Differences in Protein Profiles in Schizophrenia Prefrontal Cortex Compared to Other Major Brain Disorders

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Abstract

We are approaching the challenge of elucidating novel mechanisms in neuropsychiatric disease by using high-throughput global profiling methods, with an emphasis on pathway and functional changes. Previous studies from our laboratory have suggested a role for abnormal brain metabolism in the pathophysiology of schizophrenia. Applying these methods across disorders allows us to pinpoint unique molecular disease signatures as distinct from general indicators of compromised brain function. 2D-DIGE was used to investigate the protein profile of tissue from dorsolateral prefrontal cortex (gray and white matter) from patients with bipolar disorder, Down's syndrome, Alzheimer's disease, and control subjects. Differentially expressed proteins were sequence identified and organised according to functional category and compared to our previous findings from schizophrenia samples. Down's syndrome and Alzheimer's samples gave highly similar results to each other, with all differentially expressed proteins being upregulated, especially synaptic and cytoskeletal proteins. In the schizophrenia group, the majority of proteins were downregulated, with a substantial number involved in glucose and other metabolism, and similar results were seen in both gray and white matter. In contrast, bipolar disorder gray and white matter gave strikingly different patterns of protein expression. These data show that the protein profile of brain tissue from schizophrenic patients has distinct molecular characteristics compared to bipolar disorder and to neurodegenerative disorders. Furthermore, the changes identified by global profiling methods can be used to identify both unique and common mechanisms in major psychiatric and neurological disorders.

Key Words: Schizophrenia, Alzheimer's Disease, Bipolar Disorder, Down's Syndrome, Proteomics, Post Mortem, Prefrontal Cortex

Introduction

Schizophrenia is a severe psychiatric disorder with a worldwide prevalence of about 1%. Although much effort has been put into identifying the molecular signatures and understanding the mechanisms that underlie schizophrenia, the results are far from clear, and a consensus has yet to be reached. Recent studies using high-throughput profiling methods from our laboratory (1) and others (2-5) have indicated decreased energy metabolism in the brains of schizophrenic patients, including alterations in mitochondrial oxidative phosphorylation, at the RNA, protein and metabolite level. One concern with these data is that such findings may not reflect true disease-related changes in schizophrenia, but may be due to post-mortem artefact, the non-specific effects of chronic illness, or at best general indicators of compromised brain function, particularly as metabolic disturbances have been reported in other neuropsychiatric and neurological disorders (6, 7).

To investigate this possibility further, we have undertaken

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parallel investigations into related disorders, namely bipolar disorder and neurodegenerative disorders including Alzheimer's disease and Down's syndrome. Bipolar disorder and schizophrenia are symptomatically related neuropsychiatric disorders, both with a high rate of suicide, for which different effective medications exist. However, diagnosis of these disorders remains subjective and no clear brain pathology has been unequivocally identified (8, 9). In contrast, many neurodegenerative disorders are relatively well characterised, at the clinical, pathological, and molecular level. Recent advances have been made in both the molecular genetics and neuropathology of Alzheimer's disease (AD) (10), although it is clear that multiple etiologies exist and research has yet to yield a definitive therapy.

Down's syndrome is a broad spectrum disorder resulting from trisomy of chromosome 21, with both developmental and late-onset components including an increased incidence of psychiatric illness (11). There is a highly increased incidence of AD in Down's syndrome, with almost 100% of individuals over the age of 40 developing the neuropathological hallmarks of AD (12) and 77% suffering symptoms of dementia before the age of 69, with rapid progression (13).

It is therefore of interest to compare and contrast these related disorders, both to identify common mechanisms and to distinguish which of the findings are disease-specific.

In this study, we employed 2-dimensional fluorescence difference in-gel electrophoresis (2D-DIGE) (14, 15) to investigate the proteome in post-mortem brain of patients with Alzheimer's disease (AD), Down's syndrome (DS), bipolar disorder (BPD) and matched controls for each disorder. Studies such as these which examine large numbers of protein changes present a particular challenge in terms of data interpretation and the extraction of biologically meaningful knowledge. By applying functional annotation to each differentially expressed protein, it is possible to consider the data in terms of functional categories. Using this approach we hope to extract biological meaning which is not apparent from examining an extensive list of individual proteins. In addition, such an approach is particularly useful when comparing results between labs, methodologies, or tissue sets, as the comparison is not limited by the sensitivity or specificity of individual protein changes. In the present study, this approach is used to first examine these disorders separately and then compare each with our previously published 2D-DIGE data from schizophrenia (SZ) post-mortem brain (1).

Materials and Methods

Tissue Collection

Consent: The tissue has been collected with full informed consent obtained from a first degree relative after death in compliance with the Declaration of Helsinki. Appropriate ethical approval for the human post-mortem tissue use has been obtained from Cambridge Local Research Ethics Committee. All patient data are anonymised.

Neuropsychiatric samples: Fresh-frozen prefrontal cortex tissue (Brodmann area 9) from white and gray matter of 10 bipolar and 10 matched control individuals, together with 10 SZ individuals (1), was obtained from the Array collection of the Stanley Medical Research Institute, USA.

Neurodegenerative samples: Fresh-frozen prefrontal cortex tissue from 15 AD and 10 DS individuals was obtained from the Department of Anatomy with Radiology, Faculty of Medical and Health Science, University of Auckland (Auckland, New Zealand), and the Department of Pathology, Addenbrookes Hospital, University of Cambridge (Cambridge, UK), respectively, plus 8 controls. As tissue from the AD and DS disease groups was derived from different sources, samples from both sources were included in the neurodegenerative control set. Only gray matter was available for the study of these disorders.

Demographics

Details of case demographics are shown in Table 1. Each tissue set (neuropsychiatric and neurodegenerative) was analysed relative to its own distinct control set, in order to minimise variation in storage conditions, PMI and age.

Sample Preparation

Samples were homogenized in amidosulfobetaine 14 (ASB14) buffer (8M urea, 2%ASB14, 5mM Mg acetate, 20mM Tris base, pH 8) containing complete protease inhibitor cocktail (Roche) and phosphatase inhibitors (1mM Na pyrophosphate, 1mM Na orthovanadate, 10mM glycerophosphate, 50mM NaF and 1% Triton). Proteins were extracted by precipitation using 100mM ammonium acetate in methanol and resuspended in ASB14 buffer. The concentration of protein was determined using a detergent-compatible protein assay kit (BioRad).

2D-Fluorescence Difference Gel Electrophoresis (2D-DIGE)

2D-DIGE was performed as described in detail previously (15). The overall experimental design is illustrated in Figure 1. In short, individual protein samples (100 µg) were minimally labeled with Cy3 or Cy5 (400 pmol, GE Healthcare). In order to eliminate inter-gel experimental variation, an internal standard was used, generated from a protein pool consisting of all protein samples included in each study, in order to include the full range of proteins from this tissue. The internal standard was minimally labeled with Cy2 (400 pmol). The same standard was used for all of the neurodegenerative samples; BPD and SZ samples were run with their own internal standards.

All CyDyes were supplied as charge-balanced NHS es-

Table 1 Demographics

Summary of key demographic details of (a) neuropsychiatric samples and (b) neurodegenerative samples (mean±SD). Demographic variables of schizophrenia samples are provided for comparison.

n: number; M: male; F: female; PMI: post-mortem interval; n/a: not applicable; NOS: not otherwise specified.

