Review

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Proteomic research in psychiatry

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Abstract

Psychiatric disorders such as Alzheimer's disease, schizophrenia and mood disorders are severe and disabling conditions of largely unknown origin and poorly understood pathophysiology. An accurate diagnosis and treatment of these disorders is often complicated by their aetiological and clinical heterogeneity. In recent years proteomic technologies based on mass spectrometry have been increasingly used, especially in the search for diagnostic and prognostic biomarkers in neuropsychiatric disorders. Proteomics enable an automated high-throughput protein determination revealing expression levels, post-translational modifications and complex protein-interaction networks. In contrast to other methods such as molecular genetics, proteomics provide the opportunity to determine modifications at the protein level thereby possibly being more closely related to pathophysiological processes underlying the clinical phenomenology of specific psychiatric conditions. In this article we review the theoretical background of proteomics and its most commonly utilized techniques. Furthermore the current impact of proteomic research on diverse psychiatric diseases, such as Alzheimer's disease, schizophrenia, mood and anxiety disorders, drug abuse and autism, is discussed. Proteomic methods are expected to gain crucial significance in psychiatric research and neuropharmacology over the coming decade.

Keywords

Alzheimer's disease, anxiety disorders, autism, drug addiction, mood disorders, protein profiling, proteomics, psychiatry, schizophrenia

Abbreviations

The following abbreviations are used in this review:

2D-PAGE	two-dimensional polyacrylamide gel electrophoresis;
ABAD	Abeta binding alcohol dehydrogenase;
Abeta	amyloid beta;
AD	Alzheimer's disease;
ADH	alcohol dehydrogenase;
apo	apolipoprotein;
APP	amyloid precursor protein;
ASD	autistic spectrum disorder;
BDNF	brain-derived neurotrophic factor;
BPD	bipolar disorder;
CK	creatine kinase;
CNS	central nervous system;
CREB	cAMP response element-binding protein;
CSF	cerebrospinal fluid;
DIGE	difference gel electrophoresis;
DRP2	dystrophin related protein 2;
ELISA	enzyme-linked immunosorbent assay;
ESI	electrospray ionization;
GAPDH	glyceraldehyde-3-phosphate dehydrogenase;
GDH	glutamate dehydrogenase;
GFAP	glial fibrillary acidic protein;
GGT	gamma-glutamyltransferase;
HAB	high-anxiety-related behaviour;
HCNP-pp	hippocampal cholinergic neurostimulating peptide precursor protein;
Hp	haptoglobin;
HPLC	high-performance liquid chromatography;

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HSP	heat shock protein;
HUPO	Human Proteome Organisation;
ICAT	isotope-coded affinity tags;
Ig	immunoglobin;
iTRAQ	isobaric tag for relative and absolute protein quantification;
IV	intravenous;
LAB	low-anxiety-related behaviour;
LC-ESI MS/MS	liquid chromatography electrospray ionization tandem mass spectrometry;
LDH	lactate dehydrogenase;
MALDI-ToF	matrix-assisted laser desorption/ionization time of flight mass spectrometry;
MAP	methamphetamine;
MAPK1	mitogen-activated protein kinase 1;
MCI	mild cognitive impairment;
MDD	major depressive disorder;
MDH	malate dehydrogenase;
MK-801	[+]-5-methyl-10, 11-dihydro-5H-dibenzo-[a,d]-cyclohepten-5,10-imine hydrogen maleate;
MS	mass spectrometry;
NMDA	N-methyl-D-aspartate;
OCD	obsessive compulsive disorder;
PCR	polymerase chain reaction;
PD	Parkinson's disease;
PDH	pyruvate dehydrogenase;
PEBP	phosphatidylethanol-binding protein;
Pebp1	phosphatidylethanolamine-binding protein 1;
PK	pyruvate kinase;
PPlase	peptidyl prolyl cis-trans isomerise;
Prdx	peroxiredoxin;
prec	precursor;
PS1	presenilin 1;
qRT-PCR	quantitative reverse transcription polymerase chain reaction;
SCZ	schizophrenia/schizophrenic;
SELDI	surface-enhanced laser desorption/ionization;
SNAP	soluble N-ethylmaleimide-sensitive factor attachment protein;
SOD	superoxide dismutase;
SYT	synaptotagmin;
TR	targeted replacement;
UCH	ubiquitin carboxyl-terminal hydrolase;
UCHL1	ubiquitin carboxy-terminal hydrolase L1;
VGF	VGF nerve growth factor inducible.

Introduction

Psychiatric disorders such as AD, schizophrenia, mood and anxiety disorders, substance abuse and autism are severe and disabling diseases, and some of them are major causes of morbidity even in childhood and adolescence. In contrast to diseases adhering to strict Mendelian inheritance, mental disorders are complex, polygenetic and often poorly understood with regards to pathomechanisms and biological pathways. In most cases, it is likely that the interplay of multiple gene products with environmental factors produces a given psychiatric phenotype. Over the last decade psychiatric research has primarily focused on genomic approaches, with the human genome project syndicate producing essential progress in the understanding of mental diseases. There had been expectations by researchers and clinical practitioners of significant improvements in diagnostic and therapeutic opportunities based on the new molecular genetic data. However, to date few revolutionary tests have been developed to differentiate between similar phenotypes and states of disease, to monitor therapeutic progress or to assess the prognosis of individual patients.

The new research approach of proteomics, based on MS, has recently been applied to psychiatric research. Proteomics essentially refers to the systematic analysis of all expressed proteins. The 'proteome' describes primarily the pool of proteins encoded by the genome of an organism at a specific point in time, incorporating the set of isoforms, post-translational modifications, covalent structures and complex protein–protein interactions present therein.

Often proteomic studies are applied in tandem with transcriptomic or metabolomic approaches. Transcriptomics refers to the assessment of gene transcripts/mRNA abundance in a tissue (Hegde et al., 2003), whilst metabolomics is the quantitative and qualitative analysis of metabolites and small molecules acting in biochemical networks (Dinge et al., 2006, Godfrey et al., 2009, Oldiges et al., 2007).

In the past and at present, molecular methods such as *in situ* hybridization, qRT-PCR and blotting techniques have been performed to reveal abnormally expressed gene products in individuals affected by mental diseases. However, many of these methods are restricted to the detection of a few gene products at a time. Newer approaches, such as microarray technologies, allow for large-scale transcriptome analysis, but still have limitations. For example, post-transcriptional events, such as alternative mRNA splicing, and post-translational protein modifications increase the diversity of products that can be synthesized from a fixed number of genes. Post-transcriptional events may not be detected by mRNA arrays hampering efforts to reveal the full variety of gene expression (Carter et al., 2005). Post-translational modifications, which produce altered findings on the protein level (e.g. with altered position of protein spots in 2D-PAGE), may occur without detectable alterations of corresponding mRNA levels. These differential effects are a possible reason why MS and microarray results sometimes vary within the same study. In contrast to microarray approaches, proteomics has the great advantage of facilitating analysis at the protein level, thereby reflecting more closely the pathophysiological processes underlying the clinical phenomenology of specific psychiatric conditions. Proteomic tools allow for an automated, technology-driven large-scale mode of examination that provides the chance to determine the whole proteome in a given tissue without *a priori* assumptions about candidate molecules. The rapid development of proteomics witnessed in recent times is due to the increasing sophistication of biological MS, improvements in bioinformatics and the magnitude of data resulting from genomic sequencing of different organisms. Indeed, proteomics is complemented by functional genomics approaches that

provide complete genomic sequences, allowing for protein identification by correlation of MS measurements with sequence databases.

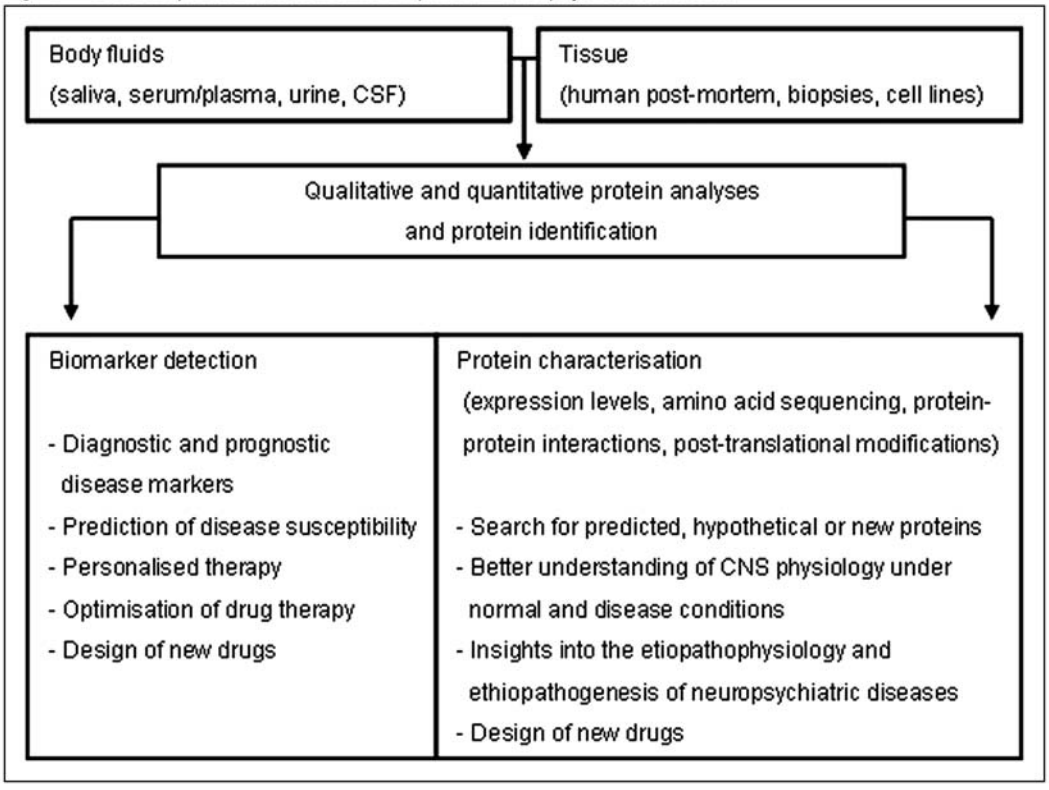
Proteomic approaches can be divided into the main fields 'expression proteomics', 'functional proteomics', 'structural proteomics' and 'interaction proteomics'. Basic research primarily deals with functional and structural aspects of proteins. However, the most common approach in psychiatry and other applied settings is the assessment of protein expression in human tissue and animal models under different conditions. As one might imagine, proteomic research in psychiatry may identify trait and state biomarkers of psychiatric diseases by comparing protein expression in patients and control subjects. The same approach is applicable to the comparison of the proteome from patients receiving different therapeutic interventions (e.g. drug versus placebo); see Figure 1. A trait marker reflects the properties of the behavioural and biological processes that play an antecedent, potentially causal role in the aetiopathophysiology of a disorder, whereas a state marker represents the state of the clinical manifestation in patients (Chen et al., 2006b). The assessment of biomarkers in easily accessible tissues such as saliva, blood, urine or CSF provides promising opportunities in diagnostic and prognostic processes. Similar phenotypes with distinct nosological entities could be compared by assessing their differential protein patterns. It might be possible to evaluate the individual prognosis of a psychiatric disease, to predict the susceptibility for a specific mental disorder and perhaps to prevent its appearance (in case of a positive family history). The analysis of proteomic profiles may further allow the prediction of drug responses/efficacy and further be used to monitor therapy. Therefore, proteomics may contribute to the development of a more personalized and specific therapy on the basis of the individual's proteome.

Using proteomic tools, the expression levels, amino acid structure, post-translational modifications (e.g. glycosylation, phosphorylation, oxidation), interactions and functions of proteins can be detected and compared between patient-derived and control post-mortem tissue samples, and also between animals models for a psychiatric disease and the respective controls. A detailed analysis of specific protein modifications in different disease stages will further contribute to a better understanding, for example by uncovering pathomechanisms causing amyloid deposits in AD or deranged cell migration, proliferation and connectivity in other severe psychiatric disorders.

Proteomics also provides the opportunity to observe the effect of different psychotropic drugs on protein expression in post-mortem tissue, *in vivo* in patients and animal models, and *in vitro* in cell lines. Findings from proteomic research can hopefully point to new drug targets for psychiatric diseases and optimize current treatment strategies (see Figure 1).

Previous proteomic studies have often found changes in a large number of proteins, with varying results across studies and the inherent problems of lack of reproducibility. One challenge of proteomic research is therefore to deal reasonably with this multitude of observed expression changes and suggested biomarkers. There are many possibly confounding

Figure 1: Possible proteomic studies with impact on neuropsychiatric diseases



factors during a proteomics project that might cause false-positive or false-negative results. From the point of view of subject selection, there is the complication of clinical heterogeneity of biologically distinct phenotypes that cannot easily be distinguished by current diagnostic criteria. From a procedural point of view, sample collection, handling and storage are sensitive and crucial steps for a successful mass spectrometric analysis of proteins. An insufficient optimization and standardization of procedures can potentially produce false-positive results (Luque-Garcia and Neubert, 2007). Characteristic of many mass spectrometric methods, digested proteins/peptides are analysed and assigned to proteins. These methodological characteristics demand a high sensitivity and specificity of mass analysers and present complex computational and statistical challenges to cut down false protein identifications (Nesvizhskii et al., 2007). The use of search algorithms (each with their own statistical 'scoring' methodology) has been suggested to obtain protein identifications with confidence. Whilst each package usually applies its own threshold for the identification of proteins from peptide fragmentation profiles, it is generally accepted that ideally more than one peptide should identify a protein from a protein digest. Furthermore the Human Proteome Organisation (HUPO) has defined guidelines for accurate protein identification and is studying mechanisms for the comparison of protein identifications from different software packages. Furthermore the inclusion of raw data (fragment spectra) of LC-ESI MS/MS is recommended as supplementary material accompanying academic publications.

In proteomic biomarker research it is certainly necessary to verify preliminary mass spectrometric findings in independent, sufficiently large sample sets and to validate results with different methods such as Western blot or ELISA to confirm relevant proteomic findings. Such validation is common in microarray studies, with Northern blots and Q-PCR being used to confirm mRNA changes. Furthermore, pilot studies may be used to calculate false-positive rates, allowing for better confidence in protein identification.

Bearing all of the above in mind, proteomics research can undoubtedly provide new insight into the study of neuropsychiatric diseases, especially in the identification of protein modifications and expression changes related to these disorders.

Proteomic methods

A proteomic analysis comprises the following main work steps (see Figure 2):

(1) protein isolation from different tissues; (2) separation and fractionation of complex protein mixtures into fractions containing fewer components; (3) the analysis of the sample components by MS; and (4) the use of specific databases for data processing.

Sample preparation

As a first work step, proteins have to be isolated from the tissue (serum/plasma, CSF, specific cells or cell lines, post-mortem tissue etc.). An optimized and standardized sample collection, handling and storage procedure is essential to minimize the risk of false-positive and false-negative results. For a review on different strategies for sample preparation in the field of biomarker research, see Luque-Garcia and Neubert (2007).

Every type of tissue is dominated by a few proteins in high concentrations. Highly abundant proteins, such as structural proteins and metabolic enzymes in the brain, or albumin, transferrin, lipoproteins and immunoglobulins in the serum, hamper mass spectrometric detection of low abundance proteins. To address these difficulties, different fractionation and enrichment procedures are required prior to mass spectrometric analysis. To reduce the sample complexity of tissue, so-called subproteomes may be obtained, focusing on membrane proteins, nuclear or cellular fractions (Vercauteren et al., 2004a). On an anatomical level, methods such as laser capture microdissection allow for the precise dissection of specific nuclei and neuronal populations in brain tissue samples (Moulédous et al., 2003). Highly abundant serum proteins may be depleted using affinity chromatography-based methods, prior to further separation. The most commonly used separation techniques for proteins are 2D-PAGE and HPLC, as well as the usage of pre-coated protein chips, centrifugal filters or magnetic beads (see Figure 2). The details and relative merits of sample preparation and protein fractionation techniques are discussed elsewhere (Freeman and Hemby, 2004; Issaq and Veenstra, 2007; Issaq et al., 2002; Luque-Garcia and Neubert, 2007; Newton et al., 2004; Tannu and Hemby, 2006).

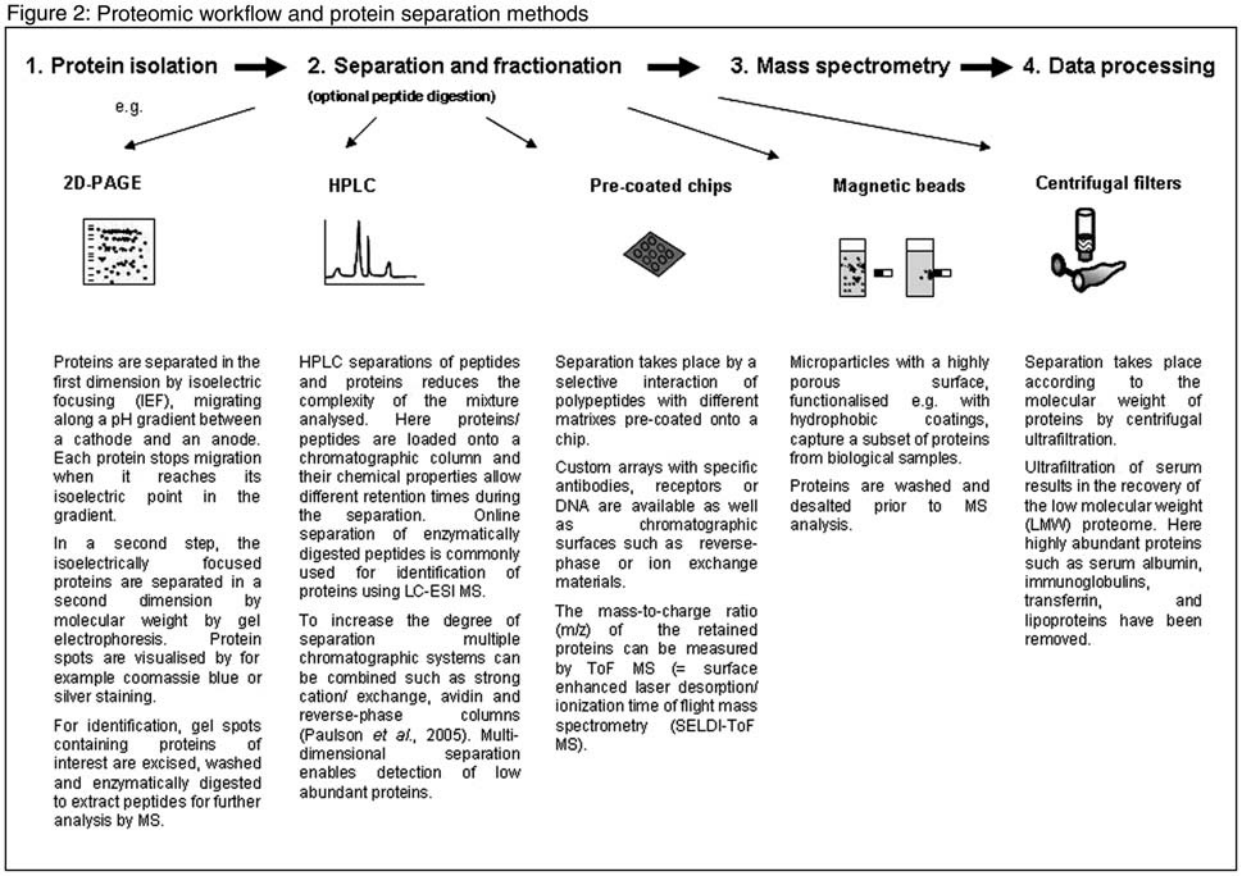
Mass spectrometry

MS has emerged as a central method to most proteomic strategies and thus in this review we are focusing on studies in which MS tools were used. Mass spectrometers for protein and peptide analysis can be configured for either ESI or MALDI, both of which are 'soft' ionization techniques that enable the transfer of intact proteins and nucleic acids into the gas phase without fragmentation (Aebersold and Mann, 2003, Domon and Aebersold, 2006); see Figure 3. Simple peptide samples may be examined with MALDI-ToF MS, while more complex mixtures may require separation using an analytical HPLC column coupled to the ESI MS systems (LC-ESI MS). The analysis of tryptic peptides of a protein extract using this method is often called shotgun proteomics. A variation of the MALDI technique, SELDI MS, has been developed. This approach is based on the retention of proteins of a sample via protein chip systems with pre-activated surfaces (e.g. with antibodies, receptors, ionic or hydrophobic material). The retained proteins are then ionized and detected by MS similar to the process using MALDI-ToF MS (Issaq et al., 2002; Merchant and Weinberger, 2000).

