Journal of proteome research

pubs.acs.org/jpr

Article

Plasma Olink Proteomics Identifies CCL20 as a Novel Predictive and Diagnostic Inflammatory Marker for Preeclampsia

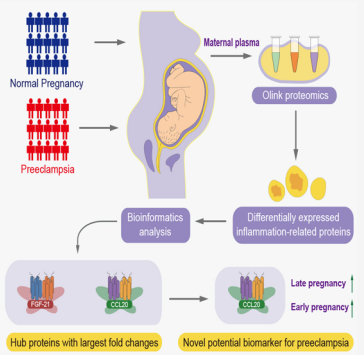
Xiufang Wang,[⊥] Ka Cheuk Yip,[⊥] Andong He,[⊥] Jinqing Tang, Shisan Liu, Ruiling Yan, Qiao Zhang,* and Ruiman Li*

Cite This: *J. Proteome Res.* 2022, 21, 2998−3006

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Inflammation is generally thought to be involved in the occurrence and development of preeclampsia (PE), but its specific effect on PE remains unclear. In the present study, the expression levels of 92 inflammation-related proteins were measured in the late pregnancy maternal plasma from patients with PE (n = 15) and normal pregnant controls (n = 15) using the Olink inflammation panel based on the highly sensitive and specific proximity extension assay technology. A total of 28 inflammation-related markers differed between the PE and control groups. Among them, fibroblast growth factor 21 (FGF-21) and cysteine−cysteine motif chemokine ligand 20 (CCL20) had the largest fold changes. We further validated the levels of CCL20 in the late (43 with PE and 44 controls) and early (37 with PE and 37 controls) pregnancy maternal plasma using enzyme-linked immunosorbent assay (ELISA). To the best of our knowledge, for the first time, CCL20 was found to be upregulated in the late and early pregnancy plasma of patients with PE and had an area under the curve (AUC) of 0.753 and 0.668, respectively. In conclusion, patients with PE had increased levels of most inflammatory markers, and CCL20 might be a novel potential predictive and diagnostic biomarker for PE.



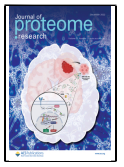
KEYWORDS: *preeclampsia, inflammation, Olink, proteomics, CCL20*

INTRODUCTION

Preeclampsia (PE) is a serious complication that affects 2−4% of the pregnant women worldwide. It is reportedly linked to ∼46 000 maternal deaths and ∼500 000 fetal and newborn deaths each year.[1] However, no clear explanation of the mechanism behind PE has been provided to date. At present, uteroplacental malperfusion caused by trophoblast invasion failure and the transformation of spiral arteries is considered an important cause for PE. This disorder is also characterized by chronic inflammation in early pregnancy, with high levels of serum cytokine expression and leukocyte activation.[2,3] Tumor necrosis factor α (TNF-α) stimulates the expression of intercellular adhesion molecule-1 in the endothelium and trophoblast cells, inducing vascular activation and dysfunction in the TNF.[4] Interleukin (IL) mediates the cluster of differentiation 81 (CD81)-triggered imbalance of maternal regulatory T-cells (Treg)/IL17-producing helper T-cells (Th17) in the trophoblast, and the upregulation of CD81 inhibits maternal Tregs, promotes Th17 cell differentiation, and disrupts the maternal immune tolerance environment involved in PE development.[5] Therefore, immune dysfunction and hyperactivation of inflammation may play essential roles in the pathogenesis of PE. Additionally, there is evidence that various circulating inflammatory molecules increase in patients with PE,[6] and some inflammatory-related proteins, such as vascular endothelial growth factor A (VEGFA)[7] and TNF,[8] may be associated with PE, but there is no specific biomarker of this complex disease. Therefore, it is necessary to identify novel biomarkers of PE.

Protein biomarkers have been central to disease prediction, diagnosis, and prevention. Advances in high-throughput proteomics allow for the simultaneous quantification of a sea of proteins; however, the huge amount of data obtained increases analysis difficulties, and false positive results also bring challenges for subsequent verification.[9] Recently, Olink technology has become popular, as it provides multiple assay panels targeted toward various disease processes. It requires small sample volumes, which is especially important when clinical samples are sparse. Additionally, it can capture a wide range of proteins across the whole dynamic range (>10 logs). A previous study has indicated that Olink proteomics displays excellent reproducibility and stability in the detection of proteins in plasma samples.[10] In the present study, Olink proteomics is used to identify differences in inflammation-related proteins between patients with PE and normal pregnant women to investigate the relationship between inflammation and PE and identify potential markers for PE.

Received: September 10, 2022
Published: October 27, 2022



MATERIALS AND METHODS

Participants

The protocol for this study was approved by the ethics committee of the First Affiliated Hospital of Jinan University, Guangzhou, China (approval number: KY-2022-043). We collected late pregnancy maternal plasma samples before delivery from 87 pregnant women, of which 43 had PE and 44 were normal pregnant controls, and early pregnancy maternal plasma samples from 74 pregnant women, including 37 with subsequent PE and 37 normal pregnant controls, in 11^{+0}–13^{+6} weeks of gestation from the First Affiliated Hospital of Jinan University. For the late pregnancy maternal plasma, PE samples were collected after the onset of the disease (generally near delivery), while the controls were collected during obstetric examination or hospitalization (usually further away from delivery) to match the PE group. For the early pregnancy maternal plasma, we prospectively collected these samples and followed up until delivery. The diagnostic criteria of PE were derived from the International Society for the Study of Hypertension in Pregnancy and are as follows: the first onset of hypertension after 20 weeks of gestation, with a systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure of ≥90 mmHg with proteinuria at 0.3 g/day, or no proteinuria with one of the following symptoms: thrombocytopenia, liver function impairment, renal function impairment, pulmonary edema or neurological abnormalities, intrauterine growth restriction, or uteroplacental insufficiency.[11] The inclusion criteria for normal pregnant controls were that there were no pregnancy complications, such as gestational or chronic hypertension, pregestational or gestational diabetes mellitus, or intrahepatic cholestasis of pregnancy.

Plasma Sample Collection

Approximately 2 mL of peripheral venous blood was collected from each patient into EDTA tubes; then, the plasma was extracted by centrifugation at 3000 rpm for 15 min and frozen in a −80 °C refrigerator for future use.

Inflammation-Related Biomarkers Analysis

Late pregnancy maternal plasma samples from PE (n = 15) and normal (n = 15) pregnant women were analyzed using the Olink Inflammation panels based on the highly sensitive and specific proximity extension assay technology, which enables 92 inflammation-related biomarkers to be analyzed simultaneously.[12] Briefly, each target protein was recognized by double antibodies and coupled with its specific complementary DNA barcode, which was subsequently quantified using a high-throughput microfluidic real-time PCR instrument, Biomark HD (Fluidigm, South San Francisco, CA). The final assay readout was presented in normalized protein expression values, which were $\log_2$-transformed.

Bioinformatics Analysis

For the Olink data, the differentially expressed proteins were obtained using the limma package,[13] with a P-value cutoff of 0.05. The R package ggplot2[14] was used to visualize heat maps and volcano plots. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were also performed using ggplot2. In the enrichment analyses, all significantly differentially expressed proteins were mapped to each term or pathway of the GO or KEGG database, and then, the GO term or KEGG pathway that was significantly enriched in differentially expressed proteins compared to the specific background was identified using a hypergeometric test.

