Placenta 101 (2020) 97–107

Contents lists available at ScienceDirect

Placenta

journal homepage: http://www.elsevier.com/locate/placenta

Global DNA methylation in placental tissues from pregnant with preeclampsia: A systematic review and pathway analysis

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ARTICLE INFO

Keywords:
DNA methylation
Early-onset pre-eclampsia
Epigenomics
Late-onset pre-eclampsia
Pathways
Pre-eclampsia
Systematic review

ABSTRACT

Pre-eclampsia (PE) is the major cause of fetal and maternal mortality and can be classified according to gestational age of onset into early-onset (EOPE, <34 weeks of gestation) and late- (LOPE, ≥34 weeks of gestation). DNA methylation (DNAm) may help to understand the abnormal placentation in PE. Therefore, we performed a systematic review to assess the role of global DNAm on pathophysiology of PE, focused on fetal and maternal tissues of placenta from pregnant with PE, including EOPE and LOPE. We searched the databases EMBASE, Medline/PubMed, Cochrane Central Register of Controlled Trials, Scopus, Lilacs, Scielo and Google Scholar, and followed the MOOSE guidelines. Moreover, we performed pathway analysis with the overlapping genes from the included studies. Twelve out of 24 included studies in the qualitative analysis considered the classification into EOPE and LOPE. We did not found heterogeneity in the criteria used for diagnosis of PE, and a few studies evaluated whether confounding factors would influence placental DNAm. Fourteen out of 24 included studies showed hypomethylation in placental tissue from pregnant with PE compared to controls. The differences in DNAm are specific to genes or differentially methylated regions, and more evident in EOPE and preterm PE compared to controls, rather than LOPE and term PE. The overlapping genes from included studies revealed pathways relevant to pathophysiology of PE. Our findings highlighted the heterogeneous results of the included studies, mainly focused on North America and China. Replication studies in different populations should use the same placental tissues, techniques to assess DNAm and pipelines for bioinformatic analysis.

1. Introduction

Preeclampsia (PE) is defined as a new-onset hypertension (systolic blood pressure (SBP) ≥ 140 mmHg and diastolic blood pressure (DBP) ≥ 90 mmHg) after 20 weeks of gestation, which may be combined with proteinuria [1]. PE affects up to 9% of all pregnancies and is the major cause of fetal and maternal mortality and morbidity [2]. PE has a heterogeneous etiology and is classified according to gestational age of onset into late- (LOPE, ≥34 weeks of gestation) and early-onset (EOPE, <34 weeks of gestation), which is considered a more severe form of PE [3–5]. However, it is unclear whether EOPE and LOPE have different etiologies and pathogenesis or are the graduation of the same condition [6,7].

DNA methylation (DNAm) is primarily restricted at context of addition of a methyl group to the C5 position of the cytosine-guanine dinucleotide (CpG) [8]. Methylation of CpG sites are naturally associated with transcriptional repression when located in gene promoters, but with increased transcription when located in gene body [9,10]. Most of the human placental methylome is hypermethylated, but 37% of it is covered by partially methylated domains that are hypomethylated and constant through gestation and between individuals [11,12].

Notably, different cell types of placenta exhibit different transcriptional, epigenetic, and morphological features, which can conceal cell-specific signals and lead to spurious associations in different DNAm studies in PE [13]. In this context, epigenomic studies examining tissue or cell-specific signatures may contribute to understand both the normal and abnormal placentation processes, Therefore, it is relevant to assess the available DNAm data in placental tissues from PE pregnant.

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https://doi.org/10.1016/j.placenta.2020.09.004
Received 27 May 2020; Received in revised form 24 August 2020; Accepted 1 September 2020
Available online 7 September 2020

In this study, we aimed to perform a systematic review to assess the role of global DNAm in the pathophysiology of PE focused on the side of placental tissue evaluated and considering the classification into EOPE and LOPE. Moreover, we performed pathway analysis with the overlapping genes found in the included studies.

2. Materials and methods

This study was conducted according to The Cochrane Handbook for Systematic Reviews of Interventions guideline [14], and results will be reported in accordance with the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) checklist [15]. The protocol of current study was registered on International Prospective Register of Systematic Reviews (PROSPERO [CRD42020161780]).

2.1. Search strategy

The search question was composed by Population, Variable, Outcome (PVO) (Population = pregnant, Variable = preeclampsia, Outcome = methylation). A literature review was conducted by searching the electronic databases EMBASE, Medline/PubMed (Medical Literature Analysis and Retrieve System Online), Cochrane Central Register of Controlled Trials (CENTRAL), Scopus, Lilacs (Latin American and Caribbean Health Sciences), Scielo and Google Scholar to identify studies published until April 2020 that investigated PE, placenta, and global DNAm. The initial search included the Medical Subject Headings (MeSH) entry terms: 'Pregnancy', 'Pre-Eclampsia' and 'DNA Methylation', which were then included for a high-sensitivity search strategy in the Medline/PubMed, as described on Supplementary Material 1.

The same terms were used to search for gray literature and conference proceedings (Google Scholar). All potentially eligible studies were considered for review, regardless the language and publication date.

2.2. Inclusion and exclusion criteria

We included case-control studies including EOPE, LOPE or PE as case group, and control groups without chronic hypertension or gestational hypertension, gestational diabetes mellitus (GDM) and other well-known risk factors. The outcome was considered as the comparison of DNAm between PE and control groups.

We excluded studies that did not report the placental tissue or that evaluated only chorionic villous tissue, trophoblast cell lines, blood cells, and whole blood. We further excluded studies without matched control groups, or with control group composed of pregnancies with complications other than PE, such as GDM.

2.3. Study selection and data extraction

Initially, the studies retrieved from the databases were input on a single electronic library and duplicates were excluded using the EndNote® software. Two reviewers (J.O.C. and I.M.C.A.C.) independently analyzed the titles and abstracts of articles retrieved, reviewed the full-text articles, and used a standard data extraction protocol. Any disagreements were solved by a third reviewer (M.R.L.). The extracted data included the sample size, study design, maternal age, gestational age, tissue evaluated, applied technique, criteria for diagnosis of PE, and classification into EOPE and LOPE.

