Prenatal Particulate Air Pollution and DNA Methylation in Newborns: An Epigenome-Wide Meta-Analysis

Olena Gruzieva,1,2 Cheng-Jian Xu,3,4,5 Paul Yousefi,6,7 Caroline Relton,6,7 Simon Kebede Merid,1 Carrie V. Breton,8 Lu Gao,8 Heather E. Volk,9,10 Jason I. Feinberg,9 Christine Ladd-Acosta,11 Kelly Bakulski,11 Charles Auffray,12 Nathanaël Lemonnier,12,13 Michelle Plaisun,14,15 Akram Ghantous,16 Zdenko Herceg,16 Tim S. Nawrot,14,17 Costanza Pizzì,18 Lorenzo Richardi,18 Franca Rusconi,19 Paolo VINUEs,15 Manolis Kogeorgas,20,22 Janine F. Félix,23-25 Liesbet Duijts,23,26 Herman T. den Dekker,23,25 Vincent W. V. Jaddoe,23,25 José L. Ruiz,27,28 Mariona Bustamante,20,22,27 Josep Maria Antó,20-22,29 Jordi Sunyer,20,22,29 Martine Vrijheid,20,22 Kristine B. Gutzkow,20 Regina Graziulevicienė,31 Carles Hernandez-Ferrer,32,33 Isabella Annesi-Maesano,34 Johanna Lepeule,34 Jean Bousquet,35,36 Anna Bergrstrom,1,2 Inger Kull,1,2,37,38 Cilla Söderhäll,39,40 Juha Kere,40-41 the Biobank-based Integrative Omics Studies (BIOS) Consortium,42 Ulrike Gehring,43 Bert Brunekreef,44,45 Allan C. Just,46 Rosalind J. Wright,47 Cheng Peng,48 Diane R. Gold,48,49 Iai Kloo,50 Dawn L. DeMeo,49 Gérard Pershagen,1,12 Gerard H. Koppelman,2,4,6 Stephanie J. London,4,6 Andrea A. Baccarelli,53,4 and Erik Melén1,2,8

1Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden
2Centre for Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden
3Groningen Research Institute for Asthma and COPD, University Medical Center Groningen, University of Groningen, Netherlands
4Department of Pediatric Pulmonology and Pediatric Allergology, University Medical Center Groningen, Beatrix Children’s Hospital, University of Groningen, Netherlands
5Department of Genetics, University Medical Center Groningen, University of Groningen, Netherlands
6MRC Integrative Epidemiology Unit, University of Bristol, Bristol, UK
7Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK
8Department of Preventive Medicine, University of Southern California Los Angeles, Los Angeles, California, USA
9Department of Mental Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA
10Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA
11Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan, USA
12European Institute for Systems Biology and Medicine (EISBM), CNRS-ENS-UCLL, Université de Lyon, Lyon, France
13Institute for Advanced Biosciences, UGA-Institut national de la santé et de la recherché médicale (Inserm), La Tronche, France
14Centre for Environmental Sciences, Hasselt University, Diepenbeek, Belgium
15MRC-PHE Centre for Environment and Health, School of Public Health, Imperial College London, London, UK
16Epigenetics Group, International Agency for Research on Cancer, Lyon, France
17Department of Public Health & Primary Care, Leuven University, Leuven, Belgium
18Cancer Epidemiology Unit, Department of Medical Sciences, University of Turin and CPO-Piemonte, Turin, Italy
19Unit of Epidemiology, Meyer Children’s University Hospital, Florence, Italy
20Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain
21Universitat Pompeu Fabra (UPF), Barcelona, Spain
22CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
23Generation R Study Group, Erasmus MC (Medical Centre), University Medical Center Rotterdam, Rotterdam, Netherlands
24Department of Epidemiology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands
25Department of Pediatrics, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands
26Department of Pediatrics, Division of Respiratory Medicine and Allergology, and Neonatology, Erasmus MC, University Medical Center, Rotterdam, Netherlands
27Centre for Genomic Regulation (CRG), Barcelona, Spain
28Instituto de Parástulos y Biomedicina López-Neyra (IPBLN), Spanish National Research Council (CSIC), Armilla, Granada, Spain
29Hospital de Mar Medical Research Institute (IMIM), Barcelona, Spain
30Norwegian Institute of Public Health, Oslo, Norway
31Department of Environmental Sciences, Vytauto Didziojo University, Kaunas, Lithuania
32Computational Health Informatics Program, Boston Children’s Hospital, Boston, Massachusetts, USA
33Epidemiology of Allergic and Respiratory Diseases Department, IPLESP, Inserm and Sorbonne University Medical School Saint-Antoine, Paris, France
34Université Grenoble Alpes, Inserm, National Institute of Health & Medical Research, CNRS, IAB, Grenoble, France
35Innovation Partnership on Active and Healthy Ageing Reference Site, MACVIA-France (Contre les Maladies Chroniques pour un Vieillissement Actif en France), Montpellier, France
36U 1168, VIMA: Ageing and Chronic Diseases Epidemiological and Public Health Approaches, Inserm Villejuif, Université Versailles St-Quentin-en-Yvelines, Montigny le Bretonneux, France
37Department of Clinical Science and Education, Södersjukhuset, Karolinska Institutet, Stockholm, Sweden
38Sachs Children’s Hospital, Stockholm, Sweden
39Department of Women’s and Children’s Health, Karolinska Institutet, Stockholm, Sweden
40Department of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden
41Folkhälsan Institute of Genetics, and Molecular Neurology Research Program, University of Helsinki, Helsinki, Finland
42School of Basic and Medical Biosciences, University of Helsinki, Helsinki, Finland
43A list of members and affiliations appears in this article’s Online Supplement
44Institute for Risk Assessment Sciences, Utrecht University, Utrecht, Netherlands

*These authors contributed equally as senior authors.
Address correspondence to O. Gruzieva, MD, PhD, Institute of Environmental Medicine, Karolinska Institutet, Nobels väg 13, SE-17177 Stockholm, Sweden. Telephone:+46 8 52480022; Email: Olena.gruzieva@ki.se
Supplemental Material is available online (https://doi.org/10.1289/EHP4522).
The authors declare they have no actual or potential competing financial interests.
Received 28 September 2018; Revised 2 May 2019; Accepted 6 May 2019; Published 31 May 2019.

Note to readers with disabilities: EHP strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in EHP articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ephponline@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.
BACKGROUND: Prenatal exposure to air pollution has been associated with childhood respiratory disease and other adverse outcomes. Epigenetics is a suggested link between exposures and health outcomes.

OBJECTIVES: We aimed to investigate associations between prenatal exposure to particulate matter (PM) with diameter <10 (PM$_{10}$) or <2.5 μm (PM$_{2.5}$) and DNA methylation in newborns and children.

METHODS: We meta-analyzed associations between exposure to PM$_{10}$ ($n = 1,949$) and PM$_{2.5}$ ($n = 1,551$) at maternal home addresses during pregnancy and newborn DNA methylation assessed by Illumina Infinium HumanMethylation450K BeadChip in nine European and American studies, with replication in 688 independent newborns and look-up analyses in 2,118 older children. We used two approaches, one focusing on single cytosine-phosphate-guanine (CpG) sites and another on differentially methylated regions (DMRs). We also related PM exposures to blood mRNA expression.

