
Perspectives in Diabetes

Nutritional Epigenomics of Metabolic Syndrome

New Perspective Against the Epidemic

Catherine Gallou-Kabani and Claudine Junien

Human epidemiological studies and appropriately designed dietary interventions in animal models have provided considerable evidence to suggest that maternal nutritional imbalance and metabolic disturbances, during critical time windows of development, may have a persistent effect on the health of the offspring and may even be transmitted to the next generation. We now need to explain the mechanisms involved in generating such responses. The idea that epigenetic changes associated with chromatin remodeling and regulation of gene expression underlie the developmental programming of metabolic syndrome is gaining acceptance. Epigenetic alterations have been known to be of importance in cancer for ~2 decades. This has made it possible to decipher epigenetic codes and machinery and has led to the development of a new generation of drugs now in clinical trials. Although less conspicuous, epigenetic alterations have also been progressively shown to be relevant to common diseases such as atherosclerosis and type 2 diabetes. Imprinted genes, with their key roles in controlling feto-placental nutrient supply and demand and their epigenetic lability in response to nutrients, may play an important role in adaptation/evolution. The combination of these various lines of research on epigenetic programming processes has highlighted new possibilities for the prevention and treatment of metabolic syndrome. *Diabetes* 54: 1899–1906, 2005

The recent explosion of the worldwide epidemic of metabolic syndrome—combining disturbances in glucose and insulin metabolism, excess predominantly abdominally distributed weight, mild dyslipidemia, and hypertension, with the subsequent development of obesity, type 2 diabetes, and cardiovascular disease—compromises progress made in reducing the morbidity and mortality of cardiovascular

disease in recent years (1). In the last 10 years, a series of studies, notably those by Hales and Barker (2), who first coined the term “fetal programming” leading to a “thrifty phenotype,” have demonstrated that these common disorders take root in early nutrition, during gestation and lactation.

A significant and increasing proportion of women (14–27%) are overweight when pregnant. Whereas the long-term effects of gestational diabetes are well documented (3,4), the consequences of metabolic syndrome in the mother, together with an unbalanced diet and metabolic disturbances during the periconceptual period, gestation, and lactation, for fetal programming, for the various critical windows of development, and during aging (5) are poorly documented and remain to be explored (6).

A recent review examined animal studies in which the fetal and postnatal environment had been manipulated by changing maternal dietary intake or modifying uterine artery flow (6). Most studies examined the consequences of protein restriction during gestation, conditions not fully matching the features of the current epidemic of metabolic syndrome. Fewer studies have dealt with the consequences of a high-carbohydrate or fat-rich diet, conditions corresponding more closely to the current epidemic of metabolic syndrome. However, it remains unclear whether metabolic syndrome can be reliably induced by the interventions made because of differences between protocols, diets (e.g., type of fatty acids), sex, and time periods examined (6).

Recent experiments have shown that the features of metabolic syndrome in the adult offspring of fat-fed rats may be acquired antenatally and during suckling. Moreover, exposure during pregnancy confers adaptive protection against endothelial dysfunction, but not against hypertension, because of maternal fat feeding during suckling (7). However, these data only partially reflect the features of metabolic syndrome because pregnant mothers were not overweight and therefore did not display the metabolic disturbances of metabolic syndrome that could also interfere with fetal/postnatal programming. Malnourished fetuses adopt several strategies to optimize their chances of survival during the neonatal period, but these strategies assume that the same type of nutritional conditions will prevail. A selective distribution of nutrients ensures that brain growth is given priority over the growth of other organs, such as the liver, muscle, and pancreas. The adaptations adopted during fetal programming may

From the Institut National de la Santé et de la Recherche Médicale (INSERM) Unit 383, Clinique Maurice Lamy, Hôpital Necker-Enfants Malades, Paris, France.

Address correspondence and reprint requests to Claudine Junien, Pharm D, PhD, INSERM Unit 383, Clinique Maurice Lamy, porte 15, Hôpital Necker-Enfants Malades, 149 rue de Sèvres, 75743 Paris, France. E-mail: junien@necker.fr.

Received for publication 17 February 2005 and accepted in revised form 29 March 2005.

GNAS, guanine nucleotide-binding protein G_sα subunit; IAP, intracisternal A particle.

© 2005 by the American Diabetes Association.

prove to be detrimental if food becomes more abundant (8). Thus, any change in conditions may have deleterious consequences.

REGULATION OF GENE EXPRESSION BY EPIGENETIC ALTERATIONS

Epigenetics, from the Greek “epi,” meaning “on,” DNA (a term first coined by Conrad Waddington in 1937), has been described as “the developmental processes by which genotype gives rise to phenotype.” Today, epigenetics may be defined as the inheritance of information based on gene expression levels rather than on gene sequence (genetics) (9). All of our tissues contain the same 30,000 genes; however, in a given tissue and at a given stage, owing to an “epigenetic code,” only a few of these genes are expressed, giving rise to the “phenotype.”

The epigenetic code comprises several levels of interconnected and interdependent codes: the DNA methylation code, the histone code (histone methylation, acetylation, and phosphorylation), and the coregulator code. These codes define a process involving the recruitment of a myriad of chromatin-remodeling complexes, enzymes, coregulators, and effectors, directing appropriate chromatin remodeling (10). Specific epigenetic patterns condition the accessibility of chromatin to transcription factors, facilitating the recognition by these factors of genes to be expressed (to various extents) and of genes to be silenced, transiently or permanently. Epigenetic chromatin marks may be propagated mitotically and, in some cases, meiotically, resulting in the stable inheritance of regulatory states. Transient nutritional stimuli occurring at critical ontogenic stages may have lasting influences on the expression of various genes by interacting with epigenetic mechanisms and altering chromatin conformation and transcription factor accessibility (11).

