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Low organic solvent exposure and combined maternal-infant genepolymorphisms affect gestational age

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ABSTRACT

Objective Little information is available on the associations of combined maternal-infant genetic susceptibility, environmental exposures, and reproductive outcomes, especially in Chinese population. This study was to investigate whether the polymorphisms of combined maternal-infant metabolic genes, *CYP1A1* Hinc□, *CYP1A1* Msp□, *GSTT1* and *GSTM1* affect the association of maternal organic solvents exposure with gestational age.

Methods A total of 1,113 mother-infant pairs were enrolled from the Beijing Yanshan Petrochemical Corporation between June 1997 and June 2002, of which 546 mothers were exposed to organic solvents and 567 were not. Multiple linear regression models were used to estimate the combined maternal-infant gene effects, and to characterize combined maternal-infant genetic susceptibility to organic solvents in relation to gestation.

Results Organic solvents exposure was significantly related to the shortened gestation (-1.2 weeks, 95% CI:-1.6,-0.9). Additionally, combined maternal-infant genotypes including Ile/Ile462-Ile/Ile462 (-0.6 weeks, 95% CI:-0.9,-0.4) in *CYP1A1* Hinc□ and absent-absent in *GSTT1* (-0.4 weeks, 95% CI:-0.9,-0.3) were significantly associated with shorter gestation. When considering both organic solvents exposure and combined maternal-infant genotypes, the largest associations were found among exposed women with absent-absent genotype (-1.5 weeks, 95% CI:-1.8,-1.2) in *GSTT1* and Ile/Ile462-Ile/Ile462 genotype (-1.5 weeks, 95% CI:-1.8,-1.2) in *CYP1A1* Hinc□, suggesting that combined genotypes would modify the effect of organic solvents exposure on gestation.

Conclusions This study demonstrates the role of combined maternal-infant genotypes in modifying the adverse effects of organic solvents exposure on gestation, and maternal-infant interaction of the 4 genes was determined.

INTRODUCTION

The duration of gestation is a key predictor of infant survival.[1] Shortened gestational age such as preterm birth is considered a major public health concern because of its high prevalence, association with mortality and morbidity, and increased cost of hospitalization and long-term disability.[2] Morken suggested that the association between birth weight lower than the population mean and spontaneous preterm birth may be evident for all gestational age groups.[3] The etiology of shortened gestational age was largely unknown, but both environmental and genetic factors and their interactions may play important roles.[4-6]

For the general population, organic solvents are widespread at work and at home.[7,8] Benzene,[9-11] toluene[12,13] and related compounds have been identified as potential reproductive toxins. Although exposure levels in most modern industries is far below the limit recommended by the Occupational Safety and Health Administration,[14] several studies suggested that benzene exposure even at low level might link to adverse reproductive outcomes such as spontaneous abortion,[11] low birth weight,[15] shortened gestational age[6] and an increased risk for congenital anomalies.[16]

However, not all women exposed to organic solvents during pregnancy have adverse reproductive outcomes,[6,17] and several studies have suggested potential reasons lie in maternal and infant genetic susceptibility.[16,18,19] Metabolism of organic solvents includes 2 phases of detoxification: 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.[20] Benzene-induced toxicity in animals is clearly mediated by its metabolism.[21] However, if there are genetic variations occurring in the metabolic enzymes, normal metabolic process will be disturbed. Some toxic adducts generate and do harm to DNA and chromosomes in the placenta and fetal cells, which ultimately lead to abnormal reproductive outcome such as decreased gestational age. The cytochrome P450 family serves as the major enzyme system in phase I metabolism in which *CYP1A1* is well-studied, and the glutathione S-transferases (GSTs) are the major phase II enzymes. Both *CYP1A1* and *GSTT* genes are highly polymorphic in the population,[22-24] which is partly responsible for the interindividual differences in the metabolic activation and detoxification pathways of these enzymes.[23,25,26] Besides, molecular epidemiological studies have demonstrated associations between genetic susceptibility in these genes and reproductive outcomes including gestational age,[6] birth weight,[17] intrauterine fetal growth restriction[27]and small-for-gestational-age births.[28]

