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Blood transcriptomic signatures associated with depression, or the risk for depression, in pregnant women from the Psychiatry Research And Motherhood - Depression (PRAM-D) study

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During pregnancy multiple biological systems undergo consistent modifications, in particular the hormonal axes and the immune system. Moreover, while it is well known that pregnant women suffering from depression show alterations in these systems, the exact underlying mechanisms are still not clear. For this reason, in this study, we explored the blood transcriptomic profile and related pathways in 41 pregnant women with a current diagnosis of depression, 23 pregnant women, who were not depressed in pregnancy but, because of a history of depressive episodes, were considered at high risk of developing antenatal depression (history-only), and 28 pregnant women who had never experienced depression in their life, including the current pregnancy. Based on resulting differentially expressed genes, we identified 28 molecular pathways modulated in depressed women compared with controls, with a main association with increased B cell activity, while history-only women showed 52 pathways differentially modulated compared with controls, involving lower cytotoxic T cell activity and higher pro-inflammatory pathways activity. Conversely, depressed women showed a differential modulation of 75 pathways, compared with history-only women, associated with increased activity of allo- and auto-immunity and pro-inflammatory pathways. Overall, our results suggest a main role of immunity within the context of perinatal depression, and of a differential modulation of specific immune processes underlying the development of depression and the associated risk.

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INTRODUCTION

Pregnancy and the early postpartum period are characterized by physical and psychological changes in the life of women, representing an important window of vulnerability for the development of different psychiatric conditions, such as mood and anxiety disorders. Indeed, recent data indicate that mood disorders during the perinatal period affect around 15–26% of women globally [1, 2], with higher prevalence observed during the COVID-19 pandemic, compared with pre-pandemic data [3]. However, according to several reports, these conditions are underdiagnosed, due to the perceived stigma, heterogeneity of assessment tools and difficulties in the diagnostic process, indicating that the real incidence could be even higher [4]. Indeed, although according to the Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-V), perinatal (or peripartum) depression is a major depressive disorder occurring during pregnancy and/or within the first four weeks postpartum [5], the identification process of affected women is challenging, since this disorder is characterized by a wide array of symptoms, and some of them, including appetite and sleep disruption, reduced concentration and tiredness, fall within the spectrum of common physiological difficulties that characterize gestation and the first year postpartum [6].

Moreover, the heterogeneity of manifestations of this condition reflects its multifactorial etiopathology, which is based on the complex interaction between environmental and biological factors [7, 8]. In particular, high perceived stress, lack of support, history of traumatic experiences such as abuse, and, most importantly, a personal or family history of mental illness (perinatal and otherwise) represent important risk factors for both antenatal and postnatal depression [8–10]. These psychosocial risk factors, combined with biological alterations, can make some women more vulnerable to develop depressive symptoms perinatally. Indeed, alterations in different biological systems, such as the hormonal and immune systems, have been widely reported in pregnant women suffering from depressive symptoms [11, 12].

In the context of hormonal systems, the hypothalamic-pituitary-adrenal (HPA) axis undergoes important modifications across the perinatal period [13] which could in turn sensitize

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women to show altered responses to stress. However, available systematic reviews of studies that have analysed cortisol levels in perinatally depressed women have reported heterogeneous results [14–17], potentially biased by the variety of biological sampling as well as by the type of clinical assessments used among studies. Despite their heterogeneity, altogether these findings suggest a potential dysregulation of the HPA axis in perinatal depression. In addition, steroid reproductive hormones, such as oestrogens and progesterone, which have neuroprotective and antidepressant properties [18–21], undergo drastic physiological changes across the perinatal period [22]. Based on available knowledge from observational [23–25] and interventional [26] studies the dramatic fluctuations of these hormones, rather than their absolute levels, could be the leading cause of the development of postpartum depressive symptoms, in a subset of women with underlying, yet still unidentified, biological vulnerability.

Furthermore, the milieu of reproductive hormones also guides an important remodelling of the immune system in pregnancy, readjusting the balance between pro-inflammatory T helper 1 (Th1) and anti-inflammatory/tolerogenic T helper 2 (Th2) cells towards a predominance of Th2 [27, 28]. An abnormal increase of the pro-inflammatory activity during pregnancy could play a pivotal role in the development of perinatal depression, in line with the theory of neuroinflammation as an underlying mechanism of depression [29]. In line with this, altered levels of peripheral pro- and anti-inflammatory factors, including interleukin (IL)-6, IL-10, tumor necrosis factor (TNF)-α and C-reactive protein, have been observed in women depressed during and after pregnancy in multiple cohorts [29–34], including studies conducted by our group [35, 36]. Interestingly, one exploratory study reported no differences in the cytokines' levels measured in plasma, but identified significantly higher levels of IL-1β, IL-18, IL-23 and IL-33 in the cerebral spinal fluid in depressed pregnant women, compared with controls [37]. This shows evidence of the actual involvement of central inflammation in perinatal depression, which may go undetected when no alterations are observed peripherally. Other proposed underlying immune mechanisms involve an altered modulation of the kynurenine pathway, which acts as a bridge among higher inflammation, neurotoxicity and lower production of serotonin [38], with studies reporting an increased activation of this pathway in postpartum depressed women [39–41]. Taken collectively, available results suggest an important involvement of the immune system, but results are mixed, due to the complex aetiology of this disorder, as well as to the different study settings.

Overall, although the available studies suggest the presence of different biological alterations occurring in association with perinatal depression, data are still heterogeneous, and also, other biological systems could be involved. Therefore, in this study, we used a hypothesis-free approach and measured the modulation of the entire transcriptome in blood samples of pregnant women at their 25th week of gestation, to have a wider picture of the underlying molecular mechanisms associated with the development of depression in pregnancy. This is a subsample of the Psychiatry and Motherhood – Depression (PRAM-D) study, where, using a hypothesis-driven approach, we had previously shown increased pro-inflammatory cytokines during pregnancy in depressed women and in women with a history of depression but not-depressed in pregnancy (history-only) compared with controls, as well as increased evening salivary cortisol and blunted salivary cortisol awakening response in the depressed women only [35, 36]. We now aim to complement these hypothesis-driven analyses with a hypothesis-free approach, by transcriptomic analyses, which ultimately may allow for the development of more insight into the underlying biological mechanisms and novel potential intervention targets.

METHODS AND MATERIALS

Study participants

As part of the PRAM-D study, women aged 18 years old and above, with a singleton pregnancy, were recruited and were administered the Structured Clinical Interview for the DSM-IV (SCID-IV), to define clinical groups based on their psychological profile, at 25 weeks gestation, a timepoint that was previously found as particularly biologically sensitive to stress response [42]. A specific description of the clinical sample has been already reported in previous studies conducted on this same cohort [35, 36, 43]. In this study, the sample consisted of $n = 28$ pregnant women who had never experienced depressive episodes in their life, including the present pregnancy (*control* group); $n = 23$ women who did not fulfil diagnostic criteria for depression in pregnancy, but were considered at high risk of depression, based on a clinical history of depressive episodes prior to present pregnancy (*history-only* group); $n = 41$ women diagnosed as depressed in the current pregnancy (*depressed* group).

Exclusion criteria were: uterine anomaly, known obstetric complications in the index pregnancy, severe or relevant chronic medical conditions, such as cardiovascular disease, metabolic or endocrine disorder (e.g., gestational diabetes and hypertension). Depressed women were excluded if presenting with any current DSM-IV diagnosis other than co-morbid anxiety disorder, having a past history of psychosis or bipolar affective disorder, or taking antidepressant medication at 25 weeks gestation (baseline).

All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. The study was approved by King's College Hospital Research Ethics Committee (approval number REC 07/Q0703/48), and all participants provided written informed consent.

The most relevant socio-demographic and clinical factors are presented in Table 1. No significant differences were observed in age across groups, whereas the distribution of pre-pregnancy body mass index (BMI) was statistically different ($p = 0.042$), mainly due to depressed women showing larger BMI than controls ($p = 0.012$) (Table 1). Similarly, Beck Depression Inventory (BDI) scores at 25th week of gestation was significantly different among groups ($p = 0.001$), with the expected higher levels in depressed women, compared with other groups. Significant differences among groups were observed also in education, marital status, cigarette use in pregnancy ($p \leq 0.05$), while employment-related variables were not discriminating factors ($p > 0.05$) and only a difference at trend level for ethnicity was observed ($p = 0.058$). Overall, depressed women were more likely to have a higher pre-pregnancy BMI, be unmarried, smoke cigarettes during pregnancy, report a lower education and be non-white, compared with other groups (Table 1). As expected, the percentage of women using antidepressants during pregnancy was significantly different both before and after the baseline of 25th week of gestation ($p < 0.05$). Indeed, no women from the control group were using antidepressants at any point in pregnancy, while the percentage of women from the depressed group was higher compared with history-only women.

