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A genome-wide association study of obstructive heart defects among participants in the National Birth Defects Prevention Study

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Abstract

Obstructive heart defects (OHDs) share common structural lesions in arteries and cardiac valves, accounting for ~25% of all congenital heart defects. OHDs are highly heritable, resulting from interplay among maternal exposures, genetic susceptibilities, and epigenetic phenomena. A genome-wide association study was conducted in National Birth Defects Prevention Study participants ($N_{discovery}$ = 3978; $N_{replication}$ = 2507), investigating the genetic architecture of OHDs using transmission/disequilibrium tests (TDT) in complete case-parental trios ($N_{discovery\_TDT}$ = 440; $N_{replication\_TDT}$ = 275) and case–control analyses separately in infants ($N_{discovery\_CCI}$ = 1635; $N_{replication\_CCI}$ = 990) and mothers (case status defined by infant; $N_{discovery\_CCM}$ = 1703; $N_{replication\_CCM}$ = 1078). In the TDT analysis, the *SLC44A2* single nucleotide polymorphism (SNP) rs2360743 was significantly

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associated with OHD ($p_{discovery} = 4.08 \times 10^{-9}$; $p_{replication} = 2.44 \times 10^{-4}$). A *CAPN11* SNP (rs55877192) was suggestively associated with OHD ($p_{discovery} = 1.61 \times 10^{-7}$; $p_{replication} = 0.0016$). Two other SNPs were suggestively associated ($p < 1 \times 10^{-6}$) with OHD in only the discovery sample. In the case–control analyses, no SNPs were genome-wide significant, and, even with relaxed thresholds ($\times$ $_{discovery} < 1 \times 10^{-5}$ and $p_{replication} < 0.05$), only one SNP (rs188255766) in the infant analysis was associated with OHDs ($p_{discovery} = 1.42 \times 10^{-6}$; $p_{replication} = 0.04$). Additional SNPs with $p_{discovery} < 1 \times 10^{-5}$ were in loci supporting previous findings but did not replicate. Overall, there was modest evidence of an association between rs2360743 and rs55877192 and OHD and some evidence validating previously published findings.

KEYWORDS
congenital heart defects, CAPN11, GWAS, National Birth Defects Prevention Study, obstructive heart defects, SLC44A2

1 | INTRODUCTION

Congenital heart defects (CHDs) are the most common and among the most serious birth defects, with a worldwide prevalence ranging from 8 to 12 per 1000 live births (Liu et al., 2019). Obstructive lesions of arteries and cardiac valves account for approximately 25% of all CHDs and include pulmonary valve stenosis and atresia, tricuspid atresia, aortic valve stenosis, coarctation of the aorta, interrupted aortic arch, Ebstein anomaly, and hypoplastic left heart syndrome (Fahed et al., 2013). These obstructive heart defects (OHDs) are often major features of the most complex forms of CHDs. Remarkable improvements have been made in prenatal diagnosis and medical and surgical management of OHDs, and survival to adulthood is now expected even for the most complex and severe defects, such as hypoplastic left heart syndrome (Triedman & Newburger, 2016). As such, infant and childhood mortality from CHDs is decreasing (Boneva et al., 2001; Centers for Disease Control and Prevention (CDC), 1998; Gilboa, Salemi, et al., 2010) and the prevalence of CHDs among adults is increasing (Gilboa, Salemi, et al., 2010; Marelli et al., 2007; Oyen et al., 2009). Long-term care of patients with CHDs can impose significant emotional and socioeconomic burdens on patients, families, caregivers, and society (Centers for Disease Control and Prevention (CDC), 2007; Hoffman, 2013; Tilford et al., 2001).

It is well accepted that the occurrence of OHDs in humans results from a sophisticated interplay among maternal exposures, environmental, genetic, and epigenetic phenomena (Botto & Correa, 2003; Vecoli et al., 2014). Multiple streams of evidence suggest that genetic factors play a significant role in the molecular embryogenesis of the heart (Pierpont et al., 2018). Although de novo mutations and copy number variation contribute to the burden of OHDs,(Hitz et al., 2012; Homsy et al., 2015; Payne et al., 2012; Zaidi et al., 2013) familial aggregation and a high heritability of OHDs has been recognized for decades (Boughman et al., 1987; Hinton et al., 2007; McBride et al., 2005). Since the completion of the Human Genome Project (Green et al., 2011), genome-wide association studies (GWAS) of millions of single nucleotide polymorphisms (SNPs) became a mainstream technique to identify genetic variants associated with OHDs (Agopian et al., 2017; Hanchard et al., 2016). In addition to GWAS, multiple efforts have investigated the role of candidate genes, pathways, and metabolites in the occurrence of OHDs. Maternal exposures, such as prepregnancy body mass index, vitamin supplements, diabetes, alcohol consumption, and environmental exposures, such as pesticide exposure, have been associated with OHDs (Botto et al., 2004; Gilboa, Correa, et al., 2010; Kopf & Walker, 2009; Yang et al., 2015).

