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Depressive symptoms in mid-pregnancy are associated with DNA methylation marks in the placenta

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ABSTRACT

Introduction: Maternal depressive symptoms during pregnancy are associated with a range of adverse neurodevelopmental outcomes in offspring. DNA methylation, influenced by both genetic and environmental factors, has been identified as a potential mechanism mediating these associations. Because the placenta serves as an interface between mother and fetus, epigenetic marks in this tissue may provide insights into how the intrauterine environment influences child outcomes. The present study aimed to determine whether maternal Edinburgh Postnatal Depression Scale (EPDS) scores in mid-pregnancy (20 + 6 to 30 + 0 gestational weeks) are associated with placental DNA methylation marks and whether these marks differ from those associated with EPDS scores in early pregnancy.

Methods: In this descriptive analysis, the DNA methylomes of placental samples collected at birth from 91 women in the FinnBrain cohort were analyzed using reduced representation bisulfite sequencing. The association of EPDS with DNA methylation of each CpG site was examined using the PQLseq.

Results: A total of 1345 CpG sites in 381 genes were identified with FDR ≤0.05, suggesting a potential association with mid-pregnancy EPDS scores. The most significant methylation marks were found in the following genes: *PTPRO, RPTOR, TBX1, SLC7A4, FBRSL1,* and *ADAMTS17*. When accounting for early pregnancy EPDS scores, 272 methylation marks remained significant. The genes were functionally enriched in biological pathways such as cell adhesion, nervous system development, and anatomical structure development. Compared with methylation marks associated with early pregnancy EPDS scores, similar methylation marks were found in 29 distinct genic regions. Further studies are needed to determine whether these methylation marks mediate the effects of maternal depressive symptoms on the fetus.

1. Introduction

The developmental origins of health and disease (DOHaD) hypothesis suggests that the perinatal environment during critical windows of early development can affect disease outcomes later in life [1]. Environmental factors and chemical exposures may lead to fetal programming of adult disease, e.g., cardiovascular disease [2,3]. This developmental plasticity appears to be at least partly mediated by

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epigenetic processes [4]. Maternal depression during pregnancy is associated with long-term effects on a child's neurodevelopmental outcomes [5], such as impaired cognitive function [6], poor motor function [7], and delayed early development [8]. Even subclinical maternal depressive symptoms increase the risk of emotional problems in the offspring [9]. Maternal depression or depressive symptoms during pregnancy predict a greater occurrence of psychiatric problems across childhood and adolescence [10–12]. Furthermore, maternal prenatal depression or depressive symptoms have been associated with variations in infants' functional brain MRI, structural MRI, or EEG [13–17]. In some studies, maternal depression during pregnancy has been associated with pregnancy complications such as premature labor [18], low birth weight [19], and preeclampsia [20]. Prenatal depression is a global issue, with prevalence varying among studies from 3% to 20% [21–24].

The placenta is one mediating link between the intrauterine environment and offspring outcomes. For example, maternal anxiety is associated with the downregulation of placental *HSD11B2* [25], and antenatal exposure to betamethasone is associated with lower methylation in the enhancer of the *FKBP5* gene [26]. Both genes have been linked to negative effects on offspring neurobehavior [27,28]. Furthermore, a few epigenome-wide association studies (EWAS) have revealed that certain methylation marks in the placenta are associated with maternal depressive symptoms [29–32]. The time of exposure may play a role [33]. A clear temporal window on when prenatal depression or depressive symptoms mediate effects on the fetus remains unclear, but mid-pregnancy is of special interest, as depressive symptoms in the second trimester alone have been associated with accelerated placental aging [34], and diminished fetal growth [35].

We previously showed that maternal depressive symptoms during early pregnancy, measured at 14 gestational weeks (GW), are associated with DNA methylation marks in the promoters or bodies of genes that are functionally enriched in neuronal and brain development [32]. The analysis was conducted with reduced representation bisulfite sequencing (RRBS), which profiles CpG-rich areas in the genome [36].

The present descriptive study aims to assess the following.

1. Are maternal Edinburgh Postnatal Depression Scale (EPDS) scores assessed at mid-pregnancy (from 20 + 6 to 30 + 0 GWs) associated with placental DNA methylation marks?
2. Are the identified methylation marks enriched in any functional pathways?
3. Does the methylation profile of these marks differ from those associated with early pregnancy depressive symptoms?

We used the penalized quasilikelihood for sequencing count data package, which implements a generalized linear mixed model to find out if CpG sites were associated with maternal EPDS scores in mid-pregnancy.

2. Materials and methods

2.1. Cohort

This study is part of the population-based FinnBrain Cohort (www.finnbrain.fi), whose recruitment procedures have been previously described [37]. The Ethics Committee of the Hospital District of Southwest Finland approved this study, and participants provided written informed consent. Maternal depressive symptoms were assessed at three time points during pregnancy, GWs 14, 24 and 34, using the validated EPDS [38,39]. This 10-item questionnaire, scored from 0 to 3 per item, produces a total score ranging from 0 to 30, with higher scores indicating more pronounced depressive symptoms. Placental samples were obtained from 252 participants with singleton pregnancies, collected from the maternal side after removing the decidua, and then cryopreserved.

Samples were excluded due to unsuccessful freezing or if the time interval between delivery and freezing exceeded 90 min. Additionally, samples from premature (<35 GW) or post-term (>42 GW) delivery or involving antenatal systemic glucocorticoid treatment were excluded. Placentas of participants with an EPDS score ≥ 10 at any of the three assessment points (n = 48) were selected. This threshold was chosen based on literature reviews as representing high depressive symptoms [40,41]. Placentas of participants with consistently low EPDS scores (<5) throughout pregnancy and who resembled those with higher EPDS scores regarding delivery mode (vaginal or cesarean section), gestational diabetes (yes or no), sex of the newborn, maternal BMI (kg/m2), and smoking status (yes/no) were subsequently included. Participants with low EPDS scores who reported anxiety or depression before pregnancy were excluded. After these steps, 96 placental samples remained and were analyzed: 48 with an EPDS score ≥ 10 at any of the three assessment points and 48 with an EPDS score <5 throughout pregnancy. To analyze the potential effects of depressive symptom exposure in mid-pregnancy on the placental epigenome, only the 92 participants who had EPDS scores available from the second assessment point (ranging from 20 + 6 to 30 + 0 GWs) were included. One placental sample was later found to have an incorrect annotation and was therefore excluded from the analysis; the remaining 91 participants were included in the final analysis (Fig. 1). Due to the sample size (n = 91), this study is descriptive. The placental DNA methylation levels and EPDS scores were analyzed as continuous variables.

Eighty-two individuals in this study overlapped with our previous study [32]. Their DNA methylation data were generated in the same batch and assay workflow, which ensured comparability across all samples and were used to identify stage-specific and common methylation marks associated with depressive symptoms. See Fig. 1 for the inclusion/exclusion process.

2.2. Tissue description

Placental tissue samples were collected from the maternal side after removal of the decidua. Shortly after delivery, research staff at Turku University Hospital obtained four samples located 2 cm from the umbilical cord insertion. Without washing, the samples were immediately snap-frozen in liquid nitrogen within 5 to 70 min post-delivery and stored at −70 °C until DNA extraction. Only one sample was used for DNA extraction.

