HHS Public Access

Author manuscript

Birth Defects Res A Clin Mol Teratol. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Birth Defects Res A Clin Mol Teratol. 2015 October ; 103(10): 834–842. doi:10.1002/bdra.23370.

Genetic Variation Affects Congenital Heart Defect Susceptibility in Offspring Exposed to Maternal Tobacco Use

Xinyu Tang[1], **Charlotte A. Hobbs**[2], **Mario A. Cleves**[2], **Stephen W. Erickson**[3], **Stewart L. MacLeod**[2], and **Sadia Malik** [,2] **the National Birth Defects Prevention Study**

[1]Biostatistics Program, Department of Pediatrics, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas

[2]Division of Birth Defects Research, Department of Pediatrics, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, Arkansas

[3]Department of Biostatistics, College of Public Health, University of Arkansas for Medical Sciences, Little Rock, Arkansas

Abstract

Background—Congenital heart defects (CHDs) are among the most prevalent and serious birth defects, occurring in 8 to 10 of every 1000 live births in the United States. Epidemiologic studies have reported an association between CHDs and maternal smoking, but it remains unknown how genes impact the susceptibility of offspring to CHDs in the presence of maternal tobacco use.

Methods—Using data from 403 case- and 219 control-parental triads enrolled in the National Birth Defects Prevention Study between 1998 and 2008, we investigated the association between CHDs and maternal and infant genetic variants involved in the tobacco metabolism and DNA repair pathways among mothers who smoked prenatally.

Results—The maternal genotypes of single nucleotide polymorphisms in the excision repair cross-complementation group 1 (*ERCC1*), poly (ADP-ribose) polymerase 2 (*PARP2*), and ERCC5 genes were identified to be significantly associated with the occurrence of CHDs in the presence of maternal tobacco use. Our analysis also revealed a moderate association between the infant genotypes of polymorphisms in the O-sialoglycoprotein endopeptidase (*OSGEP*) gene and increased risk of CHDs among mothers who smoked.

Conclusion—Our study provides evidence that maternal and infant polymorphisms within the *ERCC1*, *PARP2*, *ERCC5*, and *OSGEP* genes are associated with CHD risk in the presence of maternal tobacco use. These results may provide insight into the susceptibility of having a pregnancy affected by CHDs among women who smoke.

*Correspondence to: Sadia Malik, Division of Birth Defects, Department of Pediatrics, College of Medicine, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, 13 Children's Way, Slot 512-40, Little Rock, AR 72202. malikmail@icloud.com.

Additional Supporting Information may be found in the online version of this article.

All authors have stated that they have no conflict of interest regarding this manuscript.

Keywords

congenital heart defects; single nucleotide polymorphisms; genetic variants; tobacco use; national birth defects prevention study

Introduction

Congenital heart defects (CHDs) are among the most prevalent and serious birth defects, occurring in 8 to 10 of every 1000 live births in the United States (Boneva et al., 2001). Affected infants who survive often require repeated surgeries and lengthy hospitalizations, and many will have a lifetime of disability, imposing a significant burden on families (Reller et al., 2008; Razzaghi et al., 2014). There are several proposed risk factors for CHDs, and maternal smoking is among them (Malik et al., 2008; Alverson et al., 2011; Patel et al., 2012; Fung et al., 2013; Lee and Lupo, 2013). In the United States, an estimated 25% of reproductive-aged women smoke cigarettes, and at least 14% continue smoking during pregnancy (Tong et al., 2013; Robbins et al., 2014).

Maternal tobacco use and exposure to environmental cigarette smoke during pregnancy have been linked to intrauterine growth retardation, prematurity, perinatal mortality, and malformations (English and Eskenazi, 1992; Dejin-Karlsson et al., 1998; Misra and Nguyen, 1999; Woods and Raju, 2001; Secker-Walker and Vacek, 2003). Multiple epidemiologic studies and meta-analyses have specifically investigated the association between CHDs and maternal tobacco use (Alverson et al., 2011; Karatza et al., 2011; Patel et al., 2012; Lee and Lupo, 2013). Hackshaw et al. (2011) reviewed 172 publications from 1959 to 2010 and included 173,687 malformed cases and 11.7 million controls in a meta-analyses. They showed a significant positive association between maternal tobacco use and CHDs with an estimated odds ratio of 1.09 (95% confidence interval [CI], 1.02–1.17).

Tobacco smoke is a mixture of volatile compounds and particulate matter comprising numerous mutagenic compounds, only a few of which have been examined in isolation. Over two-thirds of all teratogenic chemicals, including tobacco metabolites are substrates for cytochrome p450 (CYP) enzymes (Guengerich and Shimada, 1991; Guengerich, 2005; Jalas et al., 2005). Fifteen CYP enzymes catalyze transformation of xenobiotic chemicals, and 10 of these play a role in tobacco metabolism (Guengerich, 2005). *CYP* genes are important in modulating the toxicity of potentially teratogenic intermediates of tobacco metabolism (Guengerich, 2005). Typically, *CYP* polymorphisms cause increased enzymatic activity and increased production of protoxicants (Ingelman-Sundberg, 2001; Wogan et al., 2004). Clubfoot has been associated with polymorphisms in cytochrome 1A2, although an association with maternal smoking was not found in this small study (Sommer et al., 2011). Shi et al. (2007) studied the association between maternal smoking and genetic variants of *CYP* and *GSTT1* and found an interaction between a *GSTT1* variant, maternal smoking and cleft palate in offspring. It is biologically plausible that polymorphisms in either the maternal or fetal genotype could alter tobacco metabolism, resulting in increased DNA damage in mother or fetus, thereby predisposing a smoking mother to having a CHD-affected infant.

