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Gene-environment interactions between air pollution and biotransformation enzymes and risk of birth defects

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Abstract

Genetic and environmental factors have been observed to influence risks for birth defects, though few studies have investigated gene-environment interactions. Our aim was to examine the interaction terms of gene variants in biotransformation enzyme pathways and air pollution exposures in relation to risk of several structural birth defects. We evaluated the role of ambient air pollutant exposure [nitrogen dioxide (NO_2), nitrogen oxide, carbon monoxide, particulate matter <10 (PM_{10}) and <2.5 ($PM_{2.5}$) microns] during pregnancy and 104 gene variants of biotransformation enzymes from infant bloodspots or buccal cells in a California population-based case-control study in 1997–2006. Cases included cleft lip with or without cleft palate (N=206), gastroschisis (N=94), tetralogy of Fallot (N=69) and dextro-transposition of the great arteries (d-TGA; N=40) and were compared to 208 nonmalformed controls. Overall, the results were not consistent, though did highlight some associations for further investigation as indicated by Wald chi-square test p-value <0.1. Increased risk of cleft lip was associated with exposure to high PM_{10} and two CYP gene variants. High $PM_{2.5}$ and the variant of *SLCO1B1* was associated with increased risk of teratology of Fallot. Higher NO_2 and two gene variants, *CYP2A6* and *SLC01B1,* were associated with increased risk of d-TGA. Results for gastroschisis were inconsistent in direction and across pollutants. These exploratory results suggest that some individuals based on their genetic background may be more susceptible to the adverse effects of air pollution.

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Authors Contribution: AMP, FL, SKH, GMS contributed to the design of the study, acquisition of data and interpretation of the data; WY performed statistical analyses; KS, CL performed genotyping analyses; FL performed air pollution exposure assessment; AMP and GMS prepared the initial draft preparation; All authors have been involved in the drafting and revising of the manuscript and have given final approval and are accountable for all aspects of the work.

Data Availability: Research data are not shared.

Keywords

congenital anomalies; cleft lip; cleft palate; gastroschisis; tetralogy of fallot; d-TGA; gene; air pollution; gene-environment; orofacial defect; heart defect

Birth defects are a leading cause of infant morbidity and mortality and affect approximately 3% of births and causes of most birth defects are largely unknown. Ambient air pollution has been associated with risk of several birth defect phenotypes, though results have not been consistent across different study populations (Hu et al., 2020). Such inconsistencies may be due to environmental or methodologic differences between studies or study populations having different susceptibilities to air pollution based on genetic variation. We hypothesize that many, if not most, birth defect etiologies are likely a combination of environmental exposures and their interaction with genetic variation.

Few studies have examined gene-environment interactions to identify those who may be more susceptible to the effects of air pollution with respect to risk of birth defects. One recent study performed a meta-prediction analysis to compare countries with different proportions of *methylene-tetrahydrofolate reductase* (*MTHFR*) polymorphisms and air pollution exposure and risk of congenital heart defects (Yang, Yang, Yu, & Shiao, 2018). This study observed percentages of the TT and CT genotypes, relative to the CC genotype of the *MTHFR* gene, were increased in countries with higher levels of air pollution, with a trend of increased congenital heart defects risks with higher levels of air pollution. Additional studies have examined gene-air pollution interactions and risk of neural tube defects (Padula et al., 2018; Wang et al., 2014). In previous work, we examined gene-air pollution interactions and risk of spina bifida and observed interactions between each of the five pollutants and several gene variants including ABCC2, SLC01B1, CYP1A1, CYP1A2, CYP2B6, CYP2C19, CYP2D6, NAT2, SLC01B1 and SLC01B3 (Padula et al., 2018).

We previously investigated gene variants related to enzyme pathways known to mediate detoxification of xenobiotic exposures. We focused on potential risks for the following structural birth defects – cleft lip with or without cleft palate, gastroschisis, tetralogy of Fallot and dextro-transposition of the great arteries (d-TGA) – with variants of genes in biotransformation enzyme pathways in combination with ambient air pollution exposures in a population-based case-control study the San Joaquin Valley of California. We selected these cases because they had at least 40 cases with genotyping data available. In our initial examination of air pollution exposure and risk of these selected birth defects, we did not find consistent associations for any air pollutants (Padula, Tager, Carmichael, Hammond, Lurmann, et al., 2013; Padula, Tager, Carmichael, Hammond, Yang, et al., 2013). Additional studies have found mixed results between air pollution and orofacial clefts, gastroschisis and congenital heart defects (Hu et al., 2020).

Methods

The California Center of the National Birth Defects Prevention Study (Reefhuis et al., 2015; Yoon et al., 2001) is a collaborative partnership between Stanford University and the California Birth Defects Monitoring Program in the Department of Public Health.

Since 1997, the Center has collected data from women residing in 8 counties (San Joaquin, Stanislaus, Merced, Madera, Fresno, Kings, Tulare, and Kern) in the San Joaquin Valley. The California Birth Defects Monitoring Program is a surveillance program that is population-based (Croen, Shaw, Jensvold, & Harris, 1991). To identify cases with birth defects, data collection staff visit all hospitals with obstetric or pediatric services, cytogenetic laboratories, and all clinical genetics prenatal and postnatal outpatient services. This study was approved by the Stanford University Institutional Review Board and the California State Committee for the Protection of Human Subjects.