2.4. Assessment of bias across studies

The risk of bias in individual studies was independently assessed by two reviewers (J.O.C. and I.M.C.A.C.) following the Newcastle-Ottawa Quality Assessment Scale, according to The Cochrane Handbook's recommendations [14]. The tool used is structured into five domains: (1) patient selection (generalization and applicability); (2) comparability of groups in the study; (3) methods for assessing outcomes (cohort studies); (4) evidence of exposure (case-control) and (5) adequate follow-up. Any disparity was solved by a third reviewer (M.R.L.).

2.5. Pathway analysis

We manually curated the overlapping genes found in the included studies (Supplementary Table 1), and interrogated them for significant well-curated signaling pathways obtained from KEGG 2019 Human Pathway [16] sorted by p-value ranking <0.5 using Enrichr [17].

3. Results

We found 988 publications in the electronic databases (Fig. 1). After exclusion of 353 duplicates, 635 articles were selected for title and abstract analysis. Of these, 515 articles were subsequently excluded for several reasons (Fig. 1), resulting in 120 studies for complete reading. Literature reviews, studies focused on the analysis of specific genes, and studies that did not specify the tissue evaluated were also excluded. Finally, 24 full-text articles remained for the systematic review [18–41] (Table 1).

3.1. Included studies

Out of the 24 articles included, 10 (41.6%) had data from North American populations, including Canada and USA [18–22,25,31,35,36,40], eight (33.3%) from China [23,26,29,30,34,37,38,41], three (12.5%) from The Netherlands [24,32,33], and other three (12.5%) from India [28], Republic of Korea [27] and Australia [39]. Among the studies, seven (29.1%) validated their results in another independent cohort [20,25,26,29,36,40,41], and eight (33.3%) presented internal validation with the same samples but using a different technique [19,21–23,30,34,35,39]. Thirteen studies (54.2%) evaluated the fetal side of placenta [18–21,24–26,32–34,36,39,40], four (16.6%) used the maternal side [22,28,29,38], and seven (29.2%) did not specify the placental side used [23,27,30,31,35,37,41]. Twelve studies (50%) considered the classification into EOPE and LOPE [20,23–25,29,32–36,40,41], and 14 (58.3%) used the technique Infinium Human Methylation 450 Bead Chip array [18–20,22,24,25,27,31–36,39] (Table 1).

Regarding the quality assessment according to Newcastle-Ottawa scale, two studies scored nine points [36,40], 11 scored eight [19,20,23–26,28,29,32,33,35] and other 11 scored seven [18,21,22,27,30,31,34,37–39,41] (Supplementary Table 2). The bioinformatic analysis of global DNAm data greatly varied among the included studies. Therefore, a meta-analysis was not possible due to the heterogeneity of placental tissues evaluated, the applied technique used to assess global DNAm, and the different methods used for bioinformatic analysis, which hindered the quantitative comparison between the included studies.

Regarding the criteria used for PE diagnosis, 21 studies (87.5%) were based in evidence of new-onset hypertension (SBP $\geq$ 140 mmHg and DBP $\geq$ 90 mmHg) and proteinuria ($\geq$0.3 g/day or $\geq$2+ dipstick in urine sample of 24 h) after 20 weeks of gestation [18–22,24–36,39–41]. Five of these studies were based in these criteria plus others, such as maternal organ dysfunction, hematological disturbances and uteroplacental dysfunction [21,27,36,39,40]. One study (4.2%) were based in new-onset hypertension (SBP $\geq$160 mmHg and DBP $\geq$110 mmHg) and significant proteinuria (42 g or 3+ in urine sample of 24 h) after 20 weeks of gestation [23]. Two studies (8.3%) did not describe the criteria used for PE diagnosis [37,38] (Table 1).

Fourteen studies (58.3%) found a decreased DNAm level in placentas from PE pregnant compared to controls [20,22,24–27,29–35,38,40]. Although 22 studies (91.6%) used paired maternal age [18–20,22–36,38–41], 18 (75%) used gestational age as covariate for the DNAm analysis [18,20–22,24–27,29,31–36,39–41]. The ratio of male/female of infants varied among studies, and 12 (50%) used gender as covariate [20–22,24,25,29,32,33,35,36,39,40].

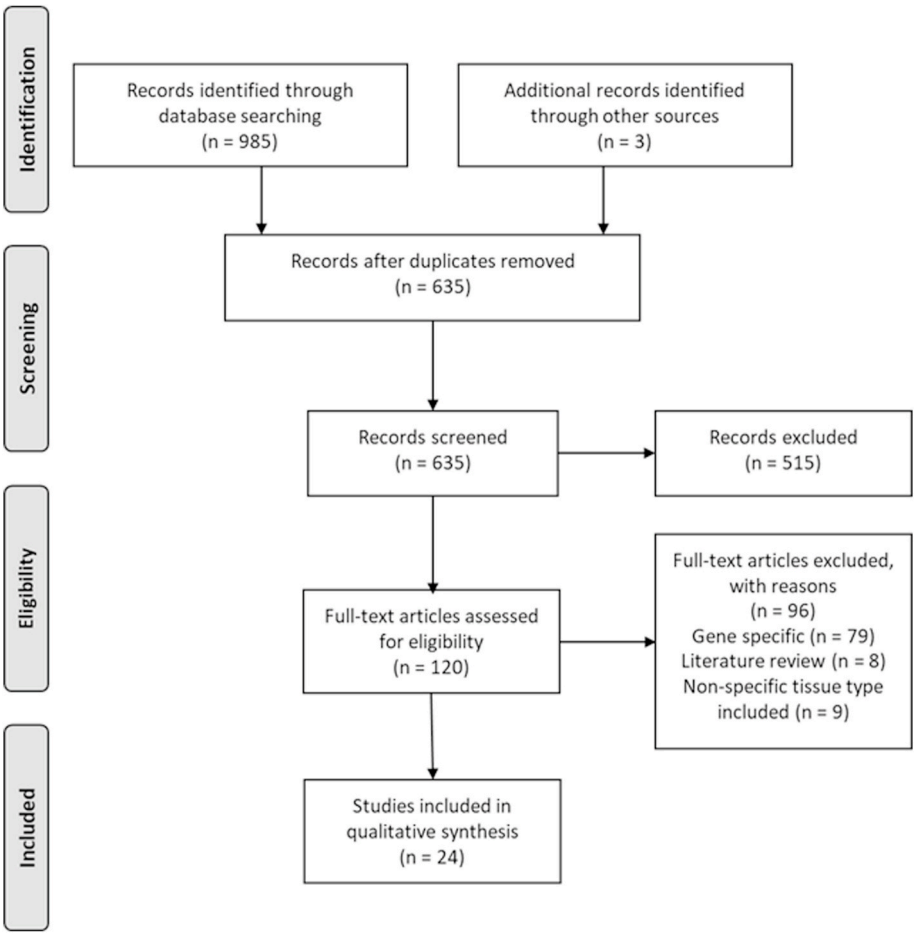

Fig. 1. Flow diagram of study selection for systematic review of published articles on the role of DNAm in placental tissue from pregnant with PE.

