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Inflammatory markers in late pregnancy in association with postpartum depression—A nested case-control study

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ABSTRACT

Recent studies indicate that the immune system adaptation during pregnancy could play a significant role in the pathophysiology of perinatal depression. The aim of this study was to investigate if inflammation markers in a late pregnancy plasma sample can predict the presence of depressive symptoms at eight weeks postpartum. Blood samples from 291 pregnant women (median and IQR for days to delivery, 13 and 7–23 days respectively) comprising 63 individuals with postpartum depressive symptoms, as assessed by the Edinburgh postnatal depression scale (EPDS ≥ 12) and/or the Mini International Neuropsychiatric Interview (M.I.N.I.) and 228 controls were analyzed with an inflammation protein panel using multiplex proximity extension assay technology, comprising of 92 inflammation-associated markers. A summary inflammation variable was also calculated. Logistic regression, LASSO and Elastic net analyses were implemented. Forty markers were lower in late pregnancy among women with depressive symptoms postpartum. The difference remained statistically significant for STAM-BP (or otherwise AMSH), AXIN-1, ADA, ST1A1 and IL-10, after Bonferroni correction. The summary inflammation variable was ranked as the second best variable, following personal history of depression, in predicting depressive symptoms postpartum. The protein-level findings for STAM-BP and ST1A1 were validated in relation to methylation status of loci in the respective genes in a different population, using openly available data. This explorative approach revealed differences in late pregnancy levels of inflammation markers between women presenting with depressive symptoms postpartum and controls, previously not described in the literature. Despite the fact that the results do not support the use of a single inflammation marker in late pregnancy for assessing risk of postpartum depression, the use of STAM-BP or the novel notion of a summary inflammation variable developed in this work might be used in combination with other biological markers in the future.

1. Introduction

Pregnancy and childbirth are life changing events. Approximately 12% of all women will suffer from depressive symptoms in the perinatal period (O'Hara and McCabe, 2013). The severity of these symptoms varies from tiredness, sleep problems, feelings of inadequacy in the new parental role, loss of appetite and loss of interests in social activity to severely depressed mood, depressive delusions, self-destructive behaviour, neglecting or harming the child and suicide (Esscher et al., 2016; Miller, 2002). Maternal depression in the perinatal period affects not only the mother but also the entire family. Studies indicate that children of mothers with perinatal depression are at increased risk of emotional problems, behavioral and psychiatric diagnoses as well as poor physical health and self-regulation (Agnafors et al., 2013; Gentile, 2017; Zijlmans et al., 2015). Maternal depression is also shown to

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be a risk factor for poor maternal-infant bonding (Dubber et al., 2015). Several risk factors have been identified for antenatal and postpartum depression (PPD), including history of depression, low socioeconomic status, stressful life events, low self-esteem, lack of social support, pregnancy and postpartum complications. The suggested biological pathways in PPD include fluctuations in hormonal and steroid levels (Brummelte and Galea, 2016; Iliadis et al., 2015a; Iliadis et al., 2015b; Skalkidou et al., 2012). The latest reviews suggest that hypothalamic-pituitary-adrenal dysregulation, genetic vulnerability and inflammatory processes represent the major biological predictors (Yim et al., 2015).

The role of inflammation in the pathogenesis of depression is increasingly acknowledged. In early studies, depressive symptoms were related to increased expression of circulating inflammatory markers, such as interleukin (IL)-6 (Maes et al., 1993). Later data has contributed to the understanding of more complex pathways pathophysiologically connected to depression; particularly, pro-inflammatory cytokines, such as IL-6, were found to activate the tryptophan metabolizing enzyme indoleamine-pyrrole 2,3-dioxygenase (IDO), causing reduced production of serotonin in the synaptic clefts and at the same time increased production of neurotoxic substances through the kynurenine pathway (Heyes et al., 1992; Stone and Darlington, 2002). One of the downstream products of the kynurenine pathway is quinolinic acid, which acts as an agonist of the N-methyl–D-aspartate (NMDA) glutamate-receptor, leading to glutamate release. Increased levels of the inflammation acute phase plasma C-reactive protein (CRP) have been also associated with altered glutamate metabolism in depressed patients (Haroon et al., 2016), whereas elevated levels of glutamate in some brain regions have been found in patients with major depression (Sanacora et al., 2004). These monoaminergic and glutamate hypotheses, focusing on inflammation, are discussed in relationship to the elevated risk for depression in patients treated with cytokines and the similarities of depression symptoms and symptoms of cytokine-induced diseases (Miller et al., 2009; Raison et al., 2006).

It has now been established that the peripheral immune response is signalling to the brain, despite previous notions of the brain as separated from local immune reactions (Galea et al., 2007). Despite the fact that cytokines usually do not pass the blood-brain barrier, they have been shown to signal to the central nervous system through humoral and neuronal routes, e.g. via activation of the vagus nerve (McCusker and Kelley, 2013). Cytokine receptors are found on neurons both peripherally and locally (Licinio and Wong, 1997), whereas the brain parenchymal macrophages, microglial cells, can produce pro-inflammatory cytokines as well as prostaglandins. The engagement of different immune-to-brain communication pathways, has been shown to initiate the production of pro-inflammatory cytokines by microglial cells (Dantzer et al., 2008).

During pregnancy, the female body needs to maintain a balance between protection against pathogens and tolerance against the semi-allogeneic fetus; this requires an adaptive change in the immune system function. This adaptation is to date not fully understood. Previous theories described an upregulation of the innate immune system and a downregulation of the adaptive immune system (Luppi, 2003), a shift from the T-helper cell type 1 (Th1) to the T-helper cell type 2 (Th2) system (Raghupathy, 1997). More recent research supports a more complex balance between the two systems and emphasizes the importance of regulatory functions (La Rocca et al., 2014; Mjosberg et al., 2010).

It is now believed that the immune system regulation during normal pregnancy follows three different phases. In analogy with open wounds pathophysiology, the first phase represents a pro-inflammatory state (Mor et al., 2011). During this phase, chemokines, cytokines and growth factors are produced in the endometrium and secreted into the cavity which are thought to have an important role in the implantation and placentation processes, altering the adhesion potential and providing chemoattraction to the blastocyst (Hannan et al., 2011). The second phase, coinciding with the rapid fetal growth period, is characterized by an anti-inflammatory state that has been associated with increase in well-being for many women (Mor et al., 2011). The placenta plays an important part in the adaptation of the maternal immune system that also includes a shift from cell-mediated immune response to humoral-mediated responses in the first two trimesters (Kumpel and Manoussaka, 2012). The third phase occurs prior to delivery, when immune cells migrate into the myometrium creating a pro-inflammatory state (Brewster et al., 2008). Increase of pro-inflammatory cytokines has been observed at the end of pregnancy, both in the cervical tissue during cervix ripening (Dubicke et al., 2010; Malmstrom et al., 2007; Sennstrom et al., 2000) as well as in the peripheral blood (Fransson et al., 2011). Many diseases of pregnancy, such as preeclampsia, gestational diabetes and preterm birth are thought to be associated with inflammation (Vannuccini et al., 2016).

Postpartum period adaptation includes stabilization of bodily systems to the non-pregnant state, but also the psychological and physiological adaptation needed to care for the baby. The inflammatory response that accelerates during labor (Sennstrom et al., 2000), continues into the postpartum period where healing and involution take place, possibly mediated through both pro- and anti-inflammatory mediators (Nilsen-Hamilton et al., 2003). The postpartum immune system has also been reported to shift to a Th1 repertoire (Elenkov et al., 2001), that has been associated with increased susceptibility for infection during the immune reconstitution in the postpartum period (Singh and Perfect, 2007). The peripartum period represents one of the few biological paradigms of dynamic states in adult life. It encompasses tremendous changes in hormonal levels, inflammatory parameters, stress tolerance and the nervous system (Kim et al., 2016). This whole period, from both somatic and psychological aspects, can be considered as a stressor per se. Stress during pregnancy has been linked to preterm birth and other adverse pregnancy outcomes possibly through interactions with the immune system (Christian, 2012; Coussons-Read et al., 2012a). Likewise, alterations in the stress-immune systems crosstalk during the pregnancy and peripartum could predispose to PPD (Corwin and Pajer, 2008).

The combination of the high prevalence of depression and the dramatic immune system changes in the perinatal period indicates a role of the inflammatory response in the development of depression. However, this is still a relatively unexplored area. Among the inflammatory markers, IL-6 is one of the most well-studied ones in the field of perinatal depression research. In the review by Osbourne and Monk (Osborne and Monk, 2013), some of the studies confirm an association of IL-6 levels with antenatal or postpartum depression, while others do not (Skalkidou et al., 2009). Other associated markers described in the literature are IL-1beta, Leukemia inhibitory factor receptor (LIF-R), Tumor necrosis factor-a (TNF-a), Interferon-gamma (IFN-gamma), or ratios of some of these. Although previous research supports a positive association between markers of inflammation and depression in the general population, the associations in pregnant groups have not always been reproduced (Osborne and Monk, 2013). Comparisons cannot easily be made, as individual studies assess the inflammation markers in different body fluids using different techniques and at different time points (Boufidou et al., 2009; Christian et al., 2009; Osborne and Monk, 2013). There are also indications of disparities between groups of women, for example higher general levels of IL-6 during pregnancy in African American women (Blackmore et al., 2014; Cassidy-Bushrow et al., 2012). Moreover, alterations in the stress-immune systems crosstalk could have different impact in

different trimesters (Coussons-Read et al., 2012a; Coussons-Read et al., 2012b).

