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Prenatal Particulate Matter/Tobacco Smoke Increases Infants' Respiratory Infections: COCOA Study

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Purpose: To investigate whether prenatal exposure to indoor fine particulate matter ($PM_{2.5}$) and environmental tobacco smoke (ETS) affects susceptibility to respiratory tract infections (RTIs) in infancy, to compare their effects between prenatal and postnatal exposure, and to determine whether genetic factors modify these environmental effects. **Methods:** The study population consisted of 307 birth cohort infants. A diagnosis of RTIs was based on parental report of a physician's diagnosis. Indoor $PM_{2.5}$ and ETS levels were measured during pregnancy and infancy. TaqMan was used for genotyping of *nuclear factor erythroid 2-related factor* (*Nrf2*) (rs6726395), *glutathione-S-transferase-pi* (*GSTP*) *1* (rs1695), and *glutathione-S-transferase-mu* (*GSTM*) *1*. Microarrays were used for genome-wide methylation analysis. **Results:** Prenatal exposure to indoor $PM_{2.5}$ increased the susceptibility of lower RTIs (LRTIs) in infancy (adjusted odds ratio [aOR]=2.11). In terms of combined exposure to both indoor $PM_{2.5}$ and ETS, prenatal exposure to both pollutants increased susceptibility to LRTIs (aOR=6.56); however, this association was not found for postnatal exposure. The *Nrf2* GG (aOR=23.69), *GSTM1* null (aOR=8.18), and *GSTP1* AG or GG (aOR=7.37) genotypes increased the combined LRTIs-promoting effects of prenatal exposure to the 2 indoor pollutants. Such effects of prenatal indoor $PM_{2.5}$ and ETS exposure were not found for upper RTIs. **Conclusions:** Prenatal exposure to both indoor $PM_{2.5}$ and ETS may increase susceptibility to LRTIs. This effect can be modified by polymorphisms in reactive oxygen species-related genes.

Key Words: Prenatal exposure; particulate matter; tobacco smoke; respiratory tract infections; polymorphism; methylation

INTRODUCTION

Environmental tobacco smoke (ETS), which consists of a mixture of gaseous and particulate pollutants, is major indoor air pollution. Indoor particulate matter (PM) is also emitted from cooking, cleaning, and other human activities as well as from smoking.[1,2] Indoor PM and ETS are major indoor air pollutants

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and may modify the effects of each other.[3,4] Therefore, it is important to understand the combined effect of indoor PM and ETS on health and to elucidate the mechanisms involved. Although there is some epidemiologic evidence on the combined effect of ETS and ambient air pollutants on childhood respiratory outcomes,[5-9] results on combined exposure to indoor PM and ETS are limited, particularly prenatal exposure.

The prenatal period is critical in terms of the later development of respiratory disorders in childhood because prenatal air pollutant exposure is associated with adverse effects on fetal growth[10] and immune responses in early life.[11-14] Prenatal and postnatal exposure involve different routes: prenatal air pollutant exposure occurs via transplacental absorption, whereas postnatal exposure occurs via the respiratory route. Therefore, prenatal air pollutant exposure may affect health via a different mechanism from postnatal exposure. Based on these results, we hypothesized that prenatal indoor PM and ETS exposure compared to postnatal exposure would more severely affect the lower respiratory tract than the upper respiratory tract. Since early-childhood respiratory disorders, especially lower-respiratory tract infections (LRTIs), can develop into chronic respiratory impairment later in life,[15-17] it is important to identify modifiable early life determinants of adverse respiratory outcomes, especially those operating in the prenatal period. However, the impact of prenatal indoor air pollutant exposure, especially the interaction between indoor PM and ETS, on the susceptibility to LRTIs remains poorly understood.

A mechanism through which PM and ETS may lead to respiratory disease is through promotion of reactive oxygen species (ROS).[18,19] The transcription factor nuclear factor erythroid 2-related factor (Nrf2) is activated by oxidative stress and leads to the transcription of antioxidant genes, such as *glutathione S-transferase-pi 1* (*GSTP1*) and *glutathione S-transferase-mu 1* (*GSTM1*). Therefore, ROS-related genes and polymorphisms may result in different responses to PM and ETS.[20] The influence of genetic variation on the association between prenatal exposure to indoor PM and/or ETS and susceptibility to RTIs in infancy remains to be studied.

Epigenetic modifications are one of the mechanisms by which prenatal exposures can affect disease later in life. DNA methylation is a well-characterized epigenetic modification, and there is evidence that it may modulate the lifelong effect of prenatal smoke exposure.[21-23]

To address these issues, a prospective birth cohort study was performed. The effect of prenatal indoor PM and/or ETS exposure on the susceptibility of RTIs in infancy was evaluated. The influence of ROS-related gene polymorphisms on RTI susceptibility in infancy was also assessed. Furthermore, whether prenatal indoor PM and ETS exposure can alter DNA methylation was investigated.

