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

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A R T I C L E   I N F O

Article history:
Received 9 September 2013
Received in revised form 7 November 2013
Accepted 7 November 2013
Available online 18 November 2013

Keywords:
Birth defects
Gene–gene interactions
Maternal genetics
Metabolic genes
Neural tube defects

A B S T R A C T

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, LEPR, 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 rs6785223 (interaction OR = 3.65, 95% CI: 2.32–5.74, p = 2.09 × 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.

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 LEPR 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; NBDDS, National Birth Defects Prevention Study; NTDs, neural tube defects; SNP, single nucleotide polymorphism; RR, risk ratio.

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1096-7192/$ – see front matter. Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.ymgme.2013.11.004
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 (NBTPS), with estimated dates of delivery between January 1, 1999 and December 31, 2007. Details of the NBTPS have been published elsewhere [27]. In brief, the NBTPS 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 NBTPS had British Pediatric Association (BPA) codes for the diagnoses anencephaly (740.0), craniorchiasis (740.1), spina bifida (741.0), and encephalocoele (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 NBTPS. 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 SLCA2 [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 NBTPS [35]. DNA was extracted from buccal cells and a standard quality control procedure was applied to each sample before they were submitted to the NBTPS sample repository [35]. To assure genotyping proficiency, high quality, and high concordance among all NBTPS 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, 20–24, ≥25 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/m²], average weight [18.5–24.9 kg/m²], overweight [25.0–29.9 kg/m²], and obese [≥30.0 kg/m²]); 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

<table>
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<th>Gene symbol</th>
<th>Ref SNP</th>
<th>Chra</th>
<th>Position</th>
<th>Allelesb</th>
<th>SNP information</th>
<th>MAF’ (CEU)</th>
<th>Selection criteria</th>
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</table>

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 \((q\text{-value})\) to control for the false discovery rate \((FDR)\) at 0.05 [44,45]. These analyses were conducted using Intercooled Stata version 12.1 \((\text{StataCorp LP, College Station, TX})\). All interactions where \(q < 0.05\) were included in step 2.

For step 2 \((i.e., \text{case-parent triad approach})\), 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 \((\text{LRT})\) statistic as twice the difference of the log likelihoods for the log-linear model that included two parameters indexing the inherited genotype \((\text{SNP1})\), two parameters indexing the maternal genotype \((\text{SNP1})\), and two interaction terms representing the product of maternal–fetal SNP1–SNP2 pairwise genotypes \((\text{SNP2} \text{ being the fetal “interacting” SNP})\) and a reduced model that excluded the interaction terms [38,46]. 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 \((e.g., \text{father not available})\) 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 \((\text{to reduce the potential for phenotypic heterogeneity}); 2) those case-parent triads where mothers did not have pre-gestational diabetes \((\text{in order to determine if these effects were independent}); and 3) those case-parent triads where mothers were not obese \((\text{in order to determine if these effects were independent of obesity})\).

3. Results

Participation in the NBDDS for the period 1999–2007 was 74% among NTD case mothers, yielding 1553 families available for analysis. Among those, 759 \((49\%)\) provided buccal brushes \((1787 \text{ individuals})\). Genotyping was performed on DNA samples derived from these 759 families. Based on quality control checks, 18 families \((2\% \text{ of families})\) 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 \((97\% \text{ of the original sample})\). 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 \((\text{those who provided buccal brushes were more likely to be non-Hispanic White compared to those who did not})\) and education \((\text{those who provided buccal brushes were more likely to have 以外の教育年数 compared to those who did not})\), data not shown.

Of the 76 interactions evaluated, five had \(q < 0.05\) \((\text{Table 3})\). Among these five, four were confirmed using log-linear models among case-parent triads \((\text{step 2})\). Our results similar when restricted to non-Hispanic White mating combinations \((\text{data not shown})\). Specifically, the following interactions were confirmed: maternal \(ENPP1\) rs1044498-fetal \(SLC2A2\) rs6785233 \((\text{interaction OR} = 3.65, \text{95\% CI:} \ 2.32–5.74, \ q = 0.001, \ \text{LRT p} = 0.00004)\) maternal \(LEP\) rs12706831-fetal \(SLC2A2\) rs6785233 \((\text{interaction OR} = 0.45, \text{95\% CI:} \ 0.29–0.71, \ q = 0.016, \ \text{LRT p} = 0.00001)\); maternal \(ENPP1\) rs1044498-fetal \(SLC2A2\) rs5400 \((\text{interaction OR} = 1.98, \text{95\% CI:} \ 1.34–2.92, \ q = 0.016, \ \text{LRT p} = 0.001)\); and maternal \(LEP\) rs2071045-fetal \(SLC2A2\) rs5400 \((\text{interaction OR} = 0.50, \text{95\% CI:} \ 0.32–0.77, \ q = 0.03, \ \text{LRT p} = 0.008)\). 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 \((\text{Table 4} \text{ for results among non-obese mothers}) [24]\).

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,
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 important to note that the maternal (ENPP1 rs5400) SNP identified in our analysis. The minor alleles of maternal LEP rs6785233 and adjacent to human ENPP1 gene have been found to be modestly associated with 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 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].

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 diabetest and obesity-related teratogenicity.

### Acknowledgments

This study was supported by the National Institute of Child Health and Development (NICHD) (H. Zhu: R21 HD 058912). It was also supported by the Centers for Disease Control and Prevention Centers.
for Excellence Award Nos. U50/CCU925286 (California) and U01/ 
DD000494 (Texas), and NIH R01 NS 050249. This research was also 
supported in part by a grant from the National Institute of Environmen-
tal Health Sciences (P30ES010126). We thank the California Depart-
ment of Public Health, Maternal Child and Adolescent Health Divi-
sion for providing surveillance data from California for this study. We 
also thank the families who participated in this study.

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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