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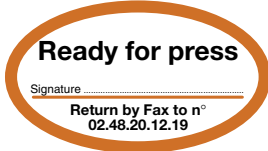
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# 'Non-Mendelian' genetics of fetal growth

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**ABSTRACT.** Mendelian genetics showed that disrupted imprinting genes can lead to phenotypic changes in pre-natal growth. It is likely that genetic determinants of fetal size may be found in imprinted genes. Mendelian genetics, however, is somewhat limited in its ability to explain the individual variability of size at birth within the 'normal' range. Fetal growth is a complex multifactorial, multigenic trait comprised of various 'sub-traits', such as body mass, fat and muscle, brain mass, head circumference, spine and limb skeletal growth. It is likely that

different genetic factors and genomic variants are responsible for the variations of these 'sub-traits', however this hypothesis needs to be tested. A study has been initiated to test the assumption that non-Mendelian genetics plays a role in the variation of human birth weight. The ultimate aim is the identification of genomic variations that are within or near certain selected candidate genes and are associated with variations of human height at birth.

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## MENDELIAN GENETICS OF FETAL GROWTH

Mendelian genetics, ie the genetics of monogenic diseases, have become an important field of pediatric endocrinology. The restriction of fetal growth offers examples of Mendelian diseases (small size for gestational age with monogenic causes). To induce pathological manifestations, mutations have to alter a gene product necessary to a function or a pathway which is itself necessary to allow normal fetal growth. The alteration of the gene product is generally qualitative (a truncated or a functionally deficient protein), more rarely quantitative (a markedly reduced expression of the protein of interest). Nonsense or missense mutations located within exons or the deletion of useful exons can result in abnormal proteins that have lost most, if not all of their function. Promoter mutations or epimutations modifying DNA methylation can result in decreased expression of the protein. Understandably, evolution has tried to eliminate disabling mutations as 'errors of nature'. For this reason, most monogenic diseases and their causative mutations are rare. Monogenic disorders can sometimes be mimicked by the experimental manipulation of the gene of interest, by altering its

RNA or protein products in a knock-out or knock-in mouse, or in a cell line.

## GENETIC VARIATION OF FETAL GROWTH THAT IS DEPENDENT ON IMPRINTING

Genomic imprinting is an epigenetic process that marks the parental origin of certain genes. Imprinting results in the monoallelic expression of a gene, at least for a given time (intrauterine life for example) and in a given tissue. The monoallelic expression depends on the parental origin of the allele. Genes that are not expressed from the maternal allele are said to be maternally imprinted (ie, maternally imprinted genes are those whose paternal allele is expressed alone). For most genes following the rules of Mendelian genetics character transmission, the parental origin of genetic information has no influence on gene expression. In contrast, genomic imprinting follows the rules of 'parent-specific gene expression'. Most imprinted genes are found clustered with other imprinted genes. This clustering of imprintable genes is likely to have functional consequences that are not yet understood. As many of the known imprinted genes are involved in the control of fetal growth, genomic imprinting might be a key component of the mechanisms underlying maternal constraint, which limits fetal size in order to allow vaginal delivery.

The first observation of an imprinted gene was obtained by the knockout of the *Igf2* gene by homologous recombination in mice (1). Fetal growth was reduced if the inactivated allele was transmit-

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ted paternally, but normal when the maternal allele was mutated. Conversely, maternal inheritance of a non-functional *Igf2r* gene results in fetal overgrowth, whereas paternal transmission of the disrupted allele leads to normal fetal size (2). Recently, Constanca et al. (3) have mutated the placenta-specific promoter of *Igf2* gene and their results were comparable to the complete knockout of *Igf2* gene (3). Newborn mice with a paternal copy of the mutated promoter showed comparable growth retardation to mice with a complete disruption of the *Igf2* gene. This indicates that a paternally expressed (maternally imprinted) gene in the placenta acts as a regulator of mouse fetal growth. This example shows that imprinted genes imply a differential role for maternally vs paternally derived genetic information, and may have different consequences on placental function. Knockouts of the paternally expressed *Igf2*, *Peg1* and insulin genes result in fetal growth restriction, whereas knockout of the maternally expressed *Igf2r* gene results in fetal overgrowth. These results support the prediction that paternally expressed (maternally silenced) genes enhance fetal growth, whereas maternally expressed (paternally silenced) genes act as growth inhibitors (4). It is fascinating that the maternal environment (nutrition, climate, light, workload, etc) could be perceived by the pregnant mother's body and generate signals that induce chemical modifications of parts of the fetal genome which are involved in regulation of fetal growth (5).