Given the complicated genetic architecture of OHDs (Chowdhury et al., 2012; Hobbs et al., 2010; Hobbs et al., 2014), it is biologically plausible that the maternal genotype may contribute to the development of these defects by influencing the prenatal environment in which the fetus develops. However, the majority of published studies focused on the inherited genetic effects on OHDs that operate through the infant genotype. Therefore, the goal of our GWAS was to identify both inherited and maternal SNPs associated with the occurrence of OHDs, utilizing a family-based transmission/disequilibrium test (TDT), as well as traditional case–control analyses conducted separately in mothers and infants enrolled in a large population-based, case–control study, the National Birth Defects Prevention Study (NBDPS).

2 | SUBJECTS AND METHODS

2.1 | Editorial policies and ethical considerations

The study was approved by the University of Arkansas for Medical Sciences institutional review board (IRB) and ethics committee. The NBDPS study protocol was approved by the IRBs at the Centers for Disease Control and Prevention and all NBDPS centers. All study participants provided informed consent; parents provided informed consent for infants.

2.2 | Study population

The NBDPS is a large, population-based, multicenter case–control study in the United States, designed to evaluate genetic and environmental factors associated with occurrence of over 30 major structural birth defects (Reefhuis et al., 2015; Yoon et al., 2001). Details of the study population, recruitment methods, and data collection have been described (Reefhuis et al., 2015; Yoon et al., 2001). For the current study, our discovery sample consisted of 3978 individuals from 861 case-parental triads (440 complete) and 1082 control-mother dyads (876 complete) identified through population-based recruitment of families in Arkansas, California, Georgia, Iowa, and Texas (Table 1). Our replication sample consisted of 2507 individuals from completed and partial triads and dyads. Specifically, 649 case-parental triads (275 complete) and 615 control-mother dyads (430 complete) were identified from population-based recruitment in Massachusetts, New York, North Carolina, and Utah (Table 1).

TABLE 1 Characteristics of participants included in an obstructive heart defect genome-wide association study, by discovery and replication sample sets, National Birth Defects Prevention Study, 1997–2011

	Discovery[a] (*N* = 3978)			Replication[a] (*N* = 2507)		
	Cases	Controls	P[b]	Cases	Controls	P[b]
Number of individuals						
Mothers	714	989		552	526	
Fathers	640	-		439	-	
Infants	666	969		471	519	
Total	2020	1958		1462	1045	
Number of families	861	1082		649	615	
Complete trios	440	-		275	-	
Maternal age at delivery, mean (*SD*)	27.82 (5.77)	26.87 (5.81)	3.14×10^{-4}	29.27 (5.57)	28.84 (5.93)	0.18
Infant sex, *N* (% of infants)[c]			0.15			0.63
Female	311 (46.70)	488 (50.36)		212 (45.01)	241 (46.44)	
Male	355 (53.30)	481 (49.64)		259 (54.99)	277 (53.37)	
Self-report maternal race/ethnicity, *N* (% of families)			9.32×10^{-6}			9.88×10^{-3}
Non-Hispanic White	544 (63.18)	611 (56.47)		514 (79.20)	446 (75.52)	
Non-Hispanic Black	49 (5.69)	69 (6.38)		34 (5.24)	43 (6.99)	
Hispanic	194 (22.53)	341 (31.52)		73 (11.25)	73 (11.87)	
Asian/Pacific Islander	22 (2.56)	29 (2.68)		7 (1.08)	18 (1.93)	
Other/Unknown	52 (6.04)	32 (2.96)		21 (3.24)	35 (5.69)	
State—Discovery, *N* (% of families)			8.38×10^{-12}			
Arkansas	317 (36.82)	294 (27.17)		-	-	
California	134 (15.56)	191 (17.65)		-	-	
Georgia	109 (12.66)	84 (7.76)		-	-	
Iowa	213 (24.74)	296 (27.36)		-	-	
Texas	88 (10.22)	217 (20.06)		-	-	
State—Replication, *N* (% of families)						8.06×10^{-25}
Massachusetts	-	-		208 (32.05)	111 (18.05)	
New York	-	-		71 (10.94)	214 (34.80)	
North Carolina	-	-		142 (21.88)	137 (22.28)	
Utah	-	-		228 (35.13)	153 (24.88)	

Abbreviation: *SD*, standard deviation.

[a]Five case families in the discovery cohort had unknown maternal race/ethnicity and age at delivery; one control infant in the replication cohort had unknown sex.

