Extracellular Matrix Proteomics in Schizophrenia and Alzheimer's Disease

Manveen K. Sethi[1] and **Joseph Zaia**[1,*]

[1] Center for Biomedical Mass Spectrometry, Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118, USA

Abstract

Brain extracellular matrix (ECM) is a highly organized system that consists of collagens, non-collagenous proteins, glycoproteins, hyaluronan and proteoglycans (PGs). Recognized physiological roles of ECM include developmental regulation, tissue homeostasis, cell migration, proliferation, differentiation, neuronal plasticity, and neurite outgrowth. Aberrant ECM structure is associated with brain neurodegenerative conditions. This review focuses on two neurodegenerative conditions, schizophrenia (Sz) and Alzheimer's disease (AD), and summarizes recent findings of altered ECM components, including PGs, glycosaminoglycans (GAGs), proteins and glycoproteins, and proteins and genes related to other brain components. The scope includes immunohistochemical, genomics, transcriptomics, proteomics and glycomics studies, and a critical assessment of current state of proteomics studies for neurodegenerative disorders. The intent is to summarize the ECM molecular alterations associated with neurodegenerative pathophysiology.

Graphical abstract
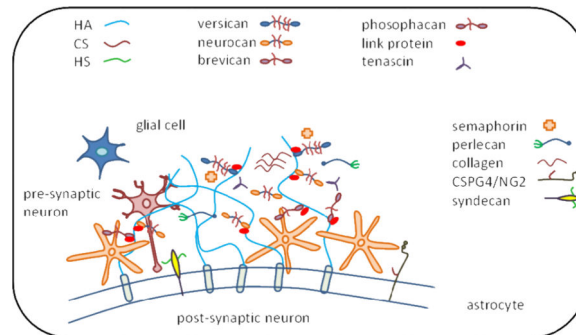
Keywords

brain extracellular matrix; proteoglycans; glycosaminoglycans; perineuronal nets; schizophrenia; Alzheimer's disease

* Corresponding author, jzaia@bu.edu.

Compliance with Ethical Standards

The authors declare no conflict of interest.

Introduction

Extracellular matrix (ECM), constitutes approximately 20% of the adult brain volume [1] and has become a focus for neurological research. Functional roles of ECM include developmental regulation, tissue homeostasis, cell migration, proliferation, and differentiation [2]. The constituents of ECM include collagenous and non-collagenous proteins, glycoproteins, hyaluronan, and proteoglycans (PGs) [2]. Proteoglycans are proteins glycosylated with glycosaminoglycan (GAG) chains. GAGs play essential roles in all areas of physiology through their biophysical properties and capacities to bind growth factors and growth factor receptors. Thus, there is a keen interest in the roles of PG neuronal functions including cell adhesion, migration, regeneration, ECM assembly, neurite outgrowth and plasticity [3]. Proteoglycan structure is regulated in a spatial and temporal manner. As a result, structures and functions of proteoglycans in ECM depend on expression of core proteins via the endoplasmic reticulum and Golgi apparatus-mediated assembly of GAG chains attached to the core protein. Because the biosynthetic reactions do not go to completion, the GAG chains on PGs are heterogeneous with respect to chain length and modifications.

Neurodegenerative diseases, including schizophrenia (Sz), Alzheimer's disease (AD), multiple sclerosis (MS), Parkinson's disease (PD), and human immunodeficiency virus dementia (HIVD), result from gradual and progressive loss of neural cells and changes in ECM components that lead to brain dysfunction [4-7]. This review focuses on changes in ECM components specifically, proteoglycans and GAGs in SZ and AD. A better understanding of these changes in ECM will help unravel the biomolecular mechanisms related to complex neurodegenerative conditions in the human brain.

GAGs are classified into five major types based on the disaccharide unit; heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), and hyaluronan (HA). HS and CS/DS are unbranched polymers composed of ~20-200 repeating disaccharide units attached to serine or threonine residue of core protein through a characteristic tetrasaccharide linker [8]. The disaccharide units contain either of two modified sugars, *N*-acetylgalactosamine (GalNAc) or *N*-acetylglucosamine (GlcNAc) and a uronic acid (either glucuronate (GlcA) or iduronate (IdoA)). The structures of the GAG classes are shown in Table 1.

The four PG classes include interstitial PGs, which are present in the ECM, basement membrane PGs, secretory granule PGs, and membrane-bound PGs [9]. Interstitial PGs are a critical component of ECM involved in stabilization and organization of collagen fibers. The interstitial PGs include small leucine-rich PGs (SLRPs) such as decorin, asporin, lumican, and biglycan [9, 10], and hyalectans, including aggrecan, versican, neurocan, and brevican [11]. The major proteoglycan found in cytoplasmic secretory granules is serglycin. It has heparin and/or CS chains attached to the core protein. It binds to several chemokines, growth factors, and inflammatory mediators, and modulates their bioactivity. Basement membrane PGs include HSPGs perlecan, agrin, collagen type XVIII, and leprecan. They mainly have HS chains attached to the core protein, except CSPG leprecan [12]. These PGs play important roles in cell adhesion, proliferation, growth factor signaling, embryogenesis, tissue

morphogenesis, and cartilage development [12-14]. The membrane-bound syndecans and glypicans are involved in processes such as cell growth and angiogenesis [15, 16]. The syndecan family consists four members, syndecans 1, 2, 3, and 4, which carry HS and/or CS chains. On the other hand, glypicans consist of six members, GPC-1, 2, 3, 4, 5, and 6 attached to the plasma membrane via a glycosyl phosphatidyl inositol anchor and carrying only HS chains. CSPG4 is a single-pass, type-1 transmembrane proteoglycan that carry one CS chain [9].

Hyalectans functions in brain ECM are related to modulation of cell adhesion and migration [17]. The C-type lectin domain of hyalectans mediates binding to other ECM proteins [18], while the EGF-like repeats make hyalectans right candidates for growth control [19]. The roles of hyalectans in neurite outgrowth are also well-recognized [20]. Studies have shown that all four hyalectans inhibit neurite outgrowth in various neuronal cell types, through the CS chains [21-23]. A recent study showed a significant reduction in neurite outgrowth *in vitro,* in the presence of ECM components, including aggrecan, brevican, and tenascin-R [24].

HA is an important component of brain ECM. It binds to growth factors and cytokines including TGF-β, TNFα, epidermal growth factor (EGF), and keratinocytes [25]. Exogenous HA induces mesenchymal properties of epithelial cells, and digestion of HA by hyaluronidase disrupts epithelial-mesenchymal transition [26, 27]. Both increase and a decrease of hyaluronidase have been observed during tumorigenesis [28].

Function and Composition of Brain ECM

As shown in Fig. 1, brain ECM consists of relatively small amounts of fibrous proteins such as collagens and fibronectin and relatively high proportion of proteoglycans and hyaluronan. Brain hyalectans include aggrecan (210 kDa protein chain, CS and KS GAGs), neurocan (136 kDa protein chain, CS and DS GAGs), brevican (145 kDa protein chain, CS GAGs) and versican (265 kDa protein chain, CS and DS GAGs) [29]. Brain SLRPs include asporin (39 kDa protein chain, CS and DS GAGs), fibromodulin (42 kDa protein chain, KS GAGs), lumican (40 kDa protein chain, KS GAGs), biglycan (42 kDa, CS and DS GAGs) and decorin (40 kDa, CS and DS GAGs) [2]. Glycoproteins including collagens, laminins, tenascin, reelin, and fibronectin are among other components of the brain ECM.

