NIKO-PETTERI NYKÄNEN

Cellular Physiology and Cell-to-Cell Propagation of Tau in Neurodegeneration:

The Impact of Late-Onset Alzheimer's Disease Susceptibility Genes

NEUROSCIENCE CENTER AND
DIVISION OF NEUROSCIENCE AND PHYSIOLOGY
DEPARTMENT OF BIOSCIENCES
FACULTY OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES
DOCTORAL PROGRAMME IN INTEGRATIVE LIFE SCIENCE
UNIVERSITY OF HELSINKI

Neuroscience Center
And
Division of Physiology and Neuroscience
Department of Biosciences
Faculty of Biological and Environmental Sciences
University of Helsinki

And
Integrative Life Science Doctoral Program

CELLULAR PHYSIOLOGY AND CELL-TO-CELL PROPAGATION OF TAU IN NEURODEGENERATION: THE IMPACT OF LATE-ONSET ALZHEIMER'S DISEASE SUSCEPTIBILITY GENES

Niko-Petteri Nykänen

ACADEMIC DISSERTATION

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Supervised by	Docent Henri Huttunen, PhD
	Neuroscience Center
	University of Helsinki
	Finland

Supervised by   Docent Henri Huttunen, PhD
                               Neuroscience Center
                               University of Helsinki
                               Finland

Thesis committee members   Docent Mikko Airavaara, PhD
                               Institute of Biotechnology
                               University of Helsinki
                               Finland

                               Docent Tomi Rantamäki, PhD
                               Neuroscience Center
                               University of Helsinki
                               Finland

Reviewed by   Docent Mikko Airavaara, PhD
                               Institute of Biotechnology
                               University of Helsinki
                               Finland

                               Docent Tarja Malm, PhD
                               A. I. Virtanen Institute for Molecular Sciences
                               University of Eastern Finland
                               Finland

Opponent   Laura Parkkinen, PhD
                               Nuffield Department of Clinical Neurosciences
                               University of Oxford
                               United Kingdom

Custos   Professor Juha Voipio, PhD
                               Department of Biosciences
                               University of Helsinki
                               Finland

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List of original publications

This Thesis is based on the following original publications, which are referred to in the text by Roman numerals:

I   Niko-Petteri Nykänen, Kai Kysenius, Prasanna Sakha, Päivi Tammela and Henri J. Huttunen (2012). γ-aminobutyric acid type A (GABAA) receptor activation modulates Tau phosphorylation. J Biol Chem. 287(9): 6743-52.

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III   Xu Yan, Niko-Petteri Nykänen, Cecilia Brunello, Annakaisa Haapasalo, Mikko Hiltunen and Henri J. Huttunen (2015). FRMD4A-cytohesin signaling modulates cellular release of Tau. Manuscript submitted.

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Author's contribution to the original publications included in the Thesis:

I: The author participated in designing the experiments, generated most of the molecular biological constructs, performed majority of the experiments (assay development, chemical library screen, mechanical experiments), contributed to the data analyses and in the writing of the manuscript.

II: The author participated in designing the experiments and conducted all the experimental procedures of the in vitro functional analyzes -section of the publication, contributed to in vitro data analyses and in the writing of the manuscript.

III: The author conducted ELISA experiments and the RNAi screen of LOAD susceptibility genes, contributed to data analyses and in the writing of the manuscript.

List of abbreviations

aa	amino acid
ABCA7	ATP-binding cassette transporte A7
AD	Alzheimer's disease
ADGC	Alzheimer's Disease Genetics Consortium
AG	argyrophilic grain
AGD	Argyrophilic grain disease
AGE	advanced glycogen endproduct
AICD	amyloid-β precursor protein intracellular domain
AIF-1	allograft inflammatory factor 1
ALS	amyotrophic lateral sclerosis
APH-1	anterior pharynx defective 1
aPKCζ	atypical protein kinase C ζ
ATP	adenoside triphosphate
APOE	apolipoprotein E
APP	amyloid-β precursor protein
Arf6	adenosine diphosphate (ADP) ribosylation factor 6
BBB	blood-brain barrier
BIN1	bridging integrator 1
bvFTD	behavioural variant frontotemporal dementia
C20ζ	C-terminal fragment of atypical protein kinase C ζ
CaMKII	calmodulin-dependent protein kinase II
CBD	corticobasal degeneration
CD2AP	CD2-associated protein
CD33	CD 33 molecule
CDK5	cyclin-dependent kinase 5
CHARGE	Cohorts for Heart and Aging in Genomic Epidemiology consortium
CJD	Creutzfeldt-Jacob disease
CK	casein kinase
CLU	clusterin
CNS	central nervous system
CR1	complement receptor 1
CSF	cerebrospinal fluid
CTE	chronic traumatic encephalopathy
CTF	carboxy-terminal fragment
DS	Down sydrome
DYRK1A	dual-specifity tyrosine (Y)-phosphorylation-related kinase 1A
EADI	European Alzheimer's Disease Initiative
EE	early endosome
EOAD	early-onset Alzheimer's disease
EPHA1	EPH receptor A1
ERK	extracellular signal-regulated kinase
EWAS	epigenome-wide association study
FKBP	FK506 binding protein
FRMD4A	FERM containing domain 4A
FTD	frontotemporal dementia

FTDP-17	frontotemporal dementia with parkinsonism linked to chromosome 17
FTLD	frontotemporal lobar degeneration
FUS	fused in sarcoma
GABA	γ-amino butyric acid
GAI	globular astrocytic inclusion
GEF	guanide exchange factor
GERAD	Genetic and Environmental Risk in Alzheimer's Disease
GFAP	glial fibrillary acidic protein
GFP	green fluorescent protein
GGI	globular glial inclusion
GGT	globular glial tauopathy
GLuc	*Gaussia princeps* luciferase
GOI	globular oligodendrocytic inclusion
GSK-3	glycogen synthase kinase 3
GWAS	genome-wide association study
GWHA	genome-wide haplotype association
HDAC6	histone deacetylase 6
HSPG	heparan sulfate proteoglycans
HTS	high-throughput screening
IGAP	International Genomics of Alzheimer's Project
iPSC	induced pluripotent stem cell
ISF	interstitial fluid
ITIM	immunoreceptor tyrosine-based inhibitory motif
JNK	c-Jun NH_2-terminal kinase (stress activated protein kinase)
LD	linkage disequilibrium
LDH	lactate dehydrogenase
LDLR	low density lipoprotein receptor
LOAD	late-onset Alzheimer's disease
LRP1	low-density lipoprotein receptor-related protein 1
lvFTD	language variant frontotemporal dementia
MAF	minor allele frequency
MAP	microtubule-associated protein
MAPK	mitogen-activated protein kinase
MAPT	microtubule-associated protein tau
MBRD	microtubule-binding repeat domain
MCI	mild cognitive impairment
MND	motor neuron disease
MS4A	membrane-spanning 4-domains subfamily A
MSA	multiple system atrophy
MT	microtubule
NCT	nicastrin
NDD	neurodegenerative disorder
NFT	neurofibrillary tangle
NGS	next-generation sequencing
NMDA	*N*-methyl-*D*-aspartate
NPH	normal pressure hydrocephalus
nSMase	neutral sphingomyelinase

O-GlcNAc	O-linked monosaccharide β-N-acetylglucosamine
OR	odds ratio
Par	partitioning-defective protein
PAR	population attributable risk
PART	primary age-related tauopathy
PB	Pick body
PCA	protein-fragment complementation assay
PD	Parkinson's disease
PGRN	progranulin
PHF	paired helical filament
PICALM	Phosphatidylinositol binding clathrin assembly protein
PiD	Pick's disease
Pin1	protein interacting with NIMA (never in mitosis A) 1
PKA	protein kinase A
PKC	protein kinase C
PLD3	phospholipase D3
PNFA	progressive nonfluent aphasia
PP	protein phosphatase
Pro	proline
PrP	prion protein
PSEN	presenilin
PSP	progressive supranuclear palsy
PTM	posttranslational modification
Ser	serine
SF	straight filament
shRNA	small hairpin ribonuclease acid
siRNA	small interfering ribonuclease acid
SNP	single-nucleotide polymorphism
SORL1	sortilin-related receptor LDLR class A repeats containing
TBI	traumatic brain injury
TDP-43	43 kDa transactive response DNA-binding protein
TGN	trans-Golgi network
Thr	threonine
TREM2	triggering receptor expressed on myeloid cells 2
VAMP2	vesicle-associated membrane protein 2 (synaptobrevin 2)
VPS10	vacuolar protein sorting 10

Abstract

Abnormal regulation of various posttranslational modifications (PTMs) of microtubule-associated protein tau induce its self-aggregation, which is a hallmark pathophysiological process of neurodegenerative diseases (NDDs) collectively called as tauopathies including Alzheimer's disease (AD) and frontotemporal dementia. Increased tau phosphorylation is a key PTM in conversion of tau into more toxic species in cells, which is regulated by interactions of various protein kinases and phosphatases. However, the exact mechanism(s) of how various combinatory PTMs affect aggregation and cell-to-cell propagation of tau are poorly understood. We developed a novel live cell reporter system based on protein-fragment complementation assay (PCA) and studied dynamic protein-protein interactions of tau in native cellular environment. The PCA was further validated on investigating cellular secretion and uptake of tau in live cells. A proof-of-concept screen was performed using PCA platform revealed several GABA$_A$ receptor activators that altered the interaction of tau-Pin1. Pin1 act as a critical facilitator of tau dephosphorylation by catalyzing the isomerization of *cis/trans* peptidyl-prolyl bond at phosphorylated Thr231-Pro motif of tau. Additionally, we showed that screen-identified GABA$_A$ receptor modulators increased tau phosphorylation at the AT8 phosphoepitope in cultures of mature primary cortical neurons and remained at elevated level 24 h after washout of the drugs. Mechanistic studies suggested that enhanced GABA$_A$ receptor-induced tau phosphorylation was associated with decreased interaction of tau and protein phosphatase 2A (PP2A) without any reduction in enzymatic activity of PP2A and involved CDK5 kinase. Furthermore, the assessment of expression and splicing status of late-onset AD (LOAD) susceptibility genes in our neuropathologically validated AD cohort of *post mortem* brain samples revealed increased expression of *MS4A6A* and decreased expression of *FRMD4A* in regards to increased AD-related neurofibrillary pathology according to Braak staging. Moreover, the expression level of *FRMD4A* was functionally associated with amyloidogenic APP processing and increased tau phosphorylation in vitro. *FRMD4A* expression levels also correlated with cellular tau secretion assessed by PCA-based assay platform using siRNA-mediated gene silencing. Subsequent mechanistic studies on secretion showed a more general involvement of cell polarity complex signaling including Par3/Par6/aPKCζ complex-induced activation of Arf6 via cytohesins. These novel connections of altered *FRMD4A* expression level in AD brain and its impact on cellular tau secretion further corroborate the suggested role of *FRMD4A* in LOAD pathogenesis and pathophysiology. Here, for the first time, we assessed a functional association between LOAD-related susceptibility gene and cell-to-cell propagation of tau, and also showed the decreased expression of *FRMD4A* related to increasing disease severity according to Braak staging.

1 Introduction

Plethora of molecular interactions governs the functions and eventually determines the fate of the cell in a highly complex cellular environment. In particular, the functionality of the cell is regulated by dynamic protein interaction network predominantly via formation of multi-protein complexes. Despite of the vast investigation effort, the majority of the protein interaction patterns are poorly understood, which is due to the immense amount of protein pathways that encompass the physiological function of the cell. Aberrations in cellular protein-protein interactions are, however, widely recognized and typically closely associated with various pathological disorders. Hence, more profound understanding of both physiological and pathophysiological protein-protein interactions maintaining the cellular integrity would provide novel insight and possible therapeutic strategies on addressing these interactions contributing to cellular dysfunction (Arkin & Wells 2004).

Protein misfolding and subsequent aggregation in the central nervous system (CNS) is a characteristic pathological feature of numerous neurodegenerative disorders (NDDs) (Lee et al., 2001; Ballatore et al., 2007). Cerebral accumulation of microtubule-associated protein tau (MAPT) defines a group of NDDs referred as tauopathies, in which intracellular tau formations called neurofibrillary tangles (NFTs) are the most prominent neuropathological hallmark inclusions. Specifically, the formation of these filamentous depositions is driven by disequilibrium of multiple posttranslational modifications (PTMs) of tau such as aberrant phosphorylation, which is the most studied and disease-associated PTM of tau in tauopathies including e.g. Alzheimer's disease (AD) and frontotemporal dementia. Importantly, although tau pathology occurs in various NDDs with additional pathologies, toxic gain-of-functions and loss of normal microtubule stabilizing function are alone sufficient to cause tau-driven neurodegeneration independently of involvement of possible concomitant pathologies.

The increased knowledge of the genetic landscape of sporadic, late-onset AD (LOAD) that represent more than 95% of all AD cases, has been mainly provided by genome-wide association studies (GWAS) (e.g. Karch & Goate 2015; Chouraki & Seshadri 2014; Lambert et al., 2013b). Given that causative factors underlying LOAD pathogenesis are mainly currently unknown, the emergence of large number of LOAD-associated susceptibility genes within the past few years have critically broaden the understanding of possible genes contributing to disease pathogenesis besides *APOE*, which is the strongest known risk factor after aging. However, the functional association of identified LOAD susceptibility genes with established AD-related pathophysiological pathways and the specific expression and splicing profiles during the disease progression remains largely elusive. Accordingly, several single-nucleotide polymorphisms (SNPs) are located either in non-coding or intergenic regions of the identified loci, and, the subtle effect of each individual gene conferring the LOAD risk are both significantly hindering the investigation of these genes in existing disease models. Nonetheless, GWAS-induced acceleration of studying the genetics of LOAD has generated a tremendous amount of opportunities for variable approaches towards targeting the pathophysiological pathways underlying the disease pathogenesis.

Accumulating evidence on prion-like spreading of various disease-specific proteins implicate an existence of a common mechanism for spreading of pathology in NDDs (Brettschneider et al., 2015; Guo & Lee 2014). A prion paradigm -hypothesis suggest that

amyloidogenic non-prion proteins, including e.g. amyloid-β (Aβ), α-synuclein, TDP-43 and superoxidase dismutase 1, huntingtin and tau in AD, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and tauopathies, respectively, may share a common or similar mechanism with prions for cell-to-cell transmission of pathology between distinct brain regions. In prion disease, such as Creutzfeldt-Jacob disease (CJD), normal cellular prion protein converts into conformationally altered misfolded protein, which is further capable of recruiting and inducing the conformational switch of their normal counterparts, a perpetual process referred as seeding fibrillization (Prusiner 1982; Aguzzi 2009; Aguzzi et al., 2008). Upon growth of prion aggregates they also fragment and spread thereby generating a loop of subsequent seeding of conformationally distinct variants, or strains. This has been also suggested to be the mechanism in non-prion proteinopathies, such as tauopathies. However, the exact molecular mechanism(s) of how and which protein species are actively secreted from the cell to extracellular space and how these protein species, or strains, are internalized by adjacent cells are poorly understood.

Demographic aging, particularly in developed societies generates increasing socio-economical burden within the next few decades caused by dementias and other NDDs. Therefore, it is of high importance to address the investigation effort towards elucidation of molecular mechanisms of pathophysiological pathways of NDDs. Since the development of novel therapeutics is expensive and time consuming and early diagnosis of NDDs in patients is highly challenging, it is essential to try to focus the research to early aberrant disease-specific protein-protein interactions. Furthermore, according to AD and related tauopathies, it is fundamental to characterize the multiple cascade-event eventually leading to tau-mediated neurodegeneration. Specifically, to investigate the contribution of expression and splicing status of currently identified LOAD susceptibility genes to disease pathogenesis and progression, and study how these genes may be functionally associated with cell-to-cell propagation of tau pathology could reveal novel therapeutic targets in treatment of these disorders.

2 Review of literature – Biology of microtubule-associated protein tau

2.1 Structure and functions

Microtubules (MTs) are components that maintain the structure of cytoskeleton organization in cells (e.g. Meraz-Rios et al., 2010). This is essential to formation of axons and dendrites that are both highly important in intracellular cargo trafficking of various molecules such as proteins, and also play a crucial role in transsynaptic signalling. Microtubule-associated protein tau (MAPT) is a major cytoskeletal-associated protein, encoded by *MAPT* gene that is located on chromosome 17q21 (Neve et al., 1986). *MAPT* contains 16 exons, from which 13 are included in the primary transcript due to an exclusion of exons 4a, 6 and 8 in the human brain (figure 2.1) (Buee et al., 2000). However, exons 4a, 6 and 8 are specific for peripheral tau expression. Constitutive tau exons 1,4, 5, 7, 9, 11, 12 and 13 are transcribed and translated into a protein unlike exons -1 (part of the promoter region) and 14, which are only transcribed but not translated (Goedert et al., 1989a).

Tau is a member of neuronal microtubule-binding protein (MAP) family with other proteins such as MAP1 and MAP2 (Iqbal et al., 2005). Tau is functionally the major MT-associated protein in neurons where its main functions are to stabilize and promote the assembly and disassembly of MTs (Weingarten et al., 1975). Tau binds to MTs via microtubule binding repeats that are located in the microtubule-binding repeat domain (MBRD) at the C-terminal part of tau (Lee et al., 1989). Microtubule-binding repeats are encoded by exons 9, 10, 11 and 12. MBRD can also be further diveded in two regions, including microtubule-binding region and acidic region, which covers the most C-teminal part of MBRD (e.g. Meraz-Rios et al., 2010; Wang & Liu 2008). The microtubule-binding region of the MBRD consists of either three (3R-tau, repeats R1, R3, R4) or four (4R-tau, repeats R1, R2, R3, R4) similar but not identical repetitive sequences of 31 or 32 amino acids (aa) residues (Lee et al., 1989; Iqbal et al., 2009). These two isoforms are due to alternative splicing of exon 10 encoding the R2-repeat (Andreadis et al., 1992; Hernandez & Avila 2007).

