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Epigenetic regulation and fetal programming

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Fetal programming encompasses the role of developmental plasticity in response to environmental and nutritional signals during early life and its potential adverse consequences (risk of cardiovascular, metabolic and behavioural diseases) in later life. The first studies in this field highlighted an association between poor fetal growth and chronic adult diseases. However, environmental signals during early life may lead to adverse long-term effects independently of obvious effects on fetal growth. Adverse long-term effects reflect a mismatch between early (fetal and neonatal) environmental conditions and the conditions that the individual will confront later in life. The mechanisms underlying this risk remain unclear. However, experimental data in rodents and recent observations in humans suggest that epigenetic changes in regulatory genes and growth-related genes play a significant role in fetal programming. Improvements in our understanding of the biochemical and molecular mechanisms at play in fetal programming would make it possible to identify biomarkers for detecting infants at high risk of adult-onset diseases. Such improvements should also lead to the development of preventive and therapeutic strategies.

Key words: fetal programming; developmental plasticity; metabolic programming; fetal environment; fetal growth; epigenetics; genomic imprinting.

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An association between early life events (environmental factors such as childhood living conditions and under-nutrition in particular) and cardiovascular disease later in life has been recognized on a number of occasions, the first as early as 1934 (reviewed by McMillen and Robinson and by Gluckman et al).[1,2]

In 1962, Neel proposed the 'thrifty genotype' hypothesis to explain the physiopathology of this association.[3] According to this hypothesis, 'thrifty' genes selected during hunter–gatherer periods, when food was scarce, would increase the capacity to store fat, placing individuals at high risk of insulin resistance in later periods when food became more abundant.

In 1992, Barker and Hales and colleagues highlighted the existence of associations between early growth patterns and chronic adult diseases.[4,5] They described associations between low birth weight and hypertension, ischaemic heart disease, glucose intolerance, insulin resistance, type-2 diabetes, hyperlipidaemia, hypercortisolaemia, obesity, obstructive pulmonary disease, and reproductive disorders in adults. They proposed the 'thrifty phenotype' hypothesis, according to which an adaptive change occurs when the fetal environment is deprived of nutrients, with optimization of the growth of key body organs (such as the brain) at the expense of other organs (such as β-cell islets). This adaptive change leads to altered postnatal metabolism, which is adapted to a poor nutritional environment in adult life, thereby enhancing survival in such an environment. These adaptations become detrimental only if nutrients are abundant in the postnatal environment.

With the 'mismatch' model of fetal programming, Gluckman and others have proposed that fetal programming is not a manifestation of pathophysiological processes. Instead, fetal programming involves adaptations made during fetal life to ensure adaptation to postnatal life, thereby maximizing subsequent health.[2,6–9] The fetus adjusts its development based on nutrient supply from the mother, in accordance with the expectation of being born into an environment that matches the one it had in utero. However, this adaptation may place the individual at risk of diseases later in life if there is a mismatch between the fetal and postnatal environments.[2,6–9] A given genotype may therefore give rise to different phenotypes depending on the environmental conditions.

This association was initially referred to as fetal programming, as these permanent adverse consequences in later life are induced by nutritional deprivation during an early critical period. The terms *developmental* or *metabolic plasticity* are now preferred.

A suboptimal fetal environment may be reflected in a low birth weight, indicative of an immediate adaptive response in the fetus to allow the fetus to survive until birth. Abnormal fetal growth is actually not constant, and the association between fetal programming and disease risk is continuous within the range of birth weights, with maximum adverse effects observed at extreme birth weights.

Many epidemiological observations have demonstrated that adaptive responses to the fetal environment can result in adverse effects later in life, but the mechanisms initiating these responses remain unclear. Recent data have strongly suggested that epigenetic processes play a key role in adaptive responses to nutritional and environmental factors during fetal and neonatal life. Epigenetic changes may involve both imprinted and non-imprinted genes.

FETAL PROGRAMMING AND POSTNATAL DISEASES: ENDOCRINE AND METABOLIC ASPECTS

There are several excellent reviews dealing with the epidemiological evidence for an association between early nutrition and the major risk factors for cardiovascular

diseases, insulin resistance, diabetes and metabolic syndrome in adult life, and addressing the critical windows during which perturbations have major effects.[1,2,10,11] In many instances, the disorders associated with suboptimal fetal growth are caused by changes in the development of key endocrine axes, with postnatal consequences for these axes, including the somatotrophic and hypothalamo–pituitary–adrenal axes and the endocrine pancreas.[1,2,12]

Growth

Maternal under-nutrition and/or impaired placental vascularization induce intrauterine growth retardation (with low birth weight and length) and early childhood growth hormone (GH) resistance, but the vast majority (90%) of infants born small for gestational age (SGA) display catch-up growth in the first few years of life.[1,13] Children with early catch-up have a higher body mass index, higher fat mass, insulin resistance, and higher systolic blood pressure during childhood and adolescence.[14–16] These findings raise questions about the management of early postnatal nutrition in SGA neonates.