ECM proteoglycans interact with brain ligands including growth factors, cell adhesion molecules, matrix components, and enzymes. This binding becomes considerably affected by variation in the GAG chains that influences the accessibility of ligands to cell surface receptors. The activity of growth factors may be either potentiated or inhibited by their interactions with ECM molecules. For example, vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) bind heparin / HS, which in turn modulates their biological activity [30, 31]. The CSPG phosphacan binds to FGFs and potentiates their mitogenic activities [32].

Among brain ECM structures, perineuronal nets (PNNs) are differentially distributed, highlighting their importance in brain physiology [24]. First described by Camillo Golgi and

Santiago Ramon y Cajal in 1890, PNNs are highly organized reticular networks composed of ECM molecules surrounding neuronal cell bodies and proximal dendrites [33]. PNNs consist of hyalectican PGs, HA, tenascin-C, tenascin-R, hyaluronan and PG binding link proteins (Haplns, Hapln1/ Crtl1, and Hapln4/ Bral2). PNNs surrounding neurons carry three main components: a hyaluronan synthase (HAS), a link protein (usually cartilage link protein-1; crtl1) and a chondroitin sulfate proteoglycan (usually aggrecan). The physiological role of PNNs are not yet well-established; however, their postnatal appearance suggests a role in restricting the development of new synaptic contacts: synapse formation and synaptic plasticity [11]. In line with this, a study showed the development of synapses in parallel with the emergence of PNNs [34]. PNNs are observed ensheathing neurons, suggesting a supportive role in providing a microenvironment for neurons [35].

Changes in Brain ECM in Neurodegenerative conditions

Schizophrenia

Schizophrenia is a serious brain disorder affecting approximately 1% of the population worldwide. The clinical symptoms of Sz appear during late adolescence or early adulthood when cognitive functions, including reasoning, thinking, and planning, achieve maturation [36]. Characterized by defects in thinking and decision-making ability, Sz is defined by positive (hallucinations and delusions), and negative (affective flattening and cognitive deficits) symptoms [37]. Currently, antipsychotic medications are the only treatment available for Sz. Treatment provides relief to a subset of Sz patients but does not appear to impact the disease outcome significantly. Thus, lack of appropriate and effective treatment demands a better understanding of neuropathophysiology of the disease, paving the way for better treatment efficacy. It has been speculated that a better understanding of critical clinical signatures that occur before the onset of symptoms and timely intervention of these early signatures may reduce or prevent the onset of severe symptoms related to Sz [38]. However, significant progress towards these ends has not been reported. Maturation of prefrontal cortex (PFC) via extensive synaptic pruning occurs during late adolescence and early adulthood, and thus, it has been hypothesized that synaptic pruning that takes place in the PFC may play a critical role in the onset of Sz [39]. Interestingly, ECM molecules regulate processes occurring during late adolescence and early adulthood, including altered GABAergic function, synaptic pruning and plasticity, axonal guidance, neuronal differentiation, and migration [5].

A number of immunohistochemical (IHC) studies indicated patterns of ECM molecular expression associated with Sz, [40-45] (Table 2A). A marked decrease of CSPG-labeled PNNs in the amygdala and entorhinal cortex of subjects with Sz was reported [41, 46-49]. The PNNs were detected using Wisteria floribunda agglutinin (WFA) lectin which is believed to bind *N*-acetylgalactosamine residue of CS in the PNNs [50]. Interestingly, treatment with chondroitinase ABC, an enzyme known to degrade CS chains, abolished the WFA staining of PNNs, indicating the specificity of WFA for CSPGs [41]. Another study reported a decrease in WFA-labeled PNNs in layer 3 and 5 of the PFC of subjects with Sz compared to normal controls [42]. Intriguingly, an increase in the number of PNNs in the PFC during postnatal development through early adulthood has been reported [42],

suggesting a parallel relationship of PNN development with the onset of Sz symptoms and indicating the contribution of altered PNN expression in the onset of the disease. A similar decrease in PNNs using antibodies against aggrecan, one of the main CSPG in the amygdala was observed for Sz subjects [44]. Another study quantified densities and fluorescence intensities of parvalbumin (PV) neurons and PNNs labeled with WFA versus an immunoreactive against major PNN protein, aggrecan in Sz and normal controls. They observed a decrease in the fluorescence intensities for both PV and PNNs labeled with WFA and aggrecan immunoreactive. However, densities of PV and PNNs were unchanged, suggesting that normal complements of PV and PNNs are preserved in Sz, but levels of PV and PNN proteins and carbohydrate moieties attached to it are dysregulated [51]. A significant reduction in numerical densities of cytoplasmic-CSPG and olfactory receptor neurons (ORNs) in Sz has been reported, suggesting a reduction of CSPG expression in mature ORNs, that may contribute to the ORN lineage dysregulation and olfactory identification abnormalities, observed in Sz [42].

Reelin, tenascin-X, fibronectin, and integrins have been extensively investigated in Sz, Table 2B. Reelin is an extracellular glycoprotein that performs diverse roles in the developing and adult brain, including regulation of neuronal migration, synapse development, synaptic plasticity, and learning and memory. In subjects with Sz, a reduced expression of both mRNA and protein levels of reelin was reported [52, 53]. Tenascin-X is the largest member of the tenascin family of ECM glycoproteins. It is involved in behavioral functions including anxiety, emotional learning and memory [54], and has been associated with Sz [55-57]. Fibronectin is a high molecular weight ECM glycoprotein that binds to integrin receptors and plays important roles in neuro- and synaptogenesis. Dysregulation of fibronectin may result in neurodevelopmental abnormalities related to the pathogenesis of Sz [58, 59]. In contrast, a study showed no significant association of fibronectin with Sz [60].

Integrins assist in cell-cell and cell-ECM interactions and play a significant role in the maintenance and modulation of neuronal synaptic activity, development of the central nervous system, and have been associated with the pathophysiology of Sz. For example, altered expression of ITGA8 and ITGB3 have been observed in Sz [61, 62]. In addition, a flow cytometry study analyzed surface expression of several glycoproteins and found a significant increase in integrin $\alpha 2_b\beta 3_a$ expression in first episode Sz patients compared with healthy controls [63].

Levels of proteolytic enzymes including matrix metalloproteases (MMPs), a disintegrin and metalloproteases (ADAMs) and a disintegrin and metalloproteases with a thrombospondin motif (ADAMTS) have been associated with Sz, Table 2B [64-68]. These enzymes mediate proteolytic ECM remodeling by interaction with cell adhesion molecules, ECM receptors, and CSPGs, and thus, play important roles in synaptic plasticity [24]. Elevated levels of MMP-9 have been reported in previous Sz studies, including those of treatment-resistant Sz patients [69, 70]. Additionally, altered mRNA expression of MMPs (MMP 16, MMP 24, MMP 25), and ADAMTS-1 and -6 has also been reported in Sz [71]. In contrast, a recent study showed no difference in the level of MMP-9 in Sz compared to healthy controls. However, this expression level was demonstrated to be sensitive to electroconvulsive

therapy, where MMP-9 levels were significantly decreased in Sz patients compared to healthy controls [72].

