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Could Epigenetics Play a Role in the Developmental Origins of Health and Disease?

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ABSTRACT: Following Barker's observations of an association between birth size and later adult diseases, considerable efforts have been made to define the characteristics of low birth weight groups in childhood. In this review, the phenotypic and biochemical characteristics during childhood of three low birth weight groups are summarized: children born following inviter fertilization (IVF), small for gestational age (SGA), or very premature. Each of these groups is likely to have been exposed to an adverse environment at different developmental stages. The triggers and mechanisms leading to programmed changes in growth, development, and metabolism of these groups of children have yet to be identified. Epigenetics has been proposed as a potential mechanism for these programmed changes through environmentally induced changes in gene expression. Data from animal models in which environmental, particularly nutritional, manipulation leads to changes in DNA methylation are presented. The relevance of these animal studies to IVF, SGA, and very premature children are discussed as are potential candidate genes that may have undergone epigenetic modification to alter growth and metabolism. (***Pediatr Res*** **61: 68R–75R, 2007**)

Over the past 30 y, pediatricians have focused on cognitive function, growth failure, and short stature as the principal health care issues facing children born SGA. In the early 1990s, Barker proposed the fetal origins hypothesis of adult disease, a simple but fundamental hypothesis in which the origins of diseases in adults begin *in utero* (1). Barker and colleagues' observations extended to the range of diseases associated with low birth weight: atherosclerosis, coronary heart disease, type 2 diabetes mellitus, syndrome X, stroke, and chronic bronchitis (2–4). These observations have been corroborated by other epidemiologic studies, including those performed in Europe and the United States (5–7).

The interest in this field has grown rapidly over the past decade. There have been in excess of 1000 publications in the field of the developmental origins of adult disease and an incorporated international society was established that exclusively focuses on this field of research—the Society for the Developmental Origins of Health and Disease. However, the most critical questions in this field remain unanswered. Firstly, which of the children who have biochemical markers of metabolic disease will go on and develop overt metabolic disease in adult life? Secondly, what are the initiating events that trigger persistent metabolic programming. Thirdly, what are the mechanisms that lead to adverse programmed metabolic changes? It is this latter question that is the focus of this review.

The low birth weight group includes those born SGA, premature, or following IVF, which is often associated with both SGA and prematurity. These three common childhood groups are likely to have been exposed to an adverse environment during different phases of early development: periconception for IVF, the last trimester of pregnancy for SGA, and the neonatal period for those born prematurely. This review will first characterize the auxological and metabolic differences of these three groups compared with normal children, then describe the epigenetic mechanisms by which these characteristics may have occurred, and finally outline gene candidates potentially involved in these phenotypic alterations. Although there is currently no published evidence linking epigenetic modification to programmed change in growth and metabolism in humans, the data from animal models and their potential relevance to these three groups will be discussed.

CHILDHOOD CLINICAL CHARACTERISTICS

SGA children. SGA children have an increased risk of short stature. Although approximately 80% of SGA children achieve a height within the normal range by 6 mo of age (8,9), short adult stature occurs in 5.2% of those with low birth weight and 7.1% of those with low birth length (8,10). As a group, SGA children fall about 4 cm short of their genetic height potential (11). Conflicting reports of abnormalities in the growth hormone–IGF-I axis of short SGA children have been published. Although reduced spontaneous growth hormone secretion has been found, this effect is likely to be minimal if corrected for age (12). Low serum IGF-I and IGFBP-3 levels have been observed but when SGA children are matched for height and body mass, these values are slightly elevated and correlated with fasting insulin levels (13). Diminished IGF-I response to growth hormone (14) suggests a partial defect in the GH receptor or postreceptor

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Abbreviations: AS, Angelman's syndrome; **BWS,** Beckwith-Wiedemann syndrome; **CpG,** cytosine phosphate guanine; **DMR,** differentially methylated region; **IVF,** *in vitro* fertilization; **PCOS,** polycystic ovarian syndrome; **SGA,** small for gestational age

pathways. Collectively, these changes are likely to be subtle and do not adequately explain the poor growth many of these children suffer during childhood.

