Neuropsychopharmacology (2012) 37, 1013–1025

www.neuropsychopharmacology.org

Cerebrospinal Fluid Biomarkers for Major Depression Confirm Relevance of Associated Pathophysiology

Claudia Ditzen*[,1], Ning Tang[2], Archana M Jastorff[1], Larysa Teplytska[1], Alexander Yassouridis[1], Giuseppina Maccarrone[1], Manfred Uhr[1], Thomas Bronisch[1], Christine A Miller[2], Florian Holsboer[1] and Christoph W Turck[1]

[1]Max Planck Institute of Psychiatry, Munich, Germany; [2]Agilent Technologies, Santa Clara, CA, USA

Individual characteristics of pathophysiology and course of depressive episodes are at present not considered in diagnostics. There are no biological markers available that can assist in categorizing subtypes of depression and detecting molecular variances related to disease-causing mechanisms between depressed patients. Identification of such differences is important to create patient subgroups, which will benefit from medications that specifically target the pathophysiology underlying their clinical condition. To detect characteristic biological markers for major depression, we analyzed the cerebrospinal fluid (CSF) proteome of depressed *vs* control persons, using two-dimensional polyacrylamide gel electrophoresis and time-of-flight (TOF) mass spectrometry peptide profiling. Proteins of interest were identified by matrix-assisted laser desorption ionization TOF mass spectrometry (MALDI-TOF-MS). Validation of protein markers was performed by immunoblotting. We found 11 proteins and 144 peptide features that differed significantly between CSF from depressed patients and controls. In addition, we detected differences in the phosphorylation pattern of several CSF proteins. A subset of the differentially expressed proteins implicated in brain metabolism or central nervous system disease was validated by immunoblotting. The identified proteins are involved in neuroprotection and neuronal development, sleep regulation, and amyloid plaque deposition in the aging brain. This is one of the first hypothesis-free studies that identify characteristic protein expression differences in CSF of depressed patients. Proteomic approaches represent a powerful tool for the identification of disease markers for subgroups of patients with major depression.

Neuropsychopharmacology (2012) **37,** 1013–1025; doi:10.1038/npp.2011.285; published online 14 December 2011

Keywords: major depression; cerebrospinal fluid biomarkers; phosphoproteins; PEDF; cystatin C; PGDS

INTRODUCTION

Currently used classification systems in psychiatry are not able to accurately display the inter-individual differences in etiology and outcome of major depressive episodes. Phenotype heterogeneity contributes to the observed differences in response and remission rates to antidepressant drug treatment between patients. Despite the availability of numerous drugs, only half of the patients afflicted with major depression respond to a first antidepressant medication, and about 30% do not reach remission even after several treatment trials (Rush *et al*, 2006; Hennings *et al*, 2009). In recent years, studies on biological differences in depressed patients have primarily focused on genetic analyses and certain brain circuits (eg, monoamine systems). In some cases, these studies revealed associations between genes or proteins and the occurrence of a major depression (Carpenter *et al*, 2004; Geracioti *et al*, 1997; Jokinen *et al*, 2008; Sullivan *et al*, 2006). Due to the complexity of the disease and the involvement of a multitude of brain systems, single markers are unlikely to have the ability to improve current diagnostic and therapeutic strategies. Although genome-wide association studies have shown that we can categorize patients as high or low number of response allele carriers to predict antidepressant treatment outcome (Ising *et al*, 2009), we still do not know which antidepressant drug a patient will benefit from. At the same time, an overall sub-classification based on individual biological features of depressed patients is still missing. To examine the complexity of pathological brain mechanisms accounting for a depressive episode, the most relevant molecular compartment to analyze is the proteome. The proteome is the entire collection of proteins encoded by the genome of an organism, including protein isoforms and posttranslational modifications (Taurines *et al*, 2011). Due to its close

*Correspondence: Dr C Ditzen, Max Planck Institute of Psychiatry, Kraepelinstr 2, Munich D-80804, Germany, Tel: +49-89-30622429, Fax: +49-89-30622562, E-mail: ditzen@mpipsykl.mpg.de
Received 22 June 2011; revised 19 September 2011; accepted 4 October 2011

proximity, cerebrospinal fluid (CSF) reflects the metabolic status and biochemical alterations of the brain. Hence, for the *in vivo* investigation of a brain disease, CSF represents the most relevant specimen (Zougman *et al*, 2008). The analysis of the CSF proteome can reveal protein differences between diseased and healthy individuals, allowing the discovery of metabolic pathways linked to the pathogenesis of psychiatric disorders (Yuan *et al*, 2002). In the present report, we are employing two complementary methods for the interrogation of the CSF proteome. Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is able to analyze intact proteins, and is a straightforward method for the detection of posttranslational modifications. Compared with 2D-PAGE, mass spectroscopy-based peptide analysis methods have greater sensitivity and reproducibilty with less material consumption (Lottspeich *et al*, 2010). This is an important aspect in the case of clinical specimens that are often of limited availability. However, the mass spectroscopy-based peptide analysis approach requires digestion of the proteins before differential analysis, resulting in an increased sample complexity. The combined application of the two complementary proteomic methods provides a comprehensive approach for biomarker discovery. In an earlier study, we have detected a 33-kDa protein with an isoelectric point of 5.2, whose expression level in CSF differed significantly between depressed patients with and without suicide attempt (Brunner *et al*, 2005). Due to a lack of material, we were unable to identify the protein. In the present paper, we report on a comprehensive hypothesis-free proteomic analysis of CSF from depressed patients. We have successfully identified several differentially expressed proteins that are discussed with regard to their potential role in the pathogenesis of unipolar depression.

MATERIALS AND METHODS

CSF Samples

The study included CSF from 12 individuals fulfilling ICD-10 criteria for a depressive episode, either as a single episode or within recurrent episodes (mean age 54 ± 16.01 years; 10 females, 2 males). A total of 12 CSF control samples were matched according to sex and age (mean age 53 ± 18.08 years; 8 females, 4 males). Participants had provided written informed consent to take part in the study (German Federal Research Ministry, Competence Nets in Medicine, subproject 1.5). Samples from individuals with additional severe systemic or central nervous system disease were excluded from the study. CSF samples with abnormal levels of glucose, lactate, total protein, cells, positive oligoclonal bands, or disturbance of the blood–brain barrier, as well as blood-contaminated samples were not considered.

Sample Preparation for 2D-PAGE

A total of 24 CSF samples were prepared for 2D-PAGE. Each sample (5 ml) was desalted and concentrated to 80 μl on a Vivaspin spin column with a molecular weight cutoff of 5 kDa (Vivascience AG, Hannover, Germany). Afterwards, immunodepletion of albumin, transferrin, IgG, IgA, antitrypsin, and haptoglobin in CSF was performed using a Multiple Affinity Removal System column (Agilent Technologies, Böblingen, Germany) as described previously (Maccarrone *et al*, 2004). To apply the same amount of protein on each gel, protein concentration of HPLC flowthrough fractions was determined using the Bradford method. Equal amounts of protein (136 μg) were then diluted in isoelectric focusing (IEF) buffer (8M urea, 2% CHAPS, 0.2% Biolyte, 100 mM DTT, 0.001% bromophenol blue) to a final volume of 300 μl.

Two-dimensional Polyacrylamide Gel Electrophoresis

Depleted CSF samples in IEF sample buffer were applied to a 17 cm, pH 3–10 Immobiline strip (BioRad, Hercules, CA). After active rehydration at 50 V for 12 h IEF, SDS polyacrylamide gel electrophoresis and gel staining were carried out as described previously (Jacob and Turck, 2008). A total of 24 gels were run, 12 each from depressed individuals and controls.

Gels were first stained with ProQ Diamond (Molecular Probes, Eugene, OR) to visualize phosphorylated proteins with a Molecular Imager FX Pro fluorescence scanner (BioRad). Subsequent total protein staining was performed with Coomassie Brilliant Blue G (Sigma Aldrich, St Louis, MO) in colloidal solution (17% (w/v) ammonium sulfate, 2% (v/v) phosphoric acid, and 34% (v/v) methanol). Coomassie-stained gels were scanned with a GS-800 densitometer (BioRad).

