

Report on the IASO Stock Conference 2006: early and lifelong environmental epigenomic programming of metabolic syndrome, obesity and type II diabetes

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Summary

Now that analysis of the organization of the human genome sequence is reaching completion, studies of the finely tuned chromatin epigenetic networks, DNA methylation and histone modifications, are required to determine how the same DNA sequence generates different cells, lineages and organs, i.e. the phenotype. Maternal nutrition, behaviour and metabolic disturbances as well as other environmental factors have been shown to have major effects on these epigenetic processes, potentially affecting the predisposition of offspring to obesity and related adult disorders. The March 2006 Stock Conference considered the latest evidence from studies in the field of obesity and other related areas that elucidate mechanisms by which the environment can modify gene expression and the resulting individual phenotype. Presentations included evaluation of the molecular basis of epigenetic memory and the nature of relevant sequence targets, windows of susceptibility, and maternal dietary and behavioural factors that determine epigenetic changes. Imprinted genes, age and tissue-related exposures, transgenerational and potential interventions were also discussed. In summary, it is clear that epigenetic alterations can no longer be ignored in evaluations of the causes of obesity and its associated disorders. There is a need for systematic large-scale epigenetic studies of obesity, employing appropriate strategies and techniques and appropriately chosen environmental factors in critical spatio-temporal windows.

Keywords: Developmental programming, epigenetics, genomic imprinting, obesity.

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Introduction

Despite significant effort, understanding of the molecular causes and mechanisms of common forms of obesity and associated disorders remains a major challenge. With the completion of the human genome sequence organization, understanding epigenetic (epiG) control of the genome is the next step towards deciphering how the same DNA sequence – under the influence of environmental factors – gives rise to different cells, lineages and organs (1). EpiG is the study of changes in gene expression which occur in the absence of mutation but which are mitotically heritable.

EpiG mechanisms are consistent with various non-Mendelian features of obesity such as the relatively high degree of discordance in monozygotic twins, males and female differences, and individual variation in normal biology and disease states.

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 developmental time windows, have persistent effects on offspring health and are even transmitted to the next generation. Recent studies have demonstrated that

common disorders like obesity, diabetes, hypertension, asthma and even schizophrenia take root in early nutrition, during gestation and lactation hence the new term 'developmental origin of health and disease' (2). Several lines of evidence now suggest that epiG could be the mechanism whereby the developmental environment influences adult phenotype (3). Thus, in addition to a number of gene variants conferring susceptibility (4), individuals with metabolic syndrome (MetS), obesity and type II diabetes (T2D) may not only show a lifelong imbalance between energy intake and energy expenditure but may also have suffered improper epiG programming during their early development due to placental insufficiency, inadequate maternal nutrition and metabolic disturbances (5).

The environment modifies epiG patterns of susceptible genes during development with permanent alterations in appetitive control, metabolic balance and fuel utilization. Moreover, epiG alterations occurring day after day and accumulating over time as age-, diet- and disease-related deteriorations intermingle with several other processes. These include chromosomal instability, telomere shortening, mitochondrial deteriorations, or the oscillatory, circadian, and seasonal rhythms with rhythmic expression of clock genes, one of which was recently identified as a histone acetyl transferase, a key player of the epiG machinery (6). These processes are known to deteriorate during progression to MetS, under the influence of oxidative stress, ageing and the folate status, leading to the relaxation (or silencing) of gene expression of a number of key genes. EpiG variation is prevalent in the population and is a risk factor for acquired diseases. Moreover, transgenerational effects (TGE), due to improperly erased epiG marks – triggered by behaviour and nutrition of previous generations – may occur.

This 2006 Stock Conference organized by David York, sponsored by International Association for the Study of Obesity, and cochaired by Peter Nathanielsz (University of Texas Health Science Center, San Antonio, TX, USA) and Claudine Junien (Inserm U 781, Paris, France), aimed to bridge the gap between epiG and environmental factors responsible for developmental programming in obesity. The conference aimed to provide examples of the achievements from other more advanced fields (e.g. cancer, atherosclerosis and maternal behaviour) to demonstrate their potential importance and evaluate their potential application to the field of obesity. The invited speakers included Lucilla Poston (King's College, London, UK), Susan Ozanne (University of Cambridge, UK), Claude Remacle (University of Louvain la Neuve, Belgium), Laura Cox (South-west Foundation for Biomedical Research, San Antonio, TX, USA), Richard Simerly (Oregon Health and Science University, Beaverton, OR, USA), Caroline McMillen (Sansom Research Institute University of South Australia, Adelaide Australia), Emma Whitelaw (Queensland Institute of

Medical Research, Australia), Robert Waterland (Baylor College of Medicine, USDA, Children's Nutrition Research Center, Houston, TX, USA), Lanlan Shen (MD Anderson Cancer Center, Houston, TX, USA), Mosche Szyf (Mc Gill University, Montreal, Canada), Gavin Kelsey (Babraham Institute, Cambridge, UK).

Discussion included a spectrum of epiG mechanisms such as metastable alleles, genomic imprinting, tissue-specific effects, paramutation, age-related epiG alterations and epiG polymorphism, as well as epiG inheritance (TGE) in animal models. Several experimental procedures used to evaluate the impact of environmental factors were considered. Finally, consideration was given to potential reversibility by epiG therapeutic and dietary approaches.

What is the molecular basis of the epigenetic memory?

Epigenetics: 'epi' from the Greek root: 'above' genetics was first coined in the 1940s by Waddington as 'the causal interactions between genes and their products which bring phenotype into being' (7). Today the term epiG is used to refer to stably maintained mitotically (and potentially meiotically) heritable patterns of gene expression that occur without changes in DNA sequence.

The epiG 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 that 'orchestrate' the genome activity together with RNA interference. The epiG codes define a process involving the recruitment of a myriad of chromatin-remodelling complexes, insulator proteins, histone exchange chaperones, enzymes, coregulators and effectors, directing appropriate chromatin remodelling (8). MicroRNAs (or siRNAs) regulate gene expression, direct silencing machinery to promoters, heterochromatin formation, and genome stability and often arise from demethylation of tandem repeats that are common in pericentromeric sequences (9). Thus the epigenome comprises components acting at the transcriptional level – DNA methylation and histone modifications – and at the post-transcriptional level – RNA interference.

