CYP1A1 Ile462Val and GSTT1 modify the effect of cord blood cotinine on neurodevelopment at 2 years of age

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ARTICLE INFO

Article history:

Received 31 January 2008

Accepted 27 May 2008

Available online 3 June 2008

Keywords:

Genetic polymorphisms

Maternal exposure

Environmental tobacco smoke

Cord blood cotinine

Children

Neurodevelopment

ABSTRACT

Maternal exposure to environmental tobacco smoke (ETS) has been reported to be associated with children's neurobehavioral development but there was no studies investigating the genetic susceptibilities to maternal ETS exposure on children's neurodevelopment. The aim of the study was to explore the modification effect of metabolic gene polymorphisms to cord blood cotinine on children's neurodevelopment at the 2 years of age. This study is one investigation of the Taiwan Birth Panel Study and a total of 145 pregnant women and their neonates were recruited between April 2004 and January 2005. We interviewed them by a structured questionnaire after delivery and collected umbilical cord blood at birth. Cotinine in umbilical cord blood as an indicator of environmental tobacco smoke was analyzed by using HPLC–MS/MS and the detection limited of this method was 0.05 ng/mL. Four metabolic genes, CYP1A1 MspI, CYP1A1 Ile462Val, GSTT1 and GSTM1 were identified. The Comprehensive Developmental Inventory for Infants and Toddlers (CDIIT) was used for assessing children's neurodevelopment at the 2 years of age accompanying with the measurement of Home Observation for Measurement of the Environment scale. We used multiple linear regression models to estimate the effects of cord blood cotinine and gene modification. Cotinine levels were significantly negatively associated with developmental quotients (DQs) of the whole test, and cognitive, language, fine-motor and social subtests of the CDIIT. Lower cognitive and language DQs were found in exposed group with absent type of GSTT1. In addition, the lowest scores in fine-motor and whole test DQs were detected in exposed group with CYP1A1 Ile462Val variant type and GSTT1 absent type. It can be concluded that CYP1A1 Ile462Val and GSTT1 metabolic genes can modify the effect of cord blood cotinine on early child neurodevelopment especially for language and fine motor development.

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1. Introduction

Maternal smoking gives adverse pregnancy outcomes including infertility (Curtis et al., 1997), spontaneous abortion (Nielsen et al., 2006), stillbirth and perinatal death (Winbo et al., 2001), inadequate fetal growth (Wang et al., 1997), and is associated with infant health after birth such as sudden infant death

syndrome (Shah et al., 2006; Ward et al., 2007). Maternal exposure to environmental tobacco smoke (ETS) is also associated with adverse health effects such as spontaneous abortion (Windham et al., 1999) and inadequate fetal growth (Dejmek et al., 2002; Jaakkola et al., 2001; Kharrazi et al., 2004).

Previous studies had examined whether maternal smoking or ETS exposure is associated with neurodevelopmental deficits in children. Maternal smoking gave adverse cognitive (Batty et al., 2006; Fried et al., 1997; Julvez et al., 2007; Lambe et al., 2006; Key et al., 2007) and behavioral development (Law et al., 2003; Orlebeke et al., 1999). On the other hand, few studies explored the effects of maternal exposure to ETS on children's cognitive development (Rauh et al., 2004) and behavioral problems

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was tested by χ^2 -test to compare the observed genotype frequencies within the study subjects.

Methods for determining genotypes GSTT1 and GSTM1 were using PCR as previously described (Comstock et al., 1990; Pemble et al., 1994). Briefly, the analysis of the GSTM1 and GSTT1 polymorphism were performed at the same time. A 110-bp fragment of the β -globulin gene was used as an internal positive control for using sense primer 5'-ACA CAACTGTGTTCACTAGC-3' and antisense primer 5'-CAACTCATCCACGTTCACC-3'. For the GSTM1 polymorphism sense primer 5'-CTGCCCTACTTGATTGATGGG-3' and antisense primer 5'-CTGGATTGTAGCAGATCATGC-3' were used. For the GSTT1 polymorphism sense primer 5'-TTCCTTACTG GTCCTCACATCTC-3' and antisense primer 5'-TCACCGGATCATGGCCAGCA-3' were used. The absent genotype was defined as the homozygous deletion of the gene.