2.3. Nucleic acid extraction and reduced representation bisulfite sequencing

Qiagen's AllPrep DNA/RNA/miRNA Universal Kit was used to isolate DNA from the placental samples. RRBS was chosen to enable high-resolution discovery and analysis of DNA methylation in CpG-rich regulatory areas. RRBS libraries were prepared from 500 ng of genomic DNA as previously described [36,42], quantified with Qubit using the Qubit™ dsDNA HS Quantification Assay Kit (Invitrogen) and quality controlled with Fragment Analyzer (Agilent). The Illumina NovaSeq 6000 platform was used to perform next-generation sequencing using 2 x 50 bp chemistry.

2.4. Raw data processing and detection of DNA methylation marks

FastQC v0.11.8 [43] and MultiQC v1.7 analyses [44] confirmed high raw data quality. Conversion efficiency, assessed using a lambda spike-in control, was >98.7% for all samples. Raw reads were trimmed with TrimGalore! v0.6.4 and mapped to the GRCh38 reference genome with Bismark v 0.22.2. (no mismatches allowed). A range of 9,523,273 – 49,702,651 aligned reads with a mapping efficiency of 67.6-78.0% were obtained for further analyses. CpG sites with ≥ 10x coverage in ≥ 90% samples were included. CpG sites in the permille with the highest coverage were excluded; 1,586,412 CpG sites were left for the analysis. Sex chromosomes were removed from the analysis, leaving 995,710 CpG

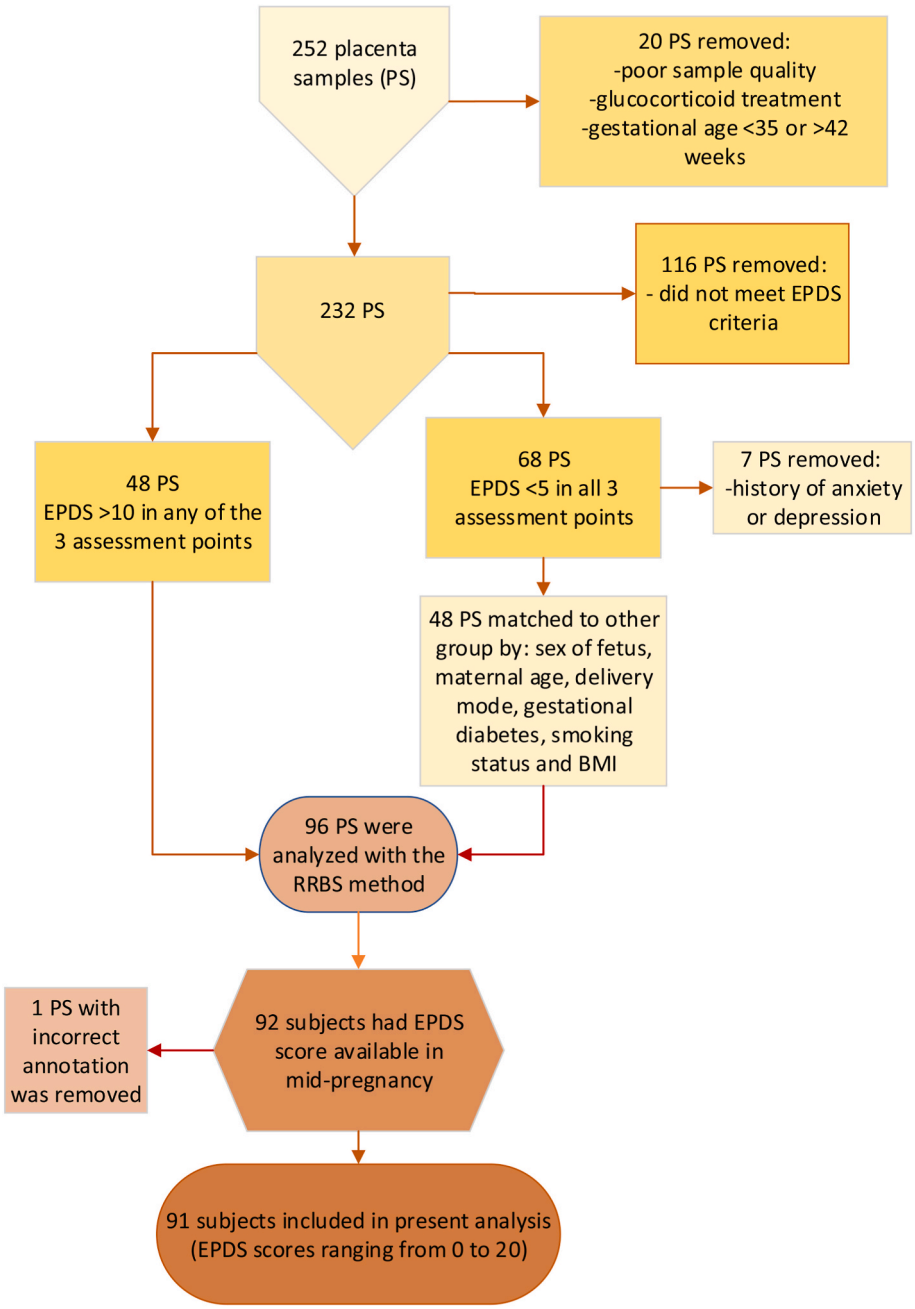

Fig. 1. Flow chart illustrating the sample selection process for this study.

sites for the analysis. Information on neighboring CpG sites was included using the radmeth algorithm (adjust -bins 1:200:2) as previously described [45,46]. Samtools v1.9 and R v3.6.2 and v4.02.2 were used. CpG sites with statistically significant (FDR $\leq$0.05) methylation associated with maternal EPDS scores are referred to as "methylation marks" in this study. The beta coefficients represent the change in the log-odds of methylation per unit increase in the continuous EPDS score, indicating the direction and statistical strength of the association.

2.5. Statistical analysis

Placental DNA methylation levels and EPDS scores (0-20) were used as continuous variables in the statistical model. The association of EPDS with DNA methylation of each CpG site was examined using the PQLseq v1.10 package [47]. In the primary analysis, the model included the following covariates as fixed effects to account for factors potentially influencing DNA methylation: maternal age (years), pre-pregnancy BMI, smoking (yes/no/quit during the first trimester), gestational diabetes (yes/no), mode of delivery (vaginal/vaginal breech/vacuum extraction/elective cesarean section/emergency cesarean section), GW at birth, sex of the offspring, and time from delivery of the placenta until the placenta sample was frozen. To examine mid-pregnancy specific EPDS association with placental DNA methylation, early pregnancy EPDS scores were included as a covariate.

2.6. Annotation and functional analyses

Annotatr package v1.16.0 [48] and GREAT tool v.4.0.4 [49] were used to localize DNA methylation marks in genic and intergenic elements. Genomic features were annotated using a hierarchical, mutually exclusive approach. Functional enrichment analysis was performed using g:Profiler version e111_eg58_p18_30541362 with default settings

[50].

To compare methylation profiles and functional pathways in mid-pregnancy with those in early pregnancy, we analyzed the methylation marks identified in our previous study [32]. Our previous study (n = 92) included the same participants, apart from five lacking the EPDS questionnaire at the second assessment point. The present study comprised four additional participants who had not answered the EPDS questionnaire at the first assessment point.

