Neurodegeneration and Alzheimer’s disease (AD). What Can Proteomics Tell Us About the Alzheimer’s Brain?*

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Neurodegenerative diseases, such as Alzheimer’s diseases (AD), are becoming more prevalent as the population ages. However, the mechanisms that lead to synapse destabilization and neuron death remain elusive. The advent of proteomics has allowed for high-throughput screening methods to search for biomarkers that could lead to early diagnosis and treatment and to identify alterations in the cellular proteome that could provide insight into disease etiology and possible treatment avenues. In this review, we have concentrated mainly on the findings that are related to how and whether proteomics studies have contributed to two aspects of AD research, the development of biomarkers for clinical diagnostics, and the recognition of proteins that can help elucidate the pathways leading to AD brain pathology. As a result of these studies, several candidate cerebrospinal fluid biomarkers are now available for further validation in different AD cohorts. Studies in AD brain and AD transgenic models support the notion that oxidative damage results in the alterations of metabolic enzymes and that mitochondrial dysfunction is central to AD neuropathology. Molecular & Cellular Proteomics 15: 10.1074/mcp.R115.053330, 409–425, 2016.

Neurodegenerative diseases are becoming more prevalent as the population ages, yet the mechanisms that lead to synapse destabilization and neuronal death remain elusive. The advent of proteomics has led to methods for high-throughput screening to search for biomarkers that can be used for the early diagnosis and treatment of various diseases and to identify alterations in the cellular proteome that can provide insight into disease etiology and potential avenues for treatment. How and why only specific classes of neurons are affected when a genetic mutation is identified in a ubiquitously expressed gene are major questions that underlie the study of neurodegeneration. Clear examples of this phenomenon are the mutations in amyloid precursor protein (APP) or presenilin 1 (PSEN1) and 2 (PSEN2) that occur in Alzheimer’s disease (AD)1, which affect learning and memory circuits (1, 2); super oxide dismutase 1 (SOD1) mutations that specifically affect motor neurons (MNs) in amyotrophic lateral sclerosis (3); huntingtin mutations that affect cortico-striatal circuits in cases of Huntington’s disease (4, 5) and Parkin and Pink1 mutations associated with autosomal recessive familial early-onset Parkinson disease targeting dopamine generating cells in the substantia nigra (6, 7).

All the neurodegenerative diseases mentioned above are characterized by neuronal dysfunction and neuronal death. However, they are distinct in terms of their genetics, pathologies, phenotypes, and treatments. Proteomics studies have been performed to analyze differentially expressed proteins in different disease paradigms, however, the diversity of models and samples that have been included in these studies are too numerous to cover in a review. We have therefore focused mainly on studies performed in AD because it is the most prevalent neurodegenerative disorder (8) and a larger number of studies have been performed using similar biological samples. Thus, in this review, we have concentrated mainly on findings related to how and whether proteomics studies have contributed to the identification of biomarkers or to our understanding of brain pathology in AD.

AD is a multifactorial and complex neurodegenerative disorder that is characterized by progressive and severe dementia with neuropsychiatric symptoms. AD is the most common cause of progressive dementia in the elderly, accounting for ~70% of all dementia cases. It affects 5–10% of the population above the age of 65 years old and 40% of people above the age of 80 years old (8). The majority of AD cases are

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1 The abbreviations used are: AD, Alzheimer’s Disease; MN, motor neuron; LC-MS/MS, liquid chromatography-tandem MS; GAPDH, glyceraldehyde 2-phosphate dehydrogenase; APP, amyloid precursor protein; NFT, neurofibrillary tangles; CSF, cerebrospinal fluid; PET, positron emission tomography; MCI, mild cognitive impairment; HC, healthy control; H-Fabps, heart fatty acid binding protein; FDR, false discovery rate; HSP, heat shock protein.
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tion for these failures is that the treatments are applied too late, when the window to ameliorate neurodegeneration in AD is no longer available. Therefore, defining the preclinical early stages of AD and identifying predictive biomarker signatures for use in diagnosing patients who are progressing from mild cognitive impairment (MCI) to early and late AD have been a major challenge and the focus of several proteomics studies (27, 32–37).

The Path Toward Discovery of New Biomarkers for AD in the CSF and Blood—The discovery of new biomarkers for neurodegenerative diseases is important because they may improve diagnosis, predict disease progression, and improve our understanding of neuropathological changes in the brain in addition to serving as therapeutic targets. Therefore, samples derived from AD patients have been a focus of explorative proteomics studies of the CSF, plasma, blood and brain (37, 38). There is a constant diffusion of proteins from the brain and spinal cord to the CSF that surrounds them, which makes the CSF an attractive target for proteomic studies, as it may reflect alterations in protein contents occurring in the brain (39). Thus, CSF samples have been a primary target when searching for biomarker candidates in AD and other neurodegenerative diseases (30, 40) supported also by the use of phospho-tau and Aβ(1–42) as diagnostic markers in the CSF.

One key advantage of using proteomics is that many proteins and protein variants (such as isoforms or post-translational modification of proteins) can be simultaneously characterized and quantified. In addition, proteomics allows for an unbiased experimental design that generates results that bring about new hypotheses for further research. Thus, science progresses in an unprejudiced direction, and this fosters the discovery of new pathways and significant biological interactions. MS has emerged as the most widely adopted technological platform for conducting proteomics studies in biomedical research. However, such broad and unbiased profiling comes at the expense of reduced sensitivity and stochastic sampling. In particular, highly abundant proteins obscure the identification of significant changes in less abundant proteins. In addition, consistent results between different laboratories have been difficult to achieve, partly as a result of the complexity and laborious nature of the methodologies being used, such as 2D-electrophoresis (2D-E) or separation chromatography prior to MS detection, and differences in sample preparation protocols. In addition, MS is limited to providing consistent and reproducible measurements of peptides; therefore, the verification of MS results using other more analytical methodologies is required (41, 42). Although 2D-E electrophoresis prior to MS detection has good resolution, dynamic range, and high throughput protein separation, there are also several caveats to use this methodology. For example, many protein spots are likely comprised of multiple proteins, it has poor protein resolution of very basic or acidic proteins and there are difficulties in electrophoresis of large and hydrophobic proteins (43). Therefore, other MS-based quantification methods have gained increasing support over the past years. Most of these methods employ differential stable isotope labeling to create a specific mass tag (43, 44). Exploratory studies to identify biomarkers for AD in the CSF have recently been performed and reviewed in recent years (37, 38). Of over one hundred potential biomarkers that have been found in previous studies, only four of them—apolipoprotein A-1 (apoA1), apolipoprotein E (apoE), prostaglandin H2 D isomerase (PTGD), and transthyretin (TTR)—were reported to be consistently down-regulated in AD CSF in at least two different studies. Differences in the methods used to select patients and the protocols used to prepare samples may account for this lack of concurrence, in addition to the limitations of MS and associated techniques, as discussed above (37).

