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Lower inflammatory markers in women with antenatal depression brings the M1/M2 balance into focus from a new direction

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High lights

- Women with antenatal depression or SSRI treatment have lower levels of a number of peripheral inflammatory markers than healthy pregnant controls.
- No difference in any of the inflammatory markers was observed between women with antenatal depression and those who were using SSRI.
- The inflammatory markers were negatively correlated with cortisone serum concentrations in controls, but not in the cases.
- Differential DNA methylation of was found for seven of these inflammatory markers in a validation cohort.

Abstract

Background: Antenatal depression and use of serotonin reuptake inhibitors (SSRI) in pregnancy have both been associated with an increased risk of poor pregnancy outcomes, such as preterm birth and impaired fetal growth. While the underlying biological pathways for these complications are poorly understood, it has been hypothesized that inflammation may be a common physiological pathway. The aim of the present study was to assess peripheral inflammatory markers in healthy women, women with antenatal depression, and in women using SSRI during pregnancy.

Methods: 160 healthy pregnant controls, 59 women with antenatal depression and 39 women on treatment with SSRIs were included. The relative levels of 92 inflammatory proteins were analyzed by proximity extension assay technology.

Results: Overall, 23 of the inflammatory markers were significantly lower in women with antenatal depression and in women on treatment with SSRIs in comparison with the healthy controls. No difference in any of the inflammatory markers was observed between women with antenatal depression and those who were using SSRI. Top three inflammatory markers that were down-regulated in women with antenatal depression were TNF-related apoptosis-inducing ligand (TRAIL), p=0.000001, macrophage colony-stimulating factor 1 (CSF-1), p=0.000004, and fractalkine (CX3CL1), p=0.000005. Corresponding inflammatory markers in SSRI users were CSF-1, p=0.000011, vascular endothelial growth factor A (VEGF-A), p=0.000016, and IL-15 receptor subunit alpha (IL-15RA), p=0.000027. The inflammatory markers were negatively correlated with cortisone serum concentrations in controls, but not in the cases. Differential DNA methylation of was found for seven of these inflammatory markers in an independent epigenetics cohort.

Conclusion: Women with antenatal depression or on SSRI treatment have lower levels of a number of peripheral inflammatory markers than healthy pregnant controls. Hypothetically, this could be due to dysregulated switch to the pro-M2 milieu that characterizes normal third trimester pregnancy. However, longitudinal blood sampling is needed to elucidate whether the presumably dysregulated M2 shift is driving the development of antenatal depression or is a result of the depression.

Key words: Antenatal depression, pregnancy, inflammatory markers, proximity extension assay, selective serotonin reuptake inhibitors

1. Introduction

Symptoms of depression are common in pregnancy, and 4-7% of pregnant women fulfill the criteria for major depressive disorder (Andersson et al., 2003; Gorman et al., 2004; Melville et al., 2010; Patkar et al., 2004). Antenatal depression and use of serotonin reuptake inhibitors (SSRI) in pregnancy have both been associated with an increased risk of poor pregnancy outcomes, such as preterm birth and impaired fetal growth. While the underlying biological mechanisms for these complications are poorly understood (Olivier et al., 2013), it has been hypothesized that inflammation may be a common physiological pathway. Many obstetric complications, including preterm birth and preeclampsia, have been associated with increased inflammatory response, and the same is true for major depression, or treatment thereof (Osborne and Monk, 2013).

During normal pregnancy the immune system undergoes numerous changes to protect the woman from pathogens while at the same time avoiding alienation of the semi-allogeneic fetus (La Rocca et al., 2014). Pregnancy-induced changes in progesterone, estradiol, leukaemic inhibitory factor, and prostaglandins are likely to be partially responsible for the switch from high levels of Type 1 T helper (Th1) cells in early pregnancy to high levels of Type 2 T helper (Th2) cells in late pregnancy (Mjosberg et al., 2010; Sykes et al., 2012). The picture may, however, be slightly more complex as other studies indicate that both Th1 and Th2 responses are limited during pregnancy, and the innate immune system may play a greater role than previously thought (Kraus et al., 2012; Luppi et al., 2002). Recently, the role of peripheral and central macrophages (microglia) in initiating and regulating pro-inflammatory and anti-inflammatory states has come into focus, with repercussion for pregnancy as well (Jones and Thomsen, 2013; Nagamatsu and Schust, 2010a, b). Macrophages are plastic cells, which can switch from the classic pro-inflammatory M1 state and associated elevated levels of tumor necrosis factor alpha (TNF-α), interleukin (IL)-6 and IL-1β to an alternative state; the M2 macrophages, induced by IL-4 and IL-13, and producing IL-10, IL-4, and transforming growth factor beta (TGF-β) (Martinez and Gordon, 2014; Wang et al., 2014). M2 macrophages are involved in wound healing and tissue remodeling tasks, with additional contributions to the metabolic performance and the endocrine signaling of the tissues (Martinez and Gordon, 2014). Early pregnancy

is characterized by an increase in M1 macrophages, however, once the placenta is developed, a shift to a predominantly pro-M2 milieu occurs, preventing fetal rejection until parturition (Brown et al., 2014). Reduced levels of pro-inflammatory cytokines, IL-2 and INF-γ are found during the second trimester (Shimaoka et al., 2000), and the pro-M2 milieu continues into the third trimester (Kraus et al., 2010). Finally, immediately prior to delivery, a last inflammatory phase is noted, characterized by high levels of pro-inflammatory cytokines both in cervical tissue (Dubicke et al., 2010; Malmstrom et al., 2007; Sennstrom et al., 2000), and in circulating blood (Fransson et al., 2012; Luppi et al., 2002). The pregnancy-induced cortisol increase also contributes to the anti-inflammatory response during the second and third trimesters. For protection of the fetus, most of the maternal cortisol is converted to inactive cortisone by the enzyme 11-beta hydroxysteroid dehydrogenase (11b-HSD) (Murphy et al., 1974), and the maternal serum cortisone to cortisol ratio is likely to reflect maternal *and* placental 11β-HSD2 activity. The inflammatory status of macrophages is controlled by a number of nuclear receptors, including the the glucocorticoid receptor, and it was recently shown that 11β-HSD1 enzyme activity is higher in M2 macrophages than in M1 macrophages, (Chinetti-Gbaguidi et al., 2012).

A bidirectional communication between the immune system and the central nervous system is essential for normal brain functions, such as initiating and regulating stress responses, emotions and behavior (Miller and Raison, 2015). Sickness behavior induced by pro-inflammatory cytokines resembles major depressive disorder, and interferon alpha (INF-α) treatment induces major depressive disorder in 25% of patients, suggesting a causal mechanism (Dantzer, 2001; Udina et al., 2012). In non-pregnant subjects, peripheral pro-inflammatory markers such as IL-6, IL-1β, IFN-α, TNF-α, and the chemokine monocyte chemoattractant protein 1 (MCP1)/chemokine (C-C motif) ligand 2 (CCL2) are increased in the blood and cerebrospinal fluid of a subgroup of patients with mood disorders versus healthy controls, both when assessed at baseline and after exposure to stressors (Dowlati et al., 2010; Jones and Thomsen, 2013; Miller et al., 2009). Similarly, the current knowledge indicates that shifts toward M1 macrophages in the M1/M2 balance may be related to depression development in the non-pregnant population (Bhattacharya et al., 2016; Jones and Thomsen, 2013; Miller and Raison, 2015).

While the inflammatory response has been extensively studied in obstetric complications such as preterm birth and preeclampsia, studies on the role of peripheral inflammatory markers in antenatal depression are thus far few. In addition, the extent of evaluated markers has, with one exception, been limited to IL-6, IL-10, IL-1β, and TNF-α (Blackmore et al., 2011; Cassidy-Bushrow et al., 2012; Christian et al., 2009; Shelton et al., 2015). Thus far, findings can at best be described as inconsistent with unchanged, increased or decreased levels of cytokines and other inflammatory markers in women suffering from antenatal depression (Blackmore et al., 2011; Cassidy-Bushrow et al., 2012; Christian et al., 2009; Shelton et al., 2015). Different immunological stages during pregnancy may potentially explain these inconsistencies.

Thus, given the limited information on how the inflammatory response in pregnancy influences the risk of antenatal depression, the aim of the present study was to assess peripheral inflammatory markers during the third trimester in healthy women, women with antenatal depression and in women using SSRI during pregnancy. A secondary aim was to evaluate the usefulness of these markers for the purpose of diagnosing antenatal depression.

2. **Material and methods**

2.1. Participants

This sub-study to the cohort study 'Biology, Affect, Stress, Imaging, and Cognition in pregnancy and the puerperium' (BASIC) consisted of 160 healthy pregnant controls, 59 women with antenatal depression and 39 women on treatment with SSRIs. BASIC is an ongoing longitudinal study that aims to investigate mood disorders in pregnancy and postpartum. All women over the age of 18 years in Uppsala County who attend their routine ultrasound in gestational week 16-18 are invited to participate in the study. Exclusion criteria for the BASIC study are (1) inability to adequately communicate in Swedish, (2) confidential personal data, and (3) pathologic pregnancy as diagnosed by routine ultrasound. Following informed consent, the participants are asked to fill in web-based questionnaires including the Swedish version of the Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1987; Rubertsson et al., 2011) in gestational week 17 and gestational week 32.

Blood samples for this study were compiled from two different sources within the BASIC cohort; i) from a psychophysiology sub-study in late pregnancy (n=205) or ii) from blood samples collected in conjunction with a planned Caesarean section (n=53).