1 (a) Neuropsychiatric disorders Patient (n)	Schizophrenia (10)	Control (10)	Bipolar (10)
Age (years)	39.6 (±11.8)	45.6 (±8.3)	39.1 (±8.0)
Gender (m/f)	8/2	7/3	7/3
PMI (hours)	28.4 (±13.3)	31.5 (±14.8)	39.1 (±19.5)
Brain pH	6.43 (±0.25)	6.49 (±0.29)	6.50 (±0.28)
Fluphenazine mg. equivalents	58950 (±60161)	n/a	5300 (±9522)
Treated with mood stabilizers (y/n)	0/10	n/a	6/4
Medicated at time of death (y/n)	9/1	n/a	5/5
Primary diagnosis	2/1/7 (paranoid/disorgan/unspec)	n/a	8/1/1 (I/II/NOS)
Psychotic features (yes/no/unknown)	10/0/0	n/a	5/3/2
1 (b) Neurodegenerative disorders Patient (n)	**Alzheimer's disease (15)**	**Control (8)**	**Down's syndrome(10)**
Age (years)	76.2 (±5.05)	63.8 (±9.10)	58.1 (±8.70)
Gender (m/f)	7/8	6/2	5/5
PMI (hours)	13.3 (±11.91)	13.9 (±8.23)	11.9 (±6.7)*

*PMI was not known for 3 of the 10 DS samples.

ters. Proteins labeled with Cy2 (pool of all samples within the experiment), Cy3, and Cy5 were mixed and separated by isoelectric focusing using 24cm nonlinear IPG DryStrips, pH 3–10 (GE Healthcare) according to the manufacturer's instructions. Proteins were further separated according to molecular weight using SDS-polyacrylamide gels (12%, Ettan DALT Twelve apparatus, GE Healthcare). Following electrophoresis, gels were scanned at appropriate wavelengths for Cy2, Cy3 and Cy5 fluorescence using a Typhoon 9400 (GE Healthcare). A representative gel image is shown in Figure 2(a).

Data Processing and Statistical Analysis

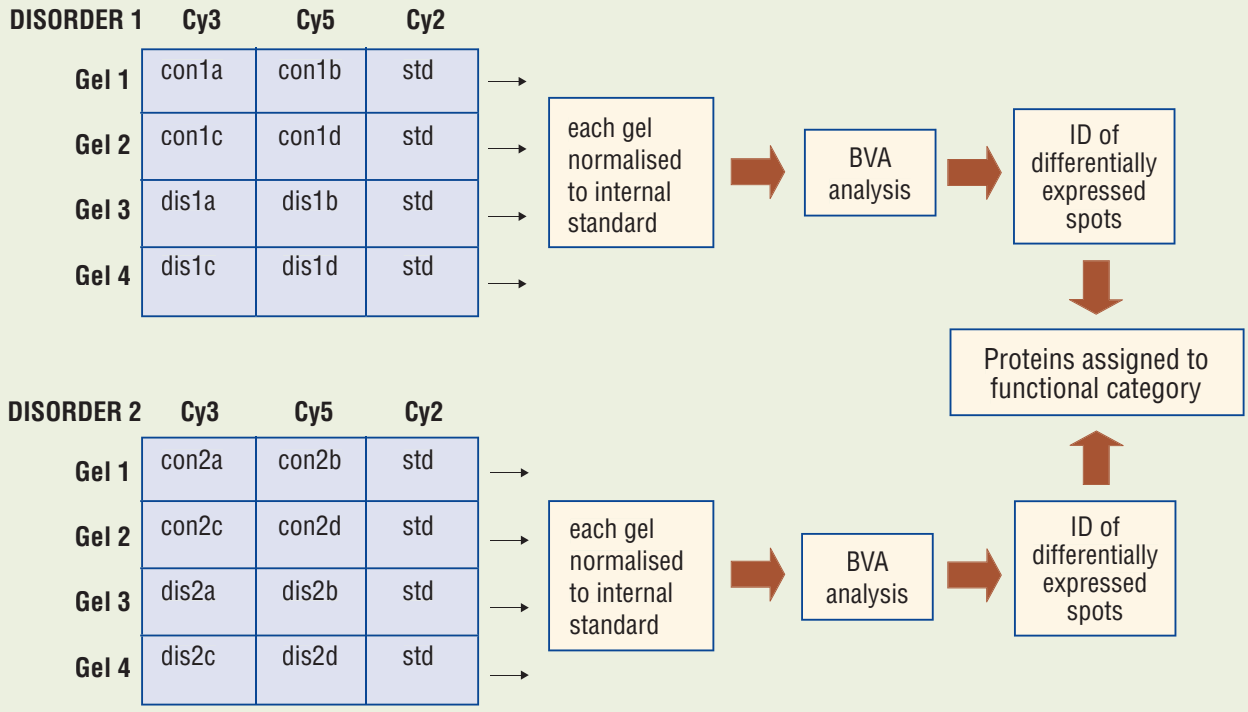
Gel images were cropped using ImageQuant V5.2 (GE Healthcare), and further analysis was carried out using DeCyder software which is a standard part of the Ettan DIGE system (14) (GE Healthcare). This software makes use of the internal standard first for intra-gel co-detection (of samples and standard), and then for inter-gel matching of the internal standard across multiple gels to ensure that the same spots in each gel are compared. DeCyder Batch Processor was used for automated detection and quantification of spots within a gel in terms of the ratios of the Cy3 and Cy5 sample volumes to the standard Cy2 volumes. The Biological Variance Analysis (BVA) module matched the spots between gels and standardized the ratios across the gels accounting for the observed differences in the Cy2 sample volumes. The BVA module was also used for statistical analysis of normalized protein abundance changes between samples (Student's t-test). Representative examples of differential expression are given in Figure 2(b). Additional analysis of the effects of demographic variables on the neurodegenerative sample set was carried out using R with Bioconductor (16, 17) as described below.

Investigation of Demographic Variables

In studies where the samples have been drawn from a heterogeneous population, it is important to take into account demographic variables which have the potential to distort the relationship between the factor of interest (i.e. presence/absence of disease) and protein expression. Using the statistics software R (17), we investigated the potential confounding effects of demographic variables recorded for our samples as follows. In most instances, demographic variables were well matched between groups. No significant differences were found in sex ratio (using Fisher's exact test), or PMI (using ANOVA) between the group means in each of the sample sets. However, group means for age

Figure 1 Schematic showing experimental design

For each disorder, each patient sample is labelled individually, protein expression in cases and controls is normalised to an internal standard, and individual proteins differentially expressed between cases and controls are identified by Biological Variance Analysis (BVA). Following identification of spots by mass spectrometry, differentially expressed proteins in each disorder were assigned to a functional category. The data from each disorder can then be qualitatively compared.



were found to vary between groups in the neurodegeneration sample set (by ANOVA p=0.000003). A post-hoc test (Tukey's honest significant difference), showed that the mean age of the AD group was significantly different from that of both the control group and the Down's syndrome group (p<0.01).

For age to be a confounding variable in this study, it should not only vary systematically between control and disease groups (as has been shown above), but it should also show some correlation with the expression of one or more proteins. We investigated the correlation of age with the expression (standardized log abundance) of each of the proteins detected in this study, using Spearman's method. The expression of 59 spots was found to be significantly correlated with age (p<0.05), out of a total of 3,862. These spots were removed from further analysis.