Maternal tobacco use during pregnancy has been shown to result in DNA damage to fetal cells (Sram et al., 1999; Shantakumar et al., 2005; Tang et al., 2006). Perera et al. (2004) found that the amount of DNA damage per unit dose of polycyclic aromatic hydrocarbons, which are prominent in tobacco smoke, was on the order of 10-fold higher in the fetus than the mother. Polycyclic aromatic hydrocarbons combine with DNA to form bulky adducts that have been shown to cause fetal aberrations in cord blood (Whyatt et al., 2001; Phillips, 2002; Perera et al., 2004). If left unrepaired, these generated DNA "damage" intermediates can cause nucleotide sequence changes that could potentially contribute to teratogenesis (Ferguson and Ford, 1997). Therefore, cells are equipped with DNA repair enzymes to repair these DNA modifications. DNA damage repair is a primary defense mechanism against mutagenic exposures (Helzlsouer et al., 1996; Friedberg and Friedberg, 2006). Among the four major repair pathways in human cells, the nucleotide-excision repair (NER) and base-excision repair (BER) pathways are the primary pathways involved in repair of DNA damage secondary to tobacco exposure (Mohrenweiser et al., 2003).

Current epidemiologic evidence has linked tobacco metabolism and DNA repair genes in mothers and risk of oral clefts in offspring (van Rooij et al., 2001; Olshan et al., 2005), but we are just beginning to understand how genes impact susceptibility of offspring to other birth defects. This study was conducted to investigate the association between CHDs and maternal and infant genetic variants in the tobacco metabolism and DNA repair pathways in the presence of maternal tobacco use. A comprehensive list of 274 single nucleotide polymorphisms (SNPs) from 10 *CYP* genes and 33 NER or BER pathways candidate genes was studied. We hypothesized that both maternal and infant genetic variants involved in tobacco metabolism and DNA repair pathways were associated with the occurrence of CHDs.

Materials and Methods

ETHICS STATEMENT

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

STUDY POPULATION

The NBDPS was a population-based case-control study designed to evaluate genetic, environmental, and behavior factors associated with the occurrence of major non-syndromic birth defects (Yoon et al., 2001). All families included in this study were recruited for the NBDPS and identified through population-based birth defects surveillance systems in 10 sites (Arkansas, California, Iowa, Massachusetts, New Jersey, New York, Texas, Georgia, North Carolina, and Utah). The case-parental and control-parental triads were only included in this study if (1) the mother completed the interview, (2) DNA samples were available, including all family members, mother, father, baby or any combination thereof, (3) the

mother had an expected date of delivery between January 1998 and January 2008, (4) the mother smoked during any of the following 6 time periods (i.e., exposure window): 3 months before pregnancy (B3), 2 months before pregnancy (B2), 1 month before pregnancy (B1), the first month of pregnancy (P1), the second month of pregnancy (P2), and the third month of pregnancy (P3), (5) the case infant was singleton live-born infant with CHD, and (6) the control infant was singleton live-born infant without any major birth defects. Cases where the pregnancy was affected by a known single gene disorder or chromosomal abnormality syndrome were excluded. We included 3-month preconception into the smoking exposure window to include many mothers who may unknowingly continue smoking in early pregnancy as up to half of all U.S. pregnancies are unplanned (Finer and Zolna, 2011, 2014). Medical records were abstracted by trained health information specialists. All diagnostic tests on cardiac NBDPS case infants were reviewed to ensure uniform criteria for diagnoses. Diagnostic tests included results from echocardiograms, surgical reports, cardiac catheterizations, and autopsies. Cases were identified using a classification system developed for NBDPS, which incorporated three dimensions of cardiac phenotype, cardiac complexity, and extracardiac anomalies (Botto et al., 2007).

DATA COLLECTION

Both case and control mothers participated in a 1-hr interview carried out by female interviewers using a computer-assisted telephone questionnaire (Yoon et al., 2001). Interviews were conducted in English or Spanish between 6 weeks and 24 months after the expected date of delivery (Yoon et al., 2001). Upon completion of the maternal interviews, case and control families received a buccal cell collection kit for collecting cheek cell samples from offspring and both parents if available (Yoon et al., 2001).

GENOTYPING AND QUALITY CONTROL

Laboratories that use NBDPS archival DNA samples must demonstrate their proficiency in genotyping techniques by passing an External Quality Assessment. The External Quality Assessment for high-throughput genotyping platforms, such as the Illumina Golden Gate assay used in this project, consists of genotyping of a single SNP in one externally supplied blood-buccal trio. Results from the laboratory must be concordant (99%) for paired blood-derived and buccal-derived DNA and between genomic and whole genome amplified DNA. In addition, inter-lab results must be concordant as well as concordance between precharacterized DNA and third-party results. Negative controls must not yield results and genotypes of trios must be consistent with Mendelian inheritance. The Arkansas Center for Birth Defects Research and Prevention has consistently scored 100% on NBDPS External Quality Assessment.

