The Nuclear Proteome of White and Gray Matter from Schizophrenia Postmortem Brains

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Keywords

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Abstract

Schizophrenia (SCZ) is a serious neuropsychiatric disorder that manifests through several symptoms from early adulthood. Numerous studies over the last decades have led to significant advances in increasing our understanding of the factors involved in SCZ. For example, mass spectrometry-based proteomic analysis has provided important insights by uncovering protein dysfunctions inherent to SCZ. Here, we present a comprehensive analysis of the nuclear proteome of postmortem brain tissues from corpus callosum (CC) and anterior temporal lobe (ATL). We show an overview of the role of deregulated nuclear proteins in these two main regions of the brain: the first, mostly composed of glial cells and axons of neurons, and the second, represented mainly by neuronal cell bodies. These samples were collected from SCZ patients in an attempt to characterize the role of the nucleus in the disease process. With the ATL nucleus enrichment, we found 224 proteins present at different levels, and 76 of these were nuclear proteins. In the CC analysis, we identified 119 present at different levels, and 24 of these

were nuclear proteins. The differentially expressed nuclear proteins of ATL are mainly associated with the spliceosome, whereas those of the CC region are associated with calcium/calmodulin signaling.

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Introduction

Schizophrenia (SCZ) is a serious, debilitating, and incurable mental disorder that affects approximately 1% of the world's population [1]. The disease normally manifests between the end of adolescence and the beginning of adulthood [2] and is characterized by a range of cognitive, behavioral, and emotional dysfunctions. SCZ is the main cause of psychiatric incapacitation [3] and, although it is usually treated as a single disease, it is likely to be a spectrum of related disorders with distinct etiology, clinical presentation, response to treatment, and development [4]. Despite its high prevalence and severity, little is known about the biochemical mechanisms involved in its development or progression, and so there are few established molecular diagnoses or specific treatments. Thus, there is currently a great interest in obtaining new knowledge about the disease and, in this arena, proteomic anal-

yses have already yielded promising results and opened up new avenues of research [5].

Proteomics is used to analyze the protein profile of a specific cell type, tissue or organism, or changes in specific proteins, using mass spectrometry as its main tool. Due to its capacity for profiling large numbers of proteins simultaneously, proteomics is currently one of the main techniques used to understand biochemical pathways and, consequently, multifactorial disorders such as SCZ [6]. In the last 2 decades, proteomics has contributed to a growing understanding of SCZ, and a number of such studies investigating this disorder have been published revealing alterations in several biochemical pathways of the central nervous system [5]. However, it is important to note that in proteomic analyses of whole tissues, it is often not possible to detect proteins that are present at low concentrations. This may occur due to the complexity of the sample and/or due to the presence of other proteins that are present at very high concentrations, which may obscure those of lower abundance. This could lead to an inability to detect proteins which have important roles in the disease process [7]. To circumvent this problem, the study of subproteomes appears as a satisfactory alternative.

Subproteomes are obtained by fractionating the proteins of a given sample into distinct groups, taking into account some specific criteria. Although there are several ways of fractionating the protein content of a sample, one alternative is the analysis of specific organelles. This type of fractionation is attractive for cellular proteome analyses, since the protein content of the organelles is less complex and represents a specific and directed set, which provides the opportunity of investigating entire protein networks to an extent that cannot be achieved using whole cell approaches [8].

In this study, we have used a protocol for separation of organelles in order to obtain samples enriched in cell nuclei. The nucleus is the largest organelle of most cells and occupies approximately 10% of its volume, although this varies according to the cell type [9]. The nucleus is the compartment where the genetic material of eukaryotic organisms is stored, and nuclear proteins constitute 10–20% of the total cellular proteins and exert important functions related to gene expression, transcriptional control, splicing, and generation of the final gene products [10]. Therefore, the importance of investigating the nuclear proteome for the understanding of any physiological or pathological process is clear, and few such studies have been carried out thus far in the field of psychiatric disorders such as SCZ.

As an added level of enrichment, we have analyzed nuclei obtained from the anterior temporal lobe (ATL) and corpus callosum (CC) regions of postmortem brains from patients and controls. Dysfunctions in the ATL have been implicated previously in SCZ, and it consists mostly of gray matter [11]. The CC is the largest white matter region of the brain, and there are morphological, electrophysiological, and neurophysiological studies showing significant involvement of this region in patients with SCZ [12–14]. Because the brain works through communication with and across the different regions, we jointly analyzed the ATL and CC nuclear proteins to provide further information on the potential role of dysfunctions of this organelle in SCZ.

Materials and Methods

Human Samples

CC and ATL samples were collected post-mortem from 12 patients who had suffered from chronic SCZ and from 8 healthy controls (Table 1). The patient samples were from the Nordbaden Psychiatric Center, Wiesloch, Germany, and the controls were from the Institute of Neuropathology, Heidelberg University, Heidelberg, Germany. Postmortem evaluations and procedures were approved by the Ethics Committee of the Heidelberg University Medical School, and both patients and controls gave written consent prior to death that their brains could be used for research purposes.

