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Obstructive Heart Defects Associated with Candidate Genes, Maternal Obesity, and Folic Acid Supplementation

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Abstract

Right-sided and left-sided obstructive heart defects (OHDs) are subtypes of congenital heart defects, in which the heart valves, arteries, or veins are abnormally narrow or blocked. Previous studies have suggested that the development of OHDs involved a complex interplay between genetic variants and maternal factors. Using the data from 569 OHD case families and 1644 control families enrolled in the National Birth Defects Prevention Study (NBDPS) between 1997 and 2008, we conducted an analysis to investigate the genetic effects of 877 single nucleotide polymorphisms (SNPs) in 60 candidate genes for association with the risk of OHDs, and their interactions with maternal use of folic acid supplements, and pre-pregnancy obesity. Applying log-linear models based on the hybrid design, we identified a SNP in methylenetetrahydrofolate reductase (*MTHFR*) gene (C677T polymorphism) with a main genetic effect on the occurrence of OHDs. In addition, multiple SNPs in betaine-homocysteine methyltransferase (*BHMT* and *BHMT2*) were also identified to be associated with the occurrence of OHDs through significant main infant genetic effects and interaction effects with maternal use of folic acid supplements. We also identified multiple SNPs in glutamate-cysteine ligase, catalytic subunit (*GCLC*) and DNA (cytosine-5-)-methyltransferase 3 beta (*DNMT3B*) that were associated with elevated risk of OHDs among obese women. Our findings suggested that the risk of OHDs was closely related to a combined effect of variations in genes in the folate, homocysteine, or glutathione/transsulfuration pathways, maternal use of folic acid supplements and pre-pregnancy obesity.

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Keywords

Obstructive heart defects; congenital heart defects; folic acid supplementation; maternal obesity; genetic variants; hybrid design

INTRODUCTION

Congenital heart defects (CHDs) are abnormalities of the heart or great vessels that are present at birth. Each year 1.35 million babies are born worldwide with CHDs [van der Linde et al., 2011]. Infant mortality accounted for 48.1% of all mortality resulting from CHDs; among those who survived the first year of life, 76.1% of deaths occurred during adulthood [Gilboa et al., 2010b]. Most published studies have focused on investigating the effects of genetic variants and/or maternal factors on the occurrence of CHDs [Botto et al., 2004; Kuehl and Loffredo, 2005; Hobbs et al., 2006a; Hobbs et al., 2006b; van Beynum et al., 2007; Gilboa et al., 2010a; Weiner et al., 2012]. Both family-based studies [Hobbs et al., 2006a; van Beynum et al., 2007] and case-control studies [Hobbs et al., 2006b; Weiner et al., 2012] revealed a significant association between the methylenetetrahydrofolate reductase (*MTHFR*) gene and the risk of CHDs. It has been suggested that maternal use of folic acid supplements within the periconceptional period is associated with a reduced risk of CHDs [Botto et al., 2004; Kuehl and Loffredo, 2005], while maternal obesity is associated with an elevated risk of CHDs [Watkins and Botto, 2001; Oddy et al., 2009; Gilboa et al., 2010a; Mills et al., 2010; Madsen et al., 2013; Brite et al., 2014].

Right-sided and left-sided obstructive heart defects (OHDs), are CHDs that occur when heart valves, arteries, or veins are abnormally narrow or blocked [Botto et al., 2007]. Among sub-phenotypes of OHDs, pulmonary stenosis, coarctation of the aorta, and aortic stenosis account for 8%, 5% and 4% of all CHDs, respectively [van der Linde et al., 2011]. Etiologies of the majority of OHDs are unknown. Most OHDs are not associated with syndromes and are thought to result from a complex interplay between environmental, behavioral, genetic, and epigenetic risk factors [Hobbs et al., 2010]. It has been demonstrated that folic acid supplementation within the periconceptional period is associated with a reduced risk of OHDs [Botto et al., 2003]. Previous studies have also shown that pre-pregnancy obesity is associated with elevated risk of some sub-phenotypes of OHDs (e.g. hypoplastic left heart syndrome and pulmonary stenosis) [Gilboa et al., 2010a; Mills et al., 2010]. It is reported that the lack of periconceptional use of vitamins or supplements that contain folic acid may be associated with an excess risk for birth defects due to diabetes mellitus [Correa et al., 2012]. We and others have also shown that even if obese women receive a standard amount of prenatal folate, they may have an increased requirement for folic acid in order to benefit from its protective effect against birth defects [Rasmussen et al., 2008; Hobbs et al., 2010; Madsen et al., 2013]. Based on this evidence, it is suspected that the observed association between obesity and birth defects may be a greater demand for folate in obese individuals. It would then follow that obese women with potential dysregulated folate metabolism or transport via genetic variation would be at differential risk. We and others have published investigations reporting that women who delivered infants with OHDs are more likely to have alterations in metabolites in pathways

involving folate, homocysteine, and glutathione [Hobbs et al., 2005a; Hobbs et al., 2005b]. These reports have led to the hypothesis that genetic variants in maternal and infant genes that encode for enzymes in these metabolic pathways may modify the risk of OHDs.

We conducted this study to evaluate the associations between OHDs and maternal and infant genetic variants in pathways related to folate, homocysteine, and glutathione metabolism, and to investigate whether the impact of such variants is modified by maternal use of folic acid supplements and pre-pregnancy obesity. A comprehensive list of 877 single nucleotide polymorphisms (SNPs) from 60 candidate genes involved in metabolic pathways (folate, homocysteine, or glutathione/transsulfuration) was studied. We hypothesized that both maternal and infant genetic variants involved in folate metabolism were associated with OHDs, and some genetic effects also interact with maternal use of folic acid supplements, or pre-pregnancy obesity through interactions between maternal/infant genes and each non-genetic factor.

MATERIALS AND METHODS

Ethics Statement

The study was approved by University of Arkansas for Medical Sciences' Institutional Review Board (IRB) and the National Birth Defects Prevention Study (NBDPS) with protocol oversight by the Centers for Disease Control and Prevention (CDC) Center for Birth Defects and Developmental Disabilities. All study subjects gave informed consent. For minors, informed written consent was obtained from their legal guardian for DNA collection.

Study Population

The NBDPS was a family-based case-control study designed to evaluate genetic, environmental, and behavior factors associated with the occurrence of major non-syndromic birth defects [Yoon et al., 2001]. In this article, we briefly describe the family recruitment for the NBDPS. Additional details have been described in a previous publication [Yoon et al., 2001]. All families included in this study were enrolled in the NBDPS between 1997 and 2008. Both cases and controls and their parents were identified through population-based birth defects surveillance systems in 10 states (Arkansas, California, Iowa, Massachusetts, New Jersey, New York, Texas, Georgia, North Carolina, and Utah). We included 586 case families with singleton live-born infants with OHDs and 1702 control families with singleton live-born infants without any major structural birth defects. Cases where the pregnancy was affected by a known single gene disorder, chromosomal abnormality, or syndrome were excluded. Medical records were abstracted by trained health information specialists. All cardiac NBDPS case infants were required to have a cardiac defect identified by one or more of the following diagnostic tests: echocardiograms, surgical reports, cardiac catherizations, and autopsies. All diagnostic tests on case infants were reviewed by a pediatric cardiologist to ensure uniform criteria for diagnoses. A classification system developed for NBDPS, which incorporated three dimensions of cardiac phenotype, cardiac complexity, and extracardiac anomalies, was used to classify cases [Botto et al., 2007]. All controls who had DNA samples available, including all family members, mother, father,

baby or any combination thereof, were included in our study. The mothers of all participating families completed interviews.

Data Collection

Both case and control mothers participated in a one-hour interview carried out by female interviewers using a computer-assisted telephone questionnaire [Yoon et al., 2001]. Interviews were conducted in English or Spanish [Yoon et al., 2001]. For the present analyses, we included interview information about two maternal factors: periconceptional folic acid supplement use, and pre-pregnancy obesity. The folic acid supplement use was defined as use for at least two months during the exposure window that was defined as one month prior to conception and two months after conception. Pre-pregnancy obesity was defined as a body mass index (BMI) ≥ 30, while normal weight was defined as a body mass index of 18.5 ≤ BMI < 25. Upon completion of the maternal interviews, case and control families received a buccal cell collection kit for collecting cheek cell samples from offspring and both parents if available [Yoon et al., 2001].

