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Early- and late-onset preeclampsia and the tissue-specific epigenome of the placenta and newborn

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ABSTRACT

Introduction: Preeclampsia (PE) carries increased risks of cardiovascular- and metabolic diseases in mothers and offspring during the life course. While the severe early-onset PE (EOPE) phenotype originates from impaired placentation in early pregnancy, late-onset PE (LOPE) is in particular associated with pre-existing maternal cardiovascular- and metabolic risk factors. We hypothesize that PE is associated with altered epigenetic programming of placental and fetal tissues and that these epigenetic changes might elucidate the increased cardiovascular- and metabolic disease susceptibility in PE offspring.
Methods: A nested case-control study was conducted in The Rotterdam Periconceptional Cohort comprising 13 EOPE, 16 LOPE, and three control groups of 36 uncomplicated pregnancies, 27 normotensive fetal growth restricted and 20 normotensive preterm birth (PTB) complicated pregnancies. Placental tissue, newborn umbilical cord white blood cells (UC-WBC) and umbilical vein endothelial cells were collected and DNA methylation of cytosine-guanine dinucleotides was measured by the Illumina HumanMethylation450K BeadChip. An epigenome-wide analysis was performed by using multiple linear regression models.
Results: Epigenome-wide tissue-specific analysis between EOPE and PTB controls revealed 5001 mostly hypermethylated differentially methylated positions (DMPs) in UC-WBC and 869 mostly hypomethylated DMPs in placental tissue, situated in or close to genes associated with cardiovascular-metabolic developmental pathways.
Discussion: This study shows differential methylation in UC-WBC and placental tissue in EOPE as compared to PTB, identifying DMPs that are associated with cardiovascular system pathways. Future studies should examine these loci and pathways in more detail to elucidate the associations between prenatal PE exposure and the cardiovascular disease risk in offspring.

Abbreviations: PE, preeclampsia; *EOPE*, early-onset preeclampsia; *LOPE*, late-onset preeclampsia; *UC-WBC*, umbilical cord white blood cells; *HUVEC*, human umbilical vein endothelial cells; *CpGs*, cytosine-guanine dinucleotides; *DMPs*, differentially methylated positions; *EWAS*, epigenome-wide association studies; *FGR*, fetal growth restriction; *PTB*, preterm birth; *OR*, odds ratio; *CI*, confidence interval; *GO-term*, gene-ontology term; *PBS*, phosphate buffered saline-solution; *MACS*, magnetic activated cell separation; *FDR*, false discovery rate; *bp*, basepairs; *IPA*, Ingenuity pathway analysis; *DAVID*, Database for Annotation, Visualization and Integrated Discovery; *PCA*, Principal Component Analysis; *non-CGI*, non-CpG island.

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1. Introduction

Preeclampsia (PE) is a major cause of maternal and fetal morbidity and mortality and is suggested to be associated with the future risk of cardiovascular- and metabolic diseases in mothers and offspring [1]. Two main disease entities have been identified. Early-onset PE (EOPE) is the more severe phenotype originating from impaired placentation during the first trimester of pregnancy; late-onset PE (LOPE) occurs in the third trimester mainly as a consequence of exposure to pre-existing maternal cardiovascular- and metabolic risk factors [2,3]. The exact pathophysiology of PE is not understood, but it is known that in EOPE inadequate spiral artery remodelling leads to ischaemia-reperfusion-type placental insults [4]. This induces an excessive production of oxidative radicals. As a result, pro-inflammatory cytokines are released into the maternal circulation inducing endothelial dysfunction that leads to the clinical symptoms of PE [4]. Excessive placental oxidative stressors are also produced in LOPE but more towards the end of pregnancy [2].

PE is considered a complex disease induced by gene- and environmental interactions [5,6]. Altered epigenetic programming of specific tissues, induced by excessive oxidative stress, has been suggested to be an underlying mechanism [5]. Impaired placental perfusion, excessive production of oxidative radicals, endothelial dysfunction and immune modifications in PE may disturb epigenetic programming in offspring tissues as well, resulting in derangements of the vascular epigenome and function in the offspring [7–10]. From this background we *hypothesize* that altered epigenetic programming of especially placental tissues is associated with the pathogenesis of PE (as cause or consequence) and with cardiovascular and metabolic risks in the offspring over the life course [11].