Nuclear Enrichment

Nuclear proteins were obtained from the ATL and CC brain tissues according to the protocol of Cox and Emili [15]. In this protocol, each sample (20 mg tissue) was homogenized in 10 volumes of buffer containing 250 mM sucrose (Sigma-Aldrich, St. Louis, MO, USA), 50 mM Tris-HCl, 5 mM MgCl₂ (Sigma-Aldrich), 1 mM dithiothreitol (Sigma-Aldrich), 250 µg spermine, and 250 µg spermidine buffer (pH = 7.4), containing 1 tablet of protease cocktail inhibitor (Roche Diagnostics, Indianapolis, IN, USA) per 25 mL buffer. The homogenate was centrifuged at 100 g for 1 min at 4°C. The supernatant was discarded. Next, 5 volumes of the same buffer was added to the sediment. The homogenate was centrifuged at 800 g for 15 min at 4°C, and the supernatant was separated for further separation of the mitochondria and stored at –80°C as CytoI. The previous step was repeated and the supernatant was named as CytoII. The pellet was homogenized in 4 mL of the same buffer mentioned above but with a concentration of 2 M sucrose. The mixture was filtered with gauze, and the filtrate was placed on 4 mL of the last buffer. The tube was centrifuged at 80,000 g for 35 min at 4°C. The pellet contained the pure nuclei. The nuclear protein pellet was dissolved in 50 mM ammonium bicarbonate (pH 8.0) prior to protein digestion.

Mass Spectrometry

Protein extracts from nuclear enrichment of ATL and CC were digested by trypsin at a ratio of 1:80 (trypsin: total protein). The resulting peptides were lyophilized and frozen at –80°C before

Table 1. Clinical data of patients and controls

Case	Age, years	Gen-der	PMI, h	pH values	Duration of disease, years	Duration of medication, years	atyptyp	CPE last dose	CPE last 10 years	Cause of death	DSM-IV	Age at onset	Last medication	Ciga-rettes	Alco-hol	Hosp	ECT
SCZ	64	F	11	6.7	48	45	3	1,536	7.7	pulmonary insufficiency	295.6	16	clozapine 500 mg, haloperidol 40 mg, ciatyl 40 mg	0	no	21	yes
SCZ	73	M	20	6.6	43	40	1	507.4	1.7	heart infarction	295.6	30	perphenazine 32 mg, promethazine 150 mg	30/ day	no	33	no
SCZ	43	M	18	6.9	22	20	2	464	2.6	heart infarction	295.6	20	zuclopethixol 40 mg, valproate 1,200 mg, tiapride 300 mg	0	no	13	no
SCZ	77	F	32	6.5	49	48	2	2,555	8.3	lung embolism	295.6	28	clozapine 400 mg, ben-peridol 25 mg, chlorprothixen 150 mg	0	no	48	yes
SCZ	76	F	17	6.8	49	47	1	300	4.9	cardio-pulmonary insufficiency	295.6	27	perazine 300 mg	0	no	30	yes
SCZ	63	F	31	6.8	40	30	3	75	1.8	heart infarction	295.6	24	olanzapine 15 mg	30/ day	no	30	yes
SCZ	92	F	37	6.9	51	48	1	100	3.4	cardio-pulmonary insufficiency	295.6	41	prothipendyl 160 mg, perazine 100 mg	0	no	51	no
SCZ	71	M	28	6.4	40	35	1	782.4	10	heart infarction	295.6	30	haloperidol 32 mg, pipamperone 40 mg	40/ day	no	12	no
SCZ	51	M	7	6.1	25	25	1	147	0.6	heart infarction	295.6	19	flupenthixol 15 mg	30/ day	no	20	no
SCZ	51	M	12	6.7	28	25	2	450	1.8	heart infarction	295.6	23	clozapine 500 mg	30/ day	no	17	no
SCZ	81	M	4	6.7	62	50	1	92.8	1.4	heart insufficiency	295.6	19	haloperidol 40 mg, prothipendyl 80 mg	20	no	48	no
SCZ	64	F	23	6.6	41	40	2	54.5	4.6	heart infarction	295.6	24	zotepine 150 mg, olanzapine 10 mg	20/ day	no	5	yes
Control	41	M	7	6.5						heart infarction				0	no		
Control	91	F	16	6.7						cardio-pulmonary insufficiency				0	no		
Control	69	F	96	6.4						lung embolism				0	no		
Control	57	M	24	6.9						heart infarction				0	no		
Control	53	M	18	7						heart infarction				0	no		
Control	63	M	13	6.5						heart infarction				0	no		
Control	66	M	16	6.8						heart infarction				0	no		
Control	79	M	24	6.4						heart infarction				0	no		

atyptyp, duration of atypical treatment/duration of treatment with typical neuroleptics during lifetime; CPE, medication calculated in chlorpromazine equivalents (mg); CPE last 10 years, the sum of medications during the last 10 years in kg; Hosp, hospitalization time in years; ECT, electroconvulsive therapy.

Table 2. Differentially expressed proteins in schizophrenia anterior temporal lobe (ATL)

