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Maternal Smoking, Xenobiotic Metabolizing Enzyme Gene Variants, and Gastroschisis Risk

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Abstract

Maternal smoking during pregnancy is one proposed risk factor for gastroschisis, but reported associations have been modest, suggesting that differences in genetic susceptibility might play a role. We included 108 non-Hispanic white and 62 Hispanic families who had infants with gastroschisis, and 1147 non-Hispanic white and 337 Hispanic families who had liveborn infants with no major structural birth defects (controls) in these analyses. DNA was extracted from buccal cells collected from infants and mothers, and information on periconceptional smoking history was obtained from maternal interviews, as part of the National Birth Defects Prevention Study. We analyzed five polymorphisms in three genes that code for enzymes involved in metabolism of some cigarette smoke constituents (*CYP1A1*, *CYP1A2*, and *NAT2)*. Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) independently for maternal smoking and maternal and infant gene variants, and to assess joint associations of maternal smoking and maternal or infant gene variants with gastroschisis. In analyses adjusted for maternal age at delivery and stratified by maternal race-ethnicity, we identified three suggestive associations among 30 potential associations with sufficient numbers to calculate ORs: *CYP1A1*2A* for non-Hispanic white mothers who smoked periconceptionally (aOR=0.38, 95% CI 0.15-0.98), and *NAT2*6* 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). This analysis does not support the occurrence of effect modification between periconceptional maternal smoking and most of the xenobiotic metabolizing enzyme gene variants assessed.

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Keywords

maternal smoking; CYP; NAT; genetic epidemiology; risk factors; gastroschisis

INTRODUCTION

Gastroschisis is a herniation of the intestines through a defect of the abdominal wall lateral to the umbilicus (usually on the right side), and it is not covered by a membrane [Ledbetter, 2012]. This congenital anomaly affects approximately 4.5 infants per 10,000 U.S. live births [Parker et al., 2010]. Many epidemiological studies have found a positive, albeit modest, association between maternal smoking during pregnancy and gastroschisis [Chabra et al., 2011; Hackshaw et al., 2011; Paranjothy et al., 2012]. Associations could be larger for specific individuals given the potential for genetic differences in maternal or fetal metabolism of chemicals in cigarette smoke.

The metabolism of chemicals in smoke occurs in two phases catalyzed by xenobiotic-metabolizing enzymes (XMEs). *CYP1A1*2A* (rs4646903) and *CYP1A2*1F* (rs762551) are functional single nucleotide polymorphisms (SNPs) reported to increase inducibility of cytochrome P-450 (CYP) activity during phase I [Georgiadis et al., 2005; Human CYP Allele Nomenclature Committee Database], and *CYP1A2*1C* (rs2069514) is a functional SNP reported to decrease inducibility of CYP activity [Human CYP Allele Nomenclature Committee Database]. Elevated CYP activity can increase the toxicity of cigarette smoke constituents that are metabolically activated to reactive intermediates by the induced enzymes [Guengerich and Shimada, 1991], although the level of susceptibility might vary dependent upon the activity of other phase I as well as phase II enzymes. *NAT2*5* (rs1801280) and *NAT2*6* (rs1799930) are functional variants reported to decrease N-acetyltransferase (NAT) activity during phase II [Consensus Human NAT Gene Nomenclature Database], resulting in prolonged exposure to toxic intermediates produced by phase I reactions [Boukouvala and Fakis, 2005].

Other studies have reported joint associations of these and other XME gene variants and exposure to cigarette smoke with risk for birth defects other than gastroschisis [Chevrier et al., 2008; Hecht et al., 2007; Lammer et al., 2004; Sommer et al., 2011] as well as joint associations of other gene variants involved in vascular disruption and exposure to cigarette smoke with risk for gastroschisis [Lammer et al., 2008; Torfs et al., 2006]. We analyzed five SNPs in three XME genes (*CYP1A1*, *CYP1A2*, and *NAT2*) in mothers and infants to assess their potential association with gastroschisis, and to assess the effect of their possible interaction with maternal smoking.

MATERIALS AND METHODS

Study Population

We used data from the National Birth Defects Prevention Study (NBDPS), a multisite, population-based, case-control study of major birth defects that included a maternal interview and self-collection of buccal (cheek) cells from each case and control infant and

his/her mother and father. Detailed methodology for the NBDPS has been published previously [Rasmussen et al., 2002; Yoon et al., 2001]. Briefly, case infants with selected major birth defects were identified using birth defects surveillance systems at the 10 participating sites. Liveborn control infants without major birth defects were randomly selected from birth certificates or birth hospital data from the same region and time period. Clinical geneticists reviewed data abstracted from medical records using standardized case definitions. Case infants with known chromosomal abnormalities or single gene disorders were excluded. Standardized computer assisted telephone interviews were conducted in English or Spanish between six weeks and 24 months after the estimated date of delivery (EDD). Women were asked about their exposures from three months before conception until delivery. Following completion of the interview, buccal cell collection kits that included cytobrushes for the mother, her child, and the child's father (two brushes per participant) were mailed. Buccal cell collection initiation varied by site, and samples were requested only from mothers whose interviews were completed after collection began. Institutional Review Boards (IRBs) at the Centers for Disease Control and Prevention (CDC) and each study site have approved the NBDPS.