During the last decades, studies have begun to increasingly focus their efforts into the identification of predictive factors, rather than underlying causes of depression; and the identification of biomarkers play a central role in this approach (Gururajan et al., 2016b). Moreover, as findings on strong predictive markers for perinatal depression are largely lacking, with few exceptions (Guintivano et al., 2014; Osborne et al., 2016), there is a need for explorative analyses that include different types of inflammatory markers or even the combination of many different markers (Osborne and Monk, 2013).

1.1. Aim

The aim of this study was to investigate if any or a combination of 92 inflammation markers assessed in late pregnancy could predict depressive symptoms postpartum. A secondary aim was to investigate, in an independent, open access sample, whether antenatal methylation levels of CpG-sites associated with the genes corresponding to markers identified would predict a postpartum depressive episode.

2. Methods

2.1. Subjects

This study is part of an ongoing longitudinal cohort project, the BASIC-study (Biology, Affection, Stress, Imaging and Cognition) (Hellgren et al., 2013; Iliadis et al., 2015b,c). All pregnant Swedish speaking women over 18 years of age, without confidential personal data, who are scheduled for routine ultrasound examination at the Uppsala University Hospital, are invited to participate in the study.

All participants were asked to fill in online questionnaires at the 17th and 32nd gestational weeks and at 6 weeks postpartum. Included in the surveys is, *inter alia*, the Edinburgh Postnatal Depression Scale (EPDS), a self-report questionnaire with 10 questions, which is widely used for depression screening in the perinatal period, exhibiting a sensitivity of 72% and specificity of 88% in the Swedish context (SBU, 2012; Cox et al., 1987; Wickberg and Hwang, 1996). A selection of participating women were invited to take part in a visit at the Women's Clinic research laboratory at the Uppsala University Hospital at the 38th gestational week and/or 8 weeks postpartum. The aim of these visits was to more thoroughly assess a group of possible cases of peripartum depression as well as a group of controls. In order to address this, and also avoid possible misclassification, only those with EPDS $\geq$ 14 in the late pregnancy and/or postpartum questionnaires, as well as a similar number of participants with EPDS < 8 were invited as possible cases and controls respectively. During the visit, most of which were held in the morning, women filled out the EPDS scale again, the Mini International Neuropsychiatric interview (MINI) was conducted, and non-fasting venous blood samples were collected.

Furthermore, all women undergoing elective caesarean section at Uppsala University Hospital were asked to participate in the study. When signing in for the caesarean section and after giving informed consent, participants were asked to fill out the EPDS-scale. Fasting blood samples were collected in the morning before the caesarean, which is performed in approximately the 38th gestational week.

For the main analysis in this nested case-control sub-study, all pregnant women who attended a visit in late pregnancy during the years 2010–2014 and those who underwent elective caesarean section were included (n = 293). All women were also assessed at six weeks postpartum via web-based questionnaires. Eligible women were Swedish speaking, non-smoking with singleton pregnancies (Fig. 1). For the two sensitivity analyses, only women who (a) did not have any significant depressive symptoms during pregnancy (i.e. <12 on EPDS and negative MINI interview) (Sensitivity analysis 1) and (b) did not report any prior history of depressive episodes (Sensitivity analysis 2) were included.

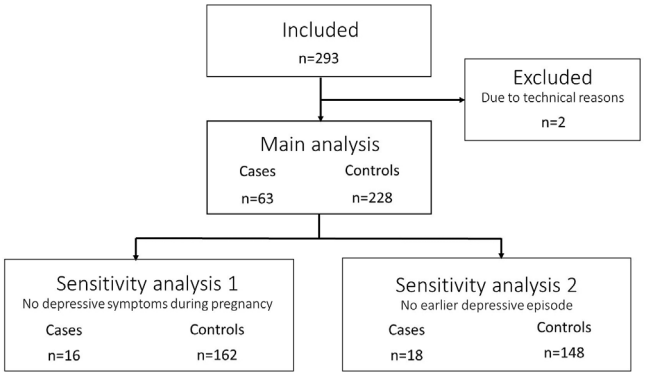

Fig. 1. Number of cases and controls in the main and sensitivity analyses.

2.2. Sample collection and analytic procedure

Coded blood samples were collected and stored at room temperature for a maximum of 1 h before centrifuged for ten minutes in 1.5 R.C.F (Relative centrifugal force). The plasma was transferred to a new tube, common for all samples, and stored at −70 °C before being sent to the Clinical Biomarker facility at SciLifeLab for analysis. Samples were thawed on ice before being transferred to 96-well plates, each consisting of 90 samples and 6 control. None of the samples used in this study had previously been thawed. Moreover, all samples were analyzed using same batch of reagents, with cases and controls evenly distributed within the plates.

The relative levels of 92 inflammatory proteins were analyzed with Proseek Multiplex Inflammation I panel using multiplex extension assay (PEA) according to the manufacturer's instructions (Olink Proteomics, Sweden) (Assarsson et al., 2014; Lundberg et al., 2011). A list of the 92 inflammation markers analyzed in the Proseek Multiplex Inflammation I panel with corresponding UniProt identities are reported elsewhere (Larsson et al., 2015). In brief, for each inflammatory protein, when a pair of DNA oligonucleotide-labeled antibody probes binds to a common target protein the DNA oligonucleotides in proximity hybridized to each other allowing a proximity-dependent DNA polymerization to form an amplifiable DNA molecule. The newly formed DNA template is subsequently amplified and quantified using BioMark™ HD real-time PCR platform (Fluidigm, South San Francisco, CA, USA). The assay has sensitivity down to fg/mL and detects relative protein values that can be used for comparison between groups, but not for absolute quantification. The plasma sample (1 mL) was mixed with 3 μL incubation mix containing 92 pairs of probes, each consisting of an antibody labeled with a unique corresponding DNA oligonucleotide. The mixture was first incubated at 4 °C overnight. Then, 96 μL extension mix containing DNA polymerase and PCR reagents was added, and the samples were incubated for 5 min at room temperature before the plate was transferred to the thermal cycler for an initial DNA extension at 50 °C for 20 min followed by 17 cycles of DNA amplification. A 96.96 Dynamic Array IFC (Fluidigm, South San Francisco, CA, USA) was prepared and primed. In a new plate, 2.8 μL of sample mixture was mixed with 7.2 μL detection mix from which 5 μL was loaded into the right side of the primed 96.96 Dynamic Array IFC. The unique primer pairs for each protein were

loaded into the left side of the 96.96 Dynamic Array IFC, and the protein expression program was run in Fluidigm Biomark reader according to the instructions for Proseek.

Each plate was run with three negative controls (buffer) and three interplate controls. Every sample was also spiked in with two incubation controls (green fluorescent protein and phycoerythrin), one extension control and one detection control. Normalization of data was performed in GenEx softwere using Olink Wizard providing normalized protein expression (NPX) data on a Log2-scale where a high protein value corresponds to a high protein concentration (Assarsson et al., 2014). In brief, the NPX is calculated in three steps from the quantification cycle (Cq) values generated in the real-time PCR: i) $\Delta Cq_{sample} = Cq_{sample} - Cq_{extensioncontrol}$, ii) $\Delta\Delta Cq = \Delta Cq_{sample} - \Delta Cq_{interplatecontrol}$, iii) NPX = Correction factor $- \Delta\Delta Cq_{sample}$. The extension control is subtracted from the Cq-value of every sample in order to correct for technical variation and the interplate control is subtracted to compensate for possible variation between runs. Finally, the NPX is calculated by normalization against a calculation correction factor.

Two samples that failed the technical quality controls were excluded, resulting in 291 blood samples analyzed. Analysis of the inflammation markers Programmed cell death 1 ligand 1 (PD-L1) and Extracellular newly identified RAGE-binding protein (EN-RAGE) were excluded from the analyses due to technical problems.

Sixteen of the 92 inflammation markers that were below LOD for more than 50% of the samples were excluded from later stages of analyses, resulting in 74 markers included in the final statistical analyses. Excluded markers were: Interleukin (IL) −1 alpha, IL-2, IL-2 receptor subunit beta, IL-4, IL-5, IL-13, IL-17A, IL-20, IL-20 receptor subunit alpha, IL-22 receptor subunit alpha 1, IL-24, IL-33, Cytokine receptor-like factor 2 (TSLP), TNF, Leukemia inhibitory factor (LIF) and Neurturin (NRTN).

2.3. Study variables

2.3.1. Exposure variables

The 74 inflammation markers that had detectable NPX values for more than 50% of the blood samples were treated as exposure variables. The samples that had NPX values below LOD were replaced with LOD/sqrt(2) (National Health and Nutrition Examination Survey, 2013). The inflammatory factors were transformed into log2(NPX + 1) to account for skewed high values and values less than one.