Table 1. Clinical Features of the Olink Cohort and the ELISA Cohort[a]

	PE group	control group	P
Olink Cohort			
sample size	N = 15	N = 15	
PE types, n (%)			
early-onset PE	7 (46.67)		
late-onset PE	8 (53.33)		
maternal age (years)	31.53 ± 5.99	30.80 ± 2.98	0.680
gestational age at delivery (weeks)	35.57 (31.43, 37.71)	39.29 (39.14, 39.57)	<0.001***
BMI (kg/m^2)	27.96 ± 3.50	25.39 ± 1.91	0.020*
primipara, n (%)	7 (46.67)	6 (40.00)	1.000
maximum SBP (mmHg)	170.00 (158.00, 180.00)	110.00 (101.00, 114.00)	<0.001***
maximum DBP (mmHg)	104.93 ± 14.54	73.07 ± 6.20	<0.001***
neonatal birth weight (kg)	2.10 ± 0.80	3.24 ± 0.27	<0.001***
sample collection time (weeks)	34.74 ± 3.34	34.30 ± 1.76	0.657
Late Pregnancy Cohort			
sample size	N = 43	N = 44	
PE types, n (%)			
early-onset PE	18 (41.86)		
late-onset PE	25 (58.14)		
maternal age (years)	31.02 ± 6.34	29.84 ± 3.43	0.280
gestational age at delivery (weeks)	35.57 (32.57, 38.00)	39.64 (39.14, 40.54)	<0.001***
BMI (kg/m^2)	27.22 ± 4.96	26.11 ± 2.42	0.190
primipara, n (%)	19 (44.20)	21 (47.70)	0.830
maximum SBP (mmHg)	166.16 ± 17.31	111.77 ± 9.00	<0.001***
maximum DBP (mmHg)	106.21 ± 11.79	74.41 ± 7.77	<0.001***
neonatal birth weight (kg)	2.23 ± 0.77	3.30 ± 0.40	<0.001***
sample collection time (weeks)	35.14 (32.14, 37.57)	34.50 (32.71, 36.57)	0.966
Early Pregnancy Cohort			
sample size	N = 37	N = 37	
PE types, n (%)			
early-onset PE	11 (29.73)		
late-onset PE	26 (70.27)		
maternal age (years)	31.32 ± 4.80	33.14 ± 4.44	0.100
gestational age at delivery (weeks)	37.43 (33.93, 38.71)	39.43 (38.57, 40.42)	<0.001***
BMI (kg/m^2)	27.10 (24.61, 29.05)	25.90 (24.33, 26.84)	0.100
primipara, n (%)	15 (40.54)	13 (35.14)	0.630
maximum SBP (mmHg)	159.16 ± 11.16	116.57 ± 9.29	<0.001***
maximum DBP (mmHg)	102.22 ± 11.77	73.78 ± 7.73	<0.001***
neonatal birth weight (kg)	2.47 ± 0.81	3.20 ± 0.30	<0.001***
sample collection time (weeks)	12.50 ± 0.67	12.44 ± 0.65	0.725

[a]***P < 0.001. The late pregnancy cohort refers to the pregnant women who provided late pregnancy plasma samples, and the early pregnancy cohort refers to the pregnant women who provided early pregnancy plasma samples for ELISA validation. Abbreviations: PE, preeclampsia; BMI, body mass index; SBP, systolic blood pressure; and DBP, diastolic blood pressure.

The results of enrichment analysis based on the background of all proteins and 92 proteins in the Olink inflammation panel

https://doi.org/10.1021/acs.jproteome.2c00544
J. Proteome Res. 2022, 21, 2998−3006

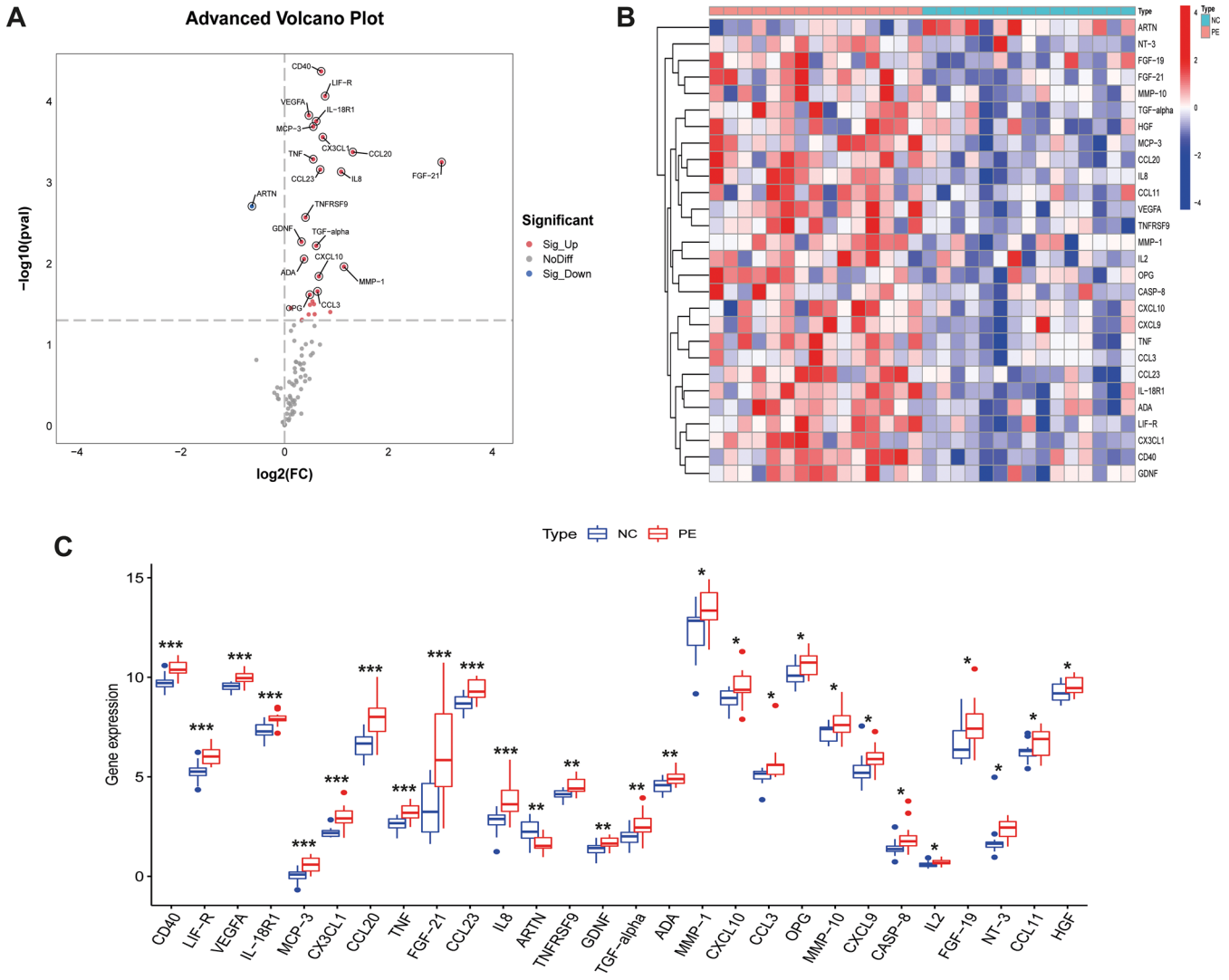


Figure 1. All differentially expressed inflammation-related biomarkers between the preeclampsia (PE) and normal groups. (A) Volcanic visualization of 92 inflammation-related biomarkers. (B) Heatmap of 28 differentially expressed proteins. (C) Box plot of the 28 inflammation-related biomarker expression. The solid dots outside the box represent observations that are 1.5 times the interquartile range on the upper and lower sides (*P < 0.05, **P < 0.01, ***P < 0.001).

were compared. Additionally, the correlation analysis of two proteins' expression was performed using Spearman's correlation. The protein−protein interaction (PPI) network of the differentially expressed proteins was constructed and visualized with Cytoscape (version 3.9.1). Additionally, receiver operating characteristic (ROC) curves were created using the ROCR package.[15]

ELISA Validation

Late pregnancy maternal plasma samples from 87 pregnant women, of which 43 had PE and 44 were normal pregnant controls, and early pregnancy maternal plasma samples from 74 pregnant women, including 37 women with subsequent PE and 37 normal pregnant controls, were used to perform ELISA analysis using the cysteine−cysteine motif chemokine ligand 20 (CCL20) ELISA kit (MB-0074A, Jiangsu Meibiao Biological Technology, China). Assays and analyses were conducted according to the manufacturer's protocol.