The endocrine and metabolic changes with SGA include reduced insulin sensitivity, lipid alterations, premature adrenarche, and polycystic ovarian syndrome. Almost all other metabolic abnormalities can be attributed to reduced insulin sensitivity and the development of insulin resistance with increasing age and obesity. The long-term consequences of insulin resistance include type 2 diabetes mellitus, coronary heart disease, cerebrovascular accident, and cancer (15). Reduced insulin sensitivity (*i.e.* insulin resistance) is present in mid-childhood and has been indirectly demonstrated in late infancy in SGA children (16–18). These observations add support to the notion that events in fetal life have led to programmed insulin resistance. Inconsistency in the site(s) of insulin resistance in SGA have been reported but they appear to include both peripheral and hepatic insulin resistance (19–22).

The reduction in insulin sensitivity is magnified by fat mass accumulation, and several studies have demonstrated that greater catch-up growth is associated with disease attributable to insulin resistance. In lower birth weight individuals, early childhood acceleration in weight gain that is sustained throughout adolescence characterizes those that go on to develop type 2 diabetes mellitus (23). A similar pattern was observed with reduced birth weight in adults who developed coronary heart disease (24). Interestingly, by 4 y of age, SGA children already have a noticeable reduction in muscle mass with a smaller increase in fat mass compared with normal birth weight children (25). Even when adjusting for fat mass and sex, SGA children have reduced leptin levels (26).

Although fasting lipids are higher in SGA children, they remain in the normal range (25,27–29). It is not until early adult life that overt dyslipidemia is evident with the metabolic syndrome seen in 2.3% of SGA compared with 0.4% of normal birth weight young adults (30).

Most, but not all studies have found that premature pubarche occurred more frequently in SGA girls characterized by elevated dehydroepiandrosterone levels and a higher risk of both ovarian hyperandrogenism and polycystic ovarian syndrome (PCOS) (31–34). As insulin resistance is a recognized association with both SGA and PCOS it is not surprising the two have been linked. Indeed insulin resistance is believed to be a prime early factor in the pathogenesis of PCOS (35).

Prematurely born children. Conceptually, there are similarities between SGA and premature children. SGA infants suffer from an adverse fetal environment during the last trimester of pregnancy whereas very premature infants (defined as birth weight $\leq$1500 g) suffer from an adverse neonatal environment in a neonatal intensive care unit during the first 3 mo of life, a time biologically equivalent to the third trimester. Therefore it is not surprising that there are similarities in the linear growth, body composition and metabolic changes during childhood observed in term SGA and very prematurely born children. Approximately 80% of children in both groups exhibit acceleration in growth to achieve a normal height by 6 mo of age. Those born very premature are approximately 0.7 SD shorter than their parents right across the height range, indicating that prematurely born children are short for their genetic height potential (36,37). Final height data from recent studies reveal conflicting results that collectively indicate that very premature children reach a height that falls short of genetic height potential by 6–8 cm (0.5–0.7 SD shorter) (38–40). Remarkably few studies have attempted to define which prenatal or neonatal events have a long-term influence on growth in those born prematurely. Very premature infants who received early neonatal dexamethasone therapy to prevent chronic lung disease were shorter than untreated children at 7–10 y of age (41). However, the mechanism in which neonatal dexamethasone therapy led to long-term poor growth is unclear.

During childhood, children born very premature exhibited low plasma IGF-I and IGFBP-3 levels compared with height and weight matched control children (42). This is in contrast with term SGA children who demonstrated elevated IGF-I levels (13). Both SGA and premature groups have unexplained elevated plasma IGF-II values (36,43). Serum IGF-II levels have been shown to be associated with fat mass in normal children, with higher levels seen in obese children (44,45). It has been proposed that the elevated plasma IGF-II levels observed in premature children play a role in the development of later obesity during adult life (44).

While detailed metabolic changes have yet to be fully characterized in very premature children, reduced insulin sensitivity has been demonstrated and is of a similar magnitude to term SGA children (46). Low protein intake in the first 3 mo of life occurred in the premature group, which was proposed as the trigger to epigenetic modification of genes involved in glucose regulation (47). Other factors such as prenatal or neonatal glucocorticoid exposure and illness in the neonatal period were not associated with insulin sensitivity (46).

