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ORIGINAL CONTRIBUTIONS

Genetic Susceptibility to Benzene and Shortened Gestation: Evidence of Gene-Environment Interaction

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This study investigated whether the association between low level benzene exposure and shortened gestation is modified by two susceptibility genes, *CYP1A1* and *GSTT1*. This report includes 542 (302 nonexposed, 240 benzene-exposed) nonsmoking and nondrinking mothers of singleton live births at Beijing Yanshan Petrochemical Corporation between June 1995 and June 1997. Epidemiologic and clinical data and blood samples were obtained from mothers. Multiple linear regression models were used to estimate the associations of benzene exposure and genetic susceptibility with gestational age, adjusting for maternal age, education, parity, stress, passive smoking, prepregnancy weight and height, and infant's sex. Without consideration of genotype, benzene exposure was associated with a decrease in mean gestational age of 0.29 (standard error (SE), 0.12) week. When stratified by the maternal *CYP1A1* genotype, the estimated decrease was 0.54 (SE, 0.12) week for the *AA* group, which was significantly greater ($p = 0.003$) than that for the *Aa/aa* group, which showed no decrease in gestational age. When both *CYP1A1* and *GSTT1* were considered, the greatest decrease was found among exposed mothers with the *CYP1A1 AA-GSTT1* absent group (0.79 (SE, 0.25) week) and the *CYP1A1 AA-GSTT1* present group (0.50 (SE, 0.22) week). Among the nonexposed, genetic susceptibility alone did not confer a significant adverse effect. This study provides evidence of gene-environment interaction and supports further assessment of the role of genetic susceptibility in the evaluation of reproductive toxins. *Am J Epidemiol* 2000;152:693–700.

benzene; cytochrome P-450 CYP1A1; environmental exposure; genes; genetic predisposition to disease; gestational age; glutathione transferase

Women are exposed to a variety of reproductive toxins, but not all who are exposed have adverse reproductive outcomes. Metabolism is essential to detoxification. It is speculated that an individual's reproductive risk associated with these toxins may be modified by genetic variation in metabolic detoxification activity (1). In cancer research, a gene-environment approach to assessing individual risk of developing cancer in the face of specific environmental exposure has afforded insight into carcinogenesis. Such an approach could also provide a research model to evaluate gene-environment interactions potentially associated with reproductive outcomes. Findings from such an investigation will

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Abbreviations: bp, base pair(s); BYPC, Beijing Yanshan Petrochemical Corporation; SD, standard deviation; SE, standard error.

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have important implications for our understanding of how environmental factors interact with genetic determinants of adverse reproductive outcomes and may lead to a better approach for prevention. In this study, we focus on benzene exposure and two susceptibility genes, *CYP1A1* and *GSTT1* (known to be responsible for the metabolism and detoxification of organic solvents) in relation to gestational age.

Benzene is a commonly used organic solvent. Solvent exposure is ubiquitous in the general population. Ashley et al. (2) measured the organic solvents in the blood of approximately 600 subjects without occupational exposure in the Third National Health and Nutrition Examination Survey. Detectable concentrations of benzene, xylene, styrene, toluene, and other volatile organic chemicals were found in most of the blood samples. Organic solvents are also commonly used in the workplace. The National Institute for Occupational Safety and Health (3) estimated that 9.8 million US workers were occupationally exposed to solvents. Organic solvents identified as potential reproductive toxins include benzene (4–6), toluene (7–9), and related compounds (10). Although the level of exposure in most modern industries is far below the limit recommended by the Occupational Safety and Health Administration (11), studies have begun to suggest that even low level occupational exposure may be linked to a broad spectrum of adverse reproductive outcomes.

