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The Developmental Environment, Epigenetic Biomarkers and Long-Term Health

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Abstract

Evidence from both human and animal studies has shown that the prenatal and early postnatal environments influence susceptibility to chronic disease in later life and suggests that epigenetic processes are an important mechanism by which the environment alters long-term disease risk. Epigenetic processes, including DNA methylation, histone modification and non-coding RNAs, play a central role in regulating gene expression. The epigenome is highly sensitive to environmental factors in early life, such as nutrition, stress, endocrine disruption and pollution, and changes in the epigenome can induce long-term changes in gene expression and phenotype. In this review we focus on how the early life nutritional environment can alter the epigenome leading to an altered susceptibility to disease in later life.

Keywords

Epigenetics; DNA methylation; developmental environment; nutrition

Introduction

The prevalence of non-communicable diseases (NCD) such as type-2 diabetes and cardiovascular disease is increasing globally at an alarming rate; the World Health Organization predicts that they will be responsible for 73% of all deaths by 2020. Much of this increase will occur in developing nations as they undergo socio-economic improvement¹. Fixed genomic variations such as single nucleotide polymorphisms and copy

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Conflicts of Interest

KMG has received reimbursement for speaking at conferences sponsored by companies selling nutritional products and KMG, PMC and KAL are part of an academic consortium that has received research funding from Abbott Nutrition, Nestec and Danone.

number variations only account for a small proportion of the variation in NCD risk². Environmental factors such as diet and level of physical activity are likely to play a major role in the development of NCDs and in particular there is growing evidence that the early life environment can play an important role in influencing the risk of developing a wide range of NCDs in later life³. The developmental environment can alter later phenotype through the altered epigenetic regulation of genes and this review focuses on the evidence that perinatal influences on epigenetic processes, particularly maternal diet, can lead to persistent phenotypic changes and an altered risk of NCDs in later life.

Early life environment and future disease risk

Following on from the proposal by Forsdahl in 1977 that undernutrition during childhood and adolescence might increase the risk of later CVD⁴, subsequent studies found an inverse relationship between birth weight and increased CVD mortality⁵ and that babies born at the highest birth weights are also at an increased risk of later NCDs^{6,7}. In such studies birthweight is thought to be a proxy measurement of the intrauterine environment, which may have been compromised through a variety of maternal, environment or placental factors⁸.

Studies of the Dutch Hunger Winter provide evidence that maternal nutrition influences offspring health in later life and suggest that the timing of the nutritional constraint is important; first trimester famine exposure increased the risk of obesity and CVD, whereas exposure in the later stages of gestation increased the risk of later insulin resistance and hypertension^{9,10}. Comparable findings are now well established in a variety of animal models where nutrition can be precisely controlled. Early animal studies focussed on the effects of global maternal undernutrition or an isocaloric low protein diet. With the growing epidemic of maternal obesity in both industrialised and developing countries, animal models have been established to investigate the effect of energy rich maternal diets on the health of the offspring¹¹⁻¹⁵. Interestingly, offspring born to mothers fed these different diets exhibit similar features to human cardio-metabolic diseases including hypertension, dyslipidemia, obesity and insulin resistance in later life. The experimental studies in animals implicate altered epigenetic regulation of genes as a major mechanism through which the developmental environment induces altered phenotypes.

Epigenetics

Epigenetic processes, such as DNA methylation and histone modifications, induce heritable changes in gene expression without a change in gene sequence¹⁶. The epigenome can therefore be regarded as a molecular record of life events, which accumulates over a lifetime. For example, monozygotic twins have been shown to be epigenetically most similar at birth but their epigenomes diverge with age at a rate that is lessened if the twins share a common environment¹⁷. Fine-tuning of phenotype by the developmental environment has adaptive value since it allows the fetus to predict and prepare for the environment to be experienced later¹⁸. Elucidation of these epigenetic processes has the potential to enable early intervention strategies to improve early development and later health and the study of epigenetic biomarkers is a rapidly advancing field.

