Paternal genetic variants and risk of obstructive heart defects: A parent-of-origin approach

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Abstract

Previous research on risk factors for obstructive heart defects (OHDs) focused on maternal and infant genetic variants, prenatal environmental exposures, and their potential interaction effects. Less is known about the role of paternal genetic variants or environmental exposures and risk of OHDs. We examined parent-of-origin effects in transmission of alleles in the folate, homocysteine, or transsulfuration pathway genes on OHD occurrence in offspring. We used data on 569 families of liveborn infants with OHDs born between October 1997 and August 2008 from the National Birth Defects Prevention Study to conduct a family-based case-only study. Maternal, paternal, and infant DNA were genotyped using an Illumina Golden Gate custom single nucleotide polymorphism (SNP) panel. Relative risks (RR), 95% confidence interval (CI), and likelihood ratio tests from log-linear models were used to estimate the parent-of-origin effect of 877 SNPs in 60 candidate genes in the folate, homocysteine, and transsulfuration pathways on the risk of OHDs. Bonferroni correction was applied for multiple testing. We identified 3 SNPs in the transsulfuration pathway and 1
SNP in the folate pathway that were statistically significant after Bonferroni correction. Among infants who inherited paternally-derived copies of the G allele for rs6812588 in the RFC1 gene, the G allele for rs1762430 in the MGMT gene, and the A allele for rs9296695 and rs4712023 in the GSTA3 gene, RRs for OHD were 0.11 (95% CI: 0.04, 0.29, P = 9.16x10^{-7}), 0.30 (95% CI: 0.17, 0.53, P = 9.80x10^{-6}), 0.34 (95% CI: 0.20, 0.57, P = 2.28x10^{-5}), and 0.34 (95% CI: 0.20, 0.58, P = 3.77x10^{-5}), respectively, compared to infants who inherited maternally-derived copies of the same alleles. We observed statistically significant decreased risk of OHDs among infants who inherited paternal gene variants involved in folate and transsulfuration pathways.

Author summary
Obstructive heart defects are birth defects that cause obstruction to the blood flow of the developing heart. Common OHDs include coarctation of the aorta, aortic stenosis and pulmonary stenosis. While there is a fair amount of literature indicating an association between maternal genetic variants and OHDs, less is known about the role of paternal genetic variants in the etiology of OHDs. We used a genotype clustering algorithm, SNP-MClust, that was developed in-house at the Arkansas Center for Birth Defects Research and Prevention to study the role of paternal genetic variants in the folate, homocysteine and transsulfuration pathways. Maternal, paternal, and infant DNA specimens were collected from participants of the National Birth Defects Prevention Study, a large population-based case-control study in the United States, and were genotyped using an Illumina Golden Gate custom single nucleotide polymorphism (SNP) panel. We identified 4 SNPs in the folate and transsulfuration pathways, rs6812588, rs1762430, rs9296695, and rs4712023, that were associated with a statistically significant decreased risk of OHDs for infants who inherited a paternally-derived copy of the variant allele compared to infants who inherited a maternal copy of the variant allele. In conclusion, we observed a significantly decreased risk and less epigenetic influence of paternal genetic variants on OHDs compared to maternally-derived variants.

Introduction
Congenital heart defects (CHDs) are the most commonly occurring group of birth defects and affect about one percent of live births in the United States annually [1, 2]. They are also the most common cause of infant mortality and lifelong morbidity [3, 4]. It has also been reported that overall survival among patients with complex heart defects is decreased with increasing age compared to healthy age-matched counterparts [3]. Although some CHDs occur in association with certain genetic syndromes (e.g., trisomy 21, 22q11 deletion, Alagille syndrome, Noonan syndrome) and teratogenic exposures (e.g., anticonvulsants, maternal pregestational diabetes), approximately 80% are of unknown etiology [5–7]. Known maternal risk factors associated with CHDs include diabetes mellitus [8], obesity [9], prenatal cigarette smoking [10–15], low blood folate concentrations [16], hyperhomocysteinemia [17], medication use [18] and genetic polymorphisms in metabolic pathways, including the folate, homocysteine, and glutathione/transsulfuration pathways [8, 9, 13–33].

