Molecular Psychiatry (2000) 5, 142–149

www.nature.com/mp

ORIGINAL RESEARCH ARTICLE

Disease-specific alterations in frontal cortex brain proteins in schizophrenia, bipolar disorder, and major depressive disorder

NL Johnston-Wilson[1], CD Sims[2], J-P Hofmann[2], L Anderson[2], AD Shore[3], EF Torrey[4], RH Yolken[1] and the Stanley Neuropathology Consortium

[1]*Stanley Division of Developmental Neurovirology, Johns Hopkins University, Baltimore, MD 21287–4933;* [2]*Large Scale Biology Corporation, Rockville, MD;* [3]*Health Services Research and Development Center, The Johns Hopkins School of Hygiene and Public Health, Johns Hopkins University, Baltimore, MD 21205;* [4]*Stanley Foundation Research Programs, NAMI Research Institute, Bethesda, MD 20814, USA*

Severe psychiatric disorders such as schizophrenia, bipolar disorder and major depressive disorder are brain diseases of unknown origin. No biological marker has been documented at the pathological, cellular, or molecular level, suggesting that a number of complex but subtle changes underlie these illnesses. We have used proteomic technology to survey postmortem tissue to identify changes linked to the various diseases. Proteomics uses two-dimensional gel electrophoresis and mass spectrometric sequencing of proteins to allow the comparison of subsets of expressed proteins among a large number of samples. This form of analysis was combined with a multivariate statistical model to study changes in protein levels in 89 frontal cortices obtained postmortem from individuals with schizophrenia, bipolar disorder, major depressive disorder, and non-psychiatric controls. We identified eight protein species that display disease-specific alterations in level in the frontal cortex. Six show decreases compared with the non-psychiatric controls for one or more diseases. Four of these are forms of glial fibrillary acidic protein (GFAP), one is dihydropyrimidinase-related protein 2, and the sixth is ubiquinone cytochrome *c* reductase core protein 1. Two spots, carbonic anhydrase 1 and fructose biphosphate aldolase C, show increase in one or more diseases compared to controls. Proteomic analysis may identify novel pathogenic mechanisms of human neuropsychiatric diseases. *Molecular Psychiatry* (2000) **5,** 142–149.

Keywords: two-dimensional gel electrophoresis; postmortem human brain; glial acidic fibrillary acidic protein; dihydropyrimidinase-related protein 2; ubiquinol cytochrome *c* reductase complex core protein 1; fructose bisphosphate aldolase C; carbonic anhydrase I

Introduction

Serious psychiatric disorders such as schizophrenia, bipolar disorder and major depressive disorder are brain diseases which are major causes of morbidity throughout the world. Epidemiological and pathological studies indicate a role for both genetic and environmental factors in the etiology and pathogenesis of these disorders, but specific disease-associated genes or pathogenic agents have not as yet been identified. There are, at present, no biological or pathological markers which are highly associated with specific psychiatric disorders.

Two-dimensional electrophoresis is an analytic method that can quantify the levels of individual protein species in complex biological samples[1] and allows comparison between diagnostic groups. This technique has been applied to the limited study of brain tissues,[2,3] and cerebrospinal fluids[4–7] obtained from individuals with schizophrenia. Recent advances in image analysis, sequencing and other proteinomic techniques allow for the more detailed analysis of protein expression in biological samples.[8,9] Recently, a large number of postmortem brain samples have been collected from well-characterized individuals with schizophrenia, bipolar disorder, and major depressive disorder as well as equivalent samples from matched, unaffected controls. The technique of two-dimensional gel electrophoresis was employed, followed by sequencing by electrospray mass spectroscopy to identify proteins that show varied levels in the frontal cortex of individuals with defined psychiatric disorders. The relationship of these proteins to clinical diagnosis and other clinical and demographic parameters was further evaluated by application of a multivariate statistical model. The conditions used allowed us to compare and evaluate the subset of proteins which were highly

Correspondence: NL Johnston-Wilson, PhD, Johns Hopkins University School of Medicine, Stanley Division of Developmental Neurovirology, Department of Pediatrics, 600 N Wolfe Street, Blalock 1111, Baltimore, MD 21287-4933, USA. E-mail: nlj@welchlink.welch.jhu.edu
Received 14 May 1999; revised and accepted 7 September 1999

abundant, relatively soluble proteins with pIs between 4–7. These methods resulted in the identification of eight disease-related changes in protein levels in the frontal cortex region of individuals with serious psychiatric diseases.

Materials and methods

Source of brain tissue
Analyses were performed on 89 postmortem human brains obtained as part of the Stanley Foundation Brain Collection. The brains were collected from designated medical examiners with the permission of the families, processed, and stored at −70°C. Diagnoses were made by two senior psychiatrists, and used DSM-IV criteria based on hospital records and telephone interviews with family members. Brain tissue was analyzed from individuals with the following diseases: schizophrenia ($n = 24$), bipolar disorder ($n = 23$), major depressive disorder without psychotic features ($n = 19$), and unaffected controls ($n = 23$). Tissue used in these studies was obtained from the frontal cortex (Brodmann Area 10) of the cerebral hemisphere which had been frozen on collection.

