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Proteomic analysis of the cerebrospinal fluid of patients with schizophrenia

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Summary. We applied proteomics technologies to analyze the cerebrospinal fluid of patients with schizophrenia. Such an analysis can result in the identification of proteins, which may play a role in the disease progress and thus lead to the discovery of clues of the etiology of schizophrenia. Cerebrospinal fluid from patients and controls was analyzed by two-dimensional gels and the proteins were identified by matrix-assisted laser desorption ionization mass spectrometry (MS) in the MS and MS/MS mode. 54 different gene products were identified, which were mainly plasma proteins. The level of apolipoprotein A-IV was significantly decreased in the schizophrenic patients compared to that in the controls. Little is known about the function of this apolipoprotein in the central nervous system. The levels of certain other proteins, like haptoglobin, fibrinogen, complement component 3, and Gc-globulin, were altered in the disease group as well, however, the changes did not reach a statistical significance.

Keywords: Apolipoprotein A-IV – Central nervous system – Cerebrospinal fluid – Proteomics – Schizophrenia

Introduction

Schizophrenia is a major mental disorder, with features of split of thoughts and behavior. The patients are incompatible with mental activity and environment. The inheritance pattern of the disease does not seem to be of Mendelian pattern. Recent view of the genetic transmission of schizophrenia is that of a multifactorial threshold model (O'Donovan and Owen, 1999; Thaker and Carpenter, 2001). Recently two strategies have dominated attempts to discover genes that underlie schizophrenia: linkage-disequilibrium studies and association studies. However, many conflicting reports have been published partially because of the disadvantage of both approaches whose establishment was based on monogenic disease and because of the complexity of the disease itself.

Compared to DNA analysis, which does not seem to be sufficient for the dissection of schizophrenia at the molecular level at the present time, and mRNA expression-based analysis, whose primary human material, namely brain tissue in schizophrenic research, is largely inaccessible since post mortem delays in primary human brain tissue affects mRNAs more readily than proteins, proteomics application appears to be more advantageous. Proteomics studies the products of genes, the functional translation of the genomic information, and only the characterization of the proteins themselves can reveal post-translational modifications such as phosphorylation, sulfation, glycosylation and give insights into protein-protein interactions and subcellular localization, thus providing clues about function. An advantage of this approach is its high throughput and the possibility to show the whole protein profile in one gel simultaneously (Fountoulakis, 2001).

The identification and characterization of central nervous system (CNS)-related proteins is important for the development of new, clinically useful neuronal markers and for studying the neurochemistry and pathogenic processes of different types of CNS disorders (Rohlff, 2001). We have applied proteomics technologies in the investigation of neurological diseases and have detected many protein level alterations in the brain of patients with Alzheimer's disease and Down syndrome (Engidawork and Lubec, 2001; Fountoulakis, 2001; Fountoulakis et al., 2002;

Greber et al., 1999). Analysis of proteins in cerebrospinal fluid (CSF) is of great diagnostic importance because of its close proximity to the brain and its clinical availability (Harrington and Merril, 1988; Andersson et al., 1994). The first 2-dimensional (2-D) map for human CSF proteins was presented by Goldman et al. (1980). Since then, more proteins were added into the human CSF database together with improvement of the 2-D gel technology and the rapid development of mass spectrometry analysis (Yun et al., 1992; Raymackers et al., 2000; Sichmann et al., 2000; Davidsson et al., 2001; Yuan et al., 2002). In the previous studies, only a limited analysis of CSF schizophrenic samples employing proteomics has been performed. Diseases involving the CNS markedly affect the protein concentration and protein pattern of the CSF (Andersson et al., 1994; Andreasen et al., 1999). The goal of the present work was to identify the gene products in the cerebrospinal fluid of schizophrenic patients and to study differential protein expression between the disease and control group.

Materials and methods

Materials

Immobilized pH gradient (IPG) strips were purchased from Amersham Biosciences (Uppsala, Sweden). Acrylamide was obtained from Serva (Heidelberg, Germany) and the other reagents for the polyacrylamide gel preparation were from Bio-Rad Laboratories (Hercules, CA, USA). CHAPS and thiourea were from Sigma (St. Louis, MO, USA). Urea and dithioerythritol were obtained from Merck (Darmstadt, Germany).

