Epigenetic mechanisms that underpin metabolic and cardiovascular diseases

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Abstract | Cellular commitment to a specific lineage is controlled by differential silencing of genes, which in turn depends on epigenetic processes such as DNA methylation and histone modification. During early embryogenesis, the mammalian genome is 'wiped clean' of most epigenetic modifications, which are progressively re-established during embryonic development. Thus, the epigenome of each mature cellular lineage carries the record of its developmental history. The subsequent trajectory and pattern of development are also responsive to environmental influences, and such plasticity is likely to have an epigenetic basis. Epigenetic marks may be transmitted across generations, either directly by persisting through meiosis or indirectly through replication in the next generation of the conditions in which the epigenetic change occurred. Developmental plasticity evolved to match an organism to its environment, and a mismatch between the phenotypic outcome of adaptive plasticity and the current environment increases the risk of metabolic and cardiovascular disease. These considerations point to epigenetic processes as a key mechanism that underpins the developmental origins of chronic noncommunicable disease. Here, we review the evidence that environmental influences during mammalian development lead to stable changes in the epigenome that alter the individual's susceptibility to chronic metabolic and cardiovascular disease, and discuss the clinical implications.

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Learning objectives

Upon completion of this activity, participants should be able to:
1. Identify the window during which the epigenome is susceptible to environmental cues in mammals.
2. List growth factors that affect the risk for adult cardiovascular and metabolic diseases.
3. Describe the animal models used to explain the epigenetic basis of adult cardiovascular disease.
4. Describe the evidence for periconceptual influences on subsequent development in humans.

Competing interests
The authors and the Journal Editor V. Heath and the CME questions author D. Lie declare no competing interests.

Introduction

Early evidence that the fetal environment influences subsequent susceptibility to chronic disorders came from experimental studies[1] and epidemiological research that showed increased rates of cardiovascular disease in historical cohorts that had experienced high infant mortality.[2] Further studies revealed an inverse relationship between birthweight and susceptibility to hypertension, cardiovascular morbidity, insulin resistance, type 2 diabetes mellitus, hyperlipidemia and obesity.[2] These observations led to the hypothesis that fetal metabolic adjustments in nutritionally adverse circumstances that aim to restrict growth and thus safeguard brain development may result in an increased risk of chronic disorders in later stages of life.[3] Yet, some data, such as those from survivors of the Dutch 'Hunger Winter' (a short-term famine in 1944–45) indicate that individuals who were exposed to adverse conditions *in utero* need not have low birthweight to exhibit adverse effects subsequently.[4] This observation is consistent with the previous finding of a continuous relationship between birthweight and risk of cardiovascular disease[5] and with recent observations that demonstrated stronger correlations between metabolic dysfunction and neonatal adiposity, leptin concentrations in the umbilical cord and maternal nutrition than with birthweight.[6,7] Other studies focused on the role of excess nutrition and rapid weight gain in infants,[8] the risk of which is increased after impaired fetal growth. Whereas most research has involved pathways through

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Key points

- Developmental plasticity enables an organism to respond to environmental cues and adjust its phenotypic development to match its environment
- Developmental plasticity is effected, at least in part, by epigenetic changes that are established in early life and modulate gene expression during development and maturity
- In mammals, the window during which the epigenome is susceptible to nutritional cues extends from conception to at least weaning
- Mismatch between the early and mature environments may result in inappropriate patterns of epigenetic changes and gene expression that increase subsequent susceptibility to metabolic and cardiovascular diseases
- The available evidence suggests that interventions to prevent metabolic and cardiovascular diseases should focus on the prenatal and early postnatal periods

which undernutrition during development translates into subsequent metabolic disease, children who are exposed to hyperglycemia *in utero* or whose mothers are obese are also at increased risk of developing metabolic disorders, especially type 2 diabetes mellitus.[9]