To overcome these problems in exploratory proteomics, targeted MS platforms have been developed as tools for biomarker validation. These focus the resources of the mass spectrometer on a defined subset of analytes (peptides) (36, 41, 42, 45). To facilitate this process, the biomarker consortium CSF proteomics project team (Alzheimer’s Association, www.alz.org) developed a targeted proteomic, multiplexed, mass spectrometry-based approach for the qualification of candidate biomarkers in the well-characterized AD neuroimaging initiative (ADNI) cohort (46). They have defined a panel of peptides that were extrapolated from previously published biomarkers studies to discriminate between AD patients and healthy controls (HC) and MCI patients and HC. In addition, they analyzed patients with MCI that did or did not progress to AD after 3 years. The panel consists of 567 peptides from 222 proteins, and the analyzed samples included 66 AD patients, 134 MCI patients, and 85 HC. Included in the panel of proteins were apoA1, apoE, PTGDs, and TTR, which are mentioned above. Of these four proteins, only TTR differentiated AD versus HC and MCI versus HC. However, when an FDR (Benjamini–Hochberg false discovery rate) correction was applied, no significant differences were found in the levels of TTR or indeed in any of the proteins analyzed in this panel (46).

More specifically, no changes were identified in the level of apoE, independent of the genotypes of the patients (46). This point has been controversial in the literature. Because apoE4 is the major risk factor for developing AD, it was expected that there would be a correlation between apoE genotypes and apoE levels in the CSF, as previously reported (37, 47). At least two different studies that used either common peptides to detect all apoE variants or peptides that were specific for each apoE variant found no differences (46, 48), strongly suggesting that apoE levels in the CSF do not differentiate AD or MCI patients from HC or patients who progress from those who do not progress to AD in the MCI population (46). More studies will be required to settle this controversy. Another interesting set of candidate biomarkers identified in the study of Spellman and colleagues were peptides belonging to the
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Heart fatty acid binding protein (H-Fabps) that were the most-altered peptides between AD and MCI patients versus HC (46). Consistent with these results, other studies have identified elevated levels of H-Fabps in the CSF of AD patients (49, 50). In addition, a correlation was found between increased H-Fabps levels and entorhinal cortex atrophy in incipient AD patients (51). However, more studies must be performed to validate the usefulness of this biomarker because, as in the analysis described above, after applying FDR correction, no significant differences were found in the levels of the peptides analyzed that corresponded to H-Fabps between populations (46). Although these studies suggest that differences in H-Fabps levels in the CSF might be related to the dysregulation of lipid homeostasis in AD (52), more studies are required to understand the role H-Fabps in brain physiology and AD neuropathology.

Two additional analyses were performed to identify peptides that differentiate or predict the progression from MCI to AD versus the lack of progression from MCI to AD (46). The result suggested that increased expression of Hemoglobin A and B and superoxide dismutase differentiate MCI patients who progress to AD from MCI patients who did not progress to AD after three years of diagnosis. This observation is consistent with studies that have suggested that blood brain barrier (BBB) dysfunction may occur at AD onset (53). These results may also arise from contamination with blood during the procedure used to obtain CSF samples. However, the authors (46) depleted the most abundant proteins from the CSF, and these proteins did not differentiate HC from AD or MCI patients. Further studies will be required to corroborate this finding. On the other hand, increased expression of neuronal pentraxin-2 (NPTX2, also known as NARP) and the neurosecretory proteins VGF and Secretogranin (SCG2) predicted progression from MCI to AD after 3 years (46). All of these proteins are secreted by neurons and are related to synaptic functions. Therefore, their increased levels in the CSF might be indicative of synaptic and neuronal loss (54–56).

This finding is consistent with earlier studies that suggested that the synaptic impairments observed in AD can be detected early in the CSF samples of MCI patients. Perrin and colleagues (2011) reported decreased levels of neuronal cell adhesion molecule (NCAM) and chromogranin A in the CSF of subjects with mild AD compared with levels in the HC group (36). They used 2D-E gels and LC-MS/MS to identify 47 proteins that were differentially expressed in mild AD patients compared with HC, and further confirm changes in 11 of these proteins by ELISA. Another study on a relatively small number of patients revealed reduced levels of a peptide that was derived from the neurosecretory protein VGF using Protein Chip Array Chip technology and SELDI-TOF-MS (57). Another study used capillary electrophoresis-MS to reveal increased levels of several synaptic proteins, including chromogranin A, and decreased levels of VGF in a group of patients in different U.S. hospitals (40). Apparently opposing results, relative to the direction of change, were found for chromogranin A in (58) and (40) and for VGF in (46) and (40, 57). These conflicting findings may be the result of differences in the criteria used to diagnose AD and MCI patients, differences in the number of patients included in the study, differences in demographics that may specifically impact chromogranin A and VGF levels in the brain, or differences in how these changes were analyzed in the CSF content. The fact that VGF was also identified in a multiplexed proteomic approach that used quantitative label-free proteomics (58) in the CSF of cognitive normal subjects to validate differences in 81 peptides suggests that peptides related to this factor may be good candidate biomarkers of cognitive decline in AD. However, validation of these results in other studies will be required.

