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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.

We utilized a 2-step approach to evaluate maternal–fetal gene–gene interactions [36]. For step 1, a case-only approach was used to screen all potential interactions (n = 76), as this design provides greater power in the assessment of gene–gene interactions compared to a case–control design or case-parent triads [37]. The case-only design has been described elsewhere [38] and has been used extensively for the assessment of gene–environment and gene–gene interactions [36,38–43]. 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. The genotypes for each SNP were classified according to the number of minor alleles present (i.e., 0, 1, 2). In the ordinal logistic regression model, the maternal genotype was treated as the dependent variable and the fetal genotype was treated as the independent variable [36,37,43]. Due to the number of comparisons, we calculated a corrected p-value (q-value) to control for the false discovery rate (FDR) at 0.05 [44,45]. These analyses were conducted using Intercooled Stata version 12.1 (StataCorp LP, College Station, TX). All interactions where $q < 0.05$ were included in step 2.

For step 2 (i.e., case-parent triad approach), maternal–fetal gene–gene interactions that were associated with NTDs in the case-only analyses (i.e., $q < 0.05$) were investigated using log-linear models for joint effects [46]. To test the no-interaction null hypothesis, we calculated a 2-degrees-of-freedom likelihood ratio test (LRT) statistic as twice the difference of the log likelihoods for the log-linear model that included two parameters indexing the inherited genotype (SNP1), two parameters indexing the maternal genotype (SNP1), and two interaction terms representing the product of maternal–fetal SNP1–SNP2 pairwise genotypes (SNP2 being the fetal "interacting" SNP) and a reduced model that excluded the interaction terms [36,46]. These analyses were run using LEM [47], a program for log-linear analysis with missing data that allows information from case-parent triads that have not been completely genotyped (e.g., father not available) to be included in the analysis for any given variant [48]. To reduce concerns regarding possible mating stratification bias [49,50], we also examined interactions among case-parent triads in which both parents were reported to be non-Hispanic White. Additionally, analyses were conducted in three subgroups: 1) those case-parent triads with spina bifida only (to reduce the potential for phenotypic heterogeneity); 2) those case-parent triads where mothers did not have pre-gestational diabetes (in order to determine if these effects were independent; and 3) those case-parent triads where mothers were not obese (in order to determine if these effects were independent of obesity).

3. Results

Participation in the NBDPS for the period 1999–2007 was 74% among NTD case mothers, yielding 1553 families available for analysis. Among those, 759 (49%) provided buccal brushes (1787 individuals). Genotyping was performed on DNA samples derived from these 759 families. Based on quality control checks, 18 families (2% of families) were excluded for being inconsistent with Mendelian inheritance at more than two genotypes. Additionally, 47 subjects were excluded for failure at more than 11 genotypes (>50%), leaving a total of 737 case-parent triads (97% of the original sample). Of those, 317 were complete triads, 313 were dyads, and 107 were monads with only one person in the family. After these quality control measures were applied, at least 95% of the samples for each variant were available; therefore the genotypes were considered of sufficiently high quality for analysis.

The distributions of key characteristics among NTD case-parent triads are presented in Table 2. Spina bifida was the most common phenotype among case subjects (n = 449, 60.9%). Furthermore, a majority of case mothers were non-Hispanic White (n = 439, 59.8%). Among case mothers, 176 were obese (25.4%), 13 had pre-pregnancy diabetes (1.8%), and 28 had gestational diabetes (4.2%). The only characteristics presented in Table 2 that were significantly different between interviewed case mothers who provided buccal brushes and those who did not were race/ethnicity (those who provided buccal brushes were more likely to be non-Hispanic White compared to those who did not) and education (those who provided buccal brushes were more likely to have >12 years of education compared to those who did not), data not shown.

Table 2
Characteristics of neural tube defect case-parent triads (n = 737), National Birth Defects Prevention Study, 1999–2007.

Characteristic	No.	%
Phenotype		
Spina bifida	449	60.9
Anencephaly	217	29.4
Encephalocele	71	9.6
Infant sex		
Male	337	47.9
Female	366	52.1
Maternal age		
<20	83	11.3
20–34	556	75.4
≥35	98	13.3
Race/ethnicity		
Non-Hispanic White	439	59.8
Non-Hispanic Black	34	4.6
Hispanic	221	30.1
Other	40	5.5
Education (years)		
<12	142	19.3
12	184	25.0
13–15	226	30.7
>15	185	25.0
Folic acid supplementation[a]		
No	351	47.6
Yes	386	52.4
Body mass index (kg/m^2)		
Underweight (<18.5)	28	4.1
Normal (18.5–24.9)	336	48.6
Overweight (25.0–29.9)	152	21.9
Obese (≥30)	176	25.4
Pre-pregnancy diabetes		
No	724	98.2
Yes	13	1.8
Gestational diabetes		
No	667	95.8
Yes	29	4.2

[a] Three months before conception through the first month of pregnancy.