Blood collection

Peripheral venous blood samples were collected from all the participants in PAXgene tubes. Samples were collected under standardized conditions, in terms of collection times (12–3 pm), technical procedures and gestational age (between 25–30 weeks). Women were not asked to fast before the blood draw. The tubes were kept at room temperature for 2 h, then frozen at −20 °C for 24 h and finally moved to a −80 °C freezer, according to the manufacturer's instructions.

RNA isolation and transcriptomic analyses

Isolation of total RNA was performed using the PAXgene blood miRNA kit according to the manufacturer's protocol (PreAnalytiX, Hombrechtikon, CHE). RNA quantity and quality were assessed by evaluation of the A260/280 and A260/230 ratios using a Nanodrop spectrophotometer (NanoDrop Technologies, Delaware, USA) and samples kept at −80 °C until processing for whole transcriptome analyses. Microarray assays were performed following the protocol described in the Affymetrix GeneChip Expression Analysis technical manual (Affymetrix, California, USA). Briefly, 100 ng of total RNA were used to synthesize cDNA with the GeneChip WT PLUS Reagent kit (ThermoFisher Scientific), which was then purified, fragmented, labelled, and hybridized onto Human Gene 2.1 ST Array Strips (ThermoFisher Scientific). Samples have been randomized and stratified in a way that each array strip included the same number of depressed, history-only

Table 1. Sociodemographic and clinical characteristics of the sample at 25 weeks of gestation.

	Controls ($n = 27–28$)	History-only ($n = 22–23$)	Depressed ($n = 40–41$)	Statistics
Age (years), mean (SD)	32.4 (4.3)	32.3 (5.2)	30.5 (6.3)	(H) = 1.208, $p = 0.546$
Ethnicity, white, n (%)	22 (78.6)	20 (86.9)	25 (60.9)	$\chi^2(2) = 5.697$, $p = 0.058$
Education, A level or higher, n (%)	27 (96.4)	17 (73.9)	28 (68.3)	$\chi^2(2) = 8.082$, $p = 0.018$
Employment status, working outside the home, n (%)	16 (57.1)	17 (73.9)	23(56.1)	$\chi^2(2) = 2.198$, $p = 0.333$
Classification of employment, professional or management, n (%)	20 (71.4)	11 (47.8)	18 (43.9)	$\chi^2(2) = 5.428$, $p = 0.066$
Marital status, married or cohabiting, n (%)	26 (92.9)	18 (78.3)	21 (51.2)	$\chi^2(2) = 14.767$, $p = 0.001$
Pre-pregnancy BMI, mean (SD)	22.1 (2.6)	23.8 (9.5)	24.4 (6.2)	(H) = 6.354, $p = 0.042$
Cigarette use in index pregnancy, n (%)	0 (0)	2 (9.09)	14 (35)	$\chi^2(2) = 13.547$, $p = 0.001$
Antidepressants before 25 weeks gestation, n (%)	0 (0)	6 (26.1)	12 (29.3)	$\chi^2(2) = 9.885$, $p = 0.007$
Antidepressants after 25 weeks gestation, n (%)	0 (0)	5 (21.7)	10 (24.4)	$\chi^2(2) = 7.917$, $p = 0.019$
BDI score at 25 weeks gestation, mean (SD)	3.4 (2.8)	5.6 (4.1)	16.7 (12.4)	(H) = 13.591, $p = 0.001$

SD standard deviation, *BMI* body mass index, *BDI* beck depression inventory, *(H)* statistics by Kruskal-Wallis H test, χ^2 statistics by Pearson's chi-square test.

and control samples. The reactions of hybridization, fluidics, and imaging were performed on the Affymetrix GeneAtlas platform (Affymetrix) instrument according to the manufacturer's protocol.

Statistical and bioinformatic analyses

Socio-demographic and clinical variables were analysed using SPSS Statistics Version 28 (IBM). Pearson's chi-square (χ^2) test of the independence of variables was used for the analysis of categorical data. Kruskal-Wallis test (H) was applied to analyse non-parametric continuous variables (age, pre-pregnancy BMI and BDI scores), followed by multiple pairwise analysis.

Raw microarray data (.CEL files) were imported and analysed within the software Partek Genomic Suite 7.0 (Partek, St. Louis, MO, USA). Principal-component analysis (PCA) was carried out to identify outliers and the statistical analysis for the evaluation of differences in gene expression levels was performed by analysis of variance (ANOVA) test using 3 comparisons: depressed vs control group, history-only vs control group, and depressed vs history-only group. As cut-offs for the identification of the differentially expressed genes, fold changes (FC) of -1.2 or $+1.2$ and p-value ≤ 0.05 were considered.

Cell-type-specificity of resulting genes was assessed by interrogating the web-based application WebCSEA (Web-based Cell-type Specific Enrichment Analysis of Genes, available at https://bioinfo.uth.edu/webcsea/) [44], to identify the top 20 general associated cell types.

Genes that resulted to be significantly modulated across groups were then analysed using the Core Analysis functionality of Ingenuity Pathway Analysis (IPA) software (Qiagen), to describe key aspects of the molecular function, biological processes and cellular components of gene products. Pathways with a cut-off of -log p-value ≥ 1.3 were considered significant and analysed to obtain a z-score of the modulation. Whenever the software was not able to provide a definite z-score for single pathways, due to limited available data, we interrogated the Molecule Activity Predictor (MAP) tool of IPA, to obtain a graphical prediction of the possible modulation of the pathways. The pathway analyses performed using IPA rely on aggregated group-level data rather than individual-level data, precluding the direct correlation with sociodemographic or clinical parameters.

RESULTS

Transcriptomic signatures and biological pathways modulated in depressed pregnant women

Our first aim was to evaluate possible differences in the blood gene expression profile and related pathways in depressed women as compared with controls. Our transcriptomic analyses identified 47 genes differentially expressed between the two groups (complete list of genes is available in the Supplementary Table S1), that, according to the cell-enrichment analysis conducted on WebCSEA, were mostly associated with B cells and plasma cells ($-\log_{10}$ p-value $= 5.512$ and $-\log_{10}$ p-value $= 4.644$, respectively), followed by innate lymphoid cells, T cells and many other innate and adaptive immune cells (Fig. 1C).

The differentially expressed genes were involved in 28 significant pathways, that as we can see from Table 2 and Fig. 1A, were mostly immune-related (47%), and especially associated with modulation of the B cell activity (almost 30% of the total; Fig. 1B) including the *PI3K Signalling in B Lymphocytes* (the most significantly associated) (Figure S1), the *B Cell Development*, the *Communication between Innate and Adaptive Immune Cells pathway*, the *IL-15 Signalling*, the *FcγRIIB Signalling in B Lymphocytes*, the *Systemic Lupus Erythematosus in B Cell Signaling Pathway*, and the *B Cell Receptor Signaling*. Since the software IPA was not able to define a z-score for any of the resulting pathways, by using the MAP tool of IPA we were able to estimate the modulation of these pathways and all the results indicated an upregulation. Other associated pathways, although less significantly so, included processes related to energy metabolism (mostly upregulated according to MAP analysis of IPA) and purine catabolism-related processes (mostly downregulated).

Transcriptomic signatures and biological pathways modulated in pregnant women with a history of depression

We were then interested in investigating the gene expression profile and pathways modulated in pregnant women with a history of depression as compared with controls. The transcriptomic analyses indicated 88 differentially expressed genes (see Table S2) in association with the risk of developing depression in pregnancy, for which the top enriched cell types were T cell, macrophage and natural killer cell (Fig. 2C).

Differentially expressed genes were involved in 52 significant pathways (Table 3, Fig. 2A), 75% of them being immune-related (Fig. 2B), including the *Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells* (Figure S2), which, was shown to be downregulated in the MAP analysis of IPA. Similarly, other immune related pathways showed strong association and negative z-scores, including the *G Protein Signaling Mediated by Tubby*, the *T Cell Receptor Signaling* (Figure S3), the *IL-4 Signaling*, the *FAK Signaling* and the *Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes*, while the *CTLA4 Signaling in Cytotoxic T Lymphocytes*, the *Pathogen Induced Cytokine Storm Signaling Pathway* and the *Tryptophan degradation to 2-amino-3-carboxymuconate Semialdehyde* pathway (Figure S4), were all upregulated in pregnant women with a history of depression, as compared with controls.

