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Conotruncal Heart Defects and Common Variants in Maternal and Fetal Genes in Folate, Homocysteine and Transsulfuration Pathways

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Abstract

Background—We investigated the association between conotruncal heart defects (CTDs) and maternal and fetal single nucleotide polymorphisms (SNPs) in 60 genes in the folate, homocysteine and pathways. We also investigated whether periconceptional maternal folic acid supplementation modified associations between CTDs and SNPs.

Methods—Participants were enrolled in the National Birth Defects Prevention Study between 1997 and 2007. DNA samples from 616 case-parental triads affected by CTDs and 1,645 control-parental triads were genotyped using an Illumina® Golden Gate custom SNP panel. A hybrid design analysis, optimizing data from case and control trios, was used to identify maternal and fetal SNPs associated with CTDs.

Results—Among 921 SNPs, 17 maternal and 17 fetal SNPs had a Bayesian false-discovery probability (BFDP) of <0.8. Ten of the 17 maternal SNPs and 2 of the 17 fetal SNPs were found within the glutamate-cysteine ligase, catalytic subunit (*GCLC*) gene. Fetal SNPs with the lowest BFDP (rs2612101, rs2847607, rs2847326, rs2847324) were found within the thymidylate synthetase (*TYMS*) gene. Additional analyses indicated that the risk of CTDs associated with candidate SNPs was modified by periconceptional folic acid supplementation. Nineteen maternal and 9 fetal SNPs had BFDP <0.8 for gene-by-environment (GxE) interactions with maternal folic acid supplementation.

Conclusions—These results support previous studies suggesting that maternal and fetal SNPs within folate, homocysteine and transsulfuration pathways are associated with CTD risk. Maternal use of supplements containing folic acid may modify the impact of SNPs on the developing heart.

Keywords

conotruncal heart defects; single nucleotide polymorphisms; oxidative stress; genetics; folic acid; gene X environment interaction

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Introduction

Congenital heart defects (CHDs) are the most prevalent structural birth defect, occurring in 8 to 11 of every 1,000 live births (Botto et al., 2001; Reller et al., 2008). CHDs include a broad range of heterogeneous cardiac malformations that may differ in etiology. Conotruncal heart defects (CTDs) comprise a subgroup of CHDs that are malformations of cardiac outflow tracts and great arteries, including truncus atreriosus, interrupted aortic arch type B, transposition of great arteries, double outlet right ventricle, conoventricular septal heart defects, tetralogy of Fallot, and pulmonary atresia with ventricular septal defect (Botto et al., 2007). These malformations share a common structural origin, being derived from cardiac neural crest cells and secondary heart field (Hutson and Kirby, 2007). CTDs account for approximately 20% to 30% of all CHDs and occur in 7 per 10,000 live births (Ferencz et al., 1985; Kuehl and Loffredo, 2005).

A minority of CTDs are associated with trisomies 13, 18, and 21, 22q11 microdeletion syndrome (Ferencz et al., 1985; Theveniau-Ruissy et al., 2008; Lammer et al., 2009), maternal diabetes (Ferencz et al., 1990) and obesity (Gilboa et al., 2010) and teratogens such as retinoic acid. For the majority of infants diagnosed with a CTD, however, the underlying cause remains elusive. Non-syndromic CTDs result from a complex interplay between genomic and epigenomic susceptibilities, and parental environmental, lifestyle, and endogenous factors (Chowdhury et al., 2012). Identification of genetic risk factors is especially challenging because maternal and fetal genetic susceptibilities may affect the intrauterine environment and contribute to CTD development (Hobbs et al., 2010; Chowdhury et al., 2012). Among first-degree relatives, the recurrence risk ratio of CTD is 11.7 (95% CI: 8.0, 17.0) (Oyen et al., 2009). It has been suggested that folic acid supplementation within the periconceptional period is protective during conotruncal area development resulting in reduced risk of conotruncal defects (Botto et al., 2004; Kuehl and Loffredo, 2005; Shaw et al., 2005; Goldmuntz et al., 2008). Data from our group and others have demonstrated that metabolites in the folate, homocysteine, and transsulfuration pathways were altered in women with pregnancies affected by septal, conotruncal and/or obstructive CHDs (Kapusta et al., 1999; Hobbs et al., 2005b; Hobbs et al., 2006; Obermann-Borst et al., 2011).

We hypothesized that common maternal and fetal genetic variants in folate, homocysteine, and transsulfuration pathways, which play key roles in one-carbon metabolism and glutathione antioxidant defense, are associated with CTDs. We examined the association between non-syndromic CTDs and 921 single nucelotide polymorphisms (SNPs) in 60 genes involved in three folate-related pathways. The current study represents the most comprehensive candidate pathway investigation of common genetic variants and CTDs to date.

Materials and Methods

Ethics Statement

The study was approved by University of Arkansas for Medical Sciences' Institutional Review Board and the 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 written consent. For minors, informed written consent was obtained from their legal guardian.

Study population and sample collection

All study subjects were enrolled in the National Birth Defects Research and Prevention Study (NBDPS), a multi-site population-based case-control study to investigate the etiology of 30 non-syndromic birth defects, creating the largest case-control study of birth defects ever conducted in the US. The study population and eligibility criteria for the NBDPS have been previously outlined (Yoon et al., 2001). Briefly, subjects were identified through 9 states' population-based birth defect surveillance systems. For the current study, cases were defined as families who delivered a singleton live birth with a CTD. 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 diagnostic tests on cardiac NBDPS case infants were reviewed by a pediatric cardiologist to ensure uniform criteria for diagnoses. Diagnostic tests included results from echocardiograms, surgical reports, cardiac catherizations, and autopsies. Using a classification system developed for NBDPS, which incorporated three dimensions of cardiac phenotype, cardiac complexity, and extracardiac anomalies (Botto et al., 2007), we included pregnancies that carried offspring diagnosed prenatally or postnatally with conotruncal defects as cases. Controls were those born within the same period as cases who had a singleton live birth without birth defects, participated in the NBDPS, and were randomly selected from birth certificate data or hospital discharge logs. Case and control mothers spoke either English or Spanish. Case and control mothers completed a computer-assisted telephone interview in which they were asked if they took folic acid containing single or multi-vitamins during three months prior to pregnancy and each month during pregnancy. All study participants for this analysis submitted buccal cells collected using cytobrushes from which DNA was isolated. For the current study, we included NBDPS participants with estimated dates of delivery between October 1997 and August 2008.

Folic Acid Supplementation

The mothers were considered to be fully exposed to folic acid containing supplement if they reported use of folic acid supplement one month prior to pregnancy and two months after pregnancy. The mothers were considered to be partially exposed if they reported use of folic acid supplement either one month prior to pregnancy or any of the two months after pregnancy. We defined the folic acid supplement users to be the families with the mothers either fully or partially exposed to the folic acid supplement.

Collection of DNA from buccal cell samples

NBDPS methods for biologic sample collection and processing are well established (Rasmussen et al., 2002). Upon interview completion, the mother receives a sample collection kit including cytobrushes to collect buccal (cheek) cell samples from mothers, infants and fathers by mail or courier. Each family returns the cytobrushes to study laboratories in mailed envelopes. Each collection tube is bar code labeled with an individual identifier clearly labeled as MOTHER, FATHER, or INFANT. The CDC laboratory logs

and tracks all samples and stores extracted DNA at the CDC storage facility (CDC and ATSDR Specimen Packaging, Inventory, and Repository).