The paucity of childhood or adolescent body composition data of those born very prematurely suggest similarities with SGA children and adolescents with increased fat mass, particularly abdominal fat by 19 y of age (48,49). However, similar to SGA cohorts, more rapid catch-up growth was associated with greater reduction in insulin sensitivity (47). Consistent with reduced insulin sensitivity, limited data also suggests that those born prematurely are at increased of premature pubarche accounting for 24% of cases presenting for specialist evaluation (33).

IVF children. Low birth weight occurs more commonly in singleton IVF infants (50,51). There is a 2.6-fold increased risk of low birth weight in term IVF infants, with a greater risk of prematurity such that 0.4% of all very low birth weight infants are conceived by IVF. There are limited conflicting data regarding the auxological and hormonal characteristics of children born following IVF. Kai *et al.* (52) found that IVF children were the same height with the same serum IGF-I levels as control children. However, the study was limited by a low participation rate, with over half the IVF children being premature, SGA, or twin, which are all conditions that constrain growth. We have found that IVF children are approximately 4 cm taller (0.5 SD) when corrected for parents' heights compared with normally conceived children (submitted for publication). IVF children had higher IGF-I to IGFBP3

ratios, higher plasma IGF-II and more favorable lipid profile than matched control children. Further studies are needed to determine whether differences in IVF practice lead to differences in phenotype or biochemical indices.

EPIGENETICS CONCEPTS

The lack of a clear mechanism that leads to sustained programmed changes in growth and metabolism following an adverse fetal or early neonatal environment led Waterland and Garza (53) to coin the term "metabolic imprinting" to focus research in the field on identifying underlying mechanisms that include nutritional regulation of gene expression. It has long been known that the phenotype of an individual is not exclusively determined by genotype. Waddington (54) introduced the term epigenetics in the late 1950s that today is interpreted as changes in gene function that occur without a changes in gene sequence. Inherent in this concept is that conformational change in chromatin can repress transcription activation of gene expression. Chromatin conformation is closely linked to methylation, which occurs on cytosine residues at CpG dinucleotides. Although about 80% of CpG in the genome are methylated, there are "islands" of CpG, usually within gene promoters, that remain unmethylated. However, during development some of these CpG islands can become methylated with consequent silencing of the associated gene (55–57). The state of CpG methylation probably regulates accessibility of the transcription machinery to regions of DNA, with methylated CpGs restricting transcription and unmethylated CpG allowing the gene to be expressed (58). The effects of cytosine methylation are mediated through numerous histone modifications such as acetylation and methylation, which cause chromatin remodeling and subsequent gene silencing as summarized in Figure 1. Possible roles for DNA methylation include maintenance of chromosomal stability and the silencing of imprinted genes, the inactive X chromosome and also of transposons.

In mammalian development, there are two main periods of epigenetic modification. During gametogenesis, genome-wide demethylation occurs followed by remethylation before fertilization. Early embryogenesis is then characterized by a second genome-wide demethylation event (59). Methylation is re-established early in embryonic life following implantation. These postfertilization demethylation and remethylation phases are likely to play a role in the removal of acquired epigenetic modifications, particularly those acquired during gametogenesis (60,61). Importantly, a subgroup of genes that carry parental methylation imprints appear to escape the second wave of demethylation.

Imprinted genes that undergo genomic or parental imprinting are among the most well-understood examples of epigenetic transcriptional modification. A subset of approximately 80 genes in humans display mono-allelic expression, *i.e.* expression only occurs from a single parental allele (62). Genomic imprinting-induced silencing of one parental allele results in mono-allelic expression from either the paternal or maternal copy of a gene. The imprint control regions for genomic imprinting usually contain a differentially methylated

