RESEARCH ARTICLE

Expression changes of proteins associated with the development of preeclampsia in maternal plasma: A case-control study

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Defective deep placentation, involving abnormal transformation of the spiral arteries in the junctional zone of the myometrium, is known to cause significant obstetric complications, such as preeclampsia (PE), fetal growth restriction, and placental infarction leading to fetal death. Serological biomarkers to predict and diagnose PE would help antenatal care and reduce obstetric complications. To discover candidate PE biomarkers, we first performed global proteomic profiling of three pairs of plasma samples obtained from pregnant women in the early second trimester, who subsequently developed PE, and controls to identify candidate proteins that were abundant in the patients. We further evaluated the changes in the expression of PE-representing proteins in stored plasma samples of a cohort that subsequently developed PE and their matched controls by MRM-MS analysis. We identified that both complement C1s subcomponent (C1S) and protein AMBP were elevated in the plasma samples of the PE cohort before the manifestation of clinical disease. We propose that these proteins may be involved in the remodeling process of the spiral arteries even before PE manifestation. These proteins can serve as potential plasma biomarkers to predict the pregnant women having an increased risk of developing PE.

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1 Introduction

The physiological transformation of the spiral arteries during pregnancy is a vascular adaptation for maximal blood

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Abbreviations: DEPs, differentially expressed proteins; **FA**, formic acid; **IPA**, ingenuity pathway analysis; **MHQA**, mass hunter quantitative analysis; **PE**, preeclampsia; **PLGEM**, power law global error model; **STN**, signal to noise

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Significance of the study

Preeclampsia (PE) is a pregnancy-specific multiorgan syndrome, which complicates 3–10% of all pregnancies. It is one of the representative causes of maternal morbidity and mortality and also contributes greatly to fetal morbidity and mortality. Predicting pregnant women at increased risk of developing PE will help antenatal care and reduce obstetric complications. Although several angiogenic or anti-angiogenic proteins have been reported to be related to PE, none of them is currently regarded as a useful biomarker of this condition. Here, we present combined global profiling and targeted proteomic data obtained from the plasma samples of pregnant women in the early second trimester, who subsequently developed PE. We found that C1S and AMBP were upregulated in the cohort that subsequently developed PE before this condition manifested. Together with the previously reported PE biomarkers, it may be possible to develop both C1S and AMBP as biomarkers for predicting PE.

perfusion to the intervillous spaces. During this transformation, vascular lumens become dilated and straightened and trophoblasts replace the muscular and elastic arterial wall with a thick layer of fibrinoid material [1]. The failure of the physiological transformation of the spiral arteries in the myometrial junctional zone can result in defective deep placentation [2]. Preeclampsia (PE) is the first-described and representative disease associated with defective deep placentation, which is characterized by the transformation of the spiral arteries in the junctional zone of the myometrium. Defective deep placentation is now considered to underlie the pathogenesis of other obstetric complications, such as fetal growth restriction, intrauterine fetal death, placental abruption, preterm labor, and premature rupture of membranes [3,4]. However, the exact cause and mechanism behind the pathogenesis of defective deep placentation are unclear. Physiological transformation of the spiral arteries is a multistep process [5]. Decidua-associated remodeling initiates morphological changes by uterine natural killer cells and various angiogenic factors [6]. Then, endovascular trophoblast-associated remodeling begins around gestational week 14 to 15, and the activity is maximized by gestational week 18 and subsequently gradually declines [7].