The detoxification of organic solvents involves two parts: phase I, in which the original nonpolar compound becomes polar and reactive, and phase II, in which the transformed polar compound is conjugated with certain endogenous functional groups such as glutathione, sulfate, glucuronide, and amino acids; thus, the end product becomes a stable hydrophilic compound that can easily be excreted (12). In humans, a significant proportion of organic solvent metabolizing enzymes are polymorphic. The expression of different host susceptibility phenotypes may explain the varying individual susceptibility to the reproductive health effects of organic solvents. The cytochrome P450 family serves as the major enzyme system in phase I metabolism. CYP1A1 is a well-studied phase I enzyme, and its polymorphism has been associated with individual cancer susceptibility (13, 14). The glutathione *S*-transferases (GSTT1 and GSTM1) are the major phase II enzymes. Our study in a Chinese population (15) showed that the *GSTT1* deletion genotype was a significant risk factor for increased sister chromatid exchange among workers exposed to benzene, thus suggesting that *GSTT1* is involved in detoxification of benzene and that its deletion puts individuals at increased risk for benzene toxicity. The *GSTM1* deletion genotype has been associated with lung cancer (16) and some noncancer diseases (17, 18). In combined phase I and phase II enzyme disorders, a 40-fold increased risk of tobacco smoke-induced lung cancer was observed in individuals with susceptible *CYP1A1* and *GSTM1* genotypes (19, 20), which suggests that phase I and phase II enzymes have a synergistic effect.

We conducted a molecular reproductive epidemiologic study in a large petrochemical plant in Beijing, China. The purpose of this report was to examine genetic susceptibility to benzene exposure in relation to length of gestation. Two susceptibility genes, *CYP1A1* (*Hin*cII) and *GSTT1*, were used to characterize genetic susceptibility and to assess gene-environment interaction, with adjustment for major covariates.

MATERIALS AND METHODS

Study site and population

This study was conducted at the Beijing Yanshan Petrochemical Corporation (BYPC), located in a suburban area of Beijing, China. BYPC, in operation since 1986, has over 80,000 employees and consists of 17 major production plants and institutes for petroleum and chemical processing. The major occupational exposures to organic solvents include benzene, toluene, styrene, and their derivatives. Since BYPC is a modern industry, the level of exposure is very low. The time-weighted average for benzene during the 8-hour shift for exposed workers ranged from 0.017 ppm to 0.191 ppm at BYPC (21). The Occupational Safety and Health Administration limit is 1 ppm as an 8-hour time-weighted average (11). The BYPC Staff Hospital, the only regional hospital that serves the community, provides prenatal care and delivery services. Eligible women were BYPC employees who gave a live singleton birth at BYPC Staff Hospital between June 1995 and June 1997.

Procedures

Eligible women were enrolled by the local medical staff. After informed consent was obtained, a previously validated questionnaire (6) was administered by trained interviewers to obtain information on demographic characteristics, cigarette smoking, alcohol consumption, occupational exposure, and medical and reproductive history. Clinical data including prepregnancy weight and height, prenatal care, and birth outcomes (including the infant's sex, gestational age, and birth weight) were obtained from medical records by a trained nurse. Blood samples were obtained from mothers via venipuncture by a skilled phlebotomist. DNA was extracted according to standard protocol (22).

Measuring gestational age

In this study, the first date of the last menstrual period recorded at the first prenatal visit was used to estimate the gestational age. The last menstrual period is accurate in this population for several reasons. In China, married couples who plan to have a child need to apply for birth permission at the local family planning administration. In essence, all births are planned, and couples will try to conceive once they have obtained birth permission. Because of the one-child policy, families are highly concerned about a healthy pregnancy and healthy babies. All the women in our study population sought prenatal care and had a pregnancy test soon after missing a menstrual period. Furthermore, the sensitivity of using gestational age to detect adverse birth outcome was increased because gestational age was calculated in exact days instead of completed weeks, the case in most epidemiologic studies.

Exposure assessment

In this study the measurement of maternal occupational exposure is based on a specialized industrial hygiene method. The details are described elsewhere (21). Briefly, maternal occupational exposure during pregnancy was assessed by industrial hygienists according to a woman's workshop, job title, and job activity. Air sample measurements for benzene, toluene, styrene, and their derivatives were obtained from major workshops during the study period. In general, pregnant employees stop working at about 28 weeks of gestation. A checklist of 55 potential reproductive hazards present in the industry was developed based on toxicologic literature. This checklist was used to identify potential exposure levels for each subject. Detailed information on the chemical process (including location maps and flow charts) in each workshop was obtained. Finally, according to a standardized algorithm developed by an industrial hygienist who was familiar with the BYPC production process but did not have knowledge of birth outcomes, the study subjects were classified into three groups: no exposure to organic solvents; exposure to benzene; and exposure to other solvents (including styrene, toluene, xylene, or their derivatives, but not benzene). In this report, we focus on two subgroups: nonexposed and benzene exposed. Although we could not completely exclude the possibility that the study subjects may have some nonoccupational source of exposure to benzene, the exposure level should be extremely low and comparable between the two groups.

Genotyping

Detection of the cytochrome P450 1A1 (CYP1A1) Hin*cII polymorphism.* The primers used in the polymerase chain reaction were intron 6 (5′-GTCTCCCTCTGGTTACAGGA-3′) and exon 7 (5′-GAAAGACCTCCCAGCGGTCA-3′) of the *CYP1A1* gene (23). Genomic DNA (50 ng) is added to a buffer containing 2.0 mM $MgCl_2$, 200 μM deoxyribonucleoside triphosphates, 300 nM primers, and 0.23 units of *Taq* polymerase in a final volume of 10 μl. After the reaction mixture is preincubated for 10 minutes at 94°C, 35 rounds of amplification are performed, consisting of denaturation at 94°C for 30 seconds, annealing at 53°C for 45 seconds, extension at 68°C for 45 seconds, and a final extension at 68°C for 1 minute. Polymerase chain reaction products are subjected to digestion by *Hinc*II for 15 hours at 37°C. The products are electrophoresed on a 2.5 percent agarose gel with ethidium bromide staining and visualized under ultraviolet light. Homozygous wild-type individuals show 139- and 32-base pair (bp) fragments, while heterozygous individuals show four bands at 139, 120, 32, and 19 bp, respectively. Homozygous rare-allele individuals show only 120-, 32-, and 19-bp bands.

Detection of the glutathione S-*transferase* (GSTT1) *deletion polymorphism.* Primers used to detect the human *GSTT1* deletion are as follows: upstream primer, 5′-TTCCTTACTGGTCCTCACATCTC-3′ (newly designed), and downstream primer, 5′-TCACCGGATCATGGCCAGCA-3′ (24). Genomic DNA (50 ng) was added to a buffer containing 2.0 mM $MgCl_2$, 200 μM deoxyribonucleoside triphosphates, 330 nM primers, and 0.23 units of *Taq* polymerase in a final volume of 10 μl. A 341-bp fragment of the *CYP1A1* gene was coamplified as a control with the following primer pair: upstream primer, 5′-GCTCCACTCACTTGACACTTCTG-3′, and downstream primer, 5′-CAGCTGCATTTGGAAGTGCT-3′. The presence of the *GSTTI* gene is indicated by an amplified 457-bp product, while the null genotype is indicated by the absence of a 457-bp band.

Statistical methods

Sequential analyses were performed to dissect genetic susceptibility to benzene in relation to the length of gestation. We first examined the maternal allele frequency of *CYP1A1* and *GSTT1* polymorphisms and maternal and infant characteristics by benzene exposure status. We then investigated whether the association between benzene exposure and shortened gestation is modified by maternal genotypes by estimating the association between benzene exposure and gestational age in total samples as well as in stratified subgroups by the specific maternal genotypes. To further assess gene-environment interaction, we examined the combined association of benzene exposure and maternal genotypes with gestational age in eight subgroups defined by benzene exposure status (no, yes) and maternal genotype for *CYP1A1 Hinc*II (*Aa/aa*, *AA*) and *GSTT1* (present, absent), using multiple linear regression analysis and the adjusted means (25). Finally, the gene-environment interaction was tested by adding a product term in the regression model. Consider a model that includes an exposure covariate X_1 that takes the value 1 if exposed and 0 otherwise and a second covariate X_2 that takes the value 1 if the individual has the high-risk genotype and 0 otherwise. We ignore the inclusion of confounding variables for the purpose of our discussion here, but they are easily added to the model below. Suppose the outcome is represented by Y. If we fit a linear regression model such as $Y = a_0 + a_1 \times X_1 + a_2 \times X_2 + a_3 \times X_1 \times X_2$, then a_1 can be interpreted as the association with exposure for individuals with the common genotype ($X_2 = 0$), and $(a_1 + a_3)$ is the corresponding association for individuals with the high-risk genotype ($X_2 = 1$). The finding that a_3 is significantly different from zero suggests that the genotype modifies the effect of exposure. It is noted that we examine here the deviation from additivity of effects. In this report, all the effect estimates were obtained by multiple linear regression models with adjustment for maternal age, education, parity, passive smoking, stress, prepregnancy weight and height, and infant's sex. The selection of the covariates was based on the current literature of the important covariates of gestational age (26), our previous studies in the Chinese population (27), and the standard statistical procedures for variable selection. All p values are two sided.

RESULTS

This analysis included 542 BYPC female workers (302 nonexposed and 240 benzene exposed) who gave live sin-

TABLE 1. Maternal and infant characteristics of the study population by benzene exposure status, Beijing, China, June 1995 to June 1997

Variables	Nonexposed		Benzene exposed		Inference statistics†	
	No.	%	No.	%	Odds ratio or difference in means	95% CI‡
Maternal characteristics						
Genotypes						
*CYP1A1 Hin*cII						
AA§	195	64.6	148	61.7	1.0	
AA/aa¶	107	35.4	92	38.7	1.1	0.8, 1.6
GSTT1						
Present	179	59.2	143	59.6	1.0	
Absent	123	40.7	97	40.4	1.0	0.7, 1.4
Age (years)						
20–24	114	38.3	97	40.9	1.0	
25–29	161	54.0	129	54.4	1.0	0.7, 1.5
≥30	23	7.7	11	4.7	0.6	0.3, 1.3
Missing	4		3			
Educational level						
≤Middle school	33	11.1	19	8.1	1.0	
High school	144	48.3	163	69.1	2.0	1.1, 3.6*
>High school	121	40.6	54	22.8	0.8	0.4, 1.5
Missing	4		4			
Parity						
0	186	62.4	139	58.9	1.0	
≥1	112	37.6	97	41.1	1.2	0.8, 1.6
Missing	4		4			
Passive smoke						
No	165	55.4	112	47.3	1.0	
Yes	133	44.6	125	52.7	1.4	1.0, 2.0
Missing	4		3			
Stress						
No	234	81.3	198	83.9	1.0	
Yes	50	17.4	31	13.1	0.7	0.5, 1.2
Unknown	12		11			
Prepregnancy weight (kg)						
Mean (SD‡)	58.3 (9.6)		58.5 (9.6)		0.26	–1.38, 1.9
Missing	5		3			
Prepregnancy height (cm)						
Mean (SD)	161.5 (5.1)		161.1 (4.9)		–0.32	–1.18, 0.54
Missing	4		3			
Infant characteristics						
Birth weight (g)						
Mean (SD)	3,466 (412)		3,377 (452)		–89	–163, –16*
Gestational age (weeks)						
Mean (SD)	39.99 (1.20)		39.76 (1.51)		–0.23	–0.45, 0.01
Preterm (<37 weeks)						
No	293	98.7	226	95.4		
Yes	4	1.3	11	4.6		
Missing	5		3			
Gender						
Male	152	51.0	126	53.6	1.0	
Female	146	49.0	109	46.4	0.9	0.6, 1.3
Missing	4		5			

* $p < 0.05$.

† Odds ratios and 95% confidence intervals for discrete variables and differences in means and 95% confidence intervals for continuous variables.

‡ CI, confidence interval; SD, standard deviation.

§ Homozygous wild-type.

¶ Heterozygous or homozygous variant.

gleton births at the BYPC Staff Hospital. Table 1 presents maternal gene allele frequency and maternal and infant characteristics by benzene exposure status. Table 1 also provides inferential statistics, that is, odds ratios and 95 percent confidence intervals for the discrete variables and differences in means and confidence intervals for the continuous variables. This is an overall low-risk population, with the majority of women at their optimal reproductive ages, most having the ideal weight for height, and none of them smoking or drinking. The mean birth weight for this sample was 3,405 (standard deviation (SD), 433) g. Ninety-seven percent of the infants were born at term. The gestational age had a near normal distribution, with a mean of 39.9 (SD, 1.3) weeks and a median of 40 weeks. The women in the benzene-exposed and nonexposed groups were similar in terms of *CYP1A1 Hin*cII and *GSTT1* allele frequency, age distribution, parity, stress, maternal prepregnancy weight and height, and infant's sex. However, the women in the exposed group were less likely to have a college education ($p = 0.029$) and more likely to be passive smokers ($p = 0.062$). The mean birth weight for the exposed group was 89 g lighter (standard error (SE), 37 g; $p = 0.017$), and the mean gestational age was 0.23 (SE, 0.12) week shorter ($p = 0.056$) than for the nonexposed group.