Selection of candidate genes and SNPs

Candidate gene selection

Sixty-two candidate genes that encode an enzyme in one of three candidate metabolic pathways were selected. Using data from the International HapMap Project, we selected a maximally informative set of haplotype tagging SNPs (htSNPs) for each of the selected 62 candidate genes using an algorithm based on the linkage disequilibrium statistic r^2 (Carlson et al., 2004). For each gene, htSNPs were chosen from the entire gene region (including introns) with additional 10-kb flanking sequences. To choose htSNPs, pairwise r^2 values were computed for each marker combination within 200 kb for loci with a minor allele frequency (MAF) >0.10 in each population studied (Chowdhury et al., 2012). In collaboration with Illumina®, htSNPs were chosen based on an Illumina® assay design score. The overall score ranges from 0–1 and is based on the predicted optimal oligonucleotide probe sequences for each marker. A set of 1536 htSNPs were selected for inclusion in a customized Illumina® GoldenGate™ genotyping panel (Illumina®, http://www.illumina.com).

In 2005–2006, during the design phase of our custom candidate gene panel, there were two genes called RFC-1 in commonly used publicly available genetic databases. It was our intent to include SNPs from the Reduced folate carrier-1 gene (*RFC-1*, now called *SLC19A1*). However, after the panel was finalized and in production at Illumina, we discovered that the *RFC-1* SNPs included in the panel were within the Replication factor C (activator 1) 1 (*RFC-1*) gene. This gene is an activator of DNA polymerase and is required for DNA synthesis and repair. Thus, the *RFC-1* genotype data presented in this report are for SNPs in the Replication factor C (activator 1) 1 gene (*RFC-1*). To validate the identity of each gene, a search was performed within the National Center for Bioinformatics databases (Benson et al., 2011).

DNA extraction and quantification

DNA was extracted from buccal cell samples using Puregene® DNA purification reagents (Qiagen®, Valencia, CA) according to the manufacturer's protocol. Genomic DNA was quantified using ABI™ (Applied Biosystems, Foster City, CA) TaqMan® RNaseP Detection Reagents using a standard curve of genomic DNA of known concentration. The standard curve samples and the genomic DNA samples from case and control subjects were subjected to an initial denaturation at 95°C for 10 min, 40 cycles of polymerase chain reaction (PCR) at 95°C for 15 sec, and 60°C for 1 min in an ABI™ PRISM® 7900HT real-time PCR instrument. DNA concentrations were calculated from the standard curve using ABI™ software.

Whole genome amplification

Genomic DNA (10–15 ng) was used as a template for whole genome amplification (WGA) using the GenomePlex® WGA kit according to the manufacturer's protocol (Sigma, St.

Louis, MO). The resultant WGA product was quantified as above, and 200 ng were used for genotyping in the Illumina® Golden Gate™ assay. The robustness of whole genome amplified DNA in microarray platforms has been previously demonstrated (Cunningham et al., 2008).

Genotyping by Illumina® Golden Gate™ Assay

SNP genotyping was conducted using 200 ng (40 ng/μL) of study subject WGA amplified DNA using Illumina's® Golden Gate™ platform (Fan et al., 2003; Fan et al., 2006). Genotype analysis was performed according to Golden Gate™ assay's protocol. BeadChips were scanned on Illumina's® BeadArray™ Reader, and initial genotype calls were generated using GenCall, Illumina's genotyping algorithm.

We found that the quality of genotype clustering varied substantially from SNP to SNP, which we attribute to the *in silico* design of the custom SNP panel without the subsequent quality checks that would be applied to a standard commercial SNP panel. The initial genotype calls along with the raw intensity data were used as inputs to SNPMClust, a bivariate Gaussian model-based genotype clustering and calling algorithm developed in-house, currently available as an R package on the Comprehensive R Archive Network (CRAN; http://cran.r-project.org/) (Chowdhury et al., 2012). After running SNPMClust, clustering and classification plots for all SNPs were visually inspected, leading to dropping a SNP from analysis or running SNPMClust under non-default settings in some cases.

A subset of Arkansas residents who completed the NBDPS was also recruited for a different study at Arkansas Children's Hospital Research Institute (Hobbs et al., 2005a) and provided both blood and buccal samples. Ninety-six participants, for whom both blood and buccal samples were available, comprised a pilot study to validate the use of WGA-buccal DNA on the custom genotyping platform. As expected, the blood-derived DNA samples, which had not undergone WGA, performed better than the WGA product. Out of 1,536 SNPs, 60 SNPs exhibited poor clustering behavior even within the blood DNA samples and were dropped from subsequent analysis. Among 94 participants for whom both DNA samples produced high call rates, and for those genotype calls in which both the blood and WGA-buccal samples passed the quality control steps described below, the concordance rate averaged 99.2%. We therefore have confidence in the fidelity of genotypes based on WGA-buccal DNA when appropriate standards are applied.

Statistical Methods

Post-genotyping Quality Control

We removed 297 individuals because they had either high no-call rates, or high rates of Mendelian inconsistency. We further applied stringent quality control measures to ensure high-quality genotypes, excluding SNPs with no-call rates > 10% (328 SNPs), Mendelian error rates > 5% (11 SNPs), MAF < 5% (204 SNPs), or significant deviation from Hardy-Weinberg Equilibrium in at least one racial group ($p < 10^{-4}$, 12 SNPs). The final dataset included 4,648 individuals (94%), each with 921 SNPs (60% of the original set of SNPs, representing 60 candidate genes), including 230 case triads, 222 case dyads, 96 case

mothers, 31 case fathers, 37 case infants, 559 control triads, 587 control dyads, 242 control mothers, 94 control fathers, and 163 control infants.

Statistical Analyses

Because the NBDPS enrolled case- and control-parental trios, we employed a hybrid log-linear model approach as suggested by Weinberg and Umbach (Weinberg and Umbach, 2005) to optimize the power of this study design. This hybrid approach uses data from both case- and control families to estimate the genetic relative risk due to maternal and fetal genotypes. We also extended this model to explore the interaction between SNPs in our candidate regions and periconceptional folic acid supplementation.

Briefly, the Weinberg/Umbach log-linear approach simultaneously estimates the contributions of maternal and fetal genotypes for a given SNP by fitting the following model:

$$ln[E(count|M,F,C,D)]=\ln(\mu_j)+\gamma I_{(D=1)}+\beta_1 I_{(D=1)}I_M+\beta_2 I_{(D=1)}I_C+ln(off), \quad [1]$$

where μ_j, j = 1, …, 6 correspond to the six possible parental mating type categories assuming mating symmetry. The indicator variable $I_{(D=1)}$ equals 1 for case families ($I_{(D=1)}$ = 1) and 0 for control families; I_M is an indicator for maternal genetic effects and equals the number of copies of the variant allele (0, 1, or 2) carried by the mother; and I_C is the corresponding indicator for fetal genetic effects. By defining the indicators for maternal and fetal genetic effects in this way, we implicitly assume multiplicative (i.e. log-additive) risk per allele. An expectation-maximization (EM) algorithm is used to estimate the relative risk in the presence of incomplete data.