2.4. Children's neurodevelopment

Children's neurodevelopment was evaluated by using the CDIIT at 2 years of age by physical therapist. The CDIIT was designed and standardized for assessing the characteristics of youngsters with ages ranged from 3 to 71 months or had developmental delays within the range (Wang et al., 1998). The CDIIT consists of cognitive, language, motor, social, and self-help subtests. The whole test includes the above five subtests. The motor subtest is further divided into gross- and fine-motor subdomains. The developmental data were collected from direct testing, observation, and self-report. Then the scores were transformed to developmental quotients (DQs) to correct the age at test. This instrument was developed from Taiwan, but acceptable concurrent validity was found with other well-established neurodevelopment measurements of child development—the Bayley scales of Infant Development-II ($r = 0.80$ – 0.97) (Liao et al., 2005b) and Peabody Developmental Motor Scales-Second Edition (Wu et al., 2005). Otherwise, the CDIIT also had acceptable test–retest reliability (intraclass correlation coefficient = 0.76 – 1.00) (Liao and Pan, 2005), internal consistency (Cronbach $\alpha = 0.75$ – 0.99) and content validity (Wang et al., 1998). In terms of construct validity, the raw scores of CDIIT increase with age (Wang et al., 1998). In addition, the diagnostic accuracy of the CDIIT for children with developmental delays was high with the area under the receiver operating characteristic curve (AUC) of 0.96 (Wang and Liao, 2007). The best cut-off point for identifying children with motor disabilities on the motor subtest of the CDIITDT was a DQ of 70 , which had 87% sensitivity and 97% specificity (Wu et al., 2005). The predictive validity for special education status was acceptable with a sensitivity of 75% and a specificity of 81% (Wang, 2005).

2.5. HOME score

The Home Observation for Measurement of the Environment (HOME) score was collected by well-trained staffs in children's home when they were 2 years of age. The HOME scale is a tool to measure the quality and quantity of caregiving of the parents to a child in the home environment at age from birth to 3 years. It contains six subscales including parental responsiveness, acceptance of child, organization of the environment, learning materials, parental involvement, and variety in experience from 45 items (Cadwell and Bradley, 1979). The higher the HOME score, the better the home environment.

2.6. Statistics

The cord blood cotinine were categorized into two groups by using 75th percent cut-off point of the distribution. The 0.16 ng/mL

cord blood cotinine level was the cut-off point to distinguish between exposed and unexposed groups (Pirkle et al., 1996). For metabolic genes, CYP1A1 MspI and CYP1A1 Ile462Val were categorized into wild and variant types. CYP1A1 MspI was grouped into TT vs. TC and CC types and the CYP1A1 Ile462Val was grouped into Ile/Ile vs. Ile/Val and Val/Val types.

We used multiple linear regression models to explore the relation between exposed and unexposed groups in CDIIT scores at 2 years of age. The modification effect of each gene was tested by combining the groups of both cord blood cotinine and gene susceptibility. Then, we explored the interaction effect of both phase I and II genes if significant modification effects existed for single genes. Potential confounders included maternal education and nationality, family income, infant sex and gestational age, HOME score, and postnatal ETS exposure. Two tailed p -values < 0.05 were considered to be statistical significance to explore the relation between exposed and unexposed groups in CDIIT scores. Due to multiple comparison tests for four metabolic genes taken into consideration on each neurodevelopment outcomes, we compared and performed each test at the 0.0125 ($=0.05/4$) level of significance. All analyses were conducted using SAS version 9.1 (SAS Institute Inc., Cary, NC).

3. Results

There were 145 maternal–newborn pairs had completed data of cotinine concentrations, DNA specimens and CDIIT test but 153 subjects lost to follow up at 2 years of age. These lost subjects had higher cord blood cotinine level, lower parental education, and family income compared to followed subjects (data not shown).

Table 1 shows the characteristic of the study subjects by ETS exposed and unexposed groups. Comparing these two groups on parental characteristics, the exposed group had higher percentage of foreign nationality mother, lower maternal education level, and lower family income. For infant characteristics, these two groups did not show significant difference in birth weight, birth length, and birth head circumference. The homozygous wild type frequencies of neonatal CYP1A1 MspI and CYP1A1 Ile462Val and the present type frequencies of neonatal GSTT1, GSTM1 were 22.8% , 52.4% , 53.1% , and 49.7% , respectively. After analysis CYP1A1 MspI and CYP1A1 Ile462Val genotypes frequencies by Hardy–Weinberg test, they were ascertained in a balance state in our study population to the heredity-balanced rule ($p = 0.16$; $p = 0.09$).