Certain growth patterns suggest a specific timing of fetal injury. A low body mass index at birth may reflect a period of malnutrition during the third trimester of pregnancy. A small head circumference (more strongly associated with blood pressure than is birth weight) reflects slow growth throughout gestation, suggesting that the injury began early in fetal development. However, although low birth weight is positively associated with blood pressure, blood pressure is highest in individuals with low birth weight and high rates of early postnatal growth. The early postnatal period is therefore also a crucial time for the development of long-term consequences of fetal growth restriction. Tissues exposed to low concentrations of insulin and insulin-like growth factor 1 (IGF1) during fetal life may develop insulin resistance as a metabolic defence against hypoglycaemia when exposed to higher concentrations of these hormones during rapid postnatal growth.[1] These abnormalities in SGA infants therefore reflect the consequences of both in utero fetal programming and the metabolic and hormonal conditions during postnatal catch-up growth.

Hippocampus–hypothalamo–pituitary–adrenal axis

The hippocampus–hypothalamo–pituitary–adrenal (HHPA) axis is functional in utero and becomes more responsive during late gestation before birth. Suboptimal intrauterine conditions alter the development of the HHPA axis, with adverse consequences not only for neonatal survival but also for adult life. Many studies in animals and humans have shown that low birth weight and prenatal stress are associated with altered HHPA axis activity in later life, and have demonstrated that the administration of glucocorticoids at critical windows of development results in impaired renal development, hypertension, glucose intolerance and insulin resistance. Whatever the original injury to the fetus, it increases the exposure of the embryo to high glucocorticoid concentrations, either derived from the maternal circulation or produced following direct stimulation of the fetal HHPA axis. The adverse effects of glucocorticoid exposure are partly related to changes in the expression of the glucocorticoid receptor and the activity of the 11β-hydroxysteroid dehydrogenase (11βHSD) isoforms 1 and 2. In maternal protein restriction models, a decrease in placental 11βHSD2 activity (this enzyme converts active glucocorticoids into their inactive metabolites) increases the transplacental transfer of maternal glucocorticoids.[1,12,17] Maternal under-nutrition

also results in changes in hippocampus, pituitary and adrenal glucocorticoid receptor content, hypothalamic corticotrophin-releasing hormone and proopiomelanocortin content and adrenal adrenocorticotrophic hormone (ACTH) receptor content.[1,12] The specific changes in the fetal HHPA axis induced by suboptimal intrauterine conditions depend on the duration of these conditions, gestational age at onset (early, late, or throughout the prenatal period), the severity and specificity of the injury (maternal hypoxaemia, caloric or protein deprivation, maternal stress, maternal glucocorticoid treatment) and the species tested. These changes result in changes to fetal HHPA axis development, affecting the set point, the secretion and bioavailability in glucocorticoids, feedback regulation and pituitary and adrenal sensitivity to acute stressors. HHPA axis activity is therefore enhanced in postnatal life, with high basal cortisol levels and an increased adrenal response to ACTH in adults.[1,12,18,19]

Prenatal glucocorticoid exposure also has adverse renal effects. Maternal undernutrition, uteroplacental insufficiency or glucocorticoid exposure result in a decrease in the number of nephrons, followed by a compensatory increase in single-nephron glomerular filtration rate, focal glomerulosclerosis and hypertension.[1] The up-regulation of sodium transporters may also contribute to sodium retention, mediating the programming of hypertension. All these effects during a critical window of active nephrogenesis lead to later changes in postnatal renal function and an increase in blood pressure.[1]