Quantitation

Quantitation using 2D-PAGE is performed using sophisticated image analysis to compare gel images for differential spot intensities of the same proteins. To reduce any gel-based variation, two-dimensional DIGE has been developed,

Figure 2: Proteomic workflow and protein separation methods

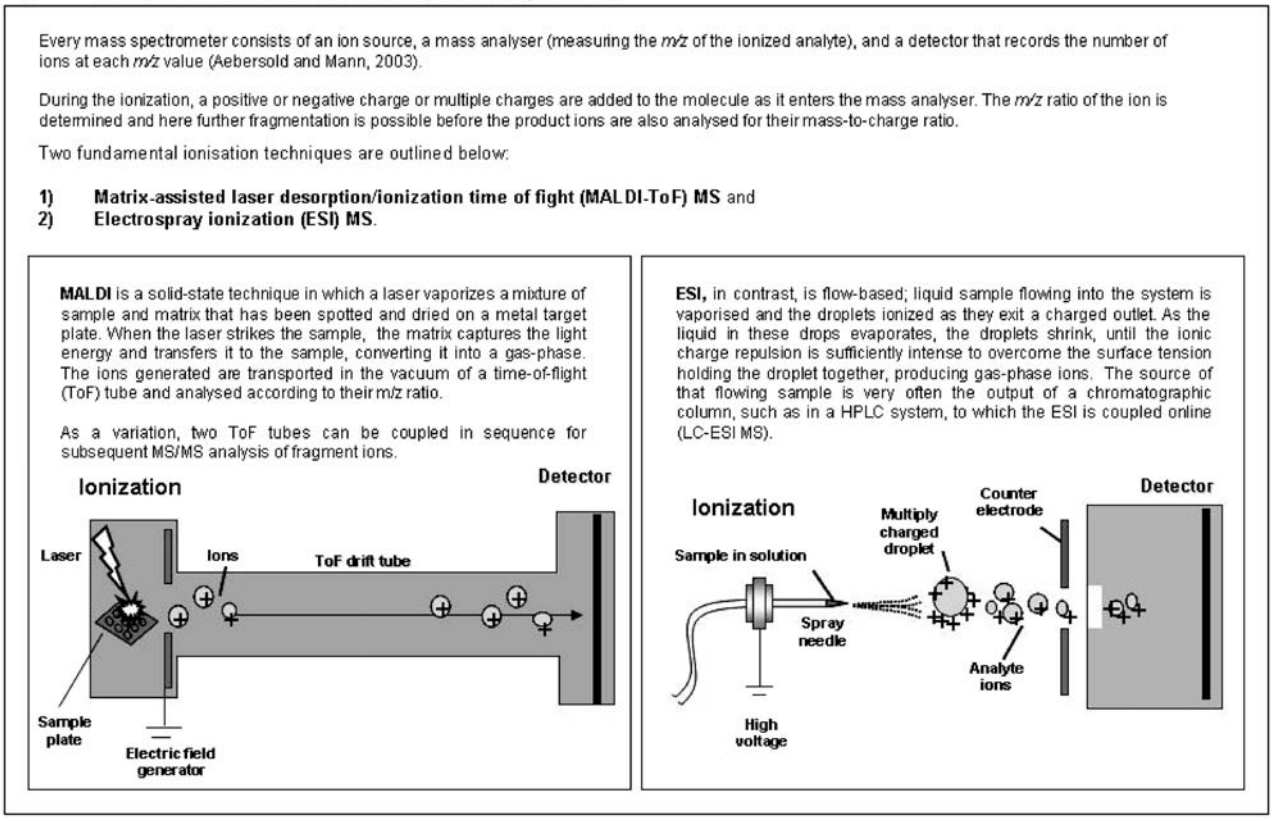


which allows multiple protein samples to be separated on a single gel through the use of fluorescent dyes (Monribot-Espagne and Boucherie, 2002; Unlu et al., 1997). Briefly, as a first step a pooled reference standard is created from all samples, which is then labelled with one of the three fluorescent dyes (cy2, cy3 or cy5). Each individual sample is then labelled with one of the remaining dyes, and mixed for equal protein loading prior to the first and second dimensional separation. The advantage of this technique is that the pooled reference standard image can be used for alignment and normalization of all of the gels for multiple comparisons, allowing for any number of gels to be compared directly. For identification of differentially expressed proteins visualized on the gel, protein spots are excised and enzymatically digested. Identification of the enzymatic peptides may be performed by LC-ESI MS/MS or by MALDI-ToF MS. Using a relatively large amount of replicates, or utilizing the DIGE technology, the quantitation of proteins is reproducible and reliable, although labour intensive (Monribot-Espagne and Boucherie, 2002; Unlu et al., 1997).

Gel-free semi-quantitative comparison of different sample groups, such as patients and controls, has been enabled using stable isotope labelling and MS (e.g. ICAT or iTRAQ) (von Haller et al., 2003; Wiese et al., 2007). Isotopic-labelling can minimize variations during the analysis as proteins of different samples are differentially labelled prior to mixing and analysis. The isotopic labels differ solely in molecular weight and allow for the relative protein quantification. However, using isotopic labelling, only a limited number of samples can be compared and replicates are therefore often pooled. In studies of AD, the comparison of CSF protein profiles with these techniques has already proven successful (Abdi et al., 2006; Choe et al., 2007; D'ascenzo et al., 2008).

Using label-free quantitation methods an unlimited number of samples can be compared (Finney et al., 2008; Ru et al., 2006). However, with the application of these 'gel-free/label-free' proteomics methods, quantitation of individual proteins is more complicated. Here several proteins are digested and the tryptic peptides are mixed; thus, quantitation of an individual peptide may not necessarily reflect levels of the parent protein. It has been reported (Tang et al., 2004) that multiple sources of variation affecting the peak intensity may be introduced during the analysis, such as differences in ESI efficiencies among different peptides and samples, as well as differences in separation for replicate runs and trypsin digestion efficiency. These issues are often peptide dependent, resulting in differences in relative abundance in peptides from the same protein, making an automated approach using all peptides identified from one protein for quantitation more difficult. Nevertheless, recently it was described that applying the correction of the peptides from the same protein across all samples can indicate whether the peptide is a good candidate for quantitation of the entire protein (Schwarz et al., 2007).

Figure 3: Principles of ionization and mass spectrometry methods



This has been shown to be particularly accurate for low abundance proteins.

Proteomic studies in psychiatric disorders

Alzheimer's disease

Cell cultures: A central hallmark of Alzheimer's pathology is the neuritic plaques (composed primarily of Abeta proteins) present in brain tissue (Kidd, 1964; Roth et al., 1966). As distinct APP isoforms might be differentially involved in AD pathogenesis, Newton et al. (2006) examined whether a MS platform was appropriate for the identification of the APP isoforms APP_{695}, APP_{751}, and APP_{770}, in an *in vitro*-model (see Table 1). Three 2D-PAGE protein spots corresponding to these three isoforms were detected. However, likely due to sample loss after in-gel digestion, only the abundant APP_{695} isoform (and not APP_{770} and APP_{751}) could be identified by subsequent MS analysis.

Another goal of *in vitro*-proteomic studies is to test the effect of drugs on protein profiles of cell lines relevant for neuropsychiatric disorders. Sultana et al. (2006c) sought to identify individual proteins that are protected by the potential AD therapeutic D609 (an inhibitor of phosphatidylcholine-specific phospholipase and a glutathione mimetic) against Abeta(1–42) induced oxidation. Pretreatment of rat neuronal cultures with this antioxidant protected GAPDH, 14-3-3 protein zeta, PK, MDH from oxidation. Post-translational modifications and expression changes of these four proteins were also reported in animal models of AD and human post-mortem studies (Cottrell et al., 2005; David et al., 2005; Korolainen et al., 2006; Schonberger et al., 2001; Shin et al., 2004; Sizova et al., 2006; Sultana et al., 2006e, 2007; Wang et al., 2005; Woltjer et al., 2005). Thus, the antioxidant effect of D609 may indicate a possible benefit of this drug in AD treatment.

Animal models: With the analysis of the proteome in rodent models, investigators have aimed at clarifying protein modifications in the early stages of AD, and have investigated possible vaccinations or drug treatments. There are several proteomic studies of rodent AD models that differ in terms of the animal models used, the analysis scheme as well as the prefractionation and MS methods (see Table 2).

Although the aetiopathology of AD dementia is currently incompletely ascertained, there is growing evidence that Abeta deposits and the reaction of the plaque surrounding tissue play major roles in disease development and progression (Haass and Selkoe, 2007; Montalto et al., 2007). With the ambition of developing a possible vaccination against Abeta deposits, transgenic mice with mutant APP and PS1 (for more details on all animal models, see Table 2), that develop Abeta deposits in brain, were immunized with Abeta attenuates (Vehmas et al., 2001). In agreement with former reports (Schenk et al., 1999) these animals developed high serum

Table 1. Alzheimer's disease: cell cultures

Cell lines	Tissue, conditions	Proteins	Methods	Reference
Retinoic acid-induced Ntera 2 cell line	Detection of APP isoforms	APP695, APP751, APP770	1D- and 2D-PAGE, Western blot, MALDI-ToF MS, LC-ESI MS/MS	Newton et al. (2006)
Rat primary neuronal cells P301L tau-expressing SH-SY5Y Neuroblastoma cells, P301L tau mice, AD human brain	Effect of xanthate D609 on protein oxidation Mice: amygdala, human: Temporal cortex; Abeta(42)-treatment	GAPDH; 14-3-3 protein zeta; PK; MDH cells: Prdx 1; transgelin-3; CTP synthase; alpha-taxilin; calcyclin binding protein; proteasome subunit alpha-4; PK M1/M2; PPlase; 14-3-3 protein epsilon; cleavage stimulation factor, 64 kDa subunit, tau variant; drebrin-like protein; UDP-glucose 6-dehydrogenase; methylcrotonyl-CoA carboxylase beta chain, mitochondrial prec; far upstream element-binding protein 1; Rho GDP dissociation inhibitor 1; HSP 90 kDa; GAPDH; valosin-containing protein; annexin A5; eukaryotic translation initiation factor 4B; heat shock cognate 71 kDa protein; 78 kDa glucose-regulated protein	2D-PAGE, MALDI-ToF MS 2D-PAGE, Western blot, MALDI-ToF/ToF MS	Sultana et al. (2006e) David et al. (2006)

antibody titres against Abeta(1–42) and showed reduced Abeta deposits in the brain, illustrating the potential validity of this approach.

In a 2004 study on the hippocampal proteome of APP/PS1 transgenic rats, more than 70 protein spots on 2D-PAGE showed a differential expression in the pre-plaque stage of the disease prior to the appearance of cognitive impairments (Vercauteren et al., 2004b); 44 of the identified proteins and their isoforms had been shown previously to play a role in learning and memory formation. The remaining proteins displayed functions in cytoskeletal and membrane stability, glucose metabolism, electron transport, signal transduction, protein/mRNA synthesis and modification as well as protein transport. At the same time Shin et al. (2004) published the cortical proteome of the Tg2578 mouse line with a mutant APP. In comparison to respective control brain they identified changes in concentrations of several proteins with functions in cellular energy metabolism (amongst others enolase 1, 2, ATP synthase alpha-chain, GAPDH, MDH, PK, aconitase, GFAP, DRP2). Furthermore in this study, increased oxidation and nitration of many proteins was observed. In a following similar study (Carrette et al., 2006), the up-regulation of enolase 1 was confirmed. Soreghan and colleagues used a non-gel-based quantitation method to assess oxidized proteins in an APP/PS1 mutant mouse model in comparison with aged wild-type mice (Soreghan et al., 2005). Fifty-nine of a total of 117 oxidatively modified proteins were specifically associated with the transgenic mice. These proteins mainly had roles described in signalling pathways, transcriptional regulation and vesicular trafficking. Gillardon et al. (2007) analysed the mitochondrial proteome of Tg2576 mice at the onset of cognitive impairment but before plaque deposition. Examination of synaptosomal and non-synaptic mitochondria revealed numerous changes in the subunit composition of the respiratory chain complexes I and III; however, the corresponding mRNA levels were not altered as measured by microarray analysis. Within this study brain mitochondria of young Tg2576 mice showed an impaired state 3 respiration and uncoupled respiration. Abeta oligomers were detected in synaptosomal fractions as was impaired glucose metabolism in the anterior cingulate cortex. So abnormalities in mitochondrial protein profiles and function could be detected in the pre-plaque state indicating that mitochondria are early targets of Abeta aggregates. Lewis et al. (2004) aimed to characterize Abeta(40) and Abeta(42) accumulation in mice, derived from the Tg2576 line, and in a double transgenic line. Abeta(1–40) and Abeta(1–42) were validated as the two main amyloid peptide species. The double mutation line showed in general higher SELDI-ToF MS peak intensities for Abeta(1–40) and Abeta(1–42), but no major qualitative alterations of the brain peptide profile: in the spectra of both mice lines truncated peptides Abeta(1–39), Abeta (1–38) and Abeta(1–37) appeared. In a further study the chemical features of another transgenic APP mouse model were examined (Esh et al., 2005). In these animals, a variety of C-terminally elongated Abeta peptides were detected in addition to Abeta(40) and Abeta(42), N-terminally truncated peptides and altered APP degradation. Sizova et al. (2006) studied the brain proteome associated with amyloid plaque deposition and identified 15 proteins which were significantly altered in a slightly different transgenic mouse to that used by Esh et al. (2005). These identified proteins play a role in glial response/inflammatory and oxidative processes, in cholesterol metabolism and neuronal signal transduction.

Aside from elevated Abeta(42) peptides, there are also increased levels of Abeta binding alcohol dehydrogenase (ABAD), which contains an intracellular binding site for Abeta. Over-expression of Abeta and ABAD in transgenic mice results in an enhanced neuronal cytotoxicity with subsequent changes in spatial learning memory (Lustbader et al., 2004). Yao et al. (2000) used this animal model to identify the antioxidant protein Prdx 2 as being consistently up-regulated in the transgenic animals as well as in human AD post-mortem brain. It was also observed that neuronal cell

Table 2. Alzheimer's disease: animal models

Animals	Tissue, conditions	Proteins	Methods	Reference
Mice, (APPSWE)x(PS1A-E9) mutation	Brain and serum, immunization with Abeta-attenuates	Abeta(1–42)	SELDI-ToF MS	Vehmas et al. (2001)
C57BL/6J mice, (ApoEtm1Unc) mutation	Hippocampus, protein oxidation	GFAP; CK B; disulfide isomerase (glucose-regulated protein, Erp61); chaperonin subunit 5; DRP2; mortalin (75 kDa glucose-regulated protein)	2D-PAGE, Western blot, MALDI-ToF/ToF MS, LC-ESI MS/MS	Choi et al. (2004)
Mice, (APP695SWE) and (APP695SWE)x (PS1A 246E) mutation; AD human brain	Mice: cortex and hippocampus, amyloid characterization	Abeta(1–42), Abeta(1–40)	Western blot, immunohistochemistry, SELDI-ToF MS	Lewis et al. (2004)
Tg2578 mice (APP K670N, M671L)	Cortex; protein profiles, protein oxidation and nitration	Proteasome complex activator subunit 2; PK; aconitase; MDH; enolase 1, 2; GFAP; alpha synuclein; GAPDH; ATP synthase alpha chain, mitochondrial prec; DRP2; collapsin response mediator protein 2; similar to zinc finger 1111; dynamin-1; laminin receptor 1; calpain 12; ATP synthase, H^+ transporting mitochondrial F1 complex, beta subunit	2D-PAGE, MALDI-ToF MS, LC-ESI MS/MS	Shin et al. (2004)
Wistar rats, (APPSWE)x (APP717)x PS1 Finnish mutation (M146L)	Hippocampus	Tyrosine 3/tryptophan 5-monooxygenase activation protein gamma, epsilon; microtubule-associated protein 1 B; neurofilament L, light polypeptide; ras-related small GTP binding protein 3A; soluble N-ethylmaleimide-sensitive factor attachment protein beta; actin beta; enolase 2; putative protein phosphatase 1, regulatory (inhibitor) subunit 7; Sds22; PEBP; guanine nucleotide-binding protein G(O), alpha subunit 2; branched chain keto acid dehydrogenase E1, beta polypeptide; copine 6; neuronal copine; 66 kDa neurofilament protein NF-66; CK B; similar to putative type 5 nonmuscle actin; DRP1, 2; similar to 10-formyltetrahydrofolate dehydrogenase; collapsin response mediator protein 2; apoE; protein phosphatase 2 alpha, regulatory subunit B; T-complex 1; seryl-tRNA synthetase; elongation factor G; heterogeneous nuclear ribonucleoprotein H1; succinate semialdehyde dehydrogenase; similar to gamma-soluble NSF attachment protein; isocitrate dehydrogenase 3 (NAD+) alpha; similar to microtubule-associated protein, RP/EB family, member 2; acetyl-Coenzyme A dehydrogenase, long chain; aspartate aminotransferase, cytoplasmic; Glutamine synthetase; PDH alpha chain 1 prec; adenylyl cyclase-associated protein 2; synapsin IIb; cystathionine beta-synthase, splice form III; collapsin response mediator protein 1; GDH; tropomyosin 1 alpha, brain; tropomyosin 3, gamma; tropomyosin 5; non-muscle alpha tropomyosin; tubulin alpha 4, beta 4, beta 5, beta 15; 66 kDa neurofilament protein; cofilin 2, muscle; NADH dehydrogenase Fe-S protein 2 (49 kDa); NADH dehydrogenase flavoprotein 2; NADH dehydrogenase flavoprotein 2; H^+ transporting ATPase, lysosomal beta 56/58 kDa, isoform 2; MDH-like enzyme; enolase 1, alpha; glycogen phosphorylase brain, muscle; PK 3, muscle; fructose-biphosphate aldolase C; aminopeptidase-B; leukotriene A4 hydrolase; dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial prec; antidepressant-related protein ADRG123; isopeptidase T; cathepsin D; constitutive photomorphogenic (COP9) homolog, subunit 4; ubiquitin-activating enzyme E1, Chr X; UMP-CMP kinase; ER-60 protease; aminopeptidase-B; aldehyde dehydrogenase, mitochondrial;	2D-PAGE, MALDI-ToF MS, LC-ESI MS/MS	Vercauteren et al. (2004b)

		mitochondrial processing peptidase alpha subunit, mitochondrial prec; HSP 60 kDa, mitochondrial prec; copine I, isoform 2; albumin; chaperonin containing TCP1, subunit 3; hippocalcin; v-crk avian sarcoma virus CT10 oncogene homolog; constitutive photomorphogenic (COP9) homolog, subunit 4; haemoglobin, alpha 1; alpha-S1 -casein		
P301L tau mice, AD human brains	Mice: whole brain homogenates, human: temporal cortex	Mice: NADH-ubiquinone oxidoreductase 30 kDa subunit; ATP synthase D chain; triosephosphate isomerase; inorganic pyrophosphatase; MDH, cytoplasmic; Prdx 3, 6; phospholipid hydroperoxide glutathione peroxidase; DRP2; glutathione S-transferase P 1; glutathione S-transferase Mu 1; synapsin I; septin 5; septin 11; stress-induced phosphoprotein 1; gamma-soluble NSF attachment protein; endoplasmic reticulum protein Erp29 prec; lipoamide acyltransferase component of branched-chain-keto acid dehydrogenase complex; 4-aminobutyrate aminotransferase, mitochondrial; ethylmalonic encephalopathy protein 1 homolog; D-tyrosyl-tRNA(Tyr) deacylase 1; growth factor receptor-bound protein 2; lamin A; lamin C and C2; paraspeckle protein 1; myeloid leukaemia factor 2; coiled-coil domain-containing protein 44	2D-PAGE, Western blot, functional mitochondrial analyses, MALDI-ToF/ToF MS	David et al. (2005)
Mice, (APP K670N, M671 L)x PS1	Protein oxidation	Adenine nucleotide translocator 2, fibroblast; growth hormone-regulated TBC protein 1; heterogeneous ribonuclear particle protein A2/B1; mitochondrial dicarboxylate carrier; Ral guanine nucleotide dissociation stimulator-like 2; UCH L4; hexokinase 2; cyclic GMP-inhibited phosphodiesterase B; calcium/calmodulin-dependent protein kinase type II alpha chain; protein salvador homolog 1; bone morphogenetic protein 4 prec; glutamate receptor, ionotropic kainate 1 prec; G protein-coupled receptor FEX; complement component 1, q subcomponent; integrin alpha-4 prec; lymphocyte antigen; tumor necrosis factor superfamily, member 9; nitric oxide synthase 2; integrin alpha-L prec; tyrosine-protein kinase HCK; macrophage-stimulating protein receptor prec; macrophage receptor types I and II; toll-like receptor 5; fibrillin 1 prec; tubulin beta 2; GFAP; stable tubule only peptide; golli-myelin basic protein prec; transcription factor B2, mitochondrial; nuclear factor of activated T cell, Rel type domain containing protein; transcription intermediary factor 1- alpha; CREB-binding protein; chromodomain-helicase-DNA-binding protein; hairy and enhancer of split 1; DNA polymerase epsilon subunit B; early develoment regulator 1; Rb1 - inducible coiled coil protein 1; CCAAT/enhancer binding protein beta; forkhead box P1; transcription factor E3; zinc finger protein HRX; homologous to mouse SKD1 and to human hVPS4; myosin heavy chain isoforms; lysosomal trafficking regulator; ADP-ribosylation factor 3, 6; Ras-related protein Rab-4A; transformed mouse 3T3 cell double minute 1; solute carrier family 45, member 2; solute carrier family 12 member 3; chloride channel 6; laminin beta-1 chain prec; calsyntenin 1; integrin beta-3 chain; otoraplin; formin 1 isoform IV; multidrug resistance protein 3; meltrin beta	Beads purification, LC-ESI MS/MS	Soreghan et al. (2005)
PDAPP mice, familial AD (APP717) mutation	Amyloid characterization	Mortalin (75 kDa glucose-regulated protein)	HPLC, Western blot, MALDI-ToF MS, SELDI-ToFMS	Esh et al. (2005)
Tg2576 (APP K670N, M671 L) and Tg2576 (APP K670N, M671L)x(PS1AE9) mice	Left cortex	Alpha-synuclein; cytochrome oxidase polypeptide Va; enolase 1; UMP-CMP kinase; ATP synthase alpha, beta chain; DRP2	2D-PAGE, Western blot, MALDI-ToF/ToF MS	Carrette et al. (2006)

(Continued)

Table 2. Continued

Animals	Tissue, conditions	Proteins	Methods	Reference
P301L tau mice, P301 L tau-expressing SH-SY5Y neuroblastoma cells, human AD brain	Mice: amygdala, human:temporal cortex; Abeta(42)-treatment	MICE: actin beta, gamma; carbonic anhydrase 2; isocitrate dehydrogenase [NADP], cytoplasmic; DRP2; proteasome subunit alpha type 3; transferrin; lactamase, beta 2; adenylate kinase isoenzyme 5; disks large homolog 4; phosphoglycerate mutase 1; synapsin-2; 75 kDa glucose-regulated protein; intracellular hyaluronan-binding protein p57; DRP5; LIM and SH3 domain protein 1; putative deoxyribonuclease TATDN1; Prdx 5, 6; transgelin-3; stromal cell-derived factor 2-like protein 1; tubulin, alpha-4, beta-2 chain; ribose-phosphate pyrophosphokinase II; myelin basic protein; vacuolar ATP synthase subunit G2; NADH-ubiquinone oxidoreductase B15 subunit; NADH dehydrogenase (ubiquinone) Fe-S protein 8; NADH-ubiquinone oxidoreductase 23 kDa subunit	2D-PAGE, Western blot, MALDI-ToF/ToF MS	David et al. (2006)
Mice, APP London (V642I)x Swedish (KM595/596NL) mutations	Cortex	Serum albumin prec; DRP2; GFAP; apoE prec; Prdx 6; olfactory marker protein; serotransferrin prec; moesin; N-ethylmaleimide sensitive fusion protein; synaptotagmin I; CaMK-II alpha subunit; dynamin 1 -like; PK M1/M2; T-complex protein 1-ACAT2; complement C1q subcomponent	2D-DIGE, Western blot, MALDI-ToF/ToF MS, LC-ESI MS/MS	Sizova et al. (2006)
Tg2578 mice (APP K670N, M671L)	Cortex and hippocampus, mitochondrial proteome	Stress-70 protein, mitochondrial; ubiquinol-cytochrome c reductase iron-sulfur subunit; glutaminase, kidney isoform; Rab GDP dissociation inhibitor beta-2; cytochrome d, heme protein, mitochondrial; NADH-ubiquinone oxidoreductase subunit 18 kDa, B8 subunit, B16.6 subunit	2D-PAGE, 2D-DIGE, Western blot, immunhistochemistry,	Gillardon et al. (2007)
Mongolian gerbils	Forebrain synaptosomes, protein oxidation under acrolein	Tropomyosin-3-gamma isoform 2; tropomyosin 5; actin beta; mitochondrial Tu translation elongation factor; voltage-dependent anion channel	2D-PAGE, Western blot, MALDI-ToF MS	Mello et al. (2007)
APOE targeted replacement mice, human AD brain	Mice: hippocampus, human: cortex and hippocampus	Mortalin (75 kDa glucose-regulated protein)	2D-DIGE, Western blot, MALDI-ToF/ToF MS	Osorio et al. (2007)
Tg ABAD/mAPP, single Tg mAPP, Tg ABAD, non-Tg mice; primary neuronal cultures of embryonic CD1 wild-type mice; AD human brains	Mice and human: cerebral cortex	Prdx 2	2D-PAGE, Western blot, immunocytochemistry, MALDI-ToF MS	Yao et al. (2007)

lines transfected with Prdx 2 were better protected against toxic Abeta levels than mock transfected cells. Therefore, it can be suggested that the up-regulated Prdx 2 expression in AD reflects part of the cellular response to protect the brain from further oxidative damage.