For the psychophysiology sub-study, women with EPDS score ≥ 13 and a random group of women with EPDS scores < 13 at gestational week 32 were invited, with the intention to oversample women with antenatal depressive symptoms. Exclusion criteria were serious pregnancy-related conditions such as severe preeclampsia, intrauterine growth restriction, gestational diabetes or twin pregnancies. Treatment with antidepressant drugs was not an exclusion criterion; any ongoing medication was recorded. Participating women visited the research laboratory at the Department of Women's and Children's Health, Uppsala University in gestational week 35-39 between January 2010 and May 2013. The visits were scheduled between 8 AM and 3 PM, with the majority starting either at 9 AM or at 1 PM, and all women had been fasting at least 90 minutes before blood samples were drawn. Out of 234 participating women in the sub-study, plasma/serum samples were available from 216 women, and these samples were used for cortisol, cortisone and inflammatory marker analyses. Presence of ongoing primary anxiety and depressive disorders was established using the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). The interview also included questions on previous depressive episodes. In women who were on treatment with SSRI but where the diagnostic interview failed to indicate the reason for treatment, no attempts were made to ascertain the reason for treatment initiation. Women with an ongoing minor (two to four symptoms persisting for at least two weeks) or an ongoing major depressive episode (at least five symptoms persisting for at least two weeks) (n=23), or a prior episode in combination with at least one EPDS score of 13 or more during pregnancy (n=31), were considered to have experienced a depressive episode during pregnancy (n=54) (with or without psychiatric comorbidities). As it was assumed that changes in the inflammatory response in the early second trimester would drive the development of depression, it was reasonable to include women who had had a depressive episode earlier in pregnancy, not only those with ongoing depression. For the purpose of the present study, women with anxiety-only disorders (n=11) were excluded. Remaining women were considered healthy controls (n=124) or were on treatment with

SSRI (n=27). Following the psychiatric interview, the women filled out the Montgomery Åsberg Depression Rating Scale (MADRS) (Montgomery and Asberg, 1979).

In addition, healthy controls (n=36), depressed cases (n=5) and women on SSRI treatment (n=12) were also sampled among the BASIC study participants who underwent an elective Caesarean section at Uppsala University Hospital. Similar exclusion criteria as in the psychophysiology study were applied. The morning before the Caesarean section, which is typically performed in gestational week 38, fasting blood samples were collected. In this part of the study, depressed cases were defined as women who had discontinued antidepressant use early in pregnancy and had EPDS scores ≥ 17 at some point during pregnancy. As no MINI-interview was done in these participants, a higher EPDS cut-off was used to increase the specificity for detection of women with major depression.

For all women, socio-demographic variables, medical and psychiatric history were derived from the BASIC questionnaires administered during pregnancy. Information regarding concomitant somatic disorders, antidepressant treatment, pregnancy complications, delivery outcome and neonatal care was collected from the medical records. The study procedures were in accordance with ethical standards for human experimentation and the study was approved by the Regional Ethical Review Board in Uppsala.

2.2. Characterization of the independent epigenetic data set

To further investigate the inflammatory markers that were of relevance in antenatal depression, changed methylation patterns for their associated genes were assessed in an independent cohort. Data for this cohort is openly available (E-GEOD-44132), and was originally published by Guintivano and colleagues (Guintivano et al., 2014). Pregnant women with a history of either major depression or bipolar disorder (I, II or not otherwise specified) were included in the study and prospectively followed during pregnancy (Guintivano et al., 2014). When blood samples were drawn during pregnancy, 19 women were depressed and remaining 31 women were euthymic. DNA methylation profiles 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.

2.3. Inflammatory markers

All samples were collected using the same type of collection tubes, with time to freezer less than 60 minutes. Stored plasma/serum samples were collected from -70°C freezers and thawed on ice before being transferred to 96-well plates, each consisting of 90 samples and 6 controls. None of the samples used in this study had previously been thawed. Moreover, all samples were analyzed using the same batch of reagents, with cases and controls evenly distributed within the plates. Analyses of the relative levels of 92 inflammatory proteins were performed at Clinical Biomarker Facility at the SciLifeLab Uppsala, using Proseek Multiplex Inflammation I (Olink Bioscience, Sweden), which is based on proximity extension assay (PEA) technology (Assarsson et al., 2014; Lundberg et al., 2011). 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 hybridize 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 μL) 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 PEA enzyme 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 according to the manufacturer's instructions. 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 software using Olink Wizard, providing normalized protein expression (NPX) data on a Log2-scale where a high protein value corresponds to a high protein concentration. In brief, the NPX is calculated in three steps from the quantification cycle (Cq) values generated in the real-time PCR: i) $\Delta Cq_{sample} = Cq_{sample} - Cq_{extension\ control}$, ii) $\Delta\Delta Cq = \Delta Cq_{sample} - \Delta Cq_{interplate\ control}$, 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.

2.4. Quantification of cortisol and cortisone

The sensitive isotope dilution supercritical fluid chromatography tandem mass spectrometry method used for quantification of maternal cortisol and cortisone levels has been described previously (Hellgren et al., 2016). In brief, 50 µL of serum was mixed with internal standard mixture, which was prepared with d_4 cortisol in methanol. After the addition of internal standard to serum samples, each sample contained 2.5 ng of d_4 cortisol. Extraction of steroids from human serum was done using 4 mL of *tert*butylmethyl ether (MTBE) as the extraction solvent. Samples were gently vortexed for 10 min and centrifuged at 1000 g for 10 min. The supernatant was collected and the solvent was evaporated under a stream of nitrogen gas. The dried samples were reconstituted with 100 µL methanol. During the extraction, the lipids were protected against oxidation by the addition of 0.05 mg/mL butylated hydroxytoluene (BHT) to the extraction solvent.

Separation of steroids was done by use of Ultra Performance Convergence Chromatography (UPC^2). The Acquity UPC^2 system from Waters Corporation, Milford, USA, equipped with a binary solvent delivery pump, an autosampler, a column oven, and a back pressure regulator was used. The analysis

was performed at 40 °C using an Acquity UPC^2 BEH column (100 mm 3.0 mm, 1.7 µm; Waters, Milford, MA, USA). The mobile phase flow rate was maintained at 3.0 mL/min with a gradient elution (eluent A, CO_2; eluent B, methanol). The gradient program was started with 2% of component B, then, a linear gradient was programmed from 2% to 17% for 2.0 min, followed by a linear gradient down to 2% B in 3.0 min, and finally it was held for 1.0 min which allowed ionic liquids to elute out from the instrument. Isocratic solvent was methanol in 0.1% formic acid with a flow rate of 0.4 mL/min. The back pressure was set at 1800 psi and the injection volume was 2.0 µL.

Steroids were identified by using a Waters Xevo TQ-S mass spectrometer (Milford, MA, USA). The data acquisition was in the positive ion electrospray ionization (+ve, ESI) mode. The desolvation gas was nitrogen, and the collision gas was argon (0.25 mL/min). The capillary voltage was 2.0 kV, the cone voltage was 30.0 V, and the source offset was 20 V. The source temperature was 150 °C and the desolvation temperature was 500 °C with the desolvation gas flow rate of 750.0 l/h. The cone gas flow was 150.0 l/h. The nebulizer gas flow at 7.0 bar. MS data were collected using two separate scan functions. The first scan function was set at low collision energy (5 eV), which provided parent ions, and the second scan function was set at high collision energy (ramped from 15 to 30 eV). The scan time for each function was set at 0.3 s. The MS data acquired in the *m/z* range of 100–1200. The precursor/product ion transitions in multiple reactions monitoring (MRM) were used for mass analysis and quantification. The pertinent mass transitions of cortisol, cortisone and internal standard were 363.05/121.2, 361.5/163.0 and 367.25/121.0, respectively. The quantification of analytes was accomplished using internal standard and the retention time of the analytes were also compared against standard steroids for peak identification. Moreover, relevant mass transitions were compared with mass transitions obtained from standard steroids. The sample analysis was performed in triplicate. The values of relative standard deviation (RSD) of intra-day repeatability were 2.53 for cortisol and 4.07 for cortisone. The RSD values for inter-day repeatability were 1.07 and 3.78 for cortisol and cortisone, respectively. All data collected in centroid mode were obtained using MasslynxNT4.1 software (Waters Corp., Milford, MA USA).

2.5. 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 450K Methylation BeadChip. The annotation file was used, for each CpG site, to define the associated gene and the distance to the closest transcriptional start site (TSS). CpG-sites were included in the subsequent analysis if annotated to any of the following genes; ADA, AXIN1, CCL11, CCL25, CD244, CD40, CD5, CSF1, CST5, CX3CL1, DNER, GDNF, IL10RB, IL15RA, IL17C, LTA, PLAU, SLAMF1, STAMBP, SULT1A1, TNFRSF9, TNFSF10, and VEGFA. We further limited the analysis to probes located within 2,000 base pairs up and downstream of the TSS, as Wagner and colleagues showed that DNA methylation and gene expression is more strongly correlated in this region (Wagner et al., 2014). After the probe exclusion steps outlined above, 168 CpG sites were investigated in the subsequent analysis.

2.6. Statistical analyses

For 18 proteins there was a lack of inflammatory protein expression in more than 50% of the individuals, these were discarded, leaving 74 proteins to be used in the statistical analyses. No difference in expression of excluded proteins between groups was noted. For handling of values below level of detection (LOD), the NPX values < LOD were replaced by LOD/square root(2). Secondly, log2(NPX+1) was calculated (instead of the log2(NPX) using the GenEx software) and used in all the following statistical analyses. This procedure was undertaken to guarantee that no values were less than zero and to avoid stretching of the data when NPX approaches zero.

Statistical analyses were performed using the IBM SPSS 22.0 (IBM, 2013) and the R-package (RCoreTeam, 2013). Comparisons of demographic data across the three groups were made by one-way ANOVA, or Chi-square tests. Primary analyses of the inflammatory markers across all three groups were made with likelihood ratio tests performed on adjusted multinomial logistic regression models, weighted to the provided population proportions of antenatal depression (10%) and SSRI use (3%), respectively. In these analyses, adjustments were made for age (continuous), body mass index

(continuous), smoking (yes/no), days left to parturition (continuous), fasting status (overnight fast or 90-minute fast), preeclampsia or hypertension (yes/no), and pre-pregnancy inflammatory or rheumatoid disorder (yes/no). These adjustments were based on significant differences between groups, or previous literature indicating their relevance for the inflammatory response (Osborne and Monk, 2013).