MATERIALS AND METHODS

Study design

Healthy newborns (n=1,733) were recruited between November 2007 and December 2013. This prospective, general population-based, birth cohort was designated as the COhort for Childhood Origin of Asthma and Allergic Diseases (COCOA); follow-up and further recruitment of this cohort is ongoing. The study methods have been detailed elsewhere.[24,25] The indoor level of fine particulate matter ($PM_{2.5}$) has been measured since 2009 for the applicants. In 608 infants, the indoor levels of $PM_{2.5}$ were evaluated between 26 and 36 weeks of pregnancy. Of these, based on the complete $PM_{2.5}$, ETS exposure, RTIs, and genotype data, 307 infants were finally included in the study (Fig. 1). Whether the 6- and 12-month-old infants had had any RTIs was determined by parental report of physician-diagnosed RTIs: "Has a doctor diagnosed RTIs in your child during the last 6 months?" Bronchiolitis, tracheobronchitis, and/or pneumonia were considered as LRTIs and common cold, sinusitis, otitis media, and/or croup as upper RTIs (URTIs).

Exposure assessment

Starting in May 2009, indoor $PM_{2.5}$ samples were collected by specialists during home visit between 26 and 36 weeks of pregnancy. In addition, $PM_{2.5}$ samples at 6 months after birth were collected in the subgroup (n=75) for the applicants. $PM_{2.5}$ concentrations were measured 3 times in the parents' bed room by using a particle discriminator (Model GT-331; SIBATA Co., Japan) with a laser light-scattering optical particle counter for 5 minutes. The mean value of 3 measurements was used for evaluation. The indoor $PM_{2.5}$ values were log-transformed and di-

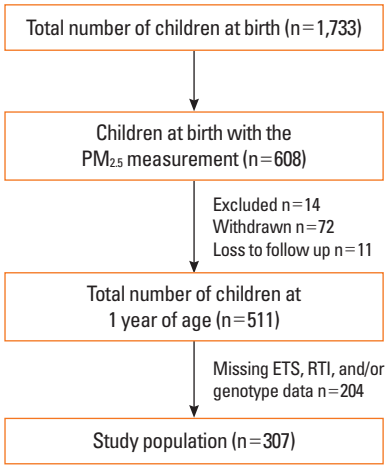

Fig. 1. Flow chart of the study population. Of infants with prenatal indoor $PM_{2.5}$ measurements (n=608), 14 met the exclusion criteria, 72 were withdrawn from the study, and 11 were lost to follow-up. An additional 204 infants were then excluded because the RTIs, prenatal ETS exposure, or genotype data were missing. ETS, environmental tobacco smoke; $PM_{2.5}$, fine particulate matter; RTIs, respiratory tract infections.

chotomized to high or low by using the median value before being entered into the regression models. Mothers were asked the following questions about their ETS exposure at home: "Have you been regularly exposed to passive smoking during your current pregnancy?"

The groups were stratified by exposure time. This led to 4 study population groups receiving the following combination of prenatal/postnatal exposures: prenatal ETS/prenatal $PM_{2.5}$, prenatal ETS/postnatal $PM_{2.5}$, postnatal ETS/prenatal $PM_{2.5}$, and postnatal ETS/postnatal $PM_{2.5}$. To assess whether the 2 indoor pollutants acted additively to increase RTI susceptibility in infancy, each group was divided into 4 groups according to their ETS exposure and whether the indoor $PM_{2.5}$ levels were high or low.

Genotyping

Genomic DNA was prepared from heparinized newborn umbilical cord blood by using a G-DEX II kit (Intron, Seoul, Korea). ROS-related genes were analyzed as follows. The *Nrf2* (rs6726395) and *GSTP1* (rs1695) polymorphisms were genotyped by using a TaqMan assay (ABI, Foster City, CA, USA). The *GSTM1* copy number was measured by real-time polymerase chain reaction (PCR). The genotyping method is detailed in the Supplemental Material.

Bisulfite conversion and genome-wide methylation array

Nine subjects were selected from the study population to undergo genome-wide methylation analysis of cord blood genomic DNA. Bisulfite conversion was performed by using the EZ DNA methylation kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. The bisulfite-converted genomic DNA was analyzed by using the Infinium Human Methylation 450 Beadchip (Illumina, San Diego, CA, USA), with >450,000 probes covering 99% of reference sequence genes, following the Illumina Infinium HD Methylation protocol. The 9 subjects and the methylation array are described in the Supplemental Material.