RESULTS: Six CpGs were significantly associated [false discovery rate (FDR) <0.05] with prenatal PM$_{10}$ and 14 with PM$_{2.5}$ exposure. Two of the PM$_{10}$-related CpGs mapped to FAM13A (cg09005156) and NOTCH4 (cg06849931) previously associated with lung function and asthma. Although these associations did not replicate in the smaller newborn sample, both CpGs were significant ($p < 0.05$) in 7- to 9-y-olds. For cg06849931, however, the direction of the association was inconsistent. Concurrent PM$_{10}$ exposure was associated with a significantly higher NOTCH4 expression at age 16 y. We also identified several DMRs associated with either prenatal PM$_{10}$ or PM$_{2.5}$ exposure, of which two PM$_{10}$-related DMRs, including H19 and MARCH11, replicated in newborns.

CONCLUSIONS: Several differentially methylated CpGs and DMRs associated with prenatal PM exposure were identified in newborns, with annotation to genes previously implicated in lung-related outcomes. https://doi.org/10.1289/EHP4522

Introduction

Many studies have reported adverse health effects of prenatal air pollution exposure in children, including adverse pregnancy outcomes, reduced lung growth, and increased risks of respiratory morbidity (Lamichhane et al. 2015; Korten et al. 2017; Horne et al. 2018). Findings from experimental models suggest that oxidative stress, inflammation, and mitochondrial dysfunction may contribute to health effects of particulate exposure, but our understanding of the involved mechanisms remains limited (Cassee et al. 2013; Niranjan and Thakur 2017). Recent studies demonstrate that environmental exposures may induce epigenetic modifications and that these changes can have long-lasting effects on gene expression and cell function (Desai et al. 2017; Gref et al. 2017). DNA methylation, the most studied epigenetic mechanism, entails cytosine modification with a methyl group at positions in DNA where a cytosine is located next to a guanine, a cytosine-phosphate-guanine (CpG) site. The crucial role of methylation in maintaining genomic stability and regulation of gene function makes it a potential mechanism by which environmental exposures contribute to the etiology of complex diseases.

Prenatal life is an important window of susceptibility to adverse effects of environmental hazards. In utero exposures may lead to epigenetic changes that influence fetal development and contribute to health outcomes throughout the life course (Barouki et al. 2018). Studies on prenatal exposures to cigarette smoke and traffic-related air pollution reported associations with modifications of the offspring epigenome (Joubert et al. 2016; Gruzieva et al. 2017). The majority of published studies investigated variability of DNA methylation in relation to air pollution either globally (i.e., overall methylation state of the genome) (Plusquin et al. 2017) or applying candidate-gene approaches (Somineni et al. 2016; Hew et al. 2015), but comprehensive evaluations of genome-wide DNA methylation patterns in children are limited (Breton et al. 2016; Gruzieva et al. 2017; Plusquin et al. 2018).

Epigenome-wide association studies (EWAS) of particulate air pollution exposure have so far been based almost exclusively on adult populations with inconclusive results. Epigenome-wide association studies of short-term exposure to particulate matter (PM) with an aerodynamic diameter of <2.5 μm (PM$_{2.5}$) reported associations with DNA methylation within genes involved in protein kinase and NFkB pathways (Jiang et al. 2014), as well as oxidative stress (Panni et al. 2016), although no robust associations could be demonstrated with long-term particulate exposure (Plusquin et al. 2017). We have previously found epigenome-wide cord blood DNA methylation differences in several mitochondria-related genes in relation to prenatal exposure to nitrogen dioxide, a marker of traffic-derived combustion pollutants (Gruzieva et al. 2017).

Earlier studies have focused on individual differentially methylated CpGs rather than differentially methylated regions (DMRs) (Breton et al. 2016; Gruzieva et al. 2017; Panni et al. 2016). DMR analysis is a statistically more powerful approach for detecting associations with exposures or health outcomes, as it uses the patterns of correlation between nearby CpGs to take advantage of the epigenomic structure (Pedersen et al. 2012; Peters et al. 2015). For the present study, we meta-analyzed genome-wide DNA methylation data in newborns in relation to maternal exposure to PM during pregnancy to identify both individual CpGs and regions of differential methylation. Furthermore, the associations found between maternal exposure to PM and cord blood DNA methylation were examined in independent data sets of newborn and older children. We also examined differences in peripheral blood gene expression for identified genes in relation to prenatal [in newborns from the Early Autism Risk Longitudinal Investigation (EARLI) cohort, $n = 119$] and current air pollution exposure [in 16-y-olds from the Barn, Allergi, Miljö, Stockholm och Epidemiologi (BAMSE) cohort in Sweden (titled Children, Allergy, Milieu, Stockholm, Epidemiology in English), $n = 244$].

Methods

Detailed information about each of the study cohorts in this analysis, including recruitment and eligibility; information about methods for measuring DNA methylation and gene expression, including quality control and normalization procedures; and detailed information about air pollution exposure estimation, are provided in Supplemental
Material. Average concentrations of PM$_{10}$ and PM$_{2.5}$ throughout pregnancy were estimated at maternal home addresses through land-use regression (LUR) or equivalent models.

**Discovery Study Population**
A total of nine European and American studies participating in the Pregnancy and Childhood Epigenetics consortium (PACE) (Felix et al. 2017) were included in the discovery meta-analysis of particulate air pollution exposure during pregnancy and newborn DNA methylation (total $N = 2,411$): INFancia y Medio Ambiente (INMA), Generation R, Southern California Children's Health Study (CHS), Early Autism Risk Longitudinal Investigation (EARLI), the PRogramming of Intergenerational Stress Mechanisms (PRISM), Project Viva, Environmental Influences on Early Ageing (ENVIRONAGE), Rhea Mother and Child Cohort in Crete, Greece (Rhea), and Piccolipiù (Table 1).

**Replication and Look-Up Study Populations**
We performed a replication analysis of the PM$_{10}$-related FDR-significant findings in a separate sample of newborns ($n = 688$) from the ALSpac project (Belton et al. 2015). A look-up association analysis of the newborn findings at older ages was based on three independent samples of 7- to 9-y-olds: a) Mechanisms of the Development of Allergy (MeDALL) comprising a pooled sample from two cohorts with uniform methylation measurements: BAMSE (Sweden) and Prevention and Incidence of Asthma and Mite Allergy (PIAMA; Netherlands), combined with an independent sample from the BAMSE cohort, BAMSE Epigene (total $N = 692$) (Xu et al. 2018); b) Human Early Life Exposome (HELIX), a pooled sample from four cohorts (total $N = 525$) (Vrijheid et al. 2014); Norwegian Mother and Child Cohort (MoBa), Etude de cohorte généraliste, menée en France sur les Déterminants pré et post naux précoce du développement psychomoteur et de la santé de l’enfant (EDEN), Kaunas Cohort, Lithuania (KAUNAS), and Born in Bradford (BiB), Bradford, UK; c) Avon Longitudinal Study of Parents and Children (ALSPAC), UK ($n = 901$); as well as on two samples of 15- to 16-y-olds: BAMSE ($n = 198$) and ALSpac ($n = 903$). Consent for blood sampling was obtained from all parents. Ethical approval for each study was granted by local institutional review boards.

**Statistical Analyses**
**Cohort-Specific Analyses.** For the cohort-specific analyses untransformed normalized methylation, beta values ($\beta$-values) were used. The $\beta$-value is a continuous variable ranging between 0 and 1, representing the ratio of the intensity of the methylated-probe signal to the total locus signal intensity. A $\beta$-value of 0 corresponds to no methylation, and a value of 1 corresponds to 100% methylation at the specific CpG site measured. All included samples were analyzed on a cohort level, except the pooled HELIX study and the pooled MeDALL study with coordinated methylation measurements, as well as air pollution exposure assessment according to a harmonized protocol.