Sample preparation

CSF samples were collected by lumbar puncture from schizophrenic patients and controls, following the guidelines of the local ethical committee. The diagnosis of schizophrenia was assigned according to CCMD-II-R (a counterpart diagnostic criterion of DSM-III-R in China) and DSM-III. The samples were stored at −80°C until assay by 2-D gel electrophoresis. The protein content was determined by using the Coomassie blue method**.** The protein concentration was approximately 0.1 mg/ml in the CSF samples from controls and 0.3 mg/ml from schizophrenic patients. After thawing, 20 μl of protease inhibitor cocktail (Complete, EDTA-free, Roche Diagnostics, Mannheim, Germany) and 5 μl of 0.2 M PMSF stock solution in DMSO were added to 1 ml of CSF. The CSF samples from 10 controls and 10 patients were pooled to form four pools for each group, containing 0.4 and 0.8 mg of total protein, respectively. To the CSF pools, 60% trichloro-acetic acid (TCA) was added to a final concentration of 20% to concentrate the protein solution and to remove salt. The samples were kept overnight on ice and centrifuged at 10,000 rpm and 4°C for 30 min. The pellet was washed with 1 ml of 90% ice-cold acetone, the mixture was kept on ice for 15 min and centrifuged as above. The supernatant was removed and the pellet was air-dried. For 2-D gel electrophoresis analysis, the pellet was dissolved in 200 μl of sample buffer consisting of 7 M urea, 2 M thiourea, 20 mM Tris pH 7.5, 4% CHAPS, 0.4% 1,4-dithioerythritol and one drop of bromophenol blue.

Two-dimensional gel electrophresis

Two-dimensional gel electrophoresis was performed essentially as reported (Langen et al., 1997). Samples were applied on immobilized pH 3–7 nonlinear gradient strips (24 cm). Focusing started at 200 V and the voltage was gradually increased to 5,000 V at 3 V/min and kept constant for a further 24 h (approximately 180,000 kVh totally). The second-dimensional separation was performed in 12% SDS-polyacrylamide gels. The gels (180 × 200 × 1.5 mm) were run at 50 mA per gel, in an ISO-DALT apparatus (Hoefer Scientific Instruments, San Francisco, CA, USA), accommodating ten gels. After protein fixation with 50% methanol, containing 5% phosphoric acid for 2 h, the gels were stained with colloidal Coomassie blue (Novex, San Diego, CA, USA) for 16 h. Excess of dye was washed out from the gels with H_2O and the gels were scanned in an Agfa DUOSCAN densitometer (resolution 400). Protein spots were quantified using the ImageMaster 2-D Elite software (Amersham Biosciences). The percentage of the volume of the spots representing a certain protein was determined in comparison with the total proteins present in the 2-D gel. The significance of changes of individual proteins between schizophrenic patients and controls was evaluated using the Student's two-tailed *t*-test.

Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS)

MALDI-MS analysis was essentially performed as described (Fountoulakis and Gasser, 2003). The spots were excised and destained with 30% acetonitrile in 50 mM ammonium bicarbonate and dried in a Speedvac evaporator. Each dried gel piece was rehydrated with 5 μl of 1 mM ammonium bicarbonate, containing 50 ng trypsin (Roche Diagnostics, Mannheim, Germany). After 16 h at room temperature, 20 μl of 50% acetonitrile, containing 0.3% trifluoroacetic acid were added to each gel piece and incubated for 15 min with constant shaking. The sample application to the Anchorchip was performed with a Cy-Well apparatus (Cybio AG, Jena, Germany). Peptide mixture (1.5 μl) was simultaneously applied with 1 μl of matrix solution, consisting of 0.025% α-cyano-4-hydroxycinnamic acid (Sigma) and the standard peptides des-Arg-bradykinin (Sigma, 904.4681 Da) and adrenocorticotropic hormone fragment 18–39 (Sigma, 2465.1989 Da) in 65% ethanol, 35% acetonitrile and 0.03% trifluoroacetic acid. Samples were analyzed in a time-of-flight mass spectrometer (Ultraflex, Bruker Daltonics, Bremen, Germany). Peptide matching and protein searches were performed automatically with the use of in-house developed software (Berndt et al., 1999). The peptide masses were compared with the theoretical peptide masses of all available proteins from all species. Monoisotopic masses were used and a mass tolerance of 0.0025% was allowed. The probability of a false positive match with a given MS-spectrum was determined for each analysis. Unmatched peptides or miscleavage sites were not considered. Analysis in the MS/MS mode was performed using the instrument's software.