A custom panel of 328 SNPs from 43 genes involved in tobacco metabolism and DNA repair pathways (i.e., NER and BER pathways) was selected for analysis. DNA was extracted from buccal cell samples using Puregene DNA purification reagents (Qigen, Valencia, CA), and genomic DNA was used as a template for whole genome amplification (WGA) using the GenomePlex WGA kit according to the manufacturer's protocol (Sigma, St. Louis, MO), as described by Hobbs et al. (2014). Genotyping was performed using 200 ng of WGA DNA on the Illumina customizable Golden Gate platform (Fan et al., 2006). An

initial set of genotype calls was generated using GenCall, Illumina's proprietary genotyping algorithm. These initial calls, plus the raw fluorescent intensity data from the microarrays, were used as inputs to SNPMClust, a bivariate Gaussian model-based genotype-clustering and -calling algorithm developed in-house at the Arkansas Center for Birth Defects Research and Prevention. SNPMClust is available as a contributed package on the Comprehensive R Archive Network and provides a likelihood-based measure of call uncertainty. As described by Hobbs et al. (2014), we observed a 99.2% genotyping concordance rate between WGA-buccal and blood-derived sources of DNA, in a pilot study of 94 women who had provided both blood and buccal samples and whose data underwent the same genotyping and quality control procedures. After running SNPMClust on the 328 SNPs, any genotype call with an uncertainty score greater than 0.5% was set to a no-call. Following this step, 43 SNPs (11.3%) with no-call rates greater than 10% were considered unreliable and dropped from analysis. This substantial number of SNPs dropped from the panel is consistent with earlier projects which have also used a Golden Gate custom-designed genotyping chip with DNA samples that had undergone WGA (Chowdhury et al., 2012; Hobbs et al., 2014; Li et al., 2014). We also excluded 11 SNPs which deviated significantly from Hardy-Weinberg equilibrium (HWE) ($p < 10^{-4}$). Hardy-Weinberg equilibrium was tested only in controls, because disequilibrium in a mixture of cases and controls could be caused by disease association. A total of 1263 individual samples were genotyped, and 133 samples were removed from analysis due to study ineligibility ($N = 5$), high no-call rates (>15%) ($N = 18$), or high rates of Mendelian inconsistency ($N = 110$), resulting in an analytical sample consisting of 1130 individuals from 403 case and 219 control families. The final analysis dataset included genotype data from 1130 (89%) individuals on 274 (72%) SNPs, with a no-call rate of 1.2%.

STATISTICAL METHODS

Descriptive statistics were expressed as mean (standard deviation) for continuous variables, and frequency (percentage) for categorical variables. Because both case-parental and control-parental triads were genotyped, a hybrid design was used for statistical analysis (Weinberg and Umbach, 2005). The use of a hybrid design allows the simultaneous evaluation of maternal and infant genetic effects, with estimates of infant genetic effects largely insensitive to population admixture. A log-linear model was fitted for each SNP as a function of mating type, case–control status, an interaction between case–control status and maternal genotype, and an interaction between case/control status and infant genotype. This model was designated to assess the main effects of maternal and infant genotypes on the risk of CHDs. When fitting the models, mating symmetry was assumed, and thus six different mating types were used. We also assumed multiplicative risk per allele (log-additive genetic effect), under which the indicators for both maternal and infant genotypes were coded as 0, 1, or 2, representing the number of copies of the variant allele.

We computed Bayesian false-discovery probabilities (BFDPs) for evaluating the chance of false-positive associations using the relative risk (RR) estimates and their 95% CIs obtained from the log-linear models (Wakefield, 2007). BFDP is used to find a balance between the relative costs of false negative and false positive; in the results section, we reported associations where BFDP < 0.80, which is a commonly used threshold suggested by

Wakefield (Wakefield, 2007; Liu et al., 2010; Oh et al., 2010; Park et al., 2010). Data were analyzed using the LEM (Vermunt, 1997) software for fitting log-linear models, R v3.1.0 (Team, 2013) for computing descriptive statistics and BFDPs, and HaploView 4.2 (Barrett, 2009) for assessing the linkage disequilibrium.

Results

POPULATION CHARACTERISTICS

The final population sample included 403 case-families and 219 control-families. Among 403 cases, there were 17 (4%) cases affected with anomalous pulmonary venous return, 14 (3%) cases affected with atrioventricular septal defects, 69 (17%) cases affected with conotruncal heart defects, 61 (15%) cases affected with left obstructive heart defects, 68 (17%) cases affected with right obstructive heart defects, and 174 (43%) cases affected by septal defects. The maternal demographics and behaviors are summarized for case and control-families respectively in Table 1. Case mothers were slightly older than the control mothers (25.9 ± 6.0 years vs. 24.9 ± 5.4 years, respectively, $p = 0.03$), but the two groups were similar with respect to all other demographic and behavioral characteristics ($p > 0.05$).

MATERNAL GENETIC VARIANTS

A total of 274 SNPs in 43 genes were included in the final analysis. The counts of SNPs in each gene and chromosome are displayed in Supplemental Table S1, which is available online. The maternal genotypes of SNP rs3212948 in the excision repair cross-complementation group 1 (*ERCC1*) gene, SNP rs3093930 in the poly (ADP-ribose) polymerase 2 (*PARP2*) gene, and SNP rs4150386 in the excision repair cross-complementation group 5 (*ERCC5*) gene were significantly associated with the occurrence of CHDs among mothers who smoked during the exposure window (Table 2, BFDP < 0.8). The risk of delivering infants with CHDs among women who smoked during the exposure window and carried CG genotype for rs3212948 was 24% less (95% CI, 6–38%) than that among women carrying the GG genotype. Accordingly, the risk among women carrying CC genotype for rs3212948 was 42% (95% CI, 12–62%) less than the risk among those carrying GG genotype. Under the multiplicative (log-linear) genetic model, the estimated RR (95% CI) for carrying 2 copies of the risk allele is the square of the estimated RR (95% CI) for carrying 1 copy of the risk allele, with 0 copies of the risk allele as the reference group. For simplicity, only the estimated RR (95% CI) for carrying 1 copy of the risk allele compared with 0 copies was reported in the following results. Similarly, the risk of delivering infants with CHDs among women who smoked during the exposure window and carried AG genotype for rs3093930 was 23% (95% CI, 6–37%) less than that among women carrying the GG genotype. The risk of delivering infants with CHDs among women carrying AC genotype for rs4150386 was estimated to be 39% (95% CI, 13–58%) less than those carrying AA genotype.