Data Analysis

Scanned images were analyzed with the help of PDQUEST software (BioRad). After normalization according to total density, spots of relevant protein intensity were detected using the automated spot detection and matching function. The automated analysis was verified and completed by manual matching. In order to find the most interesting spots, ie., those with relevant protein intensity differences between the two groups, an explorative comparison analysis based on *t*-statistics calculated by PDQUEST was performed. Differences in the most interesting protein spots were finally statistically evaluated using multivariate and univariate tests of significance (F-tests for simple effects in MANOVA). To avoid singularities, the all protein spots were first partitioned in subsets of three to five items, and then subjected to analyses of variance. A nominal level of significance of $\alpha = 0.05$ was accepted and corrected according to the Bonferroni procedure for all à-posteriori tests.

In-gel Protein Digest and Mass Spectrometry Analysis

Protein spots of interest were excised from the gel using the Proteineer Sp automated spot cutter (Bruker Daltonics GmbH, Bremen, Germany). Destaining, drying, and tryptic digestion of gel spots was carried out as previously described (Jastorff *et al*, 2009).

For matrix-assisted laser desorption ionization TOF mass spectrometry (MALDI-TOF-MS) analysis, samples were prepared by a dried droplet method on a 600-μm AnchorChip MALDI Target (Bruker Daltonics). α-cyano-4-hydroxycinnamic

was dissolved in 30% (v/v) acetonitrile and 70% (v/v) 0.1% trifluoroacetic acid to saturation. The matrix preparation was diluted 10-fold in a 2 : 1 ethanol : acetone solution. Each sample (1 μl) was spotted onto the AnchorChip sample target, and 1.5 μl of the prepared matrix solution was added to the spot. For instrument calibration, the peptide calibration standard I (Bruker Daltonics) was used. Peptide mass fingerprints were acquired using the Ultraflex I TOF/TOF mass spectrometer (Bruker Daltonics).

Protein Identification

MALDI-TOF-MS data analysis was carried out with Biotools software (Bruker Daltonics). For protein identification, the database search program Mascot (Matrix Science, London, UK) was applied to identify proteins according to their tryptic mass fingerprints. The search was carried out against NCBI non-redundant and human protein database. Mascot search was performed with a 25-ppm parent mass tolerance, and one possible tryptic miscleavage. Chemical modifications were fixed carbamidomethylation modification for cysteine and oxidation for methionine residues. Protein hits that were significant according to the Mascot score ($P<0.05$) were accepted. All protein hits were further verified by reviewing the position of the spot with regard to molecular weight and isoelectric point (pI) on the 2D-gel image.

Western Blot

Protein concentration of CSF samples was determined with the Bradford method (BioRad) and bovine serum albumin as protein standard. Proteins (2 μg) were separated on 12% SDS gels, and electrotransferred to a PVDF membrane. For IEF-immunoassays, proteins were applied to pH 3–10 Immobiline strips (BioRad) and separated by IEF as described above. Membranes were incubated with primary antibody (anti-cystatin C, 1 : 7500 (Abcam, Cambridge, UK), anti-pigment epithelium derived factor (PEDF), 1 : 5000 (Chemicon International, Hofheim, Germany), anti-prostaglandin D synthase (lipocalin-type), 1 : 5000 (Cayman Chemical, Ann Arbor, MI), anti-apolipoprotein E, 1 : 5000 (Abcam)) in 5% milk-PBS for 90 min at RT, followed by 60 min incubation at RT with a secondary antibody (IgG, 1 : 25.000 (Sigma Aldrich)). Immunocomplexes were visualized by chemoluminescense with the help of ECL Plus reagent (GE Healthcare, Piscataway, NJ) and exposed to autoradiography films. Films were scanned and processed with QuantityOne (BioRad) to quantify band and isoform signal intensities. The statistical analysis was carried out with the *t*-test. The difference was considered to be significant when $P<0.05$.

Liquid Chromatography Mass Spectrometry (LCMS) Profiling

Instrumentation. Agilent HPLC-Chip/MS system interfaced to an Agilent LC/MSD TOF mass spectrometer. The HPLC-Chip/MS system is a microfluidic chip-based device that integrates sample preparation (enrichment column), analytical separation (nanocolumn), and nanoelectrospray formation (emitter tip).

Sample Preparation. Human CSF fractions were immunodepleted using an Agilent Multiple Affinity Removal system. A 20 μg portion of each sample was then desalted using spin tubes that incorporate 5-kDa molecular weight cutoff filters (Vivaspin). The desalted samples were reduced and denatured using 50% TFE in a 50 mM ammonium bicarbonate buffer with 10 mM DTT at 95 °C for 20 min. The reduced samples were subsequently alkylated with 30 mM iodoacetamide at room temperature for 1 h, and the unreacted iodoacetamide was quenched by the addition of DTT. The samples were diluted 1 : 10 with 25 mM ammonium bicarbonate buffer, then treated with trypsin at a 1 : 25 enzyme : substrate ratio overnight at 37 °C.

Chromatographic conditions. Chromatographic separation of the digested samples was accomplished using a protein ID chip, which consists of a 40 nl enrichment column and 75 μm × 43 mm analytical column packed with ZORBAX 300SB-C18, 5-μm material. The mobile phase A was 0.1% formic acid in water and B was 90% acetonitrile + 0.1% formic acid in water. Sample was loaded on the enrichment column at 4 μl/min by the loading pump. After loading, the microvalve was switched to analysis mode, to forward-flush the sample onto the analysis column. The analytical flow rate was 300 nl/min, and the solvent gradient went from 3 to 45%B in 75 min, then to 80% B at 80 min for 2 min. Each sample was run in triplicate.

Mass spectrometer conditions. Electrospray data for the CSF samples was obtained using orthogonal nanospray in positive mode. MS conditions were as follows: drying gas temperature, 300 °C; drying gas flow, 4 l/min; capillary voltage, 1900 V, and fragmenter voltage, 150 V. The mass spectrometer was operated with a mass range of m/z 300 to 2400 U and two cycles/second. To ensure low ppm mass accuracy, the internal reference mass correction was utilized to correct for scan-to-scan variations.

Ms data analysis. Data analysis was done using Agilent MassHunter Qualitative Analysis software and Mass Profiler Professional software, designed to facilitate rapid differential analysis of samples for profiling applications. The MassHunter algorithm extracts chemically qualified molecular features from complex LC/MS TOF data sets by first finding the mass peaks in all mass spectra, and then removing the non-chromatographic chemical background. Next, peaks are clustered in RT (in seconds) and m/z to form 3-D peaks. The 3-D peaks are centroided and a peak volume determined for each peak. Related 3-D peaks (isotopes, adducts, dimers, trimers, multiple charge states) are combined and assigned a neutral mass and total volume. Mass Profiler Professional software combines capabilities for cross-sample alignment of molecular features in both the retention time and mass dimensions, including several normalization options, with statistical methods and visualization tools to aid in identification of differentially expressed features. The Profiler software compensates for scan-to-scan shifts in retention time (seconds) and measured masses (ppm). Common features are identified and cross-sample response RSD values are calculated. Results

filters can be used to reduce the number of differentially expressed biomarkers to be investigated.

RESULTS

We analyzed CSF proteomes of depressed patients and compared them to controls. For this purpose, we used a proteomic workflow that incorporates two complementary methodologies, 2D-PAGE and LCMS-based differential expression analysis.