Endocrine pancreas, insulin resistance and type-2 diabetes

Follow-up of the offspring of mothers exposed to famine in the Netherlands (Dutch winter famine of 1944–1945) has shown strongly impaired glucose tolerance in those exposed to the famine during gestation.[11] For both under-nutrition and uteroplacental insufficiency, fetal growth and birth weight are related to subsequently impaired glucose tolerance, insulin resistance, and type-2 diabetes. This suggests that adverse intrauterine conditions decreasing birth weight also impair development of the fetal endocrine pancreas.[1,10] The critical window for the intrauterine programming of glucose intolerance occurs in late gestation.[1] Nutritional disturbances may have long-term consequences for pancreatic structure and function, altering the process of β-cell development (decrease in proliferation and increase in apoptosis) and inducing an irreversible decrease in fetal β-cell mass. A decrease in β-cell number might affect the expression of pancreatic growth factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), IGF1 and IGF2.[20] The pancreatic content in IGF2, an antiapoptotic factor, is reduced in fetal and neonatal rats exposed to a low-protein diet during pregnancy, possibly due to a deficiency of particular amino acids such as taurine.[1,21,22] Moreover, fetal glucocorticoid exposure (a hallmark of fetal programming) suppresses IGF2 expression.[20]

However, a stronger insulin response to glucose administration has been observed in some animal models of low birth weight, suggesting that the intolerance in adulthood may be due to changes in the insulin sensitivity of peripheral tissues, such as liver, muscle, and adipocytes, rather than to a decrease in insulin secretion.[1,23] The activity of one glucose-utilizing enzyme (glucokinase) is decreased and that of a glucose-producing enzyme (PEPCK) is increased in liver.[1] An increase in hepatic fatty acid synthesis and a decrease in fatty oxidation are also observed, resulting in an increase in the supply of hepatic triglycerides, which may decrease skeletal muscle insulin sensitivity and spare glucose for the growth of keys organs such as the brain.[1] Fetal nutrient restriction leads to an irreversible decrease in the number of fibres in fetal muscle, resulting

in decreases in glucose uptake and glycogen synthesis and content in muscle. The impaired action of insulin is not associated with abnormal expression of either the insulin receptor or of the Glut4 glucose transporter. Instead, it probably results from an impairment downstream from the insulin receptor.[1] Skeletal muscle triglyceride content is also increased. The modifications observed in muscle may be beneficial, increasing mitochondrial lipid oxidation in skeletal muscle and subsequently resulting in glucose sparing to maintain brain growth.

INITIATING MECHANISMS

It is clear that environmental and nutritional factors during early life are determinants for postnatal and adult health, but the mechanisms by which fetal programming by environmental factors is initiated in early life remain unclear.

Genetic polymorphisms and fetal programming

The association between size at birth and risk of disease during adulthood has underpinned the genetic studies of size at birth and led to the hypothesis that determinants of size at birth may also be determinants for risk of disease in adulthood. Genetic background, including specific polymorphisms, probably plays a role in determining sensitivity to environmental and nutritional signals.

The role of the IGF system has been clearly demonstrated in fetal and placental growth,[24] and there is evidence that polymorphisms affecting genes of the IGF system (IGFI, IGFII, IGF2R) also affect birth size and postnatal height. A recent study[25] showed an association between *IGF2* polymorphisms and adult height but, surprisingly, no association with birth weight was found. The association between height and *IGF2* variants is reminiscent of the findings of a study of patients displaying Beckwith–Wiedemann syndrome (characterized by fetal and postnatal overgrowth), which demonstrated an association between some *IGF2* variants and Beckwith–Wiedemann syndrome.[26] Variations in the *IGF2R* gene (encoding the IGF2 receptor, negatively regulating IGF2 bioavailability) have also been associated with postnatal height.[27] The association between *IGF2* genetic variation and obesity has been investigated, but conflicting data have been published concerning the association between *IGF2* variants and body mass index in adults.[25,28,29] Finally, a common variant of the *H19* gene (an imprinted gene with a pattern of expression opposite to that of the *IGF2* gene) has been shown to be associated with larger birth size and higher cord blood IGF2 levels.[30] Concentrations of IGF1 in the fetus are affected by nutrient supply to the fetus and nutrient-sensitive hormones, including insulin. Nutrient restriction, particularly in late gestation, decreases IGF1 levels.[31] Fetal and cord serum IGF1 concentrations are correlated with birth weight[31], and IGF1 concentrations have been shown to be low in utero and at birth in SGA fetuses.[31] Polymorphisms of the *IGF1* gene may therefore determine sensitivity to nutritional signals. Polymorphisms of the *IGF1* gene, including a common *IGF1* promoter CA repeat, have been reported to be associated with size at birth[32–34], although this association has not been confirmed in other large population studies.[35]