Most studies have examined only maternal genes while neglecting fetal genotype and combined maternal-fetal genetic susceptibility, and little information is available on the associations of combined maternal-infant genetic susceptibility, environmental exposures, and reproductive

outcomes, especially in the Chinese population. Therefore, we hypothesized that maternal and infant gene polymorphisms of metabolic enzymes might have combined effects on the gestational age, and that the effect of exposure to organic solvents on reproductive outcomes might be modified by combined maternal-infant genes. In this report, *CYP1A1* Msp□, *CYP1A1* Hinc□, *GSTT1* and *GSTM1* polymorphisms were used to assess the combined maternal-infant genotype effects, and to characterize the combined genetic susceptibility to organic solvents in relation to gestational age.

MATERIALS AND METHODS

Study site and population

This prospective study was conducted at Beijing Yanshan Petrochemical Corporation (BYPC), located in a suburban area of Beijing, China. Beijing Yanshan Petrochemical Corporation, 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. In our study period, the time-weighted average for benzene during an 8-hour shift for exposed workers ranged from 0.017 ppm to 0.191 ppm at BYPC.[29] The Occupational Safety and Health Administration limit is 1 ppm as an 8-hour time-weighted average.[14] The BYPC Staff Hospital, the only regional hospital that serves the community, provides prenatal care and delivery services. Eligible subjects were women working at BYPC and their live singleton infants born at BYPC Staff Hospital between June 1997 and June 2002. The eligibility criteria for women in the field enrollment were as follows: live singleton infants born, full-time employed workers; newly married; aged 20 to 34 years; women were excluded if they had multiple gestation, births with major congenital defects, or a medically diagnosed gynaecological or endocrine disorder.

Procedures

From June 1995 to June 2000, we used the Chinese marriage health examination system (MHE) to identify newly-wed women in the BYPC. They were invited to take a newly married health examination in the Beijing Yanshan Child and Women Hospital, after informed consent obtained, physical examination was performed, and height and weight were measured according to a standard protocol. A structured baseline questionnaire was administered by a trained interviewer to all the women at enrollment to collect information concerning occupational exposures, personal habits such as cigarette smoking and alcohol consumption, living environment, exposure to passive smoking, dietary intake, menstrual and reproductive history, and contraceptive use.[11] Once they intended to conceive and stopped contraception, they were enrolled formally and each of them was asked to provided to daily urine sample and to keep a diary of her menstrual periods. If a woman reported a missed or late period or had early signs or symptoms of pregnancy, she was instructed to go to the affiliated hospital for a check-up and to give a urine sample to confirm a pregnancy. Once a woman was confirmed to be pregnant, she received regular prenatal care and delivery services at the designated hospitals according to standard clinical guidelines. They left their workplace in the last trimester and were followed up for pregnancy outcomes including gestational age, infant birth weight and infant's sex by the research staff. We collected 10 ml cord blood from infants and 10 ml venous blood from mothers. The buffy coats were initially stored in a -20°C designated refrigerator located on the Labor and Delivery Ward. Blood samples were transported to the Peking University Health Science Center, where DNA was extracted according to standard protocols.[30] The procedure was approved by the Institutional Review Board of Peking University Health Science Center.

Gestational age

In this study, the first date of the last menstrual period recorded at the first prenatal visit was used to calculate the gestational age by the investigators. The last menstrual period is generally 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. Because of the one-child policy, families are highly concerned about healthy pregnancy for healthy babies. All the women in our study went to the affiliated hospital for a check-up, received a HCG assay to verify pregnancy soon after missing a menstrual period and sought prenatal care. Furthermore, we calculated gestational age by day and then divided it by 7 to get gestational week number.