Table 2 presents the means and standard deviations for the total sample and for subgroups defined by maternal genotype. In addition, table 2 shows the adjusted association between benzene exposure and gestational age, where β represents the difference in mean gestational age (in weeks) between the exposed and the nonexposed in each row of the table after adjustment for the covariates listed. As estimated from the multiple linear regression model, benzene exposure was associated with a decrease in mean gestational age of 0.29 (SE, 0.12) week in the total sample. Of note, the estimated association of benzene with gestational age differed by maternal genotype in *CYP1A1 Hin*cII; benzene did not confer any adverse effect in the *Aa/aa* group (0.06 (SE, 0.18) week) but had a significantly negative effect in the *AA* group (–0.54 (SE, 0.16) week). In other words, after adjustment for the covariates, women with the *AA* genotype exhibited about a 0.60-week greater shortening of gestational age associated with exposure than did women with the *Aa/aa* genotype. A test of the interaction term between benzene exposure and the *CYP1A1 Hin*cII genotype was statistically significant ($p = 0.003$). There was a negative association between benzene exposure and gestational age in both the present and the absent genotype for *GSTT1* (–0.28 (SE, 0.15) week for the present group, –0.24 (SE, 0.21) week for the absent group). A test of the interaction term between benzene exposure and genotype in *GSTT1* was not statistically significant ($p = 0.709$).

As shown in table 3, we further assessed the association of benzene exposure and combined genotypes with gestational age, where β represents the difference in mean gestational age (in weeks) between each subgroup and the reference group. In the absence of benzene exposure, none of the maternal genotypes was significantly associated with shortened gestational age. However, in the presence of benzene exposure, there was a significant association with the *CYP1A1 AA-GSTT1* absent genotypes (–0.79 (SE, 0.25) week) and *CYP1A1 AA-GSTT1* present genotypes (–0.50 (SE, 0.22) week). For further illustration, we plotted the adjusted mean gestational age for the eight subgroups in figure 1.

DISCUSSION

In this study, we investigated the association of benzene exposure and two susceptibility genes (*CYP1A1 Hin*cII and *GSTT1*) with the length of gestation. This study has several unique features. It is one of the few studies to examine low level benzene exposure in relation to gestational age, with the consideration of the role of genetic susceptibility in assessing adverse effects of benzene on

TABLE 2. Means and standard deviations of gestational age and adjusted associations of benzene exposure with gestational age by maternal genotypes, Beijing, China, June 1995 to June 1997

Genotype	Gestational age (weeks)						Adjusted*		
	Nonexposed			Benzene exposed					
	No.	Mean	SD‡	No.	Mean	SD	β†	SE‡	*p* value
Total sample	297	39.99	1.20	237	39.76	1.51	–0.29	0.12	0.020
*CYP1A1 Hin*cII									
AA	192	39.99	1.20	147	39.53	1.59	–0.54	0.16	<0.001
Aa/aa	105	39.97	1.22	90	40.14	1.29	0.06	0.18	0.729
GSTT1									
Present	176	40.06	1.79	142	39.78	1.42	–0.28	0.15	0.063
Absent	121	39.88	1.24	95	39.74	1.63	–0.24	0.21	0.246

* Multiple linear regression models with adjustment for age (20–24, 25–29, ≥30 years), education (middle school or less, high school, above high school), parity (0, ≥1), passive smoking (no, yes), stress (no, yes), the linear and quadratic terms of prepregnancy weight and height, and infant's sex.

† β represents the difference in mean gestational age (in weeks) between exposed and nonexposed in each row of the table after adjustment for the covariates listed above.

‡ SE, standard error; SD, standard deviation.

TABLE 3. Combined associations of benzene exposure and multiple gene polymorphisms with gestational age (in weeks), Beijing, China, June 1995 to June 1997

Benzene exposure	Genotype		Crude			Adjusted*		
	*CYP1A1 Hin*cII	*GSTT1*	No.	Mean weeks	SD†	β‡	SE†	*p* value
No	*Aa/aa*	Present	62	40.09	0.99	Reference		
No	*Aa/aa*	Absent	43	39.81	1.48	–0.22	0.26	0.415
No	*AA*	Present	114	40.05	1.27	–0.01	0.21	0.975
No	*AA*	Absent	78	39.92	1.08	–0.11	0.23	0.638
Yes	*Aa/aa*	Present	48	40.06	1.45	0.01	0.26	0.964
Yes	*Aa/aa*	Absent	42	40.22	1.09	0.16	0.27	0.560
Yes	*AA*	Present	94	39.64	1.40	–0.50	0.22	0.023
Yes	*AA*	Absent	53	39.36	1.89	–0.79	0.25	0.002

* Multiple linear regression models with adjustment for age (20–24, 25–29, ≥30 years), education (middle school or less, high school, above high school), parity (0, ≥1), passive smoking (no, yes), stress (no, yes), the linear and quadratic terms of prepregnancy weight and height, and infant's sex. $R^2 = 0.08$.