Such observations have led to a reappraisal of the ways in which three key factors—genome, development and environment—influence the adult phenotype, including the individual's susceptibility to disease.[10] Although genome-wide association studies have identified some loci that influence interindividual variability in disease suscepibility, the effects of most alleles seem to be small.[11] Different ethnic groups that live in the same geographic areas and share similar environmental risks have different profiles of disease markers and prevalence, which may suggest a genetic cause for differences in disease susceptibility.[12,13] Yet, with some notable exceptions,[14] few ancestry-specific alleles have been discovered that can explain particular pathologies. Other explanations of both interindividual and ethnic differences in disease risk, therefore, need to be considered. Of note, high incidences of metabolic disease are found in those ethnic groups in which the average birthweight is low[15] or the rates of gestational diabetes and maternal obesity are high.[16]

Untangling the effects of genes from those of environmentally determined developmental processes is not straightforward. Organisms possess an evolved ability to respond to external signals by adjusting their phenotype during development to match their environment. This mechanism has been termed developmental plasticity.[17] We have discussed elsewhere how environmental cues during early life in mammals can lead to adaptive and integrated changes that relate to the anticipated adult environment, but may also give rise to disease if the environment changes or the prediction is inaccurate, which causes a mismatch between the organism and its circumstances.[18] Importantly, fetal nutrition does not equate to maternal food intake, but rather is dependent on maternal metabolism, cardiovascular function and, particularly, placental function.[19]

The long-lasting changes in developmental trajectory that underpin altered susceptibility to disease may arise, at least in part, from epigenetically mediated alterations in gene expression. Whereas compelling evidence supports both the developmental origins of health and disease and the underlying epigenetic mechanisms,[20] many features of the latter remain insufficiently understood. These elements include the differences among epigenetic mechanisms across species and between patterns of epigenetic modifications on paternal and maternal genomes, the mechanisms that regulate the establishment, stability and flexibility of epigenetic changes, and the precise connection between an epigenetic change, altered gene expression and the resultant phenotype. In this Review, we summarize the evidence in support of the theory that environmental influences, especially nutrition and stress, during mammalian development lead to permanent changes in the epigenome, which in turn increase the risk of chronic metabolic and cardiovascular diseases in later stages of life. We end by discussing implications for prevention and treatment.

Epigenetic mechanisms in development

The term epigenetics is used in this Review to refer to molecular mechanisms that establish and maintain mitotically stable patterns of gene expression, but that do not alter the genomic DNA sequence. Epigenetic mechanisms enable developing organisms to produce disparate—and stable—cellular phenotypes from the same genotype; this use of the term echoes its original use by Waddington in reference to developmental pathways.[21] Epigenetic modifications to chromatin, described in detail elsewhere,[22–26] include 5' methylation of the cytosine residue in CpG dinucleotides of DNA, covalent modifications (including methylation, acetylation, phosphorylation and ubiquitination) of histones, the proteins that package DNA into chromatin, and the gene-regulating and chromatin-organizing activities of noncoding RNAs. These epigenetic modifications change the binding of transcription activators and repressors to specific gene promoters, and/or alter the large-scale conformation and function of chromatin itself, which modulates gene expression (Figure 1). The best-studied examples of developmental epigenetic processes in mammals include X-chromosome inactivation in females and parent-specific expression of imprinted genes.[27,28] In general, DNA methylation seems to be involved in long-term silencing of gene expression, whereas histone modifications have a short-term and flexible effect, but substantial crosstalk exists between these different mechanisms.[22,29]

Epigenetic processes probably first evolved to silence retroviral invasion of the genome and then were co-opted to regulate tissue differentiation as metazoans developed.[30] Developmentally plastic responses, which are found in a wide range of metazoans,[31] and imprinting, which has a recent evolutionary origin as it is restricted to mammals and flowering plants,[32] may represent further extensions of these processes. These regulatory mechanisms have different phylogenies, although they share common epigenetic effectors, and may operate at different times during development and differ in their sensitivity to

environmental stimuli; care is, therefore, required when we extrapolate from one set of phenomena to another.