UniProt ID	Gene name	Description	Score	Mass	Pep- tides	SCZ/ CTRL	SD	Biological process	Molecular class	Molecular function
PKP2_ HUMAN	PKP2	plakophilin-2	76	97,852	2	0.05	5.154	cell adhesion	unclassified	molecular function unknown
BIN1_ HUMAN	BIN1	Myc box-dependent- interacting protein 1	175	64,887	5	0.63	1.859	cell communication and signaling	adapter molecule	receptor signaling complex scaffold activity
CADM1_ HUMAN	CADM1	cell adhesion molecule 1	102	48,935	3	0.44	4.93	cell communication and signaling	adhesion molecule	cell adhesion molecule activity
CALM_ HUMAN	CALM1	calmodulin	759	16,827	9	2.10	3.515	cell communication and signaling	calcium bind- ing protein	calcium ion binding
CDC42_ HUMAN	CDC42	cell division control protein 42 homolog	74	21,696	2	4.13	5.878	cell communication and signaling	GTPase	GTPase activity
CSRP1_ HUMAN	CSRP1	cysteine and glycine- rich protein 1	244	21,409	5	0.49	2.012	cell communication and signaling	adapter molecule	receptor signaling complex scaffold activity
CTNB1_ HUMAN	CTNNB1	catenin beta-1	71	86,069	2	0.48	1.285	cell communication and signaling	adhesion molecule	cell adhesion molecule activity
DPYL2_ HUMAN	DPYSL2	dihydropyrimidinase- related protein 2	1,892	62,711	30	2.40	2.513	cell communication and signaling	cytoskeletal associated protein	cytoskeletal protein binding
PP2BA_ HUMAN	PPP3CA	serine/threonine- protein phosphatase 2B catalytic subunit alpha isoform	325	59,335	5	1.92	9.805	cell communication and signaling	serine/ threonine phosphatase	protein serine/ threonine phosphatase activity
CANB1_ HUMAN	PPP3R1	calcineurin subunit B type 1	76	19,402	2	4.30	9.98	cell communication and signaling	regulatory/ other subunit	Phosphatase regulator activity
KAP3_ HUMAN	PRKAR2B	cAMP-dependent protein kinase type II-beta regulatory subunit	157	46,672	2	0.47	1.132	cell communication and signaling	serine/threonine kinase	protein serine/ threonine kinase activity
SEPT7_ HUMAN	SEPT7	septin-7	493	50,933	11	2.16	2.939	cell communication and signaling	cell cycle control protein	protein binding
SH3G2_ HUMAN	SH3GL2	endophilin-A1	216	40,108	4	1.76	3.614	cell communication and signaling	unclassified	molecular function unknown
SIRT2_ HUMAN	SIRT2	NAD-dependent deacetylase sirtuin-2	220	43,782	5	3.94	8.747	cell communication and signaling	cell cycle control protein	deacetylase activity
SYUB_ HUMAN	SNCB	beta-synuclein	447	14,279	6	2.05	3.512	cell communication and signaling	unclassified	molecular function unknown
1433E_ HUMAN	YWHAЕ	14-3-3 protein epsilon	595	29,326	12	0.40	4.13	cell communication and signaling	adapter molecule	receptor signaling complex scaffold activity
1433G_ HUMAN	YWHAG	14-3-3 protein gamma	1,010	28,456	18	0.56	3.229	cell communication and signaling	adapter molecule	receptor signaling complex scaffold activity
1433T_ HUMAN	YWHAQ	14-3-3 protein theta	400	28,032	7	0.41	2.421	cell communication and signaling	adapter molecule	receptor signaling complex scaffold activity
SEPT2_ HUMAN	SEPT2	septin-2	173	41,689	3	1.86	2.96	cell cycle	GTPase	GTPase activity
DPYL1_ HUMAN	CRMP1	dihydropyrimidinase- related protein 1	368	62,487	6	3.50	1.543	cell growth and/ or maintenance	enzyme: hydrolase	protein binding
DYL1_ HUMAN	DYNLL1	dynein light chain 1, cytoplasmic	141	10,530	4	1.97	8.851	cell growth and/ or maintenance	motor protein	motor activity
E41L3_ HUMAN	EPB41L3	band 4.1-like protein 3	473	121,458	13	0.56	7.25	cell growth and/ or maintenance	structural protein	structural molecule activity
FLNA_ HUMAN	FLNA	filamin-A	612	283,301	8	0.36	4.413	cell growth and/ or maintenance	anchor pro- tein	cytoskeletal anchoring activity
LMNA_ HUMAN	LMNA	lamin-A/C	387	74,380	7	0.11	3.386	cell growth and/ or maintenance	structural protein	structural molecule activity

Table 2 (continued)