[b]For maternal age at delivery, means for cases and controls were compared via two-sided two-sample t-test; for categorical variables, distributions in cases and controls were compared using Pearson's Chi-square test.

[c]Sex is unknown for one replication control sample.

Cases were singleton live-born infants affected with OHDs, including pulmonary valve stenosis or atresia, tricuspid atresia, aortic valve stenosis, coarctation of the aorta, interrupted aortic arch, Ebstein anomaly, or hypoplastic left heart syndrome (Table S1). Cases affected by or suspected to have a known single-gene disorder, chromosomal abnormality, or syndrome were excluded. Case diagnoses were confirmed by one or more of the following: echocardiograms, surgical reports, cardiac catheterization, and autopsies. Case diagnoses were then categorized using the NBDPS classification system as described by Botto et al. (2007), with three dimensions of cardiac phenotype, cardiac complexity, and extra cardiac anomalies incorporated. Briefly, each case was assigned a complexity category, which describes the phenotype as a simple heart defect, an association, or a complex form. Simple defects typically are anatomically discrete or a well-recognized single entity such as a muscular ventricular septal defect with no other cardiac involvement. Associations are common, uncomplicated combinations of heart defects. Examples of an associations include the combination of pulmonic stenosis with secundum atrial septal defect. Complex defects were those phenotypes that could not be described as simple or associations, such as those that occur as part of some single-ventricle or laterality (heterotaxy) defect (Botto et al., 2007). Controls were live-born infants without major birth defects, randomly selected from birth certificates or hospital birth logs in the same time period and geographical regions as the cases. Both cases and controls had estimated dates of delivery from October 1, 1997 through December 31, 2011.

2.3 | Data collection

An introductory packet was mailed to mothers of eligible cases and controls, no earlier than 6 weeks after their estimated dates of delivery (Reefhuis et al., 2015; Yoon et al., 2001). Approximately 10 days after the packets were mailed, mothers were contacted by female interviewers to participate in a 1-h computer-assisted telephone interview (Reefhuis et al., 2015; Yoon et al., 2001). All study materials were available in both English and Spanish. Information on demographics and behavioral factors was collected from mothers via interview (Reefhuis et al., 2015; Yoon et al., 2001). Once a mother completed the interview, a buccal cell collection kit was mailed to collect DNA specimens on her child and both parents, if possible. For the current study, we aimed to genotype mother, father, and child (case-parental triads) for case families, and mother and child (child–mother dyads) for control families. In addition to some parental nonparticipation during sample collection, some samples were lost during genotyping and quality control procedures (described below), so our final set of samples contained some incomplete case-parental triads and control-mother dyads.

2.4 | Genotyping and quality control

DNA samples from the discovery and replication studies were genotyping in the Hobbs Birth Defects Genomics Laboratory at Arkansas Children's Research Institute. In the discovery stage, we genotyped a total of 4752 DNA samples from mothers, fathers, and infants for 4,472,081 SNPs using the Illumina Infinium Omni5Exome-4 v1.0 or v1.1 Kits, according to Illumina supplied protocols. In the replication sample, 2692 samples were genotyped for 4,559,465 SNPs using the Illumina Infinium Omni5Exome-4 v1.3 Kit. Scanning of processed and stained BeadChips was accomplished on an Illumina iScan instrument, and scan image and intensity data were analyzed by use of Illumina GenomeStudio software to determine genotypes for downstream analysis. To ensure high-quality genotypes, SNPs were removed due to low call rate (<95%), minor allele frequency (MAF) < 0.0001, and deviation from Hardy–Weinberg equilibrium among controls ($p < 10^{-7}$). Samples with a call rate <95% were excluded. Checks on sex and family relationships were performed using PLINK 1.9 (Chang et al., 2015) and KING (Manichaikul et al., 2010), respectively. When discrepancies in sex and/or pedigree were present and could not be resolved, those samples were removed. SNPs with Mendelian errors were set to missing, removing them from further analysis. Following quality control, 3,930,728 SNPs remained in the discovery cohort and 3,712,478 SNPs in the replication cohort. Analyses were restricted to the 3,335,624 SNPs that passed quality control in both cohorts.

2.5 | Statistical analysis

Data were analyzed using R 3.2.2 (https://www.r-project.org) (R Core Team, 2015) and PLINK 1.9 (https://www.cog-genomics.org/plink/) (Chang et al., 2015).

