Supporting Information

Cerebrospinal fluid of preeclamptic and normotensive pregnant women compared to nonpregnant women analyzed with mass spectrometry

Coşkun Güzel[1], Caroline B. van den Berg[2], Seppe Koopman[3], Robbert Jan van Krugten[3], Marcel Stoop[1], Christoph Stingl[1], Johannes J. Duvekot[2], Theo M. Luider[1*]

1) Laboratory of Neuro-Oncology/Clinical & Cancer Proteomics, Department of Neurology, Erasmus University Medical Center Rotterdam, The Netherlands
2) Department of Obstetrics and Gynecology, Erasmus University Medical Center Rotterdam, The Netherlands
3) Department of Anesthesiology, Maasstad Hospital, Rotterdam, The Netherlands

Conflicts of interest: The authors report no conflict of interest

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

Table S1. Clinical characteristics.

General characteristics [a]				
	PE (n=30)	CO (n=30)	NP (n=29)	P-value*
Maternal age (years)	32 (29-35)	35 (32-37)	35 (29-40)	0.034
Ethnicity				
Dutch	21 (67.7%)	20 (66.7%)	21 (72.4%)	NS
Other Western	0 (0)	2(6.7%)	2 (6.9%)	
Non Western	10 (32.3%)	8 (26.7%)	6 (20.7%)	
BMI (kg/m)	25.2 (21.6-30.4)	23.2 (20.8-27.4)	24.8 (22.4-29.3)	NS
Smoking				
Yes	0 (0%)	3 (10%)	8 (27.6%)	0.006
No	26 (83.9%)	25 (83.3%)	21 (72.4	
Missing	4 (12.9%)	2 (6.6%)	0 (0%)	
Highest systolic blood pressure (mm Hg)	170 (160-170)	120 (120-130)	120 (110-126)	<0.001
Highest diastolic blood pressure (mm Hg)	110 (100-110)	75 (70-80)	71 (67-80)	
Medical history				
Chronic hypertension	4 (12.9%)	0 (0%)	0 (0%)	0.032
Diabetes mellitus type 1	2 (6.5%)	1 (3.3%)	0 (0%)	NS
Diabetes mellitus type 2	0 (0%)	0 (0%)	0 (0%)	NS
Obstetric history				
Recurrent miscarriages	1 (3.2%)	1 (3.3%)	0 (0%)	NS
PE in previous pregnancy	5 (16.1%)	4 (13.3%)	0 (0%)	NS
Current pregnancy				
Nulliparous	20 (64.5%)	13 (43.3%)	NA	NS
Gestational age sampling (days)	215 (209-254)	270 (266-272)	NA	<0.001
Gestational diabetes	1 (3.2%)	1 (3.3%)	NA	NS
Twin pregnancy	2 (6.5%)	1 (3.3%)	NA	NS
Phenotypes preeclampsia				
HELLP	7 (22.6%)	NA	NA	NA
Early-onset preeclampsia	21 (67.7%)	NA	NA	NA
Use of magnesiumsulfate during sampling	25 (83%)	NA	NA	NA
Child characteristics [b]				
Birth weight (g)	1175 (930-2440)	3193 (2860-3490)	NA	<0.001
Birth weight percentile				
<10th percentile	10 (29.0%)	2 (6.7%)	NA	0.037
10th-90th percentile	22 (67.7%)	26 (86.7%)	NA	
>90th percentile	1 (3.2%)	2 (6.7 %)	NA	
Neonatal death	0 (0%)	2 (6.7%)	NA	NS
Intrauterine fetal death	0 (0%)	1 (3.3%)	NA	NS

*a) Data are presented as median with IQR or count with percentage; b) Due to two twin pregnancies n = 32. *For comparisons between groups Kruskal-Wallis 1-way ANOVA, Chi-square tests, and Fisher's exact tests were used. Patients were excluded who meet any of the following criteria: pregnant during inclusion, preeclampsia in history, auto-immune disorders, corticosteroid use, hemoglobinopathy or clotting disorder, chronic hypertension or proteinuria, kidney disorders, neurologic disorders.*

Table S2. Differently abundant proteins (n=23) in CSF of normotensive pregnant and nonpregnant women analyzed using the Progenesis method without a Scopus score (known association with pregnancy).

