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Editorial Focus: *Epigenetic changes in gene expression*: focus on "The liver X-receptor gene promoter is hypermethylated in a mouse model of prenatal protein restriction"

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THE TERM EPIGENETICS WAS FIRST coined in 1942 by Waddington (20) to describe the interaction of genes with their environment during development that gives rise to a phenotype. Today, the term epigenetics is used when describing a phenotype that occurs in a manner outside conventional genetic interactions and refers to stable and heritable alterations in gene expression that do not involve a change in DNA sequence (9). DNA methylation is one type of epigenetic mechanism that serves as a postreplication modification and can occur in response to environmental influences (14). DNA methylation, which involves the modification of cytosines found in the dinucleotide sequence CpG (9), can activate or suppress transcription, is reversible (9), and plays a critical role in normal mammalian cell differentiation and development (14). DNA methylation is also implicated in the pathology of many age-related diseases, such as cancer (6), and, importantly, epigenetic modification of the genome can allow for stable transmission of gene activity to the next generation (9).

Developmental origins of health and disease (DOHaD) refers to the process by which the phenotype of a fetus is altered in response to environmental influences (2). The DOHaD hypothesis originated from a geographical correlation of infant mortality and ischemic heart disease (2). Based on this study, Barker (2) proposed that adverse environmental influences during early development permanently alter the body's structure, function, and metabolism in ways that lead to an increased risk for adult cardiovascular and metabolic disease. Numerous epidemiological studies now validate this association, and numerous experimental studies have investigated potential mechanisms involved in the DOHaD (1); however, the exact link between fetal life and programmed adult disease remains unclear. Although the increased risk of adult health and disease observed in a fetus exposed to environmental stresses implicates epigenetic processes as a possible link (Figure 1), few studies have directly tested this hypothesis.

The rodent model of maternal low protein is well characterized as an experimental model of DOHaD (10). Protein is key for proper fetal growth (3) and a reduction in protein content from a range of 18% to 20% to a range of 9% to 12% in the maternal diet can lead to disproportionate fetal growth, hypertension, cardiovascular disease, and metabolic programming in low-protein offspring (1, 3, 10, 19). In addition, reductions in birth weight (3), cardiovascular dysfunction (19), and programmed alterations in methylation of hepatic gene promoters (4) can extend to the next generation. Thus, the mechanism by which maternal low protein leads to DOHaD may involve epigenetic effects mediated via altered DNA methylation of key genes linked to health and disease.

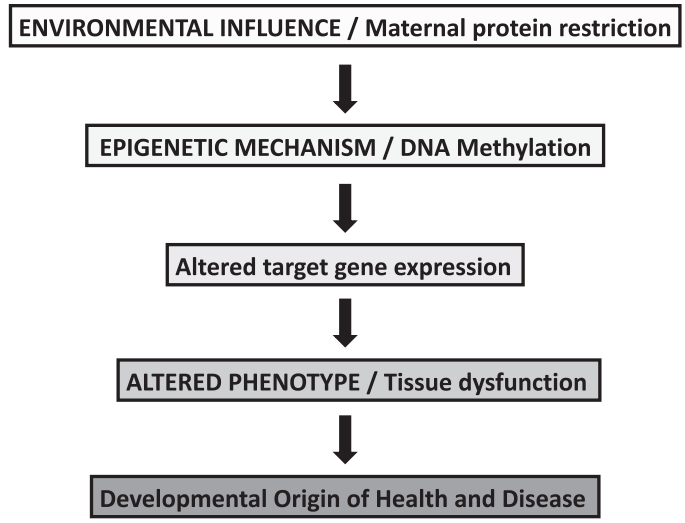

Fig. 1. DNA methylation of a gene is a type of epigenetic process that can occur in response to adverse environmental influences. Changes in gene expression associated with an increased risk for adult disease occur in response to adverse environmental influences during critical periods of development. Thus, epigenetic processes may serve as a critical link between insults during fetal life and the increased risk for adult disease.