Genotyping and Quality Control

As previously reported by Chowdhury et al [Chowdhury et al., 2012], a custom panel of 1536 SNPs from 62 genes involved in one of three candidate metabolic pathways (folate, homocysteine or glutathione/transsulfuration) or DNA synthesis/repair was selected for analysis. Genotyping was performed using 200 ng of whole genome amplified (WGA) DNA on the Illumina's customizable Golden Gate platform [Fan et al., 2006]. Initial genotype calls were generated using GenCall, Illumina's proprietary algorithm. We found that the quality of genotype clustering varied substantially from SNP to SNP on the Golden Gate custom platform, whether using blood samples or WGA-buccal samples. We therefore developed SNPMClust, a bivariate Gaussian model-based genotype calling algorithm, to complement the default Illumina GenCall algorithm, and applied very strict quality control measures. As described in greater detail by Hobbs et al [Hobbs et al., 2014], we performed a pilot study of 94 women who had provided both blood and buccal samples, and among genotype calls which passed quality control, we observed a 99.2% concordance rate between WGA-buccal DNA and blood-derived DNA. We therefore have confidence in genotypes used in our study because of our stringent quality standards.

A total of 4830 individual samples were genotyped, and 279 samples were removed from analysis due to study ineligibility, high no-call rates, or high rates of Mendelian inconsistency, resulting in an analytical sample consisting of 4551 individuals from 569 case and 1644 control families. In the pilot study, we identified 60 SNPs with poor clustering behavior even within the blood DNA samples and removed them from consideration. We also excluded 10 SNPs with Mendelian error rates > 5%, 389 SNPs with no-call rates > 10%, 192 SNPs with minor allele frequency (MAF) < 5%, and 8 SNPs that deviated significantly from Hardy-Weinberg Equilibrium (HWE) in at least one racial group ($p < 10^{-4}$). The HWE analyses were performed within each racial group using the genotype data from parents of controls. The final analysis dataset included genotype data from 4551 (94%) individuals on 877 (57%) SNPs.

Statistical Methods

Descriptive statistics were expressed as mean (standard deviation) for continuous variables, and frequency (percentage) for categorical variables. Because both case-parental and control-parental triads were genotyped, a hybrid design was used for statistical analysis [Weinberg and Umbach, 2005]. The use of a hybrid design allows (1) the evaluation of maternal and infant genetic effects simultaneously, while adjusting for the effect of each other (i.e. both maternal and infant genotypes are included in one model fitting); (2) protection of infant genetic effects from bias due to population admixture, and (3) exploration of interactions between genetic variants and non-genetic factors.

Two different sets of log-linear models were fitted. The first set was used to estimate genetic (G) effects only, whereas the second set was used to estimate gene × non-genetic (G × E) interaction effect.

G Model: a main effects model was fitted for each SNP as a function of mating type, case/control status (D), an interaction between case/control status and maternal genotype (D × M), and an interaction between case/control status and infant genotype (D × C). This model was designated to assess the main effects of maternal and infant genotypes on the risk of OHDs.

G × E Model: an interaction model was fitted for each SNP as a function of mating type, an interaction between mating types and a non-genetic factor (E), case/control status (D), an interaction for maternal genotype (D × M), an interaction for infant genotype (D × C), a three-way interaction for maternal genotype (D × E × M), and a three-way interaction for infant genotype (D × E × C). This model was designed to evaluate whether there were significant interactions between maternal and/or infant genotype and non-genetic factor.

When fitting the models, mating symmetry was assumed, and thus six different mating types were used. We also assumed multiplicative risk per allele (additive genetic effect), under which, the indicators for both maternal and infant genotypes were defined as continuous variables representing the number of copies of the variant allele (0, 1 or 2). This also implied that the relative risk (RR) of having two copies of variant allele was the square of the RR of having one copy of variant allele comparing to zero copies of variant allele. G × E Model was fitted for each E factor respectively.

We referred to the Bayesian false-discovery probabilities (BFDPs) for evaluating the chance of false-positive associations using the relative risk estimates and their 95% confidence intervals (CIs) obtained from the log-linear models [Wakefield, 2007]. BFDP is employed to find a balance between the relative costs of false negative and false positive; in the results section, we reported associations where BFDP < 0.80, which is a commonly used threshold suggested by Wakefield [Wakefield, 2007; Liu et al., 2010; Oh et al., 2010; Park et al., 2010]. Consistent with a candidate gene study of multifactorial disease, in which the risk contribution of any single SNP is expected to be moderate, we specified the prior distribution on the odds ratio for associated SNPs such that the 97.5^{th} centile was equal to 1.5, and set the prior probability of association at any given SNP at 5%. Patterns of linkage disequilibrium (LD) between significant SNPs from the same gene were constructed. Data

were analyzed using the LEM [Vermunt, 1997] software for fitting log-linear models, R v3.0.2 [Team, 2013] for computing descriptive statistics and BFDPs, and HaploView 4.2 [Barrett, 2009] for developing LD maps.

RESULTS

Population Characteristics

The final sample included 569 case families and 1,644 control-families. The distribution of the sub-phenotypes of OHDs among 569 cases is displayed in Figure 1. Table I summarizes the maternal demographics and behaviors for case and control-families respectively. Case mothers were marginally older (28.3 years vs. 27.5 years, respectively), and more likely to be overweight or obese than control mothers. The race-ethnicity distribution showed small differences with more Caucasian (non-Hispanic) and African-American case mothers than control mothers (73% vs. 69%, and 11% vs. 9%, respectively).

Genetic variants only

A total of 877 SNPs within 60 genes were included in the final analysis. Maternal genotypes for six SNPs from four genes [Cystathionine Gamma-Lyase (*CTH*); methylenetetrahydrofolate reductase (*MTHFR)*; O-6-methylguanine-DNA methyltransferase (*MGMT*), and methylenetetrahydrofolate dehydrogenase (*MTHFD1*)] were identified to be significantly associated with the occurrence of OHDs based on G Model (Table II). Similarly, infant genotypes for 24 SNPs from 13 genes [*MTHFR*; transcobalamin II (*TCN2*); betaine--homocysteine S-methyltransferase 2 (*BHMT2*); glutaredoxin (thioltransferase) (*GLRX*); betaine--homocysteine S-methyltransferase (*BHMT*); *CTH*; superoxide dismutase 2 (*SOD2*); glutamate-cysteine ligase, catalytic subunit (*GCLC*); nitric oxide synthase 3 (*NOS3*); folate receptor 1 (*FOLR1*); *MGMT; RFC1*; and 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*)] were identified to be significantly associated with the occurrence of OHDs (Table II). The maternal genotype for the SNP rs1801133 within the *MTHFR* gene was identified to be significant (BFDP=0.63). The risk of delivering infants with OHDs among women carrying one T allele for rs1801133 was estimated to be 1.28 (95% CI: 1.08–1.51) times more than that among women carrying no copy of the T allele. Accordingly, the risk among women carrying two T alleles for rs1801133 was 1.64 (95% CI: 1.17–2.28) times the risk among those carrying no copy of the T allele. As mentioned above, the estimated RR (95% CI) for carrying two copies of the risk allele was the square of the estimated RR (95% CI) for carrying one copy of the risk allele with 0 copies of the risk allele as the reference group. For simplicity, only the estimated RR (95% CI) for carrying one copy of the risk allele compared to 0 copies was reported in the following results.

The infant genotype for the same SNP was found to be significantly associated with the risk of OHDs with opposing effects. The risk of OHDs among infants carrying one T allele was estimated to be 31% (95% CI: 16%–44%) less than those carrying no copy of the T allele. Besides, infant genotypes for 3 SNPs (rs9621049, rs4820886, rs4820887) within *TCN2* gene and 5 SNPs (rs1422086, rs557302, rs625879, rs526264, rs542721) within *BHMT2* gene were found to have BFDP < 0.8. Among them, rs9621049 within *TCN2* and rs1422086

within *BHMT2* were the SNPs with the smallest BFDP within their respective gene. The infant AG genotype for rs9621049 increased the risk of OHDs by 53% (95% CI: 1.21–1.93) compared to the GG genotype (BFDP=0.27). Similarly, the risk of OHDs increased by 32% (95% CI 1.11–1.58) for infants' carrying AC genotype for rs1422086 compared to those carrying AA genotype (BFDP=0.44).