Although PE has been associated with DNA methylation changes of candidate genes in placental and newborn tissue, only a few epigenome-wide association studies (EWAS) have found new loci of interest and most of these did not examine multiple tissues per patient [12–17]. In this study we examined tissue-specific genome-wide DNA methylation of umbilical cord white blood cells (UC-WBC), placental tissue and human umbilical cord endothelial cells (HUVEC) in relation to EOPE and LOPE.

Considering that PE is complicated by fetal growth restriction (FGR) in 12% of cases and associated with iatrogenic preterm birth (PTB) in 20%, which are conditions that can independently affect epigenetic programming, we examined the epigenome of PE and uncomplicated controls but also that of controls complicated by FGR and PTB [18–20].

2. Materials and methods

2.1. Study design

Pregnant women and their newborn babies were recruited between June 2011 and June 2013 in a nested case-control study embedded in The Rotterdam Periconceptional Cohort (Predict study), at the Erasmus MC, University Medical Centre Rotterdam, the Netherlands [21]. Cases comprised of EOPE and LOPE, and unmatched controls comprised of uncomplicated pregnancies and FGR and PTB complicated pregnancies. Patients were recruited for our case-control study by two different pathways. The first pathway contains subjects that were included in the Predict study in the first trimester and developed PE later in pregnancy. The second pathway contains subjects that were admitted to our hospital because of PE after the first trimester, and were specifically recruited in the Predict Study for our case-control study. This also applies to our (un)complicated control groups.

2.2. Maternal and fetal characteristics

PE was defined according to the International Society for the Study of Hypertension in Pregnancy as gestational hypertension of at least 140/90 mmHg accompanied by a urine protein/creatinine ratio of $\geq$30 mg/mmol arising de novo after the 20th week of gestation [22]. EOPE and LOPE were defined as being diagnosed before and after 34 weeks of gestation, respectively [23]. Uncomplicated pregnancies were defined as pregnancies without the following pregnancy-specific complications: PE, gestational hypertension, FGR or PTB. FGR was defined as an estimated fetal weight below the 10th percentile for gestational age based on ultrasound measurements performed between 20 and 38 weeks gestational age [24]. Birth weight percentiles were calculated using the reference curves of the Dutch Perinatal Registry to validate birth weight <10th percentile [25]. Spontaneous preterm deliveries between 22 and 37 weeks of gestation were defined as PTB [26]. Women with HIV infection, aged <18 years, not able to read and understand the Dutch language, multiple birth pregnancies or women with pregnancies complicated by fetal congenital malformations were excluded.

Maternal and newborn characteristics were obtained from hospital medical records. All women gave written informed consent before participation and parental informed consent was obtained for the child. The research has been carried out in accordance with the Declaration of Helsinki (2013) of the World Medical Association.

2.3. Data collection

UC-WBC samples from the umbilical vein were collected with the placenta still in situ. Thereafter, placental tissue was obtained and HUVEC were isolated and stored until DNA extraction. A detailed description of the data collection, UC-WBC processing, HUVEC isolation and DNA extraction is provided in Appendix 1.

2.4. DNA methylation measurement

Isolated genomic DNA (500 ng) was treated with sodium bisulphite using the EZ-96 DNA methylation kit (Shallow) (Zymo Research, Irvine, CA, USA). Hybridization was performed following manufacturer's instructions. DNA methylation of CpGs was measured by the Illumina HumanMethylation450K BeadChip using the manufacturer's protocol (Illumina, Inc., San Diego, CA, USA) [27–29]. Data quality control and pre-processing is described in Appendix 2.

2.5. Differentially methylated position (DMP) identification

To improve statistical power, prior to further statistical analysis a selection of probes containing at least $\geq$0.05 SD variability in methylation β-values across all samples was applied for each tissue separately [30]. This resulted in a remaining total of 43,488 UC-WBC probes, 134,700 placental probes and 42,352 HUVEC probes. Methylation β-values were converted to M-values using: M-value = $\log_2$ (β-value/(1-β-value)) [31]. A multiple linear regression model was used predicting methylation M-values by disease state for EOPE and LOPE versus (un)complicated controls, adjusted for bisulphite-plate batch and gestational age as covariates for each tissue separately (R package 'CpGassoc'). Sensitivity analyses were performed for the following covariates: batch-effect of the bisulphite-plate, gestational age, birth weight, fetal gender, maternal comorbidity, mode of delivery and moment of inclusion for the study in or >1st trimester. Only gestational age and bisulphite-plate were thereafter included in our statistical model as