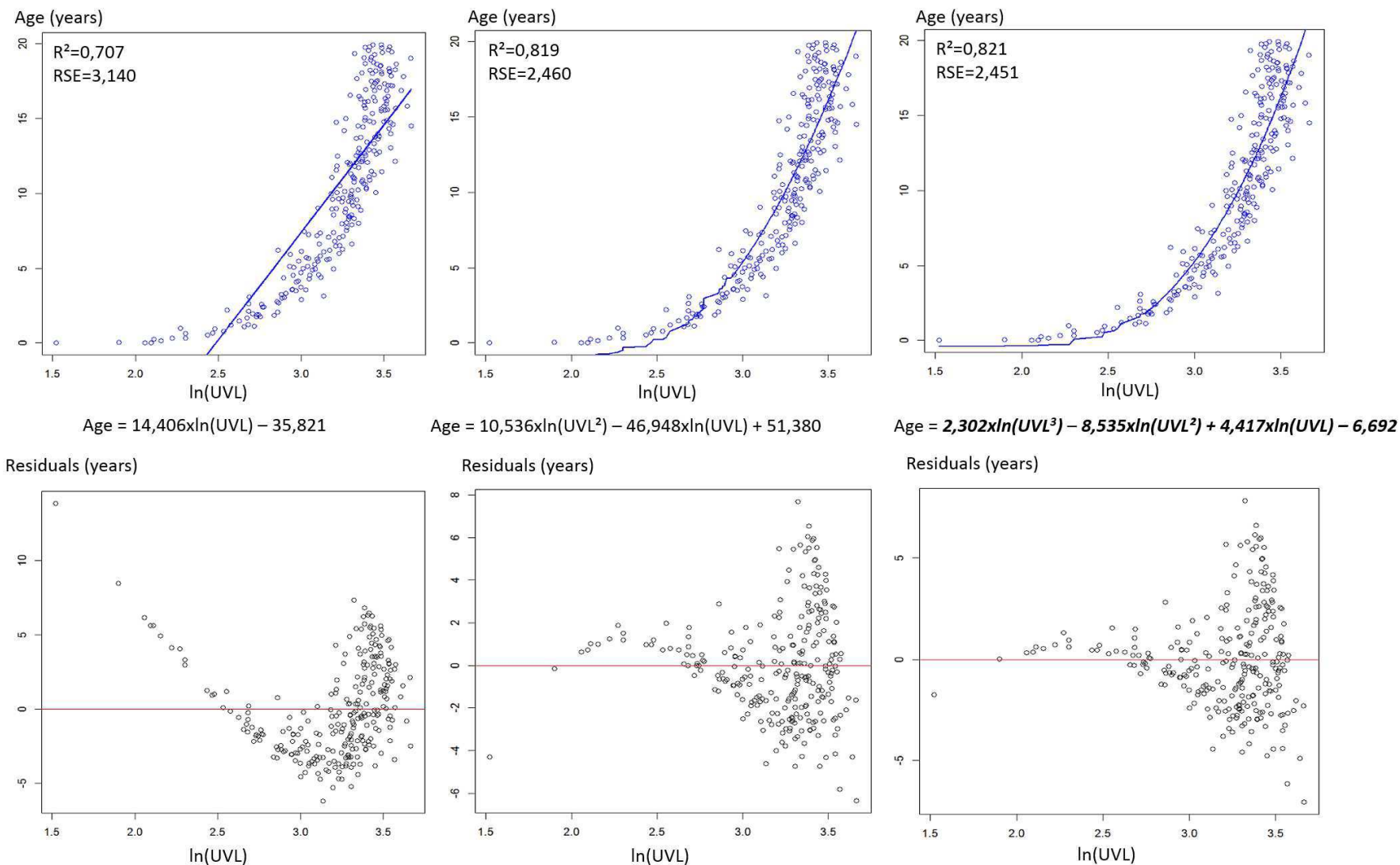


Figure E.37 Age prediction using linear, second and third degree polynomial models of age against ln(UVL) with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plots of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients



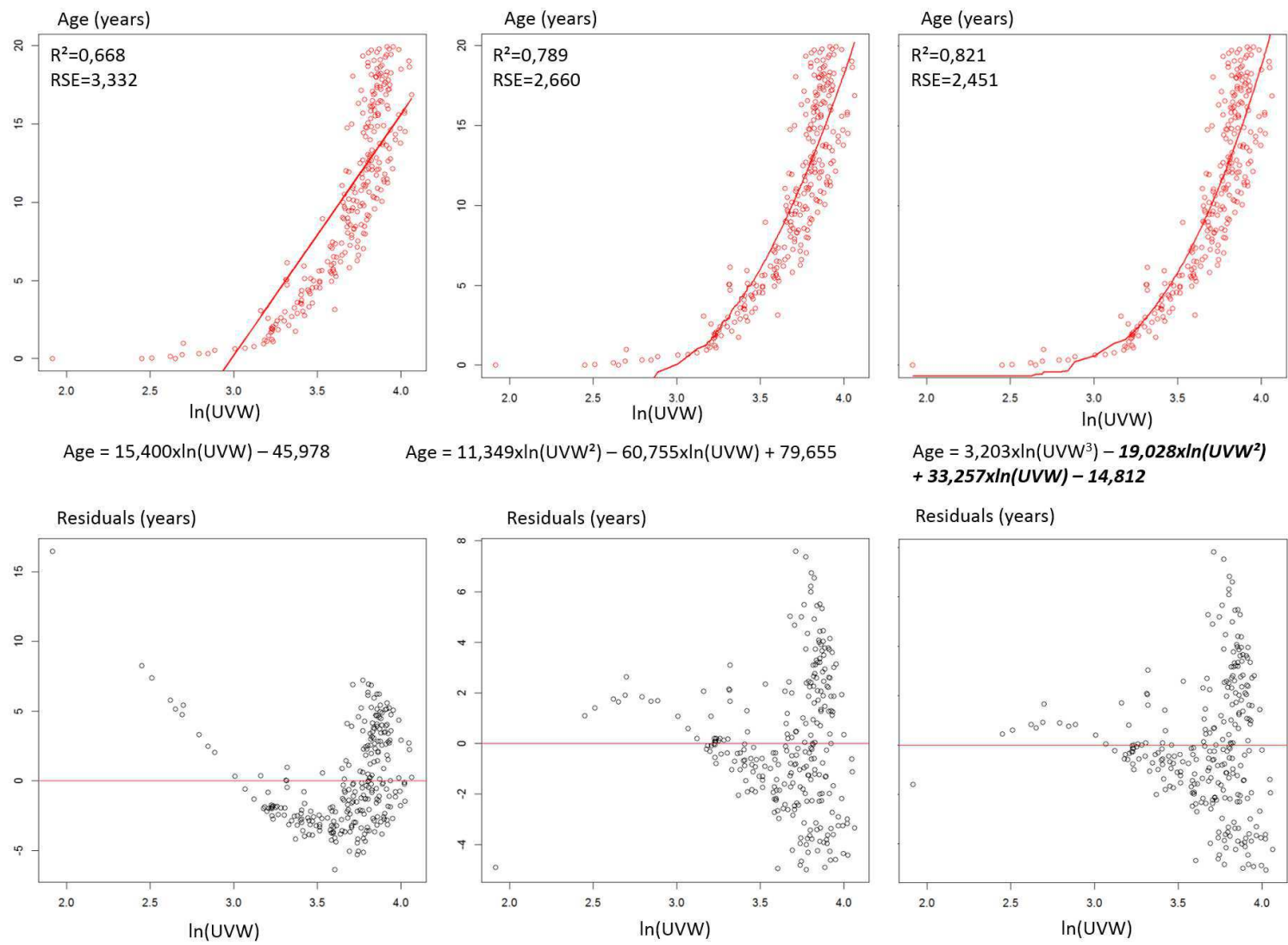


Figure E.38 Age prediction using linear, second and third degree polynomial models of age against ln(UVW) with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plots of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

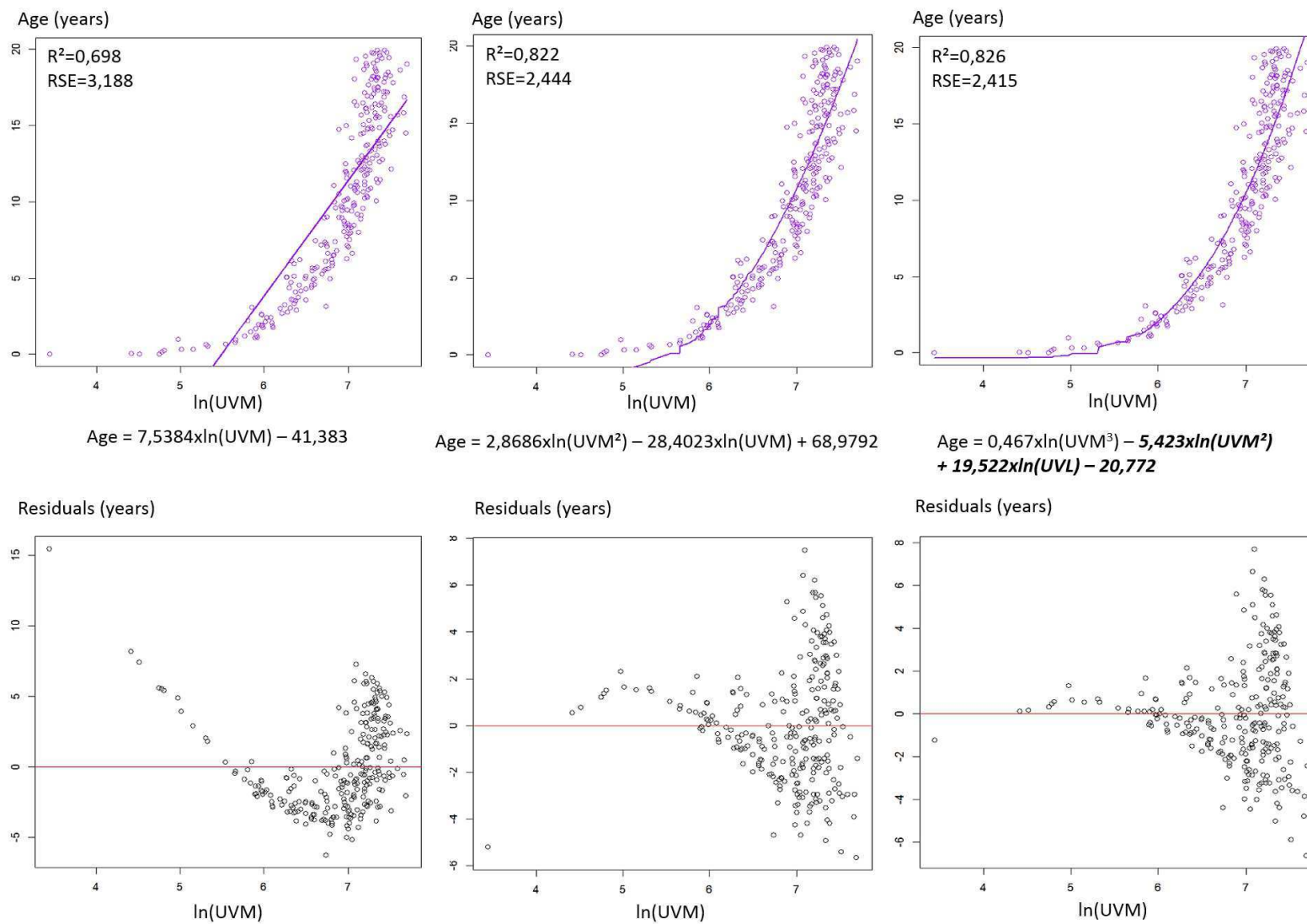


Figure E.39 Age prediction using linear, second and third degree polynomial models of age against ln(UVM) with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plots of the residuals against predictor variables (lower plots). ***Bold italic terms correspond to non-significant regression coefficients***

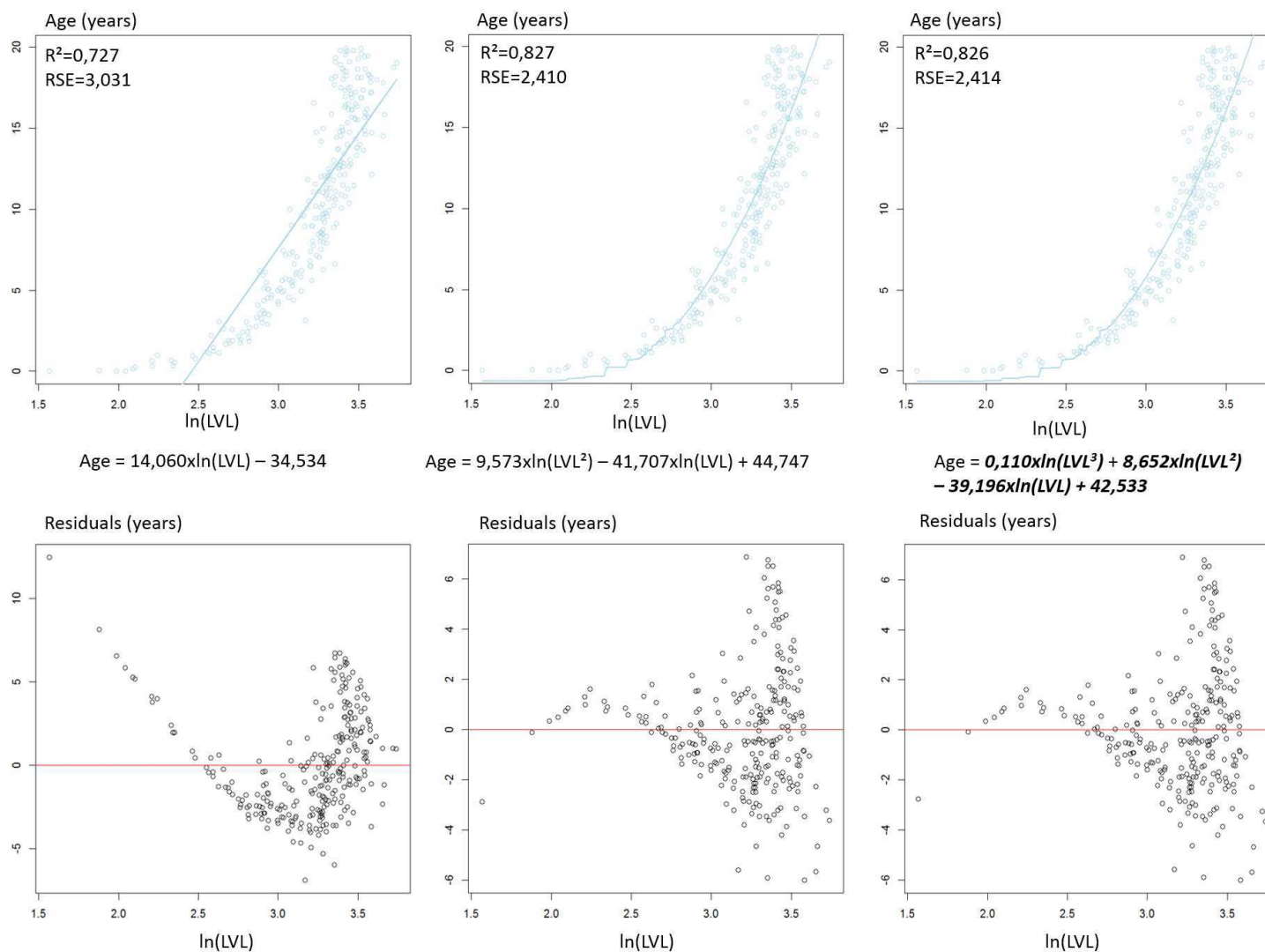


Figure E.40 Age prediction using linear, second and third degree polynomial models of age against ln(LVL) with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plots of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

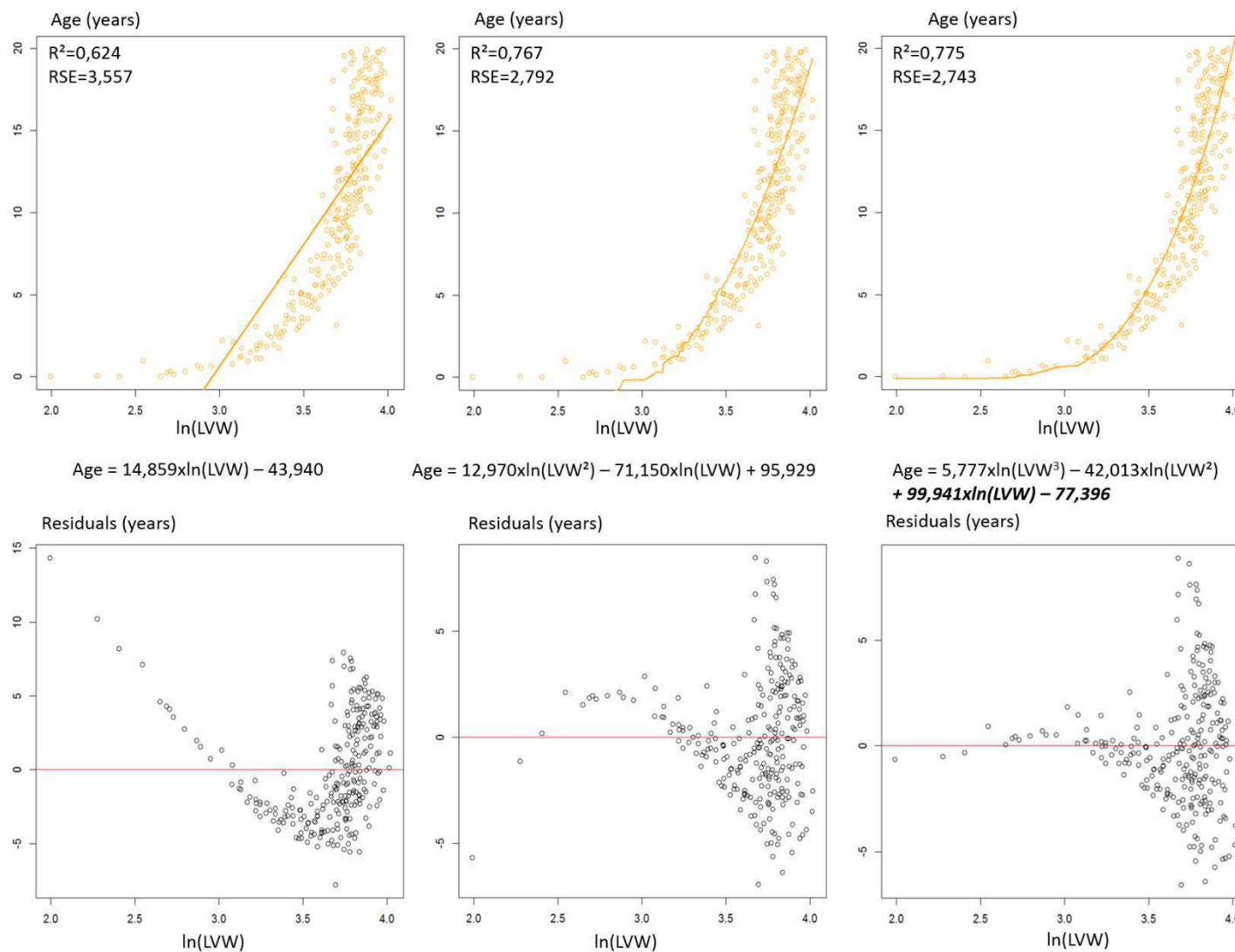


Figure E.41 Age prediction using linear, second and third degree polynomial models of age against ln(LVW) with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plots of the residuals against predictor variables (lower plots). ***Bold italic terms correspond to non-significant regression coefficients***

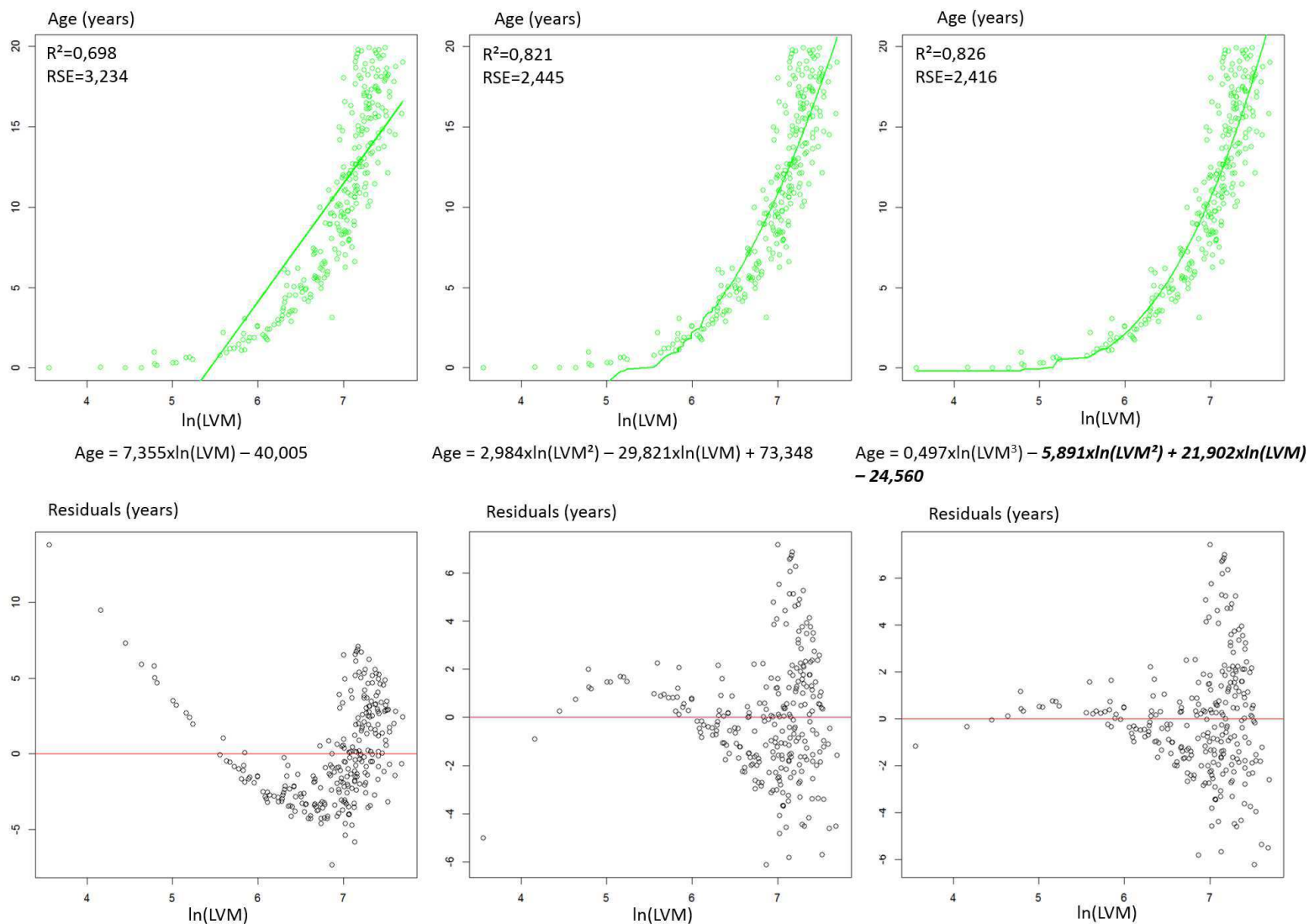


Figure E.42 Age prediction using linear, second and third degree polynomial models of age against ln(LVM) with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plots of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

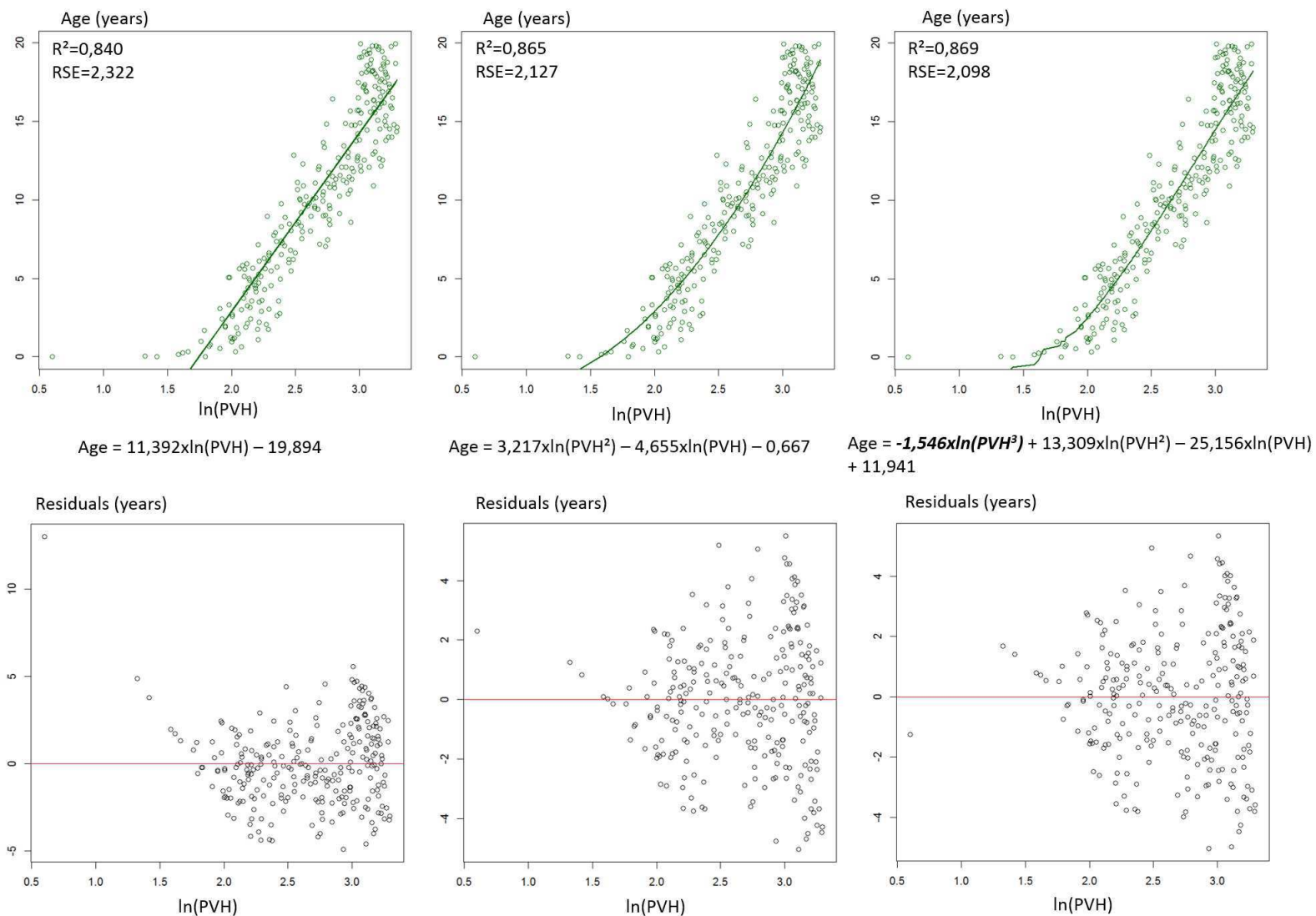


Figure E.43 Age prediction using linear, second and third degree polynomial models of age against ln(PVH) with their mathematical expressions, associated  $R^2$  and RSE values and the plots of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

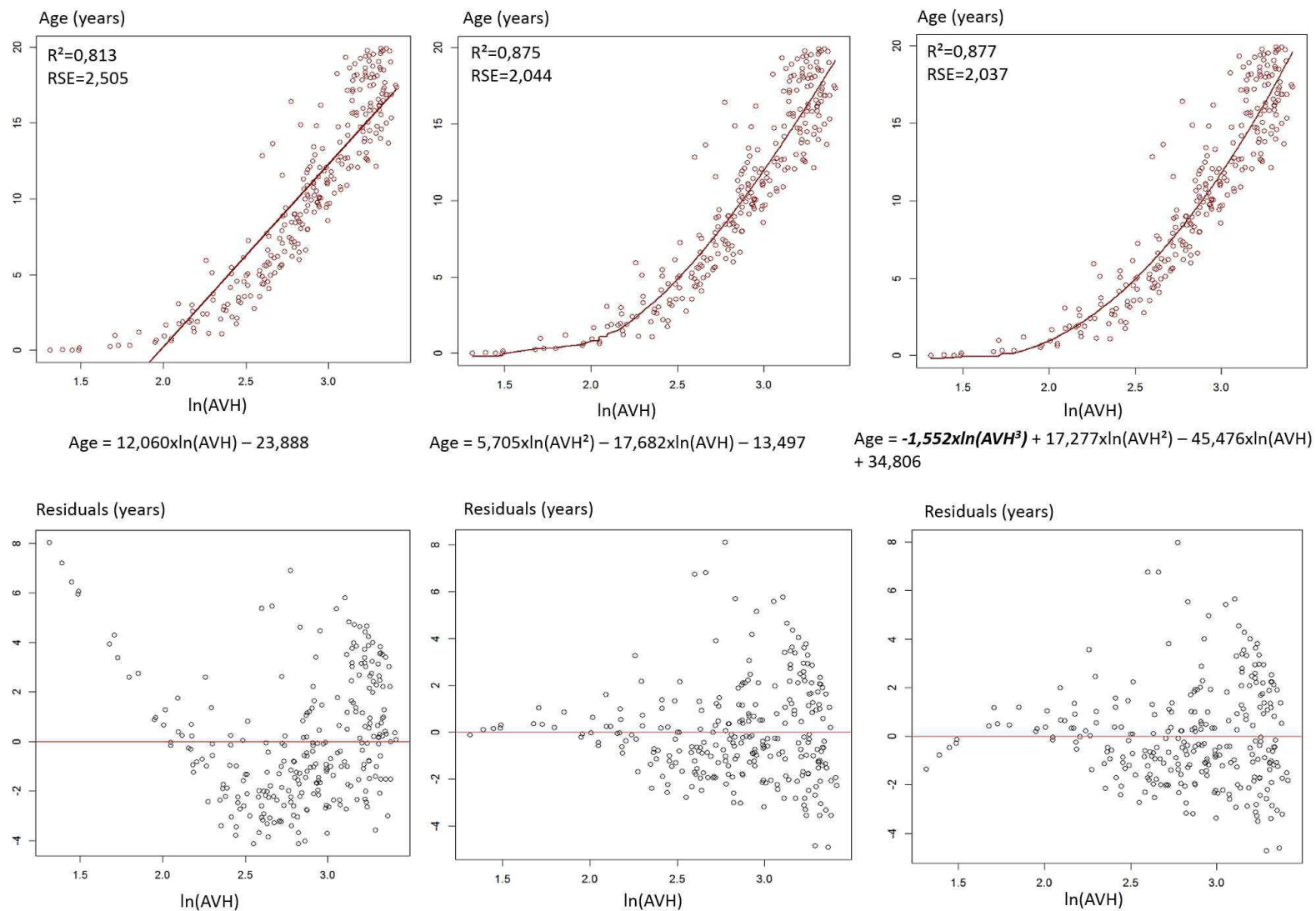


Figure E.44 Age prediction using linear, second and third degree polynomial models of age against ln(AVH) with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plots of the residuals against predictor variables (lower plots). **Italic terms correspond to non-significant regression coefficients**

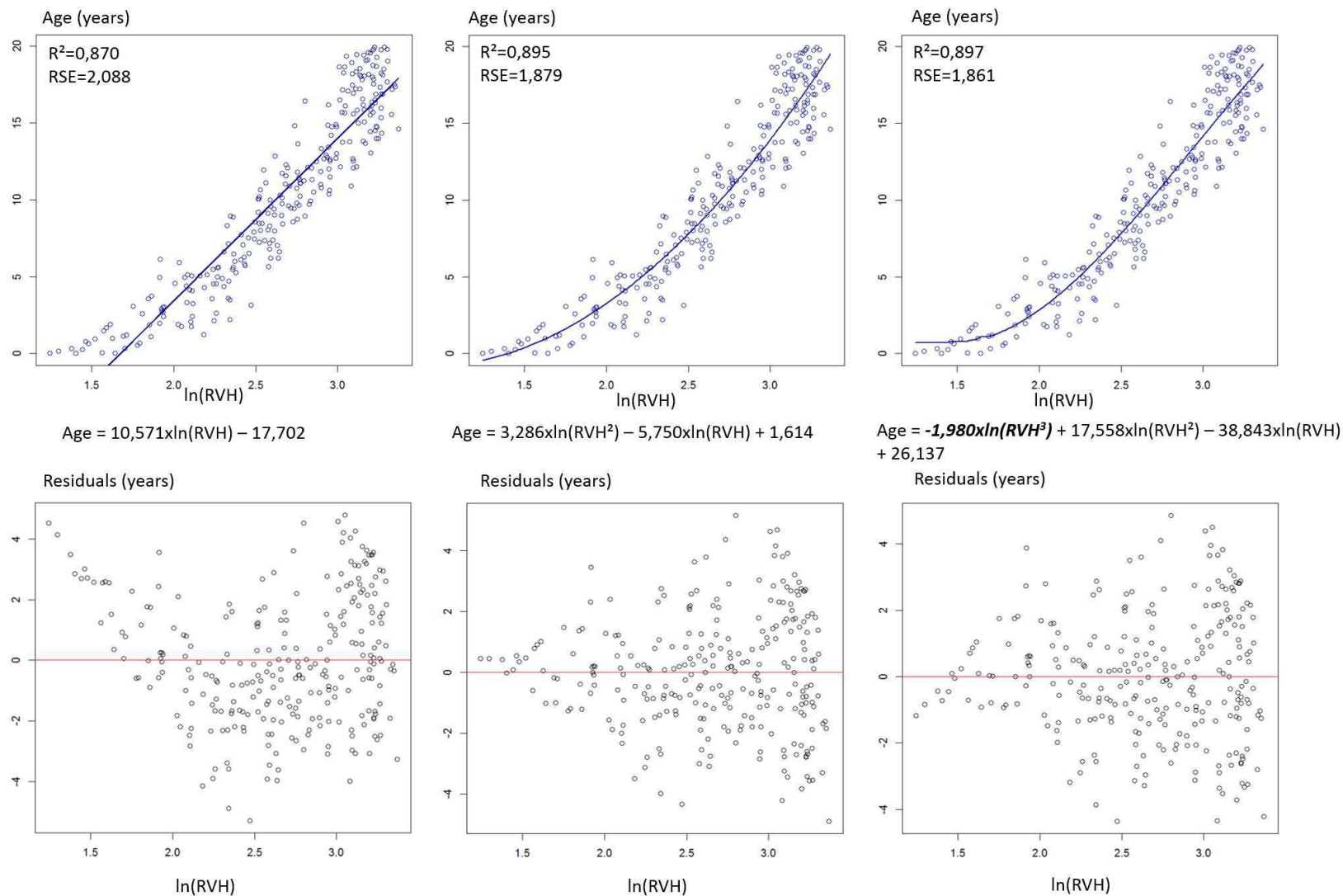


Figure E.45 Age prediction using linear, second and third degree polynomial models of age against ln(RVH) with their mathematical expressions, associated  $R^2$  and RSE values and the plots of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients



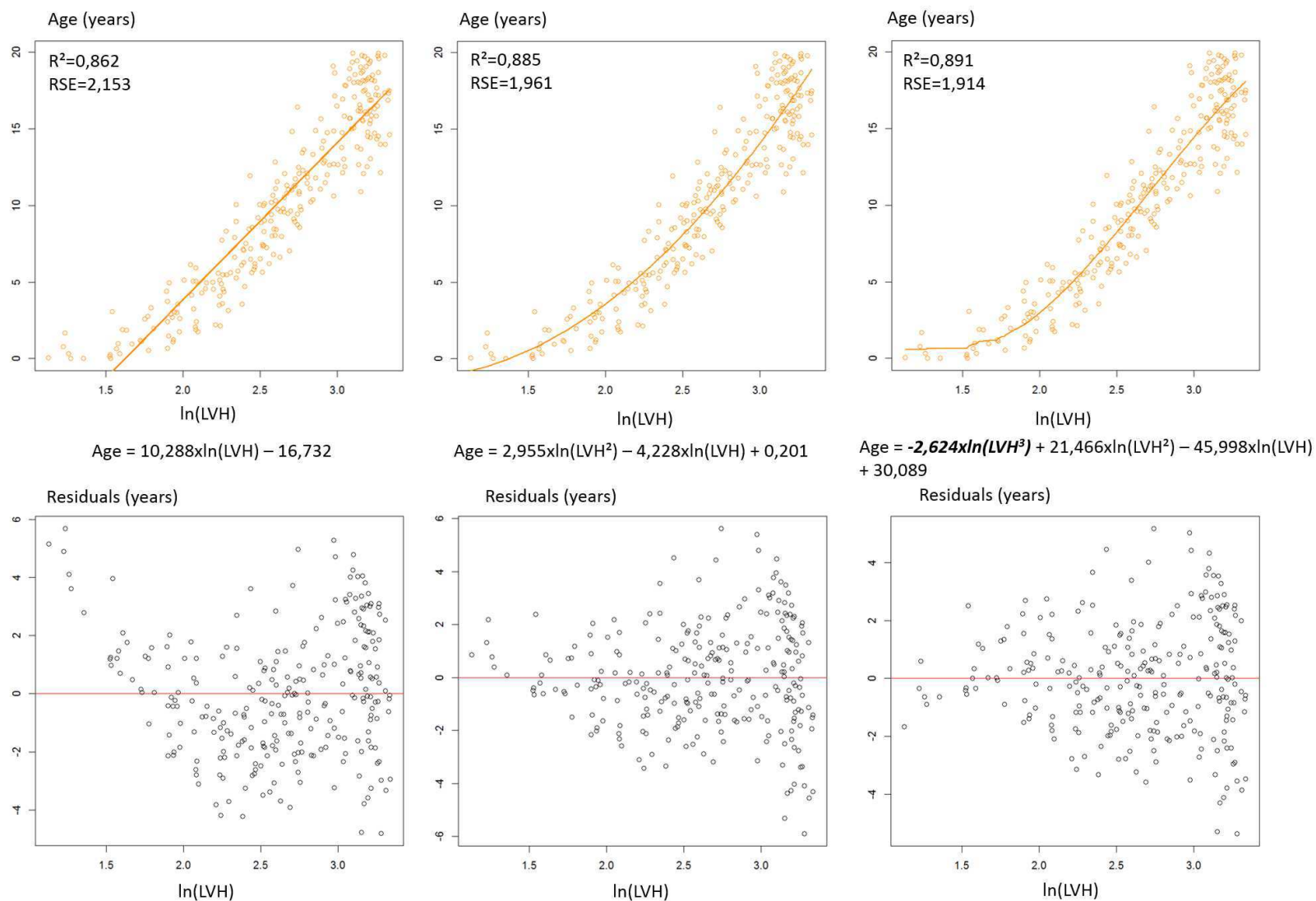


Figure E.46 Age prediction using linear, second and third degree polynomial models of age against ln(LVH) with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plots of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

## Clavicular variables

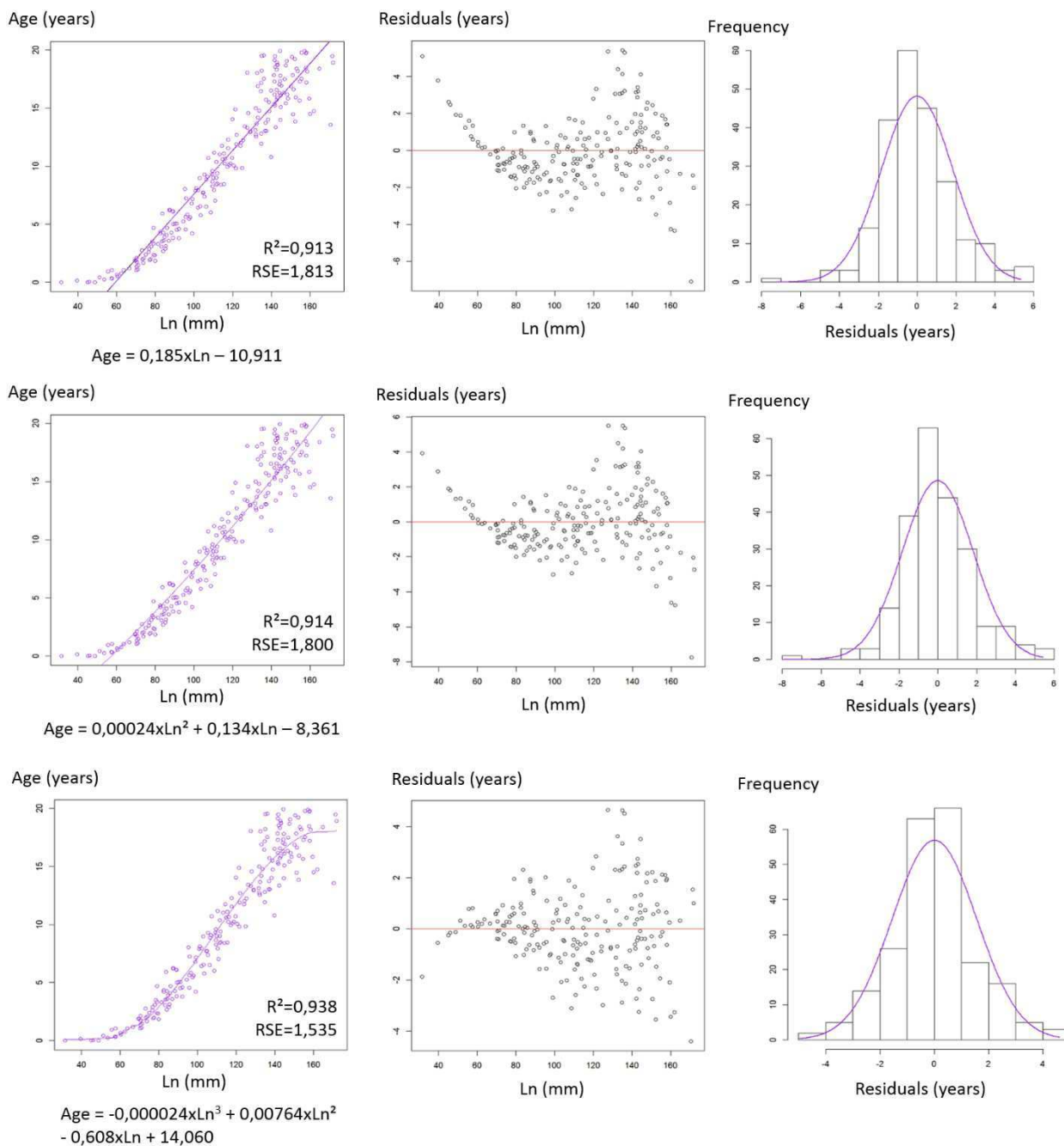


Figure E.47 Age prediction using linear, second and third degree polynomial regressions of Ln with the mathematical expressions, associated  $R^2$  and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients

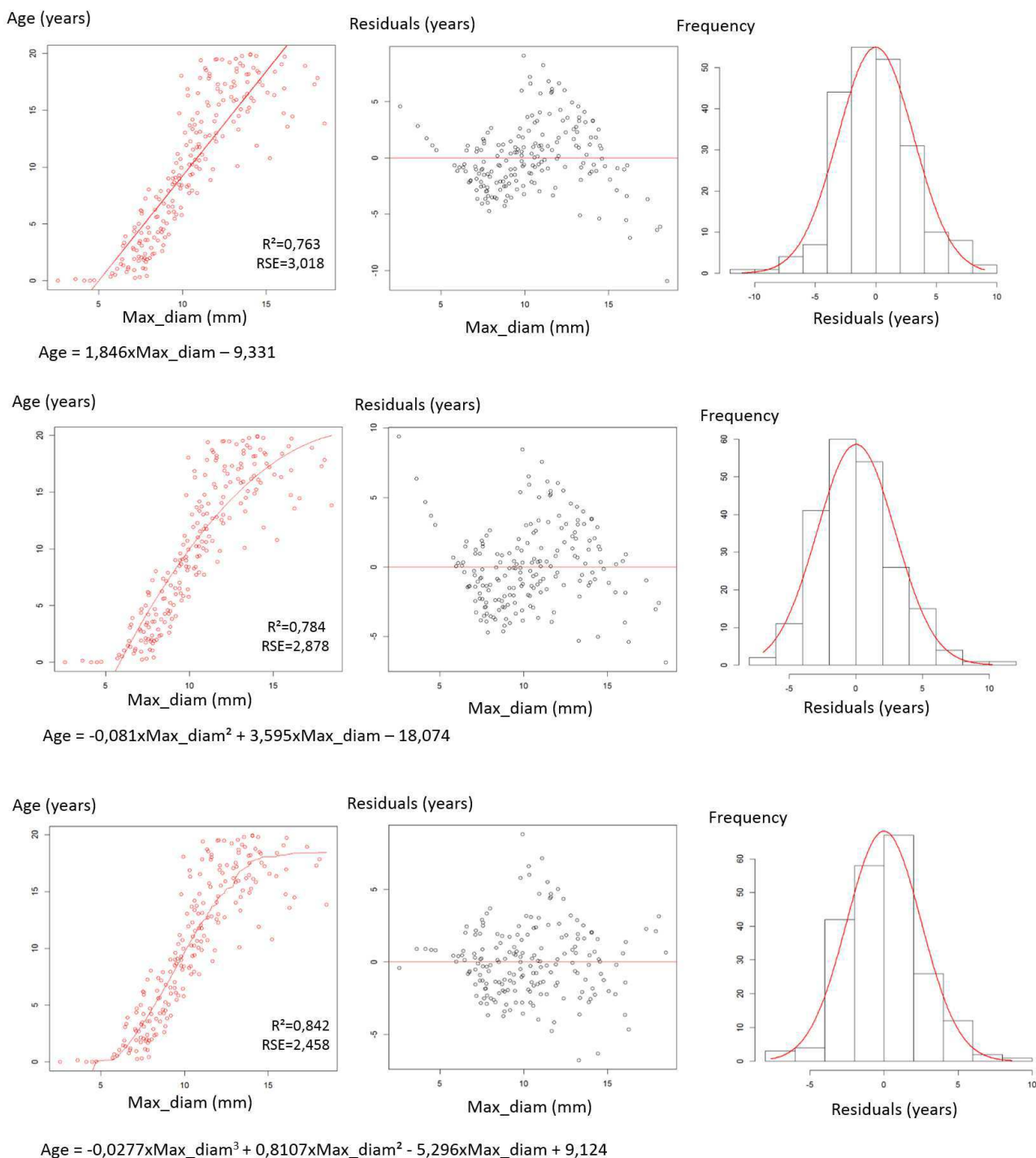


Figure E.48 Age prediction using linear, second and third degree polynomial regressions of Max\_diam with the mathematical expressions, associated R<sup>2</sup> and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. **Bold italic terms correspond to non-significant regression coefficients**

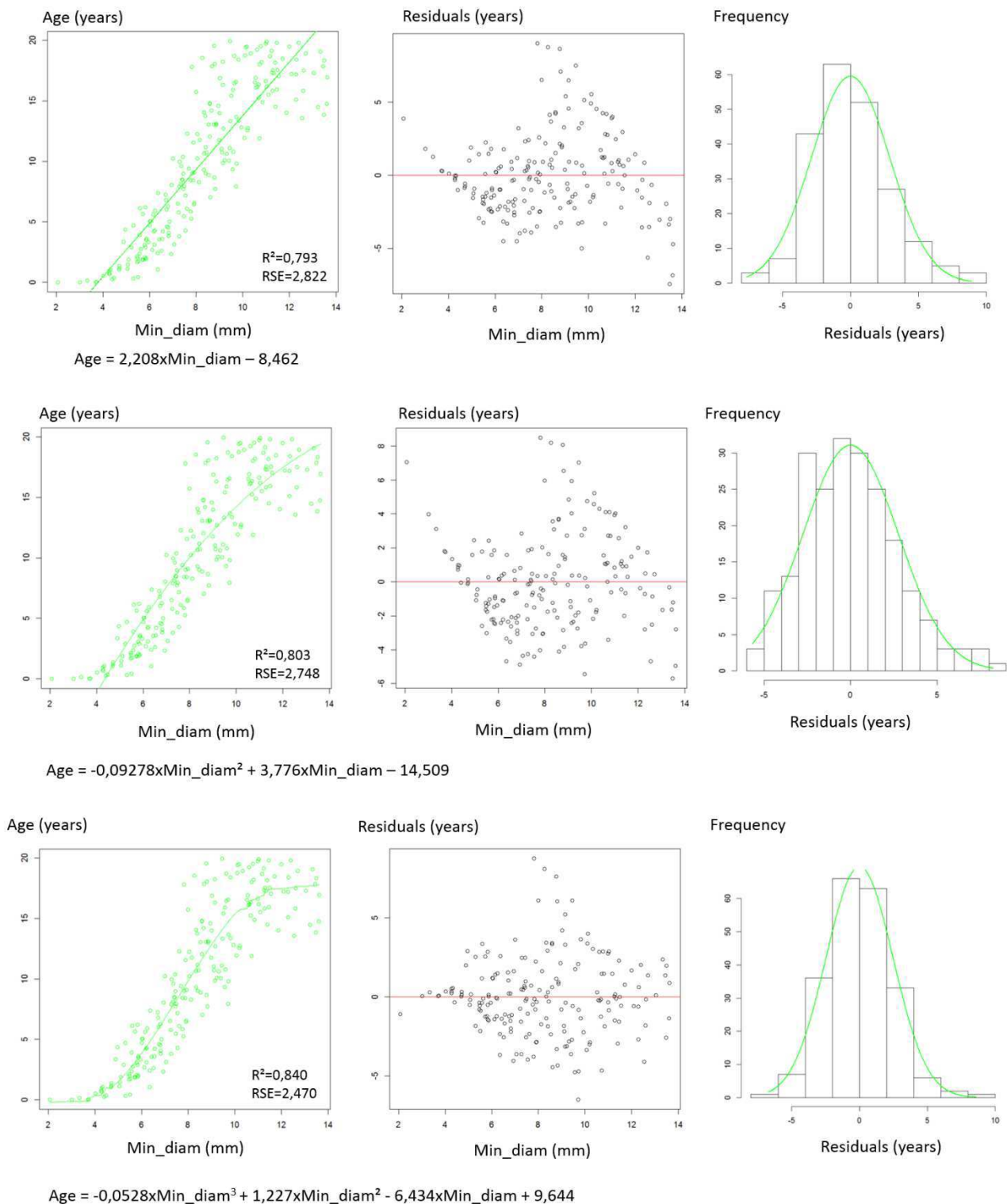


Figure E.49 Age prediction using linear, second and third degree polynomial regressions of Min\_diam with the mathematical expressions, associated R<sup>2</sup> and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients

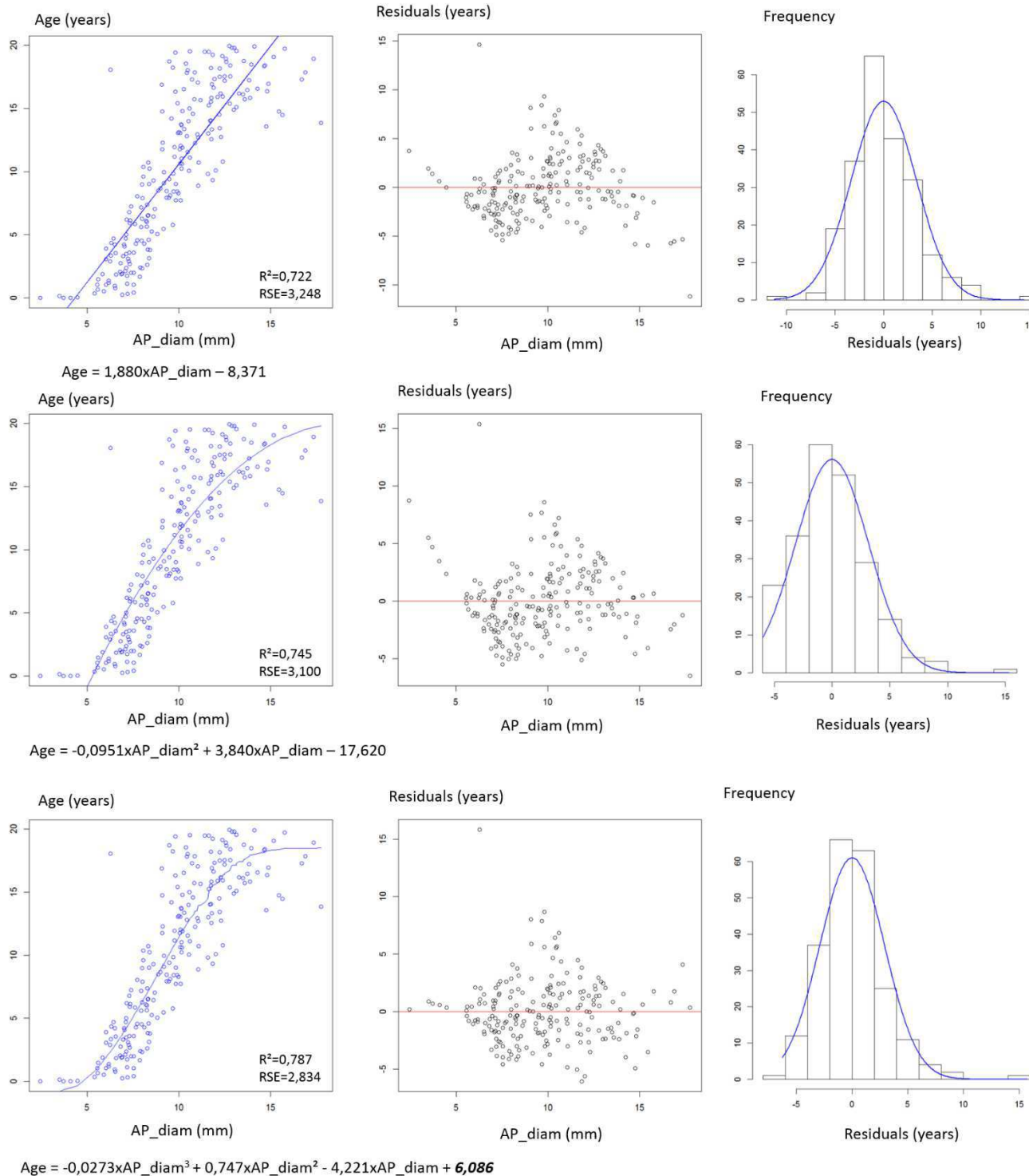
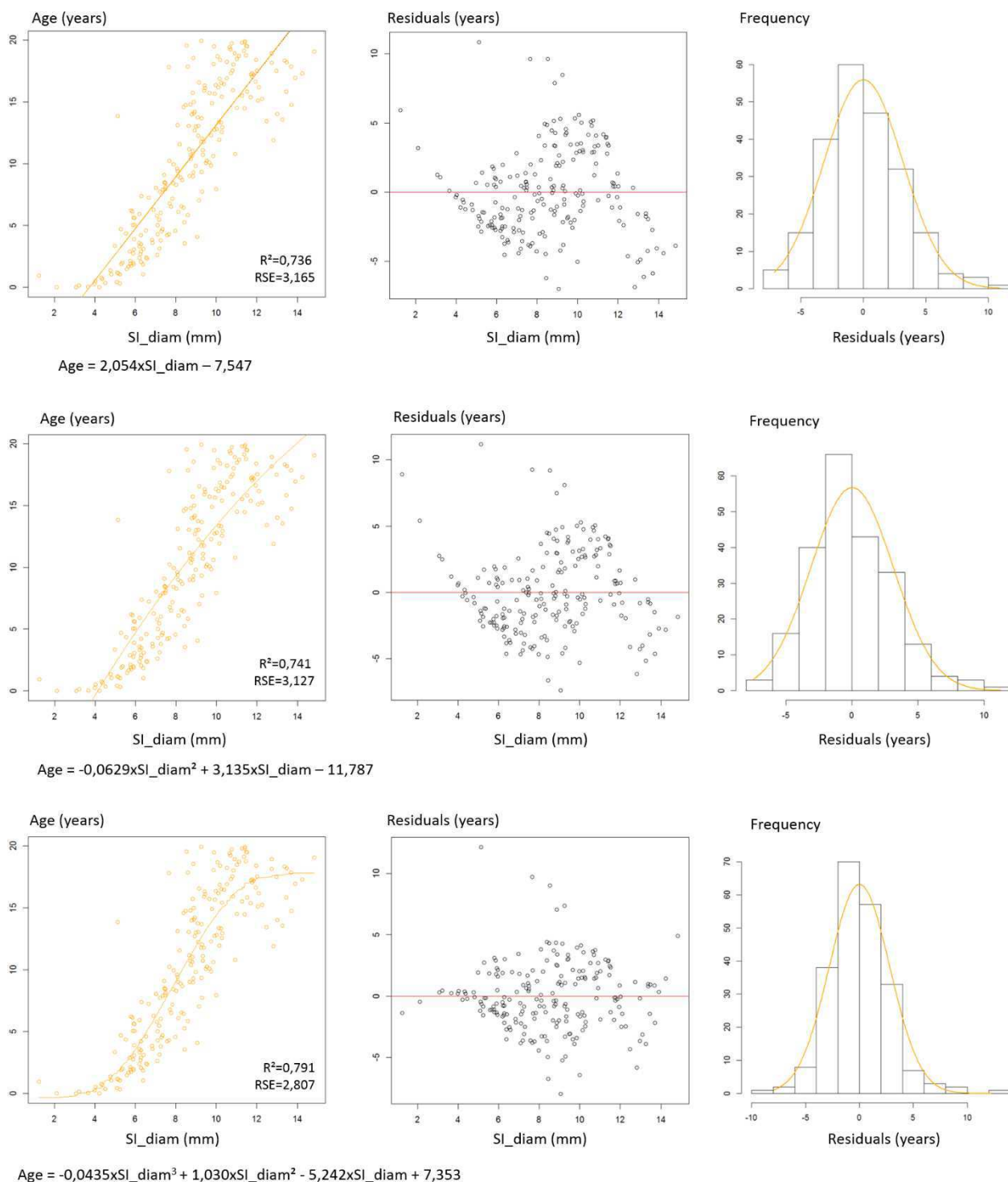