3. Results

3.1. Sample characteristics

The study included 91 participants recruited from the Southwest Finland Hospital District and the Åland Islands. The cohort was primarily of Finnish Caucasian ancestry, with one participant of other European ancestry. Forty-seven percent of the participants were primiparous. Medication use was reported by a few participants (antidepressants n = 3, inhaled corticosteroids n = 3 and antihistamines n = 2) and was not included in the analysis. Of participants with depressive symptoms (EPDS ≥ 10), 18 showed symptoms in early pregnancy only, 12 in mid-pregnancy only, and 9 at both timepoints. Demographic details of the participants are shown in Table 1.

3.2. Mid-pregnancy EPDS scores are associated with methylation marks in the placental genome

In the primary analysis, 1345 methylation marks were associated with EPDS scores in mid-pregnancy, with an FDR of ≤0.05 (Fig. 2A). Of these, 24% were located in promoters, 23% were 1 to 5 kb distance from a transcription start site (TSS), 20% were in introns, 20% in intergenic regions, 12% in exons, and 1% in the 5' untranslated region (UTR). After adjusting for covariates, higher depressive symptoms were associated with lower methylation in 62% of the methylation marks and higher methylation in 38% (full list of methylation marks in Additional File Table 1). Fig. 2B illustrates the association between EPDS scores and the methylation of CpG sites in different genomic locations.

Methylation marks were found in 133 genes with annotatr tool and 355 genes with GREAT tool. Merging regions with the same gene loci yielded a total of 379 genic regions with methylation marks. The seven most significant (by FDR) methylation marks were identified in the coding regions of *PTPRO* (protein tyrosine phosphatase receptor type O), *RPTOR* (regulatory associated protein of MTOR complex 1), *TBX1*(T-box transcription factor 1), *SLC7A4* (solute carrier family 7 member 4), *FBRSL1* (fibrosin-like 1), *ADAMTS17* (ADAM metallopeptidase with thrombospondin type 1 motif 17), and *EPHA6* (EPH receptor A6).

In conclusion, mid-pregnancy EPDS scores are associated with DNA methylation marks in the placental genome.

3.3. Covariates influenced 301 methylation marks

Of the methylation marks associated with EPDS scores assessed at mid-pregnancy, 78% were not influenced by any of the covariates used. Among the 1345 methylation marks identified, only 301 methylation marks were affected by one or more covariates. Mode of delivery influenced 99 methylation marks, of which planned cesarean section affected 20 methylation marks, including methylation marks in the genic region of *PTPRO*. A longer interval between delivery and freezing of the placenta was associated with 78 methylation marks. The sex of the fetus influenced 60 methylation marks found in the genic regions of 10 different genes. A full list of genes affected by covariates is provided in Additional File Table 2.

3.4. Methylation marks specific to mid pregnancy

Adjusting for early pregnancy EPDS scores, the PLQSeq analysis was repeated, and 272 of the methylation marks remained significant. These marks were found in the coding regions of 63 genes using the annotatr tool and the GREAT tool. The seven most significant methylation marks by FDR, were found in the areas of genes *ARHGAP23* (Rho GTPase

Table 1
Sample characteristics and mean EPDS scores across covariates. Percentages and SDs are rounded to the nearest 0.1. Education categorization: Low = Upper secondary education or lower (including high school/vocational school), Mid = University of Applied Sciences degree, High = University degree (Bachelor's, Master's, or Doctoral).

EPDS score		Early pregnancy	Mean EPDS score (SD)	Mid-pregnancy	Mean EPDS score (SD)
Total	Count	92		91	
	Median (range)	5 (0-19)	6.0 (5.0)	3 (0-20)	5.5 (4.9)
Maternal variables					
Age					
	Median (range)	31 (19-41)		31 (19-41)	
Ethnicity					
	Finnish	90 (98.9%)		90 (98.9%)	
	Other European	1 (1.1%)		1 (1.1%)	
Education					
	Low	27 (29.3%)	7.7 (5.2)	24 (26.4%)	7.3 (4.8)
	Mid	28 (30.4%)	4.4 (4.4)	37 (40.6%)	4.6 (4.2)
	High	37 (40.2%)	6.0 (4.9)	28 (30.8%)	4.7 (5.2)
	NA	0 (0%)		2 (2.2%)	13.5 (3.5)
Prepregnancy BMI					
	Median (range)	22.75 (17.22-43.63)		23.03 (17.22-43.63)	
	NA	2		2	
Maternal smoking					
	No current	81 (88.0%)	5.8 (4.8)	79 (86.8%)	5.2 (4.5)
	Cessation in 1st trimester	8 (8.7%)	8.8 (6.3)	8 (8.8%)	8.3 (7.2)
	Smoking after 1st trimester	3 (3.3%)	5.7 (4.5)	4 (4.4%)	7.5 (6.9)
Gestational diabetes					
	Yes	7 (7.6%)	5.7 (5.0)	8 (8.8%)	6.9 (6.3)
Delivery mode					
	Vaginal	66 (71.6%)	6.2 (5.2)	68 (74.7%)	6.1 (5.3)
	Planned C-section	11 (12.0%)	6.6 (4.7)	11 (12.1%)	4.7 (3.1)
	Vacuum extraction	10 (10.9%)	5.0 (4.0)	7 (7.7%)	3.9 (3.8)
	Breech presentation (vaginal delivery)	3 (3.3%)	1.7 (2.4)	3 (3.3%)	0.3 (0.6)
	C-section during planned vaginal delivery	2 (2.2%)	8.0 (5.0)	2 (2.2%)	5.0 (4.2)
Newborn variables					
Sex of offspring					
	Male	53 (57.6%)	6.4 (4.9)	51 (56.0%)	5.3 (4.4)
	Female	39 (42.4%)	5.6 (5.1)	40 (44.0%)	5.8 (5.6)
Birthweight					
	Median (range)	3490g (2365-4580g)		3410g (2365-4550g)	
Gestational age at delivery days (weeks)					
	Median (range)	39 + 3 (35 + 6 to 42 + 0)		39 + 3 (35 + 6 to 42 + 0)	
Placental					
Time to freezing (min)					
	Median (range)	40 (5-70)		38 (5-70)	