INFANT GENETIC VARIANTS

The infant genotypes of four SNPs in the O-sialoglycoprotein endopeptidase (*OSGEP*) gene were significantly associated with the occurrence of CHDs in the presence of maternal tobacco use (Table 2). The risk of CHDs among infants carrying AG genotype for

rs1320150 was estimated to be 1.39 (95% CI, 1.11–1.75) times more than those carrying AA genotype for the same SNP. In addition, infant genotypes for three other SNPs (rs938881, rs2275007, and rs883037) within the same gene were found to have similar infant genetic effects as rs1320150 on the risk of CHDs (all BFDP < 0.8). All four significant SNPs in the *OSGEP* gene were determined to be in high linkage disequilibrium ($D' \geq 0.99$).

The same analyses were replicated among Caucasians only. Similarly, we identified the maternal genotype of SNP rs3212948 in the *ERCC1* gene and the infant genotypes of the same four SNPs in the *OSGEP* gene to be significantly associated with the occurrence of CHDs (data not shown).

Discussion

Our findings indicate that multiple genetic variants in genes involved in the DNA repair pathways have modest effects on the risk of CHDs through either maternal or infant genetic effects in the presence of maternal tobacco use. These findings provide insights into the genetic susceptibility of CHDs. Among 274 CYP, NER, and BER polymorphisms, multiple SNPs were identified to influence risk of CHDs. All three SNPs with significant maternal effects were located in 3 respective genes (*ERCC1*, *PARP2*, and *ERCC5*), and the minor alleles of these SNPs were identified to be associated with decreased risk of CHDs among mothers who smoked during the exposure window. In infants, the minor alleles of four SNPs in the *OSGEP* gene were associated with increased CHD risk in the presence of maternal tobacco use.

Our study revealed associations between the risk of CHDs and polymorphisms in the *ERCC1* and *ERCC5* genes through maternal genetic main effects among mothers who smoked. The *ERCC1* gene encodes a 297 amino acid product, which comprises a subunit of the NER complex required for the incision step (Lu et al., 2014). The *ERCC5* gene encodes the 3′-endonuclease xeroderma pigmentosum G (XPG), which is involved in the excision step of NER (Garcia-Closas et al., 2006; Lu et al., 2014). Mutations in the XPG genes can lead to change in the function of DNA damage repair (Lu et al., 2014). Huang et al. (2006) showed that polymorphisms in the NER genes can modify smoking related risk of cancer; it is possible that this genetic variation may be associated with a protective effect (Huang et al., 2006). Gene–environment interactions involving maternal smoking have been implicated in the etiology of cleft palate (Beaty et al., 2011; Reiter et al., 2012), and maternal exposure to toxicants has been associated with genetic variants in the ATP-binding cassette, sub-family B (MDR/TAP), member 1 (*ABCB1*) gene, and CHD (Wang et al., 2013), but no studies have studied the association between *NER* gene-variants and CHD.

The maternal genotype of one SNP in the *PARP2* gene was identified to be significantly associated with a decreased risk of CHDs in the presence of maternal tobacco use. Poly (ADP-ribose) polymerases (*PARP*s) are enzymes involved in various cytoplasmic and nuclear processes, including inflammation, mitochondrial metabolism, DNA damage repair, and transcriptional regulation (De Vos et al., 2012; Gibson and Kraus, 2012; Liang et al., 2013). The *PARP2* gene belongs to the *PARP* superfamily (Ame et al., 1999; Szanto et al., 2014). Recent studies show that the *PARP2* gene represses transcription by decreasing the

activities or the amounts of transcription factors (Szanto et al., 2012; Liang et al., 2013). However, the molecular basis of how *PARP2* directly represses promoters and the resultant cellular outcomes remain largely unknown (Liang et al., 2013).

Our analysis also revealed a moderate association between polymorphisms in the *OSGEP* gene of infants and increased risk of CHDs among mothers who smoked during the exposure window. The *OSGEP* gene is a unique enzyme that specifically cleaves the polypeptide backbone of those membrane glycoproteins that contain clusters of O-linked sialoglycans (Abdullah et al., 1992; Ikeda et al., 2002). Based on the evidence that both the *OSGEP* and *APEX* genes exhibited the same expression pattern in several different tissues using the Northern blot analysis, it was suspected that a common regulatory mechanism may be involved in the transcription of *OSGEP* and *APEX* (Seki et al., 2002). The APEX nuclease (multifunctional DNA repair enzyme) 1 (*APEX1*) gene has been found to play a major role in cardiovascular development in zebra fish, and mutations have been found to affect cardiac looping and cause pericardial effusions (Wang et al., 2006). It has also been shown that the genetic variation in the *APEX1* gene was associated with a decreased risk of oral clefts (Olshan et al., 2005). However, there are no published genetic studies investigating associations between polymorphisms in the *OSGEP* gene with any human diseases.