2.3.2. Inflammation summary variable

In order to capture a particular woman's overall level of immune system activation in a composite manner, a summary variable was constructed, by combining information from all the inflammatory markers available. This variable represents a novel approach, used in this study for the first time. Normalized protein expressions for the 74 markers were transformed into Z-scores to account for different inflammation factor scales. Essentially, each inflammation factor became a value between +3 and −3, where +3 represents a very high score compared to the rest of the sample, and −3 represents a very low score. An average value was then used to represent whether a person has higher or lower levels of inflammation markers than the rest of the population. Each woman thus received her own mean inflammation factor Z-score in order to summarize all the 74factors. This value was then transformed into a Z-score for ease of interpretation, so that 1 unit increase corresponds to a 1 standard deviation increase in mean inflammation factor Z-score.

2.3.3. Outcome variable

The main outcome variable was depression status at 6–8 weeks postpartum. The women were classified as depressed if they scored 12 points or more in the web-based EPDS assessment (Wickberg and Hwang, 1996) at 6 weeks postpartum, (included for all women in the BASIC study), scored 12 points or more at the EPDS at the visit at the laboratory 8 weeks postpartum, or received an ongoing depression diagnosis according to the MINI interview at the same timepoint (n = 63). Otherwise, women were grouped as controls.

2.3.4. Possible confounders

Age at time of delivery, BMI at time of enrolment in maternal health care, education (grouped into high school level or higher), infant gender, history of prior depressive episodes, use of selective serotonin re-uptake inhibitors (SSRI) in late pregnancy, history of inflammatory or autoimmune diseases, days from blood sampling to delivery, fasting status at the time of blood sampling were considered as possible confounders based on the literature, and were included in the multivariable models. For the women included at the time of elective caesarean section, the possible confounder "days from blood sampling to delivery" was calculated based on the expected date of delivery.

2.4. Statistical analyses

2.4.1. Clustering

Using the hclust function in the R package ClustOfVar (Chavent et al., 2013) the 74 inflammation markers were grouped into different clusters. Membership in a cluster was decided through the cutting of a hierarchical dendrogram to generate the desired number of clusters. The number of clusters was calculated through observing the stability of partitions obtained from 2 to p-1 clusters evaluated with a bootstrap approach. The clusters were primarily used for aiding in understanding and interpreting the results of further analyses.

2.4.2. Bivariate analyses

In order to assess the existence of possible associations, bivariate analyses were performed between the outcome variable and possible confounders, as well as between the Inflammation Summary variable and possible confounders, using non-parametric bivariate correlation or Mann-Whitney U test as suited.

The modelling was approached in four different ways with progressively more complicated models (Mann-Whitney U test, logistic regression, LASSO regression and Elastic net); the aim of the complex statistical methodology was to address the complex and explorative nature of the dataset and to remove any biases that might arise from a particular modelling methodology, or from unconscious modelling choices made by the researchers.

2.4.3. Mann-Whitney U test, logistic regression and bonferroni correction

For each of the 74 exposures and additional confounders, Mann-Whitney U tests were applied to test for non-parametric associations with the outcome of interest. Crude univariate logistic regressions were then applied for the same list of exposures and confounders. For each set of analyses, the Bonferroni correction was applied to correct for multiple testing. Adjusted logistic regression analyses were also undertaken, controlling for age, BMI, education, previous depression, chronic inflammatory or rheumatic disease, days from sampling to delivery, use of SSRI medication in late pregnancy, fasting at blood sampling and infant gender.

2.4.4. LASSO and elastic-net

LASSO regression is a form of penalized regression that applies variable selection; however, when there are a number of highly collinear independent variables, tends to randomly select one. Elastic-Net is another form of penalized regression that has a tuning

variable, allowing the penalization to vary between variable selections (LASSO regression) and shrinking the coefficients of a collinear group of independent variables together (ridge regression).

For a set of exposures containing the 74 inflammation exposures, LASSO logistic regression was applied, with penalization chosen by performing LARS (least-angle regression) and stopping the addition of new variables when no significant reduction in the model variance was seen (Lockhart et al., 2014). Elastic-Net logistic regression was also applied (Zou and Hastie, 2005), choosing the penalization and tuning parameters via cross-validation with 10 replicates (penalization parameter was selected to be the largest value such that the cross-validated error was within one standard-error of the minimum – thus selecting a parsimonious model with equivalent predictive abilities).

2.4.5. Separate analysis of inflammatory markers

The Mann-Whitney *U* test, logistic regression, LASSO logistic regression and Elastic Net logistic regression were applied. Additionally, the logistic, LASSO, and Elastic-Net regressions had a further regression performed in all of the data while adjusting for the aforementioned confounders. This analysis was then repeated for those without significant depressive symptoms during pregnancy (sensitivity analysis 1) and those without history of depression (sensitivity analysis 2).

2.4.6. Inflammation summary variable analyses

As a final analysis, the dataset was restricted to the inflammation summary variable and possible confounders. As a first step, in order to assess associations with possible confounders, linear regression analyses were performed, with the inflammation summary variable as the outcome variable. Subsequently, considering depression status as the outcome variable, each variable was first run with crude logistic regression, and then a fully adjusted logistic regression including all possible confounders was implemented. To perform variable selection and protect against possible overfitting, LASSO regression was applied, with penalization chosen by performing LARS (least-angle regression) and stopping the addition of new variables when no significant reduction in the model variance was seen (Lockhart et al., 2014). This analysis was then repeated for those without significant depressive symptoms during pregnancy (sensitivity analysis 1) and those without history of depression (sensitivity analysis 2).

The level of statistical significance was set at <0.05, except for the Bonferroni analyses, where we held the family-wise error rate (alpha) at 0.05, meaning that the analysis-specific alpha was reduced to 0.05/n (where n = number of analyses performed). The statistical package R 3.2.4 was used for the analyses.

2.5. Independent epigenetic analyses

2.5.1. Characterization of the epigenetic data set

Data is openly available (E-GEOD-44132) and were originally published by Guintivano et al. Fifty-four pregnant women with a history of either Major Depression or Bipolar Disorder (I, II or NOS) were included in the study and prospectively followed during pregnancy and after delivery (Guintivano et al., 2014). DNA methylation profiles in antenatal blood were generated using the Illumina 450 K methylation beadchip, which has been made available online along with information on array batch and occurrence of a pre- and postpartum depressive episode. No other clinical variables were available.

2.5.2. CpG site annotation

The expanded annotation table by Price et al. was used for CpG site annotation (Price et al., 2013), designed for the Illumina 450 K Methylation BeadChip. The annotation file was used to, for each CpG site; define the associated gene and the distance to the closest transcriptional start site (TSS). In the initial epigenetic study, CpG-sites were included in the subsequent analysis if annotated to any of the genes that were bonferroni-significant in the main analysis (i.e. ADA, AXIN1, IL-10, STAMBP, and ST1A1). In a sub-analysis of postpartum depression in antenatal euthymic women, we included CpG sites that were annotated to the gene which was bonferroni-significant in the first sensitivity analysis (i.e. ADA). We further limited the analysis to probes located within 2000 base pairs up and downstream of the TSS, as Wagner et al. showed that DNA methylation and gene expression is higher correlated in this region (Wagner et al., 2014). After the probe exclusion steps outlined above, 29 CpG sites were investigated in the subsequent analysis.

2.5.3. Statistical analysis of the epigenetic sample

All statistical analyses for this complementary epigenetic sample study were performed in using R statistics, version 3.3.0. We aimed to investigate the association of changed antenatal methylation patterns in candidate CpG sites with postnatal depression. The ComBat function of the sva package for R was subsequently used to adjust the global DNA methylation data for batch effects (Johnson et al., 2007) and a ChAMP-based statistical procedure of the Houseman algorithm was used to adjust the methylation data for white blood cell type heterogeneity (Houseman et al., 2012). Five methylation samples were classified as cross-batch controls and were excluded from the analysis. Fifty samples remained for investigation in the subsequent analysis (among which 19 antenatally depressed), of which 27 were postpartum euthymic and 23 postpartum depressed. In the main analysis, independent samples *t*-tests were performed, contrasting methylation M-values between postpartum depressed subjects and postpartum euthymic controls, not taking antenatal depression status into account. In a sensitivity analysis, we excluded samples with antenatal depression, and contrasted methylation M-values between 20 postpartum euthymic controls and 11 postpartum depressed subjects.

3. Results

The distribution of study variables by postpartum depression status is presented in Table 1. Cases were more likely to have experienced a previous episode of depression, or to use SSRIs and to be fasting at time of the blood sampling, while they had lower median scores on the Inflammation summary variable.

The clustering bootstrap approach, with 2 to *p*-1 clusters evaluated, showed the stability of partitions to be the highest with four clusters for the NPX values of the 74 inflammation markers. The markers are grouped as depicted in Fig. 2.

3.1. Main analysis

Controls had significantly higher NPX values for 40 inflammation markers when applying the Mann Whitney U Test (Table 2 and Fig. 3), and significantly higher NPX values in the following 8 inflammation markers when applying adjusted logistic regression: Signal transducing adaptor molecule- binding protein (STAM-BP), Axin1, Adenosine deaminase (ADA), Sulfotransferase 1A1 (ST1A1), NAD-dependent deacetylase sirtuin-2 (SIRT2), Caspase 8 (CASP8), IL-10 and Monocyte chemotactic protein (MCP2; Table 2 and Fig. 3). Using the Mann-Whitney *U* test, 5 inflammation markers (STAM-BP, Axin-1, ADA, ST1A1 and IL-10) had significantly higher NPX values after controlling for multiple testing (Table 2, Fig. 3 and presented as boxplots in Supplementary Fig. S1). Of these five, STAM-BP, Axin-1 and ADA were also significantly higher in controls when using Bonferroni corrected logistic regression (Table 2). Furthermore, the plasma level of STAM-BP was higher among controls when using LASSO logistic regression (Fig. 3).

