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Maternal–fetal metabolic gene–gene interactions and risk of neural tube defects

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ABSTRACT

Single-gene analyses indicate that maternal genes associated with metabolic conditions (e.g., obesity) may influence the risk of neural tube defects (NTDs). However, to our knowledge, there have been no assessments of maternal–fetal metabolic gene–gene interactions and NTDs. We investigated 23 single nucleotide polymorphisms among 7 maternal metabolic genes (*ADRB3*, *ENPP1*, *FTO*, *LEP*, *PPARG*, *PPARGC1A*, and *TCF7L2*) and 2 fetal metabolic genes (*SLC2A2* and *UCP2*). Samples were obtained from 737 NTD case-parent triads included in the National Birth Defects Prevention Study for birth years 1999–2007. We used a 2-step approach to evaluate maternal–fetal gene–gene interactions. First, a case-only approach was applied to screen all potential maternal and fetal interactions ($n = 76$), as this design provides greater power in the assessment of gene–gene interactions compared to other approaches. Specifically, ordinal logistic regression was used to calculate the odds ratio (OR) and 95% confidence interval (CI) for each maternal–fetal gene–gene interaction, assuming a log-additive model of inheritance. Due to the number of comparisons, we calculated a corrected p-value (q-value) using the false discovery rate. Second, we confirmed all statistically significant interactions ($q < 0.05$) using a log-linear approach among case-parent triads. In step 1, there were 5 maternal–fetal gene–gene interactions with $q < 0.05$. The "top hit" was an interaction between maternal *ENPP1* rs1044498 and fetal *SLC2A2* rs6785233 (interaction OR = 3.65, 95% CI: 2.32–5.74, $p = 2.09 \times 10^{-8}$, $q = 0.001$), which was confirmed in step 2 ($p = 0.00004$). Our findings suggest that maternal metabolic genes associated with hyperglycemia and insulin resistance and fetal metabolic genes involved in glucose homeostasis may interact to increase the risk of NTDs.

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1. Introduction

Neural tube defects (NTDs) are among the most common, costly, and deadly of all human congenital anomalies whose etiologies remain largely unknown [1,2]. Maternal pre-gestational diabetes and pre-pregnancy obesity are two well-established risk factors for NTDs [3–19]. While the exact mechanisms behind these associations are unknown, it is believed that glucose homeostasis plays an important role. At the time of neural tube closure (approximately the fourth week of gestation), mothers with poorly regulated glucose levels are likely to have an altered intrauterine environment leading to abnormal organogenesis. Several genes related to glucose homeostasis have been previously identified in human and animal studies. Furthermore, genes related to glucose homeostasis have been associated with type 2 diabetes and obesity risk in genome-wide association studies (GWAS) [20–23]. Work from our group indicated an association between inherited (i.e., fetal) variation in the *UCP2* gene and NTDs [24]. *SLC2A2* is an important glucose transporter during embryonic neural tube development [25]. Additionally, we found associations between maternal genotypes in *FTO*, *TCF7L2*, and *LEP* and NTDs suggesting that maternal genetic effects may cause changes in intrauterine environment and play a role in disease risk [24]. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study has demonstrated that common genetic variants in genes such as *TCF7L2* are associated with fasting and post-challenge glucose levels during pregnancy [26]. Because of these

Abbreviations: BMI, body mass index; CATI, computer assisted telephone interview; CI, confidence interval; FDR, false discovery rate; GWAS, genome-wide association study; LRT, likelihood ratio test; NBDPS, National Birth Defects Prevention Study; NTDs, neural tube defects; SNP, single nucleotide polymorphism; RR, risk ratio.

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findings, we sought to evaluate the interactions between maternal and fetal genes related to glucose homeostasis and the risk of NTDs.

2. Materials and methods

2.1. Subjects

The study population included NTD case-parent triads (n = 737) from the National Birth Defects Prevention Study (NBDPS), with estimated dates of delivery between January 1, 1999 and December 31, 2007. Details of the NBDPS have been published elsewhere [27]. In brief, the NBDPS is a population-based case–control study of major structural birth defects. For the period 1999–2007, case infants with one or more congenital anomalies were ascertained through ten birth defects surveillance systems throughout the United States (Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah) and included live births, stillbirths, and induced pregnancy terminations. NTDs included in the NBDPS had British Pediatric Association (BPA) codes for the diagnoses anencephaly (740.0), craniorachischisis (740.1), spina bifida (741.0), and encephalocele (742.0). Abstracted data for all NTD case infants were reviewed by clinical geneticists using specific criteria, including standardized case definitions and confirmatory diagnostic procedures [28]. Infants/fetuses with known single gene disorders or chromosomal abnormalities were excluded from the NBDPS. Mothers completed a one-hour computer assisted telephone interview (CATI) in English or Spanish between 6 weeks and 2 years after the estimated date of delivery. The interview included sections on maternal conditions and illnesses, lifestyle and behavioral factors, and multivitamin use.