There are several strengths of this study including population-based ascertainment of cases, and a rigorous analytic inquiry into both the maternal and infant genetic effects on CHD risk. There are also limitations. First, there was a substantial number of SNPs removed from analysis because of poor fluorescent intensity data quality. As described above, however, we applied stringent standards which give us confidence in the accuracy of those SNPs which are included in the analysis. Second, our study was restricted to mothers who smoked prenatally and we were therefore unable to test for gene–environment interactions. In addition, the potential confounding effects from other environmental/behavioral factors were not considered. We were unable to test for paternal or environmental tobacco smoke which has been shown to have an association with CHD (Chevrier et al., 2008; Deng et al., 2013). Third, due to the relative small sample size for each sub-phenotype, the analysis was not replicated among each sub-phenotype of CHDs. Finally, the interview data were self-reported, and thus not objectively measured. Although there were potential measurement errors in the collected interview data, the measurement errors were equally distributed among case and control families, and thus the RR estimates were not biased toward either side.

Smoking-cessation programs in pregnancy have not incorporated genetic polymorphisms but have historically shown higher rates of smoking cessation by conventional methods, such as counseling (Lawrence et al., 2003; de Weerd et al., 2004). Ito et al. (2006) showed a 10.8% decrease in smoking among female patients who were told that they had a polymorphism that would increase their risk of esophageal and lung cancer if they smoked (Ito et al., 2006). We hope the results of this study will eventually add to a high risk maternal profile that would identify mothers who would benefit from preconceptional counseling. Our results provide evidence for the impact of polymorphisms in the DNA repair pathways on the etiologies of CHDs in the presence of maternal tobacco use. However, individual SNP

associations must be considered provisional and will need to be replicated in another population. Further studies, will need to incorporate functional genetics and targeted deep sequencing while improving and enhancing information collected on environmental and maternal factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the generous participation of the numerous families that made this research study possible. We also thank the Centers for Birth Defects Research and Prevention in California, Georgia, Iowa, Massachusetts, New Jersey, New York, North Carolina, Texas, and Utah for their contribution of data and manuscript review. The authors thank Dr. Andrew Olshan, Professor and Department Chair of Epidemiology, University of North Carolina, for his assistance with the proposal and grant preparation, and Ashley S. Block for assistance in preparation of the manuscript. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention.

Contract grant sponsor: Heartland Affiliate Research Committee of the American Heart Association (AHA); contract grant number: 0760053Z.

Contract grant sponsor: National Institutes of Health (NIH) Mentored Clinical Scientist Development Award (K08); contract grant number: 5K08HL090494.

Supported by the Heartland Affiliate Research Committee of the American Heart Association (AHA) under award number 0760053Z and the National Institutes of Health (NIH) Mentored Clinical Scientist Development Award (K08) under award number 5K08HL090494.

References

Abdullah KM, Udoh EA, Shewen PE, Mellors A. A neutral glycoprotease of Pasteurella haemolytica A1 specifically cleaves O-sialoglycoproteins. Infect Immun. 1992; 60:56–62. [PubMed: 1729196]

Alverson CJ, Strickland MJ, Gilboa SM, Correa A. Maternal smoking and congenital heart defects in the Baltimore-Washington Infant Study. Pediatrics. 2011; 127:e647–e653. [PubMed: 21357347]

Ame JC, Rolli V, Schreiber V, et al. PARP-2, A novel mammalian DNA damage-dependent poly (ADP-ribose) polymerase. J Biol Chem. 1999; 274:17860–17868. [PubMed: 10364231]

Barrett JC. Haploview: visualization and analysis of SNP genotype data. Cold Spring Harb Protoc. 2009; 2009:pdb ip71. [PubMed: 20147036]

Beaty TH, Ruczinski I, Murray JC, et al. Evidence for gene-environment interaction in a genome wide study of nonsyndromic cleft palate. Genet Epidemiol. 2011; 35:469–478. [PubMed: 21618603]

Boneva RS, Botto LD, Moore CA, et al. Mortality associated with congenital heart defects in the United States: trends and racial disparities, 1979–1997. Circulation. 2001; 103:2376–2381. [PubMed: 11352887]

Botto LD, Lin AE, Riehle-Colarusso T, et al. Seeking causes: classifying and evaluating congenital heart defects in etiologic studies. Birth Defects Res A Clin Mol Teratol. 2007; 79:714–727. [PubMed: 17729292]

Chevrier C, Bahuau M, Perret C, et al. Genetic susceptibilities in the association between maternal exposure to tobacco smoke and the risk of nonsyndromic oral cleft. Am J Med Genet A. 2008; 146A:2396–2406. [PubMed: 18698632]

Chowdhury S, Hobbs CA, MacLeod SL, et al. Associations between maternal genotypes and metabolites implicated in congenital heart defects. Mol Genet Metab. 2012; 107:596–604. [PubMed: 23059056]

De Vos M, Schreiber V, Dantzer F. The diverse roles and clinical relevance of PARPs in DNA damage repair: current state of the art. Biochem Pharmacol. 2012; 84:137–146. [PubMed: 22469522]

de Weerd S, Polder JJ, Cohen-Overbeek TE, et al. Preconception care: preliminary estimates of costs and effects of smoking cessation and folic acid supplementation. J Reprod Med. 2004; 49:338–344. [PubMed: 15214705]