Table 1
Distribution of study subjects by postpartum depression symptoms status and a series of background characteristics.

Variable	Controls (n = 228)	Cases (n = 63)	P-value[a]
Inflammation summary variable (median, IQR)	0.164, 1.756	−0.384, 1.238	**<0.01**
Age (years) (median, IQR)	33.0, 6.0	31.0, 6.0	0.51
Education			0.08
University/College	183 (80.3%)	44 (69.8%)	
Primary/Secondary school	45 (19.7%)	19 (30.2%)	
BMI before pregnancy			0.13
Normal (18.5–25 kg/m2)	154 (67.5%)	36 (57.1%)	
Outside of normal range	74 (32.5%)	27 (42.9%)	
Parity			0.09
0	82 (36.0%)	30 (47.6%)	
≥1	146 (64.0%)	33 (52.4%)	
Infant gender			0.43
Girl	103 (45.2%)	32 (50.8%)	
Boy	125 (54.8%)	31 (49.2%)	
Delivery mode			0.20
Vaginal or Vacuum extraction	140 (61.4%)	33 (52.4%)	
Cesarean section	88 (38.6%)	30 (47.6%)	
Inflammatory or rheumatic disease			0.69
No inflammatory or rheumatic disease	222 (97.4%)	61 (96.8%)	
Inflammatory or rheumatic disease	6 (2.6%)	2 (3.2%)	
Depression history[b]			**<0.01**
No depressive episode earlier in life	148 (65.5%)	18 (29.5%)	
Depressive episode earlier in life	78 (34.5%)	43 (70.5%)	
SSRI treatment in pregnancy			**0.010**
No treatment	205 (89.9%)	49 (77.8%)	
Treated	23 (10.1%)	14 (22.2%)	
Days from blood sampling to delivery (median, IQR)	14.0, 15.0	12.0, 17.0	0.81
Fasting at sampling			**0.021**
No	168 (73.7%)	37 (58.7%)	
Yes	60 (26.3%)	26 (41.3%)	

IQR: interquartile range, BMI: Body mass index.
Statistically significant p-values presented in bold.
[a] P-value derived from Independent *t*-test for normally distributed variables, Mann-Whitney *U* test, or Chi-square test.
[b] n = 287 included in this analysis.

Concerning possible associations between several covariates and the inflammation summary variable, significant differences were only detected for fasting at the time of blood sampling (linear regression derived B −0.67 and 95% CI −0.91 to −0.44) and history of depressive episode (B −0.60, 95% CI −0.83 to −0.37; data not shown).

In the multivariate analyses, an increase of 1 unit in STAM-BP (standard deviation among controls being 1.66) in late pregnancy was associated with a 39% decrease in the odds for postpartum depressive symptoms (Table 2). The LASSO/LARS multivariable logistic regression ranked the inflammation summary variable to be the second best variable (after earlier depression episode) in predicting depressive symptoms in the postpartum period (Table 3). However, inclusion of the inflammatory summary variable did not give a significant reduction in model covariance.

3.2. Sensitivity analysis 1

In sensitivity analysis 1, where only women with no depressive symptoms during pregnancy were included, 10 inflammation markers [ADA, OR: 0.26, 95% CI: 0.11–0.60, AXIN1, OR: 0.60, 95% CI: 0.42–0.86, CD40, OR:0.45, 95% CI: 0.23–0.85, Chemokine ligand 1 (CXCL1), OR:0.54, 95% CI: 0.3–0.81, Osteoprotegerin (OPG), OR:0.49, 95% CI: 0.26–0.92, SIRT2, OR: 0.58, 95% CI: 0.38-0.88, ST1A1, OR:0.08, 95% CI: 0.01–0.61, STAM-BP, OR:0.46, 95% CI: 0.28–0.75, and Tumor necrosis factor superfamily member 14 (TNFSF14), OR:0.34, 95% CI: 0.10–1.15] had significantly higher NPX values, while FGF-21 had lower NPX values (OR:1.21, 95% CI: 1.02–1.44) in controls in comparison to the ones who developed depressive symptoms postpartum (Fig. 3, column 1, sub-column "No preg depr"). Only one marker, ADA, remained significant after applying the Bonferroni correction (Fig. 3, column 2, sub-column "No preg depr"). LASSO and Elastic Net regressions showed no markers differing between cases and controls.

In the first sensitivity analysis, the LASSO/LARS multivariable logistic regression ranked the inflammation summary variable as the seventh best variable to predict depressive symptoms in the postpartum period, while its inclusion again did not give a significant reduction in model covariance (Crude OR: 0.68, 95% CI: 0.40–1.10, Adjusted OR: 0.97, 95% CI: 0.51–1.81 and LASSO OR: 1.00).

3.3. Sensitivity analysis 2

In sensitivity analysis 2, where only women with no history of depressive episodes were included, 15 inflammation markers had higher NPX values in controls (Fig. 3, column 1, sub-column "No earlier depr"). The three markers with the stronger effect estimates were CASP8 (OR 0.32, 95% CI: 0.09–1.10), Colony stimulating factor 1 (CSF1; OR 0.38, 95% CI: 0.13–1.12) and CD40 (OR 0.45, 95% CI: 0.25–0.81; data not shown). No markers remained significant after applying the Bonferroni correction. LASSO and Elastic Net regressions showed no markers differing between cases and controls.

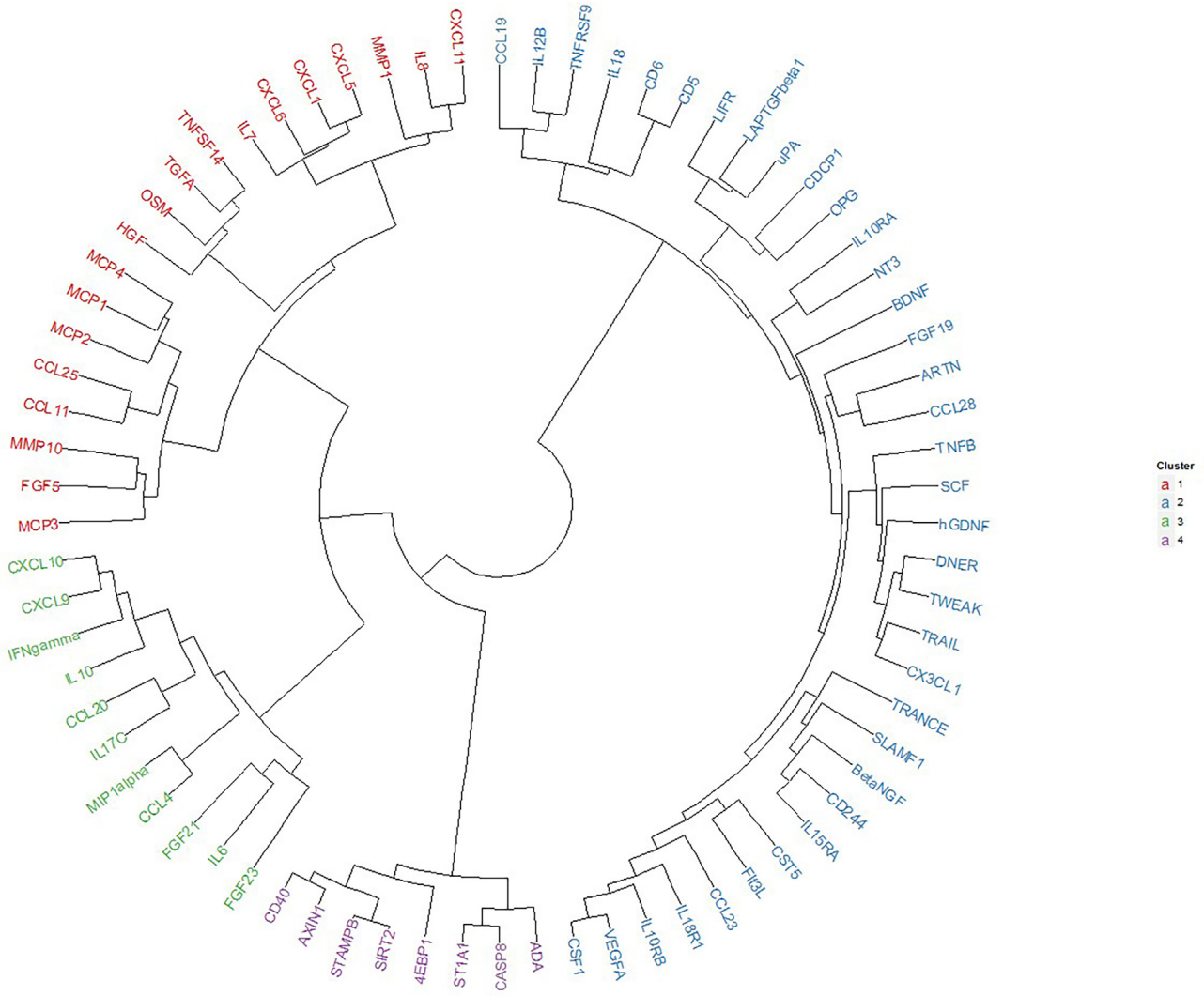

Fig. 2. Distribution of the 74 inflammation markers, grouped into 4 distinct clusters.

