

Maternal Cigarette Smoking, Metabolic Gene Polymorphism, and Infant Birth Weight

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IN THE UNITED STATES, 65% OF ALL infant deaths occur among low-birth-weight (LBW) infants (<2500 g); LBW infants account for 7.6% of all live-born infants.¹ The etiology of LBW is largely unknown, but both environmental and genetic factors may play a role.² Numerous studies have shown that maternal cigarette smoking during pregnancy is associated with reduced birth weight or increased risk of LBW.³⁻⁸ In 1997, 13.2% of US women reported smoking cigarettes during pregnancy.¹ Maternal cigarette smoking is identified as the single largest modifiable risk factor for intrauterine growth restriction in developed countries.^{9,10} However, not all women who smoke cigarettes during pregnancy have LBW infants. The reason for this variability is largely unknown, but may be related to maternal genetic susceptibility.

Tobacco smoke contains approximately 4000 compounds¹¹; the most important carcinogens in tobacco smoke are polycyclic aromatic hydrocarbons (PAHs), arylamines, and *N*-nitrosamines.¹² The ability of an individual to

Context Little is known about genetic susceptibility to cigarette smoke in relation to adverse pregnancy outcomes.

Objective To investigate whether the association between maternal cigarette smoking and infant birth weight differs by polymorphisms of 2 maternal metabolic genes: *CYP1A1* and *GSTT1*.

Design, Setting, and Participants Case-control study conducted in 1998-2000 among 741 mothers (174 ever smokers and 567 never smokers) who delivered singleton live births at Boston Medical Center. A total of 207 cases were preterm or low-birth-weight infants and 534 were non-low-birth-weight, full-term infants (control).

Main Outcome Measure Birth weight, gestation, fetal growth by smoking status and *CYP1A1* MspI (AA vs Aa and aa, where Aa and aa were combined because of small numbers of aa and similar results), and *GSTT1* (present vs absent) genotypes.

Results Without consideration of genotype, continuous maternal smoking during pregnancy was associated with a mean reduction of 377 g (SE, 89 g) in birth weight (odds ratio [OR], 2.1; 95% confidence interval [CI], 1.2-3.7). When *CYP1A1* genotype was considered, the estimated reduction in birth weight was 252 g (SE, 111 g) for the AA genotype group (n=75; OR, 1.3; 95% CI, 0.6-2.6), but was 520 g (SE, 124 g) for the Aa/aa genotype group (n=43 for Aa, n=6 for aa; OR, 3.2; 95% CI, 1.6-6.4). When *GSTT1* genotype was considered, the estimated reduction in birth weight was 285 g (SE, 99 g) (OR, 1.7; 95% CI, 0.9-3.2) and 642 g (SE, 154 g) (OR, 3.5; 95% CI, 1.5-8.3) for the present and absent genotype groups, respectively. When both *CYP1A1* and *GSTT1* genotypes were considered, the greatest reduction in birth weight was found among smoking mothers with the *CYP1A1* Aa/aa and *GSTT1* absent genotypes (-1285 g; SE, 234 g; *P*<.001). Among never smokers, genotype did not independently confer an adverse effect. A similar pattern emerged in analyses stratified by maternal ethnicity and in analyses for gestation.

Conclusions In our study, maternal *CYP1A1* and *GSTT1* genotypes modified the association between maternal cigarette smoking and infant birth weight, suggesting an interaction between metabolic genes and cigarette smoking.

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convert toxic metabolites of cigarette smoke to less harmful moieties is important for minimizing the adverse health effects of these compounds. Using PAHs as an example, the metabolic processing of PAHs in humans involves 2 phases. The phase 1 metabolism is an activation process, in which the inhaled, hydro-

phobic PAHs are converted mainly through aryl hydrocarbon hydroxylase activity into hydrophilic, reactive, electrophilic intermediates that can bind covalently to macromolecules, especially DNA.¹³ These intermediates may be more toxic than the original form. Aryl hydrocarbon hydroxylase, encoded by

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the *CYP1A1* gene, is a well-studied phase 1 enzyme and is particularly relevant to the metabolism of chemicals in cigarette smoke. The phase 2 metabolism is a detoxification process, in which these metabolic intermediates are detoxified by enzymes such as glutathione S-transferases (GSTs) or uridine diphosphate (UDP)-glucuronosyltransferase through transformation into conjugated forms that are sufficiently polar to be excreted from the body.¹⁴ *GSTT1*, encoded by the *GSTT1* gene, is a major phase 2 enzyme. There is evidence that the adverse health effects of cigarette smoke may depend on the combined effects of phase 1 and phase 2 metabolism.¹⁵⁻¹⁷

Both *CYP1A1* and *GSTT1* genes are highly polymorphic in the population¹⁸⁻²⁰ and their polymorphisms have been associated with their encoded enzyme activities.^{21,22} The expression of different host genotypes may explain varying susceptibility to the adverse health effects of cigarette smoke.