Alternative splicing of exons 2, 3 and 10 in tau mRNA results in six tau isoforms in the human brain (Andreadis et al., 1992; Goedert et al., 1989a). Exon 3 is exon 2 dependent, i.e. exon 3 is never included in the final protein if the exon 2 is absent, whereas expression of exon 2 is independent of exon 3 (Andreadis et al., 1995). These six human tau isoforms resulting from the presence or absence of alternatively spliced exons 2, 3 and 10 differ in length ranging from 351 to 441 aa (Goedert et al., 1989a; Kosik et al., 1989; Goedert et al., 1989b).
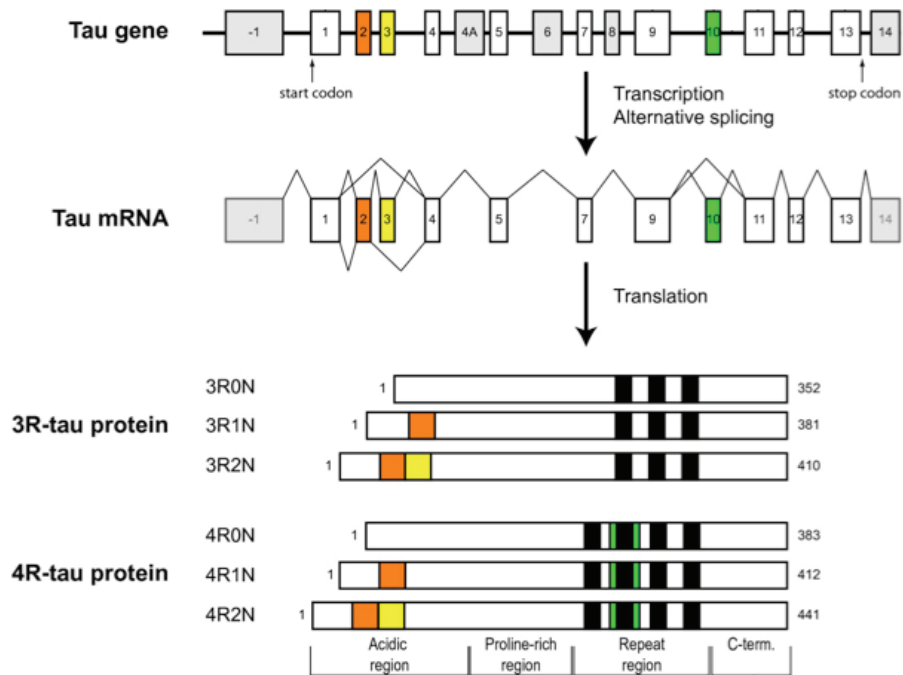
Figure 2.1. A schematic representation of the *MAPT* gene, primary transcript and domain structure of six human tau isoforms expressed in the central nervous system. The human *MAPT* gene encoding tau protein is located on chromosome 17q21. From the 16 exons of the *MAPT* gene (upper panel), 13 exons are transcribed in the primary transcript (exons 4A, 6 and 8 are specific for peripheral tau and are not present in the human brain mRNA) (middle panel). Exon -1, which is part of the promoter, and exon 14 are both transcribed but not translated into a protein. Six tau isoforms are found in the human brain produced by constitutive exons 1, 4, 5, 7, 9, 11, 12 and 13, and alternative splicing of exons 2, 3 and 10 (lower panel). Differences between the isoforms are due to presence or absence of one or two amino-terminal inserts of 29 amino acids (0N, 1N, 2N) encoded by exons 2 and 3 combined with carboxy-terminal inserts of either 3 (R1, R3 and R4) or 4 (R1-R4) repeat-regions. R2 repeat-region is encoded by exon 10. These six tau isoforms vary in lenght including 352, 381, 383, 410, 412 or 441 amino acids (with exon combinations of 2-3-10-; 2+3-10-; 2-3-10+; 2+3 10-; 2+3-10+; 2+3+10+, respectively). All tau isoforms have been found in the adult human brain, but only the shortest isoform 0N/3R, is expressed in fetal human brain. Tau protein can be divided into N-terminal acidic region, proline-rich region, microtubule-binding repeat domain (MBRD) or repeat region and C-terminus. Modified from Gendron & Petrucelli et al., 2009.

Two structurally distinct domains called projection domain and microtubule-binding repeat domain exist in tau (e.g. Meraz-Rios et al., 2010; Wang & Liu 2008). The N-terminally located projection domain is reported to project from the surface of microtubules (Hirokawa et al., 1988). Tau projection domain can be further divided into two separate regions (e.g. Meraz-Rios et al., 2010; Wang & Liu 2008), from which the C-

terminal proline rich region is positively charged. Conversely, the N-terminal projection domain region consists of predominantly acidic residues resulting in negatively charged region. Exons 2 and 3 encode 29 aa long sequences in the N-terminal projection domain, which can exist in variable patterns, i.e. the N-terminus can contain either two, one or zero 29 aa insertions (namely 2N, 1N and 0N respectively). Another region of the projection domain, called proline-rich region, modulates the length of N-terminal part of projection domain hence modifying the overall length of the protein (Buee et al., 2000). The exact function(s) of the projection domain is yet to be elucidated but it is known to interact with other cytoskeleton related proteins such as neurofilaments and plasma membrane (Brandt et al., 1995; Hirokawa et al., 1988). Furthermore, phosphorylation-dependent interaction between tau and MTs is suggested to occur via regulation of the proline-rich region (Lee et al., 2004).

All six tau isoforms are expressed in the adult human brain whereas only the shortest 0N/3R is found in the fetal human brain (Kosik et al., 1989; Goedert et al., 1989b). 2R-repeat in the 4R-tau and the presence of either one or both of the N-terminal inserts increase the microtubule-binding affinity of tau potentiating the longest 2N/4R isoform to be the most efficient in promoting MT-assembly (e.g. Lu & Kosik 2001; Alonso et al., 2001b). Besides the R2 repeat, a specific peptide sequence located at the interregion of repeats R1 and R2 is suggested as a region that most efficiently induces the MT polymerization (Goode & Feinstein 1994).

Tau has been reported to have physiological functions outside of regulation of MT dynamics. First, nuclear tau localization, where tau is found to exist in less soluble form, suggests that tau is specifically modified either posttranslationally or via interaction with other proteins (Brady et al., 1995). Secondly, induction of paired helical filament (PHF) formation is activated by binding of tau to RNA through MBRD (Kampers et al., 1996). Thirdly, tau interferes with binding of kinesin and kinesin-like proteins to MTs, which further disrupts axonal cargo transport (Tatebayashi et al., 2004) and participates in cell viability maintenance by antagonizing apoptosis (Li et al., 2007). Although tau is predominantly considered to be an axonal protein, additional role of tau in dendrites has been reported suggesting that tau mediates Aβ-toxicity in AD (Ittner et al., 2010). Specifically, Src-family tyrosine kinase Fyn interacts with tau via its projection domain. In tau knockout mice, the postsynaptic targeting of Fyn was found to be diminished, which resulted in decreased N-methyl-D-aspartate (NMDA) receptor phosphorylation that mitigated NMDA receptor-mediated excitotoxicity. This reduction of excitotoxicity subsequently decreases toxicity of Aβ (Ittner et al., 2010).

2.2   Regulation of tau phosphorylation and dephosphorylation

Like all other phosphoproteins, the biological activity of tau is also regulated by its phosphorylation status (e.g. Alonso et al., 1994; Kopke et al., 1993). In addition to tau expression, also its phosphorylation is developmentally regulated; e.g. the shortest isoform (0N/3R) in fetal and postnatal human brain has high phosphorylation status whereas in healthy adult brain it is markedly reduced (Goedert et al., 1993). Furhermore, increase in tau phosphorylation in mitotic stage of cell cycle has been observed indicating that tau phosphorylation is also functionally regulated (Delobel et al., 2002). Thus, these data implicate that both developmental and functional regulation of tau phosphorylation are

essential in modulation of MT stabilization and dynamics during normal neurite formation and other MT-associated functions (Wang & Liu 2008). Moreover, upon hypo- or hyperphosphorylation tau loses its ability to bind MTs, which indicates the importance of phosphorylation at specific sites in order to maintain high affinity to bind MTs and properly function as a MT stabilizing protein (Garcia de Ancos et al., 1993). Conversely, phosphorylation at certain sites may decrease or even prevent MT binding and stabilizing capacity of tau further inducing its self-aggregation.

Multiple protein kinases, each of which has a preference toward specific region or site, catalyze tau phosphorylation (e.g. Liu et al., 2007). Of these kinases, more than ten have been shown to phosphorylate serine (Ser) and threonine (Thr) residues in vitro. The protein kinases that participate in tau phosphorylation can be divided in two distinct groups according to their motif-specifity: proline-directed protein kinases and non-proline-directed protein kinases (e.g. Meraz-Rios et al., 2010; Wang & Liu 2008). The proline-directed protein kinases -group includes kinases such as DYRK1A, MAP-kinases, ERK1/2, CDK5 and GSK-3β, from which the latter is the most strongly associated with tau hyperphosphorylation in AD pathogenesis (e.g. Gong et al., 2005; Wang & Liu 2008; Grimes & Jope 2001). Kinases such as PKA, PKC and CaMKII are included in the non-proline-directed protein kinases. Conversely to phosphorylation, all the major protein phosphatases (PPs), with the exception of PP2C, are capable of dephosphorylating tau (Liu et al., 2005). Of these PPs including PP1, PP2A, PP2B and PP5, PP2A has the highest efficacy in tau dephosphorylation. Additionally to role of PPs and kinases in regulation of tau phosphorylation, the conformational state of tau strongly affects the rate and extent of phosphorylation (Alonso Adel et al., 2004). The changes in conformational state of tau may promote phosphorylation in two ways: tau may become a more suitable substrate for protein kinases and/or a specific conformation may reduce the efficacy of dephosphorylation by making tau less favourable substrate to PPs (Iqbal et al., 2005). Moreover, a process called tau priming phosphorylation by certain protein kinases significantly promotes the subsequent proline-directed protein kinase phosphorylation which may further promote hyperphosphorylation (Liu et al., 2004).

In the longest human brain tau isoform (2N/4R, 441 aa) there are at least 80 putative phosphorylated Ser and Thr residues (Gong et al., 2005; Wang & Liu 2008). More than 30 of these sites have been identified in PHF-tau in AD brain. The localization of these sites is predominantly concentrated in the proline-rich region (residues 172-251, numbering according to the longest isoform 2N/4R) and C-terminal region (residues 368-441), which is flanking the MBRD (Gong et al., 2005; Wang & Liu 2008; Liu et al., 2007). Additionally, some of the sites are localized in MBRD, including Ser262 (R1), Ser285 (R1-R2 inter-repeat), Ser305 (R3-R4 inter-repeat), Ser324 (R3), Ser352 (R4) and Ser356 (R4). Compared to hyperphosphorylation in other regions or sites of tau, hyperphosphorylation at sites that are located with the MBRD is suggested to have more severe effect on impairing MT stabilizing and assembly promoting function of tau, which is likely due to its diminished affinity to bind MT.

Disturbed balance between phosphorylation and dephosphorylation may result in tau hyperphosphorylation via either increased kinase activity or downregulation of PP enzyme activity or both (Gong et al., 2005). However, the sites that are essential in promoting the development of AD pathophysiology are yet poorly understood. This has lead to a debate whether tau hyperphosphorylation *per se* is sufficient alone to induce neurotoxicity and

neurofibrillary pathology observed in tauopathies. Accordingly, the combined phosphorylation of sites Thr212, Thr231 and Ser262 is reported to drive tau aggregation, disrupt MT-integrity, induce neurotoxicity and concomitant apoptosis in vitro (Alonso et al., 2010). These data provide a connection between tau hyperphosphorylation and neurofibrillary degeneration at least in vitro, and also identifies sites at which hyperphosphorylation may facilitate the conversion of tau to toxic species, which can no longer maintain its physiological functions. In addition to the above mentioned phosphorylation sites that may convert tau into toxic species, sites Ser199, Ser202, Thr205, Ser235, Ser356, Ser396, Ser404 and Ser422 have been proposed to have similar effect (Alonso Adel et al., 2004).

2.2.1 Kinases

2.2.1.1 GSK-3

Glycogen synthase kinase-3 (GSK-3) is a proline-directed Ser/Thr protein kinase, i.e. Ser or Thr preceding Pro, encoded by two distinct genes *GSK3A* and *GSK3B* resulting in two protein isoforms, GSK-3α and GSK-3β, respectively, which are abundantly expressed in the brain (Woodgett 1990). Although these two isoforms share significant sequence homology with each other, there are differences in their N- and C-terminal regions (e.g. Avila et al., 2010). GSK-3 is an essential regulatory enzyme with multiple cellular functions mediated by substrate phosphorylation, e.g. regulation of numerous physiological functions including glycogen metabolism and signal transduction (Grimes & Jope 2001).

Phosphorylation status of N-terminal serine residues 21 and 9 in GSK-3α and GSK-3β, respectively, modulate the enzymatic activity of GSK-3 kinases (Jope & Johnson 2004). GSK-3 activity is reduced by phosphorylation of these Ser residues by phopshorylation mediated by priming kinases, such as protein kinase A (PKA) (Fang et al., 2000) and Akt (Cross et al., 1995). These kinases are a part of a rather large kinase group capable of phosphorylation of these serines thereby contributing to reduction of GSK-3 activity. Conversely, the enzymatic GSK-3 activity is increased upon phosphorylation of tyrosine residues 279 and 216 in GSK-3α and GSK-3β, respectively. Additionally, several GSK-3 substrates require priming (pre-phosphorylation of neighboring sites) to be further phosphorylated by GSK-3 resulting in bidirectional GSK-3 activity regulation, i.e. the phosphorylation status of the substrate may dictate the possible subsequent phosphorylation by GSK-3. Besides the state of its specific intramolecular phosphorylation, enzymatic activity of GSK-3 kinases is modulated by their interaction with other proteins and protein complexes (Avila et al., 2010), and the subcellular localization of GSK-3 since its compartmentalization is widely distributed throughout the cell (Grimes & Jope 2001).

GSK-3, especially the GSK-3β isoform, phosphorylates multiple sites on tau and is frequently reported as one of the main kinases regulating tau phosphorylation (Ishiguro et al., 1993; Grimes & Jope 2001). It participates in regulation of phosphorylation equilibrium of tau under normal physiological conditions and strongly contributes to hyperphosphorylation in pathophysiological conditions thereby rendering the possible subsequent dissociation of tau from the MTs, which further results in disruption of the MT

network. Interestingly, the phosphorylation of GSK-3β primed tau sites seems to more strongly affect its interaction with MTs as compared with non-primed sites (Cho & Johnson 2003). Moreover, Ser9-phosphorylated GSK-3β, regardless of its reduced enzymatic activity, is suggested to be able to phosphorylate tau via a mechanism that is yet to be elucidated (Yuan et al., 2004).

2.2.1.2 CDK5

In addition to GSK-3, cyclin-dependent kinase 5 (CDK5) is considered as another main kinase that contributes to regulation of tau phosphorylation status (Cruz & Tsai 2004a). CDK5 plays an essential role in development and maintenance of CNS by phosphorylation of a vast array of substrates. Association with its non-catalytic regulatory subunits p35 and p39 is fundamental for CDK5 to obtain enzymatic activity and further maintain its normal cellular functions. Since these CDK5 activators p35 and 39 are mainly expressed in post-mitotic neurons, the CDK5 activity is predominantly enriched in CNS (Lew et al., 1994).

CDK5 deregulation induced neurotoxicity contributes to pathogenesis of several NDDs, including AD (Cruz & Tsai 2004b). Under various neurotoxic conditions induced by e.g. oxidative stress or Aβ-mediated toxicity, the CDK5 regulatory activators p35 and p39 are cleaved by calpain resulting in truncated forms p25 and p29, respectively (Patzke & Tsai 2002; Kusakawa et al., 2000). Importantly, compared to non-cleaved forms, these calpain-cleaved activators p25 and p29 are more stable in the p25/p29-CDK5 heterodimer, which prolongs its kinase activity. Moreover, both the subcellular localization between complexes p25-CDK5 and p35-CDK5 (Cruz & Tsai 2004b) and substrate specificity is altered (Patrick et al., 1999). Furthermore, tau hyperphosphorylation, induction of cytoskeletal disruption and increased neuronal apoptosis are all possible consequences of increased p25 generation and accumulation (Patrick et al., 1999). Interestingly, p25 may also bind and activate GSK-3β (Chow et al., 2014).

2.2.1.3 DYRK1A

Dual-specificity tyrosine (Y)-phosphorylation-related kinase 1A (DYRK1A) is a multifaceted enzyme related to Ser/Thr phosphorylation of tau (e.g. Ryoo et al., 2007). In addition to phosphorylation of its several protein substrates, proline-directed protein kinase DYRK1A has dual substrate specificity: Tyr321 located in the activation loop of the catalytic domain is autophosphorylated for its self-activation (Himpel et al., 2001) and phosphorylation is targeted to Ser/Thr residues of multiple proteins (Himpel et al., 2000). Interestingly, DYRK1A is suggested to prime numerous substrates such as tau to be further phosphorylated by GSK-3 (Woods et al., 2001). Yet interesting aspect connecting DYRK1A and AD is the chromosomal location of the gene that encodes DYRK1A kinase: in humans it is located on chromosome 21, which full or partial trisomy causes Down syndrome (DS) (e.g. Wiseman et al., 2009). Due to additional copy caused by chromosome 21 trisomy, the overexpression of many genes that are located there, such as gene encoding amyloid precursor protein, lead to different developmental deficits, such as impaired learning and memory and mental retardation in which DYRK1A is suggested to play an important role (Ahn et al., 2006). This is tightly connected to the fact that almost

all DS patients develop AD-like dementia and pathophysiology by the age of 40 (Park et al., 2009). Furthermore, besides its function as a tau kinase, DYRK1A is known to phosphorylate APP at Thr668 and presenilin-1, which could result in increased Aβ production and aggregation (Ryoo et al., 2008; Ryu et al., 2010).

2.2.1.4 Other proline-directed protein kinases

Mitogen-activated protein kinases (MAPKs) are a family of proline-directed Ser/Thr protein kinases, which participate in several cellular functions (e.g Munoz & Ammit 2010; Kim & Choi 2010). MAPK induced signal transduction cascades typically involve a minimum of three consecutive kinases, such as MAPK3, MAPK2 and MAPK, which subsequentally lead to phosphorylation of large number of diverse MAPK substrates. The MAPK signalling pathways can be activated either via interaction of two kinase components or formation of multiple kinase complex that is regulated by specific scaffolding proteins such as JNK-interacting proteins (Kim & Choi 2010). The most studied MAPK family members include extracellular signal-regulated kinase (ERK), c-Jun NH_2-terminal kinase (JNK, also known as stress activated protein kinase) and p38, which all exists in several isoforms (Schaeffer & Weber 1999). These kinases are involved in multiple cellular functions, including cell growth and differentiation, apoptosis and modulation of inflammation and other stress responses. Furthermore, MAPK mediated signalling pathways are known to contribute to pathogenesis of several disorders with highly diverse pathophysiological characteristics, such as cancer and NDDs like AD, Parkinson's disease and amyotrophic lateral sclerosis (ALS) (Kim & Choi 2010). Regarding to AD pathogenesis, MAPKs are reported play an essential role in several distinct AD related processes, such as regulation of β- and γ-secretases, induction of neuronal apoptosis and phosphorylation of both APP and tau (Munoz & Ammit 2010; Kim & Choi 2010). Moreover, other MAPKs (JNK, ERK and p38) are all associated with abnormal tau phosphorylation in AD (e.g. Churcher 2006). Co-localization of phosphorylation-activated p38 and tau accumulations are found in both hippocampal and cortical AD brain slices (Zhu et al., 2000) and in transgenic mice overexpressing human tau (Kelleher et al., 2007).

