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Pregnancy exposure to atmospheric pollution and meteorological conditions and placental DNA methylation

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ABSTRACT

Background: Air pollution exposure represents a major health threat to the developing foetus. DNA methylation is one of the most well-known molecular determinants of the epigenetic status of cells. Blood DNA methylation has been proven sensitive to air pollutants, but the molecular impact of air pollution on new-borns has so far received little attention.

Objectives: We investigated whether nitrogen dioxide (NO_2), particulate matter (PM_{10}), temperature and humidity during pregnancy are associated with differences in placental DNA methylation levels.

Methods: Whole-genome DNA-methylation was measured using the Illumina's Infinium HumanMethylation450 BeadChip in the placenta of 668 newborns from the EDEN cohort. We designed an original strategy using a priori biological information to focus on candidate genes with a specific expression pattern in placenta (active or silent) combined with an agnostic epigenome-wide association study (EWAS). We used robust linear regression to identify CpGs and differentially methylated regions (DMR) associated with each exposure during short- and long-term time-windows.

Results: The candidate genes approach identified nine CpGs mapping to 9 genes associated with prenatal NO_2 and PM_{10} exposure [false discovery rate (FDR) $p < 0.05$]. Among these, the methylation level of 2 CpGs located in *ADORA2B* remained significantly associated with NO_2 exposure during the 2nd trimester and whole pregnancy in the EWAS (FDR $p < 0.05$). EWAS further revealed associations between the environmental exposures under study and variations of DNA methylation of 4 other CpGs. We further identified 27 DMRs significantly (FDR $p < 0.05$) associated with air pollutants exposure and 13 DMRs with meteorological conditions.

Conclusions: The methylation of *ADORA2B*, a gene whose expression was previously associated with hypoxia and pre-eclampsia, was consistently found here sensitive to atmospheric pollutants. In addition, air pollutants were associated to DMRs pointing towards genes previously implicated in preeclampsia, hypertensive and metabolic disorders. These findings demonstrate that air pollutants exposure at levels commonly experienced in the European population are associated with placental gene methylation and provide some mechanistic insight into some of the reported effects of air pollutants on preeclampsia.

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1. Introduction

Despite significant improvements in air quality in past decades, 50% of the population in 2014 in Europe lived in areas that do not meet the World Health Organization guidelines (World Health Organization, 2006) for particulate matter < 10 µm (PM_{10}), 85% for particulate matter < 2.5 µm ($PM_{2.5}$), and 7% for nitrogen dioxide (NO_2) (Ortiz, 2017). Ambient air pollution includes gaseous pollutants, such as nitrogen oxides, sulphur dioxide, ozone, benzene, as well as particulate matter of various sizes, which are a mixture of solid and liquid droplets including black carbon, metals, and polycyclic aromatic hydrocarbons. Air pollutants exposure during pregnancy is a major health threat to children as it can cross the placenta and expose the developing fetus (Valentino et al., 2016; Wick et al., 2010). Exposure to air pollutants during pregnancy has been associated with a range of adverse health outcomes both in the short-term, including low birth weight and preterm birth and in the long term, including reduced infant lung function and neurodevelopmental disorders (Chiu et al., 2016; Clifford et al., 2016; Jedrychowski et al., 2010; Stieb et al., 2012). Air pollution pregnancy exposure is also a threat to the pregnant woman and is likely a risk factor of preeclampsia (Pedersen et al., 2014). More recently, high- or low-ambient temperatures have also been suggested to play a role in adverse pregnancy outcomes (Beltran et al., 2014; Giorgis-Allemand et al., 2017; Kloog et al., 2015).

The placenta plays a key role in fetal programming by supporting both the health of the mother and the development of the fetus. It conveys nutrients and oxygen to the fetus and regulates gas and waste exchanges as well as hormone interactions (Murphy et al., 2006). Alterations in placental physiology and function, potentially driven by epigenetic changes, may impact the health of the future individuals during their childhood as well as into their adulthood. Exposure to chemically and/or physically inappropriate environmental conditions during pregnancy can affect the placental function by modifying its epigenome (Nelissen et al., 2011). Indeed, epigenetic mechanisms control the timing and levels of gene expression, by defining the extent of their activation or by maintaining them repressed. Hence in utero environmental exposures could result in epigenetic modifications of the placenta at birth, including changes in the DNA methylation profile. As a transient organ, the placenta may provide a unique record of exposures specifically occurring during pregnancy. While several studies have reported changes in global and gene-specific methylation patterns from adult blood associated with air pollution (Madrigano et al., 2011; Panni et al., 2016), temperature and relative humidity exposure (Bind et al., 2014), the role of maternal exposure to such environmental factors on newborns DNA methylation patterns has received little attention so far. In cord blood, methylation in mitochondria-related genes was associated with NO_2 exposure during pregnancy in a recent meta–analysis (Gruzieva et al., 2017). In placenta, a few studies have linked global DNA methylation and gene candidate methylation to air pollution exposure during pregnancy (Cai et al., 2017; Janssen et al., 2013). However, no study has yet investigated the relationship between exposure to air pollutants and meteorological conditions during pregnancy and placental methylation patterns at a genome-wide scale.