Several angiogenic or anti-angiogenic proteins, such as soluble fms-like tyrosine kinase 1 (sFlt-1), soluble endoglin (sEng), placental protein 13 (PP-13), pregnancy-associated plasma protein-A (PAPP-A), neutrophil gelatinase-associated lipocalin (NGAL), placental growth factor (PlGF), and vascular endothelial growth factor (VEGF), are known to be associated with PE [8–14], and changes in their abundance occur even before the onset of clinical disease [15]. However, none of these is currently regarded as a useful biomarker of PE. Here, we used a global proteomic profiling experiment to identify potential biomarkers for predicting PE in the early second trimester using plasma samples from pregnant women who subsequently developed PE and their matched controls. We further evaluated the differentially expressed proteins (DEPs) in stored plasma samples of a cohort that subsequently developed PE and their matched controls by MRM-MS analysis to validate their diagnostic values for PE, which should help antenatal care and improve perinatal outcomes.

2 Materials and methods

2.1 Study population

A case-control study was performed with stored maternal plasma obtained in the early second trimester (gestational weeks 16–21). The study population comprised patients with a singleton pregnancy without chronic disease or pregnancy-associated complications at the time when maternal blood was collected. Patients who were subsequently diagnosed with PE were selected as cases ($n = 13$) and normotensive patients who delivered a normal-weight neonate at term without significant medical or obstetric complications were selected as controls ($n = 13$). Age (within 2 years), parity (nullipara vs. multipara), gestational age at sampling (within 1 week), and sampling time (within 6 months) were considered for the selection of controls. PE was defined as new-onset hypertension (systolic blood pressure of ≥140 mmHg and/or diastolic pressure of ≥90 mmHg on two occasions at least 4 h but not more than 7 days apart) and significant proteinuria (≥300 mg/24 h or ≥1+ on dipstick) after gestational week 20 in a previously normotensive woman [16]. A diagnosis of severe PE was based on the presence of PE plus one or more of the following criteria: persistent systolic blood pressure of ≥160 mmHg or diastolic blood pressure of ≥110 mmHg, proteinuria of ≥5 g in a 24-h urine collection or ≥3+ on dipstick in random urine samples, oliguria (<500 mL/day), cerebral or visual disturbances, pulmonary edema, epigastric or right upper-quadrant pain, impaired liver function, thrombocytopenia (<100 000 platelets/mL), or fetal growth restriction [16]. The Institutional Review Board of Seoul National University Hospital, South Korea, approved the collection of these samples and clinical information and their use for research purposes (1209-031-424).

2.2 Plasma sample preparation

Maternal blood was obtained by venipuncture and collected in tubes containing EDTA. Samples were centrifuged at 700 × *g* for 10 min, and the supernatant was then stored

in polypropylene tubes at −70°C until use. Samples with significant hemolysis were excluded. Plasma samples were immunodepleted of six highly abundant proteins (albumin, IgG, IgA, transferrin, haptoglobin, and antitrypsin) using a MARS Hu-6 column (Agilent Technologies, Santa Clara, CA, USA). Depleted plasma protein concentration was measured using the Micro BCA protein assay kit (Thermo Scientific, Rockford, IL, USA). Equal amounts of proteins were separated by SDS-PAGE and divided into ten fractions. Each protein gel fraction was subjected to in-gel tryptic digestion following the general protocol [17]. Briefly, excised protein bands were destained, reduced with 20 mM DTT, and then alkylated with 55 mM iodoacetamide. After dehydration with ACN, the proteins were digested with 12.5 ng/μL modified trypsin (Promega, Madison, WI, USA) in 50 mM ammonium bicarbonate overnight at 37°C. Peptides were extracted from the gel slices with 50% (v/v) ACN in 5% (v/v) formic acid (FA). The eluates were dried under a vacuum and stored at −20°C until use.

For MRM-MS analysis, protein was reduced with 6 M urea and 10 mM DTT, and alkylated with 30 mM iodoacetamide. The sample was then diluted to 1 M urea with 50 mM ammonium bicarbonate, and trypsin was added at a ratio of 1:50 (trypsin:protein), followed by incubation overnight at 37°C.