Transcriptomic signatures and biological pathways specifically affected in depressed pregnant women as compared with pregnant women with a history of depression

We finally investigated possible differences in genes and pathways able to discriminate pregnant women with depression in

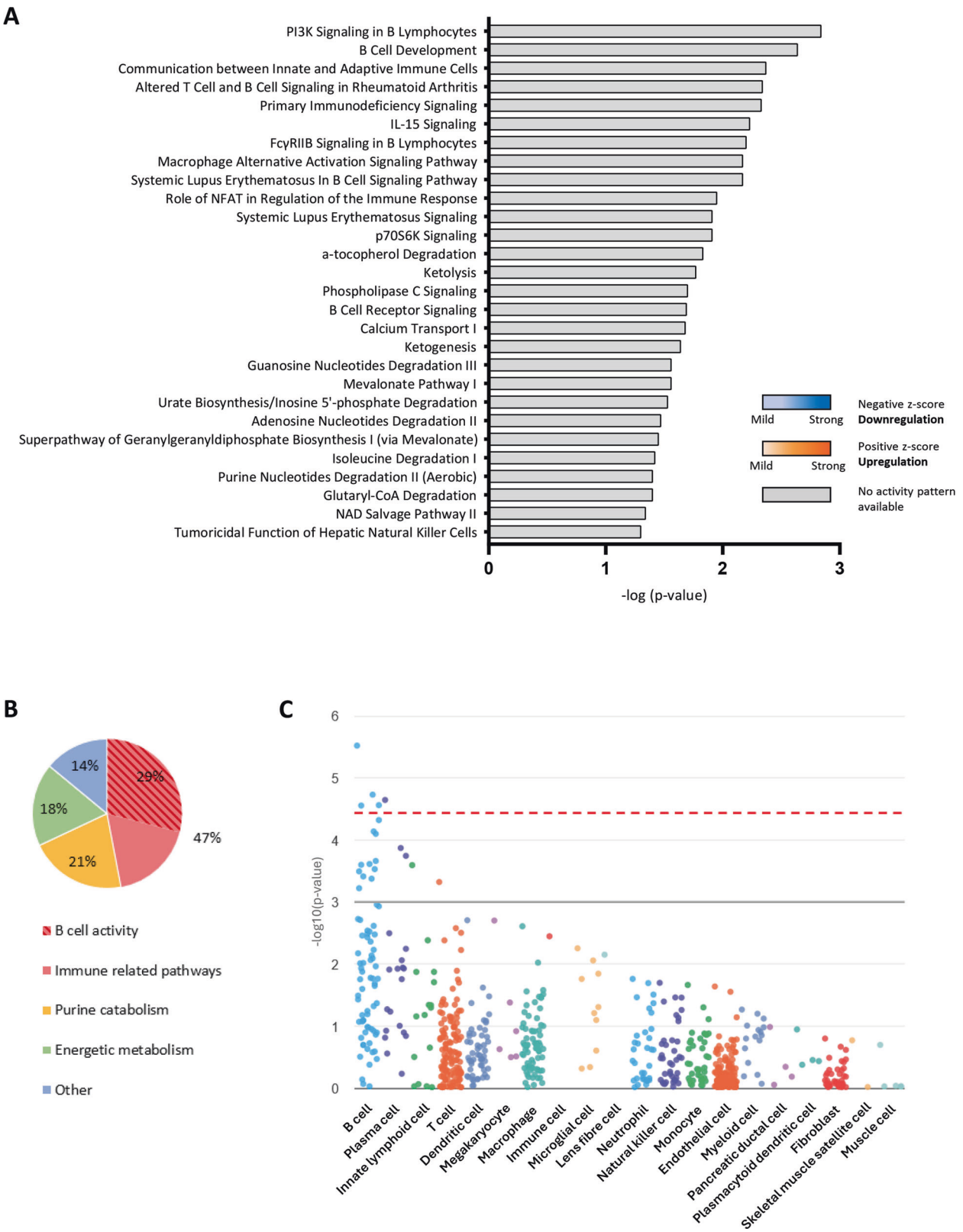

Fig. 1 Biological pathways modulated in depressed women. **A** List of pathways shown to be significantly modulated, **B** percentage of association with immune-related and other specific biological processes and **C** top 20 enriched general cell types, from depressed pregnant women as compared with controls.

Table 2. List of pathways significantly modulated in *depressed* women compared with *control* women.

	Name of pathway	-log(*p*-value)	Molecules	z-score
1	PI3K Signaling in B Lymphocytes	2.84	CD180. IGKC. IGKV1-5. IGLJ2	na
2	B Cell Development	2.64	IGKC. IGKV1-5. IGLJ2	na
3	Communication between Innate and Adaptive Immune Cells	2.37	IGKC. IGKV1-5. IGLJ2. TRAV1-2	na
4	Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	2.34	IGKC. IGKV1-5. IGLJ2. TRAV1-2	na
5	Primary Immunodeficiency Signaling	2.33	CIITA. IGKC	na
6	IL-15 Signaling	2.23	IGKC. IGKV1-5. IGLJ2	na
7	FcγRIIB Signaling in B Lymphocytes	2.2E	IGKC. IGKV1-5. IGLJ2	na
8	Systemic Lupus Erythematosus In B Cell Signaling Pathway	2.17	IGKC. IGKV1-5. IGLJ2. TRAF5	na
9	Macrophage Alternative Activation Signaling Pathway	2.17	CIITA. IGKC. PF4	na
10	Role of NFAT in Regulation of the Immune Response	1.95	IGKC. IGKV1-5. IGLJ2. TRAV1-2	na
11	p70S6K Signaling	1.91	IGKC. IGKV1-5. IGLJ2	na
12	Systemic Lupus Erythematosus Signaling	1.91	IGKC. IGKV1-5. IGLJ2. TRAV1-2	na
13	α-tocopherol Degradation	1.83	CYP4F3	na
14	Ketolysis	1.77	ACAA2	na
15	Phospholipase C Signaling	1.7	IGKC. IGKV1-5. IGLJ2. TRAV1-2	na
16	B Cell Receptor Signaling	1.69	IGKC. IGKV1-5. IGLJ2	na
17	Calcium Transport I	1.68	ANXA5	na
18	Ketogenesis	1.64	ACAA2	na
19	Mevalonate Pathway I	1.56	ACAA2	na
20	Guanosine Nucleotides Degradation III	1.56	NT5M	na
21	Urate Biosynthesis/Inosine 5′-phosphate Degradation	1.53	NT5M	na
22	Adenosine Nucleotides Degradation II	1.47	NT5M	na
23	Superpathway of Geranylgeranyldiphosphate Biosynthesis I (via Mevalonate)	1.45	ACAA2	na
24	Isoleucine Degradation I	1.42	ACAA2	na
25	Glutaryl-CoA Degradation	1.4	ACAA2	na
26	Purine Nucleotides Degradation II (Aerobic)	1.4	NT5M	na
27	NAD Salvage Pathway II	1.34	NT5M	na
28	Tumoricidal Function of Hepatic Natural Killer Cells	1.3	SERPINB9	na

-log(*p*-value) ≥ 1.3, *p*-value order; na: z-score non available.

pregnancy from those with history-only. Our transcriptomic analyses revealed 149 genes differentially expressed (see Table S3 for the entire list of genes) in depressed women, mostly related with T cell, in particular CD8-positive alpha-beta T cell, followed by macrophage and innate lymphoid cell (Fig. 3B).

The differentially resulting genes were involved in 75 pathways (Table 4, Fig. 4A), most of which (approximately 73%) were involved in the immune system (Fig. 3A). The 3 most significant resulting pathways were all related with auto- and allo-immunity, including: the *Allograft Rejection Signaling* (Figure S5), the *Autoimmune Thyroid Disease Signaling* and the *Graft-versus-Host Disease Signaling*, and, according to the MAP analysis, were upregulated. Interestingly, 29 out of the 75 pathways had a positive z-score, suggesting an upregulation, including the *Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells* (Figure S6), the *OX40 Signaling Pathway* and the *T Cell Receptor Signalling*; conversely, 4 pathways had a negative z-score, suggesting a downregulation and included the *Antiproliferative Role of TOB in T Cell Signaling*, the *CTLA4 Signaling in Cytotoxic T Lymphocytes*, the *Wound Healing Signaling Pathway* and the *IL-17 Signaling*.

DISCUSSION

To the best of our knowledge, this is the first study investigating whole transcriptome profile in a population of pregnant women with depression or with a history of depression, compared with control women. Indeed, previously available transcriptomic studies focused mainly on *postpartum* depression, and only compared depressed women versus controls [45–47], while our comparison between depressed pregnant women and pregnant women with a history of depression allows us to dissect the pathways associated with active depression vs. vulnerability to depression. Our results show the presence of an immune dysregulation in women who are affected by depression in pregnancy (*depressed* group) that differentiates them from not only healthy women (*control* group), but also women that have a history of depression during life but are not currently depressed (*history-only* group), in the same gestational period. Specifically, blood transcriptomic analyses identified 88 and 47 genes differentially modulated in the history-only and in the depressed group, compared with the control group, respectively, and 149 genes differentially modulated in the depressed group compared with the history-only group. The pathway analyses conducted on these lists resulted in 3 sets of associated pathways. In particular, for depressed women, pathway analyses showed that differentially expressed genes enriched in 28 pathways compared with controls, and 75 pathways compared with history-only women, whereas 52 pathways were enriched for differentially expressed genes in history-only women compared with controls. Of interest, most of the differentially expressed genes, as well as, the resulting associated pathways, were immune-related.

