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

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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 $<2.5$ $\mu$m (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 FAM134 (cg00905156) 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-year-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$_{10}$ and 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 $<2.5$ $\mu$m (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-year-olds from the Barn, Allergy, 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

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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 (Relton 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 four 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 (HELIK), 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 précoces 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 [rnum 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 birth indicator (s), and ancestry (in CHS). In addition, age at biosampling, municipality at birth (in BAMSE), and cohort indicator (in the pooled

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

<table>
<thead>
<tr>
<th>STUDY Country</th>
<th>Country</th>
<th>Enrollment Period</th>
<th>Total $N$</th>
<th>Total $N$ enrolled</th>
<th>Selection criteria for EWAS</th>
<th>DNA methylation measurement</th>
<th>Study reference (PMID)</th>
</tr>
</thead>
<tbody>
<tr>
<td>INMA Spain</td>
<td>1997-2008</td>
<td>3,768</td>
<td>3,768</td>
<td>Available DNA from one of the subcohorts (Saladell)</td>
<td>Illumina 450K</td>
<td><a href="http://www.proyectoinma.org/">http://www.proyectoinma.org/</a></td>
<td></td>
</tr>
<tr>
<td>EARLI USA</td>
<td>2002-2006</td>
<td>9,901</td>
<td>9,901</td>
<td>European, complete follow-up</td>
<td>Illumina 450K</td>
<td><a href="http://www.generationr.nl">www.generationr.nl</a></td>
<td></td>
</tr>
<tr>
<td>PRISM USA</td>
<td>1995-1997</td>
<td>5,341</td>
<td>5,341</td>
<td>Netherlands, non-Hispanic white</td>
<td>Illumina 450K</td>
<td><a href="http://www.projectviva.org">www.projectviva.org</a></td>
<td></td>
</tr>
<tr>
<td>CHS USA</td>
<td>2009-2012</td>
<td>322</td>
<td>322</td>
<td>NA</td>
<td>Illumina 450K</td>
<td><a href="http://www.earlistudy.org/">www.earlistudy.org/</a></td>
<td></td>
</tr>
<tr>
<td>Project Viva USA</td>
<td>2010-2016</td>
<td>1,210</td>
<td>1,210</td>
<td>Available cord blood or early/mid-childhood blood sample</td>
<td>Illumina 450K</td>
<td><a href="http://www.pace-consortium.org/">www.pace-consortium.org/</a></td>
<td></td>
</tr>
<tr>
<td>ENVIRONAGE Belgium</td>
<td>2011-2015</td>
<td>3,338</td>
<td>3,338</td>
<td>Participants resident in the municipality of Turin with enough biological material and stored biological sample</td>
<td>Illumina 450K</td>
<td><a href="http://www.piccolipiu.it">www.piccolipiu.it</a></td>
<td></td>
</tr>
<tr>
<td>Rhea Italy</td>
<td>2007-2008</td>
<td>1,590</td>
<td>1,590</td>
<td>Available 2-month follow-up data</td>
<td>Illumina 450K</td>
<td><a href="http://www.rhealife.it">www.rhealife.it</a></td>
<td></td>
</tr>
<tr>
<td>PICCOLO Italy</td>
<td>2010-2016</td>
<td>1,210</td>
<td>1,210</td>
<td>Available cord blood or early/mid-childhood blood sample</td>
<td>Illumina 450K</td>
<td><a href="http://www.pace-consortium.org/">www.pace-consortium.org/</a></td>
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<td><a href="http://www.rhealife.it">www.rhealife.it</a></td>
<td></td>
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</tbody>
</table>
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 \textit{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 nonunimodal 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 (PM\(_{2.5}\) IQR 0.8 \(\mu g/\text{m}^3\)) and RHEA (PM\(_{10}\) IQR 2.3 \(\mu g/\text{m}^3\)) cohorts and were highest for the CHS (PM\(_{2.5}\) IQR = 5.0 and PM\(_{10}\) IQR = 14 \(\mu g/\text{m}^3\)). The discovery meta-analysis of cord blood methylation in relation to prenatal exposure included 1,949 newborns for PM\(_{10}\) and 1,551 for PM\(_{2.5}\). The difference in sample sizes is due to missing prenatal PM\(_{10}\) data for Project Viva and PRISM cohorts, and missing prenatal PM\(_{2.5}\) data for the Generation R cohort. Minus \(\log_{10}(p\text{-values})\) in 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 \(p\text{-values}\) \((\lambda = 1.21\) for PM\(_{10}\) exposure and 1.37 for PM\(_{2.5}\); 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 PM\(_{10}\) exposure and DNA methylation for six CpGs, with higher PM\(_{10}\) exposure being associated with an increase in methylation for four CpGs mapping to GN2L1; 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 \(\beta\)-value per \(IQR = 5.6 \mu g/\text{m}^3\) increase in prenatal PM\(_{10}\) exposure.