Statistical analysis

Chi-square and *t* tests were used to assess the significance of differences between the groups, as appropriate. The associations between prenatal indoor $PM_{2.5}$ and/or ETS exposure and the incidence of RTIs at 12 months of age were analyzed by using multiple logistic regression. Adjustments were made for potential confounding factors, namely, maternal age at delivery, maternal body mass index, maternal educational degree, gestational age, delivery mode, infant sex, and family history of allergic diseases. The results are expressed as adjusted odds ratios (aORs) and 95% confidence intervals (CIs). All statistical analyses were performed by using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA), with a P value <0.05 considered statistically significant.

Ethics statement

The study was approved by the Institutional Review Board of Asan Medical Center (IRB No. 2008-0616), Samsung Medical Center (IRB No. 2009-02-021), Yonsei University (IRB No. 4-2008-0588), and CHA Medical Center (IRB No. 2010-010).

RESULTS

Study population characteristics

Table 1 summarizes the characteristics of the study population (n=307). The study population consisted of 171 boys and 136 girls. In total, 61.6% had been prenatally exposed to maternal ETS, and the mean indoor $PM_{2.5}$ level during pregnancy was 6.08 ± 7.64 μg/m^3. The frequencies of the *Nrf2* (rs6726395) GG, *GSTP1* (rs1695) AG or GG, and *GSTM1* null genotypes were 40.1%, 36.9%, and 56.7%, respectively. The distribution of the 2 polymorphisms was in Hardy-Weinberg equilibrium. The incidences of LRTIs and URTIs by the age of 12 months were 16.3% and 76.2%, respectively.

Table 1 also shows the characteristics of the infants who were not included for reasons shown in Fig. 1. There were no significant differences between participants and non-participants, except in gestational age and the incidence of URTIs.

Risk factors for RTIs in infancy

High $PM_{2.5}$ levels during pregnancy were independent risk factors for LRTIs in infants (aOR=2.11; 95% CI: 1.12, 3.99) (Table 2). However, none of the early-life environmental factors increased the risk of URTIs. When we compared indoor $PM_{2.5}$ levels according to RTIs and exposure time, prenatal indoor $PM_{2.5}$ levels were higher in infants with LRTIs than in those without (mean=7.21 vs 5.71, respectively; 95% CI: 4.99, 9.44 vs 4.97, 6.45, respectively; P=0.119, data not shown). These differences were not distinct according to postnatal $PM_{2.5}$ levels or the presence of URTIs.

Effects of prenatal exposure to both indoor $PM_{2.5}$ and ETS on RTIs susceptibility in infancy

Prenatal high indoor $PM_{2.5}$ and ETS exposure acted additively to increase the risk of LRTIs (aOR=6.56; 95% CI: 2.02, 21.24) in infants (Fig. 2A and Table S1). Such an additive effect was not replicated when examining the effects of exposure to prenatal $PM_{2.5}$/postnatal ETS, postnatal $PM_{2.5}$/prenatal ETS, and postnatal $PM_{2.5}$/postnatal ETS on any respiratory outcomes in infants. In addition, such additive effects were not observed for URTIs risk, regardless of exposure time (Fig. 2B and Table S1).

Effect of GSTM1, GSTP1, and Nrf2 genotypes on the relationship between prenatal indoor $PM_{2.5}$/ETS exposure and RTIs in infancy

Prenatal exposure to both high indoor $PM_{2.5}$ and ETS increased LRTIs risk in the *GSTM1* null, *GSTP1* AG or GG, and

Table 1. Characteristics of infants who were included and excluded from the study

	Participants (307)		Non-participants (1,426)		*P* value
	Number	Mean±SD or %	Number	Mean±SD or %	
Sex (male, %)	171	55.7	673	51.8	0.228
Gestational age (week)	303	39.27±1.21	1,257	39.10±1.27	0.042
Maternal age at birth (year)	307	32.69±3.44	1,296	32.56±3.43	0.535
Cesarean section delivery (%)	90/269	33.5	377/1,117	33.8	0.943
Maternal body mass index (kg/m^2)	307	20.53±2.24	1,352	20.66±2.63	0.369
Maternal education state	307		1,332		0.999
≤High school (%)	21	6.8	90	6.8	
University or college (%)	218	71.0	947	71.1	
Graduate school (%)	68	22.2	295	22.2	
Parental history of allergic disease (%)	178/295	60.3	615/1,085	56.7	0.288
Maternal ETS exposure during pregnancy (%)	189/307	61.6	668/1,084	61.6	1.000
Infantile ETS exposure at 1 year (%)	75/241	31.1	196/600	32.7	0.684
$PM_{2.5}$ during pregnancy (μg/m^3)	307	6.08±7.64	307	5.70±4.97	0.461
$PM_{2.5}$ at 6 months of age (μg/m^3)	75	5.11±7.05	245	5.40±5.33	0.699
Nrf2 GG genotype (%)	123/307	40.1	351/913	38.4	0.636
GSTP1 AG or GG genotype (%)	113/306	36.9	295/919	32.1	0.124
GSTM1 null genotype (%)	173/305	56.7	490/911	53.8	0.388
Incidence of LRTIs* at 1 year (%)	49/300	16.3	111/637	17.4	0.917
Incidence of URTIs† at 1 year (%)	234/307	76.2	450/668	67.4	0.005