Genetic variants and folic acid supplementation

Maternal use of folic acid supplements may modify the impact of either maternal or infant genetic variants, or both. The interactive effects between maternal use of folic acid supplements and genetic variants may not be evident when evaluating the main effects of genetic variants only. Although maternal uses of folic acid supplements were similar in case (58%) and control (55%) mothers, the proportion of folic acid supplement use was significantly different among different race/ethnicity groups ($p < 0.001$), with 65% (n=1004) non-Hispanic Caucasian users compared to 33% (n=68) African-American users and 33% (n=116) Hispanic users. Given the lower numbers of African-American and Hispanic women, G × E Model was fit to evaluate interaction between each SNP and folic acid supplement use among Caucasians only.

Maternal genotypes for 11 SNPs in betaine-homocysteine methyltransferase (*BHMT* and *BHMT2*) and infant genotypes for two SNPs in *BHMT* gene significantly associated with OHDs through interactions with folic acid supplement use (Table III). For example, folic acid supplement users who carried the AG genotype for rs557302 had an increased risk of delivering infants with OHDs compared to those carrying the GG genotype (RR: 1.40; 95% CI: 1.12–1.74). In contrast, no significant difference was found among women who did not use folic acid supplements (RR: 0.74, 95% CI: 0.55–1.01). Relative risks of OHDs for infants carrying AG genotype for rs557302 (compared to AA) showed that among mothers who used folic acid supplements, the risk of OHDs was reduced by 32% (95% CI: 14%–46%). Seven out of nine significant SNPs in the *BHMT2* gene were determined to be in high LD ($D' \geq 0.96$) (Fig 2). Multiple SNPs (e.g. rs557302) were also found to be significantly associated with OHD risk based on the G Models.

Genetic variants and obesity

Maternal obesity has been found to play an important role in cardiogensis and the occurrence of CHDs in many published studies [Oddy et al., 2009; Gilboa et al., 2010a; Madsen et al., 2013]. In our analyses, a main effect of the association between OHDs and pre-pregnancy obesity compared to normal weight resulted in a RR=1.37 (95% CI: 1.15–1.63; p=0.001). To evaluate how maternal obesity modified the association between OHDs and genetic variants, G × E Model was fit to evaluate the interaction between each SNP and pre-pregnancy obesity.

We identified maternal genotypes for 19 SNPs and infant genotypes for 32 SNPs that interacted with pre-pregnancy obesity to increase the risk of OHDs (Table IV and Fig 3). Among these 51 SNP × obesity interactions, there were 10 interactions for which genetic variants appeared to be protective against OHD in normal weight women yet putative among obese women. In the remaining 41 interactions, the risk of OHD was increased among obese

women who did not carry the wild type genotype. Among 19 significant maternal SNPs, rs761142 in the *GCLC* gene had the smallest interactive BFDP=0.56, 3 other SNPs on the same gene (rs572494, rs1555906, rs13212365) had a BFDP <0.80. Obese women carrying the AC genotype for rs761142 had a 57% (95% CI: 1.14–2.15) increased risk of having infants with OHDs compared to those carrying the AA genotype. The remaining significant maternal SNPs were present in the following genes: glycine N-methyltransferase (*GNMT*); DNA (cytosine-5-)-methyltransferase 3 beta (*DNMT3B*); glutathione synthetase (*GSS*); microsomal glutathione S-transferase 1 (*MGST1*); *MGMT*;, methionine adenosyltransferase I, alpha (*MAT1A*); serine hydroxymethyltransferase 1 (*SHMT1*), and; glutathione peroxidase 2 (*GPX2*).

Infant SNPs found to be significantly associated with OHDs through interactions with pre-pregnancy obesity are displayed in the bottom panel Table IV and Figure 4. We identified 11 SNPs in the *DNMT3B* gene; four SNPs in the *GCLC* gene; three SNPs in the *GLRX* gene; three SNPs in the tRNA aspartic acid methyltransferase 1 (*TRDMT1*) gene; two SNPs in the *GSS* gene, and; one SNP in the remaining genes including *MGMT*, glutathione reductase (*GSR*), glutathione S-transferase pi 1 (*GSTP1*), microsomal glutathione S-transferase 1 (*MGST1*), *BHMT*, superoxide dismutase 3 extracellular (*SOD3*), DNA-methyltransferase 3 alpha (*DNMT3A*), methionine adenosyltransferase II, alpha (*MAT2A*), *NOS3*; and methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 1, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthetase (*MTHFD1*) genes respectively. The most significant infant SNP × obesity interaction was that with the SNP rs6058893 among obese women; this SNP was within the *DNMT3B* gene and its BFDP equaled to 0.15. Among obese women, infant AG genotype for rs6058893 was associated with elevated relative risk of OHDs by 1.73 (95% CI: 1.28–2.34) compared to the GG genotype. In contrast, among normal-weight women, infants with AG genotype for rs6058893 had a reduced risk of OHDs with a RR=0.75 (95% CI: 0.58–0.97) compared to infants of normal weight women who carried the GG genotype. There was no significant main effect of any of the SNPs discussed above. Eleven out of 13 significant maternal or infant SNPs in the *DNMT3B* gene were determined to be in high LD ($D' \geq 0.9$) (Figure 5). Similar models were fitted among Caucasians only, and due to relatively smaller sample size and less power, a subset of the above SNPs was identified (data not shown). A main effect of the association between OHDs and pre-pregnancy overweight compared to normal weight resulted in a RR=1.27 (95% CI: 1.07–1.51; p=0.007). An additional G × E Model was fit to evaluate the interaction between each SNP and pre-pregnancy overweight, and the results are shown in Supplemental Table S1 (in supporting information online).

DISCUSSION

Our findings indicate that multiple genetic variants in genes involved in the folate, homocysteine or glutathione/transsulfuration pathways have modest effects on the risk of OHDs through either genetic main effects or interactions with maternal use of folic acid supplements, and pre-pregnancy obesity. These findings provide insights into the genetic susceptibility of OHDs. Among 877 SNPs involved in the folate, homocysteine, glutathione/transsulfuration pathways, multiple SNPs were identified to influence risk of OHDs. All six SNPs with significant maternal effects were located within four genes (*CTH*, *MTHFR*,

MGMT, and *MTHFD1*). Among 24 SNPs with significant infant effects, minor alleles of 16 SNPs within eight genes (*TCN2*, *BHMT2*, *BHMT*, *SOD2*, *GCLC*, *FOLR1*, and *MTRR*) were associated with increased OHD risks, while minor alleles of eight remaining SNPs within seven genes (*MTHFR*, *BHMT2*, *GLRX*, *GLRX*, *BHMT*, *NOS3*, *MGMT*, and *RFC1*) were associated with decreased OHD risks.

Further, multiple SNPs were significantly associated with risks of OHDs through their interactions with maternal obesity and folic acid supplement use. Among women who used folic acid supplements during the periconceptional period, the maternal genotype of eight SNPs within two genes (*BHMT2* and *BHMT*), and the infant genotype of one SNP within *BHMT2* genes were associated with decreased risks of OHDs. In contrast, among women who did not use supplements, five maternal SNPs within the same two genes increased the risk of OHDs.

Among women who were obese before pregnancy, maternal genotypes of 18 SNPs in nine genes (*GCLC*, *GNMT*, *GCLM*, *DNMT3B*, *MGST1*, *MGMT*, *MAT1A*, *SHMT1*, and *GPX2*) were associated with an increased risk of OHDs, while among women who had normal weight before pregnancy, maternal genotypes of only three SNPs present in three genes (*GCLC*, *GSS*, and *GPX2*) deceased the occurrence of OHDs. Similarly, infant genotypes of 28 SNPs within 14 genes (*DNMT3B*, *GCLC*, *GLRX*, *MGMT*, *GSR*, *GSTP1*, *BHMT*, *SOD3*, *TRDMT1*, *GSS*, *DNMT3A*, *MAT2A*, *NOS3*, and *MTHFD1*) increased the occurrence of OHDs, while infant genotypes of 11 SNPs within three genes (*DNMT3B*, *GLRX*, and *MGST1*) decreased the occurrence of OHDs.