3.2. Global DNAm in placenta of PE pregnant compared to controls

Seven studies did not specify which side of placental tissue was analyzed [23,27,30,31,35,37,41] (Table 1). Four studies were case-control of PE pregnant compared to control, which found heterogeneous patterns of decreased DNAm levels in PE [27,30,31,37]. Remarkably, the number of differentially methylated genes (and the % of hypomethylated) reported were 3.878 (55.2%) [30], 1.664 (60.2%) [37], 617 (80.7%) [31] and 365 (89.9%) [27].

Notably, global DNAm was greatly discordant in maternal peripheral blood and placenta from PE pregnant, with 71 and 365 differentially methylated CpGs loci, respectively [27]. A total of 48 overlapping genes were found in the included studies (Fig. 2; Supplementary Table 1A), which were related to signaling pathways relevant to pathophysiology of PE (Fig. 3A).

Two studies found that global DNAm was significantly higher in EOPE compared to controls, but not statistically higher in LOPE compared to controls [23,41]. The methylation density in the Alu and LINE-1 repeats, and *H19* presented the same results for global methylation analysis [23]. Other study found 403 differentially methylated genes (68.2% hypermethylated) in placentas of LOPE [41].

PAPPA gene was exclusively hypomethylated in EOPE in other study, while the promoter and upstream enhancer regions of *INHBA* and *FN1* were hypomethylated in EOPE and LOPE + Intrauterine growth restriction groups. For these candidate genes, a positive correlation between DNAm and gene expression in placenta was found in case, but not in control group. The DNAm of *INHBA* and *FN1* was correlated with protein levels in maternal blood in the second and third trimester of gestation in PE, respectively [35]. Moreover, potential confounding factors in the assessment of DNAm showed an association of birthweight with *INHBA* and *FN1* methylation, and gestational age with *FN1* methylation [35].

3.3. Global DNAm in fetal side of placenta from PE pregnant compared to controls

Thirteen studies examined the DNAm in fetal side of placenta [18–21,24–26,32–34,36,39,40] (Table 1). Four case-control studies compared PE to controls [18,21,26,39]. One study did not find differentially methylated CpG sites between cases and controls. However, a significant correlation was found between methylation and gestational age corrected by birthweight, but no correlation with other clinical factors [21].

The methylation profiles of genes between studies were discordant in PE. For example, while one study found that 65.5% of 296 genes were hypomethylated [26], other study found that 70.6% of 303 genes were hypermethylated [39]. Notably, only *PPARG* (hypomethylated) and *ADORA2B* (hypermethylated) were commonly found in these studies [26,39]. In maternal peripheral blood, 207 CpG sites were differentially methylated (64% hypermethylated) in PE, and approximately 75% of them were concordant and hypermethylated in placenta [18].

Eight studies included the classification into EOPE and LOPE [20,24,25,32–34,36,40]. While 192 loci were hypomethylated in EOPE, none was differentially methylated in LOPE [40]. Conversely, 248 and 275 genes were differentially methylated (74.5% and 98.9% hypomethylated) in EOPE [20,34]. Multiple genes related to stress pathways and steroid production were associated with differentially methylated CpG sites in EOPE compared to controls. *NR3C1* and *CRHBP* were

Table 1
Characteristics of the studies examining global DNA methylation in placental tissues from pregnant with preeclampsia.