Other synaptic proteins might also be valuable biomarkers for synaptic dysfunction in AD because they were identified in CSF derived from healthy donors. These include Rab3A, synaptotagmin, SNAP25, and neurogranin (59). Of these, SNAP25 and neurogranin were validated as biomarkers for synaptic degeneration in AD in studies using immunofluorescence purification coupled with MS detection (60, 61). The synaptosomal-associated protein 25 (SNAP25) mediates synaptic communication by mediating the fusion of synaptic vesicles with the plasma membrane. It is therefore an important pre-synaptic marker of functional synapses. Consistent with the idea that early AD and cognitive impairment are highly correlated with synaptic dysfunction, SNAP25 was found to be decreased in the membrane-associated fractions derived from the brains of AD patients, whereas increased levels were observed in the CSF of early AD patients compared with normal control patients (60). These results suggest that SNAP25 is a good candidate biomarker for cognitive decline. In addition, potential postsynaptic biomarkers have been studied, such as neurogranin. Neurogranin was found to be increased in the CSF of AD patients in three different cohorts, and increased neurogranin levels in MCI patients predicted progression to AD, thus indicating a possible correlation between increased CSF neurogranin levels and the rate of cognitive decline (61). Neurogranin is a small, soluble protein that is abundant in all telencephalic areas, including the cerebral cortex and the hippocampus, and it is concentrated in the dendritic spines that participate in the consolidation of memory through the regulation of calmodulin availability. Neurogranin-null mice exhibit severe deficits in spatial and emotional learning and a decrease in LTP induction (62), which is consistent with that notion that altered neurogranin levels in AD, might reflect synaptic dysfunction.

Taken together these studies suggest that H-Fabps and specific pre- and postsynaptic markers, such as SNAP25 and neurogranin, may aid in monitoring the progression of AD in patients and that they may be useful biomarkers for drug therapies that aim to modify the progression of the neurodegenerative changes observed in AD (30). However, larger
cohort studies that compare cases of MCI that progress to AD with those that do not progress to AD are required to verify that granins, VGF-derived peptides, and SNAP25 are bona fide biomarkers of cognitive decline in addition to the more accurate approximation of levels in the brain and the CSF in MCI and AD patients.

One of the problems with using CSF as a substrate in the analysis of diagnostic markers is that the lumbar puncture medical procedure used to obtain CSF is risky compared with the minimally invasive and relatively inexpensive techniques required to obtain, purify, and analyze blood and/or plasma samples. Therefore, identifying biomarkers from the plasma or blood has been pursued in different studies (63). Initial studies by Ray et al. (64) indicated that a panel of 18 proteins, including growth factors and interleukins, differentiated AD patients from control subjects with over 90% accuracy. However, these findings were not replicated using other techniques, probably because different criteria were used to classify control subjects with the same accuracy (65, 66).

To analyze the concordance among the numerous studies that have utilized blood-based samples for biomarker discovery in AD, a summary of 21 reported studies of blood-based biomarkers of AD that were limited to discovery or panel-based (over hundred proteins assayed) approaches were analyzed and validated using a proteomic Aptamer-capture array (“SOMA scan”) (63). This approach employs chemically modified nucleotides to transform a protein signal to a nucleotide signal that can be quantified on microarrays. Ninety-four of the 163 protein candidates were validated in samples from 677 subjects that were derived from various cohorts. Four candidate biomarkers were found to be associated with AD-related phenotypes in five of the cohorts: α1-antitrypsin, α2-macroglobulin, apolipoprotein E, and complement C3. In addition, AD subjects specifically replicated lower levels of pancreatic prohormone and insulin-like growth factor-binding protein 2 compared with their levels in HC subjects. Interestingly, the protein associated with the best fit for cognitive decline was clusterin (apoJ), a known amyloid chaperone (63). Similar findings complement the view that this protein may be a valuable tool for predicting AD progression using blood samples. Clusterin has been associated with increased amyloid burden, increased entorhinal cortex atrophy and rapid clinical progression in AD patients. Furthermore, longitudinal studies of 139 patients revealed that a higher level of clusterin in the plasma was correlated with a slower rate of brain atrophy and that there was a higher concentration of clusterin in specific brain regions that are most affected in AD patients (67, 68). This evidence supports the likelihood of an AD signature in blood, although more cohorts and longitudinal studies must be performed to corroborate these findings. In addition, the candidate biomarkers for AD that were derived from the CSF and blood require evaluation in other neurodegenerative diseases to determine whether they are specific to AD.

**Proteomics and the AD Brain**—In terms of neuropathology, the AD brain is characterized by amyloid plaques and neurofibrillary tangles and mutations commonly found in familial AD affect amyloid metabolism. These observations inspired the “amyloid cascade hypothesis” for neurodegeneration in AD (13). However, the accumulation of amyloid plaques is not correlated with the cognitive impairments observed in AD, and cognitive-normal subjects who exhibit amyloid plaque accumulation have been documented (69–71). On the other hand, although neurofibrillary tangles are highly correlated with cognitive impairments, mutations in tau do not cause AD. Instead, they cause frontotemporal dementia (FTD) (69, 70, 72). An extension of this hypothesis postulates that oligomeric forms of Aβ are specifically toxic to synapses and that they may initiate the molecular and cellular cascade of events that leads first to synaptic dysfunction and then to neuronal loss. These include the events that cause tau hyperphosphorylation and tangle neuropathology (14). In particular, to study characteristics of the AD brain that might shed light on the molecular cascades that lead to neurodegeneration, proteomics studies have been performed in AD postmortem tissue. Explorative proteomics studies on the pathological changes that occur in the AD brain have recently been reviewed (37, 38).

Korolainen et al., 2010 (38) reviewed more than 40 studies from 1999 to 2010 analyzing differentially expressed protein in AD compared with HC using 2D-E techniques in an attempt to analyze a consistency in differentially expressed proteins in AD brains between studies. A list of over 90 proteins with a statistically significant change was garnered from this review. Using the same pool of studies, Brinkmalm et al., 2015 (37) identified proteins—of the list published by Korolainen et al., 2010 (38)—that display altered levels in AD but that were reported in at least two different studies to have the same sign change, narrowing the list to seven proteins (List 1 in Table I). This analysis indicate that 2D-E proteomics studies have so far failed to reach a consensus for molecular markers that shed new light on molecular targets that can be used in drug therapies or to increase our understanding of the molecular events that lead to neurodegeneration. Several reasons might account for these, including the technical ones discussed above. More studies using modern techniques based on high resolution high mass accuracy mass spectrometer as published by Andreev and colleagues 2012 (73) and Musunuri and colleagues 2014 (74) (see below) will have to be performed to increase our understanding of the AD brain proteome.