We found 14 CpGs significantly associated with prenatal PM\(_{2.5}\) 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, MRI1, PSG5, C7orf50, 1.1 kb downstream of MORNI, PLAT, and ZNF695 (Table 3). The direction of the effect was negative for 11 of these CpGs, and positive for cg16253537 in FN1P1, cg01011943 in PSG5, and cg00348551 in C7orf50 in relation to higher PM\(_{2.5}\) exposure. The estimates ranged from \(-0.4\%\) to 0.3% difference in
methylation level per IQR (IQR = 2 μg/m^3) 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 F 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, COL22A1, TMCO3, SFRS8, NEUROG1, MRI1, 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
Table 2. Statistically significant CpGs (FDR \( p \leq 0.05 \)) associated with IQR increases in prenatal PM_{10} (5.6 \( \mu g/m^3 \)) 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).

<table>
<thead>
<tr>
<th>Chr</th>
<th>Position</th>
<th>Gene</th>
<th>Discovery newborns ( (n = 692) )</th>
<th>Replication newborns ( (n = 1,949) )</th>
<th>ALSPAC ( (n = 903) )</th>
<th>Replication: age 7–9 years</th>
<th>Replication: age 15–16 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>100676010</td>
<td>cg_5926255</td>
<td>SNORD96A</td>
<td>( \beta = -0.00003 )</td>
<td>( \beta = -0.00002 )</td>
<td>( \beta = 0.00001 )</td>
<td>( \beta = 0.00001 )</td>
</tr>
<tr>
<td>17</td>
<td>9595588</td>
<td>cg_21304716</td>
<td>USP43</td>
<td>( \beta = 0.00002 )</td>
<td>( \beta = 0.00001 )</td>
<td>( \beta = 0.00001 )</td>
<td>( \beta = 0.00001 )</td>
</tr>
<tr>
<td>4</td>
<td>19784463</td>
<td>cg_9901556</td>
<td>FAM13A</td>
<td>( \beta = 0.00006 )</td>
<td>( \beta = 0.00001 )</td>
<td>( \beta = 0.00001 )</td>
<td>( \beta = 0.00001 )</td>
</tr>
<tr>
<td>8</td>
<td>12456973</td>
<td>cg_84840183</td>
<td>NOTCH4</td>
<td>( \beta = 0.00001 )</td>
<td>( \beta = 0.00001 )</td>
<td>( \beta = 0.00001 )</td>
<td>( \beta = 0.00001 )</td>
</tr>
<tr>
<td>5</td>
<td>12156810</td>
<td>cg_16255613</td>
<td>P4HA2</td>
<td>( \beta = 0.00000 )</td>
<td>( \beta = 0.00000 )</td>
<td>( \beta = 0.00000 )</td>
<td>( \beta = 0.00000 )</td>
</tr>
</tbody>
</table>

Note: \( \beta \) is the estimated effect of PM_{10} exposure on DNA methylation, \( \beta \) is the estimated effect of PM_{10} exposure on DNA methylation, and \( \beta \) is the estimated effect of PM_{10} exposure on DNA methylation.

These six CpGs showed significance later in childhood in associations with prenatal PM_{10} exposure; cg20905156 (FAM13A) and cg06849931 (NOTCH4) showed increased methylation in relation to PM_{10} 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 (P4HA2) (\( p = 0.03 \)), both demonstrating the same direction of association as those in the discovery meta-analysis. In addition, cg15082635 (GNB2LI; 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 PM_{10} 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 PM_{2.5}–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 PM_{10}-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 PM_{10}-related CpGs, including one within the FAM13A gene, as well as six out of 14 PM_{2.5}-associated CpGs, were significantly associated with gene expression in \( c i s \) 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 PM_{10} exposure with expression of genes annotated to the respective CpG was detected, whereas PM_{2.5} exposure was associated with expression of ZNF695 [\( p < 0.05 \), Log fold change (LogFC) = 0.074 per 2-\( \mu g/m^3 \) increase in exposure; Table 5]. In BAMSE (\( n = 244 \)), current PM_{10} exposure at 16 y was associated with NOTCH4 (multiple transcripts, lowest \( p = 0.0001 \), LogFC = 0.05) and USP43 expression levels in peripheral blood cells (\( p < 0.05 \), LogFC = 0.05, per 5-\( \mu g/m^3 \) increase; Table 6). Among the PM_{2.5}–associated genes, C7orf50 was significantly differentially expressed in relation to current PM_{2.5} exposure (\( p = 0.03 \), LogFC = 0.02, per 2-\( \mu g/m^3 \) increase). Descriptive statistics of expression levels of genes associated with CpG methylation in response to maternal PM_{10} and PM_{2.5} 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 PM_{10} 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 RIMPS1), and “GABA synthesis, release, reuptake and degradation” (HSPA8; and RIMPS1). 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 PM2.5.

**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 PM10 and 390 with PM2.5 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 PM10 and PM2.5 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 PM10 and PM2.5 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 (Hobbs et al. 2017; Hancock et al. 2010). Research has shown that *FAM13A* interferes with the Wnt pathway, inducing β-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
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 \textit{FAM13A}, \textit{GNB2L1}, and \textit{SNORD96A}, as well as \textit{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 \textit{MR11}. Previous studies reported significant association between increased \textit{MR11} 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 \textit{H19} and \textit{MARCH11} genes were also replicated in the independent newborn data set. The \textit{H19} gene is located in an imprinted region of chromosome 11 and is expressed only from the maternally inherited chromosome. Recent evidence suggests that \textit{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 \textit{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 CHR demonstrated DNA methylation variability in newborn blood in relation to prenatal exposure to PM10.
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 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 (Kaulash 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.

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References