The differences in means of continuous variables were tested using two-sided two-sample *t*-tests, and the distributions of categorical variables were compared using Pearson's Chi-square tests. An investigation of the association between each SNP and OHD was conducted using the family-based TDT (Spielman et al., 1993) using PLINK 1.9 (Chang et al., 2015) on 440 complete case-parental triads in the discovery analysis (275 complete triads in replication). *p*-values were calculated using the exact binomial test. To investigate all potential signals, SNPs with suggestive associations ($p < 1 \times 10^{-6}$) in the discovery cohort were evaluated in the replication cohort at a Bonferroni-corrected significance threshold based on the number of significant discovery SNPs. An analysis was also performed on a combined cohort of the 715 discovery plus replication complete triads.

As we hypothesized that both infant and maternal genotypes contribute to the development of OHDs, separate case–control analyses were conducted in mothers and infants, implementing logistic regression models of additively modeled SNPs genome-wide using PLINK 1.9 (Chang et al., 2015). All models were adjusted for maternal age at delivery, genotyping kit (discovery analysis only), state of residence, infant's sex (infant analysis only), and first 10 ancestry principal components (PCs). PCs were calculated separately using PLINK 1.9 (Chang et al., 2015) for mothers and infants within each cohort using only SNPs included in each analysis (described below).

To ensure sufficient cell counts for data analysis, SNPs were only analyzed if they had MAF > 0.01 among controls included in the

TABLE 2 Single nucleotide polymorphisms (SNPs) with $p < 1 \times 10^{-6}$ for the transmission-disequilibrium test (TDT) in the discovery cohort with results from TDT in discovery, replication, and combined cohorts, National Birth Defects Prevention Study, 1997–2011

Chromosome	SNP	Position	Locus	Minor allele (effect allele)	Major allele	Cohort	*N* triads	MAF[a]	*N* allele transmitted	*N* allele untransmitted	OR	P
6	rs55877192	44,129,213	6p21.1	G	A	Discovery	396	0.080	34	93	0.37	1.61×10^{-7}
						Replication	257	0.095	33	65	0.51	1.60×10^{-3}
						Combined	**653**	**0.086**	**67**	**158**	**0.42**	$\mathbf{1.18 \times 10^{-9}}$
10	rs113920486	54,299,613	10q21.1	G	C	Discovery	374	0.023	3	31	0.10	7.66×10^{-7}
						Replication	269	0.014	6	9	0.67	0.61
						Combined	643	0.019	9	40	0.23	9.26×10^{-6}
19	**rs2360743**	**10,736,514**	**19p13.2**	**T**	**C**	**Discovery**	**377**	**0.023**	**1**	**33**	**0.03**	$\mathbf{4.08 \times 10^{-9}}$
						Replication	**250**	**0.013**	**0**	**13**	**0.00**	$\mathbf{2.44 \times 10^{-4}}$
						Combined	**627**	**0.019**	**1**	**46**	**0.02**	$\mathbf{6.82 \times 10^{-13}}$
X	rs5913271	79,731,210	Xq21.1	A	T	Discovery	401	0.039	4	34	0.12	6.04×10^{-7}
						Replication	267	0.069	11	21	0.52	0.11
						Combined	668	0.051	15	55	0.27	1.65×10^{-6}

Note: SNPs that replicated at a threshold of $0.05/4 = 0.0125$ are shown in bold font.

Abbreviations: MAF, minor allele frequency; OR, odds ratio.

[a]MAF calculated in parents of triads used in TDT.

analysis (parents for TDT, control mothers for case–control mother, and control infants for case–control infant) with genotype call rates of at least 0.95 in the included samples. To investigate all potential signals, SNPs with suggestive associations ($p < 1 \times 10^{-6}$) in the discovery cohort were evaluated in the replication cohort at a Bonferroni-corrected significance threshold based on the number of significant discovery SNPs. As no SNPs met both these thresholds in the case–control analyses, we relaxed these thresholds for the case–control analyses to consider SNPs with $p < 1 \times 10^{-5}$ in the discovery cohort, and these were considered to replicate at a threshold of $p < 0.05$ with the same direction of effect in the replication cohort.

To understand our findings in the context of current literature and knowledge, we conducted a comprehensive literature review for evidence of association between OHD and each of the SNPs identified in either the TDT ($p < 1 \times 10^{-6}$) or case–control analyses ($p < 1 \times 10^{-5}$). For each SNP, we searched for evidence of the association between OHD and the genomic region surrounding each SNP (defined as the cytoband to which each SNP is mapped) using publicly available databases, including dbSNP (https://www.ncbi.nlm.nih.gov/snp/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), PubMed (https://pubmed.ncbi.nlm.nih.gov), OMIM (http://www.omim.org/), GeneCards (https://www.genecards.org), MalaCards (https://www.malacards.org), and GTEx (https://www.gtexportal.org/home/). Research data are not shared.