In contrast, the role of paternal environmental and genetic factors on the risk of CHDs is less defined with limited literature [34]. Some studies indicate associations between young or
advanced paternal age and increased risk of atrial septal defects, ventricular septal defects, right ventricular outflow tract defects including pulmonary valve atresia, patent ductus arteriosus, and CHDs overall [35, 36]; however, other studies report no association between paternal age and risk of CHDs. Other paternal exposures associated with increased risk of CHDs include cigarette smoking, alcohol consumption, and occupational exposure to endocrine disruptors [11, 35–43].

Although the specific biological mechanisms are unclear, it is hypothesized that these exposures (paternal age, smoking, etc.) may share a similar physiologic mechanism: germline mutations and epigenetic alterations to sperm haploid DNA [10, 44–49]. Given that environmental exposures may induce changes in paternal DNA that can result in CHDs, we postulated that certain paternal genetic polymorphisms may also increase CHD risk. Numerous studies confirm that genetic polymorphisms in maternal and infant genes are directly or indirectly associated with risk of CHDs, particularly genes involved in folate, homocysteine, and transsulfuration pathways [19, 20, 50].

To our knowledge, to date (as of March 2020), only one study has assessed the influence of paternal genetic variants in folate, homocysteine, or transsulfuration pathways and CHD risk in offspring [51]. This study examined conotruncal heart defects and found less epigenetic influence on conotruncal heart defects by paternal genetic variants compared to maternal genes. No studies have assessed other groups of CHDs, such as right-sided and left-sided obstructive heart defects (OHDs). Among subtypes of OHDs, pulmonary stenosis and coarctation of the aorta account for 8% and 5% of all CHDs respectively [2], making OHDs an important group of CHDs to investigate. Moreover, studies show that women who delivered infants with OHDs were more likely to have alterations in metabolites in pathways involving folate, homocysteine, and glutathione [25, 52, 53]. Additionally, a recent study demonstrated that 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, although the focus was on genetic variants in maternal genes [54]. Whether a similar pattern of gene-environment interaction effects for paternal exposures, including alterations in pathways for paternal genes, are also responsible for causing OHDs is yet to be explored. In this study, we investigated parent-of-origin effects for genetic variants in folate, homocysteine and transsulfuration pathway genes and the occurrence of OHDs in offspring.

Results

Table 1 displays the distributions of maternal and paternal characteristics of infants born with OHDs for whom genotyping was performed. The mean maternal and paternal age at delivery were 28.3 (6.0) and 32.0 (6.7), respectively. As for race/ethnicity, 73.2% and 67.9% of mothers and fathers, respectively, were non-Hispanic white. Approximately 30% and 26% of mothers and fathers, respectively, had some college education. Among mothers, 26.1% were overweight, and 23.0% were obese. About 58% of the mothers took folic acid supplements during the periconceptional period (a month before conception through the end of the first trimester); 20.4% drank some quantity of alcohol during the entire pregnancy (date of conception through date of birth) and 13.5% smoked cigarettes during the entire pregnancy period. Among fathers, 94% were employed at the time of interview, while 18% had a birth defect or health problem at birth.

The final analysis included 877 SNPs within 60 genes. Based on Bonferroni adjustment, the statistical significance was set at \( \leq 5.70 \times 10^{-5} \). We observed a statistically significant decreased risk of OHDs for paternally-derived effects for four SNPs in three genes (Table 2): one SNP
Table 1. Summary of maternal and paternal characteristics from chi-squared analyses for mothers of infants with obstructive heart defects, The National Birth Defects Prevention Study, USA, October 1997 – August 2008 births (n = 569 case families)\(^*\).