Several genetic studies indicate abnormal CSPG expression in Sz, Table 2B. For example, increased expression of receptor phosphotyrosine phosphatase beta/zeta (RPTPbeta), a transmembrane CSPG involved in critical processes such as synaptic plasticity and learning, was observed in patients with Sz. The signaling of neuregulin-1 (NRG1), a multifunctional protein that performs an important role in the development of the central nervous system, was reduced in mice overexpressing RPTPbeta [40]. Further, an increased expression of PTPRZ1, the gene encoding RPTPbeta has been reported in the amygdala and PFC of Sz subjects [40, 73]. Altered expression of NCAN, a gene encoding CSPG neurocan, have also been reported in Sz [74, 75]. Chondroitin sulfate proteoglycan 5 has been indicated as the potential susceptibility gene for Sz, along with other integrating partners, including NR1, ERRB4, and ERBB3 [76]. A recent study combining laser capture microdissection with gene expression profiling extracted RNA from laser-captured pyramidal neurons from postmortem brains from Sz and normal control subjects. The number of genes encoding CSPGs and associated with ECM, including ACAN, VCAN, LUM, and HAPLN1 were downregulated in Sz. In addition, altered expression of genes belonging to ECM related pathways, including transforming growth factor beta (TGF β) signaling, focal adhesion and ECM-receptor interaction pathways were observed [71]. These data strongly suggest a decrease in ECM structural components and enzymes that regulate ECM-related molecules and pathways in Sz.

Proteomics studies of Sz—Proteomics studies using different methods including label-free LC-MS/MS, 2DE-MS, 2D-DIGE-MS, have revealed several common altered proteins and pathways in Sz, compared to normal control samples [77], Table 2C. Altered proteins included enolase proteins (ENO1, ENO2), heat shock proteins (HSP60, HSPA8, HSPA5), vimentin (VIM), tubulin beta chain, 14-3-3 protein zeta/delta (YWHAZ), 14-3-3 protein epsilon (YWHAE), septin 11 (SEPT11), annexin 5, 6 (ANAX5, ANAX6), protein-L-isoaspartate(D-aspartate) *O*-methyltransferase (PCMT1), galectin-1 (LGALS1), superoxide dismutase (SOD1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), peroxiredoxin-1, 5 (PRDX1, PRDX5), and protein S100B, and pathways such as energy metabolism, oxidative stress response, cell growth and maintenance, cell signaling and interaction, cellular assembly and organization, oligodendrocyte dysfunction, and cytoskeleton structure assembly [78-86]. Some serum and blood-based biomarker studies have indicated sets of proteins that correlate with Sz. Examples of these are growth factors; VEGF, EGF, FGFs and cell adhesion molecule ICAM1 [87-89].

With respect to ECM, a recent study of the cytoplasmic proteome of a corpus callosum portion of the human brain from Sz and normal control subjects illustrated 65 differentially expressed proteins, including ECM-related proteins, versican, and HAPLN2 which were observed to be downregulated in Sz patients compared to normal controls [78]. Additionally, an extensive multi- (gen-, prote-, metabolo-) omics study indicated significant upregulation of ECM related tenascin XB, but only at the gene level [79].

Glycomics studies of Sz—Given to the inherent complexity of the human brain, studying only genetic or proteomic variation may not deliver a complete picture of the pathophysiology of Sz and other neurodegenerative disorders. Since most of the ECM molecules are either proteoglycans containing GAG side chains or glycoproteins containing *N*- and/or *O*-linked glycans, understanding glycosylation changes in Sz (and other disorders) is essential, Table 2D. Previous reports have suggested aberrant expression of genes (UGT8, SGPP1, GALC, B4GALT6, SPTLC2, ASAH1) particularly related to glycosphingolipid metabolism and *N*- and *O*-linked glycan biosynthesis in subjects with schizophrenia, compared to controls [90]. Moreover, a study on blood and cerebrospinal fluid (CSF) from Sz and control subjects identified decreases in bisecting and sialylated *N*-glycosylation in Sz patients. In addition, gender-based variations of *N*-glycans, including triantennary trisialylated glycan and tetraantennary tetrasialylated glycan with a polylactosamine extension, were observed between male and female Sz patients [91]. Some studies have measured protein expression of distinct *N*-acetylglucosamine transferases (GlcNAcTs) and found decreased protein expression of UDP-GlcNAc: BetaGal Beta-1,3 GlcNAcT 8 (B3GNT8) and mannosyl (alpha-1,3-)-glycoprotein beta-1,4 GlcNAcT (MGAT4A) expression in Sz, providing further evidence for dysregulated glycosylation in Sz [92].

Alzheimer's disease

Alzheimer's disease (AD) is the leading cause of dementia and cognitive decline in aged populations. Amyloid-beta (Aβ) precursor protein (APP) is associated with the formation of amyloid plaques. Tau protein is involved in the formation of neurofibrillary tangles [93]. Studies have indicated roles of HSPGs and CSPGs in Alzheimer's disease [94-97]. HS has been shown to bind and affect aggregation, intracellular internalization and clearance of Tau and APP [98]. It also regulates APP processing by BACE1, an enzyme that cleaves β site of the amyloid protein [99]. In addition, HS enhances and stabilizes Aβ fibril formation [100], dependent on the degree of sulfation of the HS [101, 102]. Several HSPGs including perlecan, glypican, syndecan, and agrin are associated with amyloid plaques in AD [100, 103-107]. Moreover, several reports have shown perlecan in senile plaques, neurofibrillary tangles, and amyloid-laden vessels [108-110].

Among CSPGs, CS-A, CS-C, and unsulfated chondroitin are associated with lesions of AD [96], and the DSPG decorin has been associated with amyloid plaques and neurofibrillary tangles in AD [111]. Intriguingly, appican, a CSPG modified alternatively spliced isoform of APP, have been observed to express in astrocytes, but not neurons, in AD. Appican-enriched ECM serves as a better substrate for attachment of N2a neuroblastomas, pheochromocytoma PC12 cells, and primary astrocytes, than APP enriched ECM [97, 112].

With respect to ECM, the distribution patterns CSPG in cortical areas of AD and normal human brains suggest that cortical neurons associated with PNNs and large proportions of hyalectan CSPGs were largely absent from neurofibrillary changes associated with AD [113]. Another study indicated decreases in the densities of WFA labeled PNNs in AD compared to normal brain samples [114]. Contrastingly, aggrecan-based PNN's were shown to be unaffected in a transgenic (Tg2576) mouse model of AD [115]. Loss of CSPGs was reported in cells stained with lectin *Vicia villosa*, which binds to terminal *N*-acetyl

galactosamine residues present in CSPGs and/or glycoproteins, in AD compared to normal brains [116]. Taken together, ECM components, including CSPGs, and PNNs decrease in AD, and appear to play a neuroprotective role against tau pathology [117, 118]. Among other ECM components, hyaluronan positive neurons were observed to remain unchanged in AD brains, suggesting resistance of such neurons to neuropathological processes related to AD [119].