These analyses included infants of non-Hispanic white or Hispanic mothers with an EDD between October 1, 1997 and December 31, 2003. Race-ethnicity was self-reported by each mother, and infants were analyzed according to their mother's race-ethnicity. Infants of mothers of other race-ethnicities were not included because of small numbers of case infants (i.e., < 4) with mothers who reported periconceptional smoking and with analyzable buccal cell samples. Samples from mothers were removed from analyses if she reported using an egg or embryo donor. DNA samples from the infant, mother, or both were analyzed; father samples were not included in these analyses. Case infants had gastroschisis with or without other major congenital anomalies, and samples were available only if they were liveborn. Infants diagnosed with limb body wall defects were excluded from these analyses.

Smoking History

Infants and mothers were classified as exposed to periconceptional maternal smoking if the mother reported any smoking at any time in the month before or in the first three months of pregnancy, since gastroschisis occurs during the third and fourth weeks post-fertilization [Sadler and Feldkamp, 2008]. Infants and mothers were classified as unexposed if the mother did not report any smoking in the month before and in the first three months of pregnancy.

DNA Extraction

Laboratories at each participating site extracted DNA from buccal cells using a variety of methods for samples collected prior to mid-2003 [Rasmussen et al., 2002]. A laboratory atCDC extracted DNA from Georgia participant samples and from all sites after mid-2003 using a modified phenol-chloroform method [Garcia-Closas et al., 2001]. Human genomic DNA (gDNA) yields were assessed by quantitative real-time PCR using TaqMan® Ribonuclease P assays (Applied Biosystems, Foster City, CA). Specimens with DNA concentrations less than 0.1ng/μl were excluded. DNA quality and family relationships were assessed using tetranucleotide short tandem repeats (STRs) as described previously

[Gallagher et al., 2011]. DNA samples from inconsistent mother-infant pairs were excluded; consistent pairs and unpaired mothers and infants were included. Positive and negative controls were included in each DNA extraction and quantitation assay.

Genotyping Methods

We analyzed five SNPs in three genes (*CYP1A1*, *CYP1A2*, and *NAT2*) that were selected based on their effect on XME activity [Consensus Human NAT Gene Nomenclature Database; Human CYP Allele Nomenclature Committee Database], their minor allele frequencies [Packer et al., 2006], and assay success in preliminary validation studies. Appendix 1 provides more information on the selected XME gene variants. Genotyping was completed on either gDNA or whole genome amplified (WGA) products from mothers and infants using Pyrosequencing® technology (Qiagen, Valencia, CA). Methods and quality assessment results were described previously [Gallagher et al., 2011]. Replica genotyping was performed on separate days for at least 4% of specimens from each genotyping plate. For each mother-infant pair, SNPs that were inconsistent with Mendelian inheritance were removed from further analyses. Specimens with missing data for one or more SNPs were removed from further analyses. The laboratory at CDC successfully completed external quality assessment (protocols are available upon request).

Statistical Analyses

Data from control mothers were assessed for Hardy-Weinberg equilibrium by race-ethnicity for each of the five SNPs studied using Chi square tests. Mendelian errors were identified and allele frequencies were calculated using PedCheck Version 1.00 [O'Connell and Weeks, 1998] and PLINK Version 1.07 [Purcell et al., 2007]. Maternal age at delivery, alcohol use, body mass index, obesity, parity, and education were assessed as potential confounders using Chi square tests in non-Hispanic white and Hispanic control mothers separately. Maternal age at delivery was also assessed as a potential effect modifier by completing stratified analyses (< 25 years vs ≥ 25 years). Maternal age at delivery (continuous) was included in the logistic regression models.

Logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) using PASW Statistics 18, Release Version 18.0.0 (SPSS, Inc., 2009, Chicago, IL, www.spss.com). Maternal age-adjusted associations between smoking and gastroschisis were assessed, stratified by race-ethnicity. Maternal age-adjusted associations between maternal or infant XME gene variants and gastroschisis with and without stratification by maternal periconceptional smoking status were assessed separately in non-Hispanic white and Hispanic mothers and infants using dominant or recessive inheritance models. For all analyses, dominant inheritance models were used when assessing *CYP1A1*2A*, *CYP1A2*1C*, *NAT2*5*, and *NAT2*6* (i.e., persons who had one or two copies of the variant allele were combined and compared to persons who had zero copies) because small numbers of mothers and infants carrying two copies of the variant allele limited analyses of other inheritance models. Recessive inheritance models were used when assessing *CYP1A2*1F* (i.e., persons who had two copies of the variant allele were compared to persons who had zero or one copy of the variant allele combined) because small numbers of mothers and infants carrying two copies of the wild-type allele limited analyses of other

inheritance models. After stratification, analyses were completed only if there were four or more mothers or infants in each genotype category.