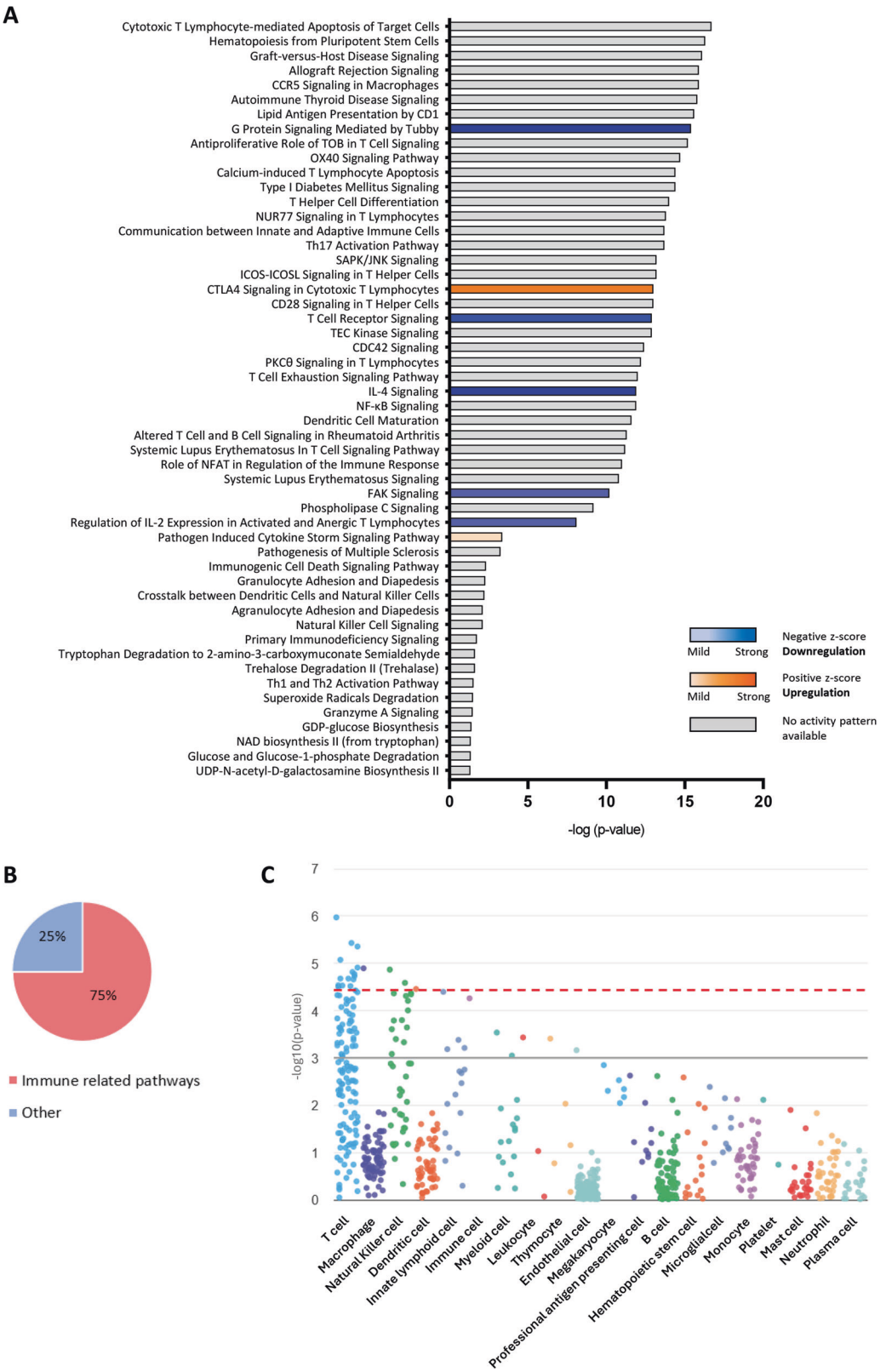

Fig. 2 Biological pathways modulated in history-only women. A List of pathways shown to be significantly modulated **B** percentage of association with immune-related biological processes and **C** top 20 enriched general cell types, from history-only as compared with controls.

The bidirectional relationship between inflammation and depressive symptoms occurring perinatally has been the subject of several investigations [29, 48] with perinatal depression being labelled as the fourth inflammatory morbidity of pregnancy (after preeclampsia, preterm birth, and gestational diabetes) [49]. To this end, data previously published on this cohort have shown that both depressed and history-only women have an increased inflammatory status compared with controls, as shown by the

Table 3. List of pathways significantly modulated in women with a *history* of depression compared with *control* women.

	Name of pathway	-log(*p*-value)	Molecules	z-score
1	Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells	16.7	CD3D, PRF1, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
2	Hematopoiesis from Pluripotent Stem Cells	16.3	CD3D, CD8A, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
3	Graft-versus-Host Disease Signaling	16.1	CD3D, PRF1, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
4	CCR5 Signaling in Macrophages	15.9	CCL5, CD3D, GNAZ, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
5	Allograft Rejection Signaling	15.9	CD3D, PRF1, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
6	Autoimmune Thyroid Disease Signaling	15.8	CD3D, PRF1, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
7	Lipid Antigen Presentation by CD1	15.6	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
8	G Protein Signaling Mediated by Tubby	15.4	CD3D, GNAZ, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	−4.123
9	Antiproliferative Role of TOB in T Cell Signaling	15.2	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
10	OX40 Signaling Pathway	14.7	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
11	Type I Diabetes Mellitus Signaling	14.4	CD3D, PRF1, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
12	Calcium-induced T Lymphocyte Apoptosis	14.4	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
13	T Helper Cell Differentiation	14.0	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
14	NUR77 Signaling in T Lymphocytes	13.8	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
15	Th17 Activation Pathway	13.7	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
16	Communication between Innate and Adaptive Immune Cells	13.7	CCL5, CD3D, CD8A, IGHV3-33, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
17	ICOS-ICOSL Signaling in T Helper Cells	13.2	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
18	SAPK/JNK Signaling	13.2	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
19	CD28 Signaling in T Helper Cells	13.0	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
20	CTLA4 Signaling in Cytotoxic T Lymphocytes	13.0	CD3D, CD8A, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	4.123
21	TEC Kinase Signaling	12.9	CD3D, GNAZ, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
22	T Cell Receptor Signaling	12.9	CD3D, CD8A, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	−3.464
23	CDC42 Signaling	12.4	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
24	PKCθ Signaling in T Lymphocytes	12.2	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na

Table 3. continued

	Name of pathway	-log(*p*-value)	Molecules	z-score
25	T Cell Exhaustion Signaling Pathway	12.0	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
26	NF-κB Signaling	11.9	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
27	IL-4 Signaling	11.9	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	−4.000
28	Dendritic Cell Maturation	11.6	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
29	Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	11.3	CD3D, IGHV3-33, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
30	Systemic Lupus Erythematosus In T Cell Signaling Pathway	11.2	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
31	Role of NFAT in Regulation of the Immune Response	11.0	CD3D, GNAZ, IGHV3-33, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
32	Systemic Lupus Erythematosus Signaling	10.8	CD3D, IGHV3-33, RNU11, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
33	FAK Signaling	10.2	ADGRE1, CCR1, CD3D, GPR174, NEDD9, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	−3.130
34	Phospholipase C Signaling	9.18	CD3D, IGHV3-33, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRGC2, TRGJP2, TRGV9	na
35	Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes	8.1	CD3D, TRAJ43, TRAJ57, TRAV1-2, TRAV8-4, TRBJ2-2, TRBJ2-3, TRBV2, TRBV28, TRBV3-1, TRGV9	−3.317
36	Pathogen Induced Cytokine Storm Signaling Pathway	3.36	AIM2, CCL5, CCR1, MLKL, PF4, PPBP, PRF1	0.378
37	Pathogenesis of Multiple Sclerosis	3.25	CCL5, CCR1	na
38	Immunogenic Cell Death Signaling Pathway	2.33	GZMK, MLKL, PRF1	na
39	Granulocyte Adhesion and Diapedesis	2.28	CCL5, CCR1, PF4, PPBP	na
40	Crosstalk between Dendritic Cells and Natural Killer Cells	2.23	CD226, KLRC4-KLRK1/KLRK1, PRF1	na
41	Natural Killer Cell Signaling	2.12	CD226, KIR2DS2, KLRC2, KLRC4-KLRK1/KLRK1	na
42	Agranulocyte Adhesion and Diapedesis	2.12	CCL5, CCR1, PF4, PPBP	na
43	Primary Immunodeficiency Signaling	1.75	CD3D, CD8A	na
44	Trehalose Degradation II (Trehalase)	1.62	HK3	na
45	Tryptophan Degradation to 2-amino-3-carboxymuconate Semialdehyde	1.62	KYNU	na
46	Th1 and Th2 Activation Pathway	1.53	CCR1, CD3D, CD8A	na
47	Superoxide Radicals Degradation	1.5	CAT	na
48	Granzyme A Signaling	1.48	H1-4, PRF1	na
49	GDP-glucose Biosynthesis	1.4	HK3	na
50	Glucose and Glucose-1-phosphate Degradation	1.36	HK3	na
51	NAD biosynthesis II (from tryptophan)	1.36	KYNU	na
52	UDP-N-acetyl-D-galactosamine Biosynthesis II	1.33	HK3	na

-log(*p*-value) ≥ 1.3, *p*-value order; na: z-score non available.