Tissue homogenization
Approximately 0.2 g of frontal lobe tissue was homogenized in 8 volumes solubilizing solution (2% NP-40 (Sigma, St Louis, MO, USA), 9 M urea (Gibco, Grand Island, NY, USA), 0.5 dithiothreitol, and 2% ampholytes (pH 8.0–10.5 Pharmacia, Piscataway, NJ, USA)) for 30 s using a Tissue Tearor at setting 2. The insoluble materials were removed by ultracentrifugation at $100\,000 \times g$, 20°C, for 30 min. Homogenates were stored at −80°C until use.

Two-dimensional gel electrophoresis
Proteins were resolved by 2-D gel electrophoresis using the 20×25 cm ISO-DALT 2-D gel system operating with 20 gels per batch. All first-dimension isoelectric focusing gels were prepared using the same batch of ampholytes (BDH 4–8A, Gallard-Schlesinger, New York, USA). Three microliters containing roughly 100 μg of solubilized brain protein were applied to each gel, and the gels run for 33 000–34 500 volt-hours using a progressively increasing voltage protocol implemented by a programmable high voltage power supply. The second dimension gels were prepared using an Angelique computer-controlled gradient casting system where the top 5% of the gel was 11% T acrylamide, and the lower 95% of the gel varied linearly from 11% to 18%. Second dimension slab gels were run in groups of 20 in thermostabilized (10°C) DALT tanks with buffer circulation.

Staining, scanning and image analysis
Slab gels were fixed and stained for protein using colloidal Coomassie Blue G-250 procedure. Staining proceeded for about 4 days in order to reach equilibrium intensity. Coomassie was chosen for being reproducibly quantitative as opposed to the equally sensitive fluorescent stains or the ten-fold more sensitive silver stain. Each slab gel was digitized in red light at 133-μm resolution, using an Eikonix 1412 scanner. Each gel was then processed using the Kepler software system, procedure PROC008b, to yield a spotlist giving position, shape and density information for each spot detected.

Master pattern construction and normalization
A master 2-D pattern of human frontal cortex was constructed using multiple 2-D images which included the common polymorphisms and additional spots encountered in the full set of brain samples (Figure 1). Spots were all identified with a number. The individual gels were then matched back to the master in two steps. First, an experienced operator manually matched about 50 protein species with their corresponding numbers from the master pattern, creating a set of reference points. Subsequently, an automated program identified all other protein species based on their relation to the manually matched spots. All spots were normalized by the sum of the densities of a small set of spots which were well resolved and present in almost all gels. A parallel set of gels was co-run with a rat liver protein preparation, and the proteins of the well characterized rat liver proteome[10] were used as molecular weight and pI standards.

Statistical analyses
The levels of individual protein species (measured as described above) were predicted by variables representing diagnostic, demographic, lifestyle, mode of death, and subsequent tissue storage characteristics using a multivariate statistical model. Over 20 such variables are known on each of these brains. Because of strong correlations with one another (colinearity) as well as a need to limit variables due to the small number of samples, the number used in the model was reduced specifically to avoid colinearity between the independent variables. The final model contained the following 10 antemortem and postmortem dependent variables that could affect protein levels in brain tissue: age (years); alcohol use (scaled 0–5); drug abuse (scaled 0–5); death by carbon monoxide poisoning (yes/no); suicide other than carbon monoxide poisoning (yes/no); diagnosis (schizophrenic, bipolar, depressed, or unaffected); body mass index (kg/m^2); brain weight (g); freezer storage interval (months); and pH of occipital lobe. Alcohol and drug abuse scales and the pH measurement methods have been described previously.[11]

Protein sequence determination
Proteins of interest were excised from the two-dimensional gels, digested with trypsin *in situ* and peptides were eluted and subjected to and sent for sequencing by electrospray mass spectrometry. The deduced peptide sequence(s) was compared against the Swiss protein database for known matches.

