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Original Contribution

Adverse Birth Outcomes Associated with Maternal Smoking and Polymorphisms in the *N*-Nitrosamine-Metabolizing Enzyme Genes *NQO1* and *CYP2E1*

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Maternal smoking during pregnancy can result in both pregnancy complications and reduced size of the fetus and neonate. Among women who smoke, genetic susceptibility to tobacco smoke also is a likely causative factor in adverse pregnancy outcomes. A prospective cohort study was conducted among 460 pregnant women who delivered live singletons in Sapporo, Japan, from 2002 to 2005. Multiple linear regression models were used to estimate associations of maternal smoking and polymorphisms in two genes encoding *N*-nitrosamine-metabolizing enzymes—NAD(P)H: quinone oxidoreductase 1 (*NQO1*) and cytochrome P-450 2E1 (*CYP2E1*)—with birth size. Among infants born to smokers with the *NQO1* homozygous wild-type allele, birth weight, birth length, and birth head circumference were significantly reduced ($p < 0.01$ for each factor). For the homozygous wild-type *CYP2E1* allele, birth weight was lower by an estimated 195 g (standard error, 55; $p < 0.001$) among smokers. These genotypes did not confer adverse effects among women who had never smoked or who quit smoking during the first trimester. The adverse effects of maternal smoking on infant birth size may be modified by maternal genetic polymorphisms in *N*-nitrosamine-metabolizing enzymes among Japanese subjects. These results may help in directing smoking cessation interventions during pregnancy, especially among susceptible women.

birth weight; cytochrome P-450 *CYP2E1*; fetal development; nitrosamines; NQO1 protein, human; pregnancy; pregnancy outcome; smoking

Abbreviations: CYP2E1, cytochrome P-450 2E1; MGB, minor groove binder; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NQO1, NAD(P)H: quinone oxidoreductase 1; PAH, polycyclic aromatic hydrocarbon; PCR, polymerase chain reaction; Pro, proline; SE, standard error; Ser, serine.

Tobacco smoke is a complex mixture of over 4,000 compounds, including polycyclic aromatic hydrocarbons (PAHs) and *N*-nitrosamines such as the tobacco-specific carcinogens 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*′-nitrosonornicotine. Both PAHs and *N*-nitrosamines are genotoxic and carcinogenic, and their metabolic activation leads to the formation of DNA adducts. Experimental and epidemiologic studies have reported an

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association between genetic susceptibility to PAHs and the risk of squamous-cell lung carcinoma and between genetic susceptibility to *N*-nitrosamines and the risk of lung adenocarcinoma (1–3).

The metabolism of xenobiotics can be classified into phase I (activation) and phase II (detoxification) reactions (4). NAD(P)H: quinone oxidoreductase 1 (NQO1) is an important enzyme with dual functions: activation or detoxification, depending on the substrate. As a detoxification enzyme, it catalyzes the two-electron reduction of quinoid compounds to the readily excreted hydroquinones, preventing the generation of reactive oxygen species and thereby protecting cells against oxidative damage. It also catalyzes the activation of some procarcinogens such as nitrosamines and heterocyclic amines, which are present in tobacco smoke (5–10). The contradictory findings of previous studies of relations between the *NQO1* C609T polymorphism, leading to an amino-acid change in codon 187 from proline (Pro) to serine (Ser), and the risk of various cancers may thus be explained by these paradoxical functions. For example, Japanese studies have reported an association between the *NQO1* Ser/Ser (TT) genotype and increased risk of cervical cancer (9) and an association between the *NQO1* Pro/Pro (CC) genotype and increased risk of lung adenocarcinoma (5).