Disruption of the balance of epigenetic networks may cause several major diseases, including cancer, syndromes involving chromosomal instabilities, and mental retardation. The importance of epigenetic changes in tumorigenesis has been acknowledged for 2 decades. However, the relevance of epigenetics to other physiopathological mechanisms in common diseases, such as metabolic syndrome, was less clear (12). Converging data are now available to support the hypothesis that, in addition to “thrifty genotype” inheritance, individuals with metabolic syndrome underwent incorrect “epigenetic programming” during fetal/postnatal development because of inadequate maternal nutrition and metabolic disturbances. These individuals may also display “transgenerational effects” because of the inheritance of epigenetic changes first experienced by their parents and/or grandparents.

EPIGENETIC PROGRAMMING DURING FETO-PLACENTAL AND POSTNATAL DEVELOPMENT

Tissues and organs are tailored in response to finely tuned genetic and epigenetic programs, by means of cycles of proliferation, differentiation, and apoptosis. Imbalance between nutrient intakes (qualitative and quantitative), metabolites, and the precise requirements of these processes, within spatiotemporal limits, can lead to 1) defective structural and functional development or even the absence of certain specialized cell types, leading to a “no return”

situation because of irreversible processes of differentiation and organogenesis and 2) changes in homeostatic processes associated with labile and potentially reversible epigenetic modifications. The impact of unbalanced diets or nutrients in animals (rat, mouse, and sheep) on several types of sequences (genes and repeated sequences) and on epigenetic programming processes throughout an individual’s lifetime, during specific periods and with their potential transmission to the next generation, is well documented (Fig. 1) (13).

Irreversible processes of differentiation and organogenesis. Protein restriction during gestation increases the rate of pancreatic apoptosis in the offspring of rats. This leads to a smaller mass of pancreatic β -cells and disturbs development of the endocrine pancreas in the next generation (14). Similarly, a high-carbohydrate diet in neonatal rats immediately induces hyperinsulinemia, which persists into adulthood without any further nutritional stimulus. Moreover, this metabolic imprinting, once established, forms a vicious cycle because high-carbohydrate female rats spontaneously transmit the high-carbohydrate phenotype to their progeny (15).

Intriguingly, however, these examples show that the same effects may result from diametrically opposed causes. These dietary interventions, which reduce placental-fetal blood flow and stunt fetal growth, have the same consequences in the long term (16). Similarly, in children and adolescents, under- and overeating may both program the lipid metabolism and other metabolic systems of these individuals for life.

Placental insufficiency is persistently associated with epigenetic alterations in the rat. Changes in hepatic one-carbon metabolism involving the folate-methionine pathway (possibly because of dietary folate depletion) and changes in the level of DNA methylation (hypomethylation) and histone acetylation are observed in the liver (17) (Fig. 1).

In the adult, changes in methylation pattern during differentiation have been described only for a few genes. Two studies showed a strict correlation between demethylation of the leptin promoter and preadipocyte differentiation into adipocytes (18,19). However, it is clear that the same mechanisms must apply to all genes involved in development and differentiation.

Finally, the accumulation of DNA methylation errors over time, through aging (5), may add to the development of type 2 diabetes by decreasing the responsiveness of genes, the expression of which must be adjusted in response to rapidly changing glucose levels. In addition, because of their inherent lability, genetically determined epigenetic mechanisms are susceptible to environmental/nutritional influences (13).

Critical spatiotemporal windows. Changes in methylation patterns in the early embryo and during development have been studied extensively for imprinted genes but only for a few nonimprinted genes, although they necessarily apply to all genes regulated during development and differentiation. As shown for the cluster of APOA1-C3-A4 genes encoding apolipoproteins (20), in all cell types, at a given stage of development, specific patterns of expression of 10s or 100s of genes corresponding to specific programs of differentiation are more or less “locked” (18).

