**DEMENTIA**

# The phosphorylation cascade hypothesis of Alzheimer's disease

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## Mini-abstract

Alzheimer's disease is characterized by amyloid β (Aβ)-induced phosphorylation of the axon-stabilizing tau protein, which causes neurodegeneration. Here, Morshed *et al*.[1] show that de-regulated phosphorylation in AD also affects other proteins and cell types in the brain, suggesting that the tau-centric view on Aβ toxicity should be revised.

## Main text

Recent large-scale proteomics studies have revealed neuronal, astrocytic, and microglial protein profile changes in Alzheimer's disease (AD) brains and biofluids.[2,3] Still, our understanding of how these changes relate to the pathognomonic amyloid β (Aβ) pathology of the disease and through which signaling pathways they are induced remain incomplete. In this issue of *Nature Aging*, Morshed *et al*.[1] take a deep dive into the phospho-proteome of human AD brains and uncover several Aβ-associated dysregulated kinase networks that may underlie many of the protein changes reported, shedding new light on the complex cellular phase of the disease.

Traditionally, AD has been characterized by the accumulation of extracellular aggregates of Aβ peptides in the brain (so-called amyloid plaques). These peptides are natural breakdown products of amyloid precursor protein (APP), which is a transmembrane protein that is highly expressed in neurons. APP has a rapid turnover and can be cleaved by several secretases in the cell membrane. The amyloidogenic

processing pathway is characterized by concerted cleavages of APP by β- and γ-secretases, which result in the release of Aβ peptides of varying C-terminal length to the extracellular matrix; the 40-43 amino acid-long forms are sticky and tend to self-aggregate into oligomers, fibrils, and plaques in the brain tissue. Amyloidogenic APP processing is very active in the endosomal vesicle recycling system of the synapse and, together with complement proteins, oligomerized Aβ may tag synapses for removal by microglia,[4] which is essential for synaptic homeostasis. In the young and healthy brain, the tagging of synapses for removal is thought to be a regulated and short-lived signal that induces phosphorylation of the axonal tau protein. The normal function of tau is to bind to and stabilize tubulin multimers of the axonal cytoskeleton. In pruning processes, tau is phosphorylated, which results in reduced affinity for tubulin and destabilization of the axon, facilitating its removal. Phosphorylated tau is normally either secreted from neurons or degraded in lysosomes. It may also be packed into inclusions for future recycling, a well-established phenomenon in hibernating mammals.[5] However, in AD, the constant exposure of neurons to aggregated Aβ (potentially via microglia and astrocytes) results in tau hyperphosphorylation and formation of intraneuronal tau inclusions, so-called neurofibrillary tangles, which gradually build up in neurons that eventually die off. This process is at the core of the traditional amyloid cascade hypothesis on AD pathogenesis.

However, due to repeated clinical trial failures of Aβ- and tau-targeting drug candidates, this rather simplified linear model (Aβ → tau → neurodegeneration → dementia) has been questioned. Instead, AD pathogenesis may involve a long and complex tissue reaction consisting of feedback and feedforward responses of astrocytes, microglia, oligodendrocytes, and the vasculature.[6] Aβ accumulation is still at the core, but instead of focusing on neuronal tau phosphorylation, researchers are increasingly paying attention to the tissue reaction around the Aβ deposits.[2,3] Since Aβ is a potent inducer of neuronal tau phosphorylation through kinase activation, how about phosphorylation events in other cell types of the brain? Could these play a role in AD neurodegeneration? This is where the study by Morshed *et al.* enters the limelight.[1]

Morshed *et al.* collected brain samples from 48 patients (AD patients and age-matched cognitively normal controls) that were divided into fractions enriched for tyrosine and serine/threonine phosphorylated peptides side by side with non-phosphorylated peptides. Multivariate and cluster analysis were applied on the data, from which six major clusters of peptides and proteins emerged, that were strongly associated with AD pathology. The authors confirm that (i) Aβ levels follow AD pathology progression in an expected way, (ii) the tau cluster, driven by phosphorylated tau peptides, is the one most strongly associated with AD, and (iii) that synapse-associated proteins, reflecting synaptic integrity, anti-correlated with microglial proteins, as well as with tau.

An unexpected finding was that oligodendrocyte-associated proteins, such as myelin and neurofilaments, exhibited a greater difference than tau between AD and controls, suggesting that oligodendrocyte pathology, which has previously been largely neglected in AD studies, is an important disease process to consider. Interestingly, the tau and oligodendrocyte clusters did not correlate, and moreover, the authors could not link the variation in the oligodendrocyte cluster to current histopathological markers for AD, indicating the need for additional markers alongside those of traditional histopathology. However, by stratifying the patients into four groups according to tau and oligodendrocyte-associated protein levels, the authors found that patients with both tau and oligodendrocyte pathologies had the highest co-occurrence of other glial and neuronal pathologies. This suggests that changes in oligodendrocyte proteins occur late in AD and in parallel to the tau pathology. Moreover, the data suggest that microgliosis precedes astrogliosis.