DNA methylation

DNA methylation is a common modification in eukaryotic organisms. Typically it involves the transfer of a methyl group to the 5' carbon position of cytosine, creating 5-methylcytosine (5-mC) ¹⁹. In mammals, methylation of cytosine mainly occurs within the dinucleotide sequence CpG, where a cytosine is immediately 5' to a guanine (the p denotes the intervening phosphate group), although non-CpG methylation is also prevalent in embryonic stem cells ²⁰. DNA methylation is a stable epigenetic mark that is transmitted through mitotic DNA replication and cell division ²¹. CpG dinucleotides are not randomly distributed throughout the genome but are clustered at the 5' end of genes in regions known as CpG islands, with hypermethylation and hypomethylation of these islands often associated with gene silencing and activation respectively ^{22, 23}. DNA methylation can act directly to block binding of transcription factors to the DNA or by recruiting a myriad of other repressive factors, such as methyl CpG binding protein 2 (MeCP2), which in turn mediate local chromatin changes ²⁴.

Methylation of CpGs is largely established during embryogenesis and the perinatal period. Following fertilisation, DNA methylation marks on the maternal and paternal genomes are largely erased (with the exception of the imprinted genes and other specific genomic regions), followed by a wave of *de novo* methylation within the inner cell mass just prior to blastocyst implantation ^{25, 26}. The *de novo* methylation of DNA is catalysed by DNA methyltransferases (DNMT) 3a and 3b ²⁶ and is maintained through mitosis by methylation of hemi-methylated DNA by DNMT1 ²⁷. This gives rise to lineage specific methylation patterns that are maintained in differentiated tissues. The failure to identify a DNA demethylase or mechanism for DNA demethylation led to the thought that DNA methylation patterns were relatively stable and generally maintained throughout life. However, this concept has now been challenged as in 2009 the existence of another epigenetic modification, 5-hydroxymethylcytosine (5hmC), was described as present in high levels in neurons and embryonic stem (ES) cells ²⁸. 5hmC arises from the oxidation of 5-mC by the enzymes of the TET family and has been proposed to act as a specific epigenetic mark opposing DNA methylation, rather than a passive intermediate in the demethylation pathway ²⁹. The high levels found in the brain and neurons indicate a role in the control of neuronal differentiation and neuronal plasticity ³⁰.

Histone modifications

In eukaryotic cells, DNA is wrapped around a core of 8 histone proteins; two molecules of each of the four histone proteins H2A, H2B, H3 and H4 combine together to form a nucleosome, the most basic unit of chromatin. Each nucleosome is composed of 146 base pairs of DNA wrapped 1.65 times around the histone core. One molecule of the fifth histone protein, H1, is bound to the DNA as it enters each nucleosome core particle and is known as the linker histone. Each nucleosome is then folded upon itself to form a 30nm chromatin fibre which is then compacted progressively into larger fibres ³¹. The folding of the chromatin is necessary to reduce the effective size of DNA but it has now become clear that the histones also play a critical role in regulating gene expression. Histone proteins contain 2 domains, a globular domain and an N terminal tail domain. The unstructured histone tails are subject to modifications including acetylation, methylation, ubiquitination, phosphorylation

and attachment of Small Ubiquitin-like Modifier proteins (SUMOylation)³². Histone modification can directly affect chromatin structure and also provide binding sites for proteins involved in gene regulation. Together, histone modifications and DNA methylation control chromatin structure and therefore the accessibility and functional role of the underlying DNA sequence³³.

Non-coding RNAs

The ENCODE project has shown that, although only 1-2% of the genome encodes for proteins, over 74% of the eukaryotic genome has functional elements³⁴. RNAs arising from these functional elements that are not transcribed have been termed non-coding RNAs (ncRNAs). They can be grouped into two classes; long ncRNAs (those longer than 200 nucleotides) and short ncRNAs (those less than 200 nucleotides, including the microRNAs (miRNAs), small interfering RNAs (siRNAs) and PIWI-interacting RNAs (piRNAs))³⁵. The ncRNAs are central components of the transcriptional regulation machinery of the cell. Short ncRNAs can induce mRNA degradation or translational repression and when targeted to the promoter region of a gene can induce both DNA methylation and repressive histone modifications³⁶. Large ncRNAs such as Xist, which plays a pivotal role in X chromosome inactivation, act by coating large regions of a chromosome creating repressive domains^{37, 38}.

Early life nutrition and the epigenome

Originally it was thought that, once established in the blastocyst, DNA methylation is largely maintained throughout the life-course. However, there is now growing evidence that the epigenome is particularly susceptible to a number of environmental factors during the prenatal and early postnatal periods and that changes during this time can lead to long-term phenotypic alterations in the offspring.