Dejin-Karlsson E, Hanson BS, Ostergren PO, et al. Does passive smoking in early pregnancy increase the risk of small-for-gestational-age infants? Am J Public Health. 1998; 88:1523–1527. [PubMed: 9772856]

Deng K, Liu Z, Lin Y, et al. Periconceptional paternal smoking and the risk of congenital heart defects: a case-control study. Birth Defects Res A Clin Mol Teratol. 2013; 97:210–216. [PubMed: 23554276]

English PB, Eskenazi B. Reinterpreting the effects of maternal smoking on infant birthweight and perinatal mortality: a multivariate approach to birthweight standardization. Int J Epidemiol. 1992; 21:1097–1105. [PubMed: 1483814]

Fan JB, Chee MS, Gunderson KL. Highly parallel genomic assays. Nat Rev Genet. 2006; 7:632–644. [PubMed: 16847463]

Ferguson LR, Ford JH. Overlap between mutagens and teratogens. Mutat Res. 1997; 396:1–8. [PubMed: 9434857]

Finer LB, Zolna MR. Unintended pregnancy in the United States: incidence and disparities, 2006. Contraception. 2011; 84:478–485. [PubMed: 22018121]

Finer LB, Zolna MR. Shifts in intended and unintended pregnancies in the United States, 2001–2008. Am J Public Health. 2014; 104(Suppl 1):S43–S48. [PubMed: 24354819]

Friedberg, EC.; Friedberg, EC. DNA repair and mutagenesis. 2nd. Washington, D.C.: ASM Press; 2006.

Fung A, Manlhiot C, Naik S, et al. Impact of prenatal risk factors on congenital heart disease in the current era. J Am Heart Assoc. 2013; 2:e000064. [PubMed: 23727699]

Garcia-Closas M, Malats N, Real FX, et al. Genetic variation in the nucleotide excision repair pathway and bladder cancer risk. Cancer Epidemiol Biomarkers Prev. 2006; 15:536–542. [PubMed: 16537713]

Gibson BA, Kraus WL. New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. Nat Rev Mol Cell Biol. 2012; 13:411–424. [PubMed: 22713970]

Guengerich, FP. Human cytochrome P450 enzymes. In: Ortiz de Montellano, P., editor. Cytochrome P450. New York: Springer; 2005. p. 377-530.

Guengerich FP, Shimada T. Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. Chem Res Toxicol. 1991; 4:391–407. [PubMed: 1912325]

Hackshaw A, Rodeck C, Boniface S. Maternal smoking in pregnancy and birth defects: a systematic review based on 173 687 malformed cases and 11.7 million controls. Hum Reprod Update. 2011; 17:589–604. [PubMed: 21747128]

Helzlsouer KJ, Harris EL, Parshad R, et al. DNA repair proficiency: potential susceptiblity factor for breast cancer. J Natl Cancer Inst. 1996; 88:754–755. [PubMed: 8637030]

Hobbs CA, Cleves MA, Macleod SL, et al. Conotruncal heart defects and common variants in maternal and fetal genes in folate, homocysteine, and transsulfuration pathways. Birth Defects Res A Clin Mol Teratol. 2014; 100:116–126. [PubMed: 24535845]

Huang WY, Berndt SI, Kang D, et al. Nucleotide excision repair gene polymorphisms and risk of advanced colorectal adenoma: XPC polymorphisms modify smoking-related risk. Cancer Epidemiol Biomarkers Prev. 2006; 15:306–311. [PubMed: 16492920]

Ikeda S, Ayabe H, Mori K, et al. Identification of the functional elements in the bidirectional promoter of the mouse O-sialoglycoprotein endopeptidase and APEX nuclease genes. Biochem Biophys Res Commun. 2002; 296:785–791. [PubMed: 12200116]

Ingelman-Sundberg M. Genetic variability in susceptibility and response to toxicants. Toxicol Lett. 2001; 120:259–268. [PubMed: 11323184]

Ito H, Matsuo K, Wakai K, et al. An intervention study of smoking cessation with feedback on genetic cancer susceptibility in Japan. Prev Med. 2006; 42:102–108. [PubMed: 16325899]

Jalas JR, Hecht SS, Murphy SE. Cytochrome P450 enzymes as catalysts of metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)21-butanone, a tobacco specific carcinogen. Chem Res Toxicol. 2005; 18:95–110. [PubMed: 15720112]

Karatza AA, Giannakopoulos I, Dassios TG, et al. Periconceptional tobacco smoking and isolated congenital heart defects in the neonatal period. Int J Cardiol. 2011; 148:295–299. [PubMed: 19951824]

Lawrence T, Aveyard P, Evans O, Cheng KK. A cluster randomised controlled trial of smoking cessation in pregnant women comparing interventions based on the transtheoretical (stages of change) model to standard care. Tob Control. 2003; 12:168– 177. [PubMed: 12773727]

Lee LJ, Lupo PJ. Maternal smoking during pregnancy and the risk of congenital heart defects in offspring: a systematic review and metaanalysis. Pediatr Cardiol. 2013; 34:398–407. [PubMed: 22886364]

Li M, Cleves MA, Mallick H, et al. A genetic association study detects haplotypes associated with obstructive heart defects. Hum Genet. 2014; 133:1127–1138. [PubMed: 24894164]

Liang YC, Hsu CY, Yao YL, Yang WM. PARP-2 regulates cell cycle-related genes through histone deacetylation and methylation independently of poly (ADP-ribosyl)ation. Biochem Biophys Res Commun. 2013; 431:58–64. [PubMed: 23291187]