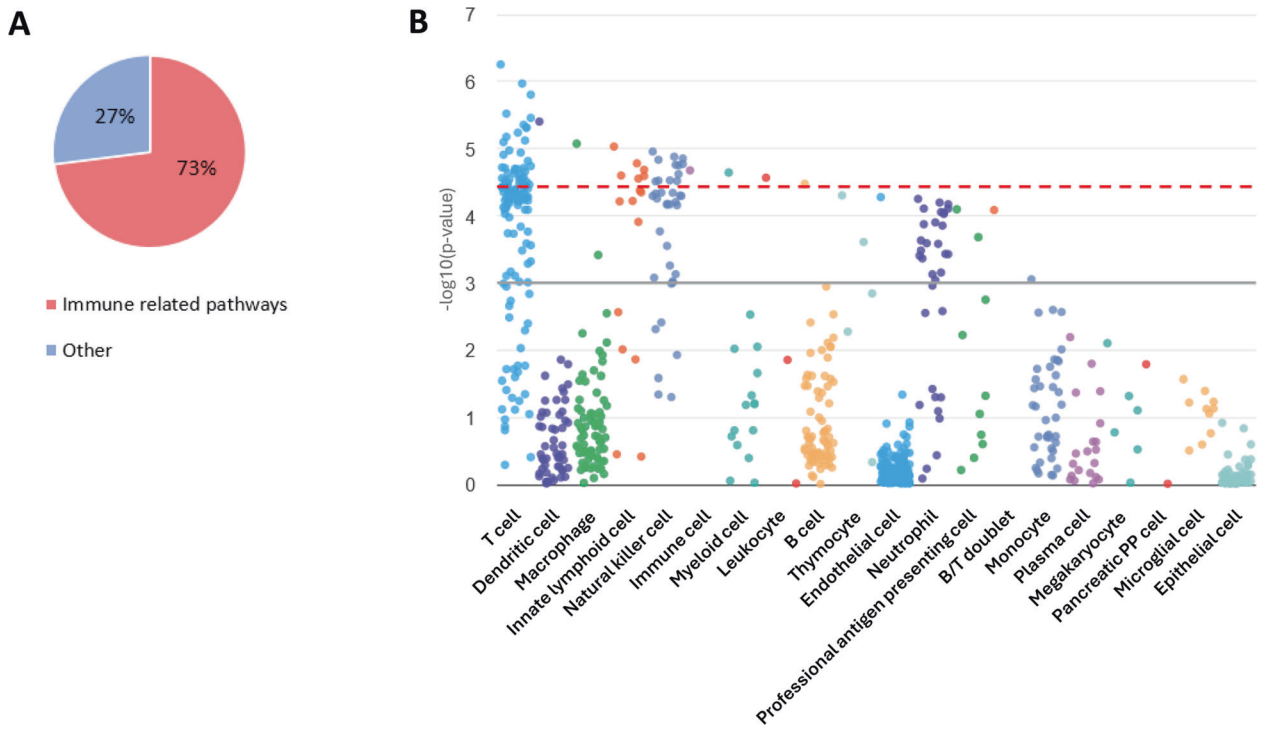

Fig. 3 Biological processes and associated cell-enrichment in depressed women as compared with history-only women. A Percentage of association with immune-related biological processes and **B** top 20 enriched general cell types, from depressed pregnant women as compared with pregnant women with a history of depression.

higher levels of circulating peripheral levels of different cytokines and immune-related molecules, especially IL-6, vascular endothelial growth factor (VEGF), and TNFα [35, 36]. In this context, based on intensity data handpicked from the panel of current microarray analyses, gene expression of *IL-6* reflects what has been reported for protein levels in our previous manuscripts, with significantly higher levels in depressed women, compared to controls, while inconsistent results were obtained for the other two molecules (See Supplementary Fig. S7). However, compared with these hypothesis-driven findings, the results from the present hypothesis-free transcriptomic analyses elucidate additional specific molecular processes and fine regulation that may not manifest in significantly altered circulating levels, due to the involvement and interaction of numerous synergic, combined and counterbalancing, mechanisms.

Indeed, in the current study, depressed women show an upregulation of B cells activity compared with controls, based on the higher expression of genes like *CD180, IGKC, IGKV1-5, IGLJ2*, and *TRAF5*. In particular, *IGKC* (Immunoglobulin kappa constant), *IGKV1-5* (Immunoglobulin kappa variable 1–5) and *IGLJ2* (immunoglobulin lambda joining 2), are all genes encoding for segments of immunoglobulins, which are released from the B cells, but also constitute the B cell receptor (BCR) that is exposed on the surface of these lymphocytes, suggesting an increased expression of the BCR (Figure S1). BCR expression and activation by specific antigens are crucial steps to promote the B cell humoral response, via modulation by other accessory receptors and transductors, including toll-like receptors (TLRs). Upon TLRs stimulation, B cells proliferate intensively and produce large amounts of cytokines and immunoglobulins [50], a mechanism that needs fine tuning. In this regard, the TNF receptor associated factor 5 (TRAF5) encoded by the *TRAF5* gene, which was shown to be upregulated in depressed women from our cohort, is a negative regulator of TLR signalling in B cells [51], and is therefore necessary to shut down the B cell response after its completion. Indeed, its absence in knock-out models results in a dramatically increased production of cytokines and IgM antibodies from B cells, but does not compromise their vitality or proliferation [51]. Similarly, CD180 (whose gene expression was shown to be upregulated in depressed women) is a cell surface accessory molecule of TLR4, and, by forming a complex together with TLR4, is able to impede the possible binding of its ligands, thus acting as a specific inhibitor of TLR4-mediated inflammatory reaction to prevent an excessive response [52].

Taken together, our findings from the transcriptomic analyses suggest that women depressed during pregnancy show a pattern of robust activation of the B cell immunity in comparison with control pregnant women, potentially *via* modulation of the TLR signalling. In line with our results, an altered B cell activation, associated with postpartum depression, was also found in a recent transcriptome-wide association study [46]. Indeed, in this study, B lymphocytes represented the cells that were mostly altered, at the transcriptome-wide level, showing 10-to-100-fold times higher number of significantly modulated transcripts in comparison with other cell types. As the authors report, based on the pathway analysis conducted on the sets of genes modulated in B cells, their results specifically implicate B cells increased activation in postpartum depression, without observing significant differences in total B cells proportions between depressed and control women. Although these results come from a cohort of women after delivery, whereas our sample is characterized by pregnant women in the second trimester, it is possible to hypothesize a common underlying signature of alterations occurring at the level of B cells immunity affecting women who are depressed perinatally, potentially occurring *via* alterations of the TLR signalling in B cells.

We further analysed the transcriptomic profile of women with a history of depressive episodes, but currently well in the index pregnancy. From our findings, women at risk of depression

Table 4. List of pathways significantly modulated in *depressed* women compared with *history-only* women.

