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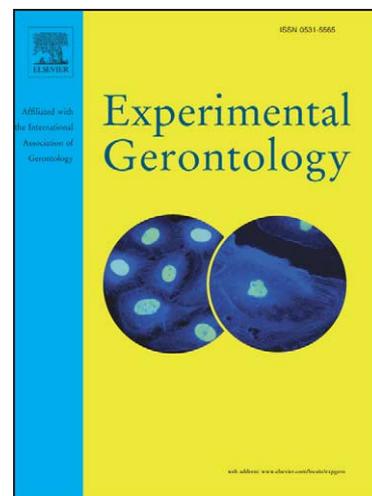
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**HUMAN LONGEVITY WITHIN AN EVOLUTIONARY PERSPECTIVE: THE
PECULIAR PARADIGM OF A POST-REPRODUCTIVE GENETICS**

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Abstract

The data we collected on the genetics of human longevity, mostly resulting from studies on centenarians, indicate that: 1. Centenarians and long-living sibpairs are a good choice for the study of human longevity, because they represent an extreme phenotype, i.e. the survival tail of the population who escaped neonatal mortality, pre-antibiotic era illnesses, and fatal outcomes of age-related complex diseases. 2. The model of centenarians is not simply an additional model with respect to well-studied organisms, and the study of humans has revealed characteristics of ageing and longevity (geographical and sex differences, role of antigenic load and inflammation, role of mtDNA variants) which did not emerge from studies in laboratory model systems and organisms. 3. All the phenotypic characteristics of nonagenarians and centenarians fit the hypothesis that ageing is a remodelling process where the body of survivors progressively adapts to internal and external damaging agents they are exposed to during several decades, largely unpredicted by evolution. 4. Such a remodelling process, which can be considered a Darwinian process occurring at the somatic level within the framework of the evolutionary constraints established by evolution for *H. sapiens* as a species may explain why the same gene polymorphism can have different (beneficial or detrimental) effects at different ages. 5. Geographic and demographic evidence suggest that longevity can be achieved by different combinations of genes, environment, and chance quantitatively and qualitatively different in different geographic areas, and that population-specific genetic factors, play a role on the longevity trait. 6. The concomitant and integrated use of new *in silico* and high throughput strategies will greatly accelerate the identification of new longevity genes in humans.

The peculiar geography and demography of centenarians

Human populations are characterized by specific gene pools that arise from the particular group's history in terms of chance (genetic drift) and environment (natural selection). Furthermore, humans are unique in having linked cultural and biological inheritance. Accordingly, human longevity is a very complex trait and its study is quite difficult and still in its infancy. A useful rule when tackling the genetics of a complex trait such as longevity is to consider an extreme phenotype. From this perspective, subjects able to reach extreme longevity, such as centenarians, have been an extraordinary model to study human longevity and healthy ageing, and to identify their biological determinants. Centenarians represent the survival tail of the population and they escaped neonatal mortality, pre-antibiotic era illnesses and they avoided or postponed most severe age-related diseases and their fatal outcomes (Franceschi et al., 1995a; Franceschi and Bonafè, 2003; Leroi et al., 2005; Franceschi et al., 2005). From a geographical point of view human longevity is not homogeneously distributed and it seems to be clustered in families enriched in long-lived parents and ancestors (Poulain et al., 2004; Guerresi et al., 2003; Willcox et al., 2006). In particular, significant differentials have been observed between Italian regions in the distribution of centenarians by sex, (femininity ratio, FR) from two women per man in the south to over eight in certain regions in the north (Passarino et al., 2002; Guerresi et al., 2003). A recent investigation suggests that the FR observed at the age of 100 in Italy is mainly due to mortality differentials among men and women over the age of 60 (Robine et al., 2006). The high mortality of men in the north and their low mortality in the south are the main explanations of why the FRs are higher in the north and lower in the south. Mortality differentials among women have only a marginal impact. The lowest FR is observed in certain parts of the Sardinia island such as the province of Nuoro, where the female-to-male ratio tends to be highly male-biased. This phenomenon appears to be mostly due a survival advantage of Sardinian men due to lower mortality for cardiovascular diseases after age 80 years (Caselli and Lipsi, 2006). We recently published the first results of a