First, we examined the associations between exposure to PM and methylation levels across the genome in each cohort separately using multiple robust linear regression [rml in the In functional analysis of expression data R package (version 3.3.2; R Core Team)] to account for potential outliers and heteroscedasticity in the data (Fox and Weisberg 2011). All analyses were adjusted for an a priori selected panel of covariates: child’s sex, maternal smoking ever during pregnancy (yes/no), cohort-specific batch indicator (s), and ancestry (in CHS). In addition, age at biosampling, municipality at birth (in BAMSE), and cohort indicator (in the pooled

---

**Table 1. Basic characteristics of cohorts included in the discovery EWAS meta-analysis.**

<table>
<thead>
<tr>
<th>STUDY</th>
<th>Country</th>
<th>Year</th>
<th>Total N</th>
<th>Enrolled</th>
<th>Permanent</th>
<th>Cohort-specific analyses</th>
<th>Air pollution exposure assessment</th>
<th>Total DNA methylation measurement</th>
<th>Study website</th>
</tr>
</thead>
<tbody>
<tr>
<td>INMA</td>
<td>Spain</td>
<td>1997-2008</td>
<td>3768</td>
<td>3120</td>
<td>available DNA from one of the subcohorts</td>
<td>LUR Illumina 450K</td>
<td>660619</td>
<td><a href="http://www.proyectoinma.org/">http://www.proyectoinma.org/</a></td>
<td></td>
</tr>
<tr>
<td>Generation R</td>
<td>Netherlands</td>
<td>2002-2006</td>
<td>9001</td>
<td>8500</td>
<td>Europeans, complete follow-up</td>
<td>LUR Illumina 450K</td>
<td>21471022</td>
<td><a href="http://www.generationr.nl">http://www.generationr.nl</a></td>
<td></td>
</tr>
<tr>
<td>EARLI</td>
<td>USA</td>
<td>2009-2012</td>
<td>232</td>
<td>232</td>
<td>NA</td>
<td>used inverse distance-squared weighting hybrid land use regression and satellite-based model</td>
<td>Illumina 450K</td>
<td>22956874</td>
<td></td>
</tr>
<tr>
<td>PRISM</td>
<td>USA</td>
<td>2012-2013</td>
<td>972</td>
<td>972</td>
<td>Random sample</td>
<td>Illumina 450K</td>
<td>24476840, 25328335</td>
<td><a href="http://www.earlistudy.org/">http://www.earlistudy.org/</a></td>
<td></td>
</tr>
<tr>
<td>Project Viva</td>
<td>USA</td>
<td>1999-2003</td>
<td>2128</td>
<td>2128</td>
<td>Available cord blood or early/mid-childhood blood sample</td>
<td>LUR Illumina 450K</td>
<td>24506846, 24506846</td>
<td><a href="http://www.piccolipiu.it">www.piccolipiu.it</a></td>
<td></td>
</tr>
<tr>
<td>ENVIRONAGE</td>
<td>Belgium</td>
<td>2010-2016</td>
<td>1210</td>
<td>1210</td>
<td>Random sample</td>
<td>Illumina 450K</td>
<td>23742113, 244506846</td>
<td><a href="http://www.limburgsgeboortecohort.be">www.limburgsgeboortecohort.be</a></td>
<td></td>
</tr>
<tr>
<td>Piccolipiù</td>
<td>Italy</td>
<td>2011</td>
<td>3338</td>
<td>3338</td>
<td>Participants resident in the municipality of Turin with enough stored biological material and 24-month follow-up data</td>
<td>Illumina 450K</td>
<td>24506846</td>
<td><a href="http://www.piccolipiu.it">www.piccolipiu.it</a></td>
<td></td>
</tr>
<tr>
<td>Rhea</td>
<td>Greece</td>
<td>2007-2008</td>
<td>1590</td>
<td>1590</td>
<td>Random sample</td>
<td>Illumina 450K</td>
<td>19711328</td>
<td><a href="http://www.piccolipiu.it">www.piccolipiu.it</a></td>
<td></td>
</tr>
</tbody>
</table>
MeDALL and HELIX sample sets) were included in the analyses of the older children. To account for potential differences in DNA methylation that may arise from variability of cell composition in whole blood (Reinius et al. 2012), we estimated cell type composition in cord blood using a reference panel of cells isolated from cord blood (leukocytes and nucleated red blood cells) (Bakulski et al. 2016), and in the older children using an adult reference panel (Reinius et al. 2012), applying the estimateCellCounts function in the minfi Bioconductor package in R (Jaffe and Irizarry 2014). We adjusted for cell composition by including these estimated cell type fractions as covariates in the multivariable linear regression.

Air pollution concentrations were entered as continuous variables without transformation. The results are presented as difference in methylation β-value per increase in average interquartile range (IQR) of PM₁₀ and PM₂.₅ exposure levels across the cohorts corresponding to 5.6 and 2.0 µg/m³, respectively.

**Meta-Analyses.** A total of 473,723 and 473,680 CpGs were included in the meta-analysis of PM₁₀ and PM₂.₅ results, respectively, after quality control filtering, as well as exclusion of probes that mapped to the X (n = 11,232) or Y (n = 4,16) chromosomes. Cohort-specific results of the cord blood EWAS were subsequently meta-analyzed using fixed-effects inverse variance weighting in version 2011-03-25, METAL (http://www.sph.umich.edu/csg/abecasis/metal/) (Willer et al. 2010). We used the False discovery rate (FDR, p < 0.05 for significance) procedure to account for multiple testing (Strimmer 2008). For replication and look-up analyses, a nominal p < 0.05 was considered statistically significant. DNA methylation sites were annotated based on data provided by Illumina (Bibikova et al. 2011).

**DMR Analyses.** Differentially methylated regions were identified using two methods available for use with meta-analysis results, comb-p (version 0.3.2), which identifies DMRs by regional clustering of low p-values from irregularly spaced p-values (Pedersen et al. 2012) and DMRcate (version 1.8.6; https://www.documentation.org/packages/DMRcate), that identifies DMRs from tunable kernel smoothing process of association signals (Peters et al. 2015). Input files for both DMR analyses were our meta-analyzed single-CpG EWAS results on newborns: regression coefficients, standard deviations, uncorrected p-values for DMRcate and uncorrected p-values and chromosomal locations for comb-p. Significant DMRs were defined based on the following criteria: a) a DMR should contain more than one probe; b) regional information can be combined from probes within 1,000 bp; c) the region showed multiple-testing corrected p < 0.01 in both methods (Sidak for comb-p and FDR for DMRcate). DMRs detected by both methods were considered significant in our analysis. Input parameters used for the DMR calling in both algorithms are provided in Table S1.

**Functional Follow-Up**

We investigated whether genes annotated to the significant CpGs were differentially expressed in cord blood in relation to air pollution exposure during pregnancy in the EARLI (n = 119) or at the time of biosampling in the BAMSE cohort (n = 244) by means of linear regression analysis. Furthermore, we analyzed the association of the FDR-significant CpG methylation with gene expression in cis (250 kb window) in 3,075 adults in the Biobank-based Integrative Omics Studies (BIOS) consortium data set (Bonder et al. 2017), and used FDR correction as threshold.