The CDIIT DQ scores at the age of 2 years were significant different in the whole test, and the cognitive, language, fine-motor, and social subtests ($p < 0.05$) between exposed and unexposed groups, as shown in Table 1. After controlling the potential confounders, the significantly adverse effects in whole test ($\beta = -7.9$; $p = 0.002$), cognitive ($\beta = -5.4$, $p = 0.04$), language ($\beta = -7.9$, $p = 0.002$), fine-motor ($\beta = -7.5$, $p = 0.004$), and social ($\beta = -7.2$, $p = 0.03$) performance remained, as shown in Table 2.

The modification effect of single gene was shown in Table 3. There were lower DQs in whole test ($\beta = -9.9$, $p = 0.004$), language subtest ($\beta = -9.2$, $p = 0.007$) and fine-motor subdomain ($\beta = -14.1$, $p < 0.0001$) of the CDIIT in the exposed group with variant type (Ile/Val + Val/Val) of CYP1A1 Ile462Val. In addition, there were lower DQs in the whole test ($\beta = -9.4$, $p = 0.006$), cognitive ($\beta = -10.2$, $p = 0.004$), language subtests ($\beta = -12.1$, $p < 0.0001$) and fine-motor subdomain ($\beta = -9.3$, $p = 0.008$) in the exposed group with absent type of GSTT1. However, there was lower DQs in the language subtest ($\beta = -10.0$, $p = 0.007$) in the exposed group with present type instead of absent type of GSTM1.

Thus, we only demonstrated the modification effect of both CYP1A1 Ile462Val and GSTT1 genes together, as shown in Table 4. The lowest DQs in language subtest was detected in the exposed

Table 1
Characteristics of the study subjects by cotinine level in cord blood

Characteristics	Cotinine level in cord blood	
	<0.16 ng/mL	0.16–14 ng/mL
Total	109	36
Mother's characteristics		
Taiwanese (%)	95.5	77.8
Age (years, mean $\pm$ S.D.)	32.7 $\pm$ 4.0	29.9 $\pm$ 4.4
Primiparae (%)	42.7	38.9
High school and below (%)	37.3	77.8
Alcohol consumption during pregnancy (%)	5.5	2.8
Family characteristics		
Annual family income <NT\$ 1,000,000 (%)	49.1	80.6
HOME ^a score at 2 years of child's age (mean $\pm$ S.D.)	41.3 $\pm$ 2.4	39.8 $\pm$ 3.0
Environmental tobacco smoke at 2 years of child's age (%)	28.0	61.3
Child's characteristics		
Male (%)	50.0	61.1
Gestational age (week, mean $\pm$ S.D.)	38.7 $\pm$ 1.5	38.7 $\pm$ 1.5
Birth weight (g, mean $\pm$ S.D.)	3224.4 $\pm$ 469.2	3186.1 $\pm$ 353.3
Birth length (cm, mean $\pm$ S.D.)	49.1 $\pm$ 2.1	48.9 $\pm$ 2.1
Birth head circumference (cm, mean $\pm$ S.D.)	33.8 $\pm$ 1.7	33.7 $\pm$ 1.4
Lead in cord blood (μ g/dL, mean $\pm$ S.D.)	1.2 $\pm$ 0.8	1.4 $\pm$ 0.7
Cotinine in cord blood (ng/mL, mean $\pm$ S.D.)	0.08 $\pm$ 0.05	0.76 $\pm$ 1.49
Child's gene polymorphisms		
CYP1A1 MspI (%)		
TT	25.7	13.9
TC	53.2	63.9
CC	21.1	22.2
CYP1A1 Ile462Val (%)		
Ile/Ile	52.3	52.8
Ile/Val	35.8	36.1
Val/Val	11.9	11.1
GSTT1 absent (%)	45.0	52.8
GSTM1 absent (%)	45.0	66.7
CDIIT ^b at 2 years of age (DQs ^c , mean $\pm$ S.D.)		
Whole test	101.5 $\pm$ 12.0	93.1 $\pm$ 12.0
Cognitive	96.2 $\pm$ 12.5	86.6 $\pm$ 12.3
Language	103.3 $\pm$ 11.9	93.2 $\pm$ 11.0
Gross-motor	86.8 $\pm$ 13.1	83.4 $\pm$ 12.6
Fine-motor	96.6 $\pm$ 11.5	89.7 $\pm$ 13.0
Social	111.1 $\pm$ 15.2	103.4 $\pm$ 14.3
Self-help	101.7 $\pm$ 14.2	102.7 $\pm$ 18.2

^a HOME, Home Observation for Measurement of the Environment.