genealogical tree reconstruction of selected centenarians aged 105 and over, from the Nuoro province (Caselli et al., 2006). An accurate centenarian age validation was performed that required a meticulous examination of numerous civil status records and parish registers. An important finding was that longevity occurs among the ascendants of the maternal branch of the family. The data used are still provisional but, should it apply to other validated cases, it would provide additional evidence of a genetic component in longevity. A more thorough examination of the data available may yield deeper insights into the role played by endogamy and consanguinity in attaining longevity.

Familiarity and genetics play a role in attaining extreme old age in humans: evidence from centenarians and their family members

Actually, an impressive and coherent series of epidemiological data from different populations (White Americans from New England, Mormons from Utah, Ashkenazi Jewish living in USA, Icelanders, Japanese from Okinawa, Netherlanders from Leiden) suggests the presence of a strong familiar and genetic component of human longevity. All these studies demonstrate that parents, siblings and offspring of long-lived subjects have a significant survival advantage and a higher probability to have been or to become long-lived, and a lower risk to undergo major age-related diseases, such as cardiovascular diseases (CVD), diabetes and cancer, when compared to appropriate selected controls. Thus, literature indicates that longevity is present in many generations of a single family in spite of the great variations in life style and life expectancy which occurred in the last century. In particular, it is interesting to note that in the most recent studies on this topic, spouses of long-lived subjects have been added as additional control group. The results indicate that this control group does not have any advantage/benefit in terms of survival and protection from the above mentioned diseases, even if they have shared with the long-lived partner most of their adult life. On the whole, these data suggest that such a familiar component can in fact be a genetic

component of longevity, and indicate that long-living subjects and families enriched in long-living members represent study groups particularly suitable to study the determinants of human longevity (Perls et al., 2002, Terry et al., 2003, 2004, Atzmon et al., 2004, Karasik et al., 2004, Ikeda et al., 2006, Schoenmaker et al., 2006)

Twin studies indicate that approximately 25% of the variation in adult lifespan is caused by genetic differences between individuals, and that genetic influences on lifespan are minimal before the age of 60 but increase after this age (Christensen et al., 2006). As the number of very old, centenarian, twins is very rare, such data on the heritability on longevity in twins do not exclude that the genetic component of extreme longevity can be even greater than the indicated 25% which, in our opinion, represents the lower estimate. In any case environment, i.e. climate, food, anthropological/cultural habits regarding nutrition and exercise, economical, working and housing conditions, as well as social and familiar support, strongly contribute to achieve the extreme limits of human lifespan. We recently performed a study on the personality of centenarians living in central Italy and preliminary data indicates that centenarians have a peculiar personality and character. On that occasion we also investigated the quality of sleep in centenarians and again we found that sleep quality is remarkable good, likely contributing to the capability to survive to extreme age (Sorbi et al., unpublished results). It is interesting to note that we know practically nothing about another component such as stochasticity that human observations in twins and animal studies indicate to play a consistent role in longevity (Rea et al., 2005).

The genetics of human longevity and the results of association studies on candidate genes: longevity genes can be population-specific

We recently reviewed the data obtained in our and other laboratories suggesting that polymorphisms of genes involved in immune response/inflammation, response to stressors including oxidative stress, insulin/IGF1 signalling pathway, among others, have a different frequency in centenarians in comparison to younger controls (young and old people) (Franceschi et

al., 2005). We also recently reviewed (Salvioli et al, 2006a) the data suggesting that both inherited and somatically-acquired variability of mitochondrial DNA (mtDNA) also play a role in human longevity, likely interacting with nuclear gene variants, within the theoretical framework that the cross-talk between these two genomes is essential for longevity as well as for other complex traits (Rose et al., 2002). It is interesting to note that, according to a large scale study, involving more than 900 subjects of different ages, the association between longevity and mtDNA haplogroup J is population specific, being evident in centenarians from northern Italy but not in those from southern Italy. In nonagenarians/centenarians from Ireland Finland and Japan mtDNA haplogroups and longevity are also associated (Niemi et al., 1998; Tanaka et al., 1998; De Benedictis et al., 1999; Ross et al., 2001; Salvioli et al., 2006a)