Figure E.50 Age prediction using linear, second and third degree polynomial regressions of AP\_diam with the mathematical expressions, associated R<sup>2</sup> and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. Bold italic terms correspond to non-significant regression coefficients



**Figure E.51** Age prediction using linear, second and third degree polynomial regressions of SI\_diam with the mathematical expressions, associated  $R^2$  and RSE values, the plots of the residuals against the predictor variable and the plots of residual distribution. ***Bold italic terms correspond to non-significant regression coefficients***

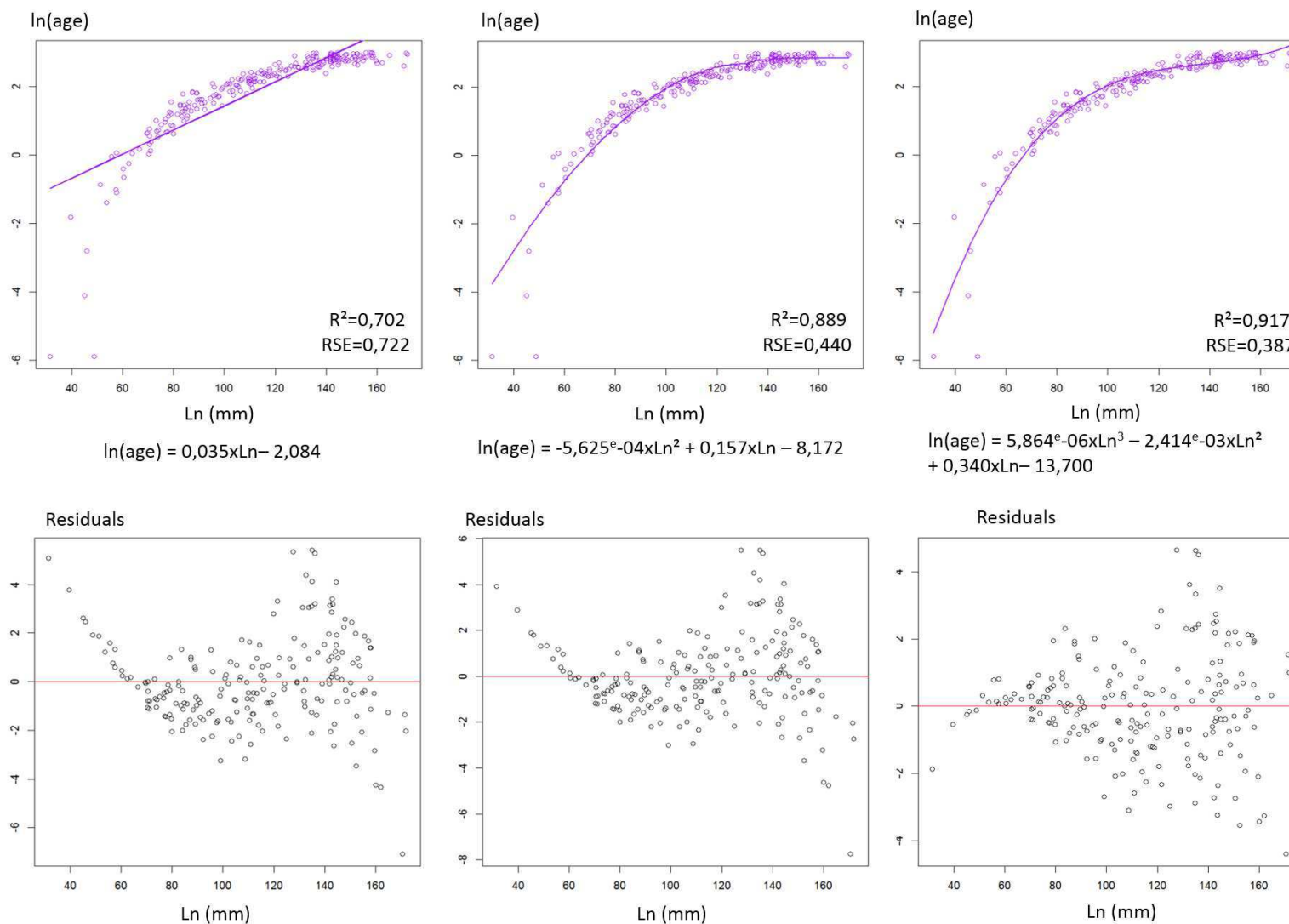


Figure E.52 Age prediction using linear, second and third degree polynomial regressions of ln(age) against Ln, with the mathematical expressions, associated R<sup>2</sup> and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

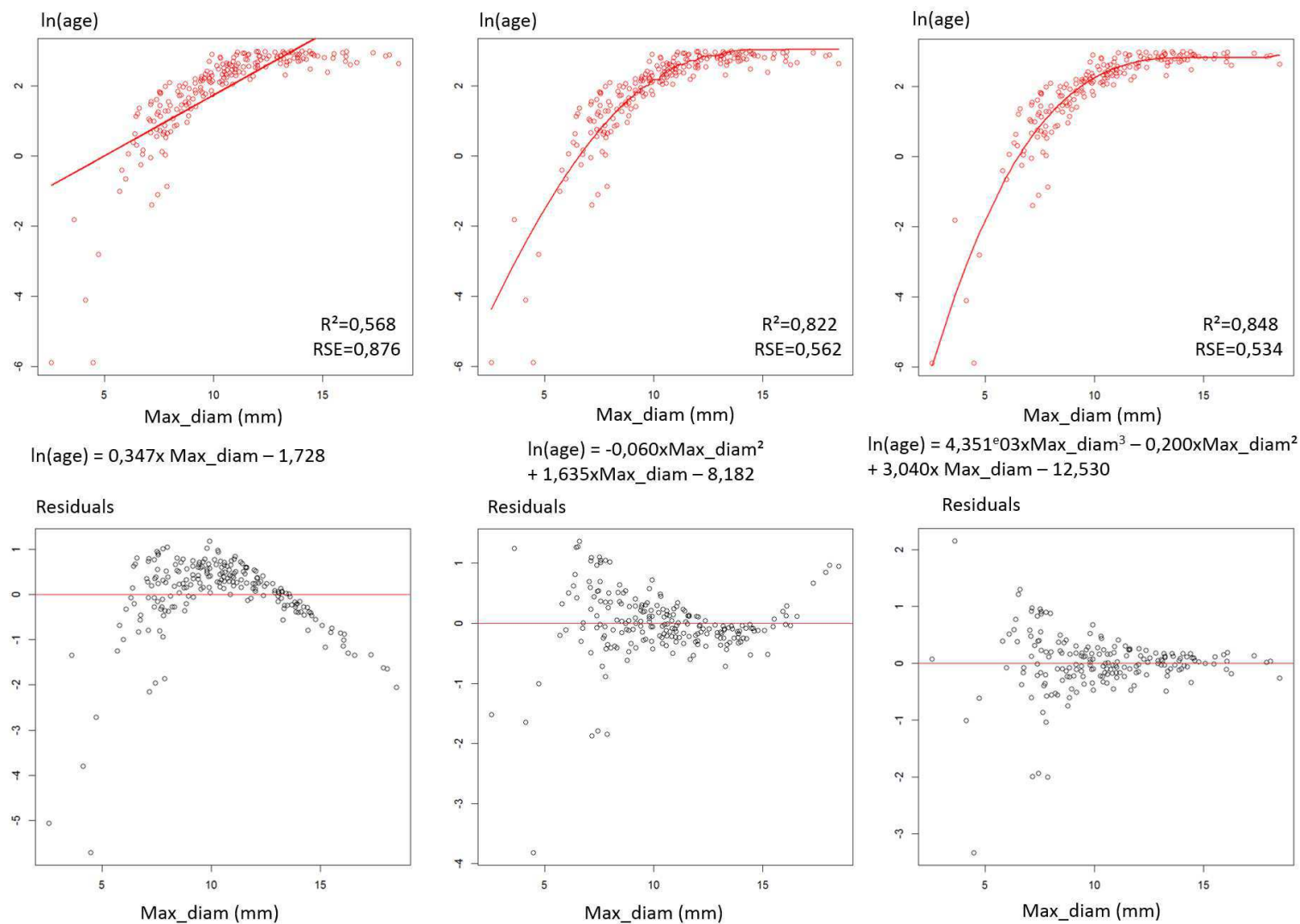


Figure E.53 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against  $\text{Max\_diam}$ , with the mathematical expressions, associated  $R^2$  and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients



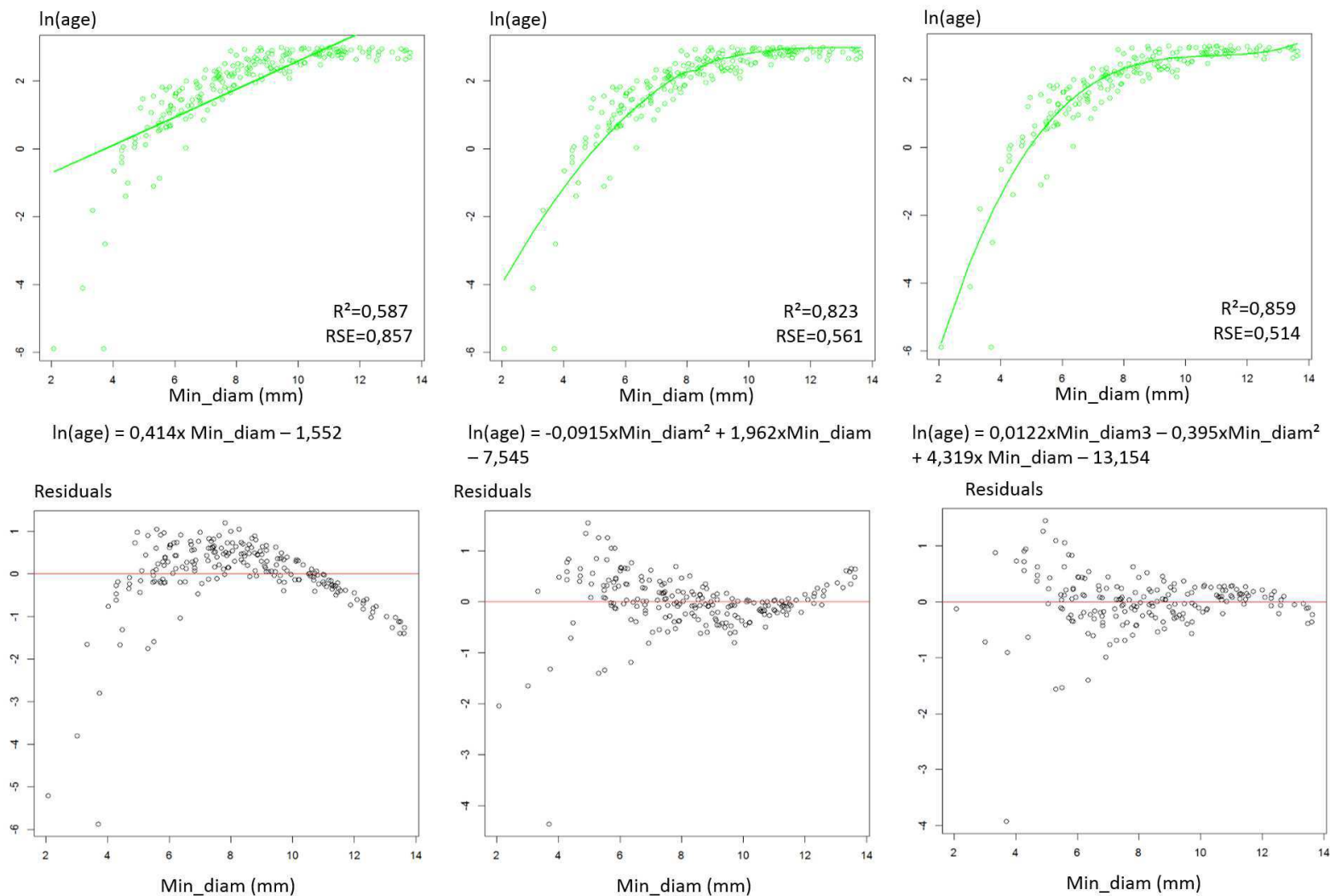


Figure E.54 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against  $\text{Min\_diam}$ , with the mathematical expressions, associated  $R^2$  and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

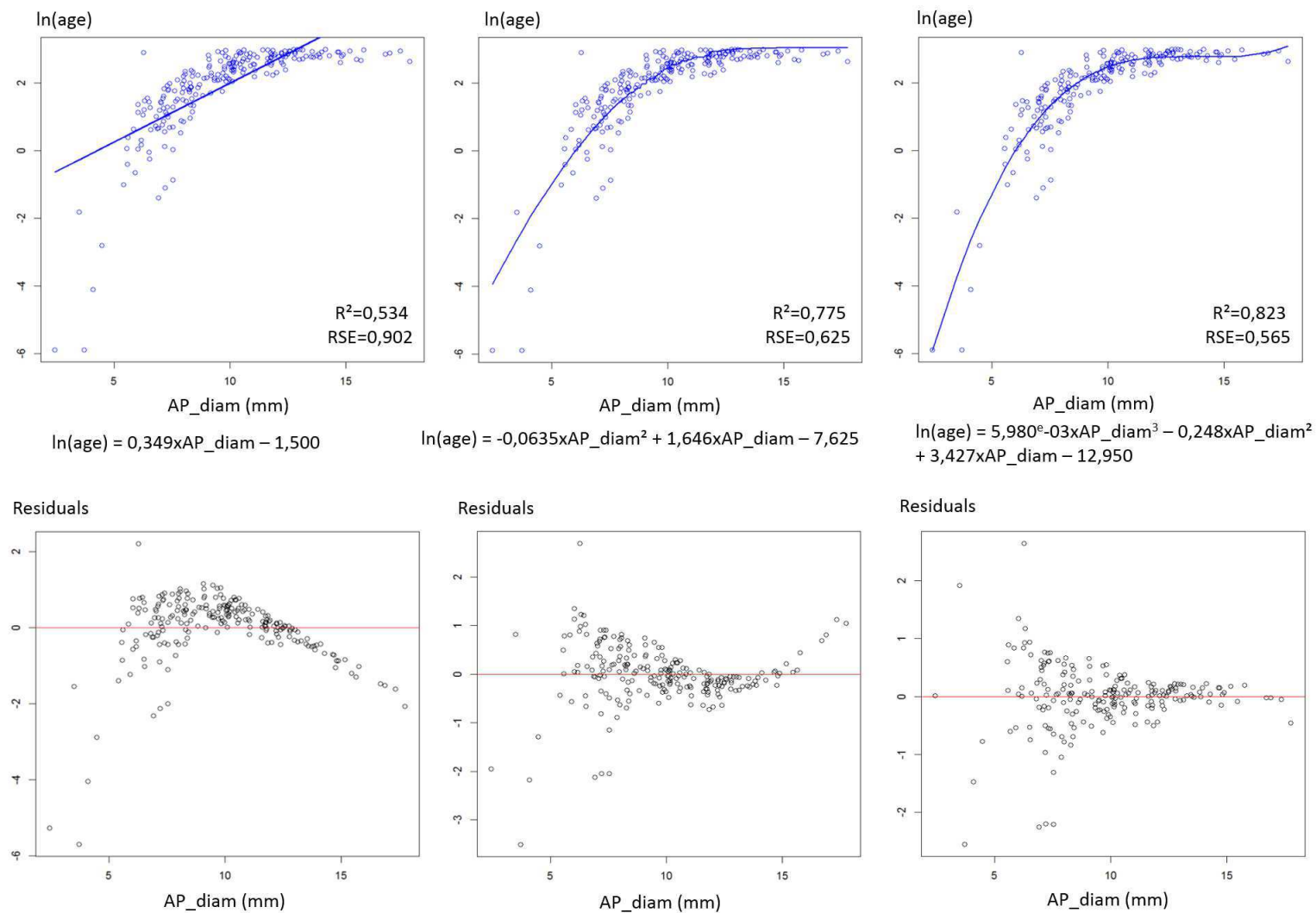


Figure E.55 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against  $\text{AP\_diam}$ , with the mathematical expressions, associated  $R^2$  and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

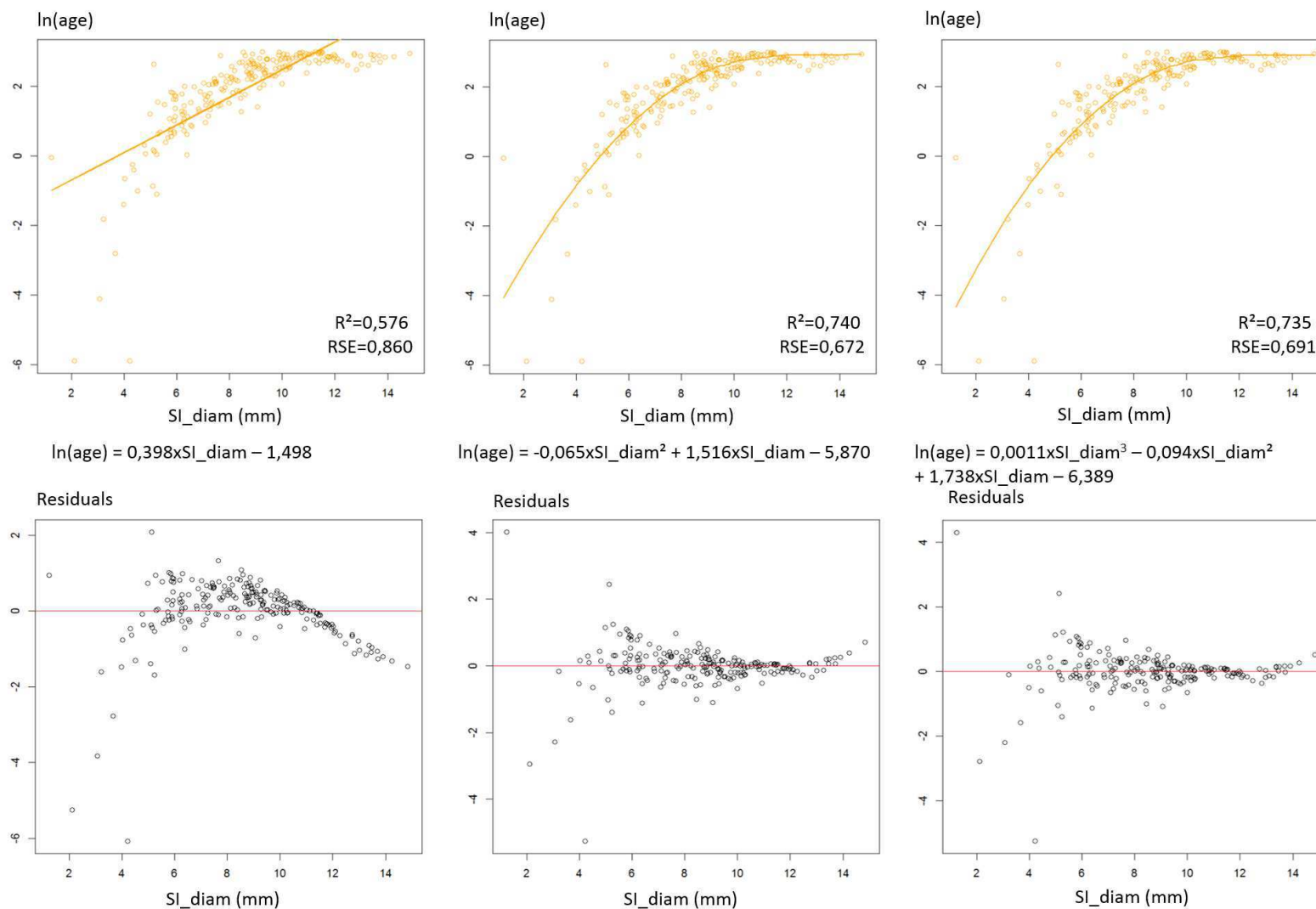


Figure E.56 Age prediction using linear, second and third degree polynomial regressions of  $\ln(\text{age})$  against  $\text{SI\_diam}$ , with the mathematical expressions, associated  $R^2$  and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

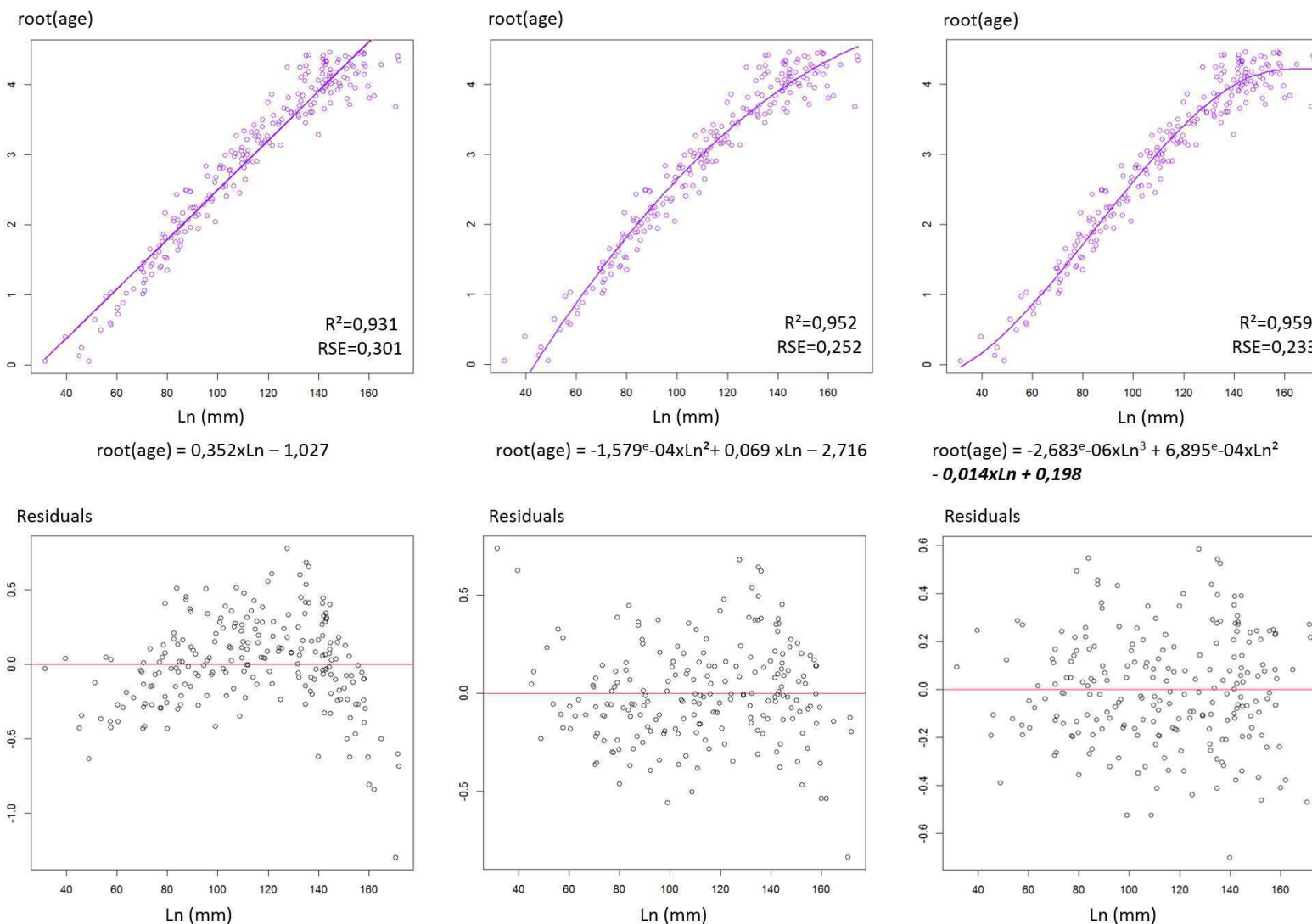


Figure E.57 Age prediction using linear, second and third degree polynomial regressions of root(age) against Ln, with the mathematical expressions, associated R<sup>2</sup> and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

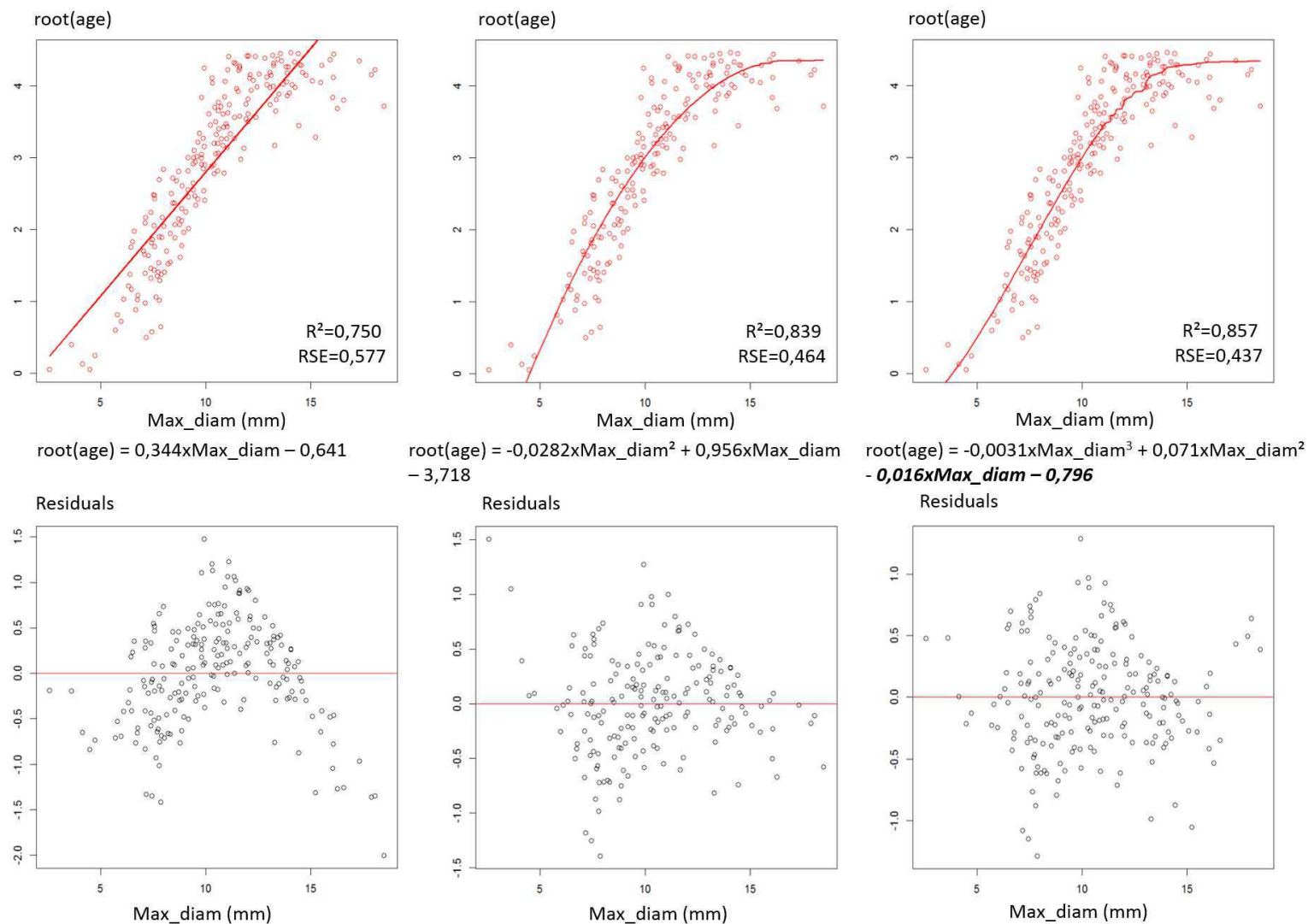


Figure E.58 Age prediction using linear, second and third degree polynomial regressions of root(age) against Max\_diam, with the mathematical expressions, associated R<sup>2</sup> and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

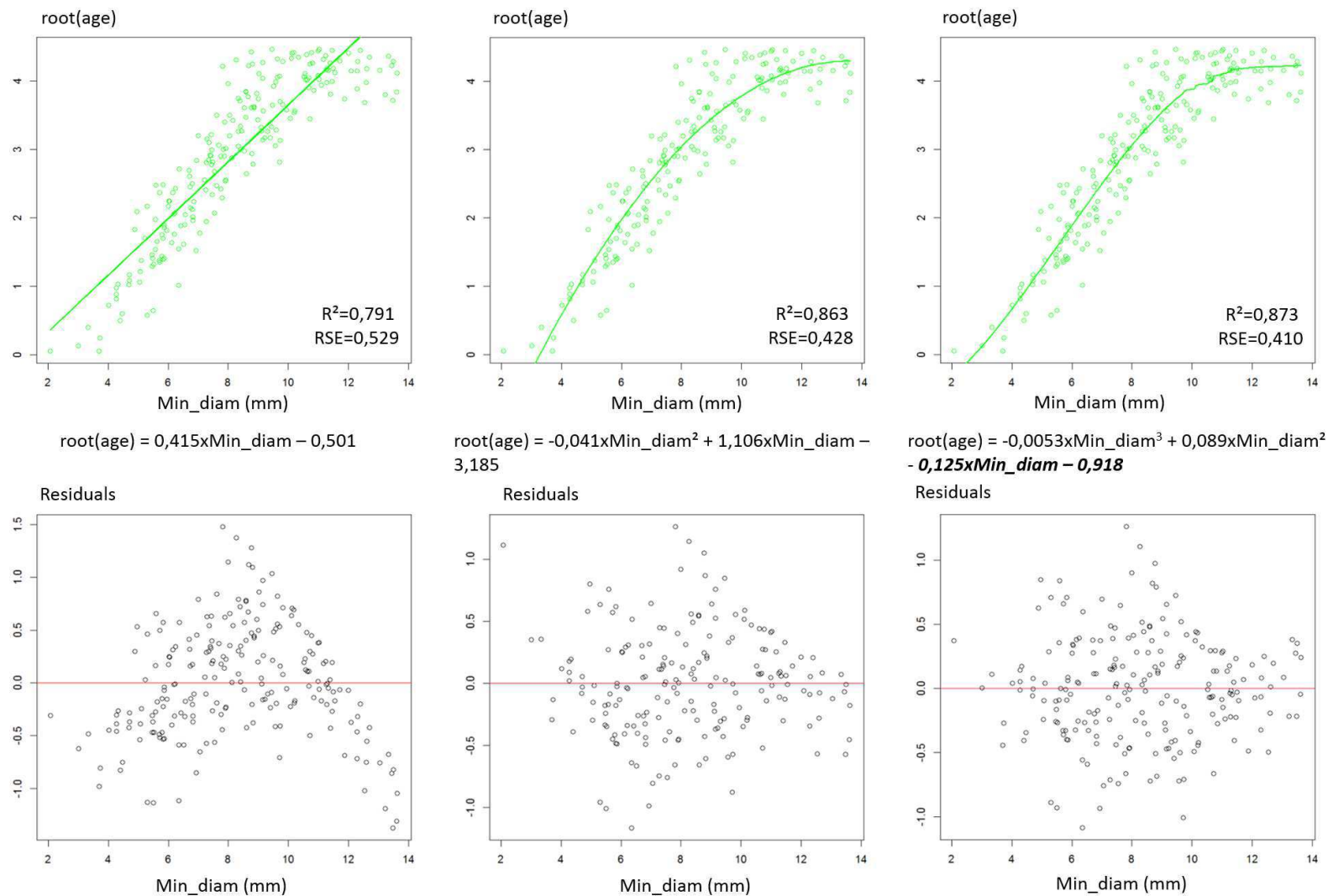


Figure E.59 Age prediction using linear, second and third degree polynomial regressions of root(age) against Min\_diam, with the mathematical expressions, associated R<sup>2</sup> and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

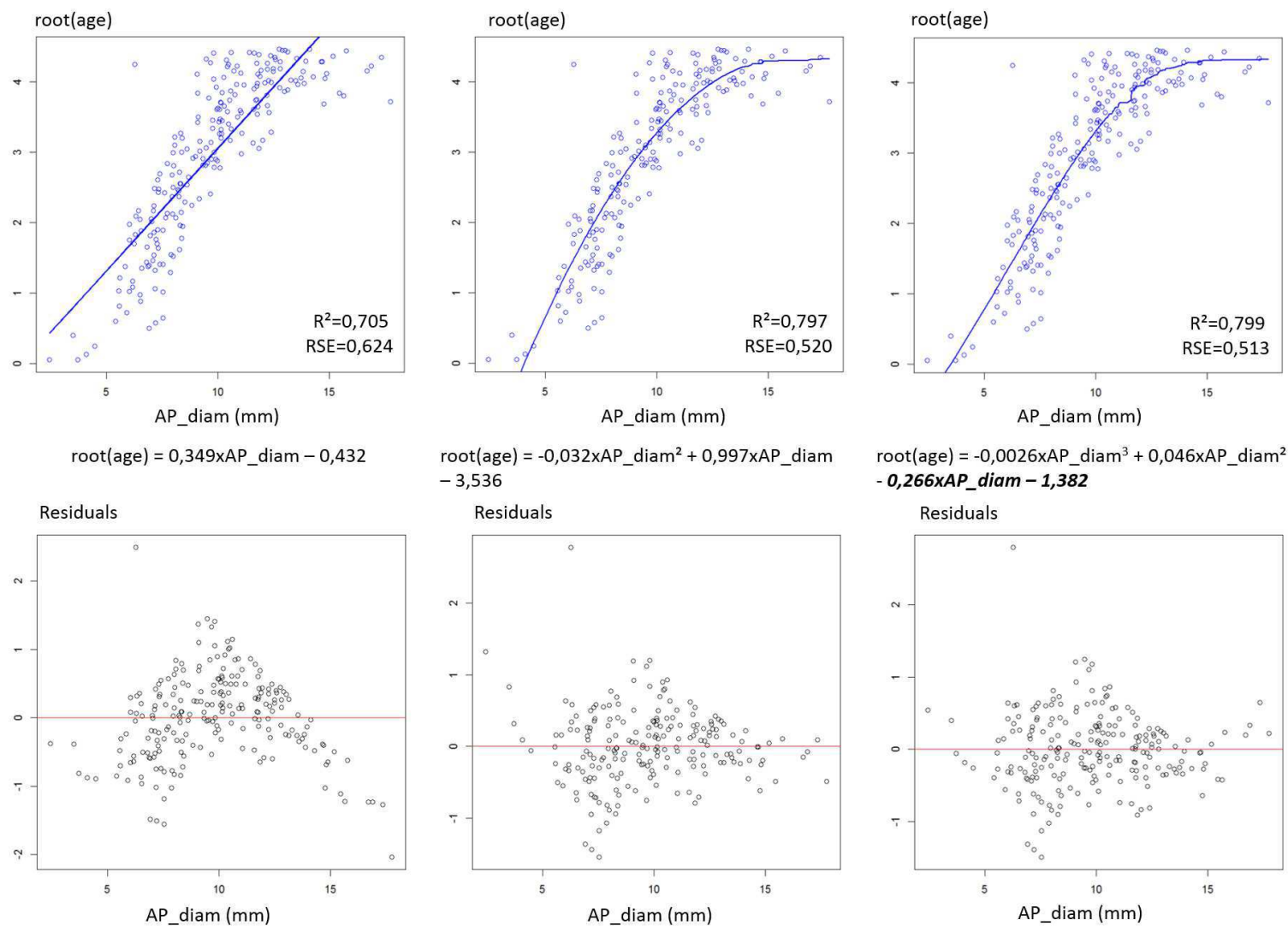


Figure E.60 Age prediction using linear, second and third degree polynomial regressions of root(age) against AP\_diam, with the mathematical expressions, associated R<sup>2</sup> and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients

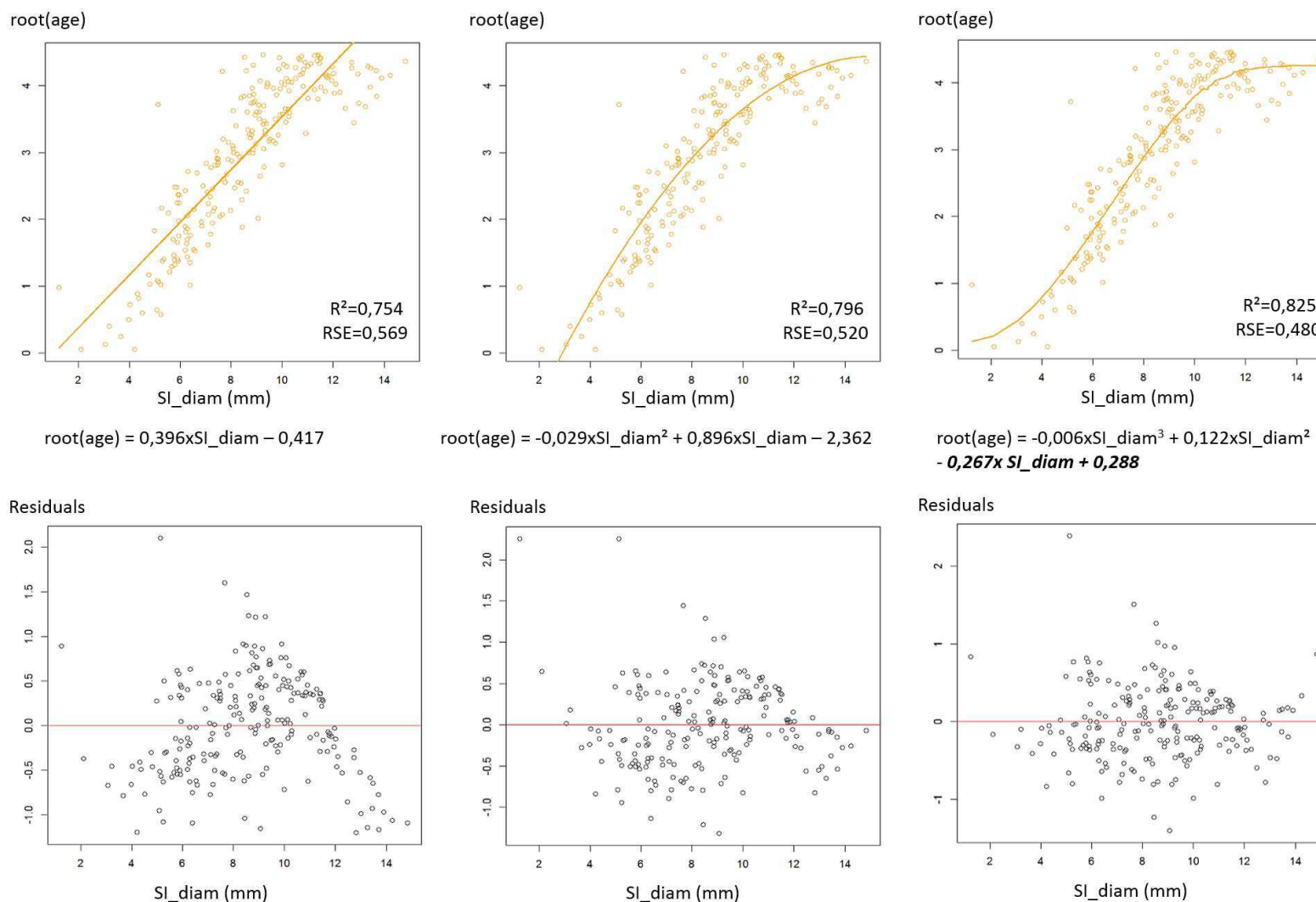


Figure E.61 Age prediction using linear, second and third degree polynomial regressions of root(age) against SI\_diam, with the mathematical expressions, associated R<sup>2</sup> and RSE values and the corresponding plot of the residuals against predictor variables (lower plots). Bold italic terms of the mathematical expressions of the model express non-significant coefficients



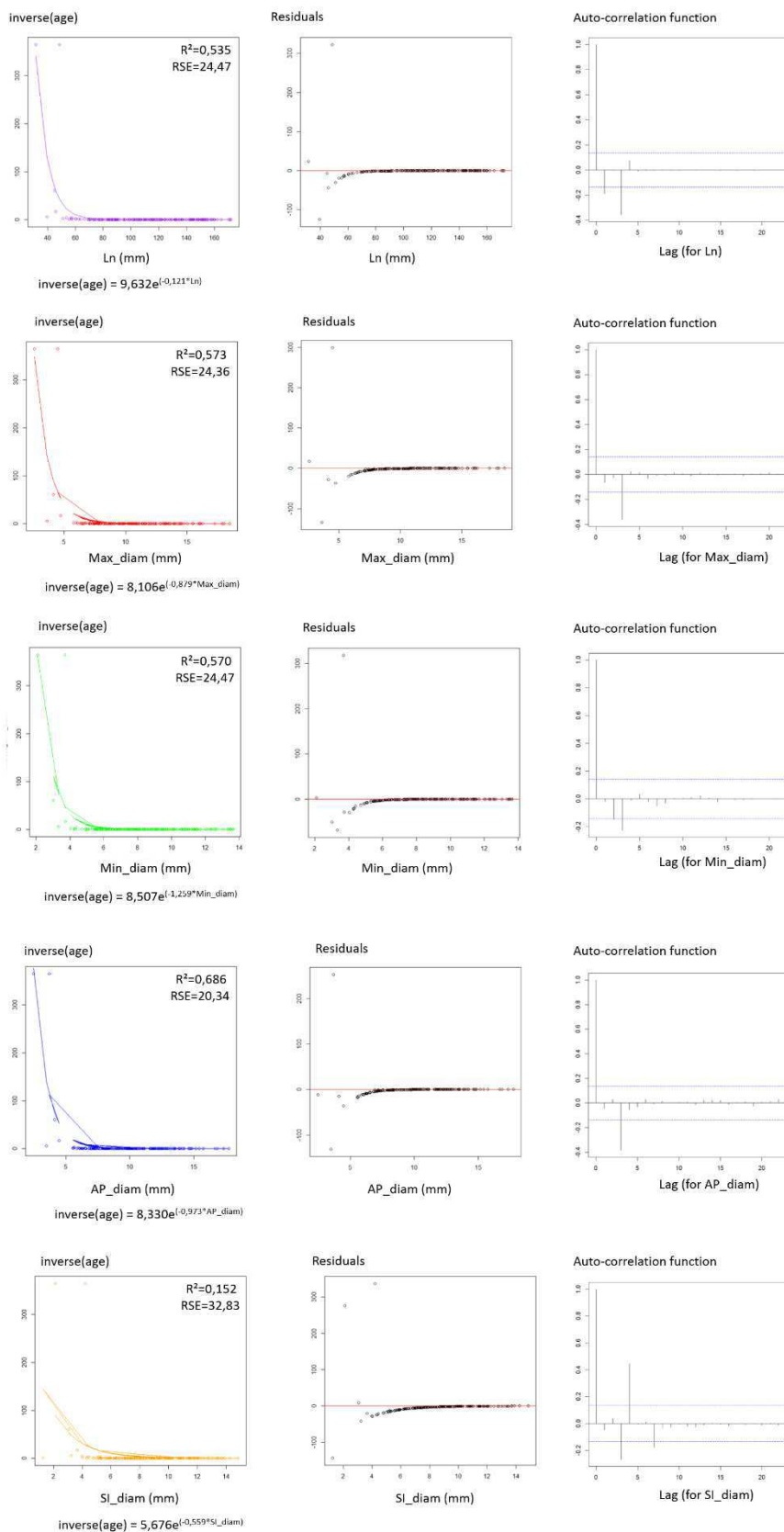


Figure E.62 Exponential regressions of the inverse of age against Ln (purple), Max\_diam (red), Min\_diam (green), AP\_diam (blue) and SI\_diam (yellow) with their mathematical expressions, associated R<sup>2</sup> and RSE values (upper plots), plot of the residuals against the predictor variables (middle plots) and the autocorrelation functions of the residuals (lower plots)

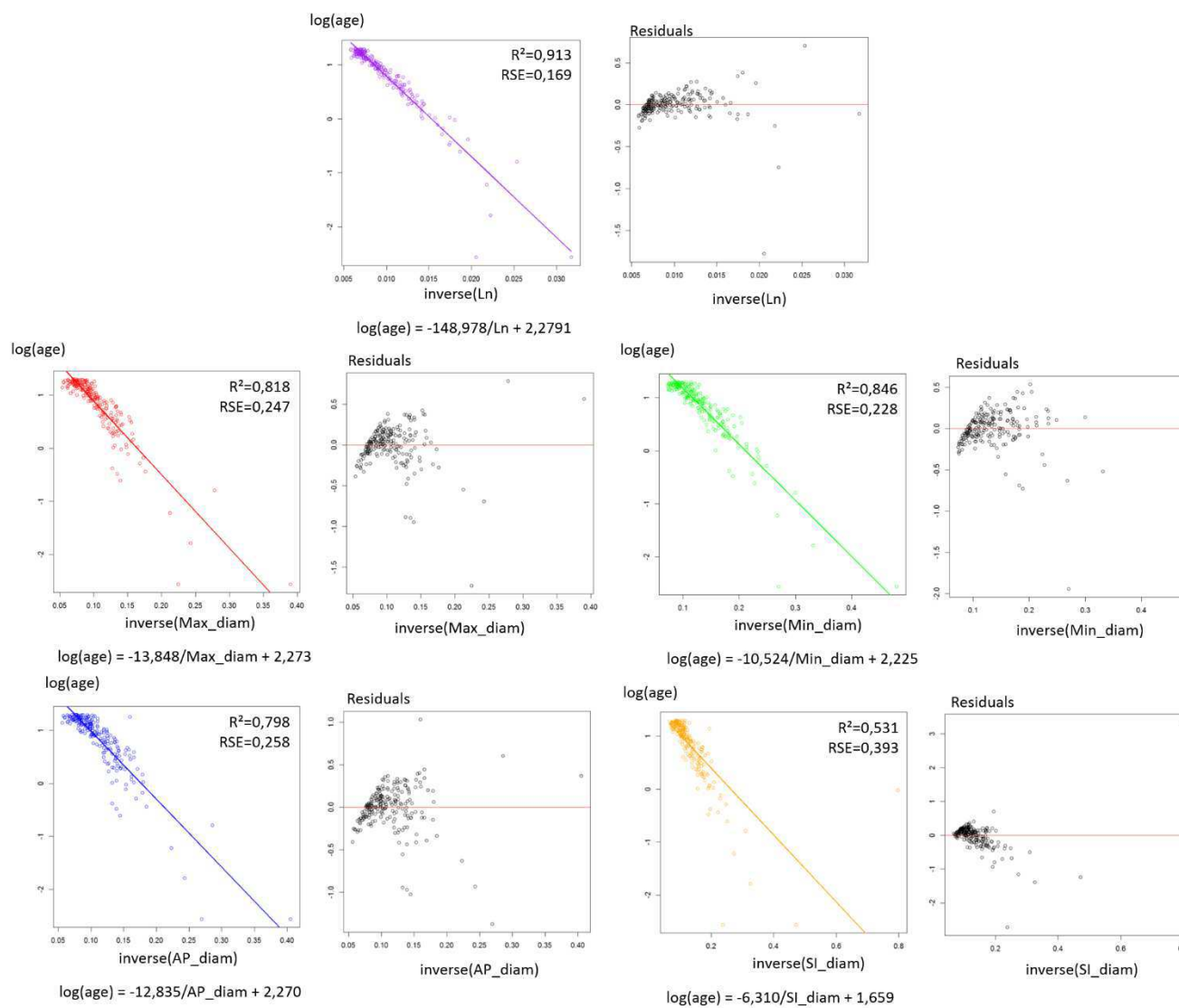


Figure E.63 Age prediction using linear regression of the decimal logarithm of age against the inverse of Ln (purple), Max\_diam (red), Min\_diam (green), AP\_diam (blue) and SI\_diam (yellow) with their mathematical expressions, associated  $R^2$  and RSE values and the plot of the residuals against predictor variables (lower plots)

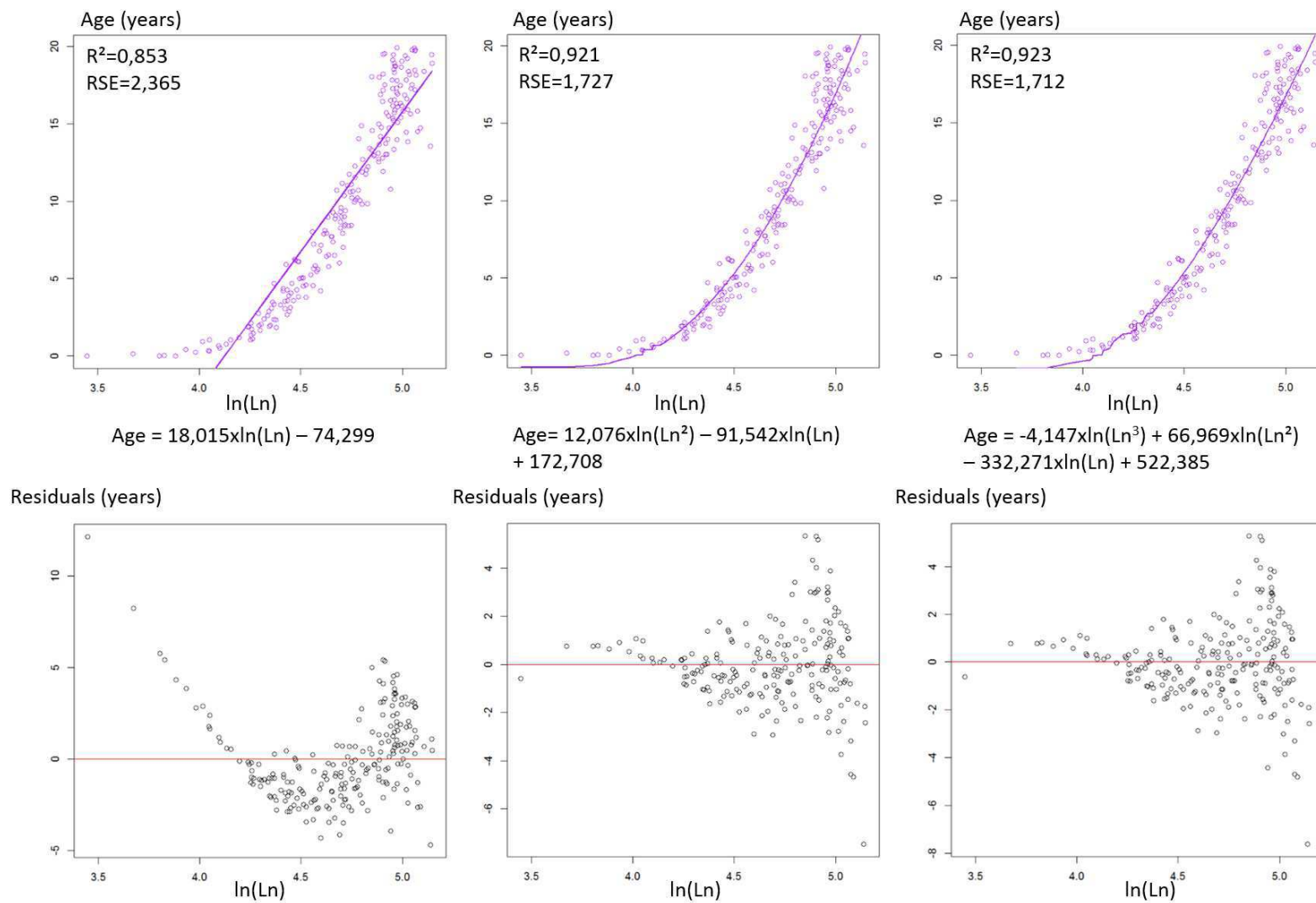


Figure E.64 Age prediction using linear, second and third degree polynomial models of age against ln(Ln) with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plots of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

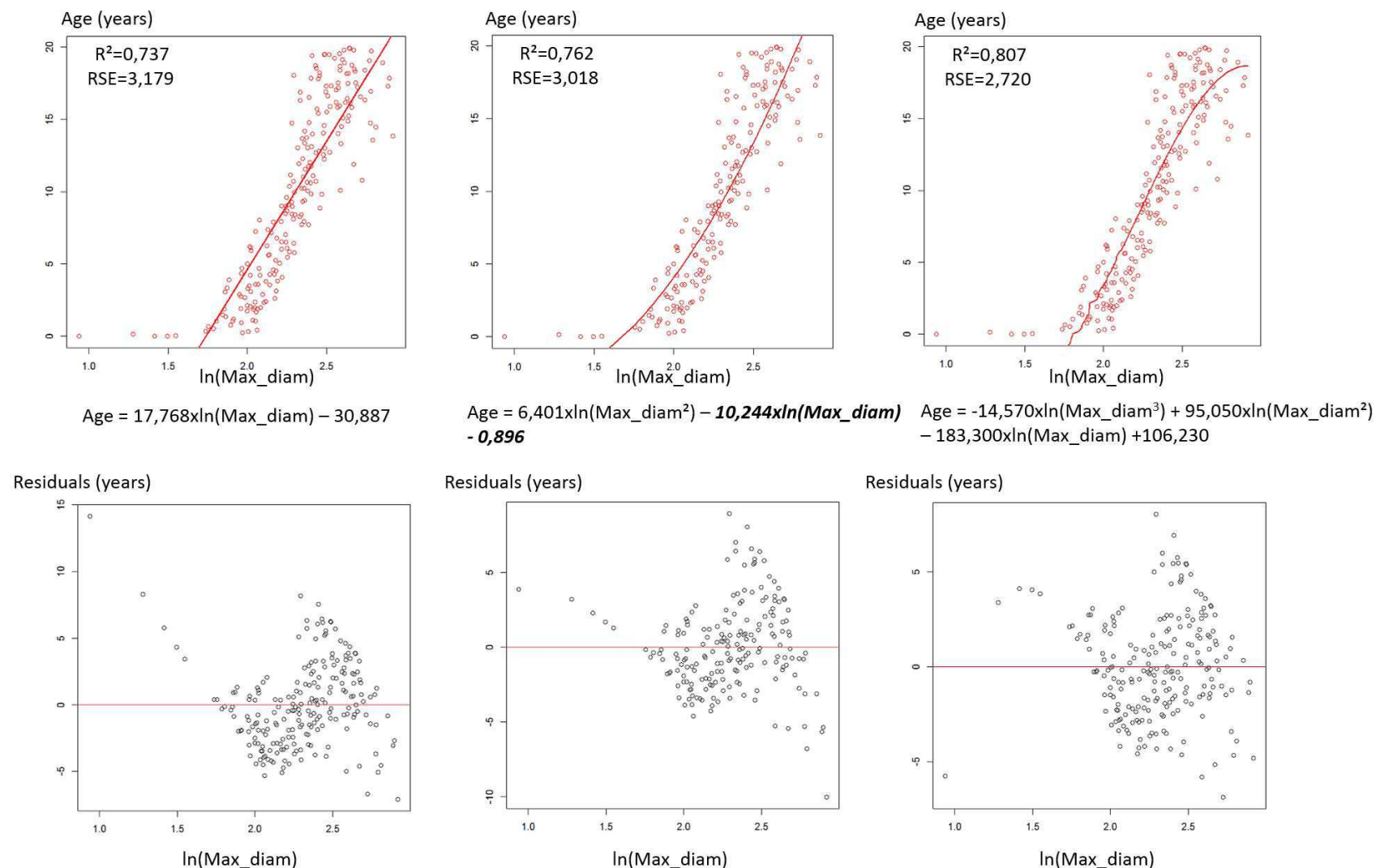


Figure E.65 Age prediction using linear, second and third degree polynomial models of age against ln(Max\_diam) with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plots of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