2.3 Mass spectrometry analysis and database search

Extracted peptide samples were suspended in 0.1% FA, loaded onto a house-packed 75-μm (inner diameter) × 12-cm C18 column and separated with a 2–35% gradient of solvent B (98% ACN, 0.1% FA in H_2O) for 65 min at a flow rate of 300 nL/min. MS spectra were recorded on a LTQ-velos mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) interfaced with a nano-HPLC (Easy-nLC; Thermo Scientific, Waltham, MA, USA). The LTQ-Velos was operated in a data-dependent mode with one survey MS scan in the mass range of 400–1400 *m/z* using an isolation width of 2.0 *m/z*, followed by five MS/MS scans using normalized collision energy of 35%. All MS/MS spectra were collected with an automatic gain control target ion setting of 10 000 ions. Precursor ions were excluded after being targeted for MS/MS fragmentation in a 30-s period. Collected MS/MS raw files were converted to mzXML files using the Trans-Proteomic Pipeline (version 4.4) and analyzed using the Sequest (version 27) algorithm in the SORCERER (Sage-N Research, Milpitas, CA, USA) platform. The search was performed using the UniProt human database (June 2014, 313 072 entries). Full tryptic specificity and up to two missed cleavage sites were allowed. Mass tolerances for precursor ions and fragment ions were set to 2.0 and 1.0 Da, respectively. Fixed modification for carbamidomethyl-cysteine and variable modifications for methionine oxidation were used. All proteins with a ProteinProphet probability of ≥99% and a PeptideProphet probability of ≥95% were identified [18]. Relative protein quantitation was accomplished using spectral counting. The MS/MS data were normalized to compare the abundances of proteins between samples using Scaffold (version 4.4.5; Proteome Software, Portland, OR, USA). The normalized spectral counts from duplicate LC/MS/MS runs were compared using the R program with power law global error model software (http://www.bioconductor.org) in order to identify DEPs [19]. We used an Ingenuity Pathway Analysis (IPA; Ingenuity Systems, Redwood City, CA, USA) tool to predict the biological functions of proteins. The mass spectrometry data have been deposited with the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository with a dataset identifier (PXD003419).

2.4 Selection of MRM peptide transitions

Peptides were analyzed on an Agilent 6490 triple quadrupole mass spectrometer (Agilent Technologies). MRM peptide transitions were determined using two scan modes: full MS/MS scan mode and unbiased Q3-ion monitoring mode [20]. The product ion scan analysis was performed in the following steps. The spectral data were acquired using the Mass Hunter acquisition software (version B.6.0; Agilent Technologies). Fragmentor voltage was fixed at 380 V and collision energy was modulated according to each target peptide precursor mass (i.e., 500−600 *m/z*, 12−17 V; 600−700 *m/z*, 18−22 V; 700−800 *m/z*, 23−30 V; and ≥800 *m/z*, 31−35 V). The MS/MS scan range was set at 400–2000 *m/z*, in accordance with the precursor ion mass and charge states, the scan time was set to 500 ms, and the dwell time of each transition was set to 20 ms. Both precursor and product ions were monitored with a unit resolution mass window (0.7 FWHM) in Q1 and Q3. For unbiased Q3-ion monitoring mode, the four most intense fragment y and/or b ions were chosen from LTQ MS/MS spectra of target peptides. Chromatographic elution profiles of each transition ion and estimation of chromatographic peak area were achieved using Mass Hunter Quantitative Analysis (MHQA) software (version B.6.0; Agilent Technologies).

2.5 Multiplxed MRM-MS analysis

The multiplexed MRM-MS analysis of peptide transitions was performed in triplicate. The integration of peak areas for MRM transitions was calculated using MHQA software. The CV of the integrated peak area for external standard peptide was determined by the ratio of the standard deviation to the mean of triplicate measurements. Each integrated peak area of MRM transition was calibrated to the external standard peptide, namely, the beta-galactosidase peptide (LNVENPK) of *E. coli*.