The other major histopathological AD hallmark, apart from amyloid deposits, is tau-containing neurofibrillary tangles. David and colleagues analysed a transgenic mouse line that expresses mutant tau protein (David et al., 2005). Proteomic techniques and functional analyses revealed that tau accumulation induces mitochondrial dysfunction, with a diminished capacity in electron transport and a significant reduction in ATP levels. Further results imply a metabolic dysregulation, modifications in the oxidative state of the brain and a synaptic pathology in the presence of the mutant tau. In a second study of this group, Abeta(42)-induced effects on the proteome were studied in the mutant tau animal model, and also in an *in vitro* cell model (David et al., 2006). Many of the differentially expressed proteins in these two models are linked to energy metabolism, stress response and protein folding, proteasome degradation, and transcriptional processes. Analogous findings were also found in human AD post-mortem brain (David et al., 2006).

Apolipoprotein (apo) E knock-out mice have also been used as animal models in AD research (Choi et al., 2004a), as studies have shown an influence of APOE gene variants on the oxidation status in the brain (Ramassamy et al., 1999). In ApoE knock-out mice an approximately two-fold increase in hippocampal protein oxidation was reported along with an elevated sensitivity for oxidation of specific proteins (GFAP, CK B, disulfide isomerase, chaperonin subunit 5, DRP2, 75 kDA glucose-regulated protein).

In another study the impact of APO gene variants on the hippocampus proteome was assessed in APOE targeted replacement (TR) mice (Osorio et al., 2007). The level of the chaperone protein 75 kDA glucose-regulated protein (mortalin) was found to be differentially expressed in APOE3 and APOE4 mice, and in human AD post-mortem tissue APOE levels were correlated with APOE genotypes APOE 3/3 and APOE 4/4. These two studies support previous findings that the apoE gene product regulates the cellular response mechanisms on oxidative stress associated with AD pathology.

In AD patients, increased levels of acrolein has been detected (Lovell et al., 2001). Acrolein (2-propen-1-al), the most reactive of the alpha, beta-unsaturated aldehydes (Esterbauer et al., 1991) is present in automobile exhaust gases, but is also endogenously produced during lipid metabolism (Adams and Klaidman, 1993). Mello et al. (2007) sought to identify oxidized proteins in gerbils after application of acrolein. Acrolein increased protein oxidation in a dose-dependent manner and the identified oxidized proteins were linked to energy metabolism, protein synthesis, neurotransmission and the cytoskeleton.

Many findings of AD animal models are in accordance with abnormalities seen in post-mortem tissue of AD patients. Therefore, animal models seem to be valid tools in AD research seeking to characterize Abeta isoforms implicated in AD and their functions, to test the effect of potential vaccinations and drug treatments and to identify oxidized proteins that play a role in the disease pathophysiology.

Post-mortem studies: As numerous studies of animal and cellular models of AD have reported an increased oxidation of proteins, proteomic approaches have been applied to post-mortem tissue to examine protein oxidation and other post-translational modifications in AD. In the AD brain an augmented oxidation (e.g. of CK B, glutamine synthetase, UCHL1, actin beta, enolase 1 and 2, phosphoglycerate mutase 1 and DRP2) as well as an increased protein nitration (e.g. of enolase 1, GAPDH, ATP synthase alpha-chain, voltage-dependent anion channel-protein, carbonic anhydrase II, triphosphate isomerase) has been observed repeatedly (Butterfield et al., 2006a; Castegna et al., 2002a,b, 2003; Choi et al., 2004b; Korolainen et al., 2002, 2006; Pamplona et al., 2005; Sultana et al., 2006a,b,e, 2007); see Table 3. For detailed reviews on this topic, see Butterfield et al. (2006b), Sultana et al. (2006d) and Butterfield and Sultana (2007). Many of the above-mentioned protein changes have been reported previously in AD animal models. As CK B, enolase 1, triphosphate isomerise, phosphoglycerate mutase 1 and GAPDH all are involved in energy metabolism and ATP synthesis, the Butterfield group has suggested that significantly impaired function of these proteins as a result of post-translational modifications would lead to compromised neuronal function (Butterfield et al., 2007). Low levels of cellular ATP at nerve terminals might, for example, lead to loss of synaptic dysfunction/loss, ultimately causing the dementia symptoms observed in AD. The oxidation targets ATP synthase alpha-chain and voltage-dependent anion channel proteins belong to the mitochondrial membrane and might play an important role in mitochondrial dysfunction and cell death in AD. Impairments in energy metabolism in AD pathology are supported by consistent reports of differentially expressed proteins directly or indirectly linked to the energy homeostasis of the cell (Kim et al., 2000, 2002; Melanson et al., 2006; Schonberger et al., 2001; Tsuji et al., 2002; Wang et al., 2005).

Amongst the proteins that exhibited changes in their oxidation status are also components of glutamate metabolism, such as glutamine synthetase and GDH (Butterfield et al., 2006b; Korolainen et al., 2006). Glutamine synthetase regulates extra neuronal glutamate levels and thereby neurotransmission. An impaired activity of this enzyme due to the observed increase in oxidation would result in an augmented neurotoxicity and neurodegeneration as glutamate synthetase converts the potential neurotoxin glutamate into glutamine. Interestingly, there is an increase in total amount of soluble GDH in AD, but a decrease in its oxidation (Korolainen et al., 2006). It is thought that the GDH pathway is mostly active when the glucose levels are low. Thus, these reports complement the findings of an altered energy metabolism in AD.

UCHL1 is part of the ubiquitin-proteasome system and plays an important role in the degradation of misfolded, damaged and short-lived proteins. An overload of this system by undegradable molecules together with a functional impairment due to oxidation might eventually result in the accumulation of abnormal proteins and in neuronal

Table 3. Alzheimer's disease: post-mortem studies

Probands	Tissue, conditions	Proteins	Methods	Reference
AD, DS	Frontal, temporal, parietal, occipital cortex, cerebellum, thalamus, caudate nucleus	DRP2	2D-PAGE, MALDI-ToF MS	Lubec et al. (1999)
AD, DS	Frontal, temporal, parietal, occipital cortex, cerebellum, thalamus, caudate nucleus	Complex III (cytochrome c oxidoreductase) core protein 1	2D-PAGE, MALDI-ToF-MS	Kim et al. (2000)
AD, DS	Frontal, temporal, parietal, occipital cortex, cerebellum, thalamus, caudate nucleus	Stathmin	2D-PAGE, MALDI-ToF-MS	Cheon et al. (2001)
AD, DS	Frontal, temporal, parietal, occipital cortex, cerebellum, thalamus, caudate nucleus	Prdx 1, 2	2D-PAGE, MALDI-ToF-MS	Kim et al. (2001)
AD	Hippocampus, temporal and enthorhinal cortex, cerebellum, cingulated gyrus, sensorimotor cortex	Vesicular fusion protein NSF; synaptotagmin; transformation sensitive protein; PEBP; DRP2; profiling II; phosphoglycerate mutase, brain; serum albumin pre; UCH L1; alpha crystallin B chain; enolase 1, 2; antioxidant protein 2; DJ-1 protein; fatty acid-binding protein, cardiac isoform; GAPDH; haemoglobin beta; succinyl CoA; 3-ketoacid-coenzyme A transferase; superoxide dismutase Cu/Zn; CK B; macrophage migration inhibitory factor; PPlase	2D-PAGE, NH2-terminal sequencing	Schonberger et al. (2001)
AD	Temporal cortex	GFAP-related	2D-PAGE, Western blot	Tsuji et al. (2001)
AD, DS	Temporal, parietal cortex, thalamus, cerebellum	Beta-SNAP; synaptotagmin I	2D-PAGE, MALDI-ToF-MS	Yoo et al. (2001a)
AD	Frontal, temporal, parietal, occipital cortex, cerebellum, thalamus, caudate nucleus	HSP 60 kDa, mitochondrial; HSP 70 kDa protein 4; heat shock cognate 71 kDa; alpha-crystallin B chain; glucose-regulated protein 75 and 94 kDa	2D-PAGE, MALDI-ToF-MS	Yoo et al. (2001b)
AD, DS	Frontal, temporal, parietal, occipital cortex, cerebellum, thalamus, caudate nucleus	Nucleoside diphosphate kinase A	2D-PAGE, Western blot, MALDI-ToF-MS	Kim et al. (2002)
AD	Temporal cortex	HSP 60 kDa, mitochondrial; guanine nucleotide-binding protein G(I)/G(S)/G(T), beta subunit 1; fatty acid-binding protein, heart; ATP synthase beta chain, mitochondrial; ATP synthase alpha subunit, mitochondrial; GFAP; calpain 2, alpha-internexin; DRP2; enolase 3	2D-PAGE, LC-ESI MS/MS	Tsuji et al. (2002)
AD	Inferior parietal lobe, protein oxidation	CK B, glutamine synthetase, UCH L1	2D-PAGE, Western blot, MALDI-ToF MS	Castegna et al. (2002a)
AD	Inferior parietal lobe, protein oxidation	DRP2; enolase 1; heat shock cognate 71 kDa	2D-PAGE, Western blot, MALDI-ToF MS	Castegna et al. (2002b)

AD	Inferior parietal lobe, protein nitration	Enolase 1, 2; triosephosphate isomerase; neuropolypeptide h3; actin beta; LDH	2D-PAGE, Western blot, MALDI-ToF MS, LC-ESI	Castegna et al. (2003)
AD, DS, PD	Frontal cortex and cerebellum	Prdx2	2D-PAGE, MALDI-ToF-MS	Krapfenbauer et al. (2003)
AD	CSF, frontal cortex, cerebellum	Abeta; novel Abeta peptide (1–45 or 2–46)	1-D PAGE, Western blot; SELDI-ToF MS	Lewczuk et al. (2003)
AD	Amyloid characterization	Amino truncated Abeta(42) peptides	Western blot, 2D-PAGE, MALDI-ToF MS, LC-ESI MS/MS	Sergeant et al. (2003)
AD, PD	Frontal cortex, protein oxidation	UCH L1 isoforms	2D-PAGE, Western blot, immunohistochemistry, MALDI-ToF MS, LC-ESI	Choi et al. (2004)
AD, C3/B6 mice with (APPSWE)x (PS1AE9) mutation	Human: frontal, temporal cortex; plaque and non-plaque areas	Abeta peptides; alpha1-antichymotrypsin; apoE; collagen type I alpha 1, type XXV; cystatin B, C; alpha-synuclein; proteoglycans; clusterin; coronin, actin-binding protein, 1A; tau; HSP 90 kDa protein 1 beta; clathrin, heavy polypeptide 1; dynamin 1; dynein, heavy polypeptide 1; GFAP; vimentin; antitrypsin; ATPase H^+ -transporting, lysosomal V0 subunit A, lysosomal V1 subunit B, D, E; ATPase H^+ -transporting, lysosomal 42 kDa, V1 subunit C1; ATPase, Ca^{++} transporting, plasma membrane 2; cathepsin D; ubiquitin-activiting enzyme E1; 14-3-3 protein beta/alpha, epsilon, zeta; phosphofructokinase, platelet; antitrypsin; cathepsin D; ubiquitin-activating enzyme E1	Laser capture microdissection, 2D-PAGE, immunohistochemistry, LC-ESI MS/MS	Liao et al. (2004)
AD	Frontal cortex, cytosolic proteome, protein	Collapsin response mediator protein 2, GFAP, unidentified protein	2D-PAGE, LC-ESI MS/MS	Kanninen et al. (2004)
AD	Hippocampus, co-immunoprecipitation with APP	N-ethylmaleimide sensitive fusion protein; syntaxin binding protein 1; dynamin 1; dynein; myosin, nonmuscle; HSP 90 alpha; heat shock cognate 71; alpha crystallin B2; cyclophilin A/cyclosporin; alpha spectrin; GFAP; tubulin beta; actin beta; neurofilament light polypeptide; myelin basic protein; 14-3-3 protein zeta; Abeta A4 prec protein-binding, family B, member 1 isoform delta E9 UCH L1; phosphoglycerate mutase A; uracil DNA glycosylase; mitogen-activated protein kinase kinase kinase 5	2D-PAGE, Western blot, immunhistochemistry, MALDI-ToF MS, LC-ESI MS/MS	Cottrell et al. (2005)
AD	Frontal cortex, GFAP characterization	GFAP isoforms	2D-PAGE, Western blot, LC-ESI MS/MS	Korolainen et al. (2005)

(Continued)

Table 3. Continued

Probands	Tissue, conditions	Proteins	Methods	Reference
AD	Frontal cortex (area 8), lipoxidative damage	Neurofilament triplet L; vimentin; tubulin alpha 1,4,6; tubulin beta 2; actin beta, gamma; GFAP; enolase 1, 2; ubiquinol-cytochrome c reductase complex core protein I; ATP synthase, beta chain; CK B; glutamine synthetase; GDH 1; guanine nucleotide-binding protein G(I)/G(S)/G(T) beta, subunit 1 or 2; HSP 60 kDa; DRP2	2D-PAGE, Western blot, gas chromatography-MS, MALDI-ToF MS	Pamplona et al. (2005)
AD	Hippocampal sector CA1, neurofibrillary tangles	GAPDH, hypothetical protein FLJ32204; vimentin; cofilin; similar to neurofilament, light polypeptide 68 kDa; myelin basic protein isoforms; microtubule-associated protein tau isoforms; tubulin isoforms; actin, cytoplasmatic 1 and 2; septin 7; alpha-internexin; fructose-biphosphate aldolase A, C; MAD, mitochondrial prec; glucose-6-phosphate isomerase; hexokinase isoforms; ATP synthase alpha/beta chain, mitochondrial prec; CK B; sodium/potassium-transporting ATPase beta-1 chain isoforms; ADP/ATP carrier protein isoforms; PK M1/M2; PK3 isoform 2; ferritin light chain; hypothetical protein DKFZp686L19147; LDH B; cytochrome C oxidase subunit IV isoform 1, mitochondrial prec; MDH, cytosolic; phosphoglycerate kinase 1; ATP synthase oligomycin sensitivity conferral protein, mitochondrial prec; carbonic anhydrase II; elongation factor 1 alpha 1; enolase 1; brain link protein-1 prec; versican core protein prec isoforms; spectrin alpha/beta chain; tenascin-R; desmoplakin, DPI; desmoglein 1 prec; galectin 7; Myc box dependent interacting protein 1 isoforms; vacuolar ATP synthase catalytic subunit A; clathrin, heavy chain 1; SH3-containing GRB2-like protein 2; syntaxin-binding protein 1, isoform 1, 2; dynamin-1; synapsin I isoforms; synapsin II; alpha-synuclein isoforms; histone isoforms; guanine nucleotide-binding protein isoforms; 2′,3′-cyclic nucleotide 3′-phosphodiesterase isoforms; annexin A5; microtubule- associated protein RP/EB family member 3; mitogen-activated protein kinase 1; calcium/calmodulin-dependent protein kinase type II alpha chain; hypothetical protein KIAA0968; Prdx 1,2,6; UCH L1; PPlase; ubiquitin A-52 residue ribosomal protein fusion product 1; similar to monoubiquitin/carboxy-extension protein fusion; ubiquitin-52 amino acid fusion protein; ubiquitin B; polyubiquitin UBC; hypothetical protein FLJ32377; similar to bA92K2.2; ubiquitin and ribosomal protein S27a prec; heat shock cognate 71 kDa; DRP2; hypothetical protein KIAA0233; similar to alpha-fetoprotein	Laser capture microdissection, immunohistochemistry, Western blot, LC-ESI MS/MS	Wang et al. (2005)
AD, prodromal AD; Tg2576 (APPSWE)	Human: temporal grey matter, mice: cortex; detergent-insoluble proteins in neurofibrillary tangles	ApoD; ApoE; catenin delta; cathepsin D; laminin; reticulon; alpha crystallin B; clathrin; actin; cofilin; destrin; dynamin I; GAPDH; spectrin; tubulin alpha, tubulin beta; collagen, type XXV, alpha 1 isoform 2 (CLAC)	1 D-PAGE, Western blot, affinity chromatography, LC-ESI MS/MS	Woltjer et al. (2005)

AD, SH-SY5Y human neuroblastoma cell line, CF-1 mice	Human: hippocampus	PEBP	2D-PAGE, Western blot, cloning, MALDI-ToF MS, LC-ESI MS/MS	Chen Q.et al. (2006)
AD	Frontal cortex, protein oxidation	Carbonic anhydrase I; MDH 1a and b; aconitase, mitochondrial; 14-3-3 protein delta, zeta; GDH; aldolase A; ATP synthase beta chain, mitochondrial	2D-PAGE, Western blot, immunohistochemistry, MALDI-ToF MS, LC-ESI MS/MS	Korolainen et al. (2006)
AD	Frontal cortex	Voltage-dependent anion-selective channel protein 1 and 3; vacuolar ATP synthase subunit d; excitatory amino acid transporter 2; voltage-dependent anion-selective channel protein 2; ubiquinol- galectin-1; NAD-dependent deacetylase sirtuin-2; CD9 antigen; 2′,3′-cyclic-nucleotide 3′-phosphodiesterase; myelin proteolipid protein; gelsolin prec; serum albumin prec; carbonic anhydrase II; myelin-oligodendrocyte glycoprotein prec; versican core protein prec; plectin 1; myelin A1 protein	Isotopic labelling, LC-ESI MS/MS	Melanson et al. (2006)
AD	Hippocampus, protein oxidation	Pplase	2D-PAGE, Western blot, MALDI-ToF MS	Sultana et al. (2006a)
AD	Hippocampus, cerebellum, protein oxidation	PPlase; phosphoglycerate mutase 1; UCH L1; DRP-2; carbonic anhydrase II; triose phosphate isomerase; enolase 1, gamma-SNAP	2D-PAGE, Western blot, immunoprecipitation, MALDI-ToF MS	Sultana et al. (2006b)
AD	Hippocampus, protein nitration	Carbonic anhydrase II; enolase 1; GAPDH; ATP synthase alpha-chain; voltage-dependent anion-channel protein-1	2D-PAGE, Western blot, immunoprecipitation, MALDI-ToF MS	Sultana et al. (2006e)
AD (PS1 mutations)	Frontal cortex, protein oxidation	UCH L1; enolase 2; actin; dimethylarginine dimethylaminohydrolase 1	2D-PAGE, Western blot, MALDI-ToF MS	Butterfield et al. (2006a)
AD, AD plus Lewy body dementia	Inferior parietal lobule, protein S-glutathionylation	Deoxyhaemoglobin; alpha-crystallin B; GAPDH; enolase 1	2D-PAGE, Western blot, MALDI-ToF MS	Newman et al. (2007)
MCI	Hippocampus and inferior parietal lobe, protein nitration	Multidrug resistance protein 3; 14-3-3 protein gamma; glutathione S-transferase M3; enolase 1; glucose-regulated protein prec; aldolase, fascin 1; HSP 70 kDa 8; Prdx 6; DRP2; MDH	2D-PAGE, Western blot, MALDI-ToF MS	Sultana et al. (2007)
AD, PD, progressive supranuclear palsy, Lewy body dementia	Frontal and temporal cortex	Abeta A4; tau, microtubule-associated isoform A and B; ubiquitin; apoE; RNA binding motif protein, X-linked-like 1	iTRAQ, ELISA, MALDI-ToF/ToF MS	Yang et al. (2007)

degeneration (Butterfield, 2004; Butterfield et al., 2006b). These suggestions are in agreement with recent proteomic findings of dysregulation of the proteasome, as well as the established finding of abnormal deubiquitination and consecutive aggregation of damaged proteins in AD dementia (Chen et al., 2006a).

Proteins involved in axonal growth and cytoskeletal regulation have also been reported to be oxidized in AD: DRP2 has been proposed to modulate neuronal plasticity in the aging brain, and altered DRP function in AD results in cytoarchitectural changes, such as reduced dendritic length (Butterfield et al., 2006b; Coleman and Flood, 1987; Lubec et al., 1999). A dysregulation of DRP2 has been repeatedly observed in human AD brain tissue (Pamplona et al., 2005; Schonberger et al., 2001; Tsuji et al., 2002). Another target of elevated oxidation, actin beta, is a key player in cytoskeletal pathways and has been shown to rapidly influence the shape of dendritic spines. Impaired function of this protein would be consistent with the observed abnormalities in synaptic plasticity and consequential reduced memory function in AD (Butterfield et al., 2006b). Expression changes or increased oxidation could be detected for several other cytoskeleton- and membrane transport-associated proteins such as actin binding protein, tau, vimentin, dynamin 1, dynein, tubulin isoforms, neurofilament triplet L or stathmin (Cheon et al., 2001; Liao et al., 2004, Pamplona et al., 2005). Cytoskeletal proteins are also potentially relevant targets for processes that cause decreased protein solubility in early AD (Wang et al., 2005; Woltjer et al., 2005).

Another prime candidate for proteomic analysis in AD is carbonic anhydrase II, an enzyme which is preferentially oxidized and catalyses the reversible hydration of CO_2, a reaction fundamental to many cellular and systemic processes including glycolysis and acid and fluid secretion. Thus, carbonic anhydrase II represents an essential enzyme for general brain function and a potentially important target for post-translational modification in AD.