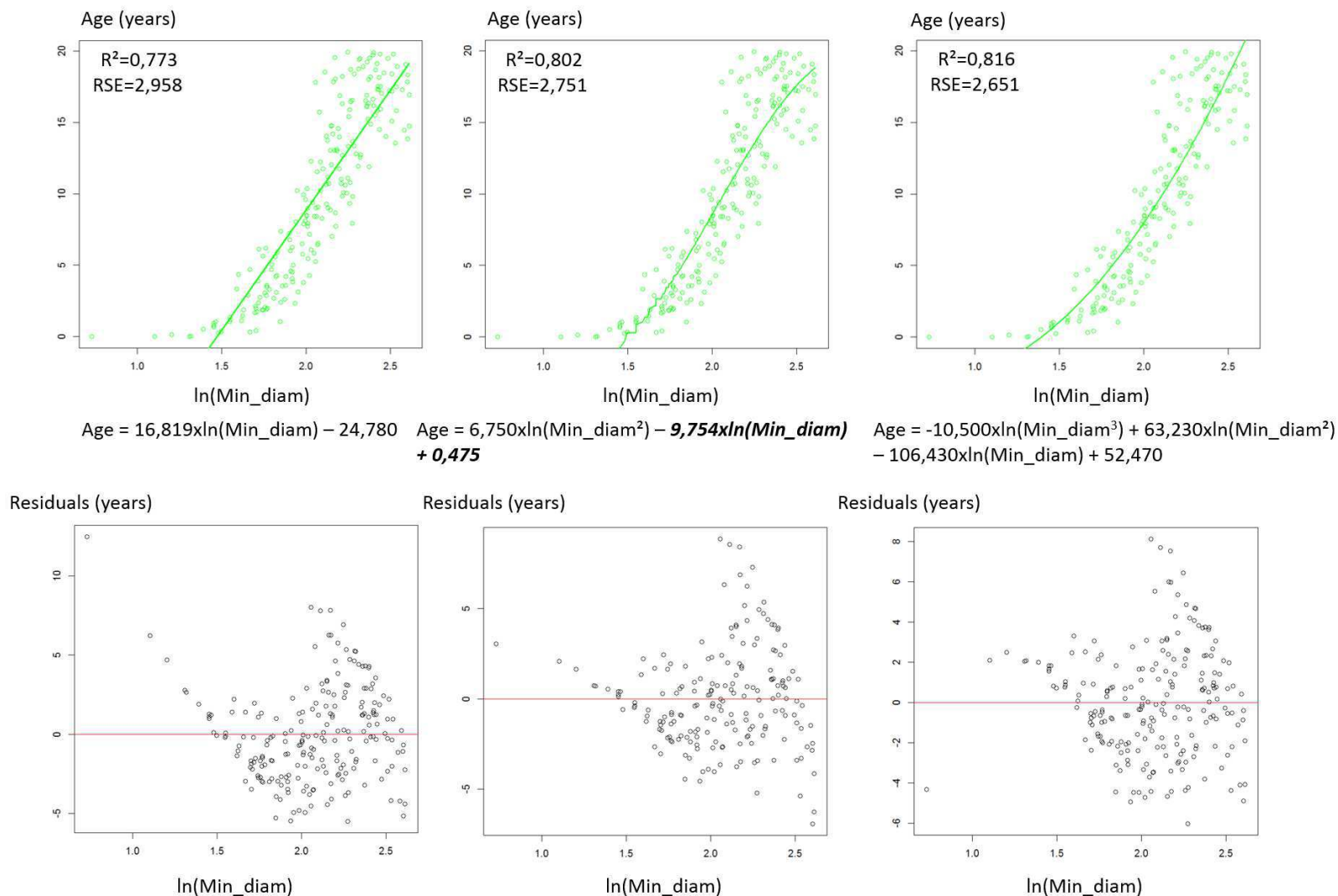


Figure E.66 Age prediction using linear, second and third degree polynomial models of age against ln(Min\_diam) with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plots of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

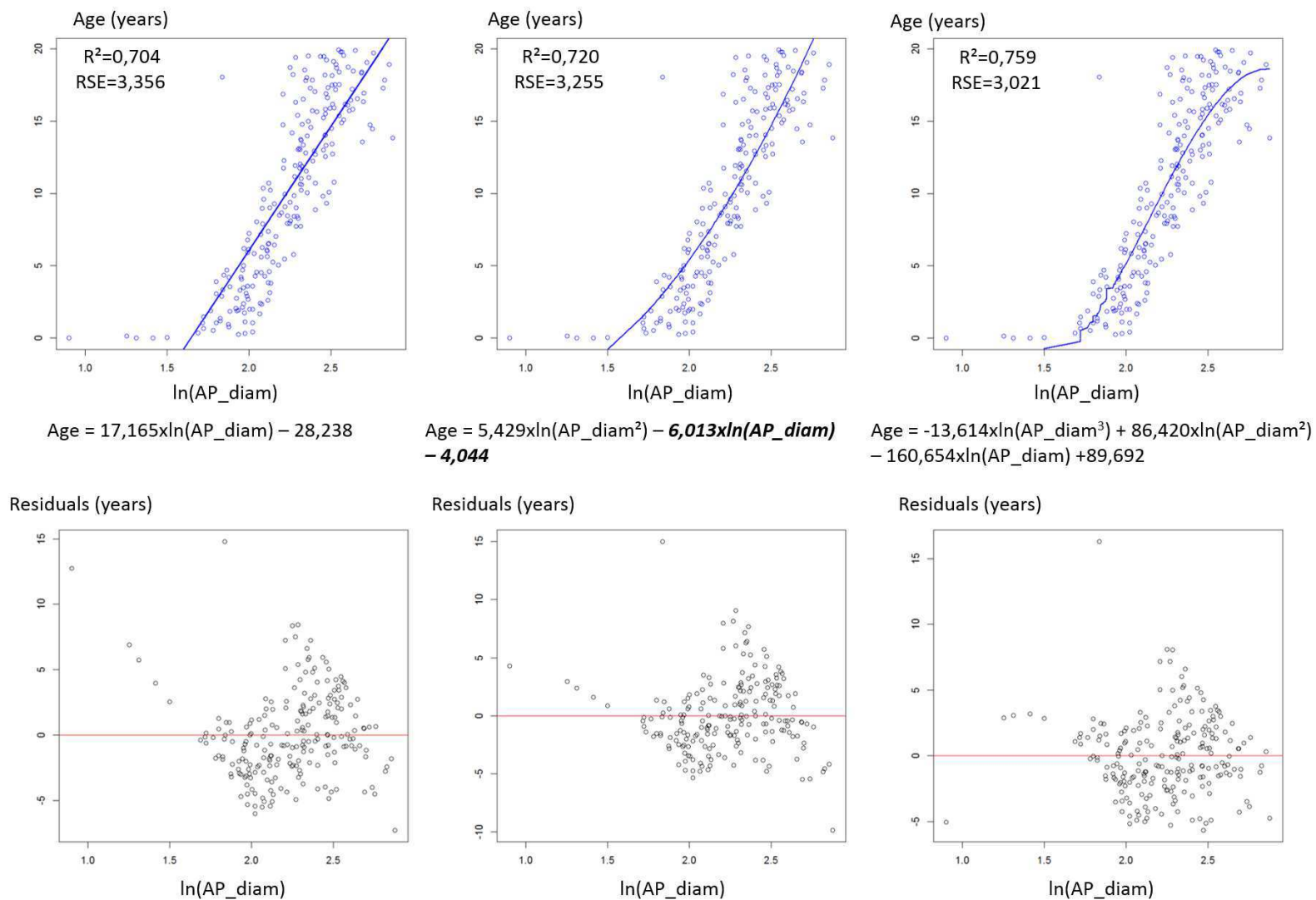


Figure E.67 Age prediction using linear, second and third degree polynomial models of age against ln(AP\_diam) with their mathematical expressions, associated R<sup>2</sup> and RSE values and the plots of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

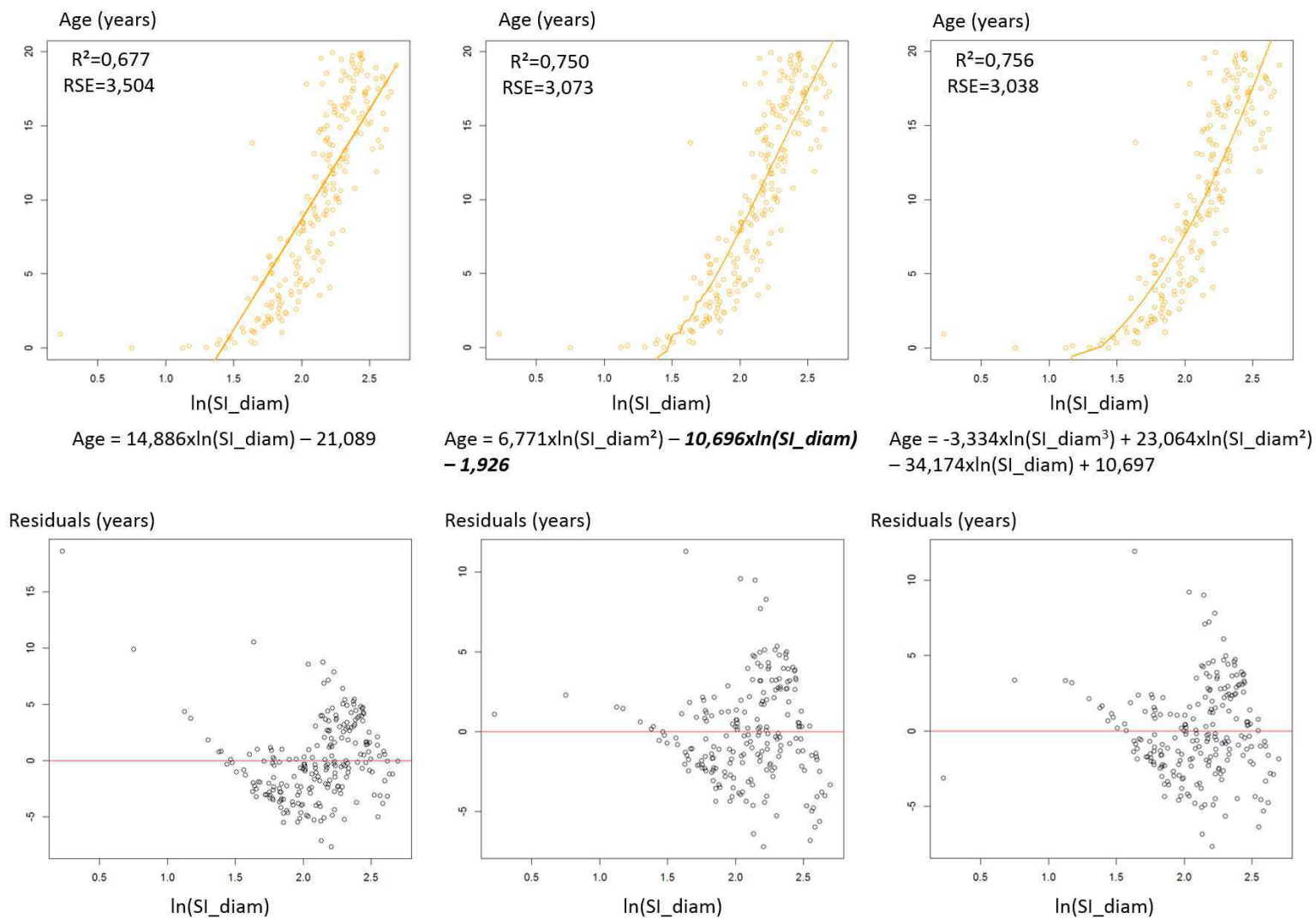


Figure E.68 Age prediction using linear, second and third degree polynomial models of age against  $\ln(SI\_diam)$  with their mathematical expressions, associated  $R^2$  and RSE values and the plots of the residuals against predictor variables (lower plots). Bold italic terms correspond to non-significant regression coefficients

## Weighted Least Squares

### Iliac variables

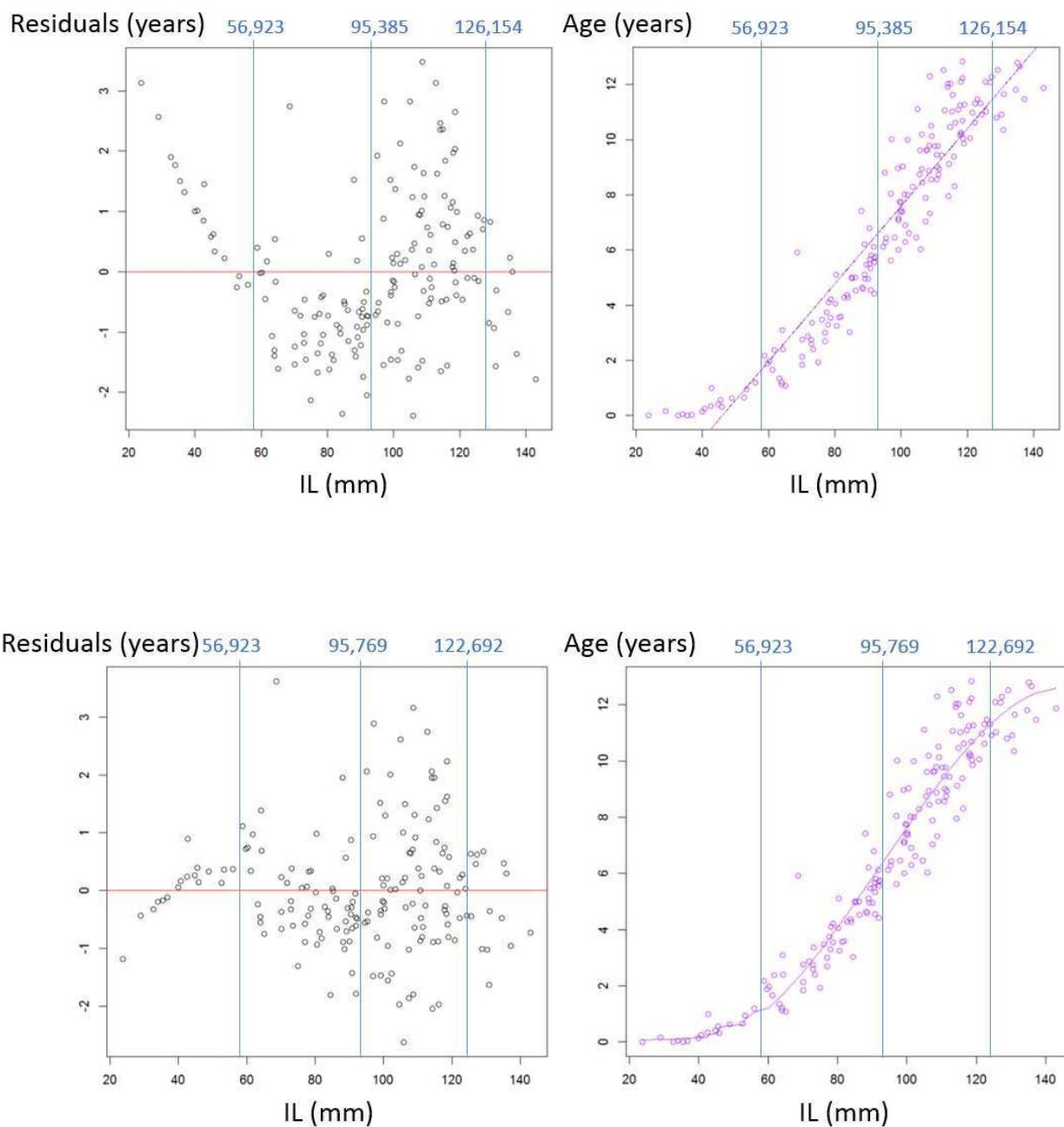


Figure E.69 The intervals of IL values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by IL. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (IL) (upper and lower right plots)



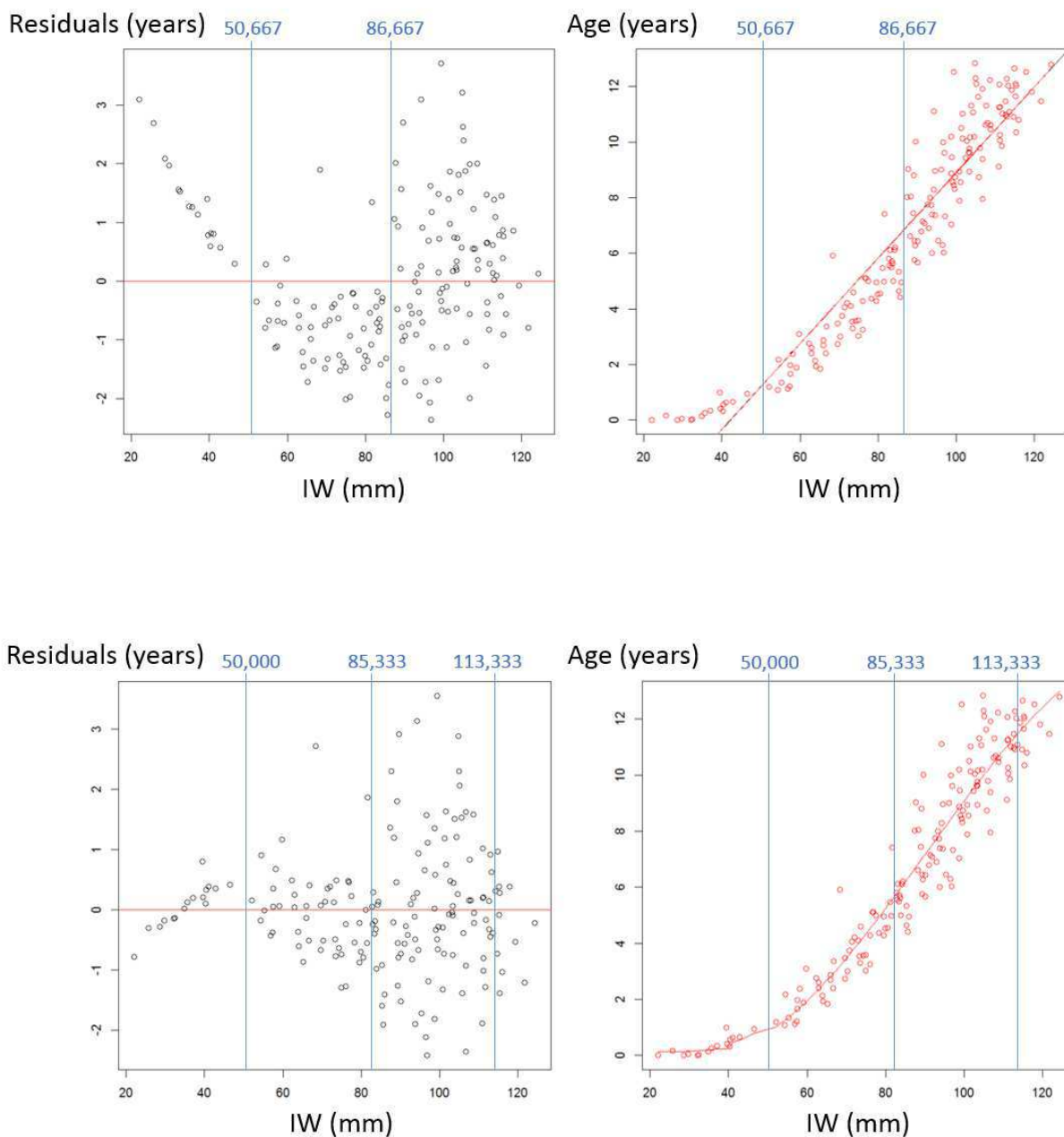


Figure E.70 The intervals of IW values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by IW. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (IW) (upper and lower right plots)

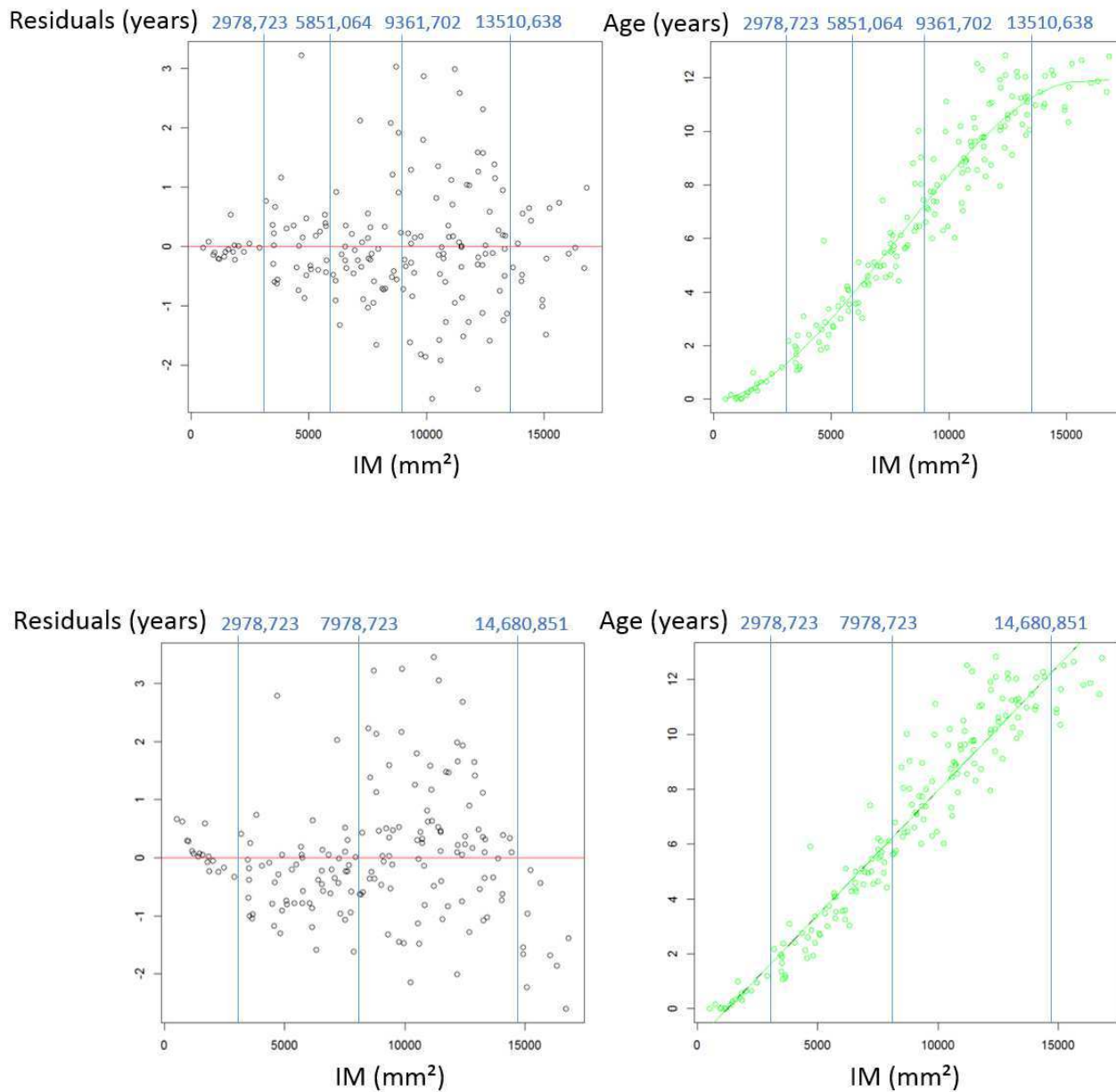


Figure E.71 The intervals of IM values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by IM. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (IM) (upper and lower right plots)

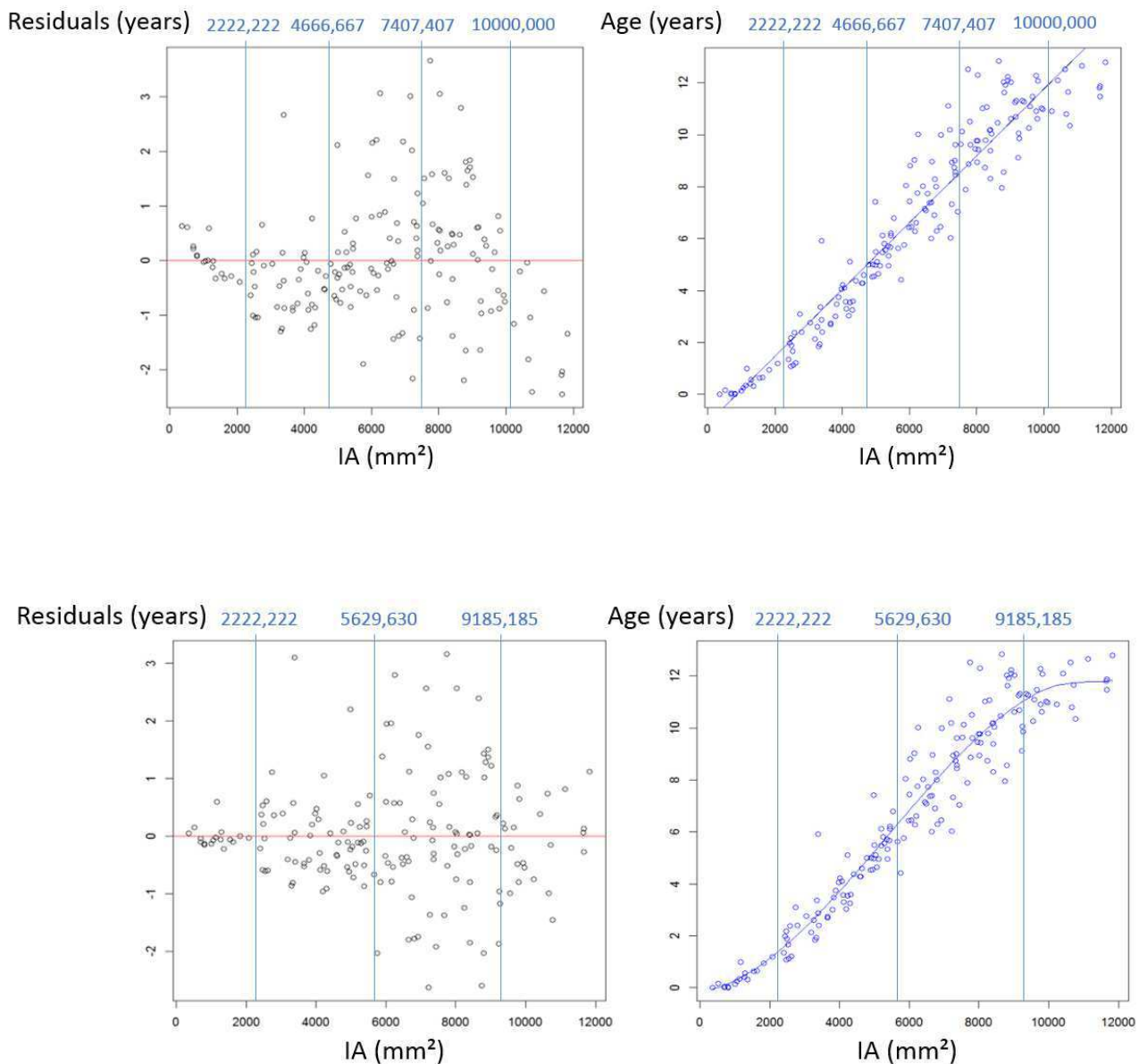


Figure E.72 The intervals of IA values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by IA. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (IA) (upper and lower right plots)

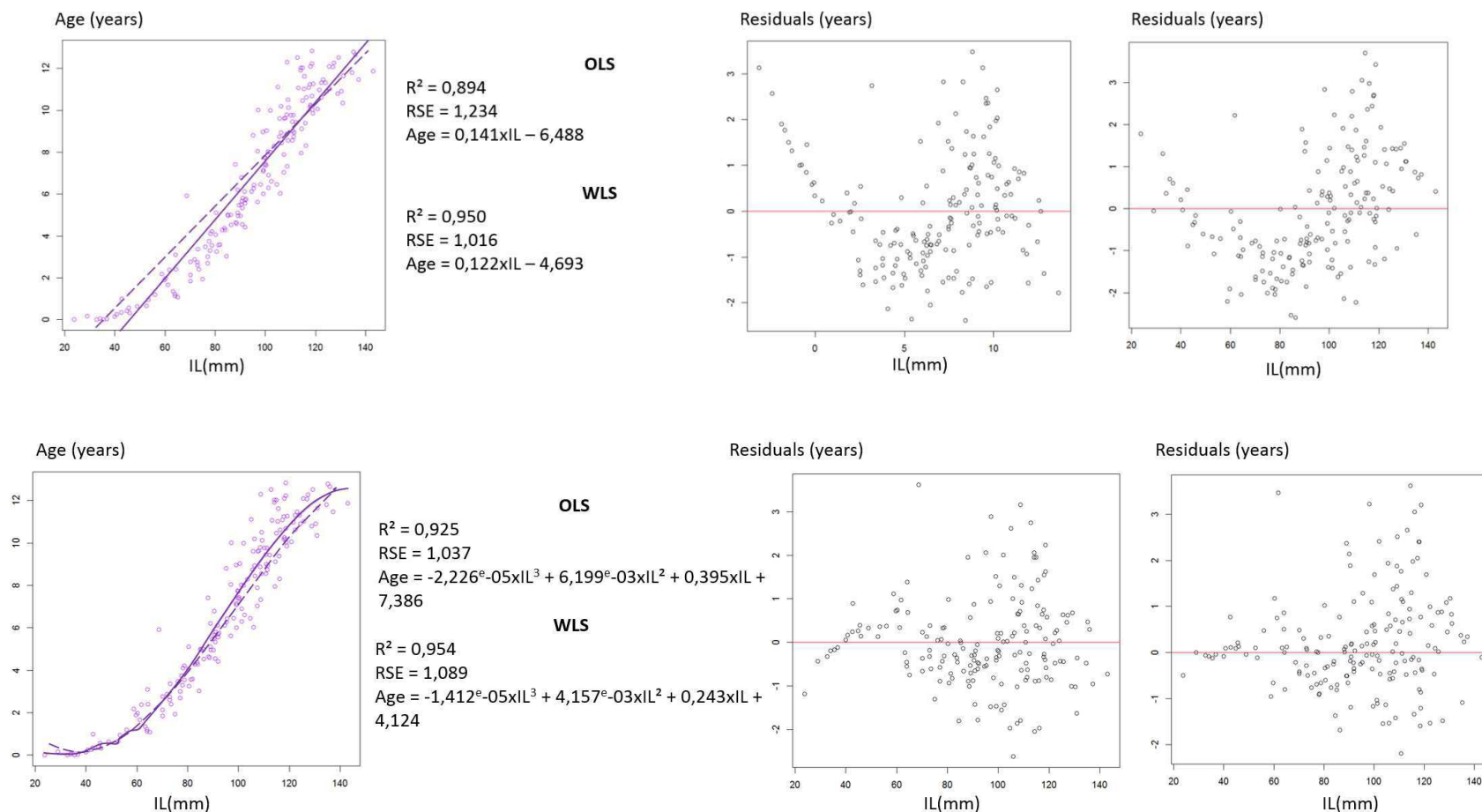


Figure E.73 Weighted Least Squares regressions of age against IL.

Upper line: Plot of age against IL, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against IL. Right plot: plot of residuals of the WLS linear regression against IL;

Lower line: Plot of age against IL, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against IL; Right plot: plot of residuals of the WLS polynomial regression against IL.

**Bold italic terms correspond to non-significant regression coefficients**

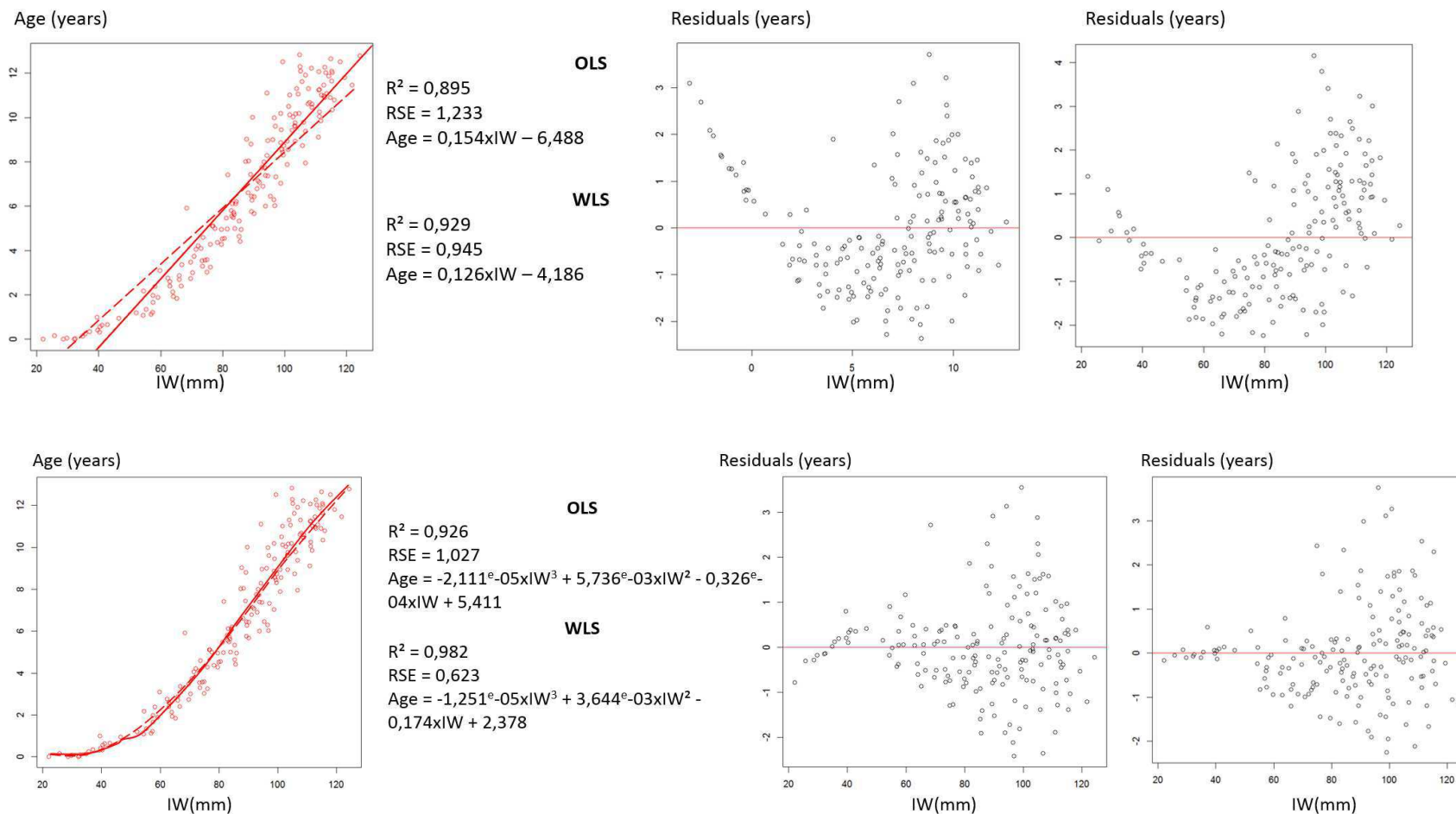


Figure E.74 Weighted Least Squares regressions of age against IW.

Upper line: Plot of age against IW, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against IW. Right plot: plot of residuals of the WLS linear regression against IW; Lower line: Plot of age against IW, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against IW; Right plot: plot of residuals of the WLS polynomial regression against IW.

**Bold italic terms correspond to non-significant regression coefficients**

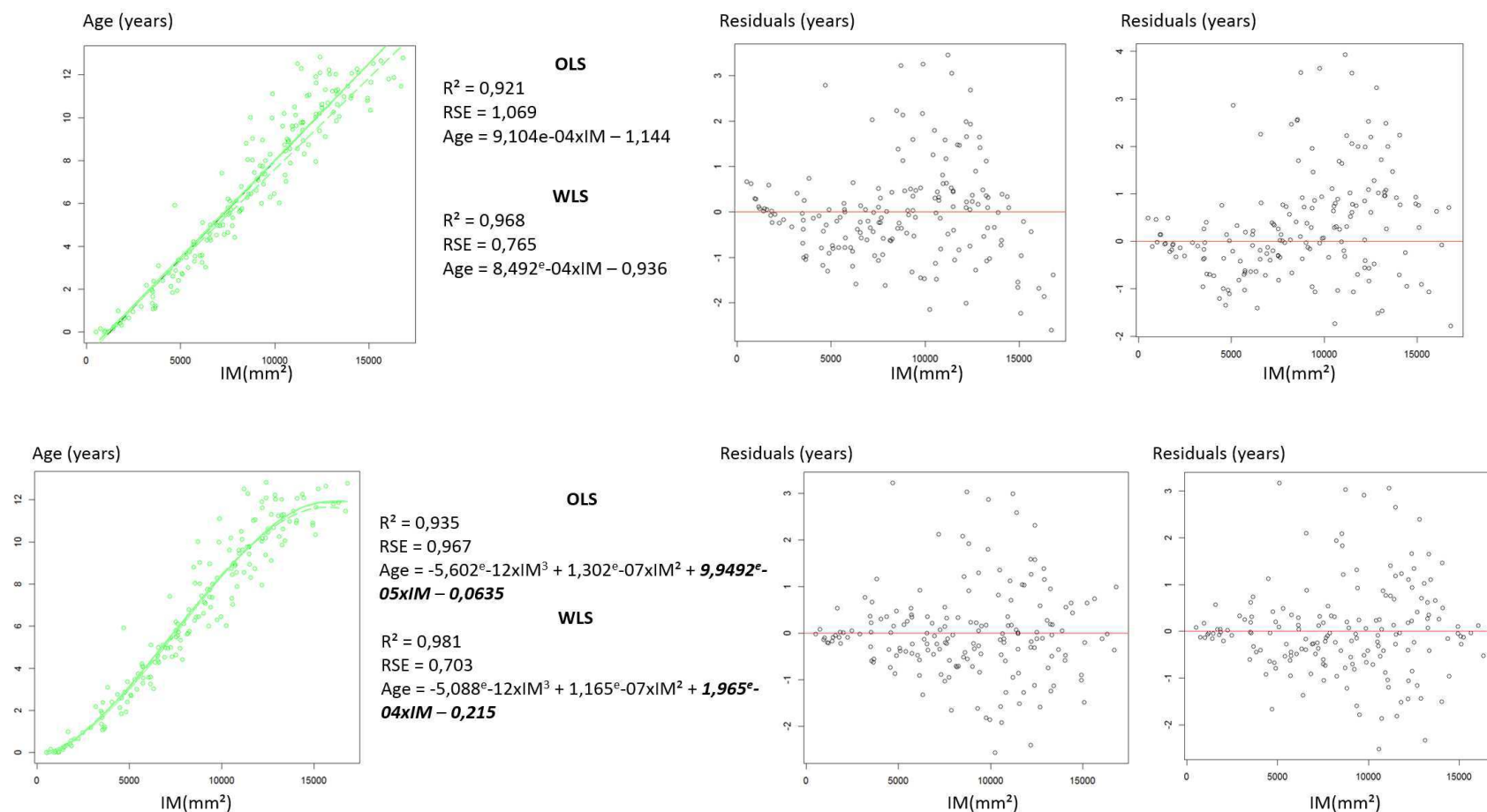


Figure E.75 Weighted Least Squares regressions of age against IM.

Upper line: Plot of age against IM, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against IM. Right plot: plot of residuals of the WLS linear regression against IM; Lower line: Plot of age against IM, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against IM; Right plot: plot of residuals of the WLS polynomial regression against IM.

Bold italic terms correspond to non-significant regression coefficients

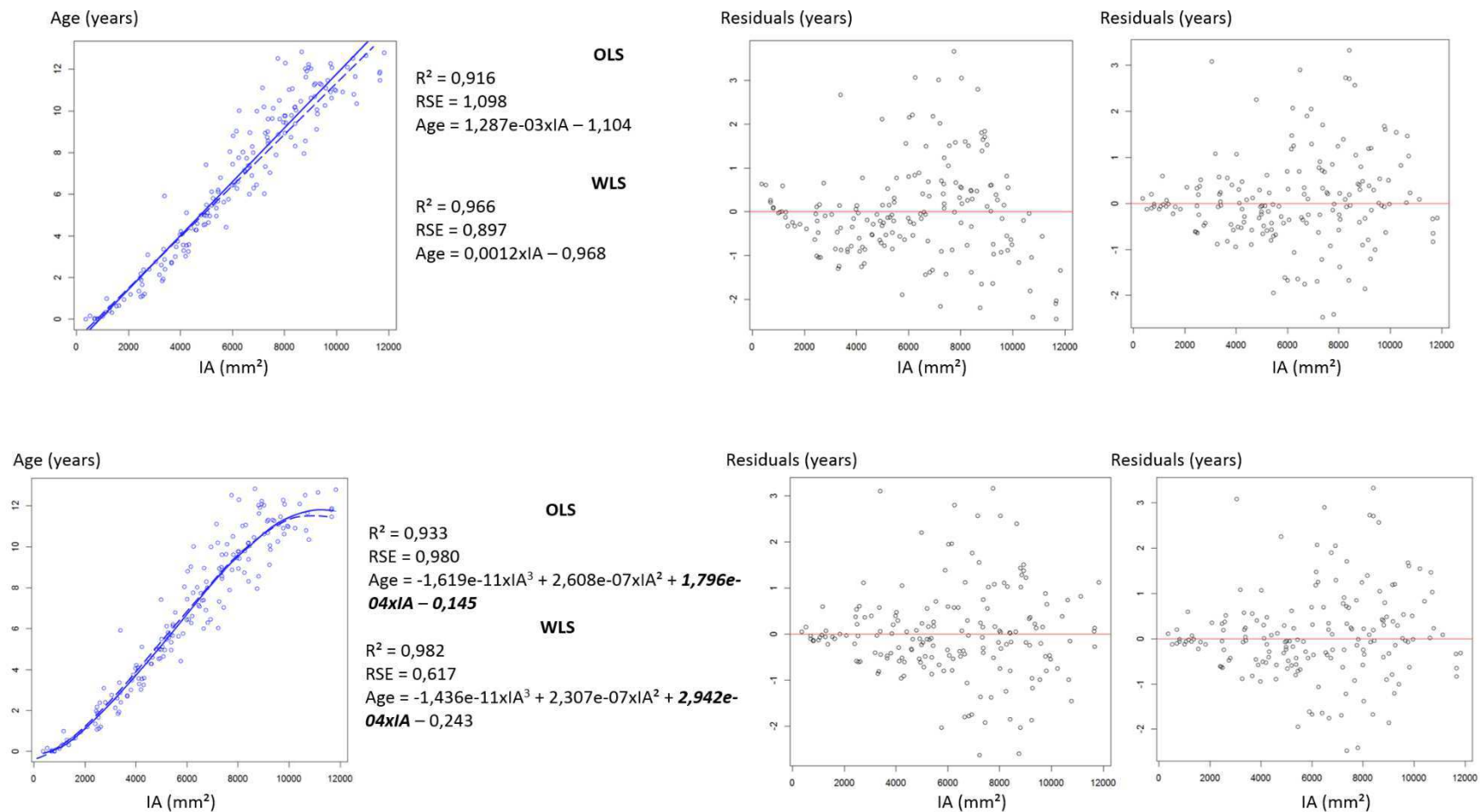


Figure E.76 Weighted Least Squares regressions of age against IA.

Upper line: Plot of age against IA, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against IA. Right plot: plot of residuals of the WLS linear regression against IA;

Lower line: Plot of age against IA, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against IA; Right plot: plot of residuals of the WLS polynomial regression against IA.

Bold italic terms correspond to non-significant regression coefficients

## Lumbar variables

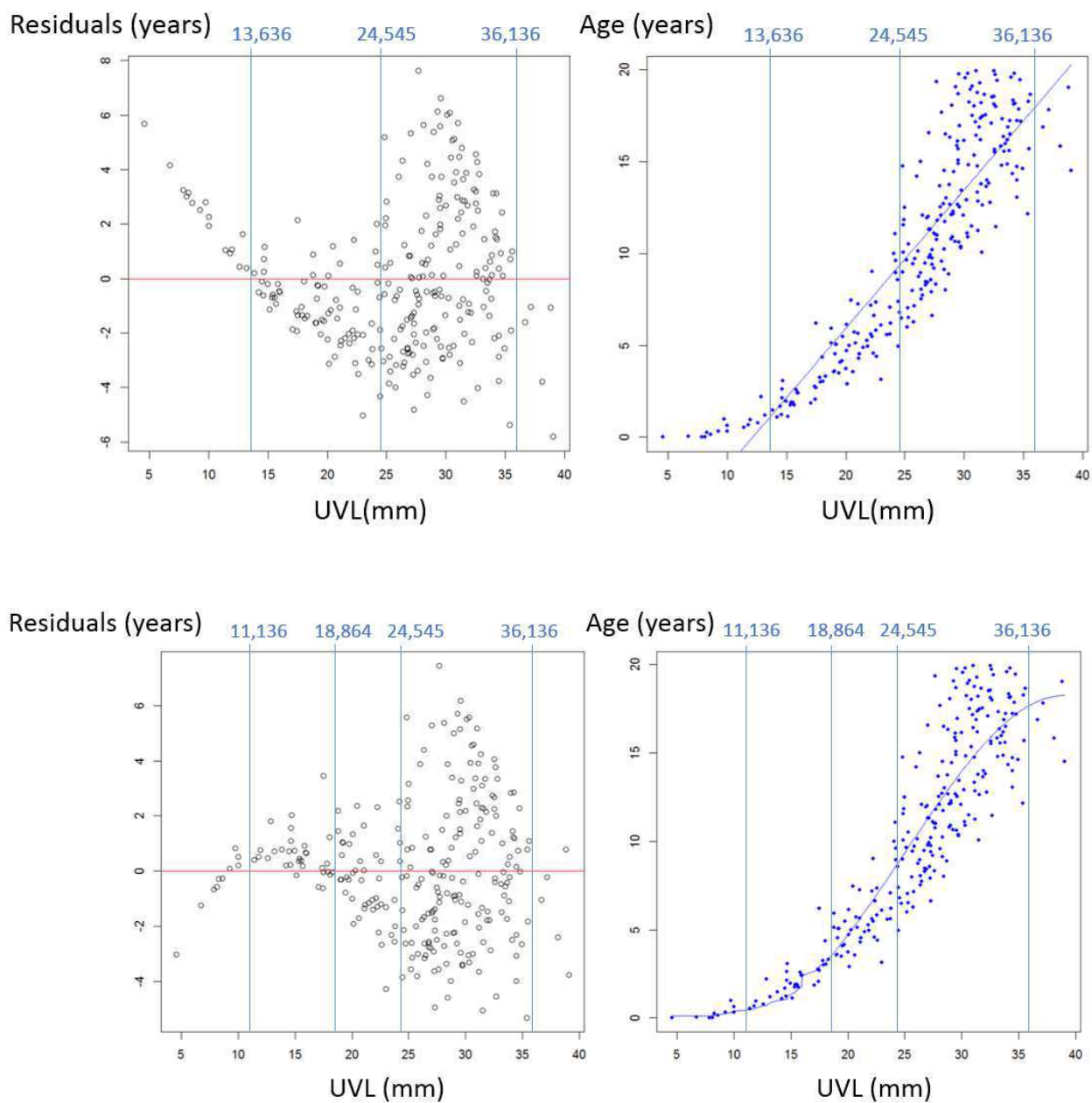


Figure E.77 The intervals of UVL values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by UVL. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (UVL) (upper and lower right plots)



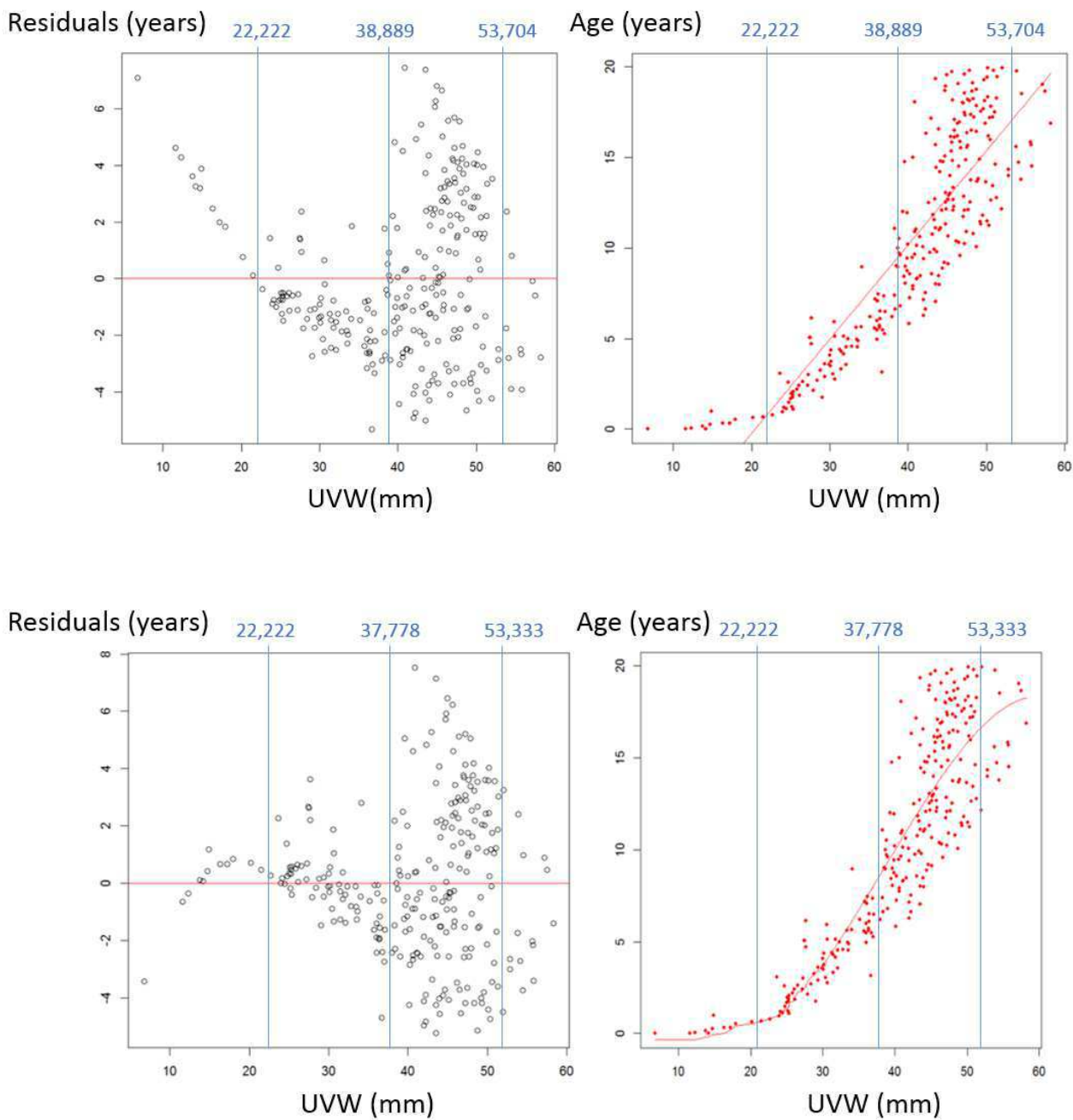


Figure E.78 The intervals of UVW values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by UVW. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (UVW) (upper and lower right plots)

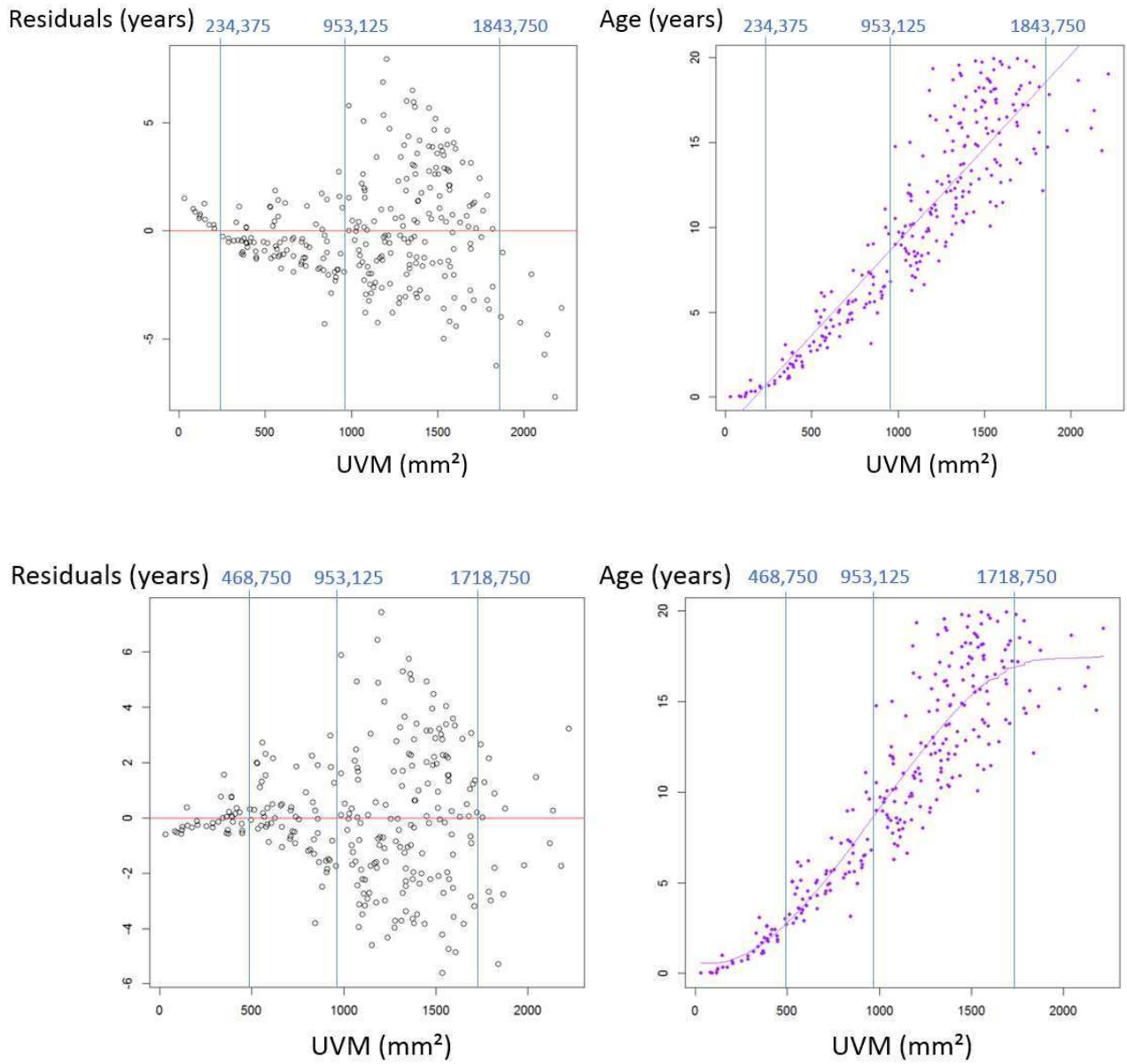


Figure E.79 The intervals of UVM values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by UVM. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (UVM) (upper and lower right plots)