^b CDIIT, Comprehensive Developmental Inventory for Infants and Toddlers.

^c DQs, developmental quotients.

group with both CYP1A1 Ile462Val wild type and GSTT1 absent type ($\beta = -12.4$, $p = 0.006$). Besides, the lowest DQs in fine-motor subdomain was detected in the exposed group with both CYP1A1 Ile462Val variant type and GSTT1 absent type ($\beta = -18.6$, $p < 0.0001$).

4. Discussion

This study showed that there were both CYP1A1 Ile462Val and GSTT1 modification effects of cotinine in cord blood on whole and fine-motor DQs. The ETS exposed group with GSTT1 absent type also had lowered cognitive and language DQs compared to unexposed group. We also indicated that cord blood cotinine could be modified by gene polymorphisms on neurodevelopment.

Table 2
Multiple linear regression models of CDIIT^a neurodevelopment at the age of 2 years by cotinine level in cord blood

CDIIT ^a (DQs ^b)	Cotinine level in cord blood (0.16–14 vs. <0.16 ng/mL)			
	Crude		Adjusted ^c	
	$\beta \pm$ S.E.	p-Value	$\beta \pm$ S.E.	p-Value
Whole test	-8.48 $\pm$ 2.31	<0.0001	-7.89 $\pm$ 2.48	0.002
Cognitive	-9.54 $\pm$ 2.39	<0.0001	-5.36 $\pm$ 2.56	0.04
Language	-10.15 $\pm$ 2.24	<0.0001	-7.85 $\pm$ 2.44	0.002
Gross-motor	-3.41 $\pm$ 2.53	0.18	-4.33 $\pm$ 2.78	0.12
Fine-motor	-6.87 $\pm$ 2.27	0.003	-7.46 $\pm$ 2.55	0.004
Social	-5.84 $\pm$ 2.25	0.01	-7.21 $\pm$ 3.24	0.03
Self-help	1.04 $\pm$ 2.93	0.72	-2.21 $\pm$ 3.07	0.47

β coefficients represent the differences of mean scores between exposed and unexposed groups before or after adjustment for the covariates. p-Values <0.05.

^a CDIIT, Comprehensive Developmental Inventory for Infants and Toddlers.

^b DQs, developmental quotients.

^c Models adjusted for maternal education and nationality, family income, infant sex and gestational age, HOME score, and postnatal ETS exposure from questionnaire.

Previous studies had found prenatal exposure to maternal smoking had been shown to induce children's poor performance on neuropsychologic including fine motor, spelling and language skills (Key et al., 2007), perception, general cognitive abilities (Batty et al., 2006; Julvez et al., 2007) and visual spatial skills, intelligence and achievement scores (Lambe et al., 2006). Animal studies also found that there were increasing motor activity associated with in utero nicotine exposure (Fung and Lau, 1988), and deficits in learning, attention and memory deficits in maze task performance (Levin et al., 1993).

The impact of maternal exposure to ETS on neurodevelopment is less clear. A recent study found adverse effect of prenatal ETS on cognitive development at 2 years of age but postnatal ETS exposure did not contribute to the developmental delay (Rauh et al., 2004). Golub et al. (2007) conducted an animal study on rhesus monkeys for maternal ETS exposure and found poor performance of visual recognition task. Our study also demonstrated an obviously effect on children's neurodevelopment including the cognitive, language and fine-motor performance as well as the general development (whole DQ of the CDIIT) (Table 2).

The development of nervous system is more vulnerable to neurotoxic agents during the embryonic and fetal stage. There are thousands of bioactive contains in cigarette and are mutagenic, neurotoxic, and fetotoxic agents, including nicotine, polycyclic aromatic hydrocarbons (PAHs), aromatic amines, carbon monoxide, 1,3-butadiene, ethylene oxide, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). Previous study had shown that these agents could pass through the placenta into the fetus (Lackmann et al., 1999).