Organic solvents exposure

In this study the measurement of maternal occupational exposure was based on a specialized industrial hygiene method developed in three steps.[29] Firstly, a walk through field study was conducted by an industrial hygienist to obtain detailed information on the production processes in each of the plants. Information was obtained on different sections of the plant known as workshops, including raw materials, end products, chemical reaction processes, and the job titles involved in the production. Location maps and flow charts of the chemical reaction processes were also prepared for each workshop. A qualitative description of typical job tasks was provided for major production workshops. Each of the plants had a different number of workshops. In total, 218 workshops were identified, and a list of 104 job titles was constructed.

Secondly, on a subset of 132 workers, two different methods of assessment of exposure were compared. For each woman in this subgroup, personal air sampling was conducted on a randomly chosen workday. Quantitative chemical measurements were performed for benzene, toluene, styrene, and xylene. If a detectable concentration of any chemical was measured, the woman was classified as exposed to the specific solvent. The second method was based on an industrial hygienist's assessment of each woman's plant, workshop, and job title information. A standardized algorithm was developed to classify each worker into either exposed or unexposed to each of the four solvents, respectively. For example, in the workshops where benzene was present as a raw material or end product, all the workers were classified as exposed to benzene. If a woman did not work primarily in such a workshop, but had a job title that required tasks in the exposed areas, she was also rated as exposed to benzene. Thus, operators who worked in such facilities as refinery, rubber plant, oil blending workshop, benzene tank farm, or phenol production workshop, were rated as exposed, and workers in the electricity control room, packing workshop, workers' union, administration office, information centre, or the library, were considered to be unexposed. The industrial hygienist's assessment was compared with the classification based on detection of chemicals from air samples. The sensitivity and specificity of the hygienist's qualitative assessment was reasonably high for benzene (0.70 and 0.62, respectively). However, sensitivity was below 0.4 for toluene, styrene, and xylene.

Thirdly, a trained interviewer obtained information on plant, workshop, and job title from all women participants. Based on the standardized algorithm developed in the validation stage, an industrial hygienist classified each woman's exposure into three categories without knowledge of birth outcomes. Women who were exposed to benzene with or without other exposures were

classified as exposed to benzene; women who were not exposed to benzene but potentially to other solvents were grouped as exposed to other solvents, both the two groups were defined as exposed group. Finally, women who were not exposed to any solvents were classified as unexposed. Further details of the exposure assessment method and validation results are described elsewhere.[31]

The industrial hygienists who performed the occupational exposure assessment were members of our research team, had no relationship with the Petrochemical Company.

Genotyping

We detected *CYP1A1* Msp□ according to Kawajiri's method,[32] which is able to detect all 3 genotypes for the polymorphism: T/T6235 (homozygous wild type), T/C6235 (heterozygous variant type), and C/C6235 (homozygous variant type). We combined T/C6235 and C/C6235 together in the later data analysis due to the small number of subjects with C/C6235.

CYP1A1 Hinc□ polymorphism was analyzed according to Katoh et al.[26] This method is able to detect all 3 possible genotypes for the polymorphism: Ile/Ile462 (homozygous wild type), Ile/Val462 (heterozygous variant type), and Val/Val462 (homozygous variant type). We combined Ile/Val462 and Val/Val462 together in the later data analysis due to the small number of subjects with Val/Val462 genotype.

The detailed method on detection of the *GSTT1* and *GSTM1* polymorphisms can be found elsewhere.[24,26] 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.

Previously sequenced genomic DNA samples were used as a positive control for the homozygous wild type, heterozygote and homozygous mutant genotypes with every PCR analysis to verify reproducibility of the RFLP-PCR and to confirm accuracy of genotype classifications. Approximately 10% of randomly selected samples were repeated for verification of the results of the genotyping assays.

Statistical analysis

We used multiple linear regression models to estimate the combined maternal-infant genotype effects, and to characterize maternal and infant genetic susceptibility to organic solvents in relation to gestational age with adjustment of major covariates, the regression coefficients were expressed by β and 95% confidence interval (CI) as comparing to each reference group. We examined general characteristics and genotype frequencies of *CYP1A1* Msp□, *CYP1A1* Hinc□, *GSTT1* and *GSTM1* of all mothers and infants, and compared the data of exposed subjects with those of nonexposed. We then investigated whether the association between organic solvents exposure and gestational age was modified by combined maternal-infant genetic effect. We examined the co-effect of organic solvents exposure and maternal-infant genotypes on gestational age in eight subgroups defined by organic solvents (no, yes), maternal genotype, and infant genotype.

