EPIGENETICS AND METABOLISM in 2014

Metabolic programming: knowns, unknowns and possibilities.

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Studies published in 2014 have helped in our understanding of the epigenetic mechanisms by which suboptimal nutritional exposures during in utero development are transmitted to subsequent generations through both the maternal and paternal line. Advances include identification of common loci that are vulnerable to *in utero* under-nutrition and over-nutrition as well as those that occur tissue-wide.

An association between birth weight and development of traditionally adult-onset diseases such as type 2 diabetes mellitus (T2DM) and cardiovascular disease in later life was first demonstrated more than two decades ago.[1] These observations led to the concept of the developmental origins of health and disease—the idea that the environment to which we are exposed during critical periods of development has a permanent effect on our tissue structure and metabolism and consequently on our long-term health.[2] Environmental factors now known to have such effects include maternal nutrition, maternal smoking, maternal stress and toxins. Despite the wealth of evidence for these 'programmed' effects, the precise mechanisms by which an event in early life can have a permanent effect on tissue function many years later, following multiple rounds of cell division, remain poorly understood. Nevertheless, epigenetic processes have emerged as a conceptually attractive mechanism in this context. The term 'epigenetics' was first used by Conrad Waddington to define the "interactions of genes with their environment which bring the phenotype into being".[3] The epigenetic processes that mediate this interaction are now recognised to include DNA methylation, covalent modifications of histone tails (including acetylation and methylation) and the effects of noncoding RNAs (such as microRNAs and long noncoding RNAs)[4]. Over the last year substantial progress has been made in determining the loci within the genome through which epigenetic programming of health and disease operates.

Initial studies in the field of epigenetic programming of health and disease focused on the detrimental effects of suboptimal experiences *in utero* that are

mediated through changes in maternal metabolism and/or behaviour. However, in the past few years, paternal factors have been shown to also have transgenerational effects on the offspring. In a study in rats, for example, paternal high-fat feeding at the time of conception was demonstrated to influence glucose tolerance and pancreatic β-cell function of female offspring.[5] A landmark study published in 2014 by Radford and colleagues provided evidence that exposure to suboptimal nutrition during fetal life leads to changes in the germ-cell DNA methylome of male offspring even when these male offspring are nourished normally from weaning.[6]. The researchers demonstrated these effects using an established mouse model of maternal under-nutrition that leads to low birth weight and glucose intolerance in male and female F1 offspring. These phenotypic differences are transmitted through the paternal line to the F2 offspring. Using methylated DNA immunoprecipitation–sequencing technology to analyse genome-wide methylation patterns, the investigators identified 111 regions in the F1 sperm from the maternally undernourished male offspring that were hypomethylated compared with the equivalent regions of sperm from control offspring. Despite these differences not being retained in late-gestation somatic tissues from F2 offspring bred from the F1 male offspring, the alterations in the adult male F1 germline might provide a mechanism by which transgenerational programming occurs through the paternal line.

Although evidence for the effects of maternal diet on the epigenome of the offspring in animal models is growing, there is a paucity of data showing such effects in humans. One of the few studies in humans to demonstrate such changes came from the analysis of individuals who were *in utero* during the Dutch Hunger Winter and whom, therefore, were exposed to extreme under-nutrition during fetal life.[7] In 2014, Dominguez-Salas and colleagues showed that more natural variation in maternal diet during early pregnancy can cause persistent changes in DNA methylation in the offspring.[8]. To demonstrate this link they analysed samples collected from children born to mothers either during the rainy (hungry) season or the dry (harvest) season. As demonstrated previously, pregnancies during these two seasons vary greatly in nutrient intake including that of key methyl donor substrates such as methionine and folate. Candidate methylation analysis of white blood cells and hair follicle samples (selected as mesodermal and ectodermal tissues, respectively) from the offspring of these mothers demonstrated increased methylation of six metastable alleles in

children who were born in the rainy season. Metastable epialleles are regions of the genome at which DNA methylation is established stochastically in the early embryo and then maintained in differentiated tissues. This approach to focus on metastable epialleles therefore negates one of the limitations of many human epigenetic studies in the field of metabolic programming. These studies have primarily been carried out in clinically accessible tissues, such as white blood cells, and the question has often been raised as to whether any changes identified are relevant to functionally important metabolic tissues and, therefore, whether they provide insight into the biological mechanisms by which epigenetic programming occurs.

Even when differences are identified in metastable epialleles in clinically accessible tissues, which reflect methylation in a tissue involved in metabolism, an important next step is to establish that these changes are functionally relevant both in terms of regulating transcription and triggering a disease process, such as development of T2DM. One approach to start to address this issue is to define the methylome of metabolically relevant tissues from an individual with the disease and compare it with that of healthy individuals. In 2014, this approach has been adopted by Dayeh and colleagues, who took a genome-wide strategy to compare DNA methylation patterns in pancreatic islets from individuals with T2DM and those from glucose-tolerant individuals.[9] The researchers identified 853 genes that showed differential methylation in islets from patients with T2DM compared with islets from control individuals. These genes included some that were previously implicated in the pathogenesis of T2DM (including *FTO* and *TCF7L2*), as well as some not previously associated with T2DM risk. Importantly, the investigators demonstrated that in at least some of the loci the methylation change was accompanied by a reciprocal change in the corresponding mRNA and that *in vitro* modulation of the transcript was associated with differences in pancreatic β-cell function.

A key question in the field of developmental programming has been finding an explanation for how a range of suboptimal exposures to diverse environmental factors in early life can lead to a common metabolic phenotypic outcomes such as T2DM in later life. Is there one common response pathway initiated when a fetus senses that it is in an inappropriate environment? What could the programming factor being sensed be, and which loci within the genome are most vulnerable to these

effects? Some of these questions have been addressed in 2014 by Quilter and colleagues, who took a genome-wide approach to compare cord-blood DNA methylation between babies who were either born to mothers who developed gestational diabetes mellitus, had experienced growth restraint *in utero*, or did not belong to one of these two groups (i.e., whose mothers did not develop gestational diabetes mellitus and who did not display any indication of *in utero* growth restraint.[10] Although sample size was small, the research team identified eight genes that were differentially methylated in the two groups exposed to a suboptimal environment *in utero* compared with the controls. These findings provide proof of principle that epigenetic programming of common genes might explain why exposure to a range of different suboptimal environments *in utero* can lead to a common metabolic phenotypic outcome, such as T2DM.

Studies in animal models and human cohorts support the now widely accepted idea that exposure to a suboptimal environment during critical periods of development can have a long-term effect on the metabolic health of an individual. Furthermore, these detrimental metabolic consequences can be transmitted to the next generation through both the maternal and the paternal line. These effects are associated with changes in epigenetic marks, such as DNA methylation, in the offspring. The next challenge to take the field forward will be to demonstrate a causal relationship between loci-specific programmed epigenetic alterations in response to an adverse early environment and metabolic disease phenotypes later in life.

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Competing interests
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Key advances

- Maternal nutrition during pregnancy alters the germline DNA methylome of male offspring in adulthood[6]
- Maternal diet in humans at the time of conception modulates DNA methylation in the offspring at metastable epialleles[8]
- Human islets from individuals with type 2 diabetes mellitus have a different methylome compared with those from glucose-tolerant individuals[9]
- Exposure to different suboptimal environments *in utero* can lead to common methylation changes in the offspring[10]