Interesting conclusions emerge from a comprehensive/critical analysis of the available data on the genetics of centenarians (Franceschi et al., 2005, 2007; Capri et al., 2006; Salvioli et al., 2006a; Christensen et al., 2006; Candore et al., 2006a):

- most of the studies so far reported have been performed in a restricted number of populations, mostly Italians and Danish, but also Japanese, French, Ashkenazi Jewish, Germans and Finnish, among others;
- in most of the studies, a limited number of polymorphisms has been considered;
- sex/gender-differences reported by some studies have not been systematically investigated. A possible reason is the low number of centenarians examined in some studies which did not allow to appreciate the possible importance of sex/gender;
- the number of genes which have been studied in more than one populations is relatively low, and the results are often discordant or difficult to compare.

To simplify such a very complex mass of data, two basically different interpretations are possible:

1. the first assumes that most of the results derived from the above-mentioned studies are likely false positive, owing to bias related to population stratification between cases (centenarians) and controls, lack of power, or other (trivial) reasons;
2. the second assumes that different/discordant findings in two (or more) different populations of centenarians and controls can be expected because different susceptibility genes may contribute to the longevity phenotype in genetically different populations.

In most of the cases we cannot decide which of the two alternatives is true, but in any case these considerations are crucial as regards replication studies, that is studies that aim to confirm in another population the associations between genetic loci and traits found in one population. Assuming that longevity can be achieved by different routes, resulting from different combinations of genes, environment, and chance that vary quantitatively and qualitatively in different geographic areas, we wonder whether replication can reasonably be expected for longevity in light of the above considerations. We are not challenging the tenet that studies on complex traits must be replicated in different populations and/or geographic areas. We are challenging the simplistic expectation that a gene polymorphism cannot be considered a longevity marker unless it is associated with longevity in all the populations tested (De Benedictis and Franceschi, 2006; Franceschi et al., 2007)

How to overcome the “cohort effect” and to take advantage from demographic data and the use of different age groups

Most of the above-mentioned limitations are common to other type of association studies on complex traits. Indeed, in the case of association studies on human longevity another limitation is present, i.e. the so called “cohort effect”, due to the unavoidable comparison of people belonging to different cohorts. The cohort effect assumes that the variation in longevity may be caused by different social and environmental factors experienced by distinct birth cohorts. We and others tried to minimize the effect of such an intrinsic limitation by: 1. using younger “controls” belonging to different age groups, including people aged around 60 years of age who are those more

close to centenarians as a cohort but also those who did not underwent any significant mortality selection; 2. creating algorithms that add demographic information to gene data; this practice aims at estimating the association between gene variants and survival/mortality parameters without generating bias due to cohort effects. The advantage of such an approach is that it increases the power of genetic analysis, besides being more correct from a methodological point of view (De Benedictis and Franceschi, 2006; Salvioli et al., 2006b; Franceschi et al., 2007).

Recently, we aimed to investigate the influence of the genetic variability of candidate genes on survival at old age in good health on the basis of a synthetic survival curve constructed using historic mortality data taken from the Italian population from 1890 onward (Passarino et al., 2007). We defined three age classes ranging from 18 to 106 years and then assembled a multinomial logistic regression model to evaluate the effect of dichotomous variables (genotypes) on the probability to be assigned to a specific category (age class). By applying the regression model to a cross-sectional dataset (10 genes; 972 subjects selected for healthy status) categorized according to age and sex we found that genetic factors influence survival at advanced age in good health in a sex- and age-specific way. Furthermore, we found that genetic variability plays a stronger role in males than in females and that, in both genders, its impact is especially important at very old ages. These results underline the age-specific effect of the gene network in modulating survival at advanced age in good health (Passarino et al., 2007).

