Epigenetics and Diabetes Risk: Not Just for Imprinting Anymore?

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Relationships of maternal obesity, diabetes, and nutrition during pregnancy with birth weight and in turn relationships between birth weight and risk of obesity and diabetes in later life have long been observed (rev. in 1). Elucidating the etiology of these relationships is challenging given that mammalian mothers impart both genes and intrauterine environment to their offspring. One factor that may play a role is epigenetics. Epigenetic effects are defined as heritable changes to DNA structure that do not involve changes to the DNA sequence. Unlike sequence changes, they can be reset or undone under certain conditions such as in early development. Mechanisms include changes in histone deacetylation and methylation of cytosines in CpG clusters (2).

An epigenetic phenomenon that is well-documented in humans and may be the first that springs to mind is genomic imprinting, whereby during germ cell development, regulatory regions of certain genes are differentially methylated and expressed depending on whether the gene is inherited from the mother or father (2). Imprinting impacts several genes, including some in which mutation of the expressed copy or disturbance of normal imprinting is involved in both most cases of the rare transient neonatal form of diabetes (3) and, based on recent evidence, apparently some cases of polygenic type 1 (4) and 2 (5) diabetes as well.

However, other factors, including environmental stimuli, can induce epigenetic changes as well (6–8); thus imprinting is not the only epigenetic mechanism potentially involved in diabetes and the related phenotypes of obesity and metabolic syndrome (9). Moreover, imprinting is not the only example of the phenomenon of parent-of-origin effects, whereby the sex of the parent influences what genes are expressed and in turn the risk for diabetes and related conditions. Parent-of-origin effects can also include mitochondrial genome inheritance, which only occurs through the maternal line, and maternal effects on the intrauterine environment (10).

There are well-characterized examples whereby maternal genes appear to influence diabetes risk via both inheritance of maternal genetic variants and effects of the maternal genetics in creating a hyperglycemic in utero environment. For example, offspring of mothers carrying an *HNF1A* mutation (maturity onset diabetes of the young 3) have an age of diabetes onset 8 years earlier than those inheriting such a mutation from their fathers (11). On the other hand, *GCK* (glucokinase) mutations increase risk for both low birth weight and diabetes (maturity onset diabetes of the young 2) when inherited from either parent but increase birth weight when carried by the mother due to the hyperglycemic environment they create (12). In contrast to *HNF1A*, *GCK* mutations have been found not to have a maternal-specific effect on future glucose tolerance, apparently due to the lack of a role of *GCK* mutations in β-cell dysfunction (13). The effects of intrauterine environment on offspring adiposity and metabolism appear to be mediated by clear physiological mechanisms and do not seem likely to require an epigenetic mechanism.

Regardless, there is documented evidence in mammals (rev. in 1) that maternal exposures such as nutrition status can influence metabolic phenotypes through epigenetic changes. Pregnant rats fed a diet moderately reduced in protein content produce obese offspring with reduced methylation of the promoter (upstream regulatory region) of the gene *PPARA*, encoding peroxisome proliferator-activated receptor (PPAR)-α, and concomitant increased expression of PPAR-α and target genes involved in fatty acid oxidation (14). The ability of neonatal leptin treatment of these offspring rats to reverse hepatic hypomethylation of *PPARA* and overexpression and prevent obesity (15) reinforces evidence for a direct causal pathway of maternal malnutrition → epigenetic fetal modification → obese offspring. Evidence for a similar phenomenon in humans has been purely epidemiological (e.g., the relationship between maternal nutrition during early pregnancy and adult adiposity observed in individuals born during the Dutch famine [16]). That grandparental nutrition status has been associated with diabetes and cardiovascular disease risk (17) hints at the potential capability of nutritional exposure to have epigenetic effects in humans, since only DNA is transmitted in this case.

Up to this point in time there has been no reported direct evidence in humans of a relationship between in utero non–imprinting-related epigenetic changes and phenotype. In the May issue of *Diabetes*, Godfrey et al. (18) report the first evidence that methylation status of gene promoters in utero affects related phenotypes later in a child's development. They selected 28 genes identified in previous animal studies (19,20) and 50 genes identified in a human expression microarray study (for a total of 78 genes) of 15 umbilical cord samples as having the highest between-subject variation in methylation. These 15 samples were part of a larger study of 78 individuals in whom both umbilical cord DNA and adiposity measures (by dual energy X-ray absorptiometry) at 9 years of age were available. They then measured the percent methylation status of these 78 gene promoters in the 78 subjects and evaluated

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the correlation with total fat mass, percent body fat, and ratio of trunk to limb fat at age 9, and selected 5 genes for further study based on initial analyses.

They found that for seven CpG promoter regions for three genes (*SOD1*, *RXRA*, and *eNOS*), percent methylation was significantly correlated with at least one of the three traits (absolute value of $r = 0.26–0.42$, $P = 0.043$ to <0.001). They then found that for one of the *RXRA* regions for which methylation was positively correlated with adiposity, methylation status was also inversely correlated with maternal carbohydrate intake, but not fat or protein intake, during early pregnancy. Finally, they attempted replication of the two strongest *RXRA* and *eNOS* signals in an independent cohort of 239 6-year-old children with both umbilical cord DNA and adiposity measurements. The *RXRA* promoter methylation status was positively correlated with adiposity in this second set with a similar effect size and significance to the original cohort.