| Characteristics                          | Maternal n (%) | Paternal n (%) |
|------------------------------------------|----------------|----------------|
| **Age at Delivery**                      |                |                |
| Mean (SD)                                | 28.3 (6.0)     | 32.0 (6.7)     |
| <35 years                                | 480 (84.4%)    | 285 (71.6%)    |
| ≥35 years                                | 89 (15.6%)     | 113 (28.4%)    |
| Missing                                  | 0              | 171            |
| **Race/Ethnicity**                       |                |                |
| Non-Hispanic white                       | 413 (73.2)     | 383 (67.9)     |
| Non-Hispanic black                       | 61 (10.8)      | 69 (12.2)      |
| Hispanic                                 | 66 (11.7)      | 77 (13.7)      |
| Other                                    | 24 (4.3)       | 25 (4.4)       |
| Missing                                  | 0              | 10             |
| **Education**                            |                |                |
| < 12 years                               | 73 (12.9)      | 81 (14.4)      |
| High school diploma or equivalent        | 139 (24.6)     | 155 (27.5)     |
| < 4 years of college education           | 170 (30.1)     | 144 (25.5)     |
| At least 4 years of college or bachelor’s degree | 182 (32.2)     | 171 (30.3)     |
| Missing                                  | 0              | 13             |
| **Mean Household Income**                |                |                |
| < $10,000                                | 70 (12.4)      | N/A            |
| $10,000 –$29,999                         | 163 (28.9)     | N/A            |
| $30,000 –$49,999                         | 129 (22.9)     | N/A            |
| ≥ $50,000                                | 180 (31.9)     | N/A            |
| Missing                                  | 22             | N/A            |
| **Body Mass Index**                      |                |                |
| Underweight (< 18.5 kg/m\(^2\))         | 14 (2.5)       | N/A            |
| Normal weight (18.5 to < 25.0 kg/m\(^2\)) | 257 (45.6)     | N/A            |
| Overweight (25.0 to < 30.0 kg/m\(^2\))  | 147 (26.1)     | N/A            |
| Obese (≥ 30.0 kg/m\(^2\))               | 130 (23.0)     | N/A            |
| Missing                                  | 16             | N/A            |
| **Periconceptional Folic Acid Supplementation** |          |                |
| No                                       | 236 (41.8)     | N/A            |
| Yes                                      | 328 (58.2)     | N/A            |
| **Alcohol Intake**\(^b\)                 |                |                |
| No                                       | 445 (78.9)     | N/A            |
| Yes                                      | 115 (20.4)     | N/A            |
| Missing                                  | 4              | N/A            |
| **Cigarette Smoking**\(^b\)              |                |                |
| No                                       | 488 (86.5)     | N/A            |
| Yes                                      | 76 (13.5)      | N/A            |
| **Cigarette Smoking in Home During First Trimester** |        |                |
| No                                       | 471 (83.5)     | N/A            |
| Yes                                      | 93 (16.5)      | N/A            |
| **Currently Employed (at Time of Interview)** |          |                |
| No                                       | N/A            | 28 (5.0)       |
| Yes                                      | N/A            | 532 (94.3)     |

(Continued)
each in replication factor C subunit1 (RFC1) and O-6-methylguanine-DNA methyltransferase (MGMT); and two SNPs in glutathione S-transferase alpha 3 (GSTA3). These genes are involved in DNA replication and repair, catalyzing transfer of methyl groups, and cellular defense.

Among infants who inherited a paternally-derived copy of the G allele for rs6812588 in the RFC1 gene, the relative risk (RR) of an OHD was 0.11 (95% confidence interval [CI]: 0.04, 0.29, Table 1. (Continued)

| Characteristics | Maternal n (%) | Paternal n (%) |
|-----------------|----------------|---------------|
| Missing information | N/A | 4 |
| Health Problem at Birth or a Birth Defect Diagnosed in Childhood? | No | N/A | 452 (80.1) |
| Yes | N/A | 100 (17.7) |
| Missing information | N/A | 12 |
| Mother Blood Relative of Baby's Father? | No | N/A | 559 (99.1) |
| Yes | N/A | 3 (0.9) |
| Missing information | N/A | 2 |

a. Data are from families for whom DNA samples were available
b. During pregnancy = date of conception to date of birth
N/A = Not Available