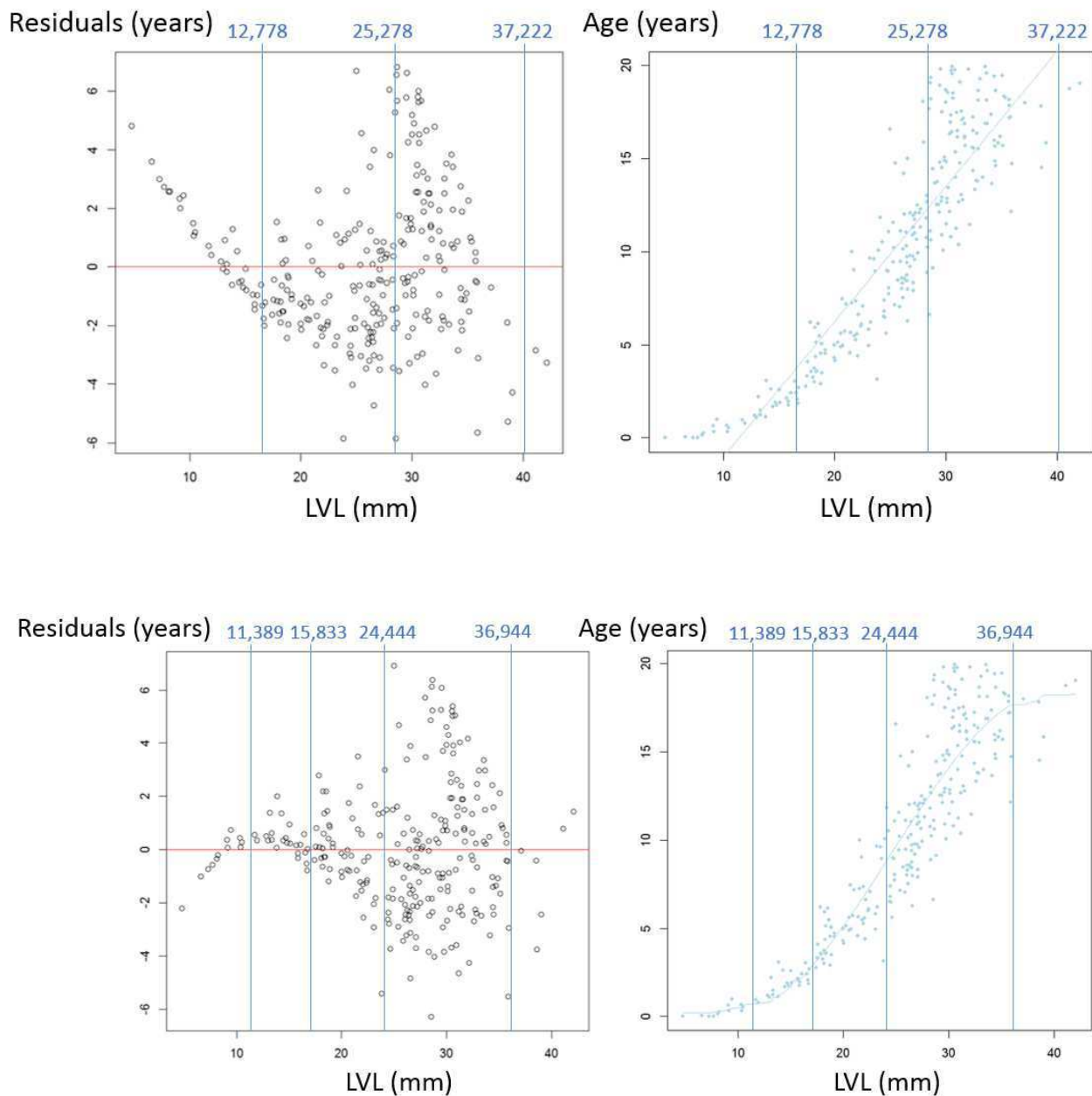


Figure E.80 The intervals of LVL values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by LVL. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (LVL) (upper and lower right plots)

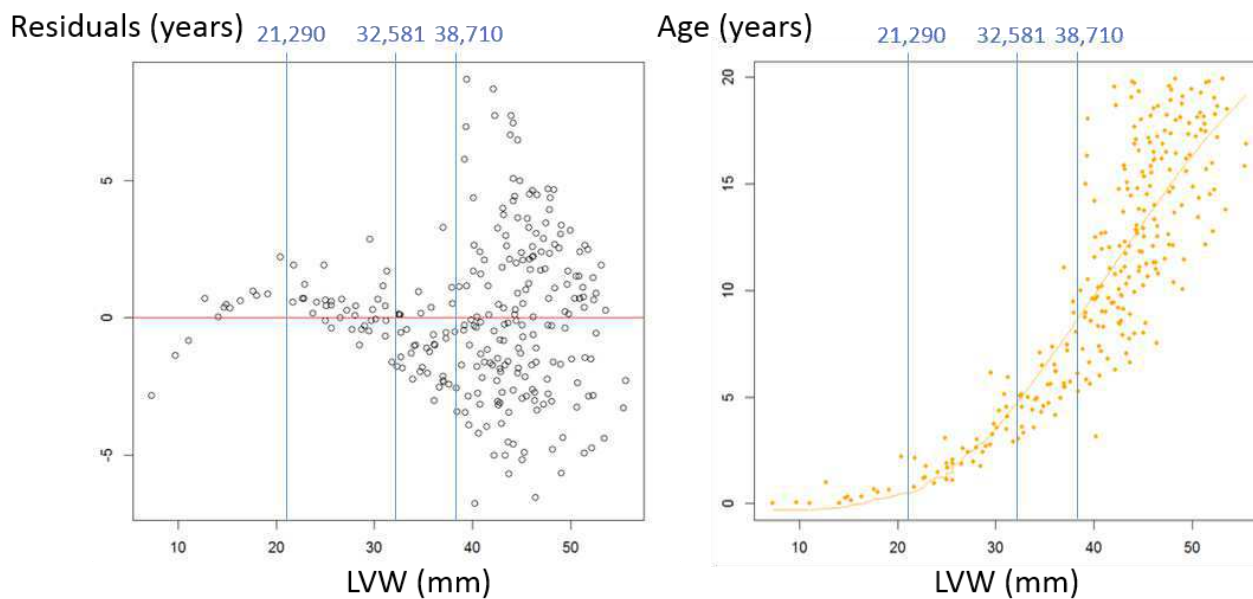
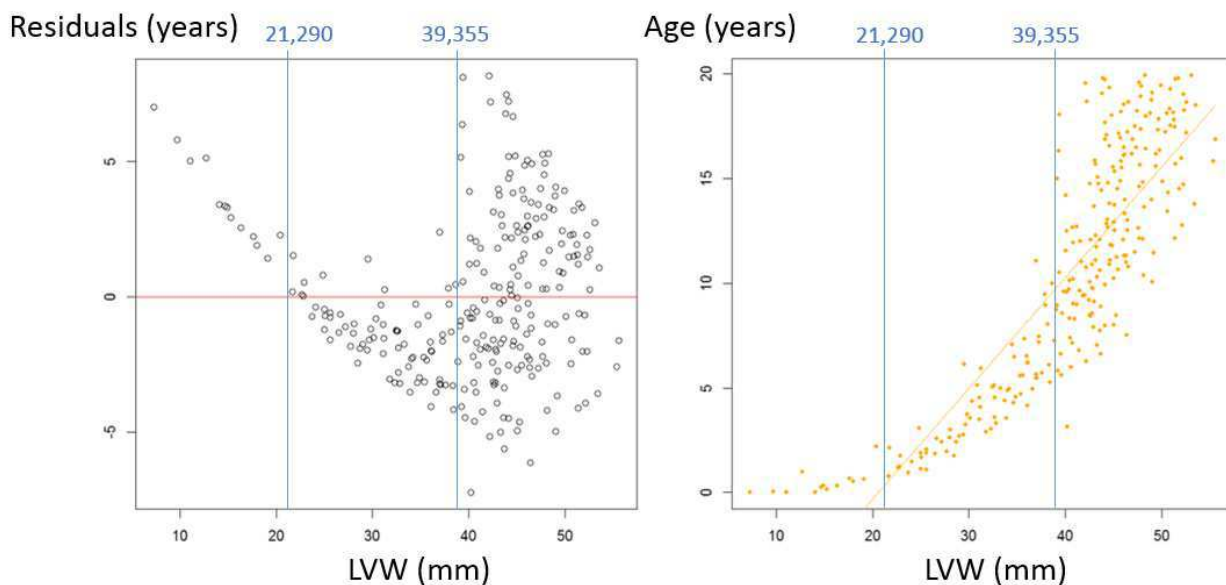


Figure E.81 The intervals of LVW values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by LVW. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (LVW) (upper and lower right plots)

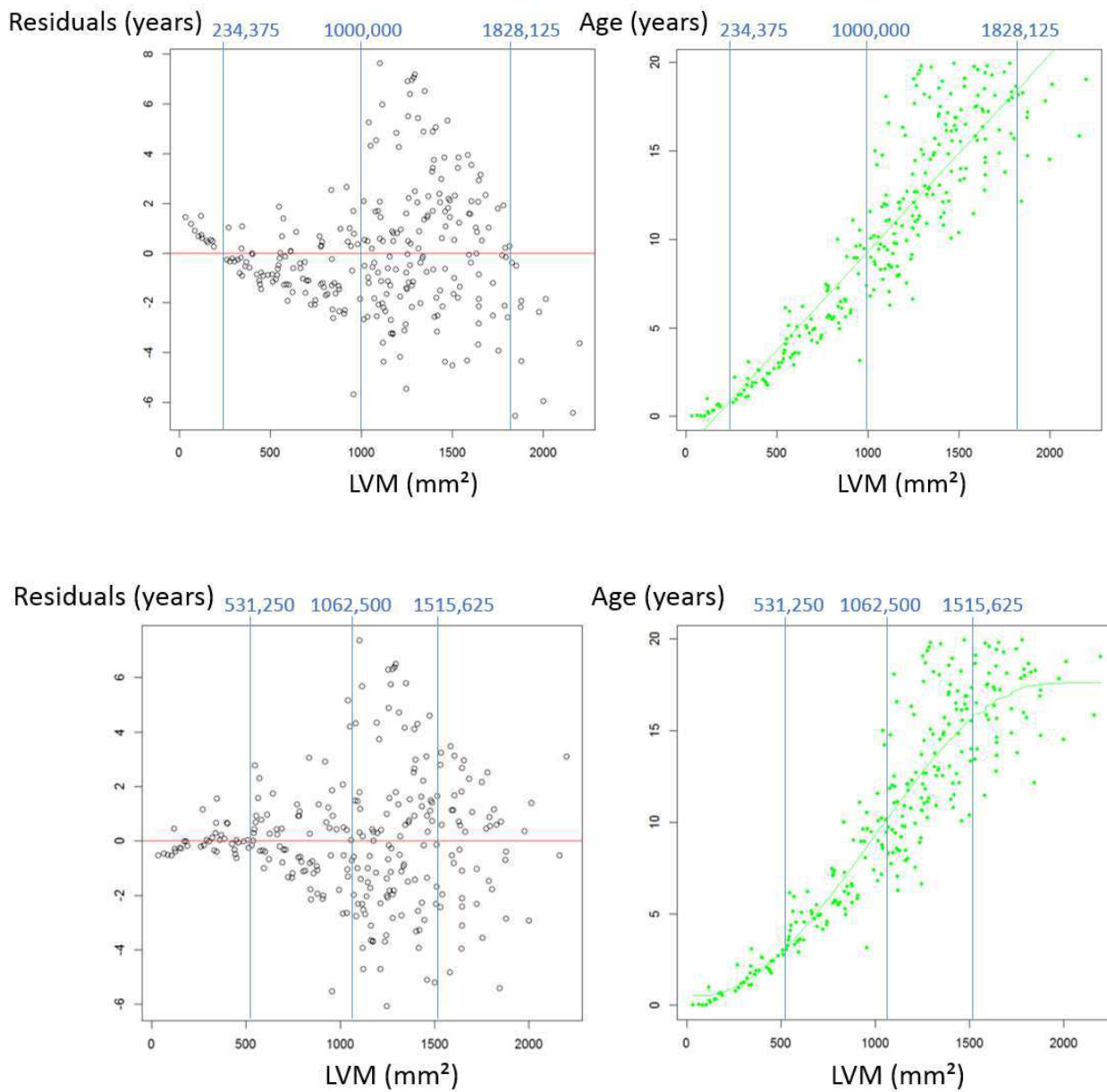


Figure E.82 The intervals of LVM values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by LVM. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (LVM) (upper and lower right plots)

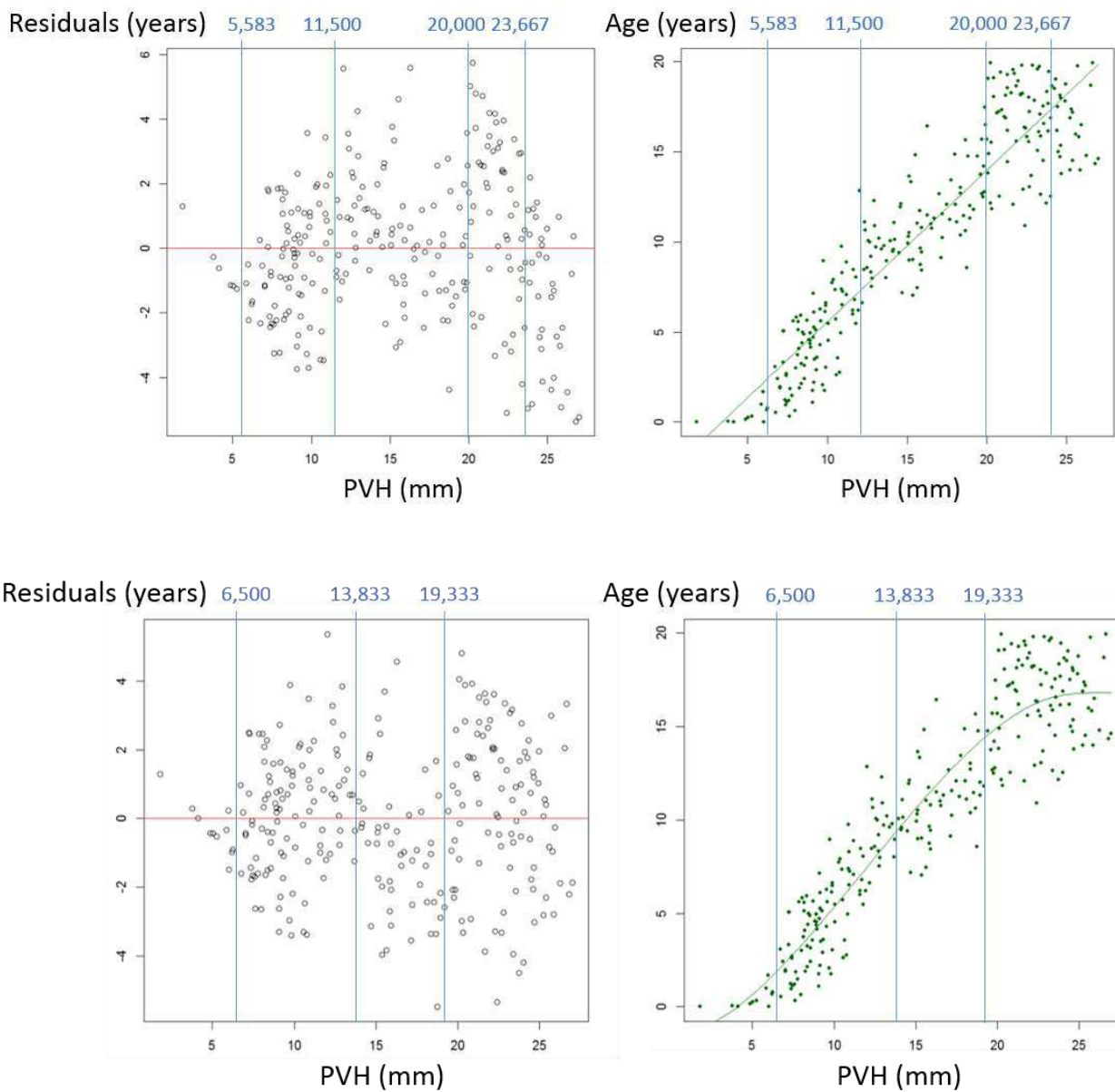


Figure E.83 The intervals of PVH values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by PVH. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (PVH) (upper and lower right plots)

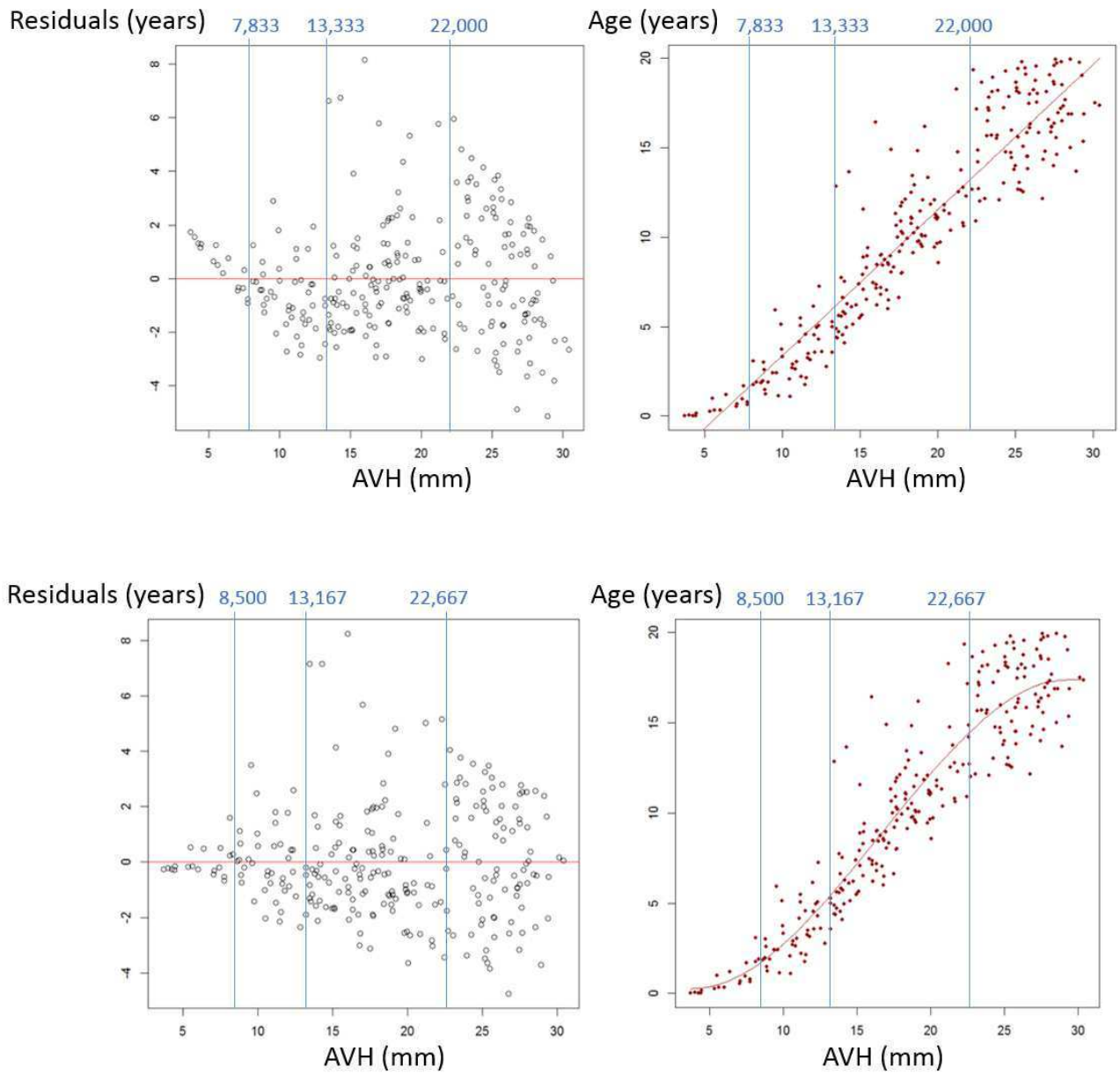


Figure E.84 The intervals of AVH values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by AVH. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (AVH) (upper and lower right plots)

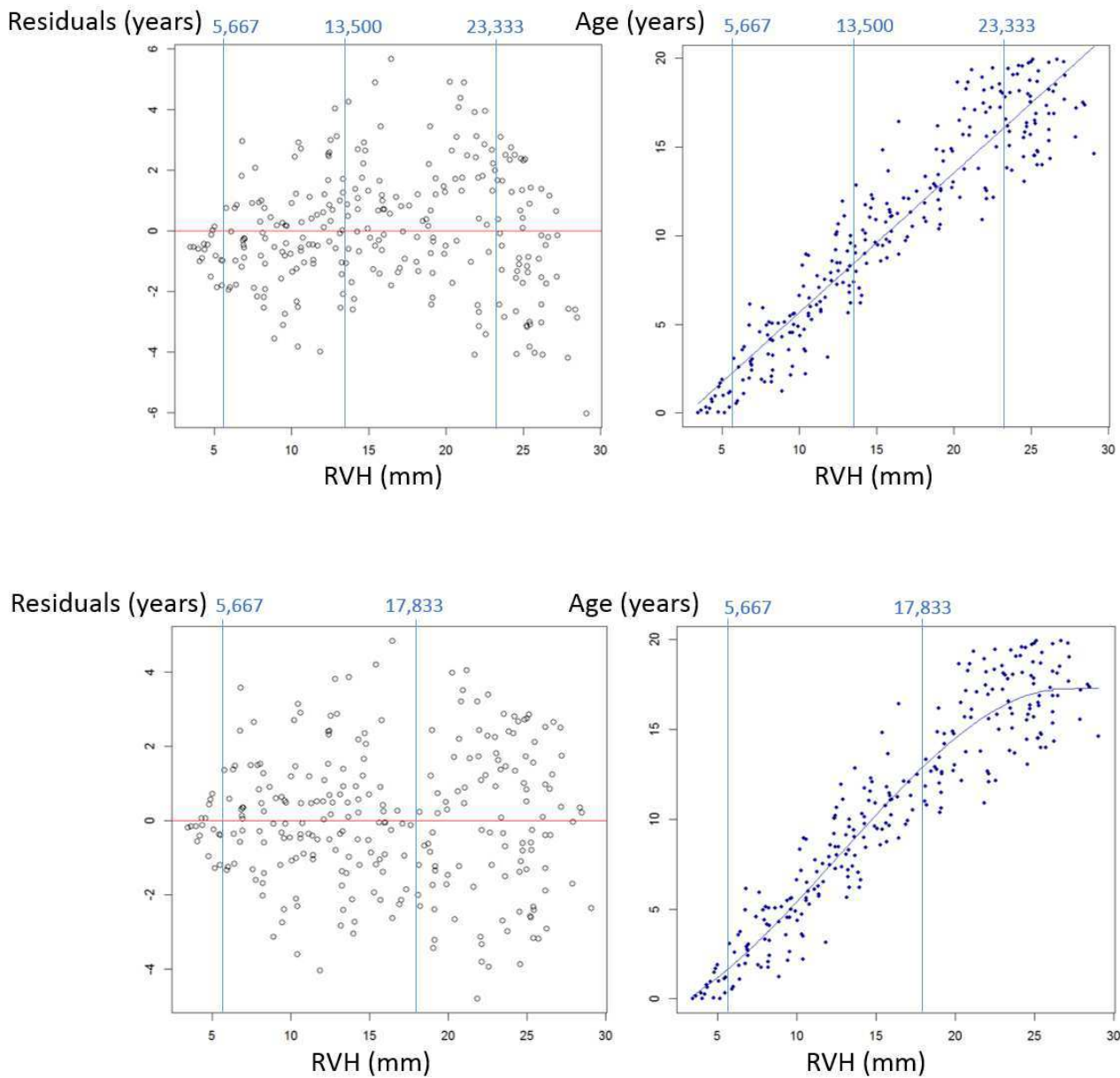


Figure E.85 The intervals of RVH values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by RVH. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (RVH) (upper and lower right plots)



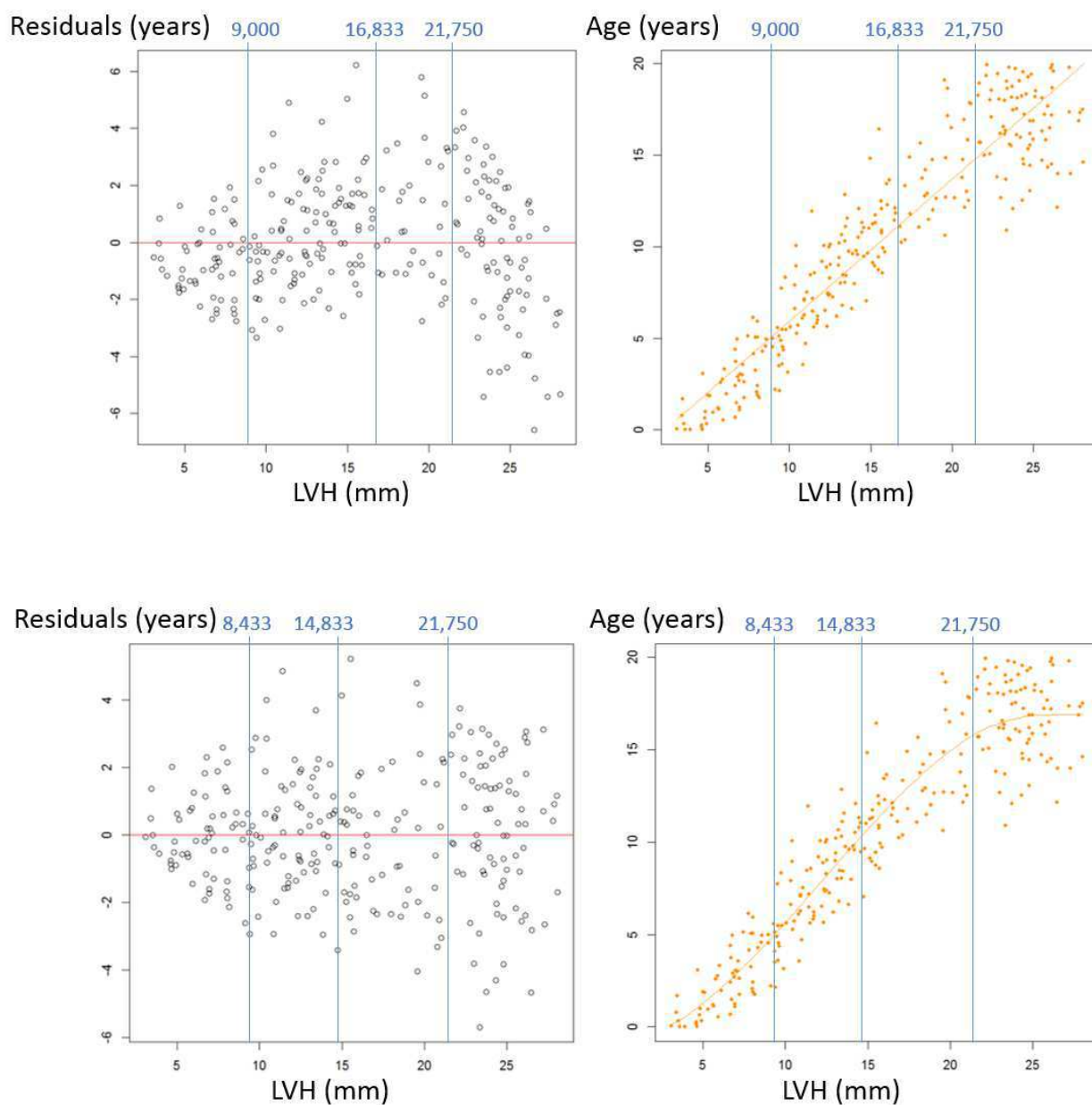


Figure E.86 The intervals of LVH values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by LVH. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (LVH) (upper and lower right plots)

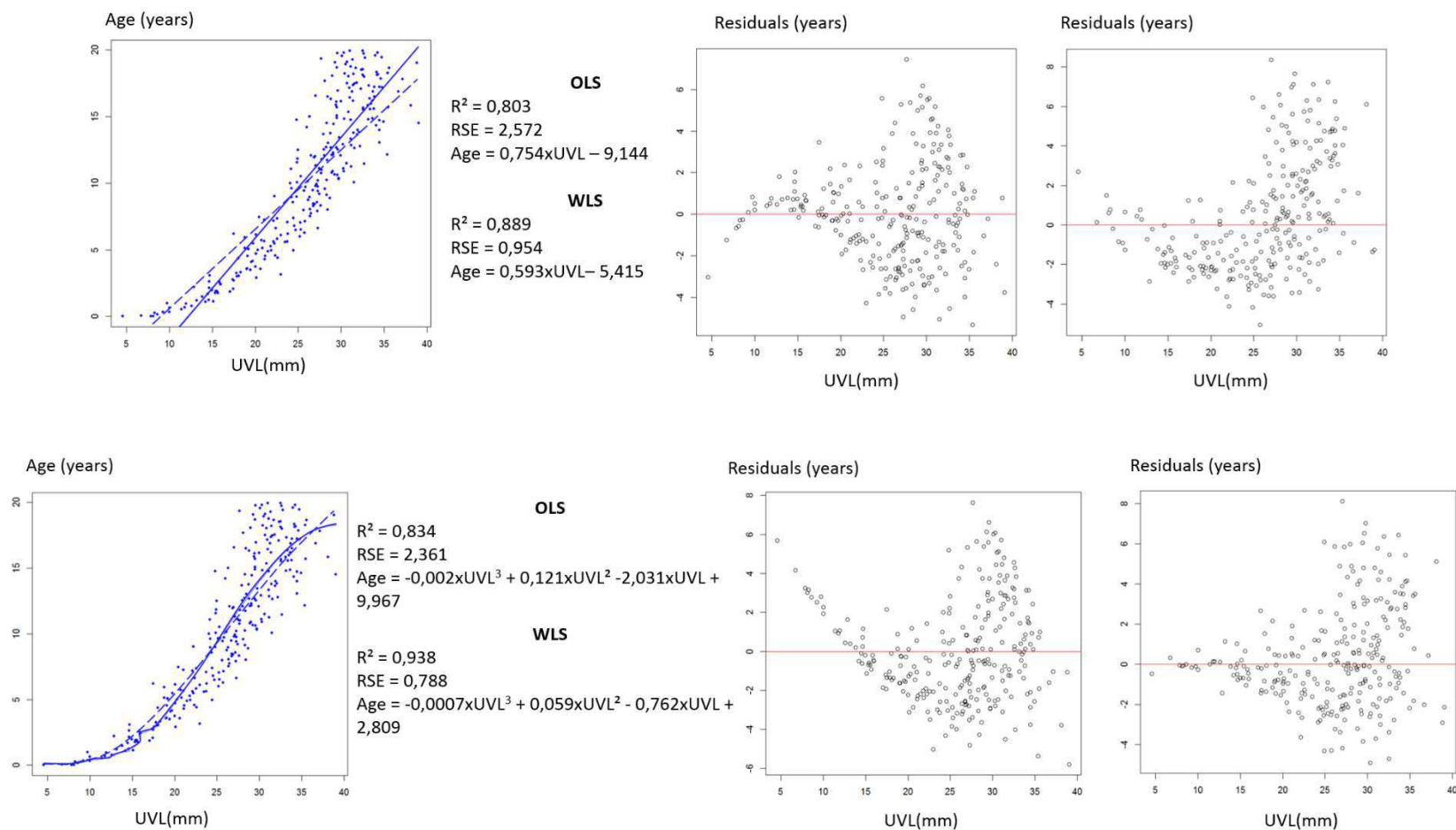


Figure E.87 Weighted Least Squares regressions of age against UVL.

Upper line: Plot of age against UVL, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against UVL. Right plot: plot of residuals of the WLS linear regression against UVL; Lower line: Plot of age against UVL, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against UVL; Right plot: plot of residuals of the WLS polynomial regression against UVL.

**Bold italic terms correspond to non-significant regression coefficients**

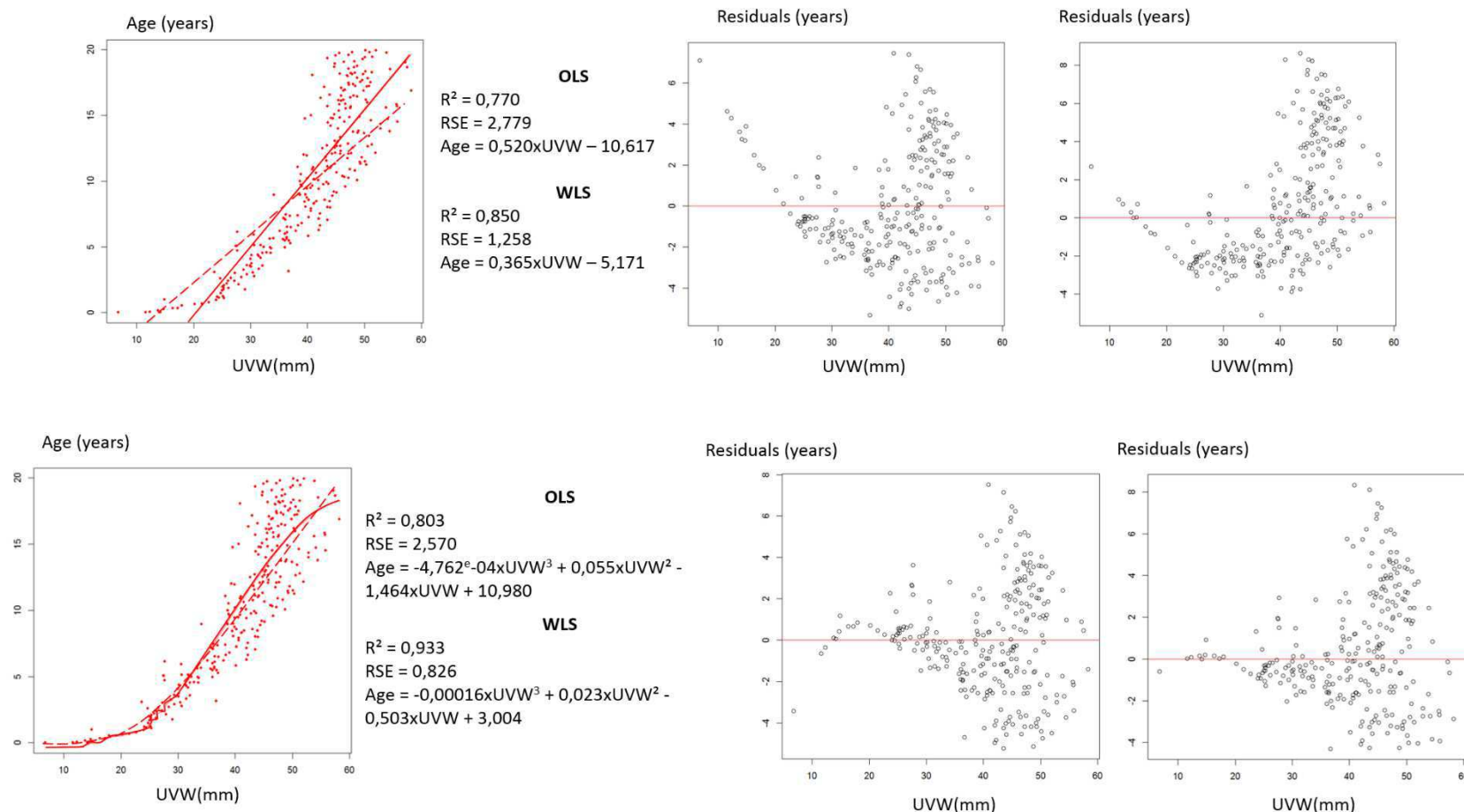


Figure E.88 Weighted Least Squares regressions of age against UVW.

Upper line: Plot of age against UVW, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against UVW. Right plot: plot of residuals of the WLS linear regression against UVW;

Lower line: Plot of age against UVW, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against UVW; Right plot: plot of residuals of the WLS polynomial regression against UVW.

**Bold italic terms correspond to non-significant regression coefficients**

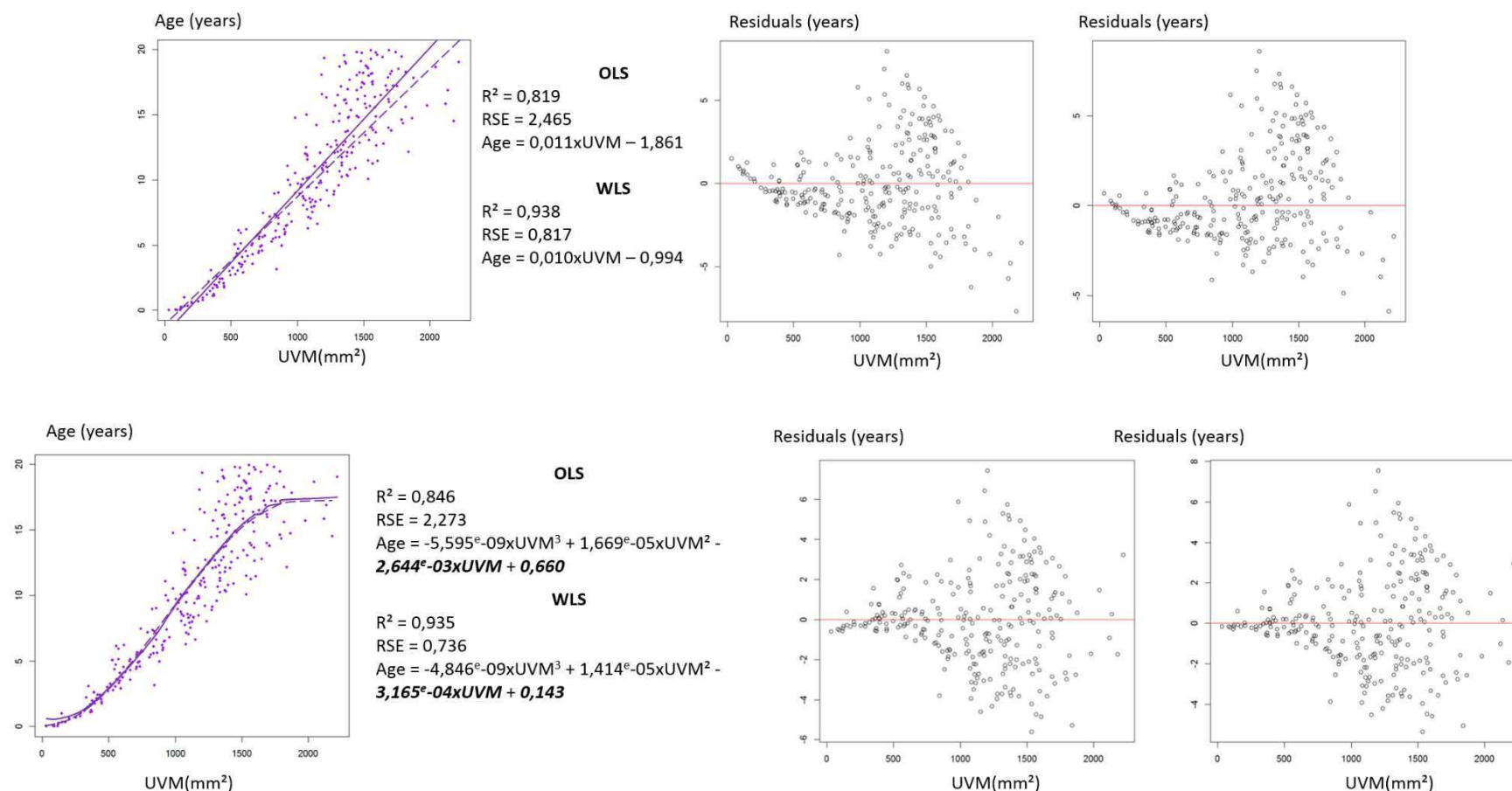


Figure E.89 Weighted Least Squares regressions of age against UVM.

Upper line: Plot of age against UVM, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against UVM. Right plot: plot of residuals of the WLS linear regression against UVM;

Lower line: Plot of age against UVM, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against UVM; Right plot: plot of residuals of the WLS polynomial regression against UVM.

**Bold italic terms correspond to non-significant regression coefficients**

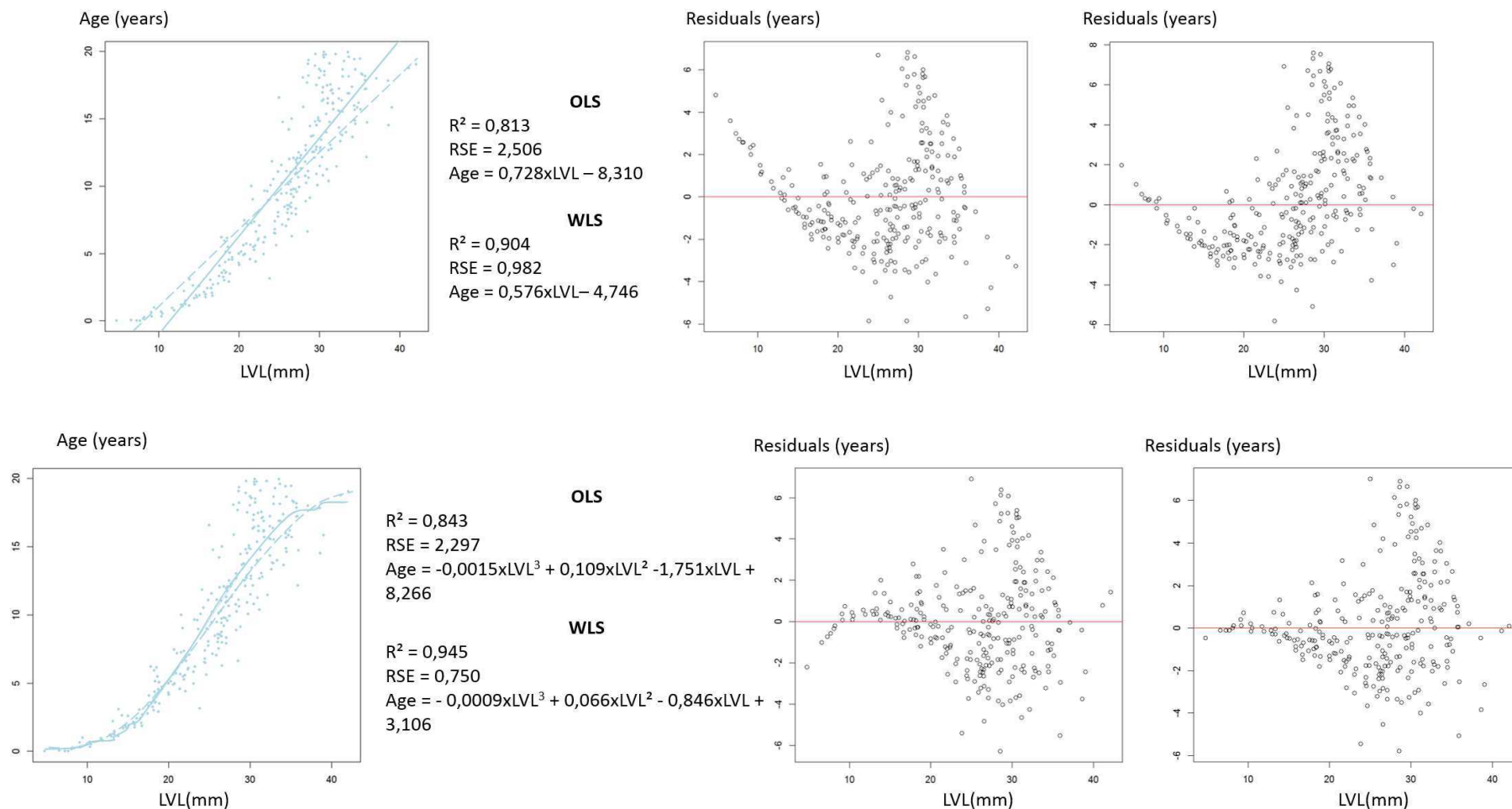


Figure E.90 Weighted Least Squares regressions of age against LVL.

Upper line: Plot of age against LVL, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against LVL. Right plot: plot of residuals of the WLS linear regression against LVL; Lower line: Plot of age against LVL, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against LVL; Right plot: plot of residuals of the WLS polynomial regression against LVL.

**Bold italic terms correspond to non-significant regression coefficients**

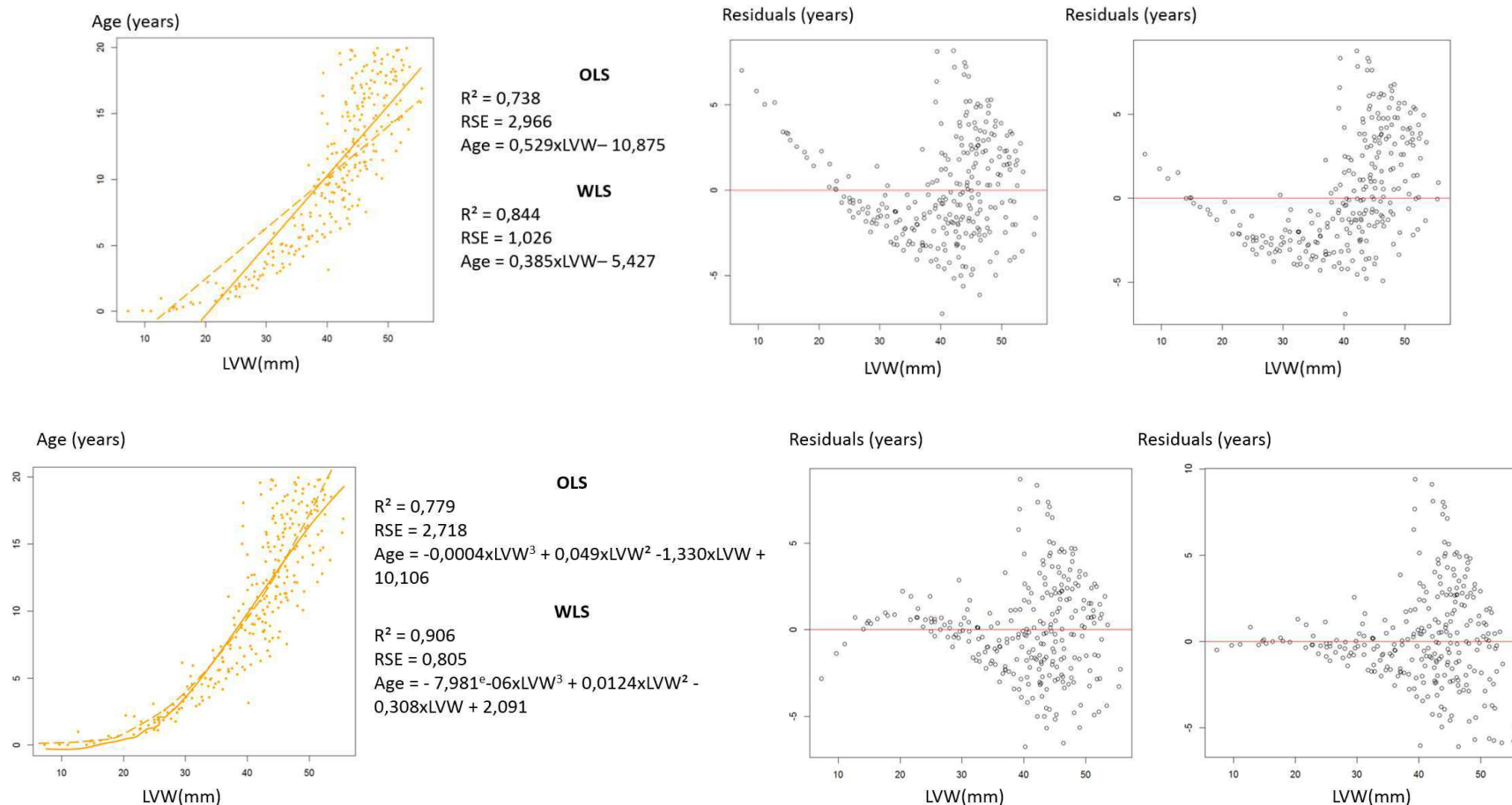


Figure E.91 Weighted Least Squares regressions of age against LVW.

Upper line: Plot of age against LVW, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against LVW. Right plot: plot of residuals of the WLS linear regression against LVW;

Lower line: Plot of age against LVW, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against LVW; Right plot: plot of residuals of the WLS polynomial regression against LVW.

**Bold italic terms correspond to non-significant regression coefficients**

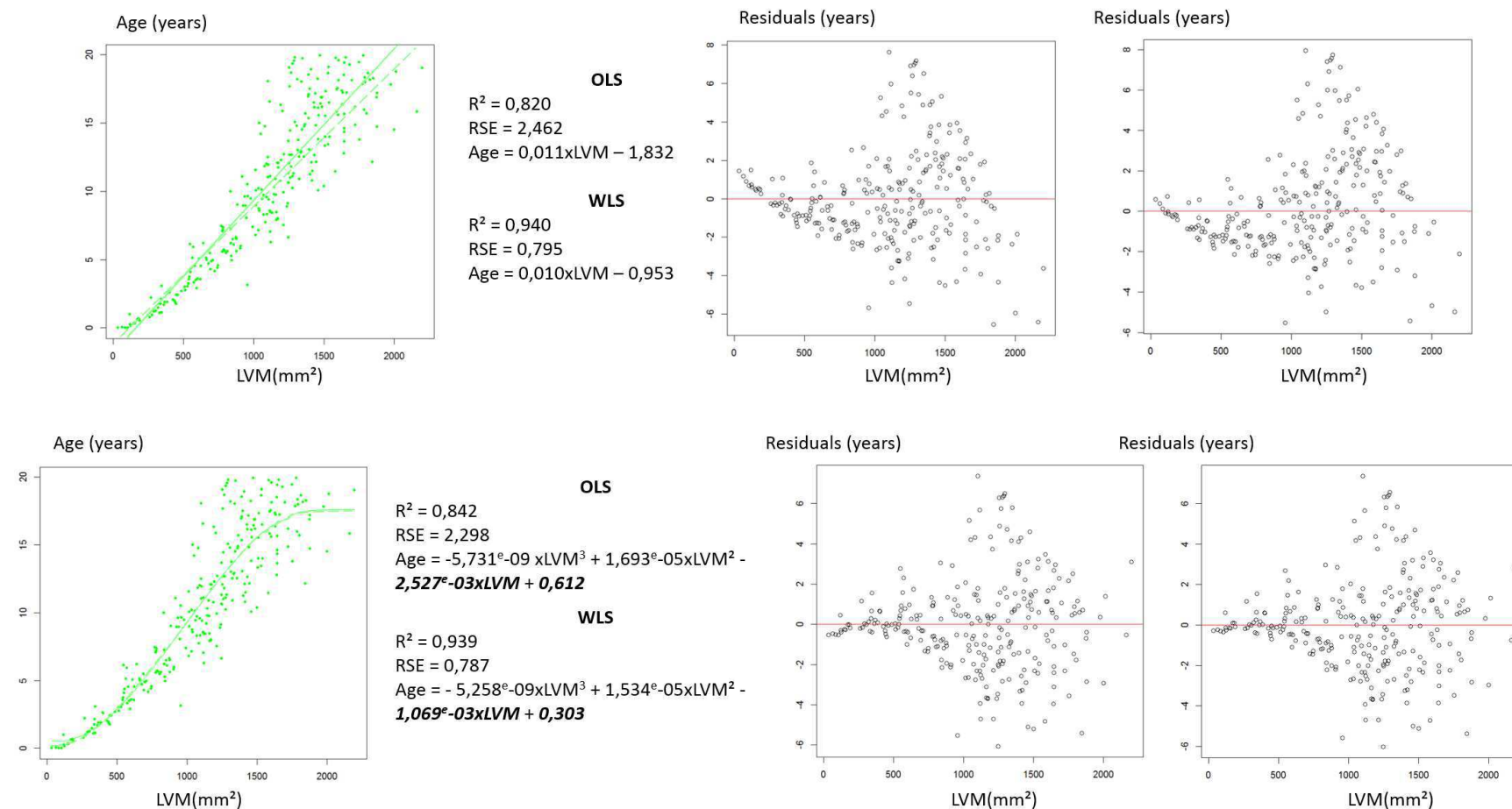


Figure E.92 Weighted Least Squares regressions of age against LVM.

Upper line: Plot of age against LVM, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against LVM. Right plot: plot of residuals of the WLS linear regression against LVM;

Lower line: Plot of age against LVM, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against LVM; Right plot: plot of residuals of the WLS polynomial regression against LVM.

Bold italic terms correspond to non-significant regression coefficients

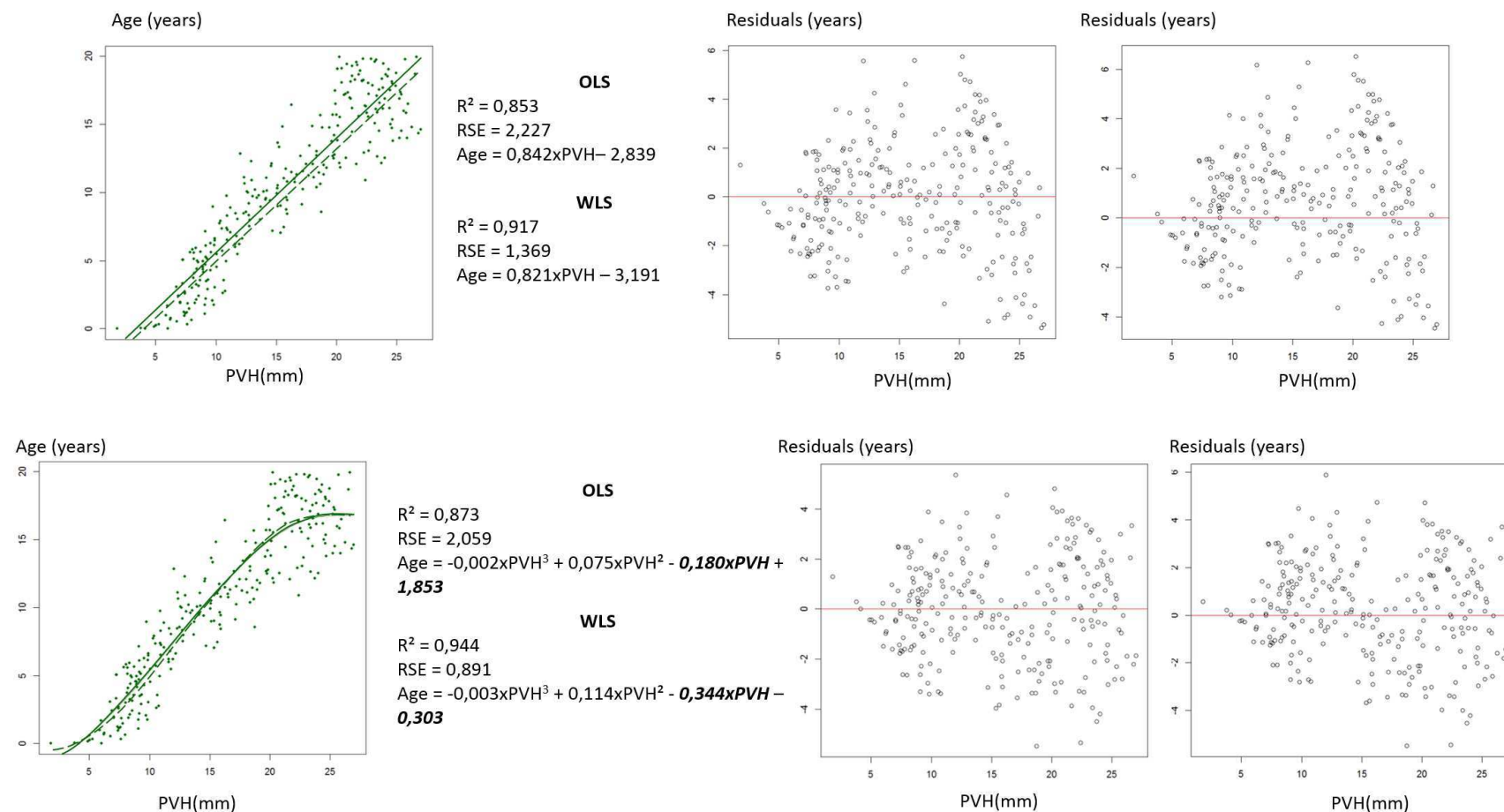


Figure E.93 Weighted Least Squares regressions of age against PVH.

Upper line: Plot of age against PVH, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against PVH; Right plot: plot of residuals of the WLS linear regression against PVH;

Lower line: Plot of age against PVH, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against PVH; Right plot: plot of residuals of the WLS polynomial regression against PVH.

