Supplemental Material

Methods

**Genotyping** The genotyping of the *Nrf2* (rs6726395) and *GSTPI* (rs1695) polymorphisms was conducted by using a TaqMan assay (ABI, Foster City, CA). The assay IDs were C_11722141_10, C_155538_1, and C_3237196_20, respectively. *GSTM1* copy number variation was measured by using real-time polymerase chain reaction (PCR) and the following primers and probes: *GSTM1* forward primer (5′-TGGACATTTC GGAGACCC ACC-3′), *GSTM1* reverse primer (5′-TCACAAATTC TGATTGATG CAGAT-3′), and probe (5′-Fam-ATGGACAACC ATATGCAAGC GGGCAT-Tamra-3′). Reactions were performed in triplicate, with RNase P as the reference gene. The final PCR volume was 5 µL, which contained 10 ng of genomic DNA and 2.5 µL TaqMan Universal PCR Master Mix, with 0.13 µL of 40X Assay Mix. The thermal cycle conditions were as follows: 50°C for 2 minutes to activate the uracil N-glycosylase and to prevent carry-over contamination, 95°C for 10 minutes to activate the DNA polymerase, followed by 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. All PCRs were performed by using 384-well plates and a 384-well Veriti Thermal Cycler (ABI). The endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System (ABI). Duplicate samples and negative controls were included to ensure genotyping accuracy. The genotyping success rate of all polymorphisms exceeded 99%.

**Subjects for genome-wide methylation array analysis** Nine cohort subjects were selected and their cord blood was subjected to genome-wide methylation analysis. Three of the subjects had not had LRTIs by the age of 12 months nor had been exposed to PM2.5 or ETS (designated as the controls). Another three had had LRTIs but had not been exposed to the indoor pollutants. The last three subjects had had LRTIs and were exposed to the indoor pollutants. A subject was considered to have been exposed to PM2.5 if the indoor PM2.5 levels measured between 26 and 36 weeks of pregnancy exceeded the mean log-transformed PM2.5 values of the study cohort. A subject was considered to have been exposed to ETS if the mother answered yes to the following question at 36 weeks gestation: “Have you been exposed to passive smoking during your current pregnancy?”

**Genome-wide methylation array** In total, 200 ng of bisulfite-converted DNA was applied per chip. The DNA was amplified, fragmented, precipitated, and resuspended with buffers from the methylation analysis kit. The resuspended DNA samples were denatured at 95°C for 20 minutes, after which they were placed in a humidified container for a minimum of 16 hours at 48°C to allow the CpG loci to hybridize to the 50-mer capture probes. After hybridization, the unhybridized and non-specifically hybridized DNA was washed away. Extension and staining were then performed by adding reagents to the Te-Flow chamber. After staining was complete, the slides were washed with low salt wash buffer, immediately coated with XC4, and then imaged on the Illumina BeadArray Reader, which is a two-color (543 nm/643 nm) confocal fluorescent scanner with 0.84-µm pixel resolution that is capable of exciting the fluorophores generated during signal amplification/staining of the allele-specific (one color) extension products on the BeadChips. The image intensities were extracted by using Illumina’s BeadScan software. The methylation status of each interrogated CpG site was represented as a β value on the basis of the fluorescent intensity ratio. The β value ranged from 0 (completely unmethylated) to 1 (completely methylated). A two-sided t test was used to assess the differences in methylation levels between two groups. The CpG loci that were differentially methylated between the two groups were selected when the delta β-value (|Δ|β) difference was > 0.20 and the P value was < 0.05. The genes bearing the different CpG loci were annotated by using the modified Fisher Exact Algorithm in DAVID. The CpG sites different between the controls and the pollutant-exposed infants with LRTIs were considered to be the result of the effects of PM2.5, ETS, and LRTIs on DNA methylation. The CpG sites different between the controls and the non-exposed infants with LRTIs were considered to be the result of LRTIs on DNA methylation. The CpG sites different between the non-exposed and exposed infants with LRTIs were considered to be the result of PM2.5 and ETS exposure on DNA methylation.

The following logic was used to identify the CpG site differences that were caused by PM2.5 and ETS exposure regardless of LRTIs: [(controls vs exposed infants with LRTIs) – (controls vs non-exposed infants with LRTIs)] ∩ (non-exposed infants with LRTIs vs exposed infants with LRTIs).

Results

**Relationship between DNA methylation patterns and prenatal indoor PM2.5/ETS exposure** Nine study subjects underwent DNA methylation analysis of their cord blood. Three had neither LRTIs nor prenatal PM2.5/ETS exposure. Another three had LRTIs but no prenatal PM2.5/ETS exposure. The remaining three had both LRTIs and prenatal PM2.5/ETS exposure. Heat maps show the differences among the three groups in terms of their DNA methylation patterns (Fig. S1 A-D). The Venn diagram shows the numbers of methylated CpG site differences among the three groups (Fig. S1 D). Thus, the subjects with both LRTIs and PM2.5/ETS exposure differed from the controls in 760 CpG sites. The subjects with LRTIs but no PM2.5/ETS exposure differed from the controls in 101
CpG sites. The subjects with both LRTIs and PM$_{2.5}$/ETS exposure differed from the subjects with LRTIs but no PM$_{2.5}$/ETS exposure in 4,550 CpG sites.

A closer analysis revealed that PM$_{2.5}$/ETS exposure was specifically associated with 15 CpG sites (Fig. S1 D). However, six of these CpG sites were excluded because they were located in intergenic regions. Table S2 summarizes the remaining nine CpG sites. Five were hypomethylated and four were hypermethylated by PM$_{2.5}$/ETS exposure.