2.2. Maternal and fetal candidate genes and single nucleotide polymorphisms (SNPs)

The selection criterion for candidate genes and SNPs was reported previously [24]. Briefly, genes and SNPs selected were those identified as being associated with type 2 diabetes or obesity in multiple GWAS studies, or those with supporting evidence from both candidate gene studies and animal models. Maternal candidate genes included in the current study were *ADRB3*, *ENPP1*, *FTO*, *LEP*, *PPARG*, *PPARGC1A*, and *TCF7L2*. Fetal candidate genes analyzed were *UCP2* and *SLC2A2* [20,25,29–34]. Information on the SNPs evaluated and the selection criteria used is listed in Table 1.

2.3. DNA samples and genotyping analysis

Buccal brushes from mothers, fathers, and infants were collected as part of the NBDPS [35]. DNA was extracted from buccal cells and a standard quality control procedure was applied to each sample before they were submitted to the NBDPS sample repository [35]. To assure genotyping proficiency, high quality, and high concordance among all NBDPS laboratories, annual evaluations are conducted to confirm the performance of each laboratory (see Supplemental material). Our laboratory at the University of Texas at Austin, Dell Pediatric Research Institute has passed all of these evaluations with a score of 100%. SNPs were assayed using TaqMan method (Life Technologies Corporation, Carlsbad, CA) and genotypes were read and discriminated on the ABI PRISM® 7900HT Sequence Detection System (Life Technologies Corporation, Carlsbad, CA).

2.4. Statistical analysis

The characteristics of cases and case mothers were summarized using counts and proportions for the following variables: phenotype (spina bifida, anencephaly, encephalocele); infant sex (male, female); maternal age (<20, 20–34, ≥35 years); maternal race/ethnicity (non-Hispanic White, non-Hispanic Black, Hispanic, other); maternal education (<12, 12, 13–15, >15 years); maternal folic acid supplementation during three months before conception through the first month of pregnancy (no, yes); maternal pre-pregnancy body mass index or BMI (underweight [<18.5 kg/m^2], average weight [18.5–24.9 kg/m^2], overweight [25.0–29.9 kg/m^2], and obese [≥30.0 kg/m^2]); and maternal pre-pregnancy diabetes (no, yes). For each analyzed polymorphism, samples for which a genotype could not be assigned and triads that had genotype combinations that were inconsistent with Mendelian inheritance were determined. For each subject, the number of genotyping failures (i.e., genotypes that could not be assigned) was determined. These analyses were performed using Intercooled Stata, version 12.1 (StataCorp LP, College Station, TX).

Table 1
Metabolic genes and SNPs included in maternal–fetal gene–gene interaction analysis.

Gene symbol	Ref SNP	Chr[a]	Position	Alleles[b]	SNP information	MAF[c] (CEU)	Selection criteria
TCF7L2	rs12255372	10	114808902	G/T	Intron	0.21	Diabetes-associated
TCF7L2	rs7903146	10	114758349	C/T	Intron	0.22	Diabetes-associated
TCF7L2	rs290487	10	114909731	C/T	Intron	0.27	Diabetes-associated
TCF7L2	rs10885390	10	114640797	T/A	Intergenic	0.24	Diabetes-associated
TCF7L2	rs3814573	10	114898093	C/T	Intron	0.40	Diabetes-associated
UCP2	rs660339	11	73689104	G/A	Missense	0.43	Obesity/diabetes
ENPP1	rs1044498	6	132172368	A/C	Missense	0.31	Insulin resistance
FTO	rs9939609	16	53820527	T/A	Intron	0.38	Obesity, BMI
FTO	rs8050136	16	53816275	C/A	Intron	0.37	Obesity, BMI
FTO	rs1421085	16	53800954	T/C	Intron	0.26	Obesity, BMI
FTO	rs17817449	16	53813367	T/G	Intron	0.35	Obesity, BMI
ADRB3	rs4994	8	37823798	T/C	Missense	0.10	Obesity, BMI
PPARG	rs1801282	3	12393125	C/G	Intron	0.06	Obesity-associated
PPARGC1A	rs8192678	4	23815662	G/A	Missense	0.30	Obesity/metabolic disorders
PPARGC1A	rs3736265	4	23814707	G/A	Missense	0.11	Obesity/metabolic disorders
LEP	rs11760956	7	127891087	G/A	Intron	0.29	tagSNP
LEP	rs12706831	7	127887068	T/G	Intron	0.46	tagSNP
LEP	rs3828942	7	127894305	G/A	Intron	0.45	tagSNP
LEP	rs2071045	7	127892980	T/C	Intron	0.26	tagSNP
LEP	rs2167270	7	127881349	G/A	5′utr	0.35	tagSNP
SLC2A2	rs11924032	3	170735099	G/A	Intron	0.31	tagSNP
SLC2A2	rs6785233	3	170756985	T/G	Intergenic	0.19	tagSNP
SLC2A2	rs5400	3	170732300	C/T	Missense	0.21	Diabetes-associated, cholesterol levels

[a] Chr (chromosome) genomic build 37.1; group term GRCh37.
[b] RefSNP alleles: reference allele/risk allele (minor allele).
[c] MAF (Minor Allele Frequency) source: 1000 genomes project.