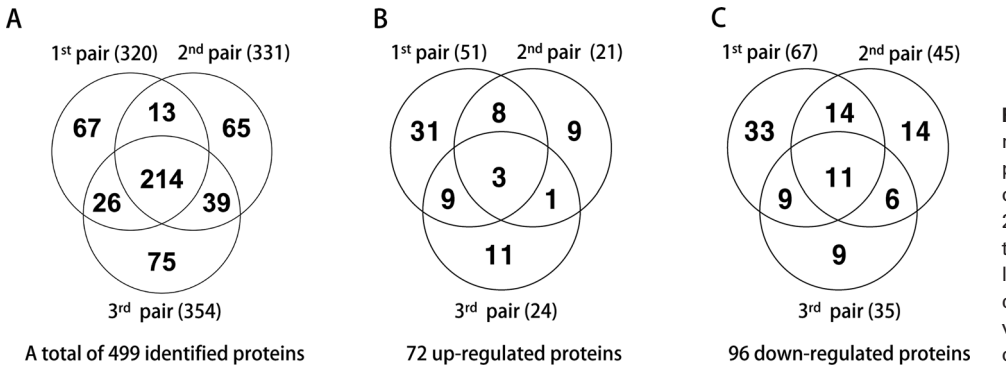

Figure 1. LC/MS profiling of maternal plasma protein with preeclampsia. The number of overlapping proteins in the 1st, 2nd, and 3rd pair of experimental groups (A). Number of overlapped up-regulated (B) and down-regulated (C) proteins (p-value ≤ 0.03) in the three pairs of PE samples.

3 Results and discussion

3.1 Study population

In this study, we selected 13 cases that subsequently developed PE and their matched normal controls ($n = 13$) from the stored samples of a cohort of pregnant women in the early second trimester. The clinical characteristics of the PE cases and controls are shown in Supporting Information Table 1A. There were no significant differences between the two groups with regard to maternal age, parity, gestational age at blood sampling, and body mass index. The characteristics of PE after diagnosis are presented in Supporting Information Table 1B. Of the patients with PE, 61.5% ($n = 8$) were diagnosed with severe PE. Almost half of the patients with PE had a growth-restricted fetus. However, no patients had impaired liver function or thrombocytopenia.

3.2 Global plasma proteomic profiling of preeclampsia

Initially, to identify proteins showing significant differences in abundance between the PE and control samples, we carried out three separate LC-MS/MS analyses for each paired sample, instead of using pooled plasma samples for a protein profiling experiment, because we only observed subtle changes in protein abundance between PE and the control samples when we used a pooled plasma sample (data not shown). However, we identified proteins with significant differences in abundance between the two groups when we used the samples from the subjects with clinically severe disease conditions among the patients. This can be explained by the premise that the physiological transformation of the spiral arteries is not an "all or nothing" concept. The range of defective deep placentation is wide, and the severity of associated diseases is diverse [21].

After the depletion of six highly abundant proteins in each pair of plasma samples, these samples were separated using SDS-PAGE, fractionated into ten gel band regions, and subjected to in-gel digestion. After duplicated LC-MS/MS analysis, we merged the protein database search results of three pairs of PE samples and identified a total of 499 proteins (95.0% peptide probability, 99.0% protein probability, minimum two peptides, FDR 0.7%) (Fig. 1A, Supporting Information Table 2). We then applied the following criteria for the DEP selection; (i) p-value ≤ 0.03, (ii) proteins that are up- or downregulated at least once among the three paired PE samples, and (iii) exclusion if protein expression changes are inconsistent in the three paired PE samples. Following these selection criteria, we identified a total of 168 DEPs. Among them, 72 proteins were upregulated (3 upregulated in all three paired samples, 18 upregulated in two paired samples, and 51 upregulated proteins in one paired sample only) (Fig. 1B, Supporting Information Table 3A), and 96 proteins were downregulated (11 in all three, 29 in two, and 56 in one paired sample only) (Fig. 1C, Supporting Information Table 3B).