Cases in the current analysis included infants or fetuses with cleft lip with or without cleft palate, gastroschisis, tetralogy of Fallot and dextro-transposition of the great arteries as confirmed by clinical, surgical, or autopsy reports. Cases recognized or strongly suspected to have single-gene conditions or chromosomal abnormalities or with identifiable syndromes were ineligible (Rasmussen et al., 2003), given their presumed distinct underlying etiology. The majority (~90%) of cases were isolated. Controls included non-malformed live-born infants randomly selected from birth hospitals to represent the population from which the cases arose. Maternal interviews were conducted by using a standardized, computer-based questionnaire, primarily by telephone, in English or Spanish, between 6 weeks and 24 months after the infant's estimated date of delivery. Estimated date of conception was derived by subtracting 266 days from the expected date of delivery. The expected date of delivery was based on self-report; if unknown, it was estimated from information in the medical records (<2% of participants).

Interviews were conducted with mothers of 75% of eligible cases and 69% of controls. The present analysis includes cases of cleft lip with or without cleft palate, gastroschisis, tetralogy of Fallot, dextro-transposition of the great arteries (d-TGA) and controls with estimated delivery dates between October 1, 1997, and December 31, 2006. Mothers with diabetes (type 1 or type 2) prior to gestation were excluded due to associations with a wide spectrum of birth defects including congenital heart defects (Correa et al., 2008). Mothers reported a full residential history from 3 months before conception through delivery, including start and stop dates for each residence. The Centers for Disease Control and Prevention geocoded the addresses by using Centrus Desktop (Pitney Bowes, Inc., Stamford, Connecticut), which combines reference street networks from Tele Atlas B. V. (′s-Hertogenbosch, Netherlands) and United States Postal Service data. Geocodes were available for the addresses of 95% of cases and 93% of controls.

For genetic experiments, DNA was derived from newborn bloodspots (infants only) or buccal samples (infant and mother of infants) that were stored in a −20°C freezer to remain stable for multiple years. A specific method to extract DNA was developed in the Lammer lab and has been used for numerous genotyping preparations in our molecular epidemiology work [*e.g.,* (Shaw, Nelson, Iovannisci, Finnell, & Lammer, 2003)]. We used this method to extract genomic (not amplified) DNA of sufficient quality and quantity from these precious bloodspots to provide use of Illumina GWAS platforms (2.5m). Genomic DNA was extracted from buccal brushes using an established protocol (NaOH extraction (Richards et al., 1993) along with the QIAquickR Purification kit (Qiagen, Valencia, CA)). Genotyping of DNA from buccal brush samples was performed on purified, unamplified genomic DNA.

Further, genotyping calls from high-density polymorphism arrays (Human660W-Quad BeadChip) are highly concordant (99.9%) between DNA derived from buccals versus blood (Dr. Charlotte Hobbs, personal communication).

The TaqMan® OpenArray® PGx Panel (derived from the PharmaADME Core Marker Set) is an efficient, easy-to-use OpenArray® plate for pharmacogenomics applications. Assays were developed to detect polymorphisms in genes encoding metabolism enzymes and associated transport proteins. The panel contained 158 assays.

For this project, we chose candidate genes whose variants are known to have altered enzyme activity or inducibility by xenobiotic compounds likely to be encountered in a pregnant woman's environment. These genes include the acetyl-*N*-transferases (*NATs, NAT1 1088, NAT11095, and NAT2*) and the glutathione *S*-transferases (*GSTM1* and *GSTT1*). The full list of gene variants is in Supplemental Material (Table A1). We also included other relevant genes like nitric oxide synthase (*NOS3*), which regulates nitric oxide production and has been associated with orofacial clefts and maternal smoking (Shaw et al., 2005).

For each gene variant, the Haploview Program (version 4.2, http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview) (Barrett, Fry, Maller, & Daly, 2005) was used to calculate minor allele frequency (MAF) and to evaluate deviations from Hardy–Weinberg equilibrium (HWE) among controls (Table A1). These analyses were conducted for all participants together and separately for native-born Hispanic, foreign-born Hispanic and non-Hispanic white mothers.

As part of the Children's Health and Air Pollution Study, ambient air pollution measurements and traffic metrics were assigned to each of the geocoded residences reported by study subjects corresponding to their first and second months of pregnancy. If there was more than 1 address during the period, exposure assignments were calculated for the number of days at each residence. Exposure assignments were made if the geocodes were within the San Joaquin Valley and were available for at least 75% of each month. Daily 24-hour averages of nitrogen dioxide (NO_2), nitrogen oxide (NO), carbon monoxide (CO), particulate matter <10 μm (PM_{10}), and particulate matter <2.5μm ($PM_{2.5}$) were then averaged over the first 2 months of pregnancy.

Further information on the exposure assessment has been published elsewhere (Padula, Tager, Carmichael, Hammond, Lurmann, et al., 2013). Briefly, ambient air quality data were acquired from the US Environmental Protection Agency's Air Quality System database. The station-specific daily air quality data were spatially interpolated by using inverse distance-squared weighting. Data from up to 4 air quality measurement stations were included in each interpolation. Owing to the regional nature of NO_2, PM_{10}, and $PM_{2.5}$ concentrations, we used a maximum interpolation radius of 50 km. NO and CO were interpolated by using a smaller maximum interpolation radius of 25 km because they are directly emitted pollutants with more spatial heterogeneity. When a residence was located within 5 km of 1 or more monitoring stations, the interpolation was based solely on the nearby values.