Author; Data; Country.	Study design; Duration (years); Study validation status	No. of patients; mean age (years mean ± standard deviation)		Mean gestational age (weeks mean ± standard deviation)		Sample size in the global analysis		Diagnosis criteria of PVO	Tissue evaluated	Applied technique
		Case	Control	Case	Control	Case	Control			
Anderson et al., 2014; USA [18]	Case-control study; NR; NR	PE: 6; 22.8 ± 1.4	6; 27.5 ± 3.65	38.4 ± 0.58	40 ± 0.49	PE: 6	6	New-onset hypertension (SBP≥ 140 mmHg or DBP≥ 90 mmHg) and proteinuria (≥+ 1 single sample or > 300 mg/24 h) after 20 weeks of gestation	Placental fetal side, white blood cells	Infinium HumanMethylation450 BeadChip array
Anton et al., 2014; USA [19]	Case-control study; 4; Internal validation	TPE: 19; 28.0 ± 8.1 PTPE: 12; 27.7 ± 7.6″	14; 27.0 ± 7.2	TPE: 38.9 ± 1.0; PTPE: 31.2 ± 4.0	39.2 ± 1.2	TPE: 19 PTPE: 12	14	New-onset hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg) and proteinuria (≥0.3 g/day or ≥2+ dipstick) after 20 weeks	Placental fetal side	Infinium HumanMethylation450 BeadChip array
Blair et al., 2013; Canada [20]	Case-control study; NR; Independent validation	EOPE: 20; 33.5 ± NR	20; 31.5 ± NR	31.8 ± NR	31.8 ± NR	EOPE: 20	20	New-onset hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg) and proteinuria (≥0.3 g/day or ≥2+ dipstick) after 20 weeks gestation	Placental fetal side	Infinium HumanMethylation450 BeadChip array
Bourque et al., 2010; Canada [21]	Case-control study; NR; Internal validation	PE: 17; NR IUGR: 13; NR PE + IUGR: 21; NR	22; NR	PE: 35.9 ± NR IUGR: 35.4 ± NR PE + IUGR: 32.5 ± NR	39.0 ± NR	PE: 4IUGR: 5	5	(1) New-onset hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg) and proteinuria (≥0.3 g/day or ≥2+ dipstick) after 20 weeks gestation (2) Sibai's criteria (3) British Eclampsia Survey Team criteria to define eclampsia	Placental fetal side	GoldenGate Methylation Cancer Panel 1 arrays
Chu et al., 2014; USA [22]	Case-control study; 10; Internal validation	PE; 24; 27.9 ± 7.2	24; 29.3 ± 5.4	35.9 ± 4.0	39.3 ± 1.2	PE: 24	24	New-onset hypertension (SBP ≥140 mm Hg and/or DBP ≥90 mm Hg) and proteinuria (≥300 mg of protein in 24 h or ≥2+ dipstick) after 20 weeks of gestation	Placental maternal side	Infinium HumanMethylation450 BeadChip array
Gao et al., 2011; China [23]	Case-control study; 2; Internal validation	EOPE: 10; 31.2 ± 5.1 LOPE: 10; 30.4 ± 3.7	24; 30.6 ± 4.1	EOPE: 32.3 ± 1.2 LOPE: 36.8 ± 2.1	38.3 ± 18.4	EOPE: 10 LOPE: 14	24	New-onset hypertension (SBP of ≥160 mmHg or DBP of ≥110 mmHg) and significant proteinuria (42 g per 24 h or ≥3+) after 20 weeks of gestation	Placenta	Immunohistochemistry
Herzog et al., 2017; The Netherlands [24]	Case-control study; 2; NR	EOPE: 13; 30.0 ± 4.7 LOPE: 16; 33.3 ± 4.5	Uncomp.: 36; 31.8 ± 5.1 FGR: 27; 29.7 ± 6.0 PTB: 20; 31.0 ± 5.1	EOPE: 30.7 ± 3.4 LOPE: 37.4 ± 1.9	Uncomp.: 39.9 ± 1.9 FGR: 38.9 ± 2.6 PTB: 35.4 ± 7.9	EOPE: 13 LOPE: 16	Uncomp.: 36 FGR: 27 PTB: 20	New-onset hypertension (SBP≥140 and DSP ≥90 mmHg) and proteinuria (≥30 mg/mmol) after the 20 weeks of gestation	Placental fetal side, UC-WBC, HUVEC	Infinium HumanMethylation450 BeadChip array
Hogg et al., 2013; Canada [25]	Case-control study; NR; Independent validation	EOPE: 19; 34.2 ± 6.0 LOPE: 18; 33.5 ± 5.5 nIUGR: 13; 34.7 ± 5.3	111; 33.10 ± 4.74	EOPE: 31.9 ± 3.3 LOPE: 37.5 ± 2.3 nIUGRn: 36.4 ± 2.3	35.1 ± 4.2	EOPE:19	19	New-onset hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg) and proteinuria (≥0.3 g/d or ≥2+ dipstick) after 20 weeks of gestation	Placental fetal side	Infinium HumanMethylation450 BeadChip array
Jia et al., 2012; China [26]	Case-control study; NR; Independent validation	PE: 9; 29.0 ± 2.9	9; 28.0 ± 2.6	35.0 ± 2.6	39.4 ± 0.2	PE:3	3	New-onset hypertension (SBP >140 mmHg and DBP >90 mmHg) with proteinuria (300 mg/24 h) after 20 weeks of gestation	Placental fetal side	Methylated DNA immunoprecipitation (MeDIP)
Kim et al., 2016; Republic of Korea [27].	Case-control study; NR; NR	PE: 12; 32.3 ± 5.4	12; 31.6 ± 2.4	33.1 ± 3.3	33.1 ± 3.3	PE:12	12	New-onset hypertension (SBP ≥ 140 mmHg or DBP ≥ 90 mmHg) and proteinuria (>300 mg/day or >2+ dipstick) or other adverse conditions after 20 weeks of gestation	Placenta and peripheral blood	Infinium HumanMethylation450 BeadChip array
			30; 22.9 ± 3.2		39.2 ± 1.2		30			

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Table 1 (*continued*)