We hypothesized that the association between maternal cigarette smoking during pregnancy and reduced birth weight or increased risk of LBW is modified by maternal genetic susceptibility. In this report, *CYP1A1* and *GSTT1* gene polymorphisms are used to characterize genetic susceptibility and to assess the interaction between metabolic genes and cigarette smoking. We chose to focus on these specific gene polymorphisms not only because such an interaction is biologically plausible, but also in light of previous research that found evidence of interaction between these gene polymorphisms and benzene exposure on gestation duration.²³ In addition, these gene variants are common in our study population, permitting us to examine gene-cigarette smoke interactions.

METHODS

Study Site and Population

Between 1998 and 2000, we conducted a molecular epidemiological study on environmental and genetic determinants of LBW (<2500 g) and preterm birth (<37 weeks' gestation) among mothers who delivered at Boston Medical Center, using a case-control design. Boston Medi-

cal Center serves a multi-ethnic population of pregnant women, many of whom are from the inner city. The overall rates of LBW and preterm birth are approximately 12% and 15% in this population compared with the national average of 7.6% and 11.8%, respectively.¹ More than 80% of study mothers had at least 1 prenatal ultrasound examination; most examinations were performed prior to 20 weeks' gestation. Cases were defined as women who delivered singleton, live, LBW or preterm infants regardless of birth weight; controls were matched for age and ethnicity and were defined as women who delivered singleton, live, term infants with birth weight 2500 g or more. Three controls were identified for every case. Multiple-gestation pregnancies (eg, twins, triplets) or newborns with major birth defects were excluded. The study protocol was approved by the Boston Medical Center Institutional Review Board and by the Massachusetts Department of Public Health.

Data Collection Procedures

All eligible cases, including those women who delivered on weekends and holidays, were approached postpartum by our research staff. The participation rate was 90% and 85% among approached eligible cases and controls, respectively. There was no significant difference between participants and nonparticipants in infant birth weight, maternal ethnicity, or other sociodemographic characteristics. After informed consent was obtained, a questionnaire interview was conducted to obtain relevant information including demographic characteristics, cigarette smoking, alcohol consumption, and medical and reproductive history. Maternal and infant medical records were reviewed to obtain clinical data including prenatal care, pregnancy complications, and birth outcomes (infant's sex, gestational age, and birth weight). A maternal blood sample was obtained and DNA was extracted according to standard protocol.²⁴

Cigarette Smoking

The information on maternal smoking was based on maternal self-reporting and

was obtained for 4 time periods: 3 months before the index pregnancy and the first, second, and third trimesters of the index pregnancy. In our study sample, mothers' data were clustered in 3 groups in terms of cigarette smoking: those who did not smoke throughout the index pregnancy; those who smoked during early pregnancy but quit smoking during the first trimester; and those who smoked continuously during the index pregnancy. Only 1 woman who did not smoke in the 3 months before pregnancy or in the first trimester began smoking in later pregnancy. None of the women who continued to smoke cigarettes in the second trimester quit smoking in the third trimester. Therefore, in the analysis, we defined "never smoker" as those women who did not smoke cigarettes during any of the 4 time periods and used never smoker as the reference group. We defined "ever smoker" as those who smoked any number of cigarettes during any of the 4 time periods. We further divided ever smokers into 2 subgroups: quitter, only smoked in the 3 months before pregnancy or during the first trimester; and continuous smokers, smoked continuously from prepregnancy to delivery. We are unable to adequately evaluate the timing of smoking in relation to birth weight given the smoking pattern in our study sample. Maternal passive smoke exposure was grouped into 2 categories based on maternal self-reporting: unexposed or exposed to 1 or more smokers at home during the index pregnancy.

Genotyping Methods

The detailed method for detection of the *CYP1A1* MspI polymorphism can be found elsewhere.²¹ This method is able to detect all 3 possible genotypes for the polymorphism: AA (homozygous wild type), Aa (heterozygous variant type), and aa (homozygous variant type). In our preliminary analysis, we evaluated 4 possible genetic models: dominant (AA=0, Aa=1, aa=1), recessive (AA=0, Aa=0, aa=1), additive (AA=0, Aa=1, aa=2), and no restriction (no assumption made). We found that the associations

between maternal smoking and infant birth weight differed considerably between the AA and Aa genotype groups (mean, -234 g; SE, 99 g vs -577 g; SE, 126 g) but were similar for the Aa and aa genotype groups (-577 g; SE, 126 g vs -508 g; SE, 238 g). Thus, our data did not suggest a recessive model. We combined the Aa and aa genotypes in the data analysis due to the small number of enrolled mothers with the aa genotype.

The detailed method on detection of the *GSTT1* deletion polymorphism can be found elsewhere.²⁰ This method is only able to detect the present (at least 1 allele present, AA or Aa) or absent (complete deletion of both alleles, aa) genotype.

Outcomes of Interest

Infant birth weight was evaluated as both a continuous and a binary (<2500 g vs ≥2500 g) variable. Gestational age was assessed in 2 ways: time since the first day of the last menstrual period and an algorithm based on last menstrual period and the result of early ultrasound (<20 weeks' gestation). This approach has been used in a large hospital-based preterm study.²³ Briefly, the last menstrual period estimate was used only if confirmed by an ultrasound within 7 days or if no ultrasound estimate was obtained; otherwise, the ultrasound estimate was used. Gestational age was analyzed both as a continuous and a binary (<37 vs ≥37 weeks' gestation) variable. Since the results were similar for gestational age based on last menstrual period vs the algorithm, we present the results based on the latter approach. We used birth weight ratio (observed birth weight/mean birth weight for gestational age) as a continuous measure of fetal growth and defined intrauterine growth restriction as birth weight ratio less than 85%, an approach used in a previous study.²⁶

Statistical Methods

We used multiple linear and logistic regression models to estimate the individual and combined associations of maternal cigarette smoking and *CYP1A1* and *GSTT1* genotypes in relation to infant birth weight, gestation, and fetal

growth with adjustment of major covariates. We first examined the association between maternal cigarette

smoking and infant birth weight without consideration of maternal genotypes. Then we investigated whether the

Table 1. Characteristics of the Study Population by Maternal Smoking Status*

Maternal Characteristics	Maternal Smoking During Pregnancy		Inference Statistics, Odds Ratio (95% Confidence Interval)
	Never (n = 567)	Ever (n = 174)	
Genotypes, %			
<i>CYP1A1</i>			
AA	57.0	58.6	1.0
Aa	34.7	35.1	1.0 (0.7-1.4)
aa	8.3	6.3	0.7 (0.4-1.5)
<i>GSTT1</i>			
Present	76.4	77.0	1.0
Absent	23.6	23.0	1.0 (0.7-1.4)
Ethnicity, %			
Black	50.1	46.0	1.0
White	10.6	35.6	3.7 (2.4-5.7)†
Hispanic	26.5	10.9	0.5 (0.3-0.8)†
Other	12.9	7.5	0.6 (0.3-1.2)
Age, %, y			
<20	10.4	14.9	1.0
20-24	26.3	28.7	0.8 (0.4-1.3)
25-29	27.5	22.4	0.6 (0.3-1.0)
≥30	35.8	33.9	0.7 (0.4-1.1)
Highest education, %			
≤Middle school	30.5	38.5	1.0
= High school	36.5	42.0	0.9 (0.6-1.3)
>High school	33.0	19.5	0.5 (0.3-0.8)†
Parity, %			
0	25.9	17.2	1.0
1	36.3	30.5	1.3 (0.8-2.1)
≥2	37.7	52.3	2.1 (1.3-3.3)†
Marital status, %			
Married	37.2	14.9	1.0
Other	62.8	85.1	3.4 (2.2-5.3)†
Passive smoking, %			
No	84.0	43.5	1.0
Yes	16.0	56.5	6.8 (4.7-10.0)†
Alcohol use, %			
No	90.4	80.4	1.0
Yes	9.6	19.6	2.3 (1.4-3.7)†
Prepregnancy height, mean (SD), m	1.6 (0.07)	1.6 (0.07)	0.006 (-0.006 to 0.018)
Prepregnancy weight, mean (SD), kg	68.0 (17.2)	67.0 (15.8)	-1.1 (-4.0 to 1.8)
Gestational age, mean (SD), wk	38.4 (3.1)	37.7 (3.6)	-0.8 (-1.3 to -0.2)†
Birth weight, mean (SD), g	3110 (775)	2830 (799)	-280.0 (-413.0 to -147.0)†
Preterm delivery, %			
No	79.4	67.8	1.0
Yes	20.6	32.2	1.8 (1.3-2.7)†
Low birth weight, %			
No	81.1	70.1	1.0
Yes	18.9	29.9	1.8 (1.3-2.7)†
Infant sex, %			
Male	49.2	56.3	1.0
Female	50.8	43.7	0.8 (0.5-1.1)

*AA indicates homozygous wild type; Aa, heterozygous variant type; aa, homozygous variant type. Odds ratios are for the discrete variables and differences in means are for the continuous variables.
†P<.01.

association between maternal cigarette smoking and birth weight was modified by maternal genotypes by estimating the association between maternal cigarette smoking and birth weight in maternal genotype groups of each gene, respectively. Furthermore, we examined the combined association of maternal cigarette smoking and maternal genotypes with birth weight in 8 subgroups. These subgroups were defined by maternal smoking status during pregnancy (never vs continuous; the quitters were excluded from the analysis due to small sample size) and by maternal genotype for *CYP1A1* (AA, Aa/aa) and *GSTT1* (present, absent). Gene-cigarette smoke interaction was also tested by adding a product term to

the regression model. Similar analysis was applied to gestational age and fetal growth. Finally, to address potential confounding by population stratification, we performed the analysis stratified by maternal ethnicity.

All the analyses were adjusted for the following potential confounders: maternal ethnicity (white, black, Hispanic, other), age (<20, 20-24, 25-29, and ≥30 years), education (≤middle school, =high school, and >high school), parity (0, 1, and ≥2), marital status (married, other), prepregnant weight and height in both linear and quadratic terms, passive smoking (no, yes), maternal self-reported alcohol use (nonusers, current users), and infant sex. All *P* values were 2-sided and defined as *P* = .05 for

statistical significance. We used statistical software SAS (SAS Institute Inc, Cary, NC) for all analyses.

RESULTS

Our analysis included a total of 741 mothers: 567 never smokers and 174 ever smokers. A total of 207 cases were preterm or LBW infants (125 were both preterm and LBW, 34 were LBW only, and 48 were preterm only) and 534 were non-LBW, full-term infants (control). As shown in TABLE 1, the never- and ever-smoking groups were similar in *CYP1A1* and *GSTT1* genotype frequencies, age distribution, maternal prepregnancy weight and height, and infant sex. However, the 2 groups differed in ethnicity, education, parity, marital status, passive smoke exposure, and alcohol use. For the ever smokers, the mean birth weight was 280 g lower (95% confidence interval [CI], -413 to -147) and the odds ratio (OR) for LBW was higher (OR, 1.8; 95% CI, 1.3-2.7) compared with the never smokers. The mean gestational age for ever smokers was 0.8 weeks shorter (95% CI, -1.3 to -0.2) and the OR of preterm birth was higher (OR, 1.8; 95% CI, 1.3-2.7).

As shown in TABLE 2, without consideration of genotype, continuous maternal smoking during pregnancy was associated with an OR of 2.1 (95% CI, 1.2-3.7) for LBW and a mean reduction of 377 g (SE, 89 g) in birth weight compared with the never smokers. When *CYP1A1* genotype was considered, the association between continuous maternal smoking and LBW differed remarkably by the genotype: the OR for LBW was 1.3 (95% CI, 0.6-2.6) among mothers with the AA genotype (n=75) but 3.2 (95% CI, 1.6-6.4) among mothers with the Aa/aa genotypes (n=43 for Aa and n=6 for aa). A similar pattern emerged when *GSTT1* genotype was considered: the OR was 1.7 (95% CI, 0.9-3.2) and 3.5 (95% CI, 1.5-8.3) for the present and absent genotypes, respectively. Consistently, when birth weight was analyzed as a continuous variable, continuous maternal smoking was associated with a mean reduction of 252 g (SE, 111 g) vs 520 g (SE, 124 g) in birth weight for the *CYP1A1*

Table 2. Adjusted Associations of Maternal Smoking During Pregnancy With Birth Weight by Maternal Genotypes*

Genotype	Maternal Smoking Group	No.	Logistic Regression Low Birth Weight (LBW)†			Multiple Linear Regression Birth Weight, g‡	
			LBW, %	Odds Ratio (95% Confidence Interval)	<i>P</i> Value	β (SE)	<i>P</i> Value
Total sample	Never	567	18.9	1.0		Referent	
	Quitter	50	24.0	1.3 (0.6-2.7)	.49	-53 (116)	.64
	Continuous	124	32.3	2.1 (1.2-3.7)	.006	-377 (89)	<.001
<i>CYP1A1</i> AA	Never	323	20.4	1.0		Referent	
	Quitter	27	25.9	1.4 (0.5-3.7)	.48	-52 (156)	.74
	Continuous	75	24.0	1.3 (0.6-2.6)	.51	-252 (111)	.02
Aa/aa	Never	244	16.8	0.8 (0.5-1.3)	.35	28 (66)	.67
	Quitter	23	21.7	0.9 (0.3-2.7)	.87	-23 (167)	.89
	Continuous	49	44.9	3.2 (1.6-6.4)	.001	-520 (124)	<.001
Interaction§	Crude			3.3 (1.4-8.0)	.009	-331 (158)	.04
	Adjusted			3.1 (1.2-7.8)	.02	-293 (157)	.06
<i>GSTT1</i> Present	Never	433	19.2	1.0		Referent	
	Quitter	38	26.3	1.3 (0.6-3.0)	.50	-125 (131)	.34
	Continuous	96	28.1	1.7 (0.9-3.2)	.08	-285 (99)	.004
Absent	Never	134	17.9	0.9 (0.5-1.5)	.66	27 (76)	.72
	Quitter	12	16.7	1.0 (0.2-4.7)	.95	222 (225)	.33
	Continuous	28	46.4	3.5 (1.5-8.3)	.004	-642 (154)	<.001
Interaction§	Crude			2.4 (0.9-6.6)	.09	-428 (184)	.02
	Adjusted			2.2 (0.8-6.3)	.14	-376 (181)	.04

*AA indicates homozygous wild type; Aa, heterozygous variant type; aa, homozygous variant type.
 †Logistic regression model: low birth weight was defined as a binary variable (<2500 g, ≥2500 g) with adjustment for maternal ethnicity (white, black, Hispanic, other), age (<20, 20-24, 25-29, ≥30 years), education (≤middle school, high school, >high school), parity (0, 1, ≥2), marital status (married, other), passive smoke exposure (no, yes), alcohol use during pregnancy (no, yes), prepregnancy weight and height in linear and quadratic terms, and infant sex.
 ‡Multiple linear regression models: birth weight was analyzed as a continuous variable with adjustment for the variables listed above. β Represents the difference in mean birth weight between smoking and never-smoking groups after adjustment for the covariates.
 §Test of interaction: a *P* value is presented for testing the null hypothesis, β = 0 in multiple linear regression models or odds ratio = 1.0 in logistic regression models for the product term, continuous smoking × genotype.

AA and Aa/aa genotypes, respectively; and a mean reduction of 285 g (SE, 99 g) vs 642 g (SE, 154 g) in birth weight for GSTT1 present and absent genotypes, respectively.

We found a similar pattern for gestational age (TABLE 3). Without consideration of genotype, continuous maternal smoking was associated with an OR of 1.8 (95% CI, 1.1-3.1) for preterm birth and a 1.0 week (SE, 0.4-week) shortening in gestation. When CYP1A1 genotype was considered, the OR was 1.5 (95% CI, 0.8-2.8) and 2.2 (95% CI, 1.1-4.4) for the AA and Aa/aa genotypes, respectively. When GSTT1 genotype was considered, the OR was 1.4 (95% CI, 0.8-2.6) and 2.8 (95% CI, 1.2-6.7) for the present and absent genotypes, respectively. When gestational age was analyzed as a continuous variable, there were reductions in mean gestational age of 0.6 (SE, 0.5) and 1.5 (SE, 0.5) weeks for the CYP1A1 AA and Aa/aa genotypes, respectively; and reductions of 0.5 (SE, 0.4) and 2.1 (SE, 0.7) week for GSTT1 present and absent genotypes, respectively.

We also examined the association of continuous maternal smoking and maternal genotype with birth weight ratio (TABLE 4). When CYP1A1 genotype was considered, the OR of intrauterine growth restriction was 1.9 (95% CI, 0.9-4.1) and 4.1 (95% CI, 2.0-8.6) for continuous smokers with AA and Aa/aa genotypes, respectively. The pattern was similar when birth weight ratio was analyzed as a continuous variable. However, this pattern was not found with stratification by GSTT1 genotype.

TABLE 5 presents the combined association of maternal cigarette smoking and CYP1A1 and GSTT1 genotypes with infant birth weight, gestational age, and birth weight ratio. There was a common pattern for the 3 outcomes. Among nonsmoking mothers, genotype alone did not confer a significant adverse effect. In the presence of maternal smoking, the greatest reduction in mean birth weight (-1285 g; SE, 234 g), gestational age (-5.2 weeks; SE, 1.0 week), and birth weight ratio (-0.120; SE, 0.048) was found among the group with the CYP1A1 Aa/aa

and GSTT1 absent genotypes. A test of interaction between maternal smoking and maternal CYP1A1 and GSTT1 genotypes was statistically significant for birth weight and for gestational age, but not for birth weight ratio.

We performed separate analyses for blacks and whites, the 2 largest ethnic subgroups in our sample with adequate numbers of smokers. The percentages of CYP1A1 AA, Aa, and aa genotypes were 58.0%, 36.3%, and 5.8%, respectively, for blacks; 73.8%, 24.6%, and 1.6%, respectively, for whites (χ^2 test, $P=.005$). The percentage of GSTT1 absent genotype was 22.5% for blacks and 18.9% for whites (χ^2 test, $P=.39$). As shown in TABLE 6 without consideration of maternal genotype, the association between maternal smoking and infant birth weight

was comparable for blacks and whites. However, when the genotype was considered, the estimated smoking effects were different between the 2 groups and gene-smoking interactions were only statistically significant in blacks.

There were 38 mothers with either gestational diabetes or diabetes mellitus in our study. Neither adjustment for diabetic status in the regression model nor exclusion of diabetic mothers from the analysis altered our results. Our data did not show significant differences in pregnancy complications (preeclampsia, eclampsia, chronic hypertension, diabetes, abruptio placentae, placenta previa, incompetent cervix, oligohydramnios, polyhydramnios, meconium in amniotic fluid) nor differences in method of delivery (vaginal vs cesarean) between never and ever

Table 3. Adjusted Associations of Maternal Smoking During Pregnancy With Gestation by Maternal Genotypes*

Genotype	Maternal Smoking Group	No.	PTB, %	Logistic Regression Preterm Birth (PTB)†		Multiple Linear Regression Gestation, wk‡	
				Odds Ratio (95% Confidence Interval)	P Value	β (SE)	P Value
Total sample	Never	567	20.6	1.0		Referent	
	Quitter	50	28.0	1.4 (0.7-2.7)	.40	-0.3 (0.5)	.58
	Continuous	124	33.9	1.8 (1.1-3.1)	.02	-1.0 (0.4)	.01
CYP1A1 AA	Never	323	21.7	1.0		Referent	
	Quitter	27	25.9	1.2 (0.5-3.0)	.74	-0.5 (0.7)	.49
	Continuous	75	30.7	1.5 (0.8-2.8)	.27	-0.6 (0.5)	.24
Aa/aa	Never	244	19.3	0.9 (0.6-1.4)	.57	0.03 (0.3)	.92
	Quitter	23	30.4	1.4 (0.5-3.7)	.51	-0.01 (0.7)	.99
	Continuous	49	38.8	2.2 (1.1-4.4)	.03	-1.5 (0.5)	.005
Interaction§	Crude			1.7 (0.7-3.9)	.25	-0.9 (0.7)	.16
	Adjusted			1.7 (0.7-4.2)	.26	-0.9 (0.7)	.17
GSTT1 Present	Never	433	21.9	1.0		Referent	
	Quitter	38	34.2	1.6 (0.7-3.4)	.24	-0.5 (0.6)	.35
	Continuous	96	30.2	1.4 (0.8-2.6)	.24	-0.5 (0.4)	.27
Absent	Never	134	16.4	0.7 (0.4-1.2)	.16	0.5 (0.3)	.12
	Quitter	12	8.3	0.3 (0.1-2.7)	.30	1.1 (1.0)	.25
	Continuous	28	46.4	2.8 (1.2-6.7)	.02	-2.1 (0.7)	.001
Interaction§	Crude			2.9 (1.1-7.8)	.04	-2.1 (0.8)	.005
	Adjusted			2.9 (1.0-8.2)	.05	-2.1 (0.8)	.007

*AA indicates homozygous wild type; Aa, heterozygous variant type; aa, homozygous variant type.
 †Logistic regression model: preterm weight was defined as a binary variable (gestational age <37, \geq 37 weeks) with adjustment for the variables listed in the second footnote to Table 2.
 ‡Multiple linear regression models: gestational age was analyzed as a continuous variable with adjustment for the variables listed above. β Represents the difference in mean gestation between smoking and never-smoking groups after adjustment for the covariates.
 §Test of interaction: a P value is presented for testing the null hypothesis, $\beta = 0$ in multiple linear regression models or odds ratio = 1.0 in logistic regression models for the product term, continuous smoking \times genotype.

smokers. Further adjustment of pregnancy complications and type of delivery in the regression analyses did not alter our results.

COMMENT

It has long been recognized that many human diseases arise from the complex interplay of environmental exposures and host susceptibilities. Our study represents the first step in investigating how genetic susceptibility modulates risk of adverse reproductive outcomes from environmental exposures such as cigarette smoke. Consistent with previous studies, we found that maternal cigarette smoking was associated with reduced birth weight and an increased risk of LBW,³⁻⁸ shortened gestation and an increased risk of preterm birth,^{8,27-29} and in-

trauterine growth restriction.^{3,9,10} Our data indicate that maternal cigarette smoking likely affects infant birth weight via both reduced fetal growth and shortened gestation. More importantly, our study shows consistent evidence that the adverse effects of maternal cigarette smoking on infant birth weight and gestational age were modified by maternal *CYP1A1* and *GSTT1* genotypes. Our data demonstrate that a subgroup of pregnant women with certain genotypes appeared to be particularly susceptible to the adverse effect of cigarette smoke, suggesting an interaction between metabolic genes and cigarette smoking.

Although there are few published data on genetic susceptibility to cigarette smoke in relation to birth weight or gestation, this susceptibility is biologically

plausible. Both *CYP1A1* and *GSTT1* genes are highly polymorphic in our study population. These gene polymorphisms have been associated with their encoded enzyme activity; *CYP1A1* MspI variant genotypes may increase enzyme activity,³⁰ while the deletion type of *GSTT1* leads to an absence of enzyme activity.³¹ There is evidence that increased *CYP1A1* enzyme activity associated with MspI variant genotype or absence of *GSTT1* enzyme activity associated with deletion genotype can be detrimental to pregnancy outcomes in the presence of cigarette smoke exposure.

Major classes of carcinogens present in cigarette smoke are converted into DNA-reactive metabolites by cytochrome P450-related enzymes and some cytochrome P450 variants have been associated with increased risk of various cancers.¹² On the other hand, the *GSTT1* enzyme is important in protecting against certain genotoxic damages, such as sister chromatid exchanges^{32,33} and the formation of hemoglobin adducts due to ethylene oxide present in tobacco smoke.³⁴ Everson et al³⁵ tested human placental specimens for DNA adducts and found that DNA adducts were almost exclusively present in those specimens from mothers who were smokers. Positive dose-response relationships were shown between levels of the smoking-related adducts and biochemical doses of maternal tobacco smoke exposure during pregnancy. Alexandrov et al³⁶ further demonstrated that the levels of benzo(a)pyrene diol-epoxide-DNA adducts and bulky DNA adducts were significantly and positively correlated with *CYP1A1* enzyme activity. A similar finding was demonstrated in another independent study.³⁷ Furthermore, Perera et al³⁸ found that newborns with elevated levels of PAH-DNA adducts had significantly decreased birth weight ($P = .05$), birth length ($P = .02$), and head circumference ($P < .001$) compared with newborns with lower adducts ($n = 135$). Consistently, our study found that smoking mothers who had *CYP1A1* MspI variant genotypes or *GSTT1* deletion genotype had lower birth weight and birth weight ratio and shorter gestational age com-

Table 4. Adjusted Associations of Maternal Smoking During Pregnancy With Fetal Growth by Maternal Genotypes*

Genotype	Maternal Smoking Group	No.	IUGR, %	Logistic Regression Intrauterine Growth Restriction (IUGR)†		Multiple Linear Regression Birth Weight Ratio‡		
				Odds Ratio (95% Confidence Interval)	P Value	β (SE)	P Value	
Total sample	Never	567	13.8	1.0		Referent		
	Quitter	50	16.0	1.3 (0.6-3.0)	.55	0.013 (0.024)	.59	
	Continuous	124	28.2	2.9 (1.6-5.2)	<.001	-0.068 (0.018)	<.001	
<i>CYP1A1</i>	AA	Never	323	14.2	1.0		Referent	
		Quitter	27	14.8	1.2 (0.4-3.9)	.75	0 (0.032)	>.99
		Continuous	75	20.0	1.9 (0.9-4.1)	.11	-0.047 (0.023)	.04
	Aa/aa	Never	244	13.1	0.9 (0.6-1.6)	.79	0.001 (0.013)	.92
		Quitter	23	17.4	1.3 (0.4-4.1)	.70	0.031 (0.034)	.37
		Continuous	49	40.8	4.1 (2.0-8.6)	<.001	-0.095 (0.025)	<.001
	Interaction§	Crude			3.0 (1.2-7.8)	.02	-0.064 (0.033)	.05
		Adjusted			2.5 (0.9-6.6)	.08	-0.055 (0.032)	.08
	<i>GSTT1</i>	Present	Never	433	13.2	1.0		Referent
Quitter			38	15.8	1.3 (0.5-3.3)	.64	0.012 (0.027)	.66
Continuous			96	28.1	3.3 (1.7-6.3)	<.001	-0.068 (0.020)	.001
Absent		Never	134	15.7	1.2 (0.7-2.2)	.46	-0.026 (0.016)	.09
		Quitter	12	16.7	1.8 (0.4-9.4)	.47	-0.009 (0.046)	.85
		Continuous	28	28.6	2.5 (0.9-6.4)	.07	-0.094 (0.032)	.003
Interaction§		Crude			0.8 (0.3-2.5)	.74	-0.015 (0.038)	.69
		Adjusted			0.6 (0.2-1.9)	.39	-0.001 (0.037)	.97

*AA indicates homozygous wild type; Aa, heterozygous variant type; aa, homozygous variant type.
 †Logistic regression model: intrauterine growth restriction was defined as a binary variable (<85%, ≥85% of birth weight ratio [observed birth weight/mean birth weight for gestation]) with adjustment for the variables listed in the second footnote to Table 2.
 ‡Multiple linear regression models: birth weight ratio was analyzed as a continuous variable with adjustment for the variables listed above. β Represents the difference in mean birth weight ratio between smoking and never-smoking groups after adjustment for the covariates.
 §Test of interaction: a P value is presented for testing the null hypothesis, β = 0 in multiple linear regression models or odds ratio = 1.0 in logistic regression models for the product term, continuous smoking × genotype.

Table 5. Combined Associations of Maternal Smoking During Pregnancy and *CYP1A1* and *GSTT1* Gene Polymorphisms With Infant Birth Weight, Gestational Age, and Birth Weight Ratio*

Maternal Subgroups			Birth Weight, g			Gestation, wk		Birth Weight Ratio	
Smoking	<i>CYP1A1</i>	<i>GSTT1</i>	No.	β (SE)	P Value	β (SE)	P Value	β (SE)	P Value
Never	AA	Present	251	Referent		Referent		Referent	
Never	AA	Absent	72	82 (102)	.42	0.9 (0.4)	.03	-0.041 (0.021)	.05
Never	Aa/aa	Present	182	56 (75)	.45	0.2 (0.3)	.46	-0.006 (0.015)	.72
Never	Aa/aa	Absent	62	12 (109)	.91	0.2 (0.5)	.64	-0.015 (0.023)	.52
Continuous	AA	Present	58	-234 (121)	.05	-0.4 (0.5)	.40	-0.048 (0.025)	.06
Continuous	AA	Absent	17	-161 (197)	.41	0.3 (0.8)	.75	-0.079 (0.041)	.05
Continuous	Aa/aa	Present	38	-251 (140)	.07	-0.01 (0.6)	.99	-0.099 (0.029)	.001
Continuous	Aa/aa	Absent	11	-1285 (234)	<.001	-5.2 (1.0)	<.001	-0.120 (0.048)	.01
Test of interaction†									
Crude				-1129 (254)	<.001	-5.5 (1.0)	<.001	-0.036 (0.053)	.50
Adjusted				-1086 (251)	<.001	-5.4 (1.0)	<.001	-0.029 (0.052)	.58

*AA indicates homozygous wild type; Aa, heterozygous variant type; aa, homozygous variant type. Multiple linear regression models with adjustment the variables listed in the second footnote to Table 2. β Represents the mean difference between each subgroup and the reference group after adjustment for the covariates. Birth weight ratio indicates observed birth weight/mean birth weight for gestation.