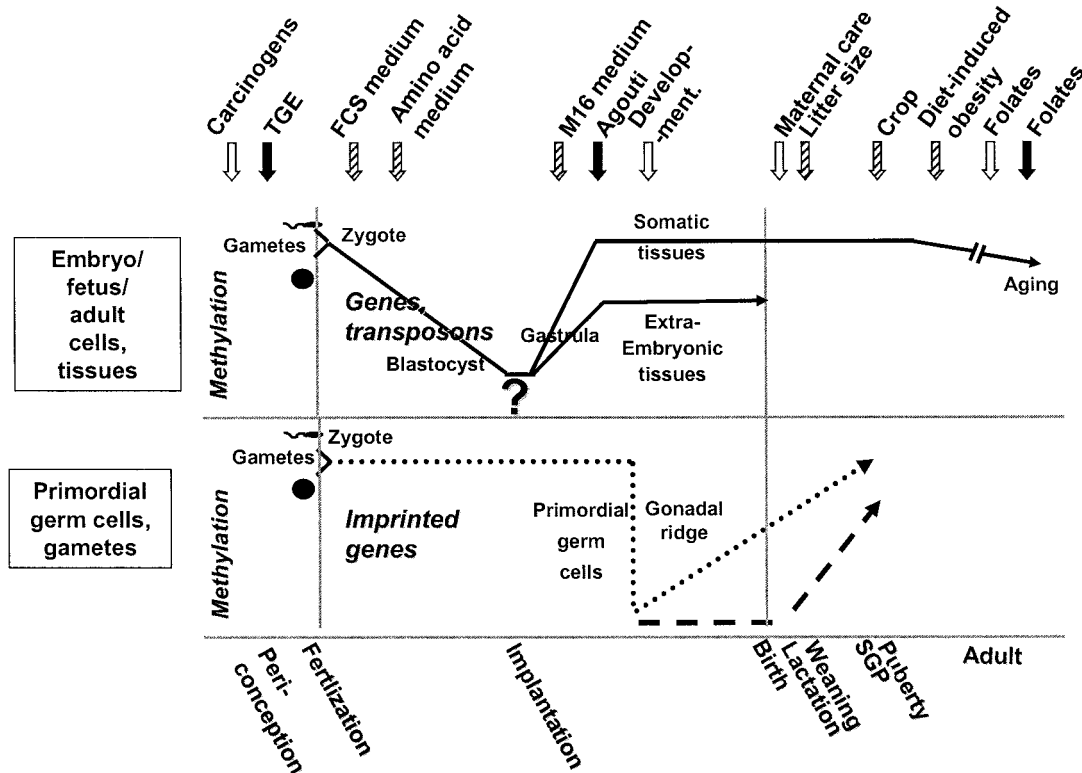


FIG. 1. Impact of nutrients or dietary intervention on DNA methylation reprogramming during early development and throughout life. *Top section:* Genes and repetitive sequences in somatic tissues and extra-embryonic tissues in the embryo/fetus/adult. After fertilization, the paternal and maternal genomes in the zygote undergo rapid demethylation at coding sequences (genes) and at repetitive sequences (transposable elements) (solid line). Active de novo methylation takes place after implantation to various extents. After implantation, the bulk of the genome becomes hypermethylated in the embryonic ectoderm and mesoderm through active de novo methylation, whereas the genome of extra-embryonic cells remains hypomethylated. There is a sequence of de novo methylation that dictates the structure and function of each somatic tissue through a finely tuned pattern involving the switching on and off of gene expression. *Bottom section:* Imprinted genes in primordial germ cells and gametes. The parental methylation imprints in imprinted genes escape this demethylation process and de novo methylation (dotted line). The imprints of imprinted genes are eliminated before primordial germ cells reach the gonadal ridge and are appropriately reinstalled during male and female gametogenesis, being completed during the slow-growth period (SGP) before puberty (dotted line for male, dashed line for female). In the male, the acquisition of DNA methylation patterns begins before birth in prospermatogonia and is completed for many sequences after birth, before the end of the pachytene stage of meiosis. In female germ cells, most gametic DNA methylation is acquired postnatally, during the oocyte growth phase, after the pachytene phase of meiosis (64). The slow-growth period is associated with the emergence of the first viable pools of spermatocytes and initiation of the programming of methylation imprints. At every stage during this cascade of epigenetic fluctuations (during both fetal development and the aging process), the nutritional balance must be optimal. The impacts of nutrients/diets are indicated by arrows; they may concern imprinted genes (▨), transposons (■), or genes (□). Arrows indicate the window during which the impact was observed. For each arrow, see the reference list for more information (13,23,68). TGE, transgenerational effects.

If there are critical spatiotemporal windows during which these programs must be completed, failure to complete these programs in time is irrecoverable (2). As suggested by two recent reports, leptin, which regulates the energetic balance through electrophysiological modulation of regulating orexigenic neurons (neuropeptide Y/agouti-related peptide) and anorexigenic neurons (pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript, α -melanocyte-stimulating hormone), also has a trophic effect on some neurons of the hypothalamus involved in the response to nutrients, and it contributes to their plasticity (21,22).

However, leptin only exerts these important effects during a narrow window in postnatal development: the neonatal period before leptin becomes involved in the acute regulation of food intake in adults (22). It is thus highly likely that levels of leptin and of particular nutritional stimuli during this precise developmental period have long-term consequences because of the inappropriate epigenetic remodeling of chromatin (11).

Epigenomic behavioral programming. A particular

epigenomic state can also be established by behavioral programming. Meaney and colleagues (23) have shown that higher levels of pup licking and grooming and arched-back nursing by rat mothers are associated with a better response to stress in adult offspring. This maternal behavior changes the epigenome of the offspring at the glucocorticoid receptor gene promoter in the hippocampus. The offspring of high-pup licking and grooming mothers display demethylation of the glucocorticoid receptor promoter, which is associated with higher levels of expression of the glucocorticoid receptor gene. These epigenetic changes in DNA methylation and histone acetylation occurred during the 1st week of life and persisted into adulthood.

However, this neonatal programming may be reversed by altering the epigenetic profile of the glucocorticoid receptor gene. Central infusion of a histone deacetylase inhibitor, trichostatin A, abolished differences between groups in histone acetylation, DNA methylation, NGFI-A (nerve growth factor inducible protein A) binding, glucocorticoid receptor expression, and hypothalamic-pitu-

itary-adrenal responses to stress, suggesting a causal relationship between epigenomic state, glucocorticoid receptor expression, and maternal effects on stress responses in the offspring (23).

IMPRINTED GENES: A PLAUSIBLE SUPPORT

For most human genes, the two alleles contribute equally to production of the gene product. In contrast, imprinted genes are monoallelically expressed (i.e., either from the paternal allele or from the maternal allele). Most imprinted genes are grouped in clusters, which may comprise reciprocally imprinted genes—some maternally and some paternally expressed—at ~15 different chromosomal sites in the human genome. To date, >70 imprinted genes have been identified of a total of 100–200 in the whole genome. These imprinted domains are regulated coordinately, via long-range mechanisms such as antisense RNA interference and methylation-sensitive boundary elements. This regulatory complexity of imprinted domains may render them particularly susceptible to environmental dysregulation via nutrition (11,24).