Image analysis of colloidal Coomassie-stained 2D-PAGE gels resulted in 213 spots that revealed quantitative differences between the two groups, with 11 being significantly different (univariate F-tests in MANOVA, $P<0.05$). Mass spectrometry analysis of the excised gel spots identified PEDF (two isoforms), apolipoprotein E precursor (ApoE), prostaglandin D2 synthase (PGDS; 21 kDa), transthyretin precursor, α-1B-glycoprotein, vitamin D-binding protein (DBP, two isoforms), cystatin C, β-2-glycoprotein, and hemopexin. In case of PEDF, ApoE, PGDS, cystatin C, and DBP, the excised spot represented a single isoform of the protein. Figure 1 shows a representative Coomassie-stained gel of CSF from a depressed patient with differentially expressed proteins indicated. Table 1 gives an overview of the identified differentially regulated proteins; Table 2 shows their molecular function and role in biological processes. Comparative analysis of the phosphoprotein-specific ProQ Diamond-stained gels indicated a difference in phosphorylation pattern between the CSF proteome of depressed and control individuals. A total of 16 differences of phosphorylated proteins were detected between the replicate groups. As ProQ Diamond is a fluorescence stain not detectable with visible light, we had difficulties cutting out the relevant gel pieces. We were only able to identify five of the differentially phosphorylated proteins by mass spectrometry. Different phosphorylation patterns were detected for heat-shock cognate 71-kDa protein, heterogeneous nuclear ribonucleoprotein H, ApoE precursor, fructose-bisphosphate aldolase C, and cAMP-dependent protein kinase catalytic subunit alpha. Figure 2 shows a representative ProQ Diamond-stained gel of CSF from a control person with differentially phosphorylated proteins indicated (see also Tables 1 and 2). To further verify our 2D-PAGE results, we additionally analyzed the depleted CSF samples by LCMS-based profiling. Successful LCMS-based differential expression analysis requires good reproducibility for both chromatography (retention time) and mass spectrometry (accurate mass), so that feature lists can be compared between different samples. The reproducibility of the replicate runs for this study was monitored by clustering the data in GeneSpring (Figure 3). Statistical analysis of the data revealed 144 target features that were significantly different between the control and depressed samples. A log2 plot of the features (Figure 4) shows the distribution of those features. Targeted MS/MS results identified all nine proteins found to be differentially expressed by 2D-PAGE. With the exception of α-1B-glycoprotein, cystatin C, and PEDF, the same direction of protein regulation was found by LCMS-based profiling and 2D-PAGE analysis. The discrepancy in regulation found for the above-named proteins is probably due to the quantitation of peptides derived from different protein isoforms during LCMS profiling and 2D-PAGE. The list of 32 corresponding proteins derived from significant different mass features found by mass spectrometry is shown in Table 1 (indicated as mass spectrometry profiling results). A summary of identified mass features is presented in Supplementary Table 1; peptide sequences and the number of peptides used for protein identification are shown in Supplementary Table 2.

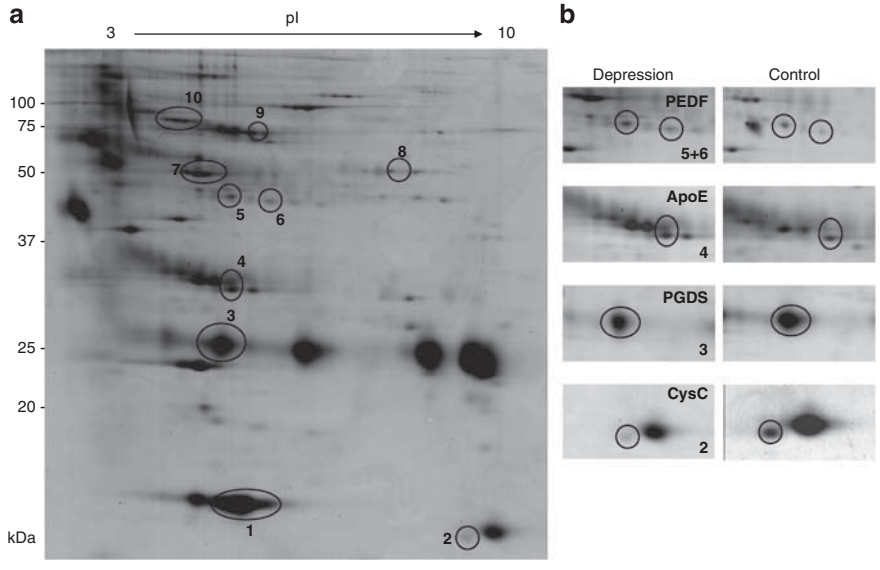

Figure 1 (a) Representative Coomassie-stained two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) gel image of cerebrospinal fluid (CSF) from a depressed patient. Differentially regulated proteins and isoforms between disease and control groups are indicated, and were identified as 1. transthyretin precursor; 2. cystatin C; 3. prostaglandin D2 synthase; 4. apolipoprotein E; 5 and 6. pigment epithelium derived factor; 7. vitamin D-binding protein; 8. β-2-glycoprotein; 9. hemopexin; and 10. alpha-1B-glycoprotein. (b) 2D-PAGE comparison of proteins that were further validated by immunoblots.

Table 1 Regulated Proteins in Cerebrospinal Fluid of Depressed Patients

No.	Protein name	Gene name	SwissProt_ID	Synonym	Method	2D-stain	Phospho-rylation	MW	Regulation in disease
1	Beta-2-glycoprotein 1	*APOH*	P02749	B2GPI; apolipoprotein H; Apo-H	2D	Coomassie	No	38298	Down
2	Vitamin D-binding protein	*GC*	P02774	DBP; VDB	2D + MSP	Coomassie	No	52964	Down
3	Transthyretin	*TTR*	P02766	Prealbumin; TBPA; TTR	2D + MSP	Coomassie	No	15887	Down
4	Prostaglandin-D2 synthase	*PTGDS*	P41222	PGDS; lipocalin-type prostaglandin-D synthase; beta-trace protein	2D	Coomassie	No	21029	Down
5	Alpha-1B-glycoprotein	*A1BG*	P04217	Alpha-1-B glycoprotein	2D + MSP	Coomassie	No	54273	Up in 2D/down in MSP
6	Heterogeneous nuclear ribonucleoprotein H	*HNRNPH1*	P31943	hnRNP H	2D	Phospho	Yes	49229	Down
7	Pigment epithelium-derived factor	*SERPINF1*	P36955	PEDF; serpin-F1; EPC-1	2D + MSP	Coomassie	Yes	46342	Up in 2D/down in MSP
8	Heat-shock cognate 71-kDa protein	*HSPA8*	P11142	Heat-shock 70-kDa protein 8	2D	Phospho	Yes	70898	Down
9	Apolipoprotein E	*APOE*	P02649	Apo-E	2D + MSP	Coomassie + Phospho	Yes	36154	Down
10	Hemopexin	*HPX*	P02790	Beta-1B-glycoprotein	2D	Coomassie	No	51676	Up
11	Cystatin-C	*CST3*	P01034	Cystatin-3; neuroendocrine basic polypeptide; gamma-trace	2D + MSP	Coomassie	No	15799	Down in 2D/up in MSP
12	Fructose-bisphosphate aldolase C	*ALDOC*	P09972	Brain-type aldolase	2D	Phospho	Yes	39456	Down
13	cAMP-dependent protein kinase catalytic subunit α	*PRKACA*	P17612	PKA C-alpha	2D	Phospho	Yes	40590	Down
14	Alpha-1-acid glycoprotein 1	*ORM1*	P02763	AGP 1; orosomucoid-1; OMD 1	MSP	—	No	23512	Down
15	Alpha-2-macroglobulin	*A2M*	P01023	Alpha-2-M; C3 and PZP-like alpha-2-macroglobulin domain-containing protein 5	MSP	—	No	163292	Down
16	Amyloid beta A4 protein	*APP*	P05067	ABPP; APPI; APP; PreA4; cerebral vascular amyloid peptide	MSP	—	Yes	86943	Up
17	Angiotensinogen	*AGT*	P01019	Serpin A8; angiotensin-1; angiotensin-2; angiotensin-3	MSP	—	No	53154	Up
18	Apolipoprotein A-I	*APOA1*	P02647	Apo-AI; apolipoprotein A1; apolipoprotein A-I (1-242)	MSP	—	Yes	30778	Down
19	Apolipoprotein A-II	*APOA2*	P02652	Apo-AII; apolipoprotein A2; Apolipoprotein A-II (1-76)	MSP	—	Yes	11175	Down
20	Apolipoprotein D	*APOD*	P05090	Apo-D	MSP	—	No	21276	Down
21	Beta-2-microglobulin	*B2M*	P61769	Beta-2-microglobulin form pI 5.3	MSP	—	No	13715	Up
22	CD44 antigen	*CD44*	P16070	Phagocytic glycoprotein I; PGP-I; HUTCH-I; extracellular matrix receptor III; ECMR-III	MSP	—	Yes	81554	Down
23	Clusterin	*CLU*	P10909	Complement-associated protein SP-40,40; CLI; NA1/NA2; apolipoprotein J	MSP	—	Yes	52495	Down
24	Collagen alpha-1 (VI) chain	*COL6A1*	P12109	COL6A1	MSP	—	No	108529	Down