*Lower respiratory tract infections: tracheobronchitis, pneumonia, and bronchiolitis; †Upper respiratory tract infections: common cold, sinusitis, otitis media, and croup.
ETS, environmental tobacco smoke; *GSTM1*, glutathione S-transferase-mu 1; *GSTP1*, glutathione S-transferase-pi 1; *Nrf2*, nuclear factor erythroid 2-related factor; $PM_{2.5}$, fine particulate matter; LRTIs, lower respiratory tract infections; URTIs, upper respiratory tract infections.

Table 2. Risk factors for respiratory tract infections in infancy

	LRTIs			URTIs		
	aOR*	95% CI	*P* value	aOR*	95% CI	*P* value
Sex (male)	1.53	(1.01, 2.31)	0.046	1.04	(0.75, 1.45)	0.799
Parental history of allergic disease	0.87	(0.58, 1.30)	0.500	0.97	(0.70, 1.35)	0.863
Gestational age	0.94	(0.80, 1.10)	0.450	0.94	(0.83, 1.08)	0.393
Cesarean section delivery	1.14	(0.74, 1.75)	0.550	0.75	(0.53, 1.06)	0.098
Higher maternal education state	1.08	(0.74, 1.58)	0.683	1.12	(0.82, 1.54)	0.468
Maternal age at birth	0.99	(0.93, 1.05)	0.713	1.06	(1.01, 1.11)	0.019
Maternal body mass index	1.04	(0.96, 1.12)	0.363	0.94	(0.88, 1.01)	0.078
Higher $PM_{2.5}$ during pregnancy	2.11	(1.12, 3.99)	0.021	1.23	(0.71, 2.15)	0.463
Higher $PM_{2.5}$ at 6 months of age	0.89	(0.37, 2.18)	0.801	1.00	(0.52, 1.94)	0.999
Maternal ETS exposure during pregnancy	1.33	(0.87, 2.04)	0.193	1.15	(0.82, 1.62)	0.414
Infantile ETS exposure at 1 year	0.87	(0.53, 1.42)	0.576	0.79	(0.53, 1.17)	0.241
Nrf2 GG genotype	1.27	(0.82, 1.97)	0.290	0.55	(0.39, 0.78)	0.001
GSTP1 AG or GG genotype	1.53	(0.98, 2.38)	0.062	0.89	(0.62, 1.28)	0.523
GSTM1 null genotype	1.69	(1.07, 2.67)	0.024	0.91	(0.64, 1.30)	0.612

*Odds ratios were adjusted for maternal age, maternal body mass index, maternal educational state, infant sex, gestational age, delivery mode, and family history of allergy.
ETS, environmental tobacco smoke; *GSTM1*, glutathione S-transferase-mu 1; *GSTP1*, glutathione S-transferase-pi 1; *Nrf2*, nuclear factor erythroid 2-related factor; $PM_{2.5}$, fine particulate matter; LRTIs, lower respiratory tract infections; URTIs, upper respiratory tract infections.