Statistical Analysis

Data are presented as the mean ± standard deviation or median (first and third quartiles) as appropriate. Statistical analyses were implemented using IBM SPSS Statistics 25 and R software (version 4.1.3). P < 0.05 was considered statistically significant.

RESULTS

Characteristics of the Participants

Table 1 shows the clinical characteristics of the participants in the Olink, late pregnancy, and early pregnancy cohorts. There were no significant differences in the maternal age, primipara proportion, and sample collection time between the two groups in the three cohorts (P > 0.05). The PE group had shorter gestations, higher maximum systolic blood pressure, and maximum diastolic blood pressure, and babies born from the PE group had lower fetal birth weights in the two cohorts (P < 0.05). Additionally, the PE group had a higher body mass index (BMI) than that of the control group in the Olink cohort (P = 0.020), but there was no significant difference in the BMI between the two groups in the late and early pregnancy cohorts (P > 0.05).

https://doi.org/10.1021/acs.jproteome.2c00544
J. Proteome Res. 2022, 21, 2998−3006

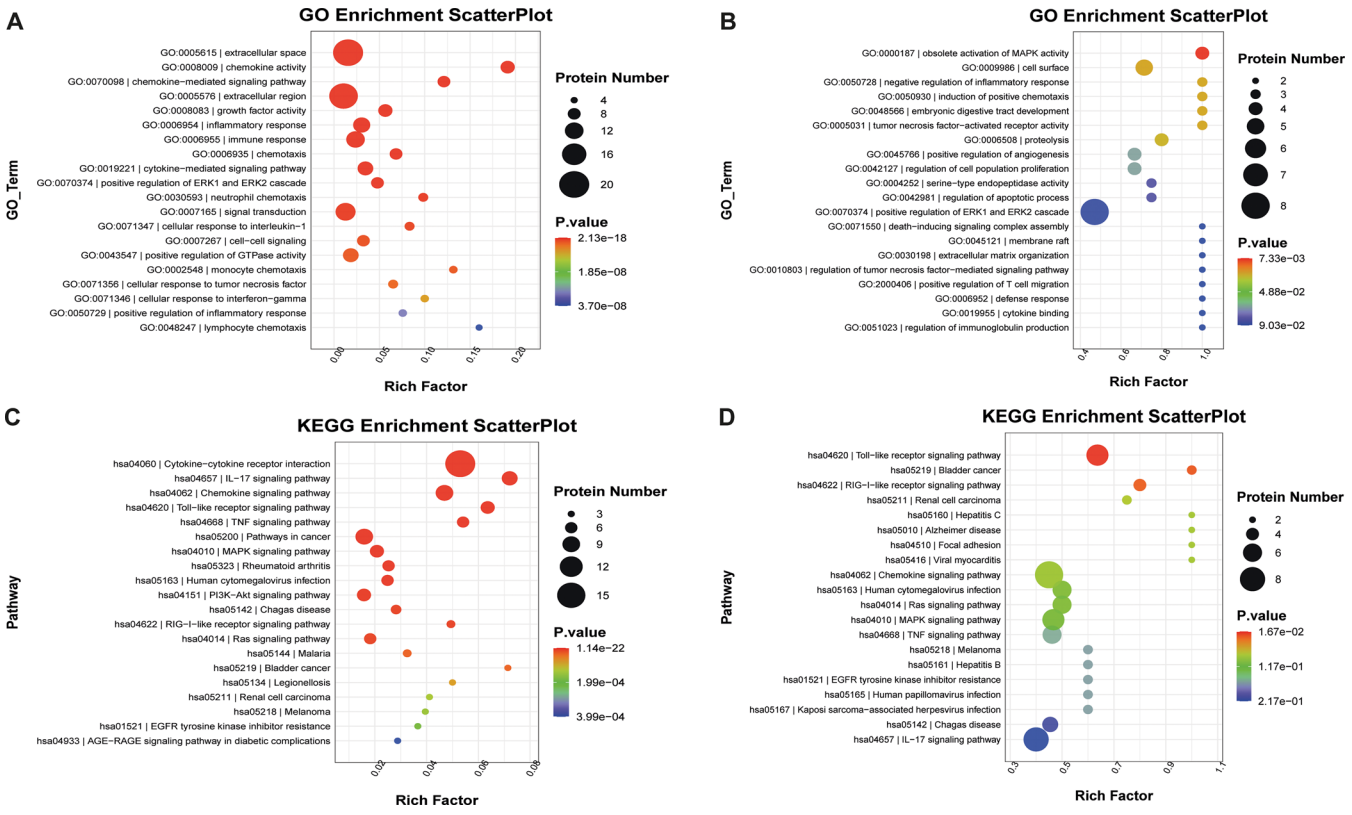


Figure 2. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of the differentially expressed inflammation-related proteins. (A) GO enrichment analysis based on the background of all annotated proteins. (B) GO enrichment analysis based on the background of 92 inflammation-related proteins. (C) KEGG enrichment analysis based on the background of all annotated proteins. (D) KEGG enrichment analysis based on the background of 92 inflammation-related proteins.

Olink Inflammation-Related Biomarker Analysis

Using Olink analysis, we evaluated and compared the expression levels of 92 inflammation-related proteins between the PE and control groups; information about these proteins is shown in Table S1. A total of 28 differentially expressed inflammation-related proteins were identified between PE and control groups, of which 27 were upregulated and only one protein was downregulated in the PE group (Figure 1A). A heatmap of these differentially expressed inflammation-related proteins is shown in Figure 1B, and the comparison of the expressions of these proteins between the PE and normal groups is shown in Figure 1C. Most of the inflammatory proteins among these proteins increased in the PE group, although some differences were not significant, which strongly echoed the results of previous studies. Among them, fibroblast growth factor 21 (FGF-21) and CCL20 had the largest fold changes, 8.13 and 2.48, respectively.