protein name	gene name	up/down in pregnant women	fold change (CO/NP)
ATP-binding cassette sub-family A member 2**	ABCA2	up	1.89
ADM (adrenomedullin)**	ADML	up	2.04
45 kDa calcium-binding protein*	SDF4	up	1.43
gamma-enolase**	ENO2	up	1.43
growth arrest-specific protein 6*/**	GAS6	up	2.50
immunoglobulin superfamily member 21*	IGSF21	up	1.43
Inositol monophosphatase 3*	IMPAD1	up	1.47
vesicular integral-membrane protein VIP36	LMAN2	up	1.64
lumican**	LUM	up	1.69
myeloid-derived growth factor*	MYDGF	up	1.96
podocalyxin-like protein 2	PODXL2	up	2.00
phosphatidylethanolamine-binding protein 1	PEPB1	up	1.61
profilin-1*/**	PFN1	up	2.33
proline-rich transmembrane protein 3	PRRT3	up	1.52
receptor-type tyrosine-protein phosphatase N2**	PTPR2	up	2.27
extracellular sulfatase Sulf-2	SULF2	up	1.49
transaldolase**	TALDO1	up	2.33
thioredoxin**	TXN	up	1.92
polyubiquitin-B*	UBB	up	2.08
vasorin*	VASN	up	1.67
complement factor D*/**	CFD	down	0.41
neuropilin-1**	NRP1	down	0.75
selenoprotein P**	SEPP1	down	0.58

CO=normotensive pregnant women.
NP= nonpregnant women.
**Significantly different protein as analyzed by Benjamini-Hochberg ($p<0.0086$) using the Scaffold method.*
*** Related to pregnancy (see Table 1).*

Table S3. Differently abundant proteins (n=62) in CSF of normotensive pregnant and nonpregnant women analyzed using the Scaffold method without a Scopus score (known association with pregnancy).

protein name	gene name	*t*-test	up/down in pregnant women	fold change (CO/NP)	pregnancy hits	total literature hits
coagulation factor XIII B chain	F13B	0.00031	up	3.09	0	166
calcium/calmodulin-dependent protein kinase type II subunit alpha	CAMK2A	0.00022	up	3.97	0	132
leucine-rich alpha-2-glycoprotein	LRG1	0.00110	up	1.54	0	117
kallikrein-6	KLK6	0.00590	down	0.90	0	112
phosphoglycerate mutase 1	PGAM1	0.00200	up	2.62	0	110
desmocollin-3	DSC3	< 0.00010	down	0.24	0	103
parvalbumin alpha	PVALB	0.00039	up	4.26	0	89
voltage-dependent calcium channel subunit alpha-2/delta-1	CACNA2D1	< 0.00010	up	1.58	0	71
complement C1q subcomponent subunit C	C1QC	0.00088	down	0.73	0	63
dihydropteridine reductase	QDPR	0.00048	up	3.46	0	60
beta-Ala-His dipeptidase	CNDP1	0.00120	up	1.34	0	59
c-type mannose receptor 2	MRC2	0.00016	up	3.26	0	57
protein FAM3C	FAM3C	0.00023	up	1.24	0	40
plasma serine protease inhibitor	SERPINA5	0.00100	down	0.68	0	36
WAP four-disulfide core domain protein 1	WFDC1	0.00400	down	0.54	0	30
Ig kappa chain C region	IGKC	0.00089	down	0.76	0	29
neurexophilin-1	NXPH1	0.00330	up	2.57	0	29
extracellular matrix protein 2	ECM2	< 0.00010	down	0.28	0	27
receptor-type tyrosine-protein phosphatase-like N	PTPRN	0.00024	up	1.84	0	27
WW domain-binding protein 2	WBP2	0.00620	up	1.93	0	27
tetranectin	CLEC3B	0.00016	down	0.67	0	26
vasorin	VASN	0.00014	up	3.62	0	24
n-acetylmuramoyl-L-alanine amidase	PGLYRP2	0.00012	down	0.73	0	23
Ig kappa chain V-III region GOL	Ig kappa chain V-III region GOL/IGKV3-20	0.00030	down	0.38	0	22
Ig kappa chain V-III region SIE	Ig kappa chain V-III region SIE/IGKV3-20	< 0.00010	down	0.27	0	22
Ig kappa chain V-III region Ti	Ig kappa chain V-III region Ti/IGKV3-20	0.00560	down	0.59	0	22
zinc transporter ZIP10	SLC39A10	0.00061	down	0.27	0	21
CD99 antigen-like protein 2	CD99L2	0.00078	up	1.29	0	19
thyroxine-binding globulin	SERPINA7	< 0.00010	up	3.01	0	17
immunoglobulin lambda-like polypeptide 5	IGLL5	0.00810	down	0.78	0	16
olfactomedin-like protein 3	OLFML3	< 0.00010	down	0.19	0	16
scrapie-responsive protein 1	SCRG1	0.00320	up	1.85	0	15