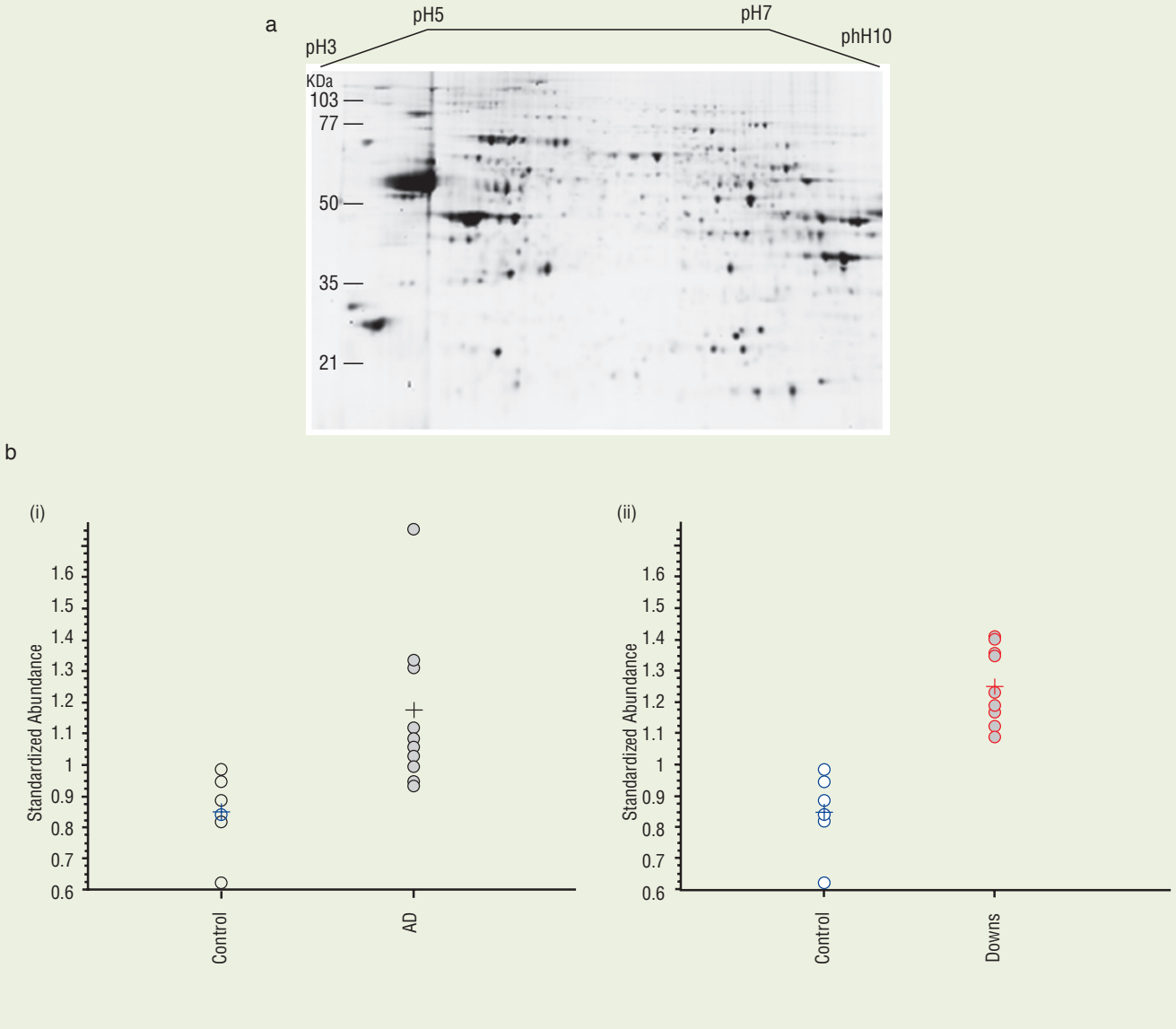
As tissue from the AD and DS disease groups was derived from different sources, samples from both sources were included in neurodegenerative control set. Principal components analysis (PCA) was used to demonstrate that there was no difference in the global protein profile of the controls from different sources.

Protein Identification and Categorization

Protein spots showing significant changes ($p \leq 0.05$) in AD, DS or BPD with respect to controls were picked from a colloidal Coomassie-stained gel. Proteins within the gel pieces were first reduced, carboxyamidomethylated, and then digested to peptides using trypsin on a MassPrepStation (Waters, Manchester, UK). The resulting peptides were applied to LC-MS/MS. For LC-MS/MS, the reverse phase liquid chromatographic separation of peptides was achieved with a PepMap C18 reverse phase, 75 µm i.d., 15-cm column (LC Packings, Amsterdam) attached to a Dionex Dual Gradient LC system attached to a QSTAR XL (Applied Biosystems, Framingham, MA, USA). Peptides were separated using a 60-minute gradient of 5% to 32% v/v acetonitrile/0.1% v/v formic acid. A typical electrospray needle

Figure 2 Representative Examples

(a) The protein profile of human pre-frontal cortex tissue separated on a 24 cm pH 3-10 non-linear strip and 12% SDS-polyacrylamide gel, and (b) differential protein expression, in this case CAPG protein in (i) AD and (ii) DS, consisting of standardized protein abundance values generated by the BVA software module (see text for details). Mean abundance values are indicated by a cross.



voltage used was 2000V. The QSTAR was operated in information dependent acquisition mode (IDA) in which one-second precursor ion scans from 400 to 1600 m/z were performed, followed by three-second product ion scans (100 to 1580 m/z) on the two most intense doubly or triply charged ions. The MS/MS fragmentation data achieved was used to search the National Center for Biotechnology Information database using the MASCOT search engine (http://www.matrixscience.com). Probability-based MASCOT scores were used to evaluate identifications. Only matches with $p < 0.05$ for random occurrence were considered significant and identifications based on a single peptide were discarded. Protein hit lists from all sample sets were combined and proteins were assigned to functional categories using Gene Ontology information (18) and literature searching.

Results

In this study, differentially expressed proteins were identified where possible by LC-MS/MS as described in Table 2. Details of dysregulated proteins in BPD and in the neurode-

Table 2 Summary of 2D-DIGE Analysis

Column 2 shows the average total number of protein spots per gel identified by BVA, of which a proportion were found to be significantly altered when compared to controls ($p<0.05$) (column 3). Some significantly altered spots were removed from the AD analysis due to the confounding effect of age (see text for details). Of the differentially expressed spots, a number were successfully identified by LC-MS/MS (column 4). Comigrating protein mixtures were eliminated from further analysis, leaving those which corresponded to a single known protein (column 5). Several proteins were identified at multiple positions on the gel suggestive of posttranslational modifications and/or different isoforms, thus the final number of individual proteins is shown in column 6. Detailed data for SZ samples can be found in Prabakaran et al 2004 (1).

