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

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Author Contributions

SRR contributed by conducting the analyses and writing the manuscript. JSW and CAH contributed to study conception and design, supervised the project, and aided in writing the manuscript. MC, GMS, WNN, EN, MMJ, PAR, XL, MLB, LEM, AFO, KL, and SB aided in data acquisition and curation, provided critical feedback, and helped shape the research, analysis, and manuscript.

The analysis has been replicated by John Witte

Web Resources

1. R, https://www.r-project.org

2. PLINK 1.9, https://www.cog-genomics.org/plink/

3. dbSNP, https://www.ncbi.nlm.nih.gov/snp/

4. ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/

5. PubMed, https://pubmed.ncbi.nlm.nih.gov

6. OMIM, http://www.omim.org/

7. GeneCards, https://www.genecards.org

8. MalaCards, https://www.malacards.org

9. GTEx, https://www.gtexportal.org/home/

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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 (CHDs). 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 ($N_{discovery}$=3,978; $N_{replication}$=2,507), 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 ($N_{discovery\_CCI}$=1,635; $N_{replication\_CCI}$=990) and mothers (case status defined by infant; $N_{discovery\_CCM}$=1,703; $N_{replication\_CCM}$=1,078). In the TDT analysis, the *SLC44A2* single nucleotide polymorphism (SNP) rs2360743 was significantly associated with OHD ($P_{discovery}$ = 4.08×10^{-9}; $P_{replication}$=2.44×10^{-4}). A *CAPN11* SNP (rs55877192) was suggestively associated with OHD ($P_{discovery}$=1.61×10^{-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 ($P_{discovery} < 1 \times 10^{-5}$ and $P_{replication} < 0.05$), only one SNP (rs188255766) in the infant analysis was associated with OHDs ($P_{discovery}$=1.42×10^{-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.

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 1,000 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, Gelb, Seidman, & Seidman, 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, Nembhard, Fixler, & Correa, 2010) and the prevalence of CHDs among adults is increasing (Gilboa, Salemi, et al., 2010; Marelli, Mackie, Ionescu-Ittu, Rahme, & Pilote, 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, Robbins, & Hobbs, 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, Pulignani, Foffa, & Andreassi, 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, Chang, Koenig, Zinn, & Garg, 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, Guyer, & National Human Genome Research Institute, 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 pre-pregnancy body mass index, vitamin supplements, diabetes, alcohol consumption, and environmental exposures, such as pesticide exposure, have been associated with OHDs (Botto, Olney, & Erickson, 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, Cleves, Karim, Zhao, & MacLeod, 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).

Subjects and Methods

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.

Study Population

The NBDPS is a large, population-based, multi-center 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 3,978 individuals from 861 case-parental triads (440 complete) and 1,082 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 2,507 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).

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 (Supplementary Table 1). 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 in 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 VSD 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.

Data Collection

An introductory packet was mailed to mothers of eligible cases and controls, no earlier than six 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 one-hour 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 non-participation 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.

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 4,752 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, 2,692 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.

Statistical Analysis

Data were analyzed using R 3.2.2 (R Core Team, 2015) and PLINK 1.9 (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, McGinnis, & Ewens, 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 ($P<1\times10^{-6}$) 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 ten 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 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 ($P<1\times10^{-6}$) 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 $P<1\times10^{-5}$ 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 ($P<1\times10^{-6}$) or case-control analyses ($P<1\times10^{-5}$). 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, ClinVar, PubMed, OMIM, GeneCards, MalaCards, and GTEx. Research data are not shared.

Results

Our final discovery study sample was comprised of 3,978 individuals from 861 case-parental triads and 1,082 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 2,507 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<1x10^{-6}, one of which attained genome-wide significance at P<5x10^{-8} (Table 2; Supplementary Figure 1). 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; Supplementary Figure 2). 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=1.18x10^{-9}; Table 2; Supplementary Figure 3). Another SNP (rs2360743), located at 19p13.2, is intronic of *SLC44A2* (MIM: 606106) and had a combined discovery plus replication OR=0.022 (P=6.82x10^{-13}; Table 2; Supplementary Figure 3).

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<1x10^{-6}, there were no SNPs associated with OHD among mothers of case infants (Table 3; Supplementary Figures 4-6), and there were only two SNPs associated with OHD among infants, neither of which replicated (Table 4; Supplementary Figures 7-9). At a more relaxed threshold of P<1x10^{-5}, there were 6 SNPs associated with OHD in mothers (Table 3; Supplementary Figures 4-6), none of which replicated, and an additional 15 SNPs associated with OHD in infants (Table 4; Supplementary Figures 7-9), 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=4.69x10^{-7}).