In the second sensitivity analysis, the LASSO/LARS multivariable logistic regression ranked the inflammation summary variable as the second best variable to predict depressive symptoms postpartum, after the use of SSRI in pregnancy. However, inclusion of the inflammation summary variable did not give a significant reduction in model covariance (Crude OR: 0.64, 95% CI: 0.39–0.99, Adjusted OR: 0.72, 95% CI: 0.41–1.22 and LASSO OR: 1.00).

Performing the analysis among only those without a history of depression or depressive symptoms during pregnancy (5 cases and 118 controls), no co-variate reaches statistical significance (Adjusted OR for the inflammation summary variable 0.49, 95%CI 0.13–1.50, LASSO/LARS OR 1.00, *p*-value 0.5, ranked as first).

3.4. Independent epigenetic sample analysis

In the independent epigenetic sample material, two CpG sites (cg23102386; cg15812873) were significantly hypomethylated in whole blood of the depressed postpartum group ($p < 0.05$; Table 4a). The results were derived by comparing methylation levels in 23 postpartum depressed and 27 postpartum euthymic women using independent samples *t*-tests, not taking antenatal depression status into account. These CpG sites are associated with STAM-BP and ST1A1.

In a final step, and after excluding women with depressive symptoms during pregnancy, we contrasted methylation levels of 11 postpartum depressed and 20 postpartum euthymic women, who were all antenatally euthymic. Five ADA associated methylation loci were studied and no individual CpG site was differentially methylated in whole blood of the depressed postpartum group (Table 4b).

4. Discussion

We aimed to study the potential association between of a wide range of inflammation markers in blood in late pregnancy and the presence of postpartum depression symptoms, via a thorough statistical approach, including sensitivity analyses and addressing issues of multiple testing and inter-correlated variables.

Out of the 74 inflammation markers assessed in late pregnancy, STAM-BP (STAM-Binding Protein, also labeled AMSH, associated molecule with the SH3 domain of STAM) was found to be significant both after the stringent Bonferroni correction and by using the LASSO analysis. An increase of 1 unit in STAM-BP (standard deviation among controls being 1.66) in late pregnancy was associated with a 39% decrease in the odds for postpartum depressive symptoms. STAM-BP is a zink-metalloprotease playing a role in cytokine-mediated intracellular signal transduction for cell growth

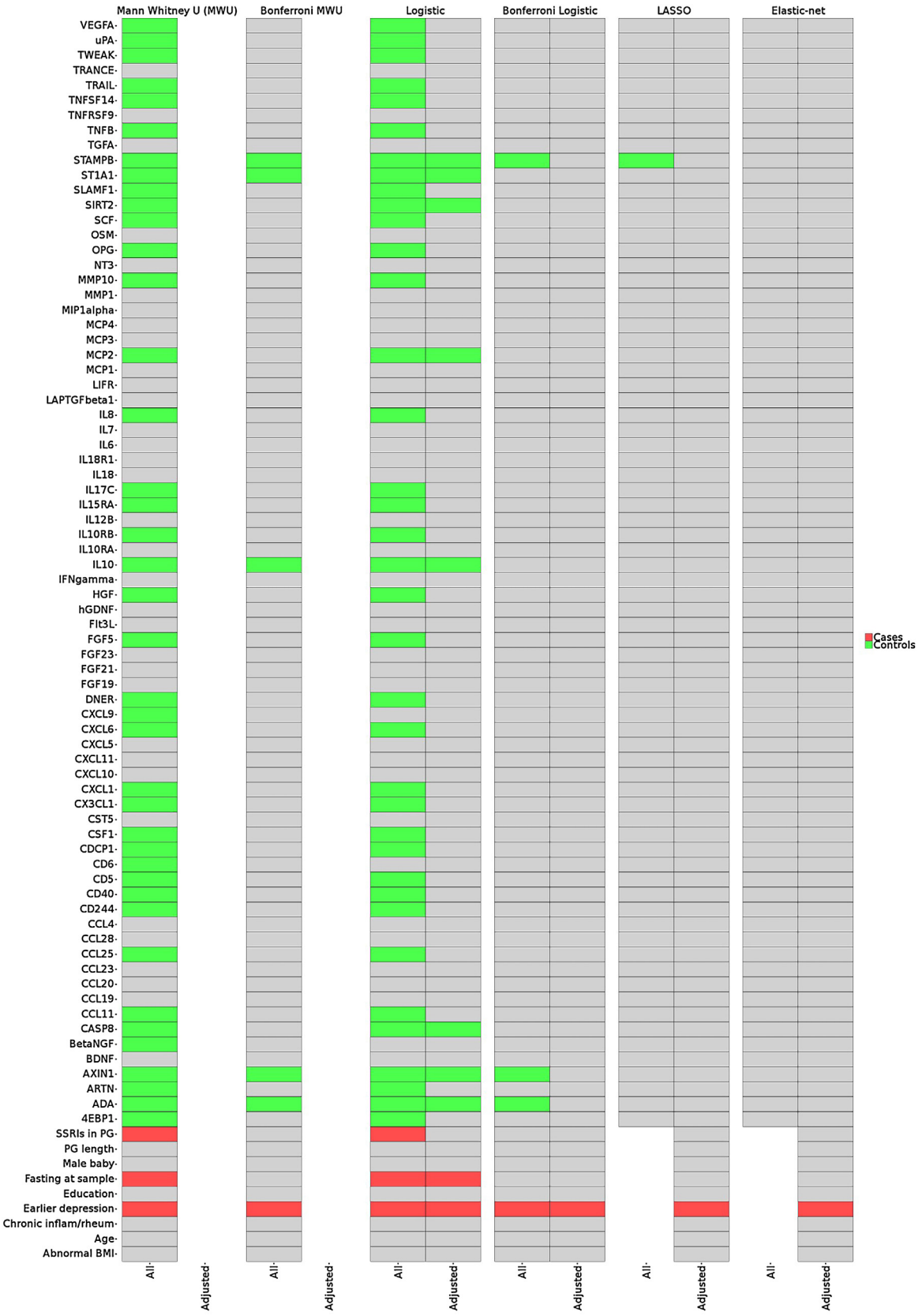

Fig. 3. Graphically presented results for differences in the 74 inflammation factors and possible confounders among cases and controls. Statistically significant differences between cases and controls are marked as **green** for markers upregulated in controls and **red** for markers upregulated in cases.
The different columns represent the different analytical methods used, (from left to right: Mann Whitney *U* test, Mann Whitney *U* test adjusted for multiple testing with Bonferroni, Logistic regression, Logistic regression adjusted for multiple testing with Bonferroni, LASSO logistic regression and Elastic net, first line), while the sub-columns represent results of the main analysis, as well as after excluding the women with depressive symptoms during pregnancy (sensitivity analysis 1), and after excluding the women with earlier depression episodes (sensitivity analysis 2).

Table 2
Mean and standard deviation (SD) of the NPX values for the inflammation markers (IF) in late pregnancy among women with postpartum depressive symptoms (cases) and controls, as well as logistic regression derived Odds Ratios (OR) and corresponding *p*-values before and after Bonferroni correction (Bonf *p*-value) for case/control status by each inflammation marker.