2.2.1.5 Non-proline-directed protein kinases

PKA (cAMP-dependent protein kinase) is a multisubstrate non-proline-directed protein kinase that is involved in several crucial cellular signaling pathways (Walsh & Van Patten 1994). Activation of PKA-catalyzed transduction pathways requires elevation of intracellular cAMP concentration and the co-localization of PKA holoenzyme with its specific substrates. Consistently with other tau kinases, PKA has been intensively studied in vitro and also reported to phosphorylate tau in vivo (Liu et al., 2004). Importantly, PKA serves as a priming kinase that prephosphorylates its substrates at specific Ser/Thr sites hence facilitating the subsequent phosphorylation by proline-directed protein kinases, such as GSK-3 and CDK5 (Wang et al., 2007; Liu et al., 2004). Moreover, PKA-mediated tau phosphorylation alone that precedes the possible sequential phosphorylation by other kinases, is suggested to result in AD -like aberrant tau phosphorylation. This phosphorylation is suggested to be duration-dependent, i.e. only the transient activation

with specific PKA-activator is capable of inducing abnormal AD -like hyperphosphorylation of tau (Wang et al., 2007; Zhang et al., 2006). Other non-proline-directed protein kinases that are implicated in regulation of tau phosphorylation include various casein kinases (CK), calcium- and calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC) (Kuret et al., 1997; Yamamoto et al., 2002; Liu et al., 2003).

2.2.1.6 Fyn

Fyn is a tyrosine (Tyr) kinase that belongs to the src tyrosine kinase family with various biological functions, such as cell adhesion signalling and regulation of neuronal function (Resh 1998). With regards to tau phosphorylation, Fyn mediated Tyr phosphorylation of tau is reported to occur after Aβ-exposure (Williamson et al., 2002) and also during the development of neurodegeneration (Lee et al., 2004). However, Tyr phosphorylation of tau may not significantly alter its MT binding properties. Interestingly, Tyr phosphorylation of Tau has been implicated to contribute to various MT-independent mechanisms in the regulation of tau, such as functioning as an indicator of tau relocalization into cellular compartments where activated kinases are abundant. Alternatively, Tyr phosphorylation of tau may serve as an indicator for activation of src family kinases that can subsequently facilitate the activation of tau phosphorylation by various Ser/Thr kinases. Importantly, additional role of Fyn in neurodegeneration has been reported. Specifically, it has been suggested that Fyn transport to dendritic postsynapse is mediated by tau, which is predominantly considered as an axonal protein, and thereby contributes to Aβ-driven synaptotoxicity via the NMDA receptor in an AD mouse model (Ittner et al., 2010). Interestingly, it was shown that postsynaptic Fyn targeting was disrupted in tau deficient mice and in mice expressing truncated tau resulting in decreased Aβ toxicity.

2.2.2 Protein phosphatases and dephosphorylation

Protein phosphatases (PPs) are responsible for dephosphorylation of tau and other substrates after phosphorylation by various kinases (Liu et al., 2005). The regulation of PP activity occurs via numerous mechanisms, such as phosphorylation of PP regulatory subunits, subcellular localization of PPs and its potential substrates and calcium (Tian & Wang 2002). In mammals, brain expression of five Ser/Thr PPs is observed, including PP1, PP2A, PP2B, PP2C and PP5 (Liu et al., 2005). Of these phosphatases, all except PP2C dephosphorylate tau at same specific sites (at least) in vitro with varying efficacy toward each specific site. The total tau dephosphorylation activity in human brain is unevenly divided between PPs 1, 2A, 2b and 5 with proportions of approximately 11%, 71%, 7% and 10%, respectively. Hence, taken together the partial PP contributions and the observed decrease of PP2A activity in AD brain, PP2A is suggested to be pathophysiologically the most important tau PP. Moreover, PP2A not only dephosphorylates tau but additionally regulates several kinases that are involved in tau phosphorylation via activation or inactivation of these kinases (Tanimukai et al., 2005).

Intracellular PP2A activity is regulated by two endogenous inhibitors I_1^{PP2A} and I_2^{PP2A} (Tanimukai et al., 2005). I_1^{PP2A} mediated inhibition of PP2A occurs via its catalytic

subunit PP2Ac. I_2^{PP2A}, which is mainly located in the nucleus in neurons, is cleaved into two fragments and further translocated into cytoplasm where it interacts with catalytic PP2A subunit and co-localizes with tau aggregates in specific regions of AD brain. Moreover, the expression level of these endogenous inhibitors of PP2A is inceased approximately 20% in AD brain, which may directly result to tau hyperphosphorylation. Interestingly, NMDA -receptor antagonist memantine, which is clinically used to treat the symptoms of AD, is reported to enhance the activity of PP2A thereby inhibiting or at least diminishing hyperphosphorylation of tau and neurofibrillary degeneration (Li et al., 2004). Additionally, compromised PP2A activity due to its increased demethylation may further reduce the ability of PP2A to dephosphorylate tau (Zhou et al., 2008). Intriguingly, sodium selenate was reported as a specific PP2A activator that decreases tau phosphorylation and mitigates functional deficits and tau driven pathology without any neurotoxic effects (van Eersel et al., 2010; Corcoran et al., 2010). Since the expression level and activity of PP2A are both significantly reduced in AD brain (Tanimukai et al., 2005; Vogelsberg-Ragaglia et al., 2001) PP2A is a potential target for drug development for AD (Liu et al., 2005; Tian & Wang 2002).

2.2.3 Tau hyperphosphorylation and neurofibrillary degeneration

The major factor that contributes to tau dysfunction and neurofibrillary degeneration in AD and related tauopathies is tau hyperphosphorylation (Iqbal et al., 2009; Alonso et al., 1994; Grundke-Iqbal et al., 1986). Conversely to normal, physiological tau phosphorylation in which tau contains 2-3 mole of phosphate per mole of tau, the phosphate level in hyperphosphorylated tau in AD brain is increased 3-4 fold (Kopke et al., 1993). There are several factors, such as disturbed phosphorylation/dephosphorylation equilibrium, altered brain glucose metabolism and Aβ-mediated pathways (although the role of Aβ is still controversial) that may contribute tau hyperphosphorylation (Gong & Iqbal 2008). The exact mechanism of hyperphosphorylated tau-induced neurofibrillary degeneration is yet to be partly elucidated regardless of the intensive investigation efforts toward the subject (e.g. Wang & Liu 2008). Nevertheless, it most probably involves both: loss of normal tau function and gain of toxic function.

Upon hyperphosphorylation tau detaches from the MTs and accumulates into two different intraneuronal tangle formations called paired helical filaments (PHFs) and straight filaments (SFs) (figure 2.2) (Iqbal et al., 1989; Alonso et al., 2001a; Grundke-Iqbal et al., 1986). In both tangle formations, tau is the predominant protein component. Formation of PHFs and SFs almost invariably results in the generation of neurofibrillary tangles (NFTs), intracellular structures detected in neuropathological analysis of AD and tauopathy brains. Besides the self-assembly process of all the six human tau isoforms into highly stable PHF/NFT aggregates, the cytosolic non-fibrillar form of hyperphosphorylated tau sequesters other MAPs (MAP1 and MAP2) and tau that is not hyperphosphorylated (Alonso et al., 1994; Alonso et al., 1997; Kopke et al., 1993). Subsequently, this toxic gain-of-function results in disruption of MT assembly and stabilization. The affinity of AD-like hyperphosphorylated tau (AD-like p-tau) to bind and sequester different tau isoforms varies (2N/4R > 1N/4R > 0N/4R and 2N/3R > 1N/3R > 0N/3R, and also 2N/4R > 2N/3R) (Alonso et al., 2001b). Thus, whereas 4R-isoforms are preferentially sequestered by AD-like p-tau, the lack of N-terminal fragments and the

absence of fourth additional MT-binding repeat R2 indicate a protective role of the shortest fetal human tau isoform against neurofibrillary degeneration. Moreover, AD-like p-tau is reported to inhibit proteasome function in cells and PHF-tau is found to be polyubiquitynated (Cripps et al., 2006), which emphasize the importance of degradation and clearance mechanisms of AD-like p-tau to prevent further accumulation of misfolded proteins and NFT formation. Importantly, AD-like p-tau disrupts axonal transport by interfering with the kinesin-like motor protein and by destabilizing MTs, which are highly important tracks in axonal transport of various cellular organelles and proteins (Tatebayashi et al., 2004). Considering the highly polarized morphology of neurons, functionality of axonal transport system is crucial for neuronal viability.
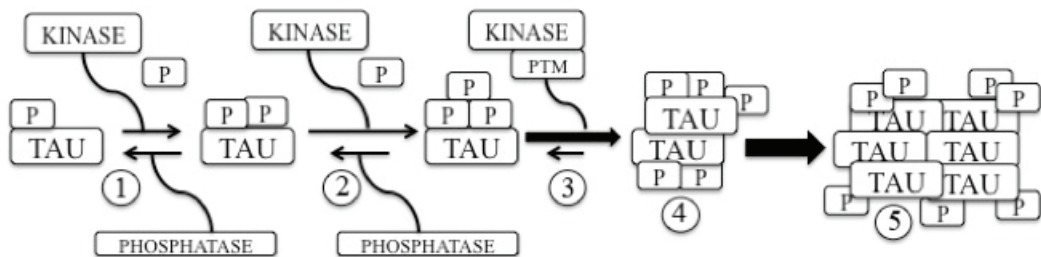
Figure 2.2. Tau phosphorylation and filament formation. Normal physiological phosphorylation and dephosphorylation cycle of tau is regulated by protein kinases and protein phosphatases (1) by either attaching a free phosphate or detaching a phosphate from tau, respectively. Tau hyperphosphorylation is a result of increased kinase activity and/or decreased phosphatase activity (2). Upon tau hyperphosphorylation and possible contribution of other posttranslational modifications (PTM), such as acetylation and truncation (3), tau typically detaches from microtubules and becomes more prone to form straight filaments (SFs)/paired helical filaments (PHFs) or prefibrils (4) subsequently leading to formation of insoluble intracellular fibrils, aggregates and neurofibrillary tangles (NFTs) (5). Normal tau phosphorylation and hyperphosphorylation are reversible processes hence hyperphosphorylation does not necessarily lead to formation of PHFs/prefibrils. Conversely, if PHFs are abundantly present in the cytosol the subsequent formation of NFTs is almost invariable.

Protein kinases are naturally the key molecules driving tau toward its hyperphosphorylated form. Indeed, phosphorylation of tau by several kinases, such as PKA, CaMKII, GSK-3β and CDK5, and by using different combinations and sequential treatments with recombinant protein kinases, "normal" tau is reported to hyperphosphorylate and self-assemble into PHFs (Wang et al., 2007). PP2A is capable of dephosphorylating all the essential sites required for self-assembly of AD-like p-tau, which further verifies its role as the major tau phosphatase. Interestingly, after dephosphorylation of AD-like p-tau by PP2A, rephosphorylation is found to occur via various kinase combinations, which can restore the self-assembling property to form

PHFs/NFTs. These results strongly implicate the reversible nature of tau hyperphosphorylation and the involvement of multiple kinases. Besides the sequential phosphorylation by specific kinase combination at certain sites, the sequential pattern of AD-associated tau phosphorylation sites has been studied in primary neurons (Bertrand et al., 2010). Similar to priming phosphorylation of tau by various kinases, the sequence of phosphorylation at specific AD-related sites could have an impact on the overall status of tau phosphorylation mediated by differential cascades. The AT8 epitope of tau, which consists of sites Ser199, Ser202 and Thr205, is considered a central mediator of various cascades regulating the priming and feedback processes and is one of the most intensively studied tau phosphorylation epitope.

2.3 Other proteins interacting with tau

2.3.1 β-Tubulin

MTs are crucial cellular elements that are involved in vast array of vital functions (Chau et al., 1998). These essential MT functions including e.g. cell motility, cell division and cell morphology, are collectively regulated by microtubule-associated proteins via stabilization of the MT structure and regulation of their assembly and disassembly dynamics. MTs are non-covalent polarized cytoskeletal polymers, which are assembled from α- and β-tubulin monomer subunits that form a dynamic heterodimer and require additional molecules to stabilize their structure (Kar et al., 2003). Binding of tau to β-tubulin subunits within the MT occurs via the repeat-region sequences located in the MBRD of tau (Amos 2004; Lee et al., 1989; Chau et al., 1998). Aberrant tau phosphorylation results in reduced ability of tau to regulate MT-dynamics, which can further lead to disruption of the MT-network and compromise axonal transport of proteins and various cellular organelles in neurons. Interestingly, β-III-tubulin, which is the predominant neuronal tubulin isoform, is also found to be hyperphosphorylated in AD brain probably due to decreased PP2A activity and/or expression (Vijayan et al., 2001). Although this process may not have a large impact in healthy human brain, the additional effect rendered by β-tubulin hyperphosphorylation to already disturbed MT-dynamics may be significant in AD brain.

2.3.2 14-3-3ζ

The 14-3-3 protein family comprises a family of small acidic regulatory molecules that interact with a large number of signaling proteins and regulate numerous cellular processes via binding to their target proteins (Fu et al., 2000). By binding to their target proteins, 14-3-3 proteins modulate and stabilize enzymatic activity and conformation of their ligands, and, function as mediators of protein-protein interactions and subcellular protein localization. Due to their abundant brain expression and wide range of ligands, 14-3-3 proteins are crucial regulators of many vital cellular functions, such as cell cycle, signal transduction and apoptosis (Fu et al., 2000; van Hemert et al., 2001). In eukaryotes, seven distinct 14-3-3 isoforms (β, ε, γ, η, σ, τ and ζ) are all encoded by indivudual genes, which are translated to highly conserved phosphoserine-binding proteins, which predominantly exist as dimers (both homo- and heterodimers) (van Hemert et al., 2001;

Yuan et al., 2004). Large diversity of mechanisms, including cellular expression level, posttranslational modifications and isoform specificity, contribute to the stringent regulation of the 14-3-3 -ligand interactions (Fu et al., 2000).

In 14-3-3ζ, the phosphorylation status of Ser58 residue (located in the dimer interface) determines whether the 14-3-3ζ is in monomeric or dimeric form (Woodcock et al., 2003). This single site has been shown to be phosphorylated by various protein kinases including Akt (protein kinase B) in vitro (Powell et al., 2002) and sphingosine-dependent kinase in vitro and in vivo (Woodcock et al., 2003). Intriguingly, the disruption of the 14-3-3ζ dimer mediated by phosphorylation of Ser58 residue does not completely inhibit its capability to bind ligands although the dimeric structure of 14-3-3ζ strongly facilitates its cellular functions.

14-3-3 proteins are known to interact with multiple molecules associated with pathways that contribute to abnormal phosphorylation of tau (Hernandez et al., 2004; Kim et al., 2004; Agarwal-Mawal et al., 2003). Importantly, a multiprotein complex containing tau, GSK-3β and 14-3-3ζ co-immunoprecipitates from brain extracts (Agarwal-Mawal et al., 2003). The exact mechanism how tau and GSK-3β are connected within the complex by 14-3-3ζ is not completely understood. Interestingly, 14-3-3ζ is suggested to mediate the interaction of tau and Ser9-phosphorylated GSK-3β thereby stimulating the GSK-3β mediated tau phosphorylation, although the 14-3-3ζ- Ser9-GSK-3β interaction does not seem to require the presence of tau (Yuan et al., 2004). However, these data have not been further confirmed. Furthermore, tau phosphorylation at Ser214 by Akt kinase is shown to significantly increase the affinity of 14-3-3ζ to bind tau resulting in reduced tau aggregation and fibril formation in vitro (Sadik et al., 2009). Additionally, it has been reported that 14-3-3ζ increases DYRK1A activity via stabilizing the active dimer structure of 14-3-3ζ (Kim et al., 2004) and that 14-3-3ζ regulates PKA-mediated tau phosphorylation (Hashiguchi et al., 2000). Due to the overlapping binding region in tau, it has been suggested that 14-3-3ζ and tubulin may compete in tau binding under specific conditions.

2.3.3 Pin1

Peptidyl-prolyl isomerases are molecules that function as a conformational switch catalyzing the *cis/trans* isomerization of peptidyl-prolyl bonds (figure 2.3) (e.g. Zhou et al., 1999; Yaffe 1997). Peptidyl-prolyl isomerases can be divided into three families: cyclophilins, FK506 binding proteins (FKBPs) and parvulins (Lu et al., 1996; Lu et al., 2002a). Of these highly conserved peptidyl-prolyl isomerase -families, Pin1 is a member of parvulins, although being the only peptidyl-prolyl isomerase capable of recognizing and subsequently isomerizing phosphorylated serine/threonine-proline sequences (pSer/Thr-Pro) (Yaffe 1997).