The early postconceptional period is a critical window for the establishment of DNA methylation patterns. In mammals, the methylation profile of the genome is reprogrammed during gametogenesis and in early embryogenesis.[29] After fertilization, rapid demethylation of the entire paternal genome occurs, except in paternally imprinted genes, heterochromatin around centromeres and some repetitive elements.[33] Of the three DNA methyl-transferases (Dnmts), Dnmt 1 is responsible for maintaining patterns of methylation, whereas Dnmt 3a and Dnmt 3b seem to be required for *de novo* methylation.[28] By contrast, the maternal genome undergoes a relatively slow demethylation.[28] Levels of methylation are lowest at the morula stage, before lineage-specific *de novo* methylation begins at the blastocyst stage. Methylation is more pronounced in the inner cell mass (which gives rise to somatic tissues) than in the trophoectoderm (the future placenta). For example, methylation of the promoter region of transcription factor Elf5 is critical for cellular commitment to the embryonic stem-cell lineage, rather than to the trophoblast lineage.[34] At a later stage of development, organisms show tissue-specific patterns of both DNA methylation[35,36] and histone modification.[37,38]

During gametogenesis, primordial germ cells lose DNA methylation before their migration to the genital ridge, after which new patterns of methylation are established (before birth in the male germ line and after birth in the female line).[33,39] This differential timing of remethylation during gametogenesis may have implications for studies of transgenerational transmission of epigenetic modifications (see below) in terms of the critical periods during which the developing progeny's gametes (F_1 generation) are vulnerable to nutritional or other influences that were imposed on the maternal (F_0) generation.

Environmental influences
Metastable epialleles in mice
The fact that epigenetic modifications might be sensitive to environmental stimuli is demonstrated by studies of metastable epialleles in mouse strains. These alleles have arisen because of retrotransposon insertions into the genome at points that affect the phenotype: epigenetic modification, and therefore expression, of the alleles can be affected by the developmental environment. In the agouti viable yellow (A^{VY}) mouse, hypomethylation of the agouti gene promoter results in increased gene expression; hence, the mouse develops a yellow coat color as well as obesity, as the agouti gene product interferes with regulation of body weight at the level of the hypothalamus. Conversely, hypermethylation decreases expression of agouti and results in mice with normal weight and brown coat color. In this strain of mice, maternal deficiency of methyl donors and cofactors (such as folate) results in agouti promoter hypomethylation, increased prevalence of obesity, and cancer, and yellow coat color in the offspring.[40] By contrast, maternal feeding of the soy isoflavone genistein
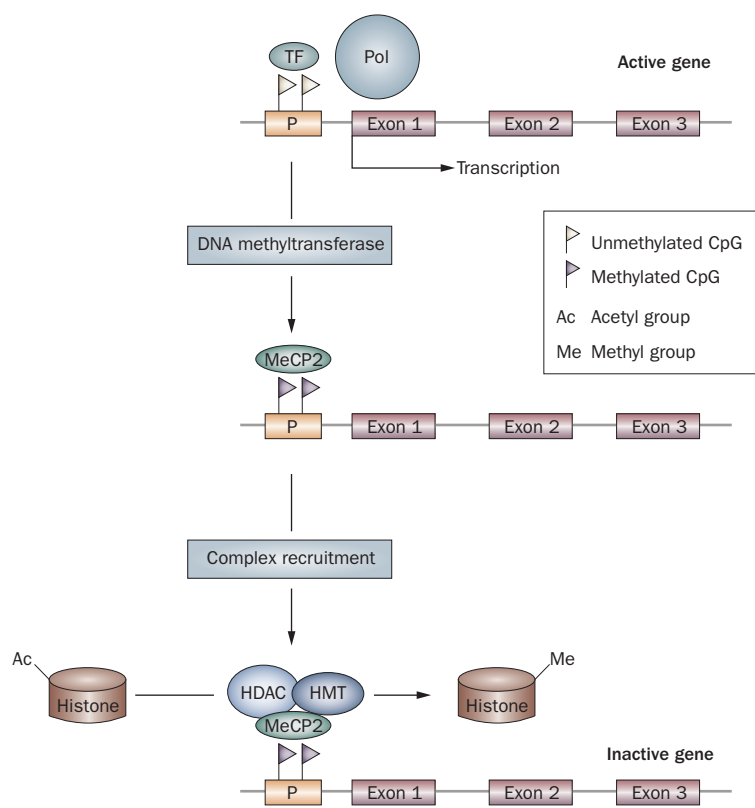
Figure 1 | Epigenetic modulation of gene expression. When CpG dinucleotides in the gene promoter are unmethylated, transcription factors and RNA polymerase can bind to their specific nucleotide sequences and transcription of coding sequences (exons) occurs. Methylation of CpGs by DNA methyltransferases leads to the binding of methyl-CpG binding protein 2, which in turn recruits the histone-modifying enzymes histone deacetylase and histone methyltransferase to form a complex that is bound to the promoter. This complex deacetylates histones and catalyzes methylation of specific lysine residues. These modifications cause the chromatin strand to adopt a 'tight' conformation, which prevents access of transcription factors and RNA polymerases to the DNA and results in the silencing of transcription. Abbreviations: HDAC, histone deacetylase; HMT, histone methyltransferase; MeCP2, methyl-CpG binding protein 2; P, promoter; Pol, RNA polymerase; TF, transcription factor.