3 | RESULTS

Our final discovery study sample was comprised of 3978 individuals from 861 case-parental triads and 1082 control-mother dyads (Table 1). Maternal age at delivery was slightly higher in cases than in controls, with a mean age of 27.82 years (standard deviation [*SD*] = 5.77) in case mothers compared with a mean age of 26.87 years (*SD* = 5.81) in control mothers ($p < 0.001$. Although there was no appreciable difference in sex between case and control infants ($p = 0.15$), there was a difference in distribution between cases and controls for self-reported maternal ethnicity as well as state of residence ($p < 0.001$ for both). Comparatively, our final replication sample consisted of 2507 individuals from 649 case-parental triads and 615 control-mother dyads (Table 1). There was no difference ($p = 0.18$) in maternal age at delivery among cases (mean = 29.27; *SD* = 5.57) compared to controls (mean = 28.84; mean = 5.93), nor was there a difference in sex between case and control infants ($p = 0.63$). However, there was a significant difference in distribution between cases and controls for both self-reported maternal ethnicity and state of residence ($p < 0.01$ for both).

A genome-wide TDT analysis was conducted among complete case triads (440 discovery; 275 replication) to assess association in the presence of genetic linkage between each SNP and OHD, as the TDT is robust to population stratification. There were four SNPs displaying associations with OHD at $p < 1 \times 10^{-6}$, one of which attained genome-wide significance at $p < 5 \times 10^{-8}$ (Table 2; S1). Two of these SNPs, including the genome-wide significant SNP, replicated at a Bonferroni-corrected significance level of 0.05/4 = 0.0125 (Table 2; Figure S2). One SNP (rs55877192), located at 6p21.1, is intronic of *CAPN11* (MIM: 604822) and had a combined discovery plus replication odds ratio (OR) = 0.42 ($p = 1.18 \times 10^{-9}$; Table 2; Figure S3). Another SNP (rs2360743), located at 19p13.2, is intronic of *SLC44A2* (MIM: 606106) and had a combined discovery plus replication OR = 0.022 ($p = 6.82 \times 10^{-13}$; Table 2; Figure S3).

Separate case–control analyses were conducted between case and control mothers and between case and control infants. The mothers-only analysis was comprised of 714 cases and 989 controls in the discovery cohort and 552 cases and 526 controls in the replication cohort (Table 1). The infant-only analysis compared 666 cases versus 969 controls in discovery and 471 cases versus 519 controls in replication (Table 1). At a suggestive threshold of $p < 1 \times 10^{-6}$, there were no SNPs associated with OHD among mothers of case infants (Table 3; Figures S4–S6), and there were only two SNPs associated with OHD among infants, neither of which replicated (Table 4; Figures S7–S9). At a more relaxed threshold of $p < 1 \times 10^{-5}$, there were six SNPs associated with OHD in mothers (Table 3; Figures S4–S6), none of which replicated, and an additional 15 SNPs associated with OHD in infants (Table 4; Figures S7–S9), one of which (rs188255766) replicated at a nominal threshold of $p < 0.05$. This SNP (rs188255766) is an intergenic variant located at 5q23.1 and had a meta-analysis OR = 0.41 ($p = 4.69 \times 10^{-7}$).

4 | DISCUSSION

We carried out a genome-wide TDT, utilizing DNA samples from parents and their infants who were affected by OHDs in the NBDPS, one of the largest case–control studies of birth defects conducted in the United States. DNA samples were available from complete and partial case-parental triads and control-maternal dyads. Our analyses utilized a family-based approach, allowing investigation of inherited genotypes. We detected four SNPs in our discovery cohort at a suggestive threshold of $p < 1 \times 10^{-6}$, two of which replicated at a Bonferroni-corrected significance level of 0.05/4 = 0.0125. Of these SNPs, one (rs55877192) was identified in *CAPN11*, a gene that is primarily expressed in the testes and not in the heart in GTEx (GTEx Consortium, 2013) and from previous evidence (Dear et al., 1999), and further research will help inform whether *CAPN11* plays a role in OHDs (i.e., abnormally expressed in the heart in patients with OHDs). The other SNP (rs2360743) is in an intronic region of *SLC44A2* and was genome-wide significant in the discovery cohort. *SLC44A2* is a choline transporter, converting choline to betaine. Mounting evidence suggests that choline exposure during pregnancy alters histone and DNA methylation in both placenta and the developing embryo. Choline regulates the concentrations of S-adenosylhomocysteine and S-adenosylmethionine, and alterations in both have been associated with CHDs (Chan et al., 2010; Chowdhury et al., 2012).

