Can Nutritional Epigenomics Explain Persistent Effects of Periconceptional Folic Acid in the Methylome?

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Nutritional epigenomics is a burgeoning field of interest given the potential of dietary factors to influence the epigenetic landscape, with potential for long-term health impacts (1,2). DNA methylation, essential for cell division and tissue specification, is a dynamic epigenetic mechanism that can be modified by certain nutritional exposures including one-carbon nutrients, (3,4) which act by altering the availability of substrates required for methylation reactions or by inhibiting the enzymes that catalyse these reactions. Folate and the metabolically related B-vitamins supply methyl groups for DNA methylation via formation of S-adenosylmethionine (SAM) within one-carbon

metabolism. Pregnancy and adolescence, periods of intense development and growth with increased demands for folate, coincide with dynamic changes in DNA methylation and thus represents a window of opportunity for gene-nutrient interactions to impact health.

The evidence for protection of the foetus against neural tube defects by maternal periconceptual folic acid (FA) supplementation from randomized controlled trials (RCTs) is conclusive, with women worldwide recommended to take supplements of 400mg FA/day in the periconception period (5). Beyond the first trimester, analysis of cord blood samples from the Folic Acid Supplementation in the Second and Third Trimesters (FASSTT) trial in pregnancy, indicated that continued supplementation affected DNA methylation of specific genes in the offspring, including those related to brain development (6,7,8). These findings offer a potential biological mechanism linking maternal folate status with offspring neurodevelopment (6,7,8) and lend support to the fact that the DNA methylome undergoes widespread change during in-utero development, however evidence of a dynamic methylation landscape beyond birth through to adolescence has not been widely investigated. Of note, one recent landmark investigation, involving a series of epigenome-wide association studies in 5019 blood samples, collected from 2348 participants of two large independent cohorts at multiple time-points from birth to late adolescence, reported that changes in methylation over time were spread across the epigenome at over half of the methylation (CpG) sites analysed (9).

Given the lack of available evidence, the study by Crider and colleagues published in this issue of the *Journal of Nutrition (10)*, exploring the long-term effect of maternal FA supplementation on DNA methylation in adolescent offspring is timely. The study contributes novel data, in a population not exposed to FA fortification, on the impact of maternal exposure to FA on the DNA methylome of offspring during adolescence. The authors were unable to detect any significant differences in DNA methylation between offspring of mothers who had taken FA in the periconceptional period and those who had not. A secondary analysis was conducted to examine

potential differences in allelic frequencies at SNPs implicated in folate metabolism. The authors also show that there are no significant differences in genotype frequencies of genes in the folate metabolising pathway between mothers and adolescents. Standard linkage disequilibrium was not possible due to lack of paternal genotype information; therefore, the authors note that caution should be taken in interpretation of these results. This study of Chinese mother-adolescent pairs highlights the complexities of studying the effect of nutrients on the dynamics of DNA methylation during periods of plasticity, given that the changes in methylation reported were not significant. The timing of FA exposure is also an important factor and varied to some extent in this study, with the majority, but not all mothers commencing FA prior to becoming pregnant. In addition, even though offspring from mothers who were supplemented with FA may have had altered DNA methylation profiles at birth, the low habitual dietary folate intake of the children and adolescents (both urban and rural) investigated may have prevented retention of methylation patterns induced by high levels of FA during pregnancy. Furthermore, maternal exposures to environmental factors, e.g. smoking (11) and BMI (12) are known to modulate DNA methylation with persistent effects in the offspring.

A number of limitations of the work were highlighted by the authors. The Infinium Human Methylation 450k array was used to compare patterns of methylation from adolescents of mothers who took FA supplements versus those who did not. The 450k microarray is enriched for CpG-dense genomic regions (CpG islands) however there is growing evidence that methylation in CpG-poor regions, such as enhancers and gene bodies, also have significant functional consequences through altering gene expression (13). Thus, to advance the study of environmental influences on the methylome, future studies should consider methods which offer greater genomic coverage such as whole genome bisulfite sequencing (WGBS) or the Infinium EPIC array which assesses over 800k sites simultaneously, thus achieving a good balance between genomic coverage and cost. The authors also discussed the use of saliva samples vs the more commonly used blood for collection of DNA as a limitation however, recent evidence suggests that saliva may, in some cases, be a better proxy for certain tissues, including brain (14). Saliva also represents a much less invasive means of accessing

DNA thus may offer a superior, alternative for future large-scale studies in targeted populations, including children and adolescents. The observational nature of this study is also a limiting factor particularly pertinent to the field of nutrigenomics, with longitudinal studies enabling the differentiation of intra-individual differences from inter-individual differences (15). Furthermore, the relatively small number of adolescents included in this study may have impacted the ability to detect changes in methylation. Future studies in large longitudinal cohorts are needed to definitively determine if methylation changes can persist through the life course, including during adolescence and early adulthood.

The current study provided a unique opportunity to investigate the impact of FA exposure *in utero* on long term epigenome wide DNA methylation patterns, in a population not exposed to FA fortification. While the study found no differences in those exposed vs not exposed to FA supplementation, the research highlights the need for further well-designed studies, including RCTs, to determine whether epigenetic changes can persist beyond early life. Explicit data regarding the timing of exposure and prevailing diet in populations being investigated are necessary to understand the pathological methylation changes associated with B-vitamin supplementation in promoting healthy development and growth. Such studies will enable persistent changes in the epigenome of offspring, throughout the life course following maternal exposures, to be tracked to help unravel disease mechanisms.

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