In humans, fetal growth restriction can result from alterations in expression of imprinted genes. A major cluster of imprinted genes occurs at chromosome 11p15. This cluster contains *IGF-2* and *H19* genes, which are reciprocally imprinted, and several genes which are under the control of a differentially methylated CpG island (*KvDMR1*). Molecular defects affecting these genes are involved in the Beckwith-Wiedemann syndrome (BWS). BWS is a syndrome of pre-natal overgrowth with macroglossia, hypoglycemia and visceromegaly. Paternal uniparental disomy for 11p15 is found in 20% of BWS patients (6). In addition, the bi-allelic expression of *IGF2* has been associated with somatic overgrowth in children having no other features of BWS (7). More recently, an epimutation has been described in some patients with Silver-Russell syndrome (SRS) (8). SRS is a syndrome of pre-natal growth retardation with body asymmetry and a small triangular facies. A demethylation (epimutation) was identified in the imprinting centre region *ICR1* of this 11p15 region. This demethylation is responsible for the biallelic expression of *H19* and the downregulation of *IGF-2* expression that probably results from a relaxation of imprinting. Ten percent of patients with BWS have a hypermethylation of the *H19* promoter

(9). This molecular mechanism is the opposite of the epimutation described in SRS patients. These observations suggest that paternally expressed and maternally silenced *IGF-2* is an important regulator of human fetal growth.

The effect of genomic imprinting is the expression of only one copy of a gene during fetal growth. This form of gene control allows the other gene copy to remain available either for critical periods of rapid post-natal growth or for functioning during tissue maturation. The predominant theory for genomic imprinting is known as the 'parental conflict theory', which focuses on the nutritional needs of the fetus that are met by the mother via the placenta (10). This theory leads to the prediction that imprinted growth enhancers will be silent in maternal derived alleles whereas imprinted growth inhibitors will be silent in paternal derived alleles. This theory has been successful at explaining the imprinting of paternally expressed enhancers (*IGF-2*, *KCNQ1OT1*) and maternally expressed inhibitors (*Igf2r*, *CDKN1C*) of pre-natal growth.

Because disrupted imprinting genes can lead to phenotypic changes in pre-natal growth, it is likely that genetic determinants of fetal size may be found in imprinted genes. It is suspected that genomic variants located within imprinted genes will be identified as one of the mechanisms underlying fetal growth variation. However, low birth weight children have higher mortality not only soon after birth but also later on in their adult life. Therefore, it is important to keep in mind that imprinting, although not the only mechanism involved in epigenetic processes, could also interact with the programming phenomena that link fetal life to post-natal environmental conditions. Changes in expression of imprinted genes could also provide a genetic link between intrauterine growth and the programming of fetal systems involved in the long-term risk of metabolic disease in adulthood, notably in the field of insulin resistance.

## NON-MENDELIAN GENETICS

Non-Mendelian genetics belong to a different world. They deal mostly with the question of how genomic variations contribute to individual differences, ie the phenotypic variation. In case of quantitative traits, people cannot be categorized as 'cases' or 'controls', they are human individuals with phenotypic variability. The extensive polymorphism of the human genome, which results in varying protein isoforms and varying protein quantities in various tissues explains much of the variation of each human trait. Humans do not have, for instance, the same birth weight, adult height, glucose level, blood pressure, muscle

or fat mass. They owe these differences in part to the physical and cultural environment in which they live, but also to their specific genomic constitution, which is different in every individual. The human genome contains approximately 10 million single nucleotide polymorphisms (SNPs), each of them with two alleles generating three genotypes (or diplotypes). The 10 millions single nucleotide polymorphisms of the human genome could in theory generate  $3^{10,000,000}$  combinations of diplotypes. This number is however a gross overestimation of human genomic diversity because many SNPs are linked to each other on the same DNA strand, with their alleles being inherited as unrecombined blocks, generating what is called linkage disequilibrium between these alleles.

**FETAL GROWTH: A MULTIFACTORIAL MULTIGENIC TRAIT**

Fetal growth is without doubt a complex trait (Fig. 1), with a partial genetic determination itself regulated by many genes. The existence of many genes important to fetal growth is demonstrated by the vast number of chromosomal abnormalities that are capable of impairing fetal growth severely. A fraction of the genes regulating fetal growth has functional SNPs (coding or regulatory). Functionally important SNPs or their variants can also be located outside of genes in the intergenic non coding DNA portions of the genome. Whether they are coding, interfering with splicing, or regulating gene expression, functional genomic SNPs should not be considered as deleterious mutations. There is no disease here, no 'errors of nature', only the natural variation of fetal growth. Nature has used genomic SNPs to shape and transmit a degree of diversity of fetal growth across individuals. To be expected to play a significant role in fetal growth at the population level, a SNP needs (a) to affect the trait in a quantitatively sufficient manner (affect size), and (b) to be present in a rather large fraction of people,