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<1x10^{-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 which is primarily expressed in the testes and not in the heart in GTEx (GTEx Consortium, 2013) and from previous evidence (Dear, Moller, & Boehm, 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 $P<1x10^{-5}$ that replicated at a nominal threshold of $P=0.05$. This intergenic SNP is located on 5q23.1, and this 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, Zhang, Ramirez, & Milewicz, 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 if 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 ~12,000kb 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 $P<1x10^{-5}$, 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, $P=6.02x10^{-6}$) in our discovery sample of 1,635 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 $P=4.72x10^{-7}$) 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 et al., 2015). We found an association (rs10763769, discovery P=7.74x10^{-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=4.48x10^{-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 ~2,000kb 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<5x10^{-8}), though this SNP and one more at P<1x10^{-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<1x10^{-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, Williams, & Tishkoff, 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Data Availability

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.

References

Agopian AJ, Goldmuntz E, Hakonarson H, Sewda A, Taylor D, & Mitchell LE (2017). Genome-wide association studies and meta-analyses for congenital heart defects. Circ Cardiovasc Genet, 10(3), e001449. doi:10.1161/circgenetics.116.001449 [PubMed: 28468790]

Boneva RS, Botto LD, Moore CA, Yang Q, Correa A, & Erickson JD (2001). Mortality associated with congenital heart defects in the United States: trends and racial disparities, 1979-1997. Circulation, 103(19), 2376–2381. doi:10.1161/01.cir.103.19.2376 [PubMed: 11352887]

Botto LD, & Correa A (2003). Decreasing the burden of congenital heart anomalies: an epidemiologic evaluation of risk factors and survival. Prog Pediatr Cardiol, 18(2), 111–121.

Botto LD, Lin AE, Riehle-Colarusso T, Malik S, Correa A, & National Birth Defect Prevention Study. (2007). Seeking causes: Classifying and evaluating congenital heart defects in etiologic

studies. Birth Defects Res A Clin Mol Teratol, 79(10), 714–727. doi:10.1002/bdra.20403 [PubMed: 17729292]

Botto LD, Olney RS, & Erickson JD (2004). Vitamin supplements and the risk for congenital anomalies other than neural tube defects. Am J Med Genet C Semin Med Genet, 125C(1), 12–21. doi:10.1002/ajmg.c.30004 [PubMed: 14755429]

Boughman JA, Berg KA, Astemborski JA, Clark EB, Mccarter RJ, Rubin JD, & Ferencz C (1987). Familial Risks of Congenital Heart Defect Assessed in a Population-Based Epidemiologic-Study. American Journal of Medical Genetics, 26(4), 839–849. doi:DOI 10.1002/ajmg.1320260411 [PubMed: 3591826]

Callewaert BL, Loeys BL, Ficcadenti A, Vermeer S, Landgren M, Kroes HY, … De Paepe AM (2009). Comprehensive clinical and molecular assessment of 32 probands with congenital contractural arachnodactyly: report of 14 novel mutations and review of the literature. Hum Mutat, 30(3), 334–341. doi:10.1002/humu.20854 [PubMed: 19006240]

Carta L, Pereira L, Arteaga-Solis E, Lee-Arteaga SY, Lenart B, Starcher B, … Ramirez F (2006). Fibrillins 1 and 2 perform partially overlapping functions during aortic development. J Biol Chem, 281(12), 8016–8023. doi:10.1074/jbc.M511599200 [PubMed: 16407178]

Centers for Disease Control and Prevention (CDC). (1998). Trends in infant mortality attributable to birth defects--United States, 1980-1995. MMWR Morb Mortal Wkly Rep, 47(37), 773–778. [PubMed: 9769135]

Centers for Disease Control and Prevention (CDC). (2007). Hospital stays, hospital charges, and in-hospital deaths among infants with selected birth defects--United States, 2003. MMWR Morb Mortal Wkly Rep, 56(2), 25–29. [PubMed: 17230142]

Chan J, Deng L, Mikael LG, Yan J, Pickell L, Wu Q, … Rozen R (2010). Low dietary choline and low dietary riboflavin during pregnancy influence reproductive outcomes and heart development in mice. Am J Clin Nutr, 91(4), 1035–1043. doi:10.3945/ajcn.2009.28754 [PubMed: 20164309]