The study by Andreev et al., 2012 (73) deserves special consideration because different measures were taken to achieve accurate results. Twenty temporal cortices of HC and AD brains (ten per condition) were sampled. The temporal cortex was chosen, as it is one of the earliest affected brain areas in AD (75). Analysis was performed using the accurate mass and time tag proteomics approach together with LC/MS (76). The study focused on two important aspects. First, selecting the best available protein extraction protocol, and
**TABLE I**

Proteins with differential expression and post-translational modifications found in proteomics studies of AD brain. List 1, is obtained from Table I in (37) and are proteins that display altered levels in AD that were identified in 2D-E proteomics studies and that were reported in at least two different studies to have the same sign change (37,38). Primary references are cited next to the protein. In List 2 are proteins that were identified and published in (73) that coincided with a similar finding in at least one other study (37,73) and also proteins that are common to List 3 (see Fig. 1). In List 3 are included proteins recently published by Musunuri and colleagues (2014) (74). Proteins in bold letters are related to the mitochondria. Proteins in red are differentially phosphorylated and oxidized in AD brain. Proteins in green are just differentially phosphorylated in AD brain. Differentially phosphorylated proteins are obtained from Table II. Oxidized proteins were obtained from Butterfield et al 2014 (82).

<table>
<thead>
<tr>
<th>List 1 (sign change) (refs)</th>
<th>List 2 (sign change) (refs)</th>
<th>List 3 (sign change)</th>
<th>List 3 (sign change)</th>
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<tr>
<td>• Aldolase A (-) (127,128)</td>
<td>• 14-3-3 protein beta/alpha (+) (74,138)</td>
<td>• Synapsin-1 (-)</td>
<td></td>
</tr>
<tr>
<td>Alpha-enolase (+) (85,129-131)</td>
<td>14-3-3 protein epsilon (+) (74,138)</td>
<td>14-3-3 protein beta/alpha (-)</td>
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<tr>
<td>Fatty acid-binding protein (-) (132,133)</td>
<td>Adenyl cyclase-associated protein 2 (+) (139)</td>
<td>14-3-3 protein epsilon (-)</td>
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<tr>
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<td>Amyloid beta A4 protein (+) (138)</td>
<td>Rho GDP-dissociation inhibitor 1 (-)</td>
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<tr>
<td>Glial fibrillary acidic protein (GFAP) (-) (134,135)</td>
<td>Calpain 2 catalytic subunit (+) (132)</td>
<td>Guanine nucleotide-binding protein G(i)G(i5)G(T) subunit β-2 (-)</td>
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<td>Peptidyl prolyl cis-trans isomerase (PIN1) (+) (130,131,136)</td>
<td>Choline-phosphate cytidylyltransferase A (+) (140)</td>
<td>Endophilin A1 (-)</td>
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<td>Clathrin heavy chain 1 (-)</td>
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<td>Ferritin heavy chain (+) (131) (74)</td>
<td>Clathrin assembly protein AP-2 α-A large chain (+)</td>
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| • Cytochrome b-1 complex subunit 1 (-) | Synaptotagmin-1 (-) | Mammalian
| • Malate dehydrogenase (+) | Synaptic vesicle glycoprotein 2A (-) | |                  |
| • Glutathione s-transferase (+) | Syntaptophysin-1 (-) | Opioid-binding protein/cell adhesion molecule (-) |                  |
| • Carbonyl anhydrase 2 (+) | Synaptic vesicle glycoprotein 2B (-) | Neurotrimin (-) |                  |
| • Acetyl-CoA acetyltransferase (+) | | |                  |
| • Alpha-enolase (+) | | |                  |
| • Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (+) (74,131) | | |                  |
| • Phosphoglycerate kinase 1 (+) | | |                  |
| • Pyruvate kinase isozymes M1/M2 (+) | | |                  |
| • Fructose-bisphosphatase aldolase C (+) | | |                  |
| • L-Lactate dehydrogenase B chain (+) | | |                  |
| • B-cell receptor-associated protein 31 (-) | | |                  |
| • Cytochrome c (-) | | |                  |
| • Peroxiredoxin-6 (+) | | |                  |
| • Peroxiredoxin-1 (+) | | |                  |
| • Peroxiredoxin-2(-) | | |                  |
| • Ferritin heavy chain (+) | | |                  |
| • Ferritin light chain (+) | | |                  |
| • Heat shock protein HSP 90-α (-) | | |                  |
| • Stress-70 protein (-) | | |                  |
| • Heat shock 70 kDa protein 1A/1B (+) | | |                  |
| • Glial fibrillary acidic protein (+) | | |                  |
| • Apolipoprotein D (+) | | |                  |
| • Syntaxin-1A (-) | | |                  |
| • Synaptotagmin-1 (-) | | |                  |
| • Synaptic vesicle glycoprotein 2A (-) | | |                  |
| • Synaptic vesicle glycoprotein 2B (-) | | |                  |
| • Synaptic vesicle glycoprotein 1 (-) | | |                  |
| • Profilin-2 (-) | | |                  |
| • Septin-5 (-) | | |                  |
| • Synaptic cell adhesion molecule 2 (-) | | |                  |
| • Synaptic vesicle glycoprotein 2A (-) | | |                  |
| • Synaptic vesicle glycoprotein 2B (-) | | |                  |
| • Synaptic vesicle glycoprotein 1 (-) | | |                  |
| • Profilin-2 (-) | | |                  |
| • Septin-5 (-) | | |                  |
| • Synaptic cell adhesion molecule 2 (-) | | |                  |

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second, improving the reliability of quantitation by using only the consensus results of four normalization methods. Under these conditions, a list of 197 consensus proteins significantly differentially expressed in AD compared with HC was created. In List 2 (in Table I) we included the proteins that were differentially expressed in (73) and coincided with a similar finding in at least one other study (the analysis was performed in Andreev and colleagues 2012 (73) and published as supplemental material in their article). In addition, common proteins published by Musunuri and colleagues 2014 (List 3 in Table I and Fig. 1) were also included. In List 3 we listed proteins recently published by Musunuri and colleagues (2014), who used shotgun multiplexed MS with stable isotope dimethyl labeling to study differentially expressed proteins in AD (ten samples) versus HC (five samples) of temporal cortex (74). Sixty-nine proteins were found to be significantly increased \(n = 37\) or decreased \(n = 32\) (74). These proteins were classified according to their biological function for example, as proteins involved in metabolic processes, inflammatory responses, oxidative stress responses, stress responses (e.g. HSP), signal transduction, synaptic functions, or apoptosis. When the functions of the increased proteins were compared with the functions of the decreased proteins, interesting features were revealed. A larger proportion of the increased proteins are involved in metabolic processes (40%) compared with those that were decreased (10%). The finding of alterations in the levels or modification of metabolic proteins associated with AD and transgenic of AD is a constant feature of several studies (see Table I and Table IV). Furthermore, proteins involved in anti-oxidative activities (14%) or in inflammatory responses (6%) were also represented. However, large proportions of the proteins that were decreased are involved in synaptic functions (24%) and signaling pathways (15%).