ie, its allelic frequency should exceed 0.10-0.20. To be frequent in the modern human population, a SNP has to be ancient, and retained and expanded throughout human history (possibly because it has been useful in a given environment). It is likely that fetal growth has been the subject of genetic selection during evolution, because of its importance for survival. Birth is clearly a critical moment of life, maybe the most critical. The perinatal period is characterized by the highest mortality risk that a human being has to face during his entire life. Size at birth seems to play a major role, as smaller or larger than normal babies have increased mortality. Evolution has stabilized human birth size within relatively narrow limits in order to reduce perinatal mortality. There is however a degree of interindividual variability. Genes important for the physiology of fetal growth can certainly be unravelled in animal models, at least for the part of physiology that is not species specific. In contrast, animal models are unlikely to tell us which human SNPs are important to shape the variations of human fetal size, because these SNPs are human. Genomic variants that determine the variations of fetal growth can only be searched for in humans. Reimplanting them through genetic manipulations in mice to demonstrate their precise physiology will be difficult, because the penetrance of their expression will have to go through the mice background (both genetic and environmental).

**FETAL GROWTH, A PHENOTYPE MADE OF SEVERAL PARTS**

Genetics studies the relationships of genes (or SNPs located outside genes) with a given phenotype. Fetal growth is not a simple homogeneous phenotype. The fetus is made of several components that are likely to have their own genetics. The quantitative trait, referred to as fetal size, measured at birth in g and cm, has to be dissected in several 'sub-traits' or components: body mass including all organs, fat and

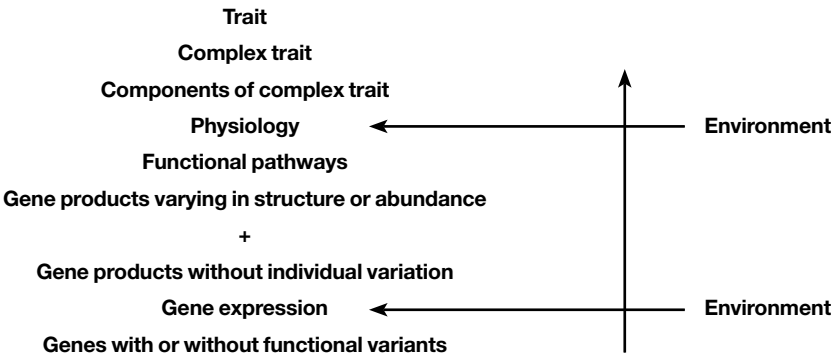


Fig. 1 - From genes to complex traits.

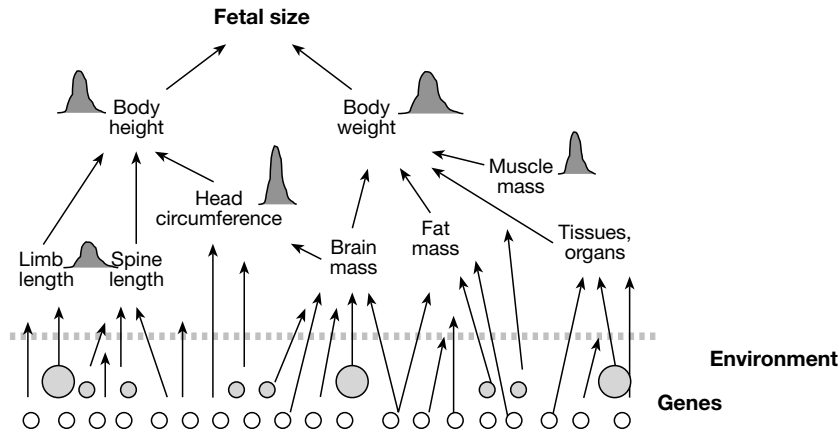


Fig. 2 - Fetal size dissected in its components. Genes that have no variant allele associated with variation of fetal size, but are nevertheless important to the determination of fetal size are in open circles. Genes that have functional variants influencing the variation of fetal size are shown as closed circles, with those having a marked influence on fetal size displayed as larger circles. Gaussian curves are shown to indicate that quantitative traits each have their own variation distribution.

muscle, brain mass, head circumference (a large part of body length), spine and limb skeletal growth. It is likely that different genetic factors and genomic variants are responsible for the variations of these 'sub-traits'. For example, gene polymorphisms that are important for the variation of skeletal growth in utero may have little to do with the storage of lipid calories, an important component (20%) of birth weight.