To identify associations between methylation levels and the expression levels of nearby genes (cis-expression quantitative trait methylation, cis-eQTM), we regressed methylation M-value on gene expression, sex, sampling age, lymphocytes percentage, monocyte percentage, and RNA Flow Cell Number. The inflation of models is corrected by using "bacon" method (van Ierssen et al. 2017). We mapped the eQTM in a window of 250 kb around the identified 5,547 CpG sites. For this analysis, we used a total of 3,075 samples for which both methylation and gene expression data were available from four cohorts: Lifelines DEEP, Rotterdam, Leiden Longevity, and Netherlands Twin Register (NTR).

To identify plausible pathways associated with air pollution exposure, we performed the over-representation analysis based on CpGs significantly associated with prenatal PM exposure in the meta-analysis at an arbitrary cutoff of p < 10⁻⁵ using ConsensusPathDB (Kamburov et al. 2013), as well as the R Bioconductor package missMethyl (version 1.10.0 gemeth function), which performs one-sided hypergeometric tests taking into account and correcting for any bias derived from the use of differing numbers of probes per gene interrogated by the array (Phipson et al. 2016).

Finally, we investigated whether previously reported differentially methylated CpGs related to in utero tobacco smoke exposure [6,073 CpGs with FDR-significance (Joubert et al. 2016)] were differentially methylated in relation to prenatal PM exposure. We performed Fisher’s exact test for overrepresentation of smoking-related CpGs among nominally significant PM-related CpGs.

We additionally examined whether our FDR-significant CpGs overlapped with the list of potentially polymorphic and cross-reactive probes provided by Chen et al. (Chen et al. 2013), and applied the dip test (Hartigan and Hartigan 1985) for two overlapping CpGs to test for non-unimodal DNA methylation distribution using an independent publicly available data set of cord blood DNA methylation samples (Barrett et al. 2013; Rojas et al. 2015).

### Results

The baseline characteristics of the study populations are presented in Table 1 (and Table S2 in the online data supplement). Exposure contrasts were smallest for the PRISM (PM₂.₅ IQR 0.8 µg/m³ and RHEA (PM₁₀ IQR 2.3 µg/m³) cohorts and were highest for the CHS (PM₂.₅ IQR 5.0 and PM₁₀ IQR 14 µg/m³). The discovery meta-analysis of cord blood methylation in relation to prenatal exposure included 1,949 newborns for PM₁₀ and 1,551 for PM₂.₅. The difference in sample sizes is due to missing prenatal PM₁₀ data for Project Viva and PRISM cohorts, and missing prenatal PM₂.₅ data for the Generation R cohort. Minus log10(p-values) for the combined analysis of CpGs across the genome in cord blood samples are presented in Figure 1. The quantile–quantile plots did not reveal any noteworthy inflation in the distribution of observed p-values (λ = 1.21 for PM₁₀ exposure and 1.37 for PM₂.₅; Figure S1). Study-specific lambdas can be found in Table S3.

**Meta-Analyses Findings**

We found epigenome-wide significant associations (FDR p < 0.05) between PM₁₀ exposure and DNA methylation for six CpGs, with higher PM₁₀ exposure being associated with an increase in methylation for four CpGs mapping to TNFRSF1A, SNORD96A, FAM13A, SRPRB, and P4HA2, and a decrease for two CpGs within USP4 and NOTCH4 (Table 2). Effect sizes were generally small, i.e., 0.1% difference in methylation β-value per IQR = 5.6 µg/m³ increase in prenatal PM₁₀ exposure.

We found 14 CpGs significantly associated with prenatal PM₂.₅ using FDR correction, positioned in or near the following genes: PLXNA4, ZNF705A, 2.5 kb downstream of C14orf2, FN1P1, COL22A1, TMCO3, SFRS8, 8.1 kb upstream of NEUROG1, MR1, PGS5, C7orf50, 1.1 kb downstream of MORN1, PLAT, and ZNF695 (Table 3). The direction of the effect was negative for 11 of these CpGs, and positive for cg16253537 in FN1P1, cg010119943 in PGS5, and cg00348551 in C7orf50 in relation to higher PM₂.₅ exposure. The estimates ranged from −0.4% to 0.3% difference in
methylation level per IQR (IQR = 2 µg/m³) increase in prenatal PM$_{2.5}$ exposure.

Two out of the 14 FDR-significant CpGs associated with prenatal PM$_{2.5}$, namely cg12193649 and cg01011943, overlapped with the list of potentially polymorphic and cross-reactive probes provided by Chen et al. (2013). However, results from the dip test applied to those two CpGs did not reveal statistically significant deviation from unimodality ($p = 0.65$ and $p = 0.99$, respectively).

Tests for heterogeneity did not display any major heterogeneity across studies: 8% and 9.9% of the examined PM$_{10}$- and PM$_{2.5}$-related CpGs, respectively, had heterogeneity $p < 0.05$, and median $I^2$ statistics for PM$_{10}$ was 0% (ranging between 0–94%) and for PM$_{2.5}$ 5.1% (ranging between 0–88.7%). No significant heterogeneity was found for any of the identified FDR-significant CpGs ($p$-values for heterogeneity ranging within 0.08–0.81; see forest plots in Figure S2).

Analyses of Differentially Methylated Regions

By applying two different methods for DMR analysis of PM$_{10}$-related results, we identified 147 significant (FDR $p < 0.01$) DMRs from DMRcate (Table S1) and 12 significant (Sidak $p < 0.01$) DMRs from comb-p (Table S2), including 11 that were significant based on both approaches (Table 4). It is interesting to note that all genome-wide significant individual CpGs identified in the discovery meta-analysis were also found within the 147 DMRs found in DMRcate, with the exception of cg06849931 located in NOTCH4.

We also found 272 significant (FDR $p < 0.01$) DMRs from DMRcate (Table S3) and 33 significant (Sidak $p < 0.01$) DMRs from comb-p (Table S4) in relation to prenatal PM$_{2.5}$ exposure, of which 15 overlapped between the two methods (Table S4). Five out of 14 genome-wide significant individual CpGs identified in the discovery meta-analysis were also seen in the DMRs, namely related to genes C7orf50, ZNF705A, PLAT, PSG5, and MRI1.

Replication and Look-Up Analyses

None of the six FDR-significant CpGs identified as differentially methylated in relation to prenatal PM$_{10}$ in our discovery meta-analysis sample of 1,949 newborns could be replicated in the 688 newborns of the ALSPAC study (Table 2). However, four out of
these six CpGs showed significance later in childhood in associations with prenatal PM10 exposure; cg06849931 (*NOTCH4*) showed increased methylation in relation to PM10 exposure during pregnancy in the combined BAMSE Epigenic and MeDALL samples \( (n = 692) \) of 7- to 9-y-olds \( (p = 0.03) \), although the direction of association for cg06849931 was opposite to the one in the discovery analysis (Table 2). Furthermore, cg06849931 was also differentially methylated in the HELIX study \( (p = 0.02) \), along with cg18640183 (*PHHA2*) \( (p = 0.03) \), both demonstrating the same direction of association as those in the discovery meta-analysis. In addition, cg15082635 (*GNB2L1; SNORD96A*) was also nominally significant in 7-to 9-y-olds from the ALSPAC study with the same direction of association \( (p = 0.02) \). None of these six associations was present in adolescents from the BAMSE \( (n = 198) \) and ALSPAC \( (n = 903) \) studies \( (p > 0.05) \). Children’s concurrent PM10 exposure at the time of biosampling was not significantly associated with any of these six CpGs \( (p > 0.05) \); see Table S5.

Among the 14 epigenome-wide significant PM2.5-associated CpGs in newborns, none appeared to be statistically significant in children and adolescents, apart from cg23270359 (*MRT1*), which was significant in the HELIX sample \( (p = 0.01) \), although the direction of association was opposite to that in the discovery meta-analysis (Table 3).