The rs1801133 SNP (i.e. C677T polymorphism) within *MTHFR* gene was identified to be significantly associated with the occurrence of OHDs, which was in concordance with several previous findings [Junker et al.; Wenstrom et al.; Lee et al., 2005; van Beynum et al., 2006; Weiner et al., 2012; Yin et al., 2012]. However, evidence of association between C677T polymorphism and CHDs has been less consistent for some other studies. A recent meta-analysis of 7697 CHD cases and 13125 controls suggested the presence of association between the C677T polymorphism and CHDs with an estimated odds ratio of 1.25 (95% CI: 1.03–1.51) but with substantial heterogeneity among contributing studies and evidence of publication bias [Mamasoula et al., 2013]. The *MTHFR* enzyme catalyzes the irreversible conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the methyl donor for homocysteine remethylation to methionine [Weiner et al., 2012]. The T allele of the *MTHFR* C677T polymorphism is associated with reduced activity of *MTHFR*, DNA hypomethylation [van der Put et al., 1998; Castro et al., 2004] and increased plasma homocysteine level [Kumar et al., 2005; DeVos et al., 2008]. Our data indicates that the maternal genotype for rs1801133 is associated with an increased risk of OHDs at an estimated RR=1.28 (95% CI: 1.08–1.51), however, the infant genotype for the same SNP is associated with a reduced risk of OHDs at an esitmated RR=0.69 (95% CI: 0.56–0.84). The reason for this seemingly conflicting data has not been investigated, but may be related to different roles for folate and one-carbon metabolism metabolic programs between the mother and the developing embryo. The two major functions of folate and one-carbon metabolism are DNA synthesis and DNA methylation. The DNA methylation pathway is also responsible for glutathione production. The mother's metabolism has to support the

developing embryo with nutrients as well as protecting the embryo from harm by environmental factors and oxidative stress. In this case, the mother's use of folate and one-carbon metabolism may be skewed toward the production of the amino acids methionine and cysteine for glutathione synthesis, which is necessary for protection of mother and embryo from harmful reactive oxygen species. The developing embryo, on the other hand, needs large quantities of deoxynucleotide triphosphate precursor pools that are required for error-free DNA synthesis. There are other published studies reporting SNPs having opposing effects [Shi et al., 2007; Adkins et al., 2008]. For example, Adkins et al [Adkins et al., 2008] found that maternal haplotypes in the insulin and IGF2 had a significant association with small for gestational age (SGA) birth, in newborns however, the opposite haplotype conferred risk of SGA. The authors conclude that these haplotypes have an opposing influence on the growth rate of the embryo. Shi et al [Shi et al., 2007] in a reanalysis of existing orofacial cleft data, investigated both fetal and maternal effects of the *IRF6* gene. They found a maternal haplotype consisting of 13 SNPs that conferred increased risk. However, in offspring analysis, the risk conferring allele for each of these SNPs was the opposite allele from the maternal risk allele. After further analysis, imprinting was discounted as a possible explanation for these opposing effects. They postulate that the unlikely result that the same haplotype could be protective against clefts in the embryo when carried by the mother, but could be deleterious when carried by the embryo could be a result of the same genes having very different functions in the mother and the developing embryo.

Our study revealed associations between OHDs and SNPs with the *BHMT*, *BHMT2* genes through both infant genetic main effects, and maternal and infant SNP× folic acid supplement use interactions. *BHMT* catalyzes the remethylation of homocysteine. *BHMT2* encodes a protein that is 73% identical to *BHMT* [Li et al., 2008]. For both *BHMT* and *BHMT2* genes, their impact on common diseases remains unknown.

The *GCLC* gene was identified to be significantly associated with elevated risk of OHDs among obese mothers. The *GCLC* gene is the rate limiting step in glutathione synthesis and is dependent on cellular availability of cysteine. Cellular glutathione protects developing embryos from harmful xenobiotic and environmental exposures [Hansen et al., 2004] creating an optimal environment for the developing embryo, and oxidative stress has been implicated in teratogenesis [Wells et al., 2009]. Nevertheless, the haplotype tagging SNPs have not been identified on the *GCLC* gene for common diseases.

Our analysis also revealed a strong association between polymorphisms in *DNMT3B* gene and increased risk of OHDs among obese mothers. The *DNMT3B* gene encodes a DNA methyltransferase which is thought to function in *de novo* methylation, rather than maintenance methylation [Shirohzu et al., 2002]. The protein localizes primarily to the nucleus and its expression is developmentally regulated [Shirohzu et al., 2002]. A positive association has been noted between mutations in the *DNMT3B* gene and immunodeficiency, centromere instability and facial anomalies (ICF) syndrome [Hansen et al., 1999; Shirohzu et al., 2002]. Our study provided preliminary evidence that polymorphisms in DNMT3B may be associated with an increased risk of OHDs in combination with pre-pregnancy obesity.

There are several strengths of this study including its large size, population-based ascertainment of cases, and a rigorous analytic inquiry into potential gene-environment interactions on OHD risk. There are also limitations. First, buccal cell collection kits in collecting DNA samples created a disparity among the quality of the DNA samples. Due to poor clustering in GenomeStudio and application of conservative standards in subsequent quality checks, the number of SNPs remained in the analysis reduced from 1534 to 877. Secondly, based on the hybrid design, the log-linear model can be extended to include more than one non-genetic factor; for example, the effects of periconceptional folic acid supplement use and pre-pregnancy obesity on the risk of OHDs can be evaluated simultaneously. However, the sample size required to test for more than 1st order interactions among non-genetic factors is substantially larger, thus we were relegated to assess the non-genetic factors independently from each other. In addition, the potential confounding effects from other environmental/behavior factors were not considered. Thirdly, none of the identified SNPs would remain statistically significant after Bonferroni correction, which is widely considered overly conservative. We report both unadjusted p-values and BFDPs. The BFDP is a Bayesian measure of the probability of false discovery, and has been widely used in candidate gene-based studies when there is a *priori* knowledge that the genes are likely to be associated with the disease. Last, due to the relatively smaller sample size for each sub-phenotype, the analysis was not replicated among each sub-phenotype of OHDs. Analyses targeted to specific sub-phenotypes may have revealed stronger associations that were muted by the overall grouped analyses.

Our results provide some evidence for gene × environment interaction in the etiologies of OHDs. However, findings are preliminary and will need to be replicated in another population. Further studies, will need to incorporate functional genetics and targeted deep sequencing while improving and enhancing information collected on environmental and maternal factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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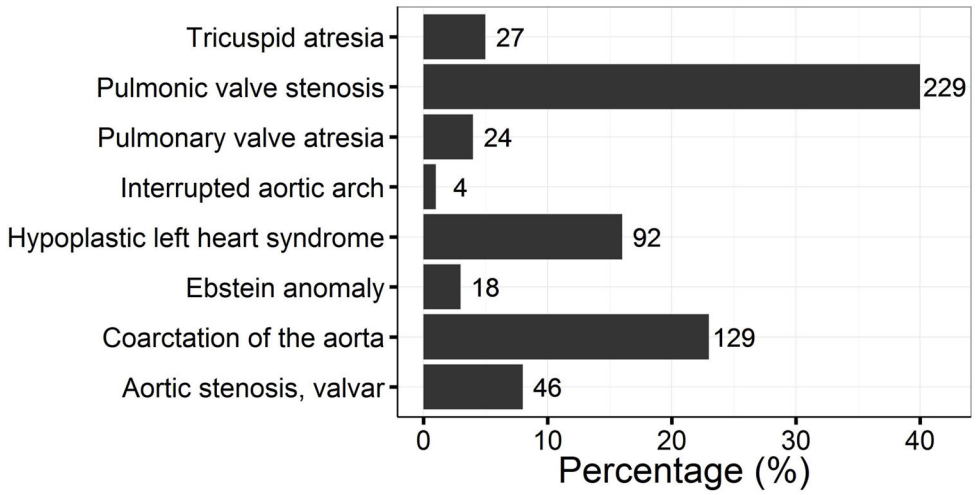

Figure 1.