Results

Two-dimensional gel electrophoresis analysis and protein identification

The CSF proteins, after concentration and salt removal by TCA precipitation, were solubilized in the

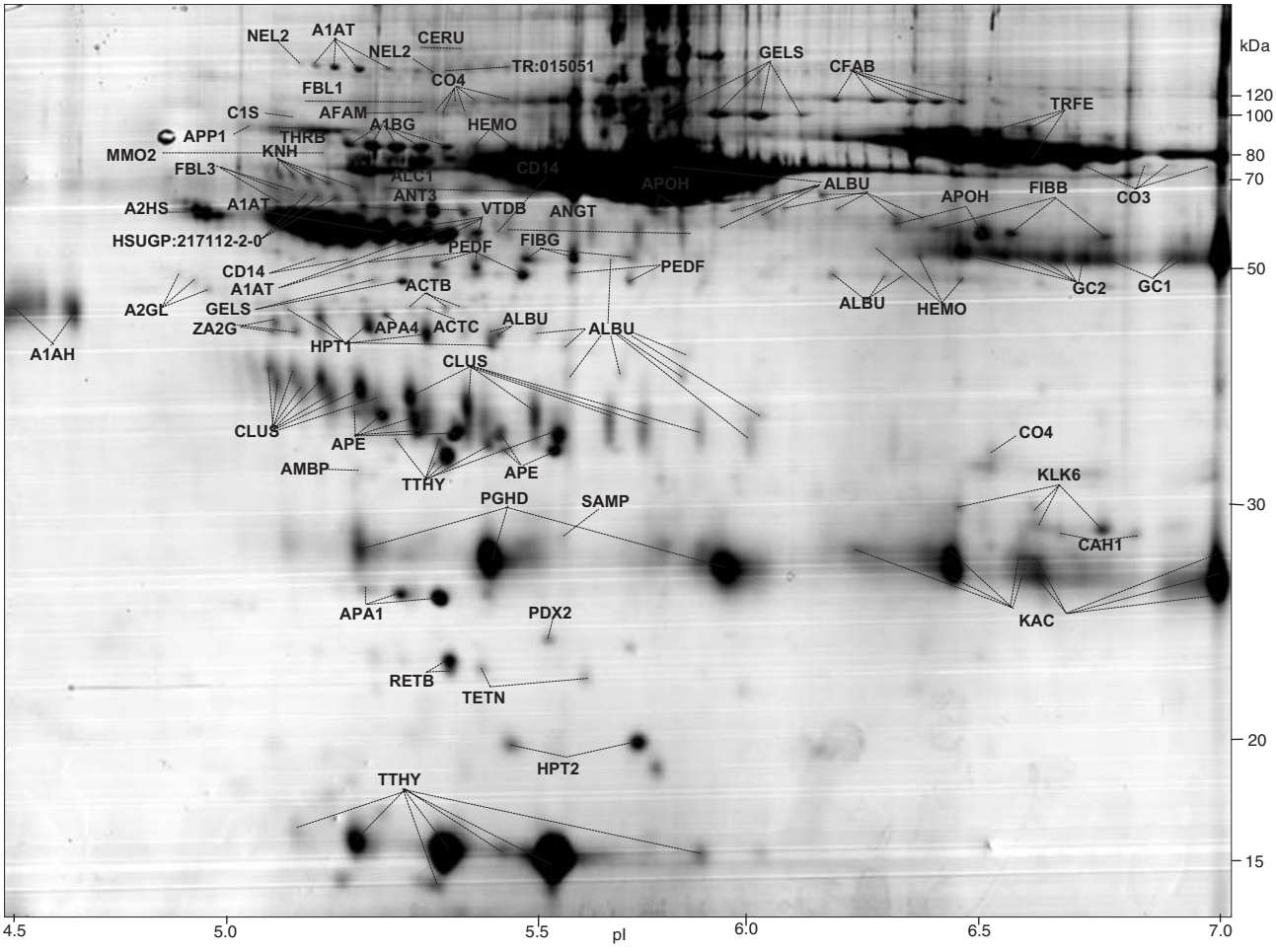

Fig. 1. Two-dimensional gel electrophoresis analysis of human CSF proteins from patients with schizophrenia. The proteins were separeted in a pH 3-7 IPG strip, followed by a 12% SDS-gel, as described under Materials and methods. The gel was stained with Coomassie blue. The identities assigned are listed in Table 1

isoelectrofocusing-compatible reagents urea, thiourea and CHAPS and analyzed by 2-D gels. The protein spots were visualized following stain with colloidal Coomassie blue. Fig. 1 shows total proteins of a CSF pool from patients with schizophrenia, analyzed on a pH 3–7 IPG strip. Most proteins are represented by multiple spots. Albumin, in addition to the large spots migrating at about 67 kDa, which are representing the full-length protein, is represented by many spots migrating at lower masses as well (Fig. 1).

The proteins were identified by MALDI-MS on the basis of peptide mass matching, following in-gel digestion with trypsin. On average, 200 spots were excised from each of four 2-D gels carrying CSF proteins. The spots of each gel were randomly selected with the goal to detect as many gene products as possible. Each excised spot was analyzed individually. The peptide masses were matched with the theoretical peptide masses of all known proteins from all species. The MS and MS/MS analysis resulted in the identification of 54 different gene products. In Table 1, the proteins identified together with the theoretical Mr and pI values are listed, as well as data from the mass spectrometry analysis, i.e. the numbers of matching and total peptides. The identification was usually based on 5 or more matches. In some cases, mainly for proteins of low molecular mass, which deliver few peptides (Fountoulakis et al., 1998), the identification was based on 4 matching peptides.

The major components were albumin, antibody chains, glycoproteins, apolipoproteins, complement chains, actin, haptoglobin, fibulin and fibrinogen. Most of the CSF proteins identified were of plasma origin or they mainly function in plasma and enter the CSF

Table 1. CSF Proteins. Proteins from the cerebrospinal fluid of controls and patients with schizophrenia were analyzed by 2-D gel electrophoresis and identified by MALDI-MS, following in-gel digestion with trypsin as described under Materials and methods. The search in protein databases was performed with in house developed software. The number of matching peptides is listed in Table 1 (matches). The spots representing the identified proteins are indicated in Fig. 1 and are designated with their abbreviated names of SWISS-PROT or the other databases. The theoretical Mr and pI values are given. Two proteins identified by search in the MS/MS mode are indicated in the column "Matches"