Two significant gene regions from the discovery PM10-related DMR analyses, including genes *H19* and *MARCH11*, were also FDR-significant in analysis of the ALSPAC newborn sample using DMRcate (replication min FDR \( p = 9.5 \times 10^{-4} \) and \( p = 3.9 \times 10^{-5} \), respectively).

### Functional Follow-Up

The top three PM10-related CpGs, including one within the *FAM13A* gene, as well as six out of 14 PM2.5-associated CpGs, were significantly associated with gene expression in cis in BIOS (Table S6).

In functional analysis of expression data from the newborns in the EARLI cohort \( (n = 119) \), no significant association of *in utero* PM10 exposure with expression of genes annotated to the respective CpG was detected, whereas PM2.5 exposure was associated with expression of *ZNF695* \( (p < 0.05, \text{Log} \text{fold change} = \text{LogFC} = 0.074 \text{ per } 2\,\mu\text{g/m}^3 \text{ increase in exposure}; \text{Table 5}) \). In BAMSE \( (n = 244) \), current PM10 exposure at 16 y was associated with *NOTCH4* (multiple transcripts, lowest \( p = 0.0001, \text{LogFC} = 0.05 \) and *USP43* expression levels in peripheral blood cells \( (p < 0.05, \text{LogFC} = 0.05, \text{ per } 5.6\,\mu\text{g/m}^3 \text{ increase}; \text{Table 6}) \). Among the PM2.5 associated genes, *C7orf50* was significantly differentially expressed in relation to current PM2.5 exposure \( (p = 0.03, \text{LogFC} = 0.02, \text{per } 2\,\mu\text{g/m}^3 \text{ increase}) \). Descriptive statistics of expression levels of genes associated with CpG methylation in response to maternal PM10 or PM2.5 exposure in the EARLI and BAMSE cohorts are provided in Table S7 and Table S8, respectively.

### Pathway Analysis

Twenty-eight of 31 unique gene identifiers extracted from the meta-analysis with PM10 exposure matched to ConsensusPathDB. Using FDR \( p < 0.05 \), six enriched pathways were identified including “Notch Signaling Pathway” (genes *NOTCH4* and *DVL2*), “Rho GTPase cycle” (*FAM13A; HMHA1; VAV2; and GMIP*), “Neurotransmitter Release Cycle” (*HSPA8; and *RIMS1*), and “GABA synthesis, release, reuptake and degradation” (*HSPA8; and *RIMS1*). In the repeated pathway analysis using gometh function in missMethyl, no statistically significant pathways were found after correction for multiple testing; however, we observed the same top significant pathways as identified by ConsensusPathDB, i.e., related to

---

**Table 2.** Statistically significant CpGs (*FDR p < 0.05*) associated with IQR increases in prenatal PM10 (5.6μg/m³) exposure in newborns (discovery meta-analysis), and replication analyses in newborns, children (age 7-9 years) and adolescents (age 15-16 years). Discovery: newborns BAMSE EpiGene + MeDALL HELIX BAMSE 16 years ALSPAC 15 years ALSPAC \( n = 692 \) ALSPAC \( n = 1,949 \) ALSPAC \( n = 903 \) \n
| Chr | Position | Gene | Direction | \( \beta \) (p-value) \n|-----|----------|------|-----------|----------------|
| 5   | 1106707010 | cg15082635 | *NOTCH4* | \( \beta \) (p-value) | \( \beta \) (p-value) |
| 7   | 9559558 | cg02304016 | *HSPA8* | \( \beta \) (p-value) | \( \beta \) (p-value) |
| 10  | 121659310 | cg06849931 | *NOTCH4* | \( \beta \) (p-value) | \( \beta \) (p-value) |

Note: A change for each meta-analysis with an IQR increase in prenatal PM10 exposure and DNA methylation in newborns (discovery meta-analysis), and replication analyses in newborns, children (age 7-9 years) and adolescents (age 15-16 years).

Functional Follow-Up

The top three PM10-related CpGs, including one within the *FAM13A* gene, as well as six out of 14 PM2.5-associated CpGs, were significantly associated with gene expression in cis in BIOS (Table S6).

In functional analysis of expression data from the newborns in the EARLI cohort \( (n = 119) \), no significant association of *in utero* PM10 exposure with expression of genes annotated to the respective CpG was detected, whereas PM2.5 exposure was associated with expression of *ZNF695* \( (p < 0.05, \text{Log} \text{fold change} = \text{LogFC} = 0.074 \text{ per } 2\,\mu\text{g/m}^3 \text{ increase in exposure}; \text{Table 5}) \). In BAMSE \( (n = 244) \), current PM10 exposure at 16 y was associated with *NOTCH4* (multiple transcripts, lowest \( p = 0.0001, \text{LogFC} = 0.05 \) and *USP43* expression levels in peripheral blood cells \( (p < 0.05, \text{LogFC} = 0.05, \text{ per } 5.6\,\mu\text{g/m}^3 \text{ increase}; \text{Table 6}) \). Among the PM2.5 associated genes, *C7orf50* was significantly differentially expressed in relation to current PM2.5 exposure \( (p = 0.03, \text{LogFC} = 0.02, \text{per } 2\,\mu\text{g/m}^3 \text{ increase}) \). Descriptive statistics of expression levels of genes associated with CpG methylation in response to maternal PM10 or PM2.5 exposure in the EARLI and BAMSE cohorts are provided in Table S7 and Table S8, respectively.

Pathway Analysis

Twenty-eight of 31 unique gene identifiers extracted from the meta-analysis with PM10 exposure matched to ConsensusPathDB. Using FDR \( p < 0.05 \), six enriched pathways were identified including “Notch Signaling Pathway” (genes *NOTCH4* and *DVL2*), “Rho GTPase cycle” (*FAM13A; HMHA1; VAV2; and GMIP*), “Neurotransmitter Release Cycle” (*HSPA8; and *RIMS1*), and “GABA synthesis, release, reuptake and degradation” (*HSPA8; and *RIMS1*). In the repeated pathway analysis using gometh function in missMethyl, no statistically significant pathways were found after correction for multiple testing; however, we observed the same top significant pathways as identified by ConsensusPathDB, i.e., related to
regulation of GTPase activity (Table S9). No significantly enriched pathways were identified for PM$_{2.5}$.

**Candidate-Gene Analysis of Smoking-Related CpGs**

Out of 6,073 FDR-significant CpGs previously reported in relation to maternal smoking exposure (Joubert et al. 2016), 359 showed nominal significance ($p < 0.05$) with prenatal PM$_{10}$ and 390 with PM$_{2.5}$ exposure, which is not more than expected by chance (Fisher’s exact test nonsignificant for overrepresentation of smoking-related CpGs among nominally significant PM-related CpGs). None of the genome-wide significant CpGs identified in our meta-analyses with PM$_{10}$ and PM$_{2.5}$ were among the 6,073 smoking-related sites.

**Discussion**

In this large-scale epigenome-wide meta-analysis evaluating the association between prenatal particulate air pollution exposure and DNA methylation in newborns, we found significant associations for PM$_{10}$ and PM$_{2.5}$ exposure during pregnancy with methylation differences in several genes of relevance for respiratory health, such as $FAM13A$ and $NOTCH4$. Some of these associations were also seen in the older children. We also identified a number of unique DMRs associated with PM exposure by applying two independent methodologies. The observed differentially methylated genes in the newborn discovery data set represent novel associations in the context of air pollution exposure. One of the top significant hits, cg00905156, localizes in the gene $FAM13A$, which has been identified in multiple genome-wide association studies (GWAS) of pulmonary function and the related phenotype of COPD (Hobs et al. 2017; Hancock et al. 2010). Research has shown that $FAM13A$ interferes with the Wnt pathway, inducing $\beta$-catenin degradation, which in turn may affect lung repair (Jiang et al. 2016). In vitro studies have also demonstrated differences in respiratory epithelial cell expression of $FAM13A$ during differentiation into pulmonary type II cells (Wade et al. 2006).

