Epigenetics and Fetal Metabolic Programming: A Call for Integrated Research on Larger Cohorts

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Fetal metabolic programming is a concept first suggested by Barker and Hales (1,2) in the early 1990s. On the basis of compelling epidemiological evidence, they hypothesized that fetal and perinatal events, such as maternal undernutrition, were central to determine one's risk to develop chronic metabolic diseases. Such conditions, including obesity, diabetes, and cardiovascular diseases, have become a very important population health concern. Since the first introduction of this concept, it has been corroborated by many animal but only few human studies (3). Therefore, a number of key issues remain to be clarified, with the most important being our knowledge of the mechanisms involved in fetal metabolic programming. In brief, more research is needed in human models of fetal metabolic programming. One such promising model is gestational diabetes mellitus (GDM).

GDM is a form of diabetes first diagnosed during pregnancy. It is the most important cause of hyperglycemia in the course of pregnancy, and its prevalence ranges from 1 to 20% (4). Regrettably, its occurrence is predicted to grow rapidly in the next years as obesity and diabetes are significant risk factors for this condition (5). In other words, GDM is prevalent, increasingly common, and predicts the development of diabetes in mothers. Maybe more importantly, GDM is also associated with a two- to fourfold increased risk for offspring to develop overweight/obesity and the metabolic syndrome, respectively (3). GDM is thus an important health issue considering that glucose metabolism impairments might arise in children as young as 3 years of age (6–8) and that up to 80% of overweight/obese children remain so at adulthood (9). Therefore, GDM is a good model to study the mechanisms involved in fetal metabolic programming and also to elucidate new mechanisms to help diagnose, treat, and prevent its consequences for the newborns and successive generations. Epigenetics is currently a very promising mechanism for fetal metabolic programming (Fig. 1) (10,11).

In the current issue of *Diabetes*, El Hajj et al. (12) report DNA methylation analyses performed in 251 cord blood and placenta samples obtained from newborns exposed or not to GDM. A total of 14 genes involved in fetal growth and development (imprinted genes; $n = 7$), glucose metabolism ($n = 4$), inflammation ($n = 1$), carcinogenesis ($n = 1$), and maintaining cellular pluripotency ($n = 1$) were analyzed in addition to two repetitive genomic elements (ALU and LINE1). The most promising results were obtained with the paternally expressed (imprinted) mouse homolog of the mesoderm-specific transcript (*MEST*) gene, which was found to be hypomethylated in cord blood and placenta samples from children exposed to GDM as compared with nonexposed samples. Interestingly, the authors also report that compared with that of normal-weight adults, the *MEST* gene was also hypomethylated in blood samples obtained from adults with morbid obesity (BMI >35 kg/m^2). The highest DNA methylation difference between groups reached 7.2% in the placenta, which is impressive when compared with the values reported in the current literature. The distinctive impact of both GDM treatments (diet vs. insulin) on the *MEST* DNA methylation profile was also tested, but no obvious interaction was observed. This might suggest that *MEST* epigenetic dysregulation took place before the GDM diagnosis and start of treatment or that none of the treatments were effective in preventing DNA methylation changes at this gene locus. Unfortunately, the functionality of the associated epivariations was not assessed, precluding stronger conclusions. Nevertheless, it remains that MEST was shown to be a factor involved in fetal growth and development and a promising candidate gene for obesity and its related metabolic complications.

As underlined by the authors, although the number of samples analyzed is reasonable when compared with other studies found in the current literature, the relatively sample size preclude any conclusion on negative results. Considering that it is important first to demonstrate that fetal environment impacts the newborn epigenome, the sample size is not a central concern as long as some positive results emerge. However, the knowledge of which genes and metabolic pathways are responsive or not to the fetal environment will increasingly become essential to understand the interplay between epigenetics and fetal metabolic programming. Accordingly, larger cohorts will need to be analyzed in order to find DNA methylation changes of smaller effect size.