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Table 2. Risk ratios and 95% confidence intervals (CIs) with p-values for paternally-derived effects for the top 20 single nucleotide polymorphisms (SNPs) identified from hybrid analyses compared to maternally-derived effects for common variants in genes involved in folate, homocysteine and transsulfuration pathways and risk of obstructive heart defects, The National Birth Defects Prevention Study, USA, October 1997 – August 2008 births (n = 569 case families).

| SNP | Referent/Risk allele | Chr | Gene | Pathway | Relative Risk (95% CI) | P-value |
|-----|----------------------|-----|------|---------|------------------------|---------|
| rs6812388 | G/A | 4 | RFC1 | Folate | 0.11 (0.04, 0.29) | 9.16 × 10^{-7} |
| rs1762430 | G/A | 10 | MGMT | Transsulfuration | 0.30 (0.17, 0.53) | 9.80 × 10^{-6} |
| rs9296995 | A/G | 6 | GSTA3 | Transsulfuration | 0.34 (0.20, 0.57) | 2.28 × 10^{-3} |
| rs4712023 | A/G | 6 | GSTA3 | Transsulfuration | 0.34 (0.20, 0.58) | 3.77 × 10^{-3} |
| rs9299871 | A/G | 10 | MGMT | Transsulfuration | 0.22 (0.10, 0.49) | 8.81 × 10^{-5} |
| rs7541359 | A/C | 1 | MTR | Homocysteine | 0.27 (0.14, 0.53) | 8.81 × 10^{-5} |
| rs7069462 | A/G | 10 | MGMT | Transsulfuration | 0.20 (0.09, 0.47) | 1.02 × 10^{-4} |
| rs2273027 | A/G | 17 | SHMT1 | Folate | 2.14 (1.46, 3.13) | 1.27 × 10^{-4} |
| rs12202200 | A/G | 6 | GSTA3 | Transsulfuration | 0.36 (0.20, 0.64) | 2.79 × 10^{-4} |
| rs6577 | C/A | 6 | GSTA2 | Transsulfuration | 0.31 (0.16, 0.60) | 2.90 × 10^{-4} |
| rs7818511 | A/G | 8 | GSR | Transsulfuration | 0.38 (0.22, 0.65) | 2.93 × 10^{-4} |
| rs600473 | C/A | 5 | BHMT | Homocysteine | 1.87 (1.34, 2.62) | 3.19 × 10^{-4} |
| rs9382157 | A/G | 6 | GSTA3 | Transsulfuration | 0.37 (0.21, 0.65) | 4.18 × 10^{-4} |
| rs1547177 | A/C | 10 | MGMT | Transsulfuration | 0.35 (0.19, 0.65) | 5.67 × 10^{-4} |
| rs2152515 | C/G | 10 | MGMT | Transsulfuration | 0.34 (0.18, 0.66) | 7.28 × 10^{-4} |
| rs2062228 | A/G | 4 | RFC1 | Folate | 0.24 (0.10, 0.56) | 1.11 × 10^{-3} |
| rs2424905 | A/G | 20 | DNMT3B | Homocysteine | 0.55 (0.38, 0.80) | 1.31 × 10^{-3} |
| rs2363641 | G/A | 14 | GSTZ1 | Transsulfuration | 1.90 (1.28, 2.80) | 1.46 × 10^{-3} |
| rs4796017 | G/A | 17 | NOS2A | Transsulfuration | 1.75 (1.23, 2.50) | 2.53 × 10^{-3} |
| rs7081756 | C/A | 10 | MAT1A | Homocysteine | 0.54 (0.36, 0.82) | 2.54 × 10^{-3} |

a. "Bolded p-values are for significant SNPs with p-value ≤ 5.70 × 10^{-5}.”