Another significant CpG site, cg06849931, is located in the $NOTCH4$ gene, which has been identified in GWAS as a genetic marker of asthma-related traits (Li et al. 2013). Recently, an
animal study proposed Notch4 as a susceptibility gene for ozone-induced lung injury (Verhein et al. 2015). Genome-wide transcriptomic analysis of lung tissue homogenates within the same study suggested that upregulation of NOTCH3 and NOTCH4 receptors may protect against inflammation. Our other observed differentially methylated CpGs reside in USP43, SRPRB, GNB2L1, SNORD96A, and a Tgf2 cytokine gene, P4HA2, GNB2L1 and P4HA2 have previously been suggested as candidate genes associated with the susceptibility and prognosis for lung cancer (Choi et al. 2015; Dong et al. 2012).

We were not able to replicate FDR-significant CpGs using a smaller independent methylation data set of newborns. However, in two out of three independent samples of school-age children, cg06849931 (NOTCH4) was found to be significantly differentially methylated in relation to prenatal PM10. The direction of association in one of these two samples was opposite, however. Also, significant differential methylation was observed for CpGs in FAMIL3A, GNB2L1, and SNORD96A, as well as P4HA2 in one out of three independent samples of school-age children, with the same direction of association as those in the discovery EWAS. Furthermore, expression of the NOTCH4 gene in BAMSE participants at 16 years of age was increased in association with concurrent exposure to PM10. Lack of replication in newborns may be attributed to generally weak effects of air pollution exposure that may be difficult to detect in a smaller sample. Furthermore, differences in exposure contrasts should be acknowledged, i.e., wide exposure range in the discovery analysis explained by inclusion of cohorts from areas with different exposure levels in comparison with the replication data set.

We found several significantly differentially methylated CpGs in relation to prenatal PM2.5 exposure, all of which were distinct from those related to prenatal PM10. Unfortunately, no independent newborn data set with PM2.5 data was available for replication analysis. Look-up analysis in older children age 7–9 y suggested association of differential methylation of cg23270359 located in MIR11. Previous studies reported significant association between increased MIR11 methylation and severe asthma (Wysocki et al. 2015).

Earlier epigenome-wide association studies of long-term particulate exposure in adults failed to demonstrate robust associations (Plusquin et al. 2017; de FC Lichtenfels et al. 2018). This failure to demonstrate robust associations may be partly explained by limited statistical power. In addition to EWAS meta-analysis based on single probes, we also investigated regions of differential methylation. Several significant findings were discovered in relation to PM exposure, and the DMR results partly overlapped between the two methods applied, as well as with the genes identified in the single probe meta-analysis. Two of the PM10-related DMRs comprising H19 and MARCH11 genes were also replicated in the independent newborn data set. The H19 gene is located in an imprinted region of chromosome 11 and is expressed only from the maternally inherited chromosome. Recent evidence suggests that H19 functions as an oncogene and inhibits the activity of tumor suppressor p53 but also plays an important role in embryonic development and growth control (Chen et al. 2018). DNA methylation levels at the H19 DMR have also been associated with being small for gestational age (Qian et al. 2016).

Our study is one of the first large-scale studies assessing the association of prenatal PM exposure on the neonatal blood methylome. Recently published EWAS meta-analysis of five cohorts (n = 1,235) did not show any association of maternal PM10 exposure during pregnancy with DNA methylation in cord blood (Plusquin et al. 2018). However, pathway analysis of top hits revealed enriched pathways relating to the GABAergic synapse, as well as NOTCH signaling, which is in line with our results. Analysis based on the CHS demonstrated DNA methylation variability in newborn blood in relation to prenatal exposure to PM10.

### Table 5. Associations between PM exposure and gene expression levels in newborn children of the EARLI cohort (n = 119).

<table>
<thead>
<tr>
<th>Chr</th>
<th>Gene</th>
<th>ProbeID</th>
<th>LogFC</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>FAMIL3A</td>
<td>16977925</td>
<td>0.025</td>
<td>0.57</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>17017814</td>
<td>−0.003</td>
<td>0.94</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>17027038</td>
<td>−0.008</td>
<td>0.90</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>17029639</td>
<td>0.063</td>
<td>0.14</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>17034630</td>
<td>−0.015</td>
<td>0.63</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>17037128</td>
<td>0.018</td>
<td>0.51</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>17039839</td>
<td>−0.050</td>
<td>0.19</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>17042335</td>
<td>−0.029</td>
<td>0.38</td>
</tr>
<tr>
<td>5</td>
<td>SNORD96A</td>
<td>17119456</td>
<td>−0.036</td>
<td>0.19</td>
</tr>
<tr>
<td>5</td>
<td>P4HA2</td>
<td>16999712</td>
<td>−0.018</td>
<td>0.57</td>
</tr>
<tr>
<td>17</td>
<td>USP43</td>
<td>16831046</td>
<td>−0.034</td>
<td>0.26</td>
</tr>
<tr>
<td>3</td>
<td>SRPRB</td>
<td>16945907</td>
<td>−0.039</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Table: Results presented per 5.6 μg/m³ increase in PM2.5 and 2 μg/m³ increase in PM10 exposure for genes annotated to FDR significant CpGs in the discovery PM10 and PM2.5 EWAS. LogFC = logarithm fold-change (one unit of the logFCs translates to a two-fold change in expression). Adjusted for sex, maternal smoking during pregnancy, and cell composition.

### Table 6. Associations between PM exposure and gene expression levels in 16-y-old children of the BAMSE cohort (n = 244).

<table>
<thead>
<tr>
<th>Chr</th>
<th>Gene</th>
<th>ProbeID</th>
<th>LogFC</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>TC6_mcf_hap5000165 hg.1</td>
<td>0.05</td>
<td>9.52E-05</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>TC6_apd_hap1000098 hg.1</td>
<td>0.06</td>
<td>9.73E-05</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>TC6_mann4000155 hg.1</td>
<td>0.06</td>
<td>9.98E-05</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>TC6_cox_hap2000190 hg.1</td>
<td>0.05</td>
<td>1.03E-04</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>TC6_ssto_hap7000159 hg.1</td>
<td>0.05</td>
<td>1.10E-04</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>TC6001564 hg.1</td>
<td>0.05</td>
<td>1.31E-04</td>
</tr>
<tr>
<td>6</td>
<td>NOTCH4</td>
<td>TC6_gbl_hap6000179 hg.1</td>
<td>0.05</td>
<td>1.42E-04</td>
</tr>
<tr>
<td>17</td>
<td>USP43</td>
<td>TC70000146 hg.1</td>
<td>0.05</td>
<td>4.98E-04</td>
</tr>
<tr>
<td>4</td>
<td>FAMIL3A</td>
<td>TC0001380 hg.1</td>
<td>−0.01</td>
<td>2.14E-01</td>
</tr>
<tr>
<td>3</td>
<td>SRPRB</td>
<td>TC0300725 hg.1</td>
<td>−0.01</td>
<td>2.89E-01</td>
</tr>
</tbody>
</table>