Disease group	Average no. of spots detected in DeCyder	Total no. of significant spots ($p<0.05$)	Total no. of identified spots	Total no. of single known proteins	Total no. of unique proteins
AD	3862	70	54	41	24
DS		137	94	70	29
BPD (gray)	2033	84	61	55	35
(white)		42	25	22	18

generative disorders are listed in Tables 3 and 4, respectively. SZ data is shown for comparison in Table 5. Protein hit lists from all four disorders were organised into functional categories using Gene Ontology information (18) and additional literature searches. The identified proteins fell into fifteen categories: vesicle trafficking, cytoskeletal, glycolysis, TCA cycle, oxidative phosphorylation, mitochondrial, other metabolism, blood regulation, protein folding, neuronal growth and development, protein synthesis, cell signalling, oxidative stress response, ubiquitination, and membrane structure. Details of individual proteins and their categories are shown in Tables 3-5 and represented graphically in Figure 3.

In this section, we present and discuss data from BPD, AD, and DS. Previously, we reported changes in protein expression in SZ which are described and discussed in detail in Prabakaran et al 2004 (1). In the final section, we discuss these major data sets in comparison with each other.

Bipolar Disorder

Both white and gray matter tissues from the DLPFC were investigated for bipolar patients and controls. Differentially expressed proteins are listed in Table 3. There is a noticeable difference between the two tissue types, with the vast majority of proteins in white matter being upregulated, including metabolic and oxidative stress markers (Figure 3, c & d). In contrast, in gray matter both up- and down-regulation of protein expression was observed with prominent protein categories including cytoskeletal and vesicle proteins, and oxidative stress and metabolic markers which were predominantly downregulated. White matter-specific changes in the bipolar brain have been pinpointed using gene expression profiling (19) as well as neuroimaging techniques (20).

In the gray matter, the data indicate a dysregulation of synaptic proteins, with the majority being upregulated. Existing data are indicative of a synaptic pathology in bipolar disorder (9), although no large scale ultrastructural study has been carried out. Cytoskeletal proteins, such as tubulin, actin, neurofilament light chain, and gelsolin are also found to be altered; this may be connected to synaptic dysregulation and/or may reflect cell atrophy and shape changes (21). Cytoskeletal proteins are also involved in myelination and glial development (22); inhibition of oligodendrocyte branching and outgrowth has been linked to increased expression of depolymerizing proteins such as gelsolin (23). Interestingly, both GFAP, an astrocytic protein, is upregulated in gray matter, and CNP, a marker of oligodendrocytes, is upregulated in both gray and white matter. Numerous studies (21, 24-26) have shown a decrease in glial cell number in bipolar disorder; however, Rajkowska et al (2001)(21) shows that glial size is increased in Brodmann area 9, with a similar trend seen in Brodmann area 24 (27). The lack of glial proliferation has been taken as evidence against a classic neurodegenerative process in BPD (21) pointing towards a more complex glial pathology, which may be connected to the prominent upregulation of differentially expressed proteins observed in white matter. One of the most consistent findings in fMRI studies of bipolar patients is a nearly 3-fold increase in the occurrence of white matter hyperintensities (WMH) (28, 29). The causes of these WMHs in bipolar disorder remain unclear, but may include astrogliosis, demyelination and loss of axons (30).

The data for proteins falling into energy metabolism categories reveal a general trend for downregulation. However, some are upregulated, such as aconitase and malate dehydrogenase, which are increased in both gray and white matter. Interestingly, proteins involved in glutamate metabolism, namely folate hydrolase, glutamate dehydrogenase, and glutamate-ammonia ligase are upregulated in BPD.

Figure 3 **Differentially expressed proteins in (a) SZ (gray matter), (b) SZ (white matter), (c) BPD (gray matter), (d) BPD (white matter), (e) AD, and (f) DS displayed according to functional category derived from GO ontologies and literature searching.**

Table 3 Bipolar Disorder (gray and white matter)

Oxidative stress	Cell signaling	Protein synthesis	Neuronal growth/dev	Protein folding	Blood regulation	Other metabolism	Oxidative phosphorylation	Mitochondrial	TCA cycle	Glycolysis	Cytoskeletal	Vesicle trafficking	Name	*Gray matter*— Av. ratio	*Gray matter*— T-test	*White matter*— Av. ratio	*White matter*— T-test	Accession number (SwissProt/ TrEMBL)	Mascot Score	Sequence coverage (%)	Max peptides
								✓	✓				Aconitase (ACO2)	1.43	0.0011	1.6	0.029	Q8TAQ6, Q99798	687	32	2
											✓		Actin (ACTB)	1.31	0.025			P02570	508	31	17
											✓		Actinin, alpha 4 (ACTN4)			1.86	0.049	O43707	128–154	7–8	6
✓					✓								**Albumin (ALB)**	2.82–2.66 (2)	0.015–0.0076	2.14–2.82 (2)	0.0014–0.015	P02768	552–687	27–37	22
						✓	✓	✓					**ATP synthase alpha chain (ATP5A)**	–1.25 to –1.46 (3)	0.004–0.03			P25705	312–567	16–21	21
			✓								✓		**Brain acid soluble protein 1 (BASP1)**	–1.8 to –1.91 (2)	0.0028–0.029			P80723	177–204	33–47	6
						✓							**Carbonyl reductase 1 (CBR1)**			1.39	0.029	P16152	100	14	2
				✓							✓		Chaperonin containing TCP1, subunit 2 (alpha) (CCT2A)	1.31	0.028	1.61	0.00025	P17987	291	21	11
				✓							✓		Chaperonin containing TCP1, subunit 2 (beta) (CCT2A)	–1.22	0.048	1.26	0.019	P78371	429	26	14
								✓	✓				Citrate synthase (CS)	–1.38	0.0019	1.68	0.014	O75390	52	3	2
			✓										Collapsin response mediator protein hCRMP-2 (CRMP2)	1.37	0.0043	1.29	0.045	Q16555	380	19	16
						✓							Creatine kinase, brain (CKB)	–1.19	0.01			P12277	362	28	21
											✓		**Cyclic-nucleotide phosphodiesterase (CNP)**	1.61–2.32 (6)	0.008–0.045	1.31	0.012	P09543	305–796	22–38	29
											✓	✓	Dynamin 1/2 (DNM1/2)	1.34	0.015			Q05193, P50570	66	3	2
										✓			Enolase, alpha (ENO1)	–1.3	0.0037			P06733	239	14	5
						✓							Folate hydrolase (FOLH1)	1.36	0.019			Q04609	146–148	11	5

Tables 3-5. Accession numbers, fold changes, and p-values for single-hit proteins that showed significant expression changes ($p \leq 0.05$) in each disorder. Fold changes represent the mean of all independent data points in each disease group. Proteins detected in more than one spot (shown in bold) may be indicative of either different isoforms and/or posttranslational modifications. The number of detected spots is noted in parentheses while the fold differences and p-values are given as a range. Multiple accession numbers are given for proteins where it was not possible to identify a single isoform. Mascot scores >50 indicate significant identity or extensive homology to sequenced proteins ($p<0.05$). The percent coverage for each polypeptide is also shown, and the maximum number of peptides per protein identified. The association of each protein with one or more functional categories is indicated by ticks on the left hand side and has been determined from publicly available gene ontology databases and literature searches.