3.3 Pathway analysis of the differentially expressed proteins

To evaluate the biological functions of DEPs, we examined 168 DEPs by IPA, which showed the enrichment of molecular functions involved in cell-to-cell signaling and interaction (56 DEPs), cellular movement (55 DEPs), protein synthesis (39 DEPs), and degradation (28 DEPs). We found that inflammatory and immune responses appeared to be the predominant functional representation. This is consistent with the distinct epidemiological features of PE, the development of which involves an immune maladaptation or the loss of maternal immune tolerance to paternally derived placental and fetal antigens. Our IPA of DEPs also revealed that 37 proteins are associated with lipid metabolism. PE is one of the diseases characterized by the absence or insufficiency of physiological remodeling of the spiral arteries in the junctional zone of the myometrium; in addition, vascular lesions are also found in patients with PE and hypertension during pregnancy [22]. Apolipoprotein M (APOM), which was downregulated, binds to high-density lipoproteins (HDLs); it is known to modulate the bioactivity of sphingosine-1-phosphate (S1P) bioactivity, which is a lipid that influences angiogenesis and endothelial

Table 1. MRM peptide transitions of the 37 target proteins

Protein name	Peptide sequence	Q1 mass	Q3 mass	Ion type	RT[a)] (min)	CE[**] (V)
Alpha-2-macroglobulin	QGIPFFGQVR	574.8	299.3	b2	11.8	17
			850.5	y7		
	FEVQVTVPK	523.8	671.5	y6	9.1	15
			770.5	y7		
Alpha-1-acid glycoprotein 1	YVGGQEHFAHLLILR	585.4	746.4	$y13^{2+}$	10.5	13
			836.2	y7		
Angiotensinogen	SLDFTELDVAAEK	719.4	975.5	y9	11.2	22
			1237.5	y11		
	VLSALQAVQGLLVAQGR	862.0	431.36	y4	15.4	27
			813.6	y8		
Antithrombin-III	VAEGTQVLELPFK	715.9	391.2	y3	12.0	20
			504.3	y4		
Apolipoprotein C-I	EFGNTLEDK	526.7	262.3	y2	5.7	11
			504.3	y4		
Apolipoprotein C-II	TYLPAVDEK	518.3	265.1	b2	7.0	11
			658.3	y6		
Apolipoprotein C-III	GWVTDGFSSLK	598.8	244.1	b2	10.9	13
			854.4	y8		
Apolipoprotein E	LGPLVEQGR	484.8	588.2	y5	6.5	13
			701.3	y6		
Apolipoprotein M	AFLLTPR	409.3	486.3	y4	9.1	8
			599.4	y5		
Ceruloplasmin	GAYPLSIEPIGVR	686.7	541.7	y5	11.4	18
			871.0	y8		
Complement C1s subcomponent	SSNNPHSPIVEEFQVPYNK	729.7	521.6	y4	9.7	18
			620.7	y5		
	TNFDNDIALVR	639.7	458.6	y4	9.5	17
			1063.3	y9		
Complement C3	VHQYFNVELIQPGAVK	921.5	471.3	y5	11.0	31
			954.6	y9		
Complement C5	NADYSYSVWK	616.8	932.5	y7	13.8	14
			1047.5	y8		
Complement component C8 beta chain	SGFSFGFK	438.7	498.3	y4	10.3	9
			585.3	y5		
Complement component C8 gamma chain	SLPVSDSVLSGFEQR	810.9	636.3	$y5^{2+}$	11.6	26
			710.9	y13		
Complement component C9	AIEDYINEFSVR	728.4	751.4	y6	12.1	20
			864.5	y7		
Complement factor I	AQLGDLPWQVAIK	719.9	841.5	y7	12.8	21
			1126.6	y10		
	VFSLQWGEVK	596.8	859.5	y7	11.4	15
			946.5	y8		
Galectin-3-binding protein	ELSEALGQIFDSQR	796.9	652.3	y5	12.3	23
			950.5	y8		
Haptoglobin	VTSIQDWVQK	602.7	675.7	y5	8.9	16
			1004.1	y8		
Hemoglobin subunit alpha	VGAHAGEYGAEALER	510.6	488.3	y4	6.0	12
			617.3	y5		
Hemoglobin subunit beta	EFTPPVQAAYQK	689.9	501.3	$y9^{2+}$	8.0	19
			1001.5	y9		
Hemopexin	RLWWLDLK	565.3	260.2	y2	12.9	13
			870.5	b6		
Highly similar to complement component C7	GGGAGFISGLTYLELDNPAGNKR	769.7	874.5	$y16^{2+}$	12.0	21
			931	$y17^{2+}$		
Histidine-rich glycoprotein	DGYLFQLLR	562.8	676.4	y5	14.0	12
			789.5	y6		
Isoform 1 of Alpha-1-antitrypsin	SVLGQLGITK	508.4	716.9	y7	8.5	11
			830.1	y8		