To assess the contribution of having any high risk XME gene variants in the mother and her infant, we also dichotomized combined gene variants from available mother-infant pairs (0 (referent group) or ≥1) for each of the five XME gene variants. These analyses were completed only when DNA was available from both a mother and her infant. If a mother or her infant carried two copies of *CYP1A2*1F*, the pair was categorized as having a high risk gene variant; for all other variant alleles (i.e., *CYP1A1*2A*, *CYP1A2*1C*, *NAT2*5*, and *NAT2*6*), if a mother or her infant carried one or two copies of the variant allele, the pair was categorized as having a high risk gene variant.

RESULTS

Interview and Buccal Cell Collection Participation Rates

The interview participation rate was 72% for all mothers of infants with gastroschisis (n=504), and 69% for all mothers of control infants (n=4949). Buccal cell samples were requested from 455 case families and 4251 control families and were submitted for the mother, infant, or both for 47% of families with gastroschisis (n=215), and 43% of control families (n=1834). After excluding families with reported maternal race-ethnicity other than non-Hispanic white or Hispanic, and specimens that did not pass quality control (i.e., STR or SNP results were inconsistent with Mendelian inheritance; DNA quantity was <0.1 ng/μl; data were missing for >1 SNP), samples from 108 non-Hispanic white case families (76 mother-infant pairs; 29 mother only; and 3 infant only), 62 Hispanic case families (36 mother-infant pairs; 22 mother only; and 4 infant only), 1147 non-Hispanic white control families (890 mother-infant pairs; 210 mother only; and 47 infant only), and 337 Hispanic control families (233 mother-infant pairs; 72 mother only; and 32 infant only) were included (Figure 1).

Study Participant Characteristics

There were some differences in selected maternal demographic and behavioral risk factors for gastroschisis among case and control infants (Table I). Mothers of infants with gastroschisis were younger, less educated, and more likely to be underweight.

Quality Control

Genotype call rates were between 99 and 100 percent for all five variants. The genotype distribution of each variant did not deviate from Hardy-Weinberg equilibrium (P>0.05) in non-Hispanic white or Hispanic mothers of control infants. The minor allele frequencies of each genetic variant in non-Hispanic white and Hispanic control mothers are listed in Appendix 1 and were consistent with reported published frequencies [Chang et al., 2009; Sherry et al., 2001; Swinney et al., 2011].

Association of Maternal Smoking and Gastroschisis

Of the potential confounders assessed, only maternal age at delivery (continuous) and maternal education (≤12 years or >12 years) were found to be associated with the XME gene

variants (Appendix 2). Because maternal age and maternal education are correlated and young maternal age at delivery is an established risk factor for gastroschisis [Rasmussen and Frias, 2008], we included only maternal age at delivery in the models. Among non-Hispanic white and Hispanic control mothers included in these genetic analyses, 20.1% and 9.8%, respectively, reported smoking in the month before pregnancy or during the first trimester. Nearly identical, elevated maternal age-adjusted ORs were observed for gastroschisis risk and exposure to maternal periconceptional smoking in both racial-ethnic groups; however, the finding was statistically significant only in non-Hispanic white mothers (aOR=2.07, 95% CI 1.33-3.23, P<0.01) (Table II).

Association of Maternal and Infant XME Gene Variants with Gastroschisis Risk

A suggestive maternal-age adjusted association of *NAT2*6* with gastroschisis was observed in Hispanic mothers (aOR=1.88, 95% CI 1.04-3.39, P=0.04) and their infants (aOR=1.93, 95% CI 0.96-3.88, P=0.07) (Table III). An age-adjusted association of *NAT2*6* with gastroschisis was not observed in non-Hispanic white mothers or their infants and adjusted associations of *CYP1A1*2A*, *CYP1A2*1C*, *CYP1A2*1F*, and *NAT2*5* with gastroschisis were not observed in mothers of either race-ethnicity or their infants (Table III). Similar results were observed in analyses stratified by maternal age at delivery (data not shown).

Modifying Effects of XME Gene Variants on the Association of Maternal Smoking and Gastroschisis

After stratifying by smoking status, a suggestive maternal age-adjusted association of *NAT2*6* with gastroschisis continued to be observed in Hispanic non-smoking mothers (aOR=2.17, 95% CI 1.12-4.19, P=0.02) and their infants (aOR=2.11, 95% CI 1.00-4.48, P=0.05); no association was observed in Hispanic smoking mothers (Table IV). No statistically significant age-adjusted associations of *NAT2*6* with gastroschisis were observed in non-Hispanic white smoking or non-smoking mothers or their infants (Table IV). A suggestive maternal age-adjusted association of *CYP1A1*2A* with gastroschisis was observed in non-Hispanic white smoking mothers (aOR=0.38, 95% CI 0.15-0.98, P=0.05) that was not observed in their infants or in non-Hispanic white non-smoking mothers or their infants (Table IV). No associations of *CYP1A1*2A* with gastroschisis were observed in Hispanic non-smoking mothers or their infants (Table IV). No statistically significant age-adjusted associations were observed between *CYP1A2*1C*, *CYP1A2*1F* or *NAT2*5* and gastroschisis (Table IV).