Table 1 Continued

No.	Protein name	Gene name	SwissProt_ID	Synonym	Method	2D-stain	Phospho-rylation	MW	Regulation in disease
25	Complement C3	*C3*	P01024	C3 and PZP-like alpha-2-macroglobulin domain-containing protein 1	MSP	—	Yes	187148	Down
26	Epididymal secretory protein E1	*NPC2*	P61916	Niemann-Pick disease type C2 protein; Human epididymis-specific protein 1	MSP	—	no	16570	Down
27	Gelsolin	*GSN*	P06396	Actin-depolymerizing factor; ADF; brevin; AGEL	MSP	—	Yes	85698	Down
28	Glutamate carboxypeptidase-like protein 2	*CNDP1*	Q96KN2	Carnosine dipeptidase 1; beta-ala-his dipeptidase	MSP	—	No	56692	Down
29	Histidine-rich glycoprotein	*HRG*	P04196	Histidine–proline-rich glycoprotein; HPRG	MSP	—	No	59578	Up
30	Homeobox protein Hox-D12	*HOXD12*	P35452	Homeobox protein Hox-4H	MSP	—	No	29031	Up
31	Neurofilament heavy polypeptide	*NEFH*	P12036	NF-H; neurofilament triplet H protein; 200 kDa neurofilament protein	MSP	—	Yes	112479	Up
32	Neuronal cell adhesion molecule	*NRCAM*	Q92823	Nr-CAM; NgCAM-related cell adhesion molecule; Ng-CAM-related; hBravo	MSP	—	Yes	143890	Up
33	Peroxisomal targeting signal 1 receptor	*PEX5*	P50542	PTS1 receptor; PTS1R; peroxisome receptor 1; PTS1-BP; peroxin-5	MSP	—	No	70865	Down
34	Plasma protease C1 inhibitor	*SERPING1*	P05155	C1 Inh; C1Inh; C1 esterase inhibitor; C1-inhibiting factor; Serpin G1	MSP	—	No	55154	Down
35	Platelet-activating factor acetylhydrolase	*PLA2G7*	Q13093	PAF acetylhydrolase; PAF 2-acylhydrolase; LDL-associated phospholipase A2	MSP	—	No	50077	Down
36	Prothrombin	*F2*	P00734	Coagulation factor II; activation peptide fragment 1 or 2	MSP	—	No	70037	Down
37	Secretogranin-1	*CHGB*	P23389	Secretogranin I; SgI; chromogranin-B; CgB; secretogranin-1 (476-566)	MSP	—	Yes	73340	Down
38	Superoxide dismutase [Cu-Zn]	*SOD1*	P00441	EC-SOD; Cu-Zn-SOD	MSP	—	Yes	15936	Down
39	Vitronectin	*VTN*	P04004	Serum-spreading factor; S-protein; V75; somatomedin-B	MSP	—	Yes	54306	Down

Abbreviations: 2D, two-dimensional gel electrophoresis; APP, amyloid precursor protein; DBP = vitamin D-binding protein; MSP, mass spectrometry-based peptide profiling; PEDF = pigment epithelium derived factor; PGDS, prostaglandin D2 synthase.

Table 2 Molecular Function of Regulated Proteins in Depression

No.	Protein name	Biological process	Molecular function	Subcellular location
1	Beta-2-glycoprotein 1	Negative regulation of angiogenesis and blood coagulation; plasminogen activation	Eukaryotic cell surface binding; glycoprotein and heparin binding; lipoprotein lipase activator activity; phospholipid binding	Secreted
2	Vitamin D-binding protein	Vitamin transport	Actin binding; vitamin D binding; vitamin transporter activity	Secreted
3	Transthyretin	Thyroid hormone-binding; transport of thyroxine from the bloodstream to the brain	Hormone activity	Secreted, cytoplasm
4	Prostaglandin-D2 synthase	Prostaglandin biosynthetic process; regulation of circadian sleep/wake cycle	Prostaglandin-D synthase activity; retinoid binding; transporter activity	rER, nucleus membrane, golgi apparatus, cytoplasm, perinuclear region, secreted
5	Alpha-1B-glycoprotein	Plasma protein of unknown function	Function unknown	Secreted
6	Heterogeneous nuclear ribonucleoprotein H	Nuclear mRNA splicing	Nucleotide binding; poly (U) RNA binding; protein binding	Nucleus, nucleoplasm
7	Pigment epithelium-derived factor	Cell proliferation; negative regulation of angiogenesis; positive regulation of neurogenesis	Serine-type endopeptidase inhibitor activity	Secreted, melanosome
8	Heat shock cognate 71 kDa protein	Membrane organization; post-Golgi vesicle-mediated transport; protein folding	ATP binding; ATPase activity, coupled; protein binding	Cytoplasm, melanosome
9	Apolipoprotein E	Anti-apoptosis; cGMP-mediated signaling; cholesterol homeostasis and transport; axon extension and neuronal synaptic plasticity; response to reactive oxygen species; synaptic transmission	Antioxidant activity; beta-amyloid, heparin, LD- and VLDL- receptor binding; metal chelating activity; phospholipid binding; tau protein binding	Secreted
10	Hemopexin	Cellular iron ion homeostasis; heme transport	Heme transporter activity; iron ion binding	Secreted
11	Cystatin-C	Defense response; fibril organization; negative regulation of blood vessel remodeling, histolysis and collagen catabolic processes	Beta-amyloid binding; cysteine-type endopeptidase inhibitor activity; protease binding	Secreted
12	Fructose-bisphosphate aldolase C	Fructose 1,6-bisphosphate metabolic process; glycolysis	Cytoskeletal protein binding; fructose-bisphosphate aldolase activity	Unknown
13	cAMP-dependent protein kinase catalytic subunit α	Activation of protein kinase A activity; hormone-mediated signaling pathway; protein kinase cascade	ATP binding; cAMP-dependent protein kinase and kinase inhibitor activity; protein kinase binding	Cytoplasm, nucleus
14	Alpha-1-acid glycoprotein 1	Acute-phase response; regulation of immune system process	Protein binding	Secreted
15	Alpha-2-macroglobulin	Negative regulation of complement activation	Enzyme binding; interleukin-1 and -8 binding; serine-type endopeptidase inhibitor activity; tumor necrosis factor binding	secreted
16	Amyloid beta A4 protein	Adult locomotory behavior; axon cargo transport; cell adhesion; cellular copper ion homeostasis; dendrite development; ionotropic glutamate receptor signaling pathway; mating behavior; mRNA polyadenylation; neuron apoptosis and remodeling; Notch signaling pathway; regulation of synapse structure and activity; regulation of translation	Acetylcholine receptor binding; DNA, heparin and metal ion binding; serine-type endopeptidase inhibitor activity	Membrane, Single-pass type I membrane protein; membrane, clathrin-coated pit
17	Angiotensinogen	Activation of phospholipase C; cell-cell and G-protein signaling, coupled to cGMP; NO mediated signal transduction; apoptosis; positive regulation of NF-kappaB transcription	Acetyltransferase activator activity; growth factor activity; serine-type endopeptidase inhibitor activity	Secreted
18	Apolipoprotein A-I	Cdc42 protein signal transduction; cholesterol homeostasis; G-protein coupled receptor protein signaling	Apolipoprotein A-I receptor binding; beta-amyloid binding; cholesterol binding; cholesterol transporter activity; HDL-receptor binding	Secreted
19	Apolipoprotein A-II	Cholesterol efflux; cholesterol homeostasis; high-density lipoprotein particle assembly	Apolipoprotein receptor binding; cholesterol binding; HDL-receptor binding; lipase inhibitor activity; phosphatidylcholine binding	Secreted
20	Apolipoprotein D	Lipid metabolic process; transport	Lipid transporter activity; protein binding; retinoid binding	Secreted