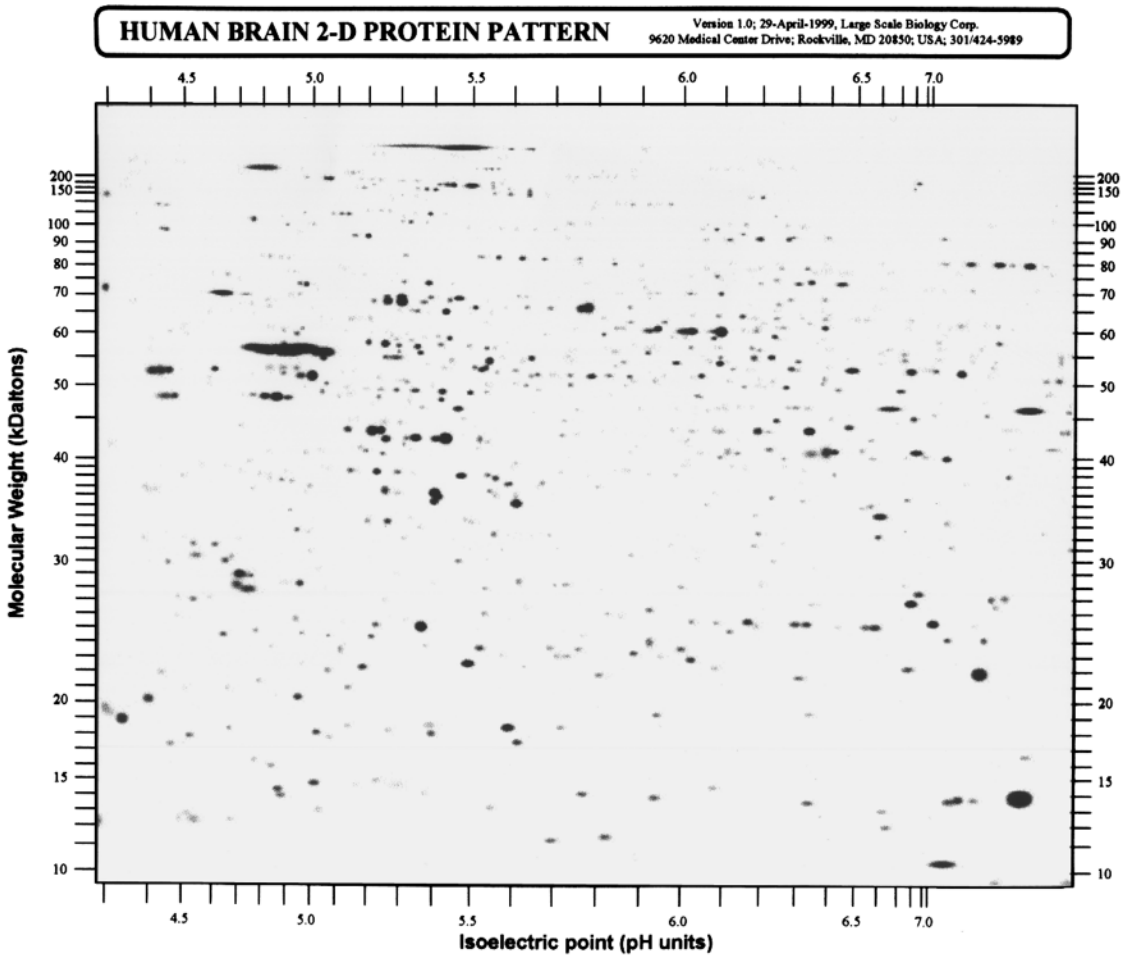

Figure 1 Master pattern of 2-D gels. A compilation of spots found on all 89 gels, with pI and molecular weight scales deduced by co-localization with rat liver standards as described in Materials and Methods.

Results

Matching and analysis of spots

A total of 217 spots were matched between all 89 gels. These proteins were quantified as described in Materials and methods. The resulting protein quantities were then analyzed in a multivariate model to eliminate the possible confounding effects of 10 different pre- and postmortem factors on the expression levels of the individual protein species.

Multivariate analysis showed that the quantities of eight distinct spots were independently related to one or more psychiatric diagnoses. Six of these (Nos. 74, 76, 119, 141, 311, and 500) exhibited a decreased level in the brains of individuals with psychiatric disorders as compared to unaffected controls while two proteins (Nos. 182 and 226) displayed increased levels compared to controls (Table 1 and Figure 2). The actual measurements of these proteins are displayed in Figure 3. This univariate analysis does not correct for the pre- and postmortem factors included in the multivariate analysis, and therefore the *P* values are different and usually higher than those reported in Table 1. These eight proteins were eluted from the 2-D gels, and tryptic peptides derived from them were sequenced by electrospray mass spectometry. The sequenced peptides were identified against known proteins in protein databanks (Table 2).

Sequence identification of proteins

Four of the protein species (76, 119, 311, and 500) that were decreased in one or more diseases were identified as glial fibrillary acidic protein (GFAP). These four spots make up the majority of a 5–6 spot charge series visible on the two-dimensional gels (Figure 2, inset). The pI and MW values of these spots are depicted in comparison to the theoretical values of the GFAP in Table 3. The center spot, 189, had been previously identified as GFAP (data not shown) and its level was not significantly altered in any of the disease states.

There were four additional protein species with diagnosis-related alterations in frontal cortex expression (Table 2). The core 1 protein of the ubiquinone cytochrome *c* reductase complex was significantly decreased and carbonic anhydrase I was significantly increased in the frontal cortex regions of individuals with depression. The pyrimidine-metabolizing enzyme

Table 1 Eight spots significantly affected by one or more diseases were selected for further study

Protein ID No.	*Increase/decrease compared to controls*	*Diseases affected (significance in multivariate analysis)*		*Other variables with significant effect at $P < 0.01$ (direction)*
74	Decrease	Depression	($P = 0.015$)	None
76	Decrease	Depression	($P = 0.0089$)	CO poisoning (decrease) Freezer interval (decrease)
119	Decrease	Depression	($P = 0.0040$)	Freezer interval (decrease)
141	Decrease	Schizophrenia	($P = 0.01$)	Other suicide (increase)
		Bipolar disorder	($P = 0.014$)	
		Depression	($P = 0.0005$)	
182	Increase	Schizophrenia	($P = 0.0023$)	Alcohol abuse (decrease)
		Bipolar disorder	($P = 0.0061$)	
		Depression	($P = 0.0009$)	
226	Increase	Depression	($P = 0.0029$)	None
311	Decrease	Schizophrenia	($P = 0.0001$)	CO poisoning (decrease)
		Depression	($P = 0.0020$)	Freezer interval (decrease)
500	Decrease	Schizophrenia	($P = 0.0011$)	Freezer interval (decrease)
		Bipolar Disorder	($P = 0.0042$)	