† SD, standard deviation; SE, standard error.

‡ β represents the difference in mean gestational age (in weeks) between each subgroup and the reference group after adjustment for the covariates listed above.

gestational age. It is based on a large number of female workers from a modern petrochemical plant where epidemiologic and clinical data were collected with a validated questionnaire and consistent methods by trained research staff and where benzene exposure was determined by extensive exposure assessment. It is an overall low-risk population (nonsmoking, nondrinking, optimal maternal age, planned pregnancy among married couples, and early prenatal care), which offers an opportunity to test the gene-benzene interaction without substantial sociodemographic and environmental confounders.

This study has revealed several interesting findings. Benzene exposure was significantly associated with shortened gestation even at a level at least five times lower than that of the Occupational Safety and Health Administration limit. However, the association was significantly modified

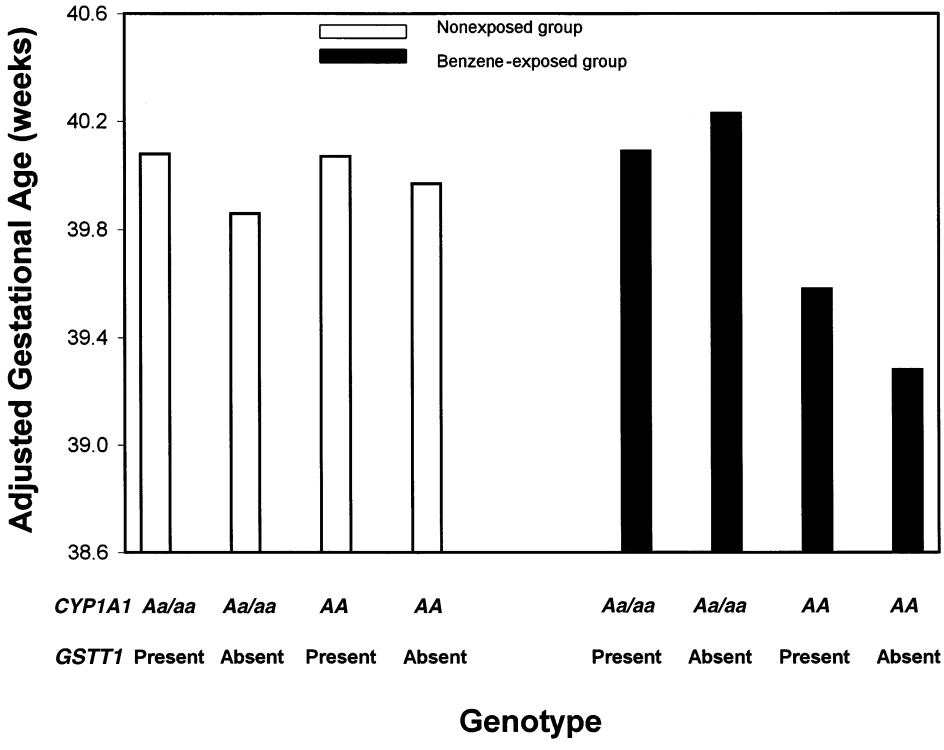

FIGURE 1. Mean gestational age by maternal benzene exposure status (no, yes) and *CYP1A1 Hin*cII (*AA* vs. *Aa/aa*) and *GSTT1* (presence vs. absence) genotypes with adjustment for maternal age, education, parity, passive smoking, stress, prepregnancy weight and height, and infant's sex, Beijing, China, June 1995 to June 1997.

by maternal genotype. The estimated association was significantly greater for the *AA* than for the *Aa/aa* allele in *CYP1A1*. When both *CYP1A1* and *GSTT1* genotypes were considered, the largest association was found among exposed mothers with *AA* in *CYP1A1* and absence in *GSTT1*. Among the nonexposed, genetic susceptibility alone did not confer a significant adverse effect.