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$p = 9.16 \times 10^{-7}$) compared to infants who inherited a maternal G allele. In the $MGMT$ gene, for infants who inherited a paternally-derived copy of the G allele for rs1762430, the RR was 0.30 (95% CI: 0.17, 0.53, $p = 9.80 \times 10^{-6}$) compared to infants who inherited the maternal G allele. Among infants who inherited a paternally-derived copy of the A allele for rs9296695 or rs4712023 in $GSTA3$, the RRs were 0.34 (95% CI: 0.20, 0.57, $p = 2.28 \times 10^{-5}$) and 0.34 (95% CI: 0.20, 0.58, $p = 3.77 \times 10^{-5}$), respectively, compared to infants who inherited the maternally-derived A allele.

Elevated, non-significant risks were observed for infants who inherited paternally-derived copies of the (i) A allele for rs2273027 in the $SHMT1$ gene in the folate pathway (RR 2.14, $p = 1.27 \times 10^{-4}$); (ii) C allele for rs600473 in the $BHMT$ gene in the homocysteine pathway (RR 1.87, $p = 3.19 \times 10^{-4}$); and (iii) G allele for rs2362641 and rs4796017 in the $GSTZ1$ and $NOS2A$ genes, respectively in the transsulfuration pathway (RR 1.90, $p = 1.46 \times 10^{-3}$ and RR 1.75, $p = 2.53 \times 10^{-3}$, respectively), compared to infants who inherited maternally-derived copies.

Fig 1 shows the distribution of RRs, identified from hybrid analyses, for obstructive heart defects in infants with paternally-derived compared to maternally-derived risk variants in genes involved in folate, homocysteine, and transsulfuration pathways, The National Birth Defects Prevention Study, USA, October 1997–August 2008 births. https://doi.org/10.1371/journal.pgen.1009413.g001

In summary, we observed a statistically significant decreased risk of OHDs among infants who inherited a paternally-derived copy of one folate and three transsulfuration pathway genes compared to maternally-derived variants.

**Discussion**

In this study, we determined the parent-of-origin effects for genetic variants in folate, homocysteine, and transsulfuration pathway genes and occurrence of OHDs in offspring. The majority of published studies have investigated the effects of maternal genetic variants and environmental factors on the occurrence of CHDs in general [12, 55–57]; however, much less
is known about the role of paternally-related genetic variants in outcomes of pregnancy, including their roles in CHD etiology [51]. We observed no statistically significant increased risk of OHDs for infants who inherited a paternally-derived copy of variant alleles in genes involved in folate, homocysteine, or transsulfuration pathways compared to infants who inherited a maternal copy of the variant allele. However, we did identify 4 SNPs (rs6812588, rs1762430, rs9296695, and rs4712023) that were associated with a statistically significant decreased risk of OHDs for infants who inherited a paternally-derived copy compared to infants who inherited a maternal copy of the variant allele (Fig 2).

One possible explanation for our results, as suggested by earlier studies, is the parent-of-origin effect in the etiology of groups of CHDs. Parent-of-origin effects arise when the phenotypic impact of an allele depends on whether it is inherited from the mother or father [58]. Although several mechanisms are proposed to cause parent-of-origin effects, genomic imprinting and trans-generational effects are the two primary mechanisms that have been widely described [58–60]. Parent-of-origin effects are often associated with the imprinting principle in which an allele of a specific gene is silenced through epigenetic mechanism when inherited from one parent and expressed when inherited from the other parent [58, 60, 61]. On the other hand, trans-generational genomic effects occur due to transmitting epigenetic information from one generation to subsequent generations in the absence of direct exposure [58, 62]. In recent years, a growing body of genome-wide association studies have successfully identified parent-of-origin effects due to genomic imprinting and trans-generational inheritance in several phenotypes, including cleft lip with/without palate [63, 64], non-syndromic orofacial clefts [65], autism spectrum disorder [59, 66], attention-deficit/hyperactivity disorder [67], body mass index [68, 69], testicular germ cell tumors [70], and schizophrenia [71]. The gene expression determined by parent-of-origin effect may result in disease. Two common examples include Prader–Willi syndrome [72] and Angelman syndrome [72, 73] in which the maternal or paternal locus in the 15q11-13 region, respectively, is either silenced or removed [60]. Only a small
number of imprinted loci encompassing a small portion of the human genome have been identified in the literature.