We hypothesized that genetic effects on CTD risk might be modulated by maternal folic acid supplements use periconceptionally. We therefore extended the log-linear approach to estimate G×E interactive effects for each SNP, where periconceptional folic acid supplementation is defined as the environmental exposure of interest. For each SNP, the following model was fitted:

$$\begin{aligned} ln[E(count|M,F,C,D,E)] &=\ln(\mu_j) \\ &+\delta_k I_{(E=1)}+\gamma I_{(D=1)}+\beta_1 I_{(D=1)}I_M \\ &+\beta_2 I_{(D=1)}I_C \\ &+\beta_3 I_{(D=1)}I_{(E=1)}I_M \\ &+\beta_4 I_{(D=1)}I_{(E=1)}I_C+ln(off), \end{aligned} \quad [2]$$

where μ_j, $I_{(D=1)}$, I_M, and I_C are defined as above, while δ_k, k= 1, …, 6 are the stratum parameters for six mating types for exposed families, and $I_{(E=1)}$ is the indicator for exposed families ($I_{(E=1)}$ = 1) and unexposed families ($I_{(E=1)}$ = 0). Based on this extended log-linear model, maternal and fetal GxE interactions can be evaluated by using a Wald test for the parameters β_3 and β_4, respectively.

$$\hat{\sum} = \begin{bmatrix} \hat{\sigma}_{1,1} & \cdots & \hat{\sigma}_{1,n} \\ \vdots & \ddots & \vdots \\ \hat{\sigma}_{n,1} & \cdots & \hat{\sigma}_{n,n} \end{bmatrix}_{n=17}.$$

Then the relative risk for maternal genetic effect among unexposed families can be estimated by $\exp(\hat{\beta_1})$ with the estimated standard error for $\hat{\beta_1}$ being $\sqrt{\hat{\sigma}_{14,14}}$. The relative risk for maternal genetic effect among exposed families can be estimated by $\exp(\hat{\beta_1} + \hat{\beta_3})$, and the standard error for $\hat{\beta_1} + \hat{\beta_3}$ can be estimated by $\sqrt{\hat{\sigma}_{14,14} + \hat{\sigma}_{16,16} + 2\,\hat{\sigma}_{14,16}}$. The relative risk for fetal genetic effect among unexposed and exposed families were estimated accordingly.

Bayesian false-discovery probability

The Bayesian false-discovery probability (BFDP) computed using results from log-linear models was considered an appropriate approach in this candidate gene study (Wakefield, 2007; Liu et al., 2010; Oh et al., 2010; Park et al., 2010; Spitz et al., 2012; Zienolddiny et al., 2013). For a specific SNP-disease association, BFDP is defined as the probability of that association being null (i.e. a false discovery), conditional on the observed data. The BFDP threshold was pre-set at 0.8; in decision theoretic terms, this threshold implies that a false nondiscovery is considered four times as costly as a false discovery. The prior probability of disease association for each candidate SNP was set at 0.05, and the prior distribution on effect size, given a true association, was a beta distribution scaled such that an odds ratio of 1.5 was the 97.5th percentile of the prior.

Software used for data analysis included SAS 9.3 (SAS Institute Inc., Cary, NC), R 2.15.0 (R Development Core Team, Vienna, Austria), LEM program (Vermunt, 1997), and HAPLOVIEW 4.2 (Barrett et al., 2005).

Results

There were a total of 2,261 families in the analytic sample including 616 case-families and 1,645 control-families. Of the 616 cases, 272 (44.2%) had tetralogy of Fallot, 252 (40.0%) had D-transposition of great arteries, 25 (4.1%) had double outlet right ventricle, 37 (6.0%) had conoventricular septal defects, 23 (3.7%) had truncus atreriosus, and 7 (1.1%) had interrupted aortic arch type B.

Maternal characteristics for case and control families are summarized in Table 1. Among women included in the analysis, the majority were non-Hispanic white (66% of cases and 69% of controls), with some college education (59% cases and 62% controls) and a normal BMI (50% cases and 55% controls). Distributions of maternal education level, body mass index, household income, and maternal use of alcohol, tobacco or multivitamins containing folic acid during the first trimester were similar between cases and controls. No statistically significant differences were found between case and control mothers except for age at delivery, 27.5 years, and 28.3 years, respectively.

CTDs and SNP Associations

Using a hybrid study design combining data from case- and control-parent trios, we estimated associations between conotruncal heart defects and each of the 921 SNPs. In analyses including only case-parental trios, untransmitted alleles from either parents provide allelic controls. In the hybrid analyses, alleles of case infants are compared with alleles of control infants providing additional statistical power (Skare et al., 2012). In the hybrid model, separate relative risks for maternal and fetal effects are estimated simultaneously in a joint model, adjusted for each other. As summarized in Table 2 and displayed in Manhattan Plot in Figure 1a, the most significant maternal SNPs were found in the glutamate-cysteine ligase, catalytic subunit (*GCLC*) gene. Specificially, 10 of 17 SNPs with a Bayesian False Discovery Probablitity (BFDP) of ≤0.80 were within the *GCLC* gene. Two fetal SNPs in the *GCLC* gene (rs10948751, rs7742367) were also significant. Of the *GCLC* SNPs, associated with CTD, several were in linkage disequilibrium with each other. Because many are in strong linkage disequilibrium they may not function independently and may tag a single causal variant or region. The remaining 7 maternal SNPs with a BFDP <0.8, were found in the methenlytetrahydrofolate synthetase (*MTHFS*), superoxide dismutase 2, mitochondrial (*SOD2*), methyltetrahydrofolate-homocysteine methyltransferase reductase (*MTRR*), glutaredoxin (thioltransferase) (*GLRX*), and betaine-homocysteine s-methyltransferase (*BHMT*) genes. Similarly, among 921 fetal SNPs evaluated and displayed in the Manhattan Plot in Figure 1b, the most significant fetal SNPs were found in the thymidylate synthetase (*TYMS*) gene. The remaining 11 fetal SNPs that had a Bayesian False Discovery Probability of ≤ 0.8 were found in 7 genes: gluthatione peroxidase 4 (*GPX4*), glutathione S-transferase mu 4 (*GSTM4*), catalase (*CAT*), O-6-methylguanine-DNA methyltransferase (*MGMT*), microsomal glutathione S-transferase 1 (*MGST1*), adenosylhomocysteinase-like 2 (*KIAA0828*), transcobalamin II (*TCN2*).

Folic acid supplementation

Maternal use of folic acid containing supplements may interact with either maternal or fetal SNPs, or both, to alter the estimated risk of CTDs. The use of folic acid containing supplements varied significantly by ethnicity with 63% (n=726) of non-Hispanic white control women reporting use compared to 20% (n=30) of African-Americans control and 33% (n=94) of Hispanic control women. Given the distribution of folic acid supplement use among individual race/ethnicity groups and the lower numbers of African-American and Hispanic women, analyses to identify folic acid-SNP interactions were restricted to non-Hispanic whites.

In Table 3, we present maternal (top panel) and fetal (bottom panel) SNPs that demonstrated significant (BFDP ≤ 0.8) interactions with maternal use of folic acid supplements. Four of the 19 maternal interactions and 6 of the 9 fetal interactions, included SNPs within Replication factor C (activator 1) 1 (*RFC-1*) gene. Furthermore, those SNPs resulting in the lowest BFDP, were within the *RFC-1* gene. Other maternal genes that included SNPs associated with CTDs among women who did not take folic acid supplements included the following: nitric oxide synthase 2, inducible (*NOS2A*), glutathione-dependent prostaglandin D synthase (*PGDS*), O-6 methylguanine-DNA methyltransferase (*MGMT*), and betaine-homocysteine methyltransferase (*BHMT2* and *BHMT*). In addition to *RFC-1*, fetal genes that

included SNPs associated with CTDs among women who were not supplement users were methenyltetrahydrofolate synthetase (*MTHFS*), and cystathionine-beta-synthase (*CBS*).

Discussion

In this population-based, case-control study, we evaluated the association between CTD risk and 921 SNPs in 60 genes in candidate pathways. To our knowledge, our study is the most comprehensive genotyping effort of common folate-related genetic variants and CTDs. We discovered multiple SNPs in fetal and maternal genes that were associated with CTD risk independent of folic acid supplement use, and some SNPs that were only associated with CTD risk when supplement use was considered.

Maternal genetic variants

The maternal SNP with the smallest BFDP (BFDP=0.15) was in the glutathione-cytosine ligase, catalytic subunit (*GCLC*) gene (rs572494). Twelve different SNPs in the *GCLC* gene were among the 34 maternal and fetal SNPs that had a BFDP ≤ 0.8. *GCLC* is the rate limiting step in glutathione synthesis and is dependent on cellular availability of cysteine. The association of SNPs in the *GCLC* gene with the risk of CTD in infants suggests that oxidative stress may be associated with CTD risk. Cellular glutathione protects developing embryos from harmful xenobiotics 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).