Ig alpha-1 chain C region	IGHA1	0.00200	down	0.83	0	13
Ig gamma-1 chain C region	IGHG1	0.00530	down	0.83	0	13
neural proliferation differentiation and control protein 1	NPDC1	< 0.00010	up	2.32	0	13
SH3 domain-binding glutamic acid-rich-like protein	SH3BGRL	0.00190	up	4.12	0	13
Ig kappa chain V-II region RPMI 6410	Ig kappa chain V-II region RPMI 6410/IGKV2-30	0.00310	down	0.58	0	12
seizure protein 6 homolog	SEZ6	0.00710	up	1.23	0	12
complement C1q tumor necrosis factor-related protein 3	C1QTNF3	0.00078	down	0.68	0	11
Ig kappa chain V-I region Mev	Ig kappa chain V-I region Mev/IGKV1-39	0.00013	down	0.36	0	11
protein FAM19A5	FAM19A5	0.00034	down	0.55	0	11
45 kDa calcium-binding protein	SDF4	0.00170	up	1.79	0	10
proline-rich acidic protein 1	PRAP1	< 0.00010	up	4.05	0	10
alpha-1-acid glycoprotein 2	ORM2	0.00620	down	0.82	0	9
ganglioside GM2 activator	GM2A	0.00650	down	0.75	0	9
Ig lambda-2 chain C regions	IGLC2	0.00023	down	0.70	0	9
immunoglobulin superfamily member 21	IGSF21	0.00018	up	2.23	0	8
inositol monophosphatase 3	IMPAD1	0.00089	up	1.54	0	8
myeloid-derived growth factor	MYDGF	0.00150	up	1.94	0	8
alpha-mannosidase 2	MAN2A1	0.00150	up	2.62	0	7
Ig lambda-3 chain C regions	IGLC3	0.00012	down	0.76	0	7
polyubiquitin-B	UBB	0.00041	up	1.45	0	6
Ig gamma-2 chain C region	IGHG2	0.00044	down	0.79	0	5
Ig kappa chain V-I region EU	Ig kappa chain V-I region EU/IGKV1-5	0.00600	down	0.76	0	5
glycoprotein endo-alpha-1,2-mannosidase-like protein	MANEAL	0.00420	up	3.08	0	4
Ig heavy chain V-III region BRO	Ig heavy chain V-III region BRO/IGHV3-13	0.00690	down	0.75	0	4
protein shisa-7	SHISA7	0.00180	up	4.28	0	4
sushi, nidogen and EGF-like domain-containing protein 1	SNED1	< 0.00010	down	0.28	0	4
v-set and transmembrane domain-containing protein 2B	VSTM2B	0.00086	up	1.31	0	3
complement C1q tumor necrosis factor-related protein 4	C1QTNF4	0.00350	down	0.58	0	2
Ig gamma-4 chain C region	IGHG4	0.00290	down	0.78	0	2
sushi domain-containing protein 5	SUSD5	0.00600	up	1.49	0	2

Proteins (n=62 out of 135) with zero counts (no association with pregnancy) for the weighted score as calculated in Table 2.
CO=normotensive pregnant women.
NP=nonpregnant women.

Table S4. Comparison list of proteins found in our previous study with the current study. The significant differentially abundant proteins in CSF of preeclamptic patients of the previous work listed below showed the same trends (i.e., up- or downregulated) in this study using the Progenesis method, except the protein Insulin-like growth factor 2. The protein Amyloid-like protein 1 was not found in the current study.

higher abundant proteins (accession number)	fold change (PE/CO)	fold change (PE/CO) current study
alpha-1-acid glycoprotein 1 (P02763)	1.54	1.56
alpha-1-antichymotrypsin (P01011)	1.44	1.43
alpha-1B-glycoprotein (P04217)	1.27	1.08
histidine-rich glycoprotein (P04196)	2.10	2.63
insulin-like growth factor 2 (P01344)	1.47	0.83
lumican (P51884)	1.54	1.19
protein AMBP (P02760)	1.80	1.59
retinol-binding protein 4 (P02753)	1.44	1.41

lower abundant proteins (accession number)	fold change (PE/CO)	fold change (PE/CO) current study
Amyloid-like protein 1 (P51693)	0.76	not found
Brain acid soluble protein 1 (P80723)	0.64	0.70
Cadherin-13 (P55290)	0.68	0.68
Cartilage acidic protein 1 (Q9NQ79)	0.93	0.79
Chromogranin-A (P10645)	0.67	0.59
Ephrin type-A receptor 4 (P54764)	0.67	0.70
Glucosidase 2 subunit beta (P14314)	0.80	0.70
Limbic system-associated membrane protein (Q13449)	0.79	0.75
Neural cell adhesion molecule (O15394)	0.82	0.83
Neurocan core protein (O14594)	0.78	0.59
Neuronal cell adhesion molecule (Q92823)	0.75	0.71
Neurosecretory protein VGF (O15240)	0.67	0.63
Neuroserpin (Q99574)	0.83	0.63
Protein kinase C-binding protein NELL2 (Q99435)	0.70	0.71
Pyruvate kinase PKM (P14618)	0.76	0.75
Seizure 6-like protein (Q9BYH1)	0.89	0.75
V-set and transmembrane domain-containing protein 2A (B5MCX6)	0.54	0.74

CO=normotensive pregnant women.
PE=preeclamptic women.