Days left to parturition was calculated as number of days between blood sampling and delivery. For women who underwent Caesarean section (typically performed in gestational week 38), days left to parturition was calculated as number of days left until estimated day of delivery according to ultrasound. Celiac disease, Crohn's disease, ulcerative colitis and multiple sclerosis were grouped as inflammatory disorders. Rheumatoid arthritis, systemic lupus erythematosus and psoriasis arthritis were considered as rheumatoid disorders. To reduce the risk of false discoveries caused by multiple testing, we used Bonferroni correction to adjust the *p*-values, and adjusted *p*-values less than 0.05 were considered significant. Significant inflammatory markers were subjected to follow-up analyses by multivariable logistic regression, using the same adjustments as above. Correlation analyses were made by Spearman Rank correlation, as self-rated depression scores were not normally distributed.

Diagnostic performance of the inflammatory markers for detection of antenatal depression was evaluated using random forest classification in ten five-fold cross-validations. Performance measures obtained from these analyses included the average (over the 50 test sets) error rate (total number of incorrect classifications), probability of miss (1–sensitivity), probability of false alarm (1–specificity), and area under the curve (AUC). The null distribution for average error rate was determined by means of N=1000 permutations. In each permutation the class identities were permuted and then the full cross validation procedure was performed. The null distribution was used to compute a permutation p-value, $p = (n + 1)/(N + 1)$, where n is the number of permutations generating an average error rate at least as high as when the data was unpermuted. Note that the smallest possible p-value is $1/(N + 1)$.

To investigate the association of changed methylation patterns in candidate CpG sites with antenatal depression, the ComBat function of the sva package for R was used to adjust the global DNA methylation data for batch effects (Johnson et al., 2007). DNA methylation measured in whole blood

is composed of different cell populations (Reinius et al., 2012). A study by Rask-Andersen et al. demonstrated that changes in leukocyte fractions could introduce considerable variability in the DNA methylation pattern which could bias downstream analyses. It is thus important to adjust the global DNA methylation pattern for white blood cell type heterogeneity (Rask-Andersen et al., 2016). A ChAMP-based statistical procedure of the Houseman algorithm was therefor 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. 50 samples remained for investigation, of which 31 were prepartum euthymic, and 19 antenatally depressed. Independent samples t-tests were performed, contrasting methylation M-values between the euthymic and depressed prepartum women. As a second step, we analyzed the results of the t-tests to identify genes with an abundance of differentially methylated CpG sites using binomial tests. P-value thresholds were set to 0.05 to stratify probes according to significant and non-significant methylation changes. Binomial tests were then performed in R using the function "binom.test", contrasting for each gene the number of nominally significant CpG sites to the total number of probes annotated to each gene not taking the direction of the methylation change into account. Genes with a binomial test p-value < 0.05 were considered significant.

3. Results

3.1. Participants

Demographic data for the study group is displayed in Table 1. The three groups; healthy controls, women with antenatal depression, and women using SSRI did not differ in terms of marital status, parity, smoking and alcohol use, or self-reported sleep disturbances. Similarly, prevalence of inflammatory diseases and obstetric complications, such as preeclampsia or hypertension, were similar between groups. However, women with antenatal depression were significantly younger, and had completed fewer years of education than controls. Their blood samples were less often taken in conjunction with a Caesarean section (and consequently following an overnight fast), and

approximately one week earlier (in relation to delivery) than the other two groups. SSRI users had shorter gestational length than women with antenatal depression, and higher pre-pregnancy body mass index (BMI) than the women in the other two groups. The most commonly used SSRIs were fluoxetine, sertraline and citalopram, used by 30.8%, 33.3% and 28.2% of women in the SSRI group, respectively. The grand majority (n = 24, 61.5%) used a starting dose of their SSRI, but nine women (23.1%) had higher doses and six women (15.4%) used lower doses. Most women in the SSRI group had used their antidepressant during the entire pregnancy (n = 33, 84.6%), but six women (15.4%) started in the second trimester. None of the participating women used neuroleptics or benzodiazepines.

As expected, self-rated depression in week 17 and 32 were highest in women with (untreated) antenatal depression and lowest in the healthy controls, with intermediate scores in SSRI users (all *p*-values between 0.05–0.001; Table 1).

3.2. Inflammatory markers differ between women with antenatal depression and healthy pregnant controls

Three blood samples were excluded due to technical problems and/or quality controls (one healthy control, one woman with antenatal depression, and one SSRI-treated woman). The adjusted multinomial regression analyses indicated that 23 of the inflammatory markers were significantly different between healthy pregnant women, depressed pregnant women, and women on SSRI treatment (Supplementary Table 1). Post hoc multivariable logistic regression analyses of the surviving inflammatory markers are displayed in Table 2. As seen in Table 2 and Supplementary Figure 1, these differences were driven by significantly lower levels of inflammatory markers in women with antenatal depression and women on SSRI treatment in comparison with controls. No difference in any of the inflammatory markers was observed between women with antenatal depression and those who were using SSRI. Also, no difference was noted between women with ongoing or earlier episode of depression during the index pregnancy, supplementary table 2. Notably, for each investigated inflammatory marker, lower levels were found in the two depressed groups. Top

three inflammatory factors that were down-regulated in women with antenatal depression were TNF-related apoptosis-inducing ligand (TRAIL), p=0.000001, macrophage colony-stimulating factor 1 (CSF-1), p=0.000004, and fractalkine (CX3CL1), p=0.000005. Corresponding inflammatory markers in SSRI users were CSF-1, p=0.000011, vascular endothelial growth factor A (VEGF-A), p=0.000016, and IL-15 receptor subunit alpha (IL-15RA), p=0.000027.

While the random forest analyses confirmed the strong group differences noted in the logistic regression analyses, none of these inflammatory markers had sufficient discriminatory power as a diagnostic marker for antenatal depression. Performance measures for models based on the 74 inflammatory proteins with no more than 50% missing values yielded an average error rate of 0.24, a sensitivity of 0.32, a specificity of 0.92, and an area under the curve (AUC) of 0.69. In the permuted model, the average error rate was 0.28 (range 0.26 – 0.30), which gives an estimated probability of <0.001. We also built models based on single inflammatory markers. For each model the mean sensitivity and specificity over 50 test sets was computed and the probability of miss (1-sensitivity) and probability of false alarm (1-specificity) for each of these proteins are plotted in Figure 1. As for the overall model, the individual inflammatory markers all suffered from low sensitivity to detect cases with antenatal depression.

3.3. The significant inflammatory markers are correlated with depression symptom severity in women with antenatal depression.

The majority of the inflammation markers that were significant in the adjusted multinomial regression model displayed a negative correlation with depression symptom severity in late pregnancy. Furthermore, the inflammatory markers also correlated with the self-reported EPDS scores obtained in gestational week 17 and 32, Table 3.

3.4. The significant inflammatory markers are correlated with cortisone and cortisone to cortisol ratio in healthy controls but not in women with antenatal depression.

No difference in cortisol, cortisone, or cortisone to cortisol ratio was noted between groups (Hellgren et al., 2016). The majority of the inflammation markers were significantly negatively correlated with cortisone or the cortisone to cortisol ratio in healthy controls. In contrast, among women with antenatal depression (with or without treatment) this pattern was not found. In this group, only one positive correlation was noted between uPA and the cortisone to cortisol ratio, Table 4.

3.5. The methylation state of 13 CpG sites is differentially methylated in women with antenatal depression

The significant inflammatory markers were further investigated in an independent, open-source cohort by comparing methylation levels in 19 women with antenatal depression and 31 euthymic pregnant women, Table 5. The methylation state of 13 CpG sites, associated with the following genes; AXIN1, CSF-1, CST5, DNER, GDNF, IL10RB, IL15RA and VEGFA, was differentially methylated in whole blood from women with antenatal depression ($p<0.05$). Subsequently, for each gene, the number of nominally significant CpG sites ($p<0.05$) to the total number of probes annotated to the same gene was analyzed by binomial tests. With four out of 11 associated CpG sites hypomethylated in women with antenatal depression, VEGFA was the only gene with a statistically significant abundance of differentially methylated CpG sites ($p<0.01$), Supplementary Table 3.

4. Discussion

The major finding of the present study was that women with antenatal depression and women on SSRI treatment had significantly lower levels of peripheral inflammatory markers than healthy pregnant controls, for as many as 23 markers. These findings are seemingly at odds with the literature in non-pregnant samples, where depression has been associated with increased levels of proinflammatory cytokines (Dantzer, 2001; Dowlati et al., 2010; Jones and Thomsen, 2013; Miller et al., 2009; Udina et al., 2012). Furthermore, none of the markers typically associated with non-pregnant depression such as IL-6, IL-1β, IFN-α, TNF-α, or MCP-1/CCL2, were altered in our cohort of women with antenatal

depression (Dowlati et al., 2010; Jones and Thomsen, 2013; Miller et al., 2009). In fact, VEGF-A, which is typically elevated in non-pregnant subjects with MDD (Carvalho et al., 2015), was lower in women with antenatal depression.

However, these results must be examined in the context of normal pregnancy and its associated alterations in immune and inflammatory responses. The majority of the inflammatory markers under investigation in this study are significantly elevated in healthy pregnancy compared with the postpartum period (17 out of 23 markers, Bränn et al, in manuscript), indeed suggesting that women who suffer from antenatal depression or who continue SSRI treatment deviate from the normal inflammatory response in pregnancy. This is further corroborated by the significant negative associations with depression severity throughout the second half of pregnancy, and the negative correlations with cortisone or the cortisone to cortisol ratio in the healthy controls. Clearly, our results suggest that depression during pregnancy is different from depression in non-pregnant populations.