Pin1 (protein interacting with NIMA (never in mitosis A) 1) is a ubiquitously expressed enzyme that has strong substrate specificity for phosphoproteins (Zhou et al., 1999). The two Pin1 structural domains have distinct functions: the N-terminal phospho-specific WW domain modulates Pin1 binding to its substrates and the C-terminal peptidyl-prolyl isomerase domain catalyzes the conformational *cis/trans* isomerization of the substrates (Lu et al., 1996; Lu et al., 2002a; Yaffe 1997). Phosphorylation of Pin1 at Ser16 residue located at pSer/Thr-Pro-binding pocket regulates the substrate binding activity by

inhibiting the WW domain-pSer/Thr-Pro motif interaction (Lu et al., 2002b). Moreover, Pin1 function is regulated by its subcellular localization, which is dependent on substrate availability. Thus, because the WW domain is required for substrate binding, the phosphorylation status of Ser16 of pSer/Thr-Pro motif affects the subcellular localization. Pin function is additionally modulated by its expression level, which is regulated by cell division cycle (Liou et al., 2002).
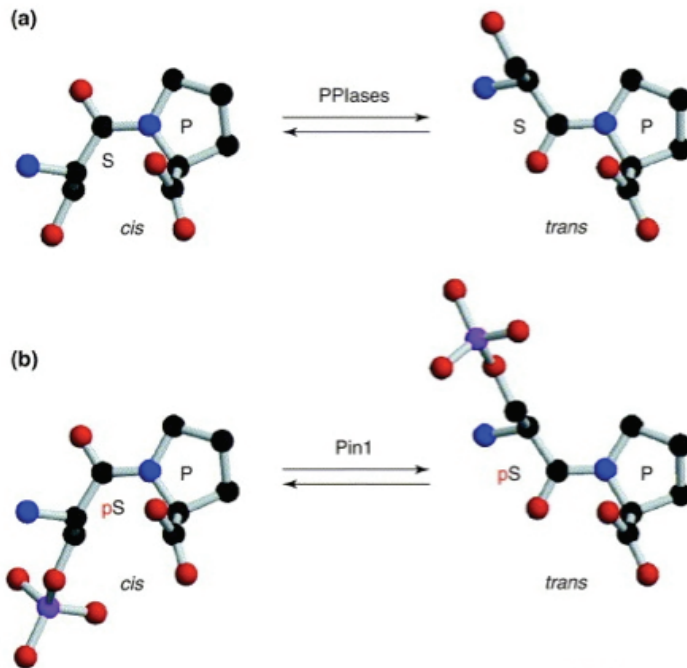
Figure 2.3. Peptidyl-prolyl *cis/trans* isomerization. Proline residues exist in two distinct isoforms potentially providing a peptidyl-prolyl bond switch in the polypeptide chain backbone, which is controlled by *cis/trans* isomerization (a). To avoid the rate limitations in the protein folding and refolding due to rather slow intrinsic spontaneous conversion between the two isomers, the conversion is catalyzed by ubiquitous peptidyl-prolyl *cis/trans* isomerases. Based on their substrate specificity, three families of peptidyl-prolyl *cis/trans* isomerases are characterized: cyclophilins, FK506 binding proteins (FKBPs) and parvulins. Parvulins are further divided into subfamilies of parvulin-type and Pin1-type peptidyl-prolyl *cis/trans* isomerases, from which Pin1 and Pin1-type peptidyl-prolyl *cis/trans* isomerases specifically isomerize phosphorylated Ser/Thr-Pro bonds (b). Isomerization of both phosphorylated and nonphosphorylated Ser/Thr-Pro motifs is essential due to a conformational specifity of numerous protein kinases and phosphatases, which phosphorylate and dephosphorylate predominantly the *trans*-form of proline bond of proteins. PPIases, peptidyl-prolyl *cis/trans* isomerases; S, serine; P, proline. Modified from Lu et al., 2002.

Pin1 is involved in several cellular processes, including regulation of cellular signaling and cell cycle progression, modulation of transcription and RNA processing, and,

contributes to neuronal survival, DNA damage response and cellular stress (Lu et al., 2002a; Lu 2004). The participation of Pin1 in a such wide array of diverse cellular processes can be at least partly explained by the vast number of its substrates. Pin1 regulation is highly stringent under normal physiological conditions and its deregulation is coherently associated with a number of human diseases with most implications in cancer and AD (Lu 2004). Importantly, the discovery of Pin1 to specifically recognize and further catalyze the pSer/Thr-Pro bond isomerization resulting in conformational change in motifs in question, introduced a novel mechanism of protein signaling and modification occurring after phosphorylation (Ranganathan et al., 1997; Yaffe 1997; Lu 2004).

Both AD hallmark proteins, tau and APP, are substrates of Pin1 (Liou et al., 2003). With regards to APP, phosphorylated Thr668-Pro motif in *cis* conformation is suggested to promote amyloidogenic APP processing and subsequent Aβ production whereas *trans* conformation seems to drive the non-amyloidogenic pathway (Pastorino et al., 2006). Furthermore, inhibition of GSK-3β by Pin1 is reported to decrease amyloidogenic APP processing (Ma et al., 2012). Specifically, Pin1 binds to phospho-Thr330-Pro motif in GSK-3β thereby inhibiting its kinase activity that results in decreased GSK-3β-mediated phosphorylation of Thr668 and APP turnover rate both in vitro and in vivo. Additionally, loss of Pin1 function is associated with altered intracellular APP localization and processing (Pastorino et al., 2012). These data implicate that upon decreased total APP levels mediated by increased protein turnover, Pin1 facilitated inhibition of GSK-3β may provide a novel mechanism in alleviating Aβ-driven AD pathophysiology.

Pin1 binds to and catalyzes the *cis/trans* isomerization of tau phosphorylated at the Thr231-Pro motif (Lu et al., 1999). Importantly, PP2A is a Pro-directed conformation-specific phosphatase that is only capable of interacting with the *trans* isomer of pSer/Thr-Pro motif (Zhou et al., 2000). Hence, this binding specifity of PP2A underlines the importance of Pin1-mediated conversion of *cis* to *trans* isomer of the tau Thr231-Pro motif enabling PP2A to dephosphorylate tau, which may further restore the ability of tau to bind and stabilize MTs (Lu et al., 1999; Zhou et al., 2000). In the case of complete Pin1 depletion or merely its reduced activity, which is seen in AD, *cis* form of pThr231-Pro motif is the prevalent isomer resulting in elevated tau hyperphosphorylation and subsequent NFT formation. Interestingly, simultaneous dephosphorylation at sites Ser16 in Pin1 and Thr231 in tau have been reported to be induced by Aβ$_{42}$ in vitro (Bulbarelli et al., 2009). Specifically, Aβ$_{42}$ induced increase in Pin1 activity was suggested to reduce or even prevent tau hyperphosphorylation. Considering the essential role of Pin1 in modulation of both APP and tau it provides a remarkable candidate target for novel AD therapies (Liou et al., 2003).

2.4 Other posttranslational modifications of tau

Tau protein can undergo a wide range of posttranslational modifications (PTMs) in addition to phosphorylation (Gong et al., 2005). These various mechanisms that modify tau posttranslationally include glycosylation, glycation, ubiquitination, polyamination, truncation, nitration (Gong et al., 2005), acetylation (Min et al., 2010) and methylation (Thomas et al., 2012). PTMs of tau under physiological and pathophysiological conditions are pivotal mechanisms in facilitating the conversion of functional tau into dysfunctional protein and inducing PHF/SF -formation, which promotes neurofibrillary degeneration in

AD and other tauopathies (Gong et al., 2005; Wang & Liu 2008). However, regardless of the crucial contribution of PTMs to tau in various NDDs, many of them are much less studied compared to tau phosphorylation.

The covalent linking of oligosaccharides to protein side chains is a process called glycosylation that is facilitated by different enzymes like glycotransferases (Gong et al., 2005; Wang & Liu 2008). The two types of glycosylation, O- and N-linked glycosylation, have a difference in the form of the glycosidic bond. N-glycosylation, in which oligosaccharides are covalently linked to the amino groups of asparagine side chains, usually occurs in rough endoplasmic reticulum and in Golgi apparatus (e.g. Gong et al., 2005). Furthermore, N-glycosylation is found to be enhanced in PHF-tau compared to normally functioning non-self-aggregated tau (Liu et al., 2002b). Additionally, abnormal glycosylation has been reported to promote hyperphosphorylation of tau via specific protein kinase pathways involving e.g. PKA, CDK5 and GSK-3β and due to decreased tau dephosphorylation by inhibition of PP2A and PP5 (Liu et al., 2002a). Importantly, aberrant glycosylation precedes hyperphosphorylation of tau and the impact of glycosylation to phosphorylation is site-specific (Gong et al., 2005; Liu et al., 2002a).

In O-glycosylation, oligosaccharides are covalently bound to Ser or Thr residues that are localized in close proximity with Pro residues (Gong et al., 2005; Wang & Liu 2008). Particularly, O-linked monosaccharide β-N-acetylglucosamine (O-GlcNAc) addition to tau Ser/Thr residues has been studied and the reduction of O-GlcNAcylation in AD brain has been reported (Liu et al., 2004; Arnold et al., 1996). This reduced O-GlcNAcylation in AD can be at least partially explained by the notion that O-GlcNAcylation and phosphorylation of tau take place in the same residues implicating that there may be a site-specific competition between these two PTMs (Liu et al., 2004). Moreover, it has been reported that increased amount of O-GlcNAc due to chemically inhibited glycoside hydrolase (O-GlcNAcase), a hydrolase that removes the O-GlcNAc from the tau Ser/Thr residues results in motor neuron loss in transgenic mouse model (JNPL3) expressing human FTDP-17 tau P301L mutant (Yuzwa et al., 2012). Intriguingly, no alterations in phosphorylation status of tau upon O-GlcNAcylation modification were found suggesting a phosphorylation-independent mechanism. In addition, the role of O-GlcNAcylation at a specific tau site was shown to significantly contribute to a reduction of tau fibrillization. However, until to date, only one O-GlcNAc site (Ser400) is identified in endogenous tau (Wang et al., 2010) and recently the protective role of O-GlcNAcylation against tau hyperphosphorylation has been questioned (Morris et al., 2015). O-GlcNAcylation is negatively regulated by compromised glucose metabolism in the brain, which may further induce tau hyperphosphorylation (Liu et al., 2004). Accordingly, reduced O-GlcNAcylation introduces an additional mechanism connecting impaired brain glucose metabolism and AD pathogenesis (Gong et al., 2005). Regardless of its role as one of the most common PTM of tau, the exact molecular mechanism(s) of glycosylation is not completely understood.

Unlike the enzymatic glycosylation, glycation is a process of nonenzymatic bonding of reducing saccharides to polypeptide amino side chains (Gong et al., 2005; Yan et al., 1994). Protein glycation-oxidation results in heterogenous and complex formations called advanced glycation end products (AGE), which have been observed to be present in PHFs/NFTs (Sasaki et al., 1998; Yan et al., 1994). Glycation of tau is also reported to be

induced by oxidative stress in neuronal cells (Yan et al., 1994) and suggested to contribute to the PHF -formation (Kuhla et al., 2007).

Tau that is located in PHFs and NFTs is polyubiquitinated in AD, unlike normal and soluble hyperphosphorylated tau (Perry et al., 1987). Ubiquitin-conjugation of damaged or misfolded proteins under normal physiological conditions directs these non-functional proteins to degradation by ATP-dependent ubiquitin-proteasome pathway (Hershko & Ciechanover 1992). Nonetheless, significant reduction of polyubiquitinated PHF-tau degradation is observed in AD, which is suggested to be at least partly due to diminished proteasome function that may lead to concomitant tau aggregation and NFT -formation (Keller et al., 2000). Besides polyubiquination, polyamination affects the rate of tau degradation (Tucholski et al., 1999). The reaction in which polyamines are added to tau is catalyzed by tissue transglutaminase. Although polyamination of tau does not have an impact on its MT -binding affinity based on in situ experiments, it aggravates the calpain protease-mediated tau cleavage and degradation in vitro.

Caspases and some other proteases execute the C-terminal truncation of tau leading to conformational changes within the protein (Yin & Kuret 2006). C-terminal cleavage of tau enhances its propensity to aggregate into PHFs/NFTs compared to full-length tau. Truncation of tau is proposed to occur at early stages of disease pathogenesis and it may be capable of inducing fibrillization of tau even in low concentrations, i.e. C-terminal truncation lowers the critical concentration of tau self-aggregation.

Tau nitration, which is shown to be site-specific toward tyrosine residues 18, 29, 197 and 394 (Reynolds et al., 2005), contributes to tau filament formation and is found to localize with NFTs before the maturation of tau inclusions in AD brain (Horiguchi et al., 2003). Tau nitrated at Tyr29 is reported to be the most affected nitration site in AD and hence suggested to contribute to pathogenesis of AD (Reynolds et al., 2006). Furthermore, nitration at Tyr29 is reported to age-dependently increase in the insoluble fraction of rTg4510 mice brain, a mouse model expressing human P301L FTDP-17 mutation that develops NFT-like pathology (Song et al., 2015).

Upon acetylation, the ubiquitin-mediated protein degradation of hyperphosphorylated tau has been reported to be inhibited (Min et al., 2010). In this study, histone acetyltransferase p300 was shown to promote tau acetylation whereas SIRT1 reverted this process by inducing deacetylation of tau. Additionally, depletion of protein deacetylase SIRT1, the expression level of which is shown to be reduced in AD (Julien et al., 2009), was suggested to induce hyperacetylation of tau leading to subsequent tau hyperphosphorylation and consequent neurofibrillary degeneration (Min et al., 2010). Importantly, this effect of hyperacetylation-driven tau hyperphosphorylation was shown to be abrogated by inducing tau deacetylation.

Since the identification of reversible tau acetylation, it has been the interest of multiple follow-up studies and has mounted further evidence on molecular mechanism of acetylation and its importance on tau function. Foremost, lysine residue 280 (K280), which is located within in the MT-binding region of tau, is pinpointed as a predominant acetylation target site that may play a pivotal role in AD pathology and other tauopathies (Cohen et al., 2011). At least partly due to its localization, tau acetylated at K280, disturb the primary function of tau by hindering its ability to bind MTs resulting in compromised MT-assembly and stabilization (Irwin et al., 2012; Cohen et al., 2011). Besides losing its normal function in modulating MTs, the toxic gain-of-function, including increased tau

fibrillization and the incremental amount of oligomerization-prone soluble tau that may lead to PHF/NFT-formation emerges due to acetylation (Irwin et al., 2012; Cohen et al., 2011). Both loss of normal function and gain of toxic function further collectively contribute to tau-mediated neurodegeneration. However, there have been some contradicting reports according on how and if tau acetylation increases tau-driven neurodegeneration, and, that which and where different deacetylases and acetyltransferases are capable of modulating tau acetylation (Noack et al., 2014; Cook et al., 2014; Cohen et al., 2011; Min et al., 2010). Recently, tau acetylation in KXGS motifs located in the MT-binding region of tau was reported to inhibit phosphorylation of these motifs and tau aggregation (Cook et al., 2014). Furthermore, the deacetylation of the KXGS motifs was reported to be driven by histone deacetylase 6 (HDAC6) (Cook et al., 2014) given that increase in its expression level correlates with increased tau burden whereas reduced HDAC6 activity was suggested to induce tau clearance (Cook et al., 2012). Additionally, the presence of HDAC6 in oligodendrocytes is reported where its inhibition contributes to tau phosphorylation and decreases its MT-binding properties (Noack et al., 2014), and, also impairs autophagic clearance of tau (Leyk et al., 2015). Hence, these data strongly implicate an important role for HDAC6 in regulation of tau acetylation although its impact on tau-mediated neurodegeneration is yet incompletely understood.

There is an ongoing debate about the exact roles and sites of various acetyltransferases (e.g. p300 and CREB binding protein) and deacetylases (e.g. HDAC6, SIRT1) that govern the acetylation/deacetylation equilibrium of tau (e.g. Cook et al., 2014; Cohen et al., 2011; Min et al., 2010). It has been also suggested that tau possesses an intrinsic acetyltransferase activity capable of self-acetylation without exogenous enzymatic activity, but this finding is yet to be further replicated and confirmed (Cohen et al., 2013). Taken together and regardless of the discrepancies of various reports, further investigation is needed to identify the site-specificity and the dynamics between acetylation and phosphorylation. This may reveal a novel targets for drug development for many tauopathies, except for argyrophilic grain disease, in which tau acetylation is not observed (Grinberg et al., 2013).

Methylation of tau at lysine residues was quite recently reported (Thomas et al., 2012). By using mass spectrometry approach, monomethylation of seven distinct lysine sites were identified from immunopurified PHFs of AD brain. Additionally, it has been reported that at least 11 lysine sites are either mono- or dimethylated in cognitively normal healthy human brain and that the methylated lysines are mainly located in the MBRD (Funk et al., 2014). Moreover, some lysine residues overlap with residues that are also acetylated and ubiquitinated suggesting a competition between different PTMs toward these sites. These findings have been verified recently in a report investigating tau PTMs by comparing endogenous tau isolated from both wild-type and hAPP mice by utilizing mass spectrometry (Morris et al., 2015). Given that not many differences in tau PTMs were detected between wild-type and human APP mice, it was suggested that tau-related neuronal dysfunction in hAPP mice is mediated by physiological tau forms. However, the enzymes that catalyze the methylation and dimethylation of tau are yet to be elucidated.

2.5 Role of tau in neurodegenerative diseases

The pathological 'umbrella' entity frontotemporal lobar degeneration (FTLD) is a group of disorders that are highly heterogenous clinically, genetically and pathologically, and share a common and prominent feature of rather specific degeneration of frontal and temporal lobes (Neary et al., 1998). The neuropathological classification of FTLDs is currently based on five distinct major subgroups, from which three are characterized by proteinaceous inclusions or deposits of specific proteins that include 43 kDa transactive response DNA-binding protein (TDP-43) in FTLD-TDP, fused in sarcoma (FTLD-FUS) and tau in FTLD-tau, a group of NDDs commonly called as tauopathies (Lashley et al., 2015).

Frontotemporal dementia (FTD), on the other hand, is a clinical term used to describe a group of dementias with early onset (under the age of 65 years) comprising the second most common cause of all dementias with the estimated prevalence of 15-22/100 000 (Onyike & Diehl-Schmid 2013). Clinical presentation of patients with FTD can be either one of the three commonly established clinical syndromes: behavioral variant FTD (bvFTD) or language variant FTD (lvFTD), which is usually divided into semantic dementia and progressive nonfluent aphasia (PNFA) (Lashley et al., 2015). In general, approximately 30-50% of all FTD cases show a positive family history (Rohrer et al., 2009). Hence, the hereditary component in FTD is rather strong. 10-20% of all FTD cases are due to mutations in three genes including *MAPT* (encoding tau), *progranulin* (*PGRN* or *Granulin* (*GRN*); both have been used in the literature) and *chromosome 9 open reading frame 72* (*C9orf72*), which encompass most of the familial cases (Rohrer et al., 2015; Rohrer et al., 2009).

The clinical symptoms between different FTD-subtypes present considerable variation. In bvFTD, symptoms are characterized by a set of behavioural alterations such as apathy, disinhibition, loss of empathy and obsessive-compulsive behaviour (Lashley et al., 2015). In addition, cognitive impairment in different cognitive domains is also developed as the disease progresses. In patients with PNFA, the symptoms usually include agrammatism, which is a form of aphasia in which the patient is not capable of speaking grammatically correctly, and/or apraxia of speech, which refers to a loss of speech level previously acquired before the disease onset. In the FTD variant of semantic dementia, anomia, i.e. difficulty to recall names or find words, is developed in addition to inability to comprehend single words.

2.5.1 Tauopathies

The predominant constituent in PHFs and SFs is hyperphosphorylated tau, which further, upon the subsequent formation of NFTs, manifest an additional neuropathological hallmark lesion in AD besides amyloid plaques (Iqbal et al., 1989; Grundke-Iqbal et al., 1986). However, the observed aggregates composed of mainly hyperphosphorylated tau subunits are not disease-specific to AD but are widely recognized in several age-related NDDs that are collectively referred as tauopathies, all of which are characterized by development of neurofibrillary degeneration associated with dementia (e.g. Buee et al., 2000; Iqbal et al., 2005). Furthermore, the mechanisms which may promote any alterations in tau metabolism that further result in NFT-formation include different PTMs,

missense mutations and altered expression of various tau isoforms or change in the total tau expression (Hernandez & Avila 2007). Importantly, the identified missense mutations in *MAPT* gene that were discovered to co-segregate with frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), unambiguously indicate the disease-causative feature of tau, i.e. abnormalities of tau *per se* are capable to result in neurofibrillary degeneration (Spillantini et al., 1998b; Hutton et al., 1998).