All the analyses were adjusted for the following potential confounders: maternal age (< 25, 25-28, and ≥ 28 years), education (elementary, middle, and high school or above), pregnancy history (no, yes), shift work (no, yes), noise exposure (no, yes), vibration exposure (no, yes), perceived stress (no, yes), passive smoking (no, yes), pre-pregnant weight and height, and infant sex.[6,15] The frequencies of the *CYP1A1* MspI and *CYP1A1* Hinc□ in these populations conformed

to the Hardy-Weinberg equilibrium. All *P* values were 2-sided and defined as $P < 0.05$ for statistical significance. We used statistical software SAS (SAS Institute Inc, Cary, NC) for all analysis.

RESULTS

A total of 1210 maternal-infant pairs were invited to participate in this study. 97 maternal-infant pairs were excluded because of failure of extracting DNA, failure of genotyping, and missing data. The final analysis included a total of 1,113 maternal-infant pairs, 546 with organic solvents exposure and 567 without maternal organic solvents exposure (Table 1). Comparing the exposed subjects with the unexposed ones, we found both groups were similar in terms of maternal age, height, weight, education, passive smoking, exposure to vibration, stress, history of pregnancy, infant' sex and preterm baby incidence rate; however, the exposed group had more shift work ($P < 0.001$) and less exposure to noise ($P < 0.001$). The mean birth weight of the exposed group was 68.4 g lighter ($P = 0.01$), and the mean gestational age was 0.9 weeks shorter ($P < 0.001$) than those of nonexposed group.

Table 1. General characteristics of organic solvent exposure group and non-exposure group.

Variable	***No exposure (N=567)***	***Exposure (N=546)***
	mean (SD†)	***mean (SD)***
Age (y)	26.77 (2.13)	26.66 (2.68)
Height before pregnancy (cm)	160.50 (5.05)	160.75 (5.29)
Weight before pregnancy (kg)	59.58 (9.47)	59.86 (9.68)
Birth weight (g)*	3451.1 (453.75)	3382.7 (46.09)
Gestational age (weeks)**	40.88 (1.39)	39.98 (1.28)
	n (%)	***n (%)***
Education:		
Elementary	61 (10.8)	68 (12.5)
Middle	324 (57.1)	323 (59.2)
High school or above	182 (32.1)	155 (28.4)
Passive smoking at home or work‡	270 (47.6)	273 (50.0)
Exposure to vibration‡	17 (3.0)	16 (2.9)
Shift work‡**	193 (34.0)	273 (50.0)
Exposure to noise‡**	108 (19.0)	61 (11.2)
Perceived stress‡	93 (16.4)	67 (12.3)
Parity (one or more)	228 (40.2)	242 (44.3)
Female baby	264 (46.6)	280 (51.3)
Preterm baby	7 (1.2)	12 (2.2)

† SD, standard deviation

‡ Passive smoking was identified by a participant's response to the question "On average, what is the number of cigarettes someone smoked indoors at home per day while you were exposed in last three months?"; Vibration, the specific question is "Are you affected by vibration in your work?". Shift work, the specific question is "Shift work or not? (0=day time work, no shift; 1=shift work)". Noise exposure, the specific question is "Do you work in a noise environment at work?". Stress mentioned in the table means physical labour stress, the specific question is "How is the intensity of labour of your work?".

* $P < 0.05$

** $P < 0.001$

For the exposed and nonexposed groups□the maternal and infant genotype frequencies were similar in *CYP1A1* Msp□, *CYP1A1* Hinc□, *GSTT1* and *GSTM1* (Table 2).

Table 2. Maternal and infant genotype frequency of exposure and non-exposure group.