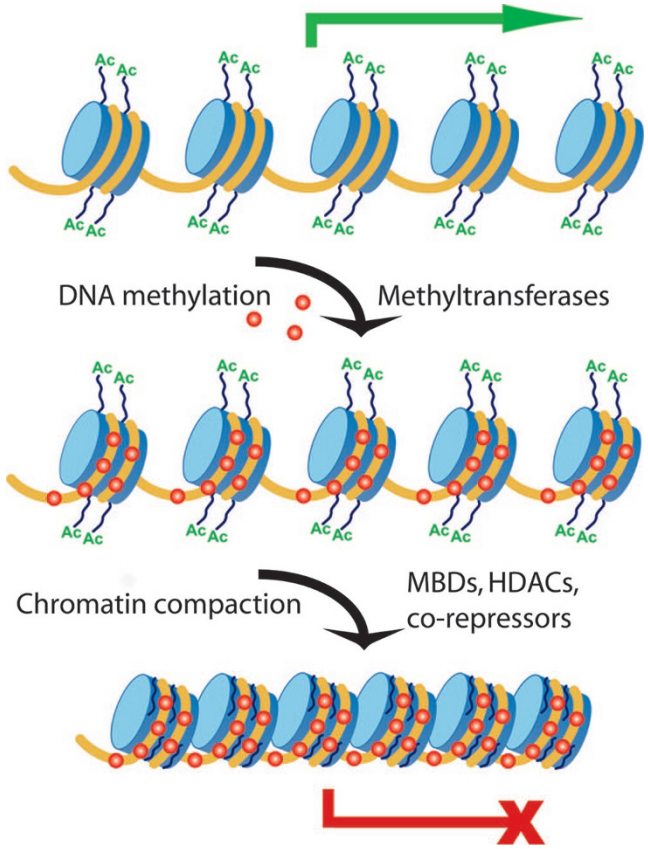

Figure 1. Epigenetic modifications in gene silencing. A series of epigenetic modifications transforms transcriptionally active regions of DNA (*top*) into inactive compact chromatin (*bottom*). DNA methylation, executed by methyltransferases, allows recruitment of methyl-binding domain proteins (MBD), which then recruit histone deacetylases (HDAC, transcriptional co-repressors and other chromatin modifying enzymes). Transcriptionally active chromatin is associated with acetylated histones, whereas inactive chromatin has methylated DNA and de-acetylated histones, as well as other histone modifications that are not shown.

CpG island in which one parental allele is methylated and the other unmethylated. A list of the phenotypes of known and likely imprinted genes are listed in Table 1. A regularly updated website of all known imprinted genes in humans and animals is available at www.otago.ac.nz/igc.

Table 1. *Human phenotypes associated with imprinted genes or parent-of-origin effects*

Phenotype	Location
Syndromes involving imprinted genes	
Beckwith-Wiedemann syndrome	11p15
Prader-Willi syndrome	15q11-q12
Angelman syndrome	15q11-q12
Silver-Russell syndrome	7p11-p13, 7q31-qter,11p15
Transient neonatal diabetes mellitus	6q24
PHP1b, Albright hereditary osteodystrophy, McCune-Albright syndrome	20q13
Syndromes that probably involve imprinted genes	
Familial nonchromaffin paraganglioma	11q13
Maternal UPD 14 syndrome	14
Paternal UPD 14 syndrome	14
Maternal UPD 2 syndrome	2
Maternal UPD 16 syndrome	16
Turner syndrome phenotypes	X

EPIGENETICS AND EARLY LIFE PERIODS

The peri-conceptual period. The concept that early nutrition or disruptive environmental influences might alter imprinting regulation is supported by extensive experimental data in rodents and observational studies in humans. Early nutrition can influence DNA methylation because mammalian one carbon metabolism, which is the source of all methyl groups for all biologic methylation reactions, is very dependent upon dietary methyl donors and cofactors (63). Several studies have begun to test the hypothesis that altered nutrition, as reflected in various culture medium during IVF, can alter methylation and expression of imprinted genes (64–66). Mouse embryos cultured in Whitten's media showed loss in methylation of the *H19* differentially methylated region (DMR) that was not seen in embryos culture in KSOM with amino acids (66). In addition, mouse embryos cultured in the presence of FCS had reduced viability, reduced body weight, decreased expression of *H19* and *IGF2,* and increased methylation of the *H19* imprinting control region when compared with controls (67). Collectively, these studies show that epigenetic alterations in the early embryo can be maintained to later stages of development.