	Name of pathway	-log(*p*-value)	Molecules	z-score
1	Allograft Rejection Signaling	30	CD28, CD3D, CD3E, CD3G, CD40LG, HLA-DQA1, IGHG1, PRF1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
2	Autoimmune Thyroid Disease Signaling	29.9	CD28, CD3D, CD3E, CD3G, CD40LG, HLA-DQA1, IGHG1, PRF1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
3	Graft-versus-Host Disease Signaling	27.3	CD28, CD3D, CD3E, CD3G, HLA-DQA1, PRF1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
4	T Helper Cell Differentiation	25.7	CD28, CD3D, CD3E, CD3G, CD40LG, HLA-DQA1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
5	Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells	25.4	CD3D, CD3E, CD3G, PRF1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	2.236
6	ICOS-ICOSL Signaling in T Helper Cells	25.3	CD28, CD3D, CD3E, CD3G, CD40LG, HLA-DQA1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAT1, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	2.000
7	Antiproliferative Role of TOB in T Cell Signaling	25.2	CD28, CD3D, CD3E, CD3G, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	−2.000
8	Type I Diabetes Mellitus Signaling	25.2	CD28, CD3D, CD3E, CD3G, HLA-DQA1, MAP2K6, PRF1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	1.342
9	Hematopoiesis from Pluripotent Stem Cells	24.7	CD3D, CD3E, CD3G, IGHG1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
10	Communication between Innate and Adaptive Immune Cells	24.6	CCL5, CCR7, CD28, CD3D, CD3E, CD3G, CD40LG, IGHG1, IGKV1-5, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
11	Lipid Antigen Presentation by CD1	24.6	CD3D, CD3E, CD3G, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
12	OX40 Signaling Pathway	24.3	CD3D, CD3E, CD3G, HLA-DQA1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	2.000
13	NUR77 Signaling in T Lymphocytes	23.8	CD28, CD3D, CD3E, CD3G, HLA-DQA1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	2.236
14	Calcium-induced T Lymphocyte Apoptosis	23.8	CD3D, CD3E, CD3G, HLA-DQA1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	2.000
15	CD28 Signaling in T Helper Cells	23.5	CD28, CD3D, CD3E, CD3G, FOS, HLA-DQA1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
16	T Cell Receptor Signaling	23.1	CD28, CD3D, CD3E, CD3G, FOS, HLA-DQA1, MAP2K6, SKAP1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	3.838
17	Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	23.1	CD28, CD3D, CD3E, CD3G, CD40LG, HLA-DQA1, IGHG1, IGKV1-5, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
18	CCR5 Signaling in Macrophages	22.7	CCL5, CD3D, CD3E, CD3G, FOS, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
19	T Cell Exhaustion Signaling Pathway	22.6	CD28, CD3D, CD3E, CD3G, EOMES, FOS, HLA-DQA1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
20	CDC42 Signaling	22.3	CD3D, CD3E, CD3G, FOS, HLA-DQA1, ITGB7, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	1.342
21	CTLA4 Signaling in Cytotoxic T Lymphocytes	22.1	CBLB, CD28, CD3D, CD3E, CD3G, FOS, HLA-DQA1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	−4.536

Table 4. continued

	Name of pathway	-log(*p*-value)	Molecules	z-score
22	Systemic Lupus Erythematosus In T Cell Signaling Pathway	22.1	CD28, CD3D, CD3E, CD3G, CD40LG, FOS, HLA-DQA1, MAP2K6, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	2.449
23	Dendritic Cell Maturation	21.8	CCR7, CD3D, CD3E, CD3G, CD40LG, HLA-DQA1, IGHG1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	2.000
24	PKCθ Signaling in T Lymphocytes	21.7	CD28, CD3D, CD3E, CD3G, FOS, HLA-DQA1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	1.890
25	G Protein Signaling Mediated by Tubby	21.6	CD3D, CD3E, CD3G, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	4.899
26	Role of NFAT in Regulation of the Immune Response	21.1	CD28, CD3D, CD3E, CD3G, FOS, HLA-DQA1, IGHG1, IGKV1-5, RCAN3, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	1.414
27	FAK Signaling	21	ADGRG1, ADGRG3, C5AR2, CCR7, CD3D, CD3E, CD3G, FOS, GPR141, GPR15, GPR171, GPR174, GPR183, IL18RAP, IL7R, ITGB7, LTB4R, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	4.218
28	NF-κB Signaling	21	CD3D, CD3E, CD3G, CD40LG, MAP2K6, TDP2, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
29	TEC Kinase Signaling	20.8	BMX, CD3D, CD3E, CD3G, FOS, ITGB7, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
30	Th17 Activation Pathway	20.7	CD3D, CD3E, CD3G, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
31	SAPK/JNK Signaling	19.9	CD3D, CD3E, CD3G, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
32	IL-4 Signaling	19.9	ARG1, CD3D, CD3E, CD3G, HLA-DQA1, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	4.707
33	Systemic Lupus Erythematosus Signaling	19.6	CD28, CD3D, CD3E, CD3G, CD40LG, FOS, IGHG1, IGKV1-5, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
34	Phospholipase C Signaling	15.8	CD3D, CD3E, CD3G, IGHG1, IGKV1-5, ITGB7, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRDC, TRDJ1, TRDJ2, TRDJ3, TRGC2, TRGV5, TRGV8, TRGV9	na
35	Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes	14.9	CD28, CD3D, CD3E, CD3G, FOS, TRAJ35, TRAJ43, TRAJ47, TRAJ48, TRAJ52, TRAJ54, TRAJ57, TRAV20, TRBJ2-2, TRBJ2-3, TRBV2, TRBV20-1, TRBV3-1, TRGV9	3.900
36	Th1 Pathway	7	CD28, CD3D, CD3E, CD3G, CD40LG, HLA-DQA1, KLRD1, MAP2K6, RUNX3	2.121
37	Th1 and Th2 Activation Pathway	5.62	CD28, CD3D, CD3E, CD3G, CD40LG, HLA-DQA1, KLRD1, MAP2K6, RUNX3	na
38	Primary Immunodeficiency Signaling	4.62	CD3D, CD3E, CD40LG, IGHG1, IL7R	
39	Crosstalk between Dendritic Cells and Natural Killer Cells	4.38	CCR7, CD28, CD40LG, KLRC4-KLRK1/KLRK1, KLRD1, PRF1	2.449
40	Phagosome Formation	4.07	ADGRG1, ADGRG3, C5AR2, CCR7, CLEC4D, CLEC4E, GPR141, GPR15, GPR171, GPR174, GPR183, IGHG1, ITGB7, LTB4R, MAP2K6	0.258
41	Th2 Pathway	3.52	CD28, CD3D, CD3E, CD3G, HLA-DQA1, RUNX3	1.342
42	Immunogenic Cell Death Signaling Pathway	3.45	DAPK2, GZMA, GZMH, HSPA6, PRF1	0.447
43	G-Protein Coupled Receptor Signaling	2.87	ADGRG1, ADGRG3, C5AR2, CCR7, FOS, GPR141, GPR15, GPR171, GPR174, GPR183, LTB4R, MAP2K6, TDP2	0.277
44	Natural Killer Cell Signaling	2.59	HCST, HSPA6, IL18RAP, KLRC3, KLRC4-KLRK1/KLRK1, KLRD1	0.447
45	S100 Family Signaling Pathway	2.57	ADGRG1, ADGRG3, C5AR2, CCR7, FOS, GPR141, GPR15, GPR171, GPR174, GPR183, IGHG1, LTB4R, MAP2K6	0.277
46	Pathogen Induced Cytokine Storm Signaling Pathway	2.57	CCL5, CD40LG, EOMES, FOS, HLA-DQA1, IL18RAP, NLRP12, PRF1	0.000
47	PFKFB4 Signaling Pathway	2.3	HK3, MAP2K6, PFKFB4	
48	Glucocorticoid Receptor Signaling	2.3	CCL5, CD3D, CD3E, CD3G, FOS, HLA-DQA1, HP, HSPA6, IL18RAP, IL7R	na
49	Polyamine Regulation in Colon Cancer	2.05	ARG1, FOS, MAP2K6	na