Genotype	*No exposure (N=567)*		*Exposure (N=546)*	
	n	***(%)***	***n***	***(%)***
Maternal				
CYP1A1 Msp				
T/T6235	233	(41.1)	215	(39.4)
T/C6235	278	(49.0)	272	(49.8)
C/C6235	56	(9.9)	59	(10.8)
CYP1A1 Hinc				
Val/Val462	24	(4.2)	29	(5.3)
Ile/Val462	191	(33.7)	177	(32.4)
Ile/Ile462	352	(62.1)	340	(62.3)
GSTT1				
Present	329	(58.0)	313	(57.3)
Absent	238	(42.0)	233	(42.7)
GSTM1				
Present	266	(46.9)	242	(44.3)
Absent	301	(53.1)	304	(55.7)
Infant				
CYP1A1 Msp				
T/T6235	201	(35.4)	192	(35.2)
T/C6235	284	(50.1)	268	(49.1)
C/C6235	82	(14.5)	86	(15.8)
CYP1A1 Hinc				
Val/Val462	15	(2.6)	23	(4.2)
Ile/Val462	201	(35.4)	205	(37.5)
Ile/Ile462	351	(61.9)	318	(58.2)
GSTT1				
Present	332	(58.6)	303	(55.5)
Absent	235	(41.4)	243	(44.5)
GSTM1				
Present	251	(44.3)	235	(43.0)
Absent	316	(55.7)	311	(57.0)

‡ Ile/Ile462 (homozygous wild type), Ile/Val462 (heterozygous variant type), and Val/Val462 (homozygous variant type); T/T6235 (homozygous wild type), T/C6235 (heterozygous variant type), and C/C6235 (homozygous variant type).

We explored the combined association of organic solvent exposure and maternal-infant genotype with gestational age (Table 3). There is a significant negative association between solvent exposure and gestational age (the exposed group compared with the unexposed one, -0.9 weeks, 95% CI:-1.1,0.7). Gestational age of each subgroup was compared with that of nonexposed and of combined present-present genotype in *GSTT1*. Similarly in *CYP1A1* Hinc□ group, non-exposed, Ile/Val□Val/Val462-Ile/Val□Val/Val462 subgroup was seen as the reference. For *GSTT1* without exposure, only the absent-present genotype without exposure was not significantly associated with shortened gestational age (-0.2 weeks, 95% CI:-0.5,0.2), significant associations were observed in all other subgroups of *GSTT1* (Table 3, figure 1). The largest association was found among exposed women with combined maternal-infant absent-absent genotype (-1.5 weeks, 95% CI:-1.8,-1.2) in *GSTT1*. For *CYP1A1* Hinc□, significant associations were observed in all subgroups and the largest association was found among exposed women with combined maternal-infant Ile/Ile462- Ile/Ile462 genotype (-1.5 weeks, 95%CI:-1.8,-1.2) (Table 3, figure 2). We did not find significant associations between exposure plus combined genotypes in *CYP1A1* Msp□/ *GSTM1* and gestational age (Data not shown).

Table 3. Adjusted combined association of organic solvent exposure and maternal-infant genotype with gestational age.