Risk for each selected birth defect associated with each infant gene variant was calculated for both the homozygotes and the heterozygotes, with homozygous wildtypes as the referent. For each gene variant, the wildtype/reference genotype was defined as the homozygous genotype with the most frequent allele among controls. Risks were estimated as odds ratios (ORs) with 95% confidence intervals (CIs) by logistic regression using SAS software (version 9.4, SAS Institute, Cary, NC).

Interaction terms for air pollution exposure (highest tertile versus lower two tertiles calculated in control group) were added to the regression analyses. Homozygous variants and heterozygotes were combined and compared to homozygous wildtypes as the referent. Wald chi-square tests were calculated for the interaction terms to determine if the subgroups were statistically different. ORs were calculated for 104 genotypes and 5 pollutants for a total of 520 comparisons for the gene-environment interaction analyses of each defect. We did not adjust for multiple comparisons given the exploratory nature of these analyses. These models were adjusted for a priori confounders including maternal race/ethnicity, vitamin use (folic acid-containing in one month before conception and first two months of pregnancy), BMI (kg/m^2, continuous), education and smoking (active and/or passive versus none). These analyses were additionally stratified by maternal use of vitamins containing folic acid, while adjusting for maternal race/ethnicity, BMI, education and smoking.

Results

The study population included 206 cases of cleft lip with or without cleft palate, 94 cases of gastroschisis, 69 cases of tetralogy of Fallot, 40 cases of dextro-transposition of the great arteries (d-TGA) and 208 controls from the San Joaquin Valley of California. The participation rate was 69% in controls and 75% in cases. Of those interviewed, 1% of controls and 3% of cases were excluded because of diabetes. Geocoding rates were 93% for controls and 95% for cases. Controls were then randomly selected from a larger sample for analysis (N=208) and 65% of cases (N=409) had genotyping performed. The demographic characteristics of each case group and controls are presented in Table 1.

Out of 158 gene loci, there were 27 loci without variation (*i.e.*, all were wildtype). An additional 27 SNPs failed the Hardy Weinberg Equilibrium among controls. Therefore, results include 104 SNPs and 540 comparisons with gene x pollutant interactions. ORs were not calculated (NC) for case/control counts less than 3.

Tables 2–5 present selected results of the gene variant-pollutant analyses with estimates of odds of each birth defect associated with combinations of genotypes and air pollution exposure compared to the referent, low pollutant exposure and common homozygous genotype (i.e., wildtype). The estimates were adjusted for maternal race/ethnicity, vitamin use, BMI, education and smoking and selection of the results were based on the p-value of the interaction term is less than 0.1 and where two of the three estimates were able to be calculated (with case and control counts ≥3). The full results are in the Supplementary Material (Tables A2–A5).

Results for cleft lip with or without cleft palate varied by direction depending on the pollutant (Table 2). High exposure to CO and NO_2 in combination with several gene variants (*ABC, CYP, DPY, GST, NAT, SLC, TPM, UGT, VKO*) showed decreased risk of cleft lip. Increased risk of cleft lip was associated with exposure to high PM_{10} and two CYP gene variants, CYP2C19 rs3758580 and CYP2D6 rs3892097, with OR=3.0; 95% CI: 1.1, 8.2 and OR=2.9; 95% CI: 1.3, 6.3, respectively.

Interaction of the gene variants and air pollutants with regard to risk of gastroschisis were inconsistent in direction and across pollutants (Table 3). None of the estimates of high air pollution and gene variants included statistically precise odds ratios, though several had ORs greater than 2 including NO and *SLCO1B1* (rs4149056 OR=2.4; 95% CI: 0.6, 10.4), PM_{10} and *CYP1A1* (rs1048943 OR=2.1 95% CI: 0.8, 5.7), and $PM_{2.5}$ and *SLCO1B1* (rs4149056 OR=3.7; 95% CI: 0.8, 17.7).

Risk of tetralogy of Fallot was associated with several pollutant gene variant combinations with ORs generally between 2 and 3, though also not statistically precise (Table 4). One notable result among those with high $PM_{2.5}$ and the variant of *SLCO1B1* (rs4149056) had a 5-fold increased risk of teratology of Fallot (OR=5.2; 95% CI: 1.3, 21.0). The remaining results were mixed.

Results of d-TGA showed gene variant-pollutant interactions in a majority of the selected results (Table 5). Increased risk of d-TGA was associated with higher NO_2 and two gene variants including *CYP2A6* (rs4986891 OR=7.8; 95% CI: 1.8, 33.7) and *SLC01B1* (rs4149056 OR= 4.3; 95% CI:1.3, 13.9).

Discussion

Our previous analyses of air pollution exposures revealed few associations and none were statistically significant with these selected defects (Padula, Tager, Carmichael, Hammond, Lurmann, et al., 2013; Padula, Tager, Carmichael, Hammond, Yang, et al., 2013). Exposures to PM_{10} and $PM_{2.5}$ were associated with increased risk of cleft lip with or without cleft palate (Padula, Tager, Carmichael, Hammond, Lurmann, et al., 2013). NO_2 and PM_{10} were associated with d-TGA and $PM_{2.5}$ was associated with d-TGA and tetraology of Fallot, though similarly not statistically significant (Padula, Tager, Carmichael, Hammond, Yang, et al., 2013).

