Protein markers for psychiatric disorders suggest new treatment strategies

Although epidemiological and pathological studies show that psychiatric disorders such as schizophrenia and bipolar disorder have a large genetic component, no specific disease-associated genes have yet been uncovered. However, a new study using proteomics (Box 1) identified five distinct proteins that could represent new targets for therapeutic intervention. All five occur in the frontal cortex of individuals with psychiatric illness at either much higher or much lower levels compared with unaffected controls.

Proteomic analysis

'Many researchers have spent years looking for these elusive molecular markers, and I was somewhat sceptical at the start of our study that proteomics would deliver,' says Robert Yolken (Johns Hopkins University, Baltimore, MD, USA), who led the study. However, he became impressed by the potential of the technique and he is now optimistic that proteomics will succeed in elucidating the molecular basis for conditions such as schizophrenia where DNA sequencing and analysis of mRNA levels with microarray systems have failed. 'The technology behind proteomics continues to develop rapidly' he notes. 'It is already possible to detect levels of proteins and differentiate between different proteins with far greater sensitivity than we were able to do even in this study,' he adds.

Yolken and colleagues used proteomics to compare postmortem brain samples from individuals who had been diagnosed with schizophrenia (n = 24),

Box 1. Focus on proteomics

Proteomics uses two-dimensional gel electrophoresis, followed by sequencing of the separated proteins by electrospray mass spectroscopy. It enables the comparison of subsets of expressed proteins among a large number of samples. Several gels were prepared using samples from 'normal' brains and a master two-dimensional (2D) pattern of the proteins present in the human frontal cortex was constructed (Fig. I). Common polymorphisms were identified and then the master was used as a reference template against which gels obtained using samples from affected brains were compared. A statistical model was used to identify differences that could be caused by lifestyle, mode of death and tissue storage conditions. Protein spots that were different in affected brains compared with normal brains that could not be explained were excised from the 2D gels and sequenced. They were then compared against a protein database for identification.

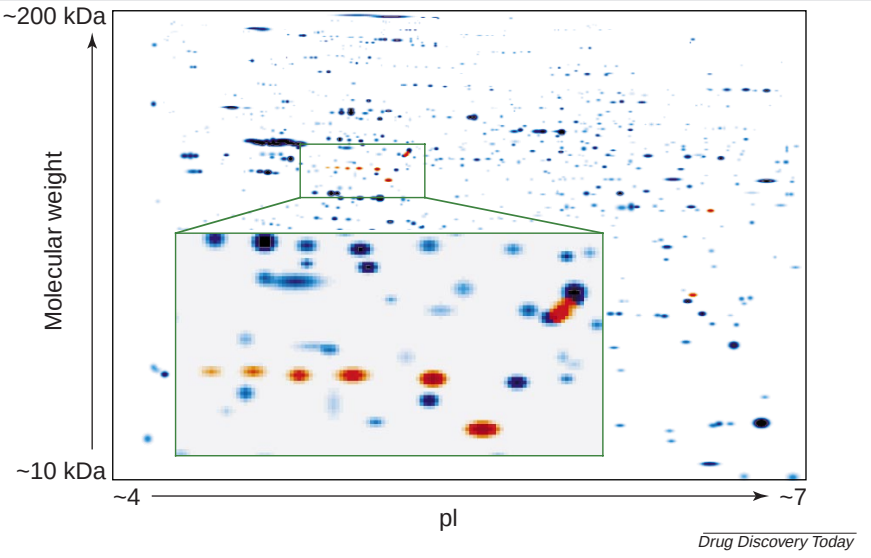

Figure I. *The two-dimensional master gel showing a magnified region; the spots in orange/brown are those that showed disease-related differences in levels. The five spots in a row are all glial fibrillary acidic protein (GFAP). The difference in migration along the pI axis is probably related to differing levels of phosphorylation between the isoforms.*
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Table 1. Relative levels of different proteins in various psychiatric disorders

Protein	Relevance to psychiatric disorder
Glial fibrillary acidic protein	Level significantly lower in frontal cortex of individuals with schizophrenia and depression.
Core 1 protein of the ubiquinone cytochrome c reductase complex	Level significantly higher in frontal cortex of individuals with depression.
Carbonic anhydrase I	Level significantly lower in frontal cortex of individuals with depression.
Dihydropyrimidinase-related protein 2 (a pyrimidine-metabolizing enzyme)	Level significantly lower in frontal cortex of individuals with schizophrenia, bipolar disorder and major depressive disorder.
Fructose biphosphate aldolase C/aspartate amino transferase*	Levels significantly higher in frontal cortex of individuals with schizophrenia, bipolar disorder and major depressive disorder.

*Both proteins present in the same protein spot but the aspartate amino transferase is thought to be a contaminant.

bipolar disorder (n = 23) and major depressive disorder with psychotic features (n = 18), and to compare them with similar samples from 23 unaffected controls. Analysis revealed eight protein spots that showed altered levels between the different diseases and the control. 'This is the first time proteomics has been applied to human brain tissue and we were thrilled to find such clear differences,' reports Yolken. Sequence analysis identified four of the spots as the same protein, glial fibrillary acidic protein (GFAP). The remaining protein spots were all different and were identified as shown in Table 1 (Ref. 1).