Table 2 Continued

No.	Protein name	Biological process	Molecular function	Subcellular location
21	Beta-2-microglobulin	Antigen processing; immune response	Protein binding	Secreted
22	CD44 antigen	Cell–cell and cell–matrix adhesion; negative regulation of apoptosis and DNA damage response; positive regulation of ERK1 and ERK2 cascade	Collagen binding; hyaluronic acid binding; receptor activity; receptor signaling protein activity	Single-pass type I membrane protein
23	Clusterin	Complement activation; innate immune response; lipid metabolic process; response to virus	Misfolded protein binding	Secreted
24	Collagen alpha-1 (VI) chain	Cell adhesion	Platelet-derived growth factor binding	Secreted, extracellular space and matrix
25	Complement C3	Complement activation; G-protein coupled receptor protein signaling; positive regulation VEGF; signal transduction	Endopeptidase inhibitor activity; receptor binding	Secreted
26	Epididymal secretory protein E1	Cholesterol efflux and homeostasis; glycolipid and intracellular cholesterol transport; phospholipid transport	Cholesterol binding; enzyme binding	Secreted
27	Gelsolin	Actin filament polymerization and severing; barbed-end actin filament capping	Actin binding; calcium ion binding	Isoform 2: cytoplasm, cytoskeleton
28	Beta-Ala-His dipeptidase	Proteolysis	Carboxypeptidase activity; metal ion binding; metallopeptidase activity; protein binding	Secreted
29	Histidine-rich glycoprotein	Blood coagulation	Cysteine-type endopeptidase inhibitor activity; heparin binding	Secreted
30	Homeobox protein Hox-D12	Regulation of transcription	Sequence-specific DNA binding; transcription factor activity	Nucleus
31	Neurofilament heavy polypeptide	Cell death; nervous system development	Function unknown	Cytoskeleton
32	Neuronal cell adhesion molecule	Axonal fasciculation; cell-cell adhesion; central nervous system development; clustering of voltage-gated sodium channels; neuron migration and differentiation; regulation of axon extension; synapse assembly	Ankyrin binding	Cell membrane; single-pass type I membrane protein
33	Peroxisomal targeting signal 1 receptor	Protein import into peroxisome matrix; protein targeting to peroxisome; protein tetramerization; protein transport	Peroxisome matrix targeting signal-1 binding; protein C-terminus binding; protein N-terminus binding	Cytoplasm, peroxisome membrane; peripheral membrane protein
34	Plasma protease C1 inhibitor	Blood circulation; blood coagulation; complement activation, classical pathway; innate immune response	Protein binding; serine-type endopeptidase inhibitor activity	Secreted
35	Platelet-activating factor acetylhydrolase	Inflammatory response; lipid catabolic process	1-alkyl-2-acetylglycerophosphocholine esterase activity; phospholipid binding	Secreted, extracellular space
36	Prothrombin	Activation of caspase activity; acute-phase response; cell surface receptor linked signaling pathway; negative regulation of astrocyte differentiation	Calcium ion binding; growth factor activity; serine-type endopeptidase activity; thrombospondin receptor activity	Secreted, extracellular space
37	Secretogranin-1	Neuroendocrine secretory granule protein; potential precursor for other biologically active peptides	Function unknown	Secreted
38	Superoxide dismutase [Cu-Zn]	Activation of MAPK; cell aging; cellular iron homeostasis; glutathione metabolic process; myelin maintenance in peripheral nervous system; negative regulation of neuron apoptosis; neurofilament cytoskeleton organization; regulation of T cell differentiation; removal of superoxide radicals; response to axon injury; response to drugs	Chaperone binding; copper and zinc ion binding; protein homodimerization activity; superoxide dismutase activity	Cytoplasm
39	Vitronectin	Cell adhesion mediated by integrin; cell-matrix adhesion; immune response; blood coagulation; regulation of endopeptidase activity	Heparin binding; integrin binding; scavenger receptor activity	Secreted, extracellular space

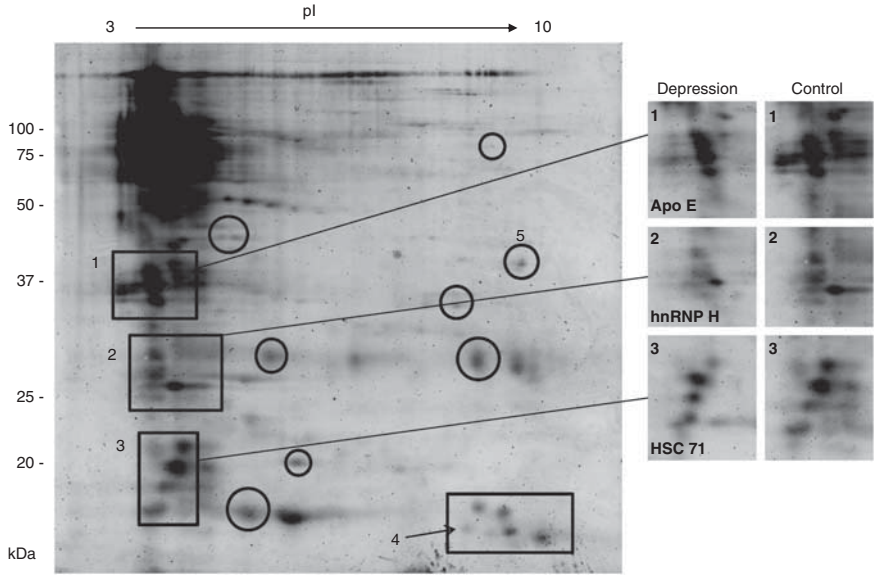

Figure 2 ProQ Diamond-stained two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) gel image of cerebrospinal fluid (CSF) from a control person. Differentially phosphorylated proteins and isoforms between disease and control groups are circled and identified proteins are numbered as 1. apolipoprotein E; 2. heterogeneous nuclear ribonucleoprotein H; 3. heat-shock cognate 71 kDa protein; 4. cAMP-dependent protein kinase catalytic subunit alpha; 5. fructose-bisphosphate aldolase C.

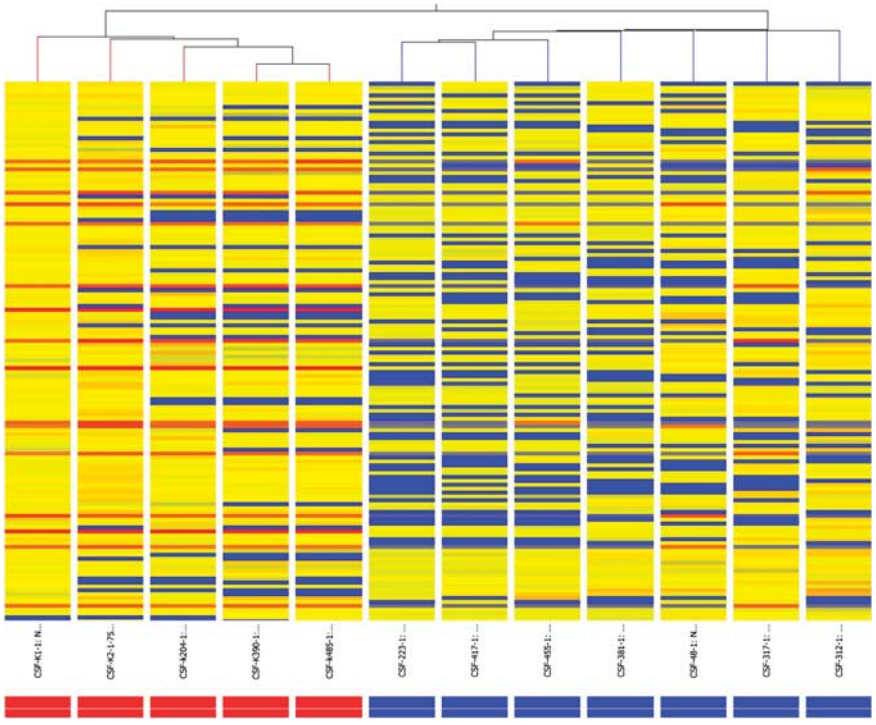

Figure 3 Hierarchical clustering tree for molecular features extracted from the liquid chromatography mass spectrometry (LCMS) analysis of depressed and control cerebrospinal fluid (CSF) samples. The clustering analysis can be used to monitor the reproducibility of the analysis (technical replicates), as well as the similarity of the biological replicates. The clustering analysis was performed for the feature list from *t*-test of features found in 100% of at least one group.