Table 3 Bipolar Disorder (gray and white matter) *(Continued)*

Oxidative stress	Cell signaling	Protein synthesis	Neuronal growth/dev	Protein folding	Blood regulation	Other metabolism	Oxidative phosphorylation	Mitochondrial	TCA cycle	Glycolysis	Cytoskeletal	Vesicle trafficking	Name	*Gray matter*— Av. ratio	*Gray matter*— T-test	*White matter*— Av. ratio	*White matter*— T-test	Accession number (SwissProt/ TrEMBL)	Mascot Score	Sequence coverage (%)	Max peptides
											✓		Gelsolin (GSN)	1.31	0.021			P06396	459	20	17
											✓		Glial fibrillary acidic protein (GFAP)	1.73	0.019			P14136	980	57	70
			✓							✓			Glucose phosphate isomerase (GPI)			1.48	0.025	P06744	58	4	3
						✓							Glutamate ammonia ligase (GLUL)	1.84	0.043			P15104	153	10	5
						✓							**Glutamate dehydrogenase (GLUD1)**	1.32 to 1.4 (2)	0.00019–0.055	1.42	0.038	P00367	147–371	11–26	13
						✓			✓				Glutamic-oxaloacetic transaminase (GOT1)	1.23	0.028			P17174	237–239	14	6
										✓			**Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)**	–1.25–1.34 (3)	0.024–0.029			P04406, P00354	193–555	23–27	27
				✓				✓					Heat shock 60kDa protein 1 (HSP60)	–1.13	0.016			P10809	663	40	18
✓				✓				✓					**Heat shock 70kDa protein (HSP70)**	–1.21 to –1.52 (3)	0.0011 –0.014	1.2–1.66 (2)	0.016–0.036	P11021, P54652, P11142	59–771	6–40	31
										✓			Lactate dehydrogenase A (LDHA)	–1.51	0.018			P00338	98–101	10	4
									✓				Malate dehydrogenase, soluble (MDH1)	1.23	0.0014	1.27	0.0081	P40925	309	21	2
												✓	N-ethylmaleimide-sensitive factor (NSF)	1.34	0.014			P46459	400	18	12
											✓		**Neurofilament light polypeptide (NFL)**	–1.99 to –2.28 (2)	0.023–0.045			P07196	643–820	29–34	24
						✓		✓	✓				Oxoacid CoA transferase (OXCT1)			1.56	0.011	P55809	111	5	3
						✓							Phosphotidylinositol transfer protein, beta (PITPNB)	–1.22	0.017			P48739	127	11	2
										✓			Pyruvate kinase (PKM2)	1.35	0.028			P14618	416	22	14
						✓							Quinoid dihydropteridine reductase (QDPR)	1.27	0.017			P09417	154	12	4
												✓	Ras-related protein Rab-1A (RAB1)			–1.56	0.0053	P11476	68	11	2

Continued on the next page

Table 3 Bipolar Disorder (gray and white matter) *(Continued)*

Oxidative stress	Cell signaling	Protein synthesis	Neuronal growth/dev	Protein folding	Blood regulation	Other metabolism	Oxidative phosphorylation	Mitochondrial	TCA cycle	Glycolysis	Cytoskeletal	Vesicle trafficking	Name	*Gray matter*— Av. ratio	*Gray matter*— T-test	*White matter*— Av. ratio	*White matter*— T-test	Accession number (SwissProt/ TrEMBL)	Mascot Score	Sequence coverage (%)	Max peptides
											✓	✓	Septin 6 (SEPT6)	1.47	0.032			Q14141	120–324	6–18	8
											✓		Spectrin (SPTA2)	–2.07	0.011			Q13813	212–215	2	4
✓								✓					Superoxide dismutase (SOD2)	–1.36	0.023			P04179	57	9	2
												✓	**Syntaxin binding protein 1 (STXBP1)**	1.27–1.64 (4)	0.013–0.049	1.65–2.61 (3)	0.00046–0.022	Q64320	153–265	7–13	8
										✓			**Transketolase (TKT)**	1.43–1.72 (2)	0.009–0.018			P29401	66–87	4–7	4
										✓			Triose phosphate isomerase (TPI)			1.24	0.037	P00938, P60174	72	8	3
											✓	✓	Tropomyosin (TMSA)	–1.49	0.0004			P09493	57	9	2
											✓		**Tubulin, beta (TUB)**	1.28–1.48 (3)	0.028–0.046			P05217, Q13748, P05218	507–517	29–37	25

Glia are primary sites of glycolysis during neuronal activity (31, 32); therefore, the dysregulation of glial markers and metabolic proteins may be linked.

A number of proteins involved in neuronal growth and development are dysregulated in BPD, with both CRMP2 and GPI being upregulated (in white matter). Mood stabilising drugs are known to have an effect on axon outgrowth and neurogenesis (33-35), thus these changes may be explained in terms of drug effects; however, as only 5 of 10 subjects were on medication at the time of death, this is unlikely to fully explain these findings.

Neurodegenerative Disorders

Proteins identified as being changed in gray matter from AD or DS patients are shown in Table 4. There is a striking similarity between the two disorders, with all proteins detected as changed being upregulated in both disease groups (Figure 3, e & f). Around 50% of individual protein changes in the DS list were also verified at the transcript level ($q<0.05$) and a further 5 showed a trend for upregulation ($q<0.15$), with only one protein found downregulated at the transcript level which had been found upregulated at the protein level (data not shown; manuscript submitted for publication). The dementing process of both DS and AD is known to have a common neuropathological endpoint, but the overwhelming similarity between the two disorders is nevertheless unexpected and suggests fundamental common mechanisms. In particular, although the AD and DS sample set show age-related differences, these are not reflected in the major disease findings. This suggests that the findings in DS are not a simple function of precocious aging, but are a representation of the end-stage pathology itself.

The most prominent categories of altered expression in these two disorders are related to vesicle trafficking and the cytoskeleton, and many of the individual proteins in these categories are known to directly interact with each other as part of SNARE-mediated exocytosis (NAPA, STXBP1, NSF) (36, 37) and clathrin-dependent endocytic vesicle recycling (CLTC, DNM1) (38). Synapsin is thought to mediate interaction between vesicles and the cytoskeleton (39); villin, moesin, and CAPG mediate interactions between the cell surface and cytoskeleton (40, 41). CAPG is related to gelsolin, which is also found upregulated in normal aging brains in cells resistant to apoptosis (42). The major structural components actin and tubulin were found upregulated, as well as their crosslinking agent coronin (39). GFAP, a marker of astrocytes activated in response to local inflammation/neuronal damage, is upregulated; this is a well-documented feature of neurodegenerative disease.