A suggestive maternal age-adjusted association of *NAT2*6* with gastroschisis was observed in non-Hispanic white (aOR=3.41, 95% CI 1.25-9.31, P=0.02) and Hispanic (aOR=3.31, 95% CI 1.42-7.75, P=0.01) non-smoking mother-infant pairs when comparing those pairs carrying one or more high risk gene variant to those pairs with no high risk gene variant (Table V). A statistically significant adjusted association of *NAT2*6* with gastroschisis was not observed in non-Hispanic white smoking mother-infant pairs (Table V). No statistically significant associations were observed in non-smoking mother-infant pairs of either race-ethnicity for the other four gene variants and were not observed in non-Hispanic white smoking mother-infant pairs for three of the four gene variants with sufficient numbers (Table V).

DISCUSSION

Our data support a statistically significant positive association between maternal periconceptional smoking and gastroschisis among non-Hispanic white mothers, and suggest that maternal *CYP1A1*2A* variants might mitigate the toxic effects of some cigarette smoke constituents for gastroschisis risk in infants of non-Hispanic white mothers. However, most of the selected XME gene variants do not act as effect modifiers for maternal smoking and gastroschisis in these data. Suggestive associations of *NAT2*6* in Hispanic non-smoking mothers and their infants were also observed. No effects were observed for *CYP1A2*1C*, *CYP1A2*1F* or *NAT2*5*.

In a broader set of NBDPS data (not limited by race or participation in the genetic portion of the study), risk factors and maternal demographics for gastroschisis cases and controls were similar [Werler et al., 2009].

Twenty percent of non-Hispanic white and almost ten percent of Hispanic mothers of control infants reported periconceptional smoking. These percentages are similar to those for all reproductive-aged women using data from the 2006 Behavioral Risk Factor Surveillance System [CDC, 2008].

Our main results on maternal smoking and gastroschisis agree with a comprehensive review of 12 studies of maternal smoking that showed a clear, albeit modest, association with gastroschisis (OR=1.50, 95% CI 1.28-1.76) [Hackshaw et al., 2011].

XME Gene Variants and Gastroschisis Risk

The elevated effect estimates observed for gastroschisis risk in Hispanic mothers and their infants who carried one or two copies of *NAT2*6* (Table III) are biologically plausible because the resulting decrease in NAT2 activity [Consensus Human NAT Gene Nomenclature Database] leads to increased susceptibility to the toxic effects of the intermediates formed in phase I reactions. *NAT2*6* has not been reported in previous studies to be associated with gastroschisis.

XME Gene Variant – Maternal Smoking Exposure Interactions and Gastroschisis

Analyses of *CYP* variants were stratified by maternal periconceptional smoking status because CYP1A1 and CYP1A2 are induced by exposure to cigarette smoke [Gunes and Dahl, 2008]. We expected individuals carrying *CYP1A1*2A* to be more susceptible to the toxic effects of chemicals in cigarette smoke because this variant has been reported to increase enzyme activity [Georgiadis et al., 2005] and lead to increased toxic intermediates; however, mothers carrying this variant who smoked periconceptionally appeared to be less likely to have an infant with gastroschisis (Table IV). The *CYP1A1*2A* fetal variant has been reported to play a protective role for oral cleft risk in children whose mothers were exposed to secondhand tobacco smoke during the first trimester [Chevrier et al., 2008]. Kurahashi and colleagues [Kurahashi et al., 2005] reported a protective effect of the maternal variant for hypospadias risk in the offspring of Japanese mothers (smoking and non-smoking); however, there was no interaction effect. In our study, this was the only

variant that had a suggestive modifying effect on maternal periconceptional smoking. *CYP1A1*2A* has not been reported in previous studies to be associated with gastroschisis.

It is unclear whether gastroschisis risk is influenced more by maternal or fetal genes or both equally. We observed suggestive adjusted associations between *NAT2*6* and gastroschisis for Hispanic and non-Hispanic white non-smoking mother-infant pairs. The suggestive associations that were consistently observed in our analyses between *NAT2*6* and gastroschisis in Hispanic families have not been reported previously. Although the variant has not been previously reported to be associated with gastroschisis, it has been associated with cleft lip with or without cleft palate [Lie et al., 2008], including reports of having a modifying effect on the association between maternal smoking and orofacial clefts [Shi et al., 2007].