There is also some evidence that genetic factors are determinants for both birth weight and type-2 diabetes. Monogenetic disorders affecting fetal insulin secretion or insulin resistance also affect fetal growth.[36,37] For example, heterozygous mutations in the glucokinase gene result in reduced fetal insulin secretion, low birth weight, and glucose intolerance in adulthood.[38] A polymorphism of the PPARγ2 gene is associated

with small size at birth and insulin resistance.[39] Discordant data have been reported regarding polymorphisms of the variable-number tandem repeat (VNTR) of the promoter of the insulin gene and birth size.[40] The insulin gene maps to the 11p15 region, which is rich in imprinted genes (such as *IGF2* and *H19*) affecting fetal growth. Associations between insulin VNTR polymorphisms and birth weight may therefore be confounded by linkage disequilibrium with other variants within the same chromosomal region. Some variants of the insulin VNTR locus are also associated with an increase in insulin secretion in the early phase of obesity and confer a predisposition to more rapid weight gain in late childhood.[41,42] The risk of early-onset obesity depends on paternal transmission of the polymorphism,[43] suggesting that genomic imprinting may be involved.

In conclusion, genetic variants probably affect growth and metabolism, but their relative roles in the modulation of adaptive responses to environmental factors and adverse consequences in adulthood remain to be determined.

Epigenetic regulation and fetal programming

Interest is increasing in the mechanisms underlying the effects of nutritional and environmental factors, together with aging, on the epigenetic regulation of genes, including imprinted genes.[44–47] Particular attention has recently focused on the role of epigenetic processes in fetal programming, with its potential consequences for susceptibility to chronic diseases in adulthood.[48,49]

Epigenetic regulation of gene expression

Epigenetics relates to stable and heritable patterns of gene expression that do not involve changes in DNA sequence.[46,50] Epigenetic mechanisms play a key role in regulating gene expression and are required to achieve the stable repression or expression of genes at defined developmental stages. One of the best-studied epigenetic marks is DNA methylation at cytosine residues of CpG dinucleotides in gene promoters, transposons, and imprinting control regions. In most cases, DNA methylation is associated with gene repression.[51] Another key aspect of chromatin organization is histone modifications, such as methylation and acetylation, affecting the N-terminal tail of histones. These two types of epigenetic modification are mechanistically linked[46] and regulate chromatin conformation, thereby also regulating the transcriptional activity or silencing of specific genomic regions. The influence of small regulatory RNA molecules is another emerging field of epigenetic gene regulation.[52,53] These epigenetic modifications constitute the link between genotype and phenotype and are referred to as the epigenotype. Epigenetic patterns are maintained throughout each cell cycle by many factors, including DNA methyltransferases, methyl-CpG binding proteins and insulator proteins (such as CTCF) and histone-modifying enzymes, making it possible to recruit chromatin-remodelling complexes to the DNA (Figure 1).[52,54]

Genomic imprinting is one of the best-understood examples of epigenetic regulation. In the same cell, one of the two parental alleles is stably repressed by epigenetic modifications, whereas the other allele is maintained in an active state. This allele-specific regulation is entirely dependent on whether the gene is inherited from the mother or from the father. Pre-existing imprint marks, such as DNA methylation, are erased in the primordial germ cells and re-established during spermatogenesis or oogenesis, depending on sex.[51,55] Many imprinted genes play key roles in fetal and placental growth and behaviour. In general, paternally expressed genes enhance growth whereas maternally expressed genes restrain growth. The health

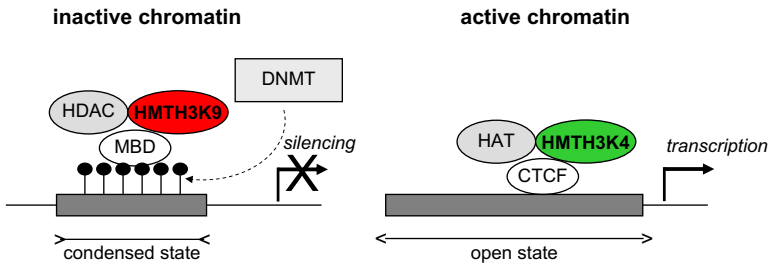

Figure 1. Epigenetic regulation of gene expression. In transcriptionally inactive regions of DNA, the CpG island recruits DNA methyltransferases (DNMT), methyl-CpG-binding proteins (MBD), histone methyltransferases (HMT) and histone deacetylases (HDAC), causing chromatin condensation and blocking transcriptional initiation. DNMTs promote DNA methylation (black lollipops). HMTs generate repressive histone methylation marks, such as lysine-9 and lysine-27 methylation on histone H3. In transcriptionally active regions of DNA, the CpG island recruits histone acetyltransferases (HAT) and HMT generating permissive histone methylation marks (such as lysine-4 methylation on histone H3). This recruitment, together with the binding of insulator proteins (such as CTCF), results in chromatin decondensation and transcriptional activity. At imprinted loci, one parental allele carries repressive epigenetic marks and the other has epigenetic modifications favouring transcription.