MGMT is a DNA repair enzyme that is thought to be involved in the prevention of DNA damage and oxidative stress, and the expression of MGMT is associated with antioxidant mechanisms [74]. Potential involvement of MGMT in the development of CHDs was previously suggested by our research group [75]. Due to the tendency of imprinted genes to cluster together, we examined regions within 500 Kb of imprinted genes to identify parent-of-origin effects [49]. Rs1762430 in MGMT has significant paternal versus maternal effects and is close to (<370bp) a known imprinted gene, GLRX3 [76]. GLRX3, a protein in the transsulfuration pathway, is thought to be involved in cell growth, organ development, and other normal processes of growth and development [77]. Therefore, findings from our study suggest that risk of OHD associated with rs1762430 in MGMT may be linked to genomic imprinting of the nearby gene GLRX3. However, future studies of this region using gene expression profiles of parental trios would help confirm the role of imprinting in OHD risk.

Genetic variation may change the response of an individual to the exposure of environmental factors. When genetic susceptibility is high, even the minimum contribution from environmental risk factors may trigger disease development. Notable effects of environmental factors have been observed in infants with RFC1 polymorphisms resulting in development of CHDs [78, 79]. RFC-1 is a protein involved in the folate pathway that is responsible for the transport of folate molecules from the circulation to peripheral cells and regulation of the delivery of 5-methyltetrahydrofolate from the endocytotic vesicle into the cytoplasm [80]. Several polymorphisms in the RFC1 gene are well studied in the literature. A previous study reported that compared to infants with A80/A80 genotype, infants with G80/G80 genotype had a non-significant increased risk of conotruncal heart defects among mothers using and not using folic acid, indicating different effects of gene-environment interaction [79]. To date, no studies have identified potential associations of rs6812588 in RFC1 and development of CHDs.

GSTA3 acts by mitigating oxidative stress in the transsulfuration pathway, which is subsequently associated with increased risk of conotruncal heart defects [53, 81]. In our study, two SNPs (rs9296695 and rs4712023) in GSTA3 were statistically significant when comparing paternal versus maternal inheritance and OHD risk. Being located in the downstream region of the gene, these SNPs might have a regulatory role on the GSTA3 gene. A study in the past has shown several maternal and fetal genotypes of SNPs in the glutathione transferase including GSTA3 to increase the impact of risk factors such as maternal obesity and tobacco use on the risk of CTDs [53]. Our findings suggest that GSTA3 might play a similar role in OHD risk as that observed for conotruncal heart defects, although further studies are warranted.

While imprinted genes tend to cluster together, our study only identified imprinted genes MGMT and GLRX3 together, and not RFC1, thus indicating a possible residual effect of RFC1 in the occurrence of OHDs. With a limited literature on comparison of maternal and paternal genetic variants on heart defects as well as potential effects of RFC1 [78, 79], our study findings warrant continued caution on the genomic imprinting effect of RFC1 on OHDs.

Our search of the published English language literature in PubMed, to date, produced only one study [51] that conducted a parent-of-origin analysis for risk of CHDs as the primary aim. In that study, Nembhard et al observed that children who inherited a paternally-derived copy of the A allele for rs7818511 in the GSR gene, or the A allele for rs17085159 or the T allele for rs12109442 in the GLRX gene, were found to be at decreased risk of developing conotruncal heart defects compared to children with the maternal copy of the same allele [51]. A study by Long et al. [82] conducted an ad hoc parent-of-origin analysis to evaluate the association between maternal SNPs in folate regulated genes and the risk of left-sided heart defects and conotruncal heart defects. In that study, the primary analysis indicated that MTR A2756G was
associated with the studied cardiac defects. Although results from parent-of-origin likelihood-ratio-test as ad hoc analysis was not statistically significant, the log-linear likelihood-ratio-test for conotruncal heart defect case triads was statistically significant. Therefore, findings from these studies further support the possibility of parent-of-origin effects in the etiology of CHDs.