The rationale of and the working hypothesis behind the association studies in centenarians

Today the available genetic methodologies allow to perform genome-wide association studies with several hundred thousands polymorphisms scanning the entire genome in order to find polymorphisms associated with a defined phenotype without any a priori hypothesis. This was not the case in previous studies performed on a limited number of candidate genes and polymorphisms. It is interesting to remind the theoretical assumptions that led us to focus of the specific genes we have investigated in Italians centenarians.

First of all it is important to remind that the first data on possible determinants of longevity in centenarians emerged from two major hypothesis, the first regarding a general conceptualization on the possible biological determinants of the ageing process, and the second originating from studies on the *immunological status* of centenarians.

In the 1989 paper one of us arrived to the conclusion that the ageing process is indirectly controlled by a variety of cellular and molecular defence mechanisms, including anti-oxidant defence, heat shock proteins, DNA repair mechanisms, and proposed the concept that they constitute a network of defence mechanisms (Franceschi et al., 1989). This hypothesis was further developed by Tom Kirkwood and Claudio Franceschi (Kirkwood and Franceschi, 1992) and constituted the theoretical basis of an international conference held in Modena in 1992, devoted to “Aging and Cellular Defence Mechanisms”.

The second hypothesis was based on the assumption that the ageing of the immune system, Immunosenescence, plays a central role in the ageing process (Franceschi et al., 1995b). Since the beginning of last decade of the last century, we systematically investigated the most parameters of the immune response and arrived to the unexpected conclusion that several parameters, such as NK cell number and activity, were remarkably conserved in centenarians and quite similar to those found in young people. We also identified the most important changes which characterize immunosenescence, i. e. a decrease of B lymphocytes and naïve T cells, a progressive increase of CD28- cytotoxic T cells and the accumulation of expanded clones of memory T cells, a shrinkage of the T cell repertoire, the lack of organ-specific autoantibodies, among others (Franceschi et al., 1995b; Cossarizza et al., 1997; Franceschi et al., 2000a). A seminal observation we made during these investigations was that peripheral blood mononuclear cells from old donors, when stimulated with mitogens, were able to secrete higher amounts of pro-inflammatory cytokines than cells from young people (Fagiolo et al., 2003). This observation was highly unexpected as the dominant paradigm at that time was that immunosenescence was a global failure of the immune system where *all* the responses were negatively affected. Our observation challenged that tenet showing that

inflammatory responses were higher and more active in old people than in young ones. Accordingly, we focused our attention on pro-inflammatory cytokines showing for example that, unexpectedly, centenarians (even those in relatively good health) had high plasma level of IL-6 (Baggio et al, 1998; Passeri et al., 2003). Incidentally, in the 1998 paper we also reported the unexpected finding that centenarians have high plasma level of Lp(a) a lipoprotein under strict genetic control which is considered a risk factor for CVD. Concomitantly, another “paradox” of centenarians emerged, i.e. their pro-coagulant status without clinical signs of thrombosis (Mari et al., 1995). These observations were made by a group working in Milan led by Daniela Mari, and were subsequently confirmed and extended (Coppola et al., 2003).

On the whole these results were a clear indication that inflammation, a fundamental response which allows animals to cope with a variety of stressors and pathogens, was up-regulated and over-stimulated in centenarians. At the same time the literature on diseases, such as atherosclerosis and Alzheimer’s disease (AD) started to understand that all these major age-related diseases shared an inflammatory background which likely played a major role in their pathogenesis (Ross et al., 1999; McGeer and McGeer., 1999) . How to reconcile these contrasting observations? On one side inflammation appeared to be a prerequisite or at least a basic component of the major killers and on the other side the centenarians, i.e. those people who were able to reach a remarkable advanced age escaping or delaying those major pathologies, were clearly “inflamed” and showed several signs of inflammation, including high levels of pro-inflammatory cytokines and coagulation factors (Mari et al., 1995; Baggio et al., 1998; Coppola et al., 2003; Passeri et al., 2003).