consequences of genomic imprinting are potentially severe: imprinted genes are functionally haploid and, thus, the phenomenon of genomic imprinting eliminates the protection that diploidy provides. Indeed, imprinted genes play a disproportionately important role in human 'epigenetic' disorders, including fetal growth disorders (Table 1).

Imprinted genes are also important in determining the growth and transfer capacity of the placenta and controlling the nutrient supply for fetal growth. Size at birth depends primarily on the nutrient supply, which in turn depends on a functional placenta. Factors governing placental development are, therefore, determinants in fetal growth retardation and its adverse outcomes. The imprinted *IGF2* gene is particularly crucial for placental growth and nutrient transfer. In mouse placenta, the *Igf2* gene

Table 1. Human disorders caused by imprinting effects.

Disorder	Growth phenotype	Parental origin	Chromosome
Silver–Russell syndrome	IUGR	Maternal	7p11.2–13, 7q32, 11p15
Beckwith–Wiedemann syndrome	Overgrowth	Paternal	11p15
Angelman syndrome		Paternal	15q11–13
Prader–Willi syndrome		Maternal	15q11–13
Transient neonatal diabetes mellitus	IUGR	Paternal	6q24
AHO, McCune Albright, PHP1B		Maternal/paternal	20q13
Familial paraganglioma		Paternal	11q13
Maternal UPD14 syndrome	IUGR	Maternal	14
Paternal UPD14 syndrome	IUGR	Paternal	14
Maternal UPD16 syndrome	IUGR	Maternal	16

AHO, Albright hereditary osteodystrophy; UPD, uniparental disomy; IUGR, intrauterine growth retardation; PHP1B, pseudohypoparathyroidism type 1b.

is expressed under control of a trophoblast-specific promoter (P0) that has been shown to be essential for the promotion of early placental growth. The *Igf2* P0 null mouse has low placental IGF2 levels but normal fetal circulating IGF2 levels. However, *Igf2* P0 null placentas are efficient, despite their small size, and can support normal fetal growth until close to term by up-regulating the transfer of amino acids and glucose.[56,57] Studies in the *Igf2* P0 knockout model, and in several other imprinted gene knockout models (*Ipl*, *H19*, *Igf2r*, *Slc38a4*) have suggested that fetal growth demand is a major determinant of placental nutrient supply and that the placenta can adjust its transfer to meet fetal demands.[58,59] There are no data associating fetal growth phenotypes with epigenetic alterations arising specifically in the placenta. However, the abnormal epigenetic regulation of genes critical for placental function is likely to cause abnormal fetal growth. Moreover, the programming effects of environmental and nutritional signals probably involve the dysregulation of imprinted genes (including the *IGF2* gene) in the placenta.

In primordial germ cells, the genome undergoes extensive demethylation, including the removal of previous parent-specific methylation marks regulating imprinted gene expression. New imprints occur during gametogenesis, in a parent-of-origin-specific manner. Within a few days of fertilization, genome-wide demethylation occurs followed by a wave of de novo methylation, both of which are resisted by imprinted loci.[60] There are therefore two critical time periods in epigenetic reprogramming: gametogenesis and early preimplantation development. DNA methylation patterns must then be maintained during the phase of rapid cellular proliferation in fetal and postnatal development. Epigenetic patterns are usually faithfully maintained during development. However, this maintenance sometimes fails, resulting in the disturbance of epigenetic patterns and human disorders. An example of the non-maintenance of epigenetic patterns is illustrated in Figure 2 for the human 11p15 imprinted region. Two fetal growth disorders with opposite phenotypes are caused by abnormal DNA methylation at a single imprinted locus.[61] Silver–Russell syndrome is characterized by intrauterine and postnatal growth retardation and is caused by a loss of DNA methylation at one of the two 11p15 imprinting control regions. This loss of methylation results in silencing of the *IGF2* gene, a key regulator of fetal growth. A gain of DNA methylation at this locus results in the up-regulation of the *IGF2* gene and Beckwith–Wiedemann syndrome, characterized by fetal overgrowth.[62]