Fetal genetic variants

Among the 17 fetal SNPs with a BFDP ≤ 0.80, the SNP with the smallest BFDP was within the thymidylate synthatase (*TYMS; rs2612101*) gene and 3 other SNPs in the *TYMS* gene were of importance. *TYMS* catalyzes the methylation of deoxyuridylate to deoxythymidylate using 5,10-methylenetetrahydrofolate as a cofactor (Gibson et al., 2011). By maintaining the dTMP pool critical for DNA replication and repair, *TYMS* is essential in actively dividing cells of the embryo (Du et al., 2006). It is plausible that SNPs in *TYMS* are critical to maintainance of metabolic requirements for cell proliferation and growth and essential to embryonic development of heart and other structures.

A recent study (Shaw et al., 2009) to determine the associating between CTDs and spina bifida, and 118 SNPs in 14 candidate genes included SNPs in the *MTHFD2* and *TYMS* genes. Statistically significant associations were observed for SNPs within the *MTHFD2 (rs702465, rs7571842)* and *TYMS (rs2847159, rs1001761, rs502396)* genes for spina bifida, but no statistically significant associations were observed for CTDs and the 118 SNPs. Zhu and colleagues investigated whether two CTD were associated with two TYMS functional variants (rs4544694: a variable number of tandem repeats polymorphism; rs16430: a 6 base-pair deletion) (Zhu et al., 2012). The investigators did not find a gene-only effect of either variant. However, among women who had low folate during the peri-conceptional period, they observed a 3.6 fold increase in CTD risk among infants who were homozygous for the 6 base-pair insertion.

The *RFC-1* gene, Replication factor C (activator 1) 1, encodes the large subunit of replication factor 3. Replication factor 3 acts as a DNA–dependent ATPase consisting of five subunits and is required for eukaryotic DNA replication and repair (Overmeer et al., 2010). The *RFC-1* gene product is required for activation of DNA polymerase and functions by binding to the primer-template junction and with PCNA, and is required for elongation of primed DNA templates by DNA polymerase delta and epsilon (Ellison and Stillman, 1998). Our observation of an association between CTD risk and multiple maternal and fetal SNPs in *RFC-1* in women who did not use folic acid supplements suggests that the impact on the developing heart of genetic variants in *RFC-1* gene, a gene active in DNA synthesis and repair, may be modified by maternal folic acid intake. This hypothesis will be a subject for further research.

Our study is limited by the inclusion of only common variants that have a minor allele frequency >5%. The release of Phase III data from the International HapMap Project and data from the 1000 Genomes Project will allow future studies to examine the impact of less common variants (Altshuler et al., 2010; Consortium, 2010). Nonsyndromic CTDs have a complex etiology and developmental mechanisms that likely includes many gene-gene and gene-environment interactions. Investigation of these interactions was beyond the scope of the current study, but is planned for future analyses. Future genetic epidemiologic studies are needed to replicate our findings. Future studies are needed to more precisely delineate the role of *MTHFD2* in the developing heart. Deep exome sequencing studies will be necessary to discover the functional SNP(s) responsible for changes in enzyme activity that may increase embryo susceptibility to development of heart defects.

Future efforts will allow for more indepth analyses of genomic regions our study identified. Other studies will be needed to replicate results to gain additional confidence in our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, Bonnen PE, de Bakker PI, Deloukas P, Gabriel SB, Gwilliam R, Hunt S, Inouye M, Jia X, Palotie A, Parkin M, Whittaker P, Chang K, Hawes A, Lewis LR, Ren Y, Wheeler D, Muzny DM, Barnes C, Darvishi K, Hurles M, Korn JM, Kristiansson K, Lee C, McCarrol SA, Nemesh J, Keinan A, Montgomery SB, Pollack S, Price AL, Soranzo N, Gonzaga-Jauregui C, Anttila V, Brodeur W, Daly MJ, Leslie S, McVean G, Moutsianas L, Nguyen H, Zhang Q, Ghori MJ, McGinnis R, McLaren W, Takeuchi F, Grossman

SR, Shlyakhter I, Hostetter EB, Sabeti PC, Adebamowo CA, Foster MW, Gordon DR, Licinio J, Manca MC, Marshall PA, Matsuda I, Ngare D, Wang VO, Reddy D, Rotimi CN, Royal CD, Sharp RR, Zeng C, Brooks LD, McEwen JE. Integrating common and rare genetic variation in diverse human populations. Nature. 2010; 467(7311):52–58. [PubMed: 20811451]

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–265. [PubMed: 15297300]

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. GenBank. Nucleic Acids Res. 2011; 39(Database issue):D32–37. [PubMed: 21071399]

Botto LD, Correa A, Erickson JD. Racial and temporal variations in the prevalence of heart defects. Pediatrics. 2001; 107(3):E32. [PubMed: 11230613]

Botto LD, Lin AE, Riehle-Colarusso T, Malik S, Correa A. Seeking causes: Classifying and evaluating congenital heart defects in etiologic studies. Birth Defects Res A Clin Mol Teratol. 2007; 79(10): 714–727. [PubMed: 17729292]

Botto LD, Olney RS, Erickson JD. Vitamin supplements and the risk for congenital anomalies other than neural tube defects. Am J Med Genet C Semin Med Genet. 2004; 125C(1):12–21. [PubMed: 14755429]

Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet. 2004; 74(1):106–120. [PubMed: 14681826]

Chowdhury S, Hobbs CA, MacLeod SL, Cleves MA, Melnyk S, James SJ, Hu P, Erickson SW. Associations between maternal genotypes and metabolites implicated in congenital heart defects. Mol Genet Metab. 2012; 107(3):596–604. [PubMed: 23059056]

Consortium GP. A map of human genome variation from population-scale sequencing. Nature. 2010; 467(7319):1061–1073. [PubMed: 20981092]

Cunningham JM, Sellers TA, Schildkraut JM, Fredericksen ZS, Vierkant RA, Kelemen LE, Gadre M, Phelan CM, Huang Y, Meyer JG, Pankratz VS, Goode EL. Performance of amplified DNA in an Illumina GoldenGate BeadArray assay. Cancer Epidemiol Biomarkers Prev. 2008; 17(7):1781–1789. [PubMed: 18628432]

Du C, Niu R, Chu E, Zhang P, Lin X. Sequence analysis and functional study of thymidylate synthase from zebrafish, Danio rerio. J Biochem. 2006; 139(5):913–920. [PubMed: 16751599]

Ellison V, Stillman B. Reconstitution of recombinant human replication factor C (RFC) and identification of an RFC subcomplex possessing DNA-dependent ATPase activity. J Biol Chem. 1998; 273(10):5979–5987. [PubMed: 9488738]

Fan JB, Chee MS, Gunderson KL. Highly parallel genomic assays. NatRevGenet. 2006; 7(8):632–644.

Fan JB, Oliphant A, Shen R, Kermani BG, Garcia F, Gunderson KL, Hansen M, Steemers F, Butler SL, Deloukas P, Galver L, Hunt S, McBride C, Bibikova M, Rubano T, Chen J, Wickham E, Doucet D, Chang W, Campbell D, Zhang B, Kruglyak S, Bentley D, Haas J, Rigault P, Zhou L, Stuelpnagel J, Chee MS. Highly parallel SNP genotyping. Cold Spring HarbSympQuantBiol. 2003; 68:69–78.