Association results also have to be observed in more than one cohort and population, and this analytical strategy will have to be applied in epigenetic epidemiology studies as well. Such studies will offer real challenges considering the difficulties in recruiting and following pregnant women throughout pregnancy and newborns throughout infancy, adolescence, and adulthood, and in large numbers (>1,000). In absence of such validation cohorts and populations, other strategies such as studying different tissues and phenotypes could prove helpful. El Hajj et al. successfully applied this strategy to show that *MEST* was hypomethylated also in blood of individuals with morbid obesity as compared with normal-weight men

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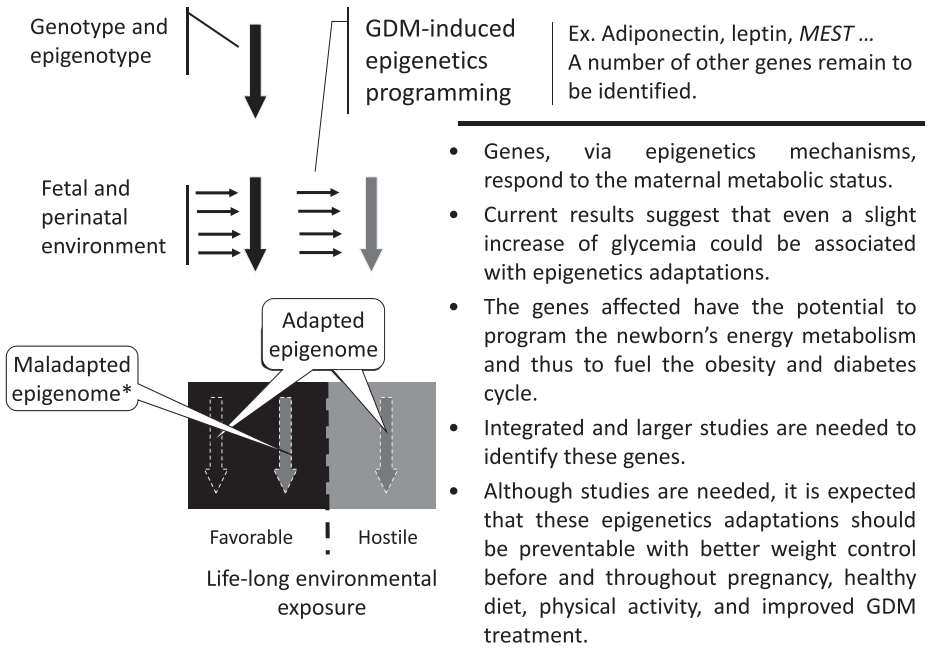

FIG. 1. Schematic epigenomic response to fetal and perinatal stimuli such as GDM exposure. *An epigenome program not well adapted to the environment (maladapted) is expected to produce metabolic changes associated with an increased risk for obesity and diabetes.

and women. In absence of validation cohorts, the analyses of complementary phenotypes such as the gene products (mRNA and protein) or newborn fetal growth and development indices (such as birth weight) would also be an acceptable strategy to confirm the initial epigenetic findings. However, transcriptomics as well as newborns' phenotype analyses are missing in the article by El Hajj et al., thus preventing any conclusion on the role of the reported *MEST* epivariations on gene transcription regulation and fetal growth and development.

The article by El Hajj et al. demonstrates that GDM exposure impacts the newborn's methylome and that these epivariations might be associated with adulthood obesity. Although the candidate gene approach will continue to prove successful (13,14), we also need more integrated studies in which the genes involved in common metabolic pathways will be analyzed together and the results interpreted as a whole. Genome-wide analysis is an interesting option to get a thoughtful coverage of the genome as the technologies (array and sequencing) are increasingly reliable and affordable, although data analysis remains challenging. Nevertheless, the next major step will clearly be to study large (>1,000) longitudinal birth cohorts from conception (first trimester of pregnancy) to adulthood and conduct randomized control trials in which mothers at high risk to develop GDM will have access to hyperglycemia prevention programs before pregnancy (or no later than the end of first trimester of pregnancy). Such studies would provide the conditions to prove that epigenetic adaptations to GDM are involved in fetal metabolic programming, and that these changes are not deterministic as it is expected they will potentially be prevented.

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