Our study has several potential limitations. First, DNA was extracted from self-collected buccal cell samples; therefore, there may be an unknown level of inconsistency in the quality of the DNA samples. However, to ensure high-quality genotypes in this study, stringent quality control measures were applied by excluding SNPs with poor clustering behavior, no-call rates >10%, Mendelian error rates >5%, minor allele frequency (MAF) <5%, or significant deviation from Hardy-Weinberg equilibrium in at least one racial group. Second, we could not validate the role of imprinting on OHD due to lack of gene expression data in the case-parental trios. Third, the cases were livebirths, so the observed decreased risks may only be representative of those cases who survived. This limits the observation of a prenatal survivor effect in this study. Fourth, heterogeneity of OHDs and the associations observed in this study might be affected by the broad outcome classification. However, OHDs for both right ventricular and left ventricular groups were combined to have a sufficient sample for meaningful interpretation. This has also been done in past studies in literature to have adequate genotyped sample for meaningful interpretations [54, 75, 83]. Fifth, our study did not include information on paternal smoking, alcohol consumption and occupation because of limited data availability and our major focus on genotyped information. A recent systematic review suggested advanced paternal age, smoking, alcohol consumption and specific occupations were associated with an increased risk of CHDs [34]. While we were able to assess paternal employment status, further studies assessing specific paternal risk factors, including smoking, alcohol consumption and occupations, in association with genetic variants would help determine risk specific to OHDs. Despite these overall limitations, our study possesses several strengths. First, in this large population-based study, our study population consisted of multiple racial/ethnic groups. Second, all the OHD cases were confirmed by pediatric cardiologists and a standard procedure for the OHD classification was used across participating study centers. Finally, in addition to exploring effects of inherited genotypes, the usage of log-linear modelling allowed us to also examine prenatal effects of maternal genotype and parent-of-origin (imprinting) effects. Furthermore log-linear models bear an advantage over other models as they can be extended to any number of alleles or loci, or any number of risk factors.

In conclusion, we observed that paternal genetic variants in certain folate and transsulfuration pathway genes contributed to a lower risk of OHDs occurrence compared to maternal variants. Future studies with larger sample size and multi-omics data are indicated to validate our findings to gain additional confidence.

Methods and materials

Ethics statement

The Institutional Review Boards (IRB) at each of the following collaborative centers of the National Birth Defects Prevention Study (NBDPS) provided approval: University of Arkansas for Medical Sciences, California Birth Defects Monitoring Program, University of Iowa, Massachusetts Department of Public Health, New Jersey Department of Health, State of New York Department of Health, University of North Carolina at Chapel Hill, Texas Department of State Health Services, and University of Utah. All study participants (including parents of minors) provided written or verbal informed consent. For the telephone interview, each participant provided a verbal consent (permission) to use their answers in the study to understand the causes of birth defects. For the DNA samples, participants also provided a signed written
consent through a form that was provided with the collection kit through mail, that also explained the study risks and benefits. The Centers for Disease Control and Prevention Institutional Review Board (IRB), along with the IRBs for each participating center, have approved the NBDPS [84, 85].

National birth defects prevention study

The NBDPS is one of the largest population-based case-control studies of birth defects conducted in the United States. and provides a unique opportunity to examine genetic, environmental, and behavior factors associated with the occurrence of major non-syndromic birth defects. Methods of the NBDPS have been previously described [84, 85], but in brief, families of case and control infants were identified from population-based birth defects surveillance systems in 10 states: Arkansas, California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah [84, 85]. The study enrolled approximately 44,000 women who were non-Hispanic (NH) white, NH-black, Hispanic, and of other races with estimated dates of delivery from October 1, 1997 through December 31, 2011. The case-only study we conducted included a subset of women with estimated dates of delivery between October 1997 and August 2008, for whom DNA specimens were available from themselves, their infants, and the infant’s father [84, 85].