Susceptibility for viral infections, such as influenza, increases during pregnancy especially in the second and third trimesters. Longitudinal studies have shown increased numbers of circulating plasmacytoid and conventional dendritic cells (also called decidual macrophages) during pregnancy (Kraus et al., 2012). In contrast, both the number and spontaneous cytokine production is reduced, in some cases up to 50%, in NK and T cells during pregnancy (Kraus et al., 2012). Macrophages set the tone for both the innate and the adaptive immunity, including the natural killer cells, by regulating cell number, differentiation, cytokine production, and activation status (Kraus et al., 2012; Martinez and Gordon, 2014; Wang et al., 2014). Based on our findings, it is hypothesized that antenatal depression is associated with an incomplete switch to the pro-M2 milieu that characterizes the second and third trimester of pregnancy (Brown et al., 2014). Importantly, results obtained in studies conducted in conjunction with delivery, which is characterized by a dramatic increase in M1 associated immunological patterns (Luppi et al., 2002), should be separated from those conducted in the weeks preceding delivery, which immunologically resembles the second trimester (Kraus et al., 2010).

In line with this reasoning, among the top down-regulated peripheral inflammatory markers, TRAIL, CSF-1, CX3CL1, VEGF-A, and IL15Rα, several have been associated with M2 macrophages. For

instance, TRAIL which is a type II transmembrane protein involved in tumor growth suppression and in the regulation of both the innate and adaptive immune responses (de Miguel et al., 2016; Gyurkovska and Ivanovska, 2016; Tisato et al., 2016), has recently been shown to be secreted by M2 macrophages (Sakhno et al., 2016). Similarly, CSF-1, a pro-inflammatory protein also known as macrophage-CSF, stimulates the differentiation and development of M2 macrophages (Lacey et al., 2012). Fractalkine/CX3CL1 is a membrane-bound or soluble chemokine expressed by neurons. Its' receptor CX3CR1 is found in the healthy brain, present on microglia (Jung et al., 2000; Reaux-Le Goazigo et al., 2013). Fractalkine has also been shown to induce M2 polarization of macrophages (Wang et al., 2014), and has a number of non-immune mechanisms, which may be of relevance in depression. For instance, fractalkine inhibits serotonergic neurotransmission by enhancing GABA activity on serotonergic neurons (Heinisch and Kirby, 2009). Besides the well-known angiogenic effects of VEGF-A, it also has neurotrophic and neuroprotective roles in the central- and peripheral nervous systems (Mackenzie and Ruhrberg, 2012), which may be of relevance for depression (Buttenschon et al., 2015). Furthermore, VEGF is linked both to TGF beta and M2 macrophages in tumor models (Solinas et al., 2009). Finally, interleukin-15 receptor alpha (IL15Rα) is one of three subunits of the receptor complex that binds the cytokine IL-15. IL-15 is expressed in most type of cells and plays an important role in immune cell functions muscle and bone growth, and adiposity. IL15Rα knockout mice show antidepressant-responsive depressive-like behavior, reduced anxiety, and impaired memory, suggesting an antidepressive effect of IL15 signaling (Pan et al., 2013; Wu et al., 2011). Although pro-inflammatory M1 markers do not differ between groups, the M1/M2 balance in the depressed group is shifted toward M1 signaling, which – in agreement with current hypotheses for depression in non-pregnant individuals – may be associated to the development of antenatal depression in vulnerable women (Bhattacharya et al., 2016; Jones and Thomsen, 2013; Miller and Raison, 2015).

In addition, several of the top down-regulated peripheral inflammatory markers have distinct actions in pregnancy. Fractalkine, VEGF-A, and IL-15Rα play important roles in blastocyst implantation and trophoblast invasion (Charnock-Jones et al., 2004; Demirci et al., 2016; Gong et al., 2014; Lobo et al.,

2004; Senger et al., 1996), and CSF-1 has been associated with preeclampsia (Li et al., 2016). VEGF-A is expressed in the human placenta throughout gestation, and is known to regulate placental angiogenesis and maternal spiral artery remodeling (Charnock-Jones et al., 2004; Senger et al., 1996), although its' precise role in adverse pregnancy outcomes such as preeclampsia, small-for-gestational-age infants, preterm birth and recurrent miscarriage seems unclear (Andraweera et al., 2012). Thus, the findings of the present study strengthen the role of inflammation as the mechanism behind the association between antenatal depression and its associated adverse pregnancy outcomes.

The list genes of CpG sites with differential methylation patterns in relation to current depression in the epigenetic data set included VEGF-A, CSF-1 and IL15Rα. Interestingly, all women in the epigenetic cohort have a history of either depression or bipolar disease, but the majority was euthymic at the time of sampling (Guintivano et al., 2014). Finding differential methylation in individuals with current depression suggests state effects on the regulation of the immune system at the epigenetic level, but replication of the methylation findings in other cohorts would be needed before generalization.

The hypothalamus-pituitary-adrenal (HPA) axis is yet another potential link between antenatal depression and the inflammatory response (Horowitz et al., 2013). While the total cortisol output is increased in pregnancy, the HPA axis is less reactive. In depressed pregnant subjects the HPA alterations seem to be regulated differently than in healthy pregnant women (Hannerfors et al., 2015; Hellgren et al., 2013; Hellgren et al., 2016; Horowitz et al., 2013; Iliadis et al., 2015). For instance, we have previously shown that women on treatment with selective serotonin reuptake inhibitors (SSRI) during pregnancy had higher corticotrophin releasing hormone (CRH) levels than healthy controls and untreated depressed women (Hannerfors et al., 2015). Furthermore, these alterations may impact the fetus because women on SSRI treatment who also had high CRH levels were at increased risk of preterm birth in comparison with healthy controls with normal CRH levels (Hannerfors et al., 2015). Also, psychiatric morbidity interacts with maternal serum cortisone to cortisol ratio in influencing fetal growth (Hellgren et al., 2016). This study shows for the first time that the interaction between the HPA and the immune system seen in healthy women is altered in women with antenatal depression

and in women on SSRI treatment. During pregnancy, most of the maternal cortisol is converted to inactive cortisone by the enzyme 11-beta hydroxysteroid dehydrogenase (11b-HSD) (Murphy et al., 1974). Importantly, the inflammatory status of macrophages is controlled by a number of nuclear receptors, including the peroxisome proliferator–activated receptors (PPARs) and the glucocorticoid receptor, and it was recently shown that 11β-HSD1 enzyme activity is higher in M2 macrophages than in M1 macrophages, (Chinetti-Gbaguidi et al., 2012). In line with this, we found that most of the top-down regulated inflammatory markers were in significant negative correlation with cortisone or the cortisone to cortisol ratio in healthy pregnant controls. In contrast, no such correlations were evident in women with antenatal depression, whether treated or not. This finding further points to a dysregulated HPA-axis response in these women, and provide a compelling link between depression, dysregulated HPA-axis, altered inflammatory response which may influence the pregnancy outcomes in these women.

Importantly, no differences in any of the inflammatory markers were noted between women with antenatal depression and those who were using SSRI. However, while in vitro experiments on murine cell lines indicate that SSRI treatment can reduce M1 activation in microglia (Su et al., 2015), SSRI treatment in humans does not consistently reduce proinflammatory cytokine levels (Hannestad et al., 2011). In fact, one study reported on increased levels of proinflammatory cytokines, IFN-γ and IL-1β, and reduced levels of anti-inflammatory cytokines, IL-10 and IL-13, in non-pregnant MDD patients after one-year treatment with SSRI (Hernandez et al., 2013). In addition, the actions of conventional antidepressants are undermined by proinflammatory cytokines, which may contribute to therapy resistance in non-pregnant MDD patients (Cattaneo et al., 2013; Uher et al., 2014). Our findings suggest that treatment with antidepressants does not normalize the inflammatory response in depressed pregnant women. Whether this is due to insufficient treatment response, as women on treatment still had higher depression scores than controls, or the treatment per se is not known. Further, as most women discontinue SSRI when they realize they are pregnancy (Ververs et al., 2006), it is also important to consider that SSRI use in pregnancy may be a representation of more severe psychiatric morbidity. Nevertheless, the similar inflammatory response between women with antenatal depression

and women on antidepressant treatment could strengthen the hypothesis that altered inflammatory response may be a common physiological pathway for the adverse outcomes associated with both antenatal depression and SSRI treatment, such as preterm birth and low birth weight (Brown et al., 2014; Osborne and Monk, 2013).

Finally, none of these inflammatory markers had sufficient discriminatory power as a diagnostic marker for antenatal depression. While specificity was high, the sensitivity for detection of cases with antenatal depression was low. In light of the attempts to discover biomarkers for MDD in non-pregnant samples, this finding is not surprising. The search for diagnostic biomarkers for MDD is still in its infancy and is complicated by the symptom-based diagnostic procedures, the multifactorial pathophysiology and the inaccessibility of the brain (Redei and Mehta, 2015). However, some progress regarding biomarkers for depression in the non-pregnant population has been made (Redei and Mehta, 2015). In fact, recent studies have shown a number of inflammatory markers to be accurate and reliable predictors of treatment response in depressed subjects (Cattaneo et al., 2016; Uher et al., 2014). Further, potentially the markers that stood out in this study may be of importance in defining clinical sub-groups of antenatal depression for advancement of the biological understanding of depression development in pregnancy. Potentially, the inflammatory markers of the present study could be combined with other markers that instead have been shown to have high sensitivity (but low specificity).

The major limitation in this study is the cross-sectional design, as this will impede any conclusions regarding causation of inflammation and antenatal depression. Longitudinal blood sampling is needed to elucidate whether the presumably dysregulated M2 shift is driving the development of antenatal depression or whether our findings are a result of the depression. Nevertheless, self-rated depression scores obtained already in gestational week 17 and 32, i.e. in advance of the blood sampling in the late third trimester, were significantly negatively correlated with most of the top down-regulated peripheral inflammatory markers, suggesting that the events driving the depression may have occurred in temporal relationship with the normal M1/M2 shift in pregnancy (Brown et al., 2014). Strengths of the study were that the great majority of cases were diagnosed by structured psychiatric interviews and

therefore we have a high specificity in the selection of depressed cases. Moreover, the technology used in this study, multiplex PEA, has demonstrated increased specificity due to dual recognition of target proteins, and high sensitivity due to the signal amplification step using amplifiable DNA reporter molecules for detection of relative protein values that can be used for comparison between groups (Assarsson et al., 2014).