†Test of interaction: a P value is presented for testing the null hypothesis, β = 0 in multiple linear regression models for the product term, continuous smoking × *CYP1A1* genotype × *GSTT1* genotype.

pared with the reference groups. Furthermore, our study found that smoking mothers who had both *CYP1A1* MspI variant genotype and *GSTT1* deletion genotype had the greatest reduction in birth weight, gestation, and birth weight ratio.

A number of methodological limitations should be considered when interpreting our results. Maternal smoking was based on self-report and thus may be subject to reporting bias. Nevertheless, studies have shown fair agreement between self-reported smoking amount and serum or urinary level of cotinine (a biochemical marker of cigarette smoke).^{5,39,40} Our results are consistent with the vast body of literature that demonstrates a detrimental effect of cigarette smoke on the fetus. Maternal genotypes were objective measurements and neither the mothers nor the research staffs were aware of maternal genotypes at the time of interview and medical record review.

Second, smoking mothers differed from never smokers in terms of ethnicity, education, parity, marital status, passive smoke exposure, and alcohol use. In the regression analyses, we adjusted for these variables. However, we cannot exclude the possibility of confounding effects by uncontrolled or inadequately controlled risk factors. For example, no attempt was made to as-

Table 6. Adjusted Associations of Maternal Smoking During Pregnancy With Birth Weight, Stratified by Maternal Genotype and Ethnicity*

Genotype	Maternal Smoking Status	Blacks			Whites		
		No.	β (SE)	P Value	No.	β (SE)	P Value
Total sample	Never	284	Referent		60	Referent	
	Continuous	54	-264 (135)	.05	52	-309 (165)	.06
<i>CYP1A1</i> AA	Never	169	Referent		47	Referent	
	Continuous	26	-5 (184)	.98	38	-219 (191)	.25
Aa/aa	Never	115	24 (99)	.81	13	63 (231)	.79
	Continuous	28	-475 (176)	.007	14	-467 (238)	.05
Interaction†	Crude		-332 (252)	.19		-275 (308)	.37
	Adjusted		-513 (246)	.04		-283 (333)	.40
<i>GSTT1</i> Present	Never	223	Referent		51	Referent	
	Continuous	39	-61 (158)	.70	41	-291 (176)	.10
Absent	Never	61	142 (117)	.22	9	-311 (264)	.24
	Continuous	15	-594 (217)	.006	11	-579 (263)	.03
Interaction†	Crude		-650 (283)	.02		-15 (343)	.96
	Adjusted		-658 (279)	.02		15 (375)	.97

*AA indicates homozygous wild type; Aa, heterozygous variant type; aa, homozygous variant type. Multiple linear regression model with adjustment for the variables listed in the second footnote to Table 2 (except ethnicity). β Represents the difference in mean birth weight between smoking and never-smoking groups after adjustment for the covariates.

†Test of interaction: a P value is presented for testing the null hypothesis, β = 0 in multiple linear regression models for the product term, continuous smoking × genotype.

sess nutritional status. There could be comorbidity between alcohol or illicit drug and tobacco use. Exclusion of alcohol or illicit drug users from the analysis did not significantly alter the results.

Third, cigarette smoke is a complex mixture of chemicals³ and other metabolic genes may be involved. This study only examined *CYP1A1* and *GSTT1* genotypes. The relative role of meta-

bolic genes vs other genes in determining genetic susceptibility to adverse reproductive outcomes of cigarette smoking is yet to be understood. Furthermore, there is a possibility of unrecognized linkage disequilibrium between the candidate marker and another gene that is the real susceptibility locus.

Population stratification is a potential issue in genetically heterogeneous

populations like that of the United States. This is an inherent weakness of a case-control study design. A family-based association study, such as transmission/disequilibrium testing, is more desirable to address this issue. Assessing the confounding of interactions is an evolving area of epidemiology. Factors that may not be confounders in a regular analysis may still change the estimate of the effect of the gene polymorphism-smoking interaction. In addition, we only examined maternal genotypes, and the role of fetal genotypes in modifying the adverse effect of cigarette smoke and maternal-fetal gene interaction remains to be determined.

The rapid advances in the Human Genome Project, bioinformatics, and biotechnology have provided unprecedented opportunities as well as challenges in understanding the genetic basis for individual differences in susceptibility to environmental exposures.⁴¹ As discussed in a recent commentary,⁴² much work remains to be done and many methodological challenges remain to be addressed in this research area. A coherent gene-environment approach, with attention to genetically susceptible populations who are disproportionately exposed to environmental reproductive hazards, may provide further insights into the etiology of intrauterine growth restriction and preterm birth and may help identify high-risk subpopulations for clinical or public health interventions.

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