In our study, *CYP1A1*2A* was the only variant that acted as an effect modifier for maternal periconceptional smoking and gastroschisis. The effects we observed in mothers and infants who were not exposed to periconceptional smoking could be due to interactions of *NAT2*6* with other exposures. Our data were analyzed separately for each race-ethnicity because of large differences in allele frequencies, which limited our ability to assess interactions. Further sub-classification of the Hispanic population was not completed, and genetic admixture within this population might have an impact on our results [Martinez, 1998]. Maternal and infant genotypes were not adjusted for each other when analyses were completed separately which could be a potential source of confounding. Other limitations included the use of self-reported maternal race-ethnicity, which was used to classify the infant race-ethnicity, and the use of self-reported smoking that did not include data on level of smoking or secondhand smoking exposures. These exploratory analyses were completed with limited numbers of families and by reporting results without correcting for multiple testing we can provide more liberal data that can better inform future studies.

Strengths of our study included the assessment of data from a large population-based, case-control study of risk factors for birth defects with both genetic and environmental exposure data and standardized case definitions.

This study focused on a small number of XME genes because of limited DNA quantity and stringent quality control. Other gene variants in the XME pathway might affect gastroschisis risk through their effect on smoking behavior (e.g. *CYP2A6* [Tyndale and Sellers, 2002]) or important detoxification reactions (e.g., glutathione S-transferase [Garlantezec et al., 2012]). Other exposures might also explain, in part, the contradictory associations with maternal smoking observed in our study. CYP1A1 and CYP1A2 are inducible by a large number of common exposures in addition to cigarette smoke, such as cruciferous vegetables [Vistisen et al., 1992], caffeine [Tantcheva-Poor et al., 1999], and charcoal-grilled food [Kall and Clausen, 1995]. Oral contraceptives [Abernethy and Todd, 1985] and apiaceous vegetables [Peterson et al., 2006] inhibit enzyme activity. NAT2 metabolizes a wide range of drugs, including isoniazid (antituberculotic), hydralazine (antihypertensive), sulfonamides (antibacterials), and caffeine [Daly, 2003; Kawamura et al., 2005].

We believe this is the first report of *CYP1A1*2A* as a possible protective variant against gastroschisis in the offspring of women who smoke during the periconceptional period and also the first report of a suggestive association between *NAT2*6* and gastroschisis risk for Hispanic non-smoking mothers and their infants. Although the sample size is small, to our knowledge, this is the largest case-control study examining genetic and non-genetic risk factors for gastroschisis that has been completed to date. Five previous studies of genetic risk factors for gastroschisis included no more than 57 case families (whereas we included 170 case families) [Cardonick et al., 2005; Feldkamp et al., 2012; Komuro et al., 2001; Lammer et al., 2008; Torfs et al., 2006]. It is challenging to conduct genetic epidemiologic analyses on such a rare birth defect, especially one that disproportionally affects younger mothers who typically have lower participation in biospecimen collection. We feel the value of these exploratory analyses is to inform studies that can build upon these methodologies, resources and results. Future studies are needed to confirm our findings with these gene variants and to investigate other exposures or other XME genes and exposure to periconceptional smoking.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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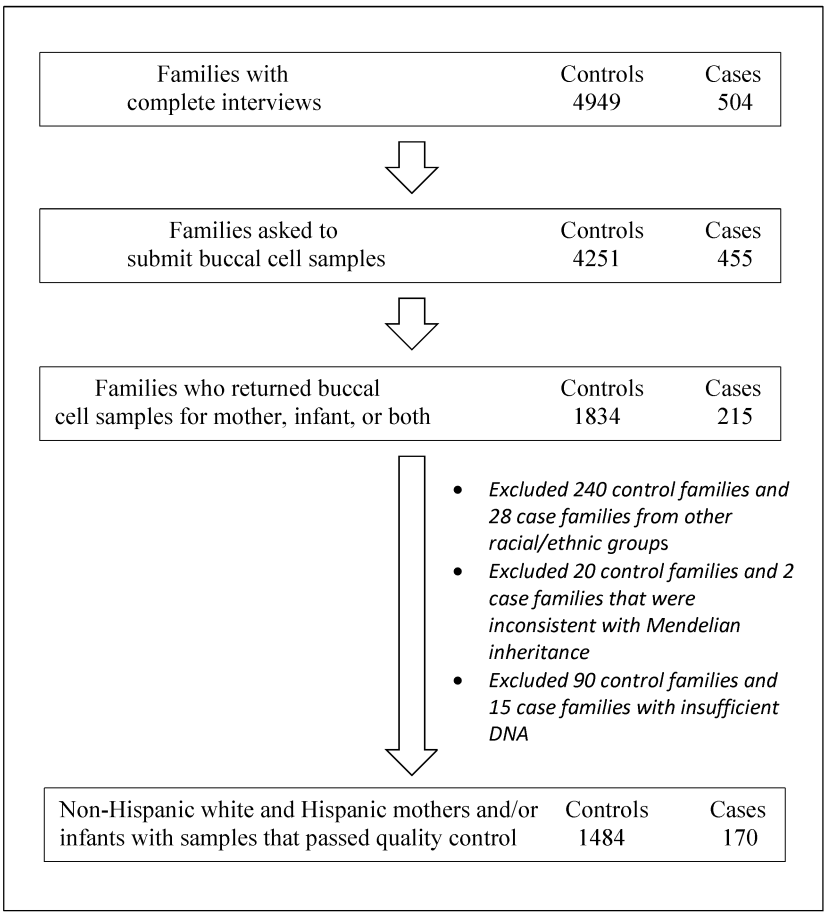

FIGURE 1.