Ferencz C, Rubin JD, McCarter RJ, Brenner JI, Neill CA, Perry LW, Hepner SI, Downing JW. Congenital heart disease: prevalence at livebirth. The Baltimore- Washington Infant Study. Am J Epidemiol. 1985; 121(1):31–36. [PubMed: 3964990]

Ferencz C, Rubin JD, McCarter RJ, Clark EB. Maternal diabetes and cardiovascular malformations: predominance of double outlet right ventricle and truncus arteriosus. Teratology. 1990; 41(3):319–326. [PubMed: 2326756]

Gibson TM, Brennan P, Han S, Karami S, Zaridze D, Janout V, Kollarova H, Bencko V, Navratilova M, Szeszenia-Dabrowska N, Mates D, Slamova A, Pfeiffer RM, Stolzenberg-Solomon RZ, Mayne ST, Yeager M, Chanock S, Rothman N, Chow WH, Rosenberg PS, Boffetta P, Moore LE. Comprehensive evaluation of one-carbon metabolism pathway gene variants and renal cell cancer risk. PLoS One. 2011; 6(10):e26165. [PubMed: 22039442]

Gilboa SM, Correa A, Botto LD, Rasmussen SA, Waller DK, Hobbs CA, Cleves MA, Riehle-Colarusso TJ, Study NBDP. Association between prepregnancy body mass index and congenital heart defects. Am J Obstet Gynecol. 2010; 202(1):51.e51–51.e10. [PubMed: 19796755]

Goldmuntz E, Woyciechowski S, Renstrom D, Lupo PJ, Mitchell LE. Variants of folate metabolism genes and the risk of conotruncal cardiac defects. Circ Cardiovasc Genet. 2008; 1(2):126–132. [PubMed: 20031554]

Hansen JM, Lee E, Harris C. Spatial activities and induction of glutamate-cysteine ligase (GCL) in the postimplantation rat embryo and visceral yolk sac. ToxicolSci. 2004; 81(2):371–378.

Hobbs CA, Cleves MA, Karim MA, Zhao W, MacLeod SL. Maternal folate-related gene environment interactions and congenital heart defects. ObstetGynecol. 2010; 116(2 Pt 1):316–322.

Hobbs CA, Cleves MA, Melnyk S, Zhao W, James SJ. Congenital heart defects and abnormal maternal biomarkers of methionine and homocysteine metabolism. Am J Clin Nutr. 2005a; 81(1):147–153. [PubMed: 15640474]

Hobbs CA, Cleves MA, Zhao W, Melnyk S, James SJ. Congenital heart defects and maternal biomarkers of oxidative stress. Am J Clin Nutr. 2005b; 82(3):598–604. [PubMed: 16155273]

Hobbs CA, Malik S, Zhao W, James SJ, Melnyk S, Cleves MA. Maternal homocysteine and congenital heart defects. JAmCollCardiol. 2006; 47(3):683–685.

Hutson MR, Kirby ML. Model systems for the study of heart development and disease. Cardiac neural crest and conotruncal malformations. SeminCell DevBiol. 2007; 18(1):101–110.

Kapusta L, Haagmans ML, Steegers EA, Cuypers MH, Blom HJ, Eskes TK. Congenital heart defects and maternal derangement of homocysteine metabolism. J Pediatr. 1999; 135(6):773–774. [PubMed: 10586186]

Kuehl KS, Loffredo CA. Genetic and environmental influences on malformations of the cardiac outflow tract. Expert Rev Cardiovasc Ther. 2005; 3(6):1125–1130. [PubMed: 16293002]

Lammer EJ, Chak JS, Iovannisci DM, Schultz K, Osoegawa K, Yang W, Carmichael SL, Shaw GM. Chromosomal abnormalities among children born with conotruncal cardiac defects. Birth Defects Res A Clin Mol Teratol. 2009; 85(1):30–35. [PubMed: 19067405]

Liu Y, Shete S, Etzel CJ, Scheurer M, Alexiou G, Armstrong G, Tsavachidis S, Liang FW, Gilbert M, Aldape K, Armstrong T, Houlston R, Hosking F, Robertson L, Xiao Y, Wiencke J, Wrensch M, Andersson U, Melin BS, Bondy M. Polymorphisms of LIG4, BTBD2, HMGA2, and RTEL1 genes involved in the double-strand break repair pathway predict glioblastoma survival. J Clin Oncol. 2010; 28(14):2467–2474. [PubMed: 20368557]

Obermann-Borst SA, van Driel LM, Helbing WA, de Jonge R, Wildhagen MF, Steegers EA, Steegers-Theunissen RP. Congenital heart defects and biomarkers of methylation in children: a case-control study. Eur J Clin Invest. 2011; 41(2):143–150. [PubMed: 20868449]

Oh SS, Chang SC, Cai L, Cordon-Cardo C, Ding BG, Greenland S, He N, Jiang Q, Kheifets L, Le A, Lee YC, Liu S, Lu ML, Mao JT, Morgenstern H, Mu LN, Pantuck A, Papp JC, Park SL, Rao JY, Reuter VE, Tashkin DP, Wang H, You NC, Yu SZ, Zhao JK, Belldegrun A, Zhang ZF. Single nucleotide polymorphisms of 8 inflammation-related genes and their associations with smoking-related cancers. Int J Cancer. 2010; 127(9):2169–2182. [PubMed: 20112337]

Overmeer RM, Gourdin AM, Giglia-Mari A, Kool H, Houtsmuller AB, Siegal G, Fousteri MI, Mullenders LH, Vermeulen W. Replication factor C recruits DNA polymerase delta to sites of nucleotide excision repair but is not required for PCNA recruitment. Mol Cell Biol. 2010; 30(20): 4828–4839. [PubMed: 20713449]

Oyen N, Poulsen G, Boyd HA, Wohlfahrt J, Jensen PK, Melbye M. Recurrence of congenital heart defects in families. Circulation. 2009; 120(4):295–301. [PubMed: 19597048]

Park SL, Bastani D, Goldstein BY, Chang SC, Cozen W, Cai L, Cordon-Cardo C, Ding B, Greenland S, He N, Hussain SK, Jiang Q, Lee YC, Liu S, Lu ML, Mack TM, Mao JT, Morgenstern H, Mu LN, Oh SS, Pantuck A, Papp JC, Rao J, Reuter VE, Tashkin DP, Wang H, You NC, Yu SZ, Zhao JK, Zhang ZF. Associations between NBS1 polymorphisms, haplotypes and smoking-related cancers. Carcinogenesis. 2010; 31(7):1264–1271. [PubMed: 20478923]

Rasmussen SA, Lammer EJ, Shaw GM, Finnell RH, McGehee RE Jr, Gallagher M, Romitti PA, Murray JC. Integration of DNA sample collection into a multi-site birth defects case-control study. Teratology. 2002; 66(4):177–184. [PubMed: 12353214]

Reller MD, Strickland MJ, Riehle-Colarusso T, Mahle WT, Correa A. Prevalence of congenital heart defects in metropolitan Atlanta, 1998–2005. J Pediatr. 2008; 153(6):807–813. [PubMed: 18657826]

Shaw GM, Iovannisci DM, Yang W, Finnell RH, Carmichael SL, Cheng S, Lammer EJ. Risks of human conotruncal heart defects associated with 32 single nucleotide polymorphisms of selected cardiovascular disease-related genes. Am J Med Genet A. 2005; 138(1):21–26. [PubMed: 16100725]

Shaw GM, Lu W, Zhu H, Yang W, Briggs FB, Carmichael SL, Barcellos LF, Lammer EJ, Finnell RH. 118 SNPs of folate-related genes and risks of spina bifida and conotruncal heart defects. BMC Med Genet. 2009; 10:49. [PubMed: 19493349]

Skare O, Jugessur A, Lie RT, Wilcox AJ, Murray JC, Lunde A, Nguyen TT, Gjessing HK. Application of a novel hybrid study design to explore gene-environment interactions in orofacial clefts. Ann Hum Genet. 2012; 76(3):221–236. [PubMed: 22497478]

Spitz MR, Gorlov IP, Dong Q, Wu X, Chen W, Chang DW, Etzel CJ, Caporaso NE, Zhao Y, Christiani DC, Brennan P, Albanes D, Shi J, Thun M, Landi MT, Amos CI. Multistage analysis of variants in the inflammation pathway and lung cancer risk in smokers. Cancer Epidemiol Biomarkers Prev. 2012; 21(7):1213–1221. [PubMed: 22573796]

Theveniau-Ruissy M, Dandonneau M, Mesbah K, Ghez O, Mattei MG, Miquerol L, Kelly RG. The del22q11.2 candidate gene Tbx1 controls regional outflow tract identity and coronary artery patterning. Circ Res. 2008; 103(2):142–148. [PubMed: 18583714]

Vermunt, JK. Log-linear models for event histories. Thousand Oakes: Sage Publications; 1997.