Table 4. continued

	Name of pathway	-log(*p*-value)	Molecules	z-score
50	Breast Cancer Regulation by Stathmin1	2.03	ADGRG1, ADGRG3, C5AR2, CCR7, GPR141, GPR15, GPR171, GPR174, GPR183, LTB4R	0.632
51	CREB Signaling in Neurons	1.97	ADGRG1, ADGRG3, C5AR2, CCR7, GPR141, GPR15, GPR171, GPR174, GPR183, LTB4R	0.632
52	Differential Regulation of Cytokine Production in Intestinal Epithelial Cells by IL-17A and IL-17F	1.93	CCL5, LCN2	
53	CD40 Signaling	1.92	CD40LG, FOS, MAP2K6	na
54	B Cell Development	1.91	HLA-DQA1, IGHG1, IGKV1-5, IL7R	na
55	IL-17A Signaling in Gastric Cells	1.83	CCL5, FOS	na
56	Role of PKR in Interferon Induction and Antiviral Response	1.82	FOS, HSPA6, MAP2K6, NLRP12	na
57	IL-10 Signaling	1.68	FOS, HLA-DQA1, IL18RAP, MAP2K6	1.000
58	Wound Healing Signaling Pathway	1.56	CCL5, CD40LG, FOS, IL18RAP, MAP2K6	−0.447
59	Arginine Degradation I (Arginase Pathway)	1.55	ARG1	na
60	IL-17A Signaling in Fibroblasts	1.51	FOS, LCN2	na
61	TGF-β Signaling	1.49	FOS, MAP2K6, RUNX3	na
62	PD-1, PD-L1 cancer immunotherapy pathway	1.47	CBLB, CD28, HLA-DQA1	na
63	Granulocyte Adhesion and Diapedesis	1.46	CCL5, CCR7, CD99, IL18RAP	na
64	Protein Citrullination	1.45	PADI4	na
65	Macrophage Classical Activation Signaling Pathway	1.44	BPI, CCL5, CD40LG, HLA-DQA1	2.000
67	IL-17 Signaling	1.41	CD40LG, FOS, LCN2, MAP2K6	−1.000
66	Trehalose Degradation II (Trehalase)	1.37	HK3	na
68	Urea Cycle	1.37	ARG1	na
69	Arginine Degradation VI (Arginase 2 Pathway)	1.37	ARG1	na
70	Macrophage Alternative Activation Signaling Pathway	1.37	ARG1, FOS, HLA-DQA1, IGHG1	1.000
71	Airway Pathology in Chronic Obstructive Pulmonary Disease	1.34	CD40LG, LCN2, PRF1	na
72	MSP-RON Signaling In Macrophages Pathway	1.34	ARG1, FOS, HLA-DQA1	na
73	Hepatic Fibrosis / Hepatic Stellate Cell Activation	1.33	CCL5, CCR7, CD40LG, IL18RAP	na
74	Agranulocyte Adhesion and Diapedesis	1.32	CCL5, CCR7, CD99, ITGB7	na
75	α-tocopherol Degradation	1.31	CYP4F3	na

-log(*p*-value) ≥ 1.3, *p*-value order; na: z-score non available.

showed, compared with controls, a modulation of several immune-related processes, including an upregulation of the "*Pathogen Induced Cytokine Storm Signaling Pathway*" and the "*Tryptophan Degradation to 2-amino-3-carboxymuconate Semialdehyde Pathway*" (mostly known as "*Kynurenine pathway*") suggesting a pro-inflammatory state also in women with a history of depression. In particular, this group of women showed a higher expression of genes encoding for two central molecules of these two pathways: the initiating sensor of the inflammasomes AIM2 – Absent In Melanoma 2 – and the enzyme kynureninase (gene *KYNU*), respectively.

Regarding the AIM2 inflammasome-related pathway, an increased activity of the inflammasomes machineries has been previously correlated with severity of depression in patients, albeit mostly in the context of the NLRP3-dependent inflammasome, the most studied and best characterized inflammasome component [53, 54], though a previous study has reported AIM2 to be the most abundant in neurons [55]. In line with our results, a previous study analysing gene expression in peripheral blood from individuals with depressive symptoms as compared with controls found higher mRNA levels of the inflammasome adaptor protein ASC, even if AIM2 expression levels were not different between groups [56]. Of note, the increased mRNA levels of the inflammasome adaptor protein ASC were driven by the expression levels found in those individuals with moderate depressive symptoms, as opposed to those with severe symptoms, and our sample of history-only women also showed slightly higher levels of depressive symptoms, compared with controls (Table 1). Therefore, although the aforementioned study [56] has not been conducted in pregnant women, some common ground in the level of symptomatology in the groups of the two studies can be observed, and the results are comparable, suggesting that inflammasomes could be implicated in the vulnerability to depressive disorders.

Regarding the kynurenine pathway (Figure S4), the observed upregulation of the gene expression of the central enzyme kynureninase (*KYNU*) indicates an activation of this mechanism and suggests the possible increase in the levels of the kynurenine metabolites (e.g. quinolinic acid, kynurenine, 3OH-anthranilic acid, and 3OHkynurenine), which are pro-inflammatory and neuro-toxic molecules, with depressogenic effects [57]. Concurrently, the increased metabolism of the precursor tryptophan through this pathway, decreases the availability of this amino-acid to be metabolized for the synthesis of serotonin, thereby lowering the levels of this neurotransmitter. Both increased activity of kynurenine-linked

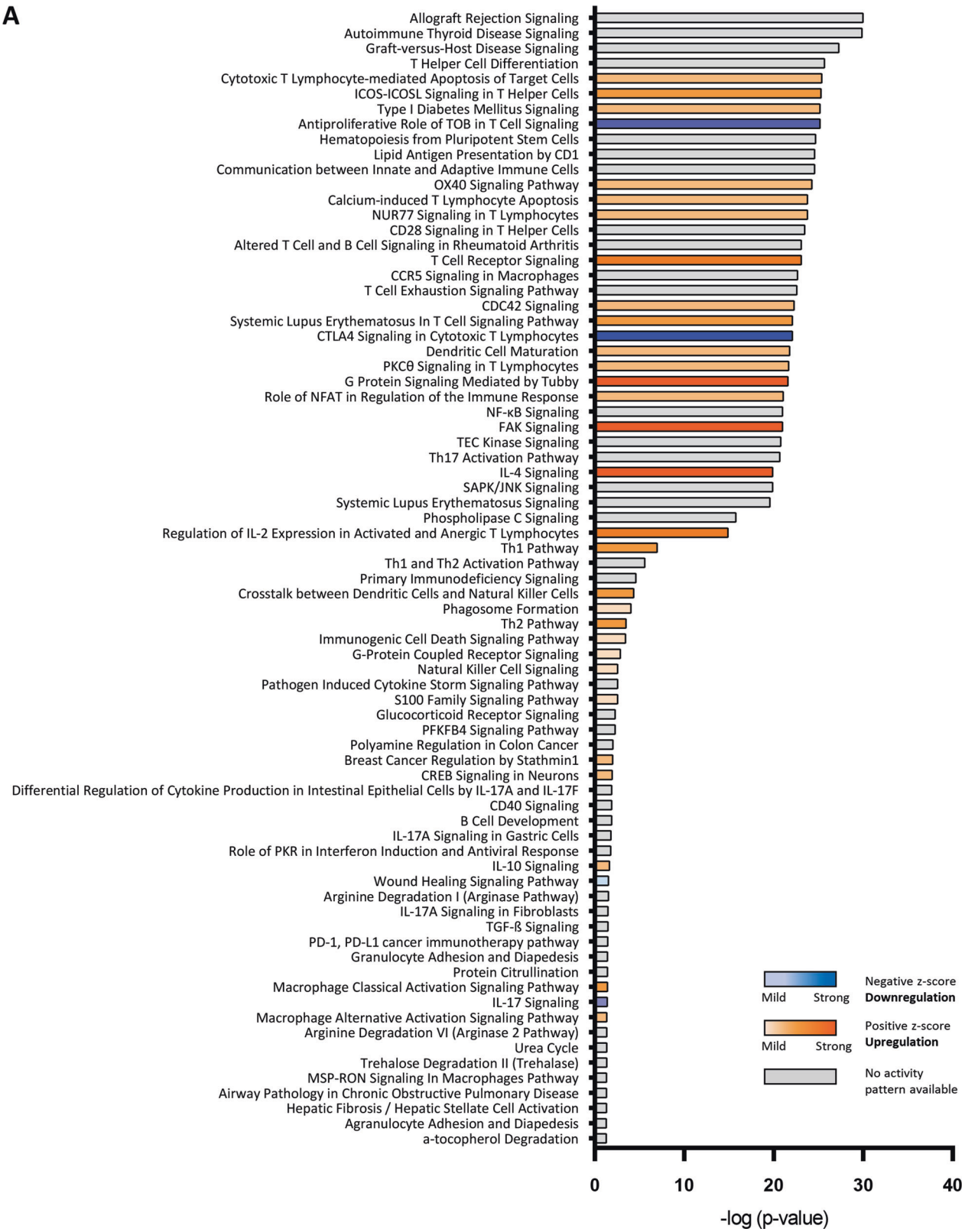

Fig. 4 Biological pathways modulated in depressed women as compared with history-only women. A List of pathways shown to be significantly modulated from depressed pregnant women as compared with pregnant women with a history of depression.

enzymes, as well as decreased levels of serotonin, are known correlates of a higher risk of developing depression [58] and have also been associated with depressive symptoms in the peripartum [59–61].