Flow Diagram of Participation by Families of Children with Gastroschisis (Cases) and Families of Children with No Major Structural Birth Defects (Controls) Who Were Included in Analyses of Genetic Data from the National Birth Defects Prevention Study, 1997-2003

TABLE I

Maternal Demographic and Behavioral Factors Among Infants with Gastroschisis (Cases) and Infants with No Major Structural Birth Defects (Controls) Who Were Included in Analyses of Genetic Data from the National Birth Defects Prevention Study, 1997-2003

	Controls (N=1484)		Cases (N=170)		
Maternal Factor	**N**	**%**	**N**	**%**	**OR (95%CI)**
Race/ethnicity					
Non-Hispanic White	1147	77.3	108	63.5	Referent
Hispanic	337	22.7	62	36.5	1.95 (1.39-2.73)
Age at delivery (years)					
< 18	40	2.7	23	13.5	2.81 (1.56-4.99)
18-19	100	6.7	48	28.2	2.35 (1.51-3.62)
20-24	323	21.8	66	38.8	Referent
25-29	391	26.3	20	11.8	0.25 (0.15-0.42)
≥30	630	42.5	13	7.6	0.10 (0.05-0.18)
Education level					
< 12 years	201	13.5	51	30.0	1.26 (0.84-1.88)
12 years	343	23.1	69	40.6	Referent
> 12 years	929	62.6	49	28.8	0.26 (0.18-0.39)
Missing	11	0.7	1	0.6	n/c
Year of estimated date of delivery					
1997-1998*	66	4.4	5	2.9	0.70 (0.24-1.73)
1999	162	10.9	19	11.2	1.09 (0.61-1.90)
2000	286	19.3	42	24.7	1.37 (0.87-2.13)
2001	262	17.7	29	17.1	1.03 (0.63-1.67)
2002	271	18.3	28	16.5	0.96 (0.58-1.57)
2003	437	29.4	47	27.6	Referent
Body mass index (kg/m^2)					
Underweight (<18.5)	66	4.4	18	10.6	2.03 (1.13-3.50)
Normal weight (18.5-24.9)	818	55.1	110	64.7	Referent

	Controls (N=1484)		Cases (N=170)		
Maternal Factor	**N**	**%**	**N**	**%**	**OR (95%CI)**
Overweight (25-29.9)	322	21.7	28	16.5	0.65 (0.41-0.99)
Obese (≥30)	229	15.4	9	5.3	0.29 (0.14-0.56)
Missing	49	3.3	5	2.9	n/c
Use of multivitamins containing folic acid from the month before through the third month of pregnancy					
Yes	1276	86.0	142	83.5	0.79 (0.52-1.23)
No	198	13.3	28	16.5	Referent
Missing	10	0.7	0	0.0	n/c

n/c= not calculated

* Data and specimen collection began with October 1997 births at 2 study sites (GA & IA); 7 study sites collected specimens for 1998 births (AR, CA, GA, IA, MA, NY, TX); 8 study sites collected specimens for 1999-2002 births (AR, CA, GA, IA, MA, NJ, NY, TX); 9 study sites collected specimens for 2003 births (AR, CA, GA, IA, MA, NY, NC, TX, UT)

TABLE II

Adjusted Associations* Between Maternal Smoking from the Month Before Through the Third Month of Pregnancy and Gastroschisis by Maternal Race-Ethnicity for Mothers of Infants with Gastroschisis (Cases), and Mothers of Infants with No Major Structural Birth Defects (Controls) Who Were Included in Analyses of Genetic Data from the National Birth Defects Prevention Study, 1997-2003

Maternal Race-Ethnicity	Periconceptional Smoking Status	Controls		Cases			
		N	%	N	%	OR (95%CI)	P
Non-Hispanic White	None	917	79.9	51	47.2	Referent	
	Any	230	20.1	57	52.8	2.07 (1.33-3.23)	<0.01
Hispanic	None	304	90.2	51	82.3	Referent	
	Any	33	9.8	11	17.7	2.14 (0.99-4.64)	0.06

* Adjusted for maternal age at delivery (continuous)

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TABLE III

Adjusted Associations* Between Selected Xenobiotic Metabolizing Enzyme Gene Variants and Gastroschisis for Non-Hispanic White and Hispanic Mothers and Infants Using Data from the National Birth Defects Prevention Study, 1997-2003.