Similarly, recent studies have shown that the process of IVF with *in vitro* manipulation in humans can produce imprinting changes like those seen in mice. An increased incidence of BWS, an overgrowth disorder associated with altered imprinting of genes including *IGF2*, has been reported in IVF offspring (68–70). In the only case control study performed, a 9-fold increased risk of BWS after IVF was found (71). Similarly, AS, a neurocognitive disorder, can be associated with loss of methylation in imprinted gene clusters and has been reported following IVF (72, 73). These epidemiologic associations between IVF and BWS or AS are strengthened by additional observations linking IVF to epigenetic changes. In 15 of 21 cases of BWS or AS following IVF, an epigenetic defect due to loss of methylation of the maternal allele was found (68–70,72,73). In a meta-analysis, 23 of the 24 cases of BWS following IVF were found to be due to hypomethylation of *KvDMR1* (74). In AS cases, hypomethylation of *SNRPN* was found to be the cause of the disorder (72). BWS or AS due to imprinting defects are the result of dramatic changes in DNA methylation. It is conceivable that less marked changes in DNA methylation will result in a far more subtle phenotype that could initially manifest as differences in growth patterns.

Recently epigenetic mutations have been identified that are common causes of Silver Russell Syndrome, a disorder characterized by very low birth weight, limb asymmetry and poor early childhood growth (75–77). Epigenetic modification of H19DMR and KvDMR1 occur in approximately 35% of children with clinical features of Silver Russell syndrome (75–77). Hypomethylation of H19 DMR1 that will lead to reduced IGF2 expression accounts for 20% of Silver Russell syndrome cases (77). Interestingly, Silver Russell syndrome and BWS may be regarded as two disorders caused by opposite (epi)genetic disturbances of the same chromosomal region displaying opposite clinical pictures.

Late gestation and early neonatal periods. Data from animal models have indicated that the epigenetic lability of imprinted genes is not limited to the early embryonic period. There are a growing number of studies that have examined the impact of late fetal and early postnatal nutrition or adverse environmental factors on methylation of both imprinted and nonimprinted genes, which has led to altered gene expression. The strongest evidence in humans linking altered fetal nutrition with programmed changes in metabolism comes from the Dutch famine study, which found that young adult subjects conceived during the famine demonstrated higher 2-h plasma glucose values following an oral glucose load than controls born before or conceived after the famine (78).

Late gestation uteroplacental artery ligation leads to intrauterine growth restriction (IUGR) and reduction in renal mass in rats. At 21 d of age, increased p53 expression and promoter hypomethylation in the kidney was found (79). Hypomethylation of the gene for p53, an apoptosis regulator in the kidney, is the proposed mechanism for late fetal renal apoptosis and loss of glomeruli in animals with IUGR (79). MacLennan *et al.* (80) have more intricately explored the relationship between one carbon metabolism and DNA methylation in the rodent late gestation uteroplacental artery ligation model. An abnormality in one carbon metabolism was found in the liver, suggesting that increased hepatic levels of s-adenosylhomocysteine reduced availability of methyl donors that led to genome wide DNA hypomethylation (80).

Treatment of mice with a global inhibitor of DNA methylation at postnatal d 11 and 14 led to dramatic alteration in allelic expression of *IGF2* (81). The effect of more subtle changes in postnatal nutrition on imprinted gene expression were examined by Waterland and Garza (82). Rats were suckled in divergent litter sizes. Smaller litter sizes led to overnutrition, whereas large litter size led to undernutrition. Immediately after weaning and in adulthood, pancreatic beta cells from the smaller litter animals displayed impaired glucose stimulated insulin secretion. DNA microarray analysis revealed altered expression of two imprinted genes (*insulin2* and *neuronatin*) and eight methylated genes found within pancreatic islet cells. These findings suggest that early postnatal diet has led to altered gene methylation and expression, reflecting enhanced epigenetic lability to early nutritional influences.