In conclusion, women with antenatal depression and women on SSRI treatment have lower levels of a number of peripheral inflammatory markers than healthy pregnant controls. Hypothetically, this could be due to dysregulated switch to the pro-M2 milieu that characterizes normal third trimester pregnancy. However, longitudinal blood sampling is needed to elucidate whether the presumably dysregulated M2 shift is driving the development of antenatal depression or is a result of the depression. In addition, no difference in any of the inflammatory markers was noted between women with antenatal depression and those who were using SSRI, potentially indicating that the underlying depression is more important than the treatment *per se*. Finally, none of the inflammatory markers under investigation had sufficient discriminatory power as a diagnostic marker for antenatal depression. Future studies will test the hypothesis that these markers may help identify a common physiological pathway and patients at risk for the adverse outcomes associated with antenatal depression and SSRI treatment.

References

Andersson, L., Sundstrom-Poromaa, I., Bixo, M., Wulff, M., Bondestam, K., aStrom, M., 2003. Point prevalence of psychiatric disorders during the second trimester of pregnancy: a population-based study. American journal of obstetrics and gynecology 189, 148-154.

Andraweera, P.H., Dekker, G.A., Roberts, C.T., 2012. The vascular endothelial growth factor family in adverse pregnancy outcomes. Hum Reprod Update 18, 436-457.

Assarsson, E., Lundberg, M., Holmquist, G., Bjorkesten, J., Thorsen, S.B., Ekman, D., Eriksson, A., Rennel Dickens, E., Ohlsson, S., Edfeldt, G., Andersson, A.C., Lindstedt, P., Stenvang, J., Gullberg, M., Fredriksson, S., 2014. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLoS One 9, e95192.

Bhattacharya, A., Derecki, N.C., Lovenberg, T.W., Drevets, W.C., 2016. Role of neuro-immunological factors in the pathophysiology of mood disorders. Psychopharmacology (Berl) 233, 1623-1636.

Blackmore, E.R., Moynihan, J.A., Rubinow, D.R., Pressman, E.K., Gilchrist, M., O'Connor, T.G., 2011. Psychiatric symptoms and proinflammatory cytokines in pregnancy. Psychosom Med 73, 656-663.

Brown, M.B., von Chamier, M., Allam, A.B., Reyes, L., 2014. M1/M2 macrophage polarity in normal and complicated pregnancy. Front Immunol 5, 606.

Buttenschon, H.N., Foldager, L., Elfving, B., Poulsen, P.H., Uher, R., Mors, O., 2015. Neurotrophic factors in depression in response to treatment. J Affect Disord 183, 287-294.

Carvalho, A.F., Kohler, C.A., McIntyre, R.S., Knochel, C., Brunoni, A.R., Thase, M.E., Quevedo, J., Fernandes, B.S., Berk, M., 2015. Peripheral vascular endothelial growth factor as a novel depression biomarker: A meta-analysis. Psychoneuroendocrinology 62, 18-26.

Cassidy-Bushrow, A.E., Peters, R.M., Johnson, D.A., Templin, T.N., 2012. Association of depressive symptoms with inflammatory biomarkers among pregnant African-American women. Journal of reproductive immunology 94, 202-209.

Cattaneo, A., Ferrari, C., Uher, R., Bocchio-Chiavetto, L., Riva, M.A., Consortium, M.R.C.I., Pariante, C.M., 2016. Absolute Measurements of Macrophage Migration Inhibitory Factor and Interleukin-1-beta mRNA Levels Accurately Predict Treatment Response in Depressed Patients. Int J Neuropsychopharmacol.

Cattaneo, A., Gennarelli, M., Uher, R., Breen, G., Farmer, A., Aitchison, K.J., Craig, I.W., Anacker, C., Zunsztain, P.A., McGuffin, P., Pariante, C.M., 2013. Candidate genes expression profile associated with antidepressants response in the GENDEP study: differentiating between baseline 'predictors' and longitudinal 'targets'. Neuropsychopharmacology 38, 377-385.

Charnock-Jones, D.S., Kaufmann, P., Mayhew, T.M., 2004. Aspects of human fetoplacental vasculogenesis and angiogenesis. I. Molecular regulation. Placenta 25, 103-113.

Chinetti-Gbaguidi, G., Bouhlel, M.A., Copin, C., Duhem, C., Derudas, B., Neve, B., Noel, B., Eeckhoute, J., Lefebvre, P., Seckl, J.R., Staels, B., 2012. Peroxisome proliferator-activated receptor-gamma activation induces 11beta-hydroxysteroid dehydrogenase type 1 activity in human alternative macrophages. Arteriosclerosis, thrombosis, and vascular biology 32, 677-685.

Christian, L.M., Franco, A., Glaser, R., Iams, J.D., 2009. Depressive symptoms are associated with elevated serum proinflammatory cytokines among pregnant women. Brain Behav Immun 23, 750-754.

Cox, J.L., Holden, J.M., Sagovsky, R., 1987. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. The British journal of psychiatry : the journal of mental science 150, 782-786.

Dantzer, R., 2001. Cytokine-induced sickness behavior: mechanisms and implications. Ann N Y Acad Sci 933, 222-234.

de Miguel, D., Lemke, J., Anel, A., Walczak, H., Martinez-Lostao, L., 2016. Onto better TRAILs for cancer treatment. Cell Death Differ 23, 733-747.

Demirci, E.K., Salamonsen, L.A., Gauster, M., 2016. The role of CX3CL1 in fetal-maternal interaction during human gestation. Cell Adh Migr, 1-8.

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., Lanctot, K.L., 2010. A meta-analysis of cytokines in major depression. Biol Psychiatry 67, 446-457.

Dubicke, A., Fransson, E., Centini, G., Andersson, E., Bystrom, B., Malmstrom, A., Petraglia, F., Sverremark-Ekstrom, E., Ekman-Ordeberg, G., 2010. Pro-inflammatory and anti-inflammatory cytokines in human preterm and term cervical ripening. J Reprod Immunol 84, 176-185.

Fransson, E., Dubicke, A., Bystrom, B., Ekman-Ordeberg, G., Hjelmstedt, A., Lekander, M., 2012. Negative emotions and cytokines in maternal and cord serum at preterm birth. Am J Reprod Immunol 67, 506-514.

Gong, X., Chen, Z., Liu, Y., Lu, Q., Jin, Z., 2014. Gene expression profiling of the paracrine effects of uterine natural killer cells on human endometrial epithelial cells. Int J Endocrinol 2014, 393707.

Gorman, L.L., O'Hara, M.W., Figueiredo, B., Hayes, S., Jacquemain, F., Kammerer, M.H., Klier, C.M., Rosi, S., Seneviratne, G., Sutter-Dallay, A.L., Group, T.-P., 2004. Adaptation of the structured clinical interview for DSM-IV disorders for assessing depression in women during pregnancy and post-partum across countries and cultures. Br J Psychiatry Suppl 46, s17-23.

Guintivano, J., Arad, M., Gould, T.D., Payne, J.L., Kaminsky, Z.A., 2014. Antenatal prediction of postpartum depression with blood DNA methylation biomarkers. Molecular psychiatry 19, 560-567.

Gyurkovska, V., Ivanovska, N., 2016. Distinct roles of TNF-related apoptosis-inducing ligand (TRAIL) in viral and bacterial infections: from pathogenesis to pathogen clearance. Inflamm Res.

Hannerfors, A.K., Hellgren, C., Schijven, D., Iliadis, S.I., Comasco, E., Skalkidou, A., Olivier, J.D., Sundstrom-Poromaa, I., 2015. Treatment with serotonin reuptake inhibitors during pregnancy is associated with elevated corticotropin-releasing hormone levels. Psychoneuroendocrinology 58, 104-113.

Hannestad, J., DellaGioia, N., Bloch, M., 2011. The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. Neuropsychopharmacology 36, 2452-2459.

Heinisch, S., Kirby, L.G., 2009. Fractalkine/CX3CL1 enhances GABA synaptic activity at serotonin neurons in the rat dorsal raphe nucleus. Neuroscience 164, 1210-1223.

Hellgren, C., Akerud, H., Skalkidou, A., Sundstrom-Poromaa, I., 2013. Cortisol awakening response in late pregnancy in women with previous or ongoing depression. Psychoneuroendocrinology 38, 3150-3154.

Hellgren, C., Edvinsson, A., Olivier, J.D., Fornes, R., Stener-Victorin, E., Ubhayasekera, S.J., Skalkidou, A., Bergquist, J., Sundstrom-Poromaa, I., 2016. Tandem mass spectrometry determined maternal cortisone to cortisol ratio and psychiatric morbidity during pregnancy-interaction with birth weight. Psychoneuroendocrinology 69, 142-149.

Hernandez, M.E., Mendieta, D., Perez-Tapia, M., Bojalil, R., Estrada-Garcia, I., Estrada-Parra, S., Pavon, L., 2013. Effect of selective serotonin reuptake inhibitors and immunomodulator on cytokines levels: an alternative therapy for patients with major depressive disorder. Clin Dev Immunol 2013, 267871.

Horowitz, M.A., Zunszain, P.A., Anacker, C., Musaelyan, K., Pariante, C.M., 2013. Glucocorticoids and inflammation: a double-headed sword in depression? How do neuroendocrine and inflammatory pathways interact during stress to contribute to the pathogenesis of depression? Modern trends in pharmacopsychiatry 28, 127-143.

Houseman, E.A., Accomando, W.P., Koestler, D.C., Christensen, B.C., Marsit, C.J., Nelson, H.H., Wiencke, J.K., Kelsey, K.T., 2012. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC bioinformatics 13, 86.

Iliadis, S.I., Comasco, E., Sylven, S., Hellgren, C., Sundstrom Poromaa, I., Skalkidou, A., 2015. Prenatal and Postpartum Evening Salivary Cortisol Levels in Association with Peripartum Depressive Symptoms. PloS one 10, e0135471.

Johnson, W.E., Li, C., Rabinovic, A., 2007. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics (Oxford, England) 8, 118-127.

Jones, K.A., Thomsen, C., 2013. The role of the innate immune system in psychiatric disorders. Mol Cell Neurosci 53, 52-62.