In order to answer this question and to solve this apparent paradox we started investigations aimed to identify a possible genetic propensity to produce high or low levels of pro-inflammatory cytokines and other pro-inflammatory molecules. Different teams of investigators were involved in this ambitious task, working in strict collaboration and sharing hypothesis and samples. In the meantime, the group of Giovanna De Benedictis, in strict collaboration with the Modena/Bologna group and the group of the Italian National Research Center on Ageing (INRCA, Ancona),

pioneered the genetic approach to the longevity trait, starting a series of investigations focused on apolipoproteins, a topic which has been thoroughly analysed in subsequent years including studies on genes coding for apolipoproteins such as APOB, APOE, APOC3 but also APOJ, genes related to stress (SOD, TH, HSP70), DNA repair (PARP), sirtuins (SIRT3) and mtDNA. Apart from the original group of Milan and Modena/Bologna the enlarged team included groups working in Ancona at (INRCA) and that of the University of Palermo led by Calogero Caruso, who focused on the opposite role played by inflammatory gene polymorphisms in longevity and CVD. This critical mass allowed us to increase the number of candidate genes to investigate and to include genes such as paroxonase (PON1) which protect LDL from oxidation, as well as those coding for anti-inflammatory cytokines such as IL-10 and TGF β 1, and genes coding for molecules crucial for the recognition of pathogens and the start of immune response such as toll receptors (TLR4) and other which plays an important role in inflammatory responses (PYRIN), α 1-antitrypsin, connexin, CCR5, COX and LOX (Candore et al., 2006a,b; Capri et al., 2006; Salvioli et al., 2006a, Franceschi et al., 2007, Vasto et al., 2007).

On the whole, these last data suggest that a pro-inflammatory genotype is unfavorable to reach extreme longevity in good health and likely favors the onset of age-related diseases such as CVD and AD which are the leading causes of mortality and disability in the elderly. Within this perspective, healthy ageing and longevity are likely the result not only of a lower propensity to mount inflammatory responses but also of an efficient anti-inflammatory network, which in unsuccessful ageing fail to fully neutralize the inflammatory processes consequent to the lifelong antigenic burden and exposure to damaging agents. Such a global imbalance can be a major driving force for frailty and common age-related pathologies, and should be addressed and studied within an evolutionary-based systems biology perspective (Franceschi et al., 2007).

Conclusions

In spite of the growing amount of genetic data regarding longevity, genetics can account only for a part of the phenomenon. This is demonstrated by different experimental evidence: i. syngenic animals have different mortality curves, suggesting the presence of a stochastic component; ii. many genetic variants considered unfavourable for longevity and associated to an increased risk of disease are found in centenarians with the same frequency observed in the general population (Franceschi et al., 2005a; Salvioli et al., 2006a). Moreover, inside the human species, gender differences are present. It is known that women live longer than men (about 5 years more) and the major part of subjects that achieve the extreme ages is composed by females. Furthermore, a positive association between genetic variants and longevity is often found only for males and rarely for both genders (Candore et al., 2006a). This suggests that longevity trait is more dependent on genetics in men than in women (Franceschi et al., 2000b). It is hypothesised that in female longevity a non-genetic component is present which is not yet completely appreciated. As a consequence, it is possible to affirm that longevity is a complex trait which depends on environmental, cultural and genetic factors. These factors are interacting with each other, as demonstrated by different studies (Gueresi et al., 2003; Carru et al., 2003) and they are also modulated by the cross talk between nuclear and mitochondrial genomes (De Benedictis et al., 1999; Bellizzi et al., 2006).