Bold italic terms correspond to non-significant regression coefficients



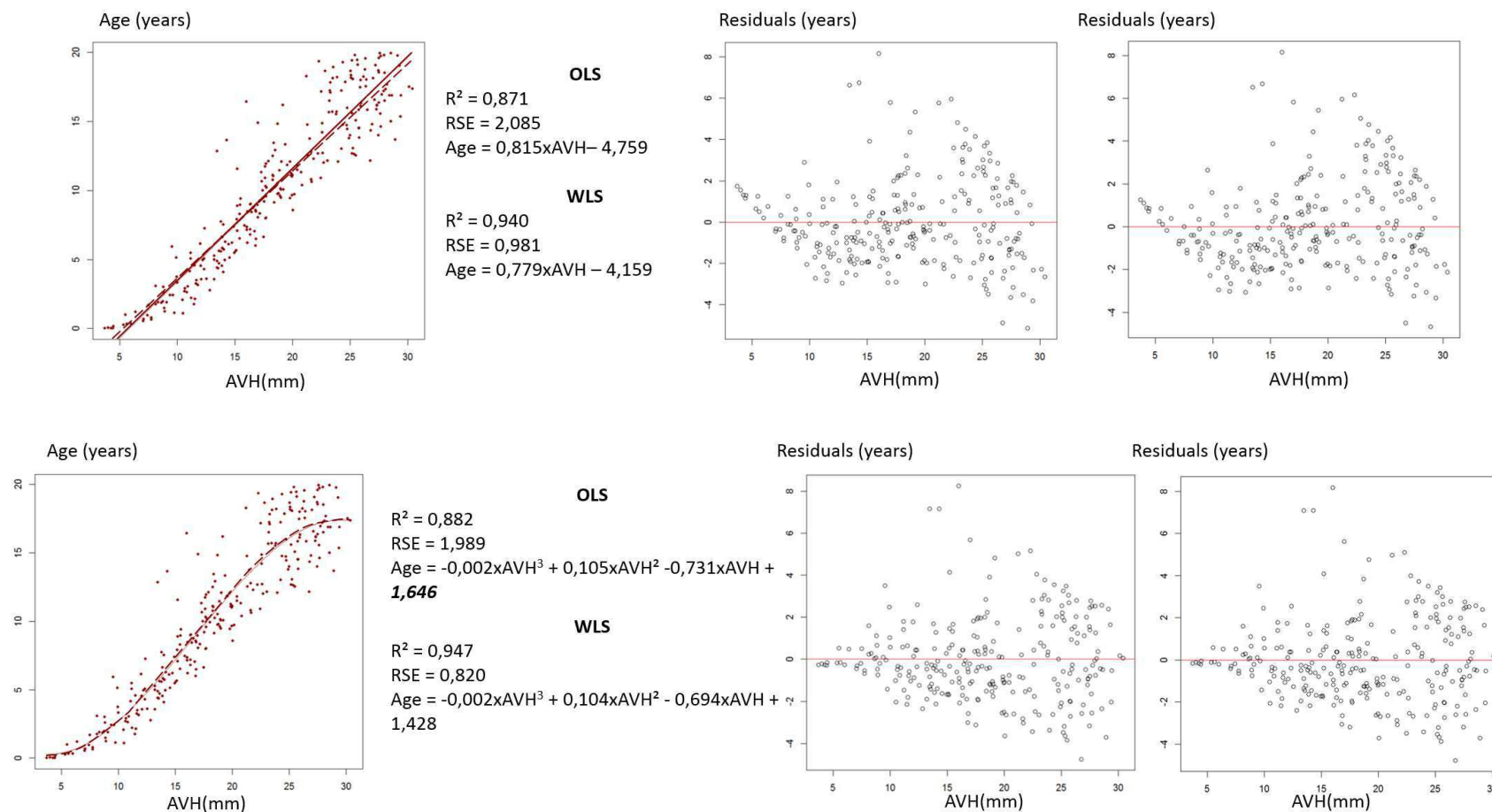


Figure E.94 Weighted Least Squares regressions of age against AVH.

Upper line: Plot of age against AVH, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against AVH. Right plot: plot of residuals of the WLS linear regression against AVH;

Lower line: Plot of age against AVH, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against AVH; Right plot: plot of residuals of the WLS polynomial regression against AVH.

Bold italic terms correspond to non-significant regression coefficients

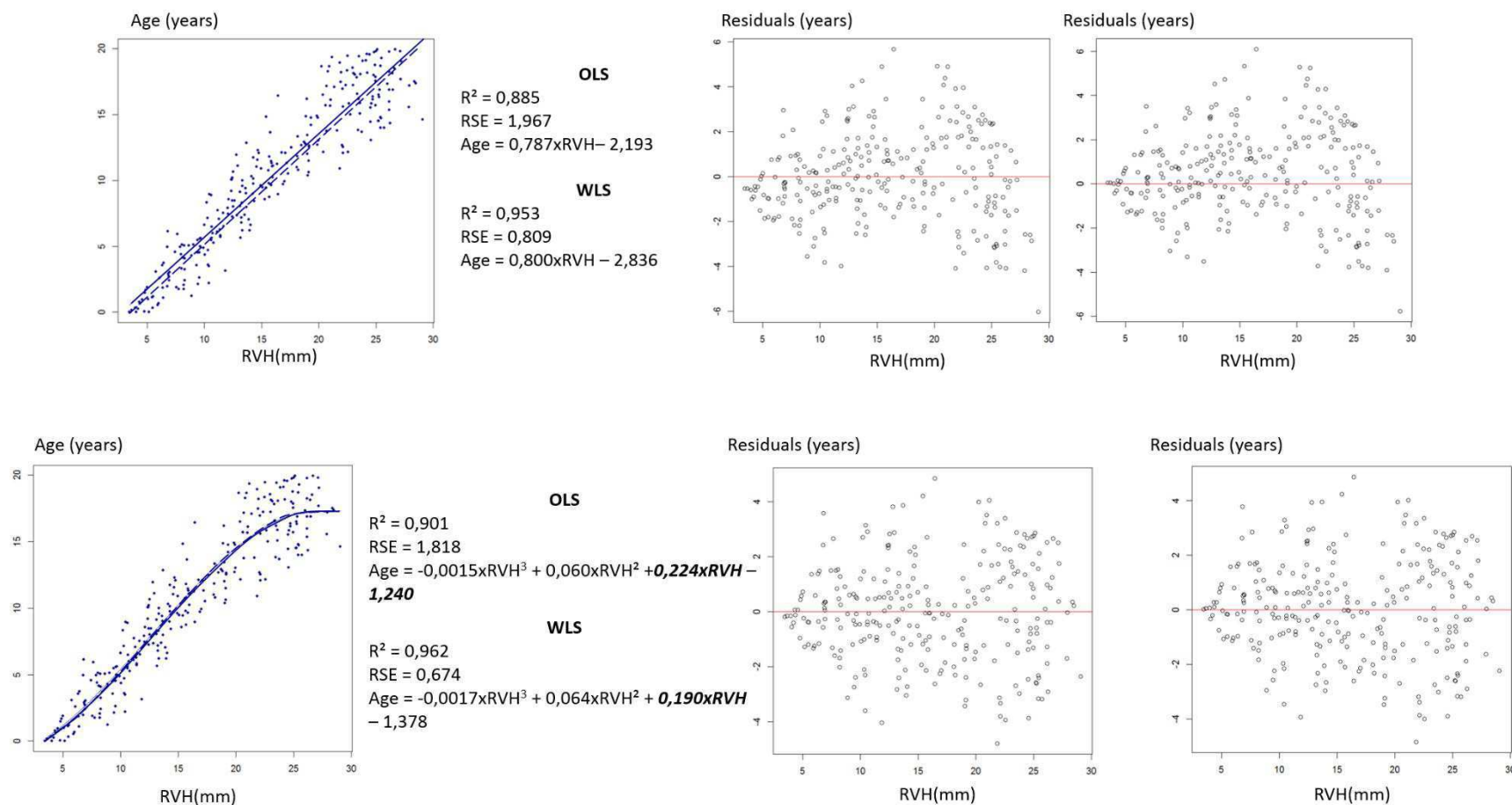


Figure E.95 Weighted Least Squares regressions of age against RVH.

Upper line: Plot of age against RVH, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against RVH. Right plot: plot of residuals of the WLS linear regression against RVH;

Lower line: Plot of age against RVH, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against RVH; Right plot: plot of residuals of the WLS polynomial regression against RVH.

Bold italic terms correspond to non-significant regression coefficients

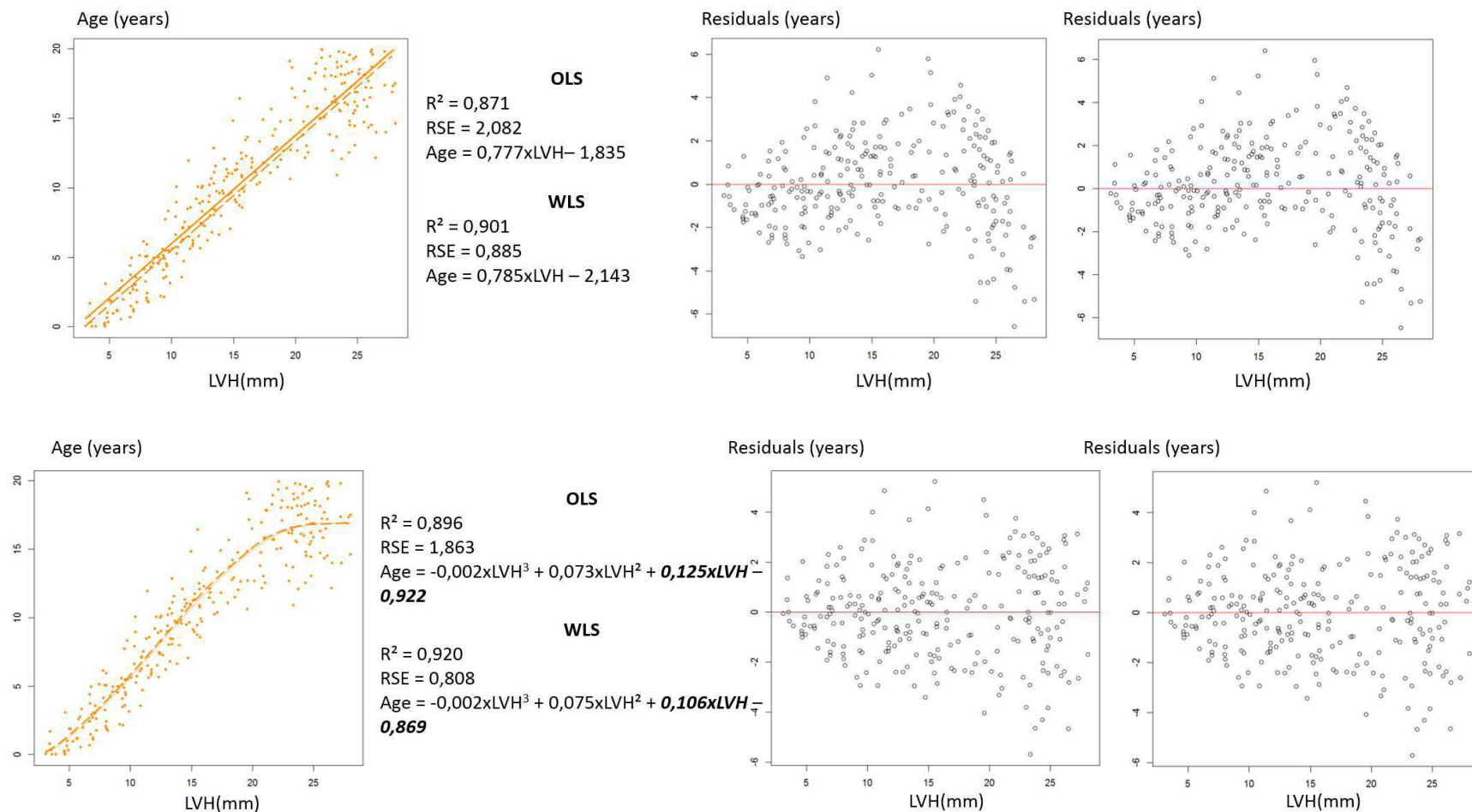


Figure E.96 Weighted Least Squares regressions of age against LVH.

Upper line: Plot of age against LVH, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against LVH. Right plot: plot of residuals of the WLS linear regression against LVH; Lower line: Plot of age against LVH, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against LVH; Right plot: plot of residuals of the WLS polynomial regression against LVH.

Bold italic terms correspond to non-significant regression coefficients

### Clavicular variables

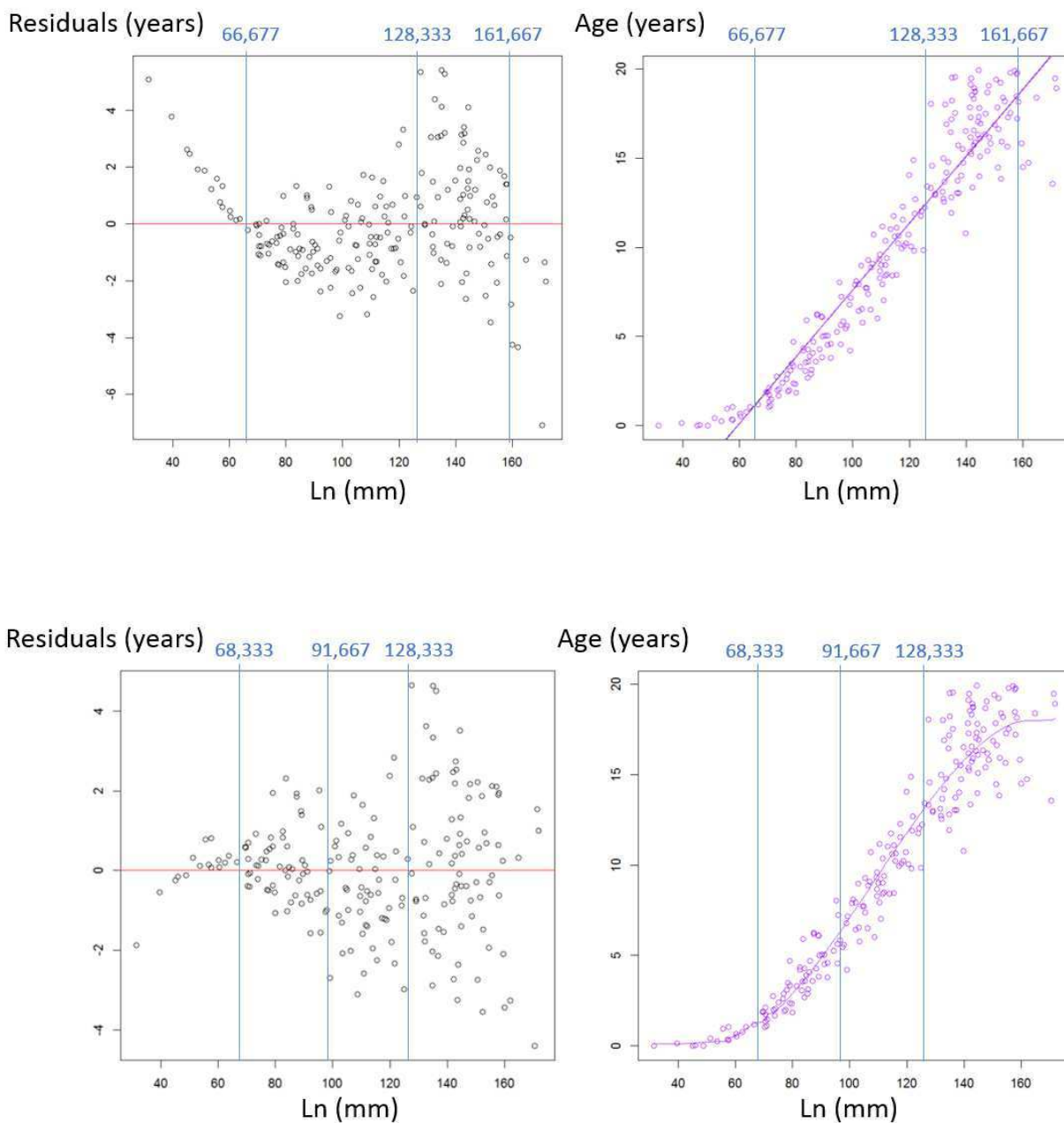


Figure E.97 The intervals of Ln values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by Ln. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (Ln) (upper and lower right plots)

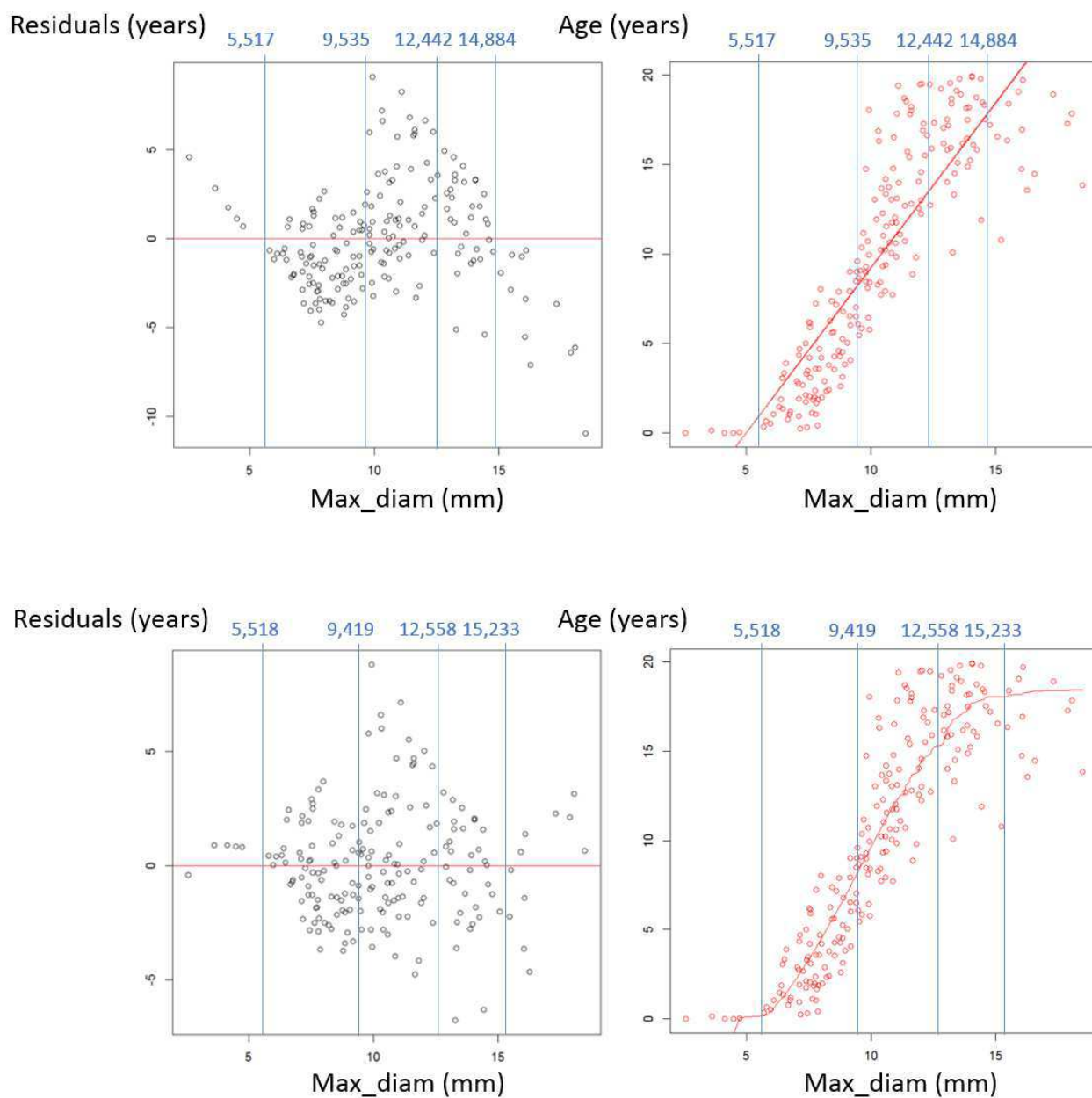


Figure E.98 The intervals of Max\_diam values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by Max\_diam. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (Max\_diam) (upper and lower right plots)

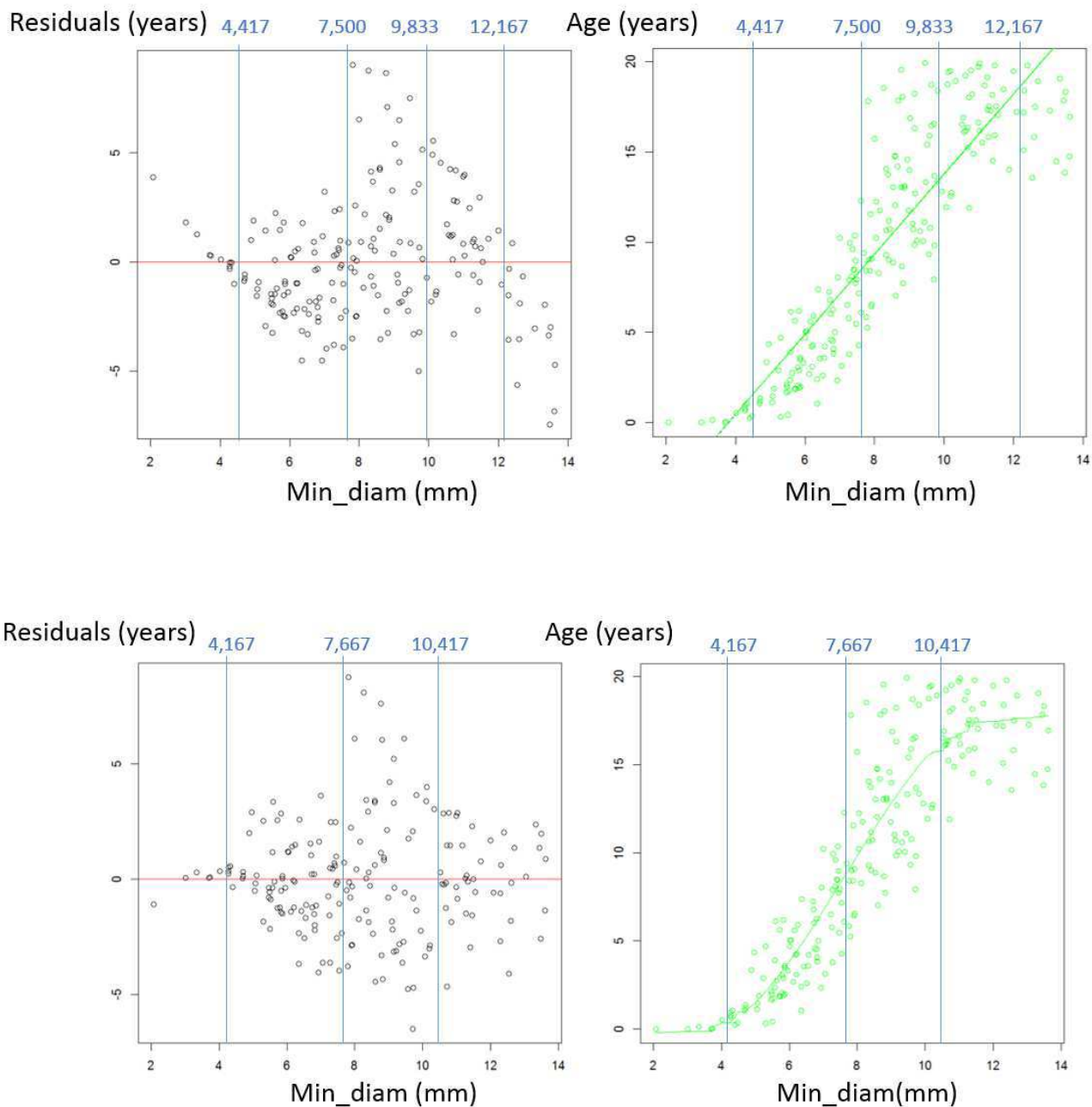


Figure E.99 The intervals of Min\_diam values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by Min\_diam. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (Min\_diam) (upper and lower right plots)

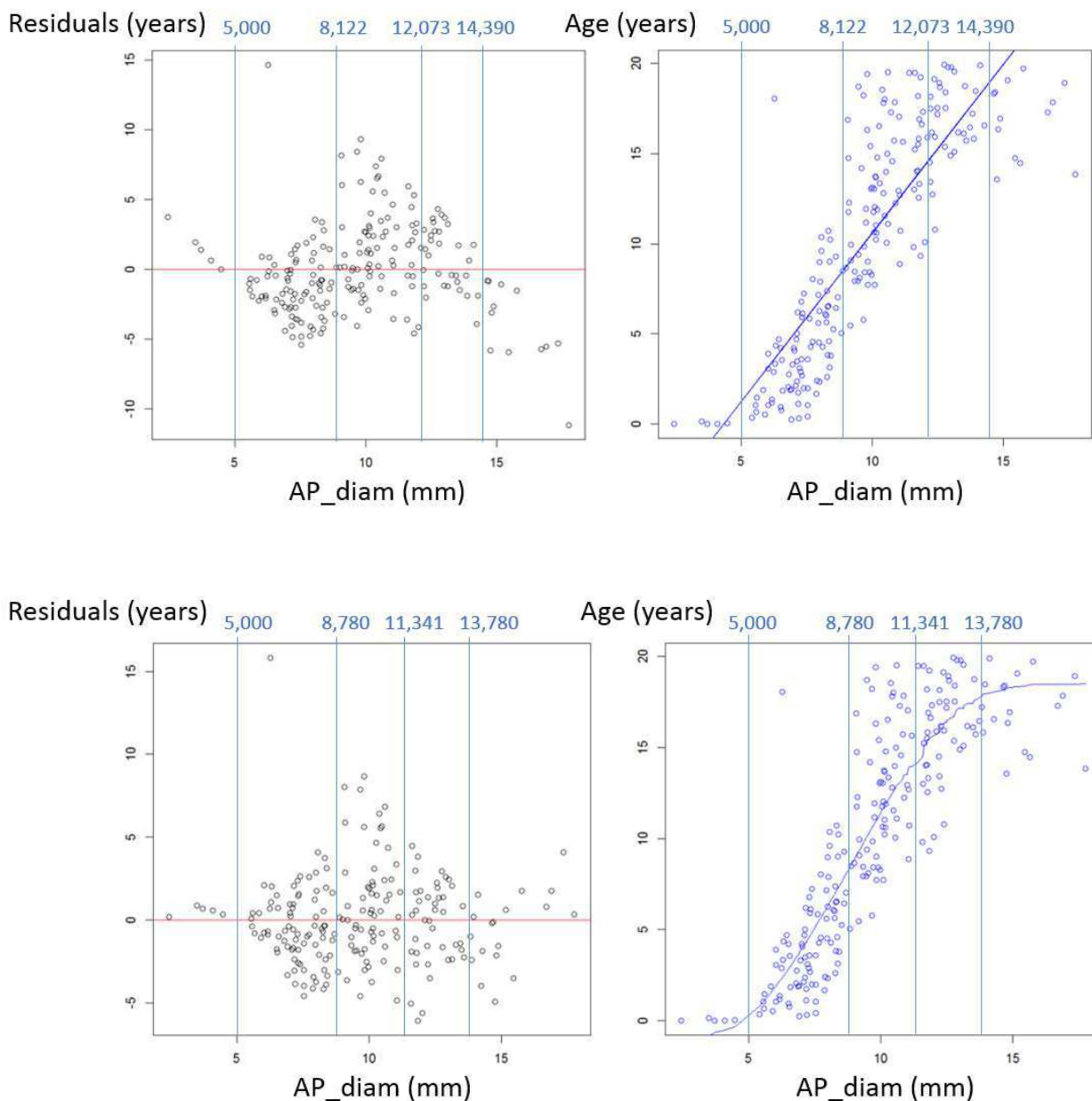


Figure E.100 The intervals of AP\_diam values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by AP\_diam. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (AP\_diam) (upper and lower right plots)

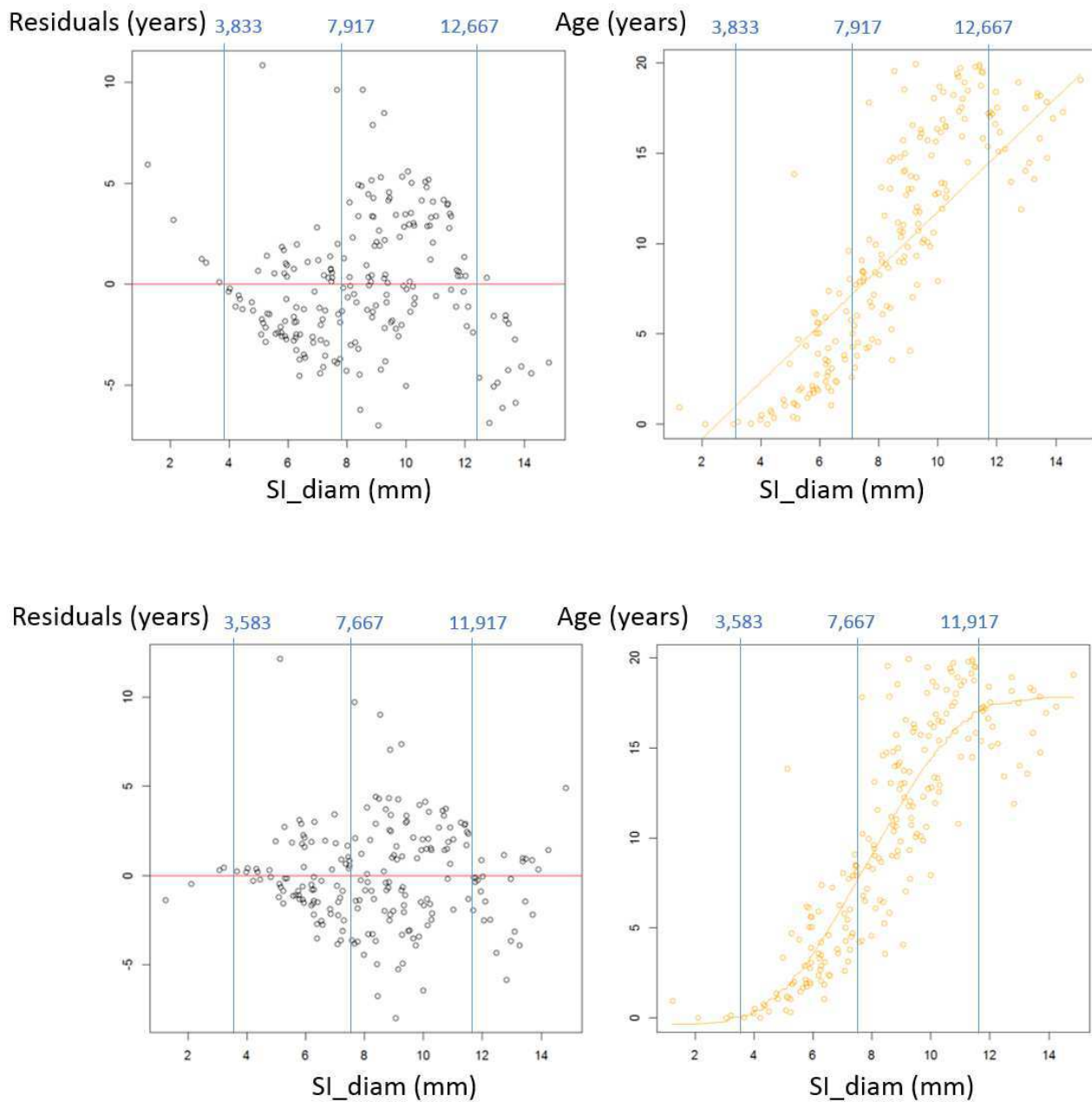


Figure E.101 The intervals of SI\_diam values determined by the changes in OLS residual pattern for the linear model (upper row) and the third degree polynomial model (lower row) of age predicted by SI\_diam. Blue values are the lower and higher values of the predictor variables that determine the intervals. The same intervals are reported on the plot of age against the predictor variable (SI\_diam) (upper and lower right plots)



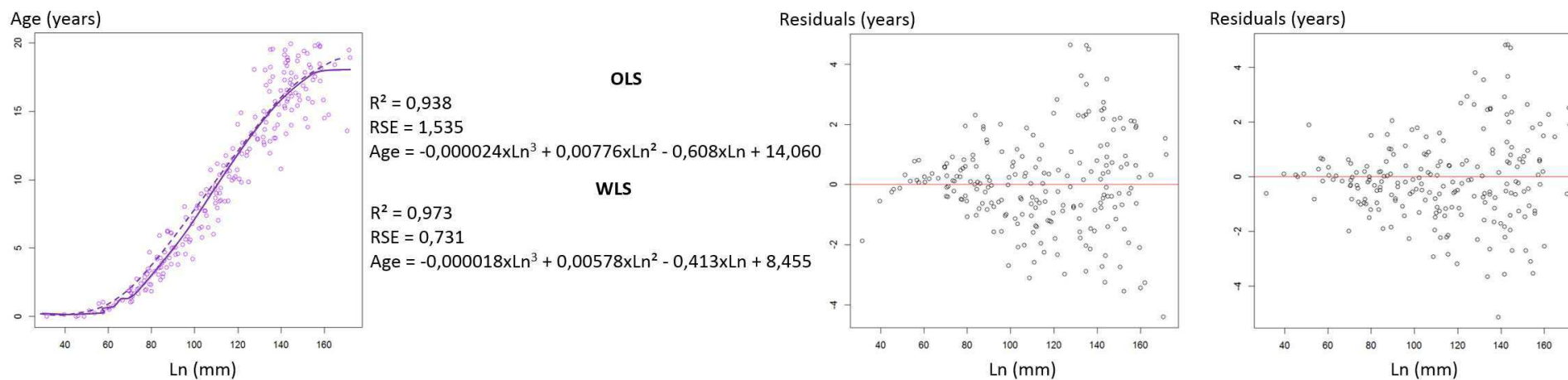
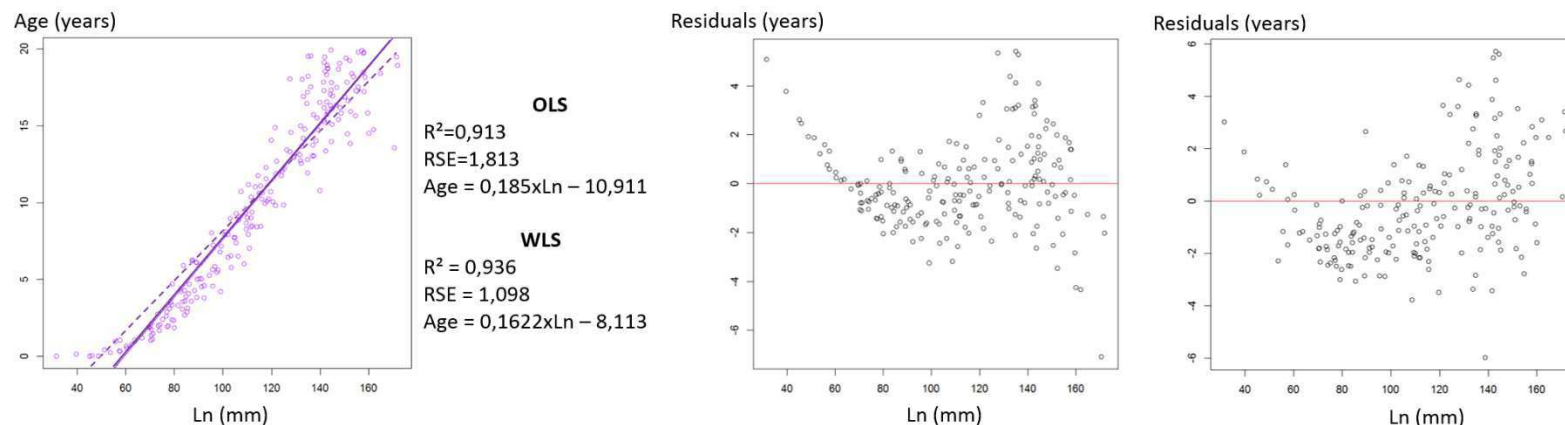


Figure E.102 Weighted Least Squares regressions of age against Ln.

Upper line: Plot of age against Ln, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against Ln; Right plot: plot of residuals of the WLS linear regression against Ln; Lower line: Plot of age against Ln, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against Ln; Right plot: plot of residuals of the WLS polynomial regression against Ln

**Bold italic terms correspond to non-significant regression coefficients**

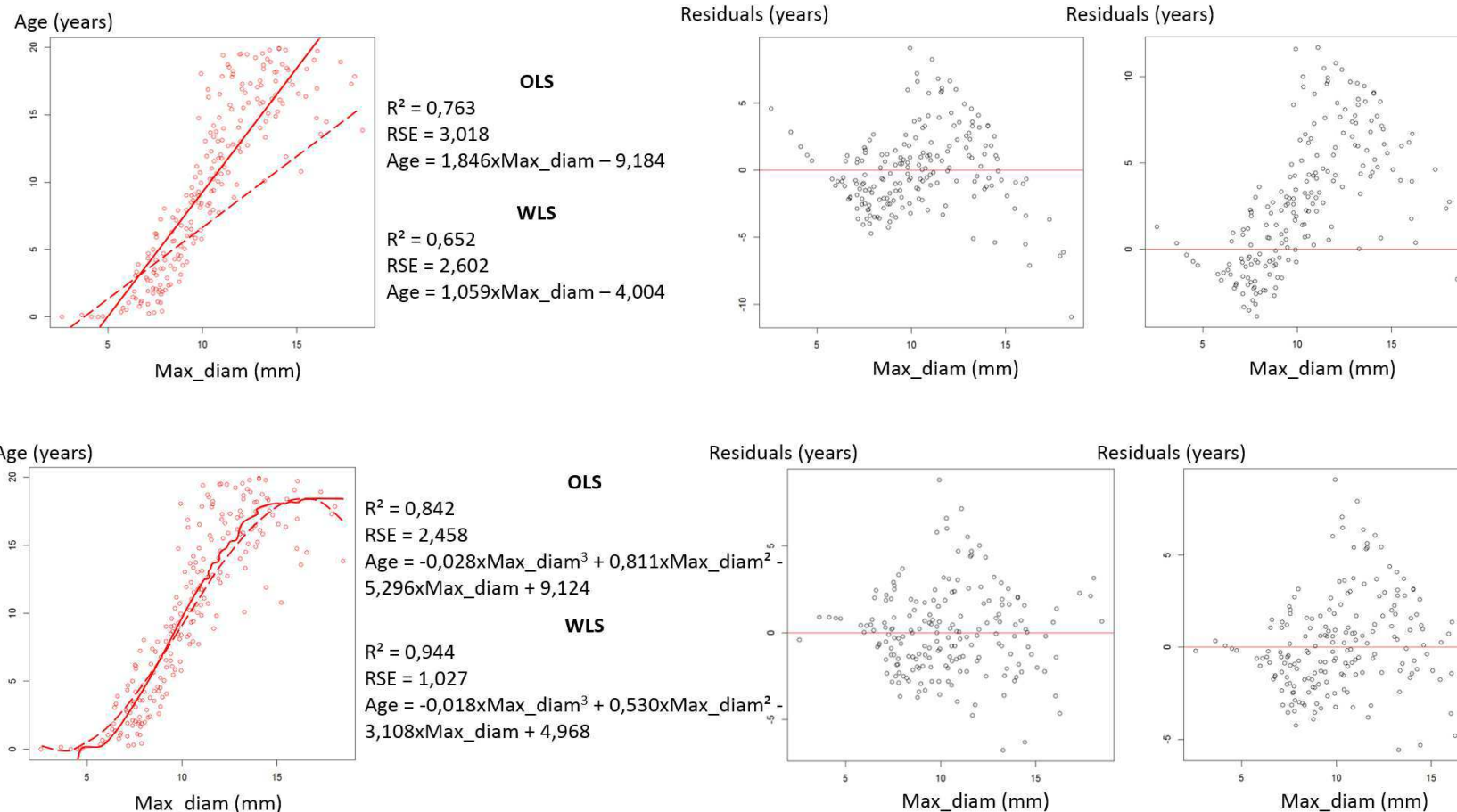


Figure E.103 Weighted Least Squares regressions of age against Max\_diam.

Upper line: Plot of age against Max\_diam, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against Max\_diam; Right plot: plot of residuals of the WLS linear regression against Max\_diam;

Lower line: Plot of age against Ln, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against Max\_diam; Right plot: plot of residuals of the WLS polynomial regression against Max\_diam

**Bold italic terms correspond to non-significant regression coefficients**

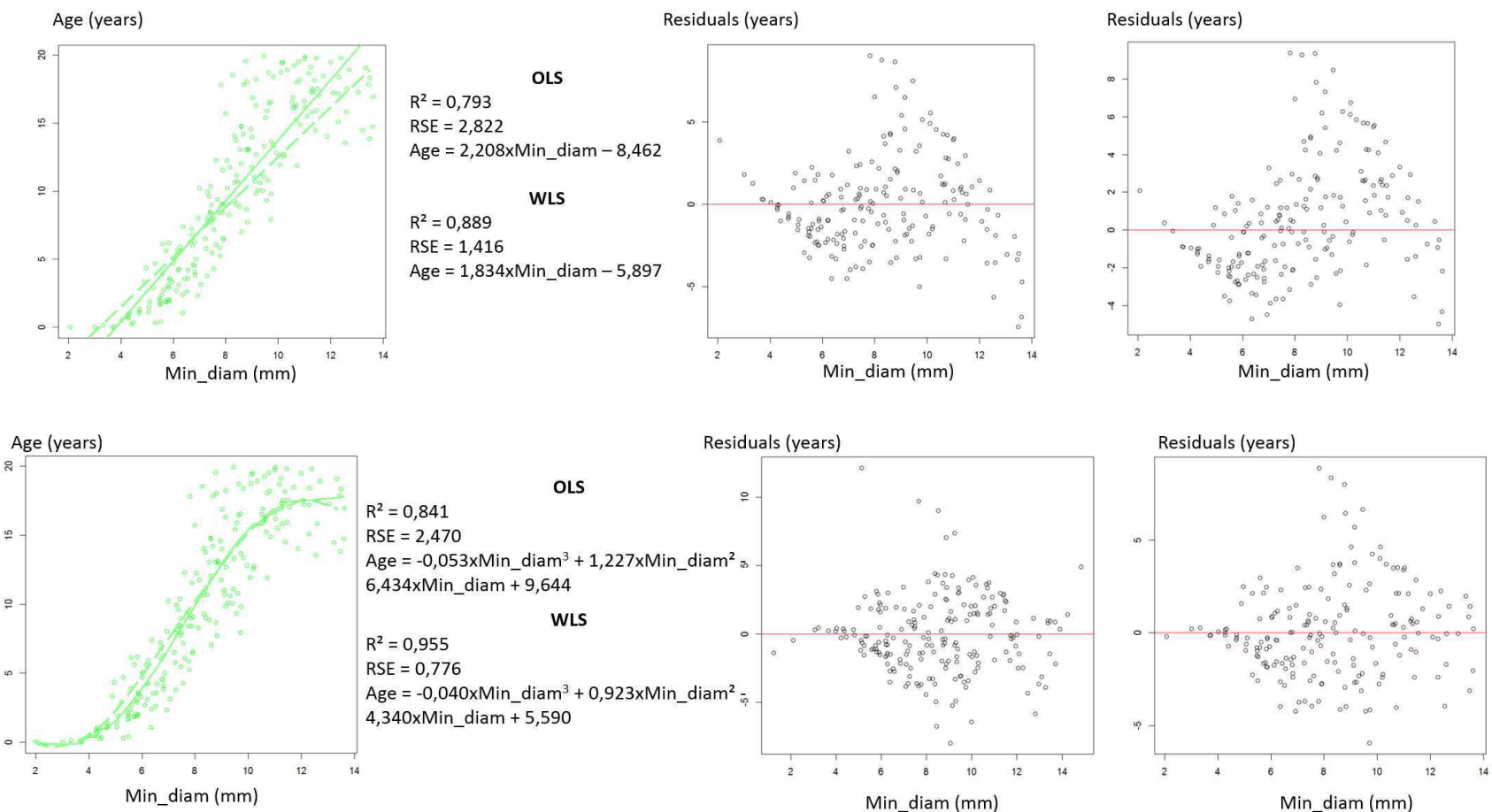


Figure E.104 Weighted Least Squares regressions of age against Min\_diam.

Upper line: Plot of age against Min\_diam, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics (R<sup>2</sup> and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against Min\_diam; Right plot: plot of residuals of the WLS linear regression against Min\_diam;

Lower line: Plot of age against Min\_diam, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics (R<sup>2</sup> and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against Min\_diam; Right plot: plot of residuals of the WLS polynomial regression against Min\_diam

**Bold italic terms correspond to non-significant regression coefficients**

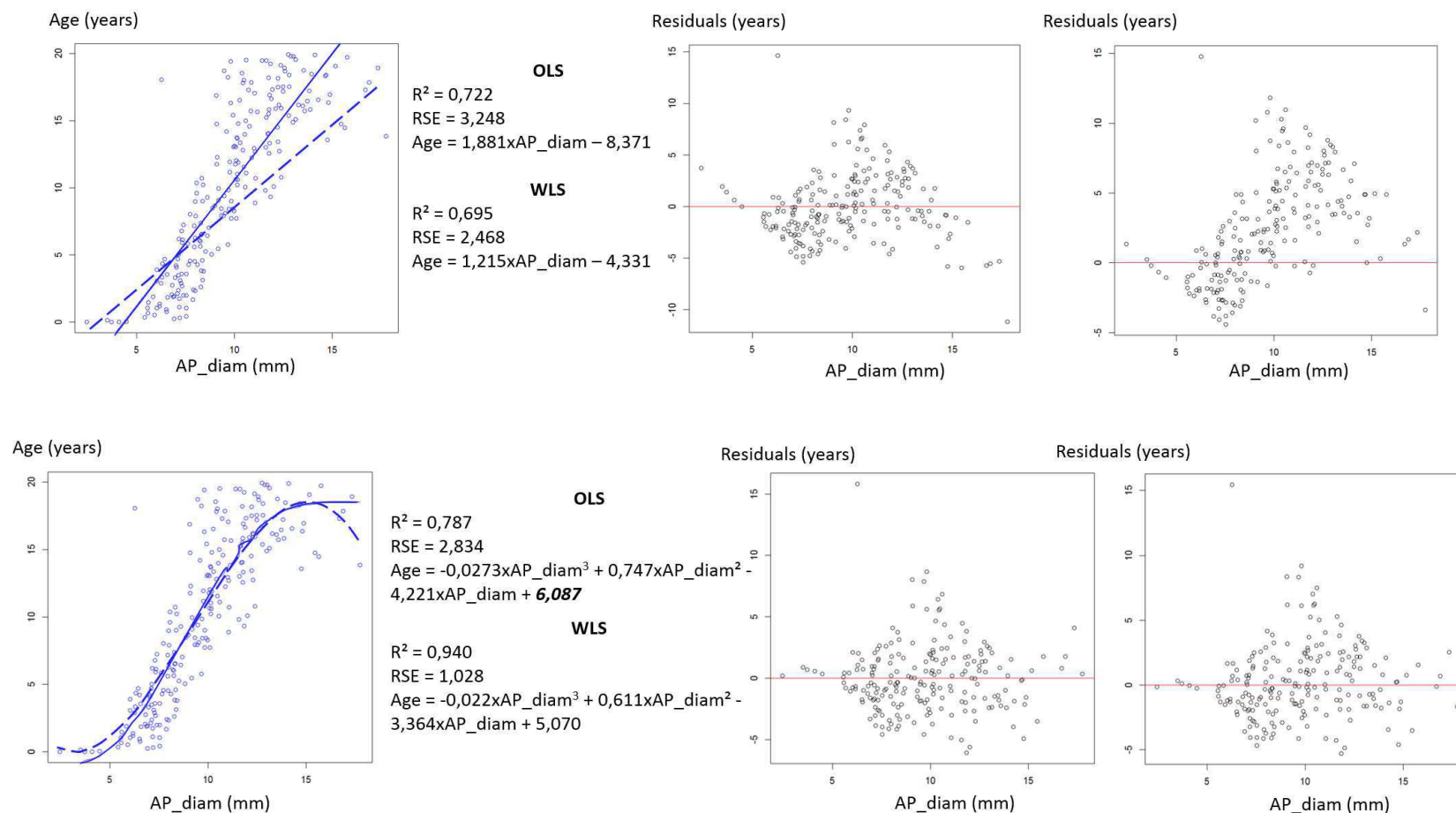


Figure E.105 Weighted Least Squares regressions of age against AP\_diam.

Upper line: Plot of age against AP\_diam, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics (R<sup>2</sup> and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against AP\_diam; Right plot: plot of residuals of the WLS linear regression against AP\_diam;

Lower line: Plot of age against AP\_diam, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics (R<sup>2</sup> and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against AP\_diam; Right plot: plot of residuals of the WLS polynomial regression against AP\_diam

Bold italic terms correspond to non-significant regression coefficients

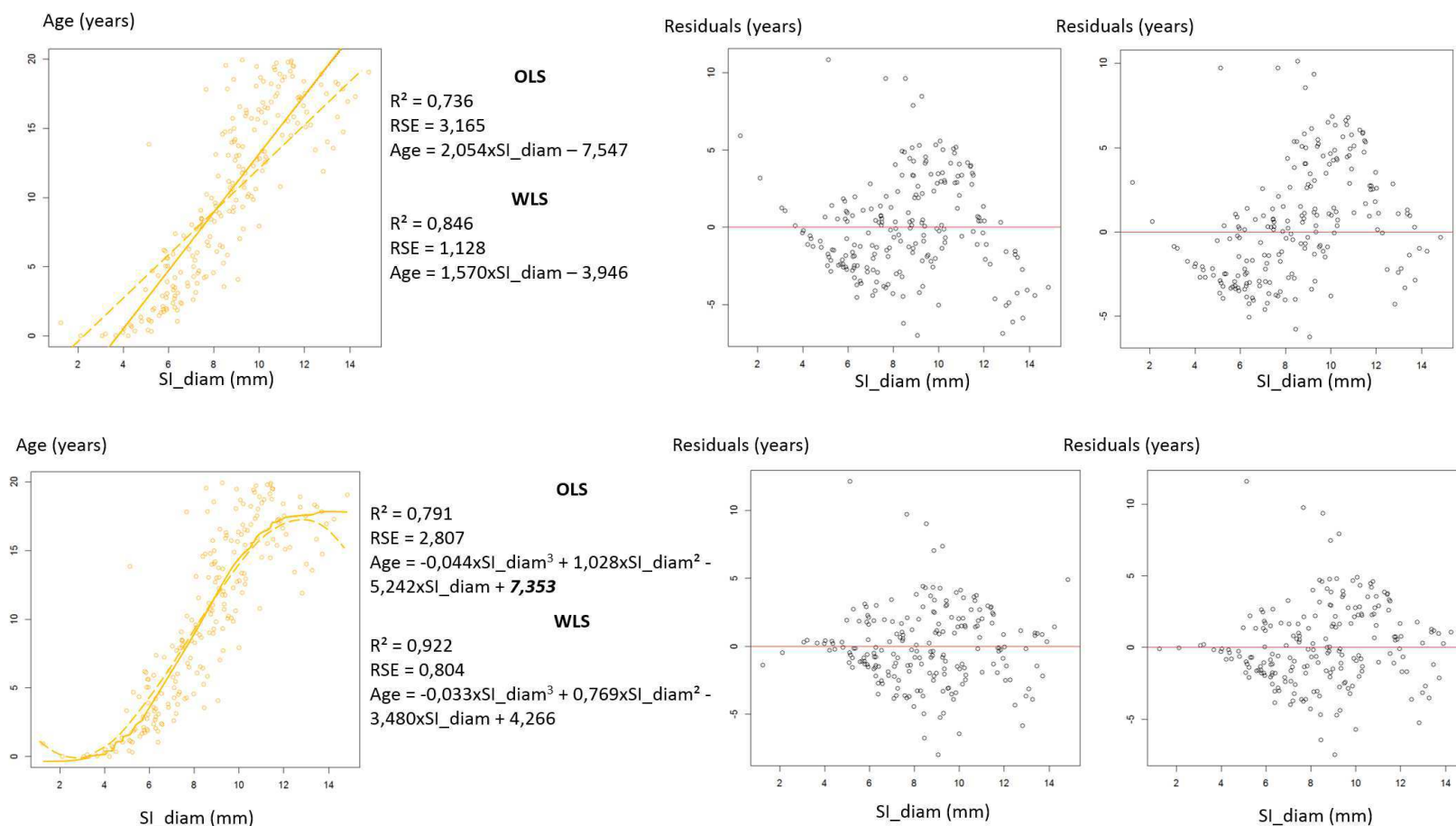


Figure E.106 Weighted Least Squares regressions of age against SI\_diam.

Upper line: Plot of age against SI\_diam, modelled by an OLS linear regression (full line) and a WLS linear regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS linear regression against SI\_diam; Right plot: plot of residuals of the WLS linear regression against SI\_diam;

Lower line: Plot of age against SI\_diam, modelled by an OLS polynomial regression (full line) and a WLS polynomial regression (dashed line), associated with their statistical characteristics ( $R^2$  and RSE), and their respective mathematical expressions. Middle plot: plot of residuals of the OLS polynomial regression against SI\_diam; Right plot: plot of residuals of the WLS polynomial regression against SI\_diam

Bold italic terms correspond to non-significant regression coefficients

## APPENDIX F

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Tables for age prediction using MARS  
models

The estimates (fitted values), minimal and maximal ages (95% PI) of the individuals are in years.

\*Negative ages can either correspond to age 0, or to individuals younger than 0 (foetuses).

Greyed values correspond to the individuals for whom real age is not included in the 95% prediction interval.

Blue values correspond to the hinge points of the MARS models.