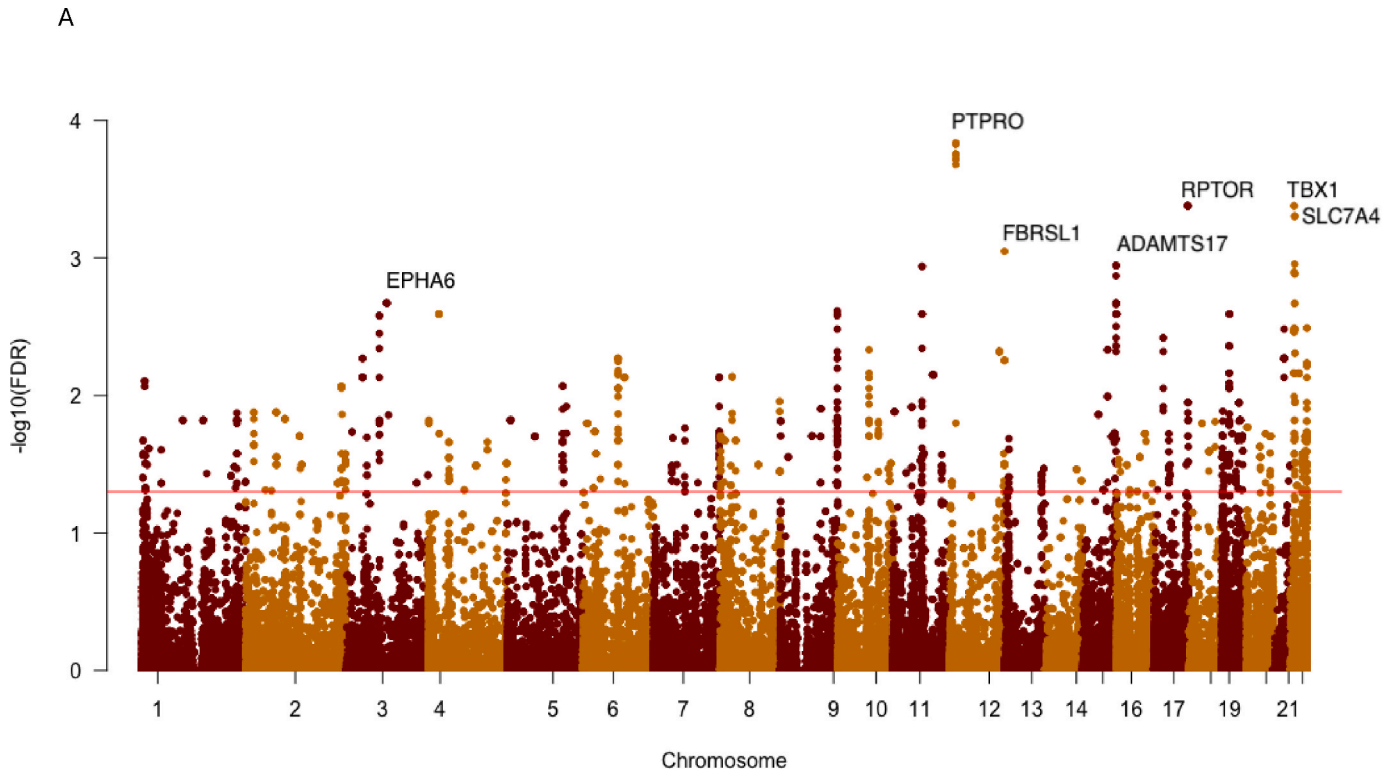

Fig. 2.A. Manhattan plot of the distribution of the methylation marks across chromosomes. The x-axis represents genomic position by chromosome, the y-axis represents statistical significance expressed as $-log_{10}$ (FDR) and the horizontal solid line indicates the threshold for significance (FDR<0.05, corresponding to a value of 1.3 on the y-axis.) The annotated genes are the 7 most significant by FDR.

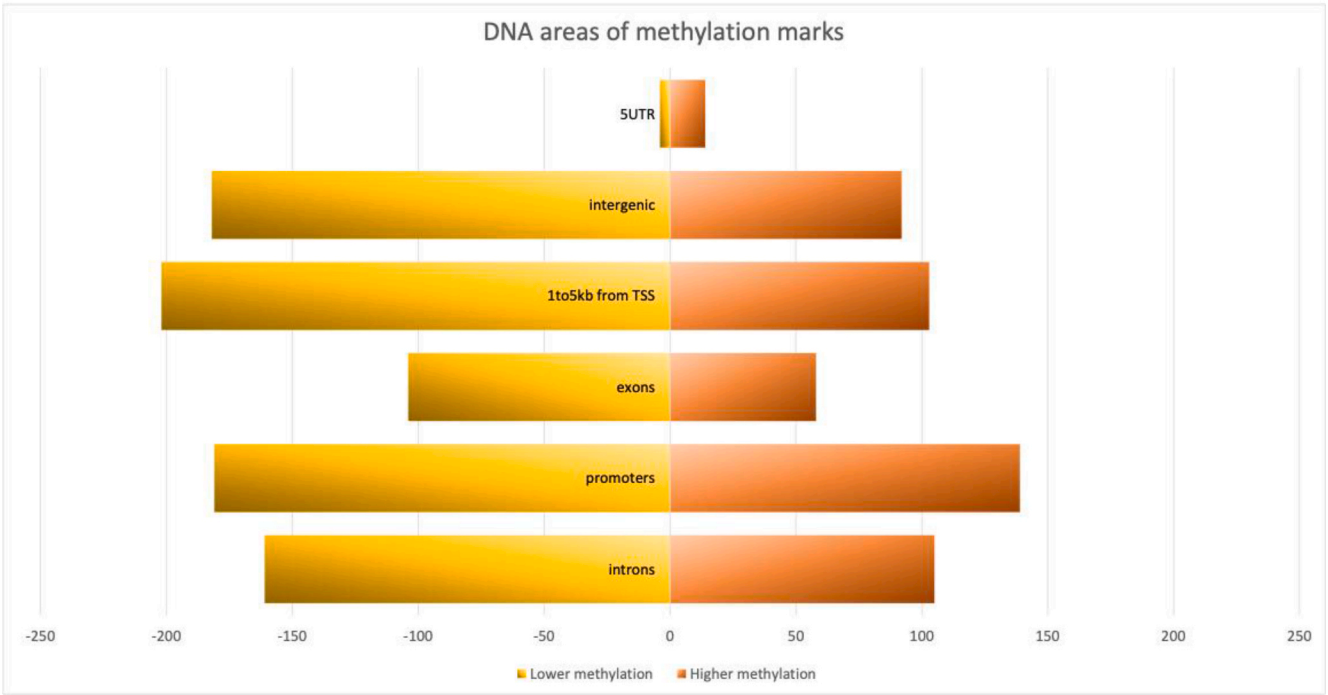

Fig. 2.B. Distribution of the significant (FDR ≤0.05) methylation marks associated with EPDS scores in mid-pregnancy in functional elements of the genome.

Activating Protein 23), *FBRSL1, PHPT1* (Phosphohistidine Phosphatase 1), *SLFN1* (Schlafen Family Member 11)*1, BICDL1* (BICD Family Like Cargo Adaptor 1), *MAL* (Mal, T Cell Differentiation Protein) and *SACK1H* (Scaffolding CK1 anchoring protein H). *ARHGAP23* is involved in signal transduction through a transmembrane receptor, *FBRSL1* enables RNA binding, *PHPT1* is involved in the dephosphorylation of

Table 2
List of gene ontology terms and their adjusted p-values.