Table 4 Neurodegenerative disorders (AD & DS)

Oxidative stress	Cell signaling	Protein synthesis	Neuronal growth/dev	Protein folding	Blood regulation	Other metabolism	Oxidative phosphorylation	Mitochondrial	TCA cycle	Glycolysis	Cytoskeletal	Vesicle trafficking	Name	AD Av. ratio	AD T-test	DS Av. ratio	DS T-test	Accession number (SwissProt/ TrEMBL)	Mascot Score	Sequence coverage (%)	Max peptides
								✓	✓				**Aconitase (ACO2)**	1.23–1.4 (3)	0.026–0.0073	1.17–1.36 (3)	0.0087–0.047	Q99798	70–786	5–26	21
											✓		**Actin (beta/gamma) (ACTB/G)**	1.27–1.31 (3)	0.012–0.035	1.37–1.55 (5)	0.000061–0.0047	P60709, P63261	86–125	6–37	11
											✓		Actin related protein 1B (ACTR1A)			1.15	0.013	P42025	61–172	3–14	3
											✓		Actin related protein 3 (ACTR3)	1.48	0.013	1.46	0.00053	P61158	129	12	5
	✓												Adenylyl cyclase associated protein (CAP1)	1.37	0.0069	1.39	0.0029	Q01518, P40123	196	14	7
✓						✓		✓					Aldehyde dehydrogenase family 4A1 (ALDH4A1)	1.43	0.0016	1.38	0.0029	P30038	150	10	5
✓						✓		✓					Aldehyde dehydrogenase family 7A1 (ALDH7A1)	1.35	0.0041	1.37–1.37 (2)	0.0033–0.016	P49419	83–136	6–12	5
												✓	**Clathrin heavy chain (CLTC)**	1.4–1.63 (2)	0.015–0.0045	2.11–2.2 (2)	0.0024–0.012	Q00610	134–308	4–7	11
			✓										Contactin (CNTN1)			1.28	0.016	Q12860	288	9	9
											✓		Coronin 1A (CORO1A)			1.5	0.0078	P31146	67	4	2
											✓	✓	**Dynamin 1/2 (DNM1)**	1.19–1.4 (4)	0.015–0.045	1.31–1.71 (8)	0.00094–0.048	Q05193, P50570	59–374	1–15	12
										✓			**Enolase (ENO1)**	1.34–1.48 (4)	0.00086–0.0052	1.18–1.52 (4)	0.0037–0.036	P06733	89–861	11–50	42
		✓											Eukaryotic translation elongation factor (EEF1G)	1.36	0.029	1.49	0.0023	P26641	103	9	4
					✓								**Fibrinogen (FGA)**	1.4	0.013	1.36–1.38 (2)	0.02–0.056	P02671	107–137	6–9	5
											✓		Gelsolin-like capping protein (CAPG)	1.39	0.00081	1.48	0.0000068	P40121	249	29	8
											✓		**Glial fibrillary acidic protein (GFAP)**	5–6.71 (2)	0.0007–0.019	1.51–4.36 (3)	0.00014–0.0063	P14136	117–980	28–57	70
						✓							Glutamate dehydrogenase (GLUD1)	1.45	0.029	1.45	0.00017	P00367	166–307	13–39	11

Continued on the next page

Table 4 Neurodegenerative disorders (AD & DS)*(Continued)*

Oxidative stress	Cell signaling	Protein synthesis	Neuronal growth/dev	Protein folding	Blood regulation	Other metabolism	Oxidative phosphorylation	Mitochondrial	TCA cycle	Glycolysis	Cytoskeletal	Vesicle trafficking	Name	*AD* Av. ratio	*AD* T-test	*DS* Av. ratio	*DS* T-test	Accession number (SwissProt/ TrEMBL)	Mascot Score	Sequence coverage (%)	Max peptides
						✓							Glutamate-ammonia ligase (GLUL)			1.42	0.0053	P15104	81	8	2
	✓												Guanine nucleotide binding protein beta subunit (GNB1)			1.4	0.00029	P04901	399	36	15
	✓												**Guanine nucleotide binding protein alpha O (GNAO1)**	1.28–1.43 (2)	0.0065–0.013	1.42–1.72 (2)	0.00045–0.0018	P29777, P09471	67–314	2–22	7
✓				✓				✓					Heat shock 70 kDa protein (HSP70)	1.68	0.019			P08107, P54652, P38646	228–518	10–24	22
									✓				Malate dehydrogenase 1 (MDH1)			1.28	0.0018	P40925, P40926	84	7	4
											✓		Moesin (MSN)	1.38	0.00053	1.34–1.51 (2)	7.3E-06–0.0088	P26038	99–215	4–12	7
												✓	N-ethylmaleimide sensitive factor attachment protein (NAPA)			1.29	0.021	Q9H115	269	28	8
												✓	N-ethylmaleimide sensitive factor (NSF)	1.5	0.0013	1.24–1.62 (6)	0.003–0.034	P46459	80–354	1–17	17
						✓							Prenyl cysteine oxidase (PCYOX1)	1.33	0.0073	1.55	0.0018	Q9UHG3, Q8N4N5	72	6	4
	✓												Protein phosphatase 2A regulatory subunit B' PR53 (PPP2R4)	1.44	0.05	1.66	0.0018	Q15173	89	7	3
	✓												Protein tyrosine phosphatase non-receptor-type substrate 1 (PTPNS1)			1.49	0.002	P78324	54	4	4
										✓			**Pyruvate kinase (PKM2)**	1.22–1.3 (3)	0.003–0.028	1.31–1.35 (2)	0.023–0.048	P14618	467–739	31–50	24
											✓		Spectrin (SPTA2)	1.48	0.0097			P02549, Q13813		6–8	16
												✓	Synapsin II (SYN2)			1.63	0.021	Q92777	143	8	4
												✓	**Syntaxin binding protein (STXBP1)**	1.3–1.3 (2)	0.03–0.032	1.34–1.65 (8)	0.00035–0.044	Q15833	68–716	7–26	21
											✓		Tubulin (TUB)			1.16	0.037	Q9BQE3	124–160	32–37	12
											✓		**Villin 2 (VIL2)**	1.38–148 (3)	0.007–0.02	1.42–1.8 (3)	0.00025–0.0026	P15311	300–529	4–23	16

One possible explanation for cytoskeletal and vesicle trafficking abnormalities may be the presence of hyperphosphorylated tau which accumulates in the form of neurofibrillary tangles in the AD brain (43). This protein aggregation interferes with axonal transport and microtubule interaction, with a subsequent accumulation of organelles in the cell body, which is followed by synaptic/neuritic degeneration (44). Amyloid precursor protein cleavage is linked with vesicle transport (45) and amyloid deposition is connected with the accumulation of large synaptic vesicles in the perikaryon (46, 47).

It is noted that these data appear to be in contradiction to the majority of studies showing overall downregulation or no change in synaptic proteins in AD (48-53) and DS (53, 54), although other studies show differing results for certain proteins (55, 56). The difference between our study and others may be to some extent explained by the heterogeneity of vesicle-associated proteins in neuronal and glial subtypes (57, 58), coupled with the precise brain region studied, and disease stage of the samples. Furthermore, the different methods used mean that careful interpretation of the data is required (e.g. tissue vs. homogenate based studies). One rigorous ultrastructural study (59) demonstrates that whilst the overall number of synapses is decreased in AD, the level of synaptic proteins in individual synapses is in fact increased in all brain regions studied, leading to a modest net increase in synaptic area. A similar morphological adaptive response has also been noted in normal aging (60). In addition, immunohistochemical analysis has pointed towards active dendritic sprouting and synaptic remodelling in certain areas of the brain in AD (61). Furthermore, the modification and sequesterization of different structural proteins may complicate interpretation of results derived from different methods (62, 63). It is important to note that increased levels of certain forms of a protein do not necessarily imply an increase in function. Post-mortem studies of degenerative disorders are not representative of the initial pathological process, but investigate the end-stage of a disease process, as the cells which are damaged by pathological processes die off and are rapidly removed from the system. The presence of upregulated proteins involved in metabolism and protein synthesis may also reflect compensatory processes occurring within surviving cells, promoting neurite outgrowth and fighting against oxidative stress, both processes which have been linked to amyloid β deposition (64). Indeed, increased activity of glycolytic enzymes has been linked with astrocytosis in AD (65). Despite the high degree of overlap between these disorders, it is equally important to note the changes which are unique to each disorder. Proteins which are upregulated in DS but not AD include contactin, a neuronal cell adhesion molecule involved in axon guidance (66), which may reflect the neurodevelopmental component of DS.