In our infant-only case–control analysis, we identified one SNP (rs188255766) with $p < 1 \times 10^{-5}$ that replicated at a nominal threshold of $p = 0.05$. This intergenic SNP is located on 5q23.1, and this

TABLE 3 Single nucleotide polymorphisms (SNPs) with $p < 1 \times 10^{-5}$ in the case–control genome-wide association study (GWAS) in mothers of case infants in the discovery cohort with results from the GWAS in the discovery and replication cohorts and the meta-analysis results, National Birth Defects Prevention Study, 1997–2011

						Discovery					Replication				Meta	
Chromosome	SNP	Position	Locus	Minor Allele[a]	Major allele	N	MAF[b]	OR	SE	p	N	MAF[b]	OR	SE	p	OR	p
2	rs4850063	4,332,775	2p25.3	G	A	1696	0.34	0.70	0.08	8.87×10^{-6}	1078	0.35	1.01	0.10	0.93	0.81	7.51×10^{-4}
5	rs7706812	160,312,017	5q34	G	T	1695	0.32	1.43	0.08	2.77×10^{-6}	1078	0.35	0.83	0.10	0.07	1.18	8.14×10^{-3}
6	rs9341603	77,534,953	6q14.1	T	C	1696	0.15	1.59	0.10	1.37×10^{-6}	1078	0.16	1.17	0.13	0.22	1.42	4.49×10^{-6}
8	rs330080	9,154,609	8p23.1	C	T	1696	0.46	0.72	0.07	6.31×10^{-6}	1077	0.46	0.97	0.09	0.77	0.81	2.00×10^{-4}
11	rs317518	110,890,363	11q23.1	C	T	1689	0.31	1.41	0.08	6.48×10^{-6}	1075	0.38	0.93	0.09	0.47	1.20	2.33×10^{-3}
16	rs9937546	73,003,719	16q22.3	C	T	1698	0.25	1.44	0.08	6.79×10^{-6}	1075	0.29	1.08	0.11	0.50	1.29	6.78×10^{-5}

Abbreviations: MAF, minor allele frequency; OR, odds ratio; SE, standard error.

[a]Minor allele is effect allele.

[b]MAF calculated in controls.

genomic region has previously been reported to be associated with fetal interrupted aortic arch and atrial septal defect. In a case report using uncultured amniocytes in a pregnancy affected by fetal aortic arch and atrial septal defect, a de novo deletion was detected involving 5q23.1–23.3, which included a compelling candidate gene, *FBN2* (MIM: 612570) (Chen et al., 2013; Putnam et al., 1995). *FBN2* is associated with congenital contractural arachnodactyly (Callewaert et al., 2009), with phenotypes including aortic root dilatation. The related gene *FBN1* (located at 15q15-21.3 [MIM: 134797]) is associated with Marfan syndrome (MIM: 154700) (Du et al., 2021), of which the phenotype includes interrupted aortic arch and atrial and ventricular septal defects, though further research is needed to determine whether there is a direct association between *FBN1* and these subphenotypes. *FBN2* is involved in the initial assembly of the aortic matrix and, with *FBN1*, helps to guide the maturation of the aortic wall during fetal development and neonatal growth (Carta et al., 2006). Although our SNP is ~12,000 kb from *FBN2*, our findings may provide additional suggestive evidence regarding the importance of this region for OHDs.

Of the remaining 16 SNPs identified in the infant-only case–control discovery analysis with $p < 1 \times 10^{-5}$, several are in genomic regions previously reported to be associated with atrial septal defects in other GWAS analyses, including 4p16 (Cordell et al., 2013; Pei et al., 2016; B. Zhao et al., 2014; L. Zhao et al., 2015). More recently, Córdova-Palomero and colleagues studied adult survivors with CHDs in the UK Biobank and were the first to report an association between 4p16 and left ventricular outflow tract obstructions among 164 affected individuals versus 332,788 unaffected individuals (Córdova-Palomera & Priest, 2019). In contrast, Cordell and colleagues did not find an association between 412 individuals with coarctation of the aorta, hypoplastic left heart syndrome, or aortic and mitral valve anomalies. We did find some evidence of association with OHDs (rs6829745, $p = 6.02 \times 10^{-6}$) in our discovery sample of 1635 infants (666 cases), although our SNP is independent from the SNPs reported by Córdova-Palomero (LD $r^2 < 0.1$). Further analyses to determine associations with a specific type of OHD with larger sample sizes may provide additional precision about this genetic locus and association with OHDs.

In a 2017, GWAS meta-analysis of inherited effects of left ventricular outflow tracts among 509 trios, Agopian and colleagues identified multiple SNPs in strong linkage disequilibrium ($r^2 > 0.49$) in an intergenic region of 6p24.3 (Agopian et al., 2017). We also found a SNP (rs35072477, discovery $p = 4.72 \times 10^{-7}$) in the same intergenic region but independent from the reported SNPs ($r^2 < 0.1$) and, as stated by Agopian and colleagues, the potential contribution of this locus to the occurrence of OHDs is unclear (Agopian et al., 2017). The 6p24.3 region has been linked to a rare autosomal dominant syndrome that includes CHDs (noncompaction of the ventricular myocardium, bradycardia, pulmonary valve stenosis, secundum atrial septal defect, left isomerism, and heterotaxy) (Wessels et al., 2008).