Our current study extends this investigation to examine if gene variants in enzyme pathways known to mediate detoxification of outside exposures may make certain people more susceptible to the effects of air pollution. An increased risk of selected birth defects was observed for women with high exposure to air pollution during the first two months of pregnancy and variants of several *CYP* and *SLC* genes with ORs ranging from 2.9 to 7.8. Increased risk of cleft lip with or without cleft palate was associated with *CYP* gene variants in combination with PM_{10} and $PM_{2.5}$, though in the unexpected direction for CO and NO_2 and several gene variants. Results for gastroschisis were not consistent, though suggestive for *CYP* and *SLC* genes in combination with high air pollution. The strongest associations were in relation to risk of the cardiac defects, tetralogy of Fallot and d-TGA.

High $PM_{2.5}$ and a variant of an *SLC* gene was associated with Tetralogy of Fallot and NO_2 in combination with gene variants of *CYP* and *SLC* genes were associated with d-TGA.

These gene pathways are involved in metabolizing both endogenous compounds and myriad xenobiotic chemicals (Nebert, 1997). Their role in detoxifying air pollutant exposures has been investigated as potential modifiers in environmental health studies (Kelada, Eaton, Wang, Rothman, & Khoury, 2003). For example, airborne polycyclic aromatic hydrocarbons (a component of particulate matter) have been associated with measures of genotoxicity of *CYP1A1* and *NAT2* genes (Kelada et al., 2003). Furthermore, several studies have reported the role of specific variants in detoxification genes in association with congenital heart malformations including *CYP1A1* and *ABCB1* (Vecoli, Pulignani, & Andreassi, 2016).

We view this investigation as exploratory even though several of the observed odds ratios were sizable and reasonably precise. Although we did not have a hypothesis as to which of the selected defects in this study may be more susceptible to gene-pollutant interactions, the direction of the results varied substantially by defect. Such caution seems prudent owing to sample sizes being relatively small, numerous comparisons being made, and a paucity of previous studies to corroborate these findings.

Few studies have examined gene-environment interactions and risk of birth defects. Previous studies have examined the interaction between smoking, which has similar constituents to air pollution, and gene variants for their combined risk of gastroschisis (Jenkins et al., 2014; Torfs, Christianson, Iovannisci, Shaw, & Lammer, 2006) and orofacial clefts (Jenkins et al., 2014; Torfs et al., 2006; Wu et al., 2012; Wu et al., 2010; Wu et al., 2014; Zeng, Wu, Zhu, Shi, & Jia, 2015). For example, decreased risk of gastroschisis was observed for non-Hispanic white mothers who smoked periconceptionally and had a variant of *CYP1A1*2A* (aOR=0.38, 95% CI 0.15–0.98). An additional gene variant of *NAT2*6* was also associated with gastroschisis for Hispanic non-smoking mothers (aOR=2.17, 95% CI 1.12–4.19) and their infants (aOR=2.11, 95% CI 1.00–4.48) (Jenkins et al., 2014). In a genome-wide association study of 550 cleft palate case-parent trios, SLC2A9 (rs3733585 and rs12508991) and WDR1 (rs6820756 and rs7699512) gave suggestive evidence of gene-environment interaction with environmental tobacco smoke among 259 Asian trios (Wu et al., 2014).

Our study examines the interaction between these gene variants related to biotransformation enzymes and air pollutant exposures and risk of four selected birth defects (cleft lip with or without cleft palate, gastroschisis, tetralogy of Fallot and dextro-transposition of the great arteries) in a well-characterized population in California. Future studies would benefit from investigation of additional gene variants and larger sample sizes to evaluate subgroups. Despite its limitations, this study exhibits detailed exposure assessment and targeted gene variant analyses. The results warrant further investigation of gene-environment interactions and risk of birth defects, specifically the selected variants in the *CYP* and *SLC* genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Demographic characteristics of Birth Defect Cases and Non-malformed Controls, California 1997–2006 (N=617).

	Controls[a] (N=208)	Cleft lip with or without cleft palate[a] (N=206)	Gastroschisis[a] (N=94)	Tetralogy of Fallot[a] (N=69)	Dextro-transposition of the great arteries[a] (N=40)
	n (%)	n (%)	n (%)	n (%)	n (%)
Maternal race/ethnicity					
White Non-Hispanic	76 (37)	68 (33)	20 (21)	18 (26)	17 (43)
US-born Hispanic	57 (27)	50 (24)	32 (34)	15 (22)	9 (23)
Foreign-born Hispanic	54 (26)	67 (33)	21 (22)	28 (41)	10 (25)
Other	21 (10)	20 (10)	19 (20)	8 (12)	4 (10)
Missing	0	1 (<1)	2 (2)	0	0
Maternal age at delivery (years)					
<20	29 (14)	21 (10)	36 (38)	4 (6)	5 (13)
20–24	61 (29)	66 (32)	37 (39)	19 (28)	16 (40)
25–29	53 (25)	59 (29)	10 (11)	22 (32)	11 (28)
30–34	39 (19)	41 (20)	10 (11)	13 (19)	7 (18)
35+	26 (13)	19 (9)	1 (1)	11 (16)	1 (2)
Maternal education (years)					
<12	62 (30)	66 (32)	35 (37)	24 (35)	10 (25)
12	52 (25)	59 (29)	40 (43)	17 (25)	10 (25)
>12	93 (45)	81 (39)	17 (18)	28 (41)	20 (50)
Missing	1 (<1)	0	2 (2)	0	0
Parity					
0	69 (33)	65 (32)	57 (61)	20 (29)	14 (35)
1	68 (33)	68 (33)	23 (24)	22 (32)	12 (30)
2+	71 (34)	73 (35)	14 (15)	27 (39)	14 (35)
Maternal Body Mass Index (kg/m^2)					
Underweight (<18.5)	4 (2)	13 (6)	5 (5)	8 (12)	2 (5)
Normal (18.5–<25)	96 (46)	93 (45)	66 (70)	24 (35)	18 (45)
Overweight (25–<30)	49 (24)	41 (20)	18 (19)	15 (22)	11 (28)