Gene ontology			
Source	Term	GO term id	Adjusted p-value
Molecular function	calcium ion binding	GO:0005509	1.13E-11
	metal ion binding	GO:0046872	4.65E-04
	cation binding	GO:0043169	9.23E-04
	small molecule binding	GO:0036094	1.37E-03
	ion binding	GO:0043167	8.89E-03
Biological process	homophilic cell adhesion via plasma membrane adhesion molecules	GO:0007156	9.45E-23
	cell-cell adhesion via plasma-membrane adhesion molecules	GO:0098742	1.24E-18
	cell-cell adhesion	GO:0098609	1.16E-14
	cell adhesion	GO:0007155	3.20E-12
	system development	GO:0048731	6.48E-07
	nervous system development	GO:0007399	7.18E-07
	developmental process	GO:0032502	6.03E-06
	multicellular organism development	GO:0007275	1.01E-05
	anatomical structure development	GO:0048856	1.73E-05
	multicellular organismal process	GO:0032501	1.05E-03
Cellular component	cell periphery	GO:0071944	2.96E-08
	plasma membrane	GO:0005886	4.20E-07
	membrane	GO:0016020	2.95E-02

histidine residues in proteins, *SLFN11* enables ATP hydrolysis and tRNA binding activity, *BICDL1* is predicted to enable dynactin and small GTPase binding activity, *MAL* encodes a highly hydrophobic integral membrane protein, and *SACK1H* encodes a protein important in tooth enamel development. Of the methylation marks found, 37% were located 1 to 5 kb from a TSS, 26% were in the promoter region, 15% were intergenic, 12% were in exons, 5% were in introns, and 5% were in the 5' UTR. Higher EPDS scores were associated with lower methylation in 57% of the methylation marks.

3.5. Gene ontology analysis revealed enrichment in cell adhesion and nervous system development, associated with mid-pregnancy EPDS scores

Functional enrichment analysis revealed significant enrichment of methylation marks in genes involved in gene ontology (GO) terms of cell adhesion, most significantly homophilic cell adhesion via plasma membrane adhesion molecules (adj. *p*-value $9.45x10^{-23}$). Other significant terms in the biological processes category included developmental processes, such as nervous system development (adj. *p*-value $7.18x10^{-7}$) and anatomical structure development (adj. *p*-value $1.73x10^{-5}$). The most significant GO term in molecular functions was calcium ion binding (adj. *p*-value $1.13x10^{-11}$). GO analysis of cellular components revealed significant enrichment in the cell periphery (adj. *p*-value $2.96x10^{-8}$) and plasma membrane (adj. *p*-value $4.20x10^{-7}$). All GO terms with adjusted *p*-values are listed in Table 2.

In conclusion, EPDS scores measured in mid-pregnancy were associated with methylation marks enriched in genes involved in structural components of the cell, and biological processes such as cell adhesion, nervous system development, and anatomical structure development.

3.6. Consistency of methylation associations with depressive symptoms in early and mid-pregnancy

We compared the beta coefficients for the 1345 CpG sites associated with EPDS scores in mid-pregnancy to those from early pregnancy. A strong positive correlation was observed (Spearman's $\rho = 0.85$, $p < 2.2 \times 10^{-16}$), supporting the consistency of epigenetic associations across these gestational time points (Fig. 3A). This suggests that many of the methylation marks associated with maternal depressive symptoms are consistent when symptoms are measured in early and mid-pregnancy.

3.7. Genes with methylation marks that associate with EPDS scores in both early and mid-pregnancy

Thirty-two genic regions found with the annotatr tool had significant methylation marks associated with EPDS scores measured in early and mid-pregnancy (Table 3). Methylation marks in the same CpG sites at both assessment points were observed in 29 genic regions, and these regions showed similar beta values. The FDRs, beta-values, and 95% confidence intervals of these 29 genes are presented in Fig. 3B. The methylation marks that had the lowest FDR associated with the EPDS scores at both assessment points were found in the genic regions of *EPHA6*, *ZNF503-AS2*, *SHROOM1,* and *SLC7A4*. Higher EPDS scores were associated with lower methylation in methylation marks in the intron region of *EPHA6*, higher methylation in methylation marks in 1 to 5 kb from the transcription start site of *ZNF503-AS2*, lower methylation in methylation marks found in intronic and exonic regions of *SHROOM1*, and lower methylation in methylation marks in the promoter and 5' untranslated region of *SLC7A4*. Among the 32 genic regions, methylation marks at *ARHGEF10* and *TBX1* were also independently associated with EPDS scores in mid-pregnancy, after accounting for early pregnancy EPDS scores as an added covariate.Table 3. List of genes with methylation marks associated with EPDS scores in early pregnancy and in mid-pregnancy. Genes with no overlapping CpG sites are indicated with an asterisk.

3.8. Functional analyses of the methylation marks associated with EPDS scores measured in early and mid-pregnancy revealed six shared biological processes

To determine whether the methylation marks at both assessment points were enriched in similar functional pathways, we performed a multi-query enrichment analysis using G:profiler. Our results revealed six similar GO terms for biological processes (Additional File Table 3). The most significant GO terms associated with EPDS scores at both assessment points were nervous system development (adj. *p*-value in early pregnancy $2.68x10^{-3}$ and mid-pregnancy 24 $7.18x10^{-7}$), multicellular organism development (adj. *p*-value respectively $3.63x10^{-3}$ and $1.01x10^{-5}$), and anatomical structure development (adj. *p*-value $1.57x10^{-4}$ and $1.73x10^{-5}$). Several GO terms related to neuronal development, such as neuron differentiation (adj. *p*-value $1.27x10^{-3}$), generation of neurons (adj. *p*-value $1.81x10^{-3}$), and neurogenesis (adj. *p*-value $8.18x10^{-3}$), were associated with maternal EPDS scores only in early pregnancy. Cell adhesion terms were the most significant terms in the biological processes category associated with mid-pregnancy EPDS scores only.

In conclusion, GO terms such as nervous system development, anatomical structure development, and multicellular organism development, were associated with EPDS scores measured in both early and mid-pregnancy. GO terms involved in neuronal development, such as neuron differentiation and the generation of neurons, were only associated with early pregnancy EPDS scores. GO terms involved with cell adhesion were only associated with mid-pregnancy EPDS scores.

4. Discussion

This descriptive study, with RRBS assessment, revealed 1345 placental methylation marks in association with mid-pregnancy EPDS scores, located in 381 genes. These genes showed functional enrichment

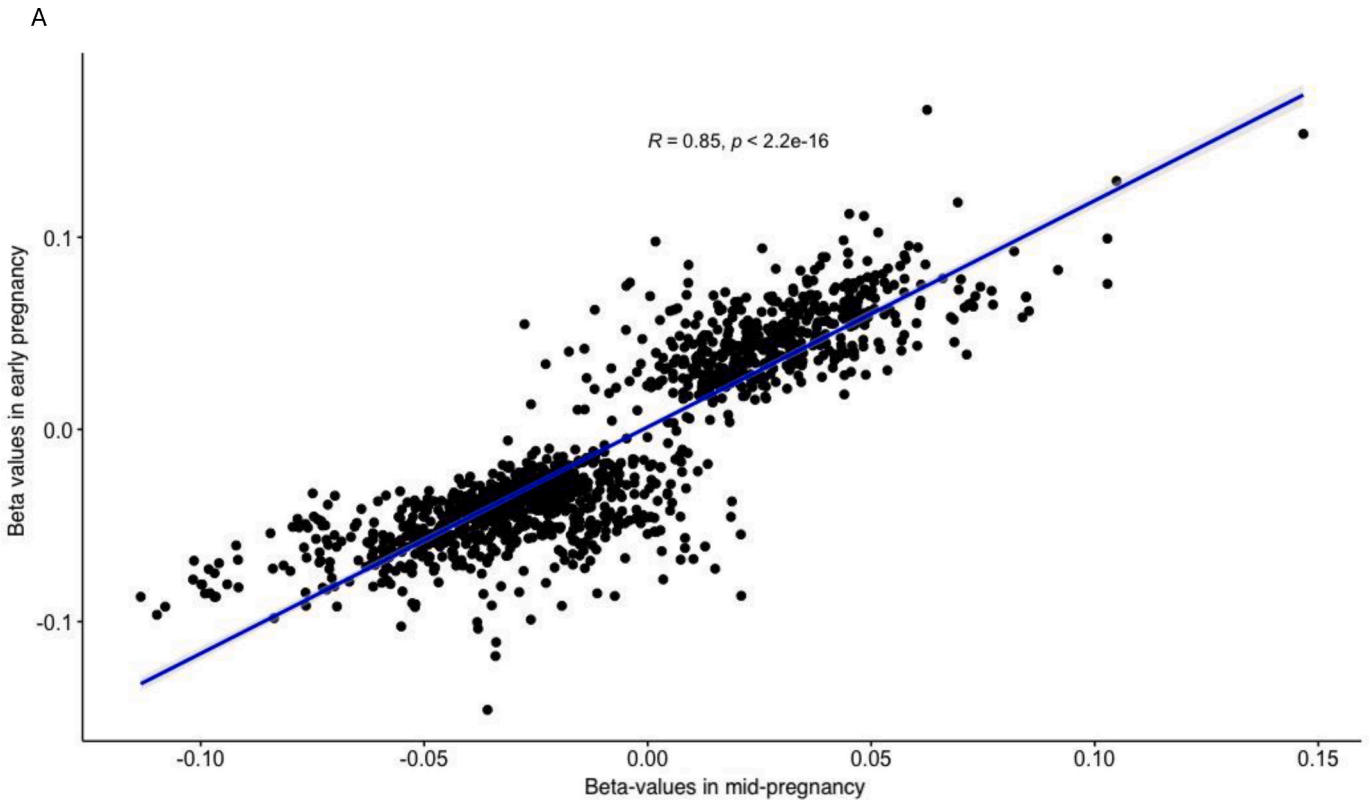

Fig. 3.A. Correlation of placental DNA methylation levels between early and mid-pregnancy. Each point represents the mean Beta value of a specific CpG site in mid-pregnancy (x-axis) and early pregnancy (y-axis). The Pearson correlation coefficient (r = 0.85) indicates the overall stability of the methylome across gestation, while the diagonal identity line (y = x) represents the theoretical position of sites with no change in methylation over time.

in cell adhesion, nervous system development, and anatomical structure development pathways. After adjusting for early pregnancy EPDS scores, 272 methylation marks remained significant, suggesting that while most of the methylation marks are explained by the depressive symptoms present in early pregnancy, a subset may reflect mid-pregnancy-specific associations. A high correlation between early- and mid-pregnancy EWAS CpG effect sizes, indicate stable methylation associations with maternal depressive symptoms, potentially reflecting persistent epigenetic regulation linked to maternal mood from early to mid-gestation. While 24% of methylation marks were in promoter regions, many were located in other regions, which may potentially influence gene regulation through diverse epigenetic mechanisms beyond simple promoter silencing. Whether these marks are associated with depressive symptoms later in pregnancy remains unclear. All findings are preliminary and require replication.

The 272 mid-pregnancy methylation marks were in the coding regions of 63 genes, several of which have been previously implicated in nervous system development or neurodevelopmental disorders. For example, *MAL* and *SCRIB* may play roles in structural neural development, as *MAL* encodes a proteolipid implicated in myelination and the development of Schwann cells in rat embryos [51,52], and *SCRIB* encodes a protein, involved in neuronal migration and cortical layering [53]. *RTN4R* and *TIAM1* have been implicated in synaptic architecture, including synapse formation, axonal elongation, and dendritic arborization in cultured human neurons [54] as well as glutamatergic synapse regulation in dentate granule neurons [55]. *RAB35* encodes a RAB protein, which has been shown to be important in brain development in mice; *RAB35* knockout mice exhibited reduced anxiety-related behavior, poor spatial memory, and disorganization of pyramidal cells in the hippocampus [56]. The *BSN* gene encodes the bassoon protein, which is highly expressed in the hippocampus and cortex of mammalian brains and is involved in synaptic vesicle trafficking [57] and has been identified as a possible candidate gene for epilepsy [58]. While these methylation signatures may have implications in neurodevelopment, they may also represent placenta-specific adaptations to maternal distress. Further functional studies are needed to determine whether these placental epigenetic changes correlate with fetal protein expression.

Furthermore, many of these 63 genes with methylation marks have previously been associated with autism spectrum disorder (ASD). For example, *FBRSL1, MTSS2, PCDHGC5, TBX1, GNB1L* and *ARHGEF10* have all been associated with ASD in various studies. Mutations in the coding region of *FBRSL1* are associated with malformation and intellectual disability syndrome, which includes autistic behavior [59]. A de novo recurrent variant of *MTSS2* has been identified as a cause of a developmental disorder with attention deficit hyperactivity disorder and ASD [60,61]. *PCDHGC5* encodes a protein that has been shown to regulate cell death in cortical inhibitory interneurons in mice [62], and imbalances in the number of cortical inhibitory neurons have been linked to ASD and schizophrenia [63]. *TBX1* and *GNB1L* are genes associated with the 22q11.2 deletion syndrome, also known as DiGeorge syndrome [64,65]. *TBX1* and *ARHGEF10* have been identified as ASD genes through mouse studies [64,66] and *GNB1L* has been identified as a candidate gene for ASD and schizophrenia in association with DiGeorge syndrome [65].

We identified 29 genes with methylation marks associated with EPDS scores measured at both assessment points. Some of these genes have been previously associated with nervous system development. *PTPRO* encodes a protein involved the differentiation and axon genesis of neurons in the central and peripheral nervous systems during gestation in animal studies [67]. *EPHA6* encodes receptor tyrosine kinases essential for neuronal development and cytoarchitecture [68], and has been demonstrated to be important for learning and memory in animal studies [69]. Whether similar marks are detectable in the developing

Table 3
List of genes with methylation marks associated with EPDS scores in early pregnancy and in mid-pregnancy. Genes with no overlapping CpG sites are indicated with an asterisk.

Genes with methylation marks associated with higher EPDS scores in early and mid-pregnancy	The most significant shared methylation mark (by FDR and CI95%) or methylation marks (if none shared) in the genic region							
	Early pregnancy				Mid-pregnancy			
	Genomic coordinates	FDR	Beta-value	95% CI	Genomic coordintes	FDR	Beta-value	95% CI
ACTN4	chr19:38663600	4.71E-02	−0.033	(-0.062–0.003)	chr19:38663612	3.12E-02	−0.039	(-0.068–0.011)
ADGRD1	chr12:131008516	1.64E-02	−0.053	(-0.092–0.015)	chr12:131008516	4.51E-02	−0.056	(-0.096–0.015)
ARHGDIA	chr17:81869841	4.51E-02	−0.034	(-0.068–0.001)	chr17:81869841	3.03E-02	−0.038	(-0.073–0.002)
ARHGEF10*	chr8:1766240	2.09E-03	−0.045	(-0.092–0.015)	chr8:1824233	1.98E-02	0.166	(0.102-0.231)
ARHGEF17	chr11:73311506	1.63E-03	−0.036	(-0.09–0.012)	chr11:73311506	2.64E-02	−0.029	(-0.055–0.004)
ARRDC4	chr15:979604501	1.41E-02	0.071	(0.019-0.122)	chr15:979604501	1.90E-02	0.057	(0.005-0.110)
BAIAP2	chr17:81094806	1.44E-02	−0.060	(-0.096–0.023)	chr17:81094806	1.34E-02	−0.063	(-0.100–0.026)
CSNK1G2	chr19:1942922	2.22E-02	−0.029	(-0.117–0.034)	chr19:1942922	1.54E-02	−0.032	(-0.050–0.013)
DDAH2	chr6:31730715	2.48E-02	−0.035	(-0.061–0.009)	chr6:31730715	2.65E-02	−0.033	(-0.059–0.007)
EBF2	chr8:26044569	1.27E-02	0.085	(0.021-0.150)	chr8:26044569	4.47E-02	0.083	(0.015-0.151)
EHBP1L1	chr11:65573508	4.88E-02	−0.031	(-0.059–0.004)	chr11:65573508	2.98E-02	−0.033	(-0.062–0.005)
EPHA6	chr3:97641565	6.47E-06	−0.077	(-0.123–0.030)	chr3:97641565	2.13E-03	−0.051	(-0.101–0.001)
ESPN	chr1:6435412	2.96E-03	−0.077	(-0.119–0.035)	chr1:6435412	8.60E-03	−0.071	(-0.115–0.027)
H2AC1	chr6:25727081	9.52E-03	−0.045	(-0.079–0.010)	chr6:25727081	4.70E-02	−0.040	(-0.076–0.004)
LOC100287837	chr11:73311643	1.63E-03	−0.048	(-0.077–0.020)	chr11:73311643	2.64E-02	−0.039	(-0.071–0.008)
MIR6821	chr22:49962884	2.41E-03	−0.027	(-0.052–0.003)	chr22:49962884	1.84E-02	−0.026	(-0.051–0.001)
MSRA	chr8:10214361	1.54E-02	−0.023	(-0.047-0.001)	chr8:10214361	4.27E-02	−0.020	(-0.044-0.003)
NFIC	chr19:3437163	4.42E-06	−0.052	(-0.087–0.017)	chr19:3437163	4.55E-02	−0.040	(-0.075–0.005)
PLXNB2*	chr22:50275872	3.41E-03	0.031	(0.002-0.060)	chr22:50287121	3.23E-03	0.070	(0.019-0.120)
PTPRO	chr12:15322833	3.09E-02	−0.052	(-0.097–0.008)	chr12:15322833	1.48E-04	−0.071	(-0.117–0.026)
SBNO2	chr19:1108583	1.64E-02	−0.078	(-0.136–0.021)	chr19:1108583	2.80E-02	−0.067	(-0.129–0.005)
SEPTIN9*	chr17:77451511	3.44E-05	−0.056	(-0.104–0.009)	chr17:77465639	3.19E-02	−0.062	(-0.096–0.028)
SHROOM1	chr5:132823227	7.66E-08	−0.063	(-0.103–0.024)	chr5:132823227	1.90E-02	−0.041	(-0.082–0.001)
SLC24A4	chr14:92490738	4.79E-02	−0.045	(-0.078–0.012)	chr14:92490738	3.45E-02	−0.037	(-0.073–0.0017)
SLC7A4	chr22:21032547	9.74E-04	−0.046	(-0.079–0.014)	chr22:21032547	3.29E-03	−0.040	(-0.076–0.004)
TBX1	chr22:19772390	2.00E-03	0.044	(0.019-0.070)	chr22:19772390	4.18E-04	0.053	(0.0257-0.081)
TMPRSS9	chr19:2424997	1.96E-03	−0.057	(-0.090–0.025)	chr19:2424997	4.86E-02	−0.042	(-0.077–0.006)
TRIM17	chr1:228416806	1.27E-03	−0.048	(-0.072–0.024)	chr1:228416806	4.34E-02	−0.036	(-0.062–0.010)
ZFR2	chr19:3821213	1.31E-02	−0.031	(-0.062–0.000)	chr19:3821465	4.16E-02	−0.031	(-0.063-0.001)
ZNF503-AS2	chr10:75399407	1.26E-03	0.075	(0.0182-0.132)	chr10:75399407	7.39E-03	0.069	(0.011-0.128)
ZNF703	chr8:37698345	4.19E-02	−0.034	(-0.067–0.000)	chr8:37698345	2.12E-02	−0.036	(-0.071–0.001)
ZRANB3	chr2:135284661	6.36E-03	−0.045	(-0.075–0.014)	chr2:135284632	3.19E-02	−0.036	(-0.068–0.004)

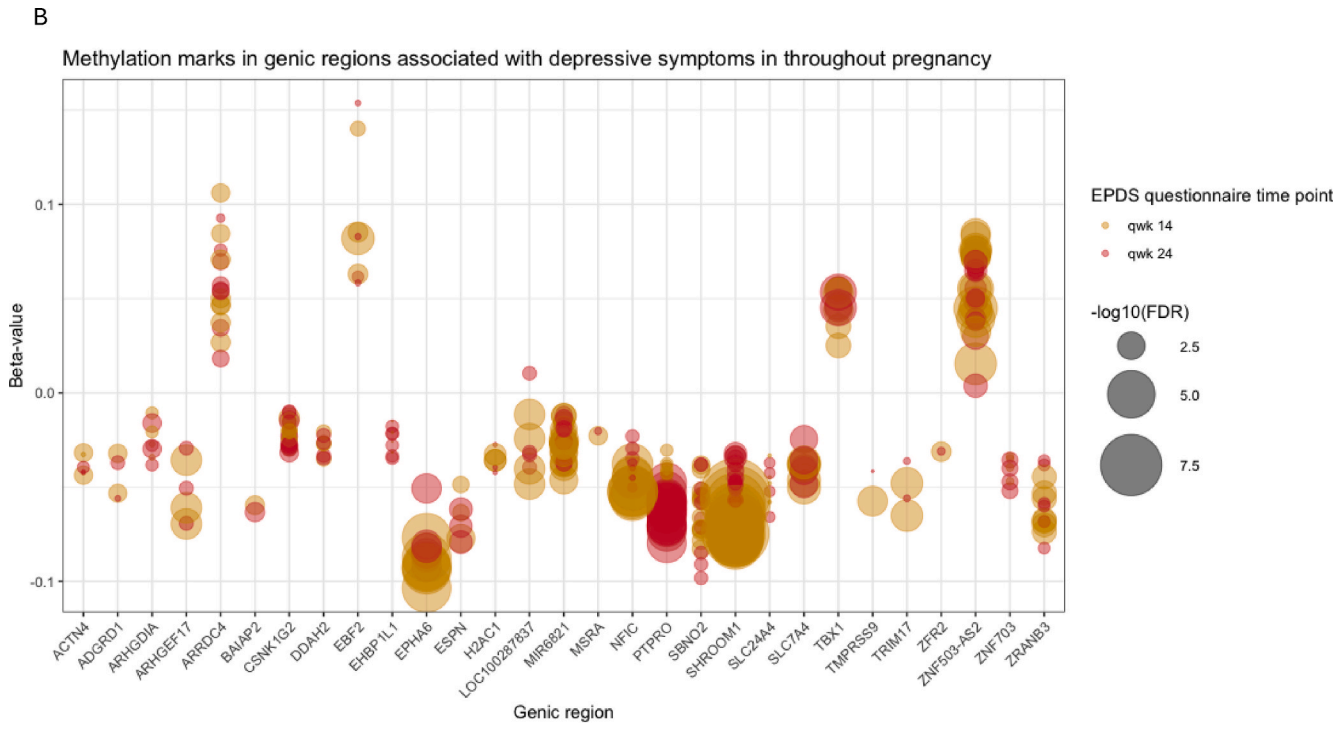

Fig. 3.B. Methylation marks associated with EPDS scores in early and mid-pregnancy.

human fetus remains unclear.