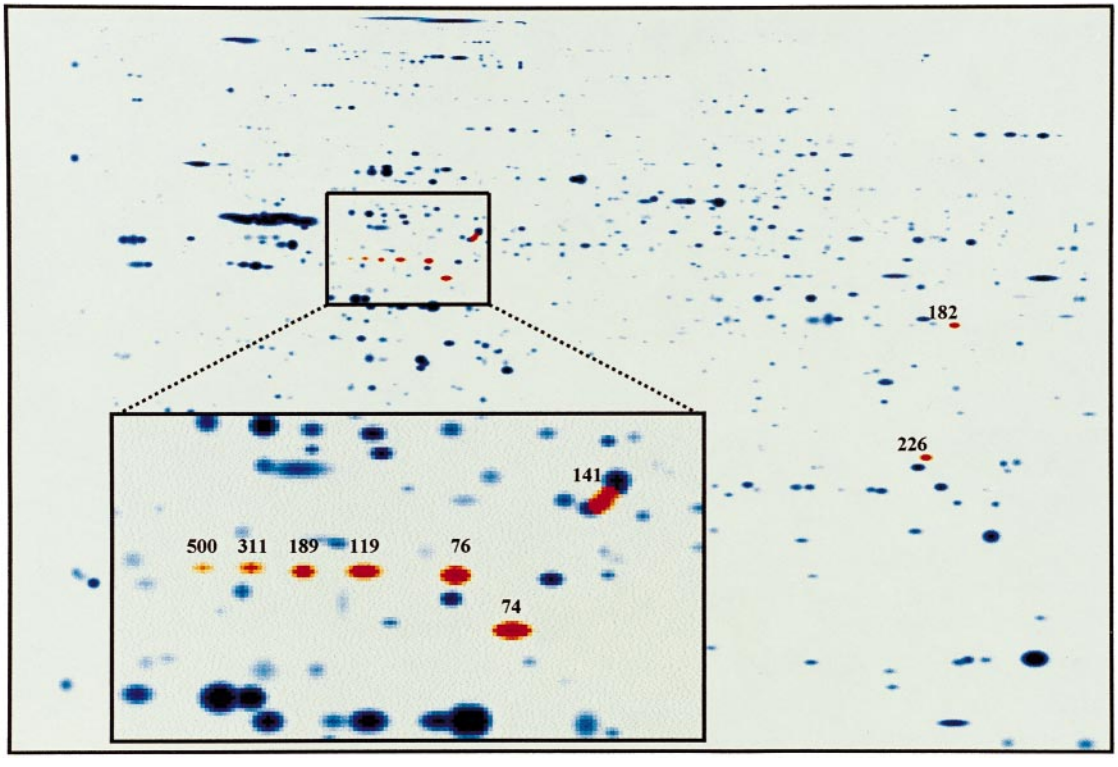

Figure 2 Spots showing disease-related variations in levels. 74-ubiquinone cytochrome *c* reductase core complex protein 1, 76, 119, 189, 311, and 500-glial fibrillary acidic protein, 141-dihydropyrimidinase-related protein 2, 182-fructose bisphosphate aldolase, 226-carbonic anhydrase I.

dihydropyrimidinase-related protein 2 was decreased in individuals with schizophrenia, bipolar disorder, and major depressive disorder. A single spot containing both fructose biphosphate aldolase C and aspartate amino transferase was found to be increased in all three diseases.

Discussion

Eight protein species displayed differences in levels which were strongly associated with at least one clinical diagnosis. The relationship between these statistically significant changes and disease were revealed by