	Controls			Cases			Mann-Whitney *U* test		Logistic regression			Adj. logistic regression[a]		
IF	N	Mean	SD	N	Mean	SD	P-value	Bonf P-value	OR	P-value	Bonf P-value	aOR	P-value	Bonf P-value
ADA	228	6.90	±0.91	63	6.43	±0.68	<0.001	0.001*	0.41	<0.001*	0.007*	0.57	0.020*	1.000
STAMPB	228	5.40	±1.66	63	4.47	±1.23	<0.001	0.002*	0.61	<0.001*	0.005*	0.70	0.007*	0.550
AXIN1	228	4.26	±2.02	63	3.14	±1.63	<0.001	0.004*	0.71	<0.001*	0.010*	0.77	0.007*	0.586
IL10	228	3.91	±1.00	63	3.50	±0.81	<0.001	0.029*	0.55	0.004	0.308	0.62	0.039*	1.000
ST1A1	228	1.26	±0.85	63	0.91	±0.39	<0.001	0.040*	0.33	0.002	0.130	0.43	0.026*	1.000
CASP8	228	1.64	±0.88	63	1.31	±0.35	0.001	0.055	0.27	0.001	0.063	0.38	0.013*	1.000
SIRT2	228	5.56	±1.93	63	4.67	±1.41	0.001	0.062	0.72	0.001	0.070	0.78	0.016*	1.000
DNER	228	9.27	±0.63	63	9.00	±0.62	0.001	0.065	0.50	0.003	0.282	0.76	0.341	1.000
CCL11	228	8.78	±0.84	63	8.46	±0.73	0.001	0.068	0.56	0.005	0.446	0.71	0.136	1.000
CD40	228	12.05	±0.94	63	11.62	±0.80	0.001	0.083	0.57	0.001	0.101	0.74	0.114	1.000
SCF	228	10.35	±0.68	63	10.03	±0.65	0.001	0.113	0.50	0.002	0.125	0.67	0.143	1.000
CCL25	228	7.56	±0.98	63	7.17	±1.05	0.002	0.174	0.66	0.007	0.600	0.82	0.235	1.000
HGF	228	9.89	±0.73	63	9.60	±0.69	0.002	0.185	0.56	0.006	0.460	0.73	0.160	1.000
MMP10	228	7.72	±1.10	63	7.30	±0.87	0.002	0.201	0.65	0.006	0.537	0.72	0.052	1.000
TWEAK	228	10.90	±0.58	63	10.66	±0.57	0.003	0.250	0.48	0.005	0.390	0.76	0.368	1.000
ARTN	228	1.80	±0.50	63	1.58	±0.41	0.003	0.290	0.37	0.002	0.192	0.63	0.201	1.000
CD5	228	4.40	±0.61	63	4.16	±0.55	0.005	0.400	0.47	0.004	0.352	0.61	0.087	1.000
CD244	228	7.53	±0.68	63	7.26	±0.62	0.006	0.490	0.52	0.005	0.407	0.75	0.292	1.000
uPA	228	15.90	±0.55	63	15.69	±0.58	0.008	0.699	0.51	0.010	0.826	0.83	0.528	1.000
CSF1	228	11.37	±0.45	63	11.22	±0.38	0.007	0.558	0.45	0.020	1.000	0.80	0.579	1.000
CX3CL1	228	7.34	±0.75	63	7.08	±0.67	0.007	0.572	0.61	0.016	1.000	0.96	0.865	1.000
MCP2	228	10.88	±1.10	63	10.49	±0.92	0.010	0.815	0.68	0.011	0.885	0.66	0.015*	1.000
TRAIL	228	10.56	±0.63	63	10.34	±0.66	0.010	0.865	0.57	0.019	1.000	0.94	0.842	1.000
OPG	228	15.25	±0.82	63	14.95	±0.73	0.011	0.881	0.63	0.011	0.895	0.76	0.195	1.000
4EBP1	228	7.30	±1.54	63	6.71	±1.52	0.014	1.000	0.77	0.008	0.662	0.81	0.055	1.000
IL8	228	6.81	±0.88	63	6.49	±0.79	0.014	1.000	0.62	0.011	0.907	0.71	0.086	1.000
BDNF	228	7.58	±5.71	63	7.38	±5.51	0.694	1.000	0.99	0.799	1.000	1.01	0.625	1.000
BetaNGF	228	1.73	±0.39	63	1.62	±0.40	0.016	1.000	0.43	0.051	1.000	0.82	0.653	1.000
CCL19	228	11.88	±1.13	63	11.86	±1.22	0.852	1.000	0.99	0.935	1.000	1.10	0.534	1.000
CCL20	228	7.42	±1.35	63	7.19	±1.07	0.395	1.000	0.86	0.221	1.000	0.85	0.232	1.000
CCL23	228	11.99	±0.80	63	11.88	±0.72	0.197	1.000	0.83	0.325	1.000	1.15	0.505	1.000
CCL28	228	5.32	±1.21	63	4.97	±1.28	0.053	1.000	0.80	0.053	1.000	1.00	0.989	1.000
CCL4	228	6.47	±0.92	63	6.26	±0.65	0.180	1.000	0.73	0.091	1.000	0.78	0.222	1.000
CD6	228	3.86	±0.79	63	3.64	±0.72	0.043	1.000	0.67	0.051	1.000	0.68	0.089	1.000
CDCP1	228	4.05	±0.80	63	3.79	±0.81	0.027	1.000	0.66	0.025	1.000	0.80	0.279	1.000
CST5	228	7.40	±0.65	63	7.24	±0.60	0.087	1.000	0.67	0.083	1.000	1.01	0.966	1.000
CXCL1	228	11.37	±1.22	63	10.94	±1.30	0.021	1.000	0.76	0.017	1.000	0.82	0.118	1.000
CXCL10	228	12.42	±1.34	63	12.44	±1.08	0.420	1.000	1.01	0.907	1.000	1.12	0.370	1.000
CXCL11	228	10.08	±1.40	63	9.84	±1.34	0.248	1.000	0.88	0.234	1.000	1.00	0.968	1.000
CXCL5	228	13.17	±1.89	63	12.87	±2.10	0.335	1.000	0.92	0.269	1.000	0.95	0.507	1.000
CXCL6	228	9.26	±1.17	63	8.84	±1.14	0.017	1.000	0.73	0.013	1.000	0.83	0.207	1.000
CXCL9	228	7.55	±1.41	63	7.22	±1.12	0.049	1.000	0.80	0.088	1.000	0.89	0.382	1.000
FGF19	228	10.27	±1.45	63	10.08	±1.26	0.325	1.000	0.91	0.360	1.000	1.12	0.350	1.000
FGF21	228	6.05	±2.56	63	6.67	±2.65	0.086	1.000	1.09	0.097	1.000	1.10	0.146	1.000
FGF23	228	3.81	±1.54	63	3.90	±1.39	0.521	1.000	1.04	0.693	1.000	1.00	0.972	1.000
FGF5	228	1.45	±0.45	63	1.35	±0.30	0.043	1.000	0.39	0.050	1.000	0.61	0.311	1.000
Flt3L	228	12.30	±0.59	63	12.18	±0.64	0.241	1.000	0.72	0.170	1.000	0.94	0.815	1.000
IFNgamma	228	1.50	±1.21	63	1.22	±0.38	0.111	1.000	0.56	0.061	1.000	0.72	0.240	1.000
IL10RA	228	1.19	±0.60	63	1.05	±0.38	0.098	1.000	0.53	0.072	1.000	0.58	0.115	1.000
IL10RB	228	8.99	±0.58	63	8.78	±0.64	0.016	1.000	0.56	0.015	1.000	0.83	0.485	1.000
IL12B	228	4.56	±0.81	63	4.56	±0.64	0.795	1.000	1.01	0.962	1.000	1.23	0.330	1.000
IL15RA	228	1.23	±0.27	63	1.14	±0.25	0.016	1.000	0.26	0.014	1.000	0.53	0.316	1.000
IL17C	228	2.76	±0.81	63	2.53	±0.57	0.049	1.000	0.64	0.041	1.000	0.74	0.221	1.000
IL18	228	10.82	±0.84	63	10.85	±0.97	0.967	1.000	1.04	0.827	1.000	1.17	0.427	1.000
IL18R1	228	9.28	±0.77	63	9.15	±0.82	0.091	1.000	0.80	0.231	1.000	1.00	0.989	1.000
IL6	228	3.47	±1.09	63	3.40	±0.86	0.629	1.000	0.93	0.619	1.000	0.84	0.311	1.000
IL7	228	3.27	±0.89	63	3.05	±0.80	0.051	1.000	0.73	0.080	1.000	0.85	0.389	1.000
LAPTGFbeta1	228	11.00	±0.77	63	10.78	±0.94	0.087	1.000	0.72	0.065	1.000	1.03	0.894	1.000
LIFR	228	7.08	±0.79	63	6.97	±0.87	0.505	1.000	0.85	0.361	1.000	1.08	0.711	1.000
MCP1	228	12.96	±0.56	63	12.95	±0.90	0.133	1.000	0.99	0.946	1.000	1.06	0.788	1.000
MCP3	228	1.52	±0.56	63	1.40	±0.44	0.165	1.000	0.60	0.131	1.000	0.65	0.257	1.000
MCP4	228	2.23	±0.62	63	2.11	±0.45	0.209	1.000	0.68	0.150	1.000	0.79	0.391	1.000
MIP1alpha	228	2.87	±0.84	63	2.77	±0.60	0.403	1.000	0.83	0.372	1.000	0.94	0.790	1.000
MMP1	228	2.56	±1.17	63	2.30	±1.04	0.084	1.000	0.81	0.114	1.000	0.81	0.143	1.000
NT3	228	2.66	±0.86	63	2.54	±0.86	0.286	1.000	0.85	0.328	1.000	1.05	0.800	1.000
OSM	228	5.49	±1.39	63	5.26	±1.08	0.160	1.000	0.88	0.229	1.000	0.86	0.237	1.000
SLAMF1	228	2.12	±0.72	63	1.92	±0.44	0.035	1.000	0.53	0.029	1.000	0.73	0.346	1.000
TGFA	228	1.38	±0.62	63	1.25	±0.22	0.381	1.000	0.51	0.116	1.000	0.53	0.125	1.000
TNFB	228	4.18	±0.70	63	3.99	±0.59	0.021	1.000	0.63	0.041	1.000	0.92	0.747	1.000
TNFRSF9	228	7.72	±0.61	63	7.66	±0.66	0.497	1.000	0.84	0.457	1.000	1.18	0.518	1.000
TNFSF14	228	1.91	±0.72	63	1.68	±0.41	0.013	1.000	0.47	0.013	1.000	0.54	0.051	1.000
TRANCE	228	3.21	±0.76	63	3.19	±0.71	0.799	1.000	0.96	0.841	1.000	1.24	0.324	1.000
VEGFA	228	14.65	±0.50	63	14.49	±0.41	0.014	1.000	0.49	0.021	1.000	0.89	0.741	1.000
hGDNF	228	2.62	±0.56	63	2.51	±0.54	0.261	1.000	0.69	0.156	1.000	1.32	0.360	1.000

[a] Adjusted for age, BMI, education, previous depression, chronic inflammatory or rheumatic disease, days from sampling to delivery, use of SSRI medication in late pregnancy, fasting at blood sampling and infant gender.