When the studies by Musunuri and colleagues (2014) and Andreev and colleagues (2012) are compared (73, 74) using a Venn diagram, ten common proteins are identified (Fig. 1) (see List 2 and 3 in Table I). Only GAPDH, a key enzyme in glycolysis, was found to be altered in all three lists compared in Table I and in Fig. 1. Two common proteins were found in List 1 and List 3 (\(\alpha\)-enolase and GFAP). Of the ten common proteins identified in List 2 and List 3, GAPDH, peroxiredoxin-1 and -6, ferritin heavy chain, annexin V, pyruvate kinase isozymes M1/M2, profilin-2, and plasma membrane calcium-transporting ATPase 3 had similar sign change (Table I and Fig. 1). One general observation is that there is a small consensus between the proteomes found. As discussed in (37, 42), there are several possible reasons for this. First, the brain is a very complex tissue that contains different types of cells and different anatomical regions that are differentially affected by AD. In studies where brain tissue was used, often few AD samples were examined (normally between four and ten), and it must be considered that individual variability can be large in different populations. An important issue is that each AD sample reflects a patient who may have other associated pathologies, such as hypertension or type two diabetes, that can act as confounders of the pathological process and that might increase interindividual variability in the protein content of the CSF and brain (77). One possible follow-up study will be to validate the group of proteins found common in (73, 74) and having the same sign change (see Fig. 1) together with proteins of List 1 (from Table I) in a larger cohort of brain samples of different AD stages using targeted MS platforms.

Several lines of evidence support the idea that A\(\beta\) oligomers are toxic to synapses and cells including proteomic studies (36, 40, 60, 61, 74). It has been proposed that A\(\beta\) oligomers interact with membrane receptors and the membrane itself to increase calcium and lipid peroxidation (14). These interactions cause oxidative stress and concomitantly increase oxidative damage to proteins, which alters their function. Oxidative stress is caused by an imbalance in the redox state of the cell that is due either to increased levels of free radicals, such as reactive oxygen species (ROS) or reactive nitrogen species, or to a decreased antioxidant response.
Free radical-mediated damage to proteins is a nonreversible phenomenon that requires clearance systems, such as the ubiquitin-proteasome system and the autophagy-lysosomal system, to remove the oxidized/dysfunctional proteins. However, clearance systems are themselves targets of oxidative damage (78–80). Therefore, the chronic accumulation of oxidized proteins eventually leads to the inhibition of the cellular processes that are used to rescue the cell from such stress.

The major source of free radicals is the mitochondrial oxidative phosphorylation pathway. A quantitative proteomics analysis of the brains of MCI and early AD patients suggested that changes in the enzymes required for the utilization of ATP in the mitochondria occur early during AD progression. These proteins include voltage-dependent anion channels (VDACs), adenine nucleotide translocase, hexokinase (which might be associated with the external membrane of mitochondria when powering glycolysis is required), and creatine kinase, which collectively control the flux of ATP to and from mitochondria (81). In this study, changes in VDAC and hexokinase were verified using Western blot analysis. Nevertheless, these results must be viewed with caution because they used pooled samples that were obtained from a limited number of AD, MCI, and HC patients. These results correlated with the results presented in Table I, wherein proteins labeled with bold letters were found to be decreased in AD brains and are known to participate in the generation of energy in the mitochondria (37, 38, 74).

Consistent with the idea that oxidative damage is central to the neurodegenerative processes associated with AD, redox proteomics methods have been used to identify proteins that undergo oxidative post-translational modifications that are detrimental to protein functions. The most relevant findings have shown major disturbances in protein homeostasis and energy production. Reactive oxygen species activity results in protein carbonylation, which can be detected using 2D-E combined with Western blot analysis incorporating antibodies against the redox modification of interest. Such analyses have revealed that proteins involved in glucose utilization and energy production are oxidized in AD and MCI brains. Increased oxidation of α-enolase, malate dehydrogenase (MDH), fructose bisphosphate aldolase A/C (FBA A/C), ATP synthase, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) has been reported. With the exception of ATP synthase, all of these proteins were found to be differentially expressed in AD brains (labeled in red in Table I). Increased oxidation of pyruvate kinase (PK) and triose phosphate isomerase (TPi) distinguished MCI from AD brains. All of these results suggest that impaired glucose metabolism is important to the progression of AD (82–85) and that altered levels of proteins related to metabolic processes are related to their oxidation. Consistent with these hypotheses, reduced glucose utilization is one of the clinical measurements used to diagnose AD-type dementia in the clinic (86).

Several kinases can be activated by oxidative stress, such as c-jun N-terminal kinase (JNK), p38, and mitogen-activated protein kinase (MAPK), and this adds a new layer of complexity to our understanding of early AD etiology (87). The hyperphosphorylation of the tau protein is thought to be central to the neuropathology observed in AD. In addition, the increased phosphorylation of other proteins, including neurofilaments, microtubule-associated protein 1B (MAP1B), collapsing response mediator protein 2 (CRMP2), and GFAP, suggests that the abnormal phosphorylation of proteins is involved in AD progression (88–91). 2D-E and Pro-Q diamond fluorescence staining, which can be used to label proteins with phosphorylated serine, threonine, and/or tyrosine residues, when coupled with MS has revealed additional proteins that show altered levels of phosphorylation. Interestingly, proteins involved in metabolism and glycolysis/glucogenesis presented altered levels of phosphorylation, suggesting that reduced glucose utilization and energy production in the brains of AD patients (92) are related to unbalanced kinase and phosphatase activities in addition to the oxidative stress process. Table II summarizes the metabolism-related proteins that showed altered levels of phosphorylation in the brains of AD patients in two different studies (90, 91). Supporting the idea that abnormal phosphorylation of metabolic proteins is related to AD pathology, metabolic-related proteins that have been found to display altered levels in AD brains have also been found to show alterations in their level of phosphorylation (Table I, proteins labeled in red and green). Interestingly, the proteins labeled in red (Table I) are the proteins that were...
found to show altered protein levels in addition to both altered oxidation and phosphorylation levels. It is tempting to speculate that these proteins might be central to the alterations in metabolic activity that are observed in AD brains. Additional studies employing techniques—such as stable isotope dilution (SID)—together with multiple reaction monitoring (MRM)-MS to quantify the levels and post-translational modifications of these proteins in a larger cohort of AD brain samples, might help to corroborate that they are truly altered protein candidates for neuropathological events triggering AD (42).