Jung, S., Aliberti, J., Graemmel, P., Sunshine, M.J., Kreutzberg, G.W., Sher, A., Littman, D.R., 2000. Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. Mol Cell Biol 20, 4106-4114.

Kraus, T.A., Engel, S.M., Sperling, R.S., Kellerman, L., Lo, Y., Wallenstein, S., Escribese, M.M., Garrido, J.L., Singh, T., Loubeau, M., Moran, T.M., 2012. Characterizing the pregnancy immune phenotype: results of the viral immunity and pregnancy (VIP) study. J Clin Immunol 32, 300-311.

Kraus, T.A., Sperling, R.S., Engel, S.M., Lo, Y., Kellerman, L., Singh, T., Loubeau, M., Ge, Y., Garrido, J.L., Rodriguez-Garcia, M., Moran, T.M., 2010. Peripheral blood cytokine profiling during pregnancy and post-partum periods. Am J Reprod Immunol 64, 411-426.

La Rocca, C., Carbone, F., Longobardi, S., Matarese, G., 2014. The immunology of pregnancy: regulatory T cells control maternal immune tolerance toward the fetus. Immunol Lett 162, 41-48.

Lacey, D.C., Achuthan, A., Fleetwood, A.J., Dinh, H., Roiniotis, J., Scholz, G.M., Chang, M.W., Beckman, S.K., Cook, A.D., Hamilton, J.A., 2012. Defining GM-CSF- and macrophage-CSF-dependent macrophage responses by in vitro models. J Immunol 188, 5752-5765.

Li, M., Piao, L., Chen, C.P., Wu, X., Yeh, C.C., Masch, R., Chang, C.C., Huang, S.J., 2016. Modulation of Decidual Macrophage Polarization by Macrophage Colony-Stimulating Factor Derived from First-Trimester Decidual Cells: Implication in Preeclampsia. Am J Pathol.

Lobo, S.C., Huang, S.T., Germeyer, A., Dosiou, C., Vo, K.C., Tulac, S., Nayak, N.R., Giudice, L.C., 2004. The immune environment in human endometrium during the window of implantation. Am J Reprod Immunol 52, 244-251.

Lundberg, M., Eriksson, A., Tran, B., Assarsson, E., Fredriksson, S., 2011. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood. Nucleic Acids Res 39, e102.

Luppi, P., Haluszczak, C., Trucco, M., Deloia, J.A., 2002. Normal pregnancy is associated with peripheral leukocyte activation. Am J Reprod Immunol 47, 72-81.

Mackenzie, F., Ruhrberg, C., 2012. Diverse roles for VEGF-A in the nervous system. Development 139, 1371-1380.

Malmstrom, E., Sennstrom, M., Holmberg, A., Frielingsdorf, H., Eklund, E., Malmstrom, L., Tufvesson, E., Gomez, M.F., Westergren-Thorsson, G., Ekman-Ordeberg, G., Malmstrom, A., 2007. The importance of fibroblasts in remodelling of the human uterine cervix during pregnancy and parturition. Mol Hum Reprod 13, 333-341.

Martinez, F.O., Gordon, S., 2014. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 6, 13.

Melville, J.L., Gavin, A., Guo, Y., Fan, M.Y., Katon, W.J., 2010. Depressive disorders during pregnancy: prevalence and risk factors in a large urban sample. Obstetrics and gynecology 116, 1064-1070.

Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry 65, 732-741.

Miller, A.H., Raison, C.L., 2015. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol 16, 22-34.

Mjosberg, J., Berg, G., Jenmalm, M.C., Ernerudh, J., 2010. FOXP3+ regulatory T cells and T helper 1, T helper 2, and T helper 17 cells in human early pregnancy decidua. Biol Reprod 82, 698-705.

Montgomery, S.A., Asberg, M., 1979. A new depression scale designed to be sensitive to change. Br J Psychiatry. 134, 382-389.

Murphy, B.E., Clark, S.J., Donald, I.R., Pinsky, M., Vedady, D., 1974. Conversion of maternal cortisol to cortisone during placental transfer to the human fetus. American journal of obstetrics and gynecology 118, 538-541.

Nagamatsu, T., Schust, D.J., 2010a. The contribution of macrophages to normal and pathological pregnancies. Am J Reprod Immunol 63, 460-471.

Nagamatsu, T., Schust, D.J., 2010b. The immunomodulatory roles of macrophages at the maternal-fetal interface. Reprod Sci 17, 209-218.

Olivier, J.D., Akerud, H., Kaihola, H., Pawluski, J.L., Skalkidou, A., Hogberg, U., Sundstrom-Poromaa, I., 2013. The effects of maternal depression and maternal selective serotonin reuptake inhibitor exposure on offspring. Frontiers in cellular neuroscience 7, 73.

Osborne, L.M., Monk, C., 2013. Perinatal depression--the fourth inflammatory morbidity of pregnancy?: Theory and literature review. Psychoneuroendocrinology 38, 1929-1952.

Pan, W., Wu, X., He, Y., Hsuchou, H., Huang, E.Y., Mishra, P.K., Kastin, A.J., 2013. Brain interleukin-15 in neuroinflammation and behavior. Neurosci Biobehav Rev 37, 184-192.

Patkar, A.A., Bilal, L., Masand, P.S., 2004. Pharmacotherapy of depression in pregnancy. Ann Clin Psychiatry 16, 87-100.

Price, M.E., Cotton, A.M., Lam, L.L., Farre, P., Emberly, E., Brown, C.J., Robinson, W.P., Kobor, M.S., 2013. Additional annotation enhances potential for biologically-relevant analysis of the Illumina Infinium HumanMethylation450 BeadChip array. Epigenetics & chromatin 6, 4.

Rask-Andersen, M., Bringeland, N., Nilsson, E.K., Bandstein, M., Olaya Bucaro, M., Vogel, H., Schurmann, A., Hogenkamp, P.S., Benedict, C., Schioth, H.B., 2016. Postprandial alterations in whole-blood DNA methylation are mediated by changes in white blood cell composition. Am J Clin Nutr 104, 518-525.

Reaux-Le Goazigo, A., Van Steenwinckel, J., Rostene, W., Melik Parsadaniantz, S., 2013. Current status of chemokines in the adult CNS. Prog Neurobiol 104, 67-92.

Redei, E.E., Mehta, N.S., 2015. The promise of biomarkers in diagnosing major depression in primary care: the present and future. Current psychiatry reports 17, 601.

Reinius, L.E., Acevedo, N., Joerink, M., Pershagen, G., Dahlen, S.E., Greco, D., Soderhall, C., Scheynius, A., Kere, J., 2012. Differential DNA methylation in purified human blood cells: implications for cell lineage and studies on disease susceptibility. PLoS One 7, e41361.

Rubertsson, C., Borjesson, K., Berglund, A., Josefsson, A., Sydsjo, G., 2011. The Swedish validation of Edinburgh Postnatal Depression Scale (EPDS) during pregnancy. Nordic journal of psychiatry 65, 414-418.

Sakhno, L.V., Shevela, E.Y., Tikhonova, M.A., Ostanin, A.A., Chernykh, E.R., 2016. The Phenotypic and Functional Features of Human M2 Macrophages Generated Under Low Serum Conditions. Scand J Immunol 83, 151-159.

Senger, D.R., Ledbetter, S.R., Claffey, K.P., Papadopoulos-Sergiou, A., Peruzzi, C.A., Detmar, M., 1996. Stimulation of endothelial cell migration by vascular permeability factor/vascular endothelial growth factor through cooperative mechanisms involving the alphavbeta3 integrin, osteopontin, and thrombin. Am J Pathol 149, 293-305.

Sennstrom, M.B., Ekman, G., Westergren-Thorsson, G., Malmstrom, A., Bystrom, B., Endresen, U., Mlambo, N., Norman, M., Stabi, B., Brauner, A., 2000. Human cervical ripening, an inflammatory process mediated by cytokines. Mol Hum Reprod 6, 375-381.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 59 Suppl 20, 22-33;quiz 34-57.

Shelton, M.M., Schminkey, D.L., Groer, M.W., 2015. Relationships among prenatal depression, plasma cortisol, and inflammatory cytokines. Biological research for nursing 17, 295-302.

Shimaoka, Y., Hidaka, Y., Tada, H., Nakamura, T., Mitsuda, N., Morimoto, Y., Murata, Y., Amino, N., 2000. Changes in cytokine production during and after normal pregnancy. Am J Reprod Immunol 44, 143-147.

Solinas, G., Germano, G., Mantovani, A., Allavena, P., 2009. Tumor-associated macrophages (TAM) as major players of the cancer-related inflammation. Journal of leukocyte biology 86, 1065-1073.

Su, F., Yi, H., Xu, L., Zhang, Z., 2015. Fluoxetine and S-citalopram inhibit M1 activation and promote M2 activation of microglia in vitro. Neuroscience 294, 60-68.

Sykes, L., MacIntyre, D.A., Yap, X.J., Teoh, T.G., Bennett, P.R., 2012. The Th1:th2 dichotomy of pregnancy and preterm labour. Mediators Inflamm 2012, 967629.

Tisato, V., Gonelli, A., Voltan, R., Secchiero, P., Zauli, G., 2016. Clinical perspectives of TRAIL: insights into central nervous system disorders. Cell Mol Life Sci 73, 2017-2027.

Udina, M., Castellvi, P., Moreno-Espana, J., Navines, R., Valdes, M., Forns, X., Langohr, K., Sola, R., Vieta, E., Martin-Santos, R., 2012. Interferon-induced depression in chronic hepatitis C: a systematic review and meta-analysis. J Clin Psychiatry 73, 1128-1138.

Uher, R., Tansey, K.E., Dew, T., Maier, W., Mors, O., Hauser, J., Dernovsek, M.Z., Henigsberg, N., Souery, D., Farmer, A., McGuffin, P., 2014. An inflammatory biomarker as a differential predictor of outcome of depression treatment with escitalopram and nortriptyline. Am J Psychiatry 171, 1278-1286.