Table: Results presented per 5.6 μg/m³ increase in PM2.5 and 2 μg/m³ increase in PM10 exposure for genes annotated to FDR significant CpGs in the discovery PM10 and PM2.5 EWAS. LogFC = logarithm fold-change (one unit of the logFCs translates to a two-fold change in expression). Adjusted for sex, maternal smoking during pregnancy, active smoking at the time of biosampling, age at biosampling, municipality at birth, doctor’s diagnosis of asthma, and cell composition.
and PM$_{2.5}$, some of which were also associated with cardiorespiratory health outcomes later in childhood, including asthma and elevated blood pressure later in childhood (Breton et al. 2016). We have also recently reported associations of NO$_2$ exposure during pregnancy with cord blood methylation differences in several genes involved in mitochondria function, and we noted that these associations with \textit{in utero} exposure persisted into early childhood (Gruzieva et al. 2017). We did not, however, observe the same associations with PM exposure in the present study. It remains to be investigated whether those associations we observed with NO$_2$ are pollutant-specific, or whether lack of overlap between NO$_2$ and PM-related findings are attributed to difference in the sources of particulate pollution in different cities and locations (Eeftens et al. 2012). This difference in sources and chemical composition of PM may also be responsible for the lack of comparability between the present results with PM$_{10}$ and PM$_{2.5}$ exposures.

Some previous EWASs have identified and replicated extensive exposure-associated epigenetic alterations, for example in relation to exposure to maternal tobacco smoke (Joubert et al. 2016). Not only is particulate air pollution a different type of exposure, but also exposure levels are generally much lower than those related to tobacco smoking, which may explain differences in the magnitude of differential methylation patterns associated with exposure. Furthermore, measurement error in assignment of exposure to maternal smoking during pregnancy is likely much lower than for air pollution. Identifying robust signals at single CpG site level for complex exposures such as long-term air pollution may also require larger sample sizes than available in the present study. In addition, all the study populations were from countries with relatively low ambient levels of particulate air pollution. Inclusion of populations with higher exposures may help identify possible effects on DNA methylation.

This study has some weaknesses. We estimated individual concentrations only for outdoor air pollution at residential addresses, which are not equivalent to personal exposure. Also, due to lack of trimester-specific prenatal exposure data, we were not able to explore the importance of exposure time windows during pregnancy. Participants likely travel to several locations throughout the day and may spend more time at locations other than their residential addresses (e.g., workplaces), which may introduce some misclassification, although most likely nondifferential and thus would generally tend to attenuate the associations. However, ambient PM$_{10}$ and PM$_{2.5}$ levels have been consistently associated with negative health effects in multiple studies, including effects on fetal and neonatal outcomes (Lamichhane et al. 2015). Our analyses included studies based in western Europe and the United States, which have relatively lower air pollution concentrations in comparison with many other places. We should also acknowledge that the study included mainly white populations, and generalizability to other ethnic groups is uncertain.

Although we adjusted our analyses for predefined important covariates, residual confounding cannot be ruled out. Another possible limitation is that we used estimated cell counts in our analyses because measured cell types or single-cell methylation data were not available in all cohorts. However, such estimated cell type adjustment has been shown to be appropriate in epidemiological settings (Kaushal et al. 2017).

Methylation signatures are tissue and cell specific (Bakulski and Fallin 2014), and therefore, selection of relevant tissues and cells is of crucial importance for epigenetic analyses. The majority of previous studies have used peripheral blood cells to examine DNA methylation patterns associated with environmental exposures; however, air pollution exposure has also been associated with DNA methylation and expression changes in placenta (Cai et al. 2017; Saenen et al. 2017; Abrahm et al. 2018), and lung epithelial cells (Clifford et al. 2017; Zhou et al. 2015). Clifford et al. reported differential methylation of CpG sites in $HOXA4$ in response to diesel exhaust following prior exposure to allergen (Clifford et al. 2017), which was also identified as DMR in our analysis with prenatal PM$_{10}$ exposure. $HOXA4$ belongs to the family of Hox genes encoding homeodomain transcription factors that determine cell and tissue identities in the developing embryo and patterning of the developing mouse lung (Packer et al. 2000).

In conclusion, our epigenome-wide meta-analysis provides suggestive evidence of newborn methylation differences in several genes with relevance for airway disease, in relation to prenatal particulate air pollution exposure. Some of these associations were also observed later in childhood. Our results also point to the importance of considering the combined effect of nearby CpGs as DMRs when evaluating the impact of exposure on DNA methylation. Further studies are warranted to establish whether this epigenetic variability could potentially explain the influence of ambient air pollution on development of respiratory outcomes.

Acknowledgments

ALSPAC: The UK Medical Research Council and the Wellcome Trust (Grant ref. 102215/2/13/2) and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors and P.Y. will serve as guarantors for the contents of this paper. A comprehensive list of grants funding is available on the ALSPAC website (http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf). This research was specifically funded by a joint grant from the UK Economic & Social and Biotechnology & Biological Sciences Research Councils (Grant ref. ES/N000498/1). ALSPAC was funded by the BBSRC (BB/I025751/1 and BB/I025263/1). Air pollution exposure assessment was funded by Public Health England as part of the MRC-PHE Centre for Environment and Health, funded also by the UK Medical Research Council (Grant ref. MR/L01341X/1). This paper does not necessarily reflect the views of Public Health England or the Department of Health.

BAMSE was supported by The Swedish Research Council, The Swedish Heart-Lung Foundation, Freemason Child House Foundation in Stockholm, MeDALL (Mechanisms of the Development of ALLergy) a collaborative project conducted within the European Union (grant agreement No. 261357), Centre for Allergy Research, Stockholm County Council (ALF), Swedish Foundation for Strategic Research (SSF) (RB08-0027), the Strategic Research Programme (SFO) in Epidemiology at Karolinska Institutet, The Swedish Research Council Formas, and the Swedish Environment Protection Agency. E.M. is supported by a grant from the European Research Council under the European Union (EU) Horizon 2020 (H2020) research and innovation programme (grant agreement number 757919, TRIBAL). O.G. is supported by Forte (Swedish Research Council for Health, Working Life and Welfare) and The Swedish Society for Medical Research.

CHS: This work was supported by NIEHS grants K01ES017801, R01ES022216, and P30ES007048.

EARLI: This work was supported by NIH grants R01ES016443, R01ES023780, and R01ES017646 as well as by Autism Speaks (AS 5938).

ENVIRONAGE: The ENVIRONAGE birth cohort is funded by the European Research Council (ERC-2012-StG-310898) and by funds of the Flemish Scientific Research Council (FWO, N1516112/G.0.873.11.N.10). The methylation assays were funded by the European Community's Seventh Framework Programme FP7/2007-2013 project EXPoOMICS (grant no. 308610). Z.H. is supported by the Exposomics EC FP7 grant (Grant agreement no. 308610). Z.H and A.G. and the Epigenetics Group at IARC are
supported by grants from the Institut National du Cancer (INCa, Plan Cancer-EVA-Inserm, France) and Association pour la Recherche sur le Cancer (ARC, France).