Cytochrome P-450 2E1 (CYP2E1) catalyzes the metabolic activation of carcinogenic nitrosamines, benzene, and low-molecular-weight compounds. Several polymorphisms in the human *CYP2E1* gene have been identified. Persons with the c2 allele of the *RsaI* (*CYP2E1*5*) polymorphism, which is located in the 5′-flanking region of *CYP2E1*, have decreased enzyme activity or noninducibility (7, 11–13). Investigators in previous epidemiologic studies have reported that this genetic polymorphism is significantly associated with decreased risk of lung cancer and bladder cancer in Asians (7, 12, 13).

Maternal smoking during pregnancy has been causally associated with adverse pregnancy outcomes, including placental abruption, placenta previa, preterm delivery, intrauterine growth retardation, and fetal death (14–24). In addition, previous studies have found an association between prenatal exposure to toxins and perinatal adverse effects. For example, NNK exposure during pregnancy has been found to increase the infant's susceptibility to pediatric lung disorders, such as bronchopulmonary dysplasia and cystic fibrosis, and the *NQO1* and *CYP2E1* polymorphisms have been found to be associated with the risk of childhood leukemia (25–27). In recent years, it has been suggested that metabolic enzymes mediating maternal genetic susceptibility to tobacco smoke might be related to adverse birth outcomes. In fact, certain maternal genetic polymorphisms in the PAH-metabolizing enzymes, such as the cytochrome P-450 1A1 (*CYP1A1*) gene or the glutathione-*S*-transferase M1 (*GSTM1*) gene, have been shown to enhance the association between maternal smoking and infant birth size (28, 29). However, few reports have been published on maternal polymorphisms in genes encoding the *N*-nitrosamine-metabolizing enzymes in relation to birth size.

To address this issue, we investigated maternal genetic polymorphisms that affect the association between maternal smoking during pregnancy and infant birth size among Japanese subjects. We characterized genetic susceptibility by analyzing polymorphisms in the genes *NQO1* and *CYP2E1*.

MATERIALS AND METHODS

Study population and data collection

A prospective cohort study was performed between July 2002 and October 2005 in Sapporo, Japan (Hokkaido Study on Environment and Children's Health). Details of the methods have been published elsewhere (29, 30). Study subjects were women who enrolled at 23–35 weeks of gestation and delivered at the Sapporo Toho Hospital. A self-administered questionnaire was completed at the time of enrollment to obtain information on relevant factors, including demographic characteristics, smoking history, alcohol consumption during pregnancy, and household income. Information obtained from maternal and infant medical records included pregnancy complications and birth outcomes (gestational age at birth, infant gender, and birth size). Women whose medical records indicated that they had pregnancy-induced hypertension, a history of diabetes mellitus, or a multiple birth were excluded from the analysis. In this study, maternal smoking status was based on maternal self-reporting and was categorized into three groups: women who did not smoke at any time during pregnancy (nonsmokers), women who quit smoking in the first trimester (quitters), and women who continued to smoke during pregnancy (smokers). Since eight women quit smoking in the second trimester and their mean infant birth size was similar to that of the smoking group (birth weight = 2,737 g (standard deviation, 440), birth length = 46.8 cm (standard deviation, 2.0), and birth head circumference = 32.8 cm (standard deviation, 1.5)), they were included in the group of smokers because of the small sample size.

This study was conducted with the informed consent of all subjects. The study protocol was approved by the institutional ethical board for human gene and genome studies of the Hokkaido University Graduate School of Medicine and by the institutional review board for gene and genome research of Health Sciences University of Hokkaido.

Genotyping methods

Maternal blood samples were collected at the time of study enrollment, and genomic DNA was extracted from lymphocytes using standard techniques (29, 31).