Analysis of Differentially Expressed Inflammation-Related Biomarkers

To further investigate the potential functions of the differentially expressed proteins, we conducted GO and KEGG enrichment analysis based on various backgrounds. Under the background of all annotated proteins, the results indicated that these proteins were enriched in several GO terms, such as signal transduction, immune response, and inflammatory response (Figure 2A), with several pathways, such as the cytokine−cytokine receptor interaction, IL-17 signaling pathway, and TNF signaling pathway (Figure 2C). When the 92 inflammation-related proteins in the Olink panel served as the background, the significant GO terms and KEGG pathways became fewer, and the results showed that these proteins were enriched in several GO terms, such as obsolete activation of MAPK activity, cell surface, and negative regulation of inflammatory response (Figure 2B), and several pathways, such as the toll-like receptor signaling pathway, bladder cancer, and RIG-I-like receptor signaling pathway (Figure 2D). Additionally, we performed a correlation analysis and characterized a PPI network to explore the interactions among the above-mentioned proteins. As shown in Figure 3, we found that C−X−C motif chemokine ligand 9 (CXCL9) and CXCL10 had the most significant positive correlation, while CCL20 and artemin (ARTN) had the most negative correlation. Furthermore, TNF and VEGFA had the highest degree scores in the PPI network, indicating they may have important roles in PE (Figure 4). We noticed that previous studies have found that they may be potential biomarkers for PE.[16,17]

Correlation between Differentially Expressed Inflammation-Related Proteins and Clinical Features

We evaluated the correlations between the 28 differentially expressed inflammation-related proteins and the clinical features of patients with PE. The results showed that higher levels of matrix metalloproteinase 10, VEGFA, CCL20, and FGF-21 were associated with the maximum systolic and diastolic blood pressure of patients with PE, while ARTN was inversely proportional to diastolic pressure. The CX3CL1 level seemed to be related to maternal proteinuria. Moreover, neurotrophin-3 (NT-3) may be proportional to the maternal age (Figure 5).

https://doi.org/10.1021/acs.jproteome.2c00544
J. Proteome Res. 2022, 21, 2998−3006

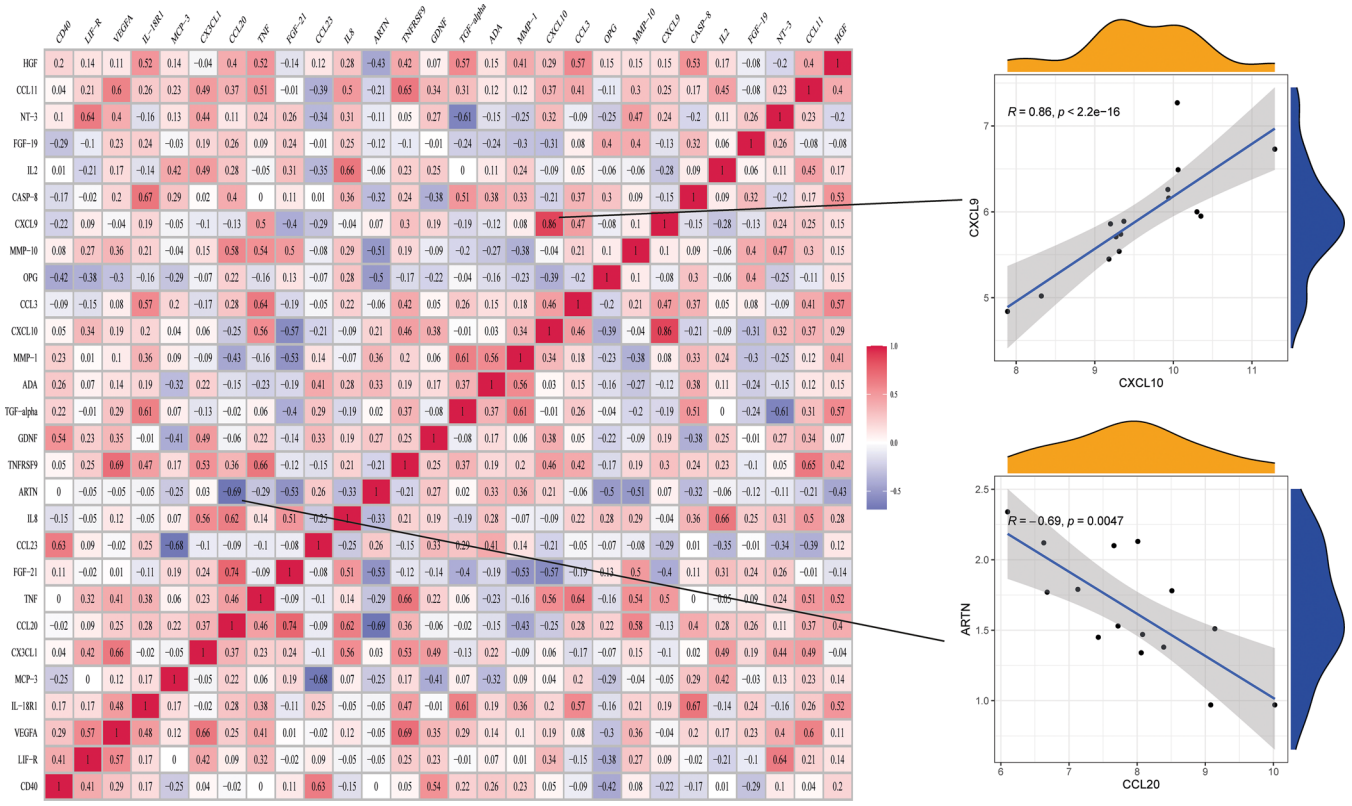


Figure 3. Correlations between the differentially expressed inflammation-related biomarkers in preeclampsia (PE) patients. The two scatterplots are visualizations of the two pairs of inflammation-related biomarkers with the highest correlation. C−X−C motif chemokine ligand 9 (CXCL9) and CXCL10 had the most significant positive correlation, and cysteine−cysteine motif chemokine ligand 20 (CCL20) and artemin (ARTN) had the most negative correlation.

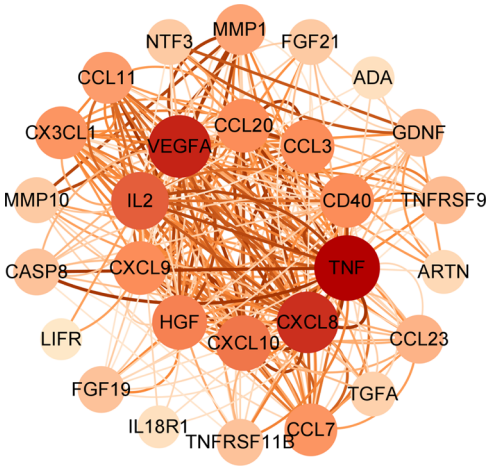


Figure 4. Protein−protein interaction (PPI) network analysis of the inflammation-related differentially expressed proteins.

ELISA Validation of CCL20 in the Late and Early Pregnancy Maternal Plasma

Based on the largest fold changes between the PE and controls groups, FGF-21 and CCL20 were identified as hub proteins. A previous study had indicated that serum FGF-21 was significantly increased in patients with PE and it may be a potential circulating biomarker for this disease.[18] However, to the best of our knowledge, CCL20 was only reported to be increased in preeclamptic decidua,[19] but its circulating levels in PE have not been reported, resulting in its unknown predictive or diagnostic value for PE. Therefore, CCL20 was selected as a novel potential predictive or diagnostic biomarker for further validation. According to the ELISA results, the CCL20 expression was significantly higher in the late pregnancy plasma of patients with PE compared with that of the controls ($P < 0.001$), which was consistent with the trend observed in the Olink data (Figure 6A), and was also higher in the early pregnancy plasma of patients with PE ($P = 0.015$) (Figure 6B). The AUC of CCL20 was 0.753 (95%CI: 0.649−0.839, $P < 0.001$), according to the ROC curve analysis based on the CCL20 concentration in the late pregnancy maternal plasma. The optimal cutoff value based on the Youden index was >435.96, with a sensitivity and specificity of 51.16 and 95.45%, respectively (Figure 6C). Additionally, the AUC of CCL20 in the early pregnancy maternal plasma was 0.668 (95% CI: 0.548−0.773, $P = 0.008$), and the optimal cutoff value based on the Youden index was >327.30, with a sensitivity and specificity of 56.76 and 75.68%, respectively (Figure 6D). Furthermore, the CCL20 expression was significantly higher in the late pregnancy maternal plasma compared with that of the early pregnancy maternal plasma in the two groups ($P < 0.001$) (Figure 6E,F).