Sub-phenotype information. Distribution of sub-phenotypes of right-sided and left-sided obstructive heart defects among 569 cases

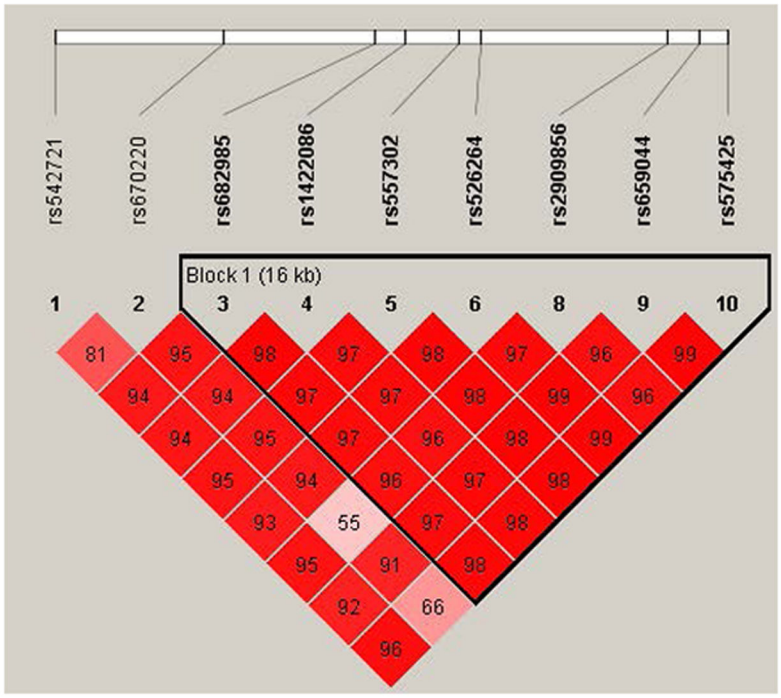

Figure 2.

LD map for significant SNPs on BHMT2 gene. The patterns of LD between SNPs on **BHMT2** gene that were identified to have significant maternal and/or infant SNP × folic acid supplementation interactions.

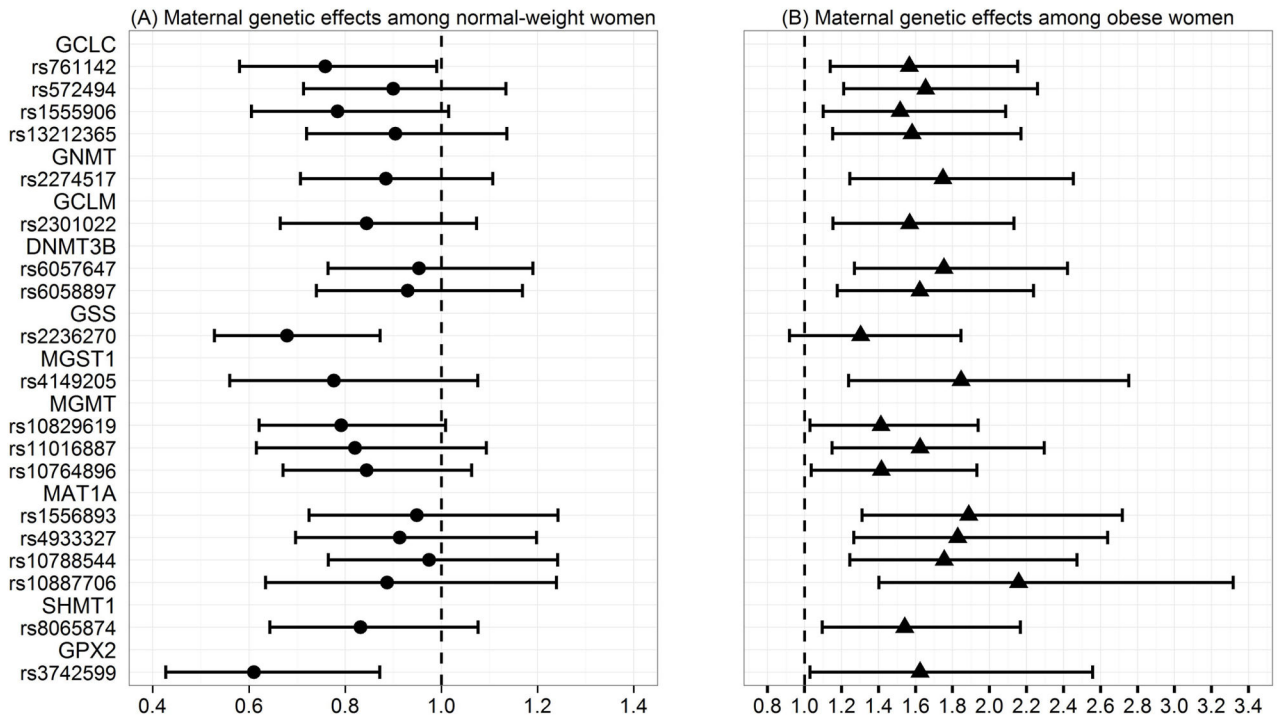

Figure 3.

Interactions between maternal genes and maternal obesity. Estimated relative risks and 95% confidence intervals for maternal SNPs with significant SNP × obesity interactions on the risk of right-sided and left-sided obstructive heart defects. (A) Maternal genetic effects among normal-weight women; and (B) Maternal genetic effects among obese women.