Tauopathies are typically divided in primary and secondary tauopathies depending whether the tau inclusions are the most predominant pathological protein depositions or if there are some other proteins that are involved in the pathogenesis of a disease (Kovacs 2015). The primary tauopathy phenotypes include e.g. progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), argyrophilic grain disease (AGD), Pick's disease (PiD), primary age-related tauopathy (previously also known as (neurofibrillary) tangle-only dementia) (PART), FTDP-17 and globular glial tauopathy (GGT), which was characterized recently (table 2.1). Besides AD, other secondary tauopathies include e.g. Down syndrome (DS) and Niemann-Pick type C disease (Buee et al., 2000). Notably, the used nomenclature for classically identified phenotypes that are associated with primary tauopathies are overlapping with the renewed classification of frontotemporal lobar degeneration (FTLD), which is a term comprising a group of several NDDs that are characterized by atrophy and disruption of predominantly frontal and temporal lobes of the brain (Cairns et al., 2007).

The neuropathological phenotypes of tauopathies, which are morphologically, biochemically and clinically heterogeneous NDDs, can be distinguished based on the differential involvement of various cell types, anatomical areas and the presence and/or absence of specific tau isoforms included in pathological tau deposits (Kovacs 2015). Moreover, tau filaments in different tauopathies are variably composed of either 4R- or 3R-tau or both, which further underlines the biochemical heterogeneity of tauopathies and currently provides the basis for *post mortem* molecular classification of specific disease by tau compositions, i.e. the major patterns of insoluble tau that can be observed from the brain samples of a specific tauopathy by Western blotting (Sergeant et al., 2005). Given that the ratio of 4R-tau to 3R-tau is normally in healthy human brain approximately 1:1 (Hong 1998), it has been suggested that it is the altered ratio of the isoforms rather than the isoform-specific toxicity that may drive the neurofibrillary degeneration of tauopathies (Wang & Liu 2008).

Table 2.1 The most common tauopathies. Average durations present the disease duration from diagnosis. In DS, disease duration presents the duration of AD. Age of onset and disease durations may vary significantly between patients.

	DISEASE	AGE OF ONSET	AVERAGE DURATION	CLINICAL SYMPTOMS	TAU RELATED BIOCHEMISTRY / NEUROPATHOLOGY	ASSOCIATED GENES / CHROMOSOMES
Primary	AGD	75-80	5	Dementia and cognitive decline; Occasional behavioural disturbances, personality changes and aggression	Predominantly 4R-tau; Oligodendroglial CB	*MAPT*
	CBD	50-70	7	Parkinsonism, asymmetric rigidity, akinesia, myoclonus (Significant variation depending on clinical syndrome)	Predominantly 4R-tau; Possible NFT:Ctx, possible NFT: S-Ctx; Astrocytic plaques; Threads: Ctx, S-Ctx, WM; Oligodendroglial CB	*MAPT*
	PSP	50-70	7	Supranuclear palsy, postural instability, mild dementia (Significant variation depending on clinical syndrome)	Predominantly 4R-tau; Possible NFT:Ctx, NFT: S-Ctx; Tufted astrocytes; Threads: Ctx, S-Ctx, WM; Oligodendroglial CB	*MAPT*
	PiD	55-65	10	Frontotemporal dementia variants; Progressive aphasia	Predominantly 3R-tau; Pick bodies; Ramified astrocytes + GOI	*MAPT*
	GGT	60	7	Frontotemporal dementia and/or frontal-lobe features and/or motor neuron disease and/or extrapyramidal features (Significant variability depending on GGT subtype)	Predominantly 4R-tau; GOI + GAI; Possible tufted astrocytes; Possible threads: Ctx, S-Ctx, WM; Possible oligodendroglial CB	*MAPT*
	PART	80	6	Variable cognitive impairment; Occasional disorientation, depression and paranoia in patients with severe cognitive impairment	Mixed 4R-tau and 3R-tau; Possible NFT:Ctx, possible NFT: S-Ctx; Possible threads: Ctx; Oligodendroglial CB	*MAPT*
Secondary	AD	EOAD >60 LOAD <65	9	Dementia and cognitive decline, behavioural changes, depression, loss of perception and judgment	Mixed 4R-tau and 3R-tau; NFT:Ctx, possible NFT: S-Ctx; Threads: Ctx	EOAD: *APP, PSEN1, PSEN2*; LOAD: *APOE* ε4
	DS	Inborn	9	Similar as in AD*	Predominantly 3R-tau; NFT:Ctx, possible NFT: S-Ctx; Threads: Ctx	Chromosome 21 trisomy

AGD, argyrophilic grain disease; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; PiD, Pick's disease; GGT, globular glial tauopathy; PART, primary age-related tauopathy; EOAD, early-onset Alzheimer's disease; LOAD, late-onset Alzheimer's disease; DS, Down syndrome; CB, coiled body; NFT, neurofibrillary tangle; Ctx, cortex; S-Ctx, subcortical; WM, white matter; GOI, globular oligodendroglial inclusion; GAI, globular astroglial inclusion; *MAPT*, microtubule-associated protein tau; *APP*, amyloid-β precurson protein; *PSEN1*, presenilin-1; *PSEN2*, presenilin-2; *APOE*, apolipoprotein E. * In DS, clinical symptoms here refer to AD in DS.

2.5.1.1 Braak staging

AD is referred as a secondary tauopathy due to the preceding amyloid plaque pathology in the disease pathogenesis. In AD, although NFTs that are composed of both 4R- and 3R-tau are primarily observed in the entorhinal cortex and hippocampus, many additional cortical and subcortical regions are also affected (Arnold et al., 1991; Braak & Braak 1991). More specifically, the sequential anatomical spreading of tau pathology between distinct brain regions during the disease progression has been established; the spreading of NFT pathology is divided into seven stages (0-VI) that is called Braak staging, which is relative to severity and affected brain areas (figure 2.4) (Braak & Braak 1991). Initially, tau aggregates are detected in locus coeruleus followed by transentorhinal cortex and anteromedial temporal lobe (Braak & Del Tredici 2011). Subsequently, tau inclusions become prominent in other areas of temporal lobe such as various hippocampal and entorhinal cortex areas and further in the basal temporal lobe and insular cortex (Braak et al., 2006; Braak & Braak 1991). Eventually, the lesions start to develop in various neocortical areas. Moreover, NFT-affected areas in AD brain have been suggested to be increased in the amount 4R-tau compared to 3R-tau (Yasojima et al., 1999). Although AD is the most common and most widely studied sporadic (secondary) tauopathy, it is still incompletely understood how tau hyperphosphorylation, filament formation and subsequent formation of NFTs and tau deposition impact on the impairment of cognition, learning and memory deficits (Wang & Liu 2008).

2.5.1.2 4R-tauopathies

In the majority of primary tauopathies 4R-tau is the predominant tau isoform observed in tau inclusions. 4R-tauopathy phenotypes include corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), argyrophilic grain disease (AGD) and globular glial tauopathy (GGT) (Kovacs 2015). Upon the first description of CBD and PSP they were initially classified, both clinically and pathologically, as separate disorders (Steele et al., 1964; Rebeiz et al., 1968). Currently, due to the overlapping pathological, clinical, genetic and biochemical features, there is a consensus that CBD and PSP may comprise a disease spectrum (Kouri et al., 2011).

CBD is characterized as a rare, sporadic and clinically heterogenous progressive NDD with age of onset ranging from early fifties to late seventies with average disease duration being 7 years (Kouri et al., 2011). The wide variety of clinical presentation, including sensory, motor, cognitive and behavioural symptoms, highly complicate the diagnosis of CBD in living patients hence underlying the importance of neuropathological examination for definitive diagnosis (Litvan et al., 1997). Although the aetiology and epidemiology of CBD remain incompletely understood, neuronal and glial (astrocytes and oligodendrocytes) 4R-tau depositions are highly prominent (Kouri et al., 2011). More specifically, neuropathological diagnostic criteria of CBD include the presence of tau-positive neuropil threads predominantly in the forebrain structures, such as gray and white matter of cortex, basal ganglia, diencephalon and rostral brainstem, and ballooned neurons (Dickson et al., 2002; Dickson 1999). Additionally, astrocytic plaques are the pathognomonic, i.e. a specific or even defining feature of particular disease, lesion in CBD (Feany & Dickson 1995). Interestingly, it was recently discovered that tau mutation

N410H in exon 13 cause CBD (Kouri et al., 2014). Moreover, the case of CBD caused by N410H had identical neuropathology with sporadic CBD in a study conducted with a cohort of autopsy-verified cases. Furthermore, an inherited tau H1 haplotype, or H1/H1 genotype in addition to H1c subhaplotype, all confer the risk for CBD (Dickson et al., 2007; Kouri et al., 2011).
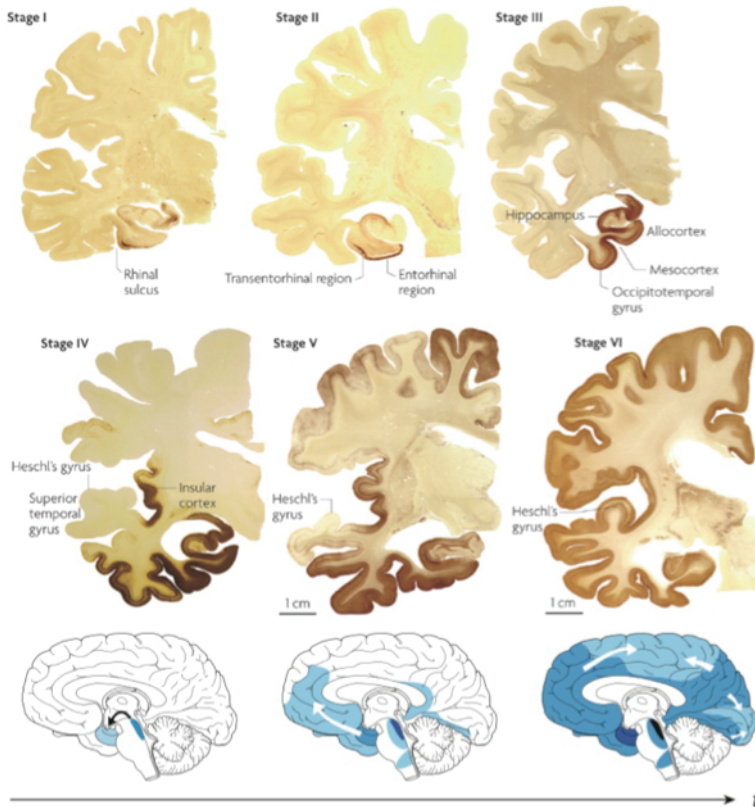
Figure 2.4. Braak staging. By examining hundreds of *post mortem* Alzheimer's disease brains it is possible to follow the average longitudinal progression of the disease. Accumulation of neurofibrillary tangle (NFT) pathology typically begins in the locus coeruleus and transentorhinal area (Braak stages I and II), subsequently progressing into limbic areas in Braak stages III and IV, and eventually reaching the neocortical and primary sensory areas in stages V and VI. NFTs were stained by using immunohistochemistry with an antibody against hyperphopshorylated tau. In the lower panel, increasing density of shading indicates increasing tau pathology over time (t). White arrows indicate the direction of NFT progression in the brain. Modified from Boutillier et al., 2007; Jucker & Walker 2011.

In CBD, the pathology is predominantly focused on circumscribed cortical atrophy and variable subcortical areas (Kouri et al., 2011). Hence, individual presentation of CBD pathology may vary significantly between numerous clinically distinct syndromes.

Generally in NDDs, the clinical syndrome represents the topography of the specific lesion whereas it is not inevitably the nature of the pathology underlying the disease. Owing to the heterogenous pathology, wide range of clinical syndromes can be exhibited in patients with CBD, including corticobasal syndrome (CBS), Richardson syndrome, which is the main clinical presentation of PSP, bvFTD, posterior cortical atrophy, progressive aphasia and AD-like dementia (Raggi et al., 2007; Josephs et al., 2006; Gorno-Tempini et al., 2004; Kertesz et al., 2000; Grimes et al., 1999; Murray et al., 2007; Bergeron et al., 1996; Williams et al., 2005; Bergeron et al., 1998). Furthermore, many NDDs present CBD mimicry, i.e. diseases resembling CBD although having different underlying pathologies, including e.g. Parkinson's disease (PD), PSP, multiple system atrophy (MSA) and AD (Kouri et al., 2011). The main features of CBS include the classical motor presentation of CBD, parkinsonism that is unresponsive to levodopa, asymmetric rigidity and akinesia along with other basal ganglia and cortical dysfunctions such as cortical sensory deficits and myoclonus, i.e. involuntary muscle twitching (Riley et al., 1990). Severe postural instability, which can lead to unexpected falls very early after the onset of the disease characterize Richardson syndrome, with additional symptoms such as dysarthria (motor speech disorder), dysphagia (difficulty to swallow), frontal cognitive defects, a lurching gait (commonly described as patient is resembling a drunken sailor or a dancing bear) and vertical supranuclear gaze palsy (Williams et al., 2005; Steele et al., 1964). Patients with primary progressive aphasia exhibit dementia disorder in which the progressive language impairment is the main disruption (Mesulam 2001).

Unlike in CBD, in which most of the tau pathology is prominent in the forebrain areas, tau pathology in PSP is predominantly present in midbrain and hindbrain structures (Dickson 1999). More specifically, NFTs, neuropil threads, tufted star-shaped astrocytes and oligodendroglial coiled bodies are the hallmark tau-related lesions that are present mainly in brain regions such as brainstem, diencephalon and basal ganglia (subthalamic nucleus, substantia nigra and globus pallidus) but also in cortical areas and in dentate nucleus of cerebellum (Dickson 1999; Williams & Lees 2009). Although PSP is a sporadic disorder, some cases with gene mutations are associated with PSP, including e.g. S305S, G303V, R5L, intron 10 +16 and deletion N296 (Stanford et al., 2000; Morris et al., 2003; Ros et al., 2005a; Ros et al., 2005b; Pastor et al., 2001; Morris et al., 2002; Williams et al., 2007b; Poorkaj et al., 2002). Moreover, similar to CBD, MAPT H1 haplotype and H1c subhaplotype are also associated with the risk of PSP (Dickson et al., 2007; Kouri et al., 2011). Macroscopically, the atrophy is normally seen in midbrain areas.

Supranuclear palsy, postural instability and mild dementia are the main characteristics of PSP syndrome, which has the disease duration and age of onset highly similar to CBD (Williams & Lees 2009). However, substantial variety is exhibited in different clinical syndromes that are associated with PSP. These various clinical phenotypes are commonly divided into typical and atypical PSP syndromes. The typical syndrome, referred as PSP-S (also known as Richardson's syndrome, PSP-RS) is the most common clinical syndrome that present PSP (Josephs 2015). However, it was shown in a recent study of 100 autopsy-confirmed PSP cases, that only 24% of all patients manifested the typical PSP-S (Respondek et al., 2014). The atypical syndromes presenting PSP include corticobasal syndrome (PSP-CBS), PD-like syndrome (PSP-P), progressive (or pure) akinesia with gait freezing (PSP-PAGF), behavioural variant frontotemporal dementia (PSP-bvFTD),

cerebellar ataxia (PSP-C) and with apraxia of speech, i.e. an oral motor disorder with difficulty to translate speech into motor plans, with or without aphasia (PSP-AOS) (Josephs 2015). Hence, upon comparison of the PSP syndromes to syndromes that are associated with CBD, there is a significant overlap between the clinical presentations of these diseases. The early clinical manifestations in PSP-P include limb bradykinesia, rigidity and occasional tremor (Williams & Lees 2009). In PSP-P patients, similar to PD, there is usually moderate or even good responsiveness to initiation of levodopa treatment especially addressing bradykinesia and rigidity (Williams et al., 2005), although the effect wears off commonly after a few years from starting the therapy (Birdi et al., 2002). PSP-PAGF is a syndrome characterized by symptoms such as progressive onset of gait disturbances (start hesitation) followed by freezing of either gait, speech or writing and unresponsiveness to levodopa (Williams et al., 2007a). Additionally, the lack of tremor, rigidity, dementia and abnormalities in the eye movement in the first 5 years since the disease onset are typical for PSP-PAGF.

The definite diagnosis is highly challenging even in autopsy, and misdiagnosis is not rare due to the significant clinical heterogeneity between different PSP-related syndromes (Williams & Lees 2009). Due to the complete lack of any therapeutics available for PSP as well as for CBD, there is an urgent need for improved biomarkers and neuroimaging techniques for earlier diagnosis, and, perhaps some novel insights for future treatment strategies as well (Josephs 2015). Intriguingly, a recent study, which is the first GWAS-based study in CBD, identified genes *MAPT* (SNP rs393152) and *MOBP* (SNP rs1768208) as genetic risk factors for both CBD and PSP (Kouri et al., 2015). Strikingly, in a separate report that was published virtually at the same time, the SNP rs1768208 was associated with elevated levels of appoptosin, which is a protein that correlates with caspase 3 activity hence affecting the caspase 3-mediated tau cleavage (Zhao et al., 2015a). Moreover, it was suggested that upregulation of appoptosin induces tau cleavage by caspase 3 resulting in PSP-associated impaired motor function and aggravated neuropathology, implicating a novel mechanism for PSP pathogenesis.