DISCUSSION

Preeclampsia is a serious pregnancy syndrome that endangers the safety of mothers, fetuses, and newborns. Although its pathological mechanism is very complex, it is widely recognized that inflammation is closely related to its occurrence and development. In the present study, we identify potential inflammation-related protein biomarkers for PE using Olink proteomics.

https://doi.org/10.1021/acs.jproteome.2c00544
J. Proteome Res. 2022, 21, 2998−3006

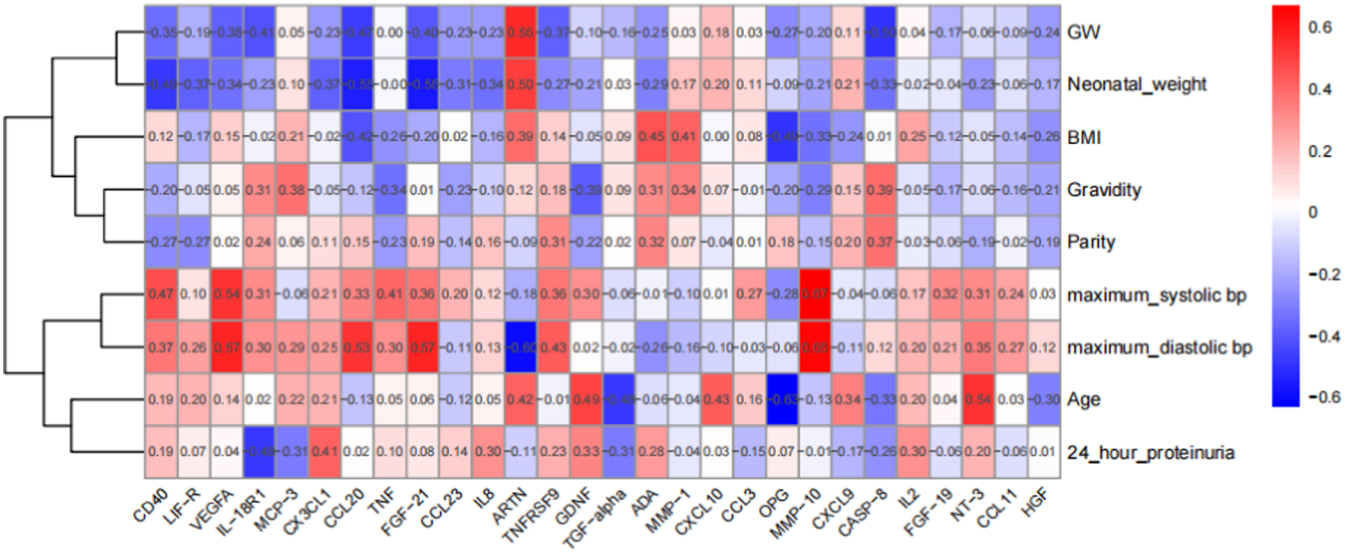


Figure 5. Correlation heatmap between the differentially expressed inflammation-related biomarkers and clinical features. Red, positively related; blue, negatively related; and white, nonrelated.

Although the precise pathogenesis of PE remains unknown, the placenta is considered to be central in its occurrence and development. In the two-stage model, poor placental perfusion leads to the release of antiangiogenic and inflammatory factors into the maternal bloodstream, which can lead to vascular endothelial injury.[11] Additionally, the placentas of patients with PE exhibit heightened inflammatory states characterized by elevated proinflammatory cytokines and chemokines, such as IL-6 and TNF.[20] A previous study indicated that inflammation-induced deficiency in vascular adaptation may be associated with PE.[21] Therefore, an aberrant maternal inflammatory response may be closely associated with PE, and inflammatory-related proteins may be biomarkers for this disease.

In the present study, we compared the expressions of inflammatory-related proteins between plasma samples from patients with PE and normal pregnant women using Olink proteomics and identified 28 differentially expressed proteins that were enriched in several inflammatory-related pathways, including the IL-17 signaling pathway and TNF signaling pathway. Lu et al.[22] indicated that the abnormal increase of serum and placental IL-17 may be associated with the formation and development of PE. Cao et al.[23] also found that serum IL-17 was significantly increased in patients with PE. However, a meta-analysis indicated that there was no evidence of a difference in the circulating levels of IL-17 between patients with PE and controls.[24] Additionally, several studies have demonstrated that TNF may be associated with PE and TNF-α may be a potential biomarker for PE.[8,25,26] More studies are required to explore the roles of the IL-17 and TNF signaling pathways in the development of PE. Additionally, other pathways, including MAPK and toll-like receptor signaling pathways, were significant after changing the background to 92 inflammation-related proteins. These bioinformatic results indicated that inflammation may be involved in the pathogenesis of PE through a multifaceted mechanism.

We further identified two hub proteins and found that CCL20 may be a novel circulating biomarker for PE, so this was selected for validation using additional plasma samples by ELISA. According to the ELISA results, the expressions of CCL20 in the late and early pregnancy maternal plasma were significantly higher in the PE groups than in the controls, which was consistent with the proteomics result. Notably, the CCL20 expression was higher in the late pregnancy maternal plasma compared with that of the early pregnancy maternal plasma, both in the PE group and the controls. A previous study found that soluble E-selectin concentrations were higher in early pregnancy in women who subsequently developed PE and were again higher in late pregnancy in women who went on to develop PE compared with those of the controls. Soluble E-selectin is an early mediator of leukocyte−endothelial adhesion that increases under various inflammatory conditions.[27] It may be due to the higher inflammation levels in the late pregnancy maternal plasma of women who developed PE. However, an increase in CCL20 in the late pregnancy maternal plasma was also found in normal pregnant controls. These changes may reflect the underlying pathophysiological processes of PE, but more exploration is required.

The novel hub protein CCL20 is also known as activation-regulated chemokine, macrophage inflammatory protein-3α, and Exodus-1. It is a chemokine that interacts with CC chemokine receptor 6 (CCR6). The ligand−receptor pair of CCL20 and CCR6 plays an important role in immune interactions and inflammation.[28] Through the binding and activation of CCR6, CCL20 can induce a powerful chemotactic response and mobilization of intracellular calcium ions. Additionally, various cytokines, such as IL-1β, IL-17, and TNF-α, can induce the expression of CCL20. Several studies have thus shown that CCL20 may be closely associated with various tumors and could be a potential diagnostic and prognostic biomarker for these tumors,[29−31] and CCR6-CCL20 plays an essential role in various autoimmune diseases.[32] Moreover, CCL20 was also involved in some inflammatory bowel diseases, such as ulcerative colitis.[33,34] However, to the best of our knowledge, there are no published reports about the relationship between CCL20 and PE. As mentioned above, PE is suspected of being closely associated with immune dysfunction and an excessive inflammatory response. Inflammation may be involved in deficient trophoblast invasion and spiral artery remodeling, also with renal structural alterations.[21] Additionally, the imbalance of Tregs and Th17 has been reportedly involved

https://doi.org/10.1021/acs.jproteome.2c00544
J. Proteome Res. 2022, 21, 2998−3006