## Iliac models

Table F-1 Mean, minimal and maximal estimated ages of the MARS prediction models using Ilium Length (IL) as predictor

Predictor variable IL (mm)	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
23.85708091	0.002777778	-0.485861212*	-1.47526214*	0.2516138
29.05951094	0.163888889	-0.194733228*	-1.10587820*	0.7194670
32.63031955	0.002777778	0.005089222	-0.87818842 *	1.0147434
33.98135991	0.061111111	0.080693441	-0.86366862*	1.0548351
35.55228483	0.016666667	0.168602399	-0.75906410 *	1.1891734
36.85146363	0.022222222	0.241304445	-0.73629996*	1.2365278
40.01486373	0.144444444	0.418328314	-0.56632473*	1.4663784
40.70134349	0.25	0.456743722	-0.57205176 *	1.4736448
42.49506107	0.341666667	0.557120157	-0.47480055*	1.6048467
42.78976592	0.988888889	0.573611841	-0.48262844*	1.6025968
44.83445668	0.4	0.688032736	-0.33444173*	1.7894845
45.63689368	0.555555556	0.73293711	-0.30868331 *	1.8304311
45.92218586	0.313888889	0.748902061	-0.27448154 *	1.8700327
48.92532727	0.625	0.916957854	-0.15405686*	2.0472995
52.63125348	0.666666667	1.124341485	0.01920868	2.2907092
53.37142812	0.947222222	1.165761658	0.07672227	2.3622324
55.99948784	1.183333333	1.31282788	0.20488400	2.5401369
58.68714193	2.175	1.463229002	0.27904316	2.6651668
59.67910432	1.888888889	1.518739218	0.36747612	2.7723751
60.27891143	1.983333333	1.552304424	0.37257761	2.7888295
61.1665427	1.672222222	1.601976269	0.45944749	2.8925001
61.69190388	2.372222222	1.631375481	0.36025822	2.8032546
63.27135973	1.35	1.71976183	0.57578780	3.0486794
63.97337987	1.219444444	1.759046878	0.59797736	3.0841565
63.98893554	1.122222222	1.759917373	0.65209488	3.1385684
64.19191155	3.086111111	1.77127591	0.40108282	2.8913982
64.30065045	2.402739726	1.777360939	0.59846846	3.0908420
65.1836934	1.075	1.826776023	0.67842206	3.1875094
68.61957763	5.913888889	2.019048104	0.68910053	3.2632208
70.06350479	1.838888889	2.099850878	1.05616878	3.6576190
70.10166003	2.138888889	2.106844733	1.03339759	3.6372133
70.2175745	2.75	2.128091856	1.03896858	3.6499704
71.73940367	2.877777778	2.407043143	1.18308721	3.8884348
72.94459892	2.744444444	2.627955432	1.29873595	4.0787997
72.97666963	2.611111111	2.633833993	1.28288406	4.0649360
73.18039824	3.355555556	2.671177447	1.25625542	4.0509375
73.50987026	2.408219178	2.731569669	1.33668405	4.1517918



Predictor variable		Estimated age		
IL (mm)	Real age	Mean estimate	Lower 95% PI	Upper 95% PI
74.92355512	1.933333333	2.990698103	1.57223387	4.4749830
76.17917784	3.483333333	3.220853748	1.71244786	4.6930393
77.03701069	3.005555556	3.378094509	1.81194689	4.8457197
77.06069236	2.691666667	3.38243536	1.82971098	4.8649519
77.55866143	3.736986301	3.47371309	1.90941623	4.9755288
77.91008051	3.291666667	3.538128207	1.95114686	5.0390456
78.22660955	4.105555556	3.596147981	1.96540435	5.0729264
78.66267561	3.538888889	3.676078889	2.02876226	5.1633182
78.77796584	4.213888889	3.697211588	2.05175677	5.1934601
80.14053245	4.065753425	3.946970048	2.25262988	5.4788056
80.32112926	5.113888889	3.980073443	2.25932707	5.4966988
80.65074208	3.25	4.040491473	2.36985819	5.6276643
81.25390084	3.575342466	4.151050474	2.44520035	5.7403993
81.93147993	3.583333333	4.275250721	2.51358597	5.8507915
82.82052548	4.284931507	4.43821277	2.67294650	6.0652684
83.81416495	4.375	4.620346885	2.80456524	6.2584878
83.86969005	4.291666667	4.630524636	2.81765360	6.2750184
84.42404882	3.038888889	4.732138599	2.96188919	6.4536215
85.04955514	4.991666667	4.846793907	2.97893132	6.5094419
85.29086267	4.983333333	4.891025577	3.04729830	6.5927688
86.16580194	5.005479452	5.051401946	3.15984508	6.7595574
86.4151451	4.533333333	5.097106547	3.21281621	6.8279866
87.99001331	7.425	5.38577989	3.42502664	7.1378310
88.28435907	4.638888889	5.439733468	3.52340112	7.2544534
88.66497361	4.588888889	5.509500113	3.59181527	7.3464638
88.9730106	6.216666667	5.565963293	3.59066457	7.3644098
88.98554604	5.122222222	5.568261039	3.59245275	7.3669751
89.06423502	4.961111111	5.582684729	3.63676589	7.4161666
89.59885687	5.461111111	5.680680914	3.72472297	7.5372676
90.03884156	4.963888889	5.761330108	3.81678268	7.6566041
90.3867714	5.491666667	5.825105648	3.84542739	7.7068188
90.45377851	6.791666667	5.837388051	3.82180914	7.6873546
90.70119961	5.666666667	5.882740339	3.85454300	7.7354273
90.79693151	4.556164384	5.900287996	3.91093192	7.7977512
90.86888308	5.341666667	5.913476719	3.90708845	7.7983683
90.91463905	5.808333333	5.921863788	3.90640009	7.8005166
91.82956491	6.109589041	6.089569698	4.07220362	8.0230410
91.91659738	5.717808219	6.10552275	4.08815131	8.0443843
91.9211216	5.569444444	6.106352039	4.08704729	8.0435607
91.99967309	4.413888889	6.120750527	4.12701998	8.0884032
92.30744872	5.769444444	6.1771658	4.15566314	8.1361269
94.59313297	6.111111111	6.596131723	4.50731693	8.6294817

Predictor variable IL (mm)	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
95.03659133	8.811111111	6.677417641	4.54071502	8.6903720
95.34956577	6.275	6.734785856	4.61938873	8.7884486
95.46427648	6.441666667	6.755812329	4.64339532	8.8195667
97.00352639	8.043835616	7.037956837	4.88905094	9.1606481
97.01332558	5.625	7.039753029	4.87280803	9.1450127
97.16002987	10.01388889	7.066643925	4.88345668	9.1647563
98.01954935	6.463888889	7.224193846	5.03536868	9.3699542
98.99046908	8.972222222	7.402163432	5.16685885	9.5616365
99.11585795	6.005555556	7.425147212	5.20436704	9.6069182
99.19066142	7.138888889	7.438858688	5.23742910	9.6446177
99.22901875	7.086111111	7.445889587	5.25353567	9.6631023
99.49486366	7.75	7.494618959	5.26037078	9.6864184
99.70240925	7.397222222	7.532662066	5.29915406	9.7380685
100.0168323	7.727777778	7.590295811	5.33439869	9.7928058
100.0406116	7.433333333	7.594654556	5.36372369	9.8236050
100.2636287	7.366666667	7.635533591	5.40924559	9.8829528
100.4797583	9.022222222	7.675150146	5.40979907	9.8969053
100.9742462	8.019444444	7.765789778	5.48354467	10.0013067
101.1802347	6.288888889	7.803547471	5.54406072	10.0745930
101.2458683	6.9	7.815578109	5.56720641	10.1018076
101.9449806	7.994444444	7.943725394	5.63190636	10.2098490
102.0032772	9.997222222	7.954411161	5.65040231	10.2319591
102.2989561	6.6	8.008609103	5.70855103	10.3084384
103.5229146	8.280555556	8.232960696	5.89292289	10.5686896
104.5515617	6.461111111	8.42151171	6.11809895	10.8576366
104.9449838	11.11111111	8.493625981	6.11318306	10.8771110
105.4562911	8.727777778	8.587348609	6.21497537	11.0106018
105.6533836	9.616666667	8.623475664	6.23067926	11.0385244
105.927544	6.035616438	8.673729265	6.33321729	11.1580590
106.1896663	10.2	8.721776283	6.30809883	11.1491908
106.2342115	8.938888889	8.729941418	6.31312177	11.1569754
106.4419275	8.455555556	8.768015761	6.35545308	11.2121840
107.3084652	7.027777778	8.926852121	6.52717726	11.4376292
107.4151606	7.891666667	8.946409388	6.51883781	11.4359043
107.6134706	9.605555556	8.982759611	6.53910131	11.4684621
107.8851764	9.633333333	9.032563284	6.56992142	11.5161266
108.4400622	9.788888889	9.134273851	6.66222298	11.6428283
108.4727324	8.863888889	9.140262299	6.70157593	11.6842066
108.6841437	12.28888889	9.17901399	6.65829613	11.6540333
108.7225336	7.336111111	9.186050859	6.73294741	11.7310646
109.1002288	8.55	9.255282389	6.77740254	11.7989349
109.1246943	10.50555556	9.259766915	6.74676074	11.7698098

Predictor variable		Estimated age		
IL (mm)	Real age	Mean estimate	Lower 95% PI	Upper 95% PI
109.2183558	10.13055556	9.276935068	6.78942046	11.8182761
110.2331394	9.761111111	9.462944902	6.95443325	12.0462004
110.6475946	8.567123288	9.53891454	7.04661645	12.1640778
110.7460762	9.452777778	9.556966217	7.03140700	12.1549737
110.8382913	8.997260274	9.573869245	7.07584585	12.2051294
111.1771048	9.772222222	9.63597376	7.12357909	12.2738674
111.3728902	8.747222222	9.671861224	7.13554342	12.2979694
111.4589386	8.941666667	9.687633895	7.16667312	12.3344337
112.2846278	9.436111111	9.838982726	7.29616893	12.5151181
112.7253821	12.51111111	9.919772989	7.32394098	12.5702147
113.2264273	11.075	10.01161457	7.42301482	12.7003509
114.0955894	12.03611111	10.17093199	7.53685669	12.8680765
114.11764	11.92777778	10.17497386	7.51967193	12.8522587
114.2193987	7.944444444	10.19362623	7.65855770	12.9974530
114.3903714	9.125	10.22496553	7.62050492	12.9699997
114.7576431	12.02777778	10.29228643	7.64350633	13.0157701
114.7975696	10.45833333	10.29960496	7.63686018	13.0115992
115.4129315	11.01666667	10.41240079	7.76235165	13.1752401
115.6364061	11.63333333	10.45336369	7.76470557	13.1914483
115.9883507	9.377777778	10.51787513	7.95859364	13.2495464
116.2218762	8.319444444	10.56068036	8.05901443	13.2598666
116.255229	10.61666667	10.56679393	8.00368274	13.1916664
117.2671857	11.08333333	10.75228559	8.35784992	13.1553927
117.7614584	11.24722222	10.84288577	8.51944334	13.1262820
117.7645026	10.17222222	10.84344378	8.59023751	13.1959016
117.9679612	10.25833333	10.88073774	8.65778613	13.1849503
118.0178316	12.09444444	10.88987898	8.58796778	13.0958905
118.352304	10.18611111	10.95118777	8.77021297	13.1490870
118.4908996	12.83888889	10.97660551	8.71673399	13.0421340
118.5051198	12.23055556	10.97734738	8.70687673	13.0307150
118.5575474	10.68888889	10.98008253	8.79635391	13.1144343
118.6930287	10.04722222	10.98715059	8.84326521	13.1464663
118.9489808	9.861111111	11.00050361	8.87895466	13.1540457
119.1461551	11.26944444	11.01079019	8.85229175	13.1057281
120.7903541	10.05277778	11.09656805	9.09668117	13.1695428
121.5717937	10.975	11.13733576	9.12881726	13.1158570
122.2050873	11.30833333	11.17037468	9.15899628	13.0764844
122.3059701	10.62777778	11.17563774	9.21112280	13.1175314
123.1088037	11.475	11.21752157	9.27950042	13.0977376
123.8868357	11.32222222	11.2581115	9.34852500	13.0813145
124.3667605	10.91111111	11.28314918	9.45055212	13.1306338
125.4773091	12.10277778	11.3410865	9.53223584	13.0903511

Predictor variable		Estimated age		
IL (mm)	Real age	Mean estimate	Lower 95% PI	Upper 95% PI
125.5480915	11.02777778	11.34477921	9.55838888	13.1087305
126.8884954	12.07777778	11.41470809	9.68998303	13.0931143
127.2259106	12.27777778	11.43231104	9.69264381	[13.0587184
128.8121977	10.79722222	11.51506763	9.93636347	13.1282235
129.1917431	12.51944444	11.53486852	9.92111675	13.0712931
130.2070382	10.90833333	11.58783646	10.07284001	13.1115113
130.779812	10.35555556	11.61771807	10.18258496	13.1583511
131.0210732	11.64722222	11.63030467	10.14051357	13.0897832
134.5973495	11.79444444	11.816879	10.54173680	13.0982407
135.2282706	12.78333333	11.84979416	10.51131550	12.9985283
135.9514014	12.65	11.88751989	10.61956272	13.0273574
137.1623701	11.45833333	11.95069613	10.91781492	13.1926146
143.054276	11.86944444	12.25807686	11.56840408	13.1961233

Table F-2 Mean, minimal and maximal estimated ages of the MARS prediction models using Ilium Width (IW) as predictor

Predictor variable IW (mm)	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
22.10177668	0.002777778	-0.47461508*	-0.89689238*	-0.22525610*
25.7288534	0.163888889	-0.24549907*	-0.71156824*	0.09472222
28.64563764	0.002777778	-0.06125098 *	-0.54449760*	0.37007763
29.71858903	0.061111111	0.00652546	-0.49055260*	0.46385563
32.12515598	0.016666667	0.15854403	-0.36009493*	0.68365641
32.41484769	0.022222222	0.17684334	-0.33724457*	0.71726149
34.8453845	0.144444444	0.33037604	-0.22807682 *	0.91666221
35.60400706	0.25	0.37829689	-0.20180921*	0.97109346
37.0473609	0.341666667	0.46947099	-0.13041319*	1.09607357
39.49544561	0.988888889	0.62411216	-0.05061308*	1.26675812
39.63374828	0.4	0.63284850	-0.01676358*	1.30574206
40.31653327	0.313888889	0.67597882	0.02231085	1.37016469
40.47137623	0.555555556	0.68575997	0.01863704	1.37223938
40.96018877	0.625	0.71663739	0.04427247	1.41602184
42.75798962	0.666666667	0.83020129	0.14087211	1.57936431
46.39969625	0.947222222	1.06024144	0.28864235	1.86233185
52.13668109	1.183333333	1.42263661	0.57539271	2.36206611
54.29810574	1.075	1.55916997	0.68700760	2.55392327
54.4601861	2.175	1.56940830	0.62057740	2.49351025
55.25549016	1.35	1.61964625	0.72934510	2.63180339
56.83487339	1.122222222	1.71941309	0.79902857	2.76012100
57.27463149	1.219444444	1.74719183	0.86255852	2.83997684
57.4423687	1.672222222	1.75778749	0.80732924	2.79097476
57.50622076	1.983333333	1.76182091	0.80976463	2.79578064
58.06341296	2.372222222	1.79701776	0.81091813	2.81761974
59.0623871	1.888888889	1.86012118	0.87553002	2.91931825
59.76166167	3.086111111	1.90429312	0.87336820	2.94311679
62.22985545	2.75	2.06020455	0.99355790	3.15493748
62.92780546	2.611111111	2.10429281	1.05209185	3.23938261
62.94771373	2.402739726	2.10555038	1.07224206	3.26027191
63.91088885	2.138888889	2.27992428	1.12790285	3.41841429
64.12574104	1.933333333	2.31882127	1.20443382	3.51780548
65.22787752	1.838888889	2.51835282	1.29074986	3.72138856
65.94556674	2.877777778	2.64828378	1.31186293	3.81886358
66.04170613	2.691666667	2.66568892	1.33936215	3.85659201
66.63094898	2.408219178	2.77236586	1.44535619	4.02528133
66.8012544	3.355555556	2.80319807	1.37792639	3.97597199
68.29455422	5.913888889	3.07354611	1.46254526	4.21947759
69.65337867	2.744444444	3.31954863	1.85105232	4.75256322

Predictor variable IW (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
69.6617987	3.483333333	3.32107300	1.78554932	4.68795611
70.25845687	3.005555556	3.42909241	1.87174508	4.83763614
70.77073316	3.736986301	3.52183527	1.91497264	4.93536977
71.45094758	4.065753425	3.64498176	2.01900775	5.11177953
72.09507698	4.213888889	3.76159539	2.10403790	5.26534488
72.97347585	4.105555556	3.92062134	2.24572797	5.50049636
73.30968514	3.538888889	3.98148890	2.30069525	5.59123623
73.48876614	3.291666667	4.01390985	2.33598562	5.64558077
73.70771334	4.588888889	4.05354820	2.31548110	5.64837217
74.32426575	3.575342466	4.16516928	2.41715428	5.81564638
74.94777525	3.583333333	4.27804988	2.51451695	5.97935030
74.98006883	3.038888889	4.28389633	2.56775595	6.03602533
76.08573632	3.25	4.48406714	2.65314824	6.23906035
76.09966106	4.291666667	4.48658809	2.68630814	6.27370184
76.73607201	5.122222222	4.60180437	2.75523726	6.41034493
76.77710252	5.113888889	4.60923256	2.70646745	6.36594075
77.39697367	4.991666667	4.72145446	2.81315908	6.53858652
78.21863216	4.375	4.87020809	2.97941377	6.79226546
79.54350727	4.284931507	5.11006440	3.16795504	7.12177311
79.6234553	4.961111111	5.12453825	3.13226668	7.09459120
79.9071219	4.533333333	5.17589345	3.20648673	7.19899330
80.55199987	4.556164384	5.29264261	3.28634389	7.34746531
81.14484712	5.461111111	5.39997208	3.31094072	7.43514093
81.54194835	4.983333333	5.47186357	3.42354559	7.58999728
81.68621215	7.425	5.49798119	3.32533768	7.50713900
82.70767692	5.808333333	5.68290788	3.49198166	7.78246657
82.98809594	5.569444444	5.73367513	3.56454652	7.88486794
83.04785522	6.109589041	5.74449400	3.51417006	7.84084985
83.41045499	5.491666667	5.81013931	3.63096489	7.99622521
83.490859	5.717808219	5.82469571	3.66604331	8.03985859
83.71683804	5.625	5.86560711	3.72338605	8.12124545
83.89441725	5.005479452	5.89775618	3.70300444	8.11975821
84.13141655	6.111111111	5.94066269	3.72630061	8.16827105
84.41305804	6.216666667	5.99165127	3.80124441	8.27318144
85.22901446	4.638888889	6.13937258	3.91326312	8.47201770
85.37347188	5.341666667	6.16552526	3.93554994	8.50967476
85.65237975	4.413888889	6.21601893	4.01585568	8.61965622
85.93877446	4.963888889	6.26786803	4.03511904	8.66939190
87.38592967	8.019444444	6.52986202	4.14083879	8.92908860
87.7358993	9.022222222	6.59322076	4.21501478	9.04050127
88.1724957	6.6	6.67226248	4.34217178	9.21411201

Predictor variable IW (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
88.36283406	8.043835616	6.70672147	4.31917108	9.21136326
89.05779532	7.433333333	6.83253774	4.45157128	9.41770717
89.22023082	8.811111111	6.86194517	4.39085419	9.37427317
89.2512774	6.463888889	6.86756587	4.43715467	9.42387700
89.33574957	5.769444444	6.88285877	4.46758879	9.46329894
89.53980529	6.275	6.91980116	4.50282345	9.52024508
89.67502328	10.01388889	6.94428112	4.43358996	9.46539875
90.06230386	6.441666667	7.01439466	4.60173148	9.67474682
90.16074412	5.666666667	7.03221636	4.62677035	9.71025971
91.03614003	6.791666667	7.19069864	4.74439769	9.92102896
91.31978671	7.138888889	7.24205023	4.79846970	10.00528090
91.79695717	7.086111111	7.32843751	4.88379367	10.14137568
92.59915098	7.75	7.47366723	4.89969583	10.24263107
92.93951591	6.9	7.53528714	4.99664837	10.37579835
93.29805002	7.994444444	7.60019640	5.04546893	10.46276684
93.54793183	7.727777778	7.64543518	5.05166583	10.49555110
93.7503637	7.397222222	7.68208359	5.16693558	10.63235955
93.85364013	6.005555556	7.70078082	5.15529601	10.63170857
94.23283947	11.11111111	7.76943134	5.04485192	10.56161119
94.33405278	8.280555556	7.78775506	5.18964869	10.71717703
94.52764941	8.972222222	7.82280393	5.20950852	10.75763550
94.55006267	7.366666667	7.82686164	5.23941297	10.78992471
95.30089339	6.461111111	7.96279256	5.38968953	11.02008946
96.12016262	8.997260274	8.11111363	5.46051243	11.17808241
96.45703126	6.288888889	8.17210057	5.59992094	11.35333365
96.58593345	9.997222222	8.19543711	5.49529786	11.23587193
96.73691704	6.035616438	8.23785166	5.69706411	11.41430374
96.91225417	9.605555556	8.28710765	5.58458622	11.27472758
97.0945056	7.336111111	8.33830602	5.75952934	11.42150384
98.73864917	7.027777778	8.80018150	6.26830561	11.67617852
98.78377922	10.2	8.81285951	6.15294759	11.55384567
98.79231092	8.863888889	8.81525625	6.22998557	11.62956508
98.9131165	9.452777778	8.84919315	6.19398352	11.57489258
98.99811909	8.55	8.87307222	6.25475690	11.62252885
99.29908525	8.455555556	8.95762012	6.35243313	11.67369091
99.35128832	12.51111111	8.97228509	6.27071132	11.58390114
99.45710783	8.319444444	9.00201208	6.43493354	11.73176901
99.66481446	8.727777778	9.06036136	6.47504726	11.73978178
100.7537642	7.891666667	9.36627089	6.82185924	11.91829710
100.8287287	8.938888889	9.38733004	6.80427822	11.88913035
101.2042347	8.567123288	9.49281779	6.94845695	11.97527481

Predictor variable IW (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
101.3057929	10.50555556	9.52134769	6.89270538	11.90382748
101.6062444	11.01666667	9.60575101	6.99301865	11.95770611
101.6236564	10.13055556	9.61064242	7.00842640	11.97042284
102.3422777	9.436111111	9.81251868	7.27510045	12.12603432
102.6487278	10.04722222	9.89860714	7.28818258	12.09175474
103.248956	9.633333333	10.06722422	7.50158985	12.21239704
103.2957346	8.941666667	10.08036534	7.61316384	12.31674141
103.3785134	9.761111111	10.10361970	7.55264953	12.24343368
103.398512	9.616666667	10.10923774	7.58397637	12.27166974
103.429249	9.772222222	10.11787242	7.58257421	12.26551720
103.5011275	10.17222222	10.13806465	7.55759364	12.22942784
103.8767825	11.30833333	10.24359426	7.57950485	12.19328176
104.2846996	11.075	10.35818700	7.80707813	12.35781165
104.6239139	10.18611111	10.45347963	7.93232646	12.43063457
104.7531434	12.83888889	10.48978299	7.77326424	12.25159999
104.9218409	12.28888889	10.53717377	7.86516433	12.31742796
105.1619608	12.09444444	10.60462864	7.98357604	12.39872927
105.5730155	11.63333333	10.63939448	8.02622222	12.42224895
105.767623	8.747222222	10.65585383	8.24488638	12.63185797
106.0139407	9.788888889	10.67668668	8.19603063	12.57154100
106.6854555	7.944444444	10.73348150	8.49089225	12.83515685
106.6966744	11.92777778	10.73443037	8.26308889	12.60683147
106.750325	9.377777778	10.73896798	8.39432465	12.73557085
107.5887779	10.61666667	10.80988195	8.43411397	12.73634672
107.7277234	11.32222222	10.82163357	8.46830016	12.76406775
108.0219121	10.68888889	10.84651522	8.58911570	12.87119460
108.6435575	12.23055556	10.89909222	8.60489700	12.85805057
108.8200558	10.45833333	10.91401995	8.65345428	12.89839534
108.8575282	10.62777778	10.91718926	8.64354602	12.88674348
110.8358859	9.125	11.08451315	9.00830005	13.15944398
111.024355	11.24722222	11.10045333	8.99243197	13.13480639
111.083621	12.07777778	11.10546588	8.96445760	13.10407435
111.1174588	10.05277778	11.10832779	9.01315285	13.15119512
111.130796	11.26944444	11.10945581	8.98099356	13.11841525
111.1347923	10.25833333	11.10979380	9.01015476	13.14739050
111.600489	9.861111111	11.14918111	9.07760087	13.19316762
111.9047245	11.02777778	11.17491249	9.13438962	13.23580020
112.5589348	10.975	11.23024374	9.21817597	13.28914596
112.704873	11.475	11.24258678	9.24605504	13.31023450
112.892029	10.91111111	11.25841591	9.28001618	13.33548721
112.9073147	12.27777778	11.25970873	9.23017803	13.28493782



Predictor variable IW (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
113.2493927	12.03611111	11.28864072	9.28003100	13.31887381
113.5851494	11.08333333	11.31703807	9.34891548	13.37213543
114.1573383	11.86944444	11.36543218	9.42218048	13.41877633
114.6915635	10.90833333	11.41061544	9.57550557	13.54724377
114.9513156	12.65	11.43258453	9.46376545	13.42341731
115.1590125	12.10277778	11.45015095	9.53363101	13.48361869
115.2642918	11.64722222	11.45905517	9.55635315	13.50144215
115.3394228	10.35555556	11.46540954	9.64551578	13.58710891
115.3700713	12.02777778	11.46800170	9.58586507	13.52603212
115.9361071	10.79722222	11.51587541	9.80549507	13.71932432
117.9394759	12.51944444	11.68531467	9.98939352	13.81000547
119.2847701	11.79444444	11.79909584	10.16877919	13.92679424
121.74948	11.45833333	12.00755402	10.77391109	14.41724251
124.3816307	12.78333333	12.23017387	10.89637730	14.41723402

Table F-3 Mean, minimal and maximal estimated ages of the MARS prediction models using Ilium Module (IM) as predictor

Predictor variable IM (mm <sup>2</sup> )	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
527.2838745	0.002777778	-0.48602216*	-1.04332767*	0.09905768
747.6678966	0.163888889	-0.31144531*	-0.90322303*	0.30504043
934.7163099	0.002777778	-0.16327521*	-0.77000834*	0.49416841
1009.87807	0.061111111	-0.10373595 *	-0.75469709*	0.53194732
1142.122696	0.016666667	0.00102142	-0.62331779*	0.70285773
1194.534581	0.022222222	0.04253941	-0.62276968*	0.71907303
1394.333312	0.144444444	0.20080965	-0.49444630*	0.90712107
1449.130921	0.25	0.24421749	-0.47007428*	0.94787342
1574.329864	0.341666667	0.34339363	-0.37480129*	1.08057139
1690.000873	0.988888889	0.43502223	-0.34495687*	1.14499268
1776.95757	0.4	0.50390484	-0.28011212*	1.23583089
1846.987894	0.555555556	0.55937925	-0.20668754*	1.33018921
1851.423334	0.313888889	0.56289278	-0.19864157*	1.33956105
2003.990641	0.625	0.68374872	-0.10535082*	1.47845785
2250.40659	0.666666667	0.87894672	0.04498834	1.70245669
2476.418053	0.947222222	1.05798133	0.18692932	1.91195795
2919.627439	1.183333333	1.40906894	0.44790961	2.30542424
3196.112671	2.175	1.62808627	0.54196709	2.48212983
3466.412388	1.983333333	1.84220375	0.75621657	2.77717842
3496.089995	1.35	1.86571282	0.78876025	2.81859346
3513.551098	1.672222222	1.87954460	0.79576323	2.83081598
3524.790361	1.888888889	1.88844777	0.78322987	2.82164231
3539.351077	1.075	1.89998202	0.85308239	2.89584738
3582.042491	2.372222222	1.93379995	0.82732544	2.88285192
3636.80305	1.122222222	1.97717844	0.93086546	3.00276119
3664.051758	1.219444444	1.99876346	0.95605219	3.03609322
3836.2153	3.086111111	2.13514253	0.95971098	3.09121585
4047.578937	2.402739726	2.30257390	1.15858507	3.35327164
4369.629511	2.75	2.55768574	1.38136140	3.67231666
4480.259402	2.138888889	2.64532103	1.48649877	3.81052398
4570.093709	1.838888889	2.71648313	1.56347678	3.91435564
4592.26167	2.611111111	2.73404344	1.53968453	3.89718993
4686.343465	5.913888889	2.80857019	1.50750027	3.89312899
4730.895633	2.877777778	2.84386211	1.63301543	4.03196187
4804.528493	1.933333333	2.90219027	1.73707022	4.15802729
4888.5424	3.355555556	2.96874175	1.76374004	4.20981090
4898.032415	2.408219178	2.97625925	1.80578007	4.25468773
5080.837771	2.744444444	3.12106821	1.92609694	4.42964954
5089.219599	2.691666667	3.12970038	1.93063978	4.43744980
5306.778552	3.483333333	3.35375720	2.10239087	4.69375069

Predictor variable IM (mm <sup>2</sup> )	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
5412.501493	3.005555556	3.46263779	2.19076926	4.82321613
5488.883332	3.736986301	3.54130094	2.21149619	4.87362730
5679.503512	4.213888889	3.73761440	2.35089927	5.08711098
5708.467603	4.105555556	3.76744357	2.37432988	5.12179789
5725.515687	3.291666667	3.78500083	2.43106607	5.18515947
5726.116983	4.065753425	3.78562008	2.39810744	5.15243452
5766.735981	3.538888889	3.82745225	2.45989379	5.23000661
6039.136519	3.575342466	4.10798862	2.68010190	5.55607758
6136.371096	3.25	4.20812732	2.77124903	5.68501293
6140.582144	3.583333333	4.21246414	2.77035791	5.68575835
6166.823576	5.113888889	4.23948933	2.75292536	5.67852399
6330.120991	3.038888889	4.40766397	2.93557615	5.92463696
6382.454986	4.291666667	4.46156104	2.96990455	5.97930389
6535.292458	4.588888889	4.61896333	3.07028016	6.13907663
6555.829338	4.375	4.64011359	3.09704124	6.17381895
6582.57818	4.991666667	4.66766134	3.09836862	6.18554172
6587.835071	4.284931507	4.67307524	3.12597271	6.21518879
6828.401269	5.122222222	4.92082647	3.32331323	6.50602041
6905.185534	4.533333333	4.99990406	3.40387791	6.61642571
6954.783119	4.983333333	5.05098298	3.43224115	6.66406403
7091.602136	4.961111111	5.19188840	3.53036297	6.81535775
7187.570894	7.425	5.29072347	3.58767610	6.90996717
7228.829741	5.005479452	5.33321460	3.68480509	7.02313055
7270.485543	5.461111111	5.37611454	3.69329975	7.04781388
7313.874415	4.556164384	5.42079932	3.72264446	7.09402079
7510.483908	6.216666667	5.62328098	3.89388961	7.34167418
7519.338594	5.808333333	5.63240013	3.91210698	7.36333273
7524.388916	4.638888889	5.63760129	3.94615787	7.39934633
7539.201728	5.491666667	5.65285652	3.91934648	7.37829163
7626.248412	6.109589041	5.74250304	4.00715449	7.49992854
7628.358858	5.569444444	5.74467652	4.00062112	7.49421535
7674.195672	5.717808219	5.79188235	4.05038283	7.56179060
7737.827698	4.963888889	5.85741488	4.09697864	7.63311568
7757.792034	5.341666667	5.87797549	4.12610818	7.67000396
7879.990936	4.413888889	6.00382413	4.23727080	7.82865667
7958.254273	6.111111111	6.08442497	4.27091646	7.89271776
8121.648865	5.625	6.25269970	4.42898458	8.11428582
8177.687649	5.666666667	6.31041220	4.46973704	8.17681661
8234.562847	6.791666667	6.36898610	4.50919271	8.23837565
8246.355122	5.769444444	6.38113057	4.52837710	8.26214286
8479.186615	8.811111111	6.62091608	4.69754504	8.52179598
8537.581554	6.275	6.68105511	4.77813384	8.62507877

Predictor variable IM (mm <sup>2</sup> )	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
8571.506506	8.043835616	6.71599331	4.79231936	8.65244852
8597.732677	6.441666667	6.74300278	4.82138888	8.69171030
8712.82794	10.01388889	6.86153561	4.88540565	8.80045648
8748.36999	6.463888889	6.89813920	4.95161854	8.88048205
8815.681956	9.022222222	6.96746158	5.01466135	8.96968427
8823.728378	8.019444444	6.97574833	4.99762858	8.95577858
8909.396312	7.433333333	7.06397492	5.09854317	9.08998625
9019.954267	6.6	7.17783493	5.18491856	9.21932771
9058.070044	7.138888889	7.21708911	5.19593504	9.24515710
9108.921984	7.086111111	7.26945986	5.24265200	9.31163663
9213.139901	7.75	7.37679047	5.34000549	9.44949226
9302.384063	6.005555556	7.46870010	5.42050988	9.56467956
9347.137129	7.397222222	7.51478981	5.46111303	9.62267507
9356.36781	7.727777778	7.52429619	5.47714818	9.64229753
9357.336356	8.972222222	7.52529366	5.44040058	9.60592634
9409.741987	6.9	7.57926450	5.52480025	9.71069238
9479.932377	7.366666667	7.65155128	5.55340952	9.76657969
9511.267899	7.994444444	7.68382270	5.58263729	9.80798537
9759.545057	6.288888889	7.93951519	5.82359260	10.14542849
9765.736095	8.280555556	7.94589114	5.78952312	10.11376503
9852.081743	9.997222222	8.03481569	5.86090814	10.21870651
9889.263808	11.11111111	8.07310827	5.89214017	10.26438858
9963.857235	6.461111111	8.14992959	5.99416503	10.39540264
10247.10404	6.035616438	8.44163618	6.23202906	10.74334472
10429.06401	9.605555556	8.62903077	6.35948717	10.94151782
10489.81655	10.2	8.69159782	6.39911168	11.00475255
10510.28169	8.727777778	8.71267420	6.43722468	11.05081891
10556.36065	7.336111111	8.76012940	6.48684515	11.11834703
10569.58603	8.455555556	8.77374979	6.49738569	11.13402733
10595.4929	7.027777778	8.80043042	6.54188711	11.18859693
10653.79458	8.997260274	8.86047341	6.55522897	11.22459653
10711.46049	8.938888889	8.91986163	6.60668262	11.29846085
10716.2719	8.863888889	8.92481675	6.63061017	11.32425826
10800.71745	8.55	9.01178445	6.66895955	11.39542567
10822.48176	7.891666667	9.03419879	6.71902387	11.45394823
10924.40265	9.616666667	9.13916377	6.76242051	11.53695433
10954.23954	9.452777778	9.16989181	6.81512210	11.60125141
11054.96368	10.505555556	9.27362429	6.89465365	11.71992733
11099.16866	10.130555556	9.31914955	6.90495787	11.74741090
11139.03183	9.633333333	9.36020332	6.94048129	11.79842633
11198.00513	8.567123288	9.42093798	7.01906673	11.89993053
11199.41194	12.51111111	9.42238681	6.96722838	11.84863891

Predictor variable IM (mm <sup>2</sup> )	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
11395.73808	9.761111111	9.62457665	7.17198850	12.12969713
11403.34043	12.28888889	9.63240606	7.15808695	12.11875009
11491.46456	9.436111111	9.72316221	7.27044298	12.26535374
11496.15832	9.788888889	9.72799616	7.28399368	12.28072858
11498.96445	9.772222222	9.73088610	7.30216372	12.29998916
11513.23294	8.941666667	9.74558075	7.30719034	12.31056094
11559.09167	8.319444444	9.79280915	7.37479466	12.39598731
11726.67453	11.01666667	9.96539723	7.45517801	12.54149829
11779.64586	8.747222222	10.01995067	7.52189147	12.62879797
11807.78395	11.075	10.04892916	7.52650799	12.64434977
12175.97268	11.92777778	10.42811464	7.80079063	13.06172139
12183.68839	10.04722222	10.43606080	7.84469911	13.10862842
12185.54858	7.944444444	10.43797655	7.90850026	13.17315249
12188.7588	10.17222222	10.44128265	7.88275907	13.14865889
12208.0841	11.63333333	10.46118514	7.86567492	13.13908512
12381.79413	9.377777778	10.64008339	8.03124979	13.37216882
12382.48126	10.18611111	10.64079105	7.99599429	13.33530914
12410.98658	12.09444444	10.67014774	8.01336321	13.28612960
12412.2942	12.83888889	10.67149441	8.03541077	13.30512439
12492.27793	10.45833333	10.75386703	8.26924695	13.35223070
12507.75801	10.61666667	10.76980946	8.30127852	13.34812251
12678.55816	9.125	10.94571092	8.66728550	13.31537976
12694.27128	11.30833333	10.96189335	8.60529058	13.21670103
12806.81296	10.68888889	11.07779633	8.81366403	13.16233487
12874.8178	12.23055556	11.14783228	8.87115991	13.06106678
12921.25621	12.03611111	11.19565767	9.01794108	13.09943292
13074.38996	11.24722222	11.23206044	9.09602927	13.09499995
13110.34487	10.25833333	11.24060760	9.21705873	13.19665392
13239.59747	12.02777778	11.27133337	9.16845197	13.07839515
13240.80706	11.26944444	11.27162091	9.19390817	13.10319952
13274.76442	9.861111111	11.27969321	9.31572398	13.20671629
13313.92559	10.62777778	11.28900256	9.30400579	13.17389481
13319.81081	11.08333333	11.29040158	9.27024828	13.13696586
13346.04678	11.32222222	11.29663837	9.29628033	13.14885979
13421.91719	10.05277778	11.31467419	9.37902018	13.19071437
13683.9916	10.975	11.37697419	9.49281712	13.16328390
13874.96209	11.475	11.42237146	9.57374580	13.14130184
14040.01593	10.91111111	11.46160786	9.64681270	13.12542405
14049.42459	11.02777778	11.46384448	9.65159080	13.12513198
14095.23353	12.07777778	11.47473412	9.64446268	13.09331820
14364.73593	12.27777778	11.53879990	9.80739625	13.11102154
14449.84301	12.10277778	11.55903145	9.87223432	13.12999686

Predictor variable IM (mm <sup>2</sup> )	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
14933.64879	10.90833333	11.67404117	10.17953556	13.17658343
14933.98475	10.79722222	11.67412103	10.18609604	13.18296287
15084.06803	10.35555556	11.70979864	10.28876968	13.20475920
15102.05121	11.64722222	11.71407358	10.22140169	13.12770038
15236.80648	12.51944444	11.74610744	10.27538148	13.10906285
15627.79245	12.65	11.83905214	10.46302364	13.08600936
16055.41389	11.79444444	11.94070579	10.78832053	13.18086837
16330.69538	11.86944444	12.00614536	10.95267053	13.19687387
16699.44724	11.45833333	12.09380460	11.20432913	13.24981840
16819.91281	12.78333333	12.12244153	11.06757851	13.04815095

Table F-4 Mean, minimal and maximal estimated ages of the MARS prediction models using Ilium Area (IA) as predictor

Predictor variable IA (mm <sup>2</sup> )	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
365.102	0.002777778	-0.51190170	-1.11825078*	0.01759304
513.707	0.163888889	-0.35929711	-0.95629571*	0.23835236
695.356	0.002777778	-0.17275918	-0.79756279*	0.46896531
700.694	0.061111111	-0.16727751	-0.77258573*	0.49605466
795.968	0.022222222	-0.06943929	-0.66710375*	0.63923736
813.005	0.016666667	-0.05194375	-0.68009766*	0.63298514
995.104	0.144444444	0.13505630	-0.51027313*	0.87486777
1057.394	0.25	0.19902278	-0.46383785*	0.94595172
1123.423	0.341666667	0.26682890	-0.39570795*	1.04020986
1166.65	0.988888889	0.31121932	-0.41401792*	1.03900518
1261.855	0.4	0.40898669	-0.26938674*	1.22130977
1293.794	0.555555556	0.44178530	-0.26256904*	1.24076601
1354.921	0.313888889	0.50455748	-0.18505494*	1.34246857
1533.458	0.625	0.68789966	-0.04926907*	1.54890303
1629.859	0.666666667	0.78689522	0.04514142	1.68146020
1818.36	0.947222222	0.98046957	0.18939728	1.90030749
2084.07	1.183333333	1.25333094	0.43637841	2.25243230
2399.241	1.35	1.57698451	0.72372316	2.66449287
2437.208	1.983333333	1.63149354	0.70991899	2.67169306
2463.473	2.175	1.66920207	0.69521427	2.67151884
2477.966	1.075	1.69000960	0.84816988	2.83249237
2492.492	1.888888889	1.71086450	0.75192171	2.74428038
2529.616	1.672222222	1.76416324	0.79342916	2.80632582
2542.328	1.122222222	1.78241380	0.89741301	2.91734229
2573.978	2.372222222	1.82785354	0.82516656	2.86260548
2612.355	1.219444444	1.88295121	0.92661999	2.98529009
2746.367	3.086111111	2.07535156	0.97149073	3.10429988
2794.661	2.402739726	2.14468702	1.08138834	3.24091502
3042.806	2.75	2.50094754	1.32470647	3.62151365
3181.178	2.138888889	2.69960753	1.50048831	3.87384660
3251.768	2.611111111	2.80095324	1.53519385	3.94760442
3295.898	1.838888889	2.86431046	1.62684791	4.06367238
3325.76	1.933333333	2.90718318	1.62800603	4.08135096
3355.785	3.355555556	2.95028992	1.64533842	4.11529400
3379.753	5.913888889	2.98470066	1.56874212	4.05195744
3384.05	2.877777778	2.99086984	1.68744992	4.17304245
3406.229	2.408219178	3.02271212	1.74017271	4.23803526
3659.875	2.691666667	3.38687040	2.01309988	4.65128622
3661.205	2.744444444	3.38877988	2.03232366	4.67124580
3799.977	3.005555556	3.58801414	2.17449227	4.89018681
3838.904	3.483333333	3.64390144	2.21251855	4.94974856
3883.068	3.736986301	3.70730747	2.24584644	5.00750915
3973.183	4.065753425	3.83668512	2.34500071	5.15651747
4021.553	4.213888889	3.90612969	2.39265009	5.23092643
4072.105	4.105555556	3.97870694	2.46138824	5.32763131

Predictor variable IA (mm <sup>2</sup> )	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
4105.992	3.575342466	4.02735833	2.52855073	5.41354099
4114.117	3.291666667	4.03902336	2.52953204	5.41901728
4190.856	3.038888889	4.14919715	2.63639019	5.56832950
4231.081	3.538888889	4.20694798	2.67175349	5.62594636
4233.919	5.113888889	4.21102248	2.63727794	5.59304087
4300.507	3.25	4.30662254	2.76157149	5.75417269
4314.264	3.583333333	4.32637340	2.77614423	5.77635617
4403.558	4.375	4.45457234	2.88120304	5.93081482
4588.396	4.284931507	4.71994333	3.08034074	6.23220987
4607.656	4.291666667	4.74759481	3.12709971	6.28962400
4645.936	4.588888889	4.80255322	3.16134072	6.34504254
4787.772	4.991666667	5.00618645	3.31748371	6.57965301
4886.169	4.533333333	5.14745453	3.46037503	6.77698020
4910.946	5.005479452	5.18302674	3.47655321	6.80686569
4953.286	4.556164384	5.24381407	3.53337560	6.88711170
4976.568	4.983333333	5.27723992	3.55851973	6.92513606
4983.055	7.425	5.28655327	3.52543919	6.89564430
5003.929	5.491666667	5.31652197	3.58114955	6.96290272
5033.67	5.122222222	5.35922097	3.61527270	7.01347939
5067.736	4.638888889	5.40812936	3.67414148	7.09119440
5123.709	4.961111111	5.48848951	3.71236368	7.16038236
5194.038	6.109589041	5.58946050	3.80486486	7.29179144
5204.157	5.461111111	5.60398830	3.81086252	7.30338720
5251.165	5.808333333	5.67147745	3.86061119	7.37914197
5281.519	5.569444444	5.71505653	3.88565779	7.42098122
5357.413	5.717808219	5.82401717	4.00498668	7.58229670
5371.578	4.963888889	5.84435379	4.03150113	7.61664761
5376.607	5.341666667	5.85157390	4.04545118	7.63337985
5423.814	5.666666667	5.91934875	4.09558951	7.70963435
5440.696	6.111111111	5.94358615	4.11300320	7.73638763
5445.579	6.216666667	5.95059665	4.10566753	7.73175336
5532.634	6.791666667	6.07558107	4.20360224	7.87784924
5661.807	5.625	6.26103410	4.37763953	8.12334850
5759.668	4.413888889	6.40153265	4.50473500	8.30458331
5838.442	5.769444444	6.51462808	4.58725525	8.43068346
5898.726	8.043835616	6.60117752	4.63554067	8.51231961
5976.235	6.441666667	6.71245680	4.73647294	8.65613194
6006.399	7.433333333	6.75576310	4.77294613	8.70929267
6024.81	8.811111111	6.78219568	4.78240284	8.72893484
6064.526	6.463888889	6.83921574	4.83656619	8.80507015
6154.478	9.022222222	6.96835938	4.91518757	8.93345540
6155.575	6.275	6.96993433	4.96605592	8.98493064
6200.363	6.6	7.03423624	5.04329102	9.08694366
6228.795	7.75	7.07505592	5.04039802	9.09978000
6256.023	10.01388889	7.11414702	5.05002152	9.12446677
6402.378	8.019444444	7.32426816	5.22692669	9.38233946



Predictor variable IA (mm <sup>2</sup> )	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
6445.205	7.138888889	7.38575467	5.31872353	9.49782935
6483.792	7.086111111	7.44115383	5.35540964	9.55586283
6542.554	7.727777778	7.52551814	5.41122749	9.64418940
6589.118	7.366666667	7.59236984	5.48377353	9.74249589
6640.782	6.005555556	7.66654358	5.56463992	9.85194420
6656.735	7.397222222	7.68944722	5.57094752	9.86707743
6670.886	8.972222222	7.70976374	5.56442214	9.86838076
6741.003	6.9	7.81043037	5.64784352	9.99059275
6763.363	8.280555556	7.84253251	5.68486339	10.03998277
6790.613	7.994444444	7.88165519	5.70133880	10.07153362
6816.28	6.288888889	7.91850518	5.77120462	10.15559911
6913.878	6.461111111	8.05862613	5.89166175	10.33005008
6932.325	9.997222222	8.08511040	5.85710456	10.30569827
7148.777	11.11111111	8.39586945	6.10063274	10.66897352
7212.534	10.2	8.48740505	6.18566595	10.78927881
7226.691	6.035616438	8.50773018	6.27384059	10.88528549
7250.889	8.938888889	8.54247113	6.27725967	10.90209155
7256.327	7.336111111	8.55027844	6.27723583	10.90507616
7325.346	8.727777778	8.64936867	6.35058691	11.01661041
7355.524	8.997260274	8.69269507	6.36533084	11.04804962
7362.164	8.55	8.70222809	6.39630570	11.08269791
7365.294	9.605555556	8.70672181	6.38278221	11.07090602
7368.389	8.455555556	8.71116529	6.39977564	11.08961168
7428.494	7.027777778	8.79745774	6.50687608	11.22996382
7525.787	9.633333333	8.93714081	6.55984536	11.33675821
7555.29	10.13055556	8.97949811	6.61282166	11.40605637
7668.939	7.891666667	9.14266341	6.75775909	11.61386748
7733.323	12.51111111	9.23509920	6.77677730	11.66850465
7752.082	8.863888889	9.26203140	6.85328476	11.75539009
7793.821	10.50555556	9.32195587	6.87485539	11.80005186
7812.486	9.616666667	9.34875312	6.91347112	11.84899357
7954.146	9.452777778	9.55213367	7.08753505	12.10142762
7999.245	9.761111111	9.61688207	7.17803887	12.21688142
8001.779	8.941666667	9.62052012	7.16421005	12.20445448
8029.255	9.772222222	9.65996728	7.19036724	12.24581213
8031.028	12.28888889	9.66251277	7.15010975	12.20653552
8048.511	9.436111111	9.68761302	7.20667555	12.27277337
8172.943	11.01666667	9.86625942	7.32978895	12.46472591
8245.827	8.747222222	9.97089861	7.48138919	12.65664754
8261.634	9.788888889	9.99359264	7.45326085	12.63726405
8296.528	11.075	10.04368978	7.49897836	12.70228587
8377.81	10.17222222	10.16038594	7.60058553	12.84886043
8399.731	8.319444444	10.19185781	7.65957926	12.91998144
8402.803	10.18611111	10.19626826	7.58886546	12.85096715
8411.533	9.377777778	10.20880188	7.62148914	12.88842050
8442.519	10.04722222	10.25328832	7.66658772	12.95066138

Predictor variable IA (mm <sup>2</sup> )	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
8620.558	10.45833333	10.50889802	7.86682565	13.24939527
8656.817	12.83888889	10.56095488	7.85118182	13.25381089
8739.558	7.944444444	10.67974572	8.05454434	13.50294796
8796.54	8.567123288	10.76155449	8.21373037	13.45929951
8806.698	12.03611111	10.77613828	8.15421717	13.36362766
8819.505	11.63333333	10.79452523	8.21120602	13.37502840
8846.873	11.92777778	10.83381733	8.26193478	13.32833738
8930.51	12.09444444	10.95389455	8.50175940	13.27044575
8932.349	12.23055556	10.95653479	8.53866926	13.30080947
9013.783	10.61666667	10.98982344	8.65371534	13.33332055
9017.584	12.02777778	10.99137722	8.60391742	13.27967024
9132.368	11.24722222	11.03829871	8.72159581	13.28101273
9157.02	10.68888889	11.04837598	8.78381516	13.31824679
9171.096	11.30833333	11.05412997	8.76716246	13.28732778
9221.787	9.125	11.07485148	8.90361024	13.37239921
9245.755	10.05277778	11.08464914	8.91385883	13.35835575
9273.456	9.861111111	11.09597277	8.90765811	13.32407951
9354.534	11.32222222	11.12911590	8.93164751	13.26589472
9408.022	11.26944444	11.15098077	8.96454641	13.24458246
9544.115	10.25833333	11.20661297	9.14971435	13.29181741
9594.951	11.08333333	11.22739375	9.14805646	13.23863622
9655.646	11.475	11.25220470	9.21059958	13.23966373
9764.539	12.27777778	11.29671806	9.29060425	13.20930315
9765.576	10.91111111	11.29714197	9.31178318	13.22943106
9805.865	10.62777778	11.31361134	9.37426283	13.25107700
9814.373	12.07777778	11.31708924	9.34696583	13.21515697
9920.612	11.02777778	11.36051770	9.46192596	13.22244174
9972.909	10.975	11.38189571	9.51800321	13.22551492
10239.178	10.90833333	11.49074136	9.74543867	13.18308140
10417.461	12.10277778	11.56362002	9.87184291	13.12879224
10619.37	12.51944444	11.64615654	10.06212618	13.11443668
10657.966	10.79722222	11.66193384	10.16992488	13.18311755
10718.943	11.64722222	11.68686007	10.19380616	13.14519742
10775.744	10.35555556	11.71007922	10.29347170	13.18729400
11125.287	12.65	11.85296568	10.44663482	12.98618826
11653.316	11.79444444	12.06881379	11.06576434	13.07014976
11660.708	11.86944444	12.07183549	11.01894285	13.01583633
11666.817	11.45833333	12.07433273	11.07496787	13.06566976
11837.305	12.78333333	12.14402495	11.06638213	12.88429101

## Lumbar models

Table F-5 Mean, minimal and maximal estimated ages of the MARS prediction models using Anterior Vertebral Height (AVH) as predictor

Predictor variable AVH (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
3.723562	0.002739726	-0.8490789*	-2.60964562*	0.8302678
4.007369	0.060273973	-0.6799807*	-2.59323561*	0.9168309
4.254699	0.016438356	-0.5326162*	-2.41454013*	1.1566628
4.425320	0.002739726	-0.4309562*	-2.32797989*	1.2853983
4.436120	0.142465753	-0.4245214*	-2.17442561*	1.4416222
5.341890	0.246575342	0.1151559	-1.89606865*	1.9438727
5.510208	0.989041096	0.2154436	-1.76513167*	2.1164156
5.622064	0.328767123	0.2820901	-1.69715628*	2.2120403
6.011112	0.336986301	0.5138933	-1.51670472*	2.4886591
6.362927	1.180821918	0.7235127	-1.38590431*	2.7064233
7.027408	0.517808219	1.1194249	-1.01150013*	3.2450779
7.071632	0.668493151	1.1457745	-0.95295862*	3.3145509
7.422028	0.947945205	1.3545481	-0.82677042*	3.5273522
7.490357	1.676712329	1.3952596	-0.81547289*	3.5555395
7.743559	0.643835616	1.5461232	-0.70152341*	3.7320771
7.753996	0.780821918	1.5523419	-0.68018908*	3.7559913
8.100399	3.084931507	1.7587364	-0.58281052*	3.9389959
8.118442	1.750684932	1.7694868	-0.49737636*	4.0288900
8.311829	1.901369863	1.8847109	-0.41080101*	4.1632679
8.625683	1.841095890	2.0717120	-0.27990694*	4.3717423
8.742312	1.953424658	2.1412021	-0.20667319*	4.4738051
8.775492	3.005479452	2.1609714	-0.25750125*	4.4311786
8.813854	2.583561644	2.1838287	-0.23834993*	4.4598126
8.866774	1.479452055	2.2153591	-0.12350774*	4.5877357
8.901761	1.230136986	2.2362050	-0.14943438*	4.5704573
9.067622	1.887671233	2.3350292	-0.02436508*	4.7365254
9.383539	2.402739726	2.5232591	0.11317258*	4.9521532
9.546815	5.915068493	2.6205424	-0.03196413*	4.8473760
9.612012	2.408219178	2.6593884	0.18666955	5.0821255
9.726493	1.120547945	2.7275990	0.30879244	5.2325466
9.911698	5.136986301	2.8379478	0.23757710	5.2071112
9.965145	3.736986301	2.8697930	0.35643910	5.3391847
9.992382	3.301369863	2.8860212	0.31946577	5.3089439
10.462642	2.063013699	3.1662127	0.66624093	5.7719608
10.500852	1.073972603	3.1889788	0.69908204	5.8142468
10.610659	2.698630137	3.2544043	0.76644540	5.9087530
10.642343	2.898630137	3.2732825	0.71878619	5.8689257
10.808200	2.602739726	3.3721037	0.88765677	6.0787938
10.899704	3.038356164	3.4575019	0.93251758	6.1590834
11.074688	4.156164384	3.6208090	1.02756365	6.3218800

Predictor variable AVH (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
11.161333	5.065753425	3.7016732	1.06063965	6.3885037
11.172248	5.454794521	3.7118598	0.98879543	6.3208856
11.182001	2.186301370	3.7209614	1.05598486	6.3918509
11.470366	1.750684932	3.9900847	1.35644905	6.8039652
11.508942	4.580821918	4.0260871	1.29133203	6.7537843
11.514203	2.142465753	4.0309973	1.31067033	6.7751596
11.636564	3.246575342	4.1451929	1.44603591	6.9579011
11.646300	3.476712329	4.1542797	1.40192393	6.9175589
11.796892	3.147945205	4.2948230	1.48130932	7.0552508
11.984205	6.120547945	4.4696374	1.62175330	7.2682193
12.024916	4.526027397	4.5076316	1.70122684	7.3634553
12.059015	3.542465753	4.5394558	1.71894147	7.3943727
12.201360	4.964383562	4.6723019	1.81186431	7.5424089
12.313306	5.065753425	4.7767783	1.88986789	7.6637561
12.319279	4.287671233	4.7823531	1.89828860	7.6744896
12.355220	7.257534247	4.8158958	1.91080810	7.7009248
12.519532	3.589041096	4.9692437	2.11071822	7.9644537
12.822359	2.747945205	5.2518638	2.30643184	8.2774167
13.191338	4.983561644	5.5962222	2.57766778	8.6915151
13.210158	3.558904110	5.6137858	2.58433772	8.7054716
13.217763	5.265753425	5.6208834	2.59457846	8.7186569
13.449986	4.863013699	5.8376114	2.72974044	8.9437318
13.455138	12.841095890	5.8424193	2.60484025	8.8208263
13.467225	4.394520548	5.8536995	2.78227245	9.0029382
13.545436	4.369863014	5.9266924	2.83012900	9.0810770
13.626500	5.600000000	6.0023469	2.94511570	9.2274502
13.636173	4.693150685	6.0113745	2.88731768	9.1733974
13.778177	5.575342466	6.1439033	2.99739418	9.3384555
13.798305	7.717808219	6.1626880	3.00960989	9.3584644
13.884786	6.605479452	6.2433982	3.02644224	9.4087806
13.888311	4.528767123	6.2466880	3.10244420	9.4861474
13.894166	5.810958904	6.2521521	3.05880554	9.4447756
13.977402	4.065753425	6.3298343	3.14384569	9.5620435
14.008989	7.512328767	6.3593139	3.14962423	9.5800521
14.045868	5.717808219	6.3937313	3.15749197	9.6021984
14.136931	4.961643836	6.4787184	3.26011453	9.7400792
14.153266	6.284931507	6.4939630	3.26975173	9.7560409
14.300358	13.654794520	6.6312406	3.29630070	9.8395416
14.456916	6.109589041	6.7773520	3.50526754	10.1091250
14.525112	5.112328767	6.8409975	3.55683624	10.1870980
14.739348	6.197260274	7.0409378	3.65107671	10.3642869
14.914265	7.391780822	7.2041832	3.80841785	10.5893529
14.949867	5.484931507	7.2374092	3.86474835	10.6594677
15.057485	5.616438356	7.3378464	3.92496019	10.7613475
15.114479	5.635616438	7.3910379	3.99004944	10.8485041

Predictor variable AVH (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
15.151645	7.893150685	7.4257235	3.99128381	10.8641283
15.204703	7.084931507	7.4752414	4.05865149	10.9520393
15.206900	11.550684930	7.4772920	4.01968336	10.9139219
15.212917	8.865753425	7.4829067	4.03060276	10.9271706
15.390809	8.276712329	7.6489288	4.17411450	11.1395592
15.401653	6.517808219	7.6590497	4.20598484	11.1756284
15.435848	8.953424658	7.6909624	4.19198712	11.1748701
15.509477	9.386301370	7.7596786	4.22966363	11.2410546
15.834874	7.441095890	8.0633631	4.50492999	11.6423093
15.988885	16.441095890	8.2070971	4.56020897	11.7572185
16.039781	7.136986301	8.2545970	4.67276571	11.8894813
16.052201	7.358904110	8.2661882	4.66636758	11.8878920
16.065109	8.449315068	8.2782348	4.64249051	11.8690127
16.089494	6.213698630	8.3009929	4.68401356	11.9199773
16.305225	6.794520548	8.5023288	4.83986710	12.1593581
16.320690	7.178082192	8.5167615	4.86580091	12.1912796
16.337508	8.997260274	8.5324577	4.87892854	12.2109190
16.376473	9.021917808	8.5688224	4.89284640	12.2399233
16.451913	8.421917808	8.6392285	4.95586083	12.3321468
16.584680	8.742465753	8.7631362	5.04204441	12.4697355
16.696831	7.027397260	8.8678041	5.13518723	12.6063014
16.773005	8.556164384	8.9388952	5.18739808	12.6880055
16.787995	6.005479452	8.9528846	5.23621141	12.7426226
16.831742	6.471232877	8.9937126	5.26108436	12.7844336
16.837040	8.463013699	8.9986572	5.25994403	12.7853447
16.936069	8.942465753	9.0910786	5.31157569	12.8753188
16.973980	8.049315068	9.1264600	5.34519195	12.9236136
17.000373	14.904109590	9.1510917	5.32116571	12.9098062
17.145564	8.315068493	9.2865947	5.52570356	13.1705596
17.280062	11.317808220	9.4121180	5.53525710	13.2321884
17.311954	10.660273970	9.4418816	5.59527574	13.3045549
17.401460	9.997260274	9.5254156	5.65483049	13.3987651
17.507840	6.616438356	9.6246967	5.75118587	13.5363088
17.526697	9.202739726	9.6422953	5.75856241	13.5509864
17.603771	11.747945210	9.7142268	5.78470662	13.6069725
17.634673	10.912328770	9.7430671	5.80513962	13.6393703
17.645476	7.980821918	9.7531491	5.84936439	13.6877777
17.677434	10.301369860	9.7829746	5.82610908	13.6768960
17.705040	9.567123288	9.8087381	5.89247029	13.7539457
17.710632	7.997260274	9.8139574	5.87326851	13.7369092
17.720231	11.928767120	9.8229153	5.85227815	13.7196351
17.722184	10.197260270	9.8247387	5.88110744	13.7492209
17.925180	12.115068490	10.0141887	6.00196653	13.9486762
18.040743	9.936986301	10.1220407	6.13861429	14.1300681
18.274443	12.504109590	10.3401472	6.29145048	14.3733892