UniProt ID	Gene name	Description	Score	Mass	Pep- tides	SCZ/ CTRL	SD	Biological process	Molecular class	Molecular function
LMNB2_ HUMAN	LMNB2	lamin-B2	173	67,762	3	0.18	5.639	cell growth and/ or maintenance	structural protein	structural molecule activity
MAP1A_ HUMAN	MAP1A	microtubule-associated protein 1A	187	306,923	4	0.35	7.653	cell growth and/ or maintenance	cytoskeletal associated protein	cytoskeletal protein binding
MARE2_ HUMAN	MAPRE2	microtubule-associated protein RP/EB family member 2	338	37,236	6	2.41	4.902	cell growth and/ or maintenance	cytoskeletal associated protein	cytoskeletal protein binding
MYH9_ HUMAN	MYH9	myosin-9	330	227,646	4	0.23	1.904	cell growth and/ or maintenance	structural protein	structural molecule activity
VIME_ HUMAN	VIM	vimentin	2,238	53,676	37	0.35	5.159	cell growth and/ or maintenance	cytoskeletal protein	structural constituent of cytoskeleton
LEG1_ HUMAN	LGALS1	galectin-1	79	15,048	2	0.65	2.212	immune response	ligand	receptor binding
LDHA_ HUMAN	LDHA	L-lactate dehydrogenase A chain	385	36,950	8	2.48	2.252	metabolism; energy pathways	enzyme: dehydrogenase	catalytic activity
COX4I_ HUMAN	COX4I1	cytochrome c oxidase subunit 4 isoform 1, mitochondrial	126	19,621	3	0.43	1.693	metabolism; energy pathways	enzyme: oxidoreductase	oxidoreductase activity
COX5B_ HUMAN	COX5B	cytochrome c oxidase subunit 5B, mitochondrial	170	13,915	4	7.19	4.156	metabolism; energy pathways	enzyme: oxidoreductase	oxidoreductase activity
GSTP1_ HUMAN	GSTP1	glutathione S-transferase P	147	23,569	2	1.64	1.268	metabolism; energy pathways	enzyme: glutathione transferase	glutathione transferase activity
NDUS8_ HUMAN	NDUFS8	NADH dehydrogenase (ubiquinone) iron- sulfur protein 8, mitochondrial	96	24,203	2	1.52	1.993	metabolism; energy pathways	enzyme: oxidoreductase	oxidoreductase activity
PDE2A_ HUMAN	PDE2A	cGMP-dependent 3',5'-cyclic phosphodiesterase	89	107,360	2	9.65	2.299	metabolism ; energy pathways	enzyme: phos- phodiesterase	phosphoric diester hydrolase activity
ODPA_ HUMAN	PDHA1	pyruvate dehydrogenase E1 component subunit alpha, somatic form, mitochondrial	219	43,952	7	0.35	5.372	metabolism; energy pathways	enzyme: dehydrogenase	catalytic activity
PGK1_ HUMAN	PGK1	phosphoglycerate kinase 1	510	44,985	11	1.86	6.605	metabolism; energy pathways	enzyme: phos- photransferase	catalytic activity
PRDX1_ HUMAN	PRDX1	peroxiredoxin-1	198	22,324	4	3.30	1.57	metabolism; energy pathways	enzyme: peroxidase	peroxidase activity
CALX_ HUMAN	CANX	calnexin	182	67,982	5	1.85	3.216	protein folding	chaperone	chaperone activity
CH60_ HUMAN	HSPD1	60-kDa heat shock protein, mitochondrial	1,089	61,187	21	1.90	2.418	protein folding; apoptosis; regulation of immune response; signal transduction	heat shock protein	heat shock protein activity
CALR_ HUMAN	CALR	calreticulin	197	48,283	2	0.31	1.984	protein metabolism	chaperone	chaperone activity
TCPD_ HUMAN	CCT4	T-complex protein 1 subunit delta	105	58,401	3	1.56	2.874	protein metabolism	chaperone	chaperone activity
DNJC5_ HUMAN	DNAJC5	DnaJ homolog subfamily C member 5	93	22,933	2	3.89	5.26	protein metabolism	chaperone	chaperone activity
HSP71_ HUMAN	HSPA1A	heat shock 70-kDa protein 1	390	70,294	8	1.84	2.307	protein metabolism	chaperone	chaperone activity
HSP72_ HUMAN	HSPA2	heat shock-related 70-kDa protein 2	485	70,263	13	1.91	2.814	protein metabolism	heat shock protein	heat shock protein activity
HSP76_ HUMAN	HSPA6	heat shock 70-kDa protein 6	328	71,440	7	1.77	2.398	protein metabolism	heat shock protein	heat shock protein activity
HSP7C_ HUMAN	HSPA8	heat shock cognate 71-kDa protein	1,156	71,082	22	1.84	2.293	protein metabolism	heat shock protein	heat shock protein activity

Table 2 (continued)

UniProt ID	Gene name	Description	Score	Mass	Pep- tides	SCZ/ CTRL	SD	Biological process	Molecular class	Molecular function
CH10_ HUMAN	HSPE1	10-kDa heat shock protein	130	10,925	3	2.31	1.134	protein metabolism	heat shock protein	heat shock protein activity
PSA4_ HUMAN	PSMA4	proteasome subunit alpha type-4	71	29,750	3	0.44	1.528	protein metabolism	ubiquitin proteasome system protein	ubiquitin-specific protease activity
SYUA_ HUMAN	SNCA	alpha-synuclein	879	14,451	12	1.88	3.76	protein metabolism	chaperone	chaperone activity
EF1A1_ HUMAN	EEF1A1	elongation factor 1-alpha 1	312	50,451	9	0.58	2.182	regulation of cell cycle	transcription regulatory protein	transcription regulator activity
H2AV_ HUMAN	H2AFV	histone H2A.V	143	13,501	2	0.17	1.264	regulation of gene expression, epigenetic	DNA-binding protein	DNA binding
BASP_ HUMAN	BASP1	brain acid soluble protein 1	1,796	22,680	24	0.51	1.805	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	transcription regulatory protein	transcription regulator activity
CAND1_ HUMAN	CAND1	cullin-associated NEDD8-dissociated protein 1	118	137,999	2	1.81	4.005	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	transcription regulatory protein	transcription regulator activity
H33_ HUMAN	H3F3A	histone H3.3	217	15,376	5	0.50	3.531	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	DNA-binding protein	DNA binding
H12_ HUMAN	HIST1H1C	histone H1.2	237	21,352	3	0.57	4.622	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	DNA-binding protein	DNA binding
H2A1B_ HUMAN	HIST1H2AB	histone H2A type 1-B/E	249	14,127	6	0.38	2.971	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	DNA-binding protein	DNA binding
H2A1D_ HUMAN	HIST1H2AD	histone H2A type 1-D	226	14,099	6	0.33	1.799	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	DNA-binding protein	DNA binding
H2B1B_ HUMAN	HIST1H2BB	histone H2B type 1-B	190	13,942	3	0.41	5.283	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	DNA-binding protein	DNA binding
H2B1C_ HUMAN	HIST1H2BC	histone H2B type 1-C/E/F/G/I	165	13,811	3	0.43	1.266	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	DNA-binding protein	DNA binding
H4_ HUMAN	HIST1H4A	histone H4	637	11,360	11	0.46	3.406	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	DNA-binding protein	DNA binding
ROA2_ HUMAN	HNRNPA2B1	heterogeneous nuclear ribonucleoproteins A2/B1	452	37,464	7	0.16	3.769	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	ribonucleo-protein	transcription factor binding
HNRPC_ HUMAN	HNRNPC	heterogeneous nuclear ribonucleoproteins C1/C2	223	33,707	2	0.24	1.52	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	RNA-binding protein	RNA binding
HNRH1_ HUMAN	HNRNPH1	heterogeneous nuclear ribonucleoprotein H	269	49,484	4	0.18	2.678	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	ribonucleo-protein	ribonucleoprotein