Of the 76 interactions evaluated, five had $q < 0.05$ (Table 3). Among these five, four were confirmed using log-linear models among case-parent triads (step 2). Our results were similar when restricted to non-Hispanic white mating combinations (data not shown). Specifically, the following interactions were confirmed: maternal *ENPP1* rs1044498-fetal *SLC2A2* rs6785233 (interaction OR = 3.65, 95% CI: 2.32–5.74, q = 0.001, LRT p = 0.00004); maternal *LEP* rs12706831-fetal *SLC2A2* rs6785233 (interaction OR = 0.45, 95% CI: 0.29–0.71, q = 0.016, LRT p = 0.00001); maternal *ENPP1* rs1044498-fetal *SLC2A2* rs5400 (interaction OR = 1.98, 95% CI: 1.34–2.92, q = 0.016, LRT p = 0.001); and maternal *LEP* rs2071045-fetal *SLC2A2* rs5400 (interaction OR = 0.50, 95% CI: 0.32–0.77, q = 0.03, LRT p = 0.008). As in our previous assessment [24], our results were similar when our analyses were restricted to 1) those case-parent triads with spina bifida only; 2) those case-parent triads where mothers did not have pre-gestational diabetes; and 3) those case-parent triads where mothers were not obese (Table 4 for results among non-obese mothers) [24].

4. Discussion

To our knowledge, this is the first study reporting maternal–fetal gene–gene interactions in metabolic genes and their associations with NTD risk. Significant interactions were identified between the fetal *SLC2A2* gene and maternal variants in *LEP* and *ENPP1* genes. Specifically,

Table 3
Top maternal–fetal metabolic gene–gene interactions associated with neural tube defects, National Birth Defects Prevention Study, 1999–2007.

Maternal SNP	Fetal SNP	Interaction OR[a]	95% CI[b]	p-Value	q-Value[c]	LRT p-value from step 2[d,e]
ENPP1 rs1044498	*SLC2A2* rs6785233	3.65	2.32–5.74	2.09E−08	0.001	0.00004
LEP rs12706831	*SLC2A2* rs6785233	0.45	0.29–0.71	0.0005	0.016	0.00001
ENPP1 rs1044498	*SLC2A2* rs5400	1.98	1.34–2.92	0.0006	0.016	0.001
LEP rs2071045	*SLC2A2* rs5400	0.50	0.32–0.77	0.0016	0.03	0.008
LEP rs2071045	*SLC2A2* rs6785233	0.46	0.27–0.77	0.0029	0.04	0.06
LEP rs12706831	*SLC2A2* rs5400	0.59	0.41–0.86	0.0059	0.08	NE
LEP rs11760956	*SLC2A2* rs6785233	0.63	0.41–0.99	0.0450	0.44	NE
LEP rs3828942	*SLC2A2* rs6785233	0.64	0.42–0.99	0.0473	0.44	NE

[a] Interaction odds ratio (OR) from step 1 (case-only analysis).
[b] Confidence interval (CI).
[c] False discovery rate q-value.
[d] Only interactions where q < 0.05 in step 1 were confirmed in step 2 (log-linear analysis in case-parent triads).
[e] Not estimated (NE).

four of the 76 interactions were q < 0.05 and were confirmed in step 2 of our analysis. The minor alleles of maternal *ENPP1* and fetal *SLC2A2* were associated with increased risk of NTDs, whereas the minor alleles of *LEP* and fetal *SLC2A2* were inversely associated with NTD risk. The direction of these associations is consistent with our previous single locus analysis [24]. Interestingly the maternal (*ENPP1* rs1044498, *LEP* rs12706831, and *LEP* rs2071045) and fetal (*SLC2A2* rs6785233 and *SLC2A2* rs5400) SNPs identified as being significant in these analyses were not significant in single locus analyses [24], suggesting the importance of evaluating factors that may not have significant "main" effects. It is noteworthy and *SLC2A2* gene is known to contribute to impaired glucose tolerance and type 2 diabetes [51]; however, we have previously evaluated potential maternal effect of *SLC2A2* SNPs and no significant association was observed [24].