There is compelling evidence that DNA methylation varies between tissues, individuals, and disease conditions in humans and various animals and that it varies with aging—in which both hyper- and hypomethylation are observed—and even in some pathological situations, such as cancer and atherosclerosis (24–26). Thus, it is possible that changing circumstances over several generations recruit alleles back into the active genome, accounting for the reversibility of adaptive changes. Genomic imprinting may act as a buffering system or “rheostat” supporting adaptation to environmental conditions by silencing or increasing the expression of monoallelically expressed genes. Failure to erase these epimutations in the germline would lead to stable transgenerational effects (27–30).

Although no formal demonstration has yet been made, there is abundant evidence from genomic (or parental) imprinting studies consistent with the hypothesis that monoallelically expressed imprinted genes are the most promising candidate genes for developmental and evolutionary modifications and transgenerational effects in response to rapid changes in the nutritional environment, such as those associated with the worldwide epidemic of metabolic syndrome, obesity, and type 2 diabetes.

- Because the epigenetic marks of imprinted genes are not erased after fertilization, the acquired changes may lead to stable transgenerational effects.
- Genomic imprinting and placentation appeared at the same time during the course of evolution of mammals (31). Placental expression has been studied for ~50% of the 70 identified imprinted genes: all imprinted genes tested were expressed in this organ (32).
- Imprinted genes in the placenta control the supply of nutrients, whereas imprinted genes in fetal compartments control nutrient demand by regulating the growth rate of the fetal tissues (31).
- Imprinted genes play an important role in the development of regions of the brain, such as the hypothalamus, that contribute to energy homeostasis regulation (28,31,33).
- The epigenetic variation transmitted by imprinted genes

is also required for postnatal metabolic adaptation to feeding (34,35).

- The epigenetic lability (metastable alleles) of imprinted genes in response to nutrients may play an important role in adaptation/evolution (13,36).
- Diabetes and obesity, two of the hallmarks of metabolic syndrome, often accompany syndromes associated with changes in imprinting (37).

Imprinted genes and fetoplacental growth: nutrient supply and demand. In the placenta, regulation of the expression of imprinted genes primarily involves the methylation of histones, which seems to be independent of DNA methylation (38). Surprisingly, the level of DNA methylation is lower in the placenta than in somatic tissues. This may account for the higher epigenetic lability of placental imprinted genes to environmental factors (Fig. 1).

Imprinted genes may play diverse roles in regulating nutrient transfer (31). First, they may affect the growth of the placenta. In keeping with sexual conflict theory, several maternally expressed genes decrease fetal and placental growth, whereas several paternally expressed genes increase it. Second, they may affect transport capacity. Several imprinted genes belong to the active amino acid transport system A, to the solute carrier family, or to the organic cation transporter family. Finally, they may regulate interactions between different cell types with fetomaternal interfaces in the placenta, or they may modulate the nutrient requirements of the fetus, mainly by controlling fetal growth.

The knockout of several imprinted genes results in the placenta being small, with paternally expressed imprinted genes, or, in placentomegaly, with maternally expressed imprinted genes (39). One of the determinants of placental size is the imprinted gene *Igf2*, which encodes the IGF-II protein. In mice, one of the control regions of *Igf2*, the P0 promoter, controls labyrinth-specific expression. Mutant fetuses in which the placenta-specific promoter for *Igf2* has been knocked out display growth retardation (31,39). These mutants display lower levels of passive and higher levels of active placental transport early in development compared with the wild type (39). The IGF-II transcribed from the placenta-specific promoter (P0) regulates development of the diffusion permeability properties of the mouse placenta. Thus, modified placental IGF-II may cause idiopathic intrauterine growth retardation in humans (40).

Imprinted genes and postnatal growth. Imprinted genes also play a key role in postnatal development. The two examples below, concerning the guanine nucleotide-binding protein G_sα subunit (GNAS) complex and two paternally imprinted genes, *Peg1* and *Peg3*, illustrate how mice models can help to unravel the manifestations of gene knockout, according to the parent of origin of the mutant allele and the age (newborn or adult) and sex of the recipient.

The GNAS complex is an imprinted domain encoding the stimulatory G-protein α-subunit (G_sα), which interacts with adenylate cyclase, and several other transcripts expressed in a tissue-specific manner from the maternal allele (gonads, pituitary gland, thyroid, kidney proximal

tubule, and adipose tissue) or from the paternal allele (brown adipose tissue).

Maternal and paternal transmission of *Gnas* knockout produce opposite effects on energy metabolism. The loss of paternal function is associated with a decrease in adiposity, hypermetabolic function, hypoglycemia, a decrease in locomotor activity, and resistance to parathyroid hormone, whereas the loss of maternal function is associated with greater adiposity. This animal model was the first in which a single genetic defect was shown to lead to opposite effects on energy metabolism, depending on the parental allele expressed (34).

Mice with mutations in a paternally expressed gene, *Gnasxl* (which encodes the unusual $G_s\alpha$ isoform $XL\alpha_s$), have low postnatal growth and survival rates. They also display a spectrum of phenotypic effects indicating that $XL\alpha_s$ controls a number of key postnatal physiological adaptations, including poor suckling; low blood glucose, insulin, and glucagon levels; and altered energy homeostasis (34). Brown adipose tissue shows a lack of lipid vesicles and impaired temperature regulation. In the brain, areas expressing $XL\alpha_s$ are important in controlling states of alertness, wakefulness, and sleep. Finally, in relationship with low levels of suckling, *Gnasxl* is expressed in all three nuclei providing motor innervation to orofacial muscles. The GNAS complex therefore plays a crucial role in postnatal adaptation to feeding (34).

Two other paternally expressed imprinted genes, *Peg3* and *Peg1*, provide a remarkable example of the synchronization of nutrient supply and demand. Gene knockout experiments suggest that epigenetic modifications may alter the phenotype of both the mother and the pups according to food availability. The synchronization of these coadaptive traits in mother and offspring by the same paternally expressed imprinted genes ensures that offspring receiving "good" maternal nurturing will themselves be both well provisioned and genetically predisposed toward "good" mothering (35).

If the fetus carries a mutation of the paternal allele of *Peg3*, the placenta is small, and fetal growth, suckling, postnatal growth, body temperature, age at weaning, and onset of puberty are all affected. If the same mutation of *Peg3* is carried by the mother, it results in impaired maternal reproductive success, a decrease in maternal care and food intake during pregnancy, and an increase in the likelihood that insufficient milk will be produced. The consequences for the offspring are poor postnatal growth and delays in weaning and in the onset of puberty (35). A lack of maternal care is also observed with the *Peg1* knockout. Mice generally eat the placenta after giving birth. However, mother mice lacking *Peg1* display behavioral quirks in that they do not eat the placenta and fail to take care of their pups (41).

These two paternally expressed imprinted genes, *Mest/Peg1* and *Peg3*, are strongly expressed in the regions in which androgenic cells accumulate: the hypothalamus, the preoptic area, and the septum. Given the key role played by hypothalamic neurons in the regulation of energy homeostasis (hunger/satiety, intake of food/fasting), these genes may play a role in neuronal programming mechanisms.

Interestingly, these two paternally expressed genes also

play a role in adult adipose tissue growth. Oligonucleotide microarray analyses showed that *Peg1* and *Peg3* are up-regulated in the adipose tissue of mice in a model of diet-induced obesity (42). In another study, the overexpression of *Peg1* appeared to enlarge adipocytes, suggesting that *Peg1* mRNA levels may be a novel marker of adipocyte size (43) (Fig. 1).

However, formal demonstration of the involvement of imprinted genes in adaptation will require a detailed analysis of epigenetic patterns in strategic areas of imprinted complexes. Increases (or decreases) in expression of an imprinted gene may be caused by variations in the expression of the monoallelically expressed allele or to a loss of imprinting, with profile changes in differential methylation regions and the expression or silencing of the two alleles. This should allow us to discriminate between these two possibilities and show whether these altered imprinted states are transmitted to subsequent generations and could play a role in adaptation.

CIRCADIAN EPIGENETIC PROGRAMMING THROUGHOUT LIFE AND AGING

Over the circadian cycle, the expression of genes is modulated by molecular signals from circadian rhythms, by nutritional signals from meals, and through the overall diversity of environmental stimuli. The accompanying epigenetic alterations are unstable and reversible in everyday life: they are necessarily transient, giving rise to a "circadian nutritional epiphenotype."

However, epigenetic alterations are not always reversible. Untargeted stochastic DNA methylation errors, such as hypomethylation associated with low levels of the main physiological donor of methyl groups, S-adenosylmethionine, have been shown to accumulate with increasing age, possibly accounting for the higher incidence of metabolic syndrome elements in older individuals (5). Moreover, the metabolism of methyl groups may be affected by diet, body weight, and environmental factors, leading to untargeted general hypomethylation of DNA in diabetic patients (44,45). A general defect in DNA methylation in type 2 diabetes is suggested by the recent observation that S-adenosylmethionine decreases in concentration in the erythrocytes of diabetic patients (44).

Genomic hypomethylation has been observed not only in cancers but also in advanced human atherosclerotic lesions, in the lesions of *Apoe*^{-/-} mice, and in rabbit aortas. In mice, recent studies have clearly demonstrated that aberrant epigenetic patterns are detectable in the genetically atherosclerosis-prone *Apoe*-null (*Apoe*^{-/-}) mouse well before the appearance of histologically detectable vascular lesions. Moreover, these alterations may be triggered by an atherogenic diet in mice and in humans (46,47).

As in cancer, regional hypermethylation has also been shown to occur in atherosclerosis. The estrogen receptor- α gene (*ER- α*) has been shown to display higher levels of methylation in atheromas than in normal aorta and in smooth muscle cells in vitro during the phenotypic switch (48).

Thus, early epigenetic alterations have been described in atherosclerosis, animals fed atherogenic fat-rich diets, type 2 diabetes, and aging (5,44,47,49,50). However, the epigenetic alterations underlying metabolic syndrome

have yet to be explored. Description of the epigenetic effects of various conditions and periods has only just begun (3–5,51).

TRANSGENERATIONAL EFFECTS AND ADAPTATION

Although it has long been thought that the epigenetic slate is wiped clean in the embryo shortly after fertilization (with the exception of imprinted genes), there are now many examples in mammals of clear transgenerational effects after nutritional intervention (protein restriction or carbohydrate-rich diet) or behavioral programming (maternal care). Similar epidemiological data have been obtained for humans, from a Swedish cohort (Fig. 1). A grandfather who was “well-nourished” before puberty may transmit a four times higher than normal risk of type 2 diabetes to his grandchildren (9,15,23,52–56).

What type of sequence could be the epigenetic support of such alterations transmitted without erasure to subsequent generations? Except for a brief period of global demethylation in the early stages of mammalian embryonic development, transposons are normally silenced by promoter CpG methylation (57) (Fig. 1). Retrotransposons are thought to be maintained in a predominantly methylated state to prevent retrotransposition events that might lead to deleterious mutations and cancer. However, transposons, such as the intracisternal A particle (IAP) retrotransposon, that escape this epigenetic silencing may interfere with the expression of neighboring genes in several ways (36,58). Thus, both transposons and imprinted genes are good candidate supports (13).