Argyrophilic grain disease (AGD) is a fairly common sporadic 4R-tauopathy with usually a very late age of onset, average being 75-80 years (Braak & Braak 1998). The characterization of AGD is based on the presence of argyrophilic grains (AGs), which are the most important pathological hallmark inclusion in AGD, pre-tangle neurons, coiled bodies in oligodendrocytes and tau-containing astrocytes. Small AGs, which are either spindle-shaped, round, button- or rod-like bodies predominantly localized in dendrites and their branches (Ikeda et al., 1995; Tolnay et al., 1998), although they have been also observed in axons (Tolnay & Clavaguera 2004). AGs most likely originate from pre-tangle neurons of the transentorhinal and entorhinal cortices, hippocampus (CA1 area), dentate gyrus, presubiculum, adjacent temporal cortex and some other cortical areas, amygdala (basolateral nuclei) and lateral tuberal nucleus of thalamus (Tolnay et al., 1998; Tolnay & Clavaguera 2004). In AGD, pre-tangle neurons are constantly observed with similar or identical distribution with AGs and show positive staining with the same phospho-specific tau antibodies that detect AGs (Ferrer et al., 2003; Tolnay & Clavaguera 2004). Pre-tangle neurons observed in AGD are highly similar and difficult to distinguish from the ones that are present in early tau depositions in AD (Bancher et al., 1989; Braak et al., 1994). Oligodendroglial coiled bodies are also constantly detected in AGD although they are highly similar to coiled bodies observed in various other tauopathies hence being

unspecific to AGD (Ikeda et al., 1998; Komori 1999; Tolnay & Clavaguera 2004). Unlike PSP-like tufted astrocytes, in AGD astrocytic tau is present more as bush-like astrocytes with thin tau immunoreactive processes, which usually are observed in clusters resembling astrocytic plaque formations of CBD (Ferrer et al., 2008). These bush-like astrocytes along with thin astrocytic plaque-like formations are prominent mainly in amygdala and temporal lobe white matter. Furthermore, ballooned neurons are frequently observed in AGD, particularly in the amygdala but also in some neocortical areas (Tolnay & Probst 1998). Moreover, NFTs and neuropil threads are variably present in AGD mostly localizing within the same areas as AGs and pre-tangle neurons and usually represent NFT pathology in the Braak stages I-III (Braak & Braak 1991; Kovacs 2015). Macroscopically, the brains of patients with AGD are normally either almost unchanged with no apparent atrophy as compared with age-matched individuals or may show minor atrophy in the frontotemporal cortex (Tolnay & Clavaguera 2004). However, in AGD cases in which the patient is demented, there is severe atrophy of the ambient gyrus, which is the junction combining amygdala and temporal lobe (Saito et al., 2002). Neuropathologically, AGD can be divided in three (or four) stages according to anatomical distribution of AGs in the brain (Saito et al., 2004; Ferrer et al., 2008).

Although the cause of AGD is currently unknown and there are no identified risk factors beside aging, the disease pathogenesis has been suggested to include the essential involvement of oxidative stress-driven activation of stress-activated tau-kinases, which further result in tau hyperphosphorylation and subsequent tau aggregation and ubiquitination of AGs in pre-tangles and NFTs (Ferrer et al., 2008). Moreover, upon incorporation of mutated ubiquitin (UBB[+1]), which is shown to be present in AG and NFT containing neurons in AGD (Fischer et al., 2003), tau degradation is inhibited resulting in structures that sequester active tau kinases leading to increased tau phosphorylation in AGs and NFTs (Ferrer et al., 2008).

Clinical symptoms of AGD typically include dementia and cognitive decline (Togo et al., 2002; Saito et al., 2002; Tolnay & Clavaguera 2004), although changes in personality, behavioural disturbances, imbalance of mood and emotions, aggression and ill temper and loss of episodic memory are occasionally observed (Ikeda et al., 2000; Braak & Braak 1998). In older AGD patients, additional symptoms including amnesia, irritability, delusions and apathy have been also reported (Togo et al., 2005). Interestingly, in study conducted with a rather small set of deceased elderly one third of cognitively normal aged (approximately 85 years old) individuals was found to have argyrophilic grain pathology (Knopman et al., 2003). Hence, it has been suggested that the presence of AG pathology in non-demented elderly may predispose or at least increase the probability for developing dementia (Josephs et al., 2008).

Globular glial tauopathies (GGTs) are a recently defined family of 4R-tauopathies presenting various combinations of different clinical features (Ahmed et al., 2013). Neuropathologically, GGTs are characterized by the presence of two distinct globular glial inclusions (GGIs, hence the name GGT): argyrophilic 4R-tau immunoreactive globular oligodendroglial inclusions (GOIs) and 4R-tau immunoreactive globular astroglial inclusions (GAIs), which are non-argyrophilic indicating that tau is in non-fully fibrillar form. Moreover, tau pathology in neurons usually exhibits as diffuse cytoplasmic inclusions, which are either globular or small (pre)tangle-like deposits (Ahmed et al., 2013; Kovacs 2015). GGTs are chararacterized as a rare sporadic tauopathies with almost

all autopsy confirmed cases showed homozygous for H1-haplotype (Tacik et al., 2015; Kovacs et al., 2008; Ahmed et al., 2011). Interestingly, a very recent study reported a novel tau mutation in a single individual associated with GGT (Tacik et al., 2015). It was shown that tau mutation K317N may directly cause GGT, and the pathogenic nature of this mutation was further showed by using K317N recombinant tau in a set of experiments, which showed decreased tubulin polymerization-promoting activity and increase in 4R-tau filament formation. Overall, at the molecular level, the formation of unconvential non-fully fibrillar tau inclusions specifically in GGTs is currently incompletely understood.

In general, brain atrophy in GGT cases is predominantly observed in temporal and frontal lobes or precentral gyrus (Ahmed et al., 2013; Tacik et al., 2015; Kovacs 2015). However, brain atrophy may differ significantly between patients ranging from very mild to significant. According to recent consensus recommendations, GGTs are classified into three subtypes, all of which present different clinical manifestations (Ahmed et al., 2013). However, there is a significant overlap in neuropathological manifestations between different types of GGTs. In type I, there is a predominant involvement of frontotemporal areas with presence of GOIs in the white matter. In cases with type II, the anatomical distribution of both GOIs and GAIs is more concentrated in motor cortex and corticospinal tract. In addition to areas in type II, frontotemporal areas are also affected in type III with an abundance of GAIs present in cortical areas together with GOIs. Noteworthy, the anatomical distribution of tau pathology (GGIs) in GGTs has strong correlation with predominating clinical symptoms (Fu et al., 2010; Kovacs et al., 2008; Ahmed et al., 2011). Importantly, GGTs may be often misdiagnosed depending on the subtype of the disease due to a significant similarities in clinical features: PiD or FTD in type I, PSP, CBD, motor neuron disease (MND) or primary lateral sclerosis (PLS) in type II and PSP, CBD or MND in type III (Ahmed et al., 2013).

2.5.1.3 3R-tauopathies

The only identified primary 3R-tauopathy to date is Pick's disease (PiD) (also called FTLD-tau with Pick bodies), in which the most characteristic features include neuronal cytoplasmic argyrophilic Pick bodies (PBs) immunoreactive for 3R-tau, circumscribed brain atrophy of the frontal and temporal lobes and widespread white matter degeneration (Mackenzie et al., 2009; Delacourte et al., 1996). Spherical or globular tau-positive PBs are predominantly present in the granule cells of the dentate gyrus, hippocampal pyramidal neurons and in temporal and/or frontal cortical regions but can be also observed in subcortical areas (Kovacs 2015). Furthermore, some of the remaining cortical neurons that exhibit ballooned shape are called Pick cells. Interestingly, it has been suggested that in PiD significant portion of 4R-tau pathology is also present (Zhukareva et al., 2002). This is, however, most likely explained either by the presence of 4R-tau in NFTs or sometimes rarely the coexisting AGD (Kovacs et al., 2013). The clinical symptoms of PiD usually manifest as FTD and progressive aphasia (Piguet et al., 2011; Kovacs et al., 2013). The age of onset is typically at early sixties and disease duration is approximately 10 years. Both the age at onset and duration of the disease shows substantial variability ranging from 42-70 years and 2-31 years, respectively, suggesting that Pick's disease may

also affect elderly. Particularly, it has been reported that disease duration may be significantly longer in patients presenting lvFTD than with bvFTD (Piguet et al., 2011).

Another disorder in which 3R-tau is the predominant isoform related to NFT-pathology is Down syndrome, where the increased ratio of 3R-tau to 4R-tau is suggested to be driven by the overexpression of DYRK1A-kinase due to the chromosome 21 trisomy (Wegiel et al., 2011; Shi et al., 2008).

2.5.1.4 Mixed 4R- and 3R-tauopathies

Primary age-related tauopathy (PART), which was formerly known also as neurofibrillary tangle-only dementia, is a primary tauopathy in which NFTs are composed of both 4R- and 3R-tau (Crary et al., 2014). NFTs observed in the brains of PART patients are identical to the NFTs seen in AD, i.e flame-shaped NFTs localized in neuronal cell bodies and apical dendrites. However, PART neuropathology is lacking amyloid plaques. The anatomical localization of NFTs in PART is mainly restricted in the structures in medial temporal lobe, basal forebrain, brainstem and olfactory structures. Clinical symptoms include changes in cognition ranging from normal to amnestic and only a small fraction of cases exhibit severe cognitive impairment. Furthermore, especially in cases with amnestic cognitive changes, disorientation, depression and paranoia may exist (Jellinger & Attems 2007). Moreover, the disease duration is usually shorter and the age of onset is higher as compared to AD (Janocko et al., 2012). Although it has been implicated that PART, especially the rare severe cases, can be associated with age-related memory impairment (Nelson et al., 2009; Ikeda et al., 1999), the full clinicopathological spectrum of PART is yet to be established given that the PART term and classification were only recently introduced (Crary et al., 2014).

2.5.1.5 Frontotemporal dementia with parkinsonism linked to chromosome 17

Unlike sporadic FTLDs, frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) is caused by mutations in two genes on chromosome 17, *MAPT* and *Progranulin* (*PGRN*), and by using this criteria FTDP-17 has been subdivided into FTDP-17 *MAPT* and FTDP-17 *PGRN* (Boeve & Hutton 2008). Since the discovery of first mutations in *MAPT* that were all associated with dementia syndrome with signs of parkinsonism in some affected individuals (Poorkaj et al., 1998; Spillantini et al., 1998b; Hutton et al., 1998), numerous additional mutations have been identified. These findings have further underlined the established disease-causative role of tau abnormalities to directly result in neurofibrillary degeneration.

Hereditary autosomal-dominant FTDP-17 *MAPT* affect both sexes equally with the avarage age at onset of symptoms being 49 years (Ghetti et al., 2015). The average age of disease onset is very similar to sporadic FTLDs together with the large range of possible appearance of various initial symptoms that may start either as early as in early twenties or in late seventies. There is a substantial divergence in life expectancy ranging from 1,5 to 26 years average being 8,5 years after the onset of symptoms (Wszolek et al., 2003; Reed et al., 2001). Intriguingly, clinical disease phenotype may vary significantly between or within families carrying the different mutations or even in individuals and families carrying the same mutation (Forman 2004; Bugiani et al., 1999). These observations

suggest that epigenetics in concert with environmental factors induce variability of phenotype manifestation.

Clinically, individuals that present fully developed syndrome normally exhibit at least two out of three main symptoms: personality and behavioural disturbances, cognitive impairment together or without motor dysfunction (Ghetti et al., 2015). The cognitive symptoms and behavioural and personality alterations in FTDP-17 *MAPT* usually fulfill the criteria for bvFTD presenting symptoms such as apathy, disinhibition, loss of empathy, hyperorality including e.g. excessive use of alcohol or drugs as well as the cognitive early stage symptoms including inattention, disorganization, impaired judgement and decision making and difficulty in initiating and completing tasks (Neary et al., 1998). In the early disease stage the orientation and memory are relatively unaffected. Also, an adynamic aphasia syndrome is often seen resulting in reduced speech that is normally caused by loss of aspects of language generation. As the disease progresses, the memory and visuaspatial function along with orientation are deteriorating eventually leading to a progressive dementia covering most of the cognitive aspects and often patients entirely lose their ability to speak (Ghetti et al., 2015). Parkinsonism is the most predominant motor symptom although in some families motor signs are completely absent or may become prominent very late in the disease progression. In case parkinsonism exhibits, in FTDP-17 *MAPT* it is characterized by postural instability, bradykinesia, absence of resting tremor and only minor or no responsiveness to levodopa treatment. However, in few cases, especially in individuals with N279K mutation in which parkinsonism is an early feature, asymmetrical resting tremor has been observed with responsiveness to levodopa (Tsuboi et al., 2002).

In the *MAPT* gene, more than 50 pathogenic mutations have been identified (figure 2.5), of which the most common include exonic mutations N279K and P301L, and +16 in intron 10 (Ghetti et al., 2015). Most of the mutations that occur in the coding region of the gene are predominantly located in the MBRD, which further deteriorate the ability of tau to maintain its normal function in MT-dynamics due to reduced MT-interaction (Hasegawa et al., 1998; Hong 1998). Moreover, these exonic mutations are missense, deletion or silent and occur in exons 1, 9-13, from which missense mutations in exons 1, 9, 11, 12 and 13 affect all human tau isoforms (Ghetti et al., 2015). The majority of intronic mutations are localized in a cluster in the 5'-splice site of the intron that is following exon 10. Together with some missense and silent mutations in exon 10, these intronic mutations have an impact on alternative mRNA splicing of exon 10 resulting in altered isoform ratios of 4R- and 3R-tau by increasing the amount of 4R-tau (D'Souza et al., 1999; Clark et al., 1998; Spillantini et al., 1998b; Hutton et al., 1998). More specifically, exonic mutations S305I, S305N and S305S, and, intronic mutations +3, +4, +11, +12, +13, +14 and +16 are responsible for destabilizing the stem-loop structure leading to increased inclusion of exon 10. The destabilizing effect of these mutations to a stem-loop structure at exon 10 5'-splice site intron junction was hypothesized upon the identification of the first *MAPT* mutations (Spillantini et al., 1998b; Hutton et al., 1998). The existence of the stem-loop structure, i.e. a structure that is suggested to sequester 5'-splice sites resulting in alternative 5'-splice site usage (Eperon et al., 1988) in tau has been further supported by additional studies (Varani et al., 1999; Grover et al., 1999; Jiang et al., 2000; Donahue et al., 2006). Moreover, exon 10 mutations occurring outside the stem-loop may enhance the splicing of exon 10, which can be regulated by either strengthened

exon splicing enhancers or weakened exon splicing silencers (D'Souza & Schellenberg 2002; D'Souza et al., 1999).
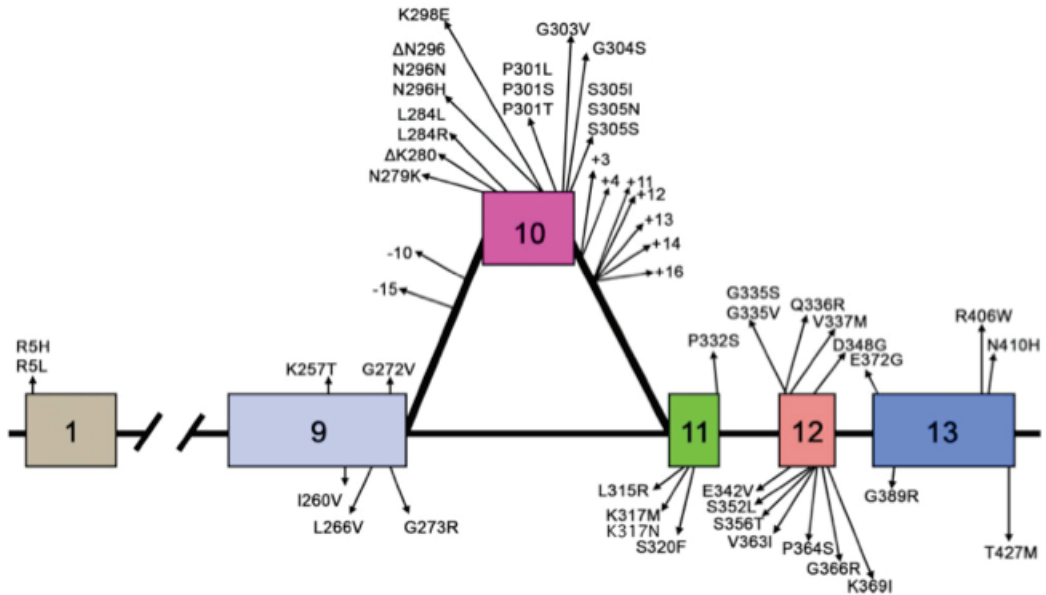

Figure 2.5. *MAPT* **gene mutations associated with frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17).** Schematic representation of *MAPT* gene regions of exons and introns (exons are numbered), where known mutations have been found. Currently, more than 50 pathological *MAPT* mutations have been identified. Modified from Ghetti et al., 2015.

The effect of coding region mutations may at least partially explain the loss-of-function of tau (Ghetti et al., 2015). The combined impact of mutations primarily affecting the RNA level is the excess production of 4R-tau, which exceeds the MT-binding affinity and the rate of MT-assembly over 3R-tau (Goedert & Jakes 1990). However, some of the exon 10 mutations, such as P301L, P301S and P301T, occur approximately in only a quarter of all tau molecules with rest being wild-type, which strongly emphasize that tau loss-of-function is not merely adequate in explaining the tau-driven neurodegeneration (Ghetti et al., 2015). Importantly, the overproduction of exon 10 may also lead to disequilibrium of the amount of possible binding sites in MTs in relation to 4R-tau, which may subsequently lead to enrichment of unbound cytoplasmic 4R-tau and eventually promote tau accumulation and aggregation. Given that the normal ratio of 4R-tau to 3R-tau isoforms in healthy human brain is approximately equimolar (Hong 1998), the disturbed balance of this ratio may directly promote tau aggregation (Gong et al., 2005). Although the FTDP-17 *MAPT* mutations have not been directly shown to influence tau phosphorylation, it has been suggested that certain missense mutations (G272V, P301L, V337M and R406W) can result in tau protein that is a better substrate for phosphorylation, which may occur faster and with higher extent by kinases compared to wild-type tau in vitro (Alonso Adel et al., 2004). Also, the conformational change induced by these mutations promotes the

sequestration of wild-type tau. Furthermore, the filament formation of tau has been reported to be reduced in the presence both 4R- and 3R-tau isoforms when compared to cases where only 4R-tau is present (Adams et al., 2010).

Unlike tauopathies, which are divided either in 4R-, 3R- or 4R- and 3R-tauopathies, FTDP-17 *MAPT* can present all different isoforms depending on the mutation (Ghetti et al., 2015). Nevertheless, as in tauopathies generally, the presence of neuronal or glial tau inclusions is invariably a neuropathological hallmark for also FTDP-17 *MAPT*. Noteworthy, tau deposits can be found in neurons or in both neurons and glia whereas glia-only deposits have not been observed. Furthermore, there is substantial variability in the structure of tau inclusion depending on the specific mutation. Tau deposits characterized with mutations V337M in exon 12 and R406W in exon 13 display high similarity to inclusions seen in AD whereas some mutations in exons 9, 11, 12, 13 present Pick body-like morphology with straight and twisted filament formations mostly composed of 3R-tau but also containing variable amounts of 4R-tau, which is the case with G389R. Additionally, prominent differences are observed in various mutations resulting in presence of variable portions of 4R- and 3R-tau in tau inclusions, e.g. V337M inclusions contain both 4R- and 3R-tau, G389R inclusions contain predominantly 3R-tau, P301L inclusions contain mostly 4R-tau with occasionally small amount of 3R-tau (figure 2.6) and mutation +3 in intron 10 contains predominantly 4R-tau. Intriguingly, various mutations may also dictate in which cell type tau inclusions are the most prominent. In particular, mutations in exons 9, 11, 12 and 13 for the most part result in tau inclusions in neurons whereas exon 1 and 10 mutations together with mutations in introns 9 and 10 mostly lead to tau deposits in both neurons and glia. In cases where glial pathology is present, tufted astrocytes and astrocytic plaques with oligodendroglial coiled bodies are normally seen. The glial depositions in FTDP-17 *MAPT* are very similar or identical to hallmark features of both PSP and CBD.