Because plaque formation characterizes AD, expression of Aβ degrading enzymes or proteases that degrade Aβ peptides are attractive therapeutic options. Examples of such enzymes include MMP-2, 3, 9, plasmin, neprilysin (NEP), and ADAM-10. ADAMs are increasingly recognized as targets for AD therapy [120]. Because it is an α-secretase involved in non-amyloidogenic cleavage of APP, upregulation of ADAM-10 mediated proteolysis could be a promising strategy to treat a neurodegenerative disorder such as AD [120]. In addition, upregulation of ADAM-10 mRNA expression in severe AD cases has been reported [121], and mutations in ADAM10 have been associated with a reduction of α-secretase in familial late-onset AD [122].

Among ECM glycoproteins, altered expression of reelin and its glycosylation patterns have been reported in CSF from AD patients [123]. A recent study showed perturbed expression of reelin and its downstream signaling members APOER2, VLDLR, and DAB1 in AD. In addition, it was demonstrated that depletion of reelin is an early event of AD pathology, occurring long before the onset of Aβ pathology, suggesting a possible role of decreased reelin expression in precipitation of AD pathology and supporting it as a potential pre-clinical marker of AD [124]. Congruently, reduced expression of reelin in the entorhinal cortex of transgenic mice and humans with AD has been reported [125]. Additionally, reelin and its signaling members have been shown to inhibit Aβ generation, promote Aβ clearance, or prevent tau hyperphosphorylation in AD pathology [126-128].

The αV integrin plays an important role in mediating synaptic dysfunction before neurodegeneration in AD [129]. Also, α2β1 and αVβ1 integrin signaling pathways mediate Aβ induced neurotoxicity in AD and may be a critical component of neurodegeneration [130]. LFA-1 integrin mediates infiltration of neutrophils to amyloid deposits in the brain. Inhibition of neutrophils or blockage of LFA-1 integrin-mediated neutrophil trafficking in a transgenic AD mouse model was shown to reduce AD neuropathology and improve memory in mice, suggesting a direct contribution of integrin-mediated neutrophil trafficking in AD pathogenesis and cognitive impairment [131]. Interestingly, APP regulates the activity of integrin β1 by binding to it. On shedding APP by treatment with alpha-secretase, the inhibitory activity of APP is removed and neurite outgrowth is triggered via integrins [132].

Proteomics studies of AD—In contrast to immunohistochemical studies that showed a reduction of ECM components and PNNs in AD, a recent proteomics study on a mouse model of AD, demonstrated significant increases in several protein components of the extracellular matrix including HAPLN1, neurocan, brevican, and tenascin-R (TNR), with increases in Aβ levels in the hippocampus. These increases in expression occurred with impairment of long-term potentiation and contextual memory, before the onset of plaque formation. Interestingly, chondroitinase ABC injection into the hippocampus restored both

long-term potentiation and contextual memory performance [133]. Another recent study used laser capture microdissection (LCM) to excise hippocampal subareas CA1 and subiculum of 40 AD brains with subsequent LC-MS/MS-based proteomics. Among 372 altered proteins, several ECM components were increased in AD, including tenascin, versican, laminin subunit beta-2, and galectin-1. In addition, increases in adhesion molecules that interact with ECM, including CD44, integrin β1, and integrin αV were observed in AD [134]. In contrast, versican and tenascin-R were significantly decreased in frontal cortex AD brain tissue [135]. Table 3, summarizes the changes in ECM and related components in AD.

Other LC-MS/MS-based proteomics studies on AD brain have identified several proteins, including microtubule-associated protein tau (MAPT), Aβ A4 protein precursor (APP), apolipoprotein E (apoE), ubiquitin carboxy-terminal hydrolase 1 (UCHL1), syntaxin-binding protein 1 (Munc-18), malate dehydrogenase (MDH2), protein piccolo (PCLO), transformation/transcription domain-associated protein (TRRAP), 14-3-3 protein zeta/delta (YWHAZ), and mucin 19 (MUC19), and pathways including signal transduction, immune response, cytoskeleton organization, lipid metabolism, energy metabolism, glycolysis, apoptosis, oxidative stress, oxidative phosphorylation and synaptic functioning, significantly altered in AD [135-141]. Further, blood plasma and serum proteomics studies from AD and control samples have been utilized to identify AD diagnostic biomarker candidates [142, 143]. For details on neuroproteomics in AD, refer to a recent review [144].

Glycomics studies of AD—Both tau and APP carry *N*- and *O*-glycosylation. Further, tau protein *N*-glycosylation occurs only in AD, and not in controls [145]. A recent study indicated GnT-III, an enzyme responsible for the formation of bisecting-GlcNAc type *N*-glycans, as a promising target for AD therapeutics. It was also shown that BACE1, an enzyme required to cleave amyloid-β (Aβ) and generate Aβ plaques in AD, is post-translationally modified with bisecting-GlcNAc type *N*-glycans in AD patients. Using knockout mouse mutants lacking GnT-III, decreased cleavage of APP by BACE1 was observed, resulting in reduced amyloid plaques and improved cognitive functions. It was suggested that due to lack of this sugar modification, BACE1 is directed towards late lysosomes, subjected to lysosomal degradation and thus less co-localized with APP [146]. Consistent with the above observation, an *N*-glycome profiling study indicated an increase in bisecting-GlcNAc type *N*-glycans and a decrease in overall sialylation in CSF from AD patients compared to normal controls [147]. The decrease in sialylated *N*-glycans in AD could be attributed to decreased sialyltransferase activity, which has been reported in serum as well as membrane and soluble fractions from postmortem brain from AD patients [148, 149]. In addition, lectin studies have shown decreased binding of WGA (wheat germ agglutinin), a lectin, that binds to sialic acid residues, in CSF of AD patients [150, 151]. Other than sialylated and bisecting *N*-glycans, reduction of NA2F, a core-fucosylated biantennary terminal galactose *N*-glycan structure, has been reported in AD compared to healthy controls, and it has been suggested to serve as suitable serum *N*-glycan marker for AD [152].

Current state of proteomics in studies of neurodegenerative disorders

Genomics and immunohistochemical analysis are widely employed to study neurodegenerative disorders [41, 42, 69, 71, 113, 114, 118]. They have provided a base of information for understanding the molecular deregulation associated with neurodegenerative disorders; however, genomics analysis does not sufficiently define the alterations at the protein level and associated biological functions. Expression and function of proteins can be modulated during transcription, translation and subsequently by post-translational modifications (PTMs), including phosphorylation, glycosylation, and acetylation. Proteomics analysis allows identification and quantification of thousands of proteins from complex mixtures, together with PTM and protein-protein interaction information, that are important to understand complete neuropathophysiology of these disorders.