Adding to the studies discussed above, label-free quantitative proteomics performed using immobilized meta-affinity chromatography (IMAC) was used to study differences in the levels of phosphopeptides between the brains of AD patients and aged-matched HC (93). In this study, 253 phosphopeptides were identified to display different levels of phosphorylation between the two groups. Approximately 21% of the altered phosphopeptides in the AD tissues were mapped to tau, as indicated by increased signal intensities compared with controls. In addition, a large group of phosphopeptides related to the endoplasmic reticulum were found to be decreased. A surprisingly large number of phosphopeptides that mapped to kinases, including TNIK, PKA, PKC, MINK, CAMKKII, MARK2, and DCLK1, were correlated with an increased intensity of phosphopeptides that belong to different chaperones, including small heat shock proteins (HSPs) such as HSP27 and CRYAB (crystallin alpha B). Interestingly, the phosphorylation of chaperones regulates their oligomeric state and provides a functional link between their increased kinase activity and the distorted functions of HSPs (93).

Proteomics and AD mice models taking into account the proteomic analyses discussed so far, we could argue that increased phosphorylation and the oxidation of proteins, particularly those related to metabolic pathways, such as glycolytic enzymes and mitochondrial proteins associated with aerobic metabolism, are altered early in the pathology of AD. Hence, the results might be confounded by postmortem procedures (normally, a brain is processed within 24 h postmortem) (94). To overcome this problem, several transgenic mouse models of AD have been developed during the past 20 years with the aim of understanding the etiology of AD. The rationale for these studies has been to express human proteins that harbor mutations causing genetic AD with the hope of recapitulating AD phenotypes in transgenic mice. Genetic AD and sporadic AD brains are indistinguishable from one another; therefore, common pathogenic mechanisms are likely involved (95).

Animal models of AD provide an opportunity to elucidate the molecular changes that occur in brain cells as the disease develops and serve as pre-clinical models to study genetic interactions and how different treatments modulate neuropathological progression. Several transgenic mice models for AD (Tg AD) have been generated (for review see (96)); however, it is important to note that none of the Tg AD models fully recapitulate the neuropathology and the behavioral deficits that are observed in AD. Nevertheless, they provide an experimentally accessible model to study the molecular processes that are related to proteins and pathological lesions of AD and that are recapitulated in Tg AD, such as Ag oligomers, memory deficits, amyloidosis, hyperphosphorylation of tau, and neurofibrillary tangles. Some of these models include Tg2576, which expresses the human 695 APP gene that encodes for the Swedish (APPsw) double mutation under the control of the hamster prion promoter (97), and the 3xTg, a triple transgenic mouse that is a knock-in for the PSEN1 gene familial AD mutation (Met146Val) that overexpresses the human APPsw with a mutated tau transgene (Pro301Leu) that has been found to cause FTD (98). Several proteomics studies have been performed in different Tg AD brains, which are summarized in Table III. Because these studies have been conducted using different Tg lines, which themselves have different genetic backgrounds and are studied at different ages, it is very difficult to generate broad conclusions from these studies. However, there are reports that support the idea that the brain changes that are observed by proteomics studies in the AD postmortem brain are indeed central to the pathological process and are recapitulated in Tg mice. For example, two different studies in Tg2576 Tg and in 3xTg mice have studied the mitochondrial proteome in early stages of disease progression before the development of amyloid plaques and tangles (in 3xTg mice) (97, 99). In Tg2576 Tg mice, significantly altered levels of proteins that belong to the electron transport chain, such as subunits of the NADH dehydrogenase complex (some of which were reduced and others were increased), and a reduction of cytochrome c were accompanied by impaired respiration by isolated mitochondria and reduced glucose utilization in the cortex (97). In 3xTg mice, deregulated proteins were observed to have functions in a wide variety of metabolic pathways, including the citric acid cycle, oxidative phosphorylation, pyruvate metabolism, glycolysis, oxidative stress, fatty acid oxidation, and recapitulated reduced levels of NADH-dehydrogenase (complex I). In addition, other complexes, such as complex IV, were also altered in 3xTg (99). This concurs with the reduced levels of complex I subunits and cytochrome c that were reported in the AD brain (see proteins labeled in bold letters in Table I and Table IV). Conversely, the Butterfield laboratory has provided evidence that indicates an early alteration of oxidatively modified proteins in the Tg AD model double knock-in APP/PSEN1. They found increased α-enolase oxidation with age, process that correlated with increased amyloid deposition (100). In the AD brain, α-enolase has been found to be both increased and oxidized (see Table I and Table II) and oxidized alpha-enolase in AD brain has reduced activity (101). Other proteins belonging to the aerobic metabolism pathway, such as pyruvate dehydrogenase and the ATP synthase alpha subunit, were also oxidized in this model (100).
The same group has analyzed differentially expressed levels of proteins in the double knock-in APP/PSEN1 Tg AD model using proteomics and treatment with the antioxidant N-acetylcysteine (NAC). Following treatment, some metabolic enzymes with reduced levels were increased. For instance, α-enolase and pyruvate kinase were reduced (suggesting that the oxidation of these proteins resulted in their degradation), whereas NAC significantly increased the levels of these enzymes (102). The discrepancies regarding the sign change of α-enolase in this Tg model, when compared with the AD brain, might be the result of the lack of neuronal cell death or strain differences because α-enolase was found to be increased in the brain of J20Tg mice (which is a double human APP mutant containing APP Swedish and Indiana mutations) (103), similar to the findings in human AD brain (Table I).