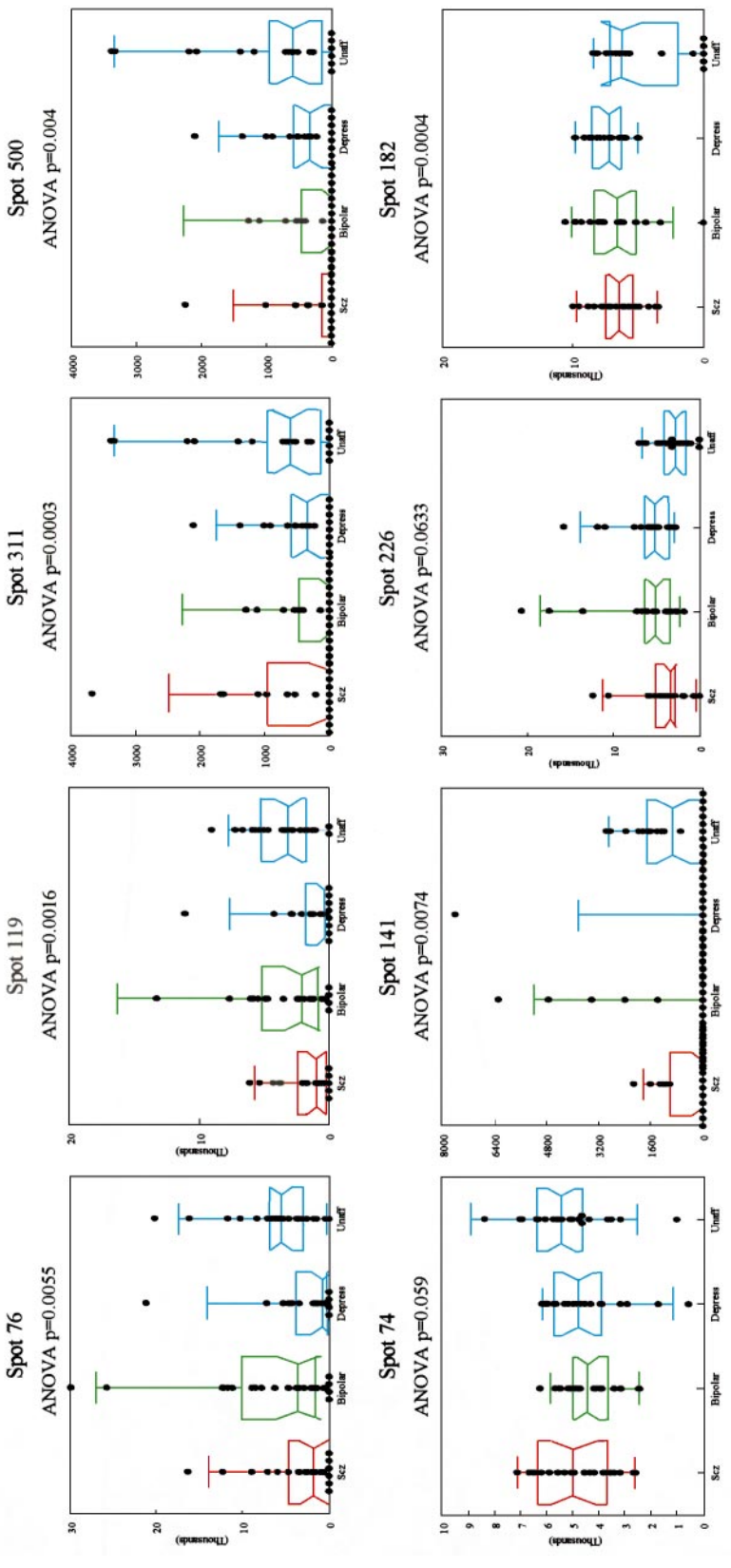

Figure 3 Box and whisker plots of protein levels for various diseases. Levels are measured in relative units as described in Materials and Methods. Boxes are drawn from the 12th to the 75th percentile of the data, with the median indicated by the line across the indentation. Error bars from the 5th to 95th percentile are also shown. ANOVA values measure the statistical differences between all groups, and were calculated on Statistica software.

Table 2 Identification of differentially expressed proteins

ID No.	*Disease related change*	*Identity[a] (Ascension No.)*	*Peptides sequenced*
74	Decreased in depression	Ubiquinol cytochrome *c* reductase complex core Protein 1 (P31930)	LSRADLTEYLSTHYK, NNGAGYFLEHLAFK
76	Decreased in depression	Glial fibrillary acidic protein (P14136)	ALAAELNQLRAK
119	Decreased in depression	Glial fibrillary acidic protein (P14136)	FADLTDAAAR, LEAENLAAYR
141	Decreased in schizophrenia, bipolar disorder and depression	Dihydropyrimidinase-related protein 2 (D78013)	VFNLYPR, MDENQFVAVTSTNAAK, MVIPGGIDVHTR
182	Increased in schizophrenia, bipolar disorder and depression	Fructose-biphosphate aldolase (P09972)	TQGLD, SALALLE[b], EEEASFNLNALNR[b′], QASAL
		Aspartate aminotransferase[c] (P17174)	RFSLLGLLPLYEH[c]
226	Increased in depression	Carbonic anhydrase I (P00915)	LLSNVEGDNAVPMQH, IINVGHSFHVNFE, LYPIANGNNQS?V
311	Decreased in schizophrenia and depression	Glial fibrillary acidic protein (P14136)	PVQTFSN, QDLA VDFSLAGA, PVQTF,
500	Decreased in schizophrenia and bipolar disorder	Glial fibrillary acidic protein (P14136)	PVQTFS, AGAL

[a]Proteins sequenced by electrospray mass spectroscopy of tryptic peptides as described in Materials and methods.
[b]Second leucine in each of these sequences is an isoleucine in the identified protein.
[c]Putative liver specific transcript, probably a contaminant.[27]

Table 3 Comparison of theoretical and measured GFAP values

	GFAP species	*PI*	*MW (Da)*
Theoretical		5.42	49 880
Measured	76	5.40	48 700
	119	5.35	49 000
	189	5.28	50 000
	311	5.23	51 000
	500	5.20	52 000

Theoretical values were obtained by entering the amino acid sequence P14136 into the pl/MW calculator at http://expasy.hcuge.ch/chzd/pi tool.html

using large sample sets combined with multivariate analysis to account for individual variation and a number of confounding pre- and postmortem factors. The conditions used to run, stain and analyze the gels allow us to study a only a small subset of all available proteins, namely the highly abundant, soluble proteins with pIs between 4–7. Despite this limitation, the identification of these proteins gives us preliminary leads into possible pathways or processes involved in these diseases. For example, there was a decrease in the expression of dihydropyrimidinase-related protein 2 (DRP-2) in the brains of individuals with schizophrenia, bipolar disorder, and major depressive disorder. The animal homologs of DRP-2 (rat CRMP-62, mouse unc-33, chick TOAD-64) are brain-specific enzymes which play a crucial role in development.[12–15] In humans, an absence of dihydropyrimidinase itself causes severe neurological impairments occurring through delayed or arrested development, and neuronal degeneration with secondary delay of myelination.[16,17] Altered levels of DRP-2 protein are thus consistent with alterations in brain development which may play a role in a wide range of neurological and psychiatric disorders.