There are many covalent epiG modifications that keep genes stably repressed or active: the best-studied epiG modification is DNA methylation, a covalent modification at many of the cytosine residues followed by a guanine residue, the CpG dinucleotide. In most cases, acquisition and maintenance of such 'CpG methylation' induces silencing of gene expression. Additionally, gene expression is determined by modifications of histones in the nucleosomes around which the DNA is wrapped. These post-translational modifications of histone proteins in turn modulate chromatin structure (8). The core histones H2A, H2B, H3 and H4 are subject to many different modifica-

tions, including acetylation, methylation and phosphorylation. In general, active genes are heavily acetylated, and methylated on histone H3 lysine 4 which regulates transcription by recruiting nucleosome remodelling enzymes and histone acetylases. Conversely, deacetylation and lys 27 methylation negatively regulate transcription by promoting a compact chromatin structure.

Chromatin modifications require a complex machinery including several entities with DNA/protein binding properties and enzymatic activities such as heterochromatin protein 1; histone deacetylase (HDAC); histone acetyl transferase; DNA methyl transferase (DNMT); histone methyl transferase; methyl binding domain proteins with demethylase activity, etc. Histone modifications influence chromatin structure in a combinatorial manner and within the context of other chromatin modulations like DNA methylation, histone exchange, histone remodelling and nuclear organization: interaction between DNA methylation and histone H3-K9 methylation form a reinforcing silencing loop. Specific epiG patterns condition chromatin accessibility to transcription factors, facilitating recognition by these factors of genes to be expressed (to various extents) and genes to be silenced, transiently or permanently in a stage- and/or tissue-specific manner.

Target genes or sequences

DNA methylation patterns and histone modifications are responsive to the environment through life. Transient nutritional stimuli occurring at critical ontogenic stages may have long-lasting influences on expression of various genes by interacting with epiG mechanisms and altering chromatin conformation and transcription factor accessibility (3,10,11). Methylation and expression are affected by environmental and genetic influences such as embryo culture conditions (12), DNMT1 overexpression (13), hyperhomocysteinaemia (11) and folate deficient or supplementation diets before and during pregnancy, in the post-natal and post-weaning period and may persist into adulthood (14,15).

There are several types of sequences associated with specific epiG makeup that can be targets of environmental factors: (i) unique genes, e.g. the glucocorticoid receptor (GR) (16), or, more likely, specific subsets of unique genes belonging to different pathways or systems (17); (ii) genes present as multiple copies such as of genes coding ribosomal RNAs (18,19); (iii) imprinted genes (IG), monoallelically expressed genes (15,20–22); (iv) transposable elements adjacent to or located within genes interfering with the expression processes (cf. Agouti^{vy}) (14,23–26) and (v) repeated sequences whether or not transposable with different roles according to their chromosomal location (cf. telomeres, centromeres) (27–29).

CpG(s) or CpNpG(s) in transcription or repressor factor binding site

Epigenetic alterations like DNA methylation can occur at a site within promoter sequences involved in differential regulation of DNA–protein interactions that are necessary for defining the three-dimensional chromatin structure necessary for gene transcription (30). One mechanism by which methylation suppresses gene expression is by inhibiting transcription factor binding to its methylated binding site or by promoting the binding of repressor factors. Differences in the DNA methylation pattern between the offspring of high- and low-licking/grooming rat mothers are associated with altered histone acetylation and transcription factor [nerve-growth factor-induced clone A (NGFI-A) binding to the GR in the hippocampus (16)]. Interestingly this specific rodent maternal behaviour during early post-natal development appears to determine the methylation status of a single CpG dinucleotide within the promoter region of the GR gene in the offspring's hippocampus.

CpG islands

In the human genome, approximately 4–8% of Cs are methylated, and 5-mC constitutes about 1% of total genome residues (31). Potentially there are 100 million CpG dinucleotide demethylation targets in the diploid mammalian genome. The dinucleotide CpG occurs once per 80 dinucleotides throughout 98% of the genome; however, the frequency is increased in 'CpG islands'. There are approximately 29 000 CpG islands in the human genome, and 50–60% of all genes contain a CpG island (31). Although CpG sequences spread throughout the genome are usually heavily methylated those occurring in CpG islands are less methylated. Genes with CpG islands can be unmethylated regardless of their transcription state. In contrast genes without CpG islands may show various methylation patterns, which do not necessarily reflect the gene's transcriptional activity (32). About 5% of CpG island loci can be methylated in a tissue-specific manner in normal human tissues (33,34). Data from Feltus *et al.* indicate that CpG islands differ in their intrinsic susceptibility to *de novo* methylation, and suggest that the propensity for a CpG island to become aberrantly methylated can be predicted based on its sequence context (35).

Genes adjacent to or containing transposable elements

A key function of epiG genome modification of eucaryotic cells is to suppress transcriptional activity of retroelements. Specific transposable elements induce epiG instability, thus allowing early diet to influence epigenotype. Proximity of such elements may render many human genes epigeneti-

cally labile as has been demonstrated for two mutant mice – the Agouti viable yellow A^{vy} and the Axin-fused Ax^{Fu} – harbouring an insertion of an intracisternal A particle (IAP) retrotransposon. There are thousands of IAP retrotransposons in mice, but many may not be transcriptionally active. The transposable elements present in humans include endogenous retroviral sequences, Alu sequences, and diverse long interspersed element-1 (LINE-1 or L1) LINE sequences, which are usually hypermethylated (36). Inter-individual variability and parent of origin DNA methylation differences at specific human Alu elements have been described (37). The sequencing of the human genome has shown that transposable elements (short interspersed elements, LINEs, etc.) account for about 35% to 40% of the genome and are found in about 4% of human genes (38). It would be interesting to determine whether these genes are involved in energy homeostasis regulation. However, no human transposable elements able to resist demethylation during pre-implantation like murine IAP sequences have yet been identified (39).

Genomically imprinted genes

For most human genes, the two alleles contribute equally to the gene product. In contrast, IG are monoallelically expressed (i.e. from either paternal or maternal allele) and thus functionally haploid. To date, some 80 genes have been found to be imprinted in humans and mice. There is compelling evidence that DNA methylation of IG varies between tissues, individuals and disease conditions in humans and various animals and with ageing – in which both hyper- and hypomethylation are observed – and even during the course of pathological processes leading to cancer or atherosclerosis (40–43).

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 IG are among the most promising targets for programming, evolutionary modifications and TGE in response to rapid changes in the nutritional environment, such as those associated with the worldwide epidemic of MetS, obesity, and T2D [for review (5)].