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, & Lee JJ (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience, 4, 7. doi:10.1186/s13742-015-0047-8 [PubMed: 25722852]

Chen CP, Huang MC, Chen YY, Chern SR, Wu PS, Chen YT, … Wang W (2013). Prenatal diagnosis of de novo interstitial deletions involving 5q23.1-q23.3 and 18q12.1-q12.3 by array CGH using uncultured amniocytes in a pregnancy with fetal interrupted aortic arch and atrial septal defect. Gene, 531(2), 496–501. doi:10.1016/j.gene.2013.09.010 [PubMed: 24036431]

Chowdhury S, Hobbs CA, MacLeod SL, Cleves MA, Melnyk S, James SJ, … Erickson SW (2012). Associations between maternal genotypes and metabolites implicated in congenital heart defects. Mol Genet Metab, 107(3), 596–604. doi:10.1016/j.ymgme.2012.09.022 [PubMed: 23059056]

Cordell HJ, Bentham J, Topf A, Zelenika D, Heath S, Mamasoula C, … Keavney BD (2013). Genome-wide association study of multiple congenital heart disease phenotypes identifies a susceptibility locus for atrial septal defect at chromosome 4p16. Nat Genet, 45(7), 822–824. doi:10.1038/ng.2637 [PubMed: 23708191]

Córdova-Palomera A, & Priest JR (2019). Association between the 4p16 genomic locus and different types of congenital heart disease: results from adult survivors in the UK Biobank. Sci Rep, 9(1), 16515. doi:10.1038/s41598-019-52969-x [PubMed: 31712678]

Dear TN, Moller A, & Boehm T (1999). CAPN11: A calpain with high mRNA levels in testis and located on chromosome 6. Genomics, 59(2), 243–247. doi:10.1006/geno.1999.5859 [PubMed: 10409436]

Du Q, Zhang D, Zhuang Y, Xia Q, Wen T, & Jia H (2021). The Molecular Genetics of Marfan Syndrome. Int J Med Sci, 18(13), 2752–2766. doi:10.7150/ijms.60685 [PubMed: 34220303]

Fahed AC, Gelb BD, Seidman JG, & Seidman CE (2013). Genetics of congenital heart disease: the glass half empty. Circ Res, 112(4), 707–720. doi:10.1161/circresaha.112.300853 [PubMed: 23410880]

Gilboa SM, Correa A, Botto LD, Rasmussen SA, Waller DK, Hobbs CA, … National Birth Defects Prevention Study. (2010). Association between prepregnancy body mass index and congenital heart defects. Am J Obstet Gynecol, 202(1), 51 e51–51 e10. doi:10.1016/j.ajog.2009.08.005 [PubMed: 19796755]

Gilboa SM, Salemi JL, Nembhard WN, Fixler DE, & Correa A (2010). Mortality resulting from congenital heart disease among children and adults in the United States, 1999 to 2006. Circulation, 122(22), 2254–2263. doi:10.1161/CIRCULATIONAHA.110.947002 [PubMed: 21098447]

Green ED, Guyer MS, & National Human Genome Research Institute. (2011). Charting a course for genomic medicine from base pairs to bedside. Nature, 470(7333), 204–213. doi:10.1038/nature09764 [PubMed: 21307933]

GTEx Consortium. (2013). The Genotype-Tissue Expression (GTEx) project. Nat Genet, 45(6), 580–585. doi:10.1038/ng.2653 [PubMed: 23715323]

Hanchard NA, Swaminathan S, Bucasas K, Furthner D, Fernbach S, Azamian MS, … McBride KL (2016). A genome-wide association study of congenital cardiovascular left-sided lesions shows association with a locus on chromosome 20. Hum Mol Genet, 25(11), 2331–2341. doi:10.1093/hmg/ddw071 [PubMed: 26965164]

Hinton RB Jr., Martin LJ, Tabangin ME, Mazwi ML, Cripe LH, & Benson DW (2007). Hypoplastic left heart syndrome is heritable. J Am Coll Cardiol, 50(16), 1590–1595. doi:10.1016/j.jacc.2007.07.021 [PubMed: 17936159]

Hitz MP, Lemieux-Perreault LP, Marshall C, Feroz-Zada Y, Davies R, Yang SW, … Andelfinger G (2012). Rare copy number variants contribute to congenital left-sided heart disease. PLoS Genet, 8(9), e1002903. doi:10.1371/journal.pgen.1002903 [PubMed: 22969434]