Outstanding experiments in the study include the deep phospho-proteome analysis and the inclusion of pTyr peptides, revealing abundant AD-associated changes in phospho-peptides that extend way beyond tau. Monitoring these phosphorylated species was crucial to measure tau pathology, to discover the downregulation of neuronal phospho-peptides, which characterizes the synaptic failure of the disease, and to provide new insight into Aβ-related regulation of different signaling factors, in particular kinases (Figure 1). Many of these are involved in plasticity (the cyclin-dependent kinases, in particular) and extracellular matrix remodeling (*e.g.*, DDR1 and PTK) processes, suggesting that they are parts of the tissue reaction around Aβ plaques.

Finally, partial least squares regression models were built to investigate which peptides best predicted AD pathology. These analyses accentuate the great complexity of the data, and that proteins in general, as well as specific phosphorylation events in particular, are associated with pathological subtypes of AD. In summary, Morshed *et al*. have produced a remarkable piece of work hinting at the complexity of pathological processes; but they have also shown that it is possible to handle such complexity by careful analysis, generating interpretable data that shed additional light on the molecular events underlying neurodegeneration in AD.[1]

The results underscore that the linear model of AD pathogenesis, linking Aβ accumulation to neuronal tau pathology, neurodegeneration, and clinical disease expression, should be revised. In contrast, the disease is associated with a plethora of changes in multiple kinase-related pathways in most cell types of the brain, which now need to be delineated experimentally. Whether this is a powerful pan-cytopathic effect of Aβ or also seen in non-Aβ tauopathies (diseases like progressive supranuclear palsy and some forms of frontotemporal dementia) will be important topics for future investigation.

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**Conflicts of interest**

Associate Professor Brinkmalm reports no disclosures. Professor Zetterberg has served at scientific advisory boards for Alector, Eisai, Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics, Nervgen, AZTherapies and CogRx, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

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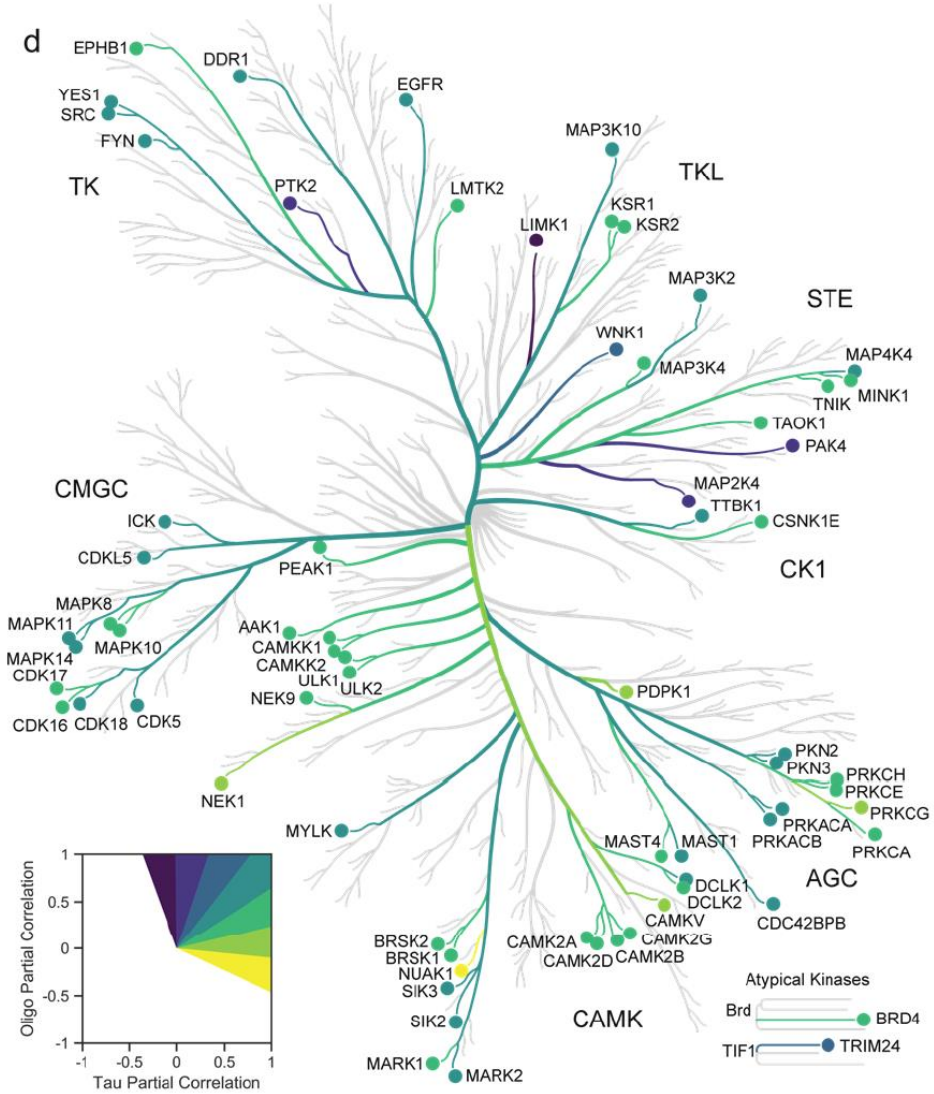


Figure 1. Kinome map of all kinases with phospho-peptides that were significantly associated with tau or oligodendrocyte cluster centroids. Branches and nodes are colored by partial correlation with tau and oligodendrocyte as shown on inset panel. From Morshed *et al*.[1]