Ascertainment of obstructive heart defects

In the NBDPS, a range of non-syndromic CHD case infants were identified following diagnostic procedures, including echocardiogram, surgical reports, cardiac catheterization, or autopsy. Each diagnostic procedure result was further reviewed by a pediatric cardiologist to ensure consistent diagnoses across the study. A classification system was developed specifically for the NBDPS and included cardiac phenotype, cardiac complexity, and extra-cardiac anomalies. Our study included left and right-sided OHDs. These were grouped together since we had limited sample size for individual phenotypes due to the overall smaller sample of genotyped CHDs. The left-sided OHDs were comprised of hypoplastic left heart syndrome, interrupted aortic arch A, coarctation of the aorta, and aortic stenosis; the right-sided OHDs were comprised of pulmonary valve stenosis, tricuspid atresia, and Ebstein anomaly.[86]

Maternal interview

After informed consent, women completed a 1-hour computer-assisted telephone interview in either English or Spanish from 6 weeks to 2 years after their estimated date of delivery. Overall interview participation rate was 67% among case women and 65% among control women. Mean number of weeks of gestation was 37.1 for cases and 38.7 for controls. Interviewers obtained information on maternal demographic characteristics and other risk factors (e.g., maternal health, pregnancy, diet/substance use, home/work, family demographics, and medication use) both before and during pregnancy. Information on the father of the infant was also collected during the interview.

DNA sample collection

Upon completing the telephone interview, families were mailed buccal cell collection kits. These kits were used to collect specimens for maternal, infant, and paternal DNA. Details on methods for DNA extraction, purification from buccal cell swabs and storage were described previously [84, 87]. In this study, a customized panel, including 1536 SNPs from 62 genes that were part of one-carbon metabolism (i.e., folate, homocysteine and glutathione/
transsulfuration pathways), was used for genotyping on Illumina’s GoldenGate platform [53, 88]. 10–15 ng of genomic DNA was used for whole genome amplification (WGA) using GenomePl WGA kit [89]. The WGA product was quantified using TaqMan RNase Reagent Kit [89]. Genotype clustering and calling was conducted using a previously developed and tested genotype calling algorithm, SNPMClust, that was developed in-house at the Arkansas Center for Birth Defects Research and Prevention [90]. To ensure high-quality genotypes, we applied stringent quality control measures and excluded SNPs with poor clustering behavior, no-call rates >10%, greater Mendelian error rates >5%, MAF <5%, or significant deviation from Hardy-Weinberg equilibrium in at least one racial group. Given the apparent lack of genome-wide data, racial group was considered as a proxy for genetic ancestry. For the current study specific to OHDs, the final dataset included 1203 individuals from 569 case families, each with 877 SNPs. Of the 569 case families, 220 (38.7%) were full trios.

Statistical methods

Summary statistics were expressed as means (standard deviation) for continuous variables, and counts (percentage) for categorical variables. To investigate the parent-of-origin effect in this case-only study, a log-linear model was fitted for the counts of each SNP as a function of mating types, maternal genetic effect, and imprinting parameter [30]. Based on the log-linear model for counts and assuming a Poisson distribution, the imprinting effect was estimated as the relative risk of an OHD in a child who inherited a paternally-derived copy of the minor allele compared to a child who inherits a copy of the minor allele from the mother. Bonferroni correction was used to adjust for multiple testing. Statistical significance level was set at \( p < 5.70 \times 10^{-5} \) based on log likelihood statistics. Data were analyzed using statistical software SAS 9.4 (SAS Institute Inc., Cary, NC) for computing descriptive statistics and PREMIM/EMIM for fitting imprinting models. EMIM uses the genotype data to perform statistical analysis, while PREMIM allows the extraction of genotype data from pedigree data files. This technique allows the estimation of complex genetic effects such as parent-of-origin (imprinting) effects [91].

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

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