Hobbs CA, Cleves MA, Karim MA, Zhao W, & MacLeod SL (2010). Maternal folate-related gene environment interactions and congenital heart defects. Obstet Gynecol, 116(2 Pt 1), 316–322. doi:10.1097/AOG.0b013e3181e80979 [PubMed: 20664391]

Hobbs CA, Cleves MA, Macleod SL, Erickson SW, Tang X, Li J, … National Birth Defects Prevention, S. (2014). Conotruncal heart defects and common variants in maternal and fetal genes in folate, homocysteine, and transsulfuration pathways. Birth Defects Res A Clin Mol Teratol, 100(2), 116–126. doi:10.1002/bdra.23225 [PubMed: 24535845]

Hoffman J (2013). The global burden of congenital heart disease. Cardiovasc J Afr, 24(4), 141–145. doi:10.5830/CVJA-2013-028 [PubMed: 24217047]

Homsy J, Zaidi S, Shen Y, Ware JS, Samocha KE, Karczewski KJ, … Chung WK (2015). De novo mutations in congenital heart disease with neurodevelopmental and other congenital anomalies. Science, 350(6265), 1262–1266. doi:10.1126/science.aac9396 [PubMed: 26785492]

Kopf PG, & Walker MK (2009). Overview of developmental heart defects by dioxins, PCBs, and pesticides. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 27(4), 276–285. doi:10.1080/10590500903310195 [PubMed: 19953399]

Liu Y, Chen S, Zuhlke L, Black GC, Choy MK, Li N, & Keavney BD (2019). Global birth prevalence of congenital heart defects 1970-2017: updated systematic review and meta-analysis of 260 studies. Int J Epidemiol, 48(2), 455–463. doi:10.1093/ije/dyz009 [PubMed: 30783674]

Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, & Chen WM (2010). Robust relationship inference in genome-wide association studies. Bioinformatics, 26(22), 2867–2873. doi:10.1093/bioinformatics/btq559 [PubMed: 20926424]

Marelli AJ, Mackie AS, Ionescu-Ittu R, Rahme E, & Pilote L (2007). Congenital heart disease in the general population: changing prevalence and age distribution. Circulation, 115(2), 163–172. doi:10.1161/CIRCULATIONAHA.106.627224 [PubMed: 17210844]

McBride KL, Pignatelli R, Lewin M, Ho T, Fernbach S, Menesses A, … Belmont JW (2005). Inheritance analysis of congenital left ventricular outflow tract obstruction malformations: Segregation, multiplex relative risk, and heritability. Am J Med Genet A, 134A(2), 180–186. doi:10.1002/ajmg.a.30602 [PubMed: 15690347]

Mitchell LE, Agopian AJ, Bhalla A, Glessner JT, Kim CE, Swartz MD, … Goldmuntz E (2015). Genome-wide association study of maternal and inherited effects on left-sided cardiac malformations. Hum Mol Genet, 24(1), 265–273. doi:10.1093/hmg/ddu420 [PubMed: 25138779]

Oyen N, Poulsen G, Boyd HA, Wohlfahrt J, Jensen PK, & Melbye M (2009). National time trends in congenital heart defects, Denmark, 1977-2005. Am Heart J, 157(3), 467–473.e461. doi:10.1016/j.ahj.2008.10.017 [PubMed: 19249416]

Payne AR, Chang SW, Koenig SN, Zinn AR, & Garg V (2012). Submicroscopic chromosomal copy number variations identified in children with hypoplastic left heart syndrome. Pediatr Cardiol, 33(5), 757–763. doi:10.1007/s00246-012-0208-9 [PubMed: 22349727]

Pei K, Huang Q, Zhang G, Lu C, Yu B, & Yang L (2016). Association between the 4p16 susceptibility locus and the risk of atrial septal defect in population from Southeast China. Pediatric Cardiology, 37(1), 120–124. doi:10.1007/s00246-015-1248-8 [PubMed: 26283177]

Pierpont ME, Brueckner M, Chung WK, Garg V, Lacro RV, McGuire AL, … Russell MW (2018). Genetic basis for congenital heart disease: revisited: a scientific statement from the american heart association. Circulation, 138(21), e653–e711. doi:10.1161/cir.0000000000000606 [PubMed: 30571578]