Table 2 (continued)

UniProt ID	Gene name	Description	Score	Mass	Pep- tides	SCZ/ CTRL	SD	Biological process	Molecular class	Molecular function
HNRH2_ HUMAN	HNRNPH2	heterogeneous nuclear ribonucleoprotein H2	293	49,517	3	0.17	2.343	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	ribonucleo- protein	RNA binding
HNRPK_ HUMAN	HNRNPK	heterogeneous nuclear ribonucleoprotein K	225	51,230	4	0.31	3.12	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	ribonucleo- protein	ribonucleoprotein
HNRPU_ HUMAN	HNRNPU	heterogeneous nuclear ribonucleoprotein U	114	91,198	3	0.41	1.193	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	ribonucleo- protein	RNA binding
MATR3_ HUMAN	MATR3	matrin-3	125	95,078	2	0.37	1.661	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	RNA-binding protein	RNA binding
NONO_ HUMAN	NONO	non-POU domain-containing octamer-binding protein	116	54,311	2	0.20	1.072	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	RNA-binding protein	RNA binding
PURA_ HUMAN	PURA	transcriptional activator protein Pur-alpha	157	35,003	2	0.41	2.617	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	transcription factor	transcription factor activity
SFRS3_ HUMAN	SFRS3	splicing factor, arginine/serine-rich 3	81	19,546	2	0.35	1.529	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	RNA-binding protein	RNA binding
SSBP_ HUMAN	SSBP1	single-stranded DNA-binding protein, mitochondrial	92	17,249	2	7.35	3.86	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	DNA-binding protein	DNA binding
SEPT5_ HUMAN	SEPT5	septin-5	327	43,206	11	0.52	5.659	signal transduction	GTPase	GTP binding
ANXA2_ HUMAN	ANXA2	annexin A2	319	38,808	5	0.46	6.797	signal transduction; cell communication	calcium-bind- ing protein	calcium ion binding
EAA1_ HUMAN	SLC1A3	excitatory amino acid transporter 1	307	59,705	6	0.22	6.411	transport	transport/cargo protein	transporter activity

Score, MASCOT Identification Score (cutoff for this dataset: 55); mass, the molecular mass of the protein (Da); peptides, number of identified peptides by mass spectrometry; SCZ/CTRL, fold change ratio between schizophrenia and control samples; SD, standard deviation among quantified peptides.

mass spectrometry analysis. Immediately prior to analysis, lyophilized peptides were dissolved in an aqueous solution of 0.1% formic acid and injected into a 2D nano high-performance liquid chromatography system (Eksigent, Dublin, CA, USA) coupled on-line to a LTQ XL-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany). The specifics of data acquisition are described in detail in Maccarrone et al. [16].

Proteome Quantification

The program used in the identification and quantification of proteins was MASCOT Distiller (Matrix Sciences, London, UK).

For the identification and quantification, this program follows a series of statistical criteria. The main test used to indicate quantitative changes between the proteins was Student's *t* in log space. This analysis assigns to each protein a *p* value of significance with regard to differences in protein levels. In addition, samples values were applied to a data normalization process. This process is based on the hypothesis that it is reasonable to expect that only a minority of the proteins in the sample will be found to be differentially expressed, considering that the overall normalization is applied in order to make the mean or median ratios in the entire dataset equal to 1. Following this logic, the data distribution is log-normal, and

Fig. 1. Biological processes related to the anterior temporal lobe (ATL) and corpus callosum (CC) differentially expressed proteins.

Fig. 2. Venn diagram comparison between differentially expressed proteins in anterior temporal lobe (ATL) and corpus callosum (CC).

the statistical test used to confirm this premise is the Shapiro-Wilk W test. If the results do not pass this test, it indicates that the values are meaningless and something has systematically gone wrong with the analysis. In these cases, the values are rejected in the normality test.

Analysis in silico

Shotgun proteomics analysis can produce high amounts of data, especially in studies of complex biological mixtures, such as postmortem brain samples. As a consequence, protein-protein interaction analysis and identification of the pathways involved are fundamental to understanding cellular phenotypes in the most complete manner possible. Due to this, we used bioinformatics tools available online in these analyses. These were: the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, <http://STRING-db.org/>), Kyoto Encyclopedia Genes and Genomes (KEGG, <http://www.genome.ad.jp/kegg/>), and Reactome (<http://reactome.org/>).

Results

In the results of the ATL nucleus enrichment, we identified a total of 4,293 unique peptides, which corresponded to 629 proteins. Of these, 224 were present at significantly different levels between the SCZ and control samples, and 76 were nuclear proteins (nuclear enrichment of 33%; Table 2). In the CC analysis, we identified 3,820 unique peptides, corresponding to 552 proteins with 119 present at different levels, and 24 of these were nuclear proteins (nuclear enrichment of 21%; Table 3). These differentially expressed proteins were analyzed using the online human protein reference database (<http://www.hprd.org/>) in order to find the biological processes and function/molecular classes with which they are related (Table 2, 3).

The differentially expressed proteins related to both the nuclei of the ATL region cells and the CC participated in biological processes related mainly to processes such as regulation of nucleobase, nucleoside, nucleotide, and nucleic acid metabolism (27% ATL, 40% CC) and cell communication and signaling (23% ATL, 36% CC) (Fig. 1). These processes are related to the main functions of the nucleus, such as gene expression, transcriptional control, splicing, and release of gene products [10].