Epigenetic reprogramming

When are the marks cleared and re-established?

Epigenetic reprogramming of the genome is an essential process that occurs during both primordial germ cell (PCG) development and early embryogenesis so that epiG marks are cleared and reset between generations. There are two possibilities for marks to be cleared and re-established: either in the germline or in early development. In fact methylation marks still retained in sperm undergo

rapid demethylation within hours of fertilization. After fertilization, the paternal and maternal genomes in the zygote undergo rapid demethylation at coding sequences (genes) and at repetitive sequences (transposable elements). In theory, epiG marks are largely erased and then reset in a lineage-specific fashion. With the exception of a brief period of global demethylation – active for the paternal haploid genome and passive for the maternal one – in the early mammalian embryo, transposons are normally silenced by promoter CpG methylation. Marks on the paternal genome are actively cleared within hours of fertilization whereas maternal genome marks are cleared over days. However, transposons like IAP sequences may escape this epiG silencing (39). In germ cells, parental methylation imprints in IG escape this demethylation process and *de novo* methylation. IG imprints are only eliminated before PCG reach the gonadal ridge and are appropriately reinstalled during male and female gametogenesis (44).

When and where are the marks laid down?

After implantation, active *de novo* methylation takes place, to various extents, depending on the part of the embryo concerned. The bulk of the genome becomes hypermethylated in the embryonic ectoderm and mesoderm, through active *de novo* methylation, whereas the genome of extraembryonic cells, such as primary endoderm and trophoblast, remain hypomethylated. There is a sequence of *de novo* methylation that dictates structure and function of each somatic tissue, through a finely tuned pattern involving the switching on and off of gene expression.

The case of somatic cell nuclear transfer (= cloning) and intrauterine growth restriction

The majority of cloned mammals derived by nuclear transfer die during gestation and display neonatal phenotypes resembling large offspring syndrome, often with respiratory and metabolic abnormalities, and have enlarged and dysfunctional placentas. Multiple investigators have reported weight increases in cloned mice. Yet because obesity was not passed on to offspring of the clones, it is unlikely to reflect any genetic changes in the clones but instead to reflect epiG abnormalities arising from inadequate nuclear reprogramming. While the paternal haploid genome undergoes rapid active demethylation and the maternal a slower passive demethylation to the same extent, the somatic donor cell demethylates partially, roughly to the same level as extraembryonic tissues (45,46). This rescue by breeding is strong evidence that the obesity effect is due to dys-regulation of IG. These anomalies are particularly evident following nuclear transfer but also

occur in embryos produced using *in vitro* culture alone (e.g. *in vitro* fertilization). The severity of the dys-regulation is greater in placental than foetal tissues, thus altering energy utilization (carbohydrate, protein and lipid metabolism) and transport function with consequences on angiogenesis, surface area, active and passive transporters. Nuclear transfer also affects higher order functions, like eating behaviour as well as foetal outcome. The reprogramming of tissues derived from the trophectoderm is incomplete. In contrast, ICM cells continue to express high levels of required methylation-related enzymes, allowing the nucleus more time to re-establish a normal methylation pattern (45,46). The ability to reprogramme the phenotype of one cell for another would essentially lead to our ability to take easily accessible material such as skin fibroblasts and reprogramme it to become tissues such as specific neuronal types, cardiac muscle, and hepatocytes (47).

Because cultured cells and transformed cells may exhibit DNA methylation differences in comparison with native cells, they do not represent an appropriate resource for the study of epiG patterns (27).

Can environmental factors influence these processes?

At every stage during the cascade of epiG fluctuations (foetal development and ageing), the nutritional balance must be 'optimal'. EpiG changes occur through life in response to environmental, behavioural, physiological and pathological signals. Moreover, environmental factors and nutrition could also have an impact on how faithfully patterns of epiG modifications are maintained throughout life (3).

Critical spatio-temporal windows

Several reports have shown that epiG programming is tightly time and space-regulated during foetal development and lactation. Developmental stages in multicellular organisms proceed according to a temporally and spatially precise pattern of gene expression. Therefore, there are critical windows for specific placental nutrient supply and foetal demand. In specific cell lineages, and at defined developmental stages, chromatin is modified in a way that leads to acquisition of constant gene repression, or activation. Methylation requires dietary methyl donors and cofactors (mammalian one carbon metabolism). During development of multicellular organisms, cells become different from one another by changing their genetic programme in response to transient stimuli. Tissue-specific patterns of CpG methylation are established during development. Long after the stimulus is gone, 'cellular memory' mechanisms enable cells to remember their chosen fate over many cell divisions (48).

Diets and genomic imprinting

The epiG effect of different weanling diets has been studied on genomically IG and on genes adjacent to or containing transposable elements using C57BL/6J females bred with Castaneus males. F1 Hybrid offspring were weaned onto a synthetic methyl-deficient diet (no methionine, choline, vit B12 or folic acid), or a synthetic control diet, or a natural control diet. Weanling diet affects the paternally expressed *Igf2* gene Loss Of Imprinting (LOI) (both synthetic diets affected LOI in kidney). When mice were fed a methyl donor-deficient diet, this led to down-regulation of the imprinted *Igf2* gene. This reduced expression correlated with hypermethylation of the paternal allele at a differentially methylated region (15).

Diets and transposable elements

The A^{vy} and $Axin^{Fu}$ phenotypes can be influenced by providing the mice with a specific diet (26,49,50). In the A^{vy} mice the mouse food was complemented with extra folic acid (folate), vitamin B12, choline and betaine which are thought to enhance methyl donor (SAM) metabolism. If non-agouti dams (a/a) are mated with male mice carrying the A^{vy} allele (A^{vy}/a), the proportion of pseudoagouti offspring carrying a methylated IAP sequence, can be increased, by feeding dams a methyl-supplemented diet during pregnancy (22,50–52). Moreover, the coat colour phenotype and A^{vy} methylation pattern persisted into adulthood (14). Similarly, maternal genistein (a soy bean phytoestrogen) alters coat colour and protects A^{vy} offspring from obesity through foetal epigenome modification (25). Thus, an environmental factor increases the probability of methylation upstream from the A^{vy} locus (14,26,52–54).