In recent years, significant advances have been made in neuroproteomics, with a large number of well-executed proteomics studies providing some common altered proteins and pathways. However, the lack of consistent results and follow-up studies remains a limiting factor. For example, a recent Sz proteomics study [78], compared their findings with previous studies [79-85], and found consistent alteration of only 30 proteins, including 14-3-3 protein zeta/delta (YWHAZ), 14-3-3 protein epsilon (YWHAE), ENO1, ENO2, SOD1, GAPDH, PRDX1, VIM, and pathways, including energy and metabolism, cytoskeletal structure and function oxidative stress response, and oligodendrocyte dysfunction; however, the expression patterns of some of the proteins were observed to be in the opposite direction.

Similarly, for AD, a review [153], compared 43 2-D proteomics studies published between 1999-2010. A total of 93 proteins were differentially expressed in 13 different brain regions. Among these, only 42 altered proteins, were found in more than one study, regardless of brain region, while only 11 proteins (APOA1, apoE, PTGDS, TTR, ALDOA, FABP3, GAPDH, ENO1, GFAP, P1N1, UCHL1) were found to alter in a similar direction, in more than one study within the same brain region. For LC-MS/MS proteomics, a study [136], compared its 197 differentially expressed proteins with previous work [140, 141, 153-156] and found only 19 common proteins, including APP, YWHAZ, PRDX1, ANXA1, and HSPA12A, altered in the same direction.

Interestingly, low concordance was observed for differential proteins, while high concordance was observed for non-differential proteins. The low concordance rate of the altered proteome among different studies leads to inconsistent conclusions regarding pathological patterns related to these disorders. It is difficult to compare proteomics studies because several points of variation can emerge based on the type of sample used, sample handling techniques, and mass spectrometry methods. As shown in Table 4, several recent proteomics studies for Sz and AD employed different brain regions, a wide range of sample handling techniques and mass spectrometry instrumentation, leading to variable data, and only a few similarities in the findings, as indicated above. Increased use of standardized methods will improve the ability to compare protein expression in brain proteomics.

The human brain is a complex structure, with cellular and ECM heterogeneity based on the brain region. Thus, studies covering different brain regions make proteomics analysis much more challenging. In addition, studies on the human brain may have been affected by post-mortem interval and differences in tissue processing procedures [157]. Nevertheless, there remains an overwhelming need to characterize protein expression in both postmortem brains and animal models [158]. A recent study indicated a significant variability in quantitative LC-MS proteomics of human brain tissue samples arise from extraction methods (72%), and other variables, including instrumental variance (16%), instrumental stability (8.4%) and proteolytic digestion (3.1%) [159]. Because of this variability, it is necessary to analyze a large cohort of biological samples to validate the findings with enough statistical significance. In addition, differences in results could also be attributed to the method of separation and fractionation. Two-dimensional gel electrophoresis (2-DE), later modified to 2-D difference in-gel electrophoresis (2-D DIGE), were two methods of choice over the past two decades. However, these gel-based techniques involve certain limitations, including low sensitivity, underrepresentation of some low abundance and low solubility membrane proteins [160-162]. Gel-free approaches using liquid chromatography directly coupled to a mass spectrometer have been employed for label-free or label-based quantitative proteomics, to overcome such limitations. Although label-based quantitative proteomics allows accurate protein profiling, the limitations of such approaches include additional time-consuming sample preparation, the requirement of higher sample amounts, the risk of incomplete labeling, and the cost of the (commercial) reagents. Label-free quantitative proteomics is, therefore, an attractive alternative since it uses a robust, reasonable accurate, straightforward and time-effective experimental workflow.

For neuroproteomics, the majority of studies have taken a discovery approach, and not all have performed orthogonal validation of the findings, Table 4. Minimal attention has been paid to ECM-related proteins and PTMs, with very few studies indicating modifications of the proteins, and ECM proteins among differentially expressed proteins. Importantly, many of these proteomics studies were lacking relevant information including complete proteome lists, peptide lists, sequence coverage, and modifications. Such information is of high relevance to the neuroproteomics community, for cross-validation and comparison of the data, to understand neuropathophysiology.

ECM-related proteins were observed to be differentially expressed in only six studies, Table 4. Intriguingly, versican, one of the hyalectans, was differentially expressed in three out of six studies [78, 134, 135], reporting differential expression of ECM proteins. Notably, all three studies were performed as shotgun proteomics experiments, with label-free LC-MS/MS analysis, using an LTQ-Orbitrap instrument. The peptide sequence coverage for versican identified by two out of the three studies (one study did not provide sequence coverage information), is shown in Fig. 2. A study [135] identified 8% sequence coverage (yellow), while another study [134], identified 17% sequence coverage (red). Major domains covered by these studies were Ig-like V-type (21-146), link 1(150-245), link 2 (251-347), EGF-like 2, calcium binding (3176-3290) and c-type lectin (3294-3354) domains. Some peptides from GAG regions (GAG-alpha; 348-1335, GAG beta; 1336-3089) were also covered, Fig.2.

Future directions

Previous proteomics studies of neurodegenerative disorders, including Sz and AD, show a lack of concordance with respect to differentially expressed proteins and associated pathways, which could be due to technical variability among different studies, including sample (number of samples, different brain regions), sample handling (2D, 2D-DIGE, LC-MS/MS), and MS (Orbitrap, Q-TOF, TOF/TOF), Table 4. Several steps can be taken to rule out such technical limitations and to obtain reliable and confident proteome profiles such as use of larger cohort of samples, robust and appropriate biological models with the correct controls, validation of protein expression levels by using a sufficient number of biological and technical replicate experiments, stringent data analysis, cross-validation of regulated proteins using orthogonal molecular techniques, e.g. western blotting, MRM, and ELISA, and use of ultra-performance high-end mass spectrometers.

Furthermore, although PTMs play important roles in regulating protein expression and related biological pathways [163], they have received very little attention in neuroproteomics. There is a need to perform in-depth analysis of global proteome data, since proteomics experiments deliver thousands of peptide fragments, and thus, there are substantial chances of finding modified peptides. Since modified peptides are present in low abundances compared to non-modified peptides, the use of enrichment methods will likely increase the relative abundances of PTM-peptides in the protein mixtures. Some examples of enrichment techniques include immunoprecipitation, affinity chromatography, and chemical derivatisation.

A plethora of altered proteins and biological pathways has been identified in neuroproteomics; but, disappointingly, only a handful of proteomics studies have identified altered ECM components. Nonetheless, some IHC and genomics studies have indicated changes in neural ECM associated with neurodegenerative conditions. Thus, given the importance of ECM components in neurodegenerative disorders, future neuroproteomics studies should target such ECM proteins for a better understanding of neuropathophysiology. At present, despite low concordance, and lack of additional information, studies have provided a large amount of information and datasets contributing to the knowledge base for understanding complex neuropathophysiology of neurodegenerative disorders.