The supposition that post-translational modifications, such as oxidation and nitration, significantly alter protein function has already been proven correct for several of the above-mentioned proteins (Butterfield et al., 2006b). Apart from protein oxidation and nitration, changes in other post-translational modifications such as S-glutathionylation, phosphorylation and glycosylation have been found in AD brain tissue (Kanninen et al., 2004; Korolainen et al., 2005, Newman et al., 2007). Therefore, proteomic approaches, combined with other study designs have been deployed successfully to test the hypothesis that increased oxidation and nitration together with other post-translational modifications are involved in the complex mechanism of brain pathology in AD disease.

Normal aging is associated with increased oxidative stress in the body and an increased probability of developing neurodegenerative disorders (Squier, 2001). Antioxidative proteins, such as peroxiredoxins and Cu/Zn SOD protects cells from oxidative damage. Peroxiredoxins are ubiquitously in all living organisms and remove cellular hydrogen peroxide and SOD catalyses the dismutation of superoxide radical to hydrogen peroxide (Kim et al., 2001). In AD post-mortem brain, elevated concentrations of Prdx 1 and 2, as well as of SOD were reported (Kim et al., 2001; Krapfenbauer et al., 2003; Melanson et al., 2006; Schonberger et al., 2001) providing evidence for compensatory response mechanisms to increased oxidative stress and cell loss in AD.

In AD post-mortem tissue altered concentrations of proteins linked to amyloidosis and cell death were found. Proteomic characterization of amyloid plaques confirmed the presence of Abeta peptides, alpha1-antichymotrypsin, apoE, collagen type XXV, cystatin C, alpha-synuclein and proteoglycans in plaques (Liao et al., 2004). Liao et al (2004) also reported an enrichment of 26 proteins of the following categories in amyloid plaques: cytoskeletal proteins, chaperones, and proteins associated with membrane trafficking, inflammation, proteolysis and phosphorylation/dephosphorylation. Sergeant et al. (2003) performed a qualitative study on aggregated Abeta peptides in human AD brain. Their results show that in the earliest stage of AD it is the amino-truncated Abeta(42) peptides that seed amyloid deposition and therefore this Abeta species seems to be instrumental in basic mechanisms of plaque formation. A dysregulation of Abeta peptides as a major hallmark of AD pathophysiology has been confirmed in human AD brain by other proteomics studies (Lewczuk et al., 2004; Yang et al., 2007). Proteomics tools, coupled with the use of laser capture micro-dissection has allowed for the characterization of proteins in neurofibrillary tangles (Wang et al., 2005). Seventy-two of 155 detected proteins could be identified leading to the discovery of new proteins that had not yet been associated with this central feature of AD neuropathology (see Table 3).

Chaperone proteins have been strongly implicated in AD as these play important roles in proper protein folding and maturation, in protein degradation and transport across membranes (Mayer and Bukau, 2005). Yoo et al. (2001b) reported aberrant expression patterns of six out of nine chaperone proteins analysed in AD: mitochondrial HSP 60 kDA, HSP 70 kDA 4, heat shock cognate 71 kDA, alpha-crystalline B chain, glucose regulated protein 75 and 94 kDA. Several further studies support the relevance of altered chaperone or chaperone-associated protein expression to AD pathogenesis: altered levels of mitochondrial HSP 60 kDA (Tsuji et al., 2002), GFAP (Liao et al., 2004; Tsuji and Shimohama, 2001; Tsuji et al., 2002); increased GFAP glycosylation (Kanninen et al., 2004), increased S-glutathionylation of alpha-cristallin B (Newman et al., 2007), increased oxidation of heat shock cognate 71 kDA (Castegna et al., 2002b), peptidyl prolyl cis-trans isomerase (PPIase) (Sultana et al., 2006a,b) and GFAP (Pamplona et al., 2005) and increased nitration of HSP 70 kDA (Sultana et al., 2007) have all been reported in AD. Furthermore HSP 90 kDA alpha, heat shock cognate 71 kDA, alpha crystallin B2, GFAP, alpha spectrin were observed in complexes with APP in human brain lysates (Cottrell et al., 2005). Given that modifications in the function of chaperones can cause major effects on the targeted proteins, these findings support the hypothesis that dysfunction of protein folding/degradation contributes to AD pathology (Cottrell et al., 2005; Sultana et al., 2006a,b).

Proteomic analysis of synaptic components have been conducted in AD post-mortem brains (Schonberger et al., 2001; Yoo et al., 2001a). Yoo et al. (2001a) found decreased beta-SNAP expression in temporal cortex of AD patients, whilst

synaptotagmin I (p65) was reduced in cerebellum, temporal and parietal cortex and synaptotagmin I (pI 7.0) was down-regulated in temporal, parietal cortex and thalamus. Schonberger et al. (2001) reported that amongst 76 differentially expressed proteins were the synaptic proteins vesicular fusion protein NSF, synaptotagmin, transformation sensitive protein and phosphatidylethanolamine-binding protein. Dysregulated synaptosomal proteins and an increased oxidation of synaptic proteins, such as gamma-SNAP (Sultana et al., 2006b) support previous reports of impaired synaptogenesis and/or synaptosomal loss as a consequence of neuronal loss in the AD brain.

CSF and peripheral biomarkers: Proteomic strategies for the detection of biomarkers for AD have already provided some preliminary data. Some groups have used a combination of 2D-PAGE and multivariate statistical methods to find a panel of biomarkers discriminating between the CSF of different patient groups without identifying proteins (Choe et al., 2002); see Table 4. Davidsson et al.'s study (2002) revealed altered intensities of 10 2D-PAGE spots between AD patients and controls, corresponding to changed levels of proapolipoprotein, apoE, beta 2-microglobulin, retinol-binding protein, transthyretin and ubiquitin. In a further study of the same group, changes in the concentrations of apolipoproteins were confirmed (Puchades et al., 2003). Puchades and colleagues also reported, amongst other things, altered levels of apoA1, apoE and apoJ. A further potential AD biomarkers pattern of five differentially expressed CSF proteins, including apoA1, was published 2006 (Castano et al., 2006). The proposed proteins (apoA1, cathepsin D, hemopexin, transthyretin and two pigment epithelium-derived factor isoforms) show potential functions in Abeta metabolism and vascular and brain physiology. A change in CSF protein expression levels was further reported for the following apolipoproteins: apoA1 (Choe et al., 2007; Korolainen et al., 2007), apoA2 (Abdi et al., 2006; Zhang et al., 2005), apoC1 (Abdi et al., 2006; Simonsen et al., 2007d), apoE (Abdi et al., 2006; Choe et al., 2007; Davidsson et al., 2002; Finehout et al., 2007), apoH (Abdi et al., 2006) and apoJ (Choe et al., 2007; Finehout et al., 2007). Apolipoproteins import cholesterol into neurons and export it, and ApoE has repeatedly been considered as linked to AD pathophysiology (for reviews, see Forero et al., 2006; Sjogren and Blennow, 2005). There are hints from APOE knock-out mice that isoforms of this apolipoprotein exert a negative effect on neural plasticity, learning and memory, activation of signalling cascades and neuronal differentiation (Hartman et al., 2001; Nathan et al., 2002; Ohkubo et al., 2001). Changes in apolipoprotein homeostasis in AD pathophysiology are further supported by human post-mortem studies (Liao et al., 2004) and the finding of a decreased apoA1 level in the serum of AD patients (Liu et al., 2006).

Apart from the often-used MALDI-ToF MS, SELDI-ToF MS was also used in several AD CSF studies. Carrette et al. (2003) proposed a combination of five polypeptides as disease markers: cystatin C, two beta 2-microglobulin isoforms, an unknown 7.7 kDA polypeptide, and a 4.8 kDA VGF polypeptide. CSF changes for beta 2-microglobulin had already been described by Davidsson et al. (2002) and Puchades et al. (2003), using different MS methods. These results were confirmed by two further studies (Hu et al., 2005; Simonsen et al., 2007b,c). As a component of the class I major histocompatibility complex, beta 2-microglobulin plays a role in the balance between membrane protein turnover and elimination (Hoekman et al., 1985). *In vitro,* partially folded beta 2-microglobulin represents a key intermediate in the generation of amyloid fibrils (Hong et al., 2002). Changes in cystatin C CSF expression were replicated by Choe et al. (2007), Hu et al. (2005) and Simonsen et al. (2007c,d). Cystatin C seems to be the main cysteine proteinase inhibitor in most human biological fluids (Grubb, 1992) and influences Abeta oligomer formation, as reported in a recent *in vitro* study (Selenica et al., 2007). It might be suggested that cystatin regulates the transformation of monomeric Abeta to larger and perhaps more toxic molecular species *in vivo*.

Abeta peptides in CSF have also been tested for their potential as disease markers. Lewczuk et al. (2003, 2004) described changes in the pattern of CSF Abeta peptides and, concordantly to some previous data, report lower CSF Abeta(42) concentration in AD. According to further data hitherto unreported Abeta peptides seem to play a role in AD pathogenesis. Maddalena et al. (2004) compared the mass profiles of CSF Abeta peptides of AD patients and healthy controls and detected the following peptides in both groups: Abeta(2–14), Abeta(1–17), Abeta(1–18), Abeta (1–33), Abeta(1–34), Abeta(1–37), Abeta(1–38), Abeta (1–39), Abeta(1–40) and Abeta(1–42). A decrease of the Abeta(1-38) level was seen in the patient group. The extent to which quantitation of CSF Abeta varies, as influenced by different analysis methods, was recently discussed by Simonsen et al. (2007a). The group compared different SELDI-ToF MS methods and ELISA for the Abeta determination. Elevated Abeta levels in test samples could only be observed by one of the SELDI-ToF arrays. These findings might explain varying results in AD research and therefore caution is demanded for the comparison of data derived from different study designs. In another SELDI-ToF MS study of this group the CSF proteome of patients with a stable MCI and patients with MCI progressing to AD were compared (Simonsen et al., 2007b). The authors identified a panel of 17 putative biomarkers for the prediction of progression from MCI to AD. The same group determined the CSF protein profiles in 95 AD patients and 72 healthy controls (Simonsen et al., 2007c). Amongst the potential marker proteins with increased concentrations were Abeta(1–38) and Abeta(1–40), the integral membrane protein 2B and the complement component C3a anaphylatoxin; amongst the down-regulated proteins were the above-mentioned beta 2-microglobulin and apoC1 and, furthermore, neurosecretory protein VGF and cystatin C. In another recent study this group verified a panel of protein biomarkers for the differentiation between AD and patients with frontotemporal dementia (Simonsen et al., 2007d). Zhang et al. (2005) were able to quantify relative levels of 163 proteins in AD patients and controls. In half of the proteins the AD/control ratio differed more than 20% (see Table 4), with the most consistent finding being up-regulation of APP and cathepsin B in AD. The abnormally expressed proteins play a role in inflammation/immune

Table 4. Alzheimer s disease: CSF biomarkers

Probands	Tissue, conditions	Proteins	Methods	Reference
AD	CSF	Panel of 9 unidentified marker protein spots	2D-PAGE, multivariate statistical methods, ELISA	Choe et al. (2002)
AD	CSF	Proapolipoprotein; apoE; beta 2-microglobulin; retinol-binding protein; transthyretin; ubiquitin	2D-PAGE, MALDI-ToF MS	Davidsson et al. (2002)
AD	CSF	Cystatin C; two beta 2-microglobulin isoforms; 7.7 kDa polypeptide; 4.8 kDa VGF polypeptide	1D-PAGE, ultrafiltration, anion exchange chromatography, SELDI-ToF MS, MALDI-ToF MS, LC-ESI MS/MS	Carrette et al. (2003)
AD	CSF, frontal cortex, cerebellum	Abeta peptide (1–45 or 2–46)	1 D-PAGE, Western blot, SELDI-ToF MS	Lewczuk et al. (2003)
AD	CSF	apoA1; apoE; apoJ; beta-trace; retinol-binding protein; kininogen prec; alpha 1-antitrypsin prec; cell cycle progression 8 protein; alpha-1 beta glycoprotein; alpha-2-HS glycoprotein; transthyretin; alpha 1-antitrypsin; beta 2-microglobulin; transferrin prec; albumin	2D-PAGE, MALDI-ToF MS, LC-ESI MS/MS	Puchades et al. (2003)
AD, neurological and psychiatric controls	CSF	Abeta peptides (3–44, 4–47, dimeric form of Abeta peptide)	ELISA, SELDI-ToF MS	Lewczuk et al. (2004)
AD	CSF	Abeta(1–38); Abeta(1–40)	SELDI-ToF MS	Maddalena et al. (2004)
MCI	CSF; analysis of intra- and inter-individual protein changes	Inter-individual changes: alpha-1 beta-glycoprotein; complement component 3 prec; apoH prec; chitinase 3-like 1; prostaglandin D2 synthase; cystatin C; thioredoxin; beta 2-microglobulin	2D-DIGE, affinity chromatography, centrifugal filters, MALDI-ToF/ToF MS, LC-ESI MS/MS	Hu et al. (2005)
AD	CSF	Abeta A4 protein prec; cathepsin B prec; similar to fem-1 homolog a; spondin 1; Ca^{2+}-dependent activator protein for secretion 2; proinsulin prec; insulin-like growth factor-binding protein 2, 6 prec; angiotensinogen prec; complement component 1, r subcomponent; complement C4 prec; Ig gamma-1 chain C region; factor VII active site mutant immunoconjugate; MEGF10 protein; neuroblastoma, suppression of tumorigenicity 1; FLJ00385 protein; tubulin alpha-2 chain; similar to tubulin alpha-3/alpha-7 chain; tubulin alpha-8 chain; alpha-2 isoform 2; histidine-rich glycoprotein prec; neuronal pentraxin I prec; alpha 2-microglobulin prec; apoA2 prec; serine (orcysteine) proteinase inhibitor; HSP 60 kDa, mitochondrial prec; selenium-binding protein 1; similar to hypothetical protein FLJ40597; alpha 2-macroglobulin prec; similar to serine; haptoglobin-1 prec; CD 14 antigen prec; neuroendocrine protein 7B2 prec; IgK chain C; protein S (alpha); complement factor B prec; neural proliferation, differentiation and control 1; heterogeneous nuclear ribonucleoprotein U isoform a; glutamate carboxypeptidase-like protein 2 prec; hemopexin prec; Antithrombin-III prec; osteoglycin preproprotein; afamin prec; dystroglycan prec;	ICAT, Western blot, LC-ESI MS/MS	Zhang et al. (2005)

		insulin-like growth factor binding protein 6 prec; chemokine ligand 16; ganglioside GM2 activator prec; ribonuclease 6 prec; beta-galactosidase-binding lectin prec; Ig alpha-2 chain C region; eukaryotic translation elongation factor 1 gamma; RCC1-like; ATP-citrate synthase; lectomedin-2; glutamyl-prolyl tRNA synthase; mimecan prec; predicted osteoblast protein; integrin-linked kinase-associated protein phosphatase 2C isoform 1; tetranectin prec; adipsin/complement factor D prec; HSP 70 kD 1A, 1B, 6; complement component C7; N-acetyllactosaminide beta-1; tetranectin; proactivator polypeptide prec; osteonectin; beta-1,3-N-acetylglucosaminyltransferase bGnT-6; vitamin D-binding protein prec; cell adhesion molecule with homology to L1CAM prec; prion protein; immunoglobulin superfamily member8; calnexin; similar to RIKEN cDNA 2410146L05; dermcidin prec; LJ00053 protein; heterogeneous nuclear ribonucleoprotein D-like; DNA-dependent protein kinase catalytic subunit; leukophysin; DEAD/H box polypeptide 9 isoform 1; metalloproteinase inhibitor 1 prec; lymphocyte antigen 75 prec; disks large-associated protein 2; KIAA1412 protein; PRO2000 protein		
AD, PD, Lewy body dementia	CSF	Brain abundant, membrane attached signal protein 1; BDNF; cell growth regulator with EF hand domain 1; chromogranin A, B; insulin-like growth factor-binding protein 5 prec; neurexin 1-alpha kallikrein 6 prec; phosphatidylcholine-sterol acyltransferase prec; ProSAAS prec; superoxide dismutase 1; transcription elongation regulator 1; vacuolar ATP synthase subunit S1 prec; cochlin prec; matrix-Gla-protein prec; spondin 1 prec; 24 kDa protein; AMBP protein prec; complement C2 prec; cytokine-like protein C17 prec; fibrinogen beta chain prec; HLA class I histocompatibility antigen, B-27, E alpha chain prec; myosin-reactive immunoglobulin heavy chain variable region;	iTRAQ, Western blot, MALDI-ToF/ToF MS	Abdi et al. (2006)
AD	CSF	apoA1; cathepsin D; hemopexin; transthyretin; two pigment epithelium-derived factor isoforms	2D-PAGE, MALDI-ToF-MS	Castano et al. (2006)
AD	CSF; Ig treatment	Albumin; clusterin/apo J; hemopexin; alpha-1 antitrypsin; apoA1, apoE; cystatin C; alpha-1 -Pi-Pittsburgh and S195a trypsin complex; antibody-antigen complex, chain C; transferrin; alpha-1	iTRAQ, Western blot, LC, MALDI-ToF/ToF MS	Choe et al. (2007); D'Ascenzo et al. 2008
AD, healthy and neurological controls	CSF	Albumin; alpha-1 antitrypsin; apoE; apoJ; complement component 3; contactin; fibrin beta; immunoglobulin heavy chain, light chain; neuronal pentraxin receptor; plasminogen; retinol-binding	2D-PAGE, LC-ESI MS/MS	Finehout et al. (2007)
AD, neurological controls	CSF, protein oxidation	Beta-trace; lambda chain prec; transthyretin; vitamin D-binding protein; apoA1; alpha-1-antitrypsin	2D-PAGE, Western blot, LC-ESI MS/MS	Korolainen et al. (2007)
AD, MCI	CSF	Fibrinogen gamma-A chain prec	2D-PAGE, MALDI-ToF MS	Lee et al. (2007)
AD	CSF; comparison of methods	Abeta (40)	ELISA, SELDI-ToF MS with antibody coated arrays, SELDI-TOF and immobilized metal affinity [copper] (IMAC30) protein chip	Simonsen et al. (2007a)

(Continued)

Table 4. Continued

Probands	Tissue, conditions	Proteins	Methods	Reference
AD, MCI	CSF	Phosphorylated osteopontin, C-terminal fragment; ubiquitin; beta 2-microglobulin; C3a anaphylatoxin des-Arg; C4a anaphylatoxin des-Arg	SELDI-ToFMS, 1D-PAGE, LC-ESI MS/MS	Simonsen et al. (2007b)
AD	CSF	Abeta(1–38); Abeta(1–40); integral membrane protein 2B, fragment; C3a anaphylatoxin; beta 2-microglobulin; apoC1; neurosecretory protein VGF, fragment; cystatin C; chromogranin A, fragment; alpha-1-antichymotrypsin; osteopontin, fragments; C3a anaphylatoxin; saposin D isoform; pancreatic ribonuclease	SELDI-ToFMS, 1D-PAGE, LC-ESI MS/MS, ELISA	Simonsen et al. (2007c)
AD, frontotemporal dementia	CSF	Cystatin C; apoC1; pancreatic ribonuclease; VGF peptide; vasostatin II; integral membrane protein; complement factor C3a	SELDI-ToF MS	Simonsen et al. (2007d)
AD, PD	CSF	alpha 1-acid glycoprotein; beta 2-glycoprotein 1 (apoH); neurexin 1	LC, MALDI-ToF/ToF MS	Pan et al. (2008)

reaction, extracellular matrix/adhesion processes, neurotransmission, signalling cascades, apoptotic and metabolic processes.

In 2007 Finehout and co-workers published a panel of CSF proteins that differentiated among AD, healthy controls and control individuals with neurological diseases. As observed in several studies, the identified proteins are linked to inflammatory processes, amyloid transport and proteolytic inhibition. This study has been criticised because it neither controlled for blood contamination in CSF nor for age-related changes in control samples (Zhang and Montine, 2007).

The occurrence of increased protein oxidation in CSF of probable AD patients with MCI was studied by Korolainen and colleagues in order to complement findings from post-mortem tissue (Korolainen et al., 2007). In general the carbonylation levels did not vary between the probable AD patients and controls subjects and only two proteins showed regulation: lambda chain was up-regulated in AD, and an unidentified protein down-regulated. However prominent difference between the carbonylation status between women and men was seen, with a higher carbonylation of Vitamin D-binding protein, apoA1 and alpha 1-antitrypsin in men.

In some proteomics CSF biomarker studies, modern labelling techniques, such as iTRAQ, have been used. Abdi, Pan and colleagues identified down-regulation of neurexin 1 (involved in pre-synaptic neurotransmitter release) and an increase of the acute-phase protein alpha 1-acid glycoprotein (Abdi et al., 2006; Pan et al., 2008). Choe and co-workers (2007) determined the change of protein profiles of two AD patients undergoing a 6-month treatment with IV Ig and 3 months of drug washout by iTRAQ labelling. In addition to an initial clinical improvement, consistent protein alteration during treatment (with opposite changes during washout) could be seen for several proteins that are supposedly linked to AD. Even though only two individuals were included, such a study design seems in general useful to reduce the risk of false-positive results, as each individual can serve as his/her own control.

Taken together, proteomic approaches have consistently identified putative AD CSF markers that play roles in amyloid homeostasis, protein transport and turnover (such as Abeta variants, transthyretin, beta 2-microglobulin, cystatin C, hemopexin), apolipoprotein metabolism (apoA1, apoC1, apoE, apoJ), inflammation reaction (alpha-1 antichymotrypsin, complement factors) and synaptic processes (VGF fragments). However, in synopsis of all results from proteomic CSF studies the variability of results is striking. Differences in sample preparation, recruited patients, applied separation and MS techniques as well as different types of used databases for protein identification might cause these controversial findings. To address this topic of inconsistency in CSF biomarkers results, Hu and colleagues studied the variability of CSF protein profiles within one person and between different individuals (Hu et al., 2005). In an undoubtedly small sample of six people, the authors discovered proteins with a significant fluctuation of abundance within the same individual. In general they observed a greater profile similarity between CSF samples from the same individual compared with samples from different individuals. Amongst the proteins with higher

intra-individual variability several had been suggested as potential AD markers (transthyretin, ubiquitin and apoE apo H precursor, alpha 2 macroglobulin, transferrin and albumin). Therefore, results from CSF biomarker studies have to be considered with caution bearing these limitations in mind.