Epigenetic regulation and environment

Monozygotic twins provide an interesting model for studies of the role of epigenetic modifications in phenotype establishment.[63] A large epigenetic study on monozygotic twins[64] recently showed that twins are epigenetically concordant at birth in most cases, and that epigenetic differences (DNA methylation and histone modifications) accumulate with age in monozygotic twins. Remarkably, the twins displaying the greatest epigenetic differences were found to be those who had lived together for the smallest amount of time. This finding indicates that the relative importance of environmental factors with respect to intrinsic factors increases during life. However, epigenetic changes that cannot be explained by environment also occur during development and are thought to be stochastic.[65] Imprinting disorders (Silver–Russell syndrome, Beckwith–Wiedemann syndrome and transient neonatal diabetes mellitus)[61,62,66,67] provide another model of phenotypic discordance caused by epigenetic differences in monozygotic twins. In these conditions, monozygotic twins are always discordant and the affected twin displays a loss of DNA methylation at imprinting control regions.

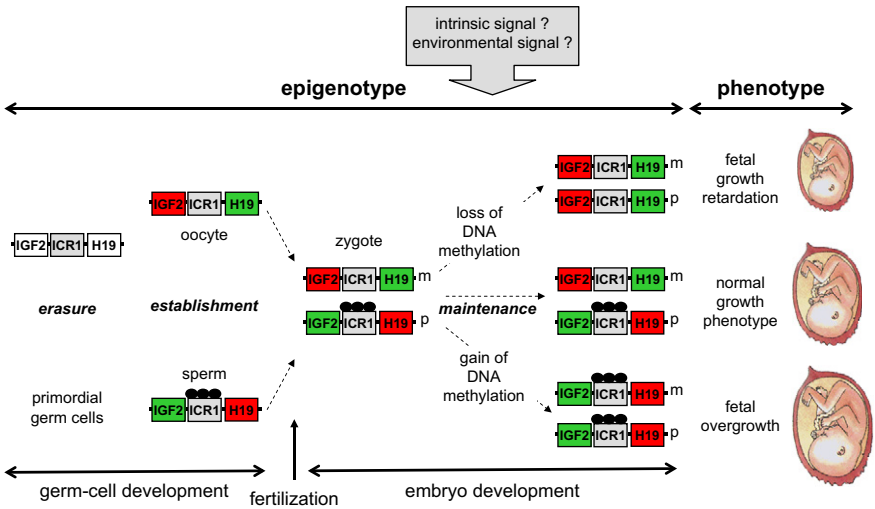

Figure 2. Two fetal growth disorders are caused by opposite epigenetic changes in the 11p15 *H19*/*IGF2* imprinted domain. The methylation marks of the 11p15 telomeric imprinting control region (ICR1) are re-established in male germ cells (black circles) after erasure in primordial germ cells. These methylation marks result in the silencing of *H19* and the expression of IGF2. Methylation at ICR1 is prevented in the female germ line, resulting in the silencing of *IGF2* and the expression of *H19*. After fertilization, the allelic DNA methylation pattern is maintained throughout development. Occasionally, a failure to maintain allelic DNA methylation marks occurs early in development (intrinsic or environmental factor). A loss of DNA methylation marks on the paternal allele results in the biallelic expression of *H19*, the silencing of *IGF2* and fetal growth retardation (Silver–Russell syndrome). Conversely, a gain of aberrant DNA methylation on the maternal allele results in the silencing of *H19*, the biallelic expression of *IGF2* and fetal overgrowth (Beckwith–Wiedemann syndrome). Silent and active genes are shown in red and green, respectively. m, maternal allele; p, paternal allele.

The epigenetic defect is thought to arise from uneven splitting of the inner cell mass and an unequal distribution of DNA methyltransferases during the twinning event.