Acknowledgements

Abbreviations

ECM	extracellular matrix
PGs	proteoglycans
GAGs	glycosaminoglycans
CS	chondroitin sulfate
HS	heparan sulfate

DS	dermatan sulfate
KS	keratan sulfate
HA	hyaluronic acid
Sz	schizophrenia
AD	Alzheimer's disease
PNNs	perineuronal nets
GalNAc	*N*-acetylgalactosamine
GlcNAc	*N*-acetylglucosamine
GlcA	glucuronic acid
IdoA	iduronic acid
1-DE	one-dimensional gel electrophoresis
2-DE	two-dimensional gel electrophoresis
2-D DIGE	two-dimensional difference in-gel electrophoresis
LC	liquid chromatography
MS	mass spectrometer
MALDI	matrix-assisted laser desorption/ionization
TOF	time-of-flight
PTMs	post-translational modifications

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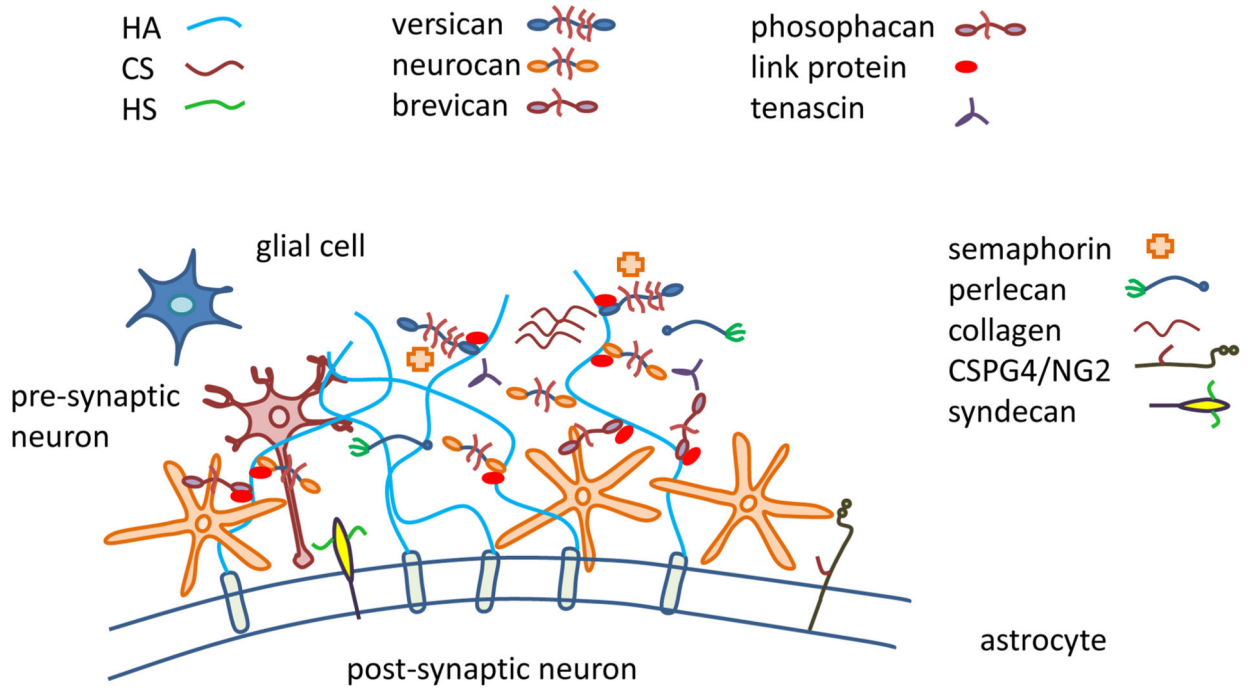
Fig. 1.
Brain extracellular matrix showing HSPGs, CSPGs, HA, collagens, and other glycoproteins