Supporting evidence

This study provides the first hard evidence for molecular markers, but several previous studies support the theory that these proteins are involved in the aetiology of psychiatric disorders:

- An absence of DRP-2 during development is known to delay myelination and produce neurological impairment[2].
- Treatment with the carbonic anhydrase inhibitor, acetazolamide, lessens symptoms in individuals in the depressive phase of bipolar disorder[3].
- Aldolase activity was found to be higher in the serum of psychiatric patients and their first-degree relatives, compared with unaffected and unrelated individuals[4].
- Subtractive cDNA libraries show an excess of mitochondrial messages in the brains of some people with schizophrenia (N.L. Johnston-Wilson and R. Yolken, unpublished observations).

The fifth protein identified, GFAP, was a component of four separate protein spots obtained in the 2D electrophoresis. All four were isoforms and differed because of posttranslational modification of the protein. 'The level of GFAP expression is known to be modulated by many factors including cytokines, hormones and growth factors throughout brain development but we do not really understand the significance of this for either normal development or psychiatric disorders,' says Yolken. Several studies have compared GFAP levels in the brains of individuals with schizophrenia with unaffected people and have found no significant disease-related alterations, but Yolken argues that the methods used – immunochemistry and 1D blotting techniques – would be unlikely to detect differences related to post-translational modification.

Future studies

Although the results are promising, the study is still very preliminary and Michael Owen (Neuropsychiatric Genetics Unit, University of Wales College of Medicine, Cardiff, UK) warns that the problem with the proteomics approach and others based on post-mortem tissue, is the existence of many confounding factors that can potentially lead to differences between groups. 'These include different time intervals from death to postmortem, different agonal states (how the person died), age and so on,' he explains. 'Also, most psychiatric patients are on psychotropic drugs and these are likely to cause changes in brain biochemistry. The groups therefore have to be closely matched for these variables. Yolken's group did make every effort to cancel out the effects of as many of the possible confounding factors as possible but', as Owen stresses, 'even the most carefully conducted study of this kind must be interpreted cautiously.'

Although there is a long way to go to understand the full implications of the five protein markers identified, part of the plan for the next couple of years will be to look at therapeutic strategies. 'One option is to look at currently available treatments to see if they affect the levels of any of these five proteins. Further down the line, if we can understand more about the differences between the diseased brain and the

healthy brain at the molecular level, it should be possible to develop drugs that are more specific in their action and perhaps less toxic,' predicts Yolken.

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Complement activation in myocardial infarction: a target for future treatments?

The discovery that complement activation plays a key role in triggering endothelial cell damage during myocardial infarction could lead to the development of new drugs for treating heart disease. Researchers in the US have also shown that monoclonal antibodies (mAbs) can inhibit the relevant complement activation pathway, known as the lectin complement pathway.

Leonard Bell, President and CEO of Alexion Pharmaceuticals (New Haven, CT, USA), which collaborated in the study[1], said the results suggest that humanized mAbs might be effective treatments for conditions such as atherosclerosis, unstable angina and heart failure. The company has licensed patent applications on the mAbs from Brigham and Women's Hospital at Harvard University (Boston, MA, USA).

Complement activation pathways

Complement proteins are present in the blood in the inactive state. After activation, the end-product of the reaction cascade is a membrane attack complex that can puncture cell membranes, causing lysis or cellular activation. Until recently, there were thought to be two complement activation pathways: the classical pathway, which requires antibodies for activation, and the alternative pathway, which does not.

In the 1990s, it became apparent that a third pathway exists[2] that is similar to the classical pathway but does not require antibodies for its activation. This pathway becomes activated when mannose-binding lectin (MBL) binds to carbohydrates on the surface of microorganisms. MBL is a large molecule (≈600 kDa) and is similar in structure to C1q, the first molecule of the classical complement cascade. As with C1q, MBL circulates in association with inactive serine protease enzymes.

Complement activation in oxidative stress

Research during the 1990s showed that complement activation occurs on the endothelial cell surface after hypoxia/reoxygenation[3]. This type of model aims to emulate what occurs in the heart during a myocardial infarction. Gregory Stahl (Associate Professor of Anesthesiology and Physiology, Brigham and Women's Hospital) said, 'We had identified that the classical pathway of complement activation was involved, and thought that all we would need to do would be to isolate the antibodies deposited on those endothelial cells and purify the antigen that they were binding to.'

Unfortunately, Stahl and his colleagues were unable to find any difference between the quantity of antibodies on the endothelial cells that had suffered oxidative stress, compared with those that had not. After searching the literature for a possible explanation, they decided to investigate whether the lectin complement pathway was involved. Only a few inhibitors of MBL were available at that time, and one such inhibitor, mannose, successfully inhibited the complement activation[4]. The team therefore designed mAbs that would inhibit MBL. Stahl added, 'We thought that these mAbs could have some therapeutic use if we could establish that this pathway plays a role in human disease.'

In a recent paper[1], Stahl and his colleagues describe how they subjected endothelial cell cultures to hypoxia/reoxygenation, before adding a source of complement proteins such as human serum. They then washed the cells before measuring MBL levels on the cell surface using an MBL-dependent C3 deposition ELISA. They found that MBL was present and the lectin complement pathway activated in cells that had undergone hypoxia/reoxygenation, but not in control cells. They also showed that novel anti-MBL mAbs could inhibit both MBL deposition and lectin complement pathway activation. Stahl said, 'These antibodies could be used to treat any disease where activation of the lectin pathway is involved, or that is

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