	Controls[a] (N=208)	Cleft lip with or without cleft palate[a] (N=206)	Gastroschisis[a] (N=94)	Tetralogy of Fallot[a] (N=69)	Dextro-transposition of the great arteries[a] (N=40)
	n (%)	n (%)	n (%)	n (%)	n (%)
Obese (≥30)	41 (20)	44 (21)	5 (5)	16 (23)	8 (20)
Missing	18 (9)	15 (7)	0	6 (9)	1 (2)
Plurality					
Singletons	207 (99.5)	198 (96)	94 (100)	67 (97)	38 (95)
Multiples	1 (0.5)	8 (4)	0	2 (3)	2 (5)
Infant sex					
Male	94 (45)	137 (66)	43 (46)	39 (57)	32 (80)
Female	114 (55)	69 (34)	51 (54)	30 (43)	8 (20)
Multi-vitamin use[b]					
No	56 (27)	71 (34)	34 (36)	27 (39)	11 (28)
Yes	148 (71)	132 (64)	58 (62)	41 (59)	29 (72)
Missing	4 (2)	3 (1)	2 (2)	1 (1)	0
Smoking[b]					
None	160 (77)	147 (71)	62 (66)	53 (77)	29 (73)
Active only	15 (7)	21 (10)	12 (13)	5 (7)	4 (10)
Passive only	18 (9)	22 (11)	13 (14)	8 (12)	6 (15)
Active and passive	15 (7)	15 (7)	7 (7)	3 (4)	1 (2)
Missing	0	1 (<1)	0	0	0
Air Pollution exposure during the first two months of pregnancy – mean (SD)					
CO (ppm)	0.6 (0.3)	0.6 (0.3)	0.5 (0.2)	0.6 (0.3)	0.6 (0.3)
NO (ppb)	15.1 (14.7)	13.7 (14.4)	11.7 (12.6)	13.4 (12.0)	13.8 (12.0)
NO_2 (ppb)	18.2 (5.6)	17.2 (5.4)	16.1 (4.9)	17.9 (5.0)	18.4 (4.4)
PM_{10} ($\mu g/m^3$)	35.5 (14.3)	36.5 (15.9)	33.1 (12.2)	38.6 (16.4)	40.3 (16.6)
$PM_{2.5}$ ($\mu g/m^3$)	19.3 (11.9)	21.1 (13.7)	17.1 (9.5)	21.7 (12.9)	21.1 (11.8)

[a] Percentages may not equal 100 owing to rounding and missing.

[b] During the month before or the first two months of pregnancy.

Table 2.

Cleft lip with or without cleft palate results adjusted for maternal race/ethnicity, vitamin use, BMI, education and smoking with p-value of the interaction term <0.1 (Reference=low air pollution defined by lower two tertiles of pollutant exposure during the first two months of pregnancy and wildtype defined as the homozygous genotype with the most frequent allele among controls).