We hypothesized that maternal exposure to air pollutants and meteorological conditions could alter the placental function through modifications of DNA methylation. Therefore we investigated the relationship of air pollutants and meteorological conditions with global DNA methylation and gene-specific methylation in placentas at birth using the Illumina 450 K array. In order to identify potentially relevant changes in genomic methylation sites related to environmental exposures, we used two complementary approaches combining a concept-driven analysis with an Epigenome-Wide Association Study (EWAS) (Fig. 1). The concept-driven analysis is an original strategy relying on the hypothesis that sensitivity to DNA methylation variations induced by environmental factors may depend on the activity of the genomic region considered (Rousseaux et al., 2013). Indeed, a change in the DNA methylation profile affecting a region involved in the epigenetic control of the expression level of a gene actively repressed or activated during pregnancy is more likely to be biologically relevant than a change occurring in another region of the genome. Therefore, based on available expression data in placenta, we identified genes whose expression is either predominant in full-term placenta or undergoes significant variations during placenta development. In addition, using our methylation data, we also established a list of genes with highly methylated CpG-rich promoters. Focusing on these genes with a specific status or pattern of expression in the placenta enabled us to explore the "basal epigenome dynamics – epigenome response" relationship in the placenta. We then used an agnostic EWAS design, in an attempt to confirm the findings of the concept-driven analysis and to identify new regions associated with maternal air pollutants exposure.

2. Methods

2.1. Study population

The EDEN mother-child cohort included 2002 pregnant women, mainly Caucasian, enrolled before 24 weeks of gestation in Nancy and Poitiers university hospitals, France, between 2003 and 2006 (Heude et al., 2015). Exclusion criteria were multiple pregnancies, pre-pregnancy diabetes, French illiteracy and planned move outside the region in the following 3 years. Residential addresses, lifestyle, demographic and medical data were collected by questionnaires and interviews during pregnancy and after delivery. Among the 1301 women for whom placenta samples were collected, we focused on 668 women. Placenta samples were collected at delivery by the midwife or the technician of the study using a standardized procedure. Samples of around 5 mm × 5 mm were carried out in the centre of the placenta on the foetal side and were immediately frozen at −80 °C. The EDEN cohort received approval from the ethics committee (CCPPRB) of Kremlin Bicêtre and from the French data privacy institution "*Commission Nationale de l'Informatique et des Libertés*" (CNIL). Written consent was obtained from the mother for herself and for the offspring.

2.2. Placental DNA methylation assessment

DNA from placental samples was extracted using the QIAsymphony instrument (Qiagen, Germany). The DNA methylation analysis was performed by the Centre National de Recherche en Génomique Humaine (CNRGH, Evry, France). The DNA samples were plated onto 96-well or 48-well plates. In total, nine plates including 64 chips were used. These plates were analyzed in 4 batches. The ratios for sex (boy/girl) and recruitment centre (Poitiers/Nancy) were balanced for each chip. Fifteen samples were measured in quadruplicates and one sample in duplicate across batches, sample plates and chips to detect technical issues such as batch effects. The Illumina's Infinium HumanMethylation450 BeadChip, representing over 485,000 individual CpG sites, was used to assess levels of methylation in placenta samples following the manufacturer's instructions (Illuminas, San Diego, CA, USA). Raw signals of 450 K BeadChips were extracted using the GenomeStudio® software (v2011.1. Illumina). The DNA methylation level of each CpG was calculated as the ratio of the intensity of fluorescent signals of the methylated alleles over the sum of methylated and unmethylated alleles (β value). All samples passed initial quality control and had on average > 98% of valid data points (detection p-value < 0.01). A refined version of the Subset Quantile Normalization (SQN) pipeline (Touleimat and Tost, 2012) including a revised annotation file (Price et al., 2013) was used for data processing, correction and normalization. Data processing and normalization did not change the density distribution of the DNA methylation levels (Fig. S1). Intensity values were corrected for potential biases in fluorescent dye intensity and background corrected using the *lumi* R package (Du et al., 2008) as implemented in the SQN pipeline. Probes potentially