Protein	Protein ID/Locus	Accession number	Protein name	Other abbreviation	pI	MW	Peptides	
							Matches	Total
CSF proteins with CNS-specific functions:								
APP1	SW:APP1_HUMAN	P51693	Amyloid-like protein 1 precursor (APLP)	APLP1	5.68	72841	9	31
KLK6	SW:KLK6_HUMAN	Q92876	Kallikrein 6 precursor (EC 3.4.21.-) (Protease M) (Neurosin) (Zyme) (SP59).	PRSS9	7.39	27522	8	14
NEL2	SW:NEL2_HUMAN	Q99435	Protein kinase C-binding protein NELL2 precursor (NEL-like protein 2) (Nel-related protein 2)	NELL2 or NRP2	5.58	96359	17	40
CSF proteins involved in signal transduction:								
ANGT	SW:ANGT_HUMAN	P01019	Angiotensinogen precursor	SERPINA8 or AGT	6.28	53405	12	16
CO3	SW:CO3_HUMAN	P01024	Complement C3 precursor	C3	6.35	188585	17	72
CD14	SW:CD14_HUMAN	P08571	Monocyte differentiation antigen CD14 precursor (LPS receptor) (LPS-R) (Myeloid cell-specific leucine-rich glycoprotein)		6.18	40681	5	19
PGHD	SW:PGHD_HUMAN	P41222	Prostaglandin-H2 D-isomerase (Prostaglandin-D synthase) (Glutathione-independent PGD synthetase) (PGD2 synthase)	PTGDS	7.70	21029	MS/MS	8
Other CSF proteins:								
217112-2-0	HSUGP:217112-2-0		Glutamate carboxypeptidase-like protein 2				8	24
A1AH	SW:A1AH_HUMAN	P19652	Alpha-1-acid glycoprotein 2 precursor (AGP 2) (Orosomucoid 2) (OMD 2)	ORM2	5.00	23603	MS/MS	10
A1AT	SW:A1AT_HUMAN	P01009	Alpha-1-antitrypsin precursor (Alpha-1 protease inhibitor) (Alpha-1-antiproteinase)	SERPINA1, PI or AAT	5.40	46878	14	22
A1BG	SW:A1BG_HUMAN	P04217	Alpha-1b-glycoprotein		5.90	52478	12	14
A2GL	SW:A2GL_HUMAN	P02750	Leucine-rich alpha-2-glycoprotein (LRG)		5.93	34553	5	13
A2HS	SW:A2HS_HUMAN	P02765	Alpha-2-hs-glycoprotein precursor (Fetuin) (Alpha-2-z-globulin)	AHSG or FETUA	5.58	40098	5	14
ACTB	SW:ACTB_HUMAN	P02570	Actin, cytoplasmic 1 (Beta-actin)		5.24	42051	5	19
ACTC	SW:ACTC_HUMAN	P04270	Actin, alpha cardiac	ACTC1	5.20	42019	5	19
AFAM	SW:AFAM_HUMAN	P43652	Afamin precursor (Alpha-albumin) (Alpha-Alb)	AFM, ALBA or ALB2	5.77	70962	6	38
ALBU	SW:ALBU_HUMAN	P02768	Serum albumin precursor	ALB	6.23	71317	19	35
ALC1	SW:ALC1_HUMAN	P01876	Ig alpha-1 chain c region	IGHA1	6.48	38485	8	11
AMBP	SW:AMBP_HUMAN	P02760	AMBP protein [Contains: Alpha-1-microglobulin (Protein HC) (Complex-forming glycoprotein, heterogeneous in charge);Inter-alpha-trypsin inhibitor]	ITIL or HCP	6.18	39886	5	17
ANT3	SW:ANT3_HUMAN	P01008	Antithrombin-III precursor (ATIII)	SERPINC1 or AT3	6.68	53025	10	28
APA1	SW:APA1_HUMAN	P02647	Apolipoprotein A-I precursor (Apo-AI)	APOA1	5.59	30758	10	17