In sum, methylation status of an *RXRA* promoter region was strongly correlated directly with childhood adiposity in two independent cohorts and inversely associated with early pregnancy carbohydrate intake in the initial cohort. Taken together, these findings suggest that effects of the in utero environment on development influence later metabolic parameters via epigenetic gene-specific promoter methylation, possibly mediated through maternal diet. That *RXRA* in particular emerged as a gene whereby methylation-modulated expression is related to adiposity lends credence to the findings given the role of *RXRA* in dimerizing with PPARs, and in turn lipid and carbohydrate metabolism (21). Notably, neither *RXRA* nor any of the genes identified in the study as having correlation of promoter methylation with childhood adiposity are imprinted genes (18).

What are the implications of this study? These data provide much needed human evidence to complement the growing body of animal data revealing that epigenetic changes occurring during gestation, possibly maternal nutrition-mediated, appear to influence adiposity and related metabolic phenotypes. Our direct knowledge of the role of epigenetic effects on human variation and disease, including obesity and potentially diabetes, is expanded beyond imprinting, and our understanding of the role of genes in obesity in general is expanded beyond the effects of fixed genetic variation. The results reveal some of the value of studying human umbilical cord tissue to understanding human variation and disease. In practical clinical terms, the findings have the potential to reinforce the importance of adequate nutrition counseling during pregnancy (as noted by the authors [18]). If the maternal dietary association is replicated and expanded, it provides a mechanism whereby maternal diet might have an impact long after birth on adiposity, which in turn would be expected to influence risk for diabetes and its complications.

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REFERENCES

1. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med 2008;359:61–73
2. Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33 (Suppl.):245–254
3. Temple IK, Mackay DJG. Diabetes mellitus, 6q24-related transient neonatal. In *GeneReviews*. Pagon RA, Bird TD, Dolan CR, Stephens K, Eds. Seattle, Washington, University of Washington, 1993
4. Wallace C, Smyth DJ, Maisuria-Armer M, Walker NM, Todd JA, Clayton DG. The imprinted DLK1-MEG3 gene region on chromosome 14q32.2 alters susceptibility to type 1 diabetes. Nat Genet 2010;42:68–71
5. Kong A, Steinthorsdottir V, Masson G, et al.; DIAGRAM Consortium. Parental origin of sequence variants associated with complex diseases. Nature 2009;462:868–874
6. Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet 2007;8:253–262
7. Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. Nature 2007;447:433–440
8. MacFarlane AJ, Strom A, Scott FW. Epigenetics: deciphering how environmental factors may modify autoimmune type 1 diabetes. Mamm Genome 2009;20:624–632
9. Murphy SK, Jirtle RL. Imprinting evolution and the price of silence. Bioessays 2003;25:577–588
10. Rampersaud E, Mitchell BD, Naj AC, Pollin TI. Investigating parent of origin effects in studies of type 2 diabetes and obesity. Curr Diabetes Rev 2008;4:329–339
11. Stride A, Shepherd M, Frayling TM, Bulman MP, Ellard S, Hattersley AT. Intrauterine hyperglycemia is associated with an earlier diagnosis of diabetes in HNF-1alpha gene mutation carriers. Diabetes Care 2002;25:2287–2291
12. Hattersley AT, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S. Mutations in the glucokinase gene of the fetus result in reduced birth weight. Nat Genet 1998;19:268–270
13. Singh R, Pearson ER, Clark PM, Hattersley AT. The long-term impact on offspring of exposure to hyperglycaemia in utero due to maternal glucokinase gene mutations. Diabetologia 2007;50:620–624
14. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. J Nutr 2005;135:1382–1386
15. Gluckman PD, Lillycrop KA, Vickers MH, et al. Metabolic plasticity during mammalian development is directionally dependent on early nutritional status. Proc Natl Acad Sci USA 2007;104:12796–12800
16. Ravelli GP, Stein ZA, Susser MW. Obesity in young men after famine exposure in utero and early infancy. N Engl J Med 1976;295:349–353
17. Kaati G, Bygren LO, Edvinsson S. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. Eur J Hum Genet 2002;10:682–688
18. Godfrey KM, Sheppard A, Gluckman PD, et al. Epigenetic gene promoter methylation at birth is associated with child's later adiposity. Diabetes 2011;60:1528–1534
19. Burdge GC, Lillycrop KA. Nutrition, epigenetics, and developmental plasticity: implications for understanding human disease. Annu Rev Nutr 2010;30:315–339
20. Lillycrop KA, Rodford J, Garratt ES, et al. Maternal protein restriction with or without folic acid supplementation during pregnancy alters the hepatic transcriptome in adult male rats. Br J Nutr 2010;103:1711–1719
21. Sugden MC, Holness MJ. Role of nuclear receptors in the modulation of insulin secretion in lipid-induced insulin resistance. Biochem Soc Trans 2008;36:891–900