Table 1. Continued

Protein name	Peptide sequence	Q1 mass	Q3 mass	Ion type	RT[a)] (min)	CE[**] (V)
Isoform 1 of Clusterin	VTTVASHTSDSDVPSGVTEVVVK	772.7	508.0	$y10^{2+}$	8.5	25
			1014.9	y10		
Isoform 1 of Complement factor H	SSNLIILEEHLK	698.4	768.4	y6	10.2	18
			881.5	y7		
Isoform 1 of C-reactive protein	GYSIFSYATK	568.8	716.4	y6	12.7	12
			916.5	y8		
Isoform 1 of N-acetyl muramoyl-L-alanine amidase	PSLSHLLSQYYGAGVAR	607.6	530.5	y6	11.4	18
			693.7	y7		
Isoform 1 of Serum albumin	LVNEVTEFAK	575.6	595.7	y5	8.4	13
			938.0	y8		
Isoform 2 of Ig mu chain C region	QVGSGVTTDQVQAEAK	809.8	989.9	y9	5.5	26
			1091.0	y10		
Plasma protease C1 inhibitor	FQPTLLTLPR	593.5	276.3	b2	11.8	14
			456.1	$y8^{2+}$		
Protein AMBP	AFIQLWAFDAVK	704.9	650.4	y6	15.2	19
			836.4	y7		
	ETLLQDFR	511.5	322.4	y2	9.9	10
			565.6	y4		
Serum paraoxonase	EVQPVELPNCNLVK	820.3	706.3	$y12^{2+}$	9.7	26
			845.0	y7		
Transthyretin	AADDTWEPFASGK	698.1	606.7	y6	9.3	18
			735.8	y7		
	GSPAINVAVHVFR	684.2	728.7	y6	10.0	18
			941.9	y8		
Vitamin D-binding protein isoform 1 precursor	THLPEVFLSK	585.8	239.1	b2	9.1	14
			819.5	y7		
Vitronectin	VDTVDPPYPR	579.8	472.7	$y8^{2+}$	6.8	13
			629.3	y5		

a) RT, retention time.
** CE, collision energy.