Maternal nutrition and developmental programming of obesity

Human epidemiologic studies have shown that prenatal nutrient deprivation followed by rapid infant growth results in offspring obesity (55). Multiple animal investigations have followed up on the human epidemiology. Maternal nutrient restriction in early ovine pregnancy increases percentage of fat in offspring (56). The predisposition to obesity results from a mismatch between the diet to which offspring are exposed early in development and nutrient availability later in development and adulthood. Thus, offspring of rats protein restricted during pregnancy but fed a normal diet during lactation become obese in adulthood (57). Mouse foetuses exposed to protein restriction *in utero* have increased predisposition to obesity as adults (58). Recent data show that catch-up growth immediately after early malnutrition should be a key point for the programming of obesity (59). Abdominal obesity, one of the key

features of MetS, appears in malnourished offspring and is aggravated by early catch-up growth. Higher rates of intra-abdominal obesity observed after growth restriction may participate to hypertension and create atherothrombotic conditions leading to the development of cardiovascular diseases (60). One of the earliest studies, in the baboon, showed that overfeeding neonatal baboons prior to weaning results in fat cell hypertrophy that is restricted to female offspring (61).

Physiological mechanisms involved in programming of obesity

Several mechanisms have been proposed for the programming of obesity as a result of suboptimal nutrient environments during development. These include altered appetite control, reduced physical activity, programmed adipocyte metabolism and mitochondrial function. Prenatal overnutrition in sheep alters appetite regulation and impairs intrahypothalamic regulatory signalling (62,63). Leptin administration on post natal days 3–13 to rats exposed to prenatal nutrient restriction reverses both the hyperphagia and obesity in adult life (64). Leptin promotes neuronal outgrowth from the arcuate nucleus to the paraventricular nucleus in the neonatal hypothalamus during the lactation period thus altering the wiring phenotype of the appetitive centres by stimulating development of appetite stimulatory neuropeptide Y projections and inhibiting appetite inhibitory projections (65). One important study demonstrated reduced activity levels in hyperphagic, obese offspring of rats undernourished in pregnancy but maintained on a rich diet after delivery (66). Thermogenesis is reduced in adult offspring of nutrient-restricted pregnant mice (67). In rats, an isoenergetic low-protein diet given throughout gestation perturbs the development of the endocrine pancreas by reducing beta-cell mass and islet vascularization at birth. Taurine, an important amino acid during development, has been found to be low in foetal and maternal plasma. When added to a low-protein diet, taurine normalizes beta-cell mass (68). Finally offspring mitochondrial dysfunction has been demonstrated following nutrition-induced development programming (69).

Imprinted genes, post-natal adaptations

Imprinted genes also play a key role in post-natal development and have enduring effects on metabolism. The GNAS complex is an imprinted domain encoding the stimulatory G-protein alpha subunit (G(s)alpha), 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). Multiple gene products of the Gnas gene

result from alternative splicing of different first exons onto a common exon 2. These products include G(s)alpha, the ubiquitously expressed G protein required for receptor-stimulated cAMP production; the neuroendocrine-specific extralarge G(s)alpha isoform (XLalphas), a paternally expressed G(s)alpha isoform; and neuroendocrine-specific protein (Nesp55), a maternally expressed chromogranin-like protein. G(s)alpha undergoes tissue-specific imprinting, being expressed primarily from the maternal allele in certain tissues. The Nespas DMR is the principal ICR at the Gnas cluster and functions bidirectionally as a switch for modulating expression of the antagonistically acting genes Gnasxl and Gnas (70). Paternal vs. maternal transmission of a G(s)alpha knockout produces opposite effects on energy metabolism (71).

Heterozygous mutation of exon 2 on the maternal (E2m+/+) or paternal (E2+/p-) allele results in opposite effects on energy metabolism. The loss of paternal function is associated with a decrease in adiposity, hypermetabolic function, hypoglycaemia, a decrease in locomotor activity and resistance to parathyroid hormone, whereas the loss of maternal function is associated with greater adiposity. +/p- and m-/++ mice have increased and decreased activation of the sympathetic nervous system respectively (72). This is the first animal model in which a single genetic defect leads to opposite effects on energy metabolism depending on parental inheritance.

XLalphas knockout mice [Gnasxl(m+/p-)] have poor suckling and perinatal lethality, implicating XLalphas as critical for post-natal feeding. They also display a spectrum of phenotypic effects indicating that XLalphas controls a number of key post-natal physiological adaptations, including poor suckling, low blood glucose, insulin and glucagon levels and altered energy homeostasis (73). Brown adipose tissue shows a lack of lipid vesicles, and impaired temperature regulation. In the brain, areas expressing (XLas) 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 post-natal adaptation to feeding (71,73). Opposing metabolic phenotypes imply functional antagonism of paternal XLas and maternal Gsa, despite similar biochemical functions on thermogenic genes, adipogenic genes, mitochondrial biogenesis, lipid oxidation. Accordingly, removal of maternal Gnas expression rescued Gnasxl deficiency showing that XLas functions antagonistically to Gsa (73).

Chen *et al.* studied the effects of G(s)alpha deficiency without disrupting other Gnas gene products by deleting G(s)alpha exon 1 (E1). The lean, hypermetabolic and insulin-sensitive E2+/p- phenotype appears to result from XLalphas deficiency, whereas loss of paternal-specific G(s)alpha expression in E1+/p- mice leads to an opposite metabolic

phenotype. The weight gain after weaning is increased for *Gnas ex2^{m/+}* compared with *Gnas ex2^{p/+}*. Thus, alternative *Gnas* gene products have opposing effects on glucose and lipid metabolism. Differences between *E1m/+* and *E1p/+* mice presumably result from differential effects on *G(s)alpha* expression in tissues where *G(s)alpha* is normally imprinted (74). The *Gnas* locus also plays an important role in adult metabolic homeostasis. Adult *Gnasxl(m+/p-)* mice had reduced fat mass and lipid accumulation in adipose tissue, with increased food intake and metabolic rates. Gene expression profiling was consistent with increased lipid metabolism in adipose tissue. *XLalphas* (or *XLN1*) is a negative regulator of sympathetic nervous system activity in mice (75). The *Nesp* transcript, expressed exclusively from the maternal allele, codes for neuroendocrine secretory protein 55 (*Nesp55*), a chromogranin-like polypeptide associated with the constitutive secretory pathway but with an unknown function. *Nesp* is expressed in restricted brain nuclei, suggesting an involvement in specific behaviours. *Nesp55*-deficient mice develop normally, excluding a role of this protein in the severe post-natal effects associated with imprinting of the *Gnas* cluster. Behavioural analysis of adult *Nesp55* mutants revealed, in three separate tasks, abnormal reactivity to novel environments independent of general locomotor activity and anxiety (76).