Wakefield J. A Bayesian measure of the probability of false discovery in genetic epidemiology studies. Am J Hum Genet. 2007; 81(2):208–227. [PubMed: 17668372]

Weinberg CR, Umbach DM. A hybrid design for studying genetic influences on risk of diseases with onset early in life. AmJHumGenet. 2005; 77(4):627–636.

Wells PG, McCallum GP, Chen CS, Henderson JT, Lee CJ, Perstin J, Preston TJ, Wiley MJ, Wong AW. Oxidative stress in developmental origins of disease: teratogenesis, neurodevelopmental deficits, and cancer. Toxicol Sci. 2009; 108(1):4–18. [PubMed: 19126598]

Yoon PW, Rasmussen SA, Lynberg MC, Moore CA, Anderka M, Carmichael SL, Costa P, Druschel C, Hobbs CA, Romitti PA, Langlois PH, Edmonds LD. The National Birth Defects Prevention Study. Public Health Rep. 2001; 116(Suppl 1):32–40. [PubMed: 11889273]

Zhu H, Yang W, Shaw N, Perloff S, Carmichael SL, Finnell RH, Shaw GM, Lammer EJ. Thymidylate synthase polymorphisms and risk of conotruncal heart defects. Am J Med Genet A. 2012; 158A(9):2194–2203. [PubMed: 22887475]

Zienolddiny S, Haugen A, Lie JA, Kjuus H, Anmarkrud KH, Kjaerheim K. Analysis of polymorphisms in the circadian-related genes and breast cancer risk in Norwegian nurses working night shifts. Breast Cancer Res. 2013; 15(4):R53. [PubMed: 23822714]

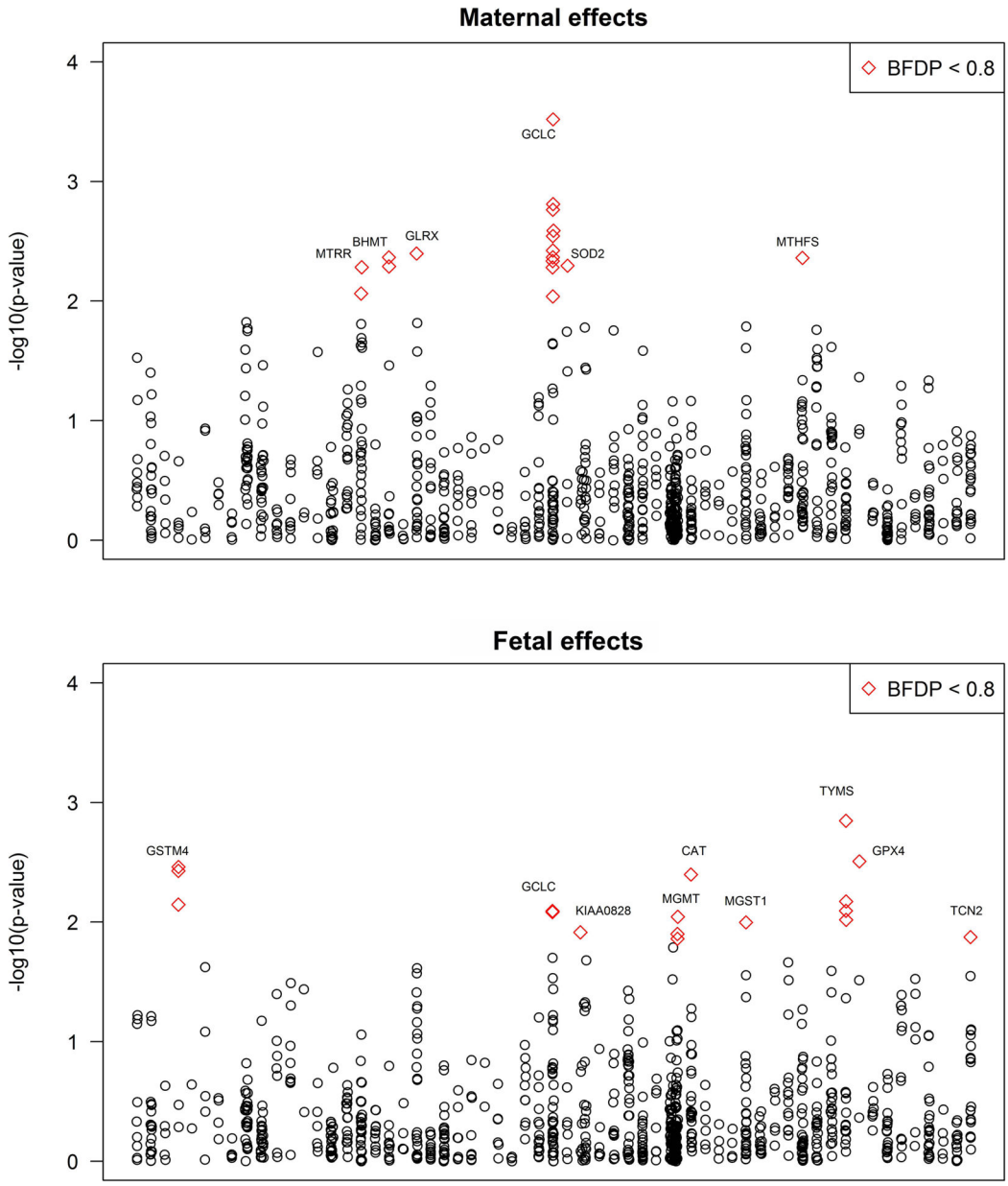

FIGURE 1.

Manhattan Plot: Maternal and infant folate-related SNPs as predictors of CTD risk. The red diamonds indicate SNPs that reached BFDP significance threshold.