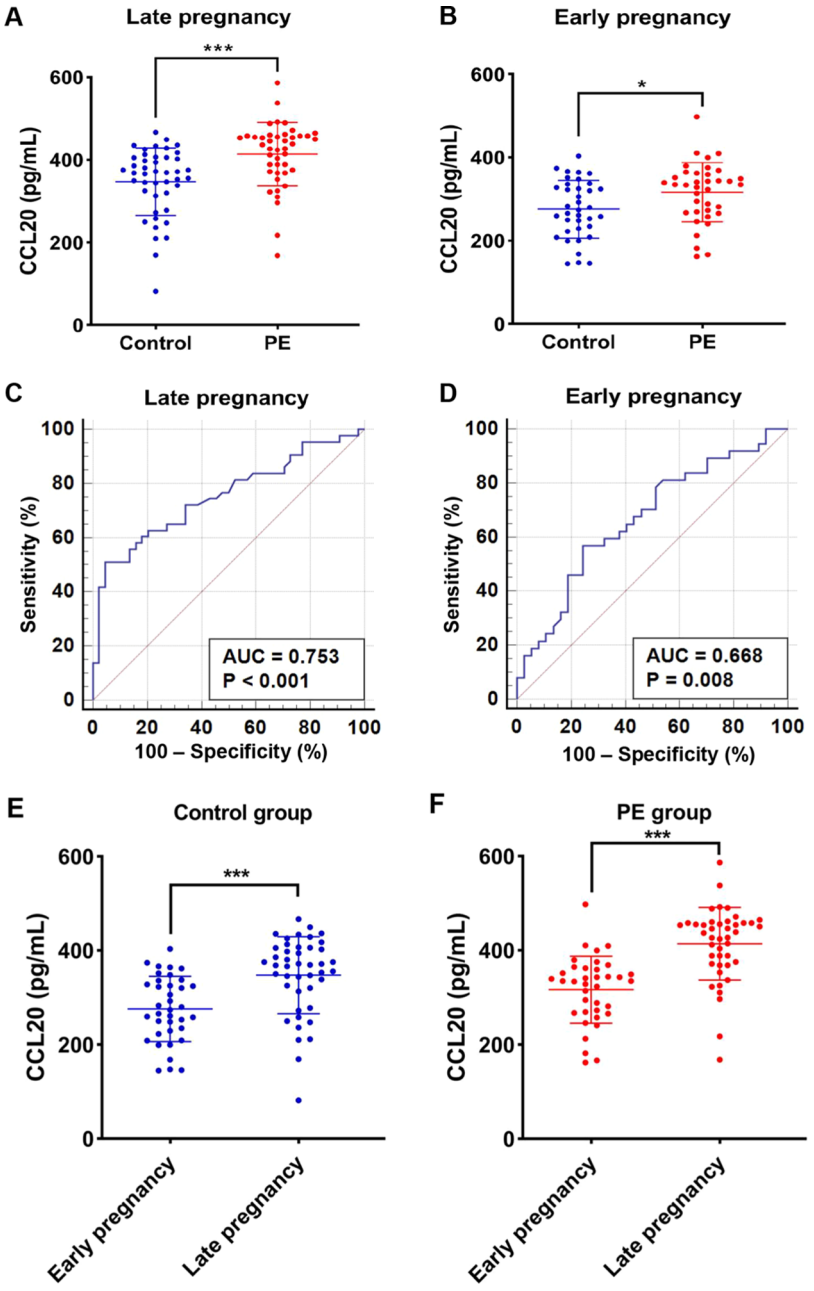


Figure 6. Expression and performance of the novel hub protein cysteine−cysteine motif chemokine ligand 20 (CCL20). (A) Validation of late pregnancy maternal plasma CCL20 levels. (B) Validation of early pregnancy maternal plasma CCL20 levels. (C) Receiver operating characteristic (ROC) curve of CCL20 in the late pregnancy maternal plasma. (D) ROC curve of CCL20 in the early pregnancy maternal plasma. (E) Comparison of the maternal plasma CCL20 expression in the early and late pregnancy of the control group. (F) Comparison of the maternal plasma CCL20 expression in the early and late pregnancy of the PE group. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

in the pathogenesis of PE.[5,35] Regarding the inflammation, CCL20 was found to be involved in the recruitment of proinflammatory Th17 and Treg to the inflammation sites, so a change in the expression of CCL20 may alter the balance between proinflammatory and regulatory responses.[32,36,37] Therefore, we naturally predict that the increase of plasma CCL20 may induce an imbalance of Tregs/Th17 in PE, but more experiments, such as trophoblast and animal experiments, are required to determine the specific pathways and mechanisms behind this. The main significance of the present study lies in the discovery that CCL20 may be a novel predictive and diagnostic marker for PE. However, there are some limitations in the present study, such as the limited sample size. Additionally, there were more late-onset PEs than early-onset PEs in the cohorts, but we could not separate the two PE types for prediction or diagnosis due to the limited number of samples. Therefore, the expression level of CCL20 might be confirmed by incorporating data from a larger sample set.

CONCLUSIONS

In the present study, we measured 92 inflammation-related proteins in the maternal plasma of patients with PE and normal pregnant women using the Olink technology and identified 28 differentially expressed inflammation-related proteins between the two groups. The immune response, inflammatory response, cytokine−cytokine receptor interaction, IL-17 signaling path-

https://doi.org/10.1021/acs.jproteome.2c00544
J. Proteome Res. 2022, 21, 2998−3006

way, and TNF signaling pathway were some of the most enriched GO terms and KEGG pathways in relation to these differentially expressed inflammation-related proteins. FGF-21 and CCL20 had the largest fold changes between the PE and control groups. To the best of our knowledge, this was the first time that CCL20 was found to be upregulated in the early and late pregnancy maternal plasma from patients with PE; therefore, CCL20 may be a potential predictive and diagnostic biomarker for PE, although its expression and role in PE need further exploration.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jproteome.2c00544.

Information of 92 inflammation-related proteins in Olink analysis (PDF)

Raw data of the Olink proteomics analysis (XLSX)

■ AUTHOR INFORMATION

Corresponding Authors

Qiao Zhang − *Institute of Molecular and Medical Virology, School of Medicine, Jinan University, Guangzhou 510632 Guangdong, China*; Email: qiaozhang1987@jnu.edu.cn

Ruiman Li − *Department of Obstetrics and Gynecology, The First Affiliated Hospital of Jinan University, Guangzhou 510630 Guangdong, China*; orcid.org/0000-0003-1239-7968; Email: hqyylrm@126.com

Authors

Xiufang Wang − *Department of Obstetrics and Gynecology, The First Affiliated Hospital of Jinan University, Guangzhou 510630 Guangdong, China*

Ka Cheuk Yip − *Department of Obstetrics and Gynecology, The First Affiliated Hospital of Jinan University, Guangzhou 510630 Guangdong, China*

Andong He − *Department of Obstetrics and Gynecology, The First Affiliated Hospital of Jinan University, Guangzhou 510630 Guangdong, China*

Jinqing Tang − *Department of Obstetrics and Gynecology, Qingyuan People's Hospital, Qingyuan 511518 Guangdong, China*

Shisan Liu − *Department of Obstetrics and Gynecology, The First Affiliated Hospital of Jinan University, Guangzhou 510630 Guangdong, China*

Ruiling Yan − *Department of Fetal Medicine, The First Affiliated Hospital of Jinan University, Guangzhou 510630 Guangdong, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jproteome.2c00544

Author Contributions

⊥X.W., K.C.Y., and A.H. contributed equally to this work. R.L., Q.Z., and R.Y. designed the study. X.W., K.C.Y., J.T., and S.L. collected the samples and the clinical information of the patients. X.W., C.Y., and A.H. performed the experiments and analyzed the data. R.L. and Q.Z. advised on the implementation of the study. X.W., K.C.Y., and A.H. wrote the article. R.Y. assisted in revising the article. All authors have read and approved the final article.