To validate the quantitative differences detected by 2D-PAGE and LCMS-based differential expression analysis, we performed immunoblotting for four representative proteins, using CSF samples from the same and independent individuals. PGDS, ApoE, PEDF, and cystatin C were analyzed. SDS gel electrophoresis does not allow to distinguish protein isoforms of similar molecular weights since they tend to run as one band. Except for cystatin C and PEDF, no differences in expression were found in the SDS gel-based western blot between depressed and control CSF samples. We therefore next performed IEF-based immunoblots that allow the separation of proteins with

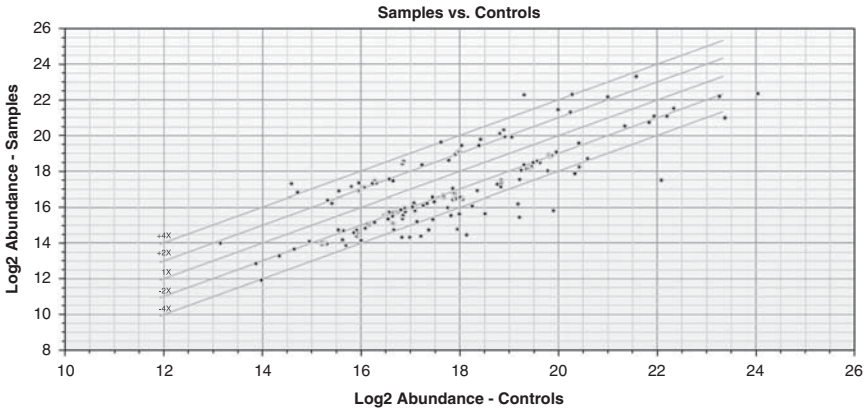

Figure 4 Log 2 plot of molecular feature abundance from cerebrospinal fluid (CSF) samples of depressed and control individuals. Features that are significantly upregulated or downregulated are shown.

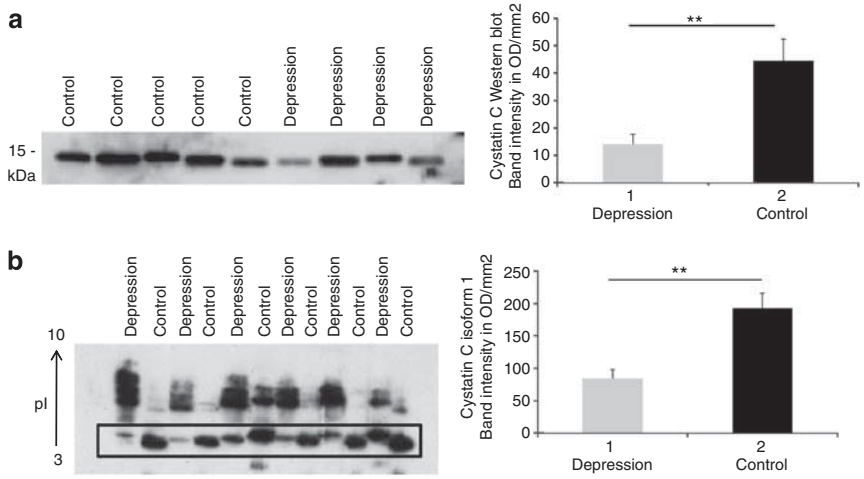

Figure 5 Cystatin C SDS gel western blot (a) and isoelectric focusing (IEF) western blot (b). Total cystatin C protein, as well as the most acidic isoform of the protein show higher expression levels in cerebrospinal fluid (CSF) from control persons ((a) + (b): $*p < 0.01$).

different isoelectric points. Using this method, we were able to quantitate individual protein isoforms and confirm the 2D-PAGE results. Figures 5, 6 and 7 show the results of the SDS- and IEF-immunoblots for cystatin C, PEDF, and PGDS, respectively.

DISCUSSION

The precise pathophysiological mechanisms leading to the clinical phenotype of a major depression still remain unclear (Krishnan and Nestler, 2010; aan het Rot *et al*, 2009; Lee *et al*, 2010). The availability of biological markers would ultimately allow to account for inter-individual variance in etiology and phenotype of depressive episodes in different individuals, and thereby improve personalized therapeutic strategies and outcome (Figure 8; Luo *et al*, 2010; Turck *et al*, 2005). The comparison of the CSF proteome of depressed patients and controls with the help of 2D-PAGE and LCMS-based differential expression analyses revealed a number of proteins that showed altered expression levels between the two groups. Interestingly, two of the identified proteins, ApoE and cystatin C, have already been implicated in other CNS diseases (Levy *et al*, 2006). Proteins that were found to be differentially regulated in depression also included PEDF, PGDS, transthyretin precursor, alpha-1B-glycoprotein, DBP, β-2-glycoprotein, and hemopexin. Among the protein markers that were only identified by LCMS-based differential expression, the neurofilament heavy polypeptide and neuronal cell adhesion molecule (NCAM) are of particular interest. Neurofilament heavy polypeptide is a protein involved in nervous system development and cell death. Neurofilaments have been discussed as biomarker candidates for neurodegenerative diseases (Petzold *et al*, 2009; Pijnenburg *et al*, 2007). NCAM is a protein involved in neuronal growth and repair (Gnanapavan *et al*, 2010). The protein's expression was found to be altered in schizophrenic patients, where it was detected as its variable alternative-spliced exon isoform in CSF (Vawter, 2000; Vawter *et al*, 2000). A selective increased expression of primarily the 120 kDa NCAM isoform has been reported in CSF from bipolar mood disorder type I and recurrent unipolar major depression patients (Poltorak *et al*, 1996). In our study, peptides of the 140-kDa NCAM isoform were found to be elevated in CSF of depressed patients. These are probably derived from the variable alternative-spliced exon isoform, which is in good agreement with previous observations.

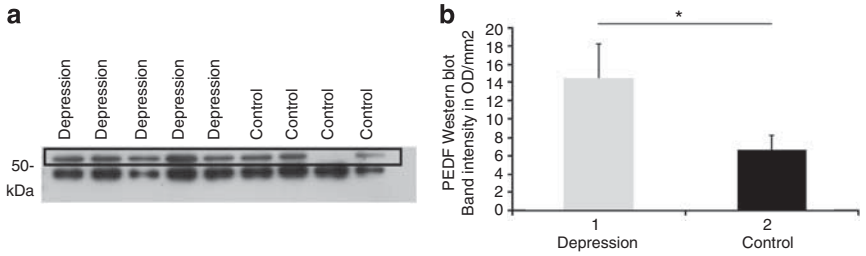

Figure 6 (a) Pigment epithelium-derived factor western blot. Two bands are detectable due to different molecular weights of the isoforms. The upper band isoform showed a significant increase in cerebrospinal fluid (CSF) of depressed individuals compared with controls (b), $*p < 0.05$.

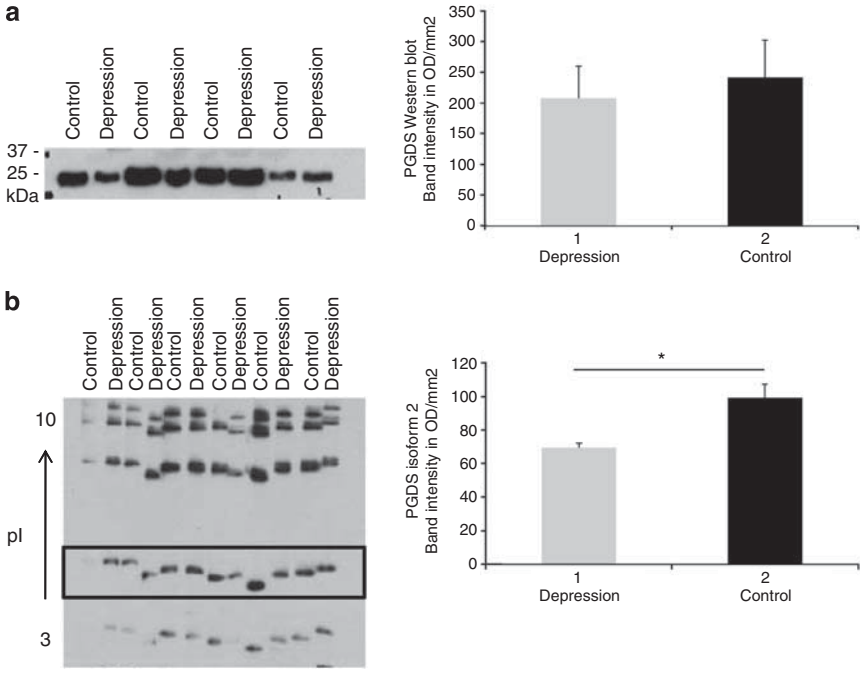

Figure 7 Prostaglandin D2 synthase SDS gel western blot (a) and isoelectric focusing (IEF) western blot. (b) Statistical analysis of western blot band intensities did not reveal a significant difference between depressed patients and controls ((a), $p = 0.134$), whereas analysis of individual isoforms showed a significant decrease of isoform 2 in depressed patients ((b), $*p < 0.05$).