#### HOW MANY GENES ARE EXPECTED TO BE IMPORTANT FOR VARIATIONS IN FETAL SIZE?

Fetal growth is a multifactorial phenomenon that is dependent on the maternal environment and on a panoply of fetal genes. But only a fraction of these genes are subjected to variation, ie, those which carry functional SNPs interfering with growth related physiology. The heritability of birth weight and height is around 0.50, indicating a significant role of genetic factors (11). It is yet impossible to know how many genes are involved in the variation of these traits. For a long time, geneticists have thought that complex traits follow the 'infinitesimal model' which proposes that a large number of genes (hundreds), each playing a minuscule role ( $<1/100^{\text{th}}$  of the overall variance), are involved in trait variation. It seems more plausible today that even very complex traits have an oligogenic control involving a few 'major' variants (12), each of them accounting for 5-10% of the variance of the trait. There are no indications yet of the number of variant alleles determining the natural variations of fetal growth in any mammal.

#### A GENETIC APPROACH OF FETAL HEIGHT

In a recent study, our group obtained birth height measurements from hundreds of children born at term to Caucasian European women after un-

eventful gestations and deliveries. Birth height was considered as a quantitative parameter following a continuous distribution (Fig. 2). Based on classical genetic data and evaluation of heritability around 0.50, it was hypothesized that 'non-Mendelian' genetics were playing a role in the variation of human birth height.

There were two options to identify the genes and variants that play a role in the individual variation of birth height. The first one is to list genes known for their role in the physiology of human fetal growth (or mammalian fetal growth) and to find the known SNPs within or near these genes using data bases or sequencing of these regions. These SNPs could *a priori* be suspected to be functional genomic variants (Gf) important to growth of fetal skeleton or to be closely linked as markers (Gm) to the functional SNPs (Gf). Finally the statistical association of fetal size with these SNPs, or blocks of SNPs (haplotypes) can be tested.

A second option is to have no *a priori* hypothesis regarding genes responsible for the skeletal growth of human fetuses, and to engage in a 'blind' genome-wide approach. This requires the use of genomic markers (Gm), hundreds of microsatellites or tens or hundreds of thousands of anonymous SNPs scattered on the whole human genome. Hundreds of pairs of siblings are needed for this purpose. The phenotypic variation of fetal height can be modelled as the intra-pair difference between the birth heights of two siblings, then tested against the similarity of genomic variation at given polymorphic sites on the genome (microsatellites or SNPs) of the two siblings.

Our study group decided to follow the first scenario, which requires the study of 400 microsatellites spanning the whole genome. At each microsatellite site, the studied siblings share 0, 1 or 2 alleles identical by descent from their parents. A variance compo-

ment method can be used to study the relationship between the intra-pair sharing of microsatellite alleles and the observed intra-pair difference of birth height. Using this approach, we found that two chromosomal regions displayed a high probability of being associated with birth height variation. These regions can be considered as 'fetal height quantitative trait locus' (QTL). Because these regions are very large (3-10 Mb) and contain hundreds of potential candidate genes, a fine-mapping approach has to be used. This method uses a dense collection of genomic markers in the regions which we previously identified as QTL. Fine mapping will keep the candidate genes to a manageable number. These genes will then be tested. The ultimate aim is the identification of genomic variations [QTNs: quantitative trait nucleotides(QTN)] which are within or near these genes, and which are associated with variations of human height at birth.

## CONCLUSIONS

Scientific evidence generated from Mendelian genetic studies can explain to some extent the genetic origins of certain growth related disorders. It is documented that fetal growth restrictions can result from alterations in expression of imprinted genes and that the major cluster of such genes occur at chromosome 11p15. Non-Mendelian genetics, however, seems to be more capable of explaining phenotypic variability. Fetal growth is a complex multifactorial, multigenic trait. This is also suggested by the large number of chromosomal abnormalities which play a role in impaired fetal growth. Evolution has stabilized human birth size within relatively narrow limits in order to reduce perinatal mortality, but with a degree of individual variability. The quantitative trait of fetal size is comprised of several 'sub-traits' or components: body mass including all organs, fat and muscle, brain mass, head circumference, spine and limb skeletal growth. It is likely that different genetic factors and genomic variants are responsible for the variations of these 'sub-traits'. A study has been initiated by our group to test the hypothesis that

non-Mendelian genetics plays a role in the variation of human birth weight. The ultimate aim is the identification of genomic variations that are within or near certain selected candidate genes and are associated with variations of human height at birth.

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