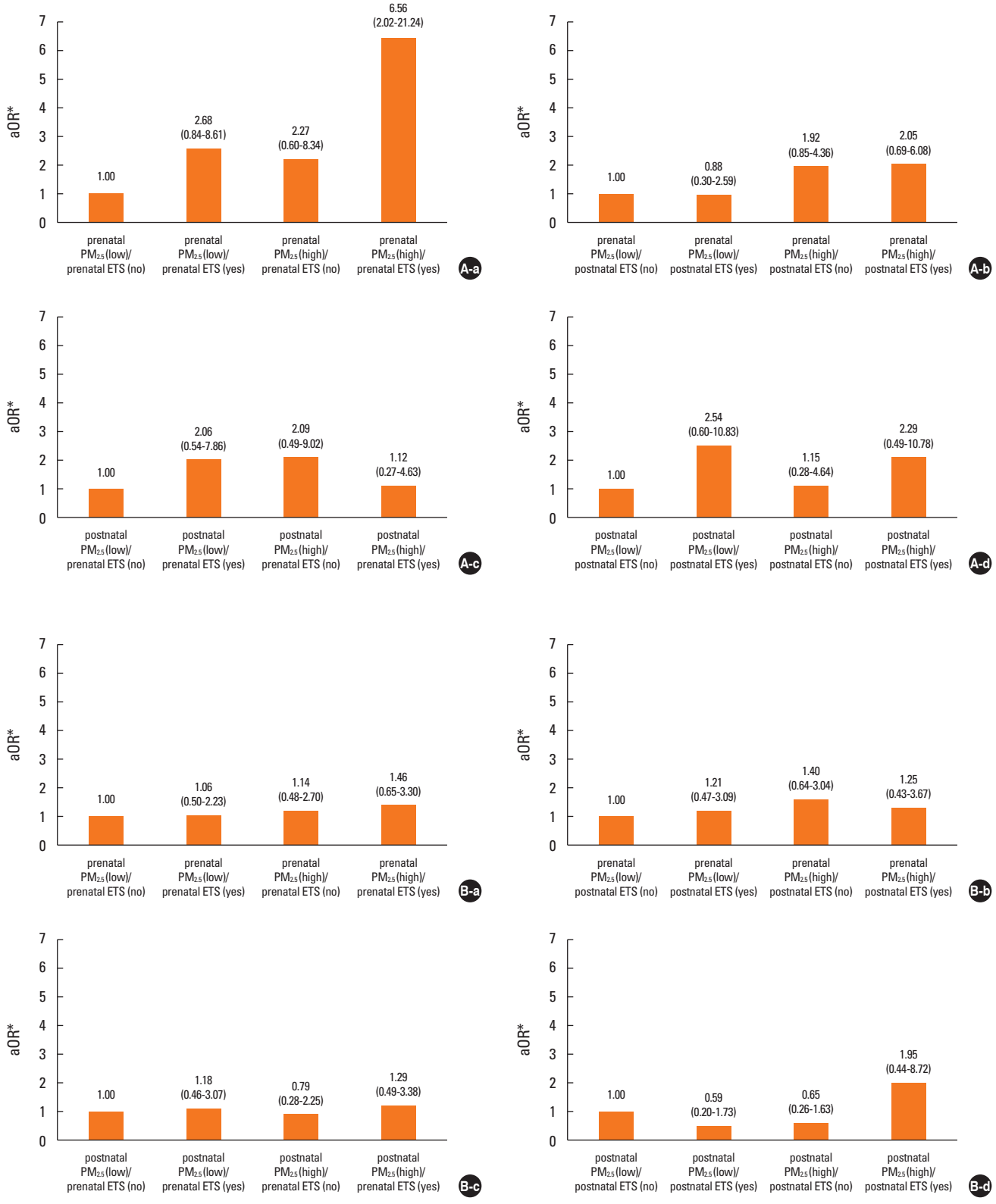

Fig. 2. Effect of combined exposure to both $PM_{2.5}$ and ETS according to exposure time on susceptibility to (A) lower and (B) upper respiratory tract infections in infancy. The groups were stratified by exposure time: (a) prenatal $PM_{2.5}$/prenatal ETS; (b) prenatal $PM_{2.5}$/postnatal ETS; (c) postnatal $PM_{2.5}$/prenatal ETS; and (d) postnatal $PM_{2.5}$/postnatal ETS. Prenatal high indoor $PM_{2.5}$ and ETS exposure acted additively to increase the risk of lower respiratory tract infections. *Adjustments for maternal age at delivery, maternal body mass index, maternal educational degree, gestational age, delivery mode, infant sex, and family history of allergy. ETS, environmental tobacco smoke; $PM_{2.5}$, fine particulate matter.

Table 3. Influence of the *glutathione S-transferase-mu 1* (*GSTM1*) copy number variation on the results of prenatal exposure to both $PM_{2.5}$ and ETS

	GSTM1 present			*GSTM1* null			*GSTM1* present			*GSTM1* null		
	LRTIs			LRTIs			URTIs			URTIs		
	No (n=112)	Yes (n=17)		No (n=137)	Yes (n=32)		No (n=36)	Yes (n=87)		No (n=37)	Yes (n=150)	
	n	*n*	aOR* (95% CI)	*n*	*n*	aOR* (95% CI)	*n*	*n*	aOR* (95% CI)	*n*	*n*	aOR* (95% CI)
$PM_{2.5}$ (Low)	29	2	1.00	34	3	1.00	6	26	1.00	10	27	1.00
ETS (No)												
$PM_{2.5}$ (Low)	37	7	1.68	45	12	4.52	11	33	0.51	12	48	2.02
ETS (Yes)			(0.24, 11.79)			(0.87, 23.60)			(0.14, 1.83)			(0.68, 6.01)
$PM_{2.5}$ (High)	13	2	2.26	28	4	1.65	4	12	0.69	9	25	1.17
ETS (No)			(0.26, 19.36)			(0.24, 11.29)			(0.13, 3.76)			(0.38, 3.57)
$PM_{2.5}$ (High)	33	6	3.75	30	13	8.18	9	32	0.57	11	33	1.86
ETS (Yes)			(0.52, 27.10)			(1.53, 43.72)			(0.14, 2.35)			(0.60, 5.75)