Similarly to the effect of different mutations that affect the composition variation of 4R- and 3R-tau depositions, the anatomical distribution of tau inclusions has been reported to vary in relation to specific mutations (Ghetti et al., 2015). However, due to lack of knowledge in early neuropathological disease progression, most of the available data concerning the distribution of tau inclusions comes from the intermediate and late stage FTDP-17 *MAPT* patients. Although the histopathological alterations characteristic to FTDP-17 *MAPT* in cerebral cortex, white matter and subcortical nuclei are visible in the intermediate disease stage, the atrophy of cerebral hemispheres may be relatively subtle. Later, in more advanced stage of disease progression, brain atrophy in different regions may be variable: prominent atrophy is usually present throughout temporal and frontal lobes and in amygdala, hippocampus and hypothalamus in addition to some other subcortical areas. Moreover, the total atrophy in temporal and frontal lobes may be asymmetrical and in the most severe cases the gyri may present in so called knife-edge appearance, i.e. the atrophy in the brain gyri is so severe that their appearance is starting to resemble sharp blade -like structures in addition to significantly enlarged lateral and third ventricles. Conversely, parietal and occipital lobes are usually not affected or at least much lesser extent of atrophy is detected. Taken together, FTDP-17 *MAPT* is highly heterogenous disorder with insidious onset that presents in multiple phenotypes and clinical symptoms, which complicates the diagnosis particularly in the early disease stage.
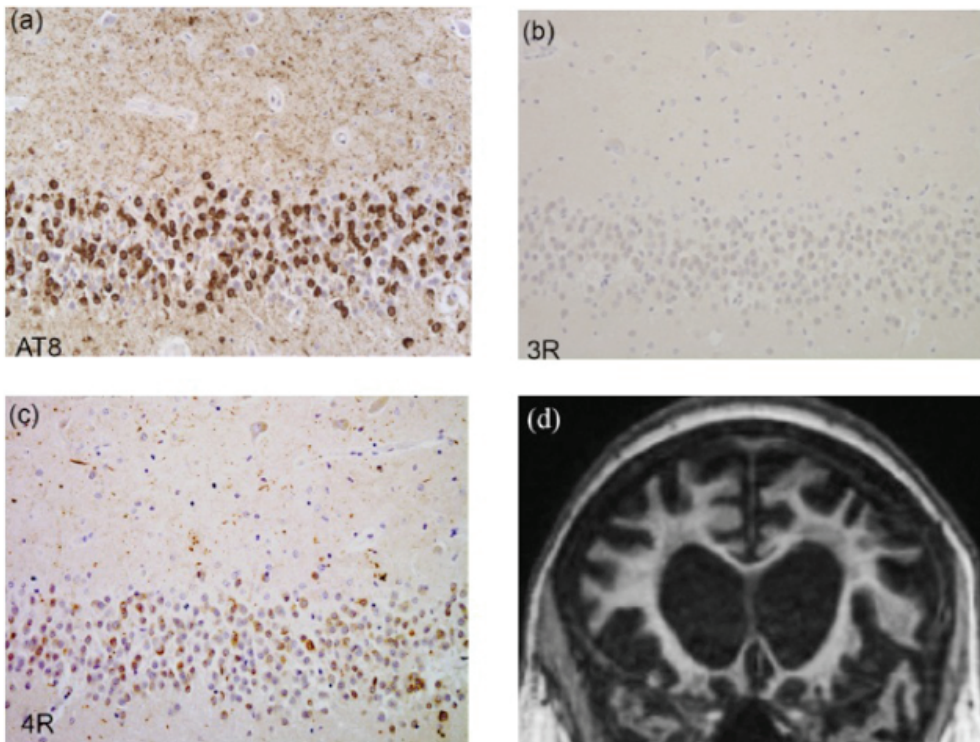
Figure 2.6. Tau pathology of a patient carrying P301L *MAPT* mutation. AT8 (hyperphosphorylated tau at Ser202 and Thr205) immunoreactivity at the dentate gyrus of the hippocampus (a). No staining is seen when labeled with 3R-tau isoform specific antibody (b) (occasional 3R-tau staining may be seen in patients with P301L mutation), whereas 4R-tau isoform specific antibody shows immunoreactivity (c). Coronal T1-weighted magnetic resonance image from a 62 year-old female with a severe behavioural variant frontotemporal dementia (d). Symptoms of the patient evolved in a period of six years. Striking bilateral atrophy is evident in the prefrontal and anterior temporal lobes and also white matter changes. Modified from Ghetti et al., 2015.

2.6 Alzheimer's disease

Alzheimer's disease (AD) is the most common form of NDD causing dementia in the elderly and estimated to cover approximately 50-75% of all dementia cases (World Alzheimer report, Alzheimer's international, 2015). AD mainly affects the elderly given that only small proportion (2-10%) of all cases is predicted to show clinical symptoms before the age of 65 years. After the age of 65 years, the increase in the prevalence and incidence of AD doubles with each 5-year age increment. It is estimated that there is 47 million people suffering from AD today, and due to demographic aging of developed societies, it is projected to rise to 74 million by the year 2030 and to reach roughly 130 million people by the year 2050, manifesting even more severe socio-economic burden that it already is today (World Alzheimer report, Alzheimer's international, 2015). However, there are recent reports suggesting that in developed countries the population

dementia risk may decline due to e.g. increased level of education and increasing success in treating the cardiovascular risk factors including e.g. hypercholesterolemia and hypertension (Wu et al., 2015; Langa 2015).

Clinical characterization of AD, which has a gradual disease onset, includes a wide variety of symptoms that include progressive impairment of memory and deterioration of cognition, behavioral changes, depression and apathy, loss of various daily functions such as perception, language and judgment, and, also physical functioning during the late stages of the disease. Although there is a strong genetic component, aging is still by far the most important risk factor for AD. Currently, there is no cure or even ways to halt the disease progression and only symptomatic treatments are available with rather mild clinical benefits. Moreover, regardless of intensive studies there are no preventive clinical methods available for AD. Notably, unambiguous diagnosis of AD is possible only *post mortem* combined with the assessment of the clinical picture of the disease progression, which strongly emphasizes the need for improvement of the possibility for early diagnosis although there has been some great advances in this field, e.g. brain imaging techniques, for the past few years.

There are two neuropathological hallmarks that characterize AD; extracellular amyloid plaques mainly composed of Aβ peptide aggregates and intraneuronal neurofibrillary tangles (NFTs) that are predominantly composed of hyperphosphorylated tau protein (figure 2.7) (Terry & Katzman 1983). Clinically, AD is divided into pre-clinical mild cognitive impairment (MCI) and mild-, moderate- and severe AD. Actually, MCI is not considered as a stage of AD but more as a pre-AD since all patients with MCI do not convert to AD. Furthermore, additional pathological assessment of the disease progression is used based on the neuroanatomical distribution of NFT-pathology within the brain called Braak staging, which are considered the lesions correlating with the severity of cognitive decline and dementia in AD (Braak & Braak 1991). The progressive loss of synapses and cholinergic neurons in the basal forebrain usually first appears in the entorhinal cortex and hippocampal areas subsequently spreading to cortical areas resulting in severe brain atrophy particularly affecting the temporal lobes. Importantly, AD can be classified into two distinct types based on the age of the symptom onset and familial history. These are familial early-onset AD (EOAD), in which the symptoms start before the age of 65 years and sporadic late-onset AD (LOAD), where the symptom onset usually occurs after the age of 65 years. Noteworthy, EOAD is estimated to represent only 1-5% of all AD cases.
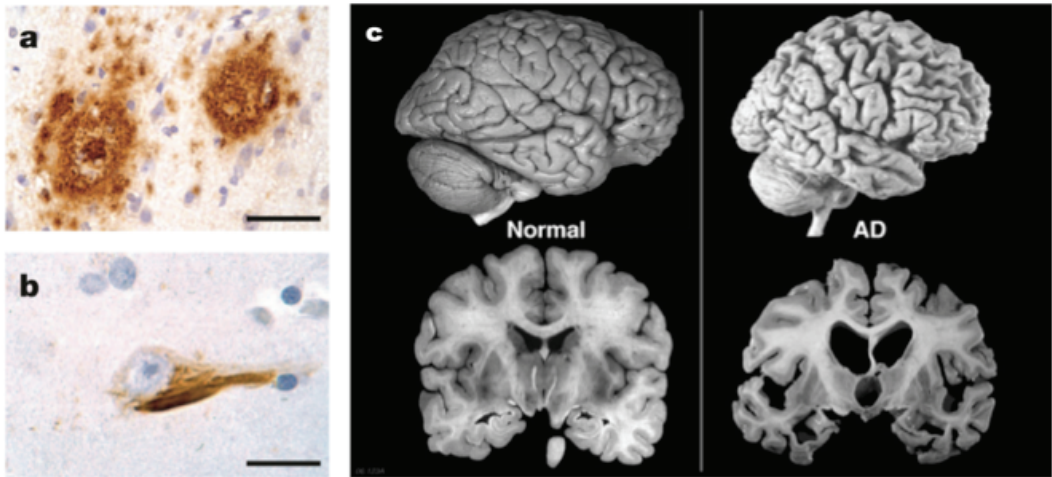
Figure 2.7. Neuropathological hallmarks of Alzheimer's disease (AD). Cortical extracellular deposits of amyloid-β (senile plaques) (a) and intraneuronal tau inclusions called neurofibrillary tangles (b) are characteristic findings in *post mortem* examination of AD patients. Scale bars 50 μm (a) and 20 μm (b). Comparison of a healthy human brain and severe AD brain (c). Due to a massive neuronal loss and gliosis, there is a substantial atrophy in the frontotemporal cortical areas and some white matter changes. Also, ventricles are significantly enlarged. (a) and (b) modified from Jucker & Walker 2013.

2.6.1 Early-onset Alzheimer's disease

Early-onset Alzheimer's disease (EOAD) is characterized by the onset of symptoms before the age of 60-65 years, strong familial background and is inherited mostly by dominant autosomal Mendelian transmission. EOAD is almost invariably caused by very rare mutations in three distinct genes, *APP*, *PSEN1* and *PSEN2,* which encode the proteins amyloid-β precursor protein, presenilin 1 and presenilin 2, respectively. Mutations in these genes are highly penetrant and nearly always result in development of early onset AD. Previously, before the identification of causative AD mutations, the presence of Aβ plaques in both AD and Down syndrome (DS) brain were discovered and further purified and characterized (Glenner & Wong 1984b; Glenner & Wong 1984a). Given that a trisomy of chromosome 21 is the cause of DS, in which almost all carriers develop AD with highly increasing incidence when reaching 40 years of age, lead to a hypothesis that mutations in genes that are located in this particular chromosome may cause AD in the absense of DS (Glenner & Wong 1984a). This suggested hypothesis was later confirmed to be correct by linkage analysis, which had been a crucially important approach in causative AD gene identification.

Linkage analyses method aims for identifying loci in which the transmission is not actually independent but seem to be more often associated with a disease than only probability would predict (e.g. Chouraki & Seshadri 2014). Specifically, linkage analysis approach utilizes the joint segregation of a specific disease and genetic markers within families or in population level. In case a genetic marker is identified in a close proximity

with causal variant(s) of a specific disease, the inheritance is preferential along with the disease at least within larger families.

After the identification of association of chromosome 21 with some forms of familial EOAD (St George-Hyslop et al., 1987), the *APP* gene was suggested as a novel candidate gene that was located in this locus (Tanzi et al., 1987; Goldgaber et al., 1987; Robakis et al., 1987; Kang et al., 1987). These results were further confirmed upon identification of association of specific *APP* mutations in different families with EOAD (Murrell et al., 1991; Mullan et al., 1992; Goate et al., 1991; Chartier-Harlin et al., 1991). Although the discovered linkage peak on chromosome 21 was verified via findings of *APP* mutations, these mutations did not sufficiently manage to completely explain all the familial EOAD cases. These doubts were confirmed by the discovery that EOAD is not genetically homogenous disorder (St George-Hyslop et al., 1990; Schellenberg et al., 1988) and further resulted in the identification of additional linkage peaks in chromosome 14 (Schellenberg et al., 1992) and chromosome 1 (Levy-Lahad et al., 1995b). Subsequently, the causative EOAD mutations were identified in the corresponding genes *PSEN1* and *PSEN2* on chromosomes 14 (Sherrington et al., 1995) and 1 (Levy-Lahad et al., 1995a), respectively.

These pivotal discoveries in EOAD genetics resulted in the formulation of a hypothesis called amyloid cascade -hypothesis stating that Aβ accumulation is the primary cause of progressive AD pathogenesis in the brain (Hardy & Higgins 1992; Hardy & Allsop 1991), which has been modified and specified over the years (Hardy & Selkoe 2002). Regardless of the ongoing debate whether the amyloid cascade -hypothesis is the leading pathogenic mechanism in AD, it has been a foundation for numerous attempts for improved diagnostics and targeted therapeutics in AD.

Aβ peptides are proteolytic cleavage products of APP protein, a type I transmembrane protein with incompletely understood physiological functions (Haass et al., 2012). Proteolytic processing of APP can occur via two competing physiological pathways: non-amyloidogenic pathway and amyloidogenic pathway. Firstly, in the non-amyloidogenic pathway, APP is cleaved by α-secretases (Sisodia et al., 1990; Esch et al., 1990) resulting in soluble APP fragment (sAPPα) and truncated C-terminal APP fragment (αCTF, also called C83) followed by an intramembrane cleavege by γ-secretase that generates APP intracellular domain (AICD) (Sastre et al., 2001; Gu et al., 2001) and p3 peptide (Haass et al., 1993). γ-secretase is a large intramembrane protease complex that consists of four distinct subunits including presenilin 1 (PS1) or presenilin 2 (PS2), which are the catalytic subunits, nicastrin (NCT), anterior pharynx defective 1 (APH-1) and PS enhancer 2 (PEN-2) (Wolfe et al., 1999; Francis et al., 2002; Yu et al., 2000), which all are essential components of full enzymatic activity of γ-secretase (Bai et al., 2015; Edbauer et al., 2003). In a competing pathway, the ectodomain of APP is first cleaved by β-secretase, mostly by BACE1 (Vassar et al., 1999) generating a soluble APP fragment (sAPPβ) and a C-terminal fragment βCTF (also known as C99). This is followed by γ-secretase cleavage, which liberates Aβ peptide that can vary in length and biochemical properties due to a variation in γ-cleavage cleavage site, from which the most frequent species are $A\beta_{40}$ and the more toxic, aggregation prone $A\beta_{42}$ (Benilova et al., 2012). Unexpectedly, a novel physiological APP processing pathway was recently identified (Willem et al., 2015). Specifically, the generation and enrichment of η-secretase mediated C-terminal APP fragment η (ηCTF) is capable to inhibit neuronal activity in hippocampus. Moreover, an

increase of ηCTFs was observed in close proximity with neuritic plaques in dystrophic neurites in both mouse model of AD and in AD human brain suggesting that physiological η-secretase activity may be altered in AD pathogenesis.

Most of the mutations in *APP*, *PSEN1* and *PSEN2* genes promote amyloidogenic processing of APP resulting in relative increase in $A\beta_{42}$ formation and the possible subsequent generation of neurotoxic Aβ oligomers (Citron et al., 1992; Scheuner et al., 1996). As postulated in the amyloid cascade -hypothesis, this observed increase of $A\beta_{42}$ caused by either increased Aβ generation, which seem to be the case in EOAD mutations, or by decreased Aβ clearance and degradation is the trigger for consequent cascade in AD pathogenesis, including tau hyperphosphorylation and NFT formation, neuronal death and eventually leading to progressive brain atrophy and dementia (e.g. Hardy & Selkoe 2002). However, although the Aβ plaque pathology seems to precede NFT pathology, it is widely established that NFTs are the primary lesions that correlate with the severity of cognitive decline and rate of dementia in AD (Buee et al., 2000; Wang & Liu 2008). Taken together, 40 mutations in *APP*, 197 in *PSEN1* and 25 in *PSEN2* genes have been reported to date with all showing autosomal dominant inheritance and complete penetrance (Chouraki & Seshadri 2014), except for one recessive APP mutation (Di Fede et al., 2009). Importantly, no mutations outside these genes have been found in EOAD, whereas any mutations in these genes have not been associated with late-onset AD. The only exception responsible here is by a single *APP* coding mutation (A673T) identified in the Icelandic population that was found to be protective against AD (Jonsson et al., 2012).

2.6.2 Late-onset Alzheimer's disease

Late-onset Alzheimer's disease (LOAD) is a complex NDD with exponentially increasing incidence after the age of 65 years and the leading cause of dementia in the elderly. In LOAD, neuropathological findings including senile plaques and NFTs are highly similar to EOAD. After aging, *APOE*, specifically the ε4 allele of *APOE*, is the strongest risk factor and simultaneously the most important genetic risk factor for LOAD (APOE chapter 2.6.3.2.1) (Bettens et al., 2013). Besides aging and genetic risk factors, a large variety of other risk factors have been suggested to contribute to pathogenesis of LOAD, including cardiovascular factors such as hypertension and hypercholesterolemia, history of head trauma, neuroinflammation and type II diabetes mellitus (Whalley et al., 2006). Conversely, some simple lifestyle choices, such as diet, physical and social activity and stimulation of cognition are associated with decreased incidence of LOAD. Recently, it was suggested that people who were overweight in midlife develop LOAD at earlier age and were associated with increased AD neuropathology (Chuang et al., 2015). Although both genetic and environmental factors contribute in the aetiology of LOAD, the exact mechanism(s) how these factors are interconnected remain poorly understood. However, it is well established that LOAD brain pathology precedes the onset of clinical symptoms by years or even decades, which underlines the importance of early diagnosis in order to initiate possible therapeutic measures in the very early stage of the disease.