From a clinical perspective, prevention of preterm delivery is of primary interest. The magnitude of reduction in gestational age documented in this study is not of major significance. However, our study findings have the following implications. From an epidemiologic perspective, since both organic solvent exposure and the susceptible genotypes are prevalent in the general population, a significant fraction of the population is at risk. The 0.5- to 0.8-week leftward shift in the gestational age distribution curve among the high-risk group of the population could lead to a significantly increased number of preterm births, thus contributing to a significant etiology fraction of preterm delivery. From an environmental and occupational health perspective, this study documented that benzene exposure, even at a level at least five times below the Occupational Safety and Health Administration limit, is significantly associated with shortened gestation. From a scientific perspective, this study supports the importance of considering genetic susceptibility in evaluation of reproductive toxins.

The biologic mechanisms by which benzene might affect gestational age remain to be determined. Shortened gestation may be due to maternal toxicity, fetal toxicity, or both. While the major known toxic effect of benzene is hematopoietic toxicity (28), it is recognized that benzene toxicity is the result of multiple benzene metabolites' affecting more than one target (29). The placental transfer of benzene has been demonstrated in animal (30) and in human (31) investigations. Animal studies have shown that benzene and other aromatic organic solvents resulted in delayed fetal development and reduced birth weight (32, 33). Epidemiologic studies have shown that maternal exposure to organic solvents as a group is consistently associated with a modest increased risk for birth defects and, somewhat less consistently, for spontaneous abortion (34). Studies also suggested benzene specifically as a potential reproductive toxin (4–6). For low level solvent exposure, only limited epidemiologic studies are available. Wilkins and Steele (35) reported that the risk of preterm delivery was increased among female veterinarians exposed to solvents. Witkowski and Johnson (36) observed an increased risk of low birth weight among women who consumed drinking water polluted by benzene or other solvents.

The biologic mechanism by which these gene polymorphisms modify the effect of benzene on gestational age is not well understood. The bulk of the evidence suggests that benzene toxicity is produced by one or more metabolites of benzene, in particular, the covalent binding to cellular macromolecules, rather than by benzene itself (37). Thus, the ability of an individual to convert toxic metabolites of benzene to less harmful moieties is important for minimizing the toxic effect of benzene. *CYP1A1* and *GSTT1/GSTM1* are known to be responsible for detoxification of organic solvents. Crofts et al. (38) found that variant genotypes at the *Hinc*II site were significantly associated with increased *CYP1A1* gene inducibility. They also observed a significant interaction between the *Hinc*II polymorphism and smoking at the mRNA level. The existence of a xenobiotic-metabolizing cytochrome P-450 system in the human feto-placental unit is now well established, and placental *CYP1A1* is highly inducible by maternal cigarette smoking (39). The glutathione *S*-transferases are a supergene family of multifunctional enzymes that catalyze the conjugation of glutathione with electrophilic substrates to reduce their toxicity. Our findings are consistent with previous studies that have shown evidence of a synergistic effect of *CYP1A1* and *GSTM1* polymorphisms in lung cancer (19, 20) and urothelial cancer (23).

When the results of this study are interpreted, several methodological limitations should be taken into account. This is a low-risk population with low-level benzene exposure as the major occupational exposure, so the generalizability of our findings to women in other populations is unknown. Since the concentration of our study subjects is in the range of 38–41 weeks of gestation, our study findings pertain to variation in the normal range of birth timing. Although it may be relevant to preterm births under certain assumptions, there is a potentially important limitation in terms of generalizing our findings to preterm birth. While we classified occupational exposure by extensive exposure assessment, a dose-response relation between benzene and gestation cannot be examined because of lack of data on individual peak and cumulative exposure. The association between genetic susceptibility to benzene and shortened gestation found in this study may be causal, may be a marker for other unknown genes or biologic pathways, or may be due to confounding by other unmeasured toxins or risk factors. Further studies are clearly needed to corroborate our findings.

In summary, we have demonstrated that benzene exposure, even at a level at least five times below the recommended limit of the Occupational Safety and Health Administration, is associated with shortened gestation. Such an association, however, is significantly modified by an individual's genotype. This study provides evidence of gene-environment interaction and supports the importance of further assessing the role of genetic susceptibility in the evaluation of reproductive toxins.

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