Discussion

Proteomic Similarities between Regions

The ATL is enriched in gray matter, while the cerebral region of the CC corresponds to the largest portion of

Table 3. Differentially expressed proteins in schizophrenia CC

UniProt ID	Gene name	Protein name	Score	Mass	Pep- tides	SCZ/ CTRL	SD	Biological process	Molecular class	Molecular function
ARF1_ HUMAN	ARF1	ADP-ribosylation factor 1	298	20,741	7	4.26	5.333	cell communication and signaling	GTPase	GTPase activity
BASP_ HUMAN	BASP1	brain acid soluble protein 1	766	22,680	14	5.96	3.633	reg. nucleic acid metabolism	transcription regulatory protein	transcription regulator activity
CALM_ HUMAN	CALM1	calmodulin	349	16,827	7	2.63	1.817	cell communication and signaling	calcium-binding protein	calcium ion binding
KCC2A_ HUMAN	CAMK2A	calcium/calmodulin- dependent protein kinase type II subunit alpha	469	54,566	10	3.08	8.707	cell communication and signaling	serine/ threonine kinase	protein serine/ threonine kinase activity
KCC2B_ HUMAN	CAMK2B	calcium/calmodulin- dependent protein kinase type II subunit beta	180	73,593	4	5.90	5.656	cell communication and signaling	serine/ threonine kinase	protein serine/ threonine kinase activity
KCC2G_ HUMAN	CAMK2G	calcium/calmodulin- dependent protein kinase type II subunit gamma	195	63,311	4	7.19	4.726	cell communication and signaling	serine/ threonine kinase	protein serine/ threonine kinase activity
CDC42_ HUMAN	CDC42	cell division control protein 42 homolog	85	21,696	2	2.09	3.534	cell communication and signaling	GTPase	GTPase activity
COF1_ HUMAN	CFL1	cofilin-1	192	18,719	6	5.54	4.899	cell growth and maintenance	cytoskeletal associated protein	cytoskeletal protein binding
CSRP1_ HUMAN	CSRP1	cysteine and glycine-rich protein 1	458	21,409	7	4.07	5.108	cell communication and signaling	adapter molecule	receptor signaling complex scaffold activity
DPYL2_ HUMAN	DPYSL2	dihydropyrimidinase- related protein 2	1,679	62,711	25	7.07	5.889	cell communication and signaling	cytoskeletal associated protein	cytoskeletal protein binding
H2AV_ HUMAN	H2AFV	histone H2A.V	422	13,501	5	0.42	2.387	reg. nucleic acid metabolism	DNA-binding protein	DNA binding
H14_ HUMAN	HIST1H1E	histone H1.4	513	21,852	11	0.12	3.04	reg. nucleic acid metabolism	DNA-binding protein	DNA binding
H31_ HUMAN	HIST1H3A	histone H3.1	353	15,509	7	0.17	8.548	reg. nucleic acid metabolism	DNA-binding protein	DNA binding
H4_ HUMAN	HIST1H4A	histone H4	979	11,360	13	0.19	3.693	reg. nucleic acid metabolism	DNA-binding protein	DNA binding
HMGB1_ HUMAN	HMGB1	high-mobility group protein B1	315	25,049	3	0.01	5.9	reg. nucleic acid metabolism	DNA-binding protein	DNA binding
ROA2_ HUMAN	HNRN- PA2B1	heterogeneous nuclear ribonucleoproteins A2/B1	325	37,464	6	0.43	5.195	reg. nucleic acid metabolism	Ribonucleo- protein	Transcription factor binding
HNRPR_ HUMAN	HNRNPR	heterogeneous nuclear ribonucleoprotein R	106	71,184	2	0.03	1.653	reg. nucleic acid metabolism	RNA-binding protein	RNA binding
HNRPU_ HUMAN	HNRNPU	heterogeneous nuclear ribonucleoprotein U	373	91,198	8	0.19	5.492	reg. nucleic acid metabolism	ribonucleo- protein	RNA binding
HP1B3_ HUMAN	HP1BP3	heterochromatin protein 1-binding protein 3	78	61,454	2	3.40	5.984	reg. nucleic acid metabolism	DNA-binding protein	DNA binding
HSP71_ HUMAN	HSPA1A	heat shock 70-kDa protein 1A/1B	310	70,294	5	1.58	1.484	protein metabolism	chaperone	chaperone activity
LMNA_ HUMAN	LMNA	prelamin-A/C	1,140	74,380	24	0.41	7.189	cell growth and maintenance	structural protein	structural molecule activity
LMNB2_ HUMAN	LMNB2	lamin-B2	207	67,762	2	0.05	2.067	cell growth and maintenance	structural protein	structural molecule activity
NPM_ HUMAN	NPM1	nucleophosmin	172	32,726	2	6.64	1.167	protein metabolism	chaperone	chaperone activity
PTMA_ HUMAN	PTMA	prothymosin alpha	177	12,196	3	10.62	2.334	cell growth and maintenance	unclassified	molecular function unknown
S100B_ HUMAN	S100B	protein S100-B	290	10,820	3	0.30	1.203	cell communication and signaling	calcium-binding protein	calcium ion binding

Score, MASCOT Identification Score (cutoff for this dataset: 55); mass, the molecular mass of the protein (Da); peptides, number of identified peptides by mass spectrometry; SCZ/CTRL, fold change ratio between schizophrenia and control samples; SD, standard deviation among quantified peptides.