leads to promoter hypermethylation, decreased prevalence of obesity and brown coat color in the offspring.[41] Similar effects of the mother's nutrition on the offspring's epigenome are also apparent in the imprinted axin-fused mouse model, in which ample maternal intake of methyl donors before and during pregnancy results in increased DNA methylation of the axin-fused gene promoter, which manifests as reduced kinking of the tail.[42] Promoter hypermethylation of this gene specifically affects the tail, which indicates that hypermethylation occurs midgestation and that epigenome plasticity is not limited to the early stages of embryonic development.

Other developmental models
Increasing evidence suggests that changes in the epigenome caused by early environmental cues are associated with increased susceptibility to metabolic disease later in

life. Most of this evidence arises from animal models, but some early supportive evidence has also emerged from studies in humans. Importantly, however, evidence for a direct causal relationship between epigenetic changes, gene expression and observable phenotype comes from studies of cultured cells, in which epigenetic status can be manipulated by knockout of components of the epigenetic machinery, or by pharmacological inhibition of DNA methylation or histone deacetylation, which lead to changes in gene expression and cell behavior that can be observed.[34,43,44] At present, epigenetic research on the developmental origins of various diseases is largely performed in animal models and a few retrospective human studies, which allow only inferential conclusions to be reached from the observed associations.

Animal models of the developmental origins of disease have attempted to mimic human pathology by alterations in maternal nutrition (energy or protein intake) during pregnancy and weaning, by impairment of placental perfusion, or by administration of glucocorticoids to the mother. These interventions were followed by examination of probable effector systems in the offspring, such as the hypothalamic–pituitary–adrenal axis, the pancreas or some parts of the cardiovascular system, and measurement of the activity of relevant metabolic pathways as well as changes in gene expression and epigenetic modifications.

Feeding a low-protein diet to pregnant rats causes hypertension and endothelial dysfunction in the offspring. These alterations are accompanied by metabolic and gene-expression changes, including overexpression of the hepatic glucocorticoid and PPARα receptors, and epigenetic changes that facilitate transcription of these receptors: for the glucocorticoid receptor, these changes include histone modifications, promoter hypomethylation and reduced expression of Dnmt1.[45] Promoter hypomethylation of PPARα is associated with reduced methylation of individual CpG dinucleotides.[46] In a similar model of a low-protein maternal diet, increased expression of the type 1B adrenal angiotensin receptor in the offspring from the first week of life was accompanied by hypomethylation of the proximal promoter of the gene encoding this receptor.[47] In another model that addressed the etiology of hypertension, uteroplacental insufficiency caused increased p53 expression in the kidneys, in association with reduced methylation of its promoter and reduced Dnmt-1 activity; the investigators concluded that increased expression of the gene that encodes p53 increased renal apoptosis and reduced the number of glomeruli.[48]