In addition to CSF studies, the serum and plasma proteome of AD patients have been analysed in the search for biomarkers (see Table 5). As an increased oxidation of proteins in AD is observed with different study designs, Choi and co-workers analysed oxidized proteins in plasma (Choi et al., 2002). They reported an oxidation of a relatively small percentage of plasma proteins, but elevated oxidation levels in AD patients compared with controls and could identify amongst these proteins fibrinogen gamma chain and alpha-1 antitrypsin isoforms. Both proteins play a role in inflammation processes and have been implicated in the aetiology of AD disease. A further study with increased oxidation of glycoproteins, including alpha-1 antitrypsin isoforms, confirmed these findings (Yu et al., 2003). Further, elevated oxidation levels were detected for transferrin and hemopexin, two proteins with major roles in iron/redox pathways. These results provide evidence for a dysregulation in heme/iron/redox homeostasis and activation of the acute phase response in AD.

In 2005 a study utilizing a proteomic approach sought to identify a mass peak pattern, not a single protein or a pattern of a few identified proteins, that could differentiate between groups with AD patients, patients with MCI and individuals without cognitive impairments (Lopez et al., 2005). In a blinded trial these authors were able to classify the named groups with the help of a protein pattern using a specific algorithm. German and colleagues used a very similar analytical platform to determine a marker pattern in the sub-set of proteins that bind to carrier molecules such as albumin (German et al., 2007). A differentiation between AD and PD patients, as well as control subjects was possible with a marker pattern of four peaks. Three of these peaks were identical to three of those observed in Lopez et al.'s (2005) study.

Liu et al. (2006) have confirmed an apolipoprotein dysregulation in AD reporting lower serum apoA1 levels in AD. Hye et al. (2006) described that image analysis of the plasma protein gel pattern alone allowed identification of AD patients with 56% sensitivity and 80% specificity. Of 15 altered gel spots, 13 contained some component of Ig or serum albumin precursor. In a very recent approach Ray et al. (2007) measured the abundance of 120 known signalling proteins by ELISA and presented a panel of 18 proteins that demarcated AD patients and controls. Furthermore these authors were able to differentiate between MCI patients that later developed AD and MCI patients without progression. The observed alterations point to a dysregulation of hematopoiesis, inflammation, neuroprotection, neurotrophic activity, phagocytosis and energy homeostasis in AD.

As the existing data shows considerable variability, further efforts are necessary to define universal standards in biomarker research so that studies become more comparable. Current results certainly have to be verified in different cohorts, addressing reproducibility, effectiveness and disease specificity. In the future the design of reliable diagnostic tests will probably embed a combination of several biomarkers.

Drug addiction

Cell cultures: One important approach in drug-dependence research is the assessment of drug effects on gene expression in different cell lines. The phenomenon of adenylate cyclase sensitization (enhanced activity of this enzyme after withdrawal of the inhibitory drug) in morphine-treated cells is thereby considered to reflect opiate dependence (Sharma et al., 1975). The aim of one proteomics study was to determine the long-term effects of morphine administration on the cellular protein profile (Moulédous et al., 2005); see Table 6. MS identification of detergent-resistant membrane raft proteins from untreated and chronically morphine-treated cells showed a 30–40% reduction of the following proteins: G protein subunits alpha i(2), alpha i(3), beta(1), beta(2) and prohibitin. Furthermore, the authors reported a correlation between G protein beta levels and adenylate cyclase sensitization, and that the G protein beta down-regulation was probably caused by degradation by the proteasome. It can be concluded that chronic morphine administration alters the neuronal balance of G proteins and adenylate cyclases, with the ubiquitin–proteoasome pathway playing a crucial role. In a second study of this group the effect of time-dependent morphine exposure (6, 24 or 72 hours) was tested (Neasta et al., 2006). Forty-five morphine-responsive proteins were identified that are involved in cell metabolism, organization of the cytoskeleton, vesicle trafficking, transcriptional regulation, protein translation, folding and degradation and cell signalling. As the protein pattern between 24 and 72 hours of drug exposure varied markedly, the authors suggested that the cellular adaptation to chronic morphine is a dynamic process. Alternatively this might reflect an artefact, as it is possible that some modulations could have been missed at some time point due to technical limitations. In agreement with several proteomic studies on rat models of morphine addiction (e.g. Bierczynska-Krzysik et al., 2006b; Kim et al., 2005; Li et al., 2006; Prokai et al., 2005) the authors found changes of the following proteins: ATP synthase beta chain, vacuolar ATP synthase subunit B, MDH, triosephosphate isomerase, rab GDP dissociation inhibitor beta, PPlase and septin-11. Thus, it seems justifiable and useful to utilize cell cultures for drug addiction research.

Animal models: One approach for using animal models for drug addiction research is to employ SELDI-ToF MS to identify a pattern of potential biomarker peaks without primarily identifying the molecules represented by these peaks. Geng et al. (2006) set up a protocol for the detection of rat brain cellular and membrane proteins (see Table 7). This group examined cocaine- versus saline-treated rats and found six potential biomarker peaks in ventral tegumental area for the discrimination of the two groups. In monkeys, even a short-time cocaine exposure was sufficient to produce selective deficits in cognitive function, enhancements in motivational processes and molecular adaptations in the orbitofrontal cortex. The modulated proteomic components were detected by 2D-DIGE and iTRAQ and mainly belonged to the classes of metabolic and cytoskeletal proteins (Krueger et al., 2005; Olausson et al., 2007).

Table 5. Alzheimer s disease: peripheral biomarkers

Probands	Tissue, conditions	Proteins	Methods	Reference
AD	Plasma, protein oxidation	Fibrinogen gamma-chain prec; alpha-1 -antitrypsin prec	2D-PAGE, Western blot, MALDI-ToF MS	Choi et al. (2002)
AD	Plasma, glycoprotein oxidation	Transferrin; hemopexin; alpha-1-antitrypsin	1D-, 2D-PAGE, affinity chromatography, Western blot, MALDI-ToFMS	Yu et al. (2003)
AD, MCI	Serum	Marker pattern (unidentified peptides/proteins)	affinity-chromatography-based technology, spin columns, MALDI-ToF MS	Lopez et al. (2005)
AD	Plasma	Serum albumin prec; Ig alpha-I chain C region; Ig kappa chain C region; Ig kappa chain V-II region TEW; Ig kappa chain V-I region Lay; Ig kappa chain V-IV region Len; Ig lambda chain C regions; Ig lambda chain V-III region LOI; Ig gamma-I chain C region; Ig mu chain C region; alpha 2-macroglobulin; desmoplakin; serum amyloid P-component prec; galectin-7; complement C4 prec; complement factor H prec; alpha 2-macroglobulin prec; ceruloplasmin prec; CFH-related protein 2 prec; inter-alpha-trypsin inhibitor heavy chain H4 prec; histone H2B.a/g/h/k/l; CD5 antigen-like prec	2D-PAGE, Western blot, LC-ESI MS/MS	Hye et al. (2006)
AD	Serum	apoA1	2D-PAGE, immunoturbidity assay, MALDI-ToF MS	Liu et al. (2006)
AD, PD	Serum, carrier protein bound sub-proteome	Marker pattern (three unidentified peptides/proteins)	Affinity-chromatography-based technology, MALDI-ToF MS	German et al. (2007)
AD, MCI	Plasma	Angiopoietin-2; chemokine 5, 7, 15, 18; chemokine 8; granulocyte-colony stimulating factor; glial-derived neurotrophic factor; epidermal growth factor; intercellular adhesion molecule-1; insulin-like growth factor-binding protein-6; interleukin 1 alpha, 3,11; platelet-derived growth factor BB; tumor necrosis factor alpha; TNF-related apoptosis-inducing ligand receptor-4; monocyte colony stimulating factor 1	Sandwich ELISA	Ray et al. (2007)

Table 6. Drug addiction: cell cultures

Cell lines	Tissue, conditions	Proteins	Methods	Reference
Recombinant human neuroblastoma SH-SY5Y clone, over-expressing the mu-opioid receptor	Chronic morphine administration	G protein subunits alpha i(2), alpha i(3), beta(1), beta(2); prohibitin	1 D-PAGE, Western blot, MALDI-ToF/ToF MS, LC-ESI MS/MS	Mouledous, et al. (2005)
Recombinant human neuroblastoma SH-SY5Y clone, overexpressing the mu-opioid receptor	Morphine administration for 6, 24 or 72 h	AFTER 48 h: ATP-dependent RNA helicase DDX1; GAPDH; bifunctional purine biosynthesis protein; vacuolar ATP synthase subunit B; RuvB-like 1; 4-trimethylaminobutyraldehyde dehydrogenase; WD-repeat protein 12; enolase 1; histone-binding protein RBBP7; eukaryotic translation initiation factor 3 subunit F; beta-succinyl CoA synthetase; galactokinase; acyl-CoA hydrolase; arsenical pump-driving ATPase; serine-threonine kinase receptor-associated protein; protein phosphatase 2A, regulatory subunit B; calponin-3; biliverdin reductase A; MDH, cytoplasmatic; annexin A5; endoplasmic reticulum protein ERp29; Ras-related protein Rab-7; AFTER 72 h: radixin; heat shock cognate 71 kDa protein; lamin A/C; T-complex protein 1, zeta subunit; DRP3; septin-11; Rab GDP dissociation inhibitor beta; mitochondrial-processing peptidase beta subunit; proliferation-associated protein 2G4; elongation factor 1-gamma; adenylosuccinate synthetase 2; elongation factor 1-gamma; tRNA-nucleotidyltransferase 1; ornithine aminotransferase; septin-2; ubiquitin-like 1 activating enzyme E1A; guanine nucleotide-binding protein, alpha inhibiting activity polypeptide 2; 3-mercaptopyruvate sulfurtransferase; MDH, cytoplasmatic; alpha-soluble NSF attachment protein; nuclear protein Hcc-1; guanine nucleotide- binding protein beta subunit 2-like 1; proteasome subunit alpha type 3; proteasome subunit beta type 6; triosephosphate isomerase	2D-PAGE, MALDI-ToF/ToF MS, LC-ESI MS/MS	Neasta et al. (2006)

As the mechanisms of nicotine addiction have not yet been completely understood, Yeom and co-workers analysed the proteome of the striatum in nicotine-addicted rat brain, as this region, together with the nucleus accumbens, is postulated to play a pathophysiological role in addiction (Yeom et al., 2005). Increases in the levels of zinc-finger binding protein-89 after repeated nicotine administration were found, and as a protein that induces growth arrest, these changes might reflect apoptosis induction by nicotine. Further proteins that are involved in cell survival and apoptotic processes were differentially regulated, such as death effector domain-containing DNA binding protein and BDNF. These findings contribute to a better understanding of the effects of nicotine administration at the molecular level. In which ways these findings can finally be translated and used for progress in nicotine addiction therapy remains open.

Kim et al. (2004, 2005), in two studies, determined the protein pattern in rat frontal cortex under chronic opioid administration (for either butorphanol tartrate or morphine) and focused on one specific post-translational modification, the phosphorylation of tyrosine residues. They reported multiple cases of altered, in most cases increased, expression of proteins with phosphorylated tyrosine in opioid-dependent rats. The abnormally expressed proteins had functions in intermediary metabolism (e.g. gluthamine synthetase), cell cytoskeleton/differentiation (e.g. tubulin, actin beta) and cell signalling (e.g. GTP-binding proteins). In another study on the effect of chronic morphine administration on rat brain, the subproteome of the synaptic plasma membrane was analysed (Prokai et al., 2005). Amongst several differentially expressed synaptic membrane proteins, the alpha 3 subunit of the Na^+/K^+ ATPase pump was identified; this enzyme had already been reported to be involved in the effects of chronic morphine dependence (Biser et al., 2002). Another group has investigated the effect of chronic morphine administration in different brain regions. In a first study they assessed the whole rat brain proteome and identified several potential dependence markers (Bierczynska-Krzysik et al., 2006a). The reported proteins are mainly cytoplasmic and mitochondrial enzymes, others belong to GTPase and gluthation S-transferase superfamilies, ATPase, asparaginase or proteasome subunit p27 families. In a further approach, this

Table 7. Drug addiction: animal models

Animals	Tissue, conditions	Proteins	Methods	Reference
Sprague-Dawley rats	Frontal cortex, butorphanol and morphine dependence	GFAP delta; GTP-binding protein alpha o; PEBP; 14-3-3 protein gamma, theta; neurofilament, light polypeptide; PK; guanine nucleotide-binding protein, alpha inhibiting 1; enolase 2, 3; aldose reductase; phosphoglycerate kinase I; fructose-biphosphate aldolase A; actin beta; tubulin alpha 1, alpha 6, beta chain, beta 5, beta chain 15; crystallin, mu; alpha-soluble NSF attachment protein; vacuolar H^+ ATPase B2; guanine deaminase; actin beta, cytoplasmic; Prdx 6; proteasome subunit type 4 prec; UMP-CMP kinase; serum albumin prec; DRP2; tuba 2 protein; glutamine synthase; aldolase C; phosphoglycerate mutase type B subunit; carbonic anhydrase 2; GAPDH; MDH 2; PPlase A; alpha-soluble NSF attachment protein; aspartate aminotransferase, cytoplasmic; ATP synthase subunit beta, mitochondrial prec; coronin, actin-binding protein 1A; ornithine aminotransferase; transketolase; fascin homolog 1, actin bundling protein	2D-PAGE, Western blot, MALDI-ToF MS	Kim et al. (2004, 2005)
Sprague-Dawley rats	Striatum, nicotin addiction	Zinc-finger-binding protein-89; 2′,3′-cyclic nucleotide 3′-phosphodiesterase 1; deoxyribonuclease 1-like 3; tandem pore domain halothane inhibited K^+ channel; brain-specific hyaluronan-binding protein; death effector domain-containing DNA binding protein; BDNF	2D-PAGE, RT-PCR, MALDI-ToF MS	Yeom et al. (2005)
Sprague-Dawley rats	Synaptic plasma membrane subproteome, morphine dependence	Triosephosphate isomerase 1; neural cell adhesion molecule; neurexin; N-ethylmaleimide-sensitive factor; neurotrimin; tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein; DRP2; beta-guanine nucleotide-binding protein; alpha-spectrin, II non-erythroid; protein kinase C and casein kinase substrate in neurons 1; clathrin; NADH dehydrogenase subunit 3; GABA transporter protein; Na^+/K^+ ATPase, alpha, beta; fructose-bisphosphate aldolase; LDH A; dynein; succinate-CoA ligase, alpha; vesicle-associated calmodulin-binding protein; adaptor protein complex AP-2; GDH 1; glutamine synthase; adaptor protein complex AP-2; HSP 60 kDa; major prion protein prec	ICAT, LC-ESI MS/MS	Prokei et al. (2005)
Sprague-Dawley rats	Hippocampus proteome; amphetamine administration, abstinence	Fructose-biphosphate aldolase C; hypothetical protein XP_206488; similar to purine-rich element-binding protein A; aspartate transaminase, cytosolic; isovaleryl coenzyme A dehydrogenase; Rab6- interacting protein 2 isoform A; hypothetical protein XP_151496; enolase 1; RIKEN cDNA 2810433K01; HSP 60 kDa 1; similar to 60S ribosomal protein L19; similar to 60S ribosomal protein L29; actin beta; similar to retrovirus-related POL polyprotein; similar to GAPDH; similar to ribosomal protein L35a; similar to NADH dehydrogenase:ubiquinone Fe-S protein 8; similar to zinc-finger protein 97; similar to ionotropic glutamate receptor NR3A2; Prdx 2; mitochondrial H-ATP synthase, subunit d	2D-DIGE, MALDI-ToF/ToF MS	Freeman, et al. (2005)
Sprague-Dawley rats	Ventral tegmental area, cytoplasmic and membrane fraction; cocaine administration	6 potential biomarker peaks	SELDI-ToF MS	Geng et al. (2006)
Wistar rats	Nucleus accumbens, repeated morphine administration	Beta-synuclein; UCH L1; mitochondrial H^+ -ATP synthase subunit d; NADH ubiquinon oxidoreductase; triosephosphate isomerase; lin-7 protein; ATP synthase beta; 14-3-3 protein gamma; fructose-biphosphate aldolase; similar to Atp6v1 e1 protein; enolase 2; actin beta; ribonuclease; angiogenin inhibitor; similar to murine homolog of human ftp-3; neurofilament light polypeptide; synapsin	2D-PAGE, MALDI-ToF/ToF MS	Li et al. (2006)

Wistar rats	Striatum, single dose MAP administration	Phosphoglycerate kinase 1; dihydrolipoamide dehydrogenase; voltage-dependent anion-selective channel protein 1; Rho GDP dissociation inhibitor alpha; Prdx 2, 5; UCH L1; actin beta, N- tropomodulin; PK M1/M2; H^+ -transporting two-sector ATPase beta chain, mitochondrial; enolase 1; 78 kDa glucose-regulated protein prec; ubiquinol-cytochrome C reductase core protein I; proteasome RN3 subunit; dynamin-like protein 1; CK B; LDH B; cystatin B; IgE-dependent histamine-releasing factor; 40S ribosomal protein SA; haptoglobin prec; dnaK-type molecular chaperone HSP72-ps1	2D-PAGE, MALDI-ToF MS	Iwazaki et al. (2006)
Wistar rats	Brain homogenate, chronic morphine administration	Ubiquinol-cytochrome C reductase ion-sulfur subunit; glutathione S-transferase P; superoxide dismutase (Mn); adenylate kinase isoenzyme; 26S proteasome non-ATPase regulatory subunit 9; 14-3-3 protein beta/alpha; Ras-related protein Rab-16; asparaginase-like sperm autoantigen; ATP synthase oligomycin sensitivity conferral protein; protein disulfide isomerase A3; transitional endoplasmic reticulum ATPase	2D-PAGE, LC-ESI MS/MS	Bierczynska-Krzysik et al. (2006a)
Wistar rats	Cortex, hippocampus, striatum; chronic morphine administration	CORTEX: similar to isoform of WD-repeat protein 1; translocase of outer mitochondrial membrane 70 homolog A; cytosolic MDH; 4-aminobutyrate aminotransferase, mitochondrial prec; aldo-keto reductase family 7, member A2; cytochrome C oxidase polypeptide Vb, mitochondrial prec; ATP synthase beta chain, mitochondrial prec; PEBP; HIPPOCAMPUS: heat shock cognate 71 kDa protein, aldose 1-epimerase; septin-6; Lon protease; STRIATUM: LDH B; F-actin capping protein beta subunit; ATPase, H^+ transporting, V1 subunit A; dynamin 1 -like protein; protein kinase C and casein kinase substrate in neurons protein 1; 3-mercaptopyruvate sulfurtransferase; endoplasmic reticulum protein ERp29 prec; superoxide dismutase Cu/Zn; Hnrpa1 protein; ATP synthase alpha chain, mitochondrial prec; proteasome subunit beta type 7 prec; superoxide dismutase (Mn), mitochondrial prec; triosephosphate isomerase; [pyruvate dehydrogenase (lipoamide)]-phosphatase 1, mitochondrial prec	2D-PAGE, MALDI-ToF/ToF MS	Bierczynska-Krzysik et al. (2006b)
Kunming mice	Hippocampus, acute and chronic morphine dependence	Fe-S protein 1 of NADH dehydrogenase 2; pyruvate dehydrogenase complex E2 subunit; LDH 2	2D-PAGE, qRT-PCR, LC-ESI MS/MS	Chen et al. (2007)
Wistar rats	Striatum; repeated morphine administration, naltrexone administration, intrahippocampal D-	Leucine aminopeptidase 3; phosphoprotein phosphatase, 1-gamma catalytic chain; methylcrotonoyl-coenzyme A carboxylase 2 (beta), enolase 1; pyridoxal kinase; Tpi1 protein; T-complex protein 1 subunit gamma; triosephosphat isomerase 1 protein; tubulin beta chain 15; annexin A11; hippocalcin, neuron-specific calcium-binding protein; DRP2; glia maturation factor beta;	2D-PAGE, MALDI-ToF MS	Iwazaki et al. (2007)
C57BL/6JBom mice	Hippocampus, postsynaptic density; chronic morphine administration	Clathrin, heavy polypeptide; Na/K -ATPase alpha 3 subunit; PK M2; 14-3-3 protein zeta; syntaxin-binding protein 1; GDH 1; glutamate-ammonia ligase; CK mitochondrial 1; 2,3 -cyclic-nucleotide 3 - phosphodiesterase 1; voltage-dependent anion channel 1	ICAT, 2D-PAGE, Western blot, immunocytochemistry, LC-ESI MS/MS	Moron et al. (2007)
Vervet monkeys	Orbito-frontal cortex, cocaine exposure	ATP synthase; glyceraldehyde-3-phosphoate dehydrogenase; triosephosphate isomerase; HSP 70 kDa; actin beta; tubulin beta (. . .)	2D-DIGE, iTRAQ, LC-ESI MS/MS	Krueger et al. (2005); Olausson et al. (2007)

(Continued)

Table 7. Continued

Animals	Tissue, conditions	Proteins	Methods	Reference
Sprague-Dawley rats	Prefrontal cortex, chronic morphine addiction	ATPase, H^+ transporting, V1 subunit B, isoform 2; similar to PK M2; NADH dehydrogenase (ubiquinone) flavoprotein 2; similar to MMRP19; similar to acetyl CoA transferase-like; dihydrolipoamide S-acetyltransferase; Acyl-Coenzyme A thioesterase 2, mitochondrial; similar to LRRGT0001; phosphoribosyl pyrophosphate synthetase 1; aconitase 2, mitochondrial; methylenetetrahydrofolate dehydrogenase, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate; similar to WD repeat domain 37; guanine nucleotide-binding protein, betapolypeptide 2-like 1; guanine nucleotide-binding protein, beta-2 subunit; cyclic nucleotide phosphodiesterase 1; annexin A4; similar to calcium/calmodulin- dependent protein kinase type II alpha chain; GDH 1; calcium/calmodulin-dependent protein kinase II alpha; CK B; Homer homolog 1; syntaxin-binding protein 1; syntaxin-binding protein 1 isoform b; synapsin IIb; synapsin 2 isoform 1; double C2, alpha; similar to actin-related protein 2/3 complex subunit 2; similar to coronin, actin-binding protein 1C; predicted actin-related protein 2 homolog; archain; internexin, alpha; lamin-A; tropomodulin 2; similar to transcriptional activator protein purine-rich single-stranded DNA-binding protein alpha; similar to zinc-finger motif enhancer binding protein 2; similar to Sfrs7 protein; DEAD (Asp-Glu-Ala-Asp) box polypeptide 1; similar to heat shock transcription factor 2-binding protein; DnaK-type molecular chaperone hst70	2D-PAGE, Western blot, MALDI-ToF	Yang et al. (2007)

group analysed the cerebral cortex, hippocampus and striatum protein profiles (Bierczynska-Krzysik et al., 2006b). In total 26 altered proteins were identified differentially in the three brain regions. In agreement with previous studies, regulation in bioenergetics pathways (reflected by a differential expression of ATP synthase), cell metabolism, protein handling, signalling and oxidative stress were seen. Li and co-workers assessed the nucleus accumbens protein profile in rats after repeated treatment with morphine (Li et al., 2006). Under repeated administration the authors reported an augmentation of neurofilaments and changes in actin beta post-translational modifications. Furthermore proteins involved in neurotransmission, energy metabolism and protein degradation were regulated. In a further study after chronic, but not acute morphine administration, the hippocampal proteome of mice showed a down-regulation of three metabolic enzymes (Fe-S protein 1 of NADH dehydrogenase 2, pyruvate dehydrogenase complex E2 subunit, LDH 2) (Chen et al., 2007). The simultaneous intake of morphine and the opioid antagonist naltrexone inhibited this down-regulation. Moron and coworkers examined mouse hippocampus after chronic morphine administration, but analysed only proteins of the post-synaptic density (Moron et al., 2007). A total of 102 proteins could be identified in this hippocampal subproteome, amongst them 10 with differential expression. Clathrin, which plays a role in endocytosis, showed increased levels to the largest extent. Further study results additionally point to a redistribution of endocytic proteins at the synapse and, as a consequence, the modulation of synaptic plasticity at excitatory synapses in the hippocampus by morphine. Yang et al. (2007) studied chronic morphine dependence on the prefrontal rat cortex proteins. The 58 identified modulated molecules belonged to the following classes: bioenergetic pathways, signal transduction, synaptic transmission, cytoskeleton, chaperones, and local synaptic protein synthetic machinery. Although most changes were described for the first time, several of the identified proteins had already been reported in morphine-dependence research (ATPase H^+ transporting V1 subunit B isoform 2, guanine nucleotide-binding protein beta polypeptide 2-like 1, guanine nucleotide-binding protein beta-2 subunit, cyclic nucleotide phosphodiesterase 1, GDH 1, calcium/calmodulin-dependent protein kinase II alpha, CK B, lamin-A) (Bierczynska-Krzysik et al., 2006b; Kim et al., 2005; Li et al., 2006; Neasta et al., 2006; Prokai et al., 2005).