There was a significant increase in the level of carbonic anhydrase I in the brains of individuals with depression. Carbonic anhydrase I is the erythrocyte form of the enzyme, while its isoform, carbonic anhydrase II, is expressed in glial cells, myelin and choroid plexus[18–20] and is also one of the principal determinants of pH fluxes within neural cells.[21] This finding is of interest in light of the report of Hayes[18] who found that treatment with the carbonic anhydrase inhibitor acetazolamide resulted in a significant improvement in symptoms in individuals in the depressive phase of bipolar disorder. Levels of carbonic anhydrase should be evaluated in other brain areas in an attempt to determine the potential utility of therapeutic interventions directed at altering the levels of this activity.

There were also increased levels of brain-specific fructose-biphosphate aldolase C in the samples obtained from individuals with schizophrenia, bipolar disorder, and unipolar depression. Others have also found aldolase abnormalities in psychiatric disease. Aldolase immunoreactivity was elevated in CSF,[22] the activity was increased in the serum of psychiatric patients in a clinical state-dependent manner[23–26] and was also abnormal in their first-degree relatives.[26] Further study of this phenomenon is warranted. Mass spectrometry sequencing of the same protein spot revealed peptides identified as aspartate aminotransferase. It appears that this protein is not expressed in brain,[27] and that it is most likely a contaminant in the preparation.

There was a statistically significant decrease in the levels of the core protein 1 of the mitochondrial ubiqui-

nol-cytochrome *c* reductase complex in the brains of individuals with depression. Subtractive cDNA libraries (Johnston-Wilson and Yolken, unpublished observations)[28] show an excess of mitochondrial messages in the brains of some individuals with schizophrenia. The role of these mitochondrial enzymes in the etiology of psychiatric disorders deserves additional consideration.

Four of the eight protein species with disease-specific differences on expression were identified as isoforms of the astrocytic glial fibrillary acidic protein (GFAP). The finding of multiple species of GFAP with different isoelectric points but similar molecular weights is probably a reflection of the presence of different levels of post-translational modification of this brain protein. Other researchers have also identified modified forms of GFAP using two-dimensional electrophoretic methods.[2,29–31]

The level of GFAP expression is modulated by many factors including cytokines, hormones and growth factors throughout brain development.[32,33] GFAP can be phosphorylated at five sites (Thr7, Ser8, Ser13, Ser17 and Ser38) in response to numerous stimuli.[34] Phosphorylation results in the disaggregation of GFAP molecules[35] and results in a protein molecule with a greater negative charge.[36] Spots 311 and 500 may represent phosphorylated forms of the protein. Alternatively, these species may represent other post-translational modifications resulting in acetylation, glycosylation or deimination,[35] which would also generate molecules which migrate in a more acidic fashion on two-dimensional gels.

Several studies have compared GFAP levels in the brains of individuals with schizophrenia and unaffected individuals. No significant disease-related alterations in GFAP have been detected by *in situ* immunohistochemistry or by one-dimensional immunoblotting methodologies.[37] However, these methods would not be likely to detect differences related to phosphorylation or other post-translational modifications detectable by two-dimensional electrophoresis. Comings *et al* 1982, employing two-dimensional electrophoretic methods, found absences of some charge variant forms of GFAP in a small group of individuals with schizophrenia and depression (~67% of analyzed samples) as compared to unaffected controls and other non-psychiatric neurological diseases (0–28%).[2]

It is not known if the alterations in the levels of the GFAP isoforms have functional significance or whether they are a reflection of disease-specific alterations in pathways controlling post-translational modification. GFAP decreases have been documented in Down's syndrome[38] and in response to tumour necrosis factor α, basic fibroblast growth factor, and glucocorticoids in cell cultures.[32,39] GFAP is known to increase dramatically in response to acute infection or neurodegeneration.[40] However, GFAP levels can be decreased in response to chronic infection with viruses such as pseudorabies,[41] varicella zoster,[42] and HIV-1.[43] Of particular interest in the case of HIV-1 is the finding that the viral envelope glycoprotein gp120 can directly inhibit the phosphorylation of GFAP.[44] The relationship between GFAP alterations, infections, and the pathophysiology of psychiatric diseases such as schizophrenia, bipolar disorder, and major depression is the subject of ongoing investigations.

Conclusion

The combination of two-dimensional electrophoresis, multivariate analysis and protein sequencing, has resulted in the identification of five proteins which are differentially expressed in the frontal cortex regions of the brains of individuals with psychiatric diseases. It is of note that some of these alterations were found in individuals with different psychiatric diagnoses and may represent features that are common to the different diseases such as non-specific markers of inflammation or a common second messenger pathway. The analyses of brain proteins by proteonomic methods is a way to identify disease-related changes in brain proteins and discover novel pathways for the diagnosis and treatment of serious human psychiatric diseases.