Generation R Study: The general design of the Generation R Study is made possible by financial support from the Erasmus Medical Center (MC), Rotterdam, the Erasmus University Rotterdam, Netherlands Organization for Health Research and Development and the Ministry of Health, Welfare and Sport. The EWAS data was funded by a grant to VWJ from Netherlands Genomics Initiative (NGI)/Netherlands Organisation for Scientific Research (NWO) Netherlands Consortium for Healthy Aging (NCHA; project no. 050-060-810), by funds from the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC. V.W.J. also received a grant from Netherlands Organization for Health Research and Development (VIDI 016.136.361) and a Consolidator Grant from the European Research Council (ERC-2014-CoG-648916). J.F.F. has received funding from the European Union’s Horizon 2020 Research and Innovation Programme under grant agreement no. 633959 (DynaHEALTH). This project received funding from the European Union’s Horizon 2020 Research and Innovation Programme (733206, LIFECYCLE).

HELIX: The research leading to these results has received funding from the European Community’s Seventh Framework Programme (FP7/2007-2009) under grant agreement no 308333 – the HELIX project. R.G. received the grant of the Lithuanian Agency for Science Innovation and Technology (No. 45 31V-66). The Norwegian Mother and Child Cohort Study (MoBa) is supported by the Ministry of Health and Care Services and the Ministry of Education and Research, NIH/NIEHS (contract no. N01-ES-75558), NIH/NINDS (grant no. 1 U01 NS 047537-01 and grant no. 2 U01 NS 047537-06A1).

INMA: This study was funded by grants from Instituto de Salud Carlos III (Red INMA G03/176), Generalitat de Catalunya-CIRIT 1999SGR 00241, and EU Commission (261357; 211250; 268479).

Piccoliipii: The study was approved and initially funded by the Italian National Centre for Disease Prevention and Control (CCM grant 2010) and by the Italian Ministry of Health (art 12 and 12bis DLgs.vo 502/92). The methylation assays were funded by the European Community’s Seventh Framework Programme FP7/2007–2013 project EXPOsOMICS (grant no. 308610). Z.H. is supported by the Exposomics EC FP7 grant (Grant agreement no: 308610). Z.H. and A.G. and the Epigenetics Group at IARC are supported by grants from the Institut National du Cancer (INCa, Plan Cancer-EVA-INserm, France) and Association pour la Recherche sur le Cancer (ARC, France).

Rhea: The methylation assays were funded by the European Community’s Seventh Framework Programme FP7/2007–2013 project EXPOsOMICS (grant no. 308610). Z.H. is supported by the Exposomics EC FP7 grant (agreement no. 308610). Z.H. and A.G. and the Epigenetics Group at IARC are supported by grants from the Institut National du Cancer (INCa, Plan Cancer-EVA-INserm, France) and Association pour la Recherche sur le Cancer (ARC, France).

PRISM: R.J.W. received funding for the PRISM cohort under R01 HL095606 and R01 HL1143396. A.C.J. is supported by R00 ES023450.

Project Viva: This Project Viva study was supported by grants from the NIH (NIH R01 HL 111108, R01 NR013945, R01 HD 034568, K24 HD069408, K23 ES022242, P01ES009825, R01AI102960, P30 ES000002) and the U.S. Environmental Protection Agency (EPA) (R832416, RD834798). This publication’s contents are solely the responsibility of the grantee and do not necessarily represent the official views of the U.S. Government, the U.S. Department of Health and Human Services or the NIH, or the EPA. Further, the EPA does not endorse the purchase of any commercial products or services mentioned in the publication.

MeDALL: The methylation study of MeDALL cohorts was funded by MEDALL, a collaborative project supported by the European Commission under the Health Cooperation Work Programme of the 7th Framework Programme (grant agreement no. 261357).

The Biobank-Based Integrative Omics Studies (BIOS) Consortium is funded by BBMRI-NL, a research infrastructure financed by the Dutch government (NWO 184.021.007).

Some of the results of the present study were previously reported in the form of a conference abstract (The 29th Annual Scientific Conference of the International Society of Environmental Epidemiology, ISEE, 2017).

ALSPAC: We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses.

BAMSE: We would like to thank all the families for their participation in the BAMSE study. In addition, we would like to thank E. Hallner, S. Nilsson, and A. Lauber at the BAMSE secretariat for invaluable support, as well as Mutation Analysis Facility (MAF) at Karolinska Institutet for genome-wide methylation analysis, and I. Delin for excellent technical assistance. The computations were performed on resources provided by SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) under Project b2014110.

CHS: We are indebted to the school principals, teachers, students, and parents in each of the study communities for their cooperation and especially to the members of the health testing field team for their efforts. We would like to express our sincere gratitude to S. Graham and R. Cooley at the California Biobank Program and Genetic Disease Screening Program within the California Department of Public Health for their assistance and advice regarding newborn bloodspots. The biospecimens and data used in this study were obtained from the California Biobank Program [SIS request number(s) 479] Section 6,555(b), 17 CCR. The California Department of Public Health is not responsible for the results or conclusions drawn by the authors of this publication.

EARLI: We thank collaborators for their help in collecting and generating the data from EARLI examined here, including C. Newschaffer, D. Fallin, I. Hertz-Picciotto, L. Croen, and F. Lurmann.

ENVIRONAGE: The authors acknowledge the participating mothers and neonates, as well as the staff of the maternity ward, midwives, and the staff of the clinical laboratory of East Limburg Hospital in Genk. We thank C. Cuenin for his help and dedication in the methylene experimental workflow and the Genetic Cancer Susceptibility Group at IARC for their assistance in these experiments.

Generation R Study: The Generation R Study is conducted by the Erasmus Medical Center (MC) in close collaboration with the School of Law and Faculty of Social Sciences of the Erasmus University Rotterdam; the Municipal Health Service Rotterdam area, Rotterdam; the Rotterdam Homecare Foundation, Rotterdam; and the Stichting Trombosediens & Artsenlaboratorium Rijnmond (STAR-MDC), Rotterdam. We gratefully acknowledge the contributions of children and parents, general practitioners, hospitals, midwives, and pharmacies in Rotterdam. The study protocol was approved by the Medical Ethical Committee of the Erasmus MC, Rotterdam. Written informed consent was obtained for all participants. The generation and management of the Illumina 450K
methylation array data (EWAS data) for the Generation R Study was executed by the Human Genotyping Facility of the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, Netherlands. We thank M. Verbiest, M. Jhamai, S. Higgins, M. Verkerk, and Dr. L. Stolk for their help in creating the EWAS database. We also thank Y. de Kluijzenaar of the Department of Urban Environment and Safety, Netherlands Organization for Applied Scientific Research (TNO), Delft, Netherlands, for help in creating the air pollution data.

HELIX: The authors would particularly like to thank all the participants for their generous collaboration.

MoBa: We are grateful to all the participating families in Norway who take part in this ongoing cohort study.


INMA: The authors would particularly like to thank all the participants for their generous collaboration. A full roster of the INMA Project Investigators can be found at http://www.proyectoinma.org/presentacion-inma/listado-investigadores/en_listado-investigadores.html.

Piccolipù: We thank all the families who took part in the study, and the Piccolipù team. We thank C. Cuenin for his help and dedication in the methylene experimental workflow and the Genetic Cancer Susceptibility Group at the International Agency for Research on Cancer (IARC) for their assistance in these experiments.

Rhea: We thank all of the participants of the Rhea study and the interviewers, statisticians, and hospital personnel for their cooperation and contribution to this study. We thank C. Cuenin for his help and dedication in the methylene experimental workflow and the Genetic Cancer Susceptibility Group at IARC for their assistance in these experiments.

Project Viva: The authors would like to thank Project Viva staff and participants. We thank collaborators for their help in directing the cohort study (E. Oken) and in collecting, generating, managing and interpreting the exposure and outcome data (J. Schwartz, B. Coull, M.-F. Hivert, S. Rifas-Shiman, and H. Gibson).