Leptin is a hormone produced and secreted by white adipose tissue and has profound effects on eating behavior, metabolic rate, endocrine function, and glucose homeostasis. Leptin deficiency in both mice and humans causes morbid obesity and diabetes, and replacement treatment leads to decreased food intake, normalized glucose homeostasis, and increased energy expenditure [32,52–55]. Two genetic markers adjacent to human *LEP* gene have been found to be modestly associated with NTDs possibly via an inherited effect, irrespective of maternal BMI [56]. In our previous study, we observed a modest increase of NTD risk (though not statistically significant) among women who carried the minor allele of SNP rs2071045 [24]; however, the functionality of this SNP is unknown.

Ectonucleotide pyrophosphate phosphodiesterase (*ENPP1*) is a membrane-bound glycoprotein that inhibits insulin receptor signaling. ENPP1 is the same protein as liver nucleotide phosphodiesterase and liver alkaline phosphodiesterase 1 and a member of a family of five enzymes (ENPP1–5) that regulate nucleotide metabolism [57]. The K121Q polymorphism (rs1044498) in exon 4 of *ENPP1* gene has been associated with insulin resistance in some populations [31] but not others [58–60]. There is evidence suggesting that this variant interacts with adiposity in modulating glucose homeostasis [61,62]; however, the possible effect of this variant on obesity remains unclear, with variable results [62–64]. We did not find a significant association between the *ENPP1* gene and NTD risk when evaluating main genetic effects in our previous assessment [24].

Table 4
Top maternal–fetal metabolic gene–gene interactions in non-obese mothers associated with neural tube defects, National Birth Defects Prevention Study, 1999–2007.

Maternal SNP	Fetal SNP	Interaction OR[a]	95% CI[b]	p-Value
ENPP1 rs1044498	*SLC2A2* rs6785233	3.24	1.83–5.74	5.18E−05
LEP rs12706831	*SLC2A2* rs6785233	0.54	0.31–0.93	0.0027
ENPP1 rs1044498	*SLC2A2* rs5400	1.78	1.12–2.86	0.0153
LEP rs2071045	*SLC2A2* rs5400	0.58	0.37–0.91	0.0259
LEP rs2071045	*SLC2A2* rs6785233	0.49	0.27–0.88	0.0172
LEP rs12706831	*SLC2A2* rs5400	0.58	0.37–0.91	0.0176
LEP rs11760956	*SLC2A2* rs6785233	0.85	0.50–1.44	0.5506
LEP rs3828942	*SLC2A2* rs6785233	0.72	0.43–1.25	0.2552

[a] Interaction odds ratio (OR) from case-only analysis.
[b] Confidence interval (CI).

At the time of neural tube closure (approximately the 4th week of gestation), an embryo receiving excessive amounts of glucose may not be able to regulate these levels, which subsequently leads to abnormal organogenesis and birth defects [25,65,66]. In mice, *Glut2* is expressed from the 8-cell stage onward [67]. Under the condition of maternal hyperglycemia, inactivation of the *Glut2* gene in mouse can protect embryos from maternal diabetes-induced NTDs [25]. Our previous study shows that fetal variants in *SLC2A2* (the human homolog of mouse *Glut2*) alone does not significantly influence NTD risk [24]. However, in this analysis, it appears as though *SLC2A2* may interact with maternal *LEP* and *ENPP1* genes to modify the risk of NTDs, suggesting that *SLC2A2* may confer sensitivity of the developing embryos under compromised intrauterine environment.

An important strength of our study is the use of data from the NBDPS, the largest population-based study of birth defects, which provided a unique opportunity to examine the interactions between maternal and fetal genes on NTD risk. The case-parent triad design is immune to population stratification bias in the assessment of fetal genotypes [50]. The log-linear modeling approach to analyses also allowed us to include data from incomplete triads (i.e., genotype data is missing for one or two individuals) [48,68]. An additional strength of the NBDPS is the extensive and standardized case review employed by clinical geneticists, which maximizes homogeneity among case groups. The main weakness of this study was the limited proportion of families with biologic samples (49%), which may limit the generalizability of our findings. In addition, clinical data such as insulin resistance or fasting blood glucose levels are not available as part of the NBDPS; therefore it is not possible to exclude mechanisms other than their associations with maternal obesity or impaired glucose homeostasis that alter the intrauterine environment. In conclusion, 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. Replication of these findings in other populations and investigation of additional genes is warranted. Furthermore, since maternal obesity and diabetes are also risk factors for other malformations [5,8,69], assessing the maternal–fetal gene–gene interactions in other birth defects will broaden our understanding of diabetes and obesity-related teratogenicity.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the California Department of Public Health.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgme.2013.11.004.

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