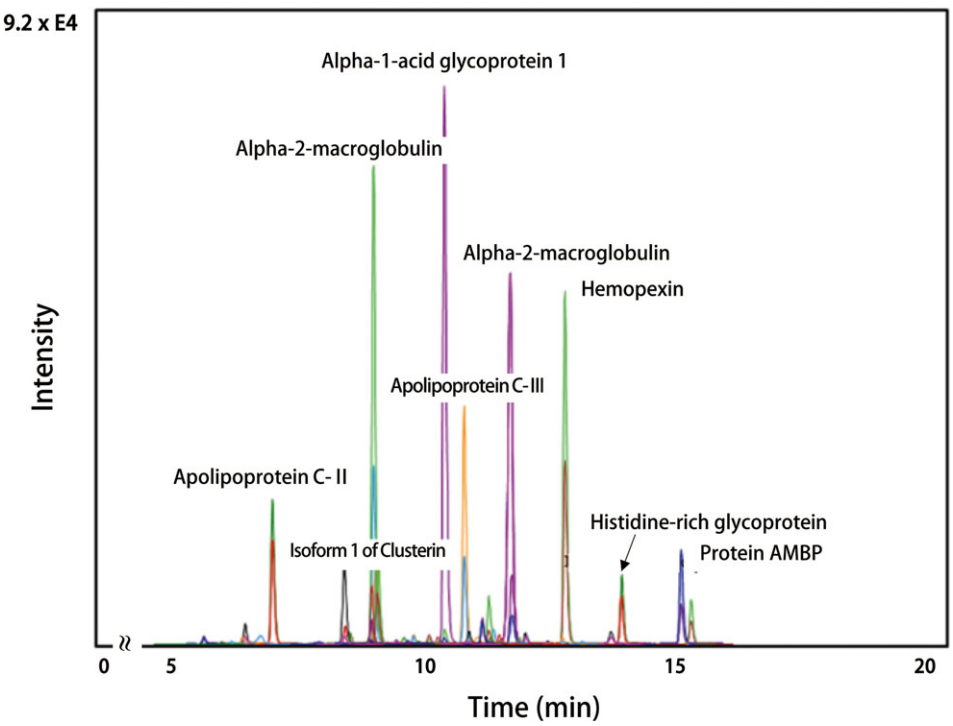

Figure 2. Multiplexed MRM-MS analysis of preeclampsia target proteins. Semi-quantitative MRM-MS analysis was performed with 43 peptides of 37 target proteins using external standard peptide, LNVENPK (beta-galactosidase), spiked into the PE sample. Extracted ion chromatograms represent the observed transition ions of 43 PE target peptides including external standard peptide.

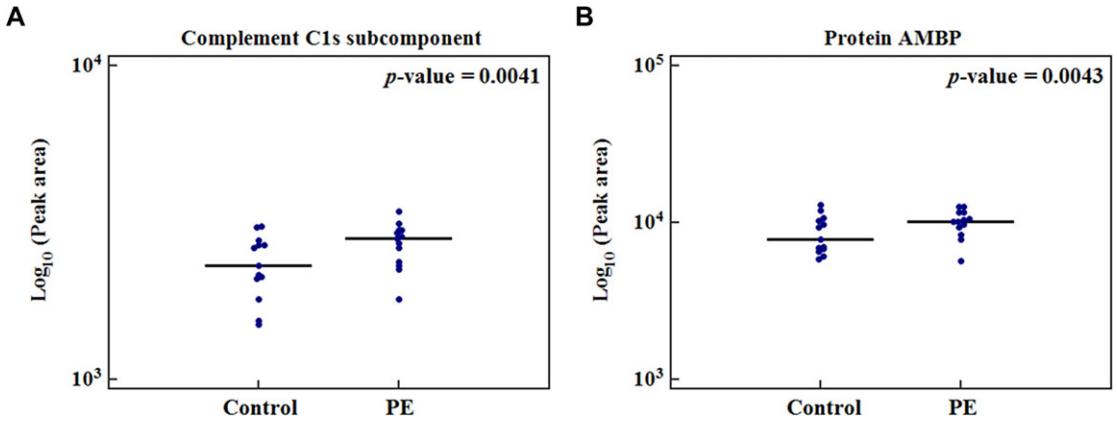

Figure 3. Interactive plots for significantly up-regulated proteins in maternal plasma of preeclampsia. Interactive plots were generated by normalized peak areas of each MRM target peptide. The protein levels of both C1S (p-value = 0.0041) (A) and AMBP (p-value = 0.0043) (B) were increased in PE plasma samples with significant values.

function [23]. Overall, apolipoproteins play an essential role in atherosclerosis by controlling inflammatory responses and lipid transport [24]. Thus, APOM identified in this study may be involved in defective deep placentation.