Notes

The authors declare no competing financial interest.

The present study was approved by the ethics committee of the First Affiliated Hospital of Jinan University (approval number: KY-2022-043).

■ ACKNOWLEDGMENTS

This work was supported by grants from the Natural Science Foundation of Guangdong Province, China (No. 2022A1515012139), the Science and Technology Projects in Guangzhou, China (No. 202201020019), the Pilot Specialist Construction Project-Obstetrics and Gynecology (No. 711008), and the Clinical Frontier Technology Program of the First Affiliated Hospital of Jinan University, China (No. JNU1AF-CFTP-2022-a01209). In addition, the authors thank LC-Bio Technology CO., Ltd., Hangzhou, China, for technical assistance with the Olink analysis.

■ ABBREVIATIONS

AUC, area under the curve; BMI, body mass index; CCL20, cysteine−cysteine motif chemokine ligand 20; CCR6, CC chemokine receptor 6; CXCL9/10, C−X−C motif chemokine ligand 9/10; FGF-21, fibroblast growth factor 21; GO, Gene Ontology; IL, interleukin; KEGG, Kyoto Encyclopedia of Genes and Genomes; NT-3, neurotrophin-3; PE, preeclampsia; PPI, protein−protein interaction; ROC, receiver operating characteristic; Th17, IL17-producing helper T-cells; TNF, tumor necrosis factor; Treg, regulatory T-cells; VEGFA, vascular endothelial growth factor A

■ REFERENCES

(1) GBD. 2015 Maternal Mortality Collaborators. Global, regional, and national levels of maternal mortality, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* **2016**, *388*, 1775−1812.

(2) Rusterholz, C.; Hahn, S.; Holzgreve, W. Role of placentally produced inflammatory and regulatory cytokines in pregnancy and the etiology of preeclampsia. *Semin. Immunopathol.* **2007**, *29*, 151−162.

(3) Abe, E.; Matsubara, K.; Ochi, H.; Ito, M.; Oka, K.; Kameda, K. Elevated levels of adhesion molecules derived from leukocytes and endothelial cells in patients with pregnancy-induced hypertension. *Hypertens. Pregnancy* **2003**, *22*, 31−43.

(4) Stubert, J.; Szewczyk, M.; Spitschak, A.; Knoll, S.; Richter, D. U.; Putzer, B. M. Adenoviral mediated expression of anti-inflammatory progranulin by placental explants modulates endothelial cell activation by decrease of ICAM-1 expression. *Placenta* **2020**, *90*, 109−117.

(5) Ding, H.; Dai, Y.; Lei, Y.; Wang, Z.; Liu, D.; Li, R.; Shen, L.; Gu, N.; Zheng, M.; Zhu, X.; Zhao, G.; Hu, Y. Upregulation of CD81 in trophoblasts induces an imbalance of Treg/Th17 cells by promoting IL-6 expression in preeclampsia. *Cell. Mol. Immunol.* **2019**, *16*, 302−312.

(6) Guney, G.; Taskin, M. I.; Tokmak, A. Increase of circulating inflammatory molecules in preeclampsia, an update. *Eur. Cytokine Network* **2020**, *31*, 18−31.

(7) He, B.; Yang, X.; Li, Y.; Huang, D.; Xu, X.; Yang, W.; Dai, Y.; Zhang, H.; Chen, Z.; Cheng, W. TLR9 (Toll-Like Receptor 9) Agonist Suppresses Angiogenesis by Differentially Regulating VEGFA (Vascular Endothelial Growth Factor A) and sFLT1 (Soluble Vascular Endothelial Growth Factor Receptor 1) in Preeclampsia. *Hypertension* **2018**, *71*, 671−680.

(8) Kumar, A.; Begum, N.; Prasad, S.; Agarwal, S.; Sharma, S. IL-10, TNF-alpha & IFN-gamma: potential early biomarkers for preeclampsia. *Cell. Immunol.* **2013**, *283*, 70−74.

(9) Gold, L.; Ayers, D.; Bertino, J.; Bock, C.; Bock, A.; Brody, E. N.; Carter, J.; Dalby, A. B.; Eaton, B. E.; Fitzwater, T.; Flather, D.; Forbes,

A.; Foreman, T.; Fowler, C.; Gawande, B.; Goss, M.; Gunn, M.; Gupta, S.; Halladay, D.; Heil, J.; Heilig, J.; Hicke, B.; Husar, G.; Janjic, N.; Jarvis, T.; Jennings, S.; Katilius, E.; Keeney, T. R.; Kim, N.; Koch, T. H.; Kraemer, S.; Kroiss, L.; Le, N.; Levine, D.; Lindsey, W.; Lollo, B.; Mayfield, W.; Mehan, M.; Mehler, R.; Nelson, S. K.; Nelson, M.; Nieuwlandt, D.; Nikrad, M.; Ochsner, U.; Ostroff, R. M.; Otis, M.; Parker, T.; Pietrasiewicz, S.; Resnicow, D. I.; Rohloff, J.; Sanders, G.; Sattin, S.; Schneider, D.; Singer, B.; Stanton, M.; Sterkel, A.; Stewart, A.; Stratford, S.; Vaught, J. D.; Vrkljan, M.; Walker, J. J.; Watrobka, M.; Waugh, S.; Weiss, A.; Wilcox, S. K.; Wolfson, A.; Wolk, S. K.; Zhang, C.; Zichi, D. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One* **2010**, *5*, No. e15004.

(10) Haslam, D. E.; Li, J.; Dillon, S. T.; Gu, X.; Cao, Y.; Zeleznik, O. A.; Sasamoto, N.; Zhang, X.; Eliassen, A. H.; Liang, L.; Stampfer, M. J.; Mora, S.; Chen, Z. Z.; Terry, K. L.; Gerszten, R. E.; Hu, F. B.; Chan, A. T.; Libermann, T. A.; Bhupathiraju, S. N. Stability and reproducibility of proteomic profiles in epidemiological studies: comparing the Olink and SOMAscan platforms. *Proteomics* **2022**, *22*, No. 2100170.

(11) Mol, B. W. J.; Roberts, C. T.; Thangaratinam, S.; Magee, L. A.; de Groot, C. J. M.; Hofmeyr, G. J. Pre-eclampsia. *Lancet* **2016**, *387*, 999−1011.

(12) 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. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One* **2014**, *9*, No. e95192.

(13) Ritchie, M. E.; Phipson, B.; Wu, D.; Hu, Y.; Law, C. W.; Shi, W.; Smyth, G. K. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* **2015**, *43*, No. e47.

(14) Villanueva, R. A. M.; Chen, Z. J. ggplot2: elegant graphics for data analysis (2nd ed.). *Meas. Interdiscip. Res. Perspect.* **2019**, *17*, 160−167.

(15) Sing, T.; Sander, O.; Beerenwinkel, N.; Lengauer, T. ROCR: visualizing classifier performance in R. *Bioinformatics* **2005**, *21*, 3940−3941.

(16) Tarca, A. L.; Romero, R.; Benshalom-Tirosh, N.; Than, N. G.; Gudicha, D. W.; Done, B.; Pacora, P.; Chaiworapongsa, T.; Panaitescu, B.; Tirosh, D.; Gomez-Lopez, N.; Draghici, S.; Hassan, S. S.; Erez, O. The prediction of early preeclampsia: Results from a longitudinal proteomics study. *PLoS One* **2019**, *14*, No. e0217273.