MFINIKSILW MCSTLIVTHA21LHKVKVGKSP PVRGSLSGKV SLPCHFSTMP TLPPSYNTSE FLRIKWSKIE VDKNGKDLKE TTVLVAQNGN IKIGQDYKGR
VSVPTHPEAV GDASLTVVKL LASDAGLYRC DVMYGIEDTQ DTVSLT146VDGV150 VFHYR AATSR YTLNFEAAQK ACLDVGAVIA TPEQLFAAYE
DGFEQCDAGW LADQTVRYPI RAPRVGCYGD KMGKAGVRTY GFRSPQETYD VYCYV243DHLDG 251DVFHLTVPSK FTFEEAAKEC ENQDARLATV
GELQAAWRNG FDQCDYGWLS DASVRHPVTV ARAQCGGGLL GVRTLYRFEN QTGFPPP347D348SR FDAYCFKPKE ATTIDLSILA ETASPSLSKE
PQMVSDRTTP IIPLVDELPV IPTEFPPVGN IVSFEQKATV QPQAITDSLA TKLPTPTGST KKPWDMDDYS PSASGPLGKL DISEIKEEVL QSTTGVSHYA
TDSWDGVVED KQTQESVTQI EQIEVGPLVT SMEILKHIPS KEFPVTETPL VTARMILESK TEKKMVSTVS ELVTTGHYGF TLGEEDDEDR TLTVGSDEST
LIFDQIPEVI TVSKTSEDTI HTHLEDLESV SASTTVSPLI MPDNNGSSMD DWEERQTSGR ITEEFLGKYL STTPFPSQHR TEIELFPYSG DKILVEGIST
VIYPSLQTEM THRRERTETL IPEMRTDTYT DEIQEEITKS PFMGKTEEEVFSGMKLSTSL SEPIHVTESS VEMTKSFDFP TLITKLSAEP TEVRDMEEDF
TATPGTTKYD ENITTVLLAH GTLSVEAATV SKWSWDEDNT TSKPLESTEP SASSKLPPAL LTTVGMNGKD KDIPSFTEDG ADEFTLIPDS TQKQLEEVTD
EDIAAHGKFT IRFQPTTSTG IAEKSTLRDS TTEEKVPPIT STEGQVYATM EGSALGEVED VDLSKPVSTV PQFAHTSEVE GLAFVSYSST QEPTTYVDSS
HTIPLSVIPK TDWGVLVPSV PSEDEVLGEP SQDILVIDQT RLEATISPET MRTTKITEGT TQEEFPWKEQ TAEKPVPALS STAWTPKEAV TPLDEQEGDG
SAYTVSEDEL LTGSERVPVL ETTPVGKIDH SVSYPPGAVT EHKVKTDEVV TLTPRIGPKV SLSPGPEQKY ETEGSSTTGF TSSLSPFSTH ITQLMEETTT
EKTSLEDIDL GSGLFEKPKA TELIEFSTIK VTVPSDITTA FSSVDRLHTT SAFKPSSAIT KKPPLIDREP GEETTSDMVI IGESTSHVPP TTLEDIVAKE TETDIDREYF
TTSSPPATQP TRPPTVEDKE 1QPPASTKFHP DINVYIIEVR ENKTG1335R1336MSDL SVIGHPIDSE SKEDEPCSEE TDPVHDLMAE ILPEFPDIIE IDLYHSEENE
EEEEECANAT DVTTTPSVQY INGKHLVTTV PKDPEAAEAR RGQFESVAPS QNFSDSSESD THPFVIAKTE LSTAVQPNES TETTESLEVT WKPETYPETS
EHFSGGEPDV FPTVPFHEEF ESGTAKKGAE SVTERDTEVG HQAHEHTEPV SLFPEESSGE IAIDQESQKI AFARATEVTF GEEVEKSTSV TYTPTIVPSS
ASAYVSEEEA VTLIGNPWPD DLLSTKESWV EATPRQVVEL SGSSSIPITE GSGEAEEDED TMFTMVTDLS QRNTTDTLIT LDTSRIITES FFEVPATTIY
PVSEQPSAKV VPTKFVSETD TSEWISSTTV EEKKRKEEEG TTGTASTFEV YSSTQRSDQL ILPFELESPN VATSSDSGTR KSFMSLTTPT QSEREMTDST
PVFTETNTLE NLGAQTTEHS SIHQPGVQEG LTTLPRSPAS VFMEQGSGEA AADPETTTVS SFSLNVEYAI QAEKEVAGTL SPHVETTFST EPTGLVLSTV
MDRVVAENIT QTSREIVISE RLGEPNYGAE IRGFSTGFPL EEDFSGDFRE YSTVSHPIAK EETVMMEGSG DAAFRDTQTS PSTVPTSVHI SHISDSEGPS
STMVSTSAFP SGEQLVTVSS SVVPVLPSAV QK FSGTASSI IDEGLGEVGT VNEIDRRSTI LPTAEVEGTK APVEKEEVKV SGTVSTNFPQ TIEPAKLWSR
QEVNPVRQEI ESETTSEEQI QEEKSFESPQ NSPATEQTIF DSQTFTETEL KTTDYSVLTT KKTYSDDKEM KEEDTSLVNM STPDPDANGL ESYTTLPEAT
EKSHFFLATA LVTESIPAEH VVTDSPIKKE ESTKHFPKGM RPTIQESDTE LLFSGLGSGE EVLPTLPTES VNFTEVEQIN NTLYPHTSQV ESTSSDKIED
FNRMENVAKE VGPLVSQTDI FEGSGGVTST TLIEILSDTG AEGPTVAPLP FSTDIGHPQN QTVRWAEEIQ TSRPQTITEQ DSNKNSSTAE INETTTSSTD
FLARAYGFEM AKEFVTSAPK PSDLYYEPSG EGSGEVDIVD SFHTSATTQA TRQESSTTFV SDGSLEKHPE VPSAKAVTAD GFPTVSVMLP LHSEQNKSSP
DPTSTLSNTV SYERSTDGSF QDRFEFEDS TLKPNRKKPT ENIIIDLDKE DKDLILTITE STILEILPEL TSDKNTIIDI DHTKPVYEDI LGMQTDIDTE VPSEPHDSND
ESNDDSTQVQ EIYEAAVNLS LTEETFEGSA DVLASYTQAT HDESMTYEDR SQLDHMGFHF TTGIPAPSTE TELDVLLPTA TSLPIPRKSA TVIPEIEGIK
AEAKALDDMF ESSTLSDGQA IADQSEIIPT LGQFERTQEE YEDKKHAGPS FQPEFSSGAE EALVDHTPYL SIATTHLMDQ SVTEVPDVME GSNPPYYTDT
TLAVSTFAKL SSQTPSSPLT IYSGSEASGH TEIPQPSALP GIDVGSSVMS PQDSFKEIHV NIEATFKPSS EEYLHITEPP SLSPDTKLEP SEDDGKPELL
EEMEASPTEL IAVEGTEILQ DFQNKTDGQV SGEAIKMFPT IKTPEAGTVI TTADEIELEG ATQWPHSTSA SATYGVEAGV VPWLSPQTSE RPTLSSSPEI
NPETQAALIR GQDSTIAASE QQVAARILDS NDQATVNPVE FNTEVATPPF SLLETSNETD FLIGINEESV EGTAIYLPG3089P DRCKMNPCLN GGTCYPTETS
YVCTCVPGYS GDQCE3125LD3217FDE CHSNPCRNGA TCVDGFNTFR CLCLPSYVGA LCE3163QDTETCD YGWHKF3176QGGC YKYFAHRRTW DAAERECRLQ
GAHLTSILSH EEQMFVNRVG HDYQWIGLND KMFEHDFRWT DGSTLQYENW RPNQPDSFFS AGEDCVVIIW HENGQWNDVP CNYHLTYTCK3290
KGTV3294ACGQPP VVENAKTFGK MKPRYEINSL IRYHCKDGFI QRHLPTIRCL GNGRWAIPKI TCMN3354PSAYQR TYSMKYFKNS SSAKDNSINT
SKHDHRWSRR WQESRR

Fig. 2.

Peptide sequence coverage for Versican protein, identified by previous studies [135] (*yellow)* and [134] (red). Domain (blue numbers), Ig-like V-type; 21-146; link 1; 150-245, link 2; 251-347, EGF-like 1; 3089-3125, EGF-like 2, calcium binding; 3127-3163, c-type lectin; 3176-3290, Sushi; 3294-3354. GAG region (green numbers), GAG-alpha; 348-1335, GAG beta; 1336-3089.

Table 1

Glycosaminoglycan repeating unit structures: hyaluronan (HA), heparan sulfate (HS, including heparin), chondroitin sulfate (CS), dermatan sulfate (DS), and keratan sulfate (KS).

GAG	Repeating unit	Modifications
Hyaluronan (HA)	GlcAβ3GlcNAcβ4	
Heparan sulfate (HS) and Heparin	GlcA/IdoAβ4/α4GlcNα4	W, X, Y = H or SO₃H Z = Ac or SO₃H or (rarely)H
Chondroitin Sulfate (CS)	GlcAβ3GalNAcβ4	W, X, Y = H or SO₃H
Dermatan Sulfate (DS)	GlcA/IdoAβ/α3GalNAcβ4	W, X, Y = H or SO₃H
Keratan Sulfate (KS)	Galβ4GlcNAcβ3	X = H or SO₃H

Table 2

Expression of ECM related components, including PNNs, PGs, glycoproteins, proteins and other ECM unrelated proteins in Schizophrenia (Sz) as reported in A) immunohistochemical studies B) Genomics studies, C) Proteomics studies, and D) Glycomics or glycosylation based studies.

A. Immunohistochemical studies	ECM component	Brain region	Change in Sz	References
	CSPG-labeled PNN (WFA labeling)	Amygdala and Entorhinal cortex	↓	[41]
	WFA-labeled PNNs	Layer 3 and 5 of the PFC	↓	[42]
	Aggrecan positive PNNs	Amygdala	↓	[44]
	Aggrecan positive PNNs and WFA labeled PNNs	Dorsolateral Prefrontal Cortex	↓	[51]
	Numerical densities of cytoplasmic-CSPG	Olfactory receptor neurons (ORNs)	↓	[42]