APA4	SW:APA4_HUMAN	P06727	Apolipoprotein A-IV precursor (Apo-AIV)	APOA4	5.17	45343	9	26
APE	SW:APE_HUMAN	P02649	Apolipoprotein E precursor (Apo-E)	APOE	5.55	36245	11	24
APOH	SW:APOH_HUMAN	P02749	Beta-2-glycoprotein I precursor (Apolipoprotein H) (Apo-H) (Activated protein C-binding protein), (APC inhibitor)	B2G1	7.86	39584	7	13
C1S	SW:C1S_HUMAN	P09871	Complement C1s component precursor (EC 3.4.21.42) (C1 esterase).		4.69	78174	11	30
CAH1	SW:CAH1_HUMAN	P00915	Carbonic anhydrase I (Carbonate dehydratase I) (CA-I)	CA1	7.14	28778	8	63
CERU	SW:CERU_HUMAN	P00450	Ceruloplasmin precursor (EC 1.16.3.1) (Ferroxidase)	CP	5.57	122982	15	38
CFAB	SW:CFAB_HUMAN	P00751	Complement factor B precursor (EC 3.4.21.47) (C3/C5 convertase) (Properdin factor B) (Glycine-rich beta glycoprotein) (GBG) (PBF2)	BF	7.03	86847	9	35
CLUS	SW:CLUS_HUMAN	P10909	Clusterin (Complement-associated protein SP-40,40) (Complement cytolysis inhibitor) (CLI) (NA1 and NA2) (Apolipoprotein J) (Apo-J)	CLU	6.23	53031	7	19
CO4	SW:CO4_HUMAN	P01028	Complement C4 precursor [Contains: C4A anaphylatoxin]	C4A or C4B	7.17	193811	11	69
FBL1	SW:FBL1_HUMAN	P23142	Fibulin-1 precursor	FBLN1	5.01	81328	5	33
FBL3	SW:FBL3_HUMAN	Q12805	EGF-containing fibulin-like extracellular matrix protein 1 precursor (Fibulin-3) (FIBL-3) (Fibrillin-like protein) (Extracellular protein S1-5)	EFEMP1, FBLN3 or FBNL	4.81	56885	5	23
FIBB	SW:FIBB_HUMAN	P02675	Fibrinogen beta chain precursor [Contains: Fibrinopeptide B].	FGB	8.26	56576	9	26
FIBG	SW:FIBG_HUMAN	P02679	Fibrinogen gamma chain precursor	FGG	5.83	50077	11	27
GC1	SW:GC1_HUMAN	P01857	Ig gamma-1 chain C region	IGHG1	8.17	36596	5	19
GC2	SW:GC2_HUMAN	P01859	Ig gamma-2 chain C region	IGHG2	7.53	36488	5	15
GELS	SW:GELS_HUMAN	P06396	Gelsolin precursor, plasma (Actin-depolymerizing factor) (ADF) (Brevin) (AGEL)	GSN	6.21	86043	9	24
HEMO	SW:HEMO_HUMAN	P02790	Hemopexin precursor (Beta-1b-glycoprotein)	HPX	7.00	52384	5	20
HPT1	SW:HPT1_HUMAN	P00737	Haptoglobin-1 precursor	HP	6.58	38940	8	20
HPT2	SW:HPT2_HUMAN	P00738	Haptoglobin-2 precursor	HP	6.57	45860	7	24
KAC	SW:KAC_HUMAN	P01834	Ig kappa chain C region	IGKC	5.74	11772	4	5
KNH	SW:KNH_HUMAN	P01042	Kininogen, hmw precursor (Alpha-2-thiol proteinase inhibitor) (Contains: bradykinin)	KNG	6.80	72983	10	16
MMO2	SW:MMO2_HUMAN	P08253	72kDa type IV collagenase precursor (72kDa gelatinase) (Matrix metalloproteinase-2) (MMP-2) (Gelatinase A) (TBE-1)	MMP2 or CLG4A	5.17	74918	6	34
O15051	TR:O15051	O15051	Hypothetical protein KIAA0343				11	55
PDX2	SW:PDX2_HUMAN	P32119	Peroxiredoxin 2 (Thioredoxin peroxidase 1) (Thioredoxin-dependent peroxide reductase 1) (TSA) (Natural killer cell enhancing factor b) (NKEF-B)	PRDX2, TDPX1 or NKEFB	5.81	22049	5	9
PEDF	SW:PEDF_HUMAN	P36955	Pigment epithelium-derived factor precursor (PEDF) (EPC-1)	SERPINF1	6.15	46471	12	19
RETB	SW:RETB_HUMAN	P02753	Plasma retinol-binding protein precursor (PRBP) (RBP)	RBP4	5.43	23195	5	12
SAMP	SW:SAMP_HUMAN	P02743	Serum amyloid P-component precursor (SAP) (9.5S alpha-1-glycoprotein)	APCS or PTX2	6.53	25485	5	10