(Tanaka et al., 1999). It was added to the Proseek Multiplex Inflammation panel as an exploratory marker as it seems to have a role in sorting and trafficking of ubiquitinated proteins (Ma et al., 2007). In cell lines of medulloblastoma, a common type of pediatric embryonal brain tumor, where the STAM-BP gene had been silenced by small interfering RNA (SiRNA), there was an accumulation of protein aggregates leading to elevated apoptotic activity (McDonell et al., 2013). Furthermore, mutations of STAM-BP have been found to cause microcephaly-capillary malformations (McDonell et al., 2013), while STAM-BP impairment has been associated with neurodegeneration (Ishii et al., 2001; Suzuki et al., 2011), indicating an important role of STAM-BP in brain homeostasis. However, the role of STAM-BP in mood disorders and its potential as a biomarker in this area is still largely unexplored.

Among the other markers found to be significantly lower among the cases compared with the controls, even after applying Bon-

Table 3
Crude, adjusted and LASSO full multivariable logistic regression derived odds ratios (ORs) and 95% Confidence Interval (CI) for postpartum depressive symptoms by the inflammation summary variables and possible confounders.

	CRUDE		ADJUSTED[b]		LASSO		
	OR	95% CI	OR	95% CI	OR	P-value	Rank
Inflammation summary variable	0.63	0.47 to 0.82	0.79	0.57 to 1.08	1.00	0.29	2
Age (years)	0.98	0.92 to 1.04	0.96	0.89 to 1.03	1.00	0.90	6
Abnormal BMI (kg/m^2)[a]	1.56	0.88 to 2.76	1.21	0.62 to 2.34	1.00	0.94	5
More than high school education	0.57	0.31 to 1.08	0.94	0.44 to 2.07	1.00	0.92	4
Chronic inflammatory or rheumatic disease	1.25	0.18 to 5.58	1.02	0.13 to 5.70	1.00	1.00	10
Fasting at sample collection	1.97	1.09 to 3.51	2.44	1.01 to 5.97	1.00	0.31	3
SSRI in pregnancy	2.55	1.20 to 5.26	1.38	0.57 to 3.23	1.00	0.88	8
Depression history	4.53	2.49 to 8.55	4.01	2.06 to 8.05	2.09	<0.01	1
Male infant	0.80	0.46 to 1.40	0.72	0.38 to 1.34	1.00	0.98	7
Days from sampling to partus	1.00	0.97 to 1.03	1.02	0.98 to 1.06	1.00	0.30	9

BMI: Body mass index, SSRI: Selective serotonin reuptake inhibitor.
[a] BMI before pregnancy outside of normal range (18.50–24.99 kg/m^2).
[b] Adjusted for age, BMI, education, previous depression, chronic inflammatory or rheumatic disease, days from sampling to delivery, use of SSRI medication in late pregnancy, fasting at blood sampling and infant gender.

Table 4a
Excerpt showing differential antenatal methylation status of candidate CpG sites in postpartum euthymic vs. postpartum depressed women.

			Antenatal Blood DNA Methylation Profiles (n = 50)				
			% DNA Methylation (SD)		Independent samples *t*-test		
Gene	Illumina ID	Distance to TSS	Postpartum Depressed (n = 23)	Postpartum Euthymic (n = 27)	t	df	P.val
STAMBP	**cg23102386**	**−775**	**87.06 (2.72)**	**88.66 (1.83)**	**−2.43**	**41.43**	**9.78E-03**
ST1A1	**cg15812873**	**−108**	**10.21 (2.29)**	**11.75 (3.70)**	**−1.82**	**47.77**	**3.78E-02**
ST1A1	cg18530748	63	15.95 (5.09)	19.21 (8.59)	−1.57	47.29	6.13E-02
STAMBP	cg04835122	14	5.66 (1.02)	6.05 (0.82)	−1.54	39.00	6.61E-02
STAMBP	cg02352181	1959	81.93 (2.19)	82.64 (2.22)	−1.15	47.13	*ns*
AXIN1	cg08577231	−1124	91.30 (0.88)	91.60 (1.61)	−1.13	41.33	*ns*
ST1A1	cg05845592	141	7.77 (4.88)	10.32 (8.39)	−0.97	46.93	*ns*
IL10	cg14284394	−1443	82.19 (1.44)	82.63 (2.02)	−0.97	46.06	*ns*
ST1A1	cg01009486	1921	87.30 (1.46)	87.61 (2.07)	−0.73	47.32	*ns*
ST1A1	cg27034150	−146	9.17 (2.47)	9.89 (3.89)	−0.68	47.87	*ns*

Cohort consists of pregnant women with a history of major depression or bipolar disorder (I, II or NOS) (E-GEOD-44132). Prior to analysis, methylation data were preprocessed and adjusted for batch effects and corrected for blood cell type heterogeneity. 5 methylation samples were classified as cross-batch controls and were excluded from the analysis. Independent samples *t*-tests were performed, contrasting methylation M-values in 23 postpartum depressed and 27 postpartum euthymic women.
Abbreviations: dfdegrees of freedom; p.valp-value; tt-statistic.

Table 4b
ADA associated methylation changes by postpartum depression in an antenatally euthymic cohort.

			Antenatal Blood DNA Methylation Profiles				
			% DNA Methylation (SD)		Independent samples *t*-test		
Gene	Illumina ID	Distance to TSS	Postpartum Depressed (n = 11)	Postpartum Euthymic (n = 20)	t	df	P.val
ADA	cg08283932	−331	3.58 (0.29)	3.78 (0.42)	−1.53	27.13	0.136
ADA	cg09030830	241	10.18 (1.46)	9.96 (1.34)	0.43	19.33	0.672
ADA	cg18704595	−581	6.67 (1.03)	6.44 (1.03)	0.06	20.62	0.554
ADA	cg06079428	−86	4.32 (0.45)	4.43 (0.43)	−0.65	19.73	0.520
ADA	cg06049791	−350	7.07 (1.23)	6.95 (0.95)	0.28	16.69	0.776

Cohort consists of pregnant women with a history of major depression or bipolar disorder (I, II or NOS) (E-GEOD-44132). Prior to analysis, methylation data were preprocessed and adjusted for batch effects and corrected for blood cell type heterogeneity. 5 methylation samples were classified as cross-batch controls and were excluded from the analysis. Samples were further excluded if exhibiting an antenatal depressive episode. Independent samples *t*-tests were thereafter performed, contrasting methylation M-values in 11 postpartum depressed and 20 postpartum euthymic women.
Abbreviations;: df, degrees of freedom; p.val, *p*-value; t, t-statistic.

ferroni correction and adjustment for confounders, only IL-10 has been studied in association with depression. IL-10 is a regulatory cytokine with anti-inflammatory properties and an inhibitory role in the synthesis of a number of cytokines (Sabat et al., 2010). Peripartum, lower levels of regulatory proteins such as IL-10 are suggested to indicate less optimal pregnancy outcomes (Sadowsky et al., 2003). Low serum concentrations of IL-10 have been reported in adults with major depression (Dhabhar et al., 2009; Eskandari et al., 2007); however a *meta*-analysis of six studies did not replicate this finding (Dowlati et al., 2010). Moreover, IL-10 has previously been studied in perinatal depression with reported lower ratio of IFN-gamma to IL-10 in depressed subjects compared to controls (Groer and Morgan, 2007). Similarly, the ratio of IL-10 to IL-8 and IL-10 to IL-6 has been linked to depressive mood (Accortt et al., 2015; Corwin et al., 2015); however the results are contradictory regarding direction. Moreover, the results from Accortt el at. (2015) refer

to the second trimester while the ones from Corwin et al. (2015) to the second week postpartum.

The other three markers (AXIN-1, ADA and ST1A1) are novel in relation to perinatal depression and studies on their potential association with depression are lacking. Axin1 is part of a complex controlling cell growth, apoptosis and development. It enhances the TGF-beta signalling, involved in immune regulation (Liu et al., 2006) and is also suggested to be involved in embryonic neuronal development (Ye et al., 2015). ADA (Adenosine deaminase) is a deaminase which has an important role in the development and maintenance of the immune system and by binding Dipeptidyl peptidase-4, ADA activates T-cells (Gines et al., 2002). ST1A1 (Sulfotransferase 1A1) is a sulfur donor that functions as a catalyzer of sulfur conjugation of, among others, neurotransmitters (Gamage et al., 2005).