A set of four proteins (PEDF, ApoE, PGDS, cystatin C) known to have important functions in brain metabolism were chosen for the validation phase of our biomarker study.

PEDF is a neurotrophic glycoprotein, which is a member of the serine protease inhibitor gene family. In neuronal cells, PEDF exhibits neuroprotective effects that seem to involve the activation of cyclic AMP-responsive element binding protein (CREB) and nuclear factor-κB (Tombran-Tink and Barnstable, 2003; Yabe *et al*, 2005). CREB has been implicated as a molecular state marker for depression and for the response to antidepressant treatment (Yamada *et al*, 2003). In this study, an increase of PEDF expression was detectable in 2D-PAGE of CSF from depressed patients. PEDF is known to activate CREB in primary neuronal cultures (Yabe *et al*, 2005). In depression, this could represent a compensation mechanism to counteract endogenous pro-apoptotic stress.

Cystatin C is a known cysteine proteinase inhibitor, which is produced by most cells including neurons (Levy *et al*, 2006). In AD, cystatin C co-localizes with amyloid-β deposits and inhibits its fibril formation. It was shown that overexpression of cystatin C in brains of amyloid precursor protein transgenic mice reduces cerebral amyloid-β-deposition (Kaeser *et al*, 2007). The CST3 Thr25 allele of CST3, which encodes cystatin C, leads to reduced cystatin C levels and promotes susceptibility to AD. In our study, we found a decrease of cystatin C, especially the pI 7.8 isoform, in 2D-PAGE of CSF from depressed patients. As decreased cystatin C levels in CSF could reflect a higher susceptibility to a neurodegenerative disease like AD, reduced levels in depressed patients could possibly indicate the presence of cognitive symptoms in depression. However, we do not have any information about amyloid plaque load in the brains of our study patients to allow correlation with cystatin C levels. PGDS, a glycoprotein with a molecular weight of approximately 26 kDa, synthesizes prostaglandin D2 (PGD2), which is one of the most potent endogenous sleep-inducing substances (Yamashima *et al*, 1997). Beside sleep induction, PGD2 is involved in sedation, nociception, and release of hormones (Eguchi *et al*, 1999; Sri Kantha *et al*, 1994; Urade and Hayaishi, 1999). Sleep disturbances

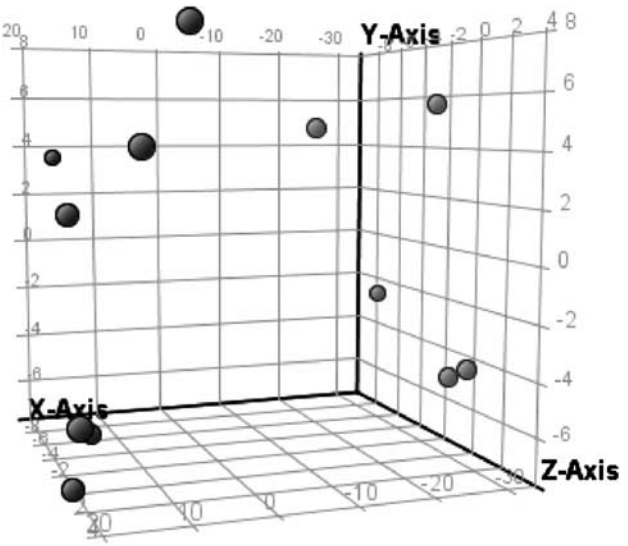

Figure 8 3D principal component analysis (PCA) for feature list from *t*-test of features found in 100% of at least one group allows patient categorization.

belong to the key symptoms of major depression. Especially, slow-wave sleep seems to be reduced in depression. PGD2 infusion into the subarachnoidal space in rat brains preferentially induced slow-wave sleep (Matsumura *et al*, 1994). We detected a decrease of PGDS isoforms in CSF of depressed patients, which could contribute to bemoaned sleep disturbances.

Apo E has an important role in lipoprotein metabolism. A homozygote constellation of the E2 allele is linked to the hyperlipoproteinemia type III, a disease that leads to an increased risk for cardiovascular diseases like stroke or heart attack (Brummer *et al*, 1998). We detected an ApoE isoform (pI ~ 6.3) that is more abundant in CSF of controls compared with depressed individuals, and probably represents an E2 or E3 isoform. The observed expression pattern could be due to interindividual differences of isoform expression according to different individual allele constellations. On the other hand, major depression has been repeatedly linked to the presence of a metabolic syndrome in afflicted persons (Jakovljevic *et al*, 2007). Patients with psychiatric disorders are known to have an increased risk of developing the metabolic syndrome, and patients with a metabolic syndrome are at a higher risk of developing a depressive episode (Capuron *et al*, 2008; Richter *et al*, 2010; Vogelzangs *et al*, 2007). The underlying mechanism linking both syndromes are still not identified, but differences in Apo E isoform expression could be one connecting link.

Next to CSF protein level differences, we also investigated CSF protein phosphorylation by using a specific gel stain. In the central nervous system, protein phosphorylation and second messenger mechanisms are linked to neurotransmission (Popoli *et al*, 2000), and altered CSF protein phosphorylation patterns have been discussed as a marker for neurodegenerative diseases (Henneman *et al*, 2009; Shaw *et al*, 2009). With the help of 2D-PAGE, we found several CSF protein phosphorylation differences between depressed patients and controls, of which five were identified (Figure 2). If and how these differences contribute to the phenotype of a major depression needs to be analyzed in future studies.

In conclusion, we detected several protein expression and phosphorylation differences in the CSF proteome of depressed patients compared with controls. With the help of mass spectrometry, we were able to identify the majority of them. A subset of the identified proteins has important roles in brain metabolism, neuronal protection, and differentiation or neurotransmission. Others, eg, PGDS, could be related to certain clinical aspects of major depression such as sleep disturbances.

The herein reported results support the notion that markers can be generated to identify subgroups of depressed patients who could share a causal mechanism. Such biomarkers are needed to match the right patient with the right antidepressant drug. Proteomics provides a promising tool for the detection of disease-specific markers for depression, and thereby has the ability to account for inter-individual molecular differences between patients and an adjusted and improved pharmacological intervention.

ACKNOWLEDGEMENTS

This study was supported by the German Federal Ministry of Science and Education (NGFN MooDS).

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

aan het Rot M, Mathew SJ, Charney DS (2009). Neurobiological mechanisms in major depressive disorder. *CMAJ* **180**: 305–313.

Brummer D, Evans D, Berg D, Greten H, Beisiegel U, Mann WA (1998). Expression of type III hyperlipoproteinemia in patients homozygous for apolipoprotein E-2 is modulated by lipoprotein lipase and postprandial hyperinsulinemia. *J Mol Med* **76**: 355–364.

Brunner J, Bronisch T, Uhr M, Ising M, Binder E, Holsboer F *et al* (2005). Proteomic analysis of the CSF in unmedicated patients with major depressive disorder reveals alterations in suicide attempters. *Eur Arch Psychiatry Clin Neurosci* **255**: 438–440.

Capuron L, Su S, Miller AH, Bremner JD, Goldberg J, Vogt GJ *et al* (2008). Depressive symptoms and metabolic syndrome: is inflammation the underlying link? *Biol Psychiatry* **64**: 896–900.

Carpenter LL, Heninger GR, Malison RT, Tyrka AR, Price LH (2004). Cerebrospinal fluid interleukin (IL)-6 in unipolar major depression. *J Affect Disord* **79**: 285–289.

Eguchi N, Minami T, Shirafuji N, Kanaoka Y, Tanaka T, Nagata A *et al* (1999). Lack of tactile pain (allodynia) in lipocalin-type prostaglandin D synthase-deficient mice. *Proc Natl Acad Sci USA* **96**: 726–730.

Geracioti TD, Loosen PT, Orth DN (1997). Low cerebrospinal fluid corticotropin-releasing hormone concentrations in eucortisolemic depression. *Biol Psychiatry* **42**: 165–174.

Gnanapavan S, Grant D, Illes-Toth E, Lakdawala N, Keir G, Giovannoni G (2010). Neural cell adhesion molecule–description of a CSF ELISA method and evidence of reduced levels in selected neurological disorders. *J Neuroimmunol* **225**: 118–122.