Post-natal programming by maternal care

There are profound maternal effects on individual differences in defensive responses and reproductive strategies in a wide range of species. Stress responses in adult rats are programmed early in life by maternal care and associated with epigenomic marking. In mammals, these effects appear to 'programme' emotional, cognitive and endocrine systems, increasing sensitivity to adversity. In highly adverse environments, such effects may be considered adaptive, increasing the offspring chances of surviving to sexual maturity; however, they have a cost in the form of an increase in the risk of various types of disease in later life (77). Maternal behaviour can direct gene offspring expression by transmitting a message concerning the nature of the environment the animal will have to live in, rather than just whether that environment is favourable or unfavourable (77).

Increased pup licking and grooming (LG) and arched-back nursing (ABN) by rat mothers altered the offspring epigenome at a hippocampal GR gene promoter. Differences in DNA methylation pattern between offspring of female rats with high and low levels of LG behaviour are associated with changes in DNA methylation, histone acetylation and transcription factor [NGFI-A binding to the GR gene in the hippocampus (16)]. This specific type of maternal behaviour during early post-natal development in rodents appears to determine the methylation status of

a single CpG dinucleotide within the offspring hippocampal GR gene promoter region. These differences emerged over the first week of life, were reversed with cross-fostering, and persisted into adulthood.

Is it possible to reverse these effects of maternal care on DNA methylation and behaviour in the adult rat? There are several substances that interfere with the epiG machinery, increasing or decreasing DNA or histone methylation, or histone acetylation. Some of these are natural nutrients or metabolites like folic acid (vit B9), SAM or methionine, others like valproic acid (VPA) or trichostatin A (TSA) are drugs. HDAC inhibitors induce replication-independent active demethylation. VPA stimulates replication-independent active demethylation in a dose-dependent manner. Central infusion of a HDAC inhibitor, TSA, or methionine can reverse both epiG states, restoring or impairing the group (high-LG and low-LG mothers) differences in histone acetylation, DNA methylation, NGFI-A binding, GR expression and hypothalamic-pituitary-adrenal corresponding reactivity to stress in adult offspring responses to stress (16,17). Altogether this suggests a causal relationship among epigenomic state, GR expression and stress responses in the adult offspring. Thus despite the inherent stability of the epigenomic marks established early in life through behavioural programming, they are potentially reversible in the adult brain. Thus increase in one amino acid (methionine) in the brain could alter DNA methylation and alter behaviour in adult brain (78).

Age and disease-related epigenetic alterations

During ageing, an epiG drift is observed, consisting in a global hypomethylation with hyper or hypomethylation of CpG in several gene promoters thus substantially altering their level of expression (2–7%). Thus epiG alterations occur and are reversed more frequently than genetic changes, often produce mosaic patterns of gene expression or silencing and can be inherited through the germline (3,79). The global loss of methyl C has been estimated to 2% or more (10%) (80–82). The age-dependent methylation change in CpG island seems infrequent. While repetitive sequences may be major constituents contributing to the age-related decrease of methylation, the hypermethylation of ribosomal DNA repeats with age in rat liver (83) may in turn lead to a decreased transcription of numerous genes.

It was thought that most epiG changes are coupled to DNA replication. However, emerging evidence that DNA methylation and chromatin modification can also occur in a replication-independent manner has challenged this notion. This epiG plasticity may facilitate changes in gene expression in response to the oscillatory, circadian and seasonal rhythms responsible for rhythmic modulation of expression of a substantial proportion of genes during the

course of the day and to environmental events throughout a cell's entire life (84).

The disruption of genomic DNA methylation patterns was the first epiG abnormality to be described in human cancer. This imbalance involves the promoter CpG island hypermethylation of tumour-suppressor genes, causing transcriptional repression, and global genomic hypomethylation, leading to chromosomal instability and reactivation of endoparasitic sequences. Local hypermethylation starts in normal cells and accumulates at a variable rate with age. Reminiscent of the inflammatory processes associated with obesity, studies in ulcerative colitis and hepatocellular cirrhosis and neoplasia revealed that chronic inflammatory states are accompanied by marked increases in CpG island methylation, perhaps as a result of increased cell turnover, in normal-appearing tissues. This confirms the hypothesis that proinflammatory exposures could account for part of the epiG variation in human populations and can be viewed as resulting in premature ageing of cells (85,86). From the study of methylation levels of the ER, MYOD1, SFRP1 (secreted frizzled-related protein 1), and MGMT genes in normal colon whether or not associated with colon cancer, it is suggested that epiG variation related to ageing, lifestyle, exposures and possibly genetic factors, is one of the modulators of acquired, age-related human diseases, including neoplasia (85,86). The repression of tumour-suppressor genes by promoter hypermethylation is also associated with a specific histone modification index (87–89).

A key step of the atherogenic process is the proliferation and migration of vascular smooth muscle cells (SMC) into the intimal layer of the arterial conduit. The phenotype of SMC, once within the intima, is known to switch from contractile to dedifferentiated. Ying *et al.* have shown that the genome of aortic SMC is responsive to environmental conditions, and that DNA methylation, in particular methylation of the oestrogen receptor alpha ($ER\alpha$), could contribute to the switch in phenotype observed in these cells (90). The oestrogen receptor beta gene ($ER\beta$) has essential roles in vascular function. $ER\beta$ KO mice have abnormal vascular tone and develop age-related hypertension. Coronary atherosclerotic tissues showed higher methylation levels than normal appearing arterial and venous tissues. In comparing $ER\beta$ methylation between plaque and non-plaque regions, $ER\beta$ methylation was more important in plaque than in normal carotid in ascending aorta, common carotid artery, and femoral artery. *In vitro* senescing aortic endothelial and SMC cell lines showed progressive methylation. Thus epiG changes in $ER\beta$ accumulate at a variable rate with age and contribute to the development of atherosclerosis (91). $ER\beta$ expression was restored in cultured vascular cells after treatment with DAC (DNMT inhibitor) or TSA (HDAC inhibitor) and even more with both inhibitors in both cell lines (91). This is an interesting model to

test the efficiency of appropriate diets/nutrients as preventing tools in patients at risk.