The first implications of participation of APOE to AD came from the observations that APOE *per se* is the protein shown to be expressed upon injury and repair of neuronal damage (Ignatius et al., 1987; Ignatius et al., 1986). This was followed by studies reporting APOE overexpression in AD (Diedrich et al., 1991) and a discovery of a linkage

peak on chromosome 19 associated in families with history of LOAD (Pericak-Vance et al., 1991). Furthermore, APOE protein was found to colocalize with both amyloid plaques and NFTs in AD (Wisniewski & Frangione 1992; Namba et al., 1991). Subsequently, the association of ε4 allele of *APOE* with both LOAD and EOAD was reported by first showing the direct high affinity binding of APOE to Aβ and the more frequent presence of ε4 in LOAD with familial background (Strittmatter et al., 1993). Secondly, the results that *APOE* ε4 allele is in fact involved in both sporadic and familial LOAD was confirmed (Saunders et al., 1993). Thirdly, the gene dosage of *APOE* ε4 in LOAD families was established and suggested that *APOE* ε4 carriers manifest an earlier disease onset and dementia than non-carriers (Corder et al., 1993), which was further confirmed in a case-control study of sporadic form of LOAD (Poirier et al., 1993). Conversely, the reduced frequency of *APOE* ε2 allele in AD was reported suggesting a protective role of ε2 allele against LOAD (Corder et al., 1994). Importantly, unlike causative mutations in *APP*, *PSEN1* and *PSEN2* in familial EOAD, *APOE* ε4 allele is a risk factor for LOAD, which is not capable alone causing the disease. In contrast, *APOE* ε4 is not a prerequisite for developing AD since approximately half of the people with AD are *APOE* ε4 non-carriers. Hence, *APOE* ε4 has been defined as a moderately penetrant genetic factor with a semi-dominant inheritance (Genin et al., 2011). Furthermore, the *APOE* genotype-dependent lifetime risks of developing AD have been estimated in non-carrier, heterozygote and homozygote in 85 year-old women to be 30%, 51% and 60%, respectively. The corresponding values in 85 year-old men are 11%, 14% and 23%, respectively. Both estimations are calculated based on current average lifetime expectancies and may be subject to change in time.

The discovery of association of *APOE* and LOAD launched a tremendous number of candidate-gene-based studies (over 1000 studies in less than 10 years) in order to identify additional risk genes for AD (Bettens et al., 2013; Chouraki & Seshadri 2014). The genes selected for a candidate gene study were usually based either on their assumed or predicted role in AD pathophysiology or linkage study-based localization within a linkage peak according to AD. Although multiple mutations in genes involved in various AD-related pathways were assessed, unfortunately these findings were rarely successfully replicated, which is most likely due to an inadequate sample size resulting in initial false-positive findings. Another shortcoming in candidate-gene-based studies capable of creating further inconsistencies in the results is the associations that are located in the non-coding region of a gene, which may by highly important in gene regulatory function but are extremely hard to study in biological pathways. However, despite of the mostly disappointing results obtained from the candidate-gene approach studies, sortilin-related receptor LDLR class A repeats (*SORL1*) gene was identified in addition to *APOE* (Rogaeva et al., 2007). Importantly, the finding has been replicated and confirmed in genome-wide association studies (GWAS) (Miyashita et al., 2013; Lambert et al., 2013b).

2.6.3 Genetic risk factors of late-onset Alzheimer's disease

2.6.3.1 Genome-wide association studies

The principle of genome-wide association studies (GWAS) is highly similar to genetic association study where frequency of alleles of specific variant are compared in disease

case and control populations (Bertram et al., 2010; Chouraki & Seshadri 2014). Unlike the genetic association studies, in which the tested variants are selected based on prior hypothesis or known pathways of a few candidate genes, the GWAS approach is largely hypothesis-free. Importantly, besides focusing on the preconceived hypothesis of certain variants and candidate genes associated with the disease, in GWAS the entire genome is covered in terms of known polymorphisms. Accordingly, this allows the detection of common variants via enhanced resolution and a larger statistical power compared to linkage analysis. These advantages make GWAS highly useful and adaptable to study the variation of common genes associated with complex disease.

Although GWAS has many advantages, the massive number of statistical testing is an importantly critical issue, which can increase the risk of false-positive results. In order to avoid false-positive GWAS signals, highly stringent criteria have been used for statistical significance in order to reproduce the results. Hence, because of the essentiality of reproducing the results to verify the observed genome-wide association, GWAS are usually organized in two distinct phases (Chouraki & Seshadri 2014). Firstly, in a genome-wide discovery stage, in which the p-value threshold is usually set to 5×10^{-8}, certain associations that reach statistical significance is established (McCarthy et al., 2008; Chouraki & Seshadri 2014). Secondly, in a replication stage the associations of selected variants are studied by utilizing de novo genotyping methodology in different independent populations (Chouraki & Seshadri 2014). To obtain the most novelty in possible discoveries from GWAS it would be optimal to include all the GWAS sample data available at given time.

The two separate GWAS phases can be further divided into multiple steps within the process (Hardy & Singleton 2009). In the first step, the single-nucleotide polymorphisms (SNPs) are genotyped genome-wide. This is almost exclusively performed on chip-based products manufactured by the two leading companies, namely Illumina and Affymetrix. Although the genotyping content of these chips differs, there is a possibility of genotype imputation[1], which may further alleviate the competition between different groups utilizing various methods, and, enhance the collaboration and comparison of the data. Finding the missing genotypes by imputation, it is crucial for forming a common set of genetic SNPs and further performing meta-analyzes when various platforms have been used (Zeggini & Ioannidis 2009).

After generating the raw SNP data set, the second step includes cleaning procedures and quality control of the data (Hardy & Singleton 2009). These procedures (i) ensure that the genotyped sex matches the sex reported for each individual sample (X and Y genotypes), (ii) measure the sample matching as a group and (iii) identify individual outliers. Outlier identification, which is based on patterns of genetic variability, enables the sample removal of the ethnically remote subjects, and, also allows the adjustment of any kind of systematic variations within or between cohorts. Next, in the third step, all the SNPs that survive the cleaning and quality control step are further tested for association trait or some specific disease. At this step, to avoid any false-positive signals and because

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[1] A statistical technique estimating the genotypes that are missing from the specific population by comparing different haplotypes of the specific population to populations with denser reference panel (i.e. predicting untyped SNPs from those SNPs that have been genotyped) (Marchini & Howie 2010; Chouraki & Seshadri 2014)

of the massive amount of statistical tests performed, a stringent p-value threshold for genome-wide statistical significance is set. Specifically, the most generally studied risk models include dominant, recessive, genomic, allelic and additive, which is the most commonly tested. The additive model is assuming that when one allele is present it is conferring an intermediate risk compared to having no or two alleles. Fourth step encompasses the selection of loci or SNPs to be replicated in an independent set of samples, which is preferably larger or at least the same size sample set tested in the genome-wide association. The selection process of loci may be based either on statistical significance, or, on statistical significance and biological plausibility together. Hence, depending on the resources available and the original study design, the amount of SNPs can vary largely, from as few as 10 to even 20000. Fifth, any combination of following three results is obtained from step four: (i) clear and unambiguous association with the selected loci with disease, (ii) no association signal is detected at all, or, (iii) association with loci and disease is detected but the magnitude of the association is insufficient to pass a predetermined threshold of statistical significance.

Sixth step, which is the first step of the replication stage of GWAS, independent cohorts are used for additional genotyping to ensure the association with disease, i.e. if the obtained signals genuinely are valid or not. Lastly, the seventh step includes multiple parts starting from data mining, in which unequivocally associated loci unveils transcripts located in and near proximity of any specific locus. Additionally, in this part all known genetic variation within the specific region are mapped. Next, further locus fine mapping is performed in order to discover any possible novel variants and to genotype untyped variants, to further validate the significance of the association with the disease. Importantly, more specific analysis of the region is performed to specify the most essential variants, the gene that is pathophysiologically most relevantly associated to disease, and, to possibly interpret the relevance on biologic effect.

For the past ten years, GWAS have been truly a major leap forward in identifying a vast number of robust genome-wide associations between the specific loci in chromosomes and highly complex human disease (Hardy & Singleton 2009; Lord et al., 2014). Highly reduced expenses and development of arrays of multiplex genotyping and whole-genome and -exome sequencing have enabled the advent of completely novel hypothesis-free approach to study genetics of human disease (Bertram et al., 2010). The revolutionary advances in microarray technology have made it now possible to assess hundreds of thousands or even millions of SNPs in a single experiment. The next-generation sequencing will hopefully further mitigate the huge workload yet ahead to more specifically understand and establish the connection of genotype and human disease.

2.6.3.1.1 Genome-wide association studies in late-onset Alzheimer's disease

When the first AD GWAS were reported, the results were initially disappointing because they only confirmed the association of APOE ε allele to AD (Li et al., 2008; Coon et al., 2007; Webster et al., 2008; Abraham et al., 2008). However, novel AD associated loci were also identified near genes *GAB2* and *PCDH11X* (Carrasquillo et al., 2009; Reiman et al., 2007), but these findings have so far turned out to be difficult to replicate in later studies (Miar et al., 2011; Wu et al., 2010; Beecham et al., 2010). Difficulties in replicating the initial findings may be due to a smaller sample size used in the replication

phase compared to the discovery phase, or, they can merely point out to be false-positive signals (Chouraki & Seshadri 2014). Additionally, a number of other AD associated loci have been reported, e.g. *ATXN1* (Bertram et al., 2008), *LMNA* (Grupe et al., 2007), *CHRNB2* (Cook et al., 2004) and *ACE* (Kehoe et al., 1999) that lack conformation by replication studies. Albeit the initial AD GWAS results were mainly disappointing they rapidly lead to initiation of multiple consortia (Chouraki & Seshadri 2014). Large international consortia, such as the European Alzheimer's Disease Initiative (EADI) and the Genetic and Environmental Risk in Alzheimer's Disease (GERAD), are pooling data from several thousands of participants across the globe.

The first convincing truly large scale studies were two back-to-back published reports in 2009 from two distinct consortia (EADI and GERAD) that showed for the first time AD associated variants other than *APOE* (Harold et al., 2009; Lambert et al., 2009). These studies identified three novel AD associated genes, i.e. *CLU*, *CR1* and *PICALM*. Importantly, these reports independently identified genome-wide significant signal in one variant of *CLU* gene. Consequently, these two studies are considered as hallmark publications for showing the first susceptibility genes for late-onset Alzheimer's disease beside the *APOE*. In the following year, a study conducted by the Cohorts for Heart and Aging in Genomic Epidemiology (CHARGE) consortium, identified different variants in *BIN1* associated with AD (Seshadri et al., 2010). These results were obtained by utilizing a three-phase approach that combines independent set of GWAS followed by two-stage in silico replication process using the GWAS data from previous EADI and GERAD consortia results. Next, the results that reached genome-wide significance after the replication were de novo genotyped in an independent Spanish cohort. Another gene, *EPHA1*, also reached genome-wide significance although the signal was significant only in the first stage of the two-stage in silico replication phase. Interestingly, this was the first study to use imputation in AD related GWAS and also the first to report results from meta-analyzes of multiple different datasets that have been previously shown to associate with AD risk genes.

Another collaboration project between three consortia (EADI, CHARGE and GERAD) discovered new variants in *ABCA7* gene associated with AD (Hollingworth et al., 2011). Simultaneously, a GWAS study from the Alzheimer's Disease Genetics Consortium (ADGC) identified association of *MS4A* gene cluster (e.g. *MS4A4A*, *MS4A6A* and *MS4A6E*) with AD (Naj et al., 2011). In addition, by combining their data, the collaboration of these two studies from different consortia managed to identify additional signal in (or near) genes *EPHA1*, *CD2AP* and *CD33* (Hollingworth et al., 2011; Naj et al., 2011). Furthermore, this study replicated the results of previous studies and validated the findings of AD associated risk genes, such as *CR1*, *CLU*, *PICALM* and *BIN1*, thereby establishing the status of these genes as AD susceptibility factors (Naj et al., 2011). Importantly, most of these associations have been replicated in different population cohorts by either using cross-replication approach, or, by utilizing targeted sequencing or genotyping of previous and novel loci discovered, thereby validating the identified signals (Carrasquillo et al., 2010; Biffi et al., 2010; Lambert et al., 2011). Notwithstanding the multiple replication studies, many previous and novel AD GWAS signals, such as *MTFDH1L* (Naj et al., 2010), *ATP5H/KCTD2* (Boada et al., 2014), *GAB2* (Reiman et al., 2007) and *PCDH11X* (Carrasquillo et al., 2009) are lacking verification and yet needs consistent replication.

The attempt to diminish or even abolish the constantly growing overlap of the individual study participants between the studies and various consortia a new mega consortium was initiated (Chouraki & Seshadri 2014). Naturally, another reason to establish this new consortium, called the International Genomics of Alzheimer's Project (IGAP), was to further facilitate novel AD GWAS discoveries. The first "mega" meta-analysis study conducted by IGAP using European cohorts across the ADGC, EADI, CHARGE and GERAD populations confirmed all the previous GWAS loci with the exception of *CD33*, which only reached genome-wide significance in the discovery phase (Lambert et al., 2013b). Moreover, new loci in or near 11 genes were identified: *CASS4, CELF1, DSG2, FERMT2, HLA-DRB5/DBR1, INPP5D, MEF2C, NME8, PTK2B, SLC24H4-RIN3, SORL1*, and *ZCWPW1*. From these loci, *SORL1* has been previously discovered and reach genome-wide significance in a different GWAS study with participants of European and Asian ancestry (Miyashita et al., 2013). These two separate studies thereby confirm the association of *SORL1* in AD. Currently, a lot of effort is used to trying to replicate these novel AD GWAS signals, and, at least *ZCWPW1* has been already confirmed to associate with AD in a study of large Spanish sample cohort (Ruiz et al., 2014b). Additionally, by using GWAS complementary method called genome-wide haplotype association (GWHA) study with three-step approach, genetic association of *FRMD4A* gene with AD was identified (Lambert et al., 2013a). Interestingly, *FRMD4A* expression has also shown to be altered in the disease progression of AD according to Braak staging (Martiskainen et al., 2015).

Despite of its robust efficacy, AD GWAS has some substantial limitations (Lord et al., 2014; Chouraki & Seshadri 2014). Firstly, in GWAS, only the signals of truly trait-associated common variants can be detected. This is mainly due to commonly used GWAS genotyping chips including these variants. At this study design, rare variants remain poorly targeted. Secondly, despite of myriad of AD associated loci that have been discovered, the variants, i.e. SNPs, underpinning these associations are yet largely unknown. Specifically, the variants within the specific locus with the strongest association usually are located in the intronic, or unfortunately even in the intergenic regions of the identified loci. Indeed, this makes interpretation of data highly complex because the causality of the factors of specific loci to AD aetiology remains largely unclear. Earlier, a theory postulating that the rare causative mutations are the principal source that underlies the association of AD GWAS variants has been mostly withered due to difficulty of explaining the connection between common coding variability and disease association regardless of the large resequencing effort (Guerreiro et al., 2010).

Regardless of a number of limitations, the advent of GWAS in AD has been a major leap forward in AD genetics. Beside *APOE*, several polymorphisms in or near genes have been identified to date by GWAS associated with late-onset AD risk, including *ABCA7, BIN1, CASS4, CD33, CD2AP, CELF1, CLU, CR1, DSG2, EPHA1, FERMT2, HLA-DRB5/DBR1, INPP5D, MS4A, MEF2C, NME8, PICALM, PTK2B, SLC24H4-RIN3, SORL1,* and *ZCWPW1* (figure 2.8) (Medway & Morgan 2014; Calero et al., 2015; Karch & Goate 2015). Moreover, the association of *FRMD4A* (Lambert et al., 2013a) and *TRIP4* (Ruiz et al., 2014b) to AD susceptibility has been reported. Although the effect size of these identified common variants conferring to AD risk is small, it has already broadened our perspective of how and which pathways and pathophysiological processes are involved in risk of developing AD (e.g. Karch & Goate 2015; Chouraki & Seshadri 2014).

In contrast to common variants, rare coding variants usually have modest or even large effect size on AD risk. In addition to rare coding variants in genes *APP*, *PSEN1* and *PSEN2* associated to early onset familial form of AD, novel loci have been reported, including *TREM2* (Jonsson et al., 2013; Guerreiro et al., 2013a) and *PLD3* (Cruchaga et al., 2014). However, replication study of the *PLD3* gene variants failed to show any significant association with AD (Hooli et al., 2015). Recently, loss-of-function variants of *ABCA7* gene were reported to confer risk of AD with fairly high effect size (Steinberg et al., 2015).
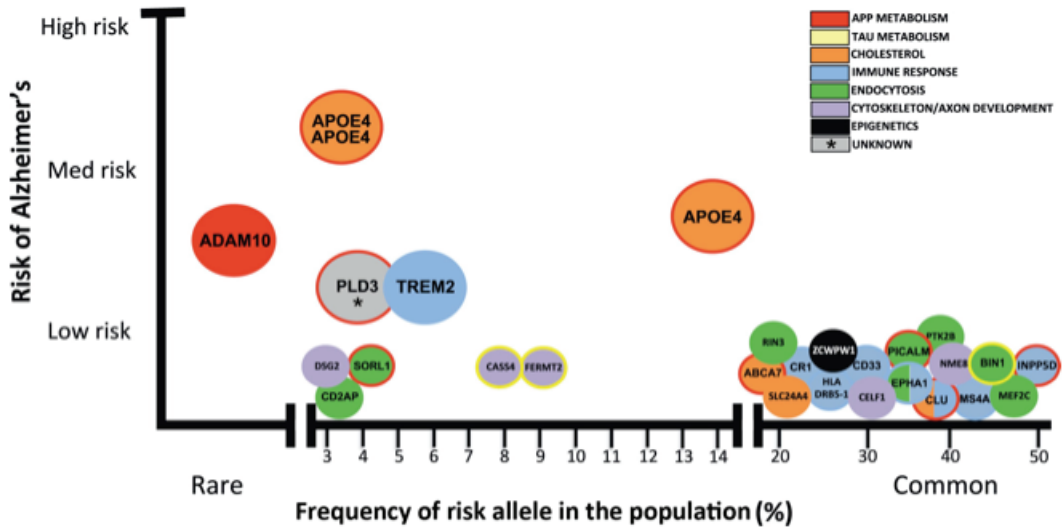
Figure 2.8. Genetic landscape of late-onset Alzheimer's disease (LOAD). Multiple genome-wide association studies during the past few years have provided an enormous amount of data regarding to genetics of LOAD. Unlike the autosomal dominant disease-causative genes *APP*, *PSEN1* and *PSEN2*, majority of the identified common LOAD susceptibility genes have a very low single contribution to LOAD pathogenesis (bottom right). Among common susceptibility genes, *APOE* ε4 (hetero- and homozygote) increase the risk from mild to moderate. Conversely, rare variants such as *TREM2* typically contribute more strongly to incidence and pathogenesis of the disease. Color-coding (top right) of the genes refers to their cellular functions; the main known cellular function is indicated with color inside the circles and additional function on the surface of the circle. However, for many susceptibility genes, their cellular functions and the possