Epigenetic modifications appear in the human placenta following anxiety and depression during pregnancy

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ABSTRACT

Introduction: The future health of the offspring can be influenced by longstanding maternal anxiety and depression disorders during pregnancy. The present study aimed to explore the effect of psychiatric disorders during pregnancy on placental epigenetics.

Methods: We measured DNA methylation patterns in term-placentas of women either suffering longstanding anxiety and depression symptoms (Index group, with overt symptoms), or a healthy population (Control, none/only mild symptoms). Whole genome DNA methylation profiling was performed using the TruSeq® Methyl Capture EPIC Library Prep Kit (Illumina, San Diego, CA, USA) for library preparation and NGS technology for genomic DNA sequencing.

Results: The results of high-throughput DNA methylation analysis revealed that the Index group had differential DNA methylation at epigenome-wide significance (p < 0.05) in 226 genes in the placenta. Targeted enrichment analyses identified hypermethylation of genes associated with psychiatric disorders (BRINP1, PUM1), and ion homeostasis (COMMD1), among others. The ECM (extracellular matrix)-receptor interaction pathway was significantly dysregulated in the Index group compared to the Control. In addition, DNA methylation/mRNA integration analyses revealed that four genes with key roles in neurodevelopment and other important processes (EPB41L4B, BMPR2, KLHL18, and UBAP2) were dysregulated at both, DNA methylation and transcriptome levels in the Index group compared to Control.

Discussion: The presented results increase our understanding of how maternal psychiatric disorders may affect the newborn through placental differential epigenome, suggesting DNA methylation status as a biomarker when aiming to design new preventive techniques and interventions.

1. Introduction

Epigenetic modifications, especially DNA methylation, have a significant influence on transcription [1,2], particularly within the regulatory regions of expressed genes, with the potential to affect gene expression without altering the DNA sequence [3].

DNA modification usually takes place on cytosines that come before guanosines in the DNA sequence [4]. These dinucleotides can be clustered in small stretches of DNA, termed CpG islands. Most CpG sites outside the CpG islands are methylated, suggesting a role in the global maintenance of the genome, while most CpG islands in gene promoters are unmethylated thereby allowing active gene transcription [5]. In general, methylation of a specific stretch of cytosines in a CpG island located in the gene’s promoter region leads to gene silencing, with the CpG island named “hypermethylated.” In contrast, a CpG island would be “hypomethylated” when a certain stretch of cytosines in the promoter...
region of a gene is not methylated, preventing that gene from being silenced. DNA methylation is widely associated with altered gene expression in response to external factors such as disease, exercise, or environmental stressors [6,7].

During pregnancy, maternal disorders linked to several environmental factors have been associated with changes in the epigenetic motives of embryonic and extraembryonic tissues and have impacted pregnancy outcomes [8,9].

Furthermore, altered DNA methylation has been observed in placental samples from women suffering from early (<34-week gestation) onset of preeclampsia compared to late-onset (>34-week gestation) or normal controls [10]. Finally, maternal nutritional status can also alter the epigenetic state of the embryonic genome, and be carried forward to subsequent developmental stages [11].

Longstanding anxiety and depression symptoms during pregnancy have been identified as an important clinical risk factor for the transmission of abnormal health and behavior to the offspring [12]. In this regard, the placenta is a key determinant of fetal health since it regulates the transfer of components from the maternal to the fetal side [9]. Recent studies from our group demonstrated that placental samples from women with self-perceived longstanding anxiety and depression symptoms exhibit not only differential expression of stress-mediating genes but also a dysregulated expression of genes ruling immune responses [13,14]. These findings suggest an association between the pregnant mother’s psychiatric problems and the placenta immune system during pregnancy which may have a considerable impact on the fetus’s health. To the best of our knowledge, no literature is available regarding possible associations between symptoms of anxiety and depression among pregnant women and DNA methylation alterations in the placenta.

The aim of the present study was, therefore, to primarily investigate alterations in DNA methylation in the term-placenta of women suffering anxiety and depression symptoms during pregnancy, compared to a healthy population. Secondly, we further aimed to determine if the alterations across the methylome could be also screened at the transcriptome level.

2. Material and methods

2.1. Experimental design

The demographic data of the patients included in this study are presented in Table 1 (source of the table [15]). Data from a total of 390 pregnant women attending an antenatal care clinic in Sweden were collected. From those 390 pregnant women, 12 patients were classified into 2 groups; the index group: women indicating symptoms of depression and anxiety (n = 6), and the control group: women with no or mild symptoms of depression and anxiety (n = 6). Within each group we selected patients with similar demographic characteristics. After birth, placentas were immediately collected, snap-frozen, and stored at −80 °C until methylation and transcriptome analyses.

The offspring’s sex among groups was recorded (index group: 3 females and 3 males; control group: 4 females and 2 males). The demographic data of the patients included in this study have already been published elsewhere [13].

2.2. Assessment of anxiety, and depression symptoms in pregnant women

The Beck’s Anxiety Inventory (BAI) and the Edinburgh Postnatal Depression Scale (EPDS) were used to measure symptoms of anxiety and depression, respectively. Both inventories are well-known, have undergone Swedish validation [16,17], and are commonly applied in both clinical and research settings. Both the EPDS and the BAI had a cut-off score of 10 to gauge the presence of at least mild anxiety and level of depression symptoms with reported specificity for identification of depression around 82%, and sensitivity close to 100%. A total of 6 women who scored >10 on both EPDS/BAI were included in the index group. A total of 6 controls who scored <10 on both EPDS/BAI were referred to as the control group. Psychological data was collected from participants on week 24–25, during childbirth and postpartum. To ensure the reliability and consistency of data collection, trained research personnel administered the BAI and EPDS according to established protocols. Participants completed the questionnaires in a private and quiet setting to minimize external influences on their responses. Women displaying pregnancy complications, including pre-eclampsia and/or preterm birth were excluded.

2.3. Collection and preparation of placenta samples

At-term placentas were placed on ice, and samples were dissected approximately 5 cm from the umbilical cord’s confluence. The approximately 2.5 cm thick villous parenchyma was then punctured to acquire 1 g samples of the fetal (including the chorionic plate), middle, and maternal areas (including the thin basal plate), which were then pooled to obtain a homogeneous placental sample composed of the different cell types derived from both the fetus and mother, snap-frozen and kept at −80 °C for later analysis.

2.4. DNA extraction

DNA isolation was performed using the DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. Approximately 25 μg of tissue was cut into small pieces and placed in a 1.5 ml microcentrifuge tube containing 180 μl Buffer ATL and disrupted with the TissueLyser II (Qiagen, Hilden, Germany). 20 μl Proteinase K was added, mixed by vortexing, and incubated at 56 °C until the tissue was completely lysed. Then 200 μl Buffer AL and 200 μl ethanol (96–100%) were added to the sample and mixed thoroughly by
vortexing. The mixture was then placed into a DNeasy Mini spin column placed in a 2 ml collection tube and centrifuged at ≥6000×g for 1 min. Then 500 μl of Buffer AW1 were added and centrifuged at ≥6000×g for 1 min followed by 500 μl of Buffer AW2 and centrifuged again at 20,000×g for 3 min to dry the DNeasy membrane. Following the centrifugation step, the DNeasy Mini spin column was placed in a clean 1.5 ml microcentrifuge tube with 200 μl Buffer AE and incubated at room temperature (RT) for 1 min, and then centrifuged at ≥ 6000×g for 1 min to elute. The total DNA obtained was quantified using a NanoDrop ND-1000 (Thermo Fisher Scientific, Fremont, CA, USA).

2.5. Whole genome DNA methylation

Whole genome DNA methylation profiling was performed using the TruSeq® Methyl Capture EPIC Library Prep Kit (Illumina, San Diego, CA, USA) for library preparation and NGS technology for genomic DNA sequencing. Briefly, 500 ng of high-quality genomic DNA were fragmented to an average size of 150–200 bp using a Covaris S2 ultrasonicator (Covaris Inc., Woburn, MA, USA). DNA was then end-repaired, adenylate-tailed, and ligated with methylated indexed adapters to create pre-capture DNA libraries. Libraries were then pooled in groups of four in equal aliquots, on which two rounds of hybridization and capture using Illumina-optimized EPIC probe sets (covering >3.3 million targeted CpG sites). The reagents included in the TruSeq Methyl Capture EPIC Library Prep Kit were used to convert hybridized products to bisulfite. The bisulfite-treated libraries were PCR-amplified for 11 cycles with Kapa HiFi HotStar Uracil + polymerase (Kapa Biosystems, London, United Kingdom). Quantity and quality control of DNA libraries were done using the Qubit DNA Assay kit (Invitrogen, Carlsbad, CA, USA) and DNA 1000 kit (Agilent, Santa Clara, CA, USA) with an Agilent 2100 Bioanalyzer, respectively. Library sequencing was performed using the NextSeq 500/550 High Output kit v2.5 (150 cycles), with a sequencing setup of 2 × 75 bp paired-end.