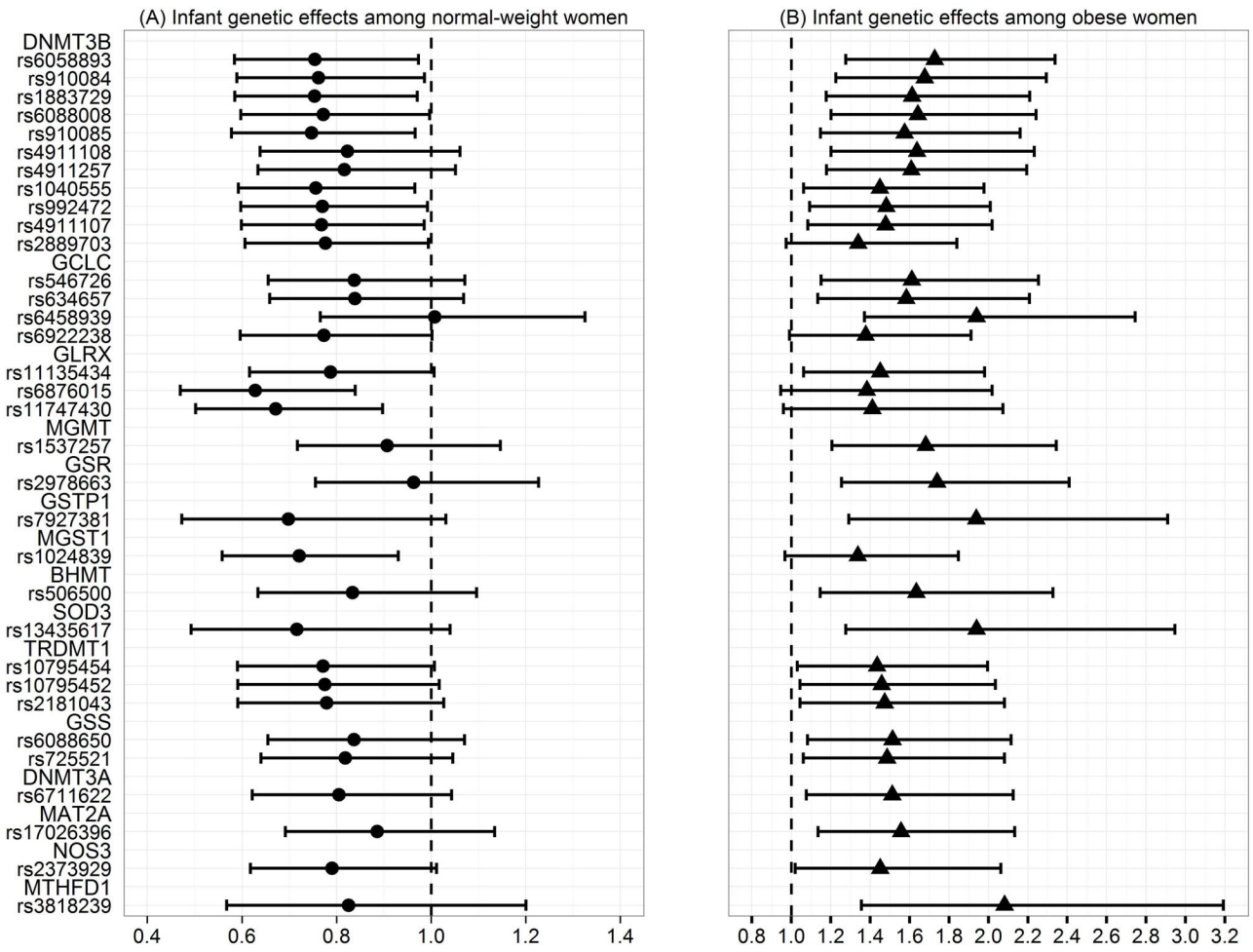

Figure 4.

Interactions between infant genes and maternal obesity. Estimated relative risks and 95% confidence intervals for infant SNPs with significant SNP × obesity interactions on the risk of right-sided and left-sided obstructive heart defects. (A) Infant genetic effects among normal-weight women; and (B) Infant genetic effects among obese women.

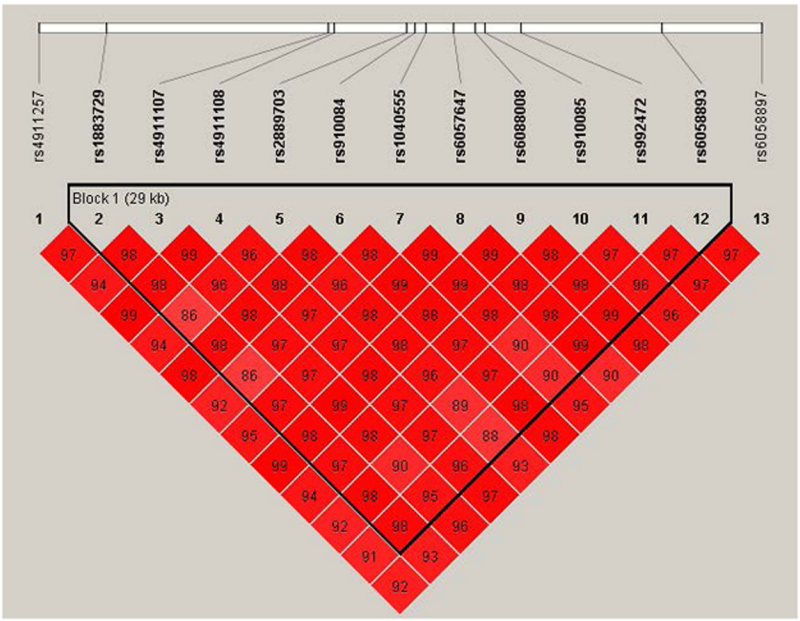

Figure 5.

LD map for significant SNPs on *DNMT3B* gene. The patterns of LD between SNPs on DNMT3B gene that were identified to have significant maternal and/or infant SNP × obesity interactions.

Table 1

Maternal Characteristics

	Case (N=569)	Control (N=1,644
Age at delivery	28.3 (6.0)	27.5 (6.0)
Mother's race		
African American	61 (11%)	144 (9%)
Caucasian	418 (73%)	1,135 (69%)
Hispanic	66 (12%)	286 (17%)
Others	24 (4%)	79 (5%)
Mother's education		
<12 years	73 (13%)	217 (13%)
High school degree or equivalent	140 (25%)	412 (25%)
1–3 years of college	174 (31%)	454 (28%)
At least 4 years of college or Bachelor degree	182 (32%)	559 (34%)
Missing information	0	2
Household income		
< $10,000	70 (13%)	236 (15%)
$10,000 to < $30,000	163 (30%)	408 (27%)
$30,000 to < $50,000	131 (24%)	348 (23%)
≥ $50,000	183 (33%)	539 (35%)
Missing information	22	113
Folic acid supplement use[1]		
Yes	332 (58%)	912 (55%)
No	237 (42%)	732 (45%)
Alcohol consumption[2]		
No	450 (80%)	1,250 (76%)
Yes	115 (20%)	390 (24%)
Missing information	4	4
Cigarette smoking[3]		
No	491 (86%)	1,356 (83%)
Yes	78 (14%)	287 (17%)
Missing information	0	1
Pre-pregnancy BMI		
Underweight (BMI < 18.5)	14 (3%)	74 (5%)
Normal weight (18.5 ≤ BMI < 25)	261 (47%)	878 (55%)
Overweight (25 ≤ BMI < 30)	148 (27%)	361 (23%)
Obese (≥ 30)	130 (24%)	281 (18%)
Missing information	16	50

Summary statistics were expressed as mean (standard deviation) for age, and frequency (percentage) for other categorical variables.

[1] Folic acid supplement use was defined as use for at least two months during the exposure window that was defined as one month prior to conception and two months after conception.

[2]Alcohol consumption is defined maternal alcohol consumption during the first trimester.

[3]Cigarette smoking is defined as maternal tobacco use during the first trimester.

Table 2

Maternal and infant SNPs associated with OHDs risk based on G Model

Significant maternal SNPs							
Chr	**Gene**	**SNP**	**Allele***	**Pathway**	**RR (95% CI)**	**p-value**	**BFDP**
1	CTH	rs535112	T/A	Homocysteine	1.29 (1.09, 1.53)	3.63×10^{-3}	0.57
1	CTH	rs515064	G/A	Homocysteine	1.27 (1.07, 1.50)	6.07×10^{-3}	0.67
1	MTHFR	rs1801133	T/C	Folate	1.28 (1.08, 1.51)	4.84×10^{-3}	0.63
10	MGMT	rs6482747	G/A	Transsulfuration	1.23 (1.04, 1.44)	1.32×10^{-2}	0.78
10	MGMT	rs4751110	G/A	Transsulfuration	1.23 (1.04, 1.44)	1.35×10^{-2}	0.79
14	MTHFD1	rs2983736	C/A	Folate	1.22 (1.04, 1.43)	1.48×10^{-2}	0.79

Significant infant SNPs							
Chr	**Gene**	**SNP**	**Allele***	**Pathway**	**RR (95% CI)**	**p-value**	**BFDP**
1	MTHFR	rs1801133	T/C	Folate	0.69 (0.56, 0.84)	1.82×10^{-4}	0.13
22	TCN2	rs9621049	A/G	Folate	1.53 (1.21, 1.93)	4.43×10^{-4}	0.27
22	TCN2	rs4820886	C/A	Folate	1.45 (1.15, 1.84)	2.02×10^{-3}	0.52
22	TCN2	rs4820887	A/G	Folate	1.43 (1.11, 1.85)	5.92×10^{-3}	0.70
5	BHMT2	rs1422086	C/A	Homocysteine	1.32 (1.11, 1.58)	1.80×10^{-3}	0.44
5	BHMT2	rs557302	G/A	Homocysteine	0.77 (0.65, 0.91)	2.91×10^{-3}	0.53
5	BHMT2	rs625879	A/C	Homocysteine	1.30 (1.09, 1.55)	3.30×10^{-3}	0.56
5	BHMT2	rs526264	A/T	Homocysteine	1.25 (1.05, 1.49)	1.07×10^{-2}	0.75
5	BHMT2	rs542721	G/C	Homocysteine	1.24 (1.04, 1.48)	1.47×10^{-2}	0.79
5	GLRX	rs6876015	G/A	Transsulfuration	0.73 (0.60, 0.90)	2.41×10^{-3}	0.51
5	GLRX	rs11747430	C/G	Transsulfuration	0.76 (0.62, 0.92)	6.46×10^{-3}	0.69
5	BHMT	rs1915706	A/G	Homocysteine	0.77 (0.64, 0.92)	3.78×10^{-3}	0.58
5	BHMT	rs490268	G/A	Homocysteine	1.29 (1.08, 1.54)	4.33×10^{-3}	0.61
5	BHMT	rs7700970	A/G	Homocysteine	1.27 (1.06, 1.52)	1.09×10^{-2}	0.76
1	CTH	rs3856027	A/G	Homocysteine	1.32 (1.09, 1.60)	4.76×10^{-3}	0.63
6	SOD2	rs5746105	G/A	Transsulfuration	1.29 (1.07, 1.55)	7.45×10^{-3}	0.70
6	SOD2	rs732498	A/G	Transsulfuration	1.27 (1.06, 1.53)	1.04×10^{-2}	0.75