Henneman WJ, Vrenken H, Barnes J, Sluimer IC, Verwey NA, Blankenstein MA *et al* (2009). Baseline CSF p-tau levels

independently predict progression of hippocampal atrophy in Alzheimer disease. *Neurology* **73**: 935–940.

Hennings JM, Owashi T, Binder EB, Horstmann S, Menke A, Kloiber S *et al* (2009). Clinical characteristics and treatment outcome in a representative sample of depressed inpatients – findings from the Munich Antidepressant Response Signature (MARS) project. *J Psychiatr Res* **43**: 215–229.

Ising M, Lucae S, Binder EB, Bettecken T, Uhr M, Ripke S *et al* (2009). A genomewide association study points to multiple loci that predict antidepressant drug treatment outcome in depression. *Arch Gen Psychiatry* **66**: 966–975.

Jacob AM, Turck CW (2008). Detection of post-translational modifications by fluorescent staining of two-dimensional gels. *Methods Mol Biol* **446**: 21–32.

Jakovljevic M, Crncevic Z, Ljubicic D, Babic D, Topic R, Saric M (2007). Mental disorders and metabolic syndrome: a fatamorgana or warning reality? *Psychiatr Danub* **19**: 76–86.

Jastorff AM, Haegler K, Maccarrone G, Holsboer F, Weber F, Ziemssen T *et al* (2009). Regulation of proteins mediating neurodegeneration in experimental autoimmune encephalomyelitis and multiple sclerosis. *Proteomics Clin Appl* **3**: 1273–1287.

Jokinen J, Samuelsson M, Nordstrom AL, Nordstrom P (2008). HPT axis, CSF monoamine metabolites, suicide intent and depression severity in male suicide attempters. *J Affect Disord* **111**: 119–124.

Kaeser SA, Herzig MC, Coomaraswamy J, Kilger E, Selenica ML, Winkler DT *et al* (2007). Cystatin C modulates cerebral beta-amyloidosis. *Nat Genet* **39**: 1437–1439.

Krishnan V, Nestler EJ (2010). Linking Molecules to Mood: New Insight into the biology of depression. *Am J Psychiatry* **167**: 1305–1320.

Lee S, Jeong J, Kwak Y, Park SK (2010). Depression research: where are we now? *Mol Brain* **3**: 8.

Levy E, Jaskolski M, Grubb A (2006). The role of cystatin C in cerebral amyloid angiopathy and stroke: cell biology and animal models. *Brain Pathol* **16**: 60–70.

Lottspeich F, Kellermann J, Keidel EM (2010). Molecular biology tools: proteomics techniques in biomarker discovery. *Scand J Clin Lab Invest Suppl* **242**: 19–22.

Luo L, Rodriguez E, Jerbi K, Lachaux JP, Martinerie J, Corbetta M *et al* (2010). Ten years of Nature Reviews Neuroscience: insights from the highly cited. *Nat Rev Neurosci* **11**: 718–726.

Maccarrone G, Milfay D, Birg I, Rosenhagen M, Holsboer F, Grimm R *et al* (2004). Mining the human cerebrospinal fluid proteome by immunodepletion and shotgun mass spectrometry. *Electrophoresis* **25**: 2402–2412.

Matsumura H, Nakajima T, Osaka T, Satoh S, Kawase K, Kubo E *et al* (1994). Prostaglandin D2-sensitive, sleep-promoting zone defined in the ventral surface of the rostral basal forebrain. *Proc Natl Acad Sci USA* **91**: 11998–12002.

Petzold A, Thompson EJ, Keir G, Quinn N, Holmberg B, Dizdar N *et al* (2009). Longitudinal one-year study of levels and stoichiometry of neurofilament heavy and light chain concentrations in CSF in patients with multiple system atrophy. *J Neurol Sci* **279**: 76–79.

Pijnenburg YA, Janssen JC, Schoonenboom NS, Petzold A, Mulder C, Stigbrand T *et al* (2007). CSF neurofilaments in frontotemporal dementia compared with early onset Alzheimer's disease and controls. *Dement Geriatr Cogn Disord* **23**: 225–230.

Poltorak M, Frye MA, Wright R, Hemperley JJ, George MS, Pazzaglia PJ *et al* (1996). Increased neural cell adhesion molecule in the CSF of patients with mood disorder. *J Neurochem* **66**: 1532–1538.

Popoli M, Brunello N, Perez J, Racagni G (2000). Second messenger-regulated protein kinases in the brain: their functional role and the action of antidepressant drugs. *J Neurochem* **74**: 21–33.

Richter N, Juckel G, Assion HJ (2010). Metabolic syndrome: a follow-up study of acute depressive inpatients. *Eur Arch Psychiatry Clin Neurosci* **260**: 41–49.

Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D *et al* (2006). Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry* **163**: 1905–1917.

Shaw LM, Vanderstichele H, Knapik-Czajka M, Clark CM, Aisen PS, Peterson RC *et al* (2009). Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Ann Neurol* **65**: 403–413.

Sri Kantha S, Matsumura H, Kubo E, Kawase K, Takahata R, Serhan CN *et al* (1994). Effects of prostaglandin D2, lipoxins and leukotrienes on sleep and brain temperature of rats. *Prostaglandins Leukot Essent Fatty Acids* **51**: 87–93.

Sullivan GM, Oquendo MA, Huang YY, Mann JJ (2006). Elevated cerebrospinal fluid 5-hydroxyindoleacetic acid levels in women with comorbid depression and panic disorder. *Int J Neuropsychopharmacol* **9**: 547–556.

Taurines R, Dudley E, Grassl J, Warnke A, Gerlach M, Coogan AN *et al* (2011). Proteomic research in psychiatry. *J Psychopharmacol* **25**: 151–196.

Tombran-Tink J, Barnstable CJ (2003). PEDF: a multifaceted neurotrophic factor. *Nat Rev Neurosci* **4**: 628–636.

Turck CW, Maccarrone G, Sayan-Ayata E, Jacob AM, Ditzen C, Kronsbein H *et al* (2005). The quest for brain disorder biomarkers. *J Med Invest* **52**(Suppl): 231–235.

Urade Y, Hayaishi O (1999). Prostaglandin D2 and sleep regulation. *Biochim Biophys Acta* **1436**: 606–615.

Vawter MP (2000). Dysregulation of the neural cell adhesion molecule and neuropsychiatric disorders. *Eur J Pharmacol* **405**: 385–395.

Vawter MP, Frye MA, Hemperly JJ, VanderPutten DM, Usen N, Doherty P *et al* (2000). Elevated concentration of N- CAM VASE isoforms in schizophrenia. *J Psychiatr Res* **34**: 25–34.

Vogelzangs N, Suthers K, Ferrucci L, Simonsick EM, Ble A, Schrager M *et al* (2007). Hypercortisolemic depression is associated with the metabolic syndrome in late-life. *Psychoneuroendocrinology* **32**: 151–159.

Yabe T, Kanemitsu K, Sanagi T, Schwartz JP, Yamada H (2005). Pigment epithelium-derived factor induces pro-survival genes through cyclic AMP- responsive element binding protein and nuclear factor kappa B activation in rat cultured cerebellar granule cells: Implication for its neuroprotective effect. *Neuroscience* **133**: 691–700.

Yamada S, Yamamoto M, Ozawa H, Riederer P, Saito T (2003). Reduced phosphorylation of cyclic AMP-responsive element binding protein in the postmortem orbitofrontal cortex of patients with major depressive disorder. *J Neural Transm* **110**: 671–680.

Yamashima T, Sakuda K, Tohma Y, Yamashita J, Oda H, Irikura D *et al* (1997). Prostaglandin D synthase (beta-trace) in human arachnoid and meningioma cells: roles as a cell marker or in cerebrospinal fluid absorption, tumorigenesis, and calcification process. *J Neurosci* **17**: 2376–2382.

Yuan X, Russell T, Wood G, Desiderio DM (2002). Analysis of the human lumbar cerebrospinal fluid proteome. *Electrophoresis* **23**: 1185–1196.

Zougman A, Pilch B, Podtelejnikov A, Kiehntof M, Schnabel C, Kumar C *et al* (2008). Integrated analysis of the cerebrospinal fluid peptidome and proteome. *J Proteome Res* **7**: 386–399.

Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)