2.6. Methylation data analysis

A flowchart of data analysis is depicted in Fig. 1. Raw sequencing reads were trimmed to remove both poor-quality calls and adapters using Trimmomatics (version 0.36) [18]. Quality-trimmed paired-end reads were analyzed with MethylSeq v1.0, which employed Bismark (v0.12.2) for reads mapping and aligned to the reference human genome (hg38) using Bowtie2 (v2.2.2). Next, the normalized data were subjected to one-way ANOVA to create a list of filtered genes with 19,767 probes which passed the significant unadjusted p-value < 0.05 and difference in the methylation levels between placenta samples from Index and Control groups of M-values ≥ 0.5 threshold criteria.

2.7. Chromosome location and biological interpretation

The methyl-seq data were first categorized according to their respective chromosome number and then organized by their location in respective chromosomes to investigate whether the methylation pattern varied in relation to chromosome location and chromosome number. The epigenome-wide enrichment analyses for GO Terms and Pathways were conducted using Partek Pathway suite (Partek) KEGG (104.0) and DAVID (v2022q3) databases.

2.8. DNA methylation/mRNA integration analyses

To explore the association between methylation levels and gene expression, we performed a DNA methylation-mRNA integration analysis (Partek Genomic Suite) using the transcriptome data from a previous study [13] on the effects of stress, anxiety and depression during pregnancy on the transcriptome of term-placenta samples. After the independent analysis of each of those data sets, we integrated the results and detected genes that showed evidence of both differential methylation and differential expression.

For this, we overlapped the significant DMGs data from the present study with significantly differentially expressed genes identified in the previous study from our group [14]. We also used this approach to determine if the genes that were identified to be hypermethylated in the present study were also downregulated in the previous study as well as genes that were hypomethylated in the present study were upregulated in the transcriptome data sets.

3. Results

3.1. DNA methylation status and differentially methylated genes in placenta samples from Index and Control groups

Following DNA methylation quantification via NGS, data were imported into the Partek Genomics Suite for total methylation and differential methylation status among groups. Following functional normalization, we identified 403 differentially methylated regions (DMR) between placental samples from the Index group versus the Control group. We found more DMR (60%) in the Index group when compared to controls. After DMR identification, we then associated those regions with nearby genes. Of the genes identified as being differentially methylated (226), 27 presented more than 10 methylated regions with nearby genes. Of the genes identified as being hypermethylated in the present study from our group [14]. We also used this approach to determine if the genes that were identified to be hypermethylated in the present study were also downregulated in the previous study as well as genes that were hypomethylated in the present study were upregulated in the transcriptome data sets.

3.2. Biological meaning of DNA methylation

To further assess the functional significance of these changes in DNA methylation between Index and Control groups, a GO and Pathway enrichment analysis was undertaken. A total of 280 GO functions had an enrichment score ≥3 (p < 0.05, Supplementary file S2). These analyses revealed that several genes involved in cell adhesion, cell motility, cell
differentiation, and proliferation were highly enriched in biological function and molecular function. The processes with the top enrichment scores included positive regulation of epithelial cell migration, cell adhesion mediated by integrins, cell surface receptor signaling pathway, cell proliferation and cell cycle regulation. The KEGG pathway database was used to identify key pathways linked to those genes with the top ranked DMR. The ECM (extracellular matrix)-receptor interaction pathway was significantly dysregulated in the Index group compared to the Control.

3.3. DNA methylation in gene promoter regions

Methylation of genes in promoter regions is of great biological interest as it is often associated with silencing of transcription and repressed gene expression. Since un-methylated CpGs are often enriched in gene promoters, we studied whether gene promoter regions were hyper-methylated (and gene expression was silenced) or un-methylated and potentially active based on the methylation levels near the transcription start site (TSS). Fig. 2 shows the distance from the methylated region to TSS in both Index and Control Groups. Genes with promoter region methylated in the Index group compared with the Control group are shown in Table 3.