A clear example of how nutrition can alter phenotype through the altered epigenetic regulation of genes is seen in studies of the honeybee. Female larvae fed on Royal Jelly for 6 days develop into fertile queen bees, whilst those fed the jelly for 3 days become sterile worker bees, even though they are genetically identical³⁹. Knockdown of DNMT3, the major DNMT in bees, increased the proportion of larvae developing into queen bees³⁹, which suggests that the effect of nutrition on developmental fate is mediated through the altered methylation of DNA.

A classic example of maternal nutrition influencing DNA methylation in mammals is in the agouti mouse model, where coat colour is influenced by the methylation status of the 5' end of the *Agouti* gene. Differences in the mother's intake of dietary methyl donors and co-factors (including folic acid, vitamin B12, betaine and choline) were shown to alter DNA methylation of the *Agouti* gene and induce differences in the coat colour of the offspring^{40, 41}.

Studies in other animal models have also shown that perturbations in maternal diet are associated with persistent metabolic changes in the offspring, accompanied by epigenetic changes in key metabolic genes or genes involved in appetite control. For example, feeding

rats a protein restricted diet during pregnancy induced hypomethylation of the glucocorticoid receptor (GR) and peroxisome proliferator activated receptor alpha (PPAR α) receptor in the livers of juvenile and adult offspring, accompanied by an increased gene expression and a persistent change in the metabolic processes that these nuclear receptors control^{42, 43}. Increased expression of PPAR α was also associated with an increase in histone marks that facilitate transcription and a decrease in those that suppress transcription¹². In contrast, global dietary restriction (giving dams a 70% reduction in total nutrient intake during pregnancy) decreased methylation and increased expression of the GR and PPAR α promoters in the offspring liver⁴⁴. Thus the effects of maternal nutrition on the epigenome of the offspring depend upon the nature of the maternal nutrient challenge.

Given the growing concern over the energy-rich Western diet a number of studies have also explored the effects of maternal high fat feeding on DNA methylation in the offspring. Maternal high fat feeding during pregnancy in rats leads to the reduced expression of FADS2, the rate-limiting enzyme in polyunsaturated fatty acid synthesis, along with altered methylation of key CpG nucleotides within its promoter in the liver of the offspring¹¹. Maternal obesity and diabetes in mice have also been reported to induce widespread changes in DNA methylation in the offspring liver⁴⁵.

It has also become apparent that the period of epigenetic plasticity may extend further into postnatal life. Overfeeding in rat pups induces hypermethylation of two CpG nucleotides within the proopiomelanocortin (POMC) promoter, which plays a key role in appetite control, and hypermethylation of the gene prevented upregulation of POMC expression despite high plasma levels of both leptin and insulin⁴⁶. Folic acid supplementation in the juvenile-pubertal period has been shown to induce hypermethylation of the PPAR α gene, with decreased PPAR α expression and levels of fatty acid β -oxidation⁴⁷, while Ly *et al.* showed that folic acid supplementation during the peri-pubertal period led to an increased risk of mammary adenocarcinomas along with a decrease in DNMT activity⁴⁸. Plasticity may also extend into adult life; feeding adult rats a diet deficient in choline, folate, methionine and vitamin B12 for 4 weeks induced hypomethylation of the proto-oncogenes c-Myc, c-Fos and c-Ras, with this effect persisting 3 weeks after re-feeding⁴⁹. Feeding adult rats a fish oil enriched diet for 9 weeks led to a transient decrease in the expression of FADS2 coupled with an increase in FADS2 promoter methylation, with the effects reversed following feeding of a standard diet for 4 weeks¹¹. So, while the epigenome may be most susceptible to environmental factors in early life, there is some plasticity maintained in later life and this offers the potential opportunity for intervention to reverse marks associated with disease risk.

Most studies have focused on identifying the effects of early life environment on changes in DNA methylation but there is now growing evidence that persistent changes can also be induced in both histone modifications and ncRNAs. Aaggard-Tillery *et al.* established an obese pregnant monkey model where the offspring were both obese and found to have site-specific alterations in fetal hepatic H3 acetylation⁵⁰. A 50% protein reduction throughout gestation increased acetylation of histones H3 and H4 in the promoter region of C/EBP β , a central regulator of energy metabolism, in the skeletal muscle of female rat offspring, along with increased gene and protein expression⁵¹. Maternal protein restriction during pregnancy

in sheep has also been shown to program marked changes in miRNA expression in both the liver⁵² and skeletal muscle⁵³ of the offspring.