*Odds ratios were adjusted for maternal age, maternal body mass index, maternal educational state, infant sex, gestational age, delivery mode, and family history of allergy.
aOR, adjusted odds ratio; CI, confidence interval; ETS, environmental tobacco smoke; $PM_{2.5}$, fine particulate matter; *GSTM1*, glutathione S-transferase-mu 1; LRTIs, lower respiratory tract infections; URTIs, upper respiratory tract infections.

Table 4. Influence of the *glutathione S-transferase-pi 1* (*GSTP1*) (rs1695) polymorphism on the results of prenatal exposure to both $PM_{2.5}$ and ETS

	GSTP1 AA			*GSTP1* AG+GG			*GSTP1* AA			*GSTP1* AG+GG		
	LRTIs			LRTIs			URTIs			URTIs		
	No (n=159)	Yes (n=29)		No (n=91)	Yes (n=20)		No (n=45)	Yes (n=151)		No (n=27)	Yes (n=86)	
	n	*n*	aOR* (95% CI)	*n*	*n*	aOR* (95% CI)	*n*	*n*	aOR* (95% CI)	*n*	*n*	aOR* (95% CI)
$PM_{2.5}$ (Low)	38	3	1.00	25	2	1.00	9	33	1.00	7	20	1.00
ETS (No)												
$PM_{2.5}$ (Low)	59	12	3.57	24	7	4.04	15	58	1.14	9	23	1.18
ETS (Yes)			(0.66, 19.17)			(0.62, 26.30)			(0.41, 3.15)			(0.29, 4.71)
$PM_{2.5}$ (High)	26	3	2.82	14	3	1.70	9	23	0.75	3	14	1.43
ETS (No)			(0.40, 19.77)			(0.19, 15.01)			(0.24, 2.34)			(0.28, 7.30)
$PM_{2.5}$ (High)	36	11	6.32	28	8	7.37	12	37	0.83	8	29	1.80
ETS (Yes)			(1.18, 33.94)			(1.12, 48.66)			(0.28, 2.43)			(0.41, 7.97)

*Odds ratios were adjusted for maternal age, maternal body mass index, maternal educational state, infant sex, gestational age, delivery mode, and family history of allergy.
aOR, adjusted odds ratio; CI, confidence interval; ETS, environmental tobacco smoke; $PM_{2.5}$, fine particulate matter; *GSTP1*, glutathione S-transferase-pi 1; LRTIs, lower respiratory tract infections; URTIs, upper respiratory tract infections.

Nrf2 GG genotypes (aOR=8.18, 95% CI: 1.53, 43.72; aOR=7.37, 95% CI: 1.12, 48.66; and aOR=23.69, 95% CI: 2.13, 263.07, respectively). Such gene–environment interactions were not observed for URTIs (Tables 3-5).

Relationship between DNA methylation patterns and prenatal indoor $PM_{2.5}$/ETS exposure

Prenatal indoor $PM_{2.5}$/ETS exposure was associated specifically with 15 CpG sites, 6 of which were located in intergenic regions. Of the remaining 9 CpG sites, 5 were hypomethylated and 4 were hypermethylated by $PM_{2.5}$/ETS exposure. This analysis is described in the Supplemental Material.

DISCUSSION

The present study showed that indoor $PM_{2.5}$ and ETS exposure may have an effect on RTIs in infants and revealed that the adverse effect may depend on the timing of the exposure. The

Table 5. Influence of the *nuclear factor erythroid 2-related factor* (*Nrf2*) (rs6726395) on the results of prenatal exposure to both $PM_{2.5}$ and ETS