Table 1. *continued*

Protein	Protein ID/Locus	Accession number	Protein name	Other abbreviation	pI	MW	Peptides	
							Matches	Total
TETN	SW:TETN_HUMAN	P05452	Tetranectin precursor (TN) (Plasminogen-kringle 4 binding protein)	TNA	5.47	22951	6	11
THRB	SW:THRB_HUMAN	P00734	Prothrombin precursor (EC 3.4.21.5) (Coagulation factor II)	F2	5.76	71474	14	23
TRFE	SW:TRFE_HUMAN	P02787	Serotransferrin precursor (Siderophilin) (Beta-1-metal binding globulin)	TF	7.06	79280	17	36
TTHY	SW:TTHY_HUMAN	P02766	Transthyretin precursor (Prealbumin) (TBPA) (TTR) (ATTR)	TTR or PALB	5.64	15991	5	6
VTDB	SW:VTDB_HUMAN	P02774	Vitamin D-binding protein precursor (DBP) (Group-specific component) (GC-globulin) (VDB)	GC	5.32	54525	16	24
ZA2G	SW:ZA2G_HUMAN	P25311	Zinc-alpha-2-glycoprotein precursor (ZN-alpha-2-glycoprotein) (ZN-alpha-2-GP)	AZGP1, ZAG or ZNGP1	5.72	34078	7	12

through the blood-CSF barrier. Three proteins of Table 1, amyloid-like protein 1, kallikrein 6 and protein kinase C-binding protein NELL2 (APP1, KLK6, and NEL2, respectively), have CNS-specific functions. Four proteins, angiotensinogen, complement C3, monocyte differentiation antigen CD14 and prostaglandin-H2 D-isomerase (ANGT, CO3, CD14, and PGHD, respectively), are involved in signal transduction and may function either in plasma, in CSF or in both fluids. About one third of the proteins of Table 1 could not be found in any current human CSF database available.

Protein quantification

Quantification of protein levels was made for each gel to detect differences between schizophrenic patients and controls. In order to correct for variations in protein loading, normalization between gels was carried out, by expressing features as a percentage of the sum of spot volume for all features detected in the gel. The most significant difference we found concerned the levels of apolipoprotein A-IV (ApoA-IV), which were significantly reduced in the disease group (average relative level 0.057) compared to the controls (average relative level 0.156) ($P < 0.03$). We also determined the levels of haptoglobin, fibrinogen, complement component 3, and Gc-globulin, which have been reported to show abnormally altered levels and might be associated with schizophrenia, as well as of other apolipoproteins, like apoA-I, apoE, apoH and apoJ, whose function has been related to apoA-IV. We did not find any change of statistical significance most likely due to the small sample size (data not shown).

Discussion

CSF samples from schizophrenic patients and controls were analyzed using two-dimensional gel electrophoresis followed by mass spectrometry and differences in the expression levels between the two groups were studied. We constructed a 2-D protein database including 54 different gene products. A negligible level difference was observed for most proteins. Only for ApoA-IV we found a difference of statistical significance.

Due to the low protein concentration in the CSF samples, we pooled the samples to meet the requirements for the loading amount for a micropreparative

analysis (about 0.4 mg protein for gels stained with Coomassie blue). Although information about individual samples could be lost because of the dilution of individual spots upon sample pooling, the pooling could also be advantageous because the individual variations can thus be subtracted and unavoidable mistakes introduced when dealing with tens of gels parallelly can be reduced. Several sample concentration methods were tried with human plasma on account of the limited CSF amounts, like TCA-, ammonium sulfate-, acetone-, chloroform/methanol-precipitation and ultrafiltration (data not shown). We finally chose TCA-precipitation followed by acetone wash to concentrate the CSF samples and remove salt prior to the first-dimensional separation.

It has been demonstrated that apolipoproteins play an important role in lipoprotein metabolism in CNS. Recent studies point to the role of apolipoproteins in degenerative and regenerative processes in the peripheral and central nervous systems, as well as in development. Apolipoprotein A-IV (ApoA-IV), a 46 kDa glycoprotein, is synthesized primarily in the intestine and secreted in plasma. The fate of ApoA-IV in plasma appears to be different in rats and humans. Whereas about 50% of ApoA-IV in rat plasma is associated with high density lipoproteins, more than 95% of ApoA-IV is found unassociated with the major plasma lipoproteins in fasting humans (Steinmetz and Utermann, 1985). No specific function has yet been assigned to ApoA-IV, and its significance in lipoprotein metabolism has remained unclear. In humans, ApoA-IV is associated with triglyceride-rich lipoproteins and high cholesterol lipoprotein (HDL), and also occurs in a lipoprotein-free form. ApoA-IV has been proposed to play a role in reverse cholesterol transport (cholesterol transport from tissues back to the liver for elimination) on the basis of in vitro properties. ApoA-IV has been shown to act *in vitro* as an activator for the plasma enzyme lecithin:cholesterol acyltransferase (LCAT), one of the activators of lipoprotein lipase (Steinmetz and Utermann, 1985), and to promote removal of cholesterol efflux from peripheral cells (Steinmetz et al., 1990).