3.4. Top-methylated chromosomes in placenta samples from women displaying self-perceived stress, anxiety, and depression during pregnancy vs. healthy patients

The methyl-seq data were first organized according to their respective chromosome number and then sorted by their location in respective chromosomes to explore if the methylation pattern varied in relation to chromosome location and chromosome number. Top-methylated chromosomes (chr 2, chr 3, chr 6, chr 9) among groups are shown in Fig. 3.

3.5. Stress-induced hypermethylation of BMPR2, EPB41L4B, KLHL18 and UBAP2 was negatively correlated with gene expression in placental samples

To explore whether hypermethylation patterns observed in the present study were associated with repressed gene expression, we related the differential methylation results to differential gene expression data from RNA-seq of the same samples from a previous study of our group [14]. Venn diagram analysis was used to generate gene lists of overlap between the DMGs observed in the present manuscript with the differentially expressed genes (DEGs) defined in the previous study. We also used this approach to determine if the genes that were identified to be hypermethylated in the Index group were downregulated in previous transcriptome analyses [14]. Four (4) genes were particularly both DM and DE in the Index group compared to the Control, including EPB41L4B, BMPR2, KLHL18, and UBAP2 (Fig. 4). The findings are graphically presented in Fig. 5.

4. Discussion

A DNA methylation sequencing approach was hereby used to examine the status of gene-specific DNA methylation in placentas recovered at term from women suffering from anxiety and depression.
Table 3
Genes with promoter region methylated in placenta samples from women displaying self-perceived anxiety, and depression during pregnancy (Index group, n = 6) compared to placental samples from healthy patients (Control group, n = 6).

<table>
<thead>
<tr>
<th>Chr</th>
<th>Gene Symbol</th>
<th>Distance to TSS</th>
<th>p-v</th>
<th>FC</th>
<th>GO Enrichment of selected Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>BMPR2</td>
<td>26014</td>
<td>0.01</td>
<td>7</td>
<td>Placental development, blood vessel remodeling, mesoderm formation</td>
</tr>
<tr>
<td>9</td>
<td>BRN1P1</td>
<td>−4431</td>
<td>0.03</td>
<td>3.9</td>
<td>Cell cycle regulation, neurogenesis</td>
</tr>
<tr>
<td>2</td>
<td>CDKL4</td>
<td>−26114</td>
<td>0.02</td>
<td>4.4</td>
<td>Regulation of cell cycle, protein phosphorylation</td>
</tr>
<tr>
<td>2</td>
<td>COMMD1</td>
<td>−7255</td>
<td>0.008</td>
<td>8</td>
<td>Protein transport, NF-kappa B transcription factor activity, HIF-1 signaling</td>
</tr>
<tr>
<td>2</td>
<td>GPR45</td>
<td>−3537</td>
<td>0.02</td>
<td>2.6</td>
<td>Intercellular mediator, hormone regulation, energetics homestasis</td>
</tr>
<tr>
<td>2</td>
<td>ITGA4</td>
<td>−6518</td>
<td>0.01</td>
<td>7</td>
<td>Regulation of local acute inflammatory response, cell-cell adhesion, T-cell migration</td>
</tr>
<tr>
<td>3</td>
<td>ITGB5</td>
<td>14560</td>
<td>0.006</td>
<td>2.1</td>
<td>Cell adhesion, cell-matrix adhesion, TGFβ signaling, cell migration</td>
</tr>
<tr>
<td>16</td>
<td>PALB2</td>
<td>26622</td>
<td>0.002</td>
<td>10</td>
<td>Apoptotic process, multicellular organism growth, mesoderm development</td>
</tr>
<tr>
<td>1</td>
<td>PUM1</td>
<td>13442</td>
<td>0.008</td>
<td>8</td>
<td>Adult locomotory behavior, posttranscriptional regulation of gene expression, posttranscriptional gene silencing, regulation of mRNA stability</td>
</tr>
<tr>
<td>4</td>
<td>SLC2A9</td>
<td>−17627</td>
<td>0.03</td>
<td>6</td>
<td>Glucose transmembrane transport, carbohydrate transport, amino acid transport</td>
</tr>
<tr>
<td>8</td>
<td>ST18</td>
<td>−32150</td>
<td>0.01</td>
<td>2.4</td>
<td>Regulation of transcription, cell proliferation</td>
</tr>
<tr>
<td>1</td>
<td>SYT2</td>
<td>−34918</td>
<td>0.03</td>
<td>6</td>
<td>Vesicle-mediated transport, cell differentiation, cellular response to calcium</td>
</tr>
<tr>
<td>7</td>
<td>TMEM130</td>
<td>6764</td>
<td>0.003</td>
<td>5.9</td>
<td>Protein binding</td>
</tr>
</tbody>
</table>

during pregnancy. To the best of our knowledge, this is the first study to examine associations between these ante-natal symptoms and epigenome-wide placental DNA methylation.