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Significant infant SNPs							
Chr	**Gene**	**SNP**	**Allele***	**Pathway**	**RR (95% CI)**	**p-value**	**BFDP**
6	SOD2	rs6912979	G/A	Transsulfuration	1.27 (1.05, 1.53)	1.31×10^{-2}	0.78
6	GCLC	rs12525474	A/G	Transsulfuration	1.39 (1.09, 1.77)	8.95×10^{-3}	0.75
7	NOS3	rs10277237	A/G	Transsulfuration	0.77 (0.62, 0.94)	1.07×10^{-2}	0.76
11	FOLR1	rs3016432	G/A	Folate	1.25 (1.05, 1.48)	1.12×10^{-2}	0.76
10	MGMT	rs10734088	G/A	Transsulfuration	0.80 (0.67, 0.95)	1.13×10^{-2}	0.76
4	RFC1	rs12644680	G/A	DNA synthesis/repair	0.74 (0.59, 0.93)	1.09×10^{-2}	0.77
5	MTRR	rs162039	A/G	Homocysteine	1.33 (1.07, 1.67)	1.15×10^{-2}	0.77

Chr: Chromosome; RR: relative risk for carrying one copy of minor allele compared to no copies; CI: confidence interval; BFDP: Bayesian false-discovery probability, BFDPs < 0.8 were considered to indicate statistical

*Allele is presented as minor/major allele in our study sample; major allele is the reference allele.

Interactions between Maternal and infant SNPs and periconceptional folic acid supplement use associated with OHDs risk based on G×E Model among Caucasians only

Significant maternal SNPs

Chr	Gene	SNP	Allele*	Pathway	Non-users RR (95% CI)	Supplement users RR (95% CI)	Interaction D×E×M† p-value	BFDP
5	BHMT2	rs557302	G/A	Homocysteine	0.74 (0.55, 1.01)	1.40 (1.12, 1.74)	6.38×10^{-04}	0.53
5	BHMT2	rs575425	A/G	Homocysteine	1.44 (1.07, 1.95)	0.76 (0.60, 0.97)	7.84×10^{-04}	0.57
5	BHMT2	rs659044	A/G	Homocysteine	1.30 (0.98, 1.74)	0.73 (0.58, 0.93)	1.82×10^{-03}	0.66
5	BHMT2	rs526264	A/T	Homocysteine	1.30 (0.97, 1.75)	0.74 (0.58, 0.93)	2.29×10^{-03}	0.68
5	BHMT2	rs682985	G/A	Homocysteine	1.33 (0.99, 1.78)	0.76 (0.60, 0.96)	2.34×10^{-03}	0.69
5	BHMT2	rs1422086	C/A	Homocysteine	1.38 (1.02, 1.88)	0.78 (0.61, 0.99)	2.84×10^{-03}	0.72
5	BHMT2	rs2909856	G/A	Homocysteine	1.37 (1.01, 1.85)	0.78 (0.61, 0.99)	2.98×10^{-03}	0.72
5	BHMT2	rs542721	G/C	Homocysteine	1.29 (0.95, 1.74)	0.77 (0.61, 0.97)	6.55×10^{-03}	0.79
5	BHMT2	rs670220	A/C	Homocysteine	1.43 (1.02, 1.99)	0.78 (0.59, 1.03)	5.02×10^{-03}	0.79
5	BHMT	rs492842	G/A	Homocysteine	1.40 (1.05, 1.89)	0.84 (0.66, 1.06)	5.11×10^{-03}	0.76
5	BHMT	rs490268	G/A	Homocysteine	1.28 (0.95, 1.72)	0.76 (0.60, 0.96)	5.87×10^{-03}	0.78

Significant infant SNPs

Chr	Gene	SNP	Allele*	Pathway	Non-users RR (95% CI)	Supplement users RR (95% CI)	Interaction D×E×C‡ p-value	BFDP
5	BHMT2	rs557302	G/A	Homocysteine	1.16 (0.85, 1.58)	0.68 (0.54, 0.86)	4.40×10^{-03}	0.75
5	BHMT2	rs575425	A/G	Homocysteine	0.74 (0.52, 1.06)	1.35 (1.06, 1.71)	4.48×10^{-03}	0.78

Chr: Chromosome; RR: relative risk for carrying one copy of minor allele compared to no copies; CI: confidence interval; BFDP: Bayesian false-discovery probability, BFDPs < 0.8 were considered to indicate statistical

*Allele is presented as minor/major allele in our study sample; major allele is the reference allele.

†Interaction D×E×M is the three-way interaction for maternal genotype (see G×E Model in Statistical Methods section for details)

‡Interaction D×E×C is the three-way interaction for infant genotype (see G×E Model in Statistical Methods section for details)

Table 4

Interactions between Maternal and infant SNPs and maternal obesity associated with OHDs risk based on G×E Model

Significant maternal SNPs								
							Interaction D×E×M[†]	
Chr	**Gene**	**SNP**	**Allele***	**Pathway**	**Normal-weight RR (95% CI)**	**Obese RR (95% CI)**	**p-value**	**BFDP**
6	GCLC	rs761142	C/A	Transsulfuration	0.76 (0.58, 0.99)	1.57 (1.14, 2.15)	4.76×10^{-4}	0.56
6	GCLC	rs572494	G/A	Transsulfuration	0.90 (0.71, 1.13)	1.65 (1.21, 2.26)	1.49×10^{-3}	0.65
6	GCLC	rs1555906	A/G	Transsulfuration	0.78 (0.61, 1.02)	1.52 (1.10, 2.09)	1.33×10^{-3}	0.67
6	GCLC	rs13212365	A/G	Transsulfuration	0.90 (0.72, 1.14)	1.58 (1.15, 2.17)	3.88×10^{-3}	0.75
6	GNMT	rs2274517	G/A	Homocysteine	0.89 (0.71, 1.11)	1.75 (1.24, 2.45)	7.23×10^{-4}	0.59
1	GCLM	rs2301022	A/G	Transsulfuration	0.84 (0.66, 1.07)	1.57 (1.15, 2.13)	1.43×10^{-3}	0.65
20	DNMT3B	rs6057647	A/T	Homocysteine	0.95 (0.76, 1.19)	1.75 (1.27, 2.42)	1.79×10^{-3}	0.68
20	DNMT3B	rs6058897	A/C	Homocysteine	0.93 (0.74, 1.17)	1.62 (1.18, 2.24)	4.39×10^{-3}	0.76
20	GSS	rs2236270	A/C	Transsulfuration	0.68 (0.53, 0.87)	1.30 (0.92, 1.85)	2.38×10^{-3}	0.74
12	MGST1	rs4149205	A/G	Transsulfuration	0.78 (0.56, 1.08)	1.85 (1.24, 2.75)	9.83×10^{-4}	0.75
10	MGMT	rs10829619	C/G	Transsulfuration	0.79 (0.62, 1.01)	1.41 (1.03, 1.94)	3.61×10^{-3}	0.75
10	MGMT	rs11016887	A/G	Transsulfuration	0.82 (0.62, 1.09)	1.62 (1.15, 2.30)	2.53×10^{-3}	0.76
10	MGMT	rs10764896	A/G	Transsulfuration	0.84 (0.67, 1.06)	1.41 (1.04, 1.93)	6.96×10^{-3}	0.79
10	MAT1A	rs1556893	C/A	Homocysteine	0.95 (0.72, 1.24)	1.89 (1.31, 2.72)	2.46×10^{-3}	0.76
10	MAT1A	rs4933327	A/G	Homocysteine	0.91 (0.70, 1.20)	1.83 (1.27, 2.64)	2.47×10^{-3}	0.77
10	MAT1A	rs10788544	A/G	Homocysteine	0.97 (0.77, 1.24)	1.75 (1.24, 2.47)	4.73×10^{-3}	0.79
10	MAT1A	rs10887706	G/A	Homocysteine	0.89 (0.63, 1.24)	2.16 (1.40, 3.32)	1.30×10^{-3}	0.79
17	SHMT1	rs8065874	A/G	Folate	0.83 (0.64, 1.08)	1.54 (1.10, 2.17)	4.04×10^{-3}	0.78
14	GPX2	rs3742599	A/C	Transsulfuration	0.61 (0.43, 0.87)	1.62 (1.03, 2.56)	8.29×10^{-4}	0.78