The first two examples of transgenerational effects in mice that are explained by epigenetic modifications concern the A^{VY} and $Axin^{Fu}$ loci. Both of these loci display expression affected by the metastable epialleles of the IAP retrotransposon. The A^{VY} allele was generated by insertion of the IAP retrotransposon into the 5' end of the A allele (13,36). Methylation at the CpG dinucleotide of the transposable IAP sequence in the agouti region varies considerably in A^{VY} mice and is inversely correlated with ectopic *agouti* expression.

This epigenetic variability results in considerable inter-individual variation in coat color, adiposity, glucose tolerance, and tumor susceptibility in isogenic $A^{VY/a}$ littermates. The proportion of pups with a phenotype corresponding to a methylated IAP depends on the mother's own phenotype and therefore on the level of methylation of the mother's own IAP sequence at the A^{VY} locus. The variable phenotypes of the offspring result from incomplete elimination of the epigenetic modification when allele A is transmitted via the maternal germ line (59,60). However, recent experiments have shown that maternal or paternal epigenetic inheritance is influenced by strain background (36).

Dietary supplementation with a methyl donor during pregnancy increases the proportion of pups carrying a methylated IAP sequence (13,36). Nutrition probably exerts its effects on methylation early in embryonic development, and these effects concern all tissues. Waterland and Jirtle (60) also showed that coat color phenotype and A^{VY} methylation pattern persisted into adulthood. Consistent with the idea of transgenerational epigenetic inheritance, the methylation status of the axin-fused ($Axin^{Fu}$) allele in mature sperm reflects the methylation status of the allele

in the somatic tissue, suggesting that this locus is not subject to epigenetic reprogramming during gametogenesis (36) (Fig. 1).

Sequencing of the human genome has shown that transposons, which comprise roughly 35–40% of the genome, are found in ~4% of human genes (61). It would be interesting to determine whether these genes are involved in energy homeostasis regulation. However, there are currently no examples of human transposable elements similar to murine IAP sequences that are able to resist demethylation during preimplantation (58).

CONCLUSIONS

Whatever the genes or sequences involved, deciphering the epigenetic patterns at stake should allow us to evaluate their potential reversibility. Once the specific epigenetic patterns corresponding to “labile” and “locked” situations are identified, these patterns should be useful for diagnosis and prognosis. They may also represent new types of target for the development of novel diets and drugs to prevent or to abolish aberrant gene silencing, which may be involved in resistance to treatment (weight loss or weight regain) (12,62,63).

However, several important questions must be resolved if inhibitors/activators of the epigenetic codes and machinery or other molecules are to be used in metabolic syndrome patients. First, will the changes produced be toxic to normal nontarget cells? The chronic administration of DNMT inhibitors to rats is associated with decreases in sperm counts and fertility, increases in preimplantation loss, and decreases in sperm DNA methylation. These new drugs may therefore have adverse effects on human germ cells (64). Second, do such therapeutic strategies for remedying the abnormal epigenetic state need to be specific for epigenetically altered genes? (65). This issue has already been addressed by the engineering of gene-specific molecules that can reactivate specific genes (66,67). Third, could drugs that have more global effects be more effective? Finally, will the effects of molecules such as antisense RNA or specific constructs (fusion constructs) be limited by the efficiency of their delivery in vivo? (23).

ACKNOWLEDGMENTS

This work was supported by a Fournier-Pharma studentship (to C.G.-K.) and by grants from INRA (Institut National de la Recherche Agronomique), INSERM (ATC-Nutrition, PRNH [Programme National de Recherches sur la Nutrition Humaine]), the Association Française des Diabétiques, and the Institut Benjamin Delessert.

REFERENCES

1. Grundy SM: Obesity, metabolic syndrome, and cardiovascular disease. *J Clin Endocrinol Metab* 89:2595–2600, 2004
2. Hales CN, Barker DJ: The thrifty phenotype hypothesis. *Br Med Bull* 60:5–20, 2001
3. Boloker J, Gertz SJ, Simmons RA: Gestational diabetes leads to the development of diabetes in adulthood in the rat. *Diabetes* 51:1499–1506, 2002
4. Dabelea D, Hanson RL, Lindsay RS, Pettitt DJ, Imperatore G, Gabir MM, Roumain J, Bennett PH, Knowler WC: Intrauterine exposure to diabetes conveys risks for type 2 diabetes and obesity: a study of discordant sibships. *Diabetes* 49:2208–2211, 2000