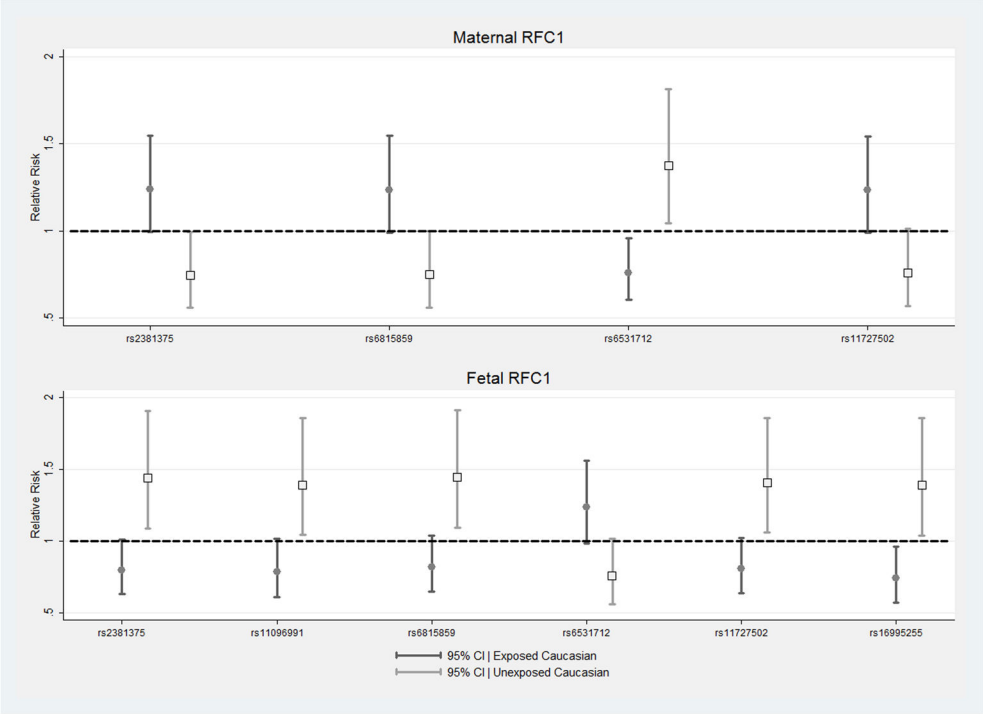

FIGURE 2.

Plot of BFDP significant SNPs on maternal and infant RFC1 for Caucasian mother families: both maternal and offspring RFC1 has significant interactive effect with folic acid; SNPs are ordered by their physical locations on the gene.

Table 1

Maternal characteristics for 616 case mothers and 1,645 control mothers

	Control (N=1,645)	Case (N=616)
Age at delivery, mean (SD)	27.5 (6.0)	28.3 (6.1)
Mother's race		
African American	143 (9%)	49 (8%)
Caucasian	1,136 (69%)	401 (66%)
Hispanic	285 (17%)	123 (20%)
Others	78 (5%)	39 (6%)
Missing information	3	4
Mother's education, N (%)		
<12 years	217 (13%)	83 (14%)
High school degree or equivalent	413 (25%)	167 (27%)
1–3 years of college	454 (28%)	173 (28%)
At least 4 years of college or Bachelor degree	559 (34%)	190 (31%)
Missing information	2	3
Household income, N (%)		
Less than 10 Thousand	236 (15%)	94 (16%)
10 to 30 Thousand	408 (27%)	150 (26%)
30 to 50 Thousand Dollars	348 (23%)	118 (20%)
More than 50 Thousand	538 (35%)	217 (37%)
Missing information	115	37
Folic acid supplementation, N (%)		
Unexposed	738 (45%)	299 (49%)
Partially exposed	359 (22%)	117 (19%)
Fully Exposed	548 (33%)	197 (32%)
Missing information	0	3
Alcohol consumption, N (%)		
Unexposed	1,251 (76%)	460 (76%)
Exposed*	390 (24%)	149 (24%)
Missing information	4	7
Cigarette smoking, N (%)		
Unexposed	1,356 (82%)	498 (81%)
Exposed*	288 (18%)	114 (19%)
Missing information	1	4
Maternal BMI, N (%)**		
Underweight (BMI <18.5)	74 (5%)	31 (5%)
Normal weight (18.5 <=BMI <25)	880 (55%)	298 (50%)
Overweight (25 <=BMI <30)	360 (23%)	141 (24%)
Obese (>=30)	281 (18%)	121 (20%)
Missing information	50	25

*Exposed drinking and smoking were defined as mothers who drank or smoked in any of the 3 months after conception

**Maternal BMI analytic categories used as defined by the National Heart, Lung, and Blood Institute and the World Health Organization

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Table 2

Maternal and fetal SNPs associated with CTDs in NBDPS population (model [1]) (RF: Referent Genotype; BFDP: Bayesian false-discovery probability; RR: relative risk for risk allele; 95% CI: 95% confidence interval for the estimated RR)

Significant maternal effects based on model [1]

Chromosome	Gene	SNP	Allele*	Pathway	RR (95% CI)	p-value	BFDP**
6	GCLC	rs572494	G/A	Transsulfuration	0.76 (0.65, 0.88)	3.02×10^{-4}	0.15
6	GCLC	rs13212365	A/G	Transsulfuration	0.77 (0.66, 0.91)	1.54×10^{-3}	0.40
6	GCLC	rs634657	A/G	Transsulfuration	1.28 (1.10, 1.48)	1.72×10^{-3}	0.42
6	GCLC	rs2397147	G/A	Transsulfuration	0.78 (0.66, 0.92)	2.57×10^{-3}	0.50
6	GCLC	rs648595	A/C	Transsulfuration	1.27 (1.08, 1.48)	2.86×10^{-3}	0.52
6	GCLC	rs546726	G/A	Transsulfuration	1.25 (1.08, 1.46)	3.78×10^{-3}	0.58
6	GCLC	rs542914	A/C	Transsulfuration	0.79 (0.67, 0.93)	4.30×10^{-3}	0.60
6	GCLC	rs12525474	A/G	Transsulfuration	0.71 (0.56, 0.90)	5.23×10^{-3}	0.68
6	GCLC	rs16883912	A/G	Transsulfuration	0.68 (0.52, 0.89)	4.62×10^{-3}	0.68
6	GCLC	rs642429	G/A	Transsulfuration	0.73 (0.57, 0.92)	9.15×10^{-3}	0.75
15	MTHFS	rs9635381	G/A	Folate	0.75 (0.62, 0.91)	4.35×10^{-3}	0.62
6	SOD2	rs6912979	G/A	Transsulfuration	1.27 (1.07, 1.49)	5.06×10^{-3}	0.63
5	MTRR	rs13183229	A/G	Homocysteine	0.79 (0.67, 0.93)	5.21×10^{-3}	0.64
5	MTRR	rs1532268	A/G	Homocysteine	0.80 (0.68, 0.95)	8.65×10^{-3}	0.72
5	GLRX	rs7700813	A/G	Transsulfuration	0.69 (0.54, 0.89)	4.00×10^{-3}	0.64
5	BHMT	rs16876512	A/G	Homocysteine	0.65 (0.49, 0.87)	4.30×10^{-3}	0.69
5	BHMT	rs6875201	G/A	Homocysteine	0.66 (0.49, 0.88)	5.14×10^{-3}	0.71

Significant fetal effects based on model [1]

Chromosome	Gene	SNP	Allele	Pathway	RR (95% CI)	p-value	BFDP***
18	TYMS	rs2612101	A/G	Folate	1.35 (1.12, 1.61)	1.42×10^{-3}	0.40
18	TYMS	rs2847607	A/G	Folate	1.29 (1.07, 1.56)	6.71×10^{-3}	0.69
18	TYMS	rs2847326	A/T	Folate	1.28 (1.07, 1.53)	8.05×10^{-3}	0.71
18	TYMS	rs2847324	G/A	Folate	1.28 (1.06, 1.54)	9.57×10^{-3}	0.74
19	GPX4	rs2302109	G/A	Transsulfuration	1.28 (1.09, 1.50)	3.11×10^{-3}	0.54

Significant fetal effects based on model [1]