Fig. 3. Analysis of Reactome of HSF1 proteins found differentially expressed in both regions by Reactome.

white matter in the brain. This results from the preponderance of neurons in the ATL [17], whereas glial cells and neuronal axons are more predominant in the CC [18]. It is important to compare proteomic profiles of these two regions of the brain mainly because this gives a more integrated view of brain function and not only what happens only with the neurons, as is typical of most other brain proteomic studies. In the comparison of these two regions, 64 of these were specific to the ATL, 13 to the CC, and 12 were common to both regions, i.e. 13.5% of the proteins are shared for the regions, as it can be seen in the Venn diagram (Fig. 2). According to analysis carried out

using the Reactome software, these latter proteins were related to cellular stress response, with a chain of reactions related to heat-shock proteins (HSPs) (p value 3.18×10^{-11}) (Fig. 3). This type of reaction is triggered by cellular stressors such as exposure to high temperatures, hypoxia, and free radicals, and these factors can cause damage to cellular proteins and induce this type of response [19–22].

Since maintaining homeostasis is important for the proper functioning of cellular metabolism, this type of stress response must be effective and coordinated [23]. For this to occur, the main molecule responsible for tran-

Fig. 4. Spliceosome of anterior temporal lobe (ATL) showing differentially expressed proteins according to program STRING.

scription-mediated stress response, the heat shock transcription factor HSF1, must be present at optimal functional levels [24, 25]. Under normal physiological conditions, this molecule is present in its inactive form, mediated by a series of protein-protein interactions. However, in the presence of stressors, this molecule becomes activated by a series of reactions, including its phosphorylation and interaction with DNA, promoting the cellular responses to stress (Fig. 3) [26–31].

The protein that mediates most of these reactions, the heat shock 70-kDa protein 1A/1B (HSPA1A), was found to be increased in the nuclear compartments of both the ATL and CC. This protein belongs to the family of heat shock protein 70 (HSP70), which has been implicated previously in SCZ [32]. A recent study showed that a polymorphism in the HSPA1A protein gene is associated

with increased risk of developing paranoid SCZ [33], and another study found increased mRNA expression of this gene in postmortem samples from the prefrontal cortex of patients with SCZ [34].

The analysis of differentially expressed proteins in the ATL nuclei showed additional changes in 4 more heat shock proteins, with 3 of these belonging to the HSP70 family: heat shock-related 70-kDa protein 2 (HSPA2), heat shock 70-kDa protein 6 (HSPA6), heat shock cognate 71-kDa protein (HSPA8), and 10-kDa heat shock protein (HSPE1). All these proteins were found at higher levels in the patients compared to controls, indicating a large response to cellular stress in the former. There is a debate about the effects displayed in the analysis of post-mortem brains in studies of psychiatric disorders. One of the main disputed points is whether or not such effects

Fig. 5. Deregulated network related to differentially expressed proteins in corpus callosum by STRING.

are a cause or consequence of the disease, or a consequence of prolonged treatment of these patients with different antipsychotic medications throughout their lives and through different stages of disease development. However, such data have appeared recurrently in proteomic data of postmortem brain of patients with SCZ and cannot be ignored. Nevertheless, corroborative studies of some format involving first-onset antipsychotic-naïve patients may help to resolve some of these issues.

Another family of proteins that were found differentially expressed in both the ATL (histone H3.3, histone H1.2, histone H2A type 1-B/E, histone H2A type 1-D, histone H2B type 1-B, histone H2B type 1-C/E/F/G/I, histone H4) and CC (histone H2A.V; histone H1.4; histone H3.1; histone H4), is the family of histone proteins. These proteins are linked to DNA, and they change the transcription mechanism of these molecules, thus modifying gene expression. Histones are also associated with neuronal functions such as synaptic plasticity, a function that is

known to be altered in patients with SCZ [35, 36]. Mass spectrometry studies have shown that posttranslational modifications in the nucleosome, which are characterized by the junction of DNA and a complex of histone proteins, regulate histone-DNA interaction, and are part of epigenetic mechanisms of genetic regulation [37]. Many recent studies associate histone dysregulation, and consequently dysregulation of epigenetic processes, with SCZ [38–40], and there has been much discussion on possible therapeutic targets based on targeted epigenetics in the treatment of this disease (reviewed in [41]). However, as more thorough epigenetic studies in postmortem brain tissue are still arduous and limited, little is known about this association.

Nuclear Proteins Altered in the ATL

In silico analysis of the proteins found at different levels in cell nuclei of the gray matter region showed that these proteins are mainly associated with spliceosome

complex (p value 5.9×10^{-6}) (online suppl. Table 1; see www.karger.com/doi/10.1159/000477299 for all online suppl. material). The spliceosome is a complex of 5 multi-megadalton ribonucleoproteins (snRNPs), which are abundant RNA-binding proteins that carry out processing of pre-mRNA transcripts. This complex removes introns of these molecules and rearranges exons, so that these conform and can give rise to the correctly configured mRNA transcripts [42]. Due to the exon rearrangement, a single pre-mRNA can give rise to different functioning mRNAs, and any perturbations of this process may lead to disease development or contribute to the severity of preexisting diseases [43].

The proteins related to this pathway that were found at altered levels in this study were heterogeneous nuclear ribonucleoproteins C1/C2 (HNRNPC), heterogeneous nuclear ribonucleoprotein K (HNRNPK), heterogeneous nuclear ribonucleoprotein U (HNRNPU), splicing factor, arginine/serine-rich 3 (SRSF3), and HSPA1A, HSPA2, HSPA6 and HSPA8, as described above. These proteins are the main constituents of the snRNP complex, which is the main component of the splicing operation (Fig. 4; KEGG).