Liu Y, Shete S, Etzel CJ, Scheurer M, et al. Polymorphisms of LIG4, BTBD2, HMGA2, and RTEL1 genes involved in the double-strand break repair pathway predict glioblastoma survival. J Clin Oncol. 2010; 28:2467–2474. [PubMed: 20368557]

Lu B, Li J, Gao Q, et al. Laryngeal cancer risk and common single nucleotide polymorphisms in nucleotide excision repair pathway genes ERCC1, ERCC2, ERCC3, ERCC4, ERCC5 and XPA. Gene. 2014; 542:64–68. [PubMed: 24582975]

Malik S, Cleves MA, Honein MA, et al. Maternal smoking and congenital heart defects. Pediatrics. 2008; 121:e810–e816. [PubMed: 18381510]

Misra DP, Nguyen RH. Environmental tobacco smoke and low birth weight: a hazard in the workplace? Environ Health Perspect. 1999; 107(Suppl 6):897–904. [PubMed: 10592147]

Mohrenweiser HW, Wilson DM III, Jones IM. Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. Mutat Res. 2003; 526:93– 125. [PubMed: 12714187]

Oh SS, Chang SC, Cai L, et al. Single nucleotide polymorphisms of 8 inflammation-related genes and their associations with smoking-related cancers. Int J Cancer. 2010; 127:2169–2182. [PubMed: 20112337]

Olshan AF, Shaw GM, Millikan RC, et al. Polymorphisms in DNA repair genes as risk factors for spina bifida and orofacial clefts. Am J Med Genet A. 2005; 135:268–273. [PubMed: 15887293]

Park SL, Bastani D, Goldstein BY, et al. Associations between NBS1 polymorphisms, haplotypes and smoking-related cancers. Carcinogenesis. 2010; 31:1264–1271. [PubMed: 20478923]

Patel SS, Burns TL, Botto LD, et al. Analysis of selected maternal exposures and non-syndromic atrioventricular septal defects in the National Birth Defects Prevention Study, 1997– 2005. Am J Med Genet A. 2012; 158A:2447–2455. [PubMed: 22903798]

Perera FP, Rauh V, Whyatt RM, et al. Molecular evidence of an interaction between prenatal environmental exposures and birth outcomes in a multiethnic population. Environ Health Perspect. 2004; 112:626–630. [PubMed: 15064172]

Phillips DH. Smoking-related DNA and protein adducts in human tissues. Carcinogenesis. 2002; 23:1979–2004. [PubMed: 12507921]

Razzaghi H, Oster M, Reefhuis J. Long term outcomes in children with congenital heart disease: National Health Interview Survey. J Pediatr. 2014; 166:119–124. [PubMed: 25304924]

Reiter R, Brosch S, Ludeke M, et al. Genetic and environmental risk factors for submucous cleft palate. Eur J Oral Sci. 2012; 120:97–103. [PubMed: 22409215]

Reller MD, Strickland MJ, Riehle-Colarusso T, et al. Prevalence of congenital heart defects in metropolitan Atlanta, 1998–2005. J Pediatr. 2008; 153:807–813. [PubMed: 18657826]

Robbins CL, Zapata LB, Farr SL, et al. Core state preconception health indicators - pregnancy risk assessment monitoring system and behavioral risk factor surveillance system, 2009. MMWR Surveill Summ. 2014; 63:1–62. [PubMed: 24759729]

Secker-Walker RH, Vacek PM. Relationships between cigarette smoking during pregnancy, gestational age, maternal weight gain, and infant birthweight. Addict Behav. 2003; 28:55–66. [PubMed: 12507527]

Seki Y, Ikeda S, Kiyohara H, et al. Sequencing analysis of a putative human O-sialoglycoprotein endopeptidase gene (OSGEP) and analysis of a bidirectional promoter between the OSGEP and APEX genes. Gene. 2002; 285:101–108. [PubMed: 12039036]

Shantakumar S, Gammon MD, Eng SM, et al. Residential environmental exposures and other characteristics associated with detectable PAH-DNA adducts in peripheral mononuclear cells in a population-based sample of adult females. J Expo Anal Environ Epidemiol. 2005; 15:482–490. [PubMed: 15856074]

Shi M, Christensen K, Weinberg CR, et al. Orofacial cleft risk is increased with maternal smoking and specific detoxification-gene variants. Am J Hum Genet. 2007; 80:76–90. [PubMed: 17160896]

Sommer A, Blanton SH, Weymouth K, et al. Smoking, the xenobiotic pathway, and clubfoot. Birth Defects Rest A Clin Mol Teratol. 2011; 91:20–28.

Sram RJ, Binkova B, Rossner P, et al. Adverse reproductive outcomes from exposure to environmental mutagens. Mutat Res. 1999; 428:203–215. [PubMed: 10517994]

Szanto M, Brunyanszki A, Kiss B, et al. Poly(ADP-ribose) polymerase-2: emerging transcriptional roles of a DNA-repair protein. Cell Mol Life Sci. 2012; 69:4079–4092. [PubMed: 22581363]

Szanto M, Brunyanszki A, Marton J, et al. Deletion of PARP-2 induces hepatic cholesterol accumulation and decrease in HDL levels. Biochim Biophys Acta. 2014; 1842:594–602. [PubMed: 24365238]

Tang D, Li TY, Liu JJ, et al. PAH-DNA adducts in cord blood and fetal and child development in a Chinese cohort. Environ Health Perspect. 2006; 114:1297–1300. [PubMed: 16882543]

Team, RC. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013.

Tong VT, Dietz PM, Morrow B, et al. Trends in smoking before, during, and after pregnancy–pregnancy risk assessment monitoring system, United States, 40 sites, 2000–2010. MMWR Surveill Summ. 2013; 62:1–19. [PubMed: 24196750]

van Rooij IA, Wegerif MJ, Roelofs HM, et al. Smoking, genetic polymorphisms in biotransformation enzymes, and non-syndromic oral clefting: a gene-environment interaction. Epidemiology. 2001; 12:502–507. [PubMed: 11505167]

Vermunt, JK. LEM: a general program for the analysis of categorical data. Tilburg, the Netherlands: Tilburg University Department of Methodology and Statistics; 1997.

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

Wang C, Xie L, Zhou K, et al. Increased risk for congenital heart defects in children carrying the ABCB1 Gene C3435T polymorphism and maternal periconceptional toxicants exposure. PloS One. 2013; 8:e68807. [PubMed: 23874772]

Wang Y, Shupenko CC, Melo LF, Strauss PR. DNA repair protein involved in heart and blood development. Mol Cell Biol. 2006; 26:9083–9093. [PubMed: 16966376]

Weinberg CR, Umbach DM. A hybrid design for studying genetic influences on risk of diseases with onset early in life. Am J Hum Genet. 2005; 77:627–636. [PubMed: 16175508]

Whyatt RM, Jedrychowski W, Hemminki K, et al. Bio-markers of polycyclic aromatic hydrocarbon-DNA damage and cigarette smoke exposures in paired maternal and newborn blood samples as a measure of differential susceptibility. Cancer Epidemiol Biomarkers Prev. 2001; 10:581–588. [PubMed: 11401906]

Wogan GN, Hecht SS, Felton JS, et al. Environmental and chemical carcinogenesis. Semin Cancer Biol. 2004; 14:473–486. [PubMed: 15489140]

Woods SE, Raju U. Maternal smoking and the risk of congenital birth defects: a cohort study. J Am Board Fam Pract. 2001; 14:330–334. [PubMed: 11572537]

Yoon PW, Rasmussen SA, Lynberg MC, et al. The National Birth Defects Prevention Study. Public Health Rep. 2001; 116(Suppl 1):32–40. [PubMed: 11889273]

TABLE 1

Summary of Maternal Demographic and Lifestyle Data among Case and Control Mothers Separately

		Case	Control
		(*N*=403)	(*N*=219)
Age at delivery (years)	Mean (SD)	25.9 (6.0)	24.9 (5.4)
Mother's race, *N* (%)			
	African American	31 (8%)	19 (9%)
	Caucasian	314 (78%)	177 (81%)
	Hispanic	35 (9%)	16 (7%)
	Others	23 (6%)	7 (3%)
Mother's education, *N* (%)			
	<12 years	82 (20%)	51 (23%)
	High school degree or equivalent	178 (44%)	81 (37%)
	1–3 years of college	99 (25%)	61 (28%)
At least 4 years of college or Bachelor degree		43 (11%)	25 (11%)
	Missing information	1	1
Household income, *N* (%)			
	< $10,000	100 (26%)	57 (27%)
	$10,000 to < $30,000	155 (41%)	77 (36%)
	$30,000 to < $50,000	62 (16%)	41 (19%)
	≥$50,000	61 (16%)	36 (17%)
	Missing information	25	8
Folic acid supplementation,[a] *N* (%)			
	Unexposed	238 (59%)	117 (53%)
	Exposed	165 (41%)	102 (47%)
Alcohol consumption,[a] *N* (%)			
	Unexposed	273 (68%)	132 (61%)
	Exposed	128 (32%)	86 (39%)
	Missing information	2	1
Maternal BMI (kg/m^2), *N* (%)			
	Underweight (BMI <18.5)	29 (7%)	18 (8%)
	Normal weight (18.5 <=BMI <25)	184 (46%)	99 (46%)
	Overweight (25 <=BMI <30)	89 (22%)	46 (21%)
	Obese (>=30)	97 (24%)	52 (24%)
	Missing information	4	4

Summary statistics were expressed as mean (standard deviation (SD)) for age, and frequency (percentage) for other categorical variables. Some percentages do not add up to 100% due to rounding.

Folic acid supplement use was defined as use for at least 2 months during the exposure window that was defined as one month prior to conception and 2 months after conception.

Alcohol consumption is defined as maternal alcohol consumption during the first trimester.

TABLE 2

Maternal and Infant SNPs Associated with CHD Risk in the Presence of Maternal Tobacco Exposure

Chromosome	Gene	SNP	Pathway	Allele[a]	RR (95% CI)	*p*-value	BFDP
Significant maternal genetic effects							
19	ERCC1	rs3212948	NER	C/G	0.76 (0.62, 0.94)	0.009136	0.74
14	PARP2	rs3093930	BER	A/G	0.77 (0.63, 0.94)	0.012453	0.78
13	ERCC5	rs4150386	NER	C/A	0.61 (0.42, 0.87)	0.007022	0.79
Significant infant genetic effects							
14	OSGEP	rs1320150	BER	G/A	1.39 (1.11, 1.75)	0.004507	0.65
14	OSGEP	rs938881	BER	A/T	1.39 (1.11, 1.76)	0.004815	0.66
14	OSGEP	rs2275007	BER	A/G	1.39 (1.11, 1.76)	0.004838	0.66
14	OSGEP	rs883037	BER	G/A	1.37 (1.09, 1.72)	0.007752	0.73

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

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