Chromosome	Gene	SNP	Allele	Pathway	RR (95% CI)	p-value	BFDP***
1	GSTM4	rs560018	G/A	Transsulfuration	0.77 (0.64, 0.92)	3.46×10^{-3}	0.57
1	GSTM4	rs542338	A/G	Transsulfuration	0.78 (0.66, 0.92)	3.73×10^{-3}	0.58
1	GSTM4	rs668413	A/C	Transsulfuration	0.80 (0.67, 0.94)	7.13×10^{-3}	0.69
11	CAT	rs12808450	A/G	Transsulfuration	0.76 (0.64, 0.92)	4.00×10^{-3}	0.60
6	GCLC	rs10948751	C/A	Transsulfuration	1.28 (1.07, 1.54)	8.07×10^{-3}	0.71
6	GCLC	rs7742367	G/A	Transsulfuration	1.28 (1.07, 1.55)	8.26×10^{-3}	0.72
10	MGMT	rs10829630	G/A	Transsulfuration	1.25 (1.06, 1.47)	9.04×10^{-3}	0.73
10	MGMT	rs3793906	A/C	Transsulfuration	0.81 (0.68, 0.96)	1.26×10^{-2}	0.78
10	MGMT	rs3829195	A/G	Transsulfuration	0.81 (0.68, 0.96)	1.38×10^{-2}	0.79
12	MGST1	rs7312090	A/G	Transsulfuration	0.78 (0.65, 0.94)	1.00×10^{-2}	0.75
7	KIAA0828	rs4728160	C/G	Homocysteine	1.28 (1.06, 1.55)	1.22×10^{-2}	0.77
22	TCN2	rs740233	G/A	Folate	0.81 (0.68, 0.96)	1.34×10^{-2}	0.78

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

** BFDP was calculated based on the estimated $\hat{\beta_1}$ and its standard error in model [1], which indicated the strength of the effect of maternal SNP on the CTDs.

*** BFDP was calculated based on the estimated $\hat{\beta_2}$ and its standard error in model [1], which indicated the strength of the effect of fetal SNP on the CTDs.

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Table 3

Risk of CTDs related to an interaction between maternal and fetal SNPs and periconceptional folic acid supplement use **only among Caucasians** (model [2]) (Chr: Chromosome; RG: Referent Genotype; BFDP: Bayesian false-discovery probability; RR: relative risk for risk allele; 95% CI: 95% confidence interval for the estimated RR)

Significant maternal effects based on model [2]								
					Non-users	**Supplement Users**	**Interactive GXE**	
Chr	**Gene**	**SNP**	**Allele***	**Pathway**	**RR (95% CI)†**	**RR (95% CI) †**	**p-value**	**BFDP****
4	RFC1	rs6531712	T/A	DNA Synthesis/ Repair	1.38 (1.05, 1.81)	0.76 (0.60, 0.96)	6.85×10^{-4}	0.50
4	RFC1	rs2381375	G/A	DNA Synthesis/ Repair	0.75 (0.56, 1.00)	1.24 (0.99, 1.55)	4.60×10^{-3}	0.74
4	RFC1	rs6815859	A/G	DNA Synthesis/ Repair	0.75 (0.56, 1.00)	1.24 (0.99, 1.54)	5.29×10^{-3}	0.76
4	RFC1	rs11727502	C/A	DNA Synthesis/ Repair	0.76 (0.57, 1.00)	1.23 (0.99, 1.54)	6.70×10^{-3}	0.78
17	NOS2A	rs2779248	G/A	Transsulfuration	1.40 (1.05, 1.87)	0.77 (0.60, 0.99)	1.61×10^{-3}	0.65
4	PGDS	rs1991316	C/A	Transsulfuration	1.69 (1.28, 2.24)	0.96 (0.74, 1.24)	1.89×10^{-3}	0.66
4	PGDS	rs724260	A/G	Transsulfuration	1.71 (1.30, 2.26)	0.98 (0.76, 1.26)	2.19×10^{-3}	0.67
4	PGDS	rs10033662	G/A	Transsulfuration	1.64 (1.25, 2.16)	0.95 (0.74, 1.22)	2.37×10^{-3}	0.68
4	PGDS	rs2289186	A/C	Transsulfuration	1.62 (1.23, 2.14)	0.93 (0.72, 1.20)	2.30×10^{-3}	0.68
4	PGDS	rs2059605	A/G	Transsulfuration	1.58 (1.20, 2.08)	0.94 (0.73, 1.20)	3.76×10^{-3}	0.73
4	PGDS	rs11097411	A/G	Transsulfuration	1.64 (1.25, 2.15)	1.00 (0.78, 1.27)	4.75×10^{-3}	0.74
4	PGDS	rs4282187	A/G	Transsulfuration	1.67 (1.26, 2.19)	1.00 (0.78, 1.28)	4.53×10^{-3}	0.75
4	PGDS	rs11727030	A/G	Transsulfuration	1.60 (1.22, 2.11)	0.97 (0.75, 1.26)	6.20×10^{-3}	0.78
10	MGMT	rs11511217	A/G	Transsulfuration	0.58 (0.39, 0.85)	1.23 (0.94, 1.59)	1.30×10^{-3}	0.72
10	MGMT	rs11016908	G/A	Transsulfuration	0.58 (0.40, 0.85)	1.15 (0.89, 1.50)	2.99×10^{-3}	0.78
10	MGMT	rs4751118	A/G	Transsulfuration	1.41 (1.07, 1.85)	0.89 (0.70, 1.12)	8.26×10^{-3}	0.79
5	BHMT2	rs542721	G/C	Homocysteine	1.38 (1.05, 1.82)	0.84 (0.66, 1.07)	6.19×10^{-3}	0.78
5	BHMT2	rs2909856	G/A	Homocysteine	1.35 (1.02, 1.79)	0.83 (0.64, 1.06)	7.27×10^{-3}	0.79
5	BHMT	rs490268	G/A	Homocysteine	1.39 (1.06, 1.82)	0.86 (0.68, 1.10)	7.62×10^{-3}	0.79

Significant fetal effects based on model [2]

					Non-users	Supplement Users	Interactive GXE	
Chr	**Gene**	**SNP**	**Allele**	**Pathway**	**RR (95% CI)** †	**RR (95% CI)** †	**p-value**	**BFDP*****
4	RFC1	rs2381375	G/A	DNA Synthesis/ Repair	1.44 (1.08, 1.91)	0.80 (0.63, 1.01)	7.90×10^{-4}	0.53
4	RFC1	rs6815859	A/G	DNA Synthesis/ Repair	1.44 (1.09, 1.91)	0.82 (0.64, 1.03)	1.14×10^{-3}	0.57
4	RFC1	rs16995255	C/G	DNA Synthesis/ Repair	1.39 (1.04, 1.86)	0.74 (0.57, 0.96)	9.53×10^{-4}	0.60
4	RFC1	rs11727502	C/A	DNA Synthesis/ Repair	1.40 (1.06, 1.86)	0.80 (0.63, 1.02)	1.53×10^{-3}	0.61
4	RFC1	rs11096991	G/A	DNA Synthesis/ Repair	1.39 (1.04, 1.86)	0.78 (0.60, 1.01)	2.30×10^{-3}	0.69
4	RFC1	rs6531712	T/A	DNA Synthesis/ Repair	0.75 (0.56, 1.02)	1.24 (0.98, 1.56)	6.60×10^{-3}	0.78
15	MTHFS	rs12438477	A/C	Folate	1.49 (1.12, 1.97)	0.83 (0.65, 1.07)	1.38×10^{-3}	0.61
21	CBS	rs1788490	G/A	Transsulfuration	1.53 (1.12, 2.10)	0.89 (0.68, 1.15)	5.63×10^{-3}	0.79
21	CBS	rs1672126	A/G	Transsulfuration	1.60 (1.17, 2.20)	0.93 (0.71, 1.21)	5.69×10^{-3}	0.79

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

** BFDP was calculated based on the estimated $\hat{\beta_3}$ and its standard error in model [2], which indicated the strength of the interactive effect of maternal SNP and folic acid supplementation on the CTDs.

*** BFDP was calculated based on the estimated $\hat{\beta_4}$ and its standard error in model [2], which indicated the strength of interactive effect of fetal SNP and folic acid supplementation on the CTDs.

† 95% CI for reported SNPs do not include one (1.00) in interval; appearance in table due to rounding of lower and upper interval value.