The father's diet can also have an effect on the epigenome and phenotype of the offspring. Offspring of fathers who were fed a low protein diet prior to mating showed widespread modest changes in DNA methylation (10-20%) compared to control offspring, including a substantial increase in methylation at an intergenic CpG island 50kb upstream of the PPAR α gene⁵⁴. *Ng et al.* also showed that a chronic paternal high-fat diet led to β -cell dysfunction in the female offspring⁵⁵. Experimental studies of paternal-diet-induced intergenerational metabolic reprogramming are an area of increasing research interest, with a *Drosophila* model showing effects mediated through histone changes and alterations in chromatin state⁵⁶.

DNA methylation is not completely erased during early embryogenesis and as such some methylated sites survive along with associated histones, providing one mechanism for epigenetic transgenerational inheritance⁵⁷. For a true transgenerational effect it must extend to the F3 generation since environmental exposure of an F0 female exposes the F1 embryo and the F2 germline present within that embryo; any traits present in the F2 generation cannot be considered a true transgenerational trait and are better described as a multigenerational effect⁵⁸. In males multigenerational exposure is limited to the F0 and F1 generations since F2 offspring from the male line are not exposed to the F0 uterine environment. Maternal undernutrition in the F0 generation can alter glucose metabolism in the third generation, despite normal nutrition during pregnancy in the F1 and F2 generations⁵⁹. There are also gender specific transgenerational effects; maternal high-fat feeding during pregnancy and lactation increased body size in F1 and F2 generations, with transmission via both the maternal and paternal lineages, however transmission of the increased body size to the F3 generation was restricted to females and transmitted through the paternal lineage only⁶⁰.

Early life nutrition and the human epigenome

Alterations have been reported in the methylation of a number of genes in DNA isolated from whole blood from individuals whose mothers were exposed to famine during the Dutch Hunger Winter. Periconceptual exposure to famine was associated with a small decrease in CpG methylation of the imprinted *IGF2* gene and an increase in methylation of leptin, *IL-10*, *MEG3* and *ABCA4*⁶¹, while late gestation famine exposure had no effect on methylation, again showing that the timing of nutritional constraint is important. These measurements were made 60 years after the famine exposure, suggesting that maternal nutritional constraint induced long-term epigenetic changes in key metabolic regulatory genes and pointing to a mechanism through which fetal famine exposure has an effect on adult metabolism. Steegers-Theunissen *et al.* have also shown altered methylation of specific CpG sites in the *IGF2* gene in the peripheral blood cells of children whose mothers did or did not take 400 μ g of folic acid per day in the periconceptual period⁶². Plasticity in the human epigenome may persist into adulthood; short-term high fat overfeeding in healthy young men was shown to induce methylation changes in over 6,000 skeletal muscle genes, with those changes only partially reversed after 6-8 weeks of a normo-caloric diet⁶³.

Epigenetic biomarkers to predict later disease risk

If the early life environment induces altered epigenetic regulation of genes then it should be possible to detect these altered epigenetic marks and use them as predictors of future metabolic capacity and disease risk. The development of new technologies to measure DNA methylation is enabling discovery of epigenetic biomarkers on a truly genome-wide scale. This increased coverage is likely to uncover many new genomic regions that contain specific epigenetic alterations outside those already studied.

In two independent cohorts Godfrey *et al.* reported that the methylation status of a single CpG site in the promoter region of the nuclear receptor *RXR α* from umbilical cord tissue was strongly related to childhood adiposity in both boys and girls, with the methylated *RXR α* CpG explaining over 25% of the variance in age and sex adjusted fat mass in children at 6 and 9-years of age ⁶⁴. Epigenetic alterations may therefore contribute a far greater proportion to an individual's risk for NCD than previously thought.