Significant infant SNPs								
							Interaction D×E×C[‡]	
Chr	**Gene**	**SNP**	**Allele***	**Pathway**	**Normal-weight RR (95% CI)**	**Obese RR (95% CI)**	**p-value**	**BFDP**
20	DNMT3B	rs6058893	A/G	Homocysteine	0.75 (0.58, 0.97)	1.73 (1.28, 2.34)	1.47×10^{-5}	0.15
20	DNMT3B	rs910084	A/G	Homocysteine	0.76 (0.59, 0.99)	1.68 (1.23, 2.29)	6.12×10^{-5}	0.29

Significant infant SNPs

Chr	Gene	SNP	Allele*	Pathway	Normal-weight RR (95% CI)	Obese RR (95% CI)	Interaction D×E×C‡ p-value	BFDP
20	DNMT3B	rs1883729	A/G	Homocysteine	0.75 (0.58, 0.97)	1.61 (1.18, 2.21)	1.07×10^{-4}	0.35
20	DNMT3B	rs6088008	G/A	Homocysteine	0.77 (0.60, 1.00)	1.64 (1.20, 2.24)	1.27×10^{-4}	0.37
20	DNMT3B	rs910085	C/A	Homocysteine	0.75 (0.58, 0.97)	1.57 (1.15, 2.16)	1.69×10^{-4}	0.41
20	DNMT3B	rs4911108	G/A	Homocysteine	0.82 (0.64, 1.06)	1.64 (1.20, 2.23)	3.97×10^{-4}	0.50
20	DNMT3B	rs4911257	G/A	Homocysteine	0.82 (0.63, 1.05)	1.61 (1.18, 2.19)	4.79×10^{-4}	0.52
20	DNMT3B	rs1040555	A/T	Homocysteine	0.76 (0.59, 0.97)	1.45 (1.06, 1.98)	6.77×10^{-4}	0.55
20	DNMT3B	rs992472	A/C	Homocysteine	0.77 (0.60, 0.99)	1.48 (1.09, 2.01)	6.78×10^{-4}	0.56
20	DNMT3B	rs4911107	A/G	Homocysteine	0.77 (0.60, 0.99)	1.48 (1.08, 2.02)	6.99×10^{-4}	0.56
20	DNMT3B	rs2889703	A/C	Homocysteine	0.78 (0.61, 0.99)	1.34 (0.97, 1.84)	5.24×10^{-3}	0.78
6	GCLC	rs546726	G/A	Transsulfuration	0.84 (0.66, 1.07)	1.61 (1.15, 2.25)	1.17×10^{-3}	0.65
6	GCLC	rs634657	A/G	Transsulfuration	0.84 (0.66, 1.07)	1.58 (1.13, 2.21)	1.44×10^{-3}	0.66
6	GCLC	rs6458939	A/C	Transsulfuration	1.01 (0.77, 1.33)	1.94 (1.37, 2.74)	2.23×10^{-3}	0.73
6	GCLC	rs6922238	C/A	Transsulfuration	0.77 (0.60, 1.00)	1.38 (0.99, 1.91)	4.79×10^{-3}	0.78
5	GLRX	rs11135434	G/A	Transsulfuration	0.79 (0.62, 1.01)	1.45 (1.06, 1.98)	1.47×10^{-3}	0.65
5	GLRX	rs6876015	G/A	Transsulfuration	0.63 (0.47, 0.84)	1.38 (0.95, 2.02)	9.85×10^{-4}	0.71
5	GLRX	rs11747430	C/G	Transsulfuration	0.67 (0.50, 0.90)	1.41 (0.96, 2.07)	2.20×10^{-3}	0.78
10	MGMT	rs1537257	A/G	Transsulfuration	0.91 (0.72, 1.15)	1.68 (1.21, 2.34)	1.55×10^{-3}	0.66
8	GSR	rs2978663	G/A	Transsulfuration	0.96 (0.76, 1.23)	1.74 (1.26, 2.41)	2.43×10^{-3}	0.71
11	GSTP1	rs7927381	A/G	Transsulfuration	0.70 (0.47, 1.03)	1.94 (1.29, 2.91)	3.27×10^{-4}	0.71
12	MGST1	rs1024839	G/A	Transsulfuration	0.72 (0.56, 0.93)	1.34 (0.97, 1.85)	2.30×10^{-3}	0.72
5	BHMT	rs506500	A/G	Homocysteine	0.83 (0.63, 1.10)	1.63 (1.15, 2.33)	2.10×10^{-3}	0.74
4	SOD3	rs13435617	A/T	Transsulfuration	0.72 (0.49, 1.04)	1.94 (1.28, 2.95)	4.58×10^{-4}	0.74
10	TRDMT1	rs10795454	C/A	Homocysteine	0.77 (0.59, 1.01)	1.43 (1.03, 2.00)	2.76×10^{-3}	0.74
10	TRDMT1	rs10795452	A/C	Homocysteine	0.78 (0.59, 1.02)	1.46 (1.04, 2.04)	2.67×10^{-3}	0.74
10	TRDMT1	rs2181043	G/A	Homocysteine	0.78 (0.59, 1.03)	1.47 (1.04, 2.08)	3.30×10^{-3}	0.77
20	GSS	rs6088650	A/G	Transsulfuration	0.84 (0.65, 1.07)	1.51 (1.08, 2.12)	3.25×10^{-3}	0.75
20	GSS	rs725521	A/G	Transsulfuration	0.82 (0.64, 1.05)	1.49 (1.06, 2.08)	3.23×10^{-3}	0.75
2	DNMT3A	rs6711622	A/G	Homocysteine	0.81 (0.62, 1.04)	1.51 (1.08, 2.13)	2.85×10^{-3}	0.75

Significant infant SNPs

Chr	Gene	SNP	Allele*	Pathway	Normal-weight RR (95% CI)	Obese RR (95% CI)	Interaction D×E×C‡ p-value	Interaction D×E×C‡ BFDP
2	MAT2A	rs17026396	G/A	Homocysteine	0.89 (0.69, 1.13)	1.56 (1.14, 2.13)	3.86×10^{-3}	0.75
7	NOS3	rs2373929	A/G	Transsulfuration	0.79 (0.62, 1.01)	1.45 (1.02, 2.06)	3.53×10^{-3}	0.76
14	MTHFD1	rs3818239	G/A	Folate	0.83 (0.57, 1.20)	2.08 (1.36, 3.19)	1.07×10^{-3}	0.78

Chr: Chromosome; RR: relative risk for carrying one copy of minor allele compared to no copies; CI: confidence interval; BFDP: Bayesian false-discovery probability, BFDPs < 0.8 were considered to indicate statistical significance

*Allele is presented as minor/major allele in our study sample; major allele is the reference allele.

†Interaction D×E×M is the three-way interaction for maternal genotype (see G×E Model in Statistical Methods section for details)

‡Interaction D×E×C is the three-way interaction for infant genotype (see G×E Model in Statistical Methods section for details)