In addition to the effects of cocaine, nicotine and opiates, the influence of psychostimulants on protein profiles was also examined by means of proteomics. Iwazaki et al. (2006) aimed at identifying protein expression profiles in the striatum after an acute low dose of MAP, as this brain region is involved in the locomotor response to this drug. Amongst the differentially expressed proteins, the authors found most proteins linked to mitochondrial function (phosphoglycerate kinase 1, ATP synthase F(1) beta chain and enolase 1). These findings are in line with previously reported MAP effects on mitochondria (Davidson et al., 2001). Further modified proteins are related to apoptotic (dynamin-like protein 1) and cytoskeletal processes (actin beta), oxidative stress (Prdx 2, 5) and protein degeneration (UCH L1) or are markers of cell damage

(CK B, LDH). It seems that these systems are regulated in the striatum by a single, small MAP dose. In a second study this group examined the increased behavioural MAP response and the striatal protein pattern during subsequent dose exposure. A repeated MAP intake caused, as described for morphine before, behavioural sensitization (MAP-induced sensitization has also been used as an animal model for MAP-induced psychosis and schizophrenia). Only two proteins overlapped between the acute dose and repeated dose study, but a congruent regulation of mitochondrial, oxidative stress and apoptotic proteins was seen, implicating neuronal stress and/or neurotoxicity. The observed expression changes of synaptic and cytoskeletal proteins could reflect modulations of pre-synaptic function/plasticity, microfilament turnover and axonal growth during MAP-induced sensitization. Assessing the impact of amphetamine on the hippocampal proteome, Freeman and colleagues compared the profile in rats naive to amphetamine, during a self-administration session, during voluntarily abstinence and after reinstatement of self-administration (Freeman et al., 2005). The authors revealed a crucial role of actin and other cytoskeletal proteins during abstinence and suggested that alterations in the neuronal tone could affect neurotransmission and finally behaviour.

It is hoped that the identification of drug-induced modifications in neuronal plasticity and signal transduction will lead to a clearer understanding of mechanisms of drug dependency, which in turn will assist in the design of specific drugs for withdrawal therapy.

Post-mortem studies: As many post-mortem and neuroimaging studies of chronically alcohol-dependent people indicate, morphological changes in brain structure (e.g. cortical or subcortical atrophy), Lewohl and colleagues were interested in cortical modifications at the level of proteins in alcohol addiction (see Table 8). This team investigated the superior frontal cortex taken from control and long-term alcohol abuse subjects (Lewohl et al., 2004). Out of an overall 182 differentially expressed proteins, 139 showed a decreased concentration in the alcoholic group. This trend of down-regulation of protein levels was confirmed in Alexander-Kaufman et al.'s study (2006) on the white matter of the dorsolateral prefrontal cortex in alcoholics. In total, 18 of the altered protein spots were identified successfully, amongst them human neuronal protein 22, alpha-internexin, transketolase, CK B, UCH L1 and GAPDH. Several of these molecules are metabolic enzymes and play an important role in energy balance. As most of these proteins show a decreased expression, the authors suggest that a depletion of these enzymes might cause impaired metabolic functions and loss of viability. In the group of former recovered alcoholic patients some of these proteins were seen adjusted to similar levels as in controls. These proteins might be essential for a partial regeneration of white matter in long-term alcohol abstinence. Studies such as this may help in understanding how alcohol consumption (and subsequent abstinence) can modify white matter plasticity and regeneration. In a 2007 study, the corpus callosum proteomes of uncomplicated and complicated alcoholics (with hepatic cirrhosis) and controls were compared in order to gain insight into possible molecular mechanisms of alcohol-induced with white matter damage in this region (Kashem et al., 2007). Fourteen of 43 identified proteins overlapped between the two alcoholic groups. Another 26 proteins only showed altered levels in the complicated group that might be influenced by the hepatic complications leading to elevated blood ammonia. Identification and classifications of the modified proteins indicated that cytoskeletal and metabolic pathways, as well as oxidative stress, lipid peroxidation and apoptosis all potentially contribute to the alcohol-induced damage in the corpus callosum. Tannu et al. (2007) examined the proteome of the nucleus accumbens in victims of cocaine overdose. Amongst the differentially expressed proteins the authors reported structural (e.g. tubulin beta), mitochondrial (e.g. ATP synthase beta-chain) and miscellaneous molecules (e.g. parvalbumin alpha), as well as proteins involved in synaptic processes (e.g. sodium channel associated protein 1) and metabolism (e.g. enolase 2). Activity alterations of the calcium-binding protein parvalbumin alpha had already been reported before in rat striatum following withdrawal from cocaine (Todtenkopf et al., 2004). For a review on gene expression data from animal models and human post-mortem studies and preliminary proteomic findings of human cocaine abuse, see Hemby (2006).

The findings of these first proteomic post-mortem studies in drug addiction research make valuable contributions to a better understanding of the multitude of protein pathways and interactions that are modulated by cocaine, alcohol and other abused drugs.

Peripheral markers: An important objective of biomarker research, in the field of drug addiction, is to find objective markers for alcohol consumption. Nomura et al. (2004) analysed the serum proteome of alcohol-dependent patients, who were hospitalized for a rehabilitation program (see Table 9). Two proteins (a 5.9 kDA fragment of fibrinogen alpha E-chain and a 7.8 kDA fragment of apoA2) were found to be down-regulated on admission and increased significantly after 1 week of alcohol abstinence. Another 28 kDA peak (representing a fragment of apoA1) was prominent on admission and decreased significantly during abstinence. The combination of these potential biomarkers initially appeared promising for the assessment of alcohol consumption as the altered protein levels were also observed in persons with GGT levels in the normal range. In a following study of the group, the diagnostic value of these markers was assessed in a sample of habitual drinkers with moderate alcohol consumption (Sogawa et al., 2007). Even in persons with GGT-levels in the normal range the relative intensities of the 5.9 and 7.8 kDA peaks differed between habitual drinkers and healthy controls with the highest sensitivity and specificity for the 5.9 kDA peak. If the 5.9 and 28 kDA peaks along with the GGT levels were considered, 96.8% of the drinkers were correctly screened with a specificity of 60.9%. These preliminary results highlight the potential of biomarkers for increased alcohol consumption.

Table 8. Drug addiction: post-mortem studies

Probands	Tissue, conditions	Proteins	Methods	Reference
Long-term alcoholics	Superior frontal cortex	Prdx 2; fatty acid-binding protein, brain; antioxidant protein 2; CK B; heat shock cognate 71 kDa protein; histidine triad nuecleotide-binding protein; voltage-dependent anion-selective channel 1; PK M1/M2; beta-synuclein; alpha-synuclein, non-A4 component of APP; tubulin, B polypeptide 4; 14-3- 3 protein isoforms; adrenomedullin; tropomyosin 2(beta); enolase 1,2; tubulin alpha; actin, chain 1; cytoplasmatic; UCH L1; HSP 70 kDa 7; heat shock cognate 71 kDa protein; pyruvate dehydrogenase E1, complement beta; tyrosine 3-monooxygenase; carbonic anhydrase II; beta-neoendorphin- dynorphin	2D-PAGE, MALDI-ToF MS, LC-ESI MS/MS	Lewohl et al. (2004)
Uncomplicated, complicated (hepatic cirrhosis), reformed alcoholics	White matter of BA9 brain region	Transketolase; DRP2; alpha-internexin; vacuolar ATP-synthase subunit B, brain isoform; NADH2 dehydrogenase (ubiquinone); CK B; fructose-bisphosphate aldolase C; GAPDH; delta- aminolevulinic acid dehydratase; UCH L1; human neuronal protein 22	2D-PAGE, MALDI-ToF MS	Alexander-Kaufman et al. (2006)
Uncomplicated, complicated (hepatic cirrhosis) alcoholics	Splenium of corpus callosum	68 kDa neurofilament; GFAP; internexin; actin beta, gamma; stathmin; profilin-2; tropomyosin-3; tubulin beta-4 chain, tubulin beta; ornithine amino transferase; CK B; glutamate carboxypeptidase 1; isocitrate dehydrogenase; ATP synthase; myo-inositol monophosphatase; apoA1; phospholipase D1; phosphoglycerate mutase; phosphoglucomutase; acetylserotonin O-methyl transferase; aldehyde reductase; 20 firritin; ubiquitin; ubiquitinin-conjugating enzyme -7; α-synuclein; DJ-1 protein; glutathione peroxidase; Prdx 6; calcineurin; annexin-5; calcineurin inhibitor; DRP2; GDP- binding protein inhibitor; GTP (alpha)-binding protein; mitogen-activated protein kinase 9; chloride intracellular channel protein; adenylate kinase; protein tyrosin kinase; protein phosphatase; protein kinase; panthothenate kinase; apoptic death agonist; sirtuin-2; cathepsin D	2D-PAGE, MALDI-ToF MS	Kashem et al. (2007)
Cocain overdose victims	Nucleus accumbens, caudate/putamen; cytosolic fraction	GFAP; tubulin beta 2; desmoplakin; 60 kDa chaperonin; sodium channel-associated protein 1; liprin-alpha 3; liprin-3; ATP synthase -chain, mitochondrial prec; fructose-bisphosphate aldolase C; enolase 2; Prdx 2; DnaJ homolog subfamily C member 7; centrosome protein cep290; hypothetical protein LOC80129; parvalbumin alpha	2D-DIGE, MALDI-ToF/ToF MS	Tannu et al. (2007)

Table 9. Drug addiction: peripheral biomarkers

Probands	Tissue, conditions	Proteins	Methods	Reference
Alcoholics	Serum	5.9 kDa fragment of fibrinogen alpha E-chain; 7.8 kDa fragment of apoA2; 28 kDa fragment of apoA1	1 D-PAGE, SELDI-ToF MS	Nomura et al. (2004); Sogawa et al. (2007)

Schizophrenia

Animal models: In schizophrenia research, MK-801-treated mice and rats are used as models for this devastating disease (Paulson et al., 2003) (see Table 10). The non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 ([+]-5-methyl-10, 11-dihydro-5H-dibenzo-[a,d]-cyclohepten-5,10-imine hydrogen maleate) is a non-competitive ion-channel blocker of the NMDA receptor (Wong et al., 1986). Activation of NMDA receptors by the excitatory neurotransmitter glutamate results in the opening of an ion channel that is non-selective for cations. In humans, NMDA-receptor antagonists induce positive and negative symptoms; in schizophrenia patients they exacerbate pre-existing symptoms (Tamminga et al., 1995). Therefore, the use of MK-801 animal models is considered reasonable in schizophrenia research. Paulson et al. (2003) assessed cortical mRNA and protein profiles in two groups of MK-801 treated rats (a short-term treated and long-term treated group) and control animals. The observed mRNA regulations of pre-synaptic proteins (such as synaptotagmin IX) are in line with previously reported synaptic disturbances in schizophrenia (Davidsson et al., 1999). Previously reported HSP 70 kDA alterations in MK-801 treated animals were also confirmed by this study (Sharp et al., 1991). Further differentially expressed proteins, including SOD, HSP 60 kDA and enolase 2, had already been linked to schizophrenia (Schwarz et al., 1998; Vermuyten et al., 1990; Yao et al., 2000). In this study marked differences in the results between mRNA and protein levels could be observed. One explanation for this discrepancy might be that 2D-PAGE is not sensitive enough to reflect small differences in protein expressions in contrast to qRT-PCR. In a further study this group determined the protein profile in the thalamus of MK-801 rats (Paulson et al., 2004b). In the short-term MK-801 treated group, the following protein changes were identical in thalamus and cerebral cortex: an increase of HSP 72 kDA and decrease of mitochondrial ATP synthase beta-subunit. Enolase 1 and HSP 60 kDA were regulated in both brain areas, but in opposite directions. Several of the modified thalamic proteins have been implicated in other schizophrenia studies (HSP 60 and 72 kDA, DRP2, aldolase C, albumin and MDH). In these two studies different protein expression pattern in the short- and long-term treatment group could be found, wherefore Paulson and colleagues focused in another study in particular on the impact of the treatment course. As in most preceding studies an acute dose MK-801 model was used and this approach was compared with 6 and 12 days of MK-801 administration (Paulson et al., 2004a). The highest number of altered proteins in the thalamus could be seen in the 6- and 12-day groups compared with the acute dosage. Most schizophrenia-linked proteins were found in the 6-day group. The majority of the regulated proteins were associated with mitochondrial function and cytoskeletal processes. In a further recent study of this group the effects of high-dose typical (haloperidol) and atypical (clozapine) antipsychotics on the cortex and thalamus proteome were examined (Paulson et al., 2007). Most proteins that were regulated by MK-801, clozapine or haloperidol, were again associated with mitochondrial function, suggesting mitochondrial dysregulation in schizophrenia pathophysiology. Another possible reason for the reproducible finding of regulated mitochondrial proteins in schizophrenia 2D-PAGE projects might be that these proteins are better represented on the gel than, for example, membrane proteins. In comparing typical and atypical antipsychotic drugs, clozapine seemed more effective in the attenuation of protein regulation by MK-801 compared with haloperidol, and haloperidol alone caused more biochemical changes. The finding that differential protein alterations are seen in Paulson's et al.'s (2004a,b, 2007) studies on the thalamic and cortex proteome could be explained by different gel and buffer systems as well as a differing MK-801 injection schedules that were used. In summary, it can be considered that the MK-801 animal model is a valuable tool in schizophrenia research, as many identified protein changes are in line with reports from human post-mortem studies.

In a 2006 study on protein expression under different antipsychotic treatments La et al. (2006) used chlorpromazine and clozapine in healthy Sprague–Dawley rats. After antipsychotic treatment, the hippocampal proteome differed in levels of MDH, Prdx 3, vacuolar ATP synthase subunit beta and mitogen-activated protein kinase kinase 1. The pattern of protein regulation differed between the typical and atypical antipsychotic, only the reduction of MDH was a consistent finding in both groups. The mitochondrial matrix protein MDH is a key enzyme in the malate shuttle system and facilitates the conversion of malate to oxaloacetate and the replenishing of oxaloacetate levels by reductive carboxylation of pyruvate (La et al., 2006). A role of this enzyme in the pathophysiology of schizophrenic psychosis is supported by the above-mentioned findings in MK-801 treated rats (Paulson et al., 2003, 2004a), by human post-mortem microarray studies (e.g. Middleton et al., 2002; Vawter et al., 2004) and also by proteomics post-mortem studies (Prabarakan et al., 2004). One could possibly conclude that neuroleptics exert their antipsychotic effect partially by targeting MDH. Such observations could in the long term be helpful in the design of more effective and specific pharmacological agents.

Recently a different animal model of schizophrenia-like behaviours, neonatal ventral hippocampal lesions in rats,

Table 10. Schizophrenia: animal models

Animals	Tissue, conditions	Proteins	Methods	Reference
Sprague-Dawley rats	Cerebral cortex, MK-801 administration	SHORT-TERM: HSP 72 kDa; stathmin; soluble superoxide dismutase; mitochondrial ATP synthase beta-subunit; PDH; actin beta; mitochondrial HSP 70 kDa; enolase 2; LONG-TERM: mitochondrial HSP 70 kDa; HSP 60, 72 kDa; enolase 1, 2; stathmin	2D-PAGE, cDNA microarray, RT-PCR, MALDI-ToF MS, LC-ESI MS/MS	Paulson et al. (2003)
Sprague-Dawley rats	Thalamus, MK-801 administration	SHORT-TERM: HSP 60, 72 kDa; similar to dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex; albumin; DRP2; guanine deaminase; enolase 1; MDH; mitochondrial aconitase; LONG-TERM: mitochondrial ATP synthase beta-subunit; alpha internexin; HSP 60 kDa, albumin; DRP2; aldolase C	2D-PAGE, MALDI-ToF MS, LC-ESI MS/MS	Paulson et al. (2004a)
Sprague-Dawley rats	Thalamus, MK-801 administration	HSP 60, 72 kDa; similar to dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex; titin; DRP2; tropomodulin 2; mitochondrial ATP synthase beta-subunit; alpha internexin; glucose-related protein 75 kDa; 3-hydroxyisobutyrate dehydrogenase; neuronal protein 22;	2D-PAGE, MALDI-ToF MS, LC-ESI MS/MS	Paulson et al. (2004b)
Sprague-Dawley rats	Hippocampus, chlorpromazine or clozapine administration	MDH; Prdx 3; vacuolar ATP synthase subunit beta; mitogen-activated protein kinase kinase 1	2D-PAGE, MALDI-ToF MS, LC-ESI MS/MS	La et al. (2006)
Sprague-Dawley rats	Thalamus, cerebral cortex; MK-801, clozapine, haloperidol administration	CORTEX: similar to RIKEN cDNA2410174K12; thioredoxin-like 1; similar to pyruvate dehydrogenase, Prdx 2; similar to inosine triphosphatase; THALAMUS: GFAP delta; similar to stomatin-like protein 2; heterogenous nuclear ribonucleoprotein; alpha-internexin; DRP2; proteasome 26S ATPase subunit 4; similar to RIKEN cDNA2410174K12; similar to pyruvate dehydrogenase beta; Prdx 6	2D-PAGE, MALDI-ToF MS, LC-ESI MS/MS	Paulson et al. (2007)
Sprague-Dawley rats	Prefrontal cortex of rats with neonatal ventral hippocampal lesions, plasma membrane and Vesicle extracts	Syntaxin-binding protein 1b; mKIAA 1045 protein; CK B; phosphatidyl ethanol amine-binding protein; neurocalcin delta; visinin-like protein 1; ATP synthase beta; clathrin lightchain b; dihydrolipoyl-lysine S-acetyl-transferase E2	2D-PAGE, Western blot, MALDI-ToF MS, LC-ESI MS/MS	Vercauteren et al. (2007)

has been investigated (Vercauteren et al., 2007). These authors have established a protocol to enrich plasma membrane- and vesicle-associated proteins in the sample. Eighteen of 392 2D-PAGE spots of prefrontal cortex proteins showed intensity variations in lesioned rats compared with controls. The majority of the dysregulated proteins were associated with various neurotransmitter systems, with major roles in plasma membrane receptor and synaptic vesicle turnover (such as syntaxin-binding protein 1b). Subproteome-based proteomic methods as utilized by Vercauteren et al. (2007), when compared with whole cell proteomic approaches, help to reduce interference from highly abundant proteins in the sample. Thus, these subproteomic approaches allow for a better analysis of low abundance proteins, thereby increasing the chance to unravel the complex protein dysregulation and interaction in schizophrenic psychosis.