Other resulting significant pathways, including the "*Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells*" and the "*T Cell Receptor Signaling*", appeared downregulated in women with a history of depression compared with controls, driven by the lower

expression levels of up to 15 genes encoding for TCR subunits, and also of 3 subunits of the TCR co-receptor CD3. The activation of the CD3-TCR complex is a necessary event for initiating the cascade of a variety of downstream processes and is the primary determinant of T cell development and activation [62]. Overall, these findings suggest a general downmodulation of T cell-mediated immunity, involving most likely the cytotoxic T lymphocytes (CTLs). These could represent compensatory mechanisms occurring in these women, underlying the capacity to maintain a subclinical state despite their clinical history. Alternatively, according to a previous study, antidepressants-free patients exhibit a less diverse TCR repertoire expressed on T cells compared with matched non-depressed individuals [63]. In line with this, an impaired activity of the T cells, characterized by a lower availability and reduced capacity to traffic to the brain, has been proposed in depression, with "activated T cells potentially playing an important neuroprotective role in the context of both stress and inflammation", by, for example, "reversing stress-induced decreases in hippocampal neurogenesis, as well as depressive-like behaviour in rodents" [64].

Overall, women who present a history of depression during their life, although currently well during pregnancy, still carry some alterations in their biological background compared with lifelong healthy control women, in particular at the level of their immune system. For this reason, these women need to be monitored with more attention during sensitive periods of their life that might elicit their latent background of vulnerability. It is of note that these women also show poorer infant neurobehavioral competencies [35] and reduced quality of mother-infant interaction [43], suggesting that these biological changes operating during pregnancy even in the absence of active depressive symptoms can influence offspring's development and postnatal emotional processes.

Lastly, when investigating the biological mechanisms differentially modulated in women who were depressed in pregnancy, compared with those with history-only, we found a list of 75 altered pathways. Interestingly, the top three resulting pathways were the "*Allograft Rejection Signaling*" (Figure S5), the "*Autoimmune Thyroid Disease Signaling*" and the "*Graft-versus-Host Disease Signaling*". These molecular pathways are all strictly related to immunity against the self (autoimmunity) and non-self (alloimmunity), which raises potential concerns when considering the context of gestation. Indeed, comparable immune mechanisms are involved in successful transplantation and pregnancy [65], and similar immunoregulatory molecules have been found to contribute to graft rejection and to spontaneous abortion [66, 67]. In our sample, depressed women showed higher transcription of genes such as *CD28*, *PRF1*, *GZMA, GZMH* and a series of genes encoding for different TCR and CD3 subunits, suggesting an increased function of T cells with cytotoxic activity such as CTLs [68, 69], which are well-known actors in the process of acute allograft rejection after transplant, via granzyme/perforin mediated mechanisms [70–72]. Of note, the role of immune cells with cytotoxic activity has been similarly identified in many studies also in the context of miscarriage, though focusing in particular on Natural Killer (NK) cells, which, as reported in a comprehensive meta-analysis, are significantly higher in women with recurrent miscarriage, as compared with controls [73].

Many studies have suggested depression and high levels of stress in pregnancy as risk factors for spontaneous abortion, in particular when this medical condition occurs recurrently. Indeed, depressive symptoms and anxiety levels after a first miscarriage have been found to be significantly increased in those women who experienced a subsequent spontaneous abortion [74, 75], and the complex interaction between stress, hormones, and immune mediators, has been proposed to underlie this correlation [76]. The analysed findings could indirectly suggest that the biology of depressed women from our sample may pose them at a higher risk of obstetric complications, and indeed we have previously shown that these depressed women [36], but not those with history-only [35], have a shorter gestational age at birth.

In addition to these biological mechanisms, other pathways resulted to be modulated from the analysis between depressed and history-only women and might provide insight on mechanisms characterizing ongoing depression *versus* vulnerability due to past episodes. For example, a consistent upregulation of many immune-related pathways was observed, while just a few were downregulated, as compared with women from the history-only group. In particular, activated pathways such as the "*Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells*" (Figure S6), the "*OX40 Signalling Pathway*", the "*FAK Signalling*", the signalling of "*Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes*", the "*Th1 pathway*", the "*Immunogenic Cell Death Signalling*" pathway, and many more, point toward a systemic pro-inflammatory type of response of the immune system in clinically depressed women. To corroborate these gene expression findings, higher levels of circulating pro-inflammatory cytokines have been found in depressed women compared with those with a history of depression, from this same cohort, as previously published [35]. These results are confirmed by other studies reporting higher levels of Th1 secreted cytokines, including IL-2, in pregnant women with depression, compared with healthy counterparts [31, 34, 59]. An imbalance in the Th1/Th2 ratio, in favour of an increase toward Th1 activity, contrary to the Th2-directed proportion of physiological pregnancy [28], has already been proposed as an underlying factor involved in perinatal depression [49]; however, the alterations between women who develop depression in pregnancy and those who are at high risk due to a previous history of depression have not been properly investigated.

Our findings suggest that an increased pro-inflammatory status and in particular a favoured Th1 polarization might occur in clinically manifested perinatal depression, a condition that is also associated with an increased risk of pregnancy loss [28, 77], in line with the aforementioned results. Moreover, our study contributes to unravelling the complexity of the determinants associated with perinatal depression, and, ultimately, might support the development of specific biomarkers for early screening and identification of vulnerable women. Furthermore, the identified pathways and genes might also lay the groundwork for the future development of personalized medicine strategies, targeting pregnant women immune system. Incorporating these findings, alongside other biological and clinical risk factors, through sophisticated machine learning algorithms, may enhance treatment customization, aiming to optimize symptom alleviation while minimizing adverse effects among distinct subsets of pregnant women. Notably, the distinct biological profile observed in women characterized for a high risk of developing depression, yet not displaying clinically significant symptoms in pregnancy (those defined as history-only), holds significance in elucidating the factors contributing to their resilience compared to other women. This knowledge can inform the development of targeted preventive interventions, fostering a deeper understanding of resilience mechanisms in perinatal mental health.

Limitations

This study has few limitations that need to be discussed.

First, as we mention in the methods section, we did not analyse the clinical and sociodemographic variables as potential confounders of the transcriptomic and pathway analyses, due to several factors. Handling the extensive data from transcriptomic analyses makes interpreting the role of demographic and clinical variables challenging without specific prior hypotheses, which would nonetheless lead to the need of multiple comparisons, increasing the likelihood of spurious findings, as well as to loss of statistical power. Moreover, the outcomes of the pathway analyses conducted on the IPA software depend on aggregated group-level data, hindering a direct correlation with sociodemographic and clinical variables. However, we provided a summary of these variables within the description of the clinical sample characteristics.

Then, among depressed and history-only women, some had used antidepressants earlier in pregnancy, even if they were not

taking antidepressants at the baseline (the 25th week of gestation), which may have affected their gene expression profile. However, due to the relatively small sample size of our cohort, excluding those women with prior antidepressants exposure would greatly affect the statistical power of the transcriptomic as well as pathway analyses, precluding us from obtaining robust, significant bioinformatic results. Future studies with larger samples will need to address this issue and also consider the potential role of previous antidepressant exposure.

CONCLUSIONS

Overall, our study has addressed an existing gap in the literature on the molecular mechanisms that underlie the presence of, as well as the vulnerability to, depression during pregnancy. We observed a modulation of a variety of immune processes, with pathways that are specific for each clinical group. In particular, depressed pregnant women showed an upregulation of B cell immunity compared with control women, and auto- and allo-immunity and pro-inflammatory mechanisms compared with history-only, while history-only women showed a combination of increased pro-inflammatory-related pathways as well as decreased T cell activation. These findings suggest that a delicate and complex fine modulation of immune-related processes is fundamentally involved in the risk for, and development of, depression in pregnancy, and its consequence on the offspring.

DATA AVAILABILITY

Cel. File data were deposited into the Gene Expression Omnibus database under accession number GSE290797.

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AUTHOR CONTRIBUTIONS

All authors substantially contributed to the conceptualisation or design of the work, reviewed the manuscript, and approved the final version for publication. MGDB: Conceptualization, Formal analysis, Data curation, Methodology, Statistical analyses, Writing – original draft, review & editing. KMSP: Conceptualization, Investigation, Data curation, Writing – review & editing. NC: Conceptualization, Formal analysis, Data curation, Methodology, Statistical analyses, Writing - review & editing. PG: Conceptualization, Formal analysis, Data curation, Methodology, Writing - review & editing. SS: Conceptualization, Data curation, Statistical analyses, Writing – review & editing. AB: Conceptualization, Investigation, Data curation, Writing - review & editing. RHB: Conceptualization, Investigation, Writing – review & editing. SC: Conceptualization, Investigation, Data curation, Writing – review & editing. ADP: Conceptualization, Investigation, Data curation, Writing – review & editing. KH: Conceptualization, Investigation, Data curation, Writing – review & editing. SO: Conceptualization, Investigation, Data curation, Writing – review & editing. SP: Conceptualization, Investigation, Data curation, Writing – review & editing. VS: Conceptualization, Investigation, Writing – review & editing. CMP: Conceptualization,

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COMPETING INTERESTS

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