Predictor variable AVH (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
18.292313	10.769863010	10.3568245	6.32105345	14.4099110
18.303417	11.109589040	10.3671878	6.34090562	14.4340626
18.344693	11.041095890	10.4057094	6.35579586	14.4649341
18.349986	13.424657530	10.4106491	6.34474683	14.4559344
18.413260	9.136986301	10.4697010	6.42604919	14.5617353
18.444492	10.238356160	10.4988492	6.45007214	14.5978509
18.478355	12.931506850	10.5304529	6.43298935	14.5938793
18.691596	10.512328770	10.7294645	6.60518630	14.8486394
18.699293	14.838356160	10.7366482	6.58121981	14.8276532
18.712019	9.764383562	10.7485249	6.62179967	14.8731603
18.773042	9.791780822	10.8054762	6.70534840	14.9803361
18.850755	11.452054790	10.8780032	6.71545721	15.0205339
18.928078	9.621917808	10.9501671	6.78678797	15.1218030
18.952369	10.128767120	10.9728371	6.80009964	15.1445196
18.990058	9.460273973	11.0080110	6.80155019	15.1605626
19.006406	10.046575340	11.0232683	6.81873016	15.1840723
19.019233	12.093150680	11.0352398	6.86512498	15.2354337
19.145982	16.186301370	11.1535311	6.89716664	15.3165504
19.189161	10.076712330	11.1938289	6.99158475	15.4276866
19.365233	13.317808220	11.3581518	7.10207328	15.6063471
19.525834	11.849315070	11.5080361	7.20555940	15.7720150
19.784145	12.076712330	11.7491112	7.40826110	16.0747305
19.794790	9.430136986	11.7590459	7.43039109	16.1009821
19.878914	11.073972600	11.8375568	7.52183480	16.2249973
19.881825	11.243835620	11.8402732	7.49868892	16.2029783
19.949919	9.638356164	11.9038232	7.53164039	16.2622945
19.966297	11.016438360	11.9191084	7.53316580	16.2701612
20.001742	11.112328770	11.9521887	7.60765698	16.3583763
20.031306	8.564383562	11.9797798	7.66109198	16.4232579
20.173745	9.706849315	12.1127140	7.75712169	16.5744374
20.407191	11.797260270	12.3305831	7.87696691	16.7846690
20.426548	11.482191780	12.3486489	7.90092634	16.8161233
20.740306	11.304109590	12.6414708	8.13105761	17.1677364
20.833297	10.364383560	12.7282564	8.20981970	17.2825028
21.179109	18.282191780	13.0509936	8.37226597	17.5788417
21.293111	14.767123290	13.1573880	8.49969543	17.7504105
21.316225	12.545205480	13.1789599	8.52857992	17.7882444
21.506539	13.753424660	13.3565754	8.71963400	18.0529851
21.599982	10.800000000	13.4437829	8.79111950	18.1606499
21.636695	12.786301370	13.4780459	8.79582629	18.1795713
21.639812	10.632876710	13.4809553	8.79934649	18.1842985
21.812682	12.273972600	13.6422900	8.90457300	18.3564572
22.179074	12.663013700	13.9842335	9.20940086	18.8031457
22.298248	19.364383560	14.0954560	9.10523171	18.7451190
22.458038	10.909589040	14.2445837	9.42490673	19.1266619

Predictor variable AVH (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
22.503360	17.175342470	14.2868812	9.34514005	19.0644430
22.570601	14.194520550	14.3496354	9.43384183	19.1791793
22.587905	14.882191780	14.3657847	9.49418671	19.2462240
22.616057	12.704109590	14.3920583	9.52268603	19.2856233
22.679250	12.027397260	14.4510344	9.58045822	19.3678627
22.806422	18.657534250	14.5697210	9.60736554	19.4440090
23.015071	12.136986300	14.7644471	9.84530577	19.7627344
23.157353	15.652054790	14.8972353	9.91732050	19.8898384
23.203389	16.490410960	14.9401994	9.86566339	19.8560056
23.230986	17.216438360	14.9659553	9.90831840	19.9093459
23.276645	17.838356160	15.0085674	9.89637082	19.9150766
23.283502	17.106849320	15.0149664	10.00051176	20.0218722
23.446982	15.715068490	15.1675381	10.06336734	20.1480246
23.473910	18.147945210	15.1926696	10.06578138	20.1608648
23.545533	18.931506850	15.2595132	10.11944336	20.2422579
23.791085	15.528767120	15.4886798	10.41396470	20.6318526
23.821125	15.709589040	15.5167159	10.41124184	20.6407609
23.868993	18.213698630	15.5613897	10.35566778	20.6037205
23.949541	12.065753420	15.6365630	10.44484957	20.7240891
24.221255	13.008219180	15.8901465	10.61189287	20.9963355
24.247077	17.241095890	15.9142457	10.56765639	20.9620969
24.370989	14.767123290	16.0298892	10.67498731	21.1174044
24.378787	19.268493150	16.0371665	10.57479306	21.0202292
24.558775	17.868493150	16.0979036	10.74794003	21.2185739
24.709185	14.742465750	16.1486598	10.80918016	21.3008711
24.731760	14.452054790	16.1562776	10.89866795	21.3935192
24.751130	15.969863010	16.1628140	10.87546616	21.3730292
24.912413	13.786301370	16.2172393	11.01357600	21.5337182
24.994582	14.005479450	16.2449672	11.02430591	21.5559515
25.041499	18.671232880	16.2607995	10.97666772	21.5148815
25.082243	18.345205480	16.2745485	10.97525965	21.5191774
25.094503	18.153424660	16.2786859	10.98443084	21.5300651
25.152353	19.430136990	16.2982073	11.01238177	21.5661148
25.153181	15.610958900	16.2984866	11.06812434	21.6219732
25.156172	12.690410960	16.2994959	11.13807240	21.6923400
25.225606	18.063013700	16.3229267	11.04674993	21.6107382
25.242931	13.052054790	16.3287729	11.15442141	21.7208350
25.329997	12.641095890	16.3581533	11.22047801	21.7990806
25.422180	19.800000000	16.3892607	11.11800542	21.7095134
25.488003	12.526027400	16.4114729	11.30307980	21.8273896
25.492356	15.090410960	16.4129416	11.25697656	21.7768429
25.623332	19.471232880	16.4571395	11.20180898	21.5879638
25.662419	19.126027400	16.4703296	11.33607233	21.6823234
25.739281	15.824657530	16.4962668	11.44514672	21.7129304
25.759684	14.509589040	16.5031519	11.53737569	21.7843300

Predictor variable AVH (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
25.766815	14.501369860	16.5055582	11.48754266	21.7272173
25.794431	13.813698630	16.5148772	11.54367520	21.7551571
25.848709	17.482191780	16.5331934	11.50785422	21.6639243
25.901566	18.002739730	16.5510299	11.58859438	21.6907037
25.923357	16.106849320	16.5583834	11.60377874	21.6836417
25.949551	16.315068490	16.5672226	11.66487718	21.7179989
26.142164	18.227397260	16.6322203	11.73805120	21.5945363
26.266640	18.758904110	16.6742247	11.88532585	21.6147355
26.339670	19.561643840	16.6988686	11.90173230	21.5565869
26.352342	17.545205480	16.7031450	11.96268073	21.6045981
26.408877	18.030136990	16.7222226	11.97001690	21.5542190
26.504329	15.830136990	16.7544330	12.14206133	21.6288176
26.742587	12.161643840	16.8348334	12.41899533	21.6625171
26.790788	14.350684930	16.8510991	12.36375919	21.5580725
26.913139	14.805479450	16.8923865	12.44156367	21.5109707
26.997438	18.136986300	16.9208332	12.46050437	21.4438520
27.128471	16.561643840	16.9650506	12.56580577	21.4153830
27.193454	19.071232880	16.9869793	12.58429234	21.3675290
27.296622	15.887671230	17.0217933	12.70735800	21.3852723
27.307691	16.183561640	17.0255285	12.75288792	21.4195019
27.343428	16.167123290	17.0375879	12.81427078	21.4444017
27.440074	18.698630140	17.0702013	12.78571978	21.3171857
27.459544	13.978082190	17.0767715	12.89856643	21.4101555
27.471648	18.550684930	17.0808559	12.84160171	21.3408343
27.472889	19.613698630	17.0812747	12.77992981	21.2778955
27.501158	16.358904110	17.0908143	12.87093685	21.3400426
27.571323	19.950684930	17.1144914	12.83978698	21.2372626
27.612667	15.550684930	17.1284431	13.05405129	21.4093191
27.654421	19.726027400	17.1425328	12.94567841	21.2583206
27.661745	14.638356160	17.1450043	12.99638866	21.3015539
27.750113	16.936986300	17.1748244	13.07436335	21.2893141
27.918530	17.205479450	17.2316568	13.19953272	21.2425493
27.946984	19.794520550	17.2412589	13.13928718	21.1532546
27.977929	16.509589040	17.2517011	13.22920275	21.2115793
27.995592	18.523287670	17.2576617	13.21037135	21.1747156
28.092471	17.334246580	17.2903536	13.36938781	21.2348294
28.194588	17.673972600	17.3248128	13.42884601	21.1900386
28.424929	16.898630140	17.4025418	13.60900309	21.1350431
28.518375	19.934246580	17.4340752	13.56046569	20.9911080
28.545169	15.008219180	17.4431170	13.79422550	21.1975139
28.634925	16.871232880	17.4734052	13.75337167	21.0650293
28.919280	13.676712330	17.5693612	14.13756868	21.1589317
29.097422	19.780821920	17.6294752	13.90932235	20.7488233
29.291586	19.049315070	17.6949961	14.16091403	20.8021954
29.371647	15.367123290	17.7220129	14.42594126	20.9854888



<b>Predictor variable</b>			<b>Estimated age</b>	
<b>AVH (mm)</b>	<b>Real age</b>	<b>Mean estimate</b>	<b>Lower 95% PI</b>	<b>Upper 95% PI</b>
29.390541	16.865753420	17.7283888	14.36083839	20.9010972
30.115444	17.517808220	17.9730079	14.90391707	20.7041322
30.385304	17.364383560	18.0640727	15.16900551	20.6937235

Table F-6 Mean, minimal and maximal estimated ages of the MARS prediction models using Right Vertebral Height (RVH) as predictor

Predictor variable RVH (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
3.475056	0.002739726	-0.2765819	-1.27864100	0.6899896
3.655323	0.142465753	-0.1155499	-1.16252981	0.9573536
3.957730	0.336986301	0.1545906	-1.00721783	1.3664009
4.072943	0.002739726	0.2575104	-0.94267182	1.5276166
4.229937	0.246575342	0.3977526	-0.92363532	1.6783786
4.322620	0.780821918	0.4805469	-0.91070446	1.7690758
4.384902	0.643835616	0.5361832	-0.83728684	1.8947509
4.574853	0.947945205	0.7058664	-0.74536486	2.1460516
4.774504	0.060273973	0.8842151	-0.65307431	2.4058599
4.814713	1.479452055	0.9201335	-0.65111374	2.4415577
4.891561	1.676712329	0.9887819	-0.61096936	2.5461815
5.014530	1.901369863	1.0986302	-0.53125019	2.7290780
5.074638	0.989041096	1.1523248	-0.51768865	2.7930734
5.163961	0.016438356	1.2321167	-0.41586737	2.9698410
5.437026	1.120547945	1.4760459	-0.28939996	3.3254241
5.476144	0.328767123	1.5109896	-0.25018195	3.3974637
5.537677	1.180821918	1.5659573	-0.27151154	3.4277638
5.755976	3.084931507	1.7609639	-0.17101796	3.7114215
5.921676	0.517808219	1.9089839	-0.07670807	3.9447623
6.002444	0.668493151	1.9811335	-0.07743149	4.0118070
6.100889	2.583561644	2.0690752	-0.02984071	4.1419989
6.316392	3.542465753	2.2615833	0.10499391	4.4576508
6.362603	1.841095890	2.3028635	0.12305074	4.5144810
6.421909	1.073972603	2.3558416	0.17459504	4.6157861
6.443960	3.736986301	2.3755403	0.12036503	4.5800585
6.771156	1.953424658	2.6678241	0.32691596	5.0611434
6.784190	4.964383562	2.6794680	0.27146614	5.0166303
6.793302	6.120547945	2.6876079	0.25505742	5.0078671
6.849772	2.898630137	2.7380521	0.35243790	5.1526285
6.875202	2.747945205	2.7607692	0.33804881	5.1595768
6.914730	2.698630137	2.7960791	0.36705054	5.2217442
6.926334	3.005479452	2.8064449	0.34800258	5.2124325
6.948587	2.402739726	2.8263240	0.39097157	5.2740734
6.973929	3.038356164	2.8489615	0.40072375	5.3050883
7.437325	4.580821918	3.2629133	0.58166767	5.8748454
7.585265	1.887671233	3.3950678	0.69405521	6.1113619
7.648615	5.915068493	3.4516587	0.69260845	6.1630694
7.753455	1.750684932	3.5453121	0.78084814	6.3392750
7.887475	4.961643836	3.6650326	0.80840350	6.4792804
7.984012	4.394520548	3.7512690	0.85268975	6.6045661
8.016657	3.301369863	3.7804308	0.90351190	6.6827791
8.042474	5.136986301	3.8034930	0.90852980	6.7094586
8.144448	4.156164384	3.8945863	0.94803464	6.8345248

Predictor variable RVH (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
8.194490	2.063013699	3.9392888	0.97966791	6.9081459
8.224775	1.750684932	3.9663426	1.00252762	6.9564165
8.251126	5.065753425	3.9898822	0.99462299	6.9706219
8.273003	4.065753425	4.0094246	1.01228235	7.0066369
8.281719	2.408219178	4.0172103	1.03842016	7.0400876
8.296953	3.246575342	4.0308191	1.05179609	7.0662458
8.661095	5.065753425	4.3561076	1.20261004	7.5225941
8.876863	1.230136986	4.5488528	1.34652295	7.8475470
8.901080	4.287671233	4.5704856	1.29523972	7.8165829
9.048456	5.112328767	4.7021372	1.36682307	8.0118229
9.314937	3.558904110	4.9401845	1.51573052	8.3843213
9.389671	5.454794521	5.0069443	1.52954720	8.4608436
9.411072	4.528767123	5.0260618	1.58381664	8.5151130
9.447268	2.142465753	5.0583956	1.60420477	8.5355012
9.570888	2.602739726	5.1688256	1.72004589	8.6513423
9.575901	4.526027397	5.1733036	1.69665363	8.6279500
9.625064	5.575342466	5.2172210	1.75519680	8.6864932
9.642682	4.983561644	5.2329593	1.76711225	8.6984087
9.697242	5.600000000	5.2816974	1.81796093	8.7492573
9.914698	4.863013699	5.4759509	2.00909973	8.9403961
10.018261	6.605479452	5.5684635	2.09703876	9.0283352
10.046002	4.369863014	5.5932448	2.11850575	9.0498022
10.225071	8.315068493	5.7532071	2.27270410	9.2040005
10.292893	7.136986301	5.8137922	2.34624521	9.2775416
10.315597	3.589041096	5.8340744	2.40170179	9.3329982
10.328884	4.693150685	5.8459432	2.38478200	9.3160784
10.397336	3.476712329	5.9070919	2.45155217	9.3828486
10.407452	2.186301370	5.9161280	2.48020247	9.4114989
10.440102	8.953424658	5.9452947	2.45890350	9.3901999
10.589348	5.484931507	6.0786160	2.60707477	9.5383712
10.596641	8.865753425	6.0851309	2.62460542	9.5559018
10.616685	5.616438356	6.1030360	2.62536562	9.5566620
10.640842	6.471232877	6.1246160	2.65292704	9.5842234
10.710105	6.005479452	6.1864886	2.71249209	9.6437885
10.803832	5.265753425	6.2702144	2.80677552	9.7380719
11.109118	6.109589041	6.5429270	3.07506927	10.0063657
11.168504	7.717808219	6.5959760	3.12431458	10.0556110
11.271924	7.084931507	6.6883613	3.21313743	10.1444338
11.347584	6.284931507	6.7559485	3.30772783	10.2390242
11.390989	5.810958904	6.7947224	3.31649807	10.2477945
11.425508	5.717808219	6.8255578	3.35787336	10.2891698
11.660780	7.512328767	7.0357262	3.56752432	10.4988207
11.698430	6.517808219	7.0693588	3.61170830	10.5430047
11.854715	3.147945205	7.2089682	3.76880102	10.7000974
12.047121	7.893150685	7.3808450	3.90752519	10.8388216

Predictor variable RVH (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
12.086022	7.441095890	7.4155955	3.94435191	10.8756483
12.133318	8.556164384	7.4578442	3.97584306	10.9071395
12.353628	9.202739726	7.6546480	4.15932027	11.0906167
12.361821	10.076712330	7.6619665	4.16052486	11.0918213
12.418983	10.046575340	7.7130289	4.22760955	11.1589060
12.439826	8.449315068	7.7316487	4.27459662	11.2058930
12.442622	7.257534247	7.7341460	4.26307303	11.1943694
12.457246	10.197260270	7.7472099	4.27277740	11.2040738
12.521401	7.997260274	7.8045189	4.34042402	11.2717204
12.528188	10.660273970	7.8105819	4.30934762	11.2406440
12.664452	8.463013699	7.9323071	4.47383665	11.4051331
12.796847	11.928767120	8.0505752	4.55817960	11.4894760
12.850678	7.358904110	8.0986624	4.64154928	11.5728457
12.928574	11.109589040	8.1682472	4.67002792	11.6013243
13.108775	8.049315068	8.3292209	4.88622883	11.8175252
13.135099	9.136986301	8.3527356	4.87707337	11.8083698
13.169194	5.635616438	8.3831931	4.93533396	11.8666304
13.240200	6.794520548	8.4466229	4.98744662	11.9187430
13.242856	7.178082192	8.4489950	4.99922072	11.9305171
13.266388	8.276712329	8.4700164	4.99772922	11.9290256
13.305096	9.997260274	8.5045939	5.01423492	11.9455313
13.326993	6.213698630	8.5241543	5.06108397	11.9923804
13.340479	9.567123288	8.5362013	5.06643834	11.9977347
13.519792	7.391780822	8.6963824	5.23459361	12.1658900
13.605699	9.386301370	8.7731231	5.29565070	12.2269471
13.636785	9.021917808	8.8008918	5.35086106	12.2821575
13.673740	7.980821918	8.8339039	5.36042580	12.2917222
13.684045	12.841095890	8.8431095	5.35607016	12.2873666
13.866337	7.027397260	9.0059505	5.54929256	12.4805890
13.955785	6.197260274	9.0858545	5.61988971	12.5511861
14.059698	6.616438356	9.1786801	5.72510561	12.6564020
14.107845	10.301369860	9.2216900	5.76784819	12.6991446
14.236551	8.997260274	9.3366631	5.87244996	12.8037464
14.282724	9.764383562	9.3779093	5.92375876	12.8550552
14.290893	11.747945210	9.3852069	5.91603103	12.8473274
14.291710	10.128767120	9.3859362	5.91870083	12.8499972
14.354545	8.421917808	9.4420668	5.99775034	12.9290467
14.575728	11.041095890	9.6396503	6.14214241	13.0734388
14.624297	11.550684930	9.6830363	6.17325920	13.1045556
14.664276	12.273972600	9.7187501	6.23367925	13.1649757
14.777069	12.093150680	9.8195076	6.30966373	13.2409601
14.907088	9.621917808	9.9356538	6.42723464	13.3585310
14.974730	10.912328770	9.9960780	6.52631424	13.4576106
15.087275	9.460273973	10.0966145	6.65305719	13.5843536
15.274540	8.564383562	10.2638987	6.78520665	13.7165031

Predictor variable RVH (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
15.393017	9.430136986	10.3697342	6.89375480	13.8250512
15.419717	14.838356160	10.3935854	6.88859912	13.8198955
15.619323	10.769863010	10.5718938	7.11238795	14.0436844
15.627455	9.791780822	10.5791581	7.11184321	14.0431396
15.672775	8.742465753	10.6196424	7.18914505	14.1204415
15.686778	11.797260270	10.6321506	7.14543333	14.0767297
15.754415	13.654794520	10.6925708	7.21624188	14.1475383
15.835583	9.638356164	10.7650784	7.29527839	14.2265748
15.837082	11.452054790	10.7664178	7.29203445	14.2233309
15.885235	11.317808220	10.8094328	7.33748659	14.2687830
15.937395	11.073972600	10.8560271	7.34874257	14.2800390
15.963945	10.238356160	10.8797448	7.39154988	14.3228463
15.987943	11.112328770	10.9011822	7.45838046	14.3896769
16.355718	11.243835620	11.2297157	7.81499954	14.7462960
16.424968	8.942465753	11.2915760	7.86462813	14.7959245
16.457314	16.441095890	11.3204713	7.85501483	14.7863112
16.743258	12.115068490	11.5759045	8.08049503	15.0117914
16.868140	9.706849315	11.6874616	8.26320891	15.1945053
16.982386	9.936986301	11.7895181	8.32618735	15.2574838
17.094822	12.076712330	11.8899569	8.43192198	15.3632184
17.187857	11.304109590	11.9730650	8.51177597	15.4430724
17.314770	10.512328770	12.0864368	8.61691772	15.5482141
17.614737	12.504109590	12.3543973	8.87730214	15.8085985
18.089146	11.016438360	12.7781870	9.33248618	16.2637826
18.113599	11.849315070	12.8000303	9.30033142	16.2316278
18.164951	13.317808220	12.8459035	9.40005897	16.3313554
18.189726	10.800000000	12.8680350	9.41560655	16.3469030
18.469876	12.663013700	13.1182930	9.64644508	16.5777415
18.728856	12.931506850	13.3496400	9.82293327	16.9020536
18.833535	12.786301370	13.4431496	9.87181134	17.0106815
18.883808	14.882191780	13.4880586	9.91272070	17.0802864
18.976272	16.186301370	13.5706562	9.85941364	17.0797567
18.982065	13.424657530	13.5758315	9.96195669	17.1856066
19.006943	12.027397260	13.5980550	9.98685950	17.2247096
19.049114	10.364383560	13.6357266	10.02156498	17.2834860
19.103234	10.632876710	13.6840722	10.08265822	17.3754707
19.133195	11.482191780	13.7108364	10.15783892	17.4677529
19.137993	12.641095890	13.7151219	10.13299786	17.4456502
19.173559	12.545205480	13.7468935	10.16452640	17.4974798
19.301976	14.194520550	13.8616085	10.14645820	17.5527110
19.336609	14.767123290	13.8925454	10.16014380	17.5861645
19.842506	14.904109590	14.3444635	10.50504484	18.2198277
19.877645	12.704109590	14.3758530	10.58391272	18.3187525
19.920793	14.767123290	14.4143973	10.52551195	18.2849804
19.938118	13.008219180	14.4298741	10.59873078	18.3680884

Predictor variable RVH (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
20.249781	18.671232880	14.7082827	10.69264955	18.6399020
20.358603	16.490410960	14.8054930	10.80208082	18.8114478
20.392608	12.136986300	14.8358700	10.94127442	18.9700513
20.580096	15.715068490	15.0033532	10.99309081	19.1288845
20.662940	17.175342470	15.0773574	11.00222069	19.1853008
20.664502	13.753424660	15.0787527	11.11648910	19.3004608
20.813004	18.282191780	15.2114092	11.03575934	19.3044946
20.920008	18.657534250	15.3069967	11.05611873	19.3859316
20.976175	15.652054790	15.3571702	11.27724575	19.6391180
21.027605	16.106849320	15.4031130	11.22930653	19.6205348
21.173483	19.364383560	15.5334252	11.24097125	19.7154651
21.350758	17.106849320	15.6917850	11.33156634	19.9072474
21.608557	13.786301370	15.9220768	11.52300562	20.2458363
21.829077	10.909589040	16.1190679	11.74116748	20.5898696
21.847276	18.931506850	16.1353249	11.52713016	20.3862200
21.914798	17.482191780	16.1956425	11.64273315	20.5403642
21.960506	18.213698630	16.2364732	11.61936642	20.5430870
22.037093	12.690410960	16.3048882	11.76789279	20.7353285
22.084566	16.936986300	16.3472961	11.70450076	20.6990338
22.098736	12.526027400	16.3599546	11.80641182	20.8090334
22.106161	15.610958900	16.3665873	11.67348794	20.6803476
22.122293	12.065753420	16.3809981	11.78882643	20.8048941
22.354908	17.216438360	16.5887929	11.76624680	20.9150892
22.472687	18.345205480	16.6940049	11.75316859	20.9692383
22.501574	19.471232880	16.7198091	11.67952211	20.9120799
22.560908	12.161643840	16.7728122	12.02111045	21.2875356
22.640381	15.824657530	16.8438054	11.86959308	21.1813809
22.743649	15.008219180	16.9360549	11.97889956	21.3496320
22.967854	18.550684930	16.9360549	11.83767930	21.2084117
23.001019	17.241095890	16.9360549	11.99443101	21.3651634
23.020843	18.147945210	16.9360549	11.98756261	21.3582950
23.164176	18.030136990	16.9360549	11.98615221	21.3568846
23.321794	17.838356160	16.9360549	12.09545546	21.4661879
23.346229	16.561643840	16.9360549	12.06015508	21.4308875
23.436677	13.813698630	16.9360549	12.30201151	21.6727439
23.460219	16.183561640	16.9360549	12.11677506	21.4875075
23.521844	19.430136990	16.9360549	12.00129366	21.3720261
23.595217	15.887671230	16.9360549	12.24416602	21.6148985
23.604191	15.090410960	16.9360549	12.18347281	21.5542052
23.632044	18.063013700	16.9360549	12.15402342	21.5247559
23.768949	13.676712330	16.9360549	12.31185808	21.6825905
23.813139	19.071232880	16.9360549	12.06732377	21.4380562
24.104656	19.126027400	16.9360549	12.05230382	21.4230362
24.191584	19.613698630	16.9360549	12.14594751	21.5166799
24.227301	18.153424660	16.9360549	12.14624847	21.5169809

Predictor variable RVH (mm)	Real age	Mean estimate	Estimated age	
			Lower 95% PI	Upper 95% PI
24.446162	19.561643840	16.9360549	12.17928714	21.5500196
24.496456	15.528767120	16.9360549	12.28474743	21.6554799
24.513234	14.452054790	16.9360549	12.37157491	21.7423073
24.542837	13.052054790	16.9360549	12.32102529	21.6917577
24.580858	15.830136990	16.9360549	12.33357816	21.7043106
24.704026	16.358904110	16.9360549	12.25681898	21.6275514
24.717038	16.167123290	16.9360549	12.36312672	21.7338592
24.801629	18.002739730	16.9360549	12.23781006	21.6085425
24.821260	19.726027400	16.9360549	12.24117217	21.6119046
24.918498	18.758904110	16.9360549	12.22158312	21.5923156
24.931220	15.709589040	16.9360549	12.28732159	21.6580540
24.941984	17.868493150	16.9360549	12.26524205	21.6359745
24.959106	17.545205480	16.9360549	12.28620830	21.6569407
24.973154	19.800000000	16.9360549	12.23909567	21.6098281
25.111771	19.934246580	16.9360549	12.23061170	21.6013441
25.195380	14.509589040	16.9360549	12.31153410	21.6822665
25.240893	14.501369860	16.9360549	12.36277056	21.7335030
25.247519	16.509589040	16.9360549	12.27990396	21.6506364
25.255678	16.315068490	16.9360549	12.35123396	21.7219664
25.323611	16.871232880	16.9360549	12.26368010	21.6344125
25.391402	18.698630140	16.9360549	12.26247875	21.6332112
25.392686	13.978082190	16.9360549	12.37257179	21.7433042
25.400120	14.805479450	16.9360549	12.37123208	21.7419645
25.438955	14.742465750	16.9360549	12.31192709	21.6826595
25.464746	19.268493150	16.9360549	12.31894347	21.6896759
25.693398	14.005479450	16.9360549	12.39996665	21.7706991
25.988846	17.334246580	16.9360549	12.40641500	21.7771474
26.139169	18.227397260	16.9360549	12.35499465	21.7257271
26.150687	19.780821920	16.9360549	12.28558960	21.6563220
26.153854	15.367123290	16.9360549	12.32753583	21.6982683
26.155889	16.865753420	16.9360549	12.38953696	21.7602694
26.160846	15.969863010	16.9360549	12.39709813	21.7678306
26.218609	14.350684930	16.9360549	12.42936280	21.8000952
26.416394	18.136986300	16.9360549	12.31392950	21.6846619
26.452195	16.898630140	16.9360549	12.36788198	21.7386144
26.572213	18.523287670	16.9360549	12.36551996	21.7362524
26.656380	19.950684930	16.9360549	12.41234953	21.7830820
27.100786	19.794520550	16.9360549	12.36337608	21.7341085
27.151056	17.673972600	16.9360549	12.44745646	21.8181889
27.183550	19.049315070	16.9360549	12.39096591	21.7616983
27.855356	15.550684930	16.9360549	12.69517643	22.0659089
27.918152	17.205479450	16.9360549	12.51708129	21.8878137
28.339727	17.517808220	16.9360549	12.54170428	21.9124367
28.482238	17.364383560	16.9360549	12.55965916	21.9303916
29.045826	14.638356160	16.9360549	12.91059388	22.2813263

Table F-7 Mean, minimal and maximal estimated ages of the MARS prediction models using Left Vertebral Height (LVH) as predictor

Predictor variable LVH (mm)	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
3.09975002	0.06027397	-0.48469734*	-3.224613358*	2.250203
3.41039397	0.78082192	-0.20071879*	-2.975561735*	2.499255
3.44386286	1.67671233	-0.17012284*	-2.972544433*	2.502272
3.52246671	0.3369863	-0.09826627*	-2.831142942*	2.643674
3.57687306	0.00273973	-0.04853010*	-2.807795070*	2.667022
3.88034984	0.00273973	0.22889651	-2.512585293*	2.962232
4.59304745	0.14246575	0.88041681	-1.839021396*	3.635795
4.60915882	0.24657534	0.89514519	-1.833907226*	3.640910
4.62899073	0.01643836	0.91327476	-1.791600998*	3.683216
4.67068127	3.08493151	0.95138661	-1.791228079*	3.683589
4.81169797	0.64383562	1.08029858	-1.670250869*	3.804566
4.85376552	0.9890411	1.11875509	-1.595738511*	3.879078
4.90026247	0.32876712	1.16126078	-1.552477702*	3.922339
4.99251583	1.90136986	1.24559520	-1.482767822*	3.992049
5.12419771	1.84109589	1.36597363	-1.372983804*	4.101833
5.31751222	0.94794521	1.54269420	-1.181459865*	4.293357
5.6208737	1.18082192	1.82001540	-0.916832279*	4.557985
5.64730044	1.12054795	1.84417370	-0.838346470*	4.636470
5.76583218	2.58356164	1.95253078	-0.789245744*	4.685571
5.89725831	2.74794521	2.07267541	-0.678597294*	4.796220
5.91265426	0.51780822	2.08674980	-0.623567888*	4.851249
6.01507135	3.30136986	2.18037549	-0.554955489*	4.919861
6.12251694	1.95342466	2.27859805	-0.476748276*	4.998069
6.65404422	4.39452055	2.76449953	0.001615037	5.476432
6.68027695	0.66849315	2.78848046	0.089528662	5.564346
6.68282292	2.40273973	2.79080788	0.068382131	5.543199
6.68763042	3.7369863	2.79520271	0.041808500	5.516625
6.74061765	1.47945206	2.84364157	0.095248776	5.570066
6.76640404	4.96438356	2.86721448	0.072476640	5.547293
6.82220916	2.89863014	2.91822934	0.181162278	5.655979
6.89848043	2.69863014	2.98795356	0.266417527	5.741234
6.93397432	1.0739726	3.02040068	0.327482946	5.802300
6.9583126	1.23013699	3.04264979	0.340368291	5.815185
6.98377244	3.03835616	3.06592418	0.335979021	5.810796
7.10207703	3.00547945	3.17407361	0.440437742	5.915255
7.14395251	3.54246575	3.21235454	0.467991637	5.942808
7.21139982	1.75068493	3.27401224	0.545143515	6.019960
7.23098742	2.60273973	3.29191845	0.536534892	6.011352
7.4488407	5.1369863	3.49107140	0.727136449	6.201953
7.74937344	6.12054795	3.76580670	0.974098976	6.448916
7.80802605	4.15616438	3.81942462	1.079781002	6.554598
7.90206652	5.06575343	3.90539274	1.157702037	6.632519
7.92534698	3.24657534	3.92667483	1.197971745	6.672789



Predictor variable	Estimated age			
	Real age	Mean estimate	Lower 95% PI	Upper 95% PI
8.00294059	2.0630137	3.99760787	1.289097568	6.763914
8.0198506	1.88767123	4.01306634	1.292765940	6.767583
8.02218714	5.91506849	4.01520231	1.250447249	6.725264
8.02560044	5.06575343	4.01832262	1.309108048	6.783925
8.045406	2.40821918	4.03642809	1.296712104	6.771529
8.14810017	1.75068493	4.13030709	1.418340336	6.893157
8.33223695	4.28767123	4.29863773	1.556457369	7.031274
8.55444439	4.58082192	4.50177109	1.771182506	7.245999
8.58882462	4.96164384	4.53320015	1.810190295	7.285007
8.96852307	4.98356164	4.88030564	2.131957100	7.606774
8.97924692	4.52876712	4.89010897	2.144038458	7.618855
9.11490805	2.18630137	5.01412507	2.305347544	7.780164
9.27194729	5.57534247	5.15768421	2.386515754	7.908433
9.34485643	3.47671233	5.22433489	2.447879745	7.991664
9.35946032	5.11232877	5.23768520	2.468073777	8.016238
9.35985446	4.06575343	5.23804551	2.453776608	8.002060
9.40559161	2.14246575	5.27985662	2.521000024	8.083001
9.45790962	4.8630137	5.32768371	2.537018308	8.114711
9.50667229	7.71780822	5.37226063	2.566547800	8.158865
9.55106881	3.5890411	5.41284619	2.648488423	8.254122
9.55232244	5.48493151	5.41399221	2.617085547	8.223095
9.5725138	4.5260274	5.43245036	2.642464687	8.254530
9.62781141	4.36986301	5.48300128	2.689475492	8.318126
9.76380699	8.31506849	5.60732313	2.757513522	8.426953
9.78530357	5.45479452	5.62697446	2.775930418	8.451817
9.90155602	3.14794521	5.73324790	2.897274293	8.608028
10.0415492	5.61643836	5.86122417	2.979351007	8.732093
10.3217522	6.60547945	6.11737485	3.190333373	9.027116
10.3383175	7.89315069	6.13251820	3.204871725	9.046622
10.4188439	10.0767123	6.20613224	3.235882266	9.101785
10.4288634	8.95342466	6.21529171	3.264759904	9.133668
10.436437	5.26575343	6.22221515	3.300221971	9.171401
10.5273296	5.71780822	6.30530569	3.346702137	9.245143
10.8026951	6.10958904	6.55703405	3.554003012	9.535033
10.8410945	3.55890411	6.59213725	3.592223636	9.584771
10.9119848	7.1369863	6.65694238	3.643702781	9.657512
10.9266246	6.28493151	6.67032557	3.696306961	9.714507
10.9337346	7.08493151	6.67682520	3.647541742	9.667874
11.0046814	4.69315069	6.74168206	3.759473142	9.801084
11.0776226	7.51232877	6.80836199	3.783912719	9.847401
11.3221182	8.44931507	7.03187036	3.944604482	10.081424
11.4010628	11.9287671	7.10403840	4.003018418	10.163515
11.4299313	6.47123288	7.13042885	4.067402717	10.236558
11.4310079	7.17808219	7.13141304	4.055155528	10.224634
11.5183225	5.6	7.21123262	4.131787008	10.327453

Predictor variable LVH (mm)	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
11.5516268	8.55616438	7.24167812	4.135428356	10.341083
11.6926975	5.8109589	7.37063943	4.263932687	10.511899
11.7504241	6.21369863	7.42341088	4.292979815	10.558260
11.7535738	6.00547945	7.42629023	4.306563283	10.572788
12.0092748	9.20273973	7.66004202	4.478340715	10.821257
12.1224055	10.0465753	7.76346173	4.560121084	10.936968
12.1900592	6.51780822	7.82530810	4.643239763	11.040378
12.2665579	8.86575343	7.89524016	4.671309139	11.091391
12.3391532	5.63561644	7.96160400	4.758173144	11.200029
12.3632933	7.44109589	7.98367192	4.741220982	11.190317
12.3938944	7.35890411	8.01164621	4.786455574	11.244730
12.4312121	9.99726027	8.04576060	4.805615043	11.275082
12.481323	8.27671233	8.09157005	4.856772300	11.341269
12.4830805	7.39178082	8.09317673	4.876343342	11.361367
12.4955289	10.1287671	8.10455653	4.867398123	11.356155
12.5142142	9.02191781	8.12163792	4.855489234	11.349850
12.6701003	7.25753425	8.26414285	5.038642563	11.579758
12.9022717	6.19726027	8.47638491	5.199989805	11.810740
13.0276713	8.99726027	8.59102029	5.278210033	11.926571
13.0745188	10.1972603	8.63384647	5.284155013	11.946567
13.0801208	9.38630137	8.63896765	5.299390760	11.963483
13.1517773	7.99726027	8.70447314	5.351095348	12.036679
13.2295644	10.660274	8.77558313	5.414174019	12.123088
13.2734238	6.79452055	8.81567769	5.489344558	12.211413
13.3062078	8.4630137	8.84564748	5.501156198	12.233058
13.4092189	8.04931507	8.93981622	5.569053304	12.331851
13.4275911	12.8410959	8.95661138	5.566974164	12.335282
13.430447	10.3013699	8.95922217	5.549904830	12.319069
13.4359852	11.109589	8.96428496	5.577824743	12.348650
13.508775	8.42191781	9.03082650	5.658747204	12.451404
13.5755882	11.5506849	9.09190457	5.668158593	12.480855
13.6475085	9.76438356	9.15765129	5.749597202	12.583864
13.8450992	6.61643836	9.33828098	5.908387400	12.801917
13.8748959	9.62191781	9.36551994	5.937728452	12.840195
13.9778906	7.98082192	9.45967368	6.000381479	12.933739
14.1075259	9.79178082	9.57818118	6.089892678	13.062131
14.2010865	9.56712329	9.66371066	6.159013865	13.159314
14.2248061	10.5123288	9.68539419	6.195173996	13.202588
14.2745313	12.0931507	9.73085104	6.199206479	13.221535
14.4558071	11.3178082	9.89656627	6.358747040	13.435445
14.4565161	10.8	9.89721442	6.338867094	13.415777
14.5733865	9.46027397	10.00405278	6.491551838	13.603515
14.5932305	11.0410959	10.02219339	6.465316784	13.583232
14.7258302	7.02739726	10.14341083	6.578977169	13.736662
14.8073594	9.63835616	10.21794163	6.641245363	13.823383

Predictor variable LVH (mm)	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
14.8963264	11.0164384	10.29927180	6.704312022	13.913133
14.9782264	14.8383562	10.37414162	6.710022449	13.943408
15.1152433	9.1369863	10.49939706	6.907888824	14.182369
15.1436174	11.2438356	10.52533556	6.869772878	14.152764
15.2247458	8.94246575	10.59949996	6.966829035	14.274152
15.2810546	11.7479452	10.65097525	7.000710588	14.324922
15.2905208	11.3041096	10.65962889	7.003909493	14.330961
15.4759159	8.74246575	10.82910991	7.154025040	14.536681
15.4821914	9.43013699	10.83484665	7.174988548	14.559527
15.5129703	16.4410959	10.86298353	7.138171536	14.531941
15.6186483	12.5041096	10.95959026	7.239728828	14.665194
15.6627407	12.0767123	10.99989783	7.279064866	14.717755
15.6709977	10.769863	11.00744604	7.300933940	14.742100
15.6767657	10.9123288	11.01271894	7.307466795	14.750363
15.7310196	8.56438356	11.06231568	7.383537736	14.842706
15.7548211	9.9369863	11.08407414	7.343272078	14.809579
15.8774335	9.70684932	11.19616154	7.487270849	14.990353
15.9896141	13.4246575	11.29871268	7.500922508	15.037651
16.0217549	12.2739726	11.32809456	7.570163019	15.116531
16.1387259	13.6547945	11.43502492	7.657424374	15.238875
16.454699	11.4520548	11.72387518	7.881700297	15.557920
16.4760881	12.1150685	11.74342821	7.915082030	15.597717
16.53254	11.8493151	11.79503431	7.936883442	15.636450
16.8220725	11.1123288	12.05971364	8.158868998	15.945274
16.8869416	10.2383562	12.11901439	8.256161863	16.062023
17.1063551	13.3178082	12.31959370	8.362081645	16.233751
17.1339689	10.3643836	12.34483716	8.419451945	16.299403
17.4008114	14.9041096	12.58877414	8.602357991	16.562343
17.4340042	11.7972603	12.61911766	8.667497596	16.637438
17.9598967	11.0739726	13.09986808	9.042627989	17.170298
18.0393526	15.6520548	13.17250356	9.056165253	17.207666
18.174436	13.7534247	13.29599146	9.200532365	17.392548
18.336806	12.7863014	13.44442378	9.329122510	17.569837
18.3550308	14.1945206	13.46108422	9.331841949	17.578023
18.4885692	12.9315069	13.58315979	9.482692743	17.768926
18.5530913	11.4821918	13.64214342	9.504267191	17.809852
18.7987821	14.7671233	13.86674431	9.712121320	18.091395
18.812515	12.0273973	13.87929840	9.650676322	18.034069
19.0259158	12.6410959	14.07438109	9.877908299	18.325306
19.521231	19.1260274	14.52717889	10.137529998	18.733486
19.5827558	10.6328767	14.58342239	10.347263415	18.961673
19.5915409	14.8821918	14.59145342	10.258069482	18.875113
19.6101793	12.7041096	14.60849195	10.332779476	18.955414
19.7338951	18.6575343	14.72158811	10.327098807	18.986839
19.7341025	17.1753425	14.72177762	10.365155903	19.024958

Predictor variable LVH (mm)	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
19.9536052	16.490411	14.92243846	10.565419874	19.291057
20.1379694	12.6630137	15.09097702	10.739690095	19.520623
20.6078163	13.7863014	15.52049267	11.138203715	20.060056
20.6571653	14.7671233	15.56560557	11.079255694	20.015909
20.7280314	16.9369863	15.63038862	11.160237419	20.118146
20.7704888	12.1369863	15.66920151	11.276929561	20.247572
20.8891298	13.0082192	15.77765850	11.295258050	20.301484
20.9942402	15.8246575	15.87374632	11.424994109	20.462746
21.0264807	12.5452055	15.90321934	11.419908026	20.467330
21.0737511	17.8684932	15.94643207	11.391427661	20.453027
21.184324	17.8383562	16.04751353	11.479186287	20.573949
21.5961736	18.2821918	16.42401029	11.815860063	21.034148
21.6798709	15.7150685	16.50052314	11.914210816	21.157602
21.6862401	18.9315069	16.50634560	11.820540474	21.065842
21.7579909	15.709589	16.57193736	11.899009949	21.165831
21.9811835	15.0082192	16.77597130	11.983511513	21.317274
22.0904963	19.3643836	16.77597130	12.024759995	21.358523
22.151084	19.9342466	16.77597130	12.032590355	21.366353
22.3286913	18.030137	16.77597130	12.057944189	21.391707
22.3433896	15.090411	16.77597130	12.136449790	21.470213
22.4136327	17.1068493	16.77597130	12.094565718	21.428329
22.4342062	18.5506849	16.77597130	12.059422260	21.393185
22.7860922	13.8136986	16.77597130	12.168665329	21.502428
22.7921085	19.4712329	16.77597130	12.057401844	21.391165
22.8987845	18.0630137	16.77597130	12.084483297	21.418246
22.9678939	16.1863014	16.77597130	12.110874850	21.444638
22.9864245	12.690411	16.77597130	12.133276623	21.467039
23.1535369	16.5616438	16.77597130	12.135355899	21.469119
23.2237956	16.1671233	16.77597130	12.094278629	21.428041
23.2464884	18.0027397	16.77597130	12.040449631	21.374212
23.2602883	16.3589041	16.77597130	12.094086188	21.427849
23.2979532	13.6767123	16.77597130	12.217762266	21.551525
23.3583229	10.909589	16.77597130	12.254657258	21.588420
23.3647178	19.0493151	16.77597130	12.091385057	21.425148
23.4084209	18.6712329	16.77597130	12.063090808	21.396854
23.5039153	19.8	16.77597130	12.094546389	21.428309
23.5582423	15.6109589	16.77597130	12.115310570	21.449073
23.6213434	15.5287671	16.77597130	12.109713765	21.443477
23.6679526	18.1534247	16.77597130	12.109172162	21.442935
23.6951567	18.7589041	16.77597130	12.067486702	21.401250
23.7302346	12.0657534	16.77597130	12.158447915	21.492211
23.754204	17.4821918	16.77597130	12.106372611	21.440135
23.8895446	19.7260274	16.77597130	12.062409526	21.396172
23.9758137	16.1068493	16.77597130	12.133931653	21.467694
23.975872	17.5452055	16.77597130	12.099696265	21.433459

Predictor variable	Estimated age			
	Real age	Mean estimate	Lower 95% PI	Upper 95% PI
24.0197292	18.1479452	16.77597130	12.070331638	21.404094
24.1326498	19.0712329	16.77597130	12.080651782	21.414415
24.229691	15.969863	16.77597130	12.132498443	21.466261
24.3071849	18.2273973	16.77597130	12.064141227	21.397904
24.3150822	12.5260274	16.77597130	12.145658307	21.479421
24.3647479	14.8054795	16.77597130	12.150815746	21.484579
24.3682939	19.5616438	16.77597130	12.054669068	21.388432
24.4126252	14.509589	16.77597130	12.185221399	21.518984
24.444266	17.2164384	16.77597130	12.115021353	21.448784
24.5769963	18.3452055	16.77597130	12.091411875	21.425175
24.6955352	19.2684932	16.77597130	12.085994437	21.419757
24.6969134	15.3671233	16.77597130	12.132335182	21.466098
24.7776939	16.1835616	16.77597130	12.130001169	21.463764
24.7796415	16.8712329	16.77597130	12.061168310	21.394931
24.7824672	13.0520548	16.77597130	12.151370367	21.485133
24.7935661	15.5506849	16.77597130	12.100916271	21.434679
24.8005818	14.4520548	16.77597130	12.126760185	21.460523
24.8630997	19.430137	16.77597130	12.082724649	21.416487
24.9960222	16.8986301	16.77597130	12.132209121	21.465972
25.0088205	15.8876712	16.77597130	12.151263011	21.485026
25.0895615	18.2136986	16.77597130	12.128577163	21.462340
25.1295738	18.5232877	16.77597130	12.099385985	21.433149
25.5175447	14.7424658	16.77597130	12.114266207	21.448029
25.530928	18.1369863	16.77597130	12.093500763	21.427264
25.5438717	16.3150685	16.77597130	12.143543929	21.477307
25.6875363	17.2410959	16.77597130	12.089039103	21.422802
25.7565666	15.830137	16.77597130	12.149547895	21.483311
25.8880758	14.3506849	16.77597130	12.147254938	21.481018
25.8925681	17.6739726	16.77597130	12.048527246	21.382290
25.9849324	16.509589	16.77597130	12.136816646	21.470579
26.0709709	19.7808219	16.77597130	12.067166523	21.400929
26.1205948	14.5013699	16.77597130	12.090777958	21.424541
26.1313988	17.2054795	16.77597130	12.118604023	21.452367
26.144725	18.6986301	16.77597130	12.112724321	21.446487
26.1782334	19.9506849	16.77597130	12.080610341	21.414373
26.2399935	19.6136986	16.77597130	12.123075882	21.456839
26.4680407	12.1616438	16.77597130	12.130697441	21.464460
26.5105331	14.0054795	16.77597130	12.148294395	21.482057
27.2008506	19.7945206	16.77597130	12.060980358	21.394743
27.2304469	17.3643836	16.77597130	12.091181364	21.424944
27.3090352	13.9780822	16.77597130	12.088056125	21.421819
27.7899036	16.8657534	16.77597130	12.036893925	21.370657
27.8709757	17.3342466	16.77597130	11.995988864	21.329752
28.0289758	17.5178082	16.77597130	11.986562888	21.320326
28.0508982	14.6383562	16.77597130	12.132965466	21.466728

Table F-8 Mean, minimal and maximal estimated ages of the MARS prediction models using Right Vertebral Height (RVH) and Left Vertebral Height (LVH) as predictors

Predictor variable		Real age	Estimated age		
RVH (mm)	LVH (mm)		Mean estimate	Lower 95% PI	Upper 95% PI
3.475056	3.576873	0.002739726	0.4525661	-1.543176698*	1.106162
3.655323	4.593047	0.142465753	1.1716319	-1.452484177*	1.944897
3.957730	3.522467	0.336986301	0.4140671	-1.337772460*	1.271515
4.072943	3.880350	0.002739726	0.6673125	-1.238666652*	1.634072
4.229937	4.609159	0.246575342	1.1830327	-1.114379735*	2.294862
4.322620	3.410394	0.780821918	0.3347621	-1.147768731*	1.379018
4.384902	4.811698	0.643835616	1.3263535	-1.056998420*	2.501339
4.574853	5.317512	0.947945205	1.6842780	-0.928138732*	3.002547
4.774504	3.099750	0.060273973	0.1149441	-0.989422700*	1.308688
4.814713	6.740618	1.479452055	2.6912965	-0.742447925*	4.235838
4.891561	3.443863	1.676712329	0.3584454	-0.861212599*	1.690212
5.014530	4.992516	1.901369863	1.4543039	-0.738786217*	2.952658
5.074638	4.853766	0.989041096	1.3561214	-0.742567867*	2.846737
5.163961	4.628991	0.016438356	1.1970661	-0.628660754*	2.795180
5.437026	5.647300	1.120547945	1.9176429	-0.479694973*	3.693760
5.476144	4.900262	0.328767123	1.3890236	-0.515252465*	3.108281
5.537677	5.620874	1.180821918	1.8989428	-0.464656199*	3.689345
5.755976	4.670681	3.084931507	1.2265672	-0.441558594*	3.012972
5.921676	5.912654	0.517808219	2.1054127	-0.211454586*	4.157337
6.002444	6.680277	0.668493151	2.6485982	-0.081450664*	4.852416
6.100889	5.765832	2.583561644	2.0015184	-0.204439541*	4.056271
6.316392	7.143953	3.542465753	2.9767045	0.038868321	5.314064
6.362603	5.124198	1.841095890	1.5474847	-0.120474141*	3.667906
6.421909	6.933974	1.073972603	2.8281197	0.084711125	5.205334
6.443960	6.687630	3.736986301	2.6538017	0.005063557	4.944344
6.771156	6.122517	1.953424658	2.2539158	0.192402173	4.715682
6.784190	6.766404	4.964383562	2.7095435	0.181420239	5.178688
6.793302	7.749373	6.120547945	3.4051128	0.193021677	5.913889
6.849772	6.822209	2.898630137	2.7490324	0.229543821	5.267892
6.875202	5.897258	2.747945205	2.0945182	0.159082307	4.516541
6.914730	6.898480	2.698630137	2.8030035	0.270915283	5.365410
6.926334	7.102077	3.005479452	2.9470726	0.296922484	5.541292
6.948587	6.682823	2.402739726	2.6503998	0.283620376	5.219361
6.973929	6.983772	3.038356164	2.8633578	0.333454732	5.490736
7.437325	8.554444	4.580821918	3.9747974	0.476334023	6.789844
7.585265	8.019851	1.887671233	3.5965079	0.693471542	6.613447
7.648615	8.022187	5.915068493	3.5981613	0.635528398	6.557224
7.753455	7.211400	1.750684932	3.0244316	0.719945144	6.044791
7.887475	8.588825	4.961643836	3.9991256	0.751783107	7.090601
7.984012	6.654044	4.394520548	2.6300354	0.784888485	5.699445
8.016657	6.015071	3.301369863	2.1778851	0.741973507	5.186158
8.042474	7.448841	5.136986301	3.1924497	0.850042557	6.349677
8.144448	7.808026	4.156164384	3.4466166	0.951157804	6.715202

Predictor variable		Real age	Mean estimate	Estimated age	
RVH (mm)	LVH (mm)			Lower 95% PI	Upper 95% PI
8.194490	8.002941	2.063013699	3.5845421	0.959701361	6.867229
8.224775	8.148100	1.750684932	3.6872600	0.998500305	7.012885
8.251126	8.025600	5.065753425	3.6005766	1.002142720	6.926351
8.273003	9.359854	4.065753425	4.5447221	1.068449245	7.910139
8.281719	8.045406	2.408219178	3.6145915	1.013824039	6.952612
8.296953	7.925347	3.246575342	3.5296352	1.008892701	6.859301
8.661095	7.902067	5.065753425	3.5131615	1.176234067	7.009505
8.876863	6.958313	1.230136986	2.8453419	1.415746887	6.554286
8.901080	8.332237	4.287671233	3.8175589	1.244925762	7.394861
9.048456	9.359460	5.112328767	4.5444432	1.471873106	8.313563
9.314937	10.841094	3.558904110	5.5928778	2.320274830	9.161965
9.389671	9.785304	5.454794521	4.8457786	1.768673321	8.610363
9.411072	8.979247	4.528767123	4.2753964	1.568148963	8.194371
9.447268	9.405592	2.142465753	4.5770866	1.696577558	8.538267
9.570888	7.230987	2.602739726	3.0382922	1.766996039	7.106261
9.575901	9.572514	4.526027397	4.6952042	1.805955670	8.647646
9.625064	9.271947	5.575342466	4.4825172	1.712050198	8.553740
9.642682	8.968523	4.983561644	4.2678080	1.676375632	8.294704
9.697242	11.518322	5.600000000	6.1018149	2.841960444	9.683650
9.914698	9.457910	4.863013699	4.7909751	1.922740918	8.764431
10.018261	10.321752	6.605479452	5.4529657	2.366527695	9.208218
10.046002	9.627811	4.369863014	4.9930660	2.086220470	8.927910
10.225071	9.763807	8.315068493	5.2001160	2.174740925	9.016431
10.292893	10.911985	7.136986301	6.0173832	2.698356590	9.540046
10.315597	9.551069	3.589041096	5.1143539	2.159000252	9.000690
10.328884	11.004681	4.693150685	6.1004399	2.839277157	9.680967
10.397336	9.344856	3.476712329	5.0291628	2.235986540	9.077676
10.407452	9.114908	2.186301370	4.8820907	2.149280256	8.990970
10.440102	10.428863	8.953424658	5.7800033	2.585232623	9.426922
10.589348	9.552322	5.484931507	5.2918692	2.371516006	9.213206
10.596641	12.266558	8.865753425	7.0845888	3.265030017	10.106720
10.616685	10.041549	5.616438356	5.6312893	2.556620678	9.398311
10.640842	11.429931	6.471232877	6.5577330	3.163723260	10.005413
10.710105	11.753574	6.005479452	6.8070913	3.372129335	10.213819
10.803832	10.436437	5.265753425	6.0033216	2.833952624	9.675642
11.109118	10.802695	6.109589041	6.4181785	3.118106222	9.959796
11.168504	9.506672	7.717808219	5.6369059	2.466976352	9.308666
11.271924	10.933735	7.084931507	6.5946110	3.219174871	10.060865
11.347584	10.926625	6.284931507	6.6336758	3.314671111	10.156361
11.390989	11.692697	5.810958904	7.1321142	3.657475327	10.499165
11.425508	10.527330	5.717808219	6.4323965	3.211036132	10.052726
11.660780	11.077623	7.512328767	6.9049948	3.481119576	10.322809
11.698430	12.190059	6.517808219	7.5963880	3.930732982	10.772423
11.854715	9.901556	3.147945205	6.3160556	3.285086121	10.126776
12.047121	10.338318	7.893150685	6.6920541	3.410388021	10.252078