B. Genomics / Transcriptomics	Gene	Symbol	Change in Sz	References
	ECM-related genes			
	Aggrecan	ACAN	↓	[71]
	Versican	VCAN	↓	[71]
	Neurocan	NCAN	↓	[71]
	Lumican	LUM	↓	[71]
	Hyaluronan and proteoglycan link protein 1	HAPLN1	↓	[71]
	Reelin	RELN	↑, ↓	[52, 53], [164]
	Tenascin XB	TNXB	↑	[79]
	Fibronectin	FN	↓	[58, 59]
	Integrin $\alpha 2_b \beta 3_a$	ITG$\alpha 2_b \beta 3_a$	↓	[63]
	Matrix metalloproteinase-9	MMP-9	↓, ↑	[72], [69, 70]
	Matrix metalloproteinase-16	MMP-16	↑	[71]
	Matrix metalloproteinase-24	MMP-24	↓	[71]
	Matrix metalloproteinase-25	MMP-25	↓	[71]
	A disintegrin and metalloproteinase with thrombospondin motifs 1	ADAMTS 1	↑	[71]
	A disintegrin and metalloproteinase with thrombospondin motifs 6	ADAMTS 6	↑	[71]
	Phosphotyrosine phosphatase beta/zeta	PTPRZ1	↓	[40, 73]
	Other proteins			
	Neuregulin-1 isoform 1	NRG1	↑	[165, 166]
	Neuregulin-1 isoform 2	NRG1	↓	[165, 166]
	Receptor tyrosine-protein kinase erbB-4	ERBB4	↑	[167]

C. Proteomics	Protein	Symbol	Change in Sz	References
	ECM-related proteins			
	Versican core protein	VCAN	↓	[78]
	Hyaluronan and proteoglycan link protein 2	HAPLN2	↓	[78]

C. Proteomics	Protein	Symbol	Change in Sz	References
	Galectin-1	LGALS1	↓	[81, 82]
	Vimentin	VIM	↑	[78, 81, 82]
	Other proteins			
	Enolase 1	ENO1	↑	[78, 83, 84]
	Enolase 2	ENO2	↑	[78, 79, 83]
	Peroxiredoxin-1, 5	PRDX1, PRDX5	↓	[78-81, 85],[84]
	Glyceraldehyde-3 -phosphate dehydrogenase	GAPDH	↓	[78-81, 85]
	Superoxide dismutase 1	SOD1	↓,↑	[78-81, 85], [84]
	Heat shock 70 kDa protein	HSPA8	↑,↓	[79, 80, 82], [84]
	Transferrin	TF	↓,↑	[79, 80], [82]
	14-3-3 protein zeta/delta	YWHAZ	↓	[78, 80, 82]
	Protein S100-B	S100B	↓	[86]
	Septin-11	SEPT11	↓	[83, 84]
	Annexin A5, 6	ANXA5, ANXA6	↓	[83, 84]

D. Glycomics / Glycosylation based studies	Glycans or glycosylated genes	Symbol	Change in Sz	References
	Bisecting *N*-glycans	-	↓	[91]
	Sialylated *N*-glycans	-	↓	[91]
	UDP-GlcNAc:BetaGal Beta-1,3 GlcNAcT 8	B3GNT8	↓	[92]
	Mannosyl (alpha-1,3-)-glycoprotein beta-1,4 GlcNAcT	MGAT4A	↓	[92]
	2-hydroxyacylsphingosine 1-beta-galactosyltransferase	UGT8	↓	[90]
	Sphingosine-1-phosphate phosphatase 1	SGPP1	↓	[90]
	Galactocerebrosidase	GALC	↓	[90]
	Beta-1, 4-galactosyltransferase 6	B4GALT6	↓	[90]
	Serine palmitoyltransferase 2	SPTLC2	↓	[90]
	Acid ceramidase	ASAH1	↓	[90]
	Galactosylceramide sulfotransferase	GAL3ST1	↓	[90]

Table 3

Expression pattern of ECM and associated components in AD.

ECM and related component	Change	References
WFA-labeled PNNs	↓	[114]
N-acetyl galactosamine residues stained by lectin *Vicia villosa*	↓	[116]
ADAM-10	↑	[121]
Reelin	↓	[125], [124]
Hyaluronan and proteoglycan link protein 1	↑	[133]
Neurocan	↑	[133]
Brevican	↑	[133]
Tenascin-R	↑,↓	[133] ,[134], [135]
Versican	↑,↓	[134], [135]
Laminin subunit beta-2	↑	[134]
Galectin-1	↑	[134]
CD44 antigen	↑	[134]
Integrin beta-1	↑	[134]
Integrin alpha-V	↑	[134]

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Table 4

Examples of proteomics studies for Sz and AD, in recent years, employing different brain regions, a wide range of sample handling techniques and mass spectrometry instruments.

Brain Region	Sample handling techniques	Mass Spectrometry	% Sequence coverage range for differential expressed proteins	Modifications Identified	Orthogonal Validation	Differential expression of ECM-related proteins observed?	References
Schizophrenia (Sz)							
Corpus Callosum (CC)	Shotgun proteomics (Label-free)	LTQ-Orbitrap XL	No information	None	None	Yes (VCAN, HAPLN2)	*Saia-Cereda et al., 2015* [78]
Dorsolateral Prefrontal Cortex	2DE	QSTAR XL	14-78%	None	None	No	*Sivagnanasundaram et al., 2007* [85]
Dorsolateral Prefrontal Cortex	2DE	MALDI-TOF/TOF	No information	None	Western blotting	No	*Martins-de-Souza et al., 2009* [80]
Mediodorsal Thalamus	2DE, iTRAQ-IEF	MALDI-TOF/TOF	No	None	Western blotting	Yes (LGALS1, VIM)	*Martins-de-Souza et al., 2010* [81]
Prefrontal Cortex	2D-DIGE	QTOF/TOF	2-56%	None	Metabolomics, genomics	No	*Prabakaran et al., 2004* [79]
Dorsolateral Prefrontal Cortex	2D-DIGE	LTQ	1-55%	None	ELISA	Yes (LGALS1, VIM)	*English et al., 2009* [82]
Hippocampus	2D-DIGE	Q-TOF	No information	None	Western blotting and ELISA	No	*Focking et al., 2011* [83]
Hippocampus	2D-DIGE	Q-TOF	No information	None	None	No	*Schubert et al., 2015* [84]
Alzheimer's disease (AD)							
Whole Brain	Shotgun proteomics (AMT tag approach)	LTQ Orbitrap	No information	None	Western blotting	No	*Andreev et al., 2012* [136]
Frontal Cortex	Resolution shotgun proteomics (Label-free)	Orbitrap	Yes [a]	None	IHC, Western blotting	None	*Gozal et al., 2009* [141]
Frontal Cortex	Resolution shotgun proteomics (Label-free)	LTQ Orbitrap	8-68%	None	Immunoblotting	Yes (VCAN, TNR)	*Donovan et al., 2012* [135]
Hippocampus	Laser capture microdissection (LCM), In-gel shotgun proteomics (Label-free)	LTQ Orbitrap	Yes [a]	None	IHC	Yes (VCAN, TNC, LGALS1, LAMB2)	*Hondius et al., 2016* [134]
Frontal Cortex	2D-DIGE	MALDI-TOF	8-59%	None	None	No	*Muller et al., 2008* [168]
Entorhinal Cortex	1DE, 2DE	MALDI-TOF	No	Increased ubiquitination	IHC, Western blotting	No	*Riederer et al., 2009* [169]
Inferior Parietal Lobule	2DE	MALDI-TOF	39-56% (for glutathionylated proteins)	Increased S-glutathionylation	None	No	*Newman et al., 2007* [170]

[a] Percent not included, peptide sequence information provided