Acknowledgements

We would like to thank the Stanley Foundation for funding this work. We would also like to thank Dr Maree Webster for her technical input, Dr Lydie Meheus of Innogenetics for sequencing and peptide IDs, and Ms Ann Cusic for the preparation of this manuscript.

References

1 O'Farrell P. High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* 1975; **250**: 4007–4021.
2 Comings DE. Two-dimensional gel electrophoresis of human brain proteins. III Genetic and non-genetic variations in 145 brains. *Clin Chem* 1982; **28**: 798–804.
3 Edgar PF, Schonberger SJ, Dean B, Faull RLM, Kydd R, Cooper GJS. A comparative proteome analysis of hippocampal tissue from schizophrenia and Alzheimer's disease individuals. *Mol Psychiatry* 1999; **4**: 173–178.
4 Harrington M, Merril CR, Torrey EF. Differences in cerebrospinal fluid proteins between patients with schizophrenia and normal persons. *Clin Chem* 1985; **31**: 722–726.
5 Merril CR, Harrington MG. Use of two-dimensional electrophoretic protein maps in studies of schizophrenia. *Schizophr Bull* 1988; **14**: 249–254.
6 Wildenauer DB, Korschenhausen D, Hoechtlen W, Ackenheil M, Kehl M, Lottspeich F. Analysis of cerebrospinal fluid from patients with psychiatric and neurological disorders by two-dimensional electrophoresis: identification of disease associated polypeptides as filbrin fragments. *Electrophoresis* 1991; **12**: 487–492.
7 Johnson G, Brane D, Block W, van Kammen DP, Gurklis J, Peters JL *et al*. Cerebrospinal fluid protein variations in common to Alzheimer's disease and schizophrenia. *Appl Theo Electrophor* 1992; **3**: 47–53.
8 James P. Of genomes and proteomes. *Biochem Biphys Res Comm* 1997; **231**: 1–6.
9 Shevchenko A, Jensen ON, Podtelejnikov AV, Sagliocco F, Wilm M, Vorm O *et al*. Linking genome and proteome by mass spectrometry: large scale identification of yeast proteins from two-dimensional gels. *Proc Natl Acad Sci USA* 1996; **93**: 14440–14445.
10 Anderson NL, Esquer-Blasco R, Hofmann J-P, Meheus L, Raymackers J, Steiner S *et al*. An updated two-dimensional gel database of