Putnam EA, Zhang H, Ramirez F, & Milewicz DM (1995). Fibrillin-2 (FBN2) mutations result in the Marfan-like disorder, congenital contractural arachnodactyly. Nat Genet, 11(4), 456–458. doi:10.1038/ng1295-456 [PubMed: 7493032]

R Core Team. (2015). R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org

Reefhuis J, Gilboa SM, Anderka M, Browne ML, Feldkamp ML, Hobbs CA, … National Birth Defects Prevention Study. (2015). The National Birth Defects Prevention Study: A review of the methods. Birth Defects Res A Clin Mol Teratol, 103(8), 656–669. doi:10.1002/bdra.23384 [PubMed: 26033852]

Sirugo G, Williams SM, & Tishkoff SA (2019). The Missing Diversity in Human Genetic Studies. Cell, 177(1), 26–31. doi:10.1016/j.cell.2019.02.048 [PubMed: 30901543]

Spielman RS, McGinnis RE, & Ewens WJ (1993). Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). Am J Hum Genet, 52(3), 506–516. [PubMed: 8447318]

Sun C, & Kontaridis MI (2018). Physiology of Cardiac Development: From Genetics to Signaling to Therapeutic Strategies. Curr Opin Physiol, 1, 123–139. doi:10.1016/j.cophys.2017.09.002 [PubMed: 29532042]

Tilford JM, Robbins JM, & Hobbs CA (2001). Improving estimates of caregiver time cost and family impact associated with birth defects. Teratology, 64 Suppl 1, S37–41. doi:10.1002/tera.1097 [PubMed: 11745843]

Triedman JK, & Newburger JW (2016). Trends in congenital heart disease: the next decade. Circulation, 133(25), 2716–2733. doi:10.1161/circulationaha.116.023544 [PubMed: 27324366]

Vecoli C, Pulignani S, Foffa I, & Andreassi MG (2014). Congenital heart disease: the crossroads of genetics, epigenetics and environment. Curr Genomics, 15(5), 390–399. doi:10.2174/1389202915666140716175634 [PubMed: 25435801]

Wessels MW, De Graaf BM, Cohen-Overbeek TE, Spitaels SE, de Groot-de Laat LE, Ten Cate FJ, … Willems PJ (2008). A new syndrome with noncompaction cardiomyopathy, bradycardia, pulmonary stenosis, atrial septal defect and heterotaxy with suggestive linkage to chromosome 6p. Hum Genet, 122(6), 595–603. doi:10.1007/s00439-007-0436-x [PubMed: 17938964]

Yang J, Qiu H, Qu P, Zhang R, Zeng L, & Yan H (2015). Prenatal alcohol exposure and congenital heart defects: a meta-analysis. PLoS One, 10(6), e0130681. doi:10.1371/journal.pone.0130681 [PubMed: 26110619]

Yoon PW, Rasmussen SA, Lynberg MC, Moore CA, Anderka M, Carmichael SL, … Edmonds LD (2001). The National Birth Defects Prevention Study. Public Health Rep, 116 Suppl 1, 32–40. doi:10.1093/phr/116.S1.32

Zaidi S, Choi M, Wakimoto H, Ma L, Jiang J, Overton JD, … Lifton RP (2013). De novo mutations in histone-modifying genes in congenital heart disease. Nature, 498(7453), 220–223. doi:10.1038/nature12141 [PubMed: 23665959]

Zhang H, Xu C, Yang R, Chen H, Kong X, Qian L, … Sun W (2015). Silencing of nodal modulator 1 inhibits the differentiation of P19 cells into cardiomyocytes. Exp Cell Res, 331(2), 369–376. doi:10.1016/j.yexcr.2014.12.016 [PubMed: 25576386]

Zhao B, Lin Y, Xu J, Ni B, Da M, Ding C, … Ma H (2014). Replication of the 4p16 susceptibility locus in congenital heart disease in Han Chinese populations. PLoS One, 9(9), e107411. doi:10.1371/journal.pone.0107411 [PubMed: 25215500]

Zhao L, Li B, Dian K, Ying B, Lu X, Hu X, … Qin L (2015). Association between the European GWAS-identified susceptibility locus at chromosome 4p16 and the risk of atrial septal defect: a case-control study in Southwest China and a meta-analysis. PLoS One, 10(4), e0123959. doi:10.1371/journal.pone.0123959 [PubMed: 25875170]

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[†] (N = 3,978)			Replication[†] (N = 2,507)		
		Cases	Controls	P[‡]	Cases	Controls	P[‡]
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)[¶]				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)	

[†] 5 case families in the discovery cohort had unknown maternal race/ethnicity and age at delivery; 1 control infant in the replication cohort had unknown sex

[‡] 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

[§] SD = standard deviation

[¶] Sex is unknown for one replication control sample

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[†]	N Allele Transmitted	N Allele Untransmitted	OR[‡]	P
6	rs55877192	44129213	6p21.1	G	A	**Discovery**	**396**	**0.080**	**34**	**93**	**0.37**	**1.61x10⁻⁷**
						Replication	**257**	**0.095**	**33**	**65**	**0.51**	**1.60x10⁻³**
						Combined	**653**	**0.086**	**67**	**158**	**0.42**	**1.18x10⁻⁹**
10	rs113920486	54299613	10q21.1	G	C	Discovery	374	0.023	3	31	0.10	7.66x10⁻⁷
						Replication	269	0.014	6	9	0.67	0.61
						Combined	643	0.019	9	40	0.23	9.26x10⁻⁶
19	rs2360743	10736514	19p13.2	T	C	**Discovery**	**377**	**0.023**	**1**	**33**	**0.03**	**4.08x10⁻⁹**
						Replication	**250**	**0.013**	**0**	**13**	**0.00**	**2.44x10⁻⁴**
						Combined	**627**	**0.019**	**1**	**46**	**0.02**	**6.82x10⁻¹³**
X	rs5913271	79731210	Xq21.1	A	T	Discovery	401	0.039	4	34	0.12	6.04x10⁻⁷
						Replication	267	0.069	11	21	0.52	0.11
						Combined	668	0.051	15	55	0.27	1.65x10⁻⁶

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

[†] MAF = minor allele frequency, calculated in parents of triads used in TDT

[‡] OR = odds ratio

Table 3.

Single Nucleotide Polymorphisms (SNPs) with P<1x10^{-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.

Chromosome	SNP	Position	Locus	Minor Allele[†]	Major Allele	Discovery					Replication					Meta	
						N	MAF[‡]	OR[§]	SE[¶]	P	N	MAF[‡]	OR[§]	SE[¶]	P	OR[§]	P
2	rs4850063	4332775	2p25.3	G	A	1696	0.34	0.70	0.08	8.87x10^{-6}	1078	0.35	1.01	0.10	0.93	0.81	7.51x10^{-4}
5	rs7706812	160312017	5q34	G	T	1695	0.32	1.43	0.08	2.77x10^{-6}	1078	0.35	0.83	0.10	0.07	1.18	8.14x10^{-3}
6	rs9341603	77534953	6q14.1	T	C	1696	0.15	1.59	0.10	1.37x10^{-6}	1078	0.16	1.17	0.13	0.22	1.42	4.49x10^{-6}
8	rs330080	9154609	8p23.1	C	T	1696	0.46	0.72	0.07	6.31x10^{-6}	1077	0.46	0.97	0.09	0.77	0.81	2.00x10^{-4}
11	rs317518	110890363	11q23.1	C	T	1689	0.31	1.41	0.08	6.48x10^{-6}	1075	0.38	0.93	0.09	0.47	1.20	2.33x10^{-3}
16	rs9937546	73003719	16q22.3	C	T	1698	0.25	1.44	0.08	6.79x10^{-6}	1075	0.29	1.08	0.11	0.50	1.29	6.78x10^{-5}

[†] Minor allele is effect allele

[‡] MAF = minor allele frequency, calculated in controls

[§] OR = odds ratio

[¶] SE = standard error

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.

Chromosome	SNP	Position	Locus	Minor Allele[†]	Major Allele	Discovery					Replication					Meta	
						N	MAF[‡]	OR[§]	SE[¶]	P	N	MAF[‡]	OR[§]	SE[¶]	P	OR[§]	P
1	rs2737665	200062389	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	35611713	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	236941567	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	137281630	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	1672096	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**	**115282058**	**5q23.1**	**T**	**C**	**1431**	**0.07**	**0.34**	**0.22**	**1.42×10^{-6}**	**897**	**0.05**	**0.56**	**0.29**	**0.04**	**0.41**	**4.69×10^{-7}**
6	rs35072477	9799050	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	137842379	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	38678740	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	98607989	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	128853643	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	30230749	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	72007984	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	23535168	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	12937344	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	47039132	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	29853202	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}

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

[†] Minor allele is effect allele

[‡] MAF = minor allele frequency, calculated in controls

[§] OR = odds ratio

[¶] SE = standard error