Pollutant[a]	Gene symbol	dbSNP ID	Odds Ratio (95% Confidence Interval)[b]		
			High air pollution + gene variant	Low air pollution + gene variant	High air pollution + wildtype
CO	SLC15A2	rs2293616	0.5 (0.2, 1.0)	0.6 (0.3, 1.1)	0.3 (0.1, 0.7)
CO	SLC15A2	rs2257212	0.5 (0.2, 1.1)	0.6 (0.3, 1.1)	0.3 (0.1, 0.7)
CO	SLC15A2	rs1143671	0.5 (0.2, 1.0)	0.6 (0.3, 1.1)	0.3 (0.1, 0.7)
CO	SLC15A2	rs1143672	0.5 (0.2, 1.1)	0.6 (0.3, 1.1)	0.3 (0.1, 0.7)
NO	ABCG2	rs2231142	0.6 (0.2, 1.4)	1.3 (0.7, 2.5)	1.2 (0.7, 2.1)
NO	DPYD	rs1801265	0.7 (0.3, 1.4)	1.2 (0.7, 2.1)	1.3 (0.7, 2.6)
NO	GSTP1	rs1695	0.7 (0.3, 1.4)	0.5 (0.3, 0.9)	0.4 (0.2, 1.0)
NO	UGT1A1	rs4124874	0.7 (0.3, 1.4)	1.1 (0.6, 2.0)	1.8 (0.7, 4.3)
NO_2	ABCC2	rs2273697	0.3 (0.1, 0.7)	0.9 (0.5, 1.6)	0.9 (0.5, 1.5)
NO_2	CYP2C19	rs17878459	NC	0.5 (0.1, 1.9)	0.6 (0.4, 0.9)
NO_2	CYP2C19	rs41291556	NC	0.6 (0.2, 2.5)	0.6 (0.4, 1.1)
NO_2	GSTP1	rs1695	0.5 (0.3, 1.1)	0.6 (0.3, 1.0)	0.3 (0.1, 0.7)
NO_2	NAT2	rs1208	0.9 (0.5, 1.7)	0.9 (0.5, 1.5)	0.3 (0.2, 0.7)
NO_2	NAT2	rs1799929	0.9 (0.5, 1.8)	0.9 (0.6, 1.6)	0.4 (0.2, 0.8)
NO_2	NAT2	rs1801280	0.9 (0.4, 1.7)	0.9 (0.5, 1.5)	0.4 (0.2, 0.8)
NO_2	SLCO1B3	rs4149117	0.7 (0.3, 1.4)	1.7 (1.0, 2.9)	1.0 (0.5, 1.8)
NO_2	TPMT	rs1800460	0.4 (0.1, 1.7)	2.8 (1.3, 6.0)	0.9 (0.5, 1.4)
NO_2	UGT2B7	rs7668258	0.6 (0.3, 1.3)	1.5 (0.9, 2.7)	1.1 (0.5, 2.5)
PM_{10}	CYP1A1	rs1799814	2.3 (0.6, 9.3)	0.4 (0.1, 1.5)	0.9 (0.6, 1.4)
PM_{10}	CYP1A1	rs1048943	1.4 (0.7, 2.9)	0.6 (0.3, 1.1)	0.7 (0.4, 1.2)
PM_{10}	CYP1A2	rs2069514	1.4 (0.7, 3.0)	0.6 (0.3, 1.1)	0.7 (0.4, 1.2)
PM_{10}	CYP2A6	rs4986891	0.5 (0.1, 1.4)	1.9 (0.8, 4.7)	1.3 (0.8, 2.1)
PM_{10}	CYP2C19	rs3758580	3.0 (1.1, 8.2)	1.1 (0.6, 2.1)	0.9 (0.5, 1.4)
PM_{10}	CYP2C8	rs10509681	1.1 (0.5, 2.5)	2.5 (1.2, 5.0)	1.3 (0.8, 2.1)
PM_{10}	CYP2D6	rs3892097	2.9 (1.3, 6.3)	1.4 (0.8, 2.5)	0.8 (0.4, 1.3)
PM_{10}	SLCO1B1	rs2306283	1.0 (0.5, 1.8)	0.7 (0.4, 1.2)	0.5 (0.2, 1.0)
PM_{10}	SLCO2B1	rs2306168	2.2 (0.7, 7.5)	0.8 (0.4, 1.6)	0.8 (0.5, 1.3)
PM_{10}	TPMT	rs1142345	0.3 (0.1, 0.9)	1.3 (0.5, 3.7)	1.3 (0.8, 2.1)
PM_{10}	TPMT	rs1800460	0.8 (0.3, 2.0)	5.3 (1.9, 14.6)	1.3 (0.8, 2.1)
$PM_{2.5}$	CYP2C19	rs4244285	3.6 (0.9, 14.0)	0.8 (0.4, 1.5)	1.2 (0.7, 2.1)

Pollutant[a]	Gene symbol	dbSNP ID	Odds Ratio (95% Confidence Interval)[b]		
			High air pollution + gene variant	Low air pollution + gene variant	High air pollution + wildtype
$PM_{2.5}$	SLC22A1	rs72552763	1.8 (0.8, 4.0)	0.7 (0.4, 1.4)	1.0 (0.5, 1.9)
$PM_{2.5}$	UGT2B7	rs7668258	1.5 (0.7, 3.3)	1.7 (0.8, 3.3)	2.6 (1.1, 6.4)

[a]Highest tertile cut-offs: CO= 0.730 ppm; NO=15.15 ppb; NO=20.15 ppb; PM10=38.80 μg/m^3; PM2.5=19.86 μg/m^3

[b]Adjusted for maternal race/ethnicity, education, BMI, folate-containing vitamin use and smoking in early pregnancy

NC = not calculated (when case or control counts were <3); rows were removed if two estimates were NC

Table 3.

Gastroschisis results adjusted for maternal race/ethnicity, vitamin use, BMI, education and smoking with p-value of the interaction term <0.1 (Reference=low air pollution defined by lower two tertiles of pollutant exposure during the first two months of pregnancy and wildtype defined as the homozygous genotype with the most frequent allele among controls).