Previous animal studies had proved fetal nicotine exposure could target specific neurotransmitter receptors in the fetal brain and discoordinate the cell replication, differentiation, and synaptic development (Slotkin, 2004). Nicotine also had adverse effects on the central nervous system through cell damage and reduced cell numbers caused by errors in cell development (Roy and Sabherwal, 1994), and was due to the reduction of the amount of oxygen available to fetal tissue by restricting the utero-placental blood flow (Suzuki et al., 1980).

PAHs have also been reported to damage the development of nervous system. Fetal toxicity from PAHs could reduce exchange of oxygen and nutritious for binding to receptors for placental growth factors (Dejmek et al., 2000), inactive apoptotic caused by DNA damage (Nicol et al., 1995) or has anti-estrogenic effect (Manchester et al., 1987). Epidemiological evidence also showed

Table 3Multiple linear regression models of CDIIT^a neurodevelopment at the age of 2 years by cotinine level in cord blood, and each of metabolic gene polymorphisms

Cotinine (ng/mL)	Gene polymorphisms	Number	CDIIT ^a (DQs ^b)						
			Whole test	Cognitive	Language	Gross-motor	Fine-motor	Social	Self-help
<0.16	CYP1A1 MspI		β coefficient						
	TT	28	Referent	Referent	Referent	Referent	Referent	Referent	Referent
	TC + CC	81	1.0	1.3	−0.2	−0.8	−0.1	−0.6	−0.9
	TT	5	−4.6	−6.4	−11.1	−2.0	−6.5	−6.3	9.3
0.16–14	TC + CC	31	−7.6	−4.2	−7.6	−5.3	−7.7	−7.8	−3.1
	CYP1A1 Ile462Val		β coefficient						
	Ile/Ile	57	Referent	Referent	Referent	Referent	Referent	Referent	Referent
	Ile/Val + Val/Val	52	−0.2	−0.6	−1.4	0.4	−3.3	0.2	0.9
0.16–14	Ile/Ile	19	−6.1	−5.4	−7.8	−1.4	−3.9	−5.3	0.4
	Ile/Val + Val/Val	17	−9.9	−5.9	−9.2	−7.2	−14.1	−9.0	−4.0
<0.16	GSTT1		β coefficient						
	Present	60	Referent	Referent	Referent	Referent	Referent	Referent	Referent
	Absent	49	1.4	−0.2	1.6	−5.9	−0.1	6.6	0.1
	Present	17	−5.0	−0.8	−1.9	−10.2	−5.7	−3.1	−2.7
0.16–14	Absent	19	−9.4	−10.2	−12.1	−4.9	−9.3	−4.4	−1.5
<0.16	GSTM1		β coefficient						
	Present	60	Referent	Referent	Referent	Referent	Referent	Referent	Referent
	Absent	49	3.9	−0.6	2.5	1.4	0.004	2.9	5.2
	Present	12	−7.9	−8.5	−10.0	−4.2	−7.3	−8.7	4.1
0.16–14	Absent	24	−5.3	−3.3	−5.0	−3.5	−7.5	−4.5	−2.2

Model adjusted for maternal education and nationality, family income, infant sex and gestational age, HOME score, and postnatal ETS exposure from questionnaire. β coefficients represent the differences of mean scores between each subgroup and the reference group after adjustment for the covariates. p -Values <0.0125 in bold.

^a CDIIT, Comprehensive Developmental Inventory for Infants and Toddlers.

^b DQs, developmental quotients.

prenatal exposure to PAHs had adverse neurodevelopment in the first 3 years of age (Perera et al., 2004).

Our results also demonstrated that both CYP1A1 Ile462Val and GSTT1 genes could modify the effect of cord blood cotinine on children's neurodevelopment. The genes also seemed to manifest their modification effects on different domains of the neurodevelopment. First, in term of whole DQ of the CDIIT, the exposed groups with variant type of CYP1A1 Ile462Val ($\beta = -9.9$, $p = 0.004$) or absent type of GSTT1 ($\beta = -9.4$, $p = 0.006$) were found lower whole DQs although the coefficient does not reach the level of significance ($p < 0.0125$) in exposed group with both gene susceptible types ($\beta = -10.1$, $p = 0.03$) (Tables 3 and 4). It could be too small numbers to detect any effect of the interaction.