Few targeted and genome-wide studies have examined the associations between EPDS scores and the placental epigenome. We found that mid-pregnancy EPDS scores were potentially associated with methylation marks in 381 genic regions. While previous studies have reported associations between methylation marks and maternal depressive symptoms in the genic regions of *HSD11B2* or *NR3C1* [27,70,71], we did not observe significant associations in these genes. Tesfaye et al. (2021) reported that methylation marks near the *CTDP1* gene in the placenta were associated with depressive symptoms measured at GW 37-43 in their EWAS [30]. We found a similar association in early pregnancy; however, no such association was observed in mid-pregnancy. Martinez et al. (2023) reported that anxiety and depressive symptoms measured at GW 24-25 were associated with methylation marks in 226 placental genes. Consistent with Martinez et al., we identified methylation marks near the *TNRC6C* gene [29], which has been suggested as a possible candidate gene in ASD [72]. In line with our gene ontology analysis, Martinez et al. reported overrepresentation of cell adhesion in the placentas of women with symptoms of anxiety and depression measured at GW 24-25, at childbirth, and postpartum [29]. Cell adhesion molecules play important roles in the proper development and plasticity of organs, particularly in the brain [73] and have also been associated with impaired trophoblast invasion in the pathogenesis of preeclampsia [74,75]. Interestingly, maternal depression has been associated with a greater risk of preeclampsia [20]. Although some of our findings were consistent with previous studies, most of the genes we identified have not been previously associated with maternal depressive symptoms. In addition to gestational age, differences in the methods used may explain the inconsistent results, as the same CpG sites were not necessarily covered, as Tesfaye et al. used the Infinium HumanMethylation450 BeadChip array and Martinez et al. used the Methyl Capture Epic [29,30]. Furthermore, the covariates controlled for have varied across studies.

Only one-fifth of the identified methylation marks were influenced by the covariates included in our analysis. Kee et al. (2022) reported associations between maternal depressive symptoms and placental methylation marks in pregnancies with female offspring in their EWAS [31]. In our study, offspring sex influenced only 60 methylation marks. However, direct comparison of specific CpG sites may be limited due to methodological differences, as Kee et al. used Infinium HumanMethylation450 BeadChip and Infinium MethylationEPIC 850K BeadChip arrays [31]. Delivery mode influenced 99 methylation marks in our study, potentially reflecting physiological stress and inflammatory cascades associated with active labor or the lack thereof in cesarean section, both of which may influence the placental epigenome. While the effects of delivery mode on the placental epigenome have not been extensively studied, differentially methylated regions have been found in cord blood DNA and peripheral blood samples of newborns born vaginally versus via cesarean section [76,77]. The interval between delivery and freezing influenced 78 methylation marks, underlining the importance of standardized tissue processing, even though sample collection and freezing within 2 h is generally considered sufficient for DNA methylation studies [78].

4.1. *Strengths and limitations*

A strength of this study is the application of RRBS, enabling the discovery of novel DNA methylation associations that might have been overlooked by predefined CpG probes in array-based screening. While our findings suggest a link between maternal depressive symptoms and the placental epigenome, several limitations should be considered. The sample size is relatively small for an EWAS, potentially reducing statistical power and inflating effect sizes; therefore, results should be interpreted as descriptive, and do not allow conclusions about causality or biological relevance. We did not measure the potential influence of these methylation marks on gene expression. Consequently, it remains unknown whether the observed methylation marks are transmitted to the fetus through changes in gene expression or placental function, or through effects on the fetal epigenome. Third, while we hypothesize that maternal depressive symptoms during mid-pregnancy influence placental programming, epigenetic patterns found in the placenta may not reflect epigenetic patterns in the fetal tissue, such as the brain. We cannot definitively state whether the observed changes are placenta-specific or reflect a systemic epigenetic response. We also cannot

exclude the possibility of reverse causality. Fourth, our EWAS is based on heterogeneous bulk placental tissue and therefore does not account for possible differences in DNA from different cell types. Shared genetic and maternal-fetal factors may contribute to the observed associations. Finally, this study relied solely on the EPDS, a self-report instrument, without objective assessments by trained clinicians or secondary depression scales. Future longitudinal studies with larger, independent cohorts should incorporate multi-method assessment batteries together with expert objective evaluation and functional validation to confirm these epigenetic signatures and clarify the direction of the observed associations.

5. Conclusion

This descriptive study found 1345 placental methylation marks potentially associated with mid-pregnancy EPDS scores. These methylation marks were in the genic region of 381 genes. After adjusting for early pregnancy EPDS scores, 272 methylation marks remained significant; these were in the genic regions of 63 genes. Many of these genes have been associated with ASD, neuronal development, depression, and other psychiatric disorders in both animal and human studies. Functional analysis revealed that methylation marks were in the genic regions of genes that regulate biological pathways involved in cell adhesion, nervous system development, and anatomical structure development. Findings need to be replicated in larger, independent cohorts. Whether these pathways and methylation marks mediate the effects of maternal depressive symptoms on the fetus remains to be elucidated in future studies.

Consent for publication

All authors consent to the publication of this manuscript.

Availability of data and materials

All Additional Files are available online. Current EU and national legislation on personal data protection of sensitive data, as well as the informed consents given by the study subjects, do not permit open data sharing of the cohort data used in this study. Investigators interested in research collaboration and obtaining access to the data are encouraged to contact the FinnBrain board (finnbrain-board@lists.utu.fi). Contact information for the board members and FinnBrain's Principal Investigators is listed on the project website: https://sites.utu.fi/finnbrain/en/contact/

The raw sequencing data cannot be publicly shared due to GDPR restrictions, as the data are potentially identifiable and therefore considered sensitive.

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CRediT authorship contribution statement

Nina Pettersson: Formal analysis, Methodology, Project administration, Visualization, Writing – original draft. **Minna K. Kyläniemi:** Formal analysis, Visualization, Writing – review & editing. **Riina Kaukonen:** Resources, Writing – review & editing. **Mikko Konki:** Resources, Writing – review & editing. **Noora M. Scheinin:** Methodology, Writing – review & editing. **Susanna Kortesluoma:** Resources, Writing – review & editing. **Linnea Karlsson:** Conceptualization, Methodology, Writing – review & editing. **Hasse Karlsson:** Conceptualization, Resources, Writing – review & editing. **Riikka Lund:** Formal analysis, Methodology, Supervision, Writing – review & editing. **Eeva Ekholm:** Methodology, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

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Abbreviations

EPDS: Edinburgh Postnatal Depression Scale, RRBS: reduced representation bisulfite sequencing, GW: gestational week, EWAS: epigenome-wide association study, GO: gene ontology, ASD: autism spectrum disorder.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2026.06.020.

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