Supporting the role of the Aβ peptide in inducing oxidative stress in Tg AD models, this group has shown that methionine 35 of the Aβ42 peptide (Met631 in APP, which was substituted in a mutated APP by a leucine) is required for in vivo induced oxidative stress in J20Tg mice (104). Consistently, for some of the altered expressed metabolic proteins that were observed in the J20Tg, such as GAPDH, substitution of methionine 631 to leucine increased the levels of this enzyme to normal (105). Decreased levels of antioxidant proteins (such as SOD2) in J20Tg Met631Leu Tg, when compared with J20Tg, suggest that oxidative stress was down-regulated because this protein is normally up-regulated by oxidative stress. When taken together, these results suggest that oxidation that is caused by Aβ oligomers is required for altered expression and modification of metabolic enzymes that are observed in Tg AD models. Consistent with this proposition, peroxiredoxin-1 and -6 have been consistently found to be up-regulated in human AD and Tg AD mouse models (Table I) and peroxiredoxin-6 is up-regulated in both AD brain and Tg AD mouse models (Table IV). Peroxiredoxin-6 is involved in redox regulation, and it has also been found to protect against oxidative injury (106). The increased levels of this protein may reflect the intent of the stressed neuron to overcome the increased oxidation of the cell that is induced by the alteration of glycolytic metabolism and altered function of the mitochondria, as discussed in the main text.

Several other proteins have been found to be up- or down-regulated in AD and Tg AD brains when compared with controls (Table IV). For example, in AD and Tg AD brains, the glycolytic enzyme fructose biphosphate aldolase C (AldoC) was found to be altered (although in opposing directions). In addition to glucose metabolism, AldoC has also been implicated in cytoskeletal protein binding (107), suggesting that AldoC may also contribute to the neuronal morphological changes that characterized AD. Another protein that has been
TABLE IV
List of proteins that were found to be differentially regulated in transgenic mouse model of AD (Tg AD) and in human AD brain (AD).

<table>
<thead>
<tr>
<th>Model</th>
<th>Protein</th>
<th>Gene</th>
<th>Sign change Tg AD</th>
<th>Reference in Tg AD</th>
<th>Sign change AD</th>
<th>Reference AD brain-human</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>3xTG AD</td>
<td>Triose phosphate isomerase</td>
<td>TPI (98)</td>
<td>(148) (+)</td>
<td>(98)</td>
<td>+</td>
<td>(131)</td>
<td>Metabolism</td>
</tr>
<tr>
<td>3xTG AD; J20 tg; APPE693delta</td>
<td>Alpha-enolase</td>
<td>ENO1</td>
<td>3x TG AD, APPE693delta (-) / J20 tg (+)</td>
<td>(98,103,148,150)</td>
<td>+</td>
<td>(74,131)</td>
<td>Metabolism</td>
</tr>
<tr>
<td>TG2576; 3xTG AD</td>
<td>NADH dehydrogenase subunits</td>
<td>NDUF</td>
<td>-</td>
<td>(97,99)</td>
<td>-</td>
<td>(37)</td>
<td>Metabolism</td>
</tr>
<tr>
<td>TG2576</td>
<td>Cytochrome c</td>
<td>cyC</td>
<td>-</td>
<td>(97)</td>
<td>-</td>
<td>(74)</td>
<td>Metabolism</td>
</tr>
<tr>
<td>3xTG AD; Thy1-APP751</td>
<td>Dihydropyrimidinase-related protein 2</td>
<td>DPYS2</td>
<td>3xTG AD (+), Thy1-APP751 (+)</td>
<td>(98,147)</td>
<td>+</td>
<td>(74)</td>
<td>Adaptor protein, cytoskeleton regulation, and axonal growth transport</td>
</tr>
<tr>
<td>3xTG AD</td>
<td>Neurofilament medium polypeptide</td>
<td>NEFM</td>
<td>-</td>
<td>(98)</td>
<td>-</td>
<td>(83,151)</td>
<td>Involved in the maintenance of neuronal caliber</td>
</tr>
<tr>
<td>3xTG AD; J20 tg</td>
<td>Rho GDP-dissociation inhibitor 1</td>
<td>ARHGDI1</td>
<td>3x TG AD (+) / J20 tg (+)</td>
<td>(98,103)</td>
<td>-</td>
<td>(74)</td>
<td>Controls function of Rho family of GTPases</td>
</tr>
<tr>
<td>3xTG AD</td>
<td>Ubiquitin carboxy-terminal hydrolase 1</td>
<td>UCHL1</td>
<td>-</td>
<td>(98)</td>
<td>+</td>
<td>(152)</td>
<td>Regulates levels of ubiquitin</td>
</tr>
<tr>
<td>J20 tg; APP/PS1 Human Double Mutant Knock-in Mice</td>
<td>Peptidyl-prolyl cis-trans isomerase</td>
<td>Pin-1</td>
<td>J20 tg (+) / APP-PS-1 (-)</td>
<td>(102,103)</td>
<td>(131) / (83) and (151) (+)</td>
<td>(74,83,131)</td>
<td>Regulates mitosis. Acts as a regulator of JNK cascade</td>
</tr>
<tr>
<td>3xTG AD; Tau (NSE/htau23); APP/PS1 Human Double Mutant Knock-in Mice</td>
<td>Glyceraldehyde-3-Phosphate Dehydrogenase</td>
<td>GAPDH</td>
<td>3x TG AD (+), APP-PS-1 (-)</td>
<td>(102,148)</td>
<td>+</td>
<td>(74)</td>
<td>Metabolism</td>
</tr>
<tr>
<td>Thy1-APP751 (Tg)</td>
<td>Glial fibrillary acidic protein</td>
<td>GFAP</td>
<td>+</td>
<td>(147)</td>
<td>+</td>
<td>(74)</td>
<td>A class-III intermediate filament</td>
</tr>
<tr>
<td>Thy1-APP751 (Tg)</td>
<td>Peroxiredoxin-6</td>
<td>PRDX6</td>
<td>+</td>
<td>(147)</td>
<td>+</td>
<td>(74)</td>
<td>Involved in redox regulation of the cell</td>
</tr>
<tr>
<td>Thy1-APP751 (Tg)</td>
<td>Serum albumin precursor</td>
<td>ALB</td>
<td>+</td>
<td>(147)</td>
<td>+</td>
<td>(74)</td>
<td>The main protein of plasma</td>
</tr>
<tr>
<td>APP/PS1 Human Double Mutant Knock-in Mice</td>
<td>Neurofilament light polypeptide</td>
<td>NEFL</td>
<td>-</td>
<td>(125)</td>
<td>-</td>
<td>(151)</td>
<td>Cytoskeleton</td>
</tr>
<tr>
<td>APP/PS1 Human Double Mutant Knock-in Mice</td>
<td>Fructose-bisphosphate aldolase C</td>
<td>ALDOC</td>
<td>-</td>
<td>(102)</td>
<td>+</td>
<td>(74)</td>
<td>Metabolism</td>
</tr>
<tr>
<td>APP/PS1 Human Double Mutant Knock-in Mice</td>
<td>Heat shock protein 90x</td>
<td>HSPOA1</td>
<td>-</td>
<td>(102)</td>
<td>-</td>
<td>(74)</td>
<td>Molecular chaperone</td>
</tr>
<tr>
<td>3xTG AD</td>
<td>Superoxide dismutase</td>
<td>SOD1</td>
<td>+</td>
<td>(148)</td>
<td>+</td>
<td>(80)</td>
<td>Antioxidant defense</td>
</tr>
<tr>
<td>3xTG AD</td>
<td>Tubulin polymerization promoting protein</td>
<td>TPPP</td>
<td>+</td>
<td>(148)</td>
<td>(74) (+); (151) (+)</td>
<td>(74,151)</td>
<td>Tubulin polymerization</td>
</tr>
<tr>
<td>3xTG AD</td>
<td>14-3-3 protein beta/alpha</td>
<td>YWHAB</td>
<td>+</td>
<td>(148)</td>
<td>-</td>
<td>(74)</td>
<td>Scaffold protein</td>
</tr>
</tbody>
</table>