Second, GSTT1 gene may modify the adverse effect of cord blood cotinine on cognitive development. The absent type of GSTT1 ($\beta = -10.2$, $p = 0.004$) were found to be associated with lower cognitive DQs (Table 3). In Table 4, exposed groups with absent type of GSTT1 group had a borderline significant ($\beta = -11.1$,

$p = 0.02$; $\beta = -8.6$, $p = 0.06$). The consistent results were found in exposed group with absent type GSTT1.

Third, GSTT1 gene may modify the adverse effect of cord blood cotinine on language development. There were lower language DQs development in exposed groups with absent type of GSTT1 ($\beta = -12.1$, $p < 0.0001$) or variant type of CYP1A1 Ile462Val ($\beta = -9.2$, $p = 0.007$) (Table 3). However, 9 out of 17 subjects in the latter group also had GSTT1 absent type. Moreover, we only found lower language DQs in exposed group with both GSTT1 absent type and CYP1A1 Ile462Val wild type ($\beta = -12.4$, $p = 0.006$) instead of variant type (Table 4).

Finally, the CYP1A1 Ile462Val and GSTT1 genes may modify the adverse effect of cord blood cotinine on fine-motor development. The lower fine-motor DQs was found in exposed group with CYP1A1 Ile462Val variant type ($\beta = -14.1$, $p < 0.0001$) or absent type of GSTT1 ($\beta = -9.3$, $p < 0.008$). The lower score was found in the exposed group with both susceptible gene types ($\beta = -18.6$, $p < 0.0001$).

Table 4Multiple linear regression models of CDIIT^a neurodevelopment at the age of 2 years by cotinine level in cord blood, and CYP1A1 Ile462Val and GSTT1 gene polymorphisms

Cotinine (ng/mL)	Gene polymorphisms		Number	CDIIT ^a (DQs ^b)						
	Phase 1	Phase 2		Whole test	Cognitive	Language	Gross-motor	Fine-motor	Social	Self-help
<0.16	CYP1A1 Ile462Val	GSTT1		β coefficient						
	Ile/Ile	Present	30	Referent	Referent	Referent	Referent	Referent	Referent	Referent
		Absent	27	0.5	0.9	4.3	−7.0	−3.0	6.8	−6.8
	Ile/Val + Val/Val	Present	30	−0.9	0.6	1.6	−1.2	−5.8	0.7	−5.9
0.16–14		Absent	22	1.5	−1.0	−0.2	−6.0	−3.1	6.9	2.4
	Ile/Ile	Present	9	−2.5	0.6	0.4	−9.5	−5.7	−0.2	−1.7
		Absent	10	−9.3	−11.2	−12.4	−0.4	−4.9	−3.4	−3.8
	Ile/Val + Val/Val	Present	8	−8.9	−2.0	−3.3	−12.4	−11.9	−5.89	−9.8
		Absent	9	−10.1	−8.6	−10.5	−10.2	−18.6	−4.7	−4.5

Model adjusted for maternal education and nationality, family income, infant sex and gestational age, HOME score, and postnatal ETS exposure from questionnaire. β coefficients represent the differences of mean scores between each subgroup and the reference group after adjustment for the covariates. p -Values <0.0125 in bold.

^a CDIIT, Comprehensive Developmental Inventory for Infants and Toddlers.

^b DQs, developmental quotients.

The phase I and phase II metabolic enzyme play an important role in detoxify smoke-derived toxic chemicals, including metabolism and detoxification process. The cytochrome P-450 1A1 (CYP1A1), the phase I gene, encoded the aryl hydrocarbon hydroxylase and ruled to metabolic chemicals such as PAHs (Anttila et al., 2001). The glutathione S-transferases (GSTs) were involved in detoxify of many chemicals (Hayes and Strange, 2000), such as the GSTM1 helped to detoxify PAHs and GSTT1 helped to detoxify of 1,3-butadiene and ethylene oxide (Fustinoni et al., 2002). The variant types of four metabolic genes in our study were high. The prevalence was similar with previous study in Taiwan (Chang et al., 2006). The high polymorphism rate raised the importance for cigarette exposure in Asian because there were large numbers of people susceptibility to the exposure.

There were lower language DQs in exposed groups with present type of GSTM1 ($\beta = -10.0$, $p = 0.007$) or absent type of GSTT1 ($\beta = -12.1$, $p < 0.0001$) (Table 3). However, 9 out of 12 subjects in the former group also had GSTT1 absent type. So the adverse effect might result from the GSTT1 absent type instead of GSTM1 present type.