Temporal alterations in lipid metabolism are noted in low-protein offspring; similar hepatic triglyceride and cholesterol content are observed at 1 mo of age (5) but increase in low-protein offspring with age relative to control (5). Hepatic lipid homeostasis is regulated by a number of nuclear receptors including the peroxisome proliferator-activated receptor-α (PPARα) and the liver X-receptor (LXR) (11). PPARα and LXR are activated by free fatty acids and cholesterol metabolites, respectively, and they modulate lipid homeostasis by activating target genes (8) that initiate the synthesis and uptake of cholesterol, fatty acids, and triglycerides (see Refs. 7 or 16 for a complete review). An earlier study by Lillycrop et al. (12) reported that a reduction in methylation of CpG dinucleotides in the PPARα nuclear receptor in 28-day-old offspring of low-protein dams is associated with an increase in expression of PPARα mRNA and its target gene, Acyl-CoA oxidase. Whether hypomethylation of the PPARα gene and increased gene expression persist into adulthood, and whether these changes in gene expression are associated with dysregulation of lipid metabolism are not addressed. However, these findings indicate that epigenetic regulation of the hepatic PPARα gene can occur in response to a fetal insult and suggests a potential

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link between adverse influences during fetal life and later adult health.

Although epigenetic processes, such as changes in gene methylation, are known mediators of transcriptional activation and repression (9), whether the specific hypomethylation pattern of the PPARα gene induced by maternal low protein in offspring can directly influence PPARα gene expression was not determined in the previous study by Lillycrop et al. (12). van Straten et al. (17) utilize the DOHaD model of maternal low protein to demonstrate epigenetic modification of another nuclear receptor critical for lipid homeostasis, the LXR. In response to prenatal exposure to low protein, a specific pattern of hypermethylation of CpG dinucleotides in the fetal liver LXR alpha gene promoter was observed at embryonic day 19.5 (E19.5) in low-protein offspring and importantly, was associated with reduced expression of the fetal hepatic LXR alpha gene (17). In addition, expression of LXR alpha target genes, which contribute to cholesterol elimination, such as the ATP-binding cassette transporters ABCG5 and ABCG8, were also reduced (17). The causal relationship between the specific pattern of CpG hypermethylation of the LXR gene identified in this study and changes in LXR gene expression was directly tested in vitro by use of pharmacological and reporter gene expression assay methodologies (17). Notably, van Straten et al. observed that the specific hypermethylation pattern of the LXR gene induced in response to maternal low protein resulted in a reduction in gene expression in vitro (17). Thus, this study provides further evidence that epigenetic effects may serve as a critical link between the fetal response to a nutritional insult and later adult disease.

However, the overall importance of epigenetic modification of a gene and the transmission of changes in gene expression into pathophysiological relevance is still not clear. In the current study by van Straten et al. (17) reduced expression of the fetal hepatic LXR gene and other genes involved in cholesterol excretion was associated with a decrease in fetal hepatic cholesterol content. In adult mice lacking the LXR alpha receptor, hepatic cholesterol is elevated in response to a dietary challenge of 2% cholesterol, suggesting that LXR alpha play a critical role in adult hepatic cholesterol homeostasis (7). Thus, suppression of fetal hepatic LXR alpha gene expression and its target genes was not associated with an increase in fetal hepatic cholesterol content. Cholesterol is critical for many processes during fetal development (15) and the fetus obtains its cholesterol from both endogenous and exogenous sources (21). Expression of rodent fetal hepatic LXR alpha peaks at E18 (15) and previous work by van Straten et al. demonstrate that LXR induced expression of hepatic ABCG5 and ABCG8 is functional in fetal mice (18). However, placental LXR and its target genes may also contribute to cholesterol homeostasis in the fetus (13), and therefore, the importance of programmed changes in the fetal hepatic LXR pathway on fetal lipid homeostasis is not yet clear. Additionally, whether programmed hypermethylation of the LXR alpha gene persists beyond fetal life is reversed, and/or contributes to changes in adult hepatic cholesterol content and later adult disease are important questions that remain to be tested.

To conclude, the study by van Straten et al. (17) provides critical evidence that modulation of a gene by an epigenetic process, such as DNA methylation in response to fetal insult can alter gene expression. Whether these specific epigenetic modifications persist long term, contribute to later reprogramming of the LXR gene and its target genes, or contribute to an increased risk for adult disease is not yet known. Moreover, whether passage of an epigenetic modification to the next generation is of pathophysiological significance remains unanswered. Hypomethylation of the hepatic PPARα gene in offspring (F1) of maternal low-protein dams persists into adulthood (12) and is transmitted to the next generation (F2) (4). Yet, hypomethylation of the PPARα gene does not translate into an increase in PPARα gene expression in F2 offspring (4). Clearly, additional studies are required to comprehensively address the importance of transgenerational effects of epigenetic mechanisms in the DOHaD. Investigation of these parameters will be critical in determining the overall importance of epigenetic processes as a potential link between fetal responses to environmental influences, the programming of adult health and disease, and the heritable risk of disease in the next generation.

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DISCLOSURES

No conflicts of interest are declared by the author.

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