Association studies can point out positive or negative association between particular allelic variants and longevity. This rather simple situation is complicated by the fact that the same variants can be associated with both longevity and age-related diseases, depending on the genetic and environmental scenario (population studied, parameters considered, etc.). This clearly indicates that it is crucial the interaction and combination among more genes and their allelic variants. Besides, available data indicate that the aging process allows the emerging of biological effects due to individual genetic differences which can be neutral or silent at younger age. This suggests that the genetics of ageing/longevity is peculiar, and likely it is not

ruled by classical genetic principles, and should thus be considered a *post-reproductive genetics*, since it regards decades of life where the strength of selection progressively fades away (De Benedictis and Franceschi, 2006). From this point of view, it is much more likely that aging is a sort of side-effect of genes playing an important role during juvenile age. As an example, it is possible that a genetic variant that increases reproductive fitness can have a detrimental effect on longevity. This phenomenon is defined as "antagonistic pleiotropy" (Williams and Nesse, 1991). It is thus evident that, if a genetic variant confers a selective advantage during young age, it will be selected even if it is unfavorable for longevity (for example by conferring a higher risk for age-related diseases). This seems to be the case for the inflammatory gene polymorphisms responsible for a higher responder status that were selected to fight infections in young age (Caruso et al., 2005; Licastro et al., 2005).

In this perspective, the apparent paradoxes emerged from the studies on centenarians can be generated not only by the lack of validated scales for centenarians, but also by the fact that a genetic variant can play different roles in young age and in old age. Indeed, one of the most important indications coming from the studies on centenarians, is that extreme longevity has unusual and largely unexpected features for which most of the current concepts in biology and genetics are inadequate (De Benedictis and Franceschi, 2006). Actually, it is clear that centenarians are part of a special biology in which different tissue and organs could not have reached the same level of senescence (a kind of mosaicism). Moreover, it will be the balance between the amount of enduring stresses and the efficacy of stress-response systems of the body that will determine how fast a tissue or body will age (Franceschi et al., 2000c; Rattan 2004).

Finally, since human aging is the emerging product of a series of different factors (environment, culture, economic conditions, stochasticity, etc.) in which genetics plays only a part, we wish to stress that it is not always possible to extrapolate and predict results to be extended to humans from animal models in which most of these variables have been

eliminated or are under control, as for example cultures of mutated *C. elegans* or KO mice. In particular, in some cases studies on animal models have led to analogous results also in humans, as in the case of Insulin/IGF-1 signaling pathway (Franceschi et al., 2005), while in other cases results obtained in animal models have not been reproducible in humans. For example, the knock out of p66^{Shc} gene leads to an increase in mice lifespan (Migliaccio et al., 1999) and consequently it has been hypothesised that centenarians should hypoexpress this gene (Purdom and Chen, 2003), while, on the contrary we observed that dermal fibroblasts from centenarians do have higher levels of p66^{Shc} mRNA and protein with respect to young and middle-aged subjects (Pandolfi et al., 2005). Thus, often it appears not sufficiently adequate the animal model for the study of human longevity (Franceschi and De Benedictis, 2006; Capri et al., 2006).

Further, it is claimed that results obtained on human populations should always be replicated. Indeed, association with particular genetic polymorphisms and longevity is reported for some population but not for others (see for a recent striking example the study of Barzilai et al., 2003 and that of Cellini et al., 2005 on CETP gene). However, this is not strange because, as underlined in this paper, human populations are characterized by specific gene pools that arise from the particular group's history in terms of chance (genetic drift) and environment (natural selection). Hence, replication cannot reasonably be expected for longevity in light of the considerations discussed in the present study (De Benedictis and Franceschi, 2006).

To conclude, there are new recent advances in the analysis of human genome which in the next future will open interesting perspectives on the study of human longevity. So, the concomitant and integrated use of new *in silico* and high throughput strategies will greatly accelerate the identification of new longevity genes in humans.

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