Pollutant[a]	Gene symbol	dbSNP ID	Odds Ratio (95% Confidence Interval)[b]		
			High air pollution + gene variant	Low air pollution + gene variant	High air pollution + wildtype
CO	CYP1A2	rs762551	0.6 (0.1, 2.1)	1.0 (0.5, 2.3)	NC
CO	CYP2C19	rs17885098	NC	1.4 (0.5, 4.3)	0.1 (0.0, 0.5)
CO	CYP2C9	rs1057910	NC	0.9 (0.2, 3.1)	0.2 (0.1, 0.5)
CO	SLCO1B3	rs7311358	0.6 (0.2, 2.3)	1.0 (0.4, 2.4)	0.1 (0.0, 0.4)
NO	ABCC2	rs717620	0.3 (0.1, 1.5)	2.4 (1.0, 5.7)	1.1 (0.5, 2.4)
NO	NAT2	rs1799929	1.0 (0.3, 2.8)	0.6 (0.3, 1.4)	0.2 (0.1, 0.7)
NO	NAT2	rs1801280	0.8 (0.3, 2.4)	0.5 (0.2, 1.3)	0.2 (0.1, 0.7)
NO	SLCO1B1	rs4149056	2.4 (0.6, 10.4)	0.8 (0.3, 2.2)	0.4 (0.2, 1.0)
NO_2	CYP1A2	rs762551	0.9 (0.3, 2.3)	0.9 (0.5, 1.9)	0.3 (0.1, 0.8)
NO_2	SLCO1B1	rs4149056	1.7 (0.5, 6.1)	0.6 (0.3, 1.5)	0.4 (0.2, 0.8)
NO_2	UGT2B15	rs1902023	0.3 (0.1, 0.8)	0.3 (0.2, 0.7)	0.2 (0.1, 0.7)
PM_{10}	CYP1A1	rs1048943	2.1 (0.8, 5.7)	0.9 (0.4, 2.0)	0.6 (0.2, 1.3)
PM_{10}	CYP1A2	rs2069514	1.3 (0.5, 3.7)	0.4 (0.2, 1.0)	0.5 (0.2, 1.2)
PM_{10}	TPMT	rs1800460	NC	6.7 (1.9, 24.2)	1.4 (0.7, 2.7)
$PM_{2.5}$	CYP1A2	rs762551	1.9 (0.7, 5.6)	0.7 (0.3, 1.6)	0.3 (0.1, 0.9)
$PM_{2.5}$	CYP2C19	rs17885098	NC	1.2 (0.3, 4.9)	0.6 (0.3, 1.4)
$PM_{2.5}$	CYP2C8	rs11572080	NC	1.3 (0.4, 4.2)	1.2 (0.5, 2.5)
$PM_{2.5}$	SLCO1B1	rs4149056	3.7 (0.8, 17.7)	0.6 (0.2, 1.5)	0.6 (0.3, 1.3)

[a] Highest tertile cut-offs: CO= 0.730 ppm; NO=15.15 ppb; NO=20.15 ppb; PM10=38.80 μg/m^3; PM2.5=19.86 μg/m^3

[b] Adjusted for maternal race/ethnicity, education, BMI, folate-containing vitamin use and smoking in early pregnancy

NC = not calculated (when case or control counts were <3); rows were removed if two estimates were NC

Table 4.

Tetralogy of Fallot results adjusted for maternal race/ethnicity, vitamin use, BMI, education and smoking with p-value of the interaction term <0.1 (Reference=low air pollution defined by lower two tertiles of pollutant exposure during the first two months of pregnancy and wildtype defined as the homozygous genotype with the most frequent allele among controls).

Pollutant[a]	Gene symbol	dbSNP ID	Odds Ratio (95% Confidence Interval)[b]		
			High air pollution + gene variant	Low air pollution + gene variant	High air pollution + wildtype
CO	ABCC2	rs717620	1.2 (0.3, 4.1)	0.5 (0.2, 1.4)	0.4 (0.2, 1.1)
CO	ABCC2	rs3740066	0.9 (0.3, 2.5)	0.7 (0.3, 1.7)	0.3 (0.1, 0.9)
CO	CYP1A1	rs1048943	0.8 (0.3, 2.5)	0.6 (0.2, 1.6)	0.4 (0.1, 1.1)
NO	CYP2A6	rs28399433	NC	2.1 (0.8, 5.5)	1.2 (0.5, 2.6)
NO	TPMT	rs1800460	2.8 (0.8, 10.1)	0.6 (0.2, 2.7)	0.6 (0.3, 1.4)
NO	UGT2B15	rs1902023	1.0 (0.4, 2.6)	0.6 (0.2, 1.3)	0.3 (0.1, 1.1)
NO_2	ABCB1	rs1045642	0.5 (0.2, 1.5)	1.5 (0.7, 3.6)	1.3 (0.4, 4.2)
NO_2	ABCB1	rs1128503	0.5 (0.1, 1.4)	1.3 (0.6, 3.1)	1.4 (0.4, 4.2)
NO_2	ABCB1	rs2032582	0.6 (0.2, 1.8)	1.7 (0.8, 3.8)	1.3 (0.5, 3.9)
NO_2	ABCC2	rs717620	1.1 (0.4, 3.2)	0.3 (0.1, 1.0)	0.3 (0.1, 0.8)
NO_2	ABCC2	rs3740066	0.7 (0.3, 1.7)	0.6 (0.3, 1.2)	0.3 (0.1, 0.9)
NO_2	CYP2A6	rs4986891	1.4 (0.3, 7.6)	NC	0.6 (0.3, 1.2)
NO_2	CYP2C19	rs12248560	0.9 (0.3, 2.7)	0.7 (0.3, 1.6)	0.4 (0.1, 0.9)
NO_2	SLCO1B3	rs4149117	0.3 (0.1, 1.3)	1.5 (0.7, 3.2)	1.0 (0.4, 2.4)
NO_2	UGT2B15	rs1902023	0.6 (0.2, 1.5)	0.5 (0.2, 1.2)	NC
PM_{10}	ABCB1	rs1045642	0.7 (0.2, 2.1)	1.6 (0.6, 4.1)	2.0 (0.6, 5.9)
PM_{10}	SLC22A2	rs316019	1.4 (0.4, 5.4)	NC	0.7 (0.4, 1.5)
PM_{10}	SLCO2B1	rs2306168	2.2 (0.5, 10.7)	0.6 (0.2, 1.7)	0.7 (0.3, 1.4)
PM_{10}	TPMT	rs1800460	0.6 (0.2, 2.5)	5.3 (1.5, 18.8)	1.2 (0.6, 2.4)
$PM_{2.5}$	ABCC2	rs717620	1.8 (0.6, 6.0)	NC	1.6 (0.7, 3.9)
$PM_{2.5}$	SLCO1B1	rs4149056	5.2 (1.3, 21.0)	0.7 (0.2, 2.2)	1.5 (0.6, 3.6)
$PM_{2.5}$	UGT2B15	rs1902023	2.5 (0.8, 7.9)	0.6 (0.2, 1.9)	0.9 (0.2, 3.4)