Organic solvents	*Genotype*		*Gestational age (weeks)*		*Expo-Geno-Ges Asso.†*	
	Maternal	*Infant*	*n*	*Mean (SD‡)*	*Adjusted β*	*(95% CI‡)*
No	***Total sample***		567	40.8 (1.4)	0.0	–
Yes			546	40.0 (1.3)	-0.9**	(-1.1, -0.7)
	GSTT1					
No	present	present	223	41.1 (1.3)	0.0	–
No	present	absent	106	40.7 (1.5)	-0.4*	(-0.7, -0.1)
No	absent	present	109	40.9 (1.2)	-0.2	(-0.5, 0.2)
No	absent	absent	129	40.5 (1.5)	-0.7**	(-0.9, -0.4)
Yes	present	present	196	40.0 (1.3)	-1.2**	(-1.5, -0.9)
Yes	present	absent	117	40.1 (1.3)	-1.0**	(-1.3, -0.7)
Yes	absent	present	107	40.1 (1.1)	-1.0**	(-1.4, -0.7)
Yes	absent	absent	126	39.7 (1.3)	-1.5**	(-1.8, -1.2)
	***CYP1A1* Hinc□**					
No	Ile/Val□Val/Val462	Ile/Val□Val/Val462	121	41.3 (1.2)	0.0	–
No	Ile/Val□Val/Val462	Ile/Ile462	94	40.9 (1.6)	-0.4*	(-0.7, -0.0)
No	Ile/Ile462	Ile/Val□Val/Val462	95	40.8 (1.3)	-0.5*	(-0.8, -0.1)
No	Ile/Ile462	Ile/Ile462	257	40.7 (1.4)	-0.6**	(-0.9, -0.3)
Yes	Ile/Val□Val/Val462	Ile/Val□Val/Val462	122	40.1 (1.4)	-1.2**	(-1.6, -0.9)
Yes	Ile/Val□Val/Val462	Ile/Ile462	84	40.0 (1.3)	-1.3**	(-1.7, -1.0)
Yes	Ile/Ile462	Ile/Val□Val/Val462	106	40.2 (1.2)	-1.1**	(-1.5, -0.8)
Yes	Ile/Ile462	Ile/Ile462	234	39.8 (1.2)	-1.5**	(-1.8, -1.2

† Expo-Geno-Ges Asso. means the combined association of organic solvent exposure and maternal-infant genotype with gestational age. Multiple linear regression models were adjusted for maternal ages (<25, 25-28, and ≥28 years), education (elementary, middle, and high school or above), parity (none, one or more), shift work (no, yes), noise exposure (no, yes), vibration exposure (no, yes), perceived stress (no, yes), passive smoking (no, yes), pre-pregnant weight and height and infant sex. Reference group for *GSTT1* is that of no exposure and of combined absent-absent genotype; reference group for CYP1A1 Hinc□ is that of no exposure and of combined Ile/Val□Val/Val462-Ile/Val□Val/Val462 genotype.

‡ SD, standard deviation; CI, confidence interval

* $P < 0.05$

** $P < 0.001$

DISCUSSION

In our study, we demonstrated that combined maternal-infant genotypes including Ile/Ile462-Ile/Ile462 in *CYP1A1* Hinc□ and absent-absent in *GSTT1* were significantly associated with the shortened gestational length. In addition, organic solvent exposure, even at a level at least five times lower than the safety level required by the Occupational Safety and Health Administration,[33] was significantly related to the shortened gestation. When considering both organic solvents exposure and combined maternal-infant genotypes, the largest associations were found among exposed women with combined absent-absent genotype in *GSTT1* and Ile/Ile462-Ile/Ile462 genotype in *CYP1A1* Hinc□, suggesting that combined maternal-infant genotypes would modify the effect of organic solvents exposure on shortened gestational age. Although in our study, the percent of preterm births is 2.2% in the exposed group while 1.2% in the unexposed one, most births were full-term births, the magnitude of reduction in gestational age in this study is not of major significance. From a clinical perspective, the shift of distribution in normal range may make no sense. But from an epidemiologic and population perspective, the change even in a normal range should be paid an attention. The calculated PAR% is 29.0%, nearly one third preterm births were attribute to occupational exposed to organic solvent. Since both low organic solvent exposure and the susceptible genotypes are prevalent in the general population, a significant fraction of the population is at risk. The shifting the distribution of gestational age at birth among the high-risk group of the population could lead to a significant increase in the number of reduced gestational age, thus contributing to a significant etiology fraction of reduced gestational age. From an environmental perspective, this study documented that low organic solvent exposure is associated with decreased gestational age, so those with organic solvent might have more opportunity to have preterm birth. From a scientific perspective, this study supports the importance of considering genetic susceptibility in evaluation of reproductive toxins. Our study has several unique features: it is one of the few studies to examine low level organic solvents exposure in relation to gestational age, with the consideration of the role of combined maternal and infant genetic susceptibility in assessing adverse effects of organic solvents on gestational age; it is based on a large sample 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 organic solvents 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 combined maternal and infant gene-organic solvent interaction without substantial sociodemographic and environmental confounders.