Wagner, J.R., Busche, S., Ge, B., Kwan, T., Pastinen, T., Blanchette, M., 2014. The relationship between DNA methylation, genetic and expression inter-individual variation in untransformed human fibroblasts. Genome biology 15, R37.

Wang, Y., Fu, Y., Xue, S., Ai, A., Chen, H., Lyu, Q., Kuang, Y., 2014. The M2 polarization of macrophage induced by fractalkine in the endometriotic milieu enhances invasiveness of endometrial stromal cells. Int J Clin Exp Pathol 7, 194-203.

Ververs, T., Kaasenbrood, H., Visser, G., Schobben, F., de Jong-van den Berg, L., Egberts, T., 2006. Prevalence and patterns of antidepressant drug use during pregnancy. Eur J Clin Pharmacol 62, 863-870.

Wu, X., Hsuchou, H., Kastin, A.J., He, Y., Khan, R.S., Stone, K.P., Cash, M.S., Pan, W., 2011. Interleukin-15 affects serotonin system and exerts antidepressive effects through IL15Ralpha receptor. Psychoneuroendocrinology 36, 266-278.

Legends to figures

Figure 1. Probability of false alarm plotted against the probability of miss for the detection of antenatal depression. For clarity reasons, not all protein names are displayed in the figure.

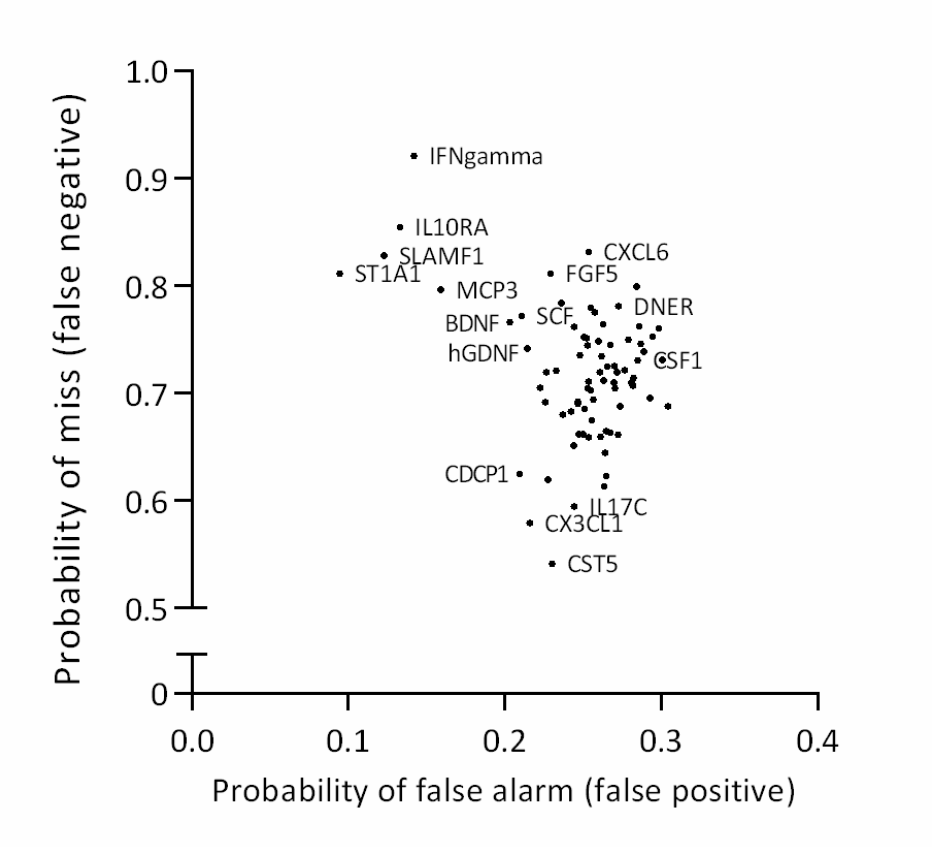

Table 1. Demographic data and clinical variables of the study group.

		n	Controls	n	Antenatal depressive disorder	n	Antidepressant treatment	*p*[a]
Age, years, Mean ± SD		160	32.4 ± 4.3	59	29.7 ± 4.5	39	32.7 ± 5.1	0.001
Pre-pregnancy BMI, kg/m2, Median (IQR)		160	23.2 (21.5 - 26.4)	59	23.3 (21.1 - 25.9)	39	25.8 (22.6 - 29.3)	0.04
Married/ cohabiting, n (%)		141	139 (98.6)	59	58 (98.3)	37	37 (100)	0.8
University education, n (%)		160	135 (84.4)	59	40 (67.8)	39	29 (74.4)	0.02
Parity, n (%)	No previous children	160	66 (41.2)	59	29 (49.2)	39	16 (41.0)	0.6
Gestational length, days, Mean ± SD		159	279.2 ± 8.4	59	281.1 ± 10.6	39	276.3 ± 8.5	0.04
Days to partus, Median (IQR)		160	14 (9 - 22)	59	20 (11 - 28)	39	11 (7 - 20)	0.001
Gender of offspring, boy, n (%)		158	81 (51.3)	58	31 (53.4)	39	24 (61.5)	0.6
Fasting at blood sampling, n (%)		160	36 (22.5)	59	5 (8.5)	39	12 (30.8)	0.02
Current smoking, n (%)		160	4 (2.5)	59	4 (6.8)	39	0	0.2
Current alcohol use, n (%)		160	2 (1.2)	59	1 (1.7)	39	0	0.8
Sleep duration, < 6 hours, n (%)		125	9 (7.2)	48	7 (14.6)	19	2 (10.5)	0.4
Inflammatory/rheumatoid disease, n (%)		160	4 (2.5)	59	0	39	1 (2.6)	0.5
Preeclampsia/hypertension, n (%)		160	9 (5.6)	59	2 (3.4)	39	3 (7.7)	0.7
Comorbid anxiety, n (%)		125	0	55	19 (34.5)	26	8 (30.8)	0.001
Pre-pregnancy psychiatric history, n (%)		160	32 (20.0)	59	27 (45.8)	39	37 (94.9)	0.001
Prior history of manic episodes, n (%)		160	1 (0.5)	59	5 (8.5)	39	2 (5.1)	0.009
Pre-pregnancy use of mood stabilizing drugs, n (%)		160	0	59	1 81.7)	39	1 (2.6)	n.c.
Prior history of psychosis, n (%)		160	0	59	1 (1.7)	39	0	n.c.
EPDS gestational weak 17, Median (IQR)		157	5 (2 - 7)	55	13 (9 – 16)	35	9 (5 – 15)	0.001
EPDS gestational weak 32, Median (IQR)		158	5 (2.75 – 8)	58	14 (13 – 16.25)	35	9 (5 – 16)	0.001

[a]Unless indicated by superscript letter, p-value denotes difference between groups. One-way ANOVA, Kruskal-Wallis test, Chi-square test or Fishers exact test. SD = Standard deviation, BMI = Body mass index, IQR = interquartile range, EPDS = Edinburgh Postnatal Depression Scale, MADRS = Montgomery Åsberg Depression Rating Scale, n.c. = not calculated

Table 2. Protein expression levels in healthy pregnant women, women with antenatal depression, and women on SSRI treatment. Data displayed as mean ± SD. Post hoc comparisons by use of multivariable logistic regression analyses.

Inflammatory marker	**n**	**Healthy pregnant**		**n**	**Antenatal depression**		**n**	**SSRI treatment**		**adjusted *p*[a] depressed vs. control**	**adjusted *p*[a] SSRI use vs. control**	**adjusted *p*[a] SSRI use vs. depressed**
		Mean	SD		Mean	SD		Mean	SD			
TRAIL	159	10.71	0.58	58	10.31	0.63	38	10.39	0.69	0.000001	0.003298	0.176027
CSF1	159	11.48	0.41	58	11.20	0.47	38	11.16	0.41	0.000004	0.000011	0.853314
CX3CL1	159	7.52	0.68	58	7.06	0.81	38	6.96	0.73	0.000005	0.000071	0.803664
CST5	159	7.55	0.71	58	7.10	0.63	38	7.05	0.55	0.000033	0.000134	0.340899
DNER	159	9.41	0.59	58	9.12	0.60	38	8.92	0.62	0.000017	0.000080	0.547355
VEGFA	159	14.74	0.45	58	14.48	0.55	38	14.39	0.45	0.000079	0.000016	0.533672
STAMPB	159	5.71	1.66	58	4.85	1.40	38	4.43	1.46	0.000193	0.000050	0.307392
CD5	159	4.52	0.58	58	4.16	0.63	38	4.12	0.51	0.000039	0.000267	0.886194
CD244	159	7.68	0.66	58	7.31	0.69	38	7.20	0.57	0.000089	0.000050	0.687181
TNFRSF9	159	7.83	0.58	58	7.41	0.67	38	7.48	0.55	0.000029	0.001092	0.819474
TNFB	159	4.33	0.68	58	3.97	0.63	38	3.94	0.61	0.000021	0.003959	0.748542
IL10RB	159	9.13	0.56	58	8.76	0.71	38	8.72	0.54	0.000048	0.000478	0.970432
CD40	159	12.26	0.91	58	11.73	0.92	38	11.52	0.80	0.000111	0.000039	0.592587
IL15RA	159	1.29	0.26	58	1.14	0.30	38	1.08	0.24	0.000098	0.000027	0.384587
hGDNF	158	2.75	0.55	58	2.44	0.56	38	2.37	0.60	0.000058	0.000567	0.415230
ST1A1	158	1.36	0.93	58	0.94	0.41	38	0.95	0.45	0.000238	0.008472	0.776118
CCL11	159	8.96	0.83	58	8.45	0.70	38	8.52	0.74	0.000108	0.004703	0.640196
ADA	159	7.04	0.91	58	6.60	0.76	38	6.42	0.86	0.001315	0.000047	0.223918
CCL25	159	7.70	1.00	58	7.05	0.88	38	7.10	1.03	0.000187	0.000863	0.638872

uPA	159	15.98	0.50	58	15.71	0.58	38	15.66	0.66	0.000223	0.001607	0.953152
AXIN1	159	4.64	2.04	58	3.70	1.84	38	3.13	1.69	0.000383	0.000284	0.669153
SLAMF1	157	2.25	0.78	58	1.92	0.49	38	1.92	0.48	0.000226	0.002224	0.892883
IL17C	159	2.87	0.82	58	2.48	0.73	38	2.33	0.45	0.000723	0.000368	0.755932

[a]Adjusted for age, body mass index, smoking, days left to parturition, fasting, preeclampsia or hypertension, and pre-pregnancy inflammatory or rheumatoid disorder.