5. Issa JP: Epigenetic variation and human disease. *J Nutr* 132:2388S–2392S, 2002
6. Armitage JA, Khan IY, Taylor PD, Nathanielsz PW, Poston L: Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? *J Physiol* 561:355–377, 2004
7. Khan IY, Dekou V, Hanson M, Poston L, Taylor PD: Predictive adaptive responses to maternal high-fat diet prevent endothelial dysfunction but not hypertension in adult rat offspring. *Circulation* 110:1097–1102, 2004
8. Ozanne SE, Hales CN: Lifespan: catch-up growth and obesity in male mice. *Nature* 427:411–412, 2004
9. Jablonka E, Lamb MJ: The changing concept of epigenetics. *Ann N Y Acad Sci* 981:82–96, 2002
10. Spotswood HT, Turner BM: An increasingly complex code. *J Clin Invest* 110:577–582, 2002
11. Waterland RA, Garza C: Potential mechanisms of metabolic imprinting that lead to chronic disease. *Am J Clin Nutr* 69:179–197, 1999
12. Egger G, Liang G, Aparicio A, Jones PA: Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 429:457–463, 2004
13. Waterland RA, Jirtle RL: Early nutrition, epigenetic changes at transposons and imprinted genes, and enhanced susceptibility to adult chronic diseases. *Nutrition* 20:63–68, 2004
14. Blondeau B, Avril I, Duchene B, Breant B: Endocrine pancreas development is altered in foetuses from rats previously showing intra-uterine growth retardation in response to malnutrition. *Diabetologia* 45:394–401, 2002
15. Srinivasan M, Aalinkel R, Song F, Patel MS: Programming of islet functions in the progeny of hyperinsulinemic/obese rats. *Diabetes* 52:984–990, 2003
16. Wu G, Bazer FW, Cudd TA, Meiningner CJ, Spencer TE: Maternal nutrition and fetal development. *J Nutr* 134:2169–2172, 2004
17. MacLennan NK, James SJ, Melnyk S, Piroozzi A, Jernigan S, Hsu JL, Janke SM, Pham TD, Lane RH: Uteroplacental insufficiency alters DNA methylation, one-carbon metabolism, and histone acetylation in IUGR rats. *Physiol Genomics* 18:43–50, 2004
18. Melzner I, Scott V, Dorsch K, Fischer P, Wabitsch M, Bruderlein S, Hasel C, Moller P: Leptin gene expression in human preadipocytes is switched on by maturation-induced demethylation of distinct CpGs in its proximal promoter. *J Biol Chem* 277:45420–45427, 2002
19. Yokomori N, Tawata M, Onaya T: DNA demethylation modulates mouse leptin promoter activity during the differentiation of 3T3-L1 cells. *Diabetologia* 45:140–148, 2002
20. Shemer R, Eisenberg S, Breslow JL, Razin A: Methylation patterns of the human apoA-I/C-III/A-IV gene cluster in adult and embryonic tissues suggest dynamic changes in methylation during development. *J Biol Chem* 266:23676–23681, 1991
21. Pinto S, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, Friedman JM, Horvath TL: Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science* 304:110–115, 2004
22. Bouret SG, Draper SJ, Simerly RB: Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science* 304:108–110, 2004
23. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ: Epigenetic programming by maternal behavior. *Nat Neurosci* 7:847–854, 2004
24. Waterland R, Garza CG: Potential for metabolic imprinting by nutritional perturbation of epigenetic gene regulation. In *Public Health Issues in Infant and Child Nutrition*. Vol. 48. Black R, Michaelson KF, Eds. New York, Lippincott Williams & Wilkins, 2002, p. 317
25. Feinberg AP: Methylation meets genomics. *Nat Genet* 27:9–10, 2001
26. Zaina S, Nilsson J: Insulin-like growth factor II and its receptors in atherosclerosis and in conditions predisposing to atherosclerosis. *Curr Opin Lipidol* 14:483–489, 2003
27. Pembrey M: Imprinting and transgenerational modulation of gene expression; human growth as a model. *Acta Genet Med Gemellol (Roma)* 45:111–125, 1996
28. Junien C: [Genomic imprinting: from tug of war to solidarity between the generations]. *Médecine/Sciences* 3:336–344, 2000 [article in French]
29. Beaudet AL, Jiang YH: A rheostat model for a rapid and reversible form of imprinting-dependent evolution. *Am J Hum Genet* 70:1389–1397, 2002
30. Young LE: Imprinting of genes and the Barker hypothesis. *Twin Res* 4:307–317, 2001
31. Constancia M, Kelsey G, Reik W: Resourceful imprinting. *Nature* 432:53–57, 2004
32. Reik W, Constancia M, Fowden A, Anderson N, Dean W, Ferguson-Smith A, Tycko B, Sibley C: Regulation of supply and demand for maternal nutrients in mammals by imprinted genes. *J Physiol* 547:35–44, 2003
33. Keverne EB: Genomic imprinting and the maternal brain. *Prog Brain Res* 133:279–285, 2001
34. Plagge A, Gordon E, Dean W, Boiani R, Cinti S, Peters J, Kelsey G: The imprinted signaling protein XL alpha s is required for postnatal adaptation to feeding. *Nat Genet* 36:818–826, 2004
35. Curley JP, Barton S, Surani A, Keverne EB: Coadaptation in mother and infant regulated by a paternally expressed imprinted gene. *Proc Biol Sci* 271:1303–1309, 2004
36. Rakyan VK, Chong S, Champ ME, Cuthbert PC, Morgan HD, Luu KV, Whitelaw E: Transgenerational inheritance of epigenetic states at the murine Axin(Fu) allele occurs after maternal and paternal transmission. *Proc Natl Acad Sci U S A* 100:2538–2543, 2003
37. Delrue MA, Michaud JL: Fat chance: genetic syndromes with obesity. *Clin Genet* 66:83–93, 2004
38. Umlauf D, Goto Y, Cao R, Cerqueira F, Wagschal A, Zhang Y, Feil R: Imprinting along the Kcnq1 domain on mouse chromosome 7 involves repressive histone methylation and recruitment of Polycomb group complexes. *Nat Genet* 36:1296–1300, 2004
39. Constancia M, Hemberger M, Hughes J, Dean W, Ferguson-Smith A, Fundele R, Stewart F, Kelsey G, Fowden A, Sibley C, Reik W: Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature* 417:945–948, 2002
40. Sibley CP, Coan PM, Ferguson-Smith AC, Dean W, Hughes J, Smith P, Reik W, Burton GJ, Fowden AL, Constancia M: Placental-specific insulin-like growth factor 2 (Igf2) regulates the diffusional exchange characteristics of the mouse placenta. *Proc Natl Acad Sci U S A* 101:8204–8208, 2004
41. Lefebvre L, Viville S, Barton SC, Ishino F, Keverne EB, Surani MA: Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene Mest. *Nat Genet* 20:163–169, 1998 [see comments]
42. Moraes RC, Blondet A, Birkenkamp-Demtroeder K, Tirard J, Ormtoft TF, Gertler A, Durand P, Naville D, Begeot M: Study of the alteration of gene expression in adipose tissue of diet-induced obese mice by microarray and reverse transcription-polymerase chain reaction analyses. *Endocrinology* 144:4773–4782, 2003
43. Takahashi M, Kamei Y, Ezaki O: Mest/Peg1 imprinted gene enlarges adipocytes and is a marker of adipocyte size. *Am J Physiol Endocrinol Metab*, 2004
44. Poirier LA, Brown AT, Fink LM, Wise CK, Randolph CJ, Delongchamp RR, Fonseca VA: Blood S-adenosylmethionine concentrations and lymphocyte methylenetetrahydrofolate reductase activity in diabetes mellitus and diabetic nephropathy. *Metabolism* 50:1014–1018, 2001
45. Maier S, Olek A: Diabetes: a candidate disease for efficient DNA methylation profiling. *J Nutr* 132:2440S–2443S, 2002
46. Hiltunen MO, Turunen MP, Hakkinen TP, Rutanen J, Hedman M, Makinen K, Turunen AM, Aalto-Setälä K, Ylä-Herttua S: DNA hypomethylation and methyltransferase expression in atherosclerotic lesions. *Vasc Med* 7:5–11, 2002
47. Lund G, Andersson L, Lauria M, Lindholm M, Fraga MF, Villar-Garea A, Ballestar E, Esteller M, Zaina S: DNA methylation polymorphisms precede any histological sign of atherosclerosis in mice lacking apolipoprotein E. *J Biol Chem*, 2004
48. Ying AK, Hassanain HH, Roos CM, Smiraglia DJ, Issa JJ, Michler RE, Caligiuri M, Plass C, Goldschmidt-Clermont PJ: Methylation of the estrogen receptor-alpha gene promoter is selectively increased in proliferating human aortic smooth muscle cells. *Cardiovasc Res* 46:172–179, 2000
49. Dong C, Yoon W, Goldschmidt-Clermont PJ: DNA methylation and atherosclerosis. *J Nutr* 132:2406S–2409S, 2002
50. Post WS, Goldschmidt-Clermont PJ, Wilhide CC, Heldman AW, Sussman MS, Ouyang P, Milliken EE, Issa JP: Methylation of the estrogen receptor gene is associated with aging and atherosclerosis in the cardiovascular system. *Cardiovasc Res* 43:985–991, 1999
51. Vickers MH, Breier BH, Cutfield WS, Hofman PL, Gluckman PD: Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. *Am J Physiol Endocrinol Metab* 279:E83–E87, 2000
52. Kaati G, Bygren LO, Edvinsson S: Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. *Eur J Hum Genet* 10:682–688, 2002
53. Martin JF, Johnston CS, Han CT, Benyshek DC: Nutritional origins of insulin resistance: a rat model for diabetes-prone human populations. *J Nutr* 130:741–744, 2000
54. Drake AJ, Walker BR, Seckl JR: Intergenerational consequences of fetal programming by in utero exposure to glucocorticoids in rats. *Am J Physiol Regul Integr Comp Physiol* 288:R34–R38, 2005
55. Patel MS, Srinivasan M: Metabolic programming: causes and consequences. *J Biol Chem* 277:1629–1632, 2002