Predictor variable		Estimated age			
RVH (mm)	LVH (mm)	Real age	Mean estimate	Lower 95% PI	Upper 95% PI
12.086022	12.363293	7.441095890	7.8950610	4.289158097	11.130848
12.133318	11.551627	8.556164384	7.4479770	3.931162584	10.772852
12.353628	12.009275	9.202739726	7.8278849	4.236201322	11.077891
12.361821	10.418844	10.076712330	6.9282480	3.395712627	10.237402
12.418983	12.122406	10.046575340	7.9258020	4.317192789	11.158883
12.439826	11.322118	8.449315068	7.4848787	4.077647075	10.919337
12.442622	12.670100	7.257534247	8.2469517	4.586127041	11.427817
12.457246	13.074519	10.197260270	8.4819214	4.712592665	11.554282
12.521401	13.151777	7.997260274	8.5550267	4.859777306	11.701467
12.528188	13.229564	10.660273970	8.6017016	4.793346383	11.635036
12.664452	13.306208	8.463013699	8.7063460	4.870905261	11.712595
12.796847	11.401063	11.928767120	7.7260310	4.107371601	10.949061
12.850678	12.393894	7.358904110	8.2950700	4.675391476	11.517081
12.928574	13.435985	11.109589040	8.8960034	5.069324475	11.911014
13.108775	13.409219	8.049315068	8.9622630	5.172625098	12.014315
13.135099	15.115243	9.136986301	9.8760615	6.002869122	12.844559
13.169194	12.339153	5.635616438	8.4254216	4.847577133	11.689267
13.240200	13.273424	6.794520548	8.9500489	5.221247966	12.062938
13.242856	11.431008	7.178082192	7.9873025	4.505868902	11.347559
13.266388	12.481323	8.276712329	8.5484849	4.863048208	11.704738
13.305096	12.431212	9.997260274	8.5415998	4.862575564	11.704265
13.326993	11.750424	6.213698630	8.1992474	4.591322667	11.433012
13.340479	14.201087	9.567123288	9.4763664	5.699306589	12.540996
13.519792	12.483081	7.391780822	8.6748994	5.044332056	11.886022
13.605699	13.080121	9.386301370	9.0187195	5.309997793	12.151688
13.636785	12.514214	9.021917808	8.7485003	5.079275001	11.920965
13.673740	13.977891	7.980821918	9.5002338	5.677091492	12.518781
13.684045	13.427591	12.841095890	9.2290678	5.466287913	12.307978
13.866337	14.725830	7.027397260	9.9483040	6.331168508	13.172858
13.955785	12.902272	6.197260274	9.0948349	5.419286757	12.260977
14.059698	13.845099	6.616438356	9.5979037	5.870379912	12.712070
14.107845	13.430447	10.301369860	9.4198156	5.698310230	12.540000
14.236551	13.027671	8.997260274	9.2870801	5.578327300	12.420017
14.282724	13.647509	9.764383562	9.5999413	5.904111828	12.745802
14.290893	15.281055	11.747945210	10.3704745	6.509754533	13.351444
14.291710	12.495529	10.128767120	9.0630316	5.315138211	12.156828
14.354545	13.508775	8.421917808	9.5665410	5.889467667	12.731157
14.575728	14.593231	11.041095890	10.1578052	6.415494800	13.257185
14.624297	13.575588	11.550684930	9.7161899	5.942480805	12.784171
14.664276	16.021755	12.273972600	10.8354778	7.027594669	13.869285
14.777069	14.274531	12.093150680	10.0940574	6.329500762	13.171191
14.907088	13.874896	9.621917808	9.9714967	6.231787907	13.073478
14.974730	15.676766	10.912328770	10.7830597	7.089345604	13.931035
15.087275	14.573387	9.460273973	10.3473438	6.659498808	13.501189
15.274540	15.731020	8.564383562	10.9052305	7.326308322	14.167998



Predictor variable		Estimated age			
RVH (mm)	LVH (mm)	Real age	Mean estimate	Lower 95% PI	Upper 95% PI
15.393017	15.482191	9.430136986	10.8414132	7.256696756	14.098387
15.419717	14.978226	14.838356160	10.6430565	6.968688466	13.810378
15.619323	15.670998	10.769863010	10.9945222	7.396697221	14.238387
15.627455	14.107526	9.791780822	10.3701918	6.747450588	13.589140
15.672775	15.475916	8.742465753	10.9344806	7.373093054	14.214783
15.686778	17.434004	11.797260270	11.7186089	7.907811070	14.749501
15.754415	16.138726	13.654794520	11.2239052	7.509029702	14.350720
15.835583	14.807359	9.638356164	10.7291538	7.229682910	14.071373
15.837082	16.454699	11.452054790	11.3726934	7.738120851	14.579811
15.885235	14.455807	11.317808220	10.6114845	6.923232819	13.764923
15.937395	17.959897	11.073972600	11.9817259	8.301471164	15.143161
15.963945	16.886942	10.238356160	11.5756121	8.077120129	14.918810
15.987943	16.822073	11.112328770	11.5572655	7.926883942	14.768574
16.355718	15.143617	11.243835620	11.0471321	7.565285120	14.406975
16.424968	15.224746	8.942465753	11.1012119	7.657901194	14.499591
16.457314	15.512970	16.441095890	11.2160286	7.581268800	14.422959
16.743258	16.476088	12.115068490	11.6444692	8.210421186	15.052111
16.868140	15.877433	9.706849315	11.4787296	8.106966893	14.948657
16.982386	15.754821	9.936986301	11.4747813	8.101147290	14.942837
17.094822	15.662741	12.076712330	11.4816133	8.087507101	14.929197
17.187857	15.290521	11.304109590	11.3930085	7.991422583	14.833112
17.314770	14.224806	10.512328770	11.1023185	7.605072618	14.446762
17.614737	15.618648	12.504109590	11.6412209	8.248251410	15.089941
18.089146	14.896326	11.016438360	11.6013682	8.352641534	15.194331
18.113599	16.532540	11.849315070	12.0582814	8.905436611	15.747126
18.164951	17.106355	13.317808220	12.2286030	9.075866520	15.917556
18.189726	14.456516	10.800000000	11.5200868	7.962120996	14.803811
18.469876	20.137969	12.663013700	13.0818152	9.976677180	17.364459
18.728856	18.488569	12.931506850	12.7090702	9.701630956	16.850840
18.833535	18.336806	12.786301370	12.6927690	9.716650204	16.855426
18.883808	19.591541	14.882191780	12.9965040	10.030022325	17.363202
18.976272	22.967894	16.186301370	16.8870114	9.637536797	19.460807
18.982065	15.989614	13.424657530	12.1837917	9.020914219	15.862604
19.006943	18.812515	12.027397260	12.8350814	10.051820434	17.281682
19.049114	17.133969	10.364383560	12.4633202	9.655027594	16.646946
19.103234	19.582756	10.632876710	13.0233145	10.393872208	17.744211
19.133195	18.553091	11.482191780	12.7992190	10.015608181	17.222517
19.137993	19.025916	12.641095890	12.9047023	10.133326695	17.407749
19.173559	21.026481	12.545205480	14.3251478	10.607514237	18.791082
19.301976	18.355031	14.194520550	13.0771558	9.865217137	17.250017
19.336609	18.798782	14.767123290	13.1976580	9.910609686	17.372536
19.842506	17.400811	14.904109590	14.2702416	9.584500749	17.732926
19.877645	19.610179	12.704109590	14.0026988	10.541281428	18.518468
19.920793	20.657165	14.767123290	13.9812022	10.944248670	18.907677
19.938118	20.889130	13.008219180	14.4805675	10.821163081	19.104206

Predictor variable		Real age	Mean estimate	Estimated age	
RVH (mm)	LVH (mm)			Lower 95% PI	Upper 95% PI
20.249781	23.408421	18.671232880	16.7588318	10.192406873	19.933636
20.358603	19.953605	16.490410960	14.4925232	10.740562799	19.031258
20.392608	20.770489	12.136986300	14.5554177	11.143169078	19.474120
20.580096	21.679871	15.715068490	16.2932986	10.924954096	20.368223
20.662940	19.734102	17.175342470	14.9612291	10.743991651	19.334679
20.664502	18.174436	13.753424660	15.8453694	10.675571516	19.832146
20.813004	21.596174	18.282191780	16.2122474	10.821106909	20.212499
20.920008	19.733895	18.657534250	15.2816434	10.870794059	19.666560
20.976175	18.039353	15.652054790	16.5823445	9.768801710	19.397072
21.027605	23.975814	16.106849320	16.5937383	11.042706943	20.678270
21.173483	22.090496	19.364383560	17.0363119	10.856308557	20.775137
21.350758	22.413633	17.106849320	17.0482840	11.179187450	21.105679
21.608557	20.607816	13.786301370	15.2199626	11.864273190	20.620561
21.829077	23.358323	10.909589040	16.7734088	11.762116999	21.512676
21.847276	21.686240	18.931506850	16.6081619	11.463538727	21.108333
21.914798	23.754204	17.482191780	16.6582198	11.415254550	21.092088
21.960506	25.089561	18.213698630	16.2696726	10.626507178	20.054654
22.037093	22.986425	12.690410960	16.8816196	11.742712870	21.562532
22.084566	20.728031	16.936986300	15.7174976	11.745286262	20.820018
22.098736	24.315082	12.526027400	16.4950218	11.805448675	21.377829
22.106161	23.558242	15.610958900	16.7152385	11.606681774	21.320010
22.122293	23.730235	12.065753420	16.6651941	11.783336586	21.464634
22.354908	24.444266	17.216438360	16.4574334	11.634247108	21.182569
22.472687	24.576996	18.345205480	16.4188131	11.623880895	21.147484
22.501574	22.792109	19.471232880	16.9381594	11.672478182	21.528485
22.560908	26.468041	12.161643840	15.8685784	12.176697001	21.348126
22.640381	20.994240	15.824657530	16.3121721	11.852237222	21.307586
22.743649	21.981183	15.008219180	17.0143923	11.897792098	21.802591
22.967854	22.434206	18.550684930	17.0422978	11.765107298	21.687767
23.001019	25.687536	17.241095890	16.0956807	11.866445054	21.183230
23.020843	24.019729	18.147945210	16.5809603	11.785522488	21.412907
23.164176	22.328691	18.030136990	17.0729993	11.786520173	21.728830
23.321794	21.184324	17.838356160	16.7852418	11.831903972	21.590037
23.346229	23.153537	16.561643840	16.8329951	12.033855594	21.822553
23.436677	22.786092	13.813698630	16.9399100	12.179497295	22.036625
23.460219	24.777694	16.183561640	16.3604163	12.085903896	21.572131
23.521844	24.863100	19.430136990	16.3355659	11.893373244	21.363695
23.595217	25.008821	15.887671230	16.2931657	12.072035202	21.515219
23.604191	22.343390	15.090410960	17.0687226	12.136328687	22.075902
23.632044	22.898785	18.063013700	16.9071200	11.976566148	21.812707
23.768949	23.297953	13.676712330	16.7909745	12.097321429	21.859124
23.813139	24.132650	19.071232880	16.5481039	12.007635848	21.613991
24.104656	19.521231	19.126027400	19.7469847	10.419563292	22.073338
24.191584	26.239993	19.613698630	15.9349330	11.934884150	21.148783
24.227301	23.667953	18.153424660	16.6833163	12.022559833	21.715456

Predictor variable		Real age	Mean estimate	Estimated age	
RVH (mm)	LVH (mm)			Lower 95% PI	Upper 95% PI
24.446162	24.368294	19.561643840	16.4795389	12.052918783	21.615389
24.496456	23.621343	15.528767120	16.6968781	12.164678415	21.866255
24.513234	24.800582	14.452054790	16.3537567	12.104845994	21.586810
24.542837	24.782467	13.052054790	16.3590275	12.168644863	21.653983
24.580858	25.756567	15.830136990	16.0755951	12.119544722	21.423474
24.704026	23.260288	16.358904110	16.8019338	12.085613634	21.854430
24.717038	23.223796	16.167123290	16.8125520	12.184462538	21.960075
24.801629	23.246488	18.002739730	16.8059491	12.002702737	21.774089
24.821260	23.889545	19.726027400	16.6188399	12.015145361	21.666774
24.918498	23.695157	18.758904110	16.6754007	12.141401691	21.829232
24.931220	21.757991	15.709589040	17.3127668	12.484193464	22.579965
24.941984	21.073751	17.868493150	17.6461434	11.679924942	21.989072
24.959106	23.975872	17.545205480	16.5937214	12.132693886	21.768246
24.973154	23.503915	19.800000000	16.7310460	12.078398238	21.801844
25.111771	22.151084	19.934246580	17.1246774	11.809833222	21.785220
25.195380	24.412625	14.509589040	16.4666399	12.209314620	21.763529
25.240893	26.120595	14.501369860	15.9696743	12.324554776	21.560690
25.247519	25.984932	16.509589040	16.0091478	12.174153412	21.435553
25.255678	25.543872	16.315068490	16.1374826	12.159339951	21.502880
25.323611	24.779641	16.871232880	16.3598497	12.118933658	21.604798
25.391402	26.144725	18.698630140	15.9626531	12.143252805	21.374894
25.392686	27.309035	13.978082190	15.6238754	12.370711260	21.385520
25.400120	24.364748	14.805479450	16.4805707	12.206725044	21.769856
25.438955	25.517545	14.742465750	16.1451430	12.199420237	21.547863
25.464746	24.695535	19.268493150	16.3843219	12.089821823	21.591349
25.693398	26.510533	14.005479450	15.8562145	12.331783650	21.495300
25.988846	27.870976	17.334246580	16.4398541	11.898432953	21.435503
26.139169	24.307185	18.227397260	17.0543907	11.960740067	21.891140
26.150687	26.070971	19.780821920	17.0230628	11.815155266	21.725504
26.153854	24.696913	15.367123290	17.0633552	12.128131638	22.064269
26.155889	27.789904	16.865753420	16.9955060	11.792288770	21.685000
26.160846	24.229691	15.969863010	17.0830330	12.067347759	22.016080
26.218609	25.888076	14.350684930	17.1749213	12.019192677	22.026737
26.416394	25.530928	18.136986300	17.5575745	11.836532055	22.088991
26.452195	24.996022	16.898630140	17.5480947	11.820522023	22.066914
26.572213	25.129574	18.523287670	17.7751639	11.662458210	22.054184
26.656380	26.178233	19.950684930	18.2006431	11.521191674	22.185242
27.100786	27.200851	19.794520550	19.7745783	10.927952991	22.599389
27.151056	25.892568	17.673972600	19.0023501	11.354026399	22.531203
27.183550	23.364718	19.049315070	17.7157740	11.956325044	22.310038
27.855356	24.793566	15.550684930	17.6028362	12.140800831	22.422229
27.918152	26.131399	17.205479450	17.7310353	11.821282046	22.184763
28.339727	28.028976	17.517808220	16.8910674	11.945124162	21.770990
28.482238	27.230447	17.364383560	16.5929883	12.213598520	21.848681
29.045826	28.050898	14.638356160	14.9921012	12.943119271	21.553566

## Clavicular model

Table F-9 Mean, minimal and maximal estimated ages of the MARS prediction models using Maximal Length (Ln) as a predictor

Predictor variable		Estimated age		
Ln (mm)	Real age	Mean estimate	Lower 95% PI	Upper 95% PI
31.48	0.002739726	-0.6906593*	-1.80129216*	0.6775544
39.44	0.161643836	-0.2183225*	-1.27724775*	1.3569575
44.92	0.016438356	0.1068541	-1.03658807*	1.7045726
45.89	0.060273973	0.1644127	-0.81781929*	1.9422733
48.62	0.002739726	0.3264076	-0.73967186*	2.0737033
51.06	0.419178082	0.4711942	-0.70288259*	2.1581151
53.66	0.246575342	0.6254751	-0.66834077*	2.2434022
55.46	0.947945205	0.7322849	-0.70067240*	2.2462019
56.72	0.364383562	0.8070518	-0.60161894*	2.3698473
57.41	1.060273973	0.8479955	-0.60467692*	2.3802564
57.46	0.328767123	0.8509625	-0.57947147*	2.4064377
60.10	0.668493151	1.0076169	-0.52569540*	2.5117398
60.46	0.517808219	1.0289788	-0.54104653*	2.5034149
62.42	0.780821918	1.1452829	-0.44951641*	2.6331992
63.62	1.049315068	1.2164894	-0.44503469*	2.6611018
66.42	1.180821918	1.3826380	-0.48682508*	2.6739602
69.29	1.890410959	1.5529403	-0.48039284*	2.7364074
69.57	1.901369863	1.5695552	-0.44883689*	2.7734282
70.32	1.035616438	1.6140593	-0.26910101*	2.9678022
70.44	2.142465753	1.6329376	-0.36287329*	2.8802392
70.46	1.364383562	1.6360839	-0.30747619*	2.9366712
70.55	1.753424658	1.6502426	-0.30779774*	2.9410067
71.00	1.120547945	1.7210361	-0.26614341*	3.0059461
71.13	1.479452055	1.7414876	-0.27871627*	3.0001000
73.11	2.739726027	2.0529789	-0.07104374*	3.3102267
73.37	1.989041096	2.0938818	0.07824594	3.4729699
73.66	1.676712329	2.1395042	0.17493468	3.5846646
74.00	2.063013699	2.1929926	0.18415746	3.6114805
75.15	2.402739726	2.3739093	0.27396177	3.7607910
76.56	2.602739726	2.5957289	0.50065037	4.0604394
76.91	2.879452055	2.6507905	0.55376603	4.1316657
77.00	1.934246575	2.6649492	0.66648920	4.2490459
77.45	1.983561644	2.7357426	0.68088756	4.2867293
77.87	3.106849315	2.8018165	0.67108605	4.2986605
78.40	3.476712329	2.8851955	0.79459911	4.4495981
78.94	3.350684932	2.9701477	0.89778559	4.5807266
78.95	2.367123288	2.9717209	0.88903679	4.5724953
78.99	4.693150685	2.9780136	0.84759250	4.5331208
79.67	2.320547945	3.0849905	1.07159937	4.7923139
79.88	1.841095890	3.1180274	1.07233674	4.8039177
81.53	3.298630137	3.3776035	1.35709089	5.1740503

Predictor variable Ln (mm)	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
82.54	4.369863014	3.5364955	1.46225642	5.3314778
82.54	4.210958904	3.5364955	1.47144467	5.3406660
83.32	3.542465753	3.6592042	1.63088911	5.5404712
83.70	5.915068493	3.7189854	1.66208424	5.5913293
84.10	4.284931507	3.7819129	1.75135265	5.7012955
84.19	2.682191781	3.7960716	1.81038848	5.7649883
84.37	3.589041096	3.8243890	1.78588708	5.7498010
84.76	3.904109589	3.8857434	1.92054751	5.9046418
85.44	3.147945205	3.9927202	2.02156075	6.0408413
85.82	4.063013699	4.0525013	2.05322581	6.0921693
86.83	3.602739726	4.2113934	2.21329045	6.3044959
87.35	6.263013699	4.2931992	2.28782824	6.4059408
87.46	6.197260274	4.3105042	2.29510152	6.4189060
88.36	4.293150685	4.4520912	2.42152531	6.5918999
88.82	6.123287671	4.5244579	2.51929002	6.7134671
89.05	3.843835616	4.5606412	2.56066620	6.7667445
89.24	6.109589041	4.5905318	2.66332137	6.8792311
89.36	5.005479452	4.6094100	2.66034985	6.8824689
89.65	4.712328767	4.6550325	2.68710909	6.9242341
90.31	5.065753425	4.7588629	2.78245797	7.0537344
91.03	5.065753425	4.8721325	2.92146135	7.2299938
91.14	4.512328767	4.8894376	2.99390801	7.3081324
92.25	4.613698630	5.0640615	3.15737489	7.5290357
92.28	3.794520548	5.0687811	3.21150308	7.5847162
94.47	5.265753425	5.4133093	3.54248841	8.0290221
95.31	8.043835616	5.5454571	3.62341028	8.1534093
95.68	4.556164384	5.6036651	3.86192594	8.4110705
95.83	5.632876712	5.6272629	3.71181984	8.2687261
95.91	7.254794521	5.6398484	3.75111667	8.3121625
97.39	5.454794521	5.8726803	4.04990147	8.6875292
97.82	5.600000000	5.9837691	4.12698941	8.8011559
98.78	6.794520548	6.2317812	4.32347839	9.0792198
99.12	4.200000000	6.3196188	4.34091158	9.1255441
99.34	7.178082192	6.3764549	4.38375146	9.1870782
100.88	7.895890411	6.7743076	4.53813922	9.4723257
101.23	8.123287671	6.8647286	4.72165861	9.6855859
102.11	7.917808219	7.0920730	4.80742046	9.8461248
102.13	6.454794521	7.0972400	4.88489865	9.9253024
103.05	8.972602740	7.3349182	4.89006929	10.0086490
103.25	6.542465753	7.3865874	5.00341343	10.1389879
103.36	5.783561644	7.4150054	5.24339893	10.3883206
104.57	7.717808219	7.7276040	5.23066367	10.4784037
104.88	7.739726027	7.8076912	5.30886707	10.5829490
105.40	7.361643836	7.9420311	5.51768025	10.8359485
105.46	9.293150685	7.9575319	5.35131517	10.6746819

Predictor variable Ln (mm)	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
106.22	6.517808219	8.1538748	5.62201886	11.0099657
106.43	8.860273973	8.2081274	5.61565108	11.0214425
106.92	9.087671233	8.3347169	5.66951291	11.1169415
107.47	10.715068490	8.4768071	5.77224739	11.2664116
108.76	6.035616438	8.8100734	6.11271524	11.7164957
109.41	9.323287671	8.9779982	6.20372185	11.8627353
109.50	8.663013699	9.0012494	6.17755897	11.8442201
109.53	8.276712329	9.0089997	6.27938594	11.9485963
110.01	9.005479452	9.1330058	6.31247264	12.0224704
110.42	11.172602740	9.2389276	6.27573806	12.0205751
110.47	7.945205479	9.2518449	6.42094224	12.1700280
110.80	7.027397260	9.3370990	6.48494654	12.2620737
111.53	9.021917808	9.5256916	6.64160094	12.4807590
111.54	10.356164380	9.5282750	6.60584465	12.4458524
111.58	8.421917808	9.5386089	6.66753413	12.5109409
112.02	8.504109589	9.6522811	6.72623519	12.6070304
112.08	9.394520548	9.6677818	6.69560723	12.5815009
113.31	11.041095890	9.9855473	6.96667381	12.9570853
113.32	9.594520548	9.9881307	6.97833624	12.9695975
114.02	8.421917808	10.1689729	7.13972575	13.1904687
114.30	11.747945210	10.2413097	7.13473102	13.2092666
114.93	10.621917810	10.4040677	7.27155340	13.3996225
115.42	11.082191780	10.5306572	7.38652112	13.5562275
115.54	8.463013699	10.5616587	7.42220113	13.6021043
115.83	10.230136990	10.6365790	7.40879913	13.6133448
116.23	10.649315070	10.7399174	7.52111559	13.7596508
117.31	9.936986301	11.0189309	7.72812175	14.0584287
117.64	11.550684930	11.1041851	7.74275876	14.1011071
118.02	10.076712330	11.2023565	7.91953860	14.3101770
118.83	10.238356160	11.4116167	8.02871432	14.4881815
119.58	10.712328770	11.6053762	8.18534212	14.7085397
119.82	14.068493150	11.6673792	8.12987699	14.6734683
120.49	10.054794520	11.8404709	8.40387216	15.0043960
121.47	14.904109590	12.0936499	8.48667659	15.1704748
121.73	9.805479452	12.1608199	8.67333531	15.3792267
121.77	11.906849320	12.1711537	8.54152252	15.2508129
122.11	12.690410960	12.2589913	8.67302954	15.4112110
124.05	11.758904110	12.7601824	9.12232670	16.0253574
124.38	12.027397260	12.8454366	9.10401855	16.0350906
124.96	9.863013699	12.9952772	9.39221430	16.3725712
126.31	13.424657530	13.3440442	9.57403215	16.6691038
127.56	13.317808220	13.6669766	9.87572784	17.0770168
127.66	18.063013700	13.6928112	9.68685235	16.8966387
128.05	14.586301370	13.7935661	9.82507637	17.0680025
129.02	12.931506850	14.0441616	10.15612541	17.4814762

Predictor variable Ln (mm)	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
129.22	13.008219180	14.0958308	10.14764408	17.4899896
131.20	16.441095890	14.6073557	10.50690472	18.0174985
131.81	13.120547950	14.7649467	10.71106747	18.2734953
131.99	15.008219180	14.8114490	10.64135152	18.2190747
132.02	12.717808220	14.8191993	10.82992437	18.4101967
132.16	12.545205480	14.8553678	10.87007933	18.4622480
132.35	13.676712330	14.9044535	10.66643798	18.2747517
132.59	18.021917810	14.9664565	10.69242768	18.3211351
133.79	14.797260270	15.2764716	11.11877217	18.8494483
133.83	16.917808220	15.2868054	11.03903217	18.7731072
134.76	12.786301370	15.5270671	11.63415501	19.4472557
134.83	11.939726030	15.5451514	11.71974629	19.5387952
134.92	17.175342470	15.5684025	11.02031443	18.8470110
135.07	18.213698630	15.6071544	11.11189958	18.9513422
135.13	19.520547950	15.6226551	11.16014424	19.0046853
136.06	19.561643840	15.8629168	11.14507271	19.0686395
136.21	17.520547950	15.8735975	11.36780094	19.2948807
136.83	13.052054790	15.9177445	11.75283578	19.6944362
136.92	13.753424660	15.9241530	11.83129060	19.7749988
137.29	15.715068490	15.9504987	11.68002379	19.6323975
138.85	14.005479450	16.0615782	11.95898143	19.9478909
139.01	14.731506850	16.0729710	11.97953676	19.9721935
139.89	16.517808220	16.1356313	12.03946755	20.0527342
141.52	15.367123290	16.2516951	12.28953738	20.3409791
141.65	16.186301370	16.2609517	12.25249424	20.3069806
141.69	17.290410960	16.2637999	12.20322033	20.2586436
141.77	15.246575340	16.2694963	12.32991941	20.3872163
142.13	18.550684930	16.2951300	12.26010956	20.3258377
142.22	13.369863010	16.3015385	12.37437510	20.4422111
142.62	18.345205480	16.3300204	12.29082284	20.3680270
142.71	15.709589040	16.3364288	12.41765234	20.4969643
142.76	16.550684930	16.3399891	12.39866932	20.4791523
142.84	18.931506850	16.3456854	12.35031458	20.4326712
143.10	15.887671230	16.3641987	12.41818377	20.5066297
143.10	18.758904110	16.3641987	12.34362952	20.4320754
143.60	13.049315070	16.3998011	12.63126760	20.7314237
143.88	13.978082190	16.4197384	12.60574902	20.7493113
144.18	17.027397260	16.4410999	12.59096893	20.7810380
144.31	16.315068490	16.4503565	12.60281903	20.8130410
144.40	17.334246580	16.4567649	12.58883060	20.8130045
144.49	19.934246580	16.4631734	12.46211109	20.7002370
144.76	17.802739730	16.4823987	12.56238826	20.8423703
144.94	15.098630140	16.4952155	12.65117245	20.9590585
145.02	16.106849320	16.5009119	12.65746774	20.9777556
145.12	16.936986300	16.5080324	12.58758312	20.9233732

Predictor variable Ln (mm)	Real age	Estimated age		
		Mean estimate	Lower 95% PI	Upper 95% PI
146.78	16.358904110	16.6262324	12.71261745	21.3057446
146.83	17.482191780	16.6297926	12.62786531	21.2287436
147.62	18.671232880	16.6860444	12.60644939	21.3297953
147.90	19.049315070	16.7059818	12.51500204	21.2817542
148.18	16.167123290	16.7259191	12.70919297	21.5193514
148.72	15.830136990	16.7643697	12.82854419	21.7224147
150.24	16.871232880	16.8726010	12.61752626	21.7470308
150.55	19.410958900	16.8946745	12.61775490	21.7953164
150.63	14.457534250	16.9003709	12.80249701	21.9924603
151.16	17.841095890	16.9381095	12.54148343	21.8136085
151.54	16.627397260	16.9651673	12.50635936	21.8373930
152.09	19.227397260	17.0043299	12.50376615	21.9200621
152.39	13.838356160	17.0256914	12.74363442	22.2064370
152.63	15.928767120	17.0427805	12.73856350	22.2385715
153.02	18.391780820	17.0705504	12.53950859	22.0999753
153.64	18.191780820	17.1146974	12.46746001	22.1240406
154.67	15.652054790	17.1880383	12.58010624	22.3963598
154.78	17.298630140	17.1958709	12.56697575	22.4002818
155.62	19.786301370	17.2556829	12.30904148	22.2725663
155.65	17.545205480	17.2578191	12.39933508	22.3675106
157.28	19.893150680	17.3738829	12.28624552	22.5071075
157.87	18.463013700	17.4158937	12.23715705	22.5494822
157.98	19.726027400	17.4237263	12.15805014	22.4874277
158.00	17.205479450	17.4251504	12.34354811	22.6760262
158.18	19.780821920	17.4379672	12.15997863	22.5203607
159.51	18.147945210	17.5326696	12.13237682	22.6989386
159.74	15.824657530	17.5490467	12.22582999	22.8280470
160.15	14.501369860	17.5782407	12.33643283	23.0022090
161.95	14.742465750	17.7064094	12.14090520	23.0857216
165.01	18.383561640	17.9242961	11.78301278	23.2021976
170.54	13.578082190	18.3180587	12.45909050	24.7355490
171.44	19.471232880	18.3821430	10.17000001	22.5859786
172.05	18.917808220	18.4255779	10.30693446	22.8174767



## APPENDIX G

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Posterior probabilities of age using  
maturation stages of the iliac bone

The posterior probabilities of age per annual age group are indicated in the tables below. The highest probability of an individual being of age A knowing he/she has a maturation stage M at a single epiphyseal site or a combination of two to four maturation stages of epiphyseal sites indicates the bone age group of the individual. The bone age group is highlighted in grey in the tables.

The highest probability can also be summed at will with the posterior probabilities of being of another age for the same stage M until a sufficient probability is obtained (e.g. 95%), associated with a precision of +/- X years.

The epiphyseal sites used in this method are indicated in the figure below.

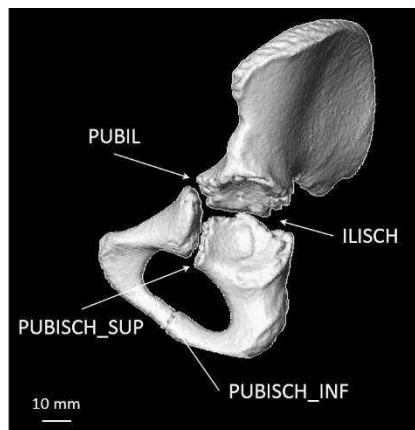


Figure G.1 The four iliac epiphyseal sites used in the study. PUBIL = Pubo-iliac site; ILISCH = Ilio-ischiatic site; PUBISCH\_SUP = Upper Pubo-ischiatic site; PUBISCH\_INF = Lower Pubo-ischiatic site

Maturation stages are defined as follows:

- 0 = no union/fusion
- 1 = partial union/fusion (or epiphyseal line still visible), including the presence of an *os acetabuli*.
- 2 = complete union/fusion (no visible line of fusion)

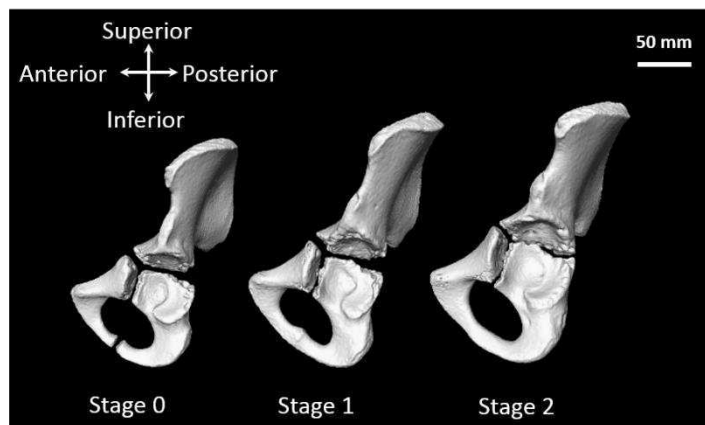


Figure G.2 Three maturation stages used in the study. Example of the PUBISCH\_INF epiphyseal site

Probabilities were calculated for both sexes combined and can be used on either of the left or right iliac bones (absence of significant bilateral asymmetry) for age estimation on dry and reconstructed bones.

Table G-1 Posterior probabilities of age per annual age group and per stage for the PUBISCH\_INF epiphysis of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination

Age group (years)	PUBISCH_INF		
	Stage=0	Stage=1	Stage=2
[0-1[	0,164	0	0
[1-2[	0,164	0	0
[2-3[	0,164	0	0
[3-4[	0,164	0	0
[4-5[	0,104	0	0
[5-6[	0,123	0	0
[6-7[	0,060	0	0
[7-8[	0,033	0	0
[8-9[	0,011	0,049	0
[9-10[	0	0,398	0
[10-11[	0	0,164	0
[11-12[	0	0,082	0
[12-13[	0	0,098	0,115
[13-14[	0	0	0,133
[14-15[	0,012	0,106	0,104
[15-16[	0	0	0,133
[16-17[	0	0,049	0,124
[17-18[	0	0,053	0,123
[18-19[	0	0	0,133
[19-20[	0	0	0,133

Table G-2 Posterior probabilities of age per annual age group and per stage for the ILISCH epiphysis of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination

Age group (years)	ILISCH		
	Stage=0	Stage=1	Stage=2
[0-1[	0,102	0	0
[1-2[	0,102	0	0
[2-3[	0,102	0	0
[3-4[	0,102	0	0
[4-5[	0,102	0	0
[5-6[	0,102	0	0
[6-7[	0,093	0	0
[7-8[	0,094	0	0
[8-9[	0,089	0,034	0
[9-10[	0,041	0,165	0
[10-11[	0,043	0,159	0
[11-12[	0,021	0,217	0
[12-13[	0,006	0,206	0,029
[13-14[	0	0,115	0,091
[14-15[	0	0,059	0,123
[15-16[	0	0,025	0,142
[16-17[	0	0,018	0,146
[17-18[	0	0	0,156
[18-19[	0	0	0,156
[19-20[	0	0	0,156

**Table G-3** Posterior probabilities of age per annual age group and per stage for the PUBIL epiphysis of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination

Age group (years)	PUBIL		
	Stage=0	Stage=1	Stage=2
[0-1[	0,090	0	0
[1-2[	0,090	0	0
[2-3[	0,090	0	0
[3-4[	0,090	0	0
[4-5[	0,090	0	0
[5-6[	0,090	0	0
[6-7[	0,090	0	0
[7-8[	0,089	0	0
[8-9[	0,084	0,020	0
[9-10[	0,060	0,108	0
[10-11[	0,057	0,119	0
[11-12[	0,047	0,153	0
[12-13[	0,028	0,162	0,032
[13-14[	0	0,135	0,101
[14-15[	0	0,124	0,107
[15-16[	0	0,135	0,101
[16-17[	0,006	0,021	0,150
[17-18[	0	0,023	0,161
[18-19[	0	0	0,173
[19-20[	0	0	0,173

**Table G-4** Posterior probabilities of age per annual age group and per stage for the PUBISCH\_SUP epiphysis of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination

Age group (years)	PUBISCH_SUP		
	Stage=0	Stage=1	Stage=2
[0-1[	0,094	0	0
[1-2[	0,094	0	0
[2-3[	0,094	0	0
[3-4[	0,094	0	0
[4-5[	0,094	0	0
[5-6[	0,094	0	0
[6-7[	0,094	0	0
[7-8[	0,094	0	0
[8-9[	0,088	0,019	0
[9-10[	0,056	0,124	0
[10-11[	0,055	0,1306	0
[11-12[	0,040	0,179	0
[12-13[	0,006	0,233	0,030
[13-14[	0	0,129	0,095
[14-15[	0	0,111	0,105
[15-16[	0	0,052	0,136
[16-17[	0,006	0	0,153
[17-18[	0	0,022	0,152
[18-19[	0	0	0,164
[19-20[	0	0	0,164

Table G-5 Posterior probabilities of age per annual age group for the different combinations of the PUBISCH\_INF and the ILISCH epiphyses of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination

Age group (years)	PUBISCH_INF - ILISCH							
	0-0	0-1	1-0	1-1	1-2	2-0	2-1	2-2
[0-1[	0,168	0	0	0	0	0	0	0
[1-2[	0,168	0	0	0	0	0	0	0
[2-3[	0,168	0	0	0	0	0	0	0
[3-4[	0,168	0	0	0	0	0	0	0
[4-5[	0,107	0	0	0	0	0	0	0
[5-6[	0,126	0	0	0	0	0	0	0
[6-7[	0,055	0	0	0	0	0	0	0
[7-8[	0,031	0	0	0	0	0	0	0
[8-9[	0,010	0,352	0,146	0,012	0	0	0	0
[9-10[	0	0	0,540	0,473	0	0	0	0
[10-11[	0	0	0,234	0,188	0	0	0	0
[11-12[	0	0	0,059	0,128	0	0	0	0
[12-13[	0	0	0,021	0,146	0,092	1*	0,468	0,027
[13-14[	0	0	0	0	0	0	0,300	0,096
[14-15[	0	0,647	0	0,045	0,414	0	0,121	0,102
[15-16[	0	0	0	0	0	0	0,0655	0,150
[16-17[	0	0	0	0,006	0,229	0	0,045	0,143
[17-18[	0	0	0	0	0,264	0	0	0,153
[18-19[	0	0	0	0	0	0	0	0,165
[19-20[	0	0	0	0	0	0	0	0,165

Table G-6 Posterior probabilities of age per annual age group for the different combinations of the PUBISCH\_INF and PUBISCH\_SUP epiphyses of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination

Age group (years)	PUBISCH_INF-PUBISCH_SUP							
	0-0	0-1	1-0	1-1	1-2	2-0	2-1	2-2
[0-1[	0,167	0	0	0	0	0	0	0
[1-2[	0,167	0	0	0	0	0	0	0
[2-3[	0,167	0	0	0	0	0	0	0
[3-4[	0,167	0	0	0	0	0	0	0
[4-5[	0,106	0	0	0	0	0	0	0
[5-6[	0,125	0	0	0	0	0	0	0
[6-7[	0,060	0	0	0	0	0	0	0
[7-8[	0,031	0	0	0	0	0	0	0
[8-9[	0,010	0,141	0,109	0,008	0	0	0	0
[9-10[	0	0	0,563	0,404	0	0	0	0
[10-11[	0	0	0,224	0,175	0	0	0	0
[11-12[	0	0	0,081	0,120	0	0	0	0
[12-13[	0	0	0,014	0,187	0,102	0,465	0,411	0,028
[13-14[	0	0	0	0	0	0	0,264	0,100
[14-15[	0	0,859	0	0,096	0,374	0	0,177	0,087
[15-16[	0	0	0	0	0	0	0,105	0,143
[16-17[	0	0	0,008	0	0,253	0,535	0	0,150
[17-18[	0	0	0	0,009	0,271	0	0,042	0,148
[18-19[	0	0	0	0	0	0	0	0,172
[19-20[	0	0	0	0	0	0	0	0,172

**Table G-7** Posterior probabilities of age per annual age group for the different combinations of the PUBISCH\_INF and the PUBIL epiphyses of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination

Age group (years)	PUBISCH_INF-PUBIL							
	0-0	0-1	1-0	1-1	1-2	2-0	2-1	2-2
[0-1[	0,166	0	0	0	0	0	0	0
[1-2[	0,166	0	0	0	0	0	0	0
[2-3[	0,166	0	0	0	0	0	0	0
[3-4[	0,166	0	0	0	0	0	0	0
[4-5[	0,106	0	0	0	0	0	0	0
[5-6[	0,125	0	0	0	0	0	0	0
[6-7[	0,060	0	0	0	0	0	0	0
[7-8[	0,033	0	0	0	0	0	0	0
[8-9[	0,010	0,132	0,094	0,009	0	0	0	0
[9-10[	0	0	0,539	0,399	0	0	0	0
[10-11[	0	0	0,211	0,182	0	0	0	0
[11-12[	0	0	0,088	0,117	0	0	0	0
[12-13[	0	0	0,062	0,148	0,105	0,813	0,255	0,029
[13-14[	0	0	0	0	0	0	0,2455	0,106
[14-15[	0	0,868	0	0,122	0,371	0	0,178	0,088
[15-16[	0	0	0	0	0	0	0,245	0,106
[16-17[	0	0	0,006	0,010	0,244	0,187	0,036	0,147
[17-18[	0	0	0	0,011	0,280	0	0,039	0,157
[18-19[	0	0	0	0	0	0	0	0,182
[19-20[	0	0	0	0	0	0	0	0,182

Table G-8 Posterior probabilities of age per annual age group for the different combinations of the PUBISCH\_SUP and the ILISCH epiphyses of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination

Age group (years)	ILISCH-PUBISCH_SUP							
	0-0	0-1	1-0	1-1	1-2	2-0	2-1	2-2
[0-1[	0,109	0	0	0	0	0	0	0
[1-2[	0,109	0	0	0	0	0	0	0
[2-3[	0,109	0	0	0	0	0	0	0
[3-4[	0,109	0	0	0	0	0	0	0
[4-5[	0,109	0	0	0	0	0	0	0
[5-6[	0,109	0	0	0	0	0	0	0
[6-7[	0,099	0	0	0	0	0	0	0
[7-8[	0,093	0	0	0	0	0	0	0
[8-9[	0,089	0,097	0,098	0,004	0	0	0	0
[9-10[	0,026	0,285	0,301	0,135	0	0	0	0
[10-11[	0,027	0,316	0,280	0,137	0	0	0	0
[11-12[	0,010	0,217	0,278	0,257	0	0	0	0
[12-13[	0,0004	0,083	0,039	0,317	0,213	0,158	0,158	0,006
[13-14[	0	0	0	0,098	0,368	0	0,274	0,062
[14-15[	0	0	0	0,043	0,209	0	0,316	0,093
[15-16[	0	0	0	0,008	0,115	0	0,171	0,139
[16-17[	0	0	0,004	0	0,094	0,841	0	0,160
[17-18[	0	0	0	0	0	0	0,080	0,17
[18-19[	0	0	0	0	0	0	0	0,184
[19-20[	0	0	0	0	0	0	0	0,184



**Table G-9 Posterior probabilities of age per annual age group for the different combinations of the ILISCH and the PUBIL epiphyses of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination**

Age group (years)	ILISCH-PUBIL							
	0-0	0-1	1-0	1-1	1-2	2-0	2-1	2-2
[0-1[	0,107	0	0	0	0	0	0	0
[1-2[	0,107	0	0	0	0	0	0	0
[2-3[	0,107	0	0	0	0	0	0	0
[3-4[	0,107	0	0	0	0	0	0	0
[4-5[	0,107	0	0	0	0	0	0	0
[5-6[	0,107	0	0	0	0	0	0	0
[6-7[	0,097	0	0	0	0	0	0	0
[7-8[	0,099	0	0	0	0	0	0	0
[8-9[	0,088	0,115	0,076	0,005	0	0	0	0
[9-10[	0,028	0,281	0,260	0,136	0	0	0	0
[10-11[	0,028	0,327	0,238	0,145	0	0	0	0
[11-12[	0,011	0,210	0,2703	0,254	0	0	0	0
[12-13[	0,002	0,066	0,152	0,255	0,224	0,485	0,081	0,007
[13-14[	0	0	0	0,118	0,388	0	0,211	0,066
[14-15[	0	0	0	0,056	0,210	0	0,262	0,094
[15-16[	0	0	0	0,026	0,085	0	0,329	0,103
[16-17[	0	0	0,003	0,003	0,092	0,515	0,054	0,158
[17-18[	0	0	0	0	0	0	0,062	0,181
[18-19[	0	0	0	0	0	0	0	0,195
[19-20[	0	0	0	0	0	0	0	0,195

**Table G-10** Posterior probabilities of age per annual age group for the different combinations of the PUBISCH\_SUP and the PUBIL epiphyses of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination

Age group (years)	PUBISCH_SUP-PUBIL							
	0-0	0-1	1-0	1-1	1-2	2-0	2-1	2-2
[0-1[	0,102	0	0	0	0	0	0	0
[1-2[	0,102	0	0	0	0	0	0	0
[2-3[	0,102	0	0	0	0	0	0	0
[3-4[	0,102	0	0	0	0	0	0	0
[4-5[	0,102	0	0	0	0	0	0	0
[5-6[	0,102	0	0	0	0	0	0	0
[6-7[	0,102	0	0	0	0	0	0	0
[7-8[	0,094	0	0	0	0	0	0	0
[8-9[	0,089	0,083	0,052	0,003	0	0	0	0
[9-10[	0,041	0,283	0,236	0,100	0	0	0	0
[10-11[	0,037	0,302	0,235	0,117	0	0	0	0
[11-12[	0,022	0,282	0,269	0,206	0	0	0	0
[12-13[	0,002	0,044	0,207	0,282	0,183	0,485	0,088	0,007
[13-14[	0	0	0	0,131	0,317	0	0,229	0,069
[14-15[	0	0	0	0,103	0,286	0	0,233	0,080
[15-16[	0	0	0	0,052	0,127	0	0,327	0,098
[16-17[	0,0004	0,006	0	0	0	0,515	0,059	0,164
[17-18[	0	0	0	0,004	0,086	0	0,062	0,175
[18-19[	0	0	0	0	0	0	0	0,203
[19-20[	0	0	0	0	0	0	0	0,203

Table G-11 Posterior probabilities of age per annual age group for the different combinations of the PUBISCH\_INF, the ILISCH and the PUBISCH\_SUP epiphyses of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination

Age group (years)	PUBISCH_INF-ILISCH-PUBISCH_SUP														
	0-0-0	0-1-1	1-0-0	1-1-0	1-0-1	1-2-0	1-1-2	1-2-1	1-2-2	1-1-1	2-1-0	2-2-2	2-2-1	2-1-2	2-1-1
[0-1[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[1-2[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[2-3[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[3-4[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[4-5[	0,107	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[5-6[	0,126	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[6-7[	0,0561	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[7-8[	0,028	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[8-9[	0,009	0,087	0,220	0,024	0,024	0	0	0	0	0,002	0	0	0	0	0
[9-10[	0	0	0,520	0,606	0,578	0	0	0	0	0,403	0	0	0	0	0
[10-11[	0	0	0,218	0,233	0,264	0	0	0	0	0,169	0	0	0	0	0
[11-12[	0	0	0,039	0,115	0,091	0	0	0	0	0,158	0	0	0	0	0
[12-13[	0	0	0,002	0,019	0,042	0,274	0,440	0,293	0,023	0,234	0,907	0,006	0,151	0,201	0,662
[13-14[	0	0	0	0	0	0	0	0	0	0	0	0,065	0,302	0,400	0,236
[14-15[	0	0,913	0	0	0	0	0,462	0,627	0,358	0,034	0	0,076	0,274	0,178	0,082
[15-16[	0	0	0	0	0	0	0	0	0	0	0	0,145	0,188	0,125	0,020
[16-17[	0	0	0	0,0009	0	0,726	0,097	0	0,288	0	0,093	0,156	0	0,095	0
[17-18[	0	0	0	0	0	0	0	0,080	0,329	0	0	0,166	0,082	0	0
[18-19[	0	0	0	0	0	0	0	0	0	0	0	0,192	0	0	0
[19-20[	0	0	0	0	0	0	0	0	0	0	0	0,192	0	0	0

Table G-12 Posterior probabilities of age per annual age group for the different combinations of the PUBISCH\_SUP, the PUBIL and the ILISCH epiphyses of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination. \*indicate probabilities obtained for one observation only

Age group (years)	PUBISCH_SUP-PUBIL-ILISCH																					
	0-0-0	0-0-1	0-1-0	0-1-1	0-1-2	0-2-1	1-0-0	1-1-0	1-0-1	1-0-2	1-2-0	1-1-2	1-2-1	1-2-2	1-1-1	2-0-0	2-1-0	2-0-1	2-2-2	2-2-1	2-1-2	2-1-1
[0-1[	0,112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[1-2[	0,112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[2-3[	0,112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[3-4[	0,112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[4-5[	0,112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[5-6[	0,112	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[6-7[	0,102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[7-8[	0,096	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[8-9[	0,086	0,193	0,147	0,017	0	0	0,146	0,010	0,017	0	0	0	0	0	0,0006	0	0	0	0	0	0	0
[9-10[	0,018	0,302	0,306	0,262	0	0	0,319	0,217	0,278	0	0	0	0	0	0,103	0	0	0	0	0	0	0
[10-11[	0,017	0,339	0,321	0,321	0	0	0,282	0,209	0,286	0	0	0	0	0	0,116	0	0	0	0	0	0	0
[11-12[	0,005	0,158	0,184	0,284	0	0	0,233	0,326	0,364	0	0	0	0	0	0,279	0	0	0	0	0	0	0
[12-13[	0,0001	0,007	0,042	0,115	1*	1*	0,019	0,238	0,054	0,695	0,914	0,402	0,270	0,075	0,362	0,469	1	0,585	0,001	0,022	0,053	0,202
[13-14[	0	0	0	0	0	0	0	0	0	0	0	0,386	0,389	0,404	0,093	0	0	0	0,043	0,178	0,286	0,291
[14-15[	0	0	0	0	0	0	0	0	0	0	0	0,179	0,203	0,241	0,038	0	0	0	0,068	0,245	0,348	0,310
[15-16[	0	0	0	0	0	0	0	0	0	0	0	0,034	0,121	0,126	0,008	0	0	0	0,096	0,398	0,178	0,181
[16-17[	0	0	0	0	0	0	0,0004	0	0,0007	0,305	0,086	0	0,016	0,154	0	0,531	0	0,414	0,165	0,073	0	0
[17-18[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,188	0,084	0,134	0,015
[18-19[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,218	0	0	0
[19-20[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,218	0	0	0

Table G-13 Posterior probabilities of age per annual age group for the different combinations of the PUBISCH\_INF, the PUBIL and the ILISCH epiphyses of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination. \*indicate probabilities obtained for one observation only

Age group (years)	PUBISCH_INF-PUBIL-ILISCH																					
	0-0-0	0-0-1	0-1-0	0-1-1	0-1-2	0-2-1	1-0-0	1-1-0	1-0-1	1-0-2	1-2-0	1-1-2	1-2-1	1-2-2	1-1-1	2-0-0	2-1-0	2-0-1	2-2-2	2-2-1	2-1-2	2-1-1
[0-1[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[1-2[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[2-3[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[3-4[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[4-5[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[5-6[	0,126	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[6-7[	0,056	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[7-8[	0,031	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[8-9[	0,009	1*	1*	0,081	0	0	0,201	0,020	0,029	0	0	0	0	0	0,002	0	0	0	0	0	0	0
[9-10[	0	0	0	0	0	0	0,527	0,563	0,574	0	0	0	0	0	0,415	0	0	0	0	0	0	0
[10-11[	0	0	0	0	0	0	0,217	0,213	0,275	0	0	0	0	0	0,183	0	0	0	0	0	0	0
[11-12[	0	0	0	0	0	0	0,045	0,121	0,088	0	0	0	0	0	0,160	0	0	0	0	0	0	0
[12-13[	0	0	0	0	0	0	0,009	0,005	0,033	1*	0,653	0,452	0,192	0,024	0,193	1*	0,980	1*	0,006	0,076	0,212	0,537
[13-14[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,069	0,228	0,422	0,287
[14-15[	0	0	0	0,919	1*	1*	0	0	0	0	0	0,455	0,665	0,356	0,045	0	0	0	0,077	0,223	0,180	0,107
[15-16[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,108	0,356	0,092	0,062
[16-17[	0	0	0	0	0	0	0	0,0008	0	0	0,347	0,093	0,064	0,278	0,001	0	0,020	0	0,154	0,054	0,094	0,007
[17-18[	0	0	0	0	0	0	0	0	0	0	0	0	0,078	0,342	0	0	0	0	0,176	0,062	0	0
[18-19[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,204	0	0	0
[19-20[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,204	0	0	0

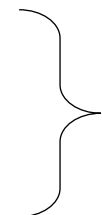
Table G-14 Posterior probabilities of age per annual age group for the different combinations of the PUBISCH\_INF, the PUBIL and the PUBISCH\_SUP epiphyses of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination. \*indicate probabilities obtained for one observation only

Age group	PUBISCH_INF-PUBIL-PUBISCH_SUP																					
	0-0-0	0-0-1	0-1-0	0-1-1	0-1-2	0-2-1	1-0-0	1-1-0	1-0-1	1-0-2	1-2-0	1-1-2	1-2-1	1-2-2	1-1-1	2-0-0	2-1-0	2-0-1	2-2-2	2-2-1	2-1-2	2-1-1
[0-1[	0,167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[1-2[	0,167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[2-3[	0,167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[3-4[	0,167	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[4-5[	0,106	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[5-6[	0,125	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[6-7[	0,060	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[7-8[	0,031	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[8-9[	0,010	1*	1*	0,026	0	0	0,153	0,014	0,021	0	0	0	0	0	0,001	0	0	0	0	0	0	0
[9-10[	0	0	0	0	0	0	0,563	0,528	0,580	0	0	0	0	0	0,347	0	0	0	0	0	0	0
[10-11[	0	0	0	0	0	0	0,212	0,217	0,255	0	0	0	0	0	0,166	0	0	0	0	0	0	0
[11-12[	0	0	0	0	0	0	0,064	0,125	0,119	0	0	0	0	0	0,147	0	0	0	0	0	0	0
[12-13[	0	0	0	0	0	0	0,007	0,115	0,022	0,288	0,653	0,342	0,044	0,026	0,241	0,803	1*	0,867	0,006	0,082	0,175	0,477
[13-14[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,072	0,246	0,349	0,255
[14-15[	0	0	0	0,974	1*	1*	0	0	0	0	0	0,572	0,535	0,320	0,095	0	0	0	0,066	0,197	0,248	0,158
[15-16[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,103	0,352	0,140	0,102
[16-17[	0	0	0	0	0	0	0,0008	0	0,002	0,711	0,347	0	0,510	0,305	0	0,197	0	0,133	0,160	0,059	0	0
[17-18[	0	0	0	0	0	0	0	0	0	0	0	0,086	0,583	0,348	0,002	0	0	0	0,169	0,062	0,088	0,007
[18-19[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,212	0	0	0
[19-20[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,212	0	0	0

Table G-15 Posterior probabilities of age per annual age group for the different combinations of the four epiphyseal sites of the iliac bone. Greyed probabilities have the highest value and correspond to the age group of the combination

Age group (years)	PUBISCH_INF-ILISCH-PUBISCH_SUP-PUBIL																			
	0-0-0-0	0-1-1-1	1-0-0-0	1-0-1-0	1-1-0-0	1-1-0-1	1-1-1-0	1-1-1-1	1-2-1-1	1-2-2-1	1-2-2-2	2-1-0-0	2-1-1-1	2-1-1-2	2-1-2-1	2-1-2-2	2-2-1-1	2-2-1-2	2-2-2-1	2-2-2-2
[0-1[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[1-2[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[2-3[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[3-4[	0,168	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[4-5[	0,107	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[5-6[	0,127	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[6-7[	0,056	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
[7-8[	0,029	0	0	0	0	0	0,003	0,0003	0	0	0	0	0	0	0	0	0	0	0	0
[8-9[	0,009	0,015	0,289	0,036	0,036	0,004	0,504	0,334	0	0	0	0	0	0	0	0	0	0	0	0
[9-10[	0	0	0,487	0,606	0,630	0,571	0,200	0,155	0	0	0	0	0	0	0	0	0	0	0	0
[10-11[	0	0	0,193	0,262	0,229	0,242	0,156	0,187	0	0	0	0	0	0	0	0	0	0	0	0
[11-12[	0	0	0,029	0,075	0,095	0,154	0,137	0,291	0	0	0	0	0	0	0	0	0	0	0	0
[12-13[	0	0	0,001	0,021	0,009	0,027	0	0	0,373	0,061	0,006	0,979	0,705	0,383	0,255	0,070	0,193	0,051	0,0206	0,001
[13-14[	0	0	0	0	0	0	0	0,033	0	0	0	0	0,209	0,425	0,423	0,435	0,322	0,315	0,191	0,045
[14-15[	0	0,985	0	0	0	0	0	0	0,613	0,717	0,282	0	0,067	0,155	0,174	0,204	0,269	0,301	0,206	0,055
[15-16[	0	0	0	0	0	0	0	0	0	0	0	0	0,018	0,037	0,132	0,136	0,200	0,196	0,426	0,101
[16-17[	0	0	0	0	0,0001	0,0002	0	0	0	0,100	0,320	0,021	0	0	0,016	0,154	0	0	0,073	0,160
[17-18[	0	0	0	0	0	0	0	0	0,0145	0,122	0,392	0	0	0	0	0	0,015	0,137	0,083	0,182
[18-19[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,227
[19-20[	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0,227

- 0-1-0-0: [8-9[
- 2-0-0-0: [12-13[
- 2-0-0-1: [12-13[
- 2-0-1-0: [12-13[
- 2-0-1-1: [12-13[
- 2-1-1-0: [12-13[



Combinations for which only one individual was observed (posterior probability = 1) and corresponding age groups