The presence of the *NQO1* and *CYP2E1* polymorphisms was determined by means of the TaqMan (Applied Biosystems, Inc., Foster City, California) polymerase chain reaction (PCR) method, using minor groove binder (MGB) probes. Briefly, to detect a polymorphism in *NQO1* involving a proline-to-serine substitution at codon 187 (C/T, rs1800566) in exon 6, we prepared a C-allele-specific MGB probe, 5′-TGT CAG TTG AGG TTC T-MGB-3′, and a T-allele-specific MGB probe, 5′-TCA GTT GAG ATT CTA AG-MGB-3′. Each of the reporters was quenched by excess MGB. Primers for PCR of the flanking region of the C/T polymorphism in *NQO1* were as follows: forward,

5′-TGC ATT TCT GTG GCT TCC AA-3′, and reverse, 5′-CTG GAG TGT GCC CAA TGC TAT A-3′. For a polymorphism in *CYP2E1* (guanine-to-cytosine transition in the 5′-flanking region, −1,294G/C, *CYP2E1*5*, rs3813864), we prepared a G-allele-specific MGB probe, 5′-ACA CTG CAC CTC TCC T-MGB-3′, and a C-allele-specific MGB probe, 5′-CAC TGC AGC TCT CCT -MGB-3′. Each of the reporters was quenched by excess MGB. Primers for PCR of the flanking region of the G/C polymorphism in *CYP2E1* were as follows: forward, 5′-GCC AAC GCC CCT TCT TG-3′, and reverse, 5′-TCA TTG GTT GTG CTG CAC CTA-3′. PCR was carried out using a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems). During PCR cycles (an initial denaturation at 95°C for 10 minutes, followed by 50°C for 2 minutes, followed by 40 cycles of 92°C for 15 seconds and 60°C for 60 seconds), the fluorescence of the products was measured using a 7500 Real-time PCR System (Applied Biosystems), resulting in the clear identification of three genotypes: the Pro/Pro (homozygous wild type), Pro/Ser (heterozygote), and Ser/Ser (homozygous mutant) genotypes of *NQO1* and the c1/c1 (homozygous wild type), c1/c2 (heterozygote), and c2/c2 (homozygous mutant) genotypes of *CYP2E1*.

Statistical analysis

Associations between variables were analyzed by analysis of variance and the chi-squared test. The multiple linear regression model was used to evaluate the individual and combined associations of maternal smoking status during pregnancy and the maternal genetic polymorphisms with infant birth weight, birth length, and birth head circumference, after adjustment for the following covariates: maternal age, height, weight before pregnancy, weight gain during pregnancy, alcohol consumption during pregnancy (total ethanol intake in g/day), parity (0 vs. $\geq$1), infant gender, gestational age at birth, and household income. The interaction between maternal genotype and smoking was also evaluated by adding a product term to the regression model. All statistical analyses were performed using SPSS for Windows, version 15.0J (Japanese version; SPSS, Inc., Chicago, Illinois).

RESULTS

Of 1,796 potentially eligible women, 514 agreed to participate (a participation rate of 29 percent). Of these, 46 were excluded from this analysis because of pregnancy-induced hypertension ($n = 24$), a history of diabetes mellitus ($n = 2$), a multiple birth ($n = 7$), a stillbirth ($n = 1$), major birth defects ($n = 3$), or dropping out of the study before delivery ($n = 9$). A further eight were excluded because blood samples could not be collected. Thus, the analysis included 460 women: 272 nonsmokers, 93 quitters, and 95 smokers. Quitters smoked an average of 13.2 (range, 2–36) cigarettes per day during the first trimester. The prevalence of smoking was 20.7 percent, which was similar to that of Sapporo City (18.7 percent) (32), and these women reported smoking an average of 13.0 (range, 1–30) cigarettes per day. The number of low-birth-weight (defined as birth weight <2,500 g) infants was 20 (4.3 percent), and the number of small-for-gestational-age (defined as birth weight <10th percentile) infants was 27 (5.9 percent).

Demographic variables and genotype frequencies for the study group are summarized in table 1. The mean infant birth weight, birth length, and birth head circumference were significantly lower in smokers than in other groups ($p < 0.01$ for each factor). The three groups differed with regard to mean maternal age, weight gain during pregnancy, parity, and household income ($p < 0.001$, $p < 0.001$, $p < 0.05$, and $p < 0.05$, respectively). Eighty-four nonsmokers (30.9 percent), 27 quitters (29.0 percent), and 33 smokers (34.7 percent) drank alcohol during pregnancy. The amount of alcohol consumed was significantly higher in smokers ($p < 0.05$); however, infant birth weight, birth length, and birth head circumference did not differ significantly between nondrinkers and drinkers ($p > 0.05$) or between nondrinking smokers and drinking smokers ($p > 0.05$). Moreover, there was no association between maternal alcohol consumption during pregnancy and the *CYP2E1* genotype with regard to the estimated reduction in birth size (data not shown). The distribution of the *NQO1* and *CYP2E1* genotypes in the 460 women satisfied Hardy-Weinberg equilibrium (chi-squared test: $p = 0.961$ and $p = 0.974$, respectively). Genotype frequencies did not differ significantly among the three groups. Because the genotype distributions and allele frequencies in this study were consistent with previous studies (9, 33–37), our participants seemed to be representative of the general Japanese population.

Multiple linear regression analysis showed that maternal smoking during pregnancy was associated with lower infant birth weight (mean reduction = 148 g (standard error (SE), 42); $p < 0.001$), birth length (mean reduction = 0.5 cm (SE, 0.2); $p < 0.05$), and birth head circumference (mean reduction = 0.5 cm (SE, 0.2); $p < 0.01$) after adjustment for the covariates (table 2). Interestingly, for the *CYP2E1* wild genotype (c1/c1), we observed statistically significant differences with regard to the estimated reduction in birth weight (mean reduction = 73 g (SE, 32); $p < 0.05$) and birth length (mean reduction = 0.4 cm (SE, 0.2); $p < 0.05$). When we performed the Bonferroni correction, infant birth weight and birth head circumference for maternal smoking status and birth length for the *CYP2E1* genotype remained significant.

Table 3 summarizes the effects of maternal smoking during pregnancy on infant birth size, as categorized by maternal metabolic genotypes. When the *NQO1* gene was considered, the mean reductions in birth weight, birth length, and birth head circumference were 199 g (SE, 58; $p < 0.01$), 0.8 cm (SE, 0.3; $p < 0.01$), and 0.7 cm (SE, 0.2; $p < 0.01$), respectively, for women with the homozygous wild-type allele (Pro/Pro) as compared with the reference group: nonsmokers with the *NQO1* variant genotype (Pro/Ser + Ser/Ser). A significant interaction between maternal genotype and continuous smoking was observed, suggesting an adverse association with infant birth weight for the *NQO1* Pro/Pro allele ($p < 0.05$). For the *CYP2E1* gene, the estimated reduction in birth weight was 195 g (SE, 55; $p < 0.001$) for women with the homozygous wild-type allele (c1/c1) as compared with the reference group: nonsmokers

TABLE 1. Characteristics of 460 mothers participating in the Hokkaido Study on Environment and Children's Health, by maternal smoking status during pregnancy, Sapporo, Japan, 2002–2005

Maternal characteristic	Maternal smoking status during pregnancy						*p* value
	Nonsmoker ($n = 272$)		Quitter ($n = 93$)		Smoker ($n = 95$)		
	Mean (SD*)		Mean (SD)		Mean (SD)		
Maternal age (years)	31.4 (4.7)		29.9 (4.9)		29.4 (5.1)		<0.001
Maternal height (cm)	158.1 (5.6)		157.8 (5.1)		158.9 (4.8)		0.309
Maternal weight before pregnancy (kg)	52.7 (8.3)		52.7 (8.9)		53.4 (8.8)		0.792
Maternal weight gain during pregnancy (kg)	10.1 (3.4)		11.9 (4.2)		11.6 (4.0)		<0.001
Maternal alcohol consumption in drinkers during pregnancy (g/day)†	1.16 (0.3–19.2)‡		1.16 (0.5–5.6)‡		2.77 (0.5–152.0)‡		0.012
Gestational age (weeks)	38.9 (1.3)		39.2 (1.2)		39.0 (1.6)		0.184
Birth weight (g)	3,078 (347)		3,138 (384)		2,961 (386)		0.003
Birth length (cm)	48.1 (1.6)		48.5 (2.0)		47.6 (2.1)		0.006
Birth head circumference (cm)	33.3 (1.2)		33.5 (1.3)		32.9 (1.5)		0.008
	No.	%	No.	%	No.	%	
Infant gender							
Male	139	51.1	46	49.5	35	36.8	
Female	133	48.9	47	50.5	60	63.2	0.053
Parity							
0	119	43.8	55	59.1	43	45.3	
≥1	153	56.3	38	40.9	52	54.7	0.034
Annual household income (millions of yen)							
<3	41	15.1	19	20.4	28	29.5	
3–<5	131	48.2	50	53.8	51	53.7	
5–<7	65	23.9	14	15.1	11	11.6	
7–<10	30	11.0	8	8.6	5	5.3	
≥10	5	1.8	2	2.2	0	0.0	0.012
Genotype							
*NQO1**,§							
Pro*/Pro	101	37.1	30	32.3	46	48.4	
Pro/Ser*	127	46.7	51	54.8	42	44.2	
Ser/Ser	44	16.2	12	12.9	7	7.4	0.065
Pro/Ser + Ser/Ser	171	62.9	63	67.7	49	51.6	
*CYP2E1**,¶							
c1/c1	161	59.2	57	61.3	60	63.2	
c1/c2	98	36.0	34	36.5	29	30.5	
c2/c2	13	4.8	2	2.2	6	6.3	0.602
c1/c2 + c2/c2	111	40.8	36	38.7	35	36.8	

* SD, standard deviation; NQO1, NAD(P)H: quinone oxidoreductase 1; Pro, proline; Ser, serine; CYP2E1, cytochrome P-450 2E1.

† 84 nonsmokers, 27 quitters, and 33 smokers. The Kruskal-Wallis test was used to estimate associations.

‡ Median (numbers in parentheses, range).

§ *NQO1* genotype was identified with the nucleotide substitution 609 C>T (Pro187Ser).

¶ *CYP2E1* genotype was identified with the nucleotide substitution −1,294 G>C.

with the *CYP2E1* variant genotype (c1/c2 + c2/c2). However, there were no statistically significant differences with regard to the estimated reduction in birth length and birth head circumference. A significant interaction between maternal genotype and continuous smoking was observed, suggesting an adverse association with infant birth length for the *CYP2E1* c1/c1 allele ($p < 0.05$). When we performed the Bonferroni correction, the results remained significant.

TABLE 2. Effects of maternal smoking during pregnancy and maternal *NQO1 and *CYP2E1** genotypes on infant birth size, Sapporo, Japan, 2002–2005**

	Birth weight (g)		Birth length (cm)		Birth head circumference (cm)	
	β† (SE*)	*p* value	β† (SE)	*p* value	β† (SE)	*p* value
Maternal smoking status during pregnancy						
Nonsmoker ($n = 272$)	Referent		Referent		Referent	
Quitter ($n = 93$)	−31 (40)	0.443	0.02 (0.2)	0.917	0.08 (0.2)	0.616
Smoker ($n = 95$)	−148 (42)	<0.001	−0.5 (0.2)	0.019	−0.5 (0.2)	0.006
NQO1 genotype						
Pro*/Ser* + Ser/Ser ($n = 283$)	Referent		Referent		Referent	
Pro/Pro ($n = 177$)	−9 (32)	0.781	−0.2 (0.2)	0.352	−0.1 (0.1)	0.442
CYP2E1 genotype						
c1/c2 + c2/c2 ($n = 182$)	Referent		Referent		Referent	
c1/c1 ($n = 278$)	−73 (32)	0.023	−0.4 (0.2)	0.014	−0.2 (0.1)	0.129

* NQO1, NAD(P)H: quinone oxidoreductase 1; CYP2E1, cytochrome P-450 2E1; SE, standard error; Pro, proline; Ser, serine.

† Adjusted for maternal age, height, weight before pregnancy, weight gain during pregnancy, alcohol consumption during pregnancy, parity, infant gender, gestational age, and household income.

The combined associations of continuous maternal smoking during pregnancy and maternal *NQO1* and *CYP2E1* genotypes were not associated with infant birth size (data not shown). Among nonsmokers and quitters, there was no correlation between adverse effects and any allele combination for either *NQO1* or *CYP2E1*.

DISCUSSION

It has been reported that a subgroup of pregnant women with certain genotypes appears to be susceptible to the adverse effects of tobacco smoking, such as an increased risk of low birth weight and preterm birth, small-for-gestational-age birth, or low birth length. This suggests an interaction between metabolic genes and maternal smoking (28, 29, 38, 39).

To our knowledge, this is the first molecular epidemiologic evidence demonstrating an impact of maternal polymorphisms in the genes encoding the *N*-nitrosamine-metabolizing enzymes NQO1 and CYP2E1 on the association between maternal smoking during pregnancy and fetal size, represented here by birth weight, birth length, and birth head circumference. The metabolic activation process leads to the formation of DNA adducts, which are an indicator of DNA damage and a biomarker that reflects individual variation in metabolism. Interindividual differences in metabolic activation pathways are partly attributable to genetic polymorphisms associated with metabolic enzymes (1–3). Activation of these pathways in the presence of compounds in tobacco smoke may be detrimental to fetal development. Bioassays have shown a number of PAHs to be transplacental carcinogens and developmental toxicants, implying that high levels of maternal PAH-DNA adducts in leukocytes might be associated with decreased infant birth size (40).

Though few studies have evaluated the association between DNA adducts derived from *N*-nitrosamines and birth size, prior research has shown higher levels of NNK metabolites in urine collected immediately after delivery from infants of smokers as compared with infants of nonsmokers, suggesting that NNK is a potent transplacental compound in bioassays; furthermore, it has implied that transplacental exposure to *N*-nitrosamines from tobacco smoke may compromise fetal growth (41).

The present study showed that pregnant women carrying the *CYP2E1* wild-type allele gave birth to babies that had significantly reduced birth weights and lengths, independent of maternal smoking status. Previous data from a mutagen-sensitivity assay indicated that women were more sensitive to the genotoxic effects of NNK than men (42), and a high frequency of lung adenocarcinoma has been reported among nonsmoking Japanese women, implying environmental tobacco smoke exposure (43, 44). Since these findings might suggest a possible gender difference in sensitivity to certain chemical compounds in tobacco smoke, further study is required to investigate an association between maternal exposure to *N*-nitrosamines and adverse birth outcomes.

The present findings showed that infant birth weight, birth length, and birth head circumference were significantly lower for mothers who smoked during pregnancy than for nonsmokers or women who quit smoking during the first trimester, after adjustment for covariates. These results are consistent with those of previous studies which have suggested that exposure to tobacco smoke later in pregnancy has a greater adverse effect on fetal development than exposure early in pregnancy (14, 15, 17–19, 21, 22, 24) and that smoking cessation during pregnancy decreases the risk of adverse birth outcomes (14, 23). Additionally, the present study found that infants whose mothers stopped smoking during the first trimester had similar birth weights, birth lengths, and birth head circumferences as those of the nonsmoking group, taking into consideration maternal

TABLE 3. Associations of maternal smoking during pregnancy with infant birth size according to maternal *NQO1 and *CYP2E1** genotypes, Sapporo, Japan, 2002–2005**

Genotype and maternal smoking status during pregnancy	Birth weight (g)		Birth length (cm)		Birth head circumference (cm)	
	β† (SE*)	*p* value	β† (SE)	*p* value	β† (SE)	*p* value
NQO1						
Pro*/Ser* + Ser/Ser						
Nonsmoker ($n = 171$)	Referent		Referent		Referent	
Quitter ($n = 63$)	−22 (49)	0.657	0.2 (0.3)	0.487	0.05 (0.2)	0.785
Smoker ($n = 49$)	−77 (55)	0.158	−0.2 (0.3)	0.581	−0.3 (0.2)	0.152
Pro/Pro						
Nonsmoker ($n = 101$)	37 (41)	0.373	0.1 (0.2)	0.527	−0.02 (0.2)	0.922
Quitter ($n = 30$)	−10 (65)	0.877	−0.2 (0.3)	0.641	0.1 (0.3)	0.657
Smoker ($n = 46$)	−199 (58)	0.001	−0.8 (0.3)	0.007	−0.7 (0.2)	0.006
Interaction‡	−159 (80)	0.048	−0.8 (0.4)	0.058	−0.3 (0.3)	0.324
CYP2E1						
c1/c2 + c2/c2						
Nonsmoker ($n = 111$)	Referent		Referent		Referent	
Quitter ($n = 36$)	17 (64)	0.797	0.5 (0.3)	0.102	0.4 (0.3)	0.139
Smoker ($n = 35$)	−170 (66)	0.010	−1.1 (0.3)	0.002	−0.6 (0.3)	0.025
c1/c1						
Nonsmoker ($n = 161$)	−65 (41)	0.116	−0.4 (0.2)	0.048	−0.1 (0.2)	0.387
Quitter ($n = 57$)	−120 (54)	0.026	−0.7 (0.3)	0.013	−0.2 (0.2)	0.284
Smoker ($n = 60$)	−195 (55)	<0.001	−0.6 (0.3)	0.045	−0.5 (0.2)	0.022
Interaction‡	40 (82)	0.628	0.9 (0.4)	0.032	0.2 (0.3)	0.482

* NQO1, NAD(P)H: quinone oxidoreductase 1; CYP2E1, cytochrome P-450 2E1; SE, standard error; Pro, proline; Ser, serine.

† Adjusted for maternal age, height, weight before pregnancy, weight gain during pregnancy, alcohol consumption during pregnancy, parity, infant gender, gestational age, and household income.

‡ Interaction in multiple linear regression models was defined as product terms for the product of the dummy independent variables: maternal smoking status (nonsmoker, quitter, or smoker) and genotype (wild or mutant). β represents the product term for smoker × wild genotype.

metabolic genotypes. The results of this study could help in directing smoking cessation interventions toward susceptible pregnant women to prevent adverse birth outcomes.

Interpretation of our results is limited by several conditions. The evaluation of exposure to tobacco smoke was indirect, and thus the possibility of reporting bias exists. Moreover, the relatively low participation rate and low frequencies of low birth weight and small-for-gestational-age birth may limit extrapolation of these results to the general population. It is possible that women who took an interest in smoking cessation or the toxicity of smoking would have participated in our study more frequently; however, we did not inform the women that smoking was harmful to health or make any intervention for smoking cessation in our questionnaire survey.

The following points should be addressed in future studies. First, a follow-up analysis of this study population should investigate the potential association between polymorphisms in genes encoding *N*-nitrosamine-metabolizing enzymes and cognitive function in childhood, because previous studies have reported that small head circumference at birth might influence brain development and/or that low birth length might be associated with behavioral problems during early childhood (23, 45, 46). Second, an examination of fetal genotypes should be undertaken to identify any maternal-fetal gene interaction that may modulate the effect of maternal smoking during pregnancy.

In conclusion, the present study suggests an important modulating role for polymorphisms in the *N*-nitrosamine-metabolizing enzyme genes *NQO1* and *CYP2E1* in concert with adverse effects of maternal smoking in infant birth size. These findings could have significant public health implications regarding the need for smoking prevention and cessation programs (both active and passive) aimed especially at susceptible women of childbearing age.

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