56. Campbell JH, Perkins P: Transgenerational effects of drug and hormonal treatments in mammals: a review of observations and ideas. *Prog Brain Res* 73:535–553, 1988
57. Yoder JA, Walsh CP, Bestor TH: Cytosine methylation and the ecology of intragenomic parasites. *Trends Genet* 13:335–340, 1997 [see comments]
58. Lane N, Dean W, Erhardt S, Hajkova P, Surani A, Walter J, Reik W: Resistance of IAPs to methylation reprogramming may provide a mechanism for epigenetic inheritance in the mouse. *Genesis* 35:88–93, 2003
59. Whitelaw E, Martin DI: Retrotransposons as epigenetic mediators of phenotypic variation in mammals. *Nat Genet* 27:361–365, 2001
60. Waterland RA, Jirtle RL: Transposable elements: targets for early nutritional effects on epigenetic gene regulation. *Mol Cell Biol* 23:5293–5300, 2003
61. International Human Genome Sequencing Consortium: Initial sequencing and analysis of the human genome. *Nature* 409:860–921, 2001
62. Brown R, Strathdee G: Epigenomics and epigenetic therapy of cancer. *Trends Mol Med* 8 (Suppl. 4):S43–S48, 2002
63. Strathdee G, Brown R: Epigenetic cancer therapies: DNA methyltransferase inhibitors. *Expert Opin Investig Drugs* 11:747–754, 2002
64. Kelly TL, Trasler JM: Reproductive epigenetics. *Clin Genet* 65:247–260, 2004
65. Jaenisch R, Bird A: Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 33 (Suppl.):245–254, 2003
66. Zhang L, Spratt SK, Liu Q, Johnstone B, Qi H, Raschke EE, Jamieson AC, Rebar EJ, Wolffe AP, Case CC: Synthetic zinc finger transcription factor action at an endogenous chromosomal site: activation of the human erythropoietin gene. *J Biol Chem* 275:33850–33860, 2000
67. Xu GL, Bestor TH: Cytosine methylation targeted to pre-determined sequences. *Nat Genet* 17:376–378, 1997
68. Mann MR, Lee SS, Doherty AS, Verona RI, Nolen LD, Schultz RM, Bartolomei MS: Selective loss of imprinting in the placenta following preimplantation development in culture. *Development* 131:3727–3735, 2004