Mitchell and colleagues conducted a GWAS on case-parental triads with left-sided congenital heart lesions and found a suggestive association with a maternal genotype at 10p11.23 (Mitchell

TABLE 4 Single nucleotide polymorphisms (SNPs) with $p < 1 \times 10^{-5}$ in the case–control genome-wide association study (GWAS) in infants in the discovery cohort with results from the GWAS in the discovery and replication cohorts and the meta-analysis results, National Birth Defects Prevention Study, 1997–2011

						Discovery					Replication					Meta	
Chromosome	SNP	Position	Locus	Minor allele[a]	Major allele	*N*	MAF[b]	OR	SE	*p*	*N*	MAF[b]	OR	SE	*p*	OR	*p*
1	rs2737665	200,062,389	1q32.1	A	G	1627	0.30	1.46	0.08	6.45×10^{-6}	989	0.29	1.05	0.11	0.66	1.30	1.09×10^{-4}
2	rs12474157	35,611,713	2p22.3	T	C	1625	0.38	1.42	0.08	9.71×10^{-6}	986	0.37	1.06	0.10	0.56	1.27	1.37×10^{-4}
2	rs78229837	236,941,567	2q37.2	A	G	1626	0.02	2.86	0.24	8.88×10^{-6}	986	0.04	0.56	0.28	0.04	1.46	3.60×10^{-2}
3	rs9813034	137,281,630	3q22.3	G	T	1631	0.44	0.70	0.08	5.57×10^{-6}	984	0.40	0.97	0.10	0.76	0.79	1.53×10^{-4}
4	rs6829745	1,672,096	4p16.3	T	G	1622	0.41	0.70	0.08	6.02×10^{-6}	970	0.38	0.98	0.10	0.88	0.79	2.24×10^{-4}
5	**rs188255766**	**115,282,058**	**5q23.1**	**T**	**C**	**1431**	**0.07**	**0.34**	**0.22**	$\mathbf{1.42 \times 10^{-6}}$	**897**	**0.05**	**0.56**	**0.29**	**0.04**	**0.41**	$\mathbf{4.69 \times 10^{-7}}$
6	rs35072477	9,799,050	6p24.3	C	T	1628	0.07	0.36	0.20	4.72×10^{-7}	988	0.04	0.99	0.25	0.98	0.53	7.76×10^{-5}
7	rs117565070	137,842,379	7q33	A	G	1612	0.08	0.47	0.16	3.02×10^{-6}	986	0.06	1.05	0.19	0.80	0.65	6.16×10^{-4}
9	rs7849958	38,678,740	9p13.1	C	A	1623	0.24	0.64	0.09	2.19×10^{-6}	983	0.18	1.22	0.12	0.11	0.81	4.77×10^{-3}
9	rs2149564	98,607,989	9q22.32	G	T	1626	0.45	1.40	0.08	9.00×10^{-6}	988	0.49	1.04	0.10	0.70	1.25	1.96×10^{-4}
9	rs10987145	128,853,643	9q33.3	T	C	1607	0.42	1.47	0.08	5.50×10^{-7}	965	0.44	1.04	0.10	0.69	1.29	2.97×10^{-5}
10	rs10763769	30,230,749	10p11.23	T	C	1631	0.13	0.57	0.13	7.74×10^{-6}	989	0.11	0.88	0.16	0.42	0.68	6.84×10^{-5}
11	rs1784257	72,007,984	11q13.4	C	T	1626	0.06	1.93	0.15	6.11×10^{-6}	989	0.05	1.24	0.21	0.31	1.68	1.69×10^{-5}
13	rs9510445	23,535,168	13q12.12	A	C	1543	0.20	1.54	0.10	7.98×10^{-6}	972	0.18	0.83	0.14	0.17	1.25	4.50×10^{-3}
16	rs7405232	12,937,344	16p13.12	T	C	1631	0.40	0.70	0.08	4.48×10^{-6}	988	0.34	1.08	0.10	0.45	0.82	1.64×10^{-3}
17	rs2291725	47,039,132	17q21.32	C	T	1613	0.45	1.42	0.08	5.59×10^{-6}	985	0.47	0.99	0.10	0.91	1.25	3.92×10^{-4}
21	rs1999317	29,853,202	21q21.3	A	G	1630	0.04	2.18	0.18	8.23×10^{-6}	989	0.04	1.32	0.25	0.27	1.85	1.87×10^{-5}

Note: SNPs that replicated at a threshold of 0.05 with same direction of effect are shown in bold.