	Nrf2 GG			*Nrf2* GA+AA			*Nrf2* GG			*Nrf2* GA+AA		
	LRTIs			LRTIs			URTIs			URTIs		
	No (n=98)	Yes (n=22)		No (n=153)	Yes (n=27)		No (n=36)	Yes (n=87)		No (n=37)	Yes (n=150)	
	n	*n*	aOR* (95% CI)	*n*	*n*	aOR* (95% CI)	*n*	*n*	aOR* (95% CI)	*n*	*n*	aOR* (95% CI)
$PM_{2.5}$ (Low)	31	2	1.00	32	3	1.00	9	24	1.00	7	29	1.00
ETS (No)												
$PM_{2.5}$ (Low)	26	10	9.66	57	9	1.19	9	27	1.20	15	54	1.09
ETS (Yes)			(0.99, 94.03)			(0.27, 5.18)			(0.36, 3.94)			(0.34, 3.53)
$PM_{2.5}$ (High)	20	2	4.27	21	4	1.19	9	15	0.66	4	22	1.65
ETS (No)			(0.31, 58.04)			(0.19, 7.31)			(0.19, 2.29)			(0.36, 7.52)
$PM_{2.5}$ (High)	21	8	23.69	43	11	2.82	9	21	0.81	11	45	1.75
ETS (Yes)			(2.13, 263.07)			(0.65, 12.31)			(0.23, 2.94)			(0.48, 6.42)

*Odds ratios were adjusted for maternal age, maternal body mass index, maternal educational state, infant sex, gestational age, delivery mode, and family history of allergy.

aOR, adjusted odds ratio; CI, confidence interval; ETS, environmental tobacco smoke; $PM_{2.5}$, fine particulate matter; Nrf2, nuclear factor erythroid 2-related factor; LRTIs, lower respiratory tract infections; URTIs, upper respiratory tract infections.

ability of indoor $PM_{2.5}$ and/or ETS to increase susceptibility to LRTIs appeared to be stronger when the exposure occurred during the prenatal period rather than the postnatal period. This study also showed that the genetic polymorphisms *GSTM1*, *GSTP1* (rs1695), and/or *Nrf2* (rs6726395) were further associated with the increased susceptibility of LRTIs in indoor $PM_{2.5}$/ETS-exposed infants. Thus, the susceptibility of LRTIs in infancy may be shaped by gene-environment interactions between ROS-related genes and prenatal indoor $PM_{2.5}$/ETS exposure. To our knowledge, this is the first study to evaluate the association between combined exposure to indoor $PM_{2.5}$/ETS and infants' susceptibility to LRTIs that is associated with exposure time and genetic susceptibility.

Most people spend as much as 90% of their time indoors, especially pregnant women and infants. Chronic exposure to indoor pollutants at home or school can increase air pollutant inhalation and significantly impact health.[26,27] The interaction between PM and ETS, the most important indoor air pollutants, modify their individual harmful effects on respiratory outcomes.[7,8] However, studies about the indoor PM concentration that would have an adverse health outcome and an interaction between PM and ETS, especially prenatal exposure, are scarce. Our study revealed the effect of indoor $PM_{2.5}$ even in the low concentration and the additive effect of indoor $PM_{2.5}$/ETS exposure during the prenatal period on the development of LRTIs in infancy.

The fetal period is critical for lung and immune development. Although some epidemiologic studies showed that prenatal PM or ETS exposure increases the risk of wheezing, asthma, and respiratory infections,[28-31] studies comparing the effect according to exposure time are limited. A few studies revealed that prenatal exposure has a stronger effect on respiratory outcomes than postnatal exposure.[32-34] We also found its stronger associations with combined exposures to indoor $PM_{2.5}$/ETS during the prenatal period than the postnatal period. However, this result must be interpreted with caution because it is difficult to clearly separate exposure periods.

Air pollutants and tobacco smoke exert their harmful effects on health by inducing oxidative stress in exposed cells and tissues.[18,19] Air pollutants can be directly absorbed to the fetal circulation and produce ROS, ultimately inducing inflammatory and oxidative stress responses in the fetal lung.[35] The fetus can also be affected indirectly by the oxidative stress and inflammatory cytokine production induced in the placenta by the pollutants.[36] Of particular interest in this regard are several intracellular antioxidant enzymes, including *GSTM1* and *GSTP1*, which defend the airway epithelium from damage caused by oxidants and inflammation. These enzymes are regulated by the transcription factor *Nrf2*, which translocates to the nucleus after oxidative stress induction.[37] These enzymes in respiratory disease pathogenesis after pollutant exposure is provided by results showing that children with the *GSTM1* null genotype are more likely to develop asthma and wheezing after prenatal ETS exposure than children with the *GSTM1* present genotype.[20] Similarly, our study showed that while both prenatal indoor $PM_{2.5}$ and ETS exposure greatly increased the incidence of LRTIs in infants, this effect was particularly marked in the infants with the *GSTM1* null, *GSTP1* (rs1695) AG or GG, or *Nrf2* (rs6726395) GG genotypes.