Transgenerational effects

Although it has long been thought that the epiG slate is wiped clean in the embryo shortly after fertilization, with the exception of IG, there are now many examples in mammals of clear TGE. This clean slate would correlate with the totipotency of the zygote. Incomplete erasure at genes associated with a measurable phenotype results in unusual patterns of inheritance from one generation to the next, termed transgenerational epiG inheritance. Epidemiological data suggesting or demonstrating the existence of TGE have been obtained for humans (3,92–100). The involvement of epiG processes has not yet been demonstrated in human foetal programming or in either of the examples of possible transmission to subsequent generations cited above. Such demonstrations would require the development of new strategies adapted to humans.

Consistent with an epiG and/or gene expression-based mechanism for transgenerational inheritance, there is increasing evidence that, nutritional intervention (caloric, iron and protein restriction or a fat-rich or carbohydrate-rich diet, betel nut consumption), endocrine disruptors, cyclophosphamide, maternal diabetes, behavioural programming (maternal care), glucocorticoids or exercise stress – during pregnancy and lactation – can affect the following generation(s) (16,24,39,50,57,100–119).

Most of these studies assumed that these TGE are the consequences of malprogramming due to an abnormal intrauterine milieu/post-natal maternal feeding or behaviour of the F1 generation. However, germline epiG inheritance may also explain these TGE (50,111). As already suggested by Campbell and Perkins and supported by data and observations published in 28 papers on TGE of drug and hormonal treatments in mammals (from 1954 to 1982), there were at least four different non-mutually exclusive possible mechanisms for induced carryover effects: (i) effects transmitted by males and through multiple generations; (ii) male transmission through a single generation; (iii) transmission through multiple female generations and (iv) progressive change while animals are kept under inducing conditions (104).

In most animal models in which the existence of TGE has been established only the first-generation animals – males and/or pregnant females – were subjected to the stimulus: endocrine disruptors, low-protein diets, betel-nut chewing, radiotherapy as used for cancer treatment, particular types of maternal behaviour, folate-deficient diets, glucocorticoids, etc. Exposure was thus limited to a single generation and little is known about the cumulative effects of exposure over several generations. In only one study carried out two decades ago, Stewart *et al.* studied colonies

of rats that had been maintained for 12 generations on diets with adequate levels of protein or marginally deficient in protein. In the malnourished colony, the proportion of 'small-for-gestational-age' offspring was 10 times higher than that for the well-nourished colony (120).

Several studies have demonstrated the existence of sex-specific effects that are still poorly understood. The sex specificity of these effects operates at different levels: (i) sex of parent transmitting the consequences of stimulus exposure and (ii) sex of offspring displaying the maternal effect or TGE. Recent studies have shown that maternal or paternal epiG inheritance may be influenced by strain background (50). Although our understanding of the fundamental biological mechanisms underlying such phenomena remains rudimentary the effects could be due to cytoplasmic, hormonal or metabolic influences or preferential influence on gametogenesis in one sex but not in the other or to gender-specific reprogramming of IG expression [reviewed in (121)].

Epigenetic inheritance

The methylation clearing is not complete and on a global DNA level, is reduced to $\pm 10\%$ (122,123). That could represent 90% of methylated genes being erased and that 10% of genes retain their methylation, or there could be numerous combinations of the two. It is also unknown what happens to the histone modifications through these phases of loss of DNA methylation signals.

What type of sequence could be the epiG 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 (124). 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 IAP, retrotransposon that escape this epiG silencing may interfere with the expression of neighbouring genes in several ways (39,50,125). The first two examples of TGE in mice explained by epiG 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 (22,50). CpG methylation of the transposable IAP sequence in the Agouti region varies considerably in A^{VY} mice and is inversely correlated with ectopic *agouti* expression. This maternal epiG effect is not the result of a maternally contributed environment. Rather, these data show that it results from incomplete erasure of an epiG modification when a silenced A^{VY} allele is passed through the female germ line, with consequent inheritance of the

epiG modification. The variable phenotypes of the offspring result from incomplete elimination of the epiG modification when allele A is transmitted via the maternal germ line. 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 (14,125). However, recent experiments have shown that maternal or paternal epiG inheritance is influenced by strain background (50).

Dietary supplementation with a methyl donor during pregnancy increases the proportion of pups carrying a methylated IAP sequence (22,50). Nutrition probably exerts its effects on methylation early in embryonic development, and these effects concern all tissues. Waterland also showed that coat colour phenotype and A^{VY} methylation pattern persisted into adulthood (14). Consistent with the idea of transgenerational epiG inheritance, the methylation status of the $Axin^{Fu}$ allele in mature sperm reflects the methylation status of the allele in the somatic tissue, suggesting that this locus is not subject to epiG reprogramming during gametogenesis (50).

The familial predisposition to T2D is mediated by both genetic and intrauterine environmental factors. In the normal course of events, maternal genes always develop in the same uterus, thus restricting studies aimed at investigating the relative contribution of these factors. Gill-Randall *et al.* have developed an embryo transfer paradigm in rats to overcome this difficulty. Interestingly, in GotoKakizaki rats, a euglycaemic intrauterine environment cannot overcome the strong genetic predisposition to diabetes. However, in Wistar rats with a low genetic risk of diabetes, exposure to hyperglycaemia *in utero* significantly increases the risk of diabetes in adult life (126). Thus a control intrauterine environment cannot overcome the effects of genetic predisposition. In contrast, even in animals with a low risk the abnormal uterine milieu becomes detrimental. These experimental data in laboratory animals are confirmed by epidemiological studies on infants of mothers suffering from diabetes or malnutrition during pregnancy (127).

Thus altogether these data support the hypothesis that abnormal glucose/insulin homeostasis may result from three different but non-exclusive causes: (i) genetic predisposition; (ii) maternal epiG inheritance and (iii) an abnormal intrauterine milieu. Interestingly, a control intrauterine environment cannot overcome the effects of the first and second ones.

The vicious cycle of mother-to-daughter transmission

The diabetic pregnancy in the offspring again induces a diabetogenic effect into the next generation, via adapta-

tions during foetal development (128). The TGE, from the diabetic mother into the third generation, fetuses and adults, is only transmitted via the maternal line: female offspring of diabetic mothers develop gestational diabetes and induce the effect in their fetuses and thereby into the next generation. Male offspring have impaired glucose tolerance, but do not transmit the effect to their offspring (100,127,129–131). Furthermore, neonatal exposure to maternal diabetes through the intake of dam's milk in rats leads to a complex malprogramming of hypothalamic orexigenic and anorexigenic circuits that are critically involved in the lifelong regulation of food intake, body weight and metabolism (132).