Post-mortem studies: Johnston-Wilson et al. (2000) compared the frontal cortex protein expression of schizophrenic (SCZ) patients with those of patients with major depression (MDD) and bipolar disorder (BPD) (see Table 11), and reported alterations in eight identified proteins. There was no specific protein change that exclusively occurred in the SCZ group. All three patient groups displayed increased levels of fructose-biphosphate aldolase C and aspartate aminotransferase and decreased concentrations of GFAP isoforms and DRP2 were observed. GFAP reductions are often observed in response to acute infections and in neurodegeneration (Murphy et al., 1995). Changes in GFAP isoforms could reflect features that are common in different psychiatric disorders (e.g. neuroinflammation). Prabakaran et al. (2004) utilized a combined transcriptomics, proteomics and metabolomics approach to investigate alterations in prefrontal cortex tissue of SCZ patients. Analyses revealed that the white and grey matter proteome differs significantly between brains of SCZ patients and controls. Amongst 50 abnormally expressed proteins, 19 were associated with mitochondrial function (e.g. PK 1 and 2, mitochondrial aconitase 2), 10 with oxidative stress (e.g. HSP 70 kDA) and a further three with peroxisomal function (MDH 1, Prdx 1 and 2). The remaining identified proteins belonged either to the class of cytoskeletal molecules or played a role in cell trafficking/turnover. A microarray approach in this study complemented these results. Clark et al. (2006) determined the proteome in anterior cingulate cortex of SCZ patients and identified 39 proteins at significantly altered levels. These proteins could be functionally classified into metabolism and oxidative stress, cytoskeletal, synaptic, signalling, trafficking and glial-specific groups with several of them having previously been described to play a role in pathophysiology of schizophrenia. The synaptosomal associated protein-25, the metabolic proteins NADH oxidoreductase and CK B, SOD, GFAP and the trafficking protein HSP 70 kDA protein had been reported before to show abnormal expression levels and/or activity in schizophrenia. Beasley et al. (2006) also focused on the anterior cingulate cortex proteome of patients with different major psychiatric diseases: SCZ, BPD, MDD. The authors identified 19 abnormally expressed proteins. In agreement with Clark et al. (2006) they reported in SCZ an increase of DRP2 and a modulation of CK B. However, whereas Clark et al. found an up-regulation of CK B, Beasley and co-workers described it as down-regulated. All of the modulated proteins play an important role in cell metabolism and cytoskeletal function and some of the modifications seem to be uniform in SCZ, MDD and BPD. In a further study on the dorsolateral prefrontal cortex in schizophrenia and BPD, an overlap of seven altered protein spots in 2D-PAGE appeared (Pennington et al., 2007). This overlap, also supported by Johnston-Wilson et al.'s (2000) study, strongly supports the hypothesis that irregularities in basic biological systems contribute to pathophysiological processes that are common in all of the three major psychiatric disorders. The clearest point of distinction between the two diseases, however, was a dominance of synaptic-associated molecules in SCZ in contrast to metabolic-linked protein changes in BPD. Mei et al. (2006) published a SELDI-ToF MS analysis of the dorsolateral prefrontal cortex proteome in schizophrenia. Protein expression profiles of schizophrenic patients and controls were compared, using an algorithm to determine a set of peaks that distinguish best between the two groups. The best models for distinction comprised a minimum of 10 peaks, with a sensitivity and specificity of about 70%. In a further proteomic study alterations in prefrontal dorsolateral cortex of SCZ and BPD patients were identified using SELDI-ToF MS and MALDI-ToF MS (Novikova et al., 2006). The majority of biomarkers with altered levels in schizophrenia were involved in cell metabolism (salvaging of ubiquitin, glycolysis, unsaturated fatty acid degradation, salvaging of adenine and methionine in the polyamine pathway, adenine and guanine recovery in purine turnover, mitochondrial energy generation, sphingomyelin degradation in lipid metabolism and glutamate turnover) and/or in intracellular signalling (Ca^{2+} cascade, G protein signalling, purine nucleoside signalling cascades, synaptic lipid signalling and multiple signalling cascades). These latter findings strengthen the theory of impaired cell signalling in schizophrenia. Other markers proteins of this study belong to the group of transcription factors and chaperones or are involved in cellular and/or neural tissue response to impairing conditions such as oxidative stress, inflammation or hypoxia.

Presently, proteomics studies in schizophrenia have revealed a number of protein systems that may be involved in the condition, with some of the proteins being identified in more than one study (such as DRP2, fructose-biphosphate aldolase C, CK B, GFAP, HSP 70 kDA protein 1). Differences in published results might be due to heterogeneous patient samples, diverse analysed brain areas and different MS methods.

CSF and peripheral biomarkers: In their proteomic CSF analysis, Jiang et al. (2003) were able to report a significantly decreased content of the 46 kDA glycoprotein apoA4 in the samples of schizophrenic patients compared with those of controls (see Table 12). The concentrations of several other proteins, such as haptoglobin, fibrinogen, complement component 3 and Gc-globulin, were also altered in the patient group, although these changes were not statistically significant. The impact of apoA4 in the CNS however is still unexplained. An analysis (Huang et al., 2006, 2007a) of CSF, serum and brain

Table 11. Schizophrenia: post-mortem studies

Probands	Tissue, conditions	Proteins	Methods	Reference
SCZ, MDD, BPD	Frontal cortex	SCZ: fructose-biphosphate aldolase C; DRP2; GFAP; aspartate aminotransferase	2D-PAGE, LC-ESI MS/MS	Johnston-Wilson et al. (2000)
SCZ	Prefrontal cortex, white and grey matter proteome	PK M1/M2; aconitase 2, mitochondrial; phosphoglycerate dehydrogenase; EH-domain containing protein 2 and 3; triosephosphate isomerase 1; hexokinase 1; tu translation elongation factor, mitochondrial; ubiquinol-cytochrome C reductase core protein 1; 58 kDa glucose regulated protein; moesin; gelsolin (amyloidosis Finnish type); MDH 1; Prdx 1, 2; HSP 70 kDa 1, 2, 5, 8, HSP 70 kDa 1- like; fructose-biphosphate aldolase A and C; GAPDH; PDH E1 component, alpha 1; NADH dehydrogenase (ubiquinone) Fe-S protein 1; tubulin alpha 1,2,6, beta 5; glutathione-S-transferase M3; gluthatione transferase omega; enolase 1, 2; leucine aminopeptidase 3; aldehyde dehydrogenase 1, family member A1; fascin homolog 1, actin-binding protein; N-ethylmaleimide sensitive fusion protein; CDC10 cell division cycle 10 homolog; glutamine synthase; actin alpha 2 smooth muscle, alpha cardiac muscle, alpha 1 sceletal muscle, beta, gamma 1; spectrin alpha; CK B; actinin alpha 4; carbonyl reductase 1, 3; quinoid dihydropteridine reductase; phosphoglycerate mutase 1,2; phospholysine phosphohistidine inorganic pyrophosphate phosphatase; UCH L1; esterase D; tyrosyl-tRNA synthetase; glycolipid transfer protein; actin-related protein 2/3 complex, subunit, 41 kDa 1A and 1B; dynamin 1 and 2; transferrin; vacuolar ATP synthase subunit E; 2′,3′- cyclic-nucleotide 3′-phosphodiesterase; brain abundant, membrane attached signal protein 1; DRP2, 4, 5; collapsin response mediator protein 1; septin 3; endophilin-A1; albumin	2D-DIGE, microarray, LC-ESI MS/MS	Prabakaran et al. (2004)
SCZ	Anterior cingulate cortex (BA 24)	N-ethylmaleimide sensitive fusion protein; synaptosomal associated protein 25; serine/threonine protein phosphatase PP1 alpha catalytic subunit; guanine nucleotide-binding protein beta polypeptide 1; PEBP; DRP 1, 2; EH-domain containing protein 3; fructose-bisphosphate aldolase C; citrate synthase; isocitrate dehydrogenase (NAD) subunit alpha; NADH oxidoreductase (ubiquinone) 1 alpha subcomplex 5, 13 kDa; acetyl CoA acetyltransferase, cytosolic; CK B; CK ubiquitous mitochondrial; biliverdin reductase B; superoxide dismutase Cu/Zn; dimethylarginine dimethylaminohydrolase 1; annexin A1, A5; hydroxyacylglutathione hydrolase (Glx II); tubulin –alpha 6 chain; stathmin; dynamin 1 brain; actin-like protein 3; dynactin subunit 2; PPlase A; HSP 70 kDa 1; prohibitin; ADP-ribosylation factor 1; GFAP; carbonic anhydrase; serotransferrin prec; mitochondrial inner membrane protein; novel protein (RP11-46F15.3) human; chromosome 2 open reading frame 32	2D-PAGE, MALDI-ToF MS	Clark et al. (2006)
SCZ, MDD, BPD	Anterior cingulated cortex	SCZ: DRP2; CK B; sorcin; aconitate hydratase, mitochondrial; fructose-biphosphate aldolase A	2D-PAGE, Western blot, MALDI-ToF MS, LC-ESI MS/MS	Beasley et al. (2006)
SCZ	Dorsolateral prefrontal cortex	Set of biomarker peaks	SELDI-ToF MS	Mei et al. (2006)
BPD, SCZ	Prefrontal dorsolateral cortex	BPD/SCZ: lymphocyte function-associated antigen 3; calmodulin-1; human mago-nashi homolog; myc-associated factor X isoform b; cyclophilin A; myelin basic protein 18590; Dickkopf homolog 2; regulator of G-protein signaling 11; UCH L1; CCAAT-box-binding transcription factor 2; HSP 60 short isoform 2; phospho-glycerate mutase 1; 14-3-3 protein epsilon; human NK2 homeobox transcription factor homolog 4; peroxisomal 2,4-dienoyl- CoA reductase 2; 5′-methyl-thioadenosine phosphatase; purine nucleoside phosphatase; bystin; ankyrin repeat domain protein 12; mitochondrial inorganic pyrophosphatase beta subunit; aldolase C; acid sphingomyelinase isoform 1; glutamine synthetase; enolase 2	SELDI-ToF MS, MALDI- ToF MS	Novicova et al. (2006)
SCZ, BPD	Dorsolateral prefrontal cortex (BA 9), grey matter	SCZ/BPD: DRP3; D-3 phosphoglycerate dehydrogenase; septin-5; SCZ: 6-phosphogluconolactonase; dynamin 1; endoplasmatic reticulum protein ERp29; enolase 2; GFAP; guanine deaminase; neurofilament triplet L protein; protein c2orf32; protein kinase C and casein kinase in neurons protein 1	2D-PAGE, Western blot, LC-ESI MS/MS	Pennington et al. (2007)

Table 12. Schizophrenia: CSF and peripheral biomarkers

Probands	Tissue, conditions	Proteins	Methods	Reference
SCZ	CSF	apoA4	2D-PAGE, MALDI-ToF MS	Jiang et al. (2003)
SCZ, MDD, AD, OCD	CSF, brain, serum	40-amino acid VGF-derived peptide (VGF23-62); transthyretin	ELISA, Western blot, SELDI-ToF MS, MALDI-ToF MS, LC-ESI MS/MS	Huang et al. (2006, 2007)
SCZ	CSF, plasma	apo E; transthyretin	2D-PAGE, Western blot MALDI-ToF MS	Wan et al. (2006)
SCZ	Plasma	Haptoglobin alpha 2-chain; haptoglobin beta-chain; alpha 1-antitrypsin; complement factor B prec	2D-PAGE, MALDI-ToF MS	Yang et al. (2006)
SCZ	CSF, brain, liver, red blood cells, serum	apoA1	2D-DIGE, ELISA, Western blot, SELDI-ToF	Huang et al. (2007)
SCZ	Plasma	Haptoglobin alpha-chain; alpha 1-antitrypsin; serum amyloid P-component; alpha 1-microglobulin; antithrombine III; vitamin D-binding protein	2D-PAGE, PCR, MALDI-ToF MS	Wan et al. (2007)
SCZ	Plasma, chlorpromazine or clozapine treatment	apoA1	2D-PAGE, MALDI-ToF MS	La et al. (2007)
SCZ	Liver, red blood cells	LIVER: retinol-binding protein; superoxide dismutase, HSP 60 kDa; selenium-binding protein; ATP synthase beta chain; aldehyde dehydrogenase; actin beta, gamma; serum albumin; acyl coenzyme A cholesterol acyl transferase; apo A1; prenylcysteine lyase; proteasome activator PA28 beta chain; GTP-binding protein (RAB/RAS like); RBC: selenium-binding protein; glutathione-S-transferase; Prdx 5, mitochondrial; HSP 70 kDa 1,2,5,8, HSP 70 kDa like 1; serum albumin; apoA1; erythroid alpha spectrin; actin, cytoplasmic 1	2D-DIGE, LC-ESI MS/MS	Prabakaran et al. (2007)

samples from drug naive first onset schizophrenic patients and control individuals revealed further significantly different protein levels. As key alterations in CSF, a 40-amino acid VGF-derived peptide was up-regulated and transthyretin at ~14 kDA along with a peptide cluster at ~6.8–7.3 kDA (that appeared co-regulated with the transthyretin cluster) were down-regulated. The authors of this study were able to replicate these specific protein/peptide changes in an independent sample set ($n = 58$). Both experiments gained a specificity of 95% and a sensitivity of 80% and 88%. In addition to the increase of VGF in CSF, analysis of eight post-mortem brains from schizophrenic patients showed up-regulated VGF levels in prefrontal cortex in four samples. To date, the function of this 40-amino acid VGF23–62 peptide in the brain is unknown. In the literature there are, however, hints of a relation of the full-length protein amongst others with synaptic plasticity and the circadian clock. Transthyretin, which is consistently down-regulated in CSF, serum and brain tissue, functions as a transporter of the thyroid hormone thyroxine as well as of retinol. A connection between the retinoid system and schizophrenia was also suggested by Wan et al. (2006), who observed a down-regulation of transthyretin tetramer, as well down-regulation of a second retinol transporter protein and apoE, in CSF of schizophrenic patients. Furthermore, after a two-month period of a valid antipsychotic treatment in hospital, the transthyretin tetramer plasma content increased significantly. These results were reported to imply an insufficient retinol transport to the brain involved in pathophysiology of schizophrenia. The same research group (Yang et al., 2006) analysed the plasma proteome of 22 patients and 20 controls and observed that 7 of the identified 66 gel spots reflect altered protein expression. An up-regulation of acute phase proteins in schizophrenic patients was noticeable for haptoglobin alpha 2-chain, haptoglobin beta-chain, alpha 1-antitrypsin and complement factor B precursor. ApoA1 and transthyretin in contrast showed significantly decreased levels. In addition, an overexpression of apoA4 was found. In a further study by Wan et al. (2007), a possible role of acute phase proteins in pathophysiology of schizophrenic psychosis was further supported by a haptoglobin alpha-chain, alpha1-antitrypsin, serum amyloid P-component and alpha 1-microglobulin up-regulation in plasma. However, the levels of transthyretin and retinol-binding protein were not altered in the investigated sample. To assess the genetic relationship between schizophrenia and acute phase proteins, Wan and colleagues (2007) tested the haptoglobin (Hp) alpha1/alpha2 (Hp 1/2) polymorphism and two single nucleotide polymorphisms of Hp, rs2070937 and rs5473, for associations in the Chinese Han population and found that in addition to the altered expression of Hp protein, a different genotype distribution was also detected with an association between Hp 1/2 and rs2070937 variants and schizophrenia. These findings lead

the authors to assume an aetiological role of the inflammatory response system in schizophrenic psychosis, in contrast to the altered acute phase protein levels being just an accompanying phenomenon of disease.

In several studies, abnormalities in the expression of apolipoproteins in schizophrenia were reported as already addressed. The above-mentioned down-regulation of apoA1 in plasma of schizophrenic patients (Yang et al., 2006) was confirmed by La et al. (2007) in a small patient group under treatment with clozapine or chlorpromazine. The authors had focused on the expression of different apolipoproteins after initially finding increased levels of the apoA1, apoA4 and apoE in plasma of Sprague–Dawley rats treated with chlorpromazine but not with clozpine. La and co-workers speculate that the down-regulation of apoA1 might be associated with the pathology of schizophrenia and that chlorpromazine increases apoA1 expression as part of its therapeutic action. A regulation of apoA1 in schizophrenia was also observed in another study (Huang et al., 2007b). Increased apoA1 levels in CSF, serum, post-mortem liver and brain tissue and red blood cells could be seen consistently through different methodological approaches. Although all these studies imply an association between the pathophysiology of schizophrenia and apolipoproteins, there are also hints that these alterations emerge just as side effect from antipsychotics or are influenced by confounding factors such as nicotine usage (Huang et al., 2007b); hence, there is still need for continuative research to clarify this topic.

In a very recent study Prabakaran et al. (2007) published corrobative data on their previous post-mortem findings (Prabakaran et al., 2004) that metabolic alterations followed by oxidative stress are linked to the disease process of schizophrenic psychosis. The group studied the proteome of liver and red blood cells and discovered six of 14 discriminating proteins in the liver and four of eight altered red blood cell proteins associated with oxidative stress. The observed changes in peripheral protein patterns, in CSF, serum or other tissues have certainly to be tested for reproducibility, but show potential to find surrogate disease markers in easily accessible tissues.

Mood disorders

Cell cultures: Cecconi et al. (2007) treated rat embryonic cortical neurons with a 3-day fluoxetine or vehicle regime (see Table 13). Amongst the identified differentially expressed proteins, cyclophilin A, 14-3-3 protein zeta/delta and 78 kDA glucose-regulated protein precursor play a role in neuroprotection, serotonin metabolism, axonal transport and chaperone machinery, respectively. Such proteomic studies on the effect of antidepressants on cell cultures together with data from animal models could point out the effect of these psychotropic substances on diverse regulatory systems, aside from serotonin and noradrenaline neurotransmission. They contribute to a better understanding of the complex mechanisms mediating the long-term effects of antidepressants.

Animal models: Since psychosocial stress is implicated as a major factor for the onset and pathophysiology of mood disorders, the authors of two recent studies (Carboni et al., 2006a; Mu et al., 2007) aimed at determining molecular changes in rat hippocampus after exposure to acute or chronic stress (see Table 14). Proteins corresponding to differentially intense 2D-PAGE spots were identified by MS. Those proteins play roles in protein folding, signal transduction and synaptic plasticity, cytoskeleton regulation and energy metabolism. In Carboni et al.'s study the animals experienced either a single stressful event or repeated exposures. In general a higher extent of molecular modifications (69 versus 30 modified spots per group) and a qualitatively different pattern was detected after repeated stress exposure as compared with a single stress event. Several of the reported protein alterations had previously been associated with stress-related responses, although some of them were observed for the first time in relation to stress. Kim and Kim (2007) used transcriptomic and proteomic approaches to assess the effects of restraint stress on the rat total cerebrum and hippocampus proteome. In this study a total of five proteins were significantly regulated by stress exposure, with the most marked being a decrease in the HCNP-pp. In an additional experiment, hippocampal cell lines were treated with the synthetic glucocorticoid dexamethasone, as the hypothalamic-pituitary-adrenal axis involvement in stress is well known. In line with the first results, HCNP-pp was significantly down-regulated under influence of this glucocorticoid. The authors speculated that reduced HCNP-pp levels might contribute to a loss of acetylcholine production in rat hippocampus.

Investigating the effects of antidepressant medication on protein profiling in the rat hippocampus, Khawaja et al. (2004) reported 33 differentially regulated proteins after a 2-week treatment with either venlafaxin or fluoxetine. Amongst these modified proteins were those involved in neurogenesis, in outgrowth/maintenance of neuronal processes and in

Table 13. Mood disorders: cell cultures

Cells lines	Tissue, conditions	Proteins	Methods	Reference
Embryonic cortical neurons (Sprague-Dawley rats)	Fluoxetine administration	14-3-3 protein zeta/delta, epsilon; 78 kDa glucose-regulated protein prec; enolase 1; histone H2B; PPlase A; putative RNA-binding protein 3; splicing factor, arginine/serine-rich 1; UCH L1	2D-PAGE, Western blot, immunocytochemistry, MALDI-ToF MS	Cecconi et al. (2007)

Table 14. Mood disorders: cell cultures/animal models

Cells/animals	Tissue, conditions	Proteins	Methods	Reference
Sprague-Dawley rats	Hippocampus; fluoxetine orvenlafaxine administration	Insulin-like growth factor I prec; glia maturation factor, beta; DRP2; PCTAIRE-motif protein kinase 3; serine protease inhibitor; probasin prec; hydroxysteroid sulfotransferase A; dimethylarginine dimethylaminohydrolase 1; Prdx6; PDH (lipoamide) beta; enolase 1; LDH beta; Ras-related protein RAB-4A; Ras-related protein RAB-1B; heat shock 10 kDa protein 1 (chaperonin 10); fatty acid binding protein 3; myelin basic protein; lymphotactin; D-dopachrome tautomerase; Adenine phosphoribosyltransferase; Glutathione S-transferase Yb3; Kruppel-like factor 9; general transcription factor IIa 2; 40 S ribosomal protein S19; 60S ribosomal protein L18A; 60 S ribosomal protein L35A; 61 S ribosomal protein L28; proteasome subunit, alpha type 2; cytochrome c oxidase subunit Vb, mitochondrial prec	2D-PAGE, MALDI-ToF MS	Khawaja et al. (2004)
Long-Evans and Sprague-Dawley rats	Rat hippocampus, stress exposure	Stress-70 protein, mitochondrial prec; 78 kDa glucose-regulated protein prec; heat-shock cognate 71 kDa protein; PPlase A; endoplasmic reticulum protein ERp29 prec; DRP2; calmodulin; phosphatidylinositol transfer protein; vacuolar ATP synthase; F-actin capping protein; alpha- internexin; GTP-binding nuclear protein Ran; GFAP; tropomyosin 1, alpha-isoform i, phosphoglycerate kinase, phosphoglycerate mutase 1; enolase 1; PK M1/M2; MDH; NADH- ubiquinone oxidoreductase 75 kDa subunit; transketolase; aconitate hydratase, mitochondrial prec; Prdx 2, 6; fructose-bisphosphate aldolase C; GDH 1, mitochondrial prec; myelin basic protein S; beta-synuclein; actin beta; prohibitin	2D-PAGE, MALDI-ToF MS	Carboni et al. (2006a)
Sprague-Dawley rats	Hippocampus, frontal cortex; fluoxetine, GR205171 orDMP696 administration	actin isoforms; Rho GDP-dissociation inhibitor 1; 14-3-3 protein zeta/delta, epsilon; GFAP; MDH, mitochondrial prec; tubulin alpha1 and beta chain; complexin-2; eukaryotic translation elongation factor 1 alpha 1; synapsin II; transgelin 3; vacuolar ATP synthase, catalytic subunit A; protein disulphide isomerase A; dynamin 1; aldose reductase; DRP2; PK; LDH B chain; ATP synthase d and e chain, mitochondrial; PK M1/M2; calreticulin precursor; 10 kDa HSP, mitochondrial; HSP 90- alpha, 90-beta; UCH L1; calcineurin subunit B isoform 1; superoxide dismutase Cu/Zn; dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial prec (E2); enolase 2; fructose-bisphosphate aldolase A; protein c20orf178 homolog; alpha-internexin; annexin A5; EF-hand domain-containing protein 2; profilin-2; stathmin; heat shock cognate 71 kDa protein; 40 S ribosomal protein SA; syntaxin-binding protein 1; cytochrome b-c1 complex subunit 1, mitochondrial prec; HSP 70 kDa 5; guanine nucleotide-binding protein, alpha o; GDP dissociation inhibitor 2; aldose reductase; aconitase 2, mitochondrial; glutathione-S-transferase	2D-PAGE, Western blot, MALDI-ToF MS	Carboni et al. (2006b)
Sprague-Dawley rats, hippocampal cell line H19-7	Cerebrum, hippocampus, serum; stress exposure	protein phosphate 2A regulatory subunit B beta 2; similar to WW domain-binding protein 11; hippocampal cholinergic neurostimulating peptide prec protein; cholinergic receptor, nicotinic, alpha polypeptide 7; similar to 1500031 N24Rik protein	2D-DIGE, Western blot, radioimmunoassay, qRT- PCR, MALDI-ToF MS	Kim and Kim, (2007)
Sprague-Dawley rats	Hippocampus, stress exposure	calreticulin prec; tropomyosin 1, alpha isoform I; dual specificity mitogen-activated protein kinase kinase 1; mitogen activated protein kinase 1; cytochrome C oxidase, subunit Va; CK mitochondrial; methylmalonate-semialdehyde dehydrogenase; aconitase 2, mitochondrial; vacuolar H^+ ATPase B2; predicted similar to coiled-coil-helix-coiled-coil-helix domain containing 6; isocitrate dehydrogenase 3 (NAD+) alpha; Rieske Fe-S protein prec transcription; heterogeneous nuclear ribonucleoprotein H1; predicted similar to RIKEN cDNA 2900041A09; tyrosine 3- monooxygenase/tryptophan 5-monooxygenase activation protein	2D-PAGE, MALDI-ToF MS	Mu et al. (2007)

neural regeneration/axonal guidance collapsin response mediator protein systems. Other modulated proteins were associated with neuronal vesicular cell trafficking and synaptic plasticity, with neurosteroidogenic responses, and possible anti-apoptotic pathway-mediated regulatory events. In a following similar study design, the rat hippocampus and frontal cortex proteome were analysed after chronic treatment with fluoxetine and two putative novel antidepressants: an NK1 receptor antagonist (GR205171) and a corticotropine-releasing factor receptor 1 antagonist (DMP696) (Carboni et al., 2006b). All treatments resulted in modified levels of actin isoforms, whereas both fluoxetine and GR205171 caused decreased synapsin II levels. Fluoxetine intake augmented extracellular signal-regulated kinase 2 and transgelin 3 and decreased vacuolar ATP synthase. After GR205171 treatment, protein disulphide isomerase A was down-regulated, whereas dynamin 1 and aldose reductase were increased. Application of DMP696 influenced PK, LDH, DRP2 and ATP synthase concentrations. Although this report revealed a specific pattern of protein modulation for each pharmacologically active compound, the authors suggested that all antidepressants share the ability of modulating neural plasticity.