Measured cotinine in blood is considered to be one of the most sensitive biomarker of nicotine for smoking exposure (Benowitz, 1999). It can distinguish not only active smoking from passive smoking, but also exposure to ETS from non-exposure (Benowitz, 1996; Nafstad et al., 1996; Pichini et al., 2000). Based on the plasma cotinine concentrations, we could avoid reporting bias or exposure misclassification.

However, because the smoking exposure was intermittent and cotinine was a biomarker of recent exposure with a half-life of approximately 20 h (Benowitz and Jacob, 1994), one of our limitations was that the cotinine measurement from cord blood was a single opportunistic measurement at delivery. In Taiwan, most pregnancy women stayed at home or even worked outside home until in labor. We believe that the time before delivering was rather short and the cord blood cotinine can be suitable as the indicator to reflect the prenatal ETS exposure. In our study, cord blood cotinine level revealed a good correlation to self-reported smoke exposure amount and smoking habits investigated in our questionnaire were relatively stable over time and the maternal and cord plasma concentrations of cotinine were highly correlated ($r = 0.71$, $p < 0.001$) (Wang et al., 2008). According to previous studies, cotinine levels remained fairly constant and at near steady-state values throughout the day (Benowitz and Jacob, 1994). Furthermore, if measurement error did occur, it tended to be toward the null and the effect of exposure was likely to be underestimated.

Another limitation was that there was probably a considerable overlap between maternal and postnatal exposure to ETS and therefore also an association between cotinine levels and postnatal exposure, which makes it very difficult to disentangle the relevance of the different time-windows. Spearman correlation coefficient was used to test the correlation between cord blood cotinine and postnatal ETS exposure. Since the correlation between cotinine in cord blood and postnatal ETS exposure by questionnaire was low ($r = 0.3$) in our study, the postnatal ETS was taken as a confounding variable and put into the model to adjust the effect. The adverse effects of cord blood cotinine on neurodevelopment remain even after adjusting for postnatal ETS exposure.

In addition, there was a negative result of postnatal ETS exposure from a prospective cohort study (Rauh et al., 2004) although children's ETS exposure from parental smoking at home might have poor cognitive performance in childhood (Johnson et al., 1999) and adolescent (Bauman et al., 1991; Yoltos et al., 2005). It was suggested there may be some residual effects from the fetus period on the nerve system thus postnatal nicotine

exposure appears to be less effect on brain development (Weitzman et al., 2002). Finally, we may underestimate the adverse effect because our lost subjects had a higher cotinine level in cord blood than followed subjects.

The effect of low socioeconomic status and/or poverty has also been shown extensively to impact neurodevelopment and has demonstrated in previous studies (Farah et al., 2006; Wood, 2003). In our study, the exposed group had a higher percentage of low socioeconomic status such as foreign-born mothers, lower maternal educational level, and lower family income. We used multivariate modeling to control any potential confounding effects from maternal education and nationality, family income, infant sex and gestational age, HOME score, and postnatal ETS exposure. There were only female infant and HOME score significantly positively associated with language DQ. Furthermore, we also used a matching method which matched each exposed subject with an unexposed subject who had same levels in maternal education and nationality, and family income. We still found cotinine levels were significantly negatively associated with DQs of the whole test, language, fine-motor and social subtests of the CDIIT ($\beta = -7.4$, $p = 0.02$; $\beta = -7.2$, $p = 0.03$; $\beta = -8.8$, $p = 0.007$; $\beta = -9.1$, $p = 0.04$) but not for cognition. The lowest score in fine-motor DQs was also detected in exposed group with both CYP1A1 Ile462Val variant type and GSTT1 absent type ($\beta = -14.5$, $p = 0.004$). The effect of socioeconomic status may not be significant when the HOME score was included into the model.

Our study demonstrates an obviously adverse effect of cord blood cotinine on early children's neurodevelopment especially for language and fine-motor development. In addition, both CYP1A1 Ile462Val and GSTT1 metabolic genes could modify the effects.

Acknowledgements

This study was partly supported by the grants from the Bureau of Health Promotion, Department of Health (BHP-PHRC-92-4 and DOH93-HP-1702), and National Science Council (NSC95-2314-B-002-269 and NSC96-2314-B-002-117-MY3), Taiwan.

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