rat liver proteins useful in gene regulation and drug effects studies. *Electrophoresis* 1995; **16**: 1977–1981.
11 Johnston NL, Cervenak J, Shore D, Torrey EF, Yolken RH, the Stanley Neuropathology Consortium. Multivariate analysis of RNA levels from postmortem human brains as measured by three different methods of RT-PCR. *J Neurosci Meth* 1997; **77**: 83–92.
12 Goshima Y, Nakamura F, Strittmatter P, Strittmatter SM. Collapsin-induced growth cone collapse mediated by an intracellular protein related to UNC-33. *Nature* 1995; **376**: 509–514.
13 Goshima Y, Kawakami T, Hori H, Sugiyama Y, Takasawa S, Hashimoto Y *et al*. A novel action of collapsin: collapsin-1 increases antereo- and retrograde axoplasmic transport independently of growth cone collapse. *J Neurobiol* 1997; **33**: 316–328.
14 Wang L-H, Strittmatter SM. A family of rat CRMP genes is differentially expressed in the nervous system. *J Neurosci* 1996; **16**: 6197–6207.
15 Hamajima N, Matsuda K, Sakata S, Tamaki N, Sasaki M, Nonaka M. A novel gene family defined by human dihydropyrimidinase and three related proteins with differential tissue distribution. *Gene* 1996; **180**: 157–163.
16 Putman CW, Rotteveel JJ, Wevers RA, van Gennip AH, Bakkeren JA, De Abreu RA. Dihydropyrimidinase deficiency, a progressive neurological disorder? *Neuropediatrics* 1997; **28**: 106–110.
17 Henderson MJ, Ward K, Simmonds HA, Duley JA, Davies PM. Dihydropyrimidinase deficiency presenting in infancy with severe developmental delay. *J Inherit Metab Dis* 1993; **16**: 574–576.
18 Hayes SG. Azetazolamide in bipolar affective disorders. *Ann Clin Psych* 1994; **6**: 91–98.
19 Giacobni E. A cytochemical study of the localization of carbonic anhydrase in the nervous system. *J Neurochem* 1962; **9**: 169–177.
20 Roussel G, Delaunoy JP, Nussbaum JL, Mandel P. Demonstration of a specific localization of carbonic anhydrase C in the glial cells of rat CNS by an immunohistochemical method. *Brain Res* 1979; **160**: 47–55.
21 Chesler M, Kaila K. Modulation of pH by neuronal activity. *TINS* 1992; **15**: 396–402.
22 Wilson VJC, Graham JG, McQueen INF, Thompson RJ. Immunoreactive aldolase C in cerebrospinal fluid of patients with neurological disorders. *Ann Clin Biochem* 1980; **17**: 110–113.
23 Meltzer H. Creative kinase and aldolase in serum: abnormality common to acute psychoses. *Science* 1968; **159**: 1368–1370.
24 Meltzer H. Increased activity of creatine phosphokinase and aldolase activity in the acute psychoses: case report. *J Psychiat Res* 1970; **7**: 249–262.
25 Coffey JW, Heath RG, Guschwan AF. Serum creatine kinase, aldolase, and copper in acute and chronic schizophrenics. *Biol Psych* 1970; **2**: 331–339.
26 Meltzer HY, Grinspoon L, Shader RI. Serum creatine phophokinase and aldolase activity in acute schizophrenic patients and their relatives. *Compr Psychiatry* 1970; **11**: 552–558.
27 Pol S, Bousquet-Lemercier B, Pave-Preux M, Pawlak A, Nalpas B, Berthelot P *et al*. Nucleotide sequence and tissue distribution of the human mitochondrial aspartate aminotransferase mRNA. *Biochem Biophys Res Commun* 1988; **157**: 1309–1315.
28 Mulcrone J, Whatley SA, Ferrier IN, Marchbanks R. A study of altered gene expression in frontal cortex from schizophrenic patients using differential screening. *Schizophr Res* 1995; **14**: 203–213.
29 Bigbee JW, Eng LF. Analysis and comparison of *in vitro* synthesized glial fibrillary acidic protein with rat CNS intermediate filament proteins. *J Neurochem* 1982; **38**: 130–134.
30 Ishida K, Kaneko K, Kubota T, Itoh Y, Miyatake T, Matsushita M *et al*. Identification and characterization of an anti-glial fibrillary acidic protein antibody with a unique specificity in a demented patient with an autoimmune disorder. *J Neurol Sci* 1997; **151**: 41–48.
31 Fujita K, Yamauchi M, Matsui T, Titani K, Takahashi H, Kato T *et al*. Increase of glial fibrillary acidic protein fragments in the spinal cord of motor neuron degeneration mutant mouse. *Brain Res* 1998; **785**: 31–40.
32 Laping NJ, Nichols NR, Day JR, Johnson SA, Finch CE. Transcriptional control of glial fibrillary acidic protein and glutamine synthetase *in vivo* shows opposite responses to corticosterone in the hippocampus. *Endocrinology* 1994; **135**: 1928–1933.
33 Norton WT, Aquino DA, Hozumi I, Chui F-C, Brosnan CF. Quantitative aspects of reactive gliosis: a review. *Neurochem Res* 1992; **17**: 877–885.
34 Tardy M, Fages C, LePrince G, Rolland B, Nunez J. Regulation of the glial fibrillary acidic protein (GFAP) and of its encoding mRNA in the developing brain and in cultured astrocytes. *Mol Aspects Dev Aging Nerv Syst* 1990; **265**: 41–52.
35 Inagaki M, Gonda Y, Nishizawa K, Kitamura S, Sato C, Ando S *et al*. Phosphorylation sites linked to glial filament disassembly *in vitro* locate in a non-alpha-helical head domain. *J Biol Chem* 1990; **265**: 4722–4729.
36 Inagaki M, Nakamura Y, Masatoshi T, Nishimura T, Inagaki N. Glial fibrillary acidic protein: dynamic property and regulation by phosphorylation. *Brain Pathol* 1994; **4**: 239–243.
37 Perrone-Bizzozero NI, Sower AC, Bird ED, Benowitz LI, Ivins KJ, Neve RL. Levels of the growth-associated protein GAP-43 are selectively increased in association cortices in schizophrenia. *Proc Natl Acad Sci USA* 1996; **93**: 14182–14187.
38 Eng L, Ghirnikar RS. GFAP and astrogliosis. *Brain Pathol* 1994; **4**: 229–237.
39 Goodison KL, Parhad IM, White CL III, Sima AF, Clark AW. Neuronal and glial gene expression in neocortex of Down's Syndrome and Alzheimer's Disease. *J Neuropath Exp Neuro* 1993; **52**: 192–198.
40 Murphy GM Jr, Lee YL, Jia XC, Yu AC, Majewska A, Song Y *et al*. Tumor necrosis factor-α and basic fibroblast growth factor decrease glial fibrillary acidic protein and its encoding mRNA in astrocyte cultures and glioblastoma cells. *J Neurochem* 1995; **65**: 2716–2714.
41 Rinaman L, Card JP, Enquist LW. Spatiotemporal responses of astrocytes, ramified microglia, and brain macrophages to central neuronal infection with pseudorabies virus. *J Neurosci* 1993; **13**: 687–700.
42 Kennedy PG, Ajor EO, Williams RK, Straus SE. Down-regulation of glial fibrillary acidic protein expression during acute lytic varicella-zoster virus infection of cultured human astrocytes. *Virology* 1994; **205**: 558–562.
43 Pulliam L, West D, Haigwood N, Swanson RA. HIV-1 envelope gp120 alters astrocytes in human brain cultures. *AIDS Res Hum Retroviruses* 1993; **9**: 439–444.
44 Levi G, Patrizio M, Bernardo A, Petrucci TC, Agresti C. Human immunodeficiency virus coat protein gp120 inhibits the β-adrenergic regulation of astroglial and microglial functions. *Proc Natl Acad Sci USA* 1993; **90**: 1541–1545.