Abbreviations: MAF, minor allele frequency; OR, odds ratio; SE, standard error.

[a]Minor allele is effect allele.

[b]MAF calculated in controls.

et al., 2015). We found an association (rs10763769, discovery $p = 7.74 \times 10^{-6}$) at the same 10p11.23 loci in the infant case–control analysis. Within this region, there are clusters of transcription factor binding sites for GATA-1, GATA-2, GABP, and others, which are known to play substantial roles in cardiogenesis.

Finally, we identified a SNP (rs7405232, discovery $p = 4.48 \times 10^{-6}$) at 16p13.12. The 16p13 locus includes *NOMO1* (MIM: 609157), a member of the transforming growth factor beta superfamily that acts through a complex network of signaling pathways during vertebrate development including cardiogenesis (Sun & Kontaridis, 2018). Investigations into the mechanism by which NOMO1 regulates Nodal signaling and cardiogenesis are revealing that silencing *NOMO1* may down regulate the expression of cardiomyocyte-specific markers and the cardiomyocyte transcriptional factors, NKX2.5, GATA4, and TBX5 (Zhang et al., 2015). NODAL modulator 1 has been tagged as an excellent candidate gene for bicuspid aorta and tricuspid insufficiency. Although our SNP is ~2000 kb away from *NOMO1*, it is possible that it affects expression via some epigenetic mechanism.

Our study has several limitations. Across all of our analyses, only one SNP in the TDT reached genome-wide significance ($p < 5 \times 10^{-8}$), though this SNP and one more at $p < 1 \times 10^{-6}$ replicated at a Bonferroni-corrected level. In the case–control analyses (maternal only and infant only), just one SNP in the infant only analysis was found to be associated with OHDs and replicated, although only at relaxed thresholds of $p < 1 \times 10^{-5}$ in analysis of the discovery cohort and $p < 0.05$ in analysis of the replication cohort. Such lack of validation reduces the confidence in our findings. However, our suggested candidates are supported by previously published evidence as described above and, thus, add some support (albeit limited) to the evidence base for these associations. Additionally, we included both right- and left-sided OHDs to maximize sample size (and, thus, potentially power). Doing so may have diluted some potential associations that are only associated with one type of OHD.

The strength of our study is that, where the majority of genetic studies are predominantly based on individuals of European ancestry (Sirugo et al., 2019), our cohort consisted of a geographically diverse, population-based, case-parental and case–control sample. The microarray data may be used by multiple investigators to test secondary hypotheses regarding gene–environment interactions and to focus on specific candidate pathways. It is possible that a gene- or pathway-based analysis will result in additional findings beyond that of a single genetic locus. Our GWAS results will reside at the Centers for Disease Control and Prevention and, when possible, will be deposited in aggregate into dbGaP. Investigators may use these data to conduct further genome-wide pathway and gene-based studies, as well as studies to identify polygenic risk scores and associations between OHDs and gene-environmental and gene-lifestyle interactions.

In summary, our trio and case–control-based analyses of common variants in a large sample of individuals affected by OHDs in both a discovery and replication cohort provide additional evidence for seven OHD candidate genes/regions. The locus found in the TDT analysis is consistent with multiple investigations, both animal and human, suggesting a role of DNA methylation in the occurrence of CHDs. The five loci in the infant-only case–control analysis found to be suggestively associated with OHDs were supported by evidence from previous GWAS analyses, case series, and/or animal studies. Our findings add to the growing evidence of the complex genetic etiology of OHDs in relation to potential CHD-related genes.

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CONFLICT OF INTERESTS

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Sara R. Rashkin contributed by conducting the analyses and writing the manuscript. John S. Witte and Charlotte A. Hobbs contributed to study conception and design, supervised the project, and aided in writing the manuscript. Mario Cleves, Gary M. Shaw, Wendy N. Nembhard, Eirini Nestoridi, Mary M. Jenkins, Paul A. Romitti, Xiang-Yang Lou, Marilyn L. Browne, Laura E. Mitchell, Andrew F. Olshan, Kevin Lomangino, and Sudeepa Bhattacharyya aided in data acquisition and curation, provided critical feedback, and helped shape the research, analysis, and manuscript.

DATA AVAILABILITY STATEMENT

The study questionnaires and process for accessing the data used in this study is described at https://www.cdc.gov/ncbddd/birthdefects/nbdps-public-access-procedures.html. The code book and analytic code may be made available upon request. To ensure that collected specimens and data are used to their full potential and to also be mindful of our role in protecting participants' privacy and rights as human subjects, aggregate genetic data and limited phenotypic/exposure data from NBDPS participants will be deposited into NIH dbGaP.

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