Does maternal obesity and/or diet during pregnancy cause metabolic imprinting in offspring, perpetuating obesity across generations? Waterland *et al.* compared offspring of obese A^y/a dams and lean a/a dams maintained on a control or a methyl-supplemented diet. The A^y allele was passed through the female germline for several generations. Maternal obesity and offspring body weight at weaning were higher in the F1 generation from A^y dams than in that from a/a dams. Mean weight increased with each successive generation in A^y/a offspring only. Thus maternal obesity during pregnancy can cause metabolic imprinting in the offspring, perpetuating obesity across generations. This transgenerational increase in obesity was tempered by the methyl-supplemented diet, suggesting a role for DNA methylation (Waterland *et al.* unpublished). Hence, the A^y mouse can be employed as a sensitive epiG biosensor to assess the effects of an environmental factor on locus-specific DNA methylation (14,26,52–54). Owing to genotype–epigenotype–environmental interactions, there are thus three factors potentially accelerating weight gain: A^y genotype, maternal obesity and methyl donor supplementation, suggesting that too many vitamins can make us fat? (15). Given the increasing proportion of women who are overweight and overfed when pregnant these results are important in terms of public health.

Neonatal female rat pups raised artificially on a high-carbohydrate (HC) milk formula during the suckling period immediately developed hyperinsulinaemia, which became chronic during the post-weaning period, when these rats were fed a laboratory diet. These rats developed obesity in adulthood. Second-generation pups born to these female rats spontaneously developed chronic hyperinsulinaemia and adult-onset obesity (HC phenotype) in the absence of dietary intervention during the suckling period. This metabolic programming, once established, also forms a vicious cycle, because HC female rats spontaneously transmit the HC phenotype to their progeny (101,133). In contrast, studies in the offspring of glucocorticoid-treated rats dams, suggest that the effects can also be transmitted through the paternal line (102).

Behavioural imprinting is perpetuated transgenerationally

TGE are cyclic, with mothers with high levels of licking/grooming behaviour teaching their female offspring to behave in a similar manner. What type of epiG mechanism can be involved? Methylation can repress gene expression by inhibiting transcription factor binding to the methylated binding site or by promoting the binding of repressors (30). Variations in maternal behaviour are associated with differences in $ER\alpha$ expression in the medial preoptic area (MPOA) and are transmitted across generations. Thus, the female offspring of high-LG mothers display high levels of $ER\alpha$ expression in the MPOA and become high-LG mothers themselves when adult (17,134). Cross-fostering studies have confirmed the association between maternal care and $ER\alpha$ expression in the MPOA. Levels of cytosine methylation in the $ER\alpha$ 1b promoter were significantly higher in the adult offspring of low-LG mothers than in the adult offspring of high-LG mothers, LG mothers. These findings suggest that maternal care is associated with cytosine methylation of the $ER\alpha$ 1b promoter, providing a potential mechanism for the programming of individual differences in $ER\alpha$ expression and maternal behaviour in female offspring (134). Thus a mother-to-daughter epiG transmission can affect somatic tissues, not necessarily germline, and result in perpetuating the effect by affecting maternal metabolism or maternal behaviour.

Dietary and therapeutic perspectives

In contrast to genetics, epiG is reversible. EpiG drugs or nutrients could be used to either activate or silence disease-related genes and their proteins. Whatever the genes or sequences involved, deciphering the epiG patterns at stake should allow us to evaluate their potential reversibility. Once the specific epiG 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) (135–137).

Is there an 'optimal methylation diet'? Can too many vitamins make us fat, depressed?

Many micronutrients and vitamins are critical for DNA synthesis/repair and maintenance of DNA methylation patterns. Folate has been most extensively investigated in this regard because of its unique function as methyl donor for nucleotide synthesis and biological methylation (DNA and histone methylation). Ingrosso *et al.* showed that total DNA hypomethylation was higher in men with hyperho-

homocysteinaemia and uraemia than in controls and allelic expression was changed in both sex-linked and imprinted genes. Folate therapy, a common method to reduce hyperhomocysteinaemia, restored DNA methylation to normal levels and corrected the patterns of gene expression (11). The methyl groups are ultimately derived from methionine. High dietary methionine intake might therefore be expected to increase DNA methylation. However, because of the circular nature of the methionine cycle, methionine excess may actually impair DNA methylation by inhibiting remethylation of homocysteine. Aberrant hypermethylation of DNA could be deleterious (54). Alcoholism, which leads to folic acid deficiency, is linked to depressive disorders and dementia and alcoholic liver disease (ALD) (138). Halsted *et al.* showed on different animal models that chronic alcohol exposure impairs folate absorption by inhibiting expression of the reduced folate carrier and decreasing the hepatic uptake and renal conservation of circulating folate. At the same time, folate deficiency accelerates alcohol-induced changes in hepatic methionine metabolism while promoting enhanced oxidative liver injury and the histopathology of ALD (139).

Using the agouti A^{vy} model, Wolff *et al.* showed that providing excess folic acid, vitamin B12, choline, and betaine to female mice before and during pregnancy shifts the coat colour of their agouti A^{vy}/a offspring towards the brown (pseudoagouti) phenotype (52). Waterland and Jirtle later demonstrated that maternal diet affects the coat colour distribution of offspring by increasing methylation at the A^{vy} metastable epiallele (14). Subsequently, the study by Waterland *et al.* in the A^{vy} mouse showed that nutritional stimuli during development, in the post-weaning period, may induce persistent changes in the epiG regulation of IG (15). Unexpectedly, however, these studies emphasize that dietary supplements may not always be beneficial, and can have aberrant effects on the epiG regulation of gene expression (54). Because locus-specific DNA hypomethylation is implicated in the aetiology of various cancers and developmental syndromes, clinical trials of 'promethylation' dietary supplements are already under way (54).

In the epiG programming model by maternal behaviour, adult rat hippocampal GR gene expression of low LG-ABN offspring is modified to the expression of high LG-ABN offspring by TSA. In contrast with TSA treatment methionine treatment causes active remethylation of the NGFI-A binding site on the GR promoter and reverses the effect of high maternal LG-ABN. L-methionine treatment of adult male offspring of high LG-ABN maternal care reverses response to stress. Thus despite the inherent stability of the epigenomic marks established early in life through behavioural programming, they are potentially reversible in the adult brain. Increase in one amino acid (methionine) in the brain could alter DNA methylation and alter behaviour in adult brain (78).