Little is known about the function of this apolipoprotein in CNS. Fukagawa et al. (1995) reported the immunocytochemical localization of ApoA-IV in tanycytes and astrocytes of the rat brain. Fujimoto et al. (1993) reported that ApoA-IV concentration in rat CSF increased markedly during ingestion of a lipid meal and this apolipoprotein infused into the third ventricle inhibited the food intake in a dose dependent manner. They proposed the effect of intravenous infusion of ApoA-IV on food intake might be mediated by acting on CNS and suggested the possible existence of specific receptors in the CNS that would respond to ApoA-IV. There are some other studies which attribute the role to ApoA-IV during degenerative and regenerative processes in the peripheral and central nervous systems, as well as during development in cell culture and animal models (Boyles et al., 1990; Goodrum et al., 1995). Meanwhile, Weinberger (1995) pointed out that many, if not most, cases of schizophrenia were caused by a defect in early brain development and schizophrenia was seen as a neurodevelopmental encephalopathy. Moreover, there are a few reports about ApoA-IV function through DNA analysis. Csaszar et al. (1997) reported an association of mutated ApoA-IV (360:His) with Alzheimer's disease. However, Merched et al. (1998) argued that this mutation is not associated with Alzheimer's disease but rather with human aging.

In this study, we found that the levels of ApoA-IV were significantly decreased in the CSF samples from schizophrenic patients compared to that from controls. It is known that the elevation of total CSF protein level is the usual occurrence in many nervous system diseases, especially infections, inflammations and tumors. The blood-CSF-barrier is partially or completely destroyed at that time, so that plasma protein can more easily than normally pass through the barrier and enter the CSF (Harrington and Merril, 1988). We also quantified the levels of the other detected apolipoproteins, apoA-I, apoE, apoH and apoJ, but no statistically significant differences were found.

It has been shown that schizophrenia is accompanied by an activation of the inflammatory response system with signs of an acute phase response. Wong et al. (1996) observed increased levels of serum α1-antitrypsin, α1-microglobulin, haptoglobin (Hp), and ceruloplasmin, and reduced levels of serum albumin and transferrin in two series of schizophrenic patients. Increased serum hemopexin levels were observed only in the acutely ill patients and decreased complement C3 levels only in the chronically ill patients. Maes et al. (1997a,b, 2001) found that schizophrenic patients had significantly higher plasma haptoglobin, complement C3 and C4, α1-acid-glycoprotein and hemopexin levels than controls and that the above disorders in acute phase reactants were more pronounced in schizophrenic than in other depressed subjects

examined simultaneously. Harrington et al. (1985) and Johnson et al. (1992) found the presence of two 40 kDa spots in 2-D gels of CSF from schizophrenic patients which were absent in the controls. These spots were later identified as fibrinogen beta chain. We detected fibrinogen beta and gamma chains in both patients and controls and failed to find any significant expression difference between the two groups.

Much work has been carried out towards measurement gene frequency of several candidate genetic markers of schizophrenia and most attention has been paid to two known acute phase proteins serum haptoglobin, transferrin and one non-acute-phase protein serum group-specific component (Gc). Brackenridge and Jones (1972) found that about 10% fewer Gc heterozygotes and 5% fewer Hp heterozygotes existed among the schizophrenic than the normal group. Rudduck et al. (1985) reported a significantly different distribution of haptoglobin types between the schizophrenic patients and the controls but found no significant difference in serum Gc. However, most of the results are conflicting and confusing and until now, no unambiguous conclusion has been made for the pathogenesis of schizophrenia. We did not find significant alterations in the levels of CSF haptoglobin, complement C3, and Gc-globulin, most likely due to the small statistic. Further studies are required with increased sample size.

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