Early embryogenesis is a critical time for epigenetic regulation, and this process is sensitive to environmental factors. Many studies have explored the epigenetic effects in embryo culture. Imprinted genes seem to be particularly sensitive to culture in vitro.[68] The composition of the culture medium and the addition of serum affect methylation and the allelic expression of imprinted genes, including the *IGF2* and *H19* genes, with consequences for fetal growth.[69–71] In-vitro culture also affects fetal growth in cattle and results in large offspring syndrome which is caused by a loss of methylation of the imprinted *IGF2R* gene.[72] In humans, the use of assisted reproductive technology (ART) has been shown to induce epigenetic alterations and to affect fetal growth and development. Several imprinting disorders, including the Beckwith–Wiedemann overgrowth disorder, occur at significantly higher frequencies in children conceived with the use of ART than in children conceived spontaneously.[73,74] The cause of these epigenetic imprinting disorders following ART remains unclear. The risk may be accounted for by subfertility of the treated couples, although various causes of infertility have been reported. Alternatively, it may be due to culture in vitro itself, although no specific aspect of the ART procedure (such as the use of a specific culture medium, the timing of embryo transfer, or the use of intracytoplasmic sperm injection)

has been found to be associated with a risk of imprinting disorders. As the first few days of embryonic development constitute a critical period of the imprinting cycle, a stochastic event is likely. Protection against demethylation may be more likely to fail after ART, resulting in an irreparable loss of imprints in one cell. The factors regulating the maintenance of allele-specific DNA methylation at imprinting control regions are largely unidentified. However, recent data have shown that in patients with Beckwith–Wiedemann syndrome, including those born following the use of ART, the DNA methylation defect involves imprinted loci other than 11p15.[75] This suggests that unfaithful maintenance of DNA methylation marks following fertilization involves the dysregulation of a *trans*-acting regulatory factor.

Epigenetic regulation and nutrition

Several studies have examined the impact of nutrition on the epigenetic regulation of both imprinted and non-imprinted genes. Most of these studies have analysed modifications of the DNA methylation pattern, the easiest epigenetic mark to study. Early nutrition can influence DNA methylation because one-carbon metabolism is dependent on dietary methyl donors and cofactors, including methionine, choline, folic acid and vitamin B12.[76] The availability of dietary methyl donors and cofactors is therefore critical during ontogenic periods. Aberrant changes in the DNA methylation of genes important for fetal programming, such as the glucocorticoid receptor gene or the *IGF2* gene, are caused by protein restriction diet, and some of these changes can be prevented by dietary supplementation with cofactors.

Protein restriction in pregnant rats induces persistent loss of DNA methylation and greater expression of some hepatic genes (glucocorticoid receptor and *PPAR*α) in the offspring.[77] The epigenetic change, caused by a decrease in DNMT1 activity,[78] can be prevented by folate supplementation.[77] Although not directly regulated by nutrition, maternal behaviour also programs the epigenetic regulation (DNA methylation and histone acetylation) of the glucocorticoid receptor gene in the hippocampus and determines the stress responses of the offspring.[79,80] Moreover, stress responses are modified by the administration of histone deacetylase inhibitor.[79] Uteroplacental insufficiency in rats increases renal apoptosis through a loss of methylation of the *p53* promoter, resulting in an increase in *p53* gene expression.[81] This increase may play a role in nephronic reduction. Changes in the intrauterine environment associated with uteroplacental insufficiency also affect DNA methylation and histone acetylation in the liver, due to changes in one-carbon metabolism.[76]

Another model for the nutritional control of epigenetic modifications is the yellow Agouti (A^{vy}) mouse. The Agouti gene encodes a molecule involved in the production of a yellow pigment in melanocytes. The A^{vy} allele results from the insertion of a transposon upstream from the Agouti gene which promotes constitutive ectopic Agouti expression, leading to a yellow coat and adult obesity. The degree of methylation of the transposon is inversely correlated with ectopic Agouti expression. It has been elegantly shown that maternal methyl supplementation with cofactors (such as vitamin B12 or folates)[82] or other nutritional factors (such as phyto-oestrogen)[83] increases the methylation of the transposon, resulting in a brown coat colour and protection against obesity. Transposon sequences account for about 40% of the human genome, but it remains unknown whether a mechanism like that at work in the Agouti model affects human disease susceptibility.

As discussed above, the environment of the early embryo can affect establishment and/or maintenance of the epigenetic regulation of imprinted genes. The lability of

imprinted genes is not limited to the early embryonic period. It was recently demonstrated that a methyl-donor-deficient diet in postnatal life permanently affects the expression of *IGF2*, resulting in growth retardation.[84] This suggests that the effects of nutrition are not limited to the fetal stage and that nutrition during postnatal development can permanently alter the epigenetic regulation of imprinted genes. In humans, diet has been shown to affect the DNA methylation status of patients with hyperhomocysteinaemia. This disease is characterized by the accumulation of S-adenosylhomocysteine (an inhibitor of DNA methyltransferases). This leads to lower levels of DNA methylation and a shift from mono- to bi-allelic expression for some imprinted genes (including *H19*). Folate supplementation normalizes DNA methylation levels and restores mono-allelic expression of the *H19* gene.[85]