In addition, our results revealed that the expression levels of complement components in PE were increased compared with those in the control samples. Complement factors are known for their involvement in the regulation of angiogenic factors. Following complement activation, angiogenic factors are dysregulated, which causes placental dysfunction [25]. It has also been reported that an inflammatory response develops due to tissue damage and subsequent immunological activation including the complement system. Compared with those in a normal pregnancy, endothelial cell dysfunction and systemic activation of the inflammatory response are exaggerated in PE [26]. Defective placentation associated with inflammation can be considered to be the cause of PE [27]. We found that the levels of AMBP were also elevated in PE. AMBP, a type of lipocalin transport protein, is known to be involved in the processes of inflammatory response and is known to be elevated in maternal plasma in patients with PE [28].

3.4 MRM-MS analysis of differentially expressed proteins

To validate the DEPs identified by the proteome profiling experiment, we conducted targeted quantitative LC/MS/MS analysis for selected proteins. We selected 37 target proteins associated with the pathophysiology of PE, such as trophoblastic invasion, vascular remodeling, maternal immune response, and endothelial cell activation. We designed 86 MRM peptide transitions of 43 peptides for 37 target proteins, including their retention times, ion types, and required collision energy (Table 1). Prior to the MRM-MS analysis, we evaluated the analytical performance of our MRM-MS assay by estimating the linearity of quantitative measurement and the assay reproducibility. This was accomplished by measuring different concentrations of external standard peptide (LNVENPK, beta-galactosidase peptide, m/z 407.2) spiked into the plasma samples. The quantitative calibration curve showed good linearity (R^2 = 0.9973) with a reproducible CV in the given standard peptide concentrations (Supporting Information Fig. 1). With 86 predetermined MRM transitions for the target proteins, we analyzed 13 paired PE plasma samples spiked with the beta-galactosidase peptide in triplicate. As shown in Fig. 2, the CV of each MRM target peptide was less than 20% in the entire set of analyzed samples and the variation in chromatographic retention time was less than 1%. A total of 37 proteins were quantified by integrating the peak areas of their peptide transitions, followed by normalization with spiked external standard peptide transitions (Supporting Information Table 4). We found that six proteins: C1S, haptoglobin (HP), hemoglobin subunit alpha (HBA), hemoglobin subunit beta (HBB), isoform 1 of C-reactive protein (CRP), and AMBP, were elevated (≥1.2-fold change) in the plasma samples of women who subsequently developed PE. Among them, two proteins, C1S (p-value = 0.041) and AMBP (p-value = 0.043), were found to be significantly elevated in the women who subsequently developed PE (Fig. 3).

4 Concluding remarks

Large-scale quantitative proteomic analysis followed by MRM-MS-based targeted quantitative proteomic analysis of plasma samples from a cohort with PE in the early second trimester enabled the identification of a number of DEPs. Among these, C1S and AMBP were significantly elevated in the plasma of the women who subsequently developed PE compared with those in the control subjects. We propose that C1S and AMBP are associated with the development of

PE, although further confirmation of this by a study with a large cohort is required before such proteins can be utilized for the prediction of PE. Defective deep placentation is now considered to be commonly associated with the pathogenesis of major obstetric complications such as PE, fetal growth restriction, intrauterine fetal death, placental abruption, preterm labor, and premature rupture of membranes. In addition, the early second trimester, which is the period when we obtained the plasma samples, is the time when deep placentation occurs. The findings of this study can be applied to other obstetric complications associated with defective deep placentation, besides PE. If proteins are found to be abundant in disorders with any obstetric complications compared with those in normal controls, they may be useful for understanding the mechanism of defective deep placentation. We expect that these identified proteins may have potential as biomarkers for obstetric complications associated with defective deep placentation.

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The authors have declared no conflict of interest.

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