Uteroplacental insufficiency was also used to generate a rodent model of pancreatic β-cell dysfunction that develops diabetes in adulthood. The molecular lesion that underlies this pathology is underexpression of the pancreas-specific transcription factor encoded by *Pdx1*, and the study traced the ontogeny of the underlying epigenetic changes. Neonatal (and reversible) histone modifications that reduce *Pdx1* expression were followed in adulthood, after the animals developed diabetes, by methylation of the CpG island in the *Pdx1* promoter

and permanent gene silencing.[43] Neonatal changes in hepatic gene expression following uteroplacental insufficiency in rats also correlate with changes in the binding of acetylated histone H3 to the respective promoters.[49] Histone modifications were also proposed to be responsible for decreased expression of GLUT4 (a membrane glycoprotein that facilitates glucose transport, and is involved in the development of glucose intolerance) in rat pups whose dams were fed 50% of their normal daily food intake through midpregnancy to lactation. These changes, which persisted into adulthood, were proposed to be adaptive and to arise in response to the decreased production of pancreatic insulin.[50] In a model that is perhaps more relevant to the current nutritional situation in high-income countries, a maternal high-fat diet in primates leads to impaired lipid metabolism in the fetus in association with increased histone H3 acetylation and decreased histone deacetylase activity (both of which indicate increased transcription), together with increased expression of several relevant genes.[51]

As described above, manipulation of nutrition, particularly with respect to supply of one-carbon substrates that act as methyl-group donors and the associated cofactors, can affect epigenetic modifications to chromatin and the subsequent phenotype. Two studies, one experimental and one epidemiological, are of interest in this respect. In sheep, periconceptional restriction of maternal folate, vitamin B_{12} and methionine intake leads to widespread changes in the fetal epigenome and to offspring that, in spite of similar birthweights to those of control animals, become fatter and more resistant to insulin and have higher blood pressure by adult age.[52] In humans, maternal levels of serum folate during pregnancy positively correlate with the offspring's adiposity and insulin resistance at 6 years of age, whereas levels of serum vitamin B_{12} negatively correlate with insulin resistance. The greatest degree of insulin resistance was observed in children whose mothers were both folate-replete and vitamin B_{12}-deficient during pregnancy.[53]

Epigenetic changes in metabolic disease

Epigenetic processes are involved in some of the immediate manifestations of chronic noncommunicable diseases. For example, decreased histone H3 methylation (an epigenetic mark associated with repression of transcription), together with increased expression of proinflammatory genes, has been proposed to underlie the sustained proinflammatory phenotype of vascular smooth muscle cells that is seen in diabetic animals even after normalization of glycemia.[54] Similarly, transient hyperglycemia causes persistent expression of proatherogenic genes, which is underpinned by specific changes in histone H3 methylation in vascular endothelial cells.[55]

In the skeletal muscle of people who carry a single nucleotide polymorphism that creates a CpG site in the promoter of the respiratory-chain component gene *NDUFB6*, DNA methylation of this site negatively correlates with gene expression and insulin sensitivity.
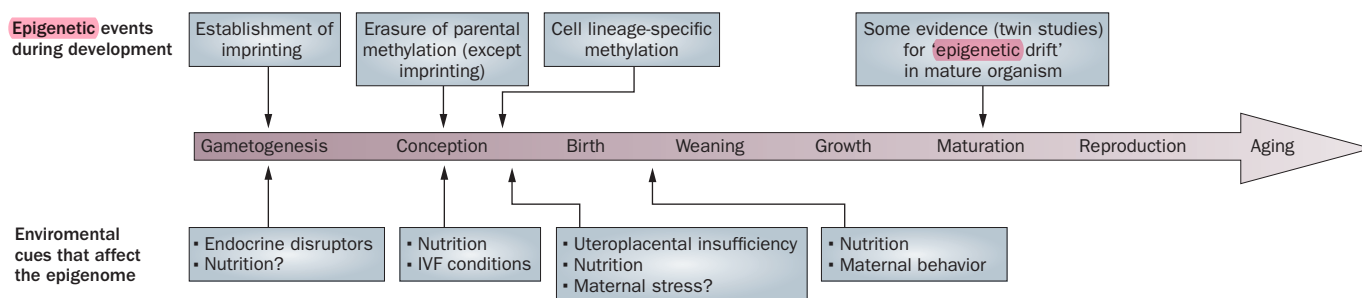
Figure 2 | Environmental sensitivity of the epigenome throughout life. The top row indicates normal reprogramming of the epigenome during gametogenesis, fertilization and development. The bottom row indicates the environmental cues that affect the epigenome and have late-life consequences, and the stages of life at which they act. Sensitivity of the epigenome to the environment (represented by shading of the arrow) is likely to decrease during life as growth slows. Abbreviation: IVF, *in vitro* fertilization.