EPIGENETICS AND THE PLACENTA

The placenta contains all known imprinted genes and it has been suggested that they play roles in total placental growth as well as differential growth of specific cell types and activities of certain transporters (83–85). Overall, therefore, they may play a significant part in determining placental nutrient delivery. It has been deduced from studies with knockout mice that the maternally expressed genes appear to abrogate placental growth whereas the paternally expressed genes enhance placental growth. There is evidence that imprinted genes may also regulate an organic cation transporter and components of the system A amino acid transporter (83–87). In particular, the placental labyrinth-specific PO transcript of the paternally

imprinted Igf2 gene in mice has been suggested as a powerful regulator of placental growth that can alter placental efficiency and placental nutrient permeability (88).

It has now been demonstrated also that nonimprinted genes key to placental growth and function as well as mechanisms of pregnancy maintenance and parturition are under epigenetic control. For instance the activity of a gene for a critical enzyme in prostaglandin biosynthesis (prostaglandin H synthase-2) can be modified by alteration of histone acetylation status and DNA methylation status in human placental explants (89). This effect was tissue-specific with effects on amnion but not adjacent chorio-decidua. Moreover, similar alterations result in massive changes in IL-1β output by human placental explants (90). These inflammatory mediator substances have substantial effects on uteroplacental hemodynamics and are critical in mechanisms of labour both at term and preterm. The regulatory mechanisms extend back in pregnancy to at least the time of implantation since recent studies have demonstrated clearly that the implantation process is regulated epigenetically (91).

Presently, there are no direct data linking placental epigenetic changes to changes in and regulation of fetal phenotype at birth or later in life. We believe that such linkages are not only possible but highly likely. It is known that knockouts of key genes in mice can result in abnormal placentation and subsequently altered growth and development (*e.g.* growth restricted fetuses) that can also be fatal. We hypothesize that similar effects occur with abnormal epigenetic regulation of critical genes in the placenta. Testing of this hypothesis will require initially experiments in which key genes (*e.g.* DNA methyltransferases) are modified in a uterine-specific (preferably placental-specific) and gestational age–specific manner. Thereafter, similar experiments with epigenetically altered critical genes can determine specific regulatory pathways.

DIETARY FOLATE AND DNA METHYLATION

Folate and/or methyl group dietary studies provide the most compelling data for the interaction of nutrients with DNA methylation, because these dietary elements are directly involved in one carbon metabolism. The sole metabolic function of all co-enzymatic forms of folate is to transfer one carbon units for reaction such as methylation. Folate deficiency affects DNA stability through two principal pathways: DNA hypomethylation and DNA synthesis and repair (92). In humans, the major source of methyl groups in humans comes from methionine and to a lesser extent choline. Pregnancy, fetal, and early neonatal life are periods in which there is a high demand for folate and supplementation is usually given to preterm infants once full oral feeding is established. Serial plasma folate measurements in 140 preterm infants fell progressively to very low levels by the second to third week of life, at which time full oral feeding was achieved and folate supplementation was introduced (93). Those not supplemented continued to have low serum folate levels. Therefore, premature infants are at risk of a period of up to several weeks of folate deficiency that could conceivably lead to DNA hypomethylation.

Effects of dietary methyl donors (methionine and choline) and folate on DNA methylation have been reported in rodent and human studies. For example, rats fed a diet deficient in methionine and choline exhibited a change from normal DNA methylation to global DNA hypomethylation that included specific gene hypomethylation (94,95). A folate-deficient diet introduced to healthy rats led to DNA hypomethylation in the brain (96). Further support for the important role of methyl donor sufficiency in changing DNA methylation long term was identified in a study of methyl supplementation in two strains of pregnant mice. Supplementation led to enhanced DNA methylation of an LTR element with a mutant agouti gene creating a leaner phenotype of the offspring (97).