Epigenetic biomarkers, unlike genetic biomarkers, are thought to have some tissue specificity. However, it is often impractical to study diseased and/or the appropriate tissues for epigenetic studies, so accessible surrogate tissues such as umbilical cord and blood are often used instead, with a further advantage being that these biomarkers can then be measured repeatedly. Although DNA methylation patterns can be tissue specific a number of studies have also now shown inter-tissue methylation correlations. For some genomic regions methylation appears to be largely independent of the tissue of origin, while for other regions there is a tissue-specific dependence ⁶⁵. Methylation across differentially methylated regions for the imprinted genes *H19*, *MEST* and *PEG10* did not significantly differ across a range of tissues (buccal, brain, eye intestine, liver, lung, muscle and umbilical cord blood) ⁶⁶. The methylation levels of a number of non-imprinted genes measured in blood were also equivalent in buccal cells despite these cell types originating from different germ layers (mesoderm and ectoderm respectively) ⁶⁷. Methylation changes induced by maternal diet can be similar in the umbilical cord and liver ⁶⁸. These studies suggest that methylation levels in more readily available tissues, such as blood, buccal or umbilical cord, may provide useful proxy markers of methylation in metabolically relevant tissues.

However, data showing that some DNA methylation marks can be dynamically regulated in response to postnatal environmental stimuli mean that much work will need to be done to demonstrate the utility of perinatal epigenetic predictive markers. For example, an acute burst of physical activity induced hypomethylation of the peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (*PGC1 α*) and mitochondrial transcription factor A (*TFAM*) promoters in muscle tissue ⁶⁹. One study that examined DNA methylation stability over time in children found that the methylation levels of the genes *MAOA*, *DRD4* and *SLC6A4* was highly dynamic between the ages of 5 and 10 years ⁷⁰. In contrast, Clarke-Harris *et al.* reported year on year stability of 7 CpG sites within the *PGC1 α* promoter in peripheral blood cells in children from 5 to 14 years of age ⁷¹, suggesting that for these CpG sites methylation levels are set up in early life and stability maintained. Moreover, 7 of the CpG sites analysed at age 5-7 years were predictive of adiposity in the children at ages 9 to 14, which is further evidence that developmentally induced methylation marks may be

significant contributors to later phenotype. The differences in the stability of the PGC1 α methylation between the two studies may reflect differences in the location of the CpGs or tissue specific differences between blood and muscle (PGC1 α has a muscle specific transcript, although it is unclear which promoter region was analysed by Barres *et al.*). Whether the changes in methylation in response to exercise occur on top of a developmentally induced methylation change is also not known.

Interventions and developmental epigenetic changes

A number of intervention studies suggest that the effects of the early life environment on the epigenome and phenotype can be prevented and/or reversed. In their rat model of maternal protein restriction Lillycrop *et al.* showed that supplementation of the restricted diet with glycine or folic acid prevented hepatic GR and PPAR α hypomethylation and with altered metabolic phenotype⁴², suggesting that impaired 1-carbon metabolism plays a central role in this model. In neonatal rats whose mothers were exposed to a 70% in utero global nutrient restriction postnatal leptin treatment reversed promoter hypermethylation and increased expression of PPAR α and GR in the liver⁴⁴. Thus, it may be possible to not only identify those at risk of developing later disease, through the use of epigenetic biomarkers early in life, but to develop intervention strategies which target and reverse these epigenetic changes.

Conclusions

There is now considerable evidence that our genotype and later environment are not the only determinant of non-communicable disease risk, and that epigenetic marks induced by the early life environment are associated with altered gene expression patterns in important metabolic tissues, leading to altered susceptibility to disease in later life.

Demonstration of a role for altered epigenetic regulation of genes in the development of NCDs together with the identification of potential epigenetic biomarkers of future disease risk raises the possibility of preventive medicine. Individuals identified as at risk at an early stage in the life course could receive nutritional or lifestyle interventions, allowing a more effective strategy of preventive treatment. This would both improve quality of life and reduce the economic burden associated with current treatment strategies. Further understanding of the mechanisms by which nutrition can modify the epigenome and the periods of epigenetic susceptibility will aid development of novel intervention strategies to reverse this current global epidemic of NCDs.

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Figure 1.

DNA methylation and histone modifications are two epigenetic modifications which determine the chromatin structure and the ability for genes to be transcribed. The processes interact and can lead to open chromatin and gene transcription or to closed chromatin and gene silencing. DNMT – DNA methyltransferase

Figure 2.

Epigenetic biomarkers; detecting early life epigenetic biomarkers which are associated with disease risk could lead to stratified interventions and reduced susceptibility to chronic disease. ncRNA – non-coding RNA