Author; Data; Country.	Study design; Duration (years); Study validation status	No. of patients; mean age (years mean ± standard deviation)		Mean gestational age (weeks mean ± standard deviation)		Sample size in the global analysis		Diagnosis criteria of PVO	Tissue evaluated	Applied technique
		Case	Control	Case	Control	Case	Control			
Kulkarni et al., 2011; India [28]	Case-control study; 2; NR	TPE: 30; 22.3 ± 3.0 PTPE: 27; 24.00 ± 3.7		TPE: 38.8 ± 0.9 PTPE: 34.0 ± 1.6		TPE: 30 PTPE: 27		New-onset hypertension (SBP >140 mmHg and DBP >90 mmHg) with proteinuria (>1þ or 300 mg/24 h) after 20 weeks of gestation	Placental maternal side	Methylamp Global DNA Methylation Quantification Kit
Li et al.,2020; China [29]	Case-control study; 1; Independent validation	EOPE: 20; 31.6 ± 4.6	TB: 20; 32.8 ± 5.1PB: 20; 31.5 ± 4.9	32.8 ± 1.4	TB: 39.1 ± 0.7; PB: 33.7 ± 1.7	EOPE: 4	TB:4; PB:4	New-onset hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg) and proteinuria (≥0.3 g/day or ≥2+ dipstick) after 20 weeks of gestation	Placental maternal side	Infinium HumanMethylation850 BeadChip array
Liu et al., 2014; China [30].	Case-control study; NR; Internal validation	PE: 27; 30.1 ± 2.7 GDM: 28; 30.8 ± 1.4	30; 29.7 ± 1.8	PE: 37.7 ± 0.9 GDM: 36.2 ± 0.8	36.4 ± 0.5	PE: 27 GDM: 28	30	New-onset hypertension (SBP ≥ 140 and DBP ≥90 mmHg) and proteinuria (≥2+ or ≥ 300 mg in 24 h) after 20 weeks of gestation	Placenta	385 K Human CpG Island plus Promoter arrays
Martin et al., 2015; USA [31]	Case-control study; NR; NR	PE: 19; 28.4 ± NR	17; 28.2 ± NR	38.6 ± NR	32.8 ± NR	PE:19	17	New onset hypertension (≥140/ 90 mmHg) and proteinuria (>300 mg of protein in a 24 h or protein/creatinine ratio of 0.3 mg/dL) after 20 weeks of gestation	Placenta	Infinium HumanMethylation450 BeadChip array
Van Den Berg et al., 2017; The Netherlands [32]	Case-control study; NR; NR	EOPE: 13; 30.0 ± 4.7 LOPE: 16; 33.3 ± 4.5	Uncomp.: 36; 31.8 ± 5.1 FGR: 27; 29.7 ± 6.0 PTB: 20; 31.0 ± 5.1	EOPE: 30.7 ± 3.4 LOPE: 37.4 ± 1.9	Uncomp.: 39.9 ± 1.9 FGR: 38.9 ± 2.6 PTB: 35.4 ± 7.9	EOPE: 13 LOPE: 16	Uncomp.: 36 FGR: 27 PTB: 20	New-onset hypertension (SBP≥140 and DBP ≥90 mmHg) and proteinuria (≥30 mg/mmol) after 20 weeks of gestation	Placental fetal side, UCL, HUVEC	Infinium HumanMethylation450 BeadChip array
Van Den Berg et al., 2020; The Netherlands [33].	Case-control study; NR; NR	EOPE: 13; 30.0 ± 4.7 LOPE: 16; 33.3 ± 4.5	Uncomp.: 36; 31.8 ± 5.1 FGR: 27; 29.7 ± 6.0 PTB: 20; 31.0 ± 5.1	EOPE: 30.7 ± 3.4 LOPE: 37.4 ± 1.9	Uncomp.: 39.9 ± 1.9 FGR: 38.9 ± 2.6 PTB: 35.4 ± 7.9	EOPE: 13 LOPE: 16	Uncomp.: 36 FGR: 27 PTB: 20	New-onset hypertension (SBP≥140 and DBP ≥90 mmHg) and proteinuria (≥30 mg/mmol) after 20 weeks of gestation	Placental fetal side, UC-WBC, HUVEC	Infinium HumanMethylation450 BeadChip array
Wang et al., 2019; China [34]	Case-control study; NR; Internal validation	EOPE: 30; 31.23 ± 5.26	30; 30.1 ± 4.0	33.7 ± 3.5	39.1 ± 2.3	EOPE:20	20	New-onset hypertension (SBP ≥140 mmHg and DBP ≥90 mmHg) and proteinuria (≥300 mg/day from 24 h) after 20 weeks of gestation	Placental fetal side	Infinium HumanMethylation450 BeadChip array
Wilson et al., 2015; Canada [35]	Case-control study; NR; Internal validation	EOPE: 20; NR LOPE: 11; NR LOPE + IUGR: 8; NR IUGR: 10; NR	37; NR	NR	NR	EOPE: 20 LOPE: 11 LOPE + IUGR: 8 IUGR: 10	37	New-onset hypertension (SBP >140 and DBP >90 mm Hg) and proteinuria (>300 g/day) after 20 weeks gestation	Placenta	Infinium HumanMethylation450 BeadChip array
Wilson et al., 2018; Canada [36]	Case-control study; NR; Independent validation	EOPE: 22; 33.3 ± NR LOPE: 18; 34.0 ± NR IUGR: 11; 34.3 ± NR	PTC: 24; 32.5 ± NR TC: 19; 34.9 ± NR	EOPE: 32.0 ± NR LOPE: 37.4 ± NR IUGR: 36.6 ± NR	PTC: 32.6 ± NR TC: 38.4 ± NR	EOPE: 22 LOPE: 18 IUGR: 11	PTC: 24 TC: 19	New-onset hypertension (BSP >140 mmHg and >90 mmHg) and proteinuria (>300 mg/day) after 20 weeks gestation ii) HELLP syndrome without hypertension or proteinuria; or iii) eclamptic seizure without previous hypertension or proteinuria	Placental fetal side	Infinium HumanMethylation450 BeadChip array
Xuan et al., 2016; China [37]	Case-control study; NR; NR	PE: 6; 29.8 ± NR	6; 30.2 ± NR	38.0 ± NR	39.5 ± NR	PE: 6	6	Not reported	Placenta	NimbleGen Human DNA Methylation 3 × 720 K CpG Island Plus RefSeq Promoter Microarray
Yan et al., 2013, China [38]	Case-control study; 1; NR	PE: 30; 28.5 ± 3.8	30; 27.9 ± 3.0	36.1 ± 2.3	39.2 ± 0.8	PE:5	5	Not reported	Placental maternal side	Agilent Human CpG Island Microarray
				35.0 ± 0.8	39.0 ± 0.2	PE:8	16			

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Table 1 (*continued*)

Author; Data; Country.	Study design; Duration (years); Study validation status	No. of patients; mean age (years mean ± standard deviation)		Mean gestational age (weeks mean ± standard deviation)		Sample size in the global analysis		Diagnosis criteria of PVO	Tissue evaluated	Applied technique
		Case	Control	Case	Control	Case	Control			
Yeung et al., 2016; Australia [39]	Case-control study; 13; Internal validation	PE: 8; 28.0 ± 2.0	16; 32.0 ± 1.0					New-onset hypertension (SBP ≥140 mmHg and/or DSP ≥90 mmHg) and proteinuria (2 on dipstick or 300 mg/24 h) or renal insufficiency, liver disease, neurological problems, and hematological disturbances after 20 weeks of gestation	Placental fetal side	Infinium HumanMethylation450 BeadChip array
Yuen et al., 2010; Canada [40]	Case-control study; NR; Independent validation	EOPE: 4; 33.3 ± NR LOPE: 4; 36.4 ± NR IUGR: 4; 37.3 ± NR	EOPE: 4; 36.0 ± NR LOPE: 5; 37.2 ± NR C: 5; 35.9 ± NR	EOPE: 31.0 ± NR LOPE 38.0 ± NR IUGR 33.0 ± NR	EOPE: 29.6 ± NR LOPE: 38.0 ± NR C: 37.8 ± NR	EOPE: 4 LOPE: 4 IUGR: 4	EOPE: 4 LOPE: 5 C: 5	(1) New-onset hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg) and proteinuria (≥0.3 g/day or ≥2+ dipstick) after 20 weeks gestation (2) Sibai's criteria (3) British Eclampsia Survey Team criteria to define eclampsia	Placental fetal side	Illumina GoldenGate Methylation Cancer Panel I array
Zhu et al., 2015; China [41]	Case-control study; NR; Independent validation	LOPE: 20; 27.7 ± NR	20; 26.7 ± NR	38.2 ± NR	38.7 ± NR	LOPE: 20	20	New-onset hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg) and proteinuria (≥0.3 g/day or ≥2+ dipstick) after 20 weeks of gestation	Placenta	Methylated DNA immunoprecipitation + deep sequencing

Abbreviations: C, control; EOPE, early-onset PE; FGR, normotensive fetal growth restricted; GDM, gestational diabetes mellitus; HUVEC, human umbilical vein endothelial cells; IUGR, intrauterine growth restriction; NR, Not reported; LOPE, late-onset PE; nIUGR, normotensive Intrauterine growth restriction; PE, preeclampsia; PTB, normotensive preterm birth; PTC, preterm control; PTPE, preterm preeclampsia; TC, term control; TPE, term preeclampsia; UCL, umbilical cord leukocytes; UC-WBC, umbilical cord white blood cells; Uncomp., Uncomplicated; USA, United States of America.

hypermethylated, while regions associated with *CRH*, *CYP11A1*, *HSD3B1*, *TEAD3* and *CYP19* were hypomethylated in EOPE [25].