In some patients with severe depression, intracellular folic acid deficiencies, in the absence of anaemia, are associated with central nervous system deficiencies in monoamine metabolites (140). Methionine treatment, useful in depression and dementia (141,142) can exacerbate psychosis in most schizophrenic and manic patients (143). In contrast, valproate, a drug producing hypomethylated DNA, reduces such symptoms in a mouse treated with L-methionine that models some of the molecular neuropathologies detected in schizophrenia, including the hypermethylation of reelin and glutamic acid decarboxylase gene promoter CpG islands and the down-regulation of expression (144). Hence nutritional supplementation in adulthood can in some cases correct for genetically based epiG deficiencies, hinting at the importance of an 'optimal methylation diet' (145). Recent developments in epigenomic approaches that survey locus-specific DNA methylation on a genome-wide scale offer broader opportunities to assess the effects of high methionine intake on mammalian epigenomes (54).

Alleviating malprogramming by diet?

There are now a few examples testing whether transfer of the malprogramming phenotype, due to high-fat or HC diets, to the progeny could be reversed or attenuated by maternal nutritional interventions. Recent data suggest that an appropriate dietary fatty-acid profile and intake during the periconceptual/gestation/lactation period helps the female offspring to cope with deleterious intrauterine conditions. Gallou-Kabani *et al.* investigated whether reducing fat intake during the periconceptual/gestation/lactation period, in mice mothers with high-fat diet (HFD)-induced obesity, could be used to modify foetal/neonatal MetS programming positively, thereby preventing MetS. Sensitivity/resistance to the HFD differed significantly between generations and sexes. Despite having free access to the HFD, a significantly higher proportion of female mice were no longer hyperphagic, remained lean, with normal insulin sensitivity and normal glycemia, but mild hypercholesterolemia and glucose intolerance, thus displaying an adapted 'satiety phenotype' (117). Srinivasan *et al.* have previously shown that artificial rearing of newborn female rat pups on a HC milk formula resulted in chronic hyperinsulinaemia and adult-onset obesity (HC phenotype) and that the maternal HC phenotype was transmitted to their progeny (2-HC rats) because of foetal development in the HC female rat. Modification of the intrauterine environment in HC female rats was achieved by pair feeding them to the amount of diet consumed by age-matched control rats from the time of their weaning. This mild dietary restriction reversed their HC phenotype and also prevented the development of the HC phenotype in their progeny (133). As shown by Waterland *et al.* maternal obesity during preg-

nancy can cause metabolic imprinting in the a/a offspring of A^{vy}/a obese mice, perpetuating obesity across generations. This transgenerational increase in obesity was tempered by the methyl-supplemented diet, suggesting a role for DNA methylation (Waterland *et al.* unpublished) (22). Similarly, maternal genistein protected A^{vy} offspring from obesity through modification of the foetal epigenome (25). Hence, the A^{vy} mouse can be employed as a sensitive epiG biosensor to assess the effects of dietary methionine supplementation on locus-specific DNA methylation.

Pharmacoeigenomics: could epigenetically inactivated genes be pharmacologically activated?

Epigenetic states might determine response to drugs. In the emerging field of pharmacoeigenomics, phase I, II and III clinical trials with DNMT1 and HDAC inhibitors (5-aza-Cytidine, Zebularine, SAHA, TSA) are underway. A hallmark of cancer is a paradoxical aberration of DNA methylation patterns, a global loss of DNA methylation, that coexists with regional hypermethylation of certain genes. Specific 'histone index' are also observed in human and mouse tumours (88). DNA methylation inhibitors can block tumour growth but can also induce prometastatic genes (146). There is therefore an urgent need to dissociate between these two contradictory effects to enable use of methylation inhibitors therapeutically (88). Chromatin acetylation and DNA methylation are found in a dynamic interrelation and the consequences of HDAC inhibitors are not limited to changes in histone acetylation but they also bring about a change in the state of modification of DNA (147). Thus the combined use of several agents represents an interesting therapeutical tool, provided side-effects are carefully identified (148).

In addition, non-epiG drugs might have epiG effects. As shown with a recent 'Folate polyp' trial of colon polyp prevention using aspirin (ASA) vs. folate, DNA methylation is potentially a target for disease prevention. The clinical data showed that ASA protects, while folate borderline increases polyp recurrences. The methylation of the ER gene and LINE-repeated sequences was measured at exit colonoscopy at 3 years: the percentage of methylation of the ER gene was much lower in patients treated with ASA compared with placebo, and gradually increased in patients with no recurrence, recurrence of adenoma to recurrence of serrated adenoma. The percentage of LINE methylation increases by folate status quartile (85).

Adipogenesis is dependent on the sequential activation of several transcription factors. VPA has been used for decades in the treatment of epilepsy, and is also effective as a mood stabilizer and in migraine therapy. As a histone deacetylase (HDAC) inhibitor, VPA induces widespread epiG reprogramming which also involves demethylation of specific genes (149). VPA inhibits mouse 3T3 L1 and

human pre-adipocyte differentiation. TSA also inhibited adipogenesis, whereas the VPA analogue valpromide, which does not possess HDAC inhibitory effects, did not prevent adipogenesis. These data highlight an interesting role for HDAC activity in adipogenesis that can be blocked by treatment with VPA and/or TSA (150).

Conclusion

The importance of understanding how gene expression is programmed in early life and throughout life, under the influence of the environment needs no further demonstration. This corresponds to the new field of epigenomics. While it is clear that epiG modifications are mitotically heritable, the fidelity of this process is not well understood. Inheritance through more than one generation of meioses is even less well studied. So it remains unclear to what extent epiG changes contribute to phenotypic variation in natural populations. How might such evidence be obtained? What are the features of phenotypes that might suggest an epiG component? The answers to such questions must come from studies designed specifically to detect subtle, stochastically determined phenotypic variation and epiG tissue specificity of epiG marks in suitable animal models (1,107).

Understanding epiG alterations as a driving force in obesity and associated disorders opens new fields of research on mechanisms underlying development of appetite regulation/glucose homeostasis, in epidemiology, risk assessment, and treatment. Molecular studies of obesity would benefit from adding an epiG perspective. Candidate gene approaches and epigenomic approaches are required to identify human metastable epialleles to characterize epiG changes associated with programming and with disease. Knowledge of altered gene and cellular mechanisms is essential to the development of new therapeutic approaches and interventions for prevention and clinical management of obesity and its related conditions.

Conflict of Interest Statement

No conflict of interest was declared.

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