ROS reacts with lipids, proteins, and DNA, resulting in cell membrane damage, alteration of gene and protein expression,

and even cell death.[18,37] Secondary mediators generated by oxidant reactions with lipids, proteins, and other biomolecules contribute to the toxic effects of pollutants. Oxidative stress also induces MAP kinase and NF-κB activation, which may ultimately produce a variety of proinflammatory mediators. Proinflammatory mediators from the airway epithelium play a critical role in the pathogenesis of several pulmonary diseases. Previous experimental studies supported the association between air pollutants and oxidative stress by demonstrating that antioxidant pretreatment attenuates oxidative stress and airway epithelial cell injury induced by air pollutants.[38-40]

Prenatal exposure to environmental factors may affect disease susceptibility later in life by inducing epigenetic changes. A cross-sectional study of children under 18 years of age revealed that air pollutants and ETS both associate with significantly increased DNA methylation and decreased transcription of interferon gamma (IFN-γ) in T-effector cells and forkhead box transcription factor 3 (Foxp3) in T-regulatory cells.[41] Interestingly, *GSTM1* and *GSTP1* polymorphisms alter the ability of prenatal tobacco smoke exposure to induce global DNA methylation.[21] Although the sample size in our experiment was too small to make firm conclusions, our data suggest that the ability of prenatal indoor $PM_{2.5}$ and ETS exposure to promote LRTIs in infancy may be due to DNA methylation alterations; the 9 CpG sites whose methylation was significantly altered by $PM_{2.5}$/ETS exposure were in subjects with LRTIs (Table S2 and Fig. S1). Further studies on this issue are required.

This study has several limitations. First, it was not possible to clearly distinguish between the effects of prenatal and postnatal exposure or exposure that persisted during both the pre- and postnatal periods. Further studies on the effect of indoor PM and ETS exposure during specific prenatal and postnatal periods may help identify the mechanisms involved. Second, the RTIs and ETS data were derived from questionnaires, and the indoor $PM_{2.5}$ levels were measured only 1 day between 26 and 36 weeks of pregnancy and at 6 months of age. Therefore, an information bias could not be excluded. Although questionnaires may misclassify ETS exposure, previous studies have shown a fairly good correlation between self-reported ETS exposure and biomarkers of ETS exposure.[5,42-44] Future studies may gain greater sensitivity by using more objective and precise measures of RTIs, and smoke and indoor PM exposure. The third limitation is the relatively small study population, which is because the indoor $PM_{2.5}$ measurements started later in the COCOA study, and these data were thus only available for about one-third of the whole COCOA cohort. However, it is unlikely that the addition of indoor $PM_{2.5}$ measurements to the protocol introduced a selection bias because the study participants and non-participants did not differ significantly in terms of their characteristics. An increase in the sample size would be likely to lead to more consistent and significant data. The fourth limitation is that we only selected 1 polymorphism from each gene. However, these polymorphisms have been shown in several studies to contribute to asthma susceptibility.[20,45-47]

An important strength of our study is its prospective design: the indoor $PM_{2.5}$ and ETS exposure data and the data on many potential confounders were collected before the children were born. This is likely to have markedly reduced the study bias. An additional strength is that the $PM_{2.5}$ measurement was performed at home. This direct measurement of residential indoor $PM_{2.5}$ probably estimates the actual exposure levels more accurately than other indirect methods. Finally, we investigated genotypic data for *GSTM1*, *GSTP1*, and *Nrf2* to determine gene–environment interactions between both $PM_{2.5}$/ETS exposure and LRTIs. These results imply that air pollutant exposure should be reduced, especially in genetically susceptible infants, and support a mechanism for oxidative stress in inducing adverse respiratory outcomes by air pollutants.

It should be noted that there was an important difference between previous studies and ours, namely, that maternal ETS exposure was considered in our study. It was not possible to evaluate the effect of maternal active smoking because the active smoking rate of Korean women is low: only 11.4% of the COCOA cohort mothers had smoked before their pregnancy, of whom only 1 continued to smoke during pregnancy. Thus, maternal ETS exposure was more likely to be an important source of pollutant exposure in our cohort than maternal smoking.

CONCLUSIONS

Indoor $PM_{2.5}$ and ETS exposure increases susceptibility to LRTIs in infants. This effect was particularly marked when the exposure occurred in the prenatal period. Moreover, the effect was modified by ROS-related gene polymorphisms. Along with studies suggesting that acute LRTIs in early life is associated with a long-standing susceptibility to all forms of lung disease, including asthma,[15-17] our study highlights the importance of health intervention strategies that focus on the indoor environment in the prenatal period. Additional analyses of genetic and epigenetic variants may help individualize such strategies. Further studies of gene–environment interactions and epigenetic mechanisms that shape the effect of air pollutants on susceptibility to LRTIs and chronic lung diseases are warranted, along with studies assessing the association between LRTIs in infancy and the development of chronic lung diseases, such as asthma.

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