The comparison between EOPE and normotensive preterm births (PB) revealed 697 differentially methylated genes (67% hypomethylated) in placenta of EOPE. One differentially methylated CpG was found in EOPE compared to uncomplicated pregnancies controls and normotensive pregnancies with fetal growth restricted in placental tissue [24]. Significant differences in CpG methylation of circadian clock genes were found to be tissue-specific, in umbilical cord leukocytes (31), placenta (7), and HUVEC (1).

In placental tissue, the circadian clock genes *AKT1*, *BHLHE41*, *CSKN1E*, *PRDX1*, and *RORA* were hypomethylated in EOPE and significantly different from spontaneous PB [32]. The *CDH13*, *IGF2BP2* and *LSAMP* genes were also hypomethylated in placental tissue of EOPE and different from spontaneous PB. Notably, *CDH13* was hypermethylated in umbilical cord white blood cells of EOPE, and it was differentially methylated in EOPE compared to all study groups (uncomplicated controls, fetal growth restriction and PB) [33]. Other studies using the same set of samples found no difference in LOPE compared to all groups [24,31–33].

Noteworthy, six studies that examined the fetal side of placenta in EOPE or LOPE compared to controls had similar conclusions [20,24,25,32–34], and the number and methylation status of genes or regions are described above. In summary, five of these studies showed a pattern of hypomethylation in EOPE [20,24,32–34]. Differentially methylated CpGs sites were found in EOPE compared to controls [20,25,34]. EOPE were also different from spontaneous PB, fetal growth restriction and uncomplicated controls, and these differences were more evident when EOPE was compared to spontaneous PB controls [32,33]. Moreover, EOPE differed from spontaneous PB controls but not from fetal growth restriction or uncomplicated controls [24]. Notably, the differentially methylated sites were associated with cardiovascular system, stress pathways, steroid production and circadian clock genes. These findings suggest that EOPE have an increased placental dysregulation of DNAm, and support the hypothesis that EOPE and LOPE have different etiologies.

Most the 1.703 CpG sites were hypomethylated in EOPE compared to PB. Only five sites were differentially methylated between LOPE and term controls, which were not unique to LOPE [36]. Three studies showed that DNAm is affected by gestational age and fetus gender, which is a potential bias for DNAm analysis [20,25,40]. A total of 21

Fig. 2. Overlapping genes found in the included studies from PE pregnant compared to control groups (not specified the side of placental tissue evaluated).

overlapping genes were found among these studies (Fig. 4; Supplementary Table 1B), which were related to relevant pathways for PE (Fig. 3B).

Only one study defined preterm and term delivery for PE [19], and found 229 differentially methylated genes between controls and PE (term and preterm, 89.6% hypermethylated in PE) but none differentially methylated in term PE. Nevertheless, 1.448 differentially methylated genes were found between control and preterm PE (91.8% hypermethylated). Moreover, 118 differentially methylated genes were found between term and preterm PE (91.6% hypermethylated in term PE) [19].

3.4. Global DNAm in maternal side of placenta from PE pregnant compared to controls

Four studies considered the maternal side of placenta for DNAm analysis, and compared PE pregnant [22,29,38], term and preterm PE [28] to control, and one compared PE pregnant to PB and term birth (TB) [29].

While altered methylation levels were reported for 23 genes (52% hypermethylated) in PE [38], 10 hypomethylated CpG sites were identified in PE, and 49 differentially methylated CpG sites (78% hypomethylated) in EOPE [22]. The mean of global DNAm was higher in preterm and term PE compared to control, but the increase was significant only for term PE. Global DNAm was significant associated with SBP and DBP in term PE [28].

Global DNAm levels in placentas of PE were similar to PB, and the levels for PE and PB were higher than TB and, therefore, placental

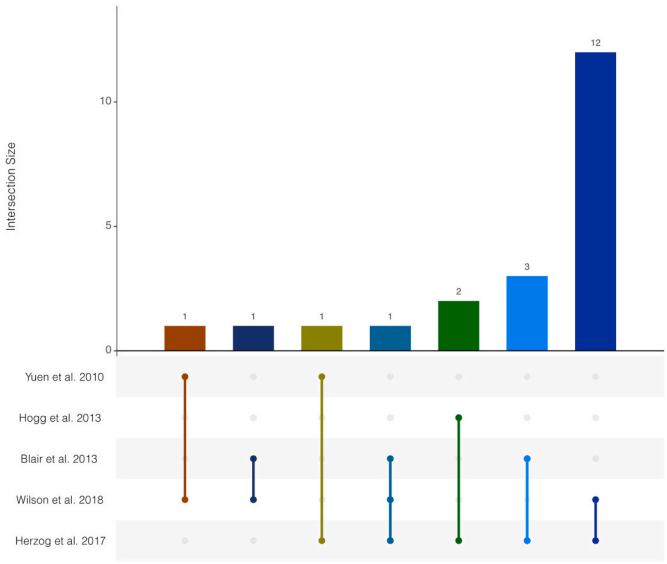

Fig. 4. Overlapping genes in the included studies in fetal side of placenta from EOPE and LOPE compared to control groups.

methylation levels were related to gestational age. Moreover, 2.400 differentially methylated genes were found between PE and TB (75.7% hypermethylated in PE), and 808 differentially methylated genes between PE and PB (68.8% hypomethylated in PE). Finally, 3.969

