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Intersection of complex genetic traits affecting maternal metabolism, fetal metabolism, and neural tube defect risk: Looking for needles in multiple haystacks ☆

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Neural tube defects (NTDs) are among the most common birth defects (1 in 1000 live births); while their etiology is still not well understood, current evidence indicates that both genetic and environmental factors are involved [1-3]. Although animal models have been valuable for elucidating molecular processes that regulate neural tube formation [4-7], specific genes responsible for NTDs in humans have been difficult to identify. This could be because NTDs (which themselves are multifaceted, depending on manifestation and location) are complex genetic traits, because of modifier gene effects on Mendelian traits, or because of environmental effects (maternal folic acid status, teratogens, maternal disease), or all of the above. Added to this, environmental factors (such as folic acid deficiency, anticonvulsant medications, maternal diabetes or obesity) themselves can cause NTDs [8-13]. Indeed, maternal pregestational diabetes, in which the intrauterine environment is altered due to increased delivery of glucose to the embryo, is a major cause of neural tube, and other birth defects in humans and in animal models [10,14]. On the other hand, animal models have demonstrated that penetrance of diabetic teratogenesis is dependent on the strain background of the embryo [15-17], indicating that modifier genes or polymorphisms of genes leading to hyperglycemia-induced NTDs influence susceptibility to environmental factors. Maternal genes also contribute to NTD risk, separate from their contribution to the off-spring genotype because they influence the intrauterine environment to which the embryo is exposed [18,19].

Recent findings from genome-wide association studies (GWAS) indicate that obesity, insulin resistance, and type 2 diabetes are influenced by sequence variants at multiple genetic loci [20,21]. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study was a multi-center study involving 25,505 women that investigated the correlation of mid-gestation glucose tolerance testing with pregnancy outcomes such as birth weight and pregnancy and neonatal complications [22]. Notably, the HAPO Study found that many of the genetic variants that GWAS found to be associated with gestational and type 2 diabetes were associated with maternal glucose levels and inflammatory markers, even in the absence of overt diabetes [23-26]. Furthermore, metabolomic analysis of serum of mothers with high fasting plasma glucose levels showed abnormalities in many metabolites that are associated

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with insulin resistance [27]. While the glucose tolerance and serum testing of mothers in the HAPO study took place mid-gestation, when the insulin resistance of pregnancy begins to manifest and after the period of gestation at which NTDs arise, these studies support the hypothesis that maternal genes associated with glucose metabolism can affect circulating macro- and micronutrients and inflammatory cytokines that affect the uterine environment.

Previously, Huiping Zhu and colleagues investigated several single nucleotide polymorphisms (SNPs) among nine genes that had been found to be associated with type 2 diabetes or obesity in multiple GWAS among participants in the National Birth Defects Prevention Study. They evaluated maternal and offspring genetic effects and found five significant maternal effects [28]. In this issue of *Molecular Genetics and Metabolism*, this group further investigated interaction of maternal and offspring metabolic genes and NTD risk among 737 case-parent triads in the National Birth Defects Prevention Study [29]. They examined SNPs among seven maternal metabolic genes and two offspring (fetal) genes. Four significant interactions were observed among case-parent triads, with increased risk between a variant of maternal *ENPP1* and two variants of fetal *SLC2A2*, and decreased risk between two variants of maternal *LEP* and the same two variants of fetal *SCL2A2*. The strongest interactions were between a maternal variant of *ENPP1* and a fetal variant of *SCL2A2*. It is noteworthy that these maternal and offspring SNPs were not found to be significant in single locus analysis [28]. Interestingly, the interactions were similar even in case-parent triads in which the mothers did not have pre-gestational diabetes or were not obese. This suggests that subtle difference in maternal fuel metabolism, even without reaching a threshold for clinical diagnosis of obesity or diabetes, influences not only the metabolic environment of the embryo, but also how the embryo handles the altered fuel sources due to the fact that its genetic background determines its susceptibility to NTDs.

So, what do these genes encode, and how might they influence metabolism and risk for NTDs? *ENPP1* encodes ectonucleotide pyrophosphatase/phosphodiesterase 1, a membrane-bound glycoprotein that inhibits insulin signaling in hepatocytes, skeletal muscle, adipocytes, and pancreatic β cells [30]. *ENPP1* is also expressed in tissues not thought to be involved in insulin action, such as osteoblasts, where it promotes differentiation and mineralization [31]. *LEP* encodes the hormone, leptin, which is secreted by white adipose tissue and acts on the hypothalamus and peripheral organs to regulate satiety and energy homeostasis, and contributes to regulation of the hypothalamic–pituitary–gonadal axis in females [32,33]. Thus, both *ENPP1* and *LEP* variants have the potential to affect circulating nutrients delivered to the embryo as well as proinflammatory cytokines that could increase inflammation in the uterine environment.

SCL2A2 encodes the glucose transporter, Glut2, which is expressed by embryos, as well as by pancreatic β cells and the liver [34,35]. Glut2 is a high K_M (17 mM) glucose transporter; thus at normal glucose concentrations (approximately 5 mM), it is not an efficient glucose transporter, but at high glucose concentrations (such as those to which pancreatic β cells and the liver are exposed following a meal) it transports glucose efficiently. Mouse embryos that are *Scl2a2*^{+/-} or *Scl2a2*^{-/-} are resistant to maternal hyperglycemia-induced NTDs [34]. We recently showed that high glucose media induces oxidative stress and inhibits expression of *Pax3*, a gene required for neural tube closure, in murine embryonic stem cells that express functional Glut2 glucose transporters but not in those that lack Glut2 [36]. This indicates that *SCL2A2* variants expressed by embryos could increase susceptibility to maternal hyperglycemia-induced NTDs by increasing glucose transport into embryo cells, thereby inducing oxidative stress and suppressing essential neural tube gene expression. However, as noted above, the gene-gene interactions were not related to maternal pre-gestational diabetes or obesity. Furthermore, because the K_M of Glut2 for glucose is about 3 times higher than normal circulating glucose concentration, it would not be an efficient transporter of glucose

to the embryo in normoglycemic pregnancies. Therefore, while maternal glucose levels in the first trimester of the NTD pregnancies were not known, it is unlikely that increased insulin resistance that might be caused by *ENPP1* gain-of-function variants would cause maternal blood glucose levels to rise sufficiently to increase glucose transport to embryos via Glut2. On the other hand, we noticed that *Scl2a2*^{+/-} and *Scl2a2*^{-/-} embryos were produced at less than predicted Mendelian frequencies in both hyperglycemic and control mice [34]. This suggests that Glut2-mediated transport of a solute other than glucose is important for early embryo survival. Glut2 is also a low K_M (0.8 mM) glucosamine transporter [37]. Thus, it is possible that *SCL2A2* variants affect NTD risk due to differential transport of glucosamine or other solutes.

It should be noted that *SCL2A2* variants were not found to be significant in single locus analysis [28]. However, since the embryo had to have inherited the risk-associated *SCL2A2* variant from either the mother or the father, it would be of interest to investigate whether there is an interaction of maternal *SCL2A2* variants with maternal-offspring *ENPP1*–*SCL2A2* or *LEP*–*SCL2A2* variants.

The significance of this paper is that it provides, for the first time, an explanation for genetic susceptibility to NTDs due to specific maternal and fetal combinations of genes related to fuel metabolism. More work will still be needed to determine the nutrients, metabolites, or proinflammatory cytokines responsible, and how, on a molecular and biochemical level, these NTDs occur.

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