In elderly people who carry this polymorphism, DNA methylation is increased, and thus insulin sensitivity is decreased.[56] Similar relationships between age, DNA methylation of promoters, protein expression, glucose metabolism and diabetogenic potential have been reported for another respiratory-chain component gene, *COX7A1*, in humans[57] and for hepatic glucokinase in rats.[58]

Window of epigenetic lability
The importance of the periconceptional period in later metabolic dysfunction is illustrated by retrospective studies in humans who were prenatally exposed to famine during the Dutch Hunger Winter. Although those who were exposed to famine periconceptionally and in the first trimester of pregnancy did not have lower birthweights than unexposed individuals, as adults they experienced a high prevalence of obesity and coronary heart disease, whereas individuals who were exposed in later stages of gestation had reduced birthweight and experienced a different constellation of metabolic dysfunction, including insulin resistance and hypertension.[4] Individuals who were exposed during the periconceptional period also showed decreased methylation of specific CpG dinucleotides in the imprinted gene for *IGF2* when measured nearly 60 years after, which indicates that early-life nutrition can cause persistent changes in the human epigenome.[59]

Some evidence that supports the importance of the periconceptional period for subsequent development also comes from studies in children who were conceived by assisted reproductive technologies.[60] This population has an increased incidence of imprinting disorders, of which the best known is Beckwith–Wiedemann syndrome (BWS), characterized by asymmetrical somatic overgrowth and increased susceptibility to developmental tumors. The imprinting defect in BWS involves a gene cluster on chromosome 11p that contains several growth-related genes, including the paternally expressed *IGF2* and the maternally expressed gene *CDKN1C*, a negative regulator of cell proliferation and a putative tumor-suppressor gene. These findings underline the need for careful control of the environmental conditions for oocyte maturation and embryo culture.

Some genes, for example *IGF2*, also show postnatal epigenetic lability. Postweaning mice fed a diet that is deficient in methyl donors have permanent loss of imprinting and dysregulation of expression of this gene.[61] In rats, different patterns of maternal attention in the immediate postnatal period result in differential methylation of the promoters of the nonimprinted glucocorticoid and estrogen receptors in some brain regions.[62,63]

Malleability of epigenetic modifications to environmental influences has also been established by twin studies in humans. Although monozygotic twins have an identical genotype, they are usually not phenotypically identical: a commonly observed feature is discordance in the frequency or onset of diseases. These differences may, at least in part, be a manifestation of epigenetic changes that occur over their lifetimes. Many studies on monozygotic twins have reported an association between epigenetic differences and discordance in diseases, such as BWS[64] and bipolar disorder.[65] Patterns of global and locus-specific DNA methylation are similar in 3-year-old monozygotic twin pairs, but substantially different in 50-year-old twin pairs.[66] This difference means a fourfold increase in differentially expressed genes in the adult twin pairs. The influence of age has also been demonstrated with *COX7A1*, which encodes a component of the mitochondrial oxidative phosphorylation cascade. Elderly adult twins showed much higher methylation of the promoter of *COX7A1* than did young adult twins, and the investigators related the correspondingly low gene expression to metabolic dysfunction in increased age.[57]

Inheritance of epigenetic marks
Changes in the epigenome during development may be passed on to subsequent generations. Evidence from rodent studies shows that nutritional and endocrinological interventions in pregnant animals (F_0) result in phenotypic and/or epigenetic changes that persist for at least two generations (F_1 and F_2).[67–70] Whether this persistence represents true transgenerational transmission, or simply changes in the germ cells of the F_1 generation that were exposed to nutritional and/or endocrinological interventions *in utero*, remains unclear. Transgenerational

inheritance of environmentally malleable epigenetic modifications in the well-studied mouse models of metastable epialleles also remains controversial.[71,72] Although some epidemiological data demonstrate nongenomic inheritance of disease risk in humans (such as that from the Dutch Hunger Winter studies),[69,73] our current knowledge of the role of epigenetic factors is very limited, because delineating the specific contributions of the genome versus those of the epigenome of an individual is difficult. Nevertheless, occasional reports suggest transgenerational epigenetic inheritance does occur in humans, such as the inheritance of a germ-line epimutation in the promoter of the mismatch-repair gene *MLH1*.[74]