The five markers discussed above, along with SIRT2, MCP2, and CASP8, also showed significant differences between cases and controls after adjusting for history of depressive episodes, use of SSRI in pregnancy and fasting at blood sampling in logistic regression. SIRT2 is an enzyme that has been suggested, in animal studies, to be involved in the modulation of depressive behaviour (Liu et al., 2015). MCP2 is a chemokine that has been linked to fetal inflammation and preterm labor (Jacobsson et al., 2005), a finding that might give further insights in the link between antenatal depression and preterm labor (Grote et al., 2010). Lastly, CASP8 comprises a cysteine protease involved in the immune system by activating T-cells, B-cells and Natural killer cells and also participates in differentiation of macrophages. It is of interest to note that STAM-BP, Axin1, ADA, ST1A1, SIRT2, and CASP8 were all assigned to the same cluster, indicating a degree of co-variance. Interestingly, among those, only STAM-BP was selected by the LASSO model to have a predictive value. On the other hand, IL-10 and MCP2 were found in separate clusters.

Besides the eight inflammation markers discussed above, another 32 of the 74 markers analyzed, were significantly higher in the control group in the univariate analyses before controlling for multiple comparisons. Some of these markers, for example IL-8, receptors for IL-17 and Vascular endothelial growth factor (VEGF), have been associated with depression in earlier research (Anderson et al., 2013; Gururajan et al., 2016a). It is of interest that all of the 40 markers associated with postpartum depression in our analysis were lower in depressed women, following a common pattern irrespectively of their pro- or anti-inflammatory properties. This was confirmed even in the further step, combining all markers into a summary variable, which was ranked by LASSO as the second best variable in predicting postpartum depression symptoms in our dataset. A previous study has also shown lower levels of inflammatory markers for the first days postpartum among women that later presented with depressive symptoms (Corwin et al., 2015). This indicates that pregnant women prone to developing depression postpartum may be characterized by less robust immune activation, or a dysfunctional shift of inflammatory activity, during pregnancy. In other words, it could be possible that in pregnant women, the reinstatement or even further activation of the immune response before labor is protective against depression, and required for a healthy state of mind. It is though very important to note that this variable was ranked only as seventh once women with symptoms during pregnancy were excluded, rendering it less valuable in cases of new-onset postpartum depression. New studies in the field with larger samples, addressing new-onset postpartum depression, might consider using similar novel constructs or combining such a construct with other markers.

In 2013, a literature review of 18 articles addressing human inflammatory markers and perinatal depression, stresses the importance of separating the cases with onset during pregnancy and those with postpartum onset (Osborne and Monk, 2013). Excluding women with depression symptoms during pregnancy in this study made the inflammation summary variable fall from the second to the seventh best variable for prediction of postpartum depressive symptoms postpartum. This might be due to a power problem in our dataset, but could also support the theory of a different pathophysiological mechanism for depressive symptoms arising after delivery, perhaps not as related to inflammation as depression during pregnancy. It is important to note that it seems like the association between the above mentioned inflammation markers and postpartum depression symptoms primarily depends on women with depressive symptoms already during pregnancy, who continue to be depressed postpartum.

To the best of our knowledge, there are only a few other studies investigating the association between inflammatory markers in late pregnancy and depressive symptoms postpartum. The existing literature on perinatal depression and inflammation are hard to draw conclusions from, since scales used for examining depression vary widely. Further, the time points of inflammation marker assessment and the examination of mood, differ broadly in the existing studies; from early first trimester to second and third trimester to days before delivery and hours, days and weeks after delivery up to months after delivery (Maes et al., 2000; Maes et al., 1999; Maes et al., 2001; Schmeelk et al., 1999). One study reported a positive correlation between CRP and depressive symptoms during pregnancy, but a negative correlation between CRP levels and depressive symptoms one to five days postpartum, and no correlation between CRP levels and depressive symptoms five to six weeks postpartum (Scrandis et al., 2008). However, this study used the Hamilton Depression Rating Scale-Seasonal Affective Disorder (SIGH-SAD) to assess mood, a tool not designed to assess symptoms of perinatal depression, in a quite small sample setup.

Another recent study on African American women measured IL-6 and IL-10 levels as well as the ratio IL-6/IL-10 during the second pregnancy trimester and assessed the association with postpartum scores on the EPDS (Accortt et al., 2015). No direct association was found between the inflammatory markers and EPDS scores; however, women with low pregnancy levels of vitamin D in combination with higher levels of IL-6 or IL-6/IL-10 ratios were more likely to report elevated depressive symptoms. Among the 74 markers analyzed in this study, IL-6 and IFN-gamma have previously been associated with depression (Tsao et al., 2006). In the present study, these markers were not significantly different between cases and controls in the main analyses. However, IFN-gamma levels were higher in controls after exclusion of women undergoing caesarean section. This might be due to an alteration of IFN-gamma levels in these women for unknown reasons.

A newly published study assessed mood and inflammatory markers (IL-6, IL-1B, TNF-a, IL-8, IFN-gamma and IL-10) and cortisol levels in 152 women prior to delivery and at six time points postpartum and found decreased levels of pro-inflammatory TNF-a to be associated with PPD symptoms at all postpartum time-points (Corwin et al., 2015). The authors argue that decreased inflammation in depressed women could point to a more cortisol sensitive immune reactivity in the depressed group. A direct comparison with the current study is not feasible, as the samples in that study were collected postpartum, and in the current study TNF was one of the markers we had to exclude from our analyses due to missing NPX values for more than 50% of the samples. It is on the other hand important to note that inflammatory markers, such as cytokines, have been suggested to interact with the HPA-axis to influence mood status and that the timing of different actions during pregnancy and postpartum might be of significant importance (Corwin et al., 2015). Analysis of the current material according to depression during pregnancy interestingly shows that in non-depressed patients, there is a correlation between cortisol levels and inflammation markers, which is absent among depressed

patients (Edvinsson, manuscript, personal communication). Such complex interactions could also explain the inconclusive findings for inflammatory markers and perinatal depression. It should be also noted that findings regarding the association of peripheral inflammatory markers with central nervous system disorders should however be viewed with caution, as there are differences in immune response in the brain and the periphery.

In this study, we have investigated a large number of different inflammation markers in a relatively large sample size and even performed a sensitivity analysis in an attempt to differentiate between depression present already during pregnancy and depression that develops after delivery. Furthermore, we have introduced the novel notion of a summary inflammation variable in an attempt to collectively depict the immune system's activation level. We used the EPDS scale, which is the most widely used screening tool for perinatal depression, but also, and in most cases, strengthened the assessment by complementing this with the use of the MINI interview.

The PEA (Assarsson et al., 2014), similarly to other DNA-assisted proximity assay technologies (Darmanis et al., 2011; Fredriksson et al., 2002; Kamali-Moghaddam et al., 2010), provides detection of proteins with high sensitivity due to utilization of amplifiable DNA oligonucleotides conjugated to affinity binders. The requirement of multi- recognition events increases the assay specificity. Due to neglectable cross-reactivity, these technologies allow high multiplicity in order to simultaneously analyze a large number of proteins in as low as one μl of sample volume. This study explored a particularly wide range of inflammatory biomarkers. While some of the identified markers that differed between the groups were reported in previous research, some others represented novel findings. Further studies are needed to evaluate the potential importance of such novel markers as STAM-BP, as well as the combined inflammation summary variable, which might represent a novel way to reflect possible dysfunctional reactivity of the immune system in peripartum depressed women. A considerable strength of the study is the replication of the protein-level findings for STAM-BP and ST1A1 also in relation to the methylation status of loci in the respective genes.

This study also presents with some limitations. Many important inflammation factors, such as many interleukins, TNF and LIF-R, were excluded from our analyses due to technical reasons. Several studies have focused on CRP as an inflammatory marker but CRP was not included in the Proseek inflammatory panel due to technical limitations. Also, only NPX values and not actual levels of markers are used in this study. The study sample, recruited via the BASIC study, includes a significant proportion of highly educated women that are slightly older and have relatively fewer pregnancy complications than the overall pregnant population, while most are of Scandinavian or European decent; this needs to be taken into account when considering the generalizability of the findings. No information on income was available. Screening for depression with the MINI interview was not possible among all participants, and the assessment of depressive symptoms was made solely through the self-reported EPDS. Therefore, the outcome measure was in most cases significant depression symptoms postpartum, based on an EPDS cut-off score of 12 points, and not a clinical depression diagnosis. The presence of significant anxiety symptoms among controls cannot be excluded. There were significant problems with statistical power for a possible sensitivity analysis including only women without any depression history or depression symptoms during pregnancy. Lastly, because of significant co-linearity between fasting at sampling, time of sampling and caesarean delivery, we only included fasting status in the adjusted models.

5. Conclusions

This study explores perinatal depression in association with the immune system from a novel angle. A different inflammatory pattern in late pregnancy was identified among women with depressive symptoms postpartum. This study provides data for a better understanding of the pathophysiology of perinatal depression and might contribute in the search of biomarkers for early identification of those at risk for postpartum depression; future studies in larger samples, focusing of STAM-BP and other inflammatory factors shown to differ between cases and controls in our study, alone or in combination with other biological and epidemiological markers, should be encouraged. Sub-group analyses among those non-depressed during pregnancy should be considered. Postpartum depression is a condition with severe consequences not only for the new mother, but the family as a whole. Timely identification of women at risk for postpartum depression has important practical clinical implications and should be pursued, especially in settings where pregnant women are in frequent contact with the health care system.

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Conflict of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2017.02.029.

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