Eight of the proteins belonging to the hnRNP family were found deregulated in a study of oligodendrocyte cells treated with clozapine, an antipsychotic used in the treatment of SCZ [44]. Moreover, studies of silencing and super-expression of proteins showed a crucial role of hnRNP proteins in the myelination of neurons by oligodendrocytes, independently of the indirect regulation of quaking proteins as previously proposed [45, 46]. This dysfunction in myelination was related to dysregulation of the synaptic connection [47–49]. One of these proteins was HNRNPC, which was found at decreased levels in SCZ patients compared to controls in this study. It is known that the regulation process of myelination undergoes a precise control dependent on the alternative splicing [45], and if the hnRNP protein complex is deregulated, this may cause aberrant alternative splicing, as reported in neurodegenerative and neuropsychiatric diseases such as frontotemporal dementia with Parkinsonism, amyotrophic lateral sclerosis and SCZ [50–52].

The deregulation of hnRNP proteins, in addition to being related to oligodendrocyte cells, is also related to the dysfunction of the neurotransmitter system. In a study performed using polymerase chain reaction analysis of SCZ prefrontal cortex tissue, alterations in splicing of mRNA molecules related to the GABAergic system were found [51]. Another study has shown that proteins related to dopamine receptors are also regulated by alter-

native splicing and that perturbed splicing of these proteins can cause dysfunctions in the dopaminergic system [53]. These systems are known to be altered in patients with SCZ [54]. Thus far, there are only a few recent studies that correlate perturbations in splicing and SCZ. However, the converging evidence suggests that this could play a major role in the disease process and therefore warrants further study, particularly accounting for effects on both neuronal and oligodendrocyte cells.

It should be considered that some of the alterations may be related to heterogeneity of the postmortem tissues including potential differences in cell types and cell density. Therefore, it may not be possible to identify which specific cell types the changes are associated with. However, it is likely that the changes are associated with glial cells when analyzing white matter and with neurons when analyzing gray matter, considering the natural abundance of such cells in these tissues. It should also be considered that data generated using postmortem tissue has other disadvantages such as potential differences in postmortem intervals or agonal states. Therefore, functional analyses, such as super-expression and knockout studies, should be done to test hypotheses resulting from such investigations using postmortem tissues. For example, microdissection of specific cell types could be used to assess significant cell-specific differences between patients and controls.

Nuclear Proteins of White Matter (CC)

After enriching the pathways of the 24 proteins found differentially expressed in the nucleus of the CC region, only 4 proteins were present in all of the enriched pathways (online suppl. Table 2) and were present in the same interaction network (Fig. 5). These 4 proteins were calmodulin (CALM1), calcium/calmodulin-dependent protein kinase type II subunit alpha (CAMK2A), calcium/calmodulin-dependent protein kinase type II subunit beta (CAMK2B), and calcium/calmodulin-dependent protein kinase type II subunit gamma (CAMK2G), which are all calcium dependent for their activation and response, as well as being constituents of the serine/threonine protein kinase family, which has been widely associated with metabolic events such as muscle contraction, cellular metabolism/proliferation, gene expression, and neurotransmitter release (reviewed in [55]).

The correlation between deregulation of calcium and SCZ began in the 1970s [56], and several studies confirming and discussing various aspects of this correlation have been performed. Among the many hypotheses that correlate calcium signaling to SCZ, there is the deregulation

of the dopaminergic system, which is one of the hypotheses of SCZ development of the disease [57]. Calcium participates in the uptake of dopamine by synaptic vesicles and, in this process, calcium is associated with CaMKII proteins [26]. In this study, we found increased levels of the alpha, beta, and gamma CAMK2 proteins, suggesting that there may be a high uptake of dopamine by synaptic vesicles and, consequently, a greater release of the neurotransmitter in the synaptic cleft [54]. The major focus in most studies in SCZ has been on the proteins of neuronal cells, as can be seen in the results presented above. However, one study quantified the calmodulin proteins in nuclei of neuronal and glial cells and showed that the abundance of these molecules is greater in the latter cell type [58]. Even so, little research has been done investigating the role of the deregulation of calmodulin proteins in glial cells.

A few recent studies have associated calcium/calmodulin-dependent protein with glial cells in functions such as actin cytoskeleton remodeling, glutamate and glycine transport, and regulation of neurotrophic factors [59–62]. This involves the activation of the PI3K protein which, according to Pérez-Garcia et al. [62], is regulated by calmodulin proteins associated with calcium. The process of activation of the PI3K protein involves its autophosphorylation, which was found to be reduced in a recent study of postmortem CC from patients with SCZ performed by our group [63]. Given that neurotrophic factors are decreased in patients [64], we can associate the increased amount of CAMK found in this study to a form of cellular compensation which aims to normalize the activation of the neurotrophic factors. Another possible association is that the high concentration of CaMK proteins may desensitize the PI3K activation process, causing the observed disruption in this pathway.

Also present in the network identified above are the nucleic acid metabolism-related proteins, which are proteins of the HSP family, and proteins linked to the genetic transcription, which belong to the family of hnRNPs.

These results are related to those found in the ATL region, showing a possible relationship between the deregulation of the white and gray matter, with changes in cell stress and regulation of genetic expression.

Conclusion

The results found in this study provide an overview of the participation of nuclear proteins in the pathophysiology of the disease. When it comes to the gray matter, the results point to the dysregulation of the spliceosome, an area which has not been investigated previously. In the case of the white matter, the role of calcium/calmodulin protein deregulation in glial cells has only been partly explored in previous studies, and may be related to altered production of neurotrophic factors. These results represent the first in-depth study comparing effects on gray and white matter in SCZ and lay the groundwork for further studies in this area to help increase our understanding of this complex disease. This could lead to identification of novel biomarkers and drug targets which, in turn, may result in development of newer and better treatment options for people suffering from this disease for improved therapeutic outcomes.

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Disclosure Statement

The authors declare no conflict of interest.

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