		Control	Case	OR (95% CI)	P	Control	Case	OR (95% CI)	P
		Infants				Mothers			
Gene Variant**		Non-Hispanic White							
*CYP1A1*2A*	TT	734	58	Referent		862	86	Referent	
	TC+CC	203	21	1.19 (0.67-2.12)	0.55	238	19	0.63 (0.36-1.11)	0.11
*CYP1A2*1C*	GG	906	72	Referent		1062	101	Referent	
	GA+AA	29	7	1.72 (0.65-4.51)	0.27	37	4	1.11 (0.34-3.60)	0.87
*CYP1A2*1F*	CC+CA	465	38	Referent		545	46	Referent	
	AA	471	41	1.15 (0.70-1.91)	0.58	554	59	1.41 (0.91-2.18)	0.13
*NAT2*5*	TT	272	24	Referent		332	27	Referent	
	TC+CC	665	55	1.18 (0.68-2.04)	0.55	768	76	1.21 (0.73-1.98)	0.46
*NAT2*6*	GG	459	37	Referent		541	44	Referent	
	GA+AA	478	42	1.03 (0.62-1.70)	0.92	558	61	1.38 (0.89-2.15)	0.15
		Hispanic							
*CYP1A1*2A*	TT	95	14	Referent		117	20	Referent	
	TC+CC	169	26	1.12 (0.54-2.30)	0.76	187	38	1.18 (0.64-2.16)	0.60
*CYP1A2*1C*	GG	132	16	Referent		147	22	Referent	
	GA+AA	133	24	1.59 (0.79-3.21)	0.19	158	36	1.54 (0.85-2.78)	0.16
*CYP1A2*1F*	CC+CA	125	14	Referent		157	25	Referent	
	AA	140	26	1.92 (0.93-3.96)	0.08	148	33	1.40 (0.78-2.50)	0.26
*NAT2*5*	TT	126	17	Referent		142	23	Referent	
	TC+CC	139	23	1.26 (0.63-2.53)	0.51	163	35	1.46 (0.81-2.64)	0.21
*NAT2*6*	GG	172	21	Referent		207	32	Referent	
	GA+AA	93	19	1.93 (0.96-3.88)	0.07	98	26	1.88 (1.04-3.39)	0.04

* Adjusted for maternal age at delivery (continuous)

** Dominant mode of inheritance used for *CYP1A1*2A, CYP1A2*1C, NAT2*5*, and *NAT2*6*; Recessive mode of inheritance used for *CYP1A2*1F*

TABLE IV

Adjusted Associations* Between Maternal Smoking from the Month Before Through the Third Month of Pregnancy and Selected Xenobiotic Metabolizing Enzyme Gene Variants and Gastroschisis for Non-Hispanic White and Hispanic Mothers and Infants Using Data from the National Birth Defects Prevention Study, 1997-2003.

		Control	Case	aOR (95% CI)	P	Control	Case	aOR (95% CI)	P
Variant Alleles**	Maternal Smoking	Infants				Mothers			
		Non-Hispanic White							
*CYP1A1*2A*									
Absent	None	598	25	Referent		681	37	Referent	
Present	None	166	13	1.56 (0.73-3.36)	0.25	192	13	1.00 (0.48-2.05)	1.00
Absent	Any	136	33	Referent		181	49	Referent	
Present	Any	37	8	0.89 (0.36-2.17)	0.79	46	6	0.38 (0.15-0.98)	0.05
*CYP1A2*1C*									
Absent	None	744	33	Referent		841	47	Referent	
Present	None	20	5	3.19 (0.93-10.98)	0.07	32	3	n/c	
Absent	Any	162	39	Referent		221	54	Referent	
Present	Any	9	2	n/c		5	1	n/c	
*CYP1A2*1F*									
Absent	None	385	15	Referent		452	21	Referent	
Present	None	379	23	1.80 (0.87-3.72)	0.11	421	29	1.69 (0.90-3.19)	0.10
Absent	Any	80	23	Referent		93	25	Referent	
Present	Any	92	18	0.74 (0.36-1.52)	0.42	133	30	1.04 (0.56-1.95)	0.89
*NAT2*5*									
Absent	None	226	12	Referent		265	11	Referent	
Present	None	538	26	1.20 (0.56-2.58)	0.64	608	39	1.57 (0.75-3.30)	0.23
Absent	Any	46	12	Referent		67	16	Referent	
Present	Any	127	29	1.09 (0.50-2.40)	0.83	160	37	0.95 (0.48-1.88)	0.89
*NAT2*6*									
Absent	None	381	14	Referent		437	20	Referent	
Present	None	383	24	1.52 (0.73-3.14)	0.26	435	30	1.55 (0.82-2.92)	0.17