Discussion

Comparison with Schizophrenia Data

The pattern of differential protein expression in SZ and BPD is complex in both cases. Although the BPD data show deficits in glucose metabolism, in common with SZ, not all proteins are universally downregulated, and there is upregulation of synaptic vesicle processing and some metabolic pathways, in particular glutamate metabolism. BPD shows upregulation of some proteins involved in oxidative stress response, particularly in the white matter. This shows some similarity with the data from AD and DS, which we believe may be representative of a compensatory mechanism. In addition, there is upregulation of some proteins involved in neuronal growth and development, in contrast to SZ where these proteins are predominantly downregulated. This suggests that neurons in BPD may be undergoing more neurotrophic processes compared to SZ patients. Abnormalities of neuroplasticity and cellular resilience have been proposed to underlie both schizophrenia (67-69) and bipolar disorder (70). However, differences in the expression of genes for cell survival/death between BPD and SZ have recently been demonstrated in the hippocampus, with a greater involvement of apoptosis-related genes in BPD (71). The interplay between these processes may have a role in the induction of different mood states in BPD (72). Additionally, if the above findings do indeed represent greater cellular adaptability in BPD, they may underpin the better prognosis and social and intellectual functioning for sufferers of BPD over SZ patients.

Of particular note are the gray/white matter differences in SZ and BPD. In schizophrenia similar changes are seen in both gray and white matter, but in BPD the changes are rather different in the two tissue types, with virtually all proteins being upregulated in white matter, in contrast to the gray matter in which numerous proteins are downregulated. This is in keeping with neuroimaging data suggesting that in BPD specific abnormalities are found in white matter (29).

In both AD and DS, all altered proteins are upregulated, which may be indicative of compensatory mechanisms in the brain which aim to counteract the inevitable progression of neuronal loss. This is primarily reflected in the upregulation of synaptic vesicle processing, and in glucose and other metabolic pathways. In SZ, the prominent direction of change is downregulation; this may reflect a general deficit in glucose metabolism (1-4, 73) which in turn affects synaptic activity and neuronal plasticity, in keeping with data showing reduced neuronal size in SZ (8, 27, 74-76). There is little overlap between the proteomic signature of the neurodegenerative disorders and SZ. This is an interesting ob-

Table 5 Schizophrenia (gray and white matter)

Membrane structure	Ubiquination	Oxidative stress	Cell signaling	Protein synthesis	Neuronal growth/dev	Protein folding	Blood regulation	Other metabolism	Oxidative phosphorylation	Mitochondrial	TCA cycle	Glycolysis	Cytoskeletal	Vesicle trafficking	Name	*Gray matter*— Av. ratio	*Gray matter*— T-test	*White matter*— Av. ratio	*White matter*— T-test	Accession number (SwissProt/ TrEMBL)	Mascot Score	Sequence coverage (%)	Max peptides
										✓	✓				**Aconitase (ACO2)**	–1.25 to –1.29 (2)	0.035–0.043	–1.37 to –1.41 (3)	0.000011–0.0012	Q99798	65–723	5–33	22
													✓		**Actin (ACTA1)**					P02568	222–316	16–29	17
													✓		**Actin (ACTA2)**					P03996	222–369	16–29	26
													✓		**Actin (ACTAC)**	–1.15 to –1.55 (5)	0.011–0.048	–1.18	0.026	P04270	222–316	16–29	17
													✓		**Actin (ACTB)**					P02570	193–369	15–26	26
													✓		**Actin (ACTG1)**					P02571	259–518	18–42	22
													✓		**Actinin (ACTN4)**	–1.3 to –1.61 (2)	0.0055–0.0074	1.58	0.026	O43707	155–234	4–8	5
		✓				✓									Albumin	–1.69	0.0063			P02768	240–261	14	8
		✓					✓		✓						Aldehyde dehydrogenase (ALDH1)	–1.28	0.014			P00352	294	14	6
												✓			**Aldolase (ALDA/C)**	–1.25	0.027	–1.24 to –1.47 (5)	0.000086–0.035	P04075, P09972	367–611	39–56	26
														✓	ATPase, lysosomal, H+ transporting, 31kDa V1 subunit E (ATP6V1E1)			–1.33	0.0059	P36543	336	33	17
					✓								✓		**Brain abundant membrane attached signal protein 1 (BASP1)**	2.03 to 2.4 (2)	0.0089–0.035			P80723	177–204	33–47	6
								✓							**Carbonyl reductase (CBR1/3)**			–1.23 to –1.41 (2)	0.0029–0.03	P16152, O75828	103–387	11–39	16
			✓												**Cell division cycle 10 homolog (CDC10)**	–1.22 to –1.36 (2)	0.028–0.038			Q16181	128–214	11–18	8
								✓							**Creatine kinase (CKBB)**			–1.24 to –1.3 (3)	0.0014–0.0023	P12277	96–193	9–12	34
													✓		**2'-3-cyclic nucleotide phosphodiesterase (CNP)**	–1.28 to –1.63 (7)	0.0024–0.032	–1.41	0.0069	P09543	54–853	2–51	31
					✓										**Dihydropyrimidinase-like (CRMP1)**					Q14194	321	16	9
					✓										**Dihydropyrimidinase-like (CRMP2)**					Q16555	100-486	10–33	11
													✓	✓	**Dynamin (DNM1/2)**	–1.17	0.033	1.48 to 1.62 (2)	0.01–0.034	Q05193, P50570	150–186	5–6	6
			✓											✓	EH-domain containing 3 (EHD2)	1.29	0.048			Q9NZN4	69–141	4	2
												✓			**Enolase (ENO1/2)**	–1.24 to –1.61 (2)	0.034–0.0035	–1.29 to –1.53 (5)	0.0031–0.043	P09104, P06733	167–610	11–37	22