(17) Zhao, X.; Chen, S.; Zhao, C.; Xia, F. Maternal Immune System and State of Inflammation Dictate the Fate and Severity of Disease in Preeclampsia. *J. Immunol. Res.* **2021**, *2021*, No. 9947884.

(18) Stepan, H.; Kley, K.; Hindricks, J.; Kralisch, S.; Jank, A.; Schaarschmidt, W.; Schrey, S.; Ebert, T.; Lossner, U.; Kratzsch, J.; Bluher, M.; Stumvoll, M.; Richter, J.; Fasshauer, M. Serum levels of the adipokine fibroblast growth factor-21 are increased in preeclampsia. *Cytokine* **2013**, *62*, 322−326.

(19) Huang, S. J.; Chen, C. P.; Schatz, F.; Rahman, M.; Abrahams, V. M.; Lockwood, C. J. Pre-eclampsia is associated with dendritic cell recruitment into the uterine decidua. *J. Pathol.* **2008**, *214*, 328−336.

(20) Aggarwal, R.; Jain, A. K.; Mittal, P.; Kohli, M.; Jawanjal, P.; Rath, G. Association of pro- and anti-inflammatory cytokines in preeclampsia. *J. Clin. Lab. Anal.* **2019**, *33*, No. e22834.

(21) Cotechini, T.; Komisarenko, M.; Sperou, A.; Macdonald-Goodfellow, S.; Adams, M. A.; Graham, C. H. Inflammation in rat pregnancy inhibits spiral artery remodeling leading to fetal growth restriction and features of preeclampsia. *J. Exp. Med.* **2014**, *211*, 165−179.

(22) Lu, D.; Peng, Q.; Chen, D.; Chen, X.; Jiang, M. Expression imbalance of IL-17/IL-35 in peripheral blood and placental tissue of pregnant women in preeclampsia. *Taiwan. J. Obstet. Gynecol.* **2020**, *59*, 409−414.

(23) Cao, W.; Wang, X.; Chen, T.; Zhu, H.; Xu, W.; Zhao, S.; Cheng, X.; Xia, L. The Expression of Notch/Notch Ligand, IL-35, IL-17, and Th17/Treg in Preeclampsia. *Dis. Markers* **2015**, *2015*, No. 316182.

(24) Deng, Z.; Zhang, L.; Tang, Q.; Xu, Y.; Liu, S.; Li, H. Circulating levels of IFN-gamma, IL-1, IL-17 and IL-22 in pre-eclampsia: A systematic review and meta-analysis. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **2020**, *248*, 211−221.

(25) Mohammadpour-Gharehbagh, A.; Jahantigh, D.; Eskandari, M.; Eskandari, F.; Rezaei, M.; Zeynali-Moghaddam, S.; Teimoori, B.; Salimi, S. The role of TNF-alpha and TLR4 polymorphisms in the placenta of pregnant women complicated by preeclampsia and in silico analysis. *Int. J. Biol. Macromol.* **2019**, *134*, 1205−1215.

(26) Ampey, A. C.; Dahn, R. L.; Grummer, M. A.; Bird, I. M. Differential control of uterine artery endothelial monolayer integrity by TNF and VEGF is achieved through multiple mechanisms operating inside and outside the cell - Relevance to preeclampsia. *Mol. Cell. Endocrinol.* **2021**, *534*, No. 111368.

(27) Carty, D. M.; Anderson, L. A.; Freeman, D. J.; Welsh, P. I.; Brennand, J. E.; Dominiczak, A. F.; Delles, C. Early pregnancy soluble E-selectin concentrations and risk of preeclampsia. *J. Hypertens.* **2012**, *30*, 954−959.

(28) Schutyser, E.; Struyf, S.; Van Damme, J. The CC chemokine CCL20 and its receptor CCR6. *Cytokine Growth Factor Rev.* **2003**, *14*, 409−426.

(29) Fan, T.; Li, S.; Xiao, C.; Tian, H.; Zheng, Y.; Liu, Y.; Li, C.; He, J. CCL20 promotes lung adenocarcinoma progression by driving epithelial-mesenchymal transition. *Int. J. Biol. Sci.* **2022**, *18*, 4275−4288.

(30) Wang, D.; Yuan, W.; Wang, Y.; Wu, Q.; Yang, L.; Li, F.; Chen, X.; Zhang, Z.; Yu, W.; Maimela, N. R.; Cao, L.; Wang, D.; Wang, J.; Sun, Z.; Liu, J.; Zhang, Y. Serum CCL20 combined with IL-17A as early diagnostic and prognostic biomarkers for human colorectal cancer. *J. Transl. Med.* **2019**, *17*, 253.

(31) Chen, W.; Qin, Y.; Wang, D.; Zhou, L.; Liu, Y.; Chen, S.; Yin, L.; Xiao, Y.; Yao, X. H.; Yang, X.; Ma, W.; Chen, W.; He, X.; Zhang, L.; Yang, Q.; Bian, X.; Shao, Z. M.; Liu, S. CCL20 triggered by chemotherapy hinders the therapeutic efficacy of breast cancer. *PLoS Biol.* **2018**, *16*, No. e2005869.

(32) Meitei, H. T.; Jadhav, N.; Lal, G. CCR6-CCL20 axis as a therapeutic target for autoimmune diseases. *Autoimmun. Rev.* **2021**, *20*, No. 102846.

(33) Skovdahl, H. K.; Damas, J. K.; Granlund, A. V. B.; Ostvik, A. E.; Doseth, B.; Bruland, T.; Mollnes, T. E.; Sandvik, A. K. C-C Motif Ligand 20 (CCL20) and C-C Motif Chemokine Receptor 6 (CCR6) in Human Peripheral Blood Mononuclear Cells: Dysregulated in Ulcerative Colitis and a Potential Role for CCL20 in IL-1beta Release. *Int. J. Mol. Sci.* **2018**, *19*, 3257.

(34) Kaser, A.; Ludwiczek, O.; Holzmann, S.; Moschen, A. R.; Weiss, G.; Enrich, B.; Graziadei, I.; Dunzendorfer, S.; Wiedermann, C. J.; Murzl, E.; Grasl, E.; Jasarevic, Z.; Romani, N.; Offner, F. A.; Tilg, H. Increased expression of CCL20 in human inflammatory bowel disease. *J. Clin. Immunol.* **2004**, *24*, 74−85.

(35) Miller, D.; Motomura, K.; Galaz, J.; Gershater, M.; Lee, E. D.; Romero, R.; Gomez-Lopez, N. Cellular immune responses in the pathophysiology of preeclampsia. *J. Leukoc. Biol.* **2022**, *111*, 237−260.

(36) Li, Q.; Laumonnier, Y.; Syrovets, T.; Simmet, T. Recruitment of CCR6-expressing Th17 cells by CCL20 secreted from plasmin-stimulated macrophages. *Acta Biochim. Biophys. Sin.* **2013**, *45*, 593−600.

(37) Yamazaki, T.; Yang, X. O.; Chung, Y.; Fukunaga, A.; Nurieva, R.; Pappu, B.; Martin-Orozco, N.; Kang, H. S.; Ma, L.; Panopoulos, A. D.; Craig, S.; Watowich, S. S.; Jetten, A. M.; Tian, Q.; Dong, C. CCR6 regulates the migration of inflammatory and regulatory T cells. *J. Immunol.* **2008**, *181*, 8391−8401.

https://doi.org/10.1021/acs.jproteome.2c00544
J. Proteome Res. 2022, 21, 2998−3006