The strongest argument linking folate deficiency with metabolic gene hypomethylation was established in rat offspring whose mothers were fed a low-protein diet during pregnancy (98). DNA methylation of the glucocorticoid receptor was lower and gene expression higher than offspring from control-fed animals (98). Interestingly, the addition of folic acid to the low protein diet prevented this change in methylation and expression from occurring. This observation may have relevance to the insulin resistance seen in children born SGA or prematurely. Activation of the glucocorticoid receptor leads to glucocorticoid induced insulin resistance in liver and in skeletal muscle (99,100).

Data from animal studies indicate that folate or methyl group deficiency during late fetal life or early postnatal life produces stable long-term total DNA and specific gene hypomethylation. Conversely, studies in adult rats and humans suggest that folate- or methyl group–deficient diets lead to DNA hypomethylation that reverses with resumption of a normal diet (94,95,101). Collectively these observations suggest that there is an unexplained ontogenic window in which nutritional deficiency leads to persistent DNA hypomethylation during fetal and early neonatal life that does not exist in adults.

CANDIDATE GENES

There are a number of epigenetically modifiable gene candidates that could play a role in programmed changes in growth and insulin sensitivity in IVF, SGA, and prematurely born children. These include imprinted genes, since imprinting is known to be disrupted in some IVF children, but also nonimprinted genes involved in growth and metabolism.

Candidate genes that might explain the taller stature and elevated IGF-II levels seen in our IVF children include the growth genes *IGF2* and *CDKN1C* (*p57KIP2*), which are controlled by methylation at *H19* DMR and KvDMR1, respectively. Conversely, changes in methylation at the putatively polymorphically imprinted *IGF2 receptor* gene locus could lead to decreased IGF-II activity and small size at birth due to increased numbers of the sink IGF-II receptor (102,103). Similarly overexpression of *RASGRF1*, which regulates postnatal growth and is known to be imprinted in rodents, could explain the taller stature and higher IGF-I levels in IVF children. Conversely, underexpression of *RASGRF1*, which is

known to be imprinted in rodents, could explain poor childhood growth in SGA and prematurely born children (104).

Other candidates include leptin, in which reduced levels play a critical role in the development of the metabolic syndrome in rats (insulin resistance, hypertension and dyslipidemia) (105). Newborn SGA rats treated with a short course of leptin displayed a normal adult phenotype without any biochemical evidence of metabolic syndrome (105). Therefore, there appears to be a critical window in early life in which under nutrition causing aberrant methylation of *LEP* could lead to persistent insulin resistance and development of adult obesity and hypertension. This is yet another plausible mechanism whereby children born prematurely could develop insulin resistance.

GRB10 is an imprinted gene that acts to inhibit insulin and IGF-I receptor signalling (106). It has been established that *GRB10* is not the cause of Russell Silver syndrome, a discrete syndromal cause of SGA children (107). *GRB10* hypomethylation during the fetal or early neonatal periods could cause insulin resistance, poor growth, and abnormalities in the GH/IGF-I axis as observed in SGA and premature children.

SUMMARY

There are persistent alterations in growth and metabolism in SGA, very premature, and IVF children. These changes, which appear to be programmed from early life environmental exposure, must involve altered cellular function and probably altered gene expression. However, the trigger(s) and mechanism(s) that lead to these programmed changes have yet to be elucidated in humans. There is compelling evidence from animal studies that environmental factors such as altered nutrition lead to epigenetic changes and altered gene expression, however, it is unclear whether these changes lead to metabolic disease. Future studies in animal models will better define the long-term disease risks of epigenetic changes following specific nutritional or environmental manipulation. Although animal models of SGA or IVF cannot be easily extrapolated to humans and animal models of prematurity do not yet exist, using the available data we hypothesize that an adverse embryonic, fetal, or neonatal environment is responsible for epigenetic modification leading to the growth and metabolic changes observed in later childhood. Further studies are needed to examine candidate genes for methylation changes and in particular establish methylation patterns in different tissues. Accessing target tissues remains one of the major limitations in human studies. Limited current data support epigenetic change as a mechanism behind the observed phenotypic changes in IVF, SGA, and prematurely born cohorts. Epigenetics is likely to become a major focus of attention for developmental biologists in establishing mechanisms that link adverse early life events with later adult disease.

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