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consistently observed to have increased levels in human AD and Tg AD brains is GAPDH (Table I and Table IV); however, as discussed below, this may be part of the neuropathological process observed in AD brain because GAPDH has been found to be hyperphosphorylated and oxidized in the AD brain (Table I). GAPDH is a multifunctional protein that has roles in glycolysis, transcriptional activation, apoptosis, and transport (108–110) and, upon oxidative stress it binds Siah (110) and undergoes nuclear translocation. Nuclear GAPDH has been found to induce p53-dependent transcription of apoptotic genes (111). In addition, recently, it has been found that GAPDH increased the aggregation of the Aβ peptide and is colocalized and co-immunoprecipitated with Aβ peptide in the 3xTg AD mouse model (112). When considered together, these results suggest that oxidized GAPDH might be involved in neurodegeneration in the AD brain. Additionally, they indicate the relevance of proteomics studies to find differentially modified proteins because they confer knowledge of their function under the disease conditions.

The brains of AD patients are characterized by early damage of synapses, retrograde degeneration of axons, and the eventual atrophy of dendrites (1, 12). Two different proteins that are related to the control of cytoskeleton dynamics and function are found to be altered in AD and Tg AD brains (Table IV). One of these proteins is the dihydropyrimidinase-related protein 2 (DPYSL2), which is a cytosolic protein that is also known as CRMP2 and shows increased levels in AD and Tg brains (Table IV). CRMP2 binds tubulin and promotes microtubule assembly at the plus-end and interacts with the Rac1-associated protein (Sra-1/WASP) family verprolin-homologous protein (WAVE1) complex to regulate actin filament stability and the growth cone (113, 114). CRMP2 has been found to be hyperphosphorylated in the AD brain, and phosphorylation of CRMP2 inhibits its activity, thereby reducing axonal growth (90, 115). Most likely, increased CRMP2 levels in AD reflect inactive CRMP2 that may underlie neuronal morphological changes that are observed in the AD brain. Another cytoskeleton dynamics related protein that has been found to have altered levels in AD and Tg AD brains is Rho GDP dissociation inhibitor 1 (RhoGDI) (Table IV). This protein regulates the activation of the Rho family of GTPases that are key proteins for the regulation of actin dynamics polymerization (116).

Finally, the ubiquitin carboxy-terminal hydrolase 1 (UCHL1) has been shown to be reduced in both AD and Tg AD brains (Table IV). UCHL1 most likely regulates the availability of monomeric ubiquitin by preventing its degradation (117). Mutations in the UCHL1 gene have been found to be associated with neurodegeneration (118, 119), and the overexpression of UCHL1 results in increased levels of ubiquitin (120). Conversely, the genetic deletion of UCHL1 decreases long-term potentiation, and its inhibition increases dendritic spine size, but reduces its density (121, 122). These results suggest that UCHL1 may also contribute to the alteration in the morphology of neurons in the AD brain, thereby impairing circuitry functioning.

The idea that oxidative damage and mitochondrial dysfunction is central to AD pathology has garnered support throughout the years (123, 124), and proteomic analyses that have been performed in the AD post mortem brain and Tg AD animal models support these findings. To obtain further insights into this process and to understand the order of AD pathological events, proteomics studies in Tg AD model mouse might be of great interest. As performed with the analysis of CSF in a cohort of AD patients and discussed in the main text, similar studies could be performed using a multiplexed MS-based approach for a panel of proteins related to oxidative stress and mitochondria homeostasis. By analyzing the progressive development of neuropathology and cognitive changes in the Tg AD mice models at early stages and after the onset of pathological changes, further insight might be gained. It will be important to combine these studies with the study of the oxidative and phosphorylation state of the proteins with differential expression levels in the disease state because these experiments confer additional information about the functionality of relevant proteins. It will also be interesting to compare different Tg mouse models. For instance, the APP693delta, which contains the human APP 695 harboring the APP Glu693 delta mutation under the mouse prion promoter, produces Aβ oligomers without plaque deposition (125). Therefore, this model is interesting for the study of the chronic effect of Aβ generation in the brain, whereas other models, such as TgCRND8, are aggressive amyloid plaque generators and show amyloid formation beginning at 3 months of age (96).

These studies suggest a possible scenario for AD development, where increased cellular oxidative state is initiated by the interaction of Aβ oligomers with membrane and specific receptors that impair glucose utilization and mitochondrial metabolism. The increase in oxidative damage to proteins and the reduction in energy supply may have a particularly strong impact on glutamatergic synapses in the early stages of AD. The soluble form of Aβ is secreted in an activity-dependent manner at these synapses, and it has therefore been suggested that glutamatergic neurotransmission can be modulated by Aβ (14). In addition, glutamatergic synapses may be particularly susceptible because the increased Ca**+** influx that is associated with glutamatergic receptors may increase oxidative stress in these neurons (126). These processes may result in the increased activity of kinases and the dysregulation of HSPs, which supports the catabolism of damaged proteins. This hinders both the ubiquitin proteasome system and the lysosomal-autophagy process, resulting in the early loss of synaptic contacts.

Targeting these processes may provide therapeutic interventions and future studies (124). However, this is easier said than done, as the affected pathways are highly complex and...
regulated. Future research is necessary to test the feasibility of these possibilities.

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