The mechanisms by which epigenetic information (whether in terms of environmentally imposed methylation patterns or genomic imprinting) survives the reprogramming that occurs during embryogenesis and gametogenesis remain unresolved. A role for small RNAs in spermatozoa has been proposed for the non-Mendelian inheritance of one trait in the mouse, but the wider applicability of this mechanism remains uncertain.[75] Alternatively, as demonstrated in the studies of epigenetic changes in the brains of infant mice that are induced by maternal behaviour,[62,63] the epigenetic modification may not survive gametogenesis, but the behavior that induces the change might be re-established in each generation. Similarly, reduced size or function of the uterus during pregnancy may result in a uterus of decreased size in female offspring,[76] which in turn will have small offspring; both generations might have similar epigenetic changes without germ-line transmission having occurred.

Conclusions

Early-life events cause changes in the epigenome that are associated with increased disease susceptibility. The available data are now beginning to provide a molecular basis for epidemiological and experimental evidence that shows that the early period of life is critical in determining ensuing susceptibility to chronic noncommunicable diseases, such as obesity, type 2 diabetes mellitus and cardiovascular dysfunction.

Developing organisms seem to have a wide window of susceptibility to epigenetic changes (Figure 2). Clearly, the periconceptional period is particularly important, as shown by the sensitivity to suboptimal nutrition during this developmental stage,[52,59,77,78] in which widespread reprogramming of the epigenome occurs.[33] Nutritional constraint later in pregnancy[4,79] and/or postnatal overnutrition that leads to rapid growth,[8,79] as well as maternal–fetal overnutrition,[80] also cause metabolic dysfunction later in life, and epigenetic changes relevant to each of these situations have been reported.[51,61,81]

These observations have a number of clinical and public-health implications. The growing awareness of the importance of the periconceptional period, when suboptimal nutrition can have long-lasting effects without causing any change in birthweight,[4,59] underscores the importance of healthy nutrition during the prepregnancy period—a time when nutrition may be unbalanced, even in women in high-income countries.[82] A related finding, the demonstration that the balance of micronutrients that affect one-carbon metabolism during pregnancy affects the subsequent metabolic health of the offspring,[53] has implications for the design of nutritional supplementation programs.

The realization that the early-life environment can cause measurable and stable changes in the epigenome that parallel, and may contribute to, subsequent disease susceptibility has two related consequences. First, although genomic information has added little to clinical risk factors in the prediction of the onset of type 2 diabetes mellitus,[83,84] epigenomic markers, such as methylation patterns in specific gene promoters, may enable the identification of individuals who will have increased susceptibility to chronic disease in adulthood because of adverse factors in their early environment. Second, identification of such individuals may allow the prevention of disease, either by lifestyle modification or by active nutritional or pharmacological intervention. Our work in animal models has demonstrated that the adverse effects of impaired early-life nutrition and the associated epigenetic changes can be prevented[85] or reversed[81,86] by nutritional interventions (such as folate supplementation) or endocrinological interventions (such as neonatal leptin administration). Maternal overnutrition may contribute to transgenerational amplification of obesity in humans;[87] in the agouti mouse model, such female-line transmission of obesity is abrogated by dietary supplementation with substrates and cofactors of DNA methylation.[88] The phenotypic and epigenetic consequences of intrauterine growth retardation for pancreatic development and adult-onset diabetes mellitus can be reversed by early treatment with a histone deacetylase inhibitor, a drug class that has been already well studied in clinical oncological trials.[43] Such observations lay the groundwork for a new approach to the prevention of chronic, adult-onset diseases.

Review criteria

For this Review we selected full-text papers published in English. PubMed and Scopus were searched in November 2008 and again in February 2009 using the terms "cardiovascular disease", "chromatin", "developmental origins of [health and] disease", "developmental plasticity", "differentiation", "DNA methylation", "epigenetics", "epigenome", "gene expression", "histone", "imprinting", "obesity", "transgenerational" and "type 2 diabetes".

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