[a] Highest tertile cut-offs: CO= 0.730 ppm; NO=15.15 ppb; NO=20.15 ppb; PM10=38.80 μg/m^3; PM2.5=19.86 μg/m^3

[b] Adjusted for maternal race/ethnicity, education, BMI, folate-containing vitamin use and smoking in early pregnancy

NC = not calculated (when case or control counts were <3); rows were removed if two estimates were NC

Table 5.

Dextro-Transposition of the Great Arteries results adjusted for maternal race/ethnicity, vitamin use, BMI, education and smoking with p-value of the interaction term <0.1 (Reference=low air pollution defined by lower two tertiles of pollutant exposure during the first two months of pregnancy and wildtype defined as the homozygous genotype with the most frequent allele among controls).

Pollutant[a]	Gene symbol	dbSNP ID	Odds Ratio (95% Confidence Interval)[b]		
			High air pollution + gene variant	Low air pollution + gene variant	High air pollution + wildtype
CO	UGT2B7	rs7662029	NC	1.3 (0.5, 3.7)	1.6 (0.4, 5.9)
NO_2	ABCC2	rs3740066	1.5 (0.6, 4.0)	0.6 (0.2, 1.6)	0.6 (0.2, 2.0)
NO_2	CYP2A6	rs4986891	7.8 (1.8, 33.7)	NC	0.9 (0.4, 2.2)
NO_2	NAT2	rs1208	1.4 (0.5, 3.8)	0.6 (0.2, 1.6)	0.5 (0.1, 1.8)
NO_2	NAT2	rs1801280	1.5 (0.5, 4.4)	0.8 (0.3, 2.0)	0.4 (0.1, 1.6)
NO_2	SLC15A2	rs2293616	1.5 (0.4, 5.5)	2.0 (0.7, 5.9)	2.9 (0.8, 10.1)
NO_2	SLC15A2	rs1143671	1.5 (0.4, 5.5)	2.0 (0.7, 6.1)	2.9 (0.8, 10.1)
NO_2	SLC15A2	rs1143672	1.3 (0.3, 4.8)	1.9 (0.6, 5.6)	3.0 (0.9, 10.6)
NO_2	SLCO1B1	rs4149056	4.3 (1.3, 13.9)	0.5 (0.2, 1.8)	0.7 (0.3, 1.7)
NO_2	UGT2B7	rs7668258	0.4 (0.1, 1.6)	1.2 (0.4, 3.2)	2.7 (0.9, 8.5)
PM_{10}	CYP1A1	rs1048943	0.9 (0.3, 3.3)	NC	0.7 (0.3, 1.6)
PM_{10}	CYP1A2	rs2069514	1.4 (0.4, 5.0)	NC	0.4 (0.2, 1.2)
PM_{10}	SLC22A1	rs628031	0.9 (0.3, 2.6)	0.3 (0.1, 1.0)	0.6 (0.2, 1.8)
PM_{10}	SLCO1B1	rs4149056	2.5 (0.8, 7.3)	0.6 (0.2, 2.1)	0.7 (0.3, 1.8)
PM_{10}	SLCO2B1	rs2306168	4.4 (0.8, 25.6)	0.8 (0.2, 2.8)	0.9 (0.4, 2.1)
PM_{10}	TPMT	rs1142345	NC	4.3 (1.2, 15.5)	1.7 (0.7, 3.7)
PM_{10}	TPMT	rs1800460	NC	4.8 (1.1, 20.8)	1.5 (0.7, 3.4)
$PM_{2.5}$	CYP2D6	rs3892097	3.2 (0.9, 10.8)	0.5 (0.1, 2.1)	0.7 (0.2, 2.4)
$PM_{2.5}$	UGT2B7	rs7668258	0.6 (0.1, 2.8)	1.6 (0.5, 5.2)	3.2 (0.8, 12.8)

[a] Highest tertile cut-offs: CO= 0.730 ppm; NO=15.15 ppb; NO=20.15 ppb; PM10=38.80 $\mu g/m^3$; PM2.5=19.86 $\mu g/m^3$

[b] Adjusted for maternal race/ethnicity, education, BMI, folate-containing vitamin use and smoking in early pregnancy

NC = not calculated (when case or control counts were <3); rows were removed if two estimates were NC