Although there were few published data on combined maternal-infant genetic susceptibility to organic solvents exposure in relation to gestational age, this susceptibility is biologically plausible. Benzene suggested specifically as a potential reproductive toxin.[9,11] Its toxicity was produced by one or more metabolites of benzene, in particular the covalent binding to cellular macromolecules, rather than by benzene itself, the intermediates probably being more toxic than

the original form.[34] Some animal and human studies observed that benzene could be transferred through placenta and further affect the development of infants.[35,36] The cytochrome P450 family and the glutathione S-transferases (GSTs) serve as the major enzymes in the 2-phase detoxification of organic solvents. Alexandrov *et al* found that the levels of benzo(a)pyrene diol-epoxide-DNA adducts and bulky DNA adducts were significantly and positively correlated with CYP1A1 enzyme activity.[37] The GSTT1 enzyme is important in protecting against certain genotoxic damages, such as sister chromatid exchanges and the formation of hemoglobin adducts.[38,39] Furthermore, infant with growth retardation and/or premature birth were more likely to have higher frequencies of adducts, DNA damage and chromosomal aberrations in the placenta and fetal cells.[40] Interindividual differences in susceptibility to adverse reproductive outcomes of organic solvents exposure are in part attributable to different maternal and infant genotypes associated with these enzymes. *CYP1A1* MspI variant genotypes may increase enzyme activity,[41] while the deletion type of *GSTT1* leads to an absence of enzyme activity.[32,41] Both CYP1A1 genes and GSTs *(GSTT1 and GSTM1)* genes are highly polymorphic in our study population. Thus, we may suggest that metabolic enzyme genetic polymorphisms resulting in changes of enzyme activity play an important role in the adverse reproductive outcomes due to organic solvents exposure. Consistently, our study found that both maternal and infants' *CYP1A1* Hinc□ Ile/Ile462 genotype, absent genotypes of *GSTT1* were significantly associated with shorter gestational age. Furthermore, the greatest reductions of gestational age were found among organic solvents exposed subjects with combined maternal-infant genotype Ile/Ile462-Ile/Ile462 in *CYP1A1* Hinc□ and absent-absent in *GSTT1*, which further suggested the effects of organic solvents on shortened gestational age were modified by combined maternal-infant genetic susceptibility. So when we examined the genetic susceptibility to gestational age, not only maternal but also infant genetic susceptibility should be taken into account. Claire *et al* reported that the complete *GSTM1* deletion, the partial *GSTT1* deletion and homozygous *CYP1A1*2A* in newborns were significantly associated with small-for-gestational age birth.[28] Our previous study also found that both infant and maternal *CYP1A1* Msp□ C/C6235 genotypes could increase the risk for low birth weight by using a triad design.[18]

Nevertheless, some limitations of our study should be noted. Firstly, the generalization of our findings to other populations might be limited as population stratification is common in genetic epidemiology, in our study, the genetic backgrounds of the subjects were heterogeneity, and more methods such as the pedigree investigation could be employed to evaluate our discovery. Secondly, since most of our study subjects had gestational age ranging from 38 to 41 weeks, we should take caution with the findings to interpret the preterm birth. Thirdly, in our study, the ultimate exposure contrast for analysis is "exposed vs. unexposure". Other refined exposure information such exposure probability, duration, intensity and frequency were not concerned. Finally, molecular mechanism for gestational age is extremely complex, and we couldn't exclude other biologic pathways as well as confounding by other potential toxins we hadn't measured. Further studies are needed to make better interpretation.

In a word, low level organic solvents exposure was significantly related to the shortened gestational age. The combined maternal-infant genotypes of Ile/Ile462-Ile/Ile462 in *CYP1A1*

Hinc□ and absent-absent in *GSTT1* were associated with shortened gestation, and further combined effects on gestation with organic solvents were also observed.

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Effort-reward imbalance and incidence of low back and neck injuries in San Francisco transit operators

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Notes