Table 5 Schizophrenia (gray and white matter) *(Continued)*

Membrane structure	Ubiquination	Oxidative stress	Cell signaling	Protein synthesis	Neuronal growth/dev	Protein folding	Blood regulation	Other metabolism	Oxidative phosphorylation	Mitochondrial	TCA cycle	Glycolysis	Cytoskeletal	Vesicle trafficking	Name	*Gray matter*— Av. ratio	*Gray matter*— T-test	*White matter*— Av. ratio	*White matter*— T-test	Accession number (SwissProt/ TrEMBL)	Mascot Score	Sequence coverage (%)	Max peptides
								✓						✓	Esterase D (ESD)	–1.35	0.02			P10768	245	32	9
														✓	Fascin (FSCN1)	–1.26	0.039			Q16658	389	20	8
													✓		Gelsolin (GSN)	–1.42	0.0028	–1.36	0.025	P06396	332–459	19–20	17
								✓							Glutamate-ammonia ligase (GLUL)			–1.52	0.0017	P15104	153	10	5
		✓						✓							Glutathione S-transferase (GSTM3/GSTO1)			–1.19	0.044	P21266, P78417	94–143	14	4
												✓			**Glyceraldehyde 3-phosphate dehydrogenase (GAPD)**			–1.32 to –1.5 (5)	0.000086–0.016	P00354, P04406	162–279	23–29	34
✓															Glycolipid transfer protein (GLTP)			–1.34	0.0042	Q9NZD2	159	8–14	3
		✓				✓			✓						**Heat shock 70kDa protein family (HSPA1)**					P08107	520	24	22
		✓				✓			✓						**Heat shock 70kDa protein family (HSPA1L)**	–1.31 to –1.56 (3)	0.012–0.039	–1.23 to –1.41 (4)	0.00086–0.032	P34931	520	24	22
		✓				✓			✓						**Heat shock 70kDa protein family (HSPA2)**					P54652	465–564	23–28	20
		✓				✓			✓						**Heat shock 70kDa protein family (HSPA5)**					P11021	59	6	3
		✓				✓			✓						**Heat shock 70kDa protein family (HSPA8)**					P11142	507	28	17
												✓			Hexokinase (HK1)	–1.31	0.035	1.71	0.01	P19367	165	8	7
								✓							Leucine aminopeptidase (LAP3)			–1.25	0.026	P28838	201	11	6
											✓				Malate dehydrogenase (MDH1)	–1.29	0.00094	–1.55	0.00027	P40925	79	6	2
													✓		Moesin (MSN)			1.19	0.029	P26038	115	4	3
														✓	N-ethylmaleimide-sensitive factor (NSF)			1.62	0.011	P46459	141–153	8	6
		✓						✓							Peroxiredoxin (PRDX1)			–1.6	0.024	Q06830	89	14	4
		✓						✓							Peroxiredoxin 2 (PRDX2)	–1.28	0.02	–1.38	0.000032	P32119	124	22	7

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Table 5 Schizophrenia (gray and white matter) *(Continued)*

Membrane structure	Ubiquination	Oxidative stress	Cell signaling	Protein synthesis	Neuronal growth/dev	Protein folding	Blood regulation	Other metabolism	Oxidative phosphorylation	Mitochondrial	TCA cycle	Glycolysis	Cytoskeletal	Vesicle trafficking	Name	*Gray matter*— Av. ratio	*Gray matter*— T-test	*White matter*— Av. ratio	*White matter*— T-test	Accession number (SwissProt/ TrEMBL)	Mascot Score	Sequence coverage (%)	Max peptides
					✓			✓							Phosphoglycerate dehydrogenase (PHGDH)			–1.3	0.004	O43175	75	4	2
												✓			Phosphoglycerate mutase (PGAM1/2)			–1.18	0.026	P18669, P15259	95	15	5
								✓							Phospholysine phosphohistidine inorganic pyrophosphate phosphatase (LHPP)			–1.25	0.0067	Q9H008	102	12	3
			✓			✓									Protein disulphide isomerase (PDIA3)			–1.27	0.0068	P30101	247	20	8
										✓		✓			Pyruvate dehydrogenase (PDHA1)			–1.48	0.0051	P08559	387	22	14
												✓			**Pyruvate kinase M1 (PKM)**	–1.29 to –1.58 (4)	0.00045–0.0074	–1.3	0.039	P14618, P14786	278-807	14–48	35
								✓							Quinoid dihydropteridine reductase (QDPR)	–1.36	0.021			P09417	154	12	4
			✓										✓	✓	Septin 3 (SEPT)	–1.2	0.022	–1.19	0.0063	Q9UH03	53	6	2
			✓		✓									✓	SH3-domain GRB2-like 2 (SH3GL2)			–1.32	0.014	Q99962	68	6	2
													✓		**Spectrin (SPTA2)**	–1.41	0.012	–1.29 (2)	0.037–0.048	Q13813	104-512	2–9	20
															Transferrin (TF)	1.39	0.007	–1.17 to –1.22 (2)	0.0094–0.02	P02787	63–126	7–9	6
												✓			Triose phosphate isomerase (TPI)			–1.47	0.0036	P00938	72	8	3
				✓						✓					Tu translation elongation factor (TUFM)			–1.4	0.0043	P49411	430	23	10
													✓		**Tubulin (TUBA1)**					P05209	109-283	11–20	9
													✓		**Tubulin (TUBA2)**					Q13748	90	13	3
													✓		**Tubulin (TUBA6)**	–1.24 to –1.61 (2)	0.034–0.0035	–1.29 to –1.53 (5)	0.0031–0.043	Q9BQE3	90–283	9–20	9
													✓		**Tubulin (TUBB5)**					P05218	248–294	18–25	11
				✓											Tyrosyl-tRNA synthetase (YARS)	–1.3	0.021			P54577	340	17	10
									✓	✓					Ubiquinol-cytochrome c reductase (UQCRC1)	–1.44	0.016	–1.35	0.0023	P31930	143	11	5
	✓														Ubiquitin thiolesterase (UCHL1)			–1.41	0.0022	P09936	141	18	7

servation, as neurodegeneration is one of the many factors that have been proposed to underlie schizophrenia symptoms (77), and indeed, there is some symptomatic overlap between the disorders: cognitive impairment is present in SZ, as well as in AD and DS (78), and there is a high prevalence of psychotic symptoms in patients with AD (79). Nonetheless, numerous neuropathological studies have shown an absence of neurodegenerative and AD-like changes in SZ brain (80), and the data presented in this study further highlight the different mechanisms in play. The processes of metabolic deficit and increased oxidative stress in the schizophrenic brain appear distinct from the classic processes of neurodegeneration and cellular aging as found in AD and DS.

Findings in each disorder point towards the involvement of different cell types, e.g. alterations in vesicle trafficking proteins can be assumed to be derived primarily from neurons, although other changes are found in cell-type-specific proteins such as CNP, a marker of oligodendrocytes. This is a perennial problem in high-throughput studies which utilise tissue homogenate as the starting material. Future studies are planned using laser capture microdissection to examine the contribution of individual cell types to the data. One additional caveat with this approach is that although the 2D-DIGE method is a highly sensitive method in terms of fold changes observable, it is only capable of detecting a fraction of the proteome. In particular, information about membrane-bound proteins such as neurotransmitter receptors is not revealed using the methodology described here. Studies aimed at examining the proteome of membrane and other subcellular fractions in these disorders by quantitative mass spectrometry are underway in the laboratory. Further directed research is also required to fully characterise the roles of individual proteins in the pathogenesis of these disorders. Only by the combination of a number of different methods will we be able to build up a full picture of the underlying mechanisms.

Concluding Remarks

By using 2D-DIGE methodology, we have identified characteristic protein expression profiles for four major neuropsychiatric and neurodegenerative disorders. Although some individual proteins were found to be altered in parallel across all disease groups, organising the altered proteins into functional groupings reveals characteristic proteomic signatures for each disorder. In this study, neurodegenerative disorders can be distinguished from neuropsychiatric disorders on the basis of protein expression patterns. AD and DS show a very similar profile, with some subtle differences, which is in keeping with expectation given the similar neuropathology of the two disorders. In comparing the two degenerative disorders with BPD and with SZ, few, if any, categories show the same pattern between disease groups. Thus, the metabolic deficits demonstrated previously in SZ appear to be specific to this disorder and are not replicated in post-mortem studies of related disorders using identical methodology. Further work is required to fully characterise the roles of individual proteins in the pathogenesis of these disorders.

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