Intergenerational transmission of epigenetic patterns and disease risk

Several animal studies, most of them involving nutritional or hormonal manipulation, have demonstrated intergenerational effects of fetal programming.[86,87] For example, dietary manipulations (protein restriction) of birth weight in rats have shown that continued poor maternal nutrition produces amplified effects on birth weight through a number of generations.[88] These effects persist for approximately three generations, despite a return to normal nutrition.[88] Fetal exposure to excessive glucocorticoid concentrations results in low birth weight and subsequent adult hyperinsulinaemia and hyperglycaemia due to an increase in hepatic phosphoenolpyruvate carboxykinase activity, with these adverse effects resolving only in the third generation.[89] The effects of fetal programming may therefore not be limited to the first generation; some environmental effects appear to be passed on through subsequent generations.

Such intergenerational inheritance may be mediated at least in part by epigenetic mechanisms involving the regulation of imprinted and non-imprinted genes.[86,87,90] The DNA methylation pattern is normally cleared on passage through the germ line and reset some time after fertilization. For transgenerational epigenetic inheritance to occur, epigenetic marks must occasionally be inefficiently erased at one of the two critical periods (gametogenesis or early preimplantation development).[90] The Agouti mouse provides a model for transgenerational epigenetic inheritance. The inheritance of coat colour in the offspring depends on the coat colour of the mother, but not that of the father.[91] This inheritance does not depend on the maternal environment but instead results from the transmission of an epigenetic mark on the A^{vy} allele in the female germ line. Resistance to reprogramming has been described at transposon sequences, at which the erasure of epigenetic marks such as DNA methylation is not always complete after fertilization.[92] Another model of intergenerational transmission is provided by pregnant rats treated with an endocrine disruptor (anti-androgenic or oestrogenic compound) during the period of testis development in the offspring. Maternal treatment results in male fertility defects, not only in the first generation but also in the next three generations, despite the absence of in utero exposure to the endocrine disruptor.[93] These fertility defects are correlated with changes in DNA methylation in the testis.

The concept of transgenerational epigenetic transmission in humans remains controversial. However, a few human disorders, such as the Prader–Willi imprinting syndrome or exposure to diethylstilboestrol (with transmission through the maternal line to the third generation), suggest that epigenetic inheritance may play a role in human disorders.[87,90,94]

CONCLUSION

The human fetus responds to maternal under-nutrition, placental dysfunction, maternal stress, and other environmental influences by changing its physiological development and slowing its growth. These adaptive processes result in a higher risk of chronic adult diseases. At the molecular level, this is reflected in transcriptional changes in metabolic and growth pathways. There is some evidence that some of these changes are achieved by alteration of the epigenetic regulation of genes. Transposons and the regulatory regions of imprinted genes seem to be particularly sensitive to environmental and nutritional signals.[44] Moreover, epidemiological studies have also suggested that fetal programming effects may not be limited to the first generation, and that epigenetic mechanisms may account, at least in part, for transgenerational non-genomic inheritance.

Many important questions remain unresolved. Other endocrine axes, such as the gonadotrophic reproductive axis, might be vulnerable to exposure to androgens and oestrogens at specific times, with potential adverse effects on fertility later in life. The physiological mechanisms of early postnatal catch-up growth in SGA patients are poorly understood. As postnatal catch-up may worsen the metabolic dysfunction and increase the risk of type-2 diabetes in later life, it is important to design re-nutrition programs so as to avoid subsequent deleterious effects. The administration of synthetic glucocorticoids to women at risk of preterm delivery entails a risk of disease in later life for the offspring. Moreover, SGA infants may have weak stress responses before and immediately after delivery. There is a need to optimize glucocorticoid treatment before delivery and to provide support for SGA children in the immediate postnatal period.

A better understanding of patterns of human plasticity in response to early nutrition and other environmental factors should also make it possible to identify markers for recognizing, early in postnatal life, children at risk of adverse effects. Such markers will be particularly useful for children displaying no fetal growth retardation. Improvements in our understanding should also help to guide therapeutic interventions for promoting health during childhood (such as adjusting early-life nutritional interventions and promoting physical exercise) without compromising it later in life. Moreover, recent animal studies suggest that fetal programming can be reversed.[95] Such information might be applicable to humans, based on the need for a match between the fetal and postnatal environment.

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