The results from the present study revealed that symptoms of anxiety and depression during pregnancy evoke a great hypermethylation response in genes with a clear influence on a variety of fundamental processes during cell development, such as cell differentiation and maturation, as well as cell adhesion and migration.

However, the consequences of DNA methylation strongly depend on the region of the gene that is hyper-or hypomethylated [19]. We observed a remarkable difference in DNA methylation patterns concerning the distance to the TSS. One possible explanation for the observed differences could be the involvement of distal regulatory elements, such as enhancers or insulators, that might influence the methylation state of nearby CpG sites. Epigenetic modifications at distal regulatory regions have been shown to impact gene expression by modulating chromatin accessibility and interacting with promoter regions [20]. Another factor that may contribute to the observed differences is the tissue-specific nature of DNA methylation patterns [21]. The placenta is a dynamic and specialized tissue with unique epigenetic features that support its vital role during pregnancy. It is possible that the observed differences in methylation patterns at specific genomic locations are associated with placenta-specific regulatory mechanisms. Modifications in DNA methylation across the promoter region of a gene affects the transcription of genes [22]. DNA hypermethylation in a promoter region, e.g. the region of DNA upstream of a gene where relevant proteins such as RNA polymerase and transcription factors, begin to transcribe, is an important epigenetic mechanism for the dysregulation of expression of these genes in a context-dependent manner [23].

In the present study, we identified 11 genes that were hypermethylated within their promoter regions in the Index group compared to Controls. Within this subset of hyper-methylated genes within the promoter region, several are of potential interest regarding psychiatric functions.

BRN1P1 (BMP/RA-inducible neural-specific protein 1) DNA hypermethylation within the promoter region was observed in the Index group relative to controls, suggesting repressed transcription for this gene. BRN1P1 proteins have been widely associated with neurogenesis and cell cycle control [24,25] since they have the potential to suppress cell cycle progression in neural stem cells. BRN1P1 in particular has been implicated in neurodevelopmental disorders [26]. Kobayashi et al. found that BRN1P1 plays multiple roles in the development and maintenance of adult hippocampal circuitry, such as regulation of neurogenesis and neuronal differentiation [27]. In addition, BRN1P1 knock-out mice showed an increase in the population of immature neurons and several abnormal behaviors such as an increase in locomotor activity, and poor social interaction, among others [28]. Certain hippocampal circuits are postulated to be impaired in a number of neurological conditions, including Alzheimer’s disease, temporal lobe epilepsy, schizophrenia, and mood disorders [29]. These findings indicate that BRN1P1 hypermethylation linked to maternal psychiatric disorders during pregnancy might trigger neurodevelopmental disorders in the offspring.

Similarly, COMMD1 (Copper metabolism gene domain) hypermethylation was observed in the Index group compared to Controls. Loss of function of COMMD1 (which is usually upregulated in the human placenta across pregnancy) leads to intracellular copper (Cu2+) overload [30]. This finding is of particular interest due to the essentiality of metal ion homeostasis for proper brain function so severe neurological symptoms and cognitive diseases can be avoided [31]. For instance, elevated Cu2+ in the blood is associated with the autism spectrum syndrome [32] and has been suggested as a diagnostic biomarker. Moreover, COMMD1 deficiency in mice leads to embryonic lethality related to dysregulated placenta vascularization via hypoxia-inducible factor 1 activity [33]. In addition, COMMD1 is acknowledged as important for NF-kB signaling, sodium transport, and XIAP signaling, suggesting that COMMD1 is a regulator of multiple, important cellular processes. In sum, COMMD1 has a role in several biological functions of relevance, and therefore variations in its functionality may have an impact on the offspring’s health and in particular their brain function.

Similarly, Pumilio RNA binding family member 1 gene (PUM1), which encodes the Pumilio1 protein is distinguished by the presence of an RNA-binding Pumilio homology domain that regulates the mRNA stability and inhibits translation by interacting with its target mRNA. [34]. Mutations of this gene are observed in neurodegenerative diseases, such as spinocerebellar ataxia [35]. The hypermethylation observed in the present study in patients suffering from anxiety or depression during pregnancy provides further evidence for a pathogenic role of PUM1 mutations, especially regarding mental disorders found during fetal development.