Post-mortem studies: Johnston-Wilson et al. (2000) assessed the proteome of the frontal cortex in MDD, BPD and schizophrenia. The authors reported common alterations in all three patient groups for some identified proteins (see the schizophrenia post-mortem section and also Tables 11 and 15). Specific changes in MDD were observed for two mitochondrial proteins: ubiquinol-cytochrome-C reductase complex core protein 1 (reduction) and carbonic anhydrase 1 (elevation). BPD patients, however, did not show any specific protein regulations. In 2006 two further groups published results on the post-mortem protein pattern in BPD, MDD and schizophrenia. In one the dorsolateral prefrontal cortex was examined (Novikova et al., 2006), and in another the anterior cingulate cortex was screened (Beasley et al., 2006). None of the 24 SELDI-ToF biomarker peaks in Novikova et al.'s study was unique to one of the examined disease group (BPD or SCZ). In BPD seven marker proteins had altered levels; all were up-regulated, and two were affected exclusively in BPD: Dickkopf homolog 2 as a member of Wnt cascade and myelin basic protein 18590 that is involved in myelin formation by oligodendrocytes. The remaining BPD-affected proteins (CCAAT-box-binding transcription factor 2, peroxisomal 2, 4-dienoyl- CoA reductase 2, bystin, ankyrin repeat domain protein 12 and aldolase C) were also found to be altered in schizophrenic psychosis samples. It can again be suggested that both BPD and schizophrenia are associated with complex multifactorial molecular changes, including gene transcription, cell signalling, lipid and glucose metabolism. Beasley et al. (2006) found 19 proteins that were differentially expressed. The identified changes provide support for the hypothesis that cytoskeletal and mitochondrial dysfunction are important components of the neuropathology in common of all of these three major psychiatric disorders. In BPD or/and MDD patient samples the levels of the following mitochondrial proteins were altered: GDH, mitochondrial prec (in BPD); succinyl coenzyme A:3-ketoacid CoA transferase (in BPD and MDD); ATP synthase beta-chain, mitochondrial prec (in MDD); fructose-biphosphate aldolase A (in MDD); MDH, mitochondrial prec (in MDD). In depression, for example, as one of the proteins with cytoskeletal function transgelin 3 that co-locates with actin, tubulin isoforms and microtubule-associated proteins was down-regulated. DRP1 and DRP2, which play a role as cytosolic phosphoproteins in processes such as axon guidance, neuronal migration and differentiation as well as signal transduction, were increased in MDD and schizophrenia (with the same trend in BPD). To summarize, in all of these studies of the cortical proteomes of MD, BPD and schizophrenia patients, an overlap of protein alterations was observed. Common irregularities in mitochondrial and metabolic function, in signalling and cytoskeletal processes might represent common biological features involved in the pathophysiology of all of these major psychiatric diseases.

Depression is often associated with suicidal behaviour. In a recent post-mortem comparative study the prefrontal cortex proteome of suicide victims and controls was assessed (Schlicht et al., 2007). Five 2D-PAGE proteins spots differed significantly in intensities between both groups, the following three appearing only in suicide victims: manganese superoxide dismutase, alpha-crystallin B chain and GFAP. Manganese superoxide dismutase, as a major antioxidant enzyme, protects cells against oxidative stress, alpha-crystallin B chain belongs to the low molecular HSP proteins and GFAP is reported to be involved in astrocytic activation in gliosis. Whether the observed expression changes in proteins connected with glial function, neurodegeneration and neuronal injury have a real impact upon suicidal behaviour, or whether they just occurred as an effect of previous medication of the suicide victims, has to be ascertained.

Peripheral biomarkers: In a recent biomarker study, Huang et al. (2006) examined primarily the CSF proteome of schizophrenic patients, but also examined the CSF proteome of several MDD patients. Interestingly, MDD and schizophrenic psychoses shared an up-regulation of a 40-amino acid VGF-derived peptide. The prominent differences between the MDD and schizophrenia group however were a distinct specific down-regulation of a secretogranin II 529–566 peptide in samples from depression patients and decreased levels of transthyretin in schizophrenia. In this study the CSF of five patients with obsessive compulsive disorder was also included, although the authors could not report a significant difference between protein patterns in this patient group compared with the control group. Searching for suicide biomarkers, Brunner et al. (2005) compared the CSF protein profile of unmedicated depressed patients with and without prior suicide attempts. The authors discovered a 33 kD protein with an isoelectric point of 5.2 the expression levels of which differed significantly in patients with and without suicide attempts in the past. Unfortunately the authors were not able to identify this protein due to lack of material. However, this result together with Schlicht et al.'s (2007) reports on the prefrontal cortex

Table 15. Mood disorders: post-mortem studies

Probands	Tissue, conditions	Proteins	Methods	Reference
MDD, BPD, SCZ	Frontal cortex	MDD: GFAP; ubiquinol-cytochrome-C reductase complex core protein 1; fructose-biphosphate aldolase C; DRP2; carbonic anhydrase 1; aspartate aminotransferase; BPD: fructose-biphosphate aldolase C; GFAP; DRP2	2D-PAGE, LC-ESI MS/MS	Johnston-Wilson et al. (2000)
MDD, BPD, SCZ	Anterior cingulated cortex	MDD: albumin; trypsin III prec; DRP1; DRP2; vacuolar ATPase B subunit; succinyl-CoA:3-ketoacid- coenzyme A transferase 1, mitochondrial; glutamine synthetase; guanine nucleotide-binding protein, beta subunit; ATP synthase subunit beta, mitochondrial; tubulin alpha; sorcin; fructose-biphosphate aldolase A; MDH mitochondrial prec; carbonic anhydrase 1; transgelin 3; BPD: succinyl-CoA:3- ketoacid-coenzyme A transferase 1, mitochondrial; tubulin beta; GDH mitochondrial prec	2D-PAGE, MALDI-ToF MS, LC-ESI MS/MS	Beasley et al. (2006)
BPD, SCZ	Prefrontal dorsolateral cortex	BPD/SCZ: lymphocyte function-associated antigen 3; calmodulin-1; human mago-nashi homolog; myc-associated factor X isoform b; cyclophilin A; myelin basic protein 18590; Dickkopf homolog 2; regulator of G-protein signaling 11; UCH L1; CCAAT-box-binding transcription factor 2; HSP 60 short isoform 2; phospho-glycerate mutase 1; 14-3-3 protein epsilon; human NK2 homeobox transcription factor homolog 4; peroxisomal 2,4-dienoyl- CoA reductase 2; 5′-methyl-thioadenosine phosphatase; purine nucleoside phosphatase; bystin; ankyrin repeat domain protein 12; mitochondrial inorganic pyrophosphatase beta subunit; aldolase C; acid sphingomyelinase isoform 1; glutamine synthetase; enolase 2	SELDI-ToF MS, MALDI-ToFMS	Novicova et al. (2006)
Suicide victims	Prefrontal cortex	Manganese superoxide dismutase; alpha-crystallin B chain; GFAP	2D-PAGE, MALDI-ToF MS	Schlicht et al. (2007)
SCZ, BPD	Dorsolateral prefrontal cortex (BA 9), grey matter	SCZ/BPD: DRP3; D-3 phosphoglycerate dehydrogenase; septin-5; BPD: 4-trimethylaminobutyraldehyde dehydrogenase; ADH; aldehyde dehydrogenase 1A1; aldehyde dehydrogenase family 7 member A1; internexin alpha; tubulin alpha, beta; ATP synthase beta, delta, gamma chain; ATPase (transitional ER); cadherin-24 prec; (i) collagen alpha-1 (ii) chain prec; cytosol aminopeptidase; cytosolic acyl coenzyme A thioester hydrolase; D-3-phosphoglycerate dehydrogenase; dihydrolipoyl dehydrogenase; enolase 2; synuclein gamma; inorganic pyrophosphate; isocitrate dehydrogenase subunit alpha; kinesin light chain 2; mitochondrial processing subunit peptidase beta subunit; neurofilament triplet M protein; NG,NG-dimethylarginine demethylaminohydrolase 1; Prdx2, 6; probable ubiquitin ligase protein mycbp2; pyridoxal phosphate phosphatase; secernin 1; septin 5, 6, 11; serotransferrin; serum albumin; stress 70 protein; succinyl CoA ligase beta chain; T-complex 1, beta, zeta subunit; ubiquinol-cytochrome C reductase complex; vacuolar ATP synthase subunit B, brain isoforms	2D-PAGE, Western blot, LC-ESI MS/MS	Pennington et al. (2007)

Table 16. Mood disorders: CSF biomarkers

Probands	Tissue, conditions	Proteins	Methods	Reference
Suicide victims	CSF	33 kD protein, isoelectric point of 5.2	2D-PAGE, image analysis	Brunneret al. (2005)
MDD, SCZ, OCD, AD	CSF, serum, brain tissue	MDD: 40-amino acid VGF-derived peptide; secretogranin II 529-566 peptide	ELISA, Western blot, SELDI-ToF MS, MALDI-ToF MS, LC-ESI MS/MS	Huang et al. (2006)

Table 17. Anxiety disorders: animal models

Animals	Tissue, conditions	Proteins	Methods	Reference
High-, and low-anxiety-related behavior CD1 mice	Hypothalamus, amygdala, cortex, red blood cells	Glyoxalase 1	2D-PAGE, Western blot, microarray, LC-ESI MS/MS	Kromer et al. (2005)
High-, and low-anxiety-related behavior CD1 mice; human blood	Mice: hypothalamus, amygdala, cortex, red blood cells; human: white blood cells	Glyoxalase 1, enolase phosphatase	2D-PAGE, Western blot, LC-ESI MS/MS	Ditzen et al. (2006)

proteome in suicide victims seem to be a promising first step for a more precise choice of candidate genes in suicide research. See Table 16 for a summary of CSF biomarkers for mood disorders.

Anxiety disorders

Animal models: In the analysis of anxiety-like behaviour, intrastrain breeding approaches in mice play an important role in studying particular behavioural traits. One work group (Ditzen et al., 2006; Kromer et al., 2005) compared the proteome of mouse lines with HAB and LAB (see Table 17). Different tissues were analysed with proteomics and Western blot analyses (hypothalamus, amygdala, cortex and red blood cells) as well as using microarray studies (hypothalamic paraventricular nucleus). The authors identified a consistent increase in expression of glyoxalase 1 in LAB mice compared with HAB mice. Although it has been reported that this enzyme plays a role in the detoxification of methylglyoxal (a potent cytotoxic metabolite) its function is not entirely clear. Whether glyoxalase 1 expression can be used as a valid and reliable biomarker to reflect anxiety-related behaviour has to be more properly assessed in further behavioural and protein studies. It also remains to be determined whether glyoxalase 1 is more then a byproduct and contributes aetiologically to anxiety-related traits.

Autistic spectrum disorder

Post-mortem studies: Junaid et al. (2004) searched for molecular changes in the grey matter of eight ASD brains (see Table 18). They reported post-translational modifications in the form of an increased polarity of glyoxalase 1 in 2D-PAGE in the autistic samples. Sequencing of this more acidic protein revealed a single nucleotide polymorphism causing an AlaGlu exchange. A possible role of this gene for the aetiology of autism was confirmed by the finding of a reduced glyoxalase 1 activity in the brain lysates and a further population association study. The final impact of different isoforms of glyoxalase 1 on autistic features and/or on the above-mentioned anxiety-related behaviour is still unclear and needs further studies.

Peripheral biomarkers: To date no disease markers for the diagnosis of autism have been validated and diagnostic procedures are mainly based on the observation of behavioural abnormalities. However, a reliable protein biomarker would facilitate an early and exact ASD diagnosis, a crucial precondition for an early behaviour-modifying therapeutical approach. Recently Corbett et al. (2007) analysed the serum proteome in a group of autistic children aged four to six years for differentially expressed proteins (see Table 18). The authors of this LC-MS/MS study reported altered levels of the following apolipoprotein and complement factors: apoB100, complement factor H related protein, complement C1q and fibronectin 1. The findings of this first MS-based study on peripheral markers are in line with former studies, that revealed alterations in immune modulating and inflammation systems (e.g. complement) in ASD patients (Molloy et al., 2006; Nelson et al., 2006; Zimmerman et al., 2005).

Discussion

Our overview on widely used proteomic methods and currently available data reflects new developments and opportunities in modern neuropsychiatric research. Proteomic analysis of brain compartments and peripheral tissue, of cell and animal models, is now a promising tool to better understand the complexity of brain disorders and drug effects as well as to search for corresponding early disease markers.

Despite the encouraging progress in proteomic technologies this approach is still in a developmental stage with pitfalls and hurdles to overcome. In general, in a given tissue, a proteome of millions of proteins could be expected, but so far only a small fraction has been detected with current proteomic methods. One reason for this is that certain characteristics of brain molecules complicate their analysis. Proteins of interest in CNS are often transmembrane- and membrane-associated proteins, including ion channels, G proteins and receptors.

Table 18. Autistic spectrum disorder: post-mortem studies and peripheral biomarkers

Approach	Tissue, conditions	Proteins	Methods	Reference
Post-mortem	Total brain grey matter	glyoxalase I	2D-PAGE, LC-ESI MS/MS	Junaid et al. (2004)
Peripheral biomarkers	Serum	apoB100 prec; complement factor H-related protein prec; complement C1q subcomponent, C chain prec; fibronectin 1 isoform 1 preproprotein, cold-insoluble globulin	spin filters, LC-ESI MS/MS	Corbett et al. (2007)
Peripheral biomakers	Serum	10KDa peak in MALDI spectrum	magnetic beads, MALDI	Taurines et al., (2009)

These molecules unfortunately are rather insoluble. To give consideration to these limitations, proteomic methods in neuropsychiatric research have to be specially adapted. The widely used 2D-PAGE imposes clear limitations in representation and detection of these insoluble proteins, as well as for the detection of low abundance gene products, proteins of small size, hydrophobic, acidic and basic proteins, all of which often escape 2D-PAGE analyses. Furthermore, neuropeptides and proteins only exist in very low concentrations, in addition, the amount of available brain and CSF tissue is limited.

Every type of tissue is dominated by few proteins in high concentrations that hamper mass spectrometric detection of low abundance proteins. To address these difficulties, different fractionation and enrichment procedures are inevitable prior to mass spectrometric analysis, as explained in more detail in the methods section. A sophisticated and efficient sample prefractionation and the recovery of more homogenous sample fractions/subproteomes prior to MS will eventually lead to the reward of higher information yield. Apart from adapting 2D-PAGE methods, non-gel-based techniques such as LC-ESI MS will gain in importance as they seem more suitable for the analysis of interesting protein populations in brain research. As a progressive method, nanoscale capillary LC-ESI MS systems actually allow the detection of very small protein or peptide amounts.

In addition to advances in prefractionation methods, improvements in the reliability of mass spectrometers are desirable to allow proteomics to enter in a clinical setting where only small tissue samples are available. Strong efforts are also needed in the field of proteomics data processing. A systematic study in 2005 tested various algorithms currently in use for MS/MS data analysis (Kapp et al., 2005). The authors of this study compared five search algorithms with respect to their sensitivity and specificity, and evaluated them based on specified false-positive rates. As the algorithms still displayed weaknesses in sensitivity or specificity, the authors suggested the use of multiple (at least two) search algorithms to reduce the number of false-positive identifications. According to these results there is still room for improvement regarding proteomic software tools.

With the rapid growth in the research area of proteomics vast quantities of biological data (especially in association with other 'omics' approaches such as metabolomics or lipidomics) are generated. It is crucial to think about efficient data-mining technologies and the establishment of international, public databases. As a general goal, multicentre collaborations are desirable to obtain large-scale data from multiple levels of analysis and to integrate these data. This requires a joint effort and collaborations between clinical researchers of the medical sector and academia from chemistry, biochemistry, cell biology and molecular genetics alongside specialists in bioinformatics and statistics. 'SwissProt' represents an already available database that provides reliable protein sequences associated with a high level of annotation (such as protein function, domains structure, post-translational modifications, variants, etc.), a minimal level of redundancy, the usage of standardized nomenclature and a high level of integration with other databases. In this context also HUPO has to be mentioned. This project (see http://www.HUPO.org) aims to identify all protein isoforms in cells (in health and disease state) of organisms such as humans or mice. In the sub-project the Human Brain Proteomics Project (HBPP), the human and mouse brain proteome shall be characterized and the data will be compared with mouse models of human disease and to relevant autopsy materials from human neurodegenerative diseases. A further important aim of the HUPO is to define standards concerning the handling, exchange and dissemination of proteomics data as no gold standard exists up to date to guide investigators and enhance comparability of proteomics and biomarker studies. For AD, at a 2003 consensus panel, researchers gave the following guidelines for biomarker research in this dementia: the ideal biomarker for AD should aim to detect a fundamental feature of neuropathology and should be validated in neuropathologically confirmed cases; the diagnostic sensitivity should be >80% for detecting AD and the specificity of >80% for distinguishing other dementias; it should be reliable, reproducible, non-invasive, simple to perform and inexpensive. For biomarker validation it is recommended to confirm the findings by at least two independent studies conducted by qualified investigators and the results published in peer-reviewed journals. Furthermore, it would be especially useful if the biomarker could also capture the beneficial effect of disease-modifying therapy (Ho et al., 2005).

Despite all of the above-mentioned difficulties and challenges, proteomic technologies provide rapid progress and immense benefits and already now create important biological data in neuropsychiatric research. Proteomic approaches allow a large-scale high-throughput qualitative and quantitative protein analysis complementing other traditional methods used in molecular genetics. Proteomics has the advantage

of being relatively unbiased without *a priori* assumptions about differences between sample groups. It is a powerful research field that can reveal the function of so far uncharacterized proteins and generate new hypotheses to improve the understanding of the basic physiology of CNS under normal and disease conditions. In contrast to molecular genetic studies, proteomics has the great advantage of analysing processes at the protein level, thereby possibly being closer to the pathophysiological processes underlying the clinical phenomenology of specific psychiatric conditions. This is a crucial point as there exist more proteins than genes due to modifications of gene products by alternative splicing and post-translational modifications of the proteins expressed. Analyses on the protein level become even more important because of the already-mentioned reports that mRNA levels and protein concentrations correlate insufficiently and it seems not possible to predict protein expression levels from quantitative mRNA data (Gygi et al., 1999). For some genes in this study the protein levels varied by more than 20-fold with constant mRNA levels. As discussed in the introduction, on the one hand technical issues can contribute to an imperfect correlation, whilst on the other hand these differences can be attributed to the increasing complexity of gene products from the gene to the protein level by alternative RNA splicing and post-translational modifications. Current mRNA analysis methods are not set up to systematically capture different mRNA splice variants, whereas proteomic approaches are able to identify these variants (Hegde et al., 2003). As diverse isoforms of proteins can differ in their molecular characteristics and finally their function, it is crucial to distinguish between these variants by proteomic methods. Through amino acid sequencing, detection of post-translational modifications and protein–protein interactions of molecules involved in neuronal transmission and signal cascades, a better context-based functional understanding of cellular protein networks in neuropsychiatric disorders will emerge. The detailed knowledge, concerning molecular pathways *in vivo* together with proteomics data of drug effects *in vitro*, could facilitate the discovery of new potential drug targets and the design of more specific medications with fewer side effects.

As already mentioned for several psychiatric disorders, proteomic studies present the immense opportunity to identify surrogate biomarkers in easily accessible tissue for early disease detection, perhaps disease prevention, and the differentiation of stages and similar phenotypes of distinct nosological entities. Furthermore, biomarkers are suitable for a personal drug-monitoring scheme. The assessment of the patients' individual protein profile before and in the course of medication might in the future allow a prediction of drug response and an adequate treatment modification. Ideally the marker recovery should be simple and non-invasive enough to perform in easily accessible tissue whose gene expression profile is similar to more inaccessible CNS tissues. In a recent study the gene expression patterns in blood and brain were compared (Sullivan et al., 2006). The authors found significant expression similarities in whole blood and multiple CNS tissues with a median correlation of about 0.5. About half of a set of schizophrenia candidate genes were found in both blood and prefrontal cortex. The authors concluded that surrogate marker search in blood is a useful tool when it has been determined that the relevant genes are expressed in both tissues.

After a successful identification of disease biomarkers proteomic technologies will hopefully overcome the obstacle of designing clinically useful and easily applicable laboratory or bedside tests.

Conclusion

Proteomic high-throughput technologies based on MS are increasingly used as a valuable tool in psychiatric research. This approach provides the opportunity to analyse and identify the complexity and dynamics of pathophysiological processes in neuropsychiatric disorders at the protein level. Understanding the molecular mechanisms of synaptic transmission, protein–protein interactions and signalling cascades will provide crucial insights into brain diseases, allowing the search for diagnostic and prognostic biomarkers as well as new therapeutic targets.

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