Table 3. Spearman rank correlation between significant inflammatory markers and self-rated depression.

Inflammatory marker	**EPDS Gestational week 17 (n=244)**		**EPDS Gestational week 32 (n=248)**		**MADRS Gestational week 36 (n=182)**	
	rho	***p***	***rho***	***p***	***rho***	***p***
TRAIL	**-0.189**	0.0030	**-0.171**	0.0071	**-0.252**	0.0003
CSF1	**-0.238**	0.0002	**-0.217**	0.0006	**-0.202**	0.0042
CX3CL1	**-0.224**	0.0004	**-0.221**	0.0004	**-0.244**	0.0005
CST5	**-0.303**	0.0000	**-0.289**	0.0000	**-0.285**	0.0000
DNER	**-0.170**	0.0077	**-0.221**	0.0004	**-0.274**	0.0001
VEGFA	**-0.221**	0.0005	**-0.181**	0.0041	**-0.191**	0.0067
STAMPB	**-0.262**	0.0000	**-0.183**	0.0038	**-0.227**	0.0012
CD5	**-0.203**	0.0014	**-0.137**	0.0305	**-0.203**	0.0039
CD244	**-0.232**	0.0003	**-0.215**	0.0007	**-0.251**	0.0003
TNFRSF9	**-0.174**	0.0066	-0.116	0.0684	**-0.170**	0.0159
TNFB	**-0.173**	0.0068	**-0.204**	0.0012	**-0.209**	0.0030
IL10RB	**-0.196**	0.0021	**-0.189**	0.0028	**-.174**	0.0140
CD40	**-0.237**	0.0002	**-0.175**	0.0058	**-0.233**	0.0009
IL15RA	**-0.274**	0.0000	**-0.252**	0.0001	**-0.297**	0.0000
hGDNF	**-0.184**	0.0039	**-0.142**	0.0259	**-0.193**	0.0062
ST1A1	**-0.232**	0.0003	**-0.202**	0.0014	**-0.238**	0.0007
CCL11	**-0.266**	0.0000	**-0.273**	0.0000	**-0.231**	0.0010

ADA	**-0.235**	0.0002	**-0.189**	0.0027	**-0.277**	0.0001
CCL25	**-0.170**	0.0078	**-0.190**	0.0027	**-0.176**	0.0129
uPA	**-0.187**	0.0033	**-0.188**	0.0029	**-0.165**	0.0193
AXIN1	**-0.181**	0.0045	-0.1188	0.07	**-0.195**	0.0056
SLAMF1	**-0.231**	0.0003	**-0.221**	0.0005	**-0.245**	0.0005
IL17C	**-0.210**	0.0010	**-0.200**	0.0015	**-0.244**	0.0005

EPDS = Edinburgh Postnatal Depression Scale, MADRS = Montgomery Åsberg Depression Rating Scale.

Table 4. Spearman rank correlations between significant inflammatory markers and levels of cortisol, cortisone and the quotient of cortisone and cortisol.

	Healthy controls n = 120						**Antenatal depression and SSRI treatment n = 74**					
Inflammatory marker	**Cortisol**		**Cortisone**		**Cortisone/ Cortisol**		**Cortisol**		**Cortisone**		**Cortisone/ Cortisol**	
	rho	***p***	**rho**	***p***	**rho**	***p***	**rho**	***p***	**rho**	***p***	**rho**	***p***
TRAIL	**-0.253**	**0.005**	**-0.290**	**0.001**	-0.127	0.168	-0.047	0.692	0.012	0.919	0.123	0.297
CSF1	-0.056	0.540	**-0.316**	**0.001**	**-0.332**	**0.001**	0.001	0.992	0.099	0.402	0.177	0.132
CX3CL1	-0.079	0.390	-0.150	0.102	-0.117	0.205	-0.067	0.571	-0.031	0.790	0.090	0.445
CST5	-0.163	0.075	**-0.363**	**0.001**	**-0.275**	**0.002**	0.044	0.707	0.051	0.664	0.050	0.671
DNER	-0.076	0.410	**-0.267**	**0.003**	**-0.197**	**0.031**	0.045	0.703	0.004	0.972	-0.004	0.974
VEGFA	-0.122	0.183	**-0.319**	**0.001**	**-0.311**	**0.001**	0.103	0.382	0.140	0.233	0.106	0.369
STAMPB	-0.049	0.598	**-0.202**	**0.027**	**-0.259**	**0.004**	0.066	0.574	0.151	0.200	0.183	0.119
CD5	**-0.205**	0.025	**-0.318**	**0.001**	**-0.183**	**0.045**	-0.040	0.736	-0.118	0.316	0.036	0.759
CD244	-0.087	0.345	**-0.344**	**0.001**	**-0.342**	**0.001**	0.164	0.163	0.088	0.458	-0.048	0.683
TNFRSF9	-0.163	0.075	**-0.183**	**0.045**	-0.082	0.375	-0.087	0.463	0.040	0.732	0.190	0.105
TNFB	-0.148	0.107	**-0.275**	**0.002**	**-0.187**	**0.041**	-0.103	0.382	-0.146	0.214	0.011	0.928
IL10RB	-0.140	0.128	**-0.219**	**0.016**	-0.130	0.157	-0.072	0.543	0.003	0.983	0.186	0.113

CD40	-0.086	0.348	**-0.253**	**0.005**	**-0.251**	**0.006**	0.128	0.278	0.187	0.110	0.118	0.317
IL15RA	-0.110	0.233	**-0.260**	**0.004**	**-0.224**	**0.014**	0.059	0.618	-0.022	0.852	0.005	0.964
hGDNF	**-0.211**	0.021	**-0.260**	**0.004**	-0.150	0.101	0.036	0.759	0.163	0.165	0.200	0.088
ST1A1	-0.021	0.821	**-0.211**	**0.021**	**-0.311**	**0.001**	0.114	0.331	0.056	0.634	-0.025	0.833
CCL11	-0.059	0.519	-0.126	0.172	-0.119	0.197	0.012	0.920	-0.115	0.330	-0.108	0.362
ADA	-0.114	0.216	-0.171	0.062	-0.147	0.109	-0.083	0.480	-0.044	0.709	0.109	0.353
CCL25	-0.021	0.817	-0.051	0.581	-0.085	0.356	-0.100	0.395	-0.010	0.931	0.167	0.155
uPA	**-0.221**	0.015	**-0.185**	**0.043**	0.003	0.971	-0.142	0.227	0.054	0.649	**0.297**	0.010
AXIN1	-0.030	0.748	**-0.249**	**0.006**	**-0.309**	**0.001**	0.128	0.277	0.170	0.148	0.131	0.267
SLAMF1	-0.102	0.268	**-0.211**	**0.021**	**-0.247**	**0.007**	-0.020	0.866	-0.046	0.699	0.051	0.667
IL17C	-0.172	0.060	**-0.247**	**0.007**	-0.144	0.117	0.053	0.652	-0.021	0.859	-0.069	0.557

Table 5. Excerpt showing differentially methylated CpG sites in peripartum depression.

			Antenatal Blood DNA Methylation Profiles (n=50)				
			% DNA Methylation (SD)		**Independent samples t-test**		
Gene	**Illumina ID**	**Distance to TSS**	**Antenatal depression (n=19)**	**Healthy controls (n=31)**	**t**	**df**	***p***
IL15RA	cg13730379	-435	78.10 (2.19)	80.27 (3.07)	-2.97	47.94	4.60E-03
CSF1	cg20815701	831	18.24 (1.74)	16.79 (1.87)	2.76	43.06	8.49E-03
AXIN1	cg03330454	-346	9.29 (0.92)	8.59 (0.78)	2.78	35.63	8.66E-03
VEGFA	cg00539360	-809	87.40 (1.49)	88.44 (1.46)	-2.46	39.87	1.85E-02
VEGFA	cg01298514	-1138	90.29 (1.16)	91.12 (1.02)	-2.45	35.67	1.92E-02
DNER	cg20755820	516	9.21 (0.81)	9.85 (1.00)	-2.42	42.97	1.96E-02
GDNF	cg21590264	74	11.12 (1.16)	11.89 (1.02)	-2.41	33.24	2.17E-02
IL15RA	cg08497766	273	12.79 (0.79)	13.37 (0.91)	-2.35	41.11	2.34E-02

IL10RB	cg00765428	-33	5.74 (0.75)	5.28 (0.52)	2.30	29.83	2.87E-02
GDNF	cg07128111	-376	12.74 (1.29)	11.90 (1.27)	2.21	40.40	3.28E-02
VEGFA	cg17055274	1114	8.00 (0.70)	8.68 (1.28)	-2.17	47.97	3.47E-02
VEGFA	cg17866984	-178	9.52 (0.73)	9.97 (0.79)	-2.04	39.51	4.78E-02
CST5	cg02882813	302	84.80 (1.45)	85.68 (1.73)	-2.03	45.46	4.86E-02

Cohort consists of pregnant women with a history of major depression or bipolar disorder (I, II, or not otherwise specified) (E-GEOD-44132). Prior to analysis, methylation data were preprocessed and adjusted for batch effects and corrected for blood cell type heterogeneity. Five methylation samples classified as cross-batch controls and were excluded from the analysis. Independent samples t-tests were performed, contrasting methylation M-values in 19 women with antenatal depression and 31 euthymic women. Abbreviations: df, degrees of freedom; p.val, p-value; t, t-statistic.