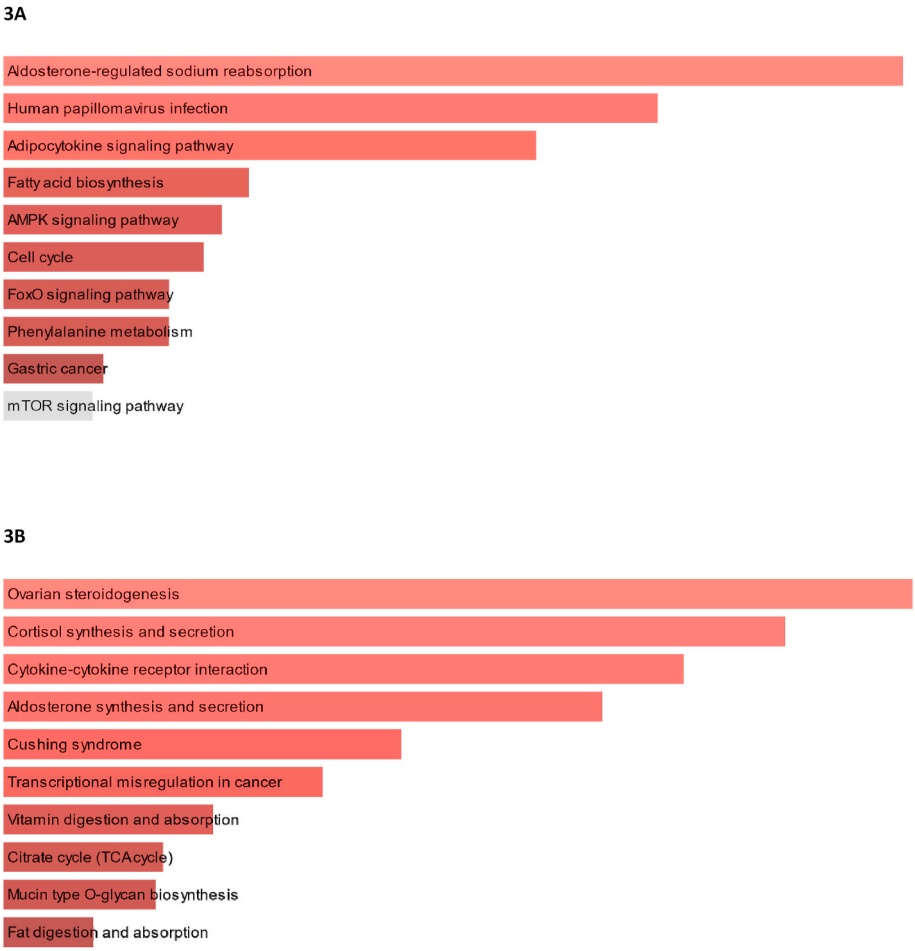

Fig. 3. Pathway analysis of the overlapping genes found among the included studies. **(A)** For studies performed with placental tissue from PE pregnant compared to controls (not specified the side of placental tissue). **(B)** For studies performed with fetal side of placenta from EOPE and LOPE compared to controls.

differentially methylated genes were found between PB and TB (80.4% hypermethylated in PB) [29].

4. Discussion

PE account for up to 26% of maternal deaths worldwide and is the main complication of pregnancy [3]. Therefore, it is important to understand the mechanisms that may lead to PE. This study is the first systematic review that assessed the role of global DNAm in the pathophysiology of PE, with focus on both maternal and fetal placental tissues. Our novel findings highlighted that the included studies show highly heterogeneous results, and that the differences between PE and controls are specific to genes or differentially methylated regions. These differences are more evident when EOPE and preterm PE were compared to controls, rather than LOPE and term PE. Most of the studies have a pattern of hypomethylation in placental tissue from PE pregnant compared to controls. Conversely, the placental methylome of normal pregnancy is hypermethylated outside the partially methylated domains [12].

It is important to highlight that tissues from the fetal or maternal side of placenta may account for the heterogeneous results of DNAm found among the studies included in this systematic review. We have also included studies that did not specify which side of placental tissue was analyzed, but their results were considered in a separate section. Therefore, the design of upcoming studies should focus on this methodological issue, which could help the understanding of the effects of different levels of DNAm on the different sides of placenta.

4.1. Confounding factors of DNA methylation in PE

The heterogeneous findings can be due to the multifactorial nature of PE, biological variation, study design and/or statistical analysis. Studies that examined whether confounding factors (age and fetal gender) would affect DNAm are discordant: some found an effect of maternal age and gestational age [20,22,31,35,39,40,42], but others did not [21]; some showed an effect of fetal gender [21,22,39,40,42], but another did not [21].

Placental DNAm showed a difference according to gestation stages [43], with a hypomethylation in early pregnancy and an increased methylation in later pregnancy [44]. A progressive increase of DNAm levels were found from the first to third trimesters, mainly in genes related to immune regulators, which reflect the placental immune modulation during pregnancy [45]. Therefore, the ideal design is to perform experiments with samples matched for both maternal and gestational age or follow-up studies to avoid and exclude this potential bias, and the exclusion of probes located in the sex chromosomes is required during the analysis, mainly in the X chromosome.

Genetic variation in different populations is another confounding factor that can alter DNAm in specific regions. For instance, probes that overlap with single nucleotide polymorphisms (SNPs) are usually excluded during bioinformatic analysis of global DNAm. However, SNPs that occur within CpG dinucleotide (CpG-SNPs) may lead to alteration of methylation in a region and thereby affect gene expression [46]. The correlation of CpG-SNPs with complex diseases is well-described [47], including type 2 diabetes [48] and cancer [49]. However, the association between CpG-SNPs and PE has not been examined. Further studies focused on potential CpG-SNPs and promoter region methylation may help to interpret findings from candidate gene association studies in PE for *NAMPT* [50,51], *NOS2* [52], *NOS3* [53] and *TIMP1* [54]. Potential CpG-SNPs of these candidate genes were also studied in subgroup of PE who were nonresponsive to antihypertensive therapy, and these follow-up epigenetic studies may further help to reveal targets for PE therapy [55,56].

4.2. Use of DNA methylation as a prognostic marker in PE

The placenta is a complex temporary organ that provides the fetal development, and composed by several number of cells and exhibiting regional variations. Placenta is divided into maternal and fetal sides, and the later carry the paternal genome [57]. A cohort analysis found that 35% of the genetic predisposition to PE is attributed to maternal characteristics and 20% to fetal effects that include the paternal genome, which suggest a genetic influence on PE development. The remaining is attributed to couple, environment and unmeasured factors [58].

DNAm is a tissue-specific epigenetic mark [59]. Notably, it is difficult to interpret DNAm data obtained from different pregnant related tissues and cell cultures. For example, only EOPE is related with levels of DNAm of circadian clock and clock-controlled genes in placental and newborn tissues. The same samples of placenta showed a decrease of differentially methylated CpG sites compared to umbilical cord leukocytes [32]. Approximately 75% of differentially methylated CpG sites overlapped between maternal white blood cell in first trimester compared to fetal side of placenta [18]. However, placental tissue showed an increase in differentially methylated CpG loci compared to peripheral blood at delivery [27].

Unfortunately, studies including the maternal side of placenta are scarce, and the DNAm data in the maternal and fetal sides of placenta showed highly discordant results. Therefore, to establish which placental side would be most suitable for studies focused on diagnostic markers for PE is unclear. Moreover, it is difficult to extrapolate the results from placenta to a description of diagnostic markers based on DNAm in the maternal blood. Further studies comparing DNAm between the maternal side of placenta and peripheral blood throughout gestation could help to establish novel diagnostic markers for PE.

4.3. Role of DNA methylation in the pathophysiology of PE

Our review highlighted a differential DNAm pattern in EOPE and preterm PE. These findings suggest that methylation have a pathophysiological role on early stages of pregnancy, and that placental epigenetic dysregulation may affect the initial steps of these early severe forms of PE. Indeed, EOPE was shown to exhibit a more severe form of PE [5], and a remarkable placental dysfunction [4].

The causes of PE are heterogeneous. Usually, there is a failure in the remodeling of the spiral uterine arteries by the trophoblastic cells, which can be triggered by an exacerbated immune response at the maternal-fetal interface [60]. These events are associated to poor placental perfusion, leading to physiological changes and gene expression in response to hypoxia and reoxygenation [61].

Transcriptional and epigenetic mechanisms control placental development and cytotrophoblast differentiation, and are activated by oxygen levels during pregnancy [62]. In primary cultures of human cytotrophoblasts and syncytiotrophoblasts, CpGs sites became hypermethylated in cytotrophoblasts exposed for 24 h to <1% oxygen. However, these same sites became hypomethylated upon differentiation of cytotrophoblast into syncytiotrophoblasts [63], and they showed hypomethylation in EOPE [20]. These findings suggest an imbalance of these cells in the expression of hypoxia-related genes in PE.

The number of overlapping genes among studies is low, ranging from 48 in PE versus controls in maternal side of placenta to 21 in EOPE, LOPE versus control in fetal side of placenta, and only *LIMCH1* is repeated in three studies. Some genes are well characterized in hypoxia and trophoblast invasion (*TERT*, *ALDH1A3*, *IRS1*), which are crucial during PE development. Other gene families frequently appear and are differentially methylated between study groups, such as *CXCL, SERPIN* and *TMEM*, which are involved in angiogenesis, inflammation, migration, cell proliferation, and invasion in types of cancer [64–66].

Altered methylation of specific genes was shown in PE (Supplementary Table 1). *LEP* [67], *PAPPA2* [68–70] and *YWHAQ* [71] showed increased expression and decreased DNAm in preeclamptic placentas.

Additionally, LEP and PAPPA2 protein was altered in maternal serum before the onset of PE symptoms [72,73], and had an increased expression in placentas from PE pregnant from the third trimester [42]. A decreased expression of *PLXNB1* in preeclamptic placentas may be responsible for the deficiency in Met signaling and in PE development [74].

The increased expression of *HSF1* in endothelial cells from term PE suggests a possible protective role as stress specific natural adaptive response against the generated stress [75,76]. *CRH*, *CYP11A1, TEAD3* showed an increased DNAm in preeclamptic placentas, suggesting a hormonal involvement in PE [25]. Moreover, the high expression of *CYP11A1* induces trophoblast autophagy, inhibits trophoblastic invasion and proliferation, as well as increases apoptosis [77,78].

Altered serum levels and placental tissue expression of CXC chemokines, including *CXCL9*, *CXCL10* and *CXCL12*, which participate in several processes triggered by PE, such as neovascularization, embryonic development and inflammatory responses, suggest their role in pathogenesis of PE [79]. Notably, these genes are related to ovarian steroidogenesis, cortisol synthesis and secretion, and cytokine-cytokine receptor interaction (Fig. 3B).

The pathway related to the overlapping genes is already described on process associated with PE, as trophoblast invasion. During pregnancy, AMP-activated protein kinase (AMPK) is necessary for the correct placental differentiation, nutrient transportation, maternal and fetal energy homeostasis, and protection of the fetal membrane. This activation is required for placental differentiation and vasodilation of uterine artery. Therefore, AMPK deficiency induces poor placentation, which results in angiogenic imbalance [80].

Many signaling pathways are involved in PE and are affected by oxidative stress, such as forkhead transcription factors of the O class (FOXO) family. Oxidative stress is responsible for the initiation or progression of pathological process in female reproduction, such as PE. The normal level of reactive oxygen species plays an important regulatory role through various signaling transduction pathways in folliculogenesis, corpus luteum oocyte maturation and feto-placental development, and FOXO is a bond of the different signaling pathway, playing an important role in signaling networks [81]. Insufficient spiral arteries remodeling in PE was associated to higher placental oxidative stress and the generation of oxidized fatty acids [82], as well as an increase of placental dimethyl acetal fatty acid [83], leptin, chemerin and fatty acid binding protein-4 in all pregnancy trimesters and forms of the disease [84].

5. Conclusion

In this systematic review, we found that there are significant differences on global DNA methylation levels between PE and controls, and a pronounced effect on DNAm of specific genes in PE, especially in EOPE and preterm PE. However, these studies should be replicated using the same placental tissues, and the same techniques and pipelines for bioinformatic analysis, in order to reduce variations between the studies. Biological variation cannot be avoided, so it is important to carry out studies in different populations, since the available results are mainly focused on samples from North America and China, and studies from literature have already shown the role of CpG-SNPs on epigenomic changes.

Authors contributions

JOC, IMCA, JAGT, KBG, and MRL have made substantial contributions to the conception or design of the work. All authors have made contributions to the analysis or interpretation of data; All authors have drafted the manuscript or revised it critically for important intellectual content; All authors have read and approved the final version.

Funding

This work was supported by the National Council for Scientific and Technological Development (CNPq/Brazil) [Grant #312599/2019-6], Minas Gerais Research Foundation (FAPEMIG/Brazil) [APQ-01960-18] and the Coordination for the Improvement of Higher Education Personnel (CAPES/Brazil) [Finance Code 001].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2020.09.004.

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