Variant Alleles**	Maternal Smoking	Control	Case	aOR (95% CI)	P	Control	Case	aOR (95% CI)	P
		Infants				Mothers			
		Non-Hispanic White							
Absent	Any	78	23	Referent		104	24	Referent	
Present	Any	95	18	0.69 (0.34-1.41)	0.30	123	31	1.17 (0.63-2.16)	0.63
		Hispanic							
*CYP1A1*2A*									
Absent	None	85	13	Referent		108	17	Referent	
Present	None	153	22	0.97 (0.45-2.10)	0.94	168	30	1.09 (0.56-2.13)	0.80
Absent	Any	10	1	Referent		9	3	Referent	
Present	Any	16	4	n/c		19	8	n/c	
*CYP1A2*1C*									
Absent	None	117	14	Referent		133	18	Referent	
Present	None	122	21	1.51 (0.71-3.20)	0.28	144	29	1.48 (0.76-2.85)	0.25
Absent	Any	15	2	Referent		14	4	Referent	
Present	Any	11	3	n/c		14	7	1.75 (0.42-7.40)	0.44
*CYP1A2*1F*									
Absent	None	114	12	Referent		147	20	Referent	
Present	None	125	23	2.10 (0.96-4.59)	0.06	130	27	1.61 (0.84-3.09)	0.15
Absent	Any	11	2	Referent		10	5	Referent	
Present	Any	15	3	n/c		18	6	0.65 (0.15-2.84)	0.57
*NAT2*5*									
Absent	None	113	16	Referent		130	20	Referent	
Present	None	126	19	1.07 (0.51-2.24)	0.86	147	27	1.36 (0.70-2.61)	0.36
Absent	Any	13	1	Referent		12	3	Referent	
Present	Any	13	4	n/c		16	8	n/c	
*NAT2*6*									
Absent	None	157	18	Referent		189	25	Referent	
Present	None	82	17	2.11 (1.00-4.48)	0.05	88	22	2.17 (1.12-4.19)	0.02
Absent	Any	15	3	Referent		18	7	Referent	

Variant Alleles**	Maternal Smoking	Control	Case	aOR (95% CI)	P	Control	Case	aOR (95% CI)	P
		Infants				Mothers			
		Non-Hispanic White							
Present	Any	11	2	n/c		10	4	1.03 (0.24-4.40)	0.97

n/c= not calculated because of low numbers (< 4)

*Adjusted for maternal age at delivery (continuous)

**Dominant mode of inheritance used for *CYP1A1*2A, CYP1A2*1C, NAT2*5*, and *NAT2*6*; Recessive mode of inheritance used for *CYP1A2*1F*

TABLE V

Adjusted Associations* Between Maternal Smoking from the Month Before Through the Third Month of Pregnancy and Selected Xenobiotic Metabolizing Enzyme Gene Variants (Maternal and Infant Combined) and Gastroschisis for Non-Hispanic White and Hispanic Mothers and Their Infants Using Data from the National Birth Defects Prevention Study, 1997-2003.

Gene Variant**		Maternal Smoking	Non-Hispanic White				Hispanic			
			Control	Case	aOR (95% CI)	P	Control	Case	aOR (95% CI)	P
*CYP1A1*2A*	Absent	None	496	22	Referent		44	6	Referent	
	Present	None	224	15	1.20 (0.57-2.54)	0.64	167	25	1.17 (0.44-3.14)	0.76
	Absent	Any	118	28	Referent		7	0	Referent	
	Present	Any	52	11	0.81 (0.36-1.83)	0.61	14	5	n/c	
*CYP1A2*1C*	Absent	None	686	32	Referent		71	8	Referent	
	Present	None	34	5	2.19 (0.66-7.20)	0.20	141	23	1.56 (0.64-3.76)	0.33
	Absent	Any	158	37	Referent		11	0	Referent	
	Present	Any	10	2	n/c		10	5	n/c	
*CYP1A2*1F*	Absent	None	251	9	Referent		84	9	Referent	
	Present	None	469	28	1.89 (0.82-4.41)	0.14	128	22	1.67 (0.71-3.90)	0.24
	Absent	Any	51	18	Referent		6	1	Referent	
	Present	Any	118	21	0.61 (0.29-1.29)	0.19	15	4	n/c	
*NAT2*5*	Absent	None	114	4	Referent		76	7	Referent	
	Present	None	606	33	1.88 (0.60-5.92)	0.28	136	24	1.82 (0.73-4.55)	0.20
	Absent	Any	21	4	Referent		8	0	Referent	
	Present	Any	149	34	1.46 (0.45-4.79)	0.53	13	5	n/c	
*NAT2*6*	Absent	None	261	5	Referent		117	9	Referent	
	Present	None	459	32	3.41 (1.25-9.31)	0.02	95	22	3.31 (1.42-7.75)	0.01
	Absent	Any	58	14	Referent		10	3	Referent	
	Present	Any	111	25	0.97 (0.45-2.07)	0.93	11	2	n/c	

n/c= not calculated because of low numbers (< 4)

*Adjusted for maternal age at delivery (continuous)

**Dominant mode of inheritance used for *CYP1A1*2A*, *CYP1A2*1C*, *NAT2*5*, and *NAT2*6*; Recessive mode of inheritance used for *CYP1A2*1F*