In addition, DNA methylation/mRNA integration analyses revealed a common dysregulation of 4 genes with key roles in pregnancy. BMPR2, BMP11, LHX1, LHL18, and UBA2 show enriched hypermethylation in the present study and were associated with decreased gene expression in the Index group in a previous study from our laboratory using the same placenta samples [14].

The Bone Morphogenetic Protein Receptor 2 (BMPR2) gene is involved in numerous developmental processes, including cell growth, apoptosis, and differentiation, and is essential for post-implantation physiology and fertility. Recent studies revealed that treatment of mouse embryos with BMPR2 inhibitors, impaired development of the
extraembryonic compartment, and born mice were visibly smaller than those from control mice [36]. Moreover, the absence of uterine \textit{BMPR2} signaling suppressed IL-15, VEGF, angiopoietin, and Corin signaling and resulted in abnormal vascular development and placental abruption, thereby placing \textit{BMPR2} as a key regulator of several physiologic signaling pathways and events at the maternal-fetal interface [37].

Prenatal maternal psychiatric symptoms were also associated with the hypermethylation of multiple probes located in the gene \textit{EPB41L4B}. This gene is crucial for the cytoskeleton modifications brought on by steroid-induced cell differentiation and development [38,39]. It is also involved in the glucocorticoid receptor (GR) pathway, the main target for the stress-related steroid hormone cortisol [40]. Although glucocorticoids are fundamental for fetal development and survival, exposure to excess glucocorticoids during pregnancy in instance to relation to maternal psychiatric symptoms can be harmful to fetal development and growth [41]. Given our findings and the data mentioned above, it is conceivable that \textit{EPB41L4B} contributes to placental alterations brought on by the pregnant mother’s mental condition which could have a negative impact on neonatal outcomes.

On the other hand, \textit{UBAP2} (Ubiquitin-binding associated protein 2) and \textit{KLHL18} (Kelch-like protein 18) are also hypermethylated and downregulated in placenta samples from the Index group. These genes are known to regulate collagen solubility for the remodeling of the extracellular matrix (ECM), cervical softening, and fetal membrane activation [42]. It is known that the strength of fetal membranes relays on collagen fiber synthesis, which appears lowered in cases of membrane rupture [43]. These alterations can lead to recognized perinatal risks such as infection and inflammation. Interestingly, the ECM-receptor interaction pathway was significantly hypermethylated in the Index group compared to the Control. Several studies suggest activation of integrins by ECM ligands results in the stimulation of multiple pathways that can generate ROS, which can be beneficial or detrimental.
in a context-dependent manner [44].

4.1. Limitations of the study

In light of the complex nature of depression and the epigenetic mechanisms involved, we acknowledge that the number of samples analyzed in this study is relatively limited. While we made efforts to carefully select patients within the two groups to ensure meaningful comparisons, we recognize that this study represents a preliminary exploration of a complex and multifaceted subject. As such, the results presented here provide a window into the potential epigenetic differences associated with depression, but further investigations with larger and more diverse cohorts are warranted to validate and extend these findings.

In addition, only 4 genes were found both hypermethylated and differentially expressed in the presently studied population. This would suggest that the abnormal DNA methylation observed might not necessarily be linked to dysregulated gene expression but rather affect gene expression patterns in a later stage of life, resulting in impaired physiological functions.

5. Conclusions

Taken together, the results from the present study demonstrate that exposure to stress, with self-perceived anxiety or depression during pregnancy alters the DNA methylation status of particular genes in the term placenta, genes with key roles in important physiological functions. Epigenetic modifications in the placenta may serve as a mechanism linking environmental cues to placental pathology and, may help predict the risk of offspring disease. Therefore, DNA methylation may serve as a biomarker of the intrauterine and extrauterine environment and may help predict the risk of adult disease programmed in utero.

Author contributions


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Ethics approval

All methods were performed in accordance with the relevant guidelines and regulations and informed consent was obtained from all participants. The Regional Ethical Review Board in Linköping (Dnr 2011/499-31, 2013/355-32) and the Swedish Ethical Review Authority (EPM), Uppsala, both gave ethical permissions (Dnr 2020–05429). Information was handled anonymously. In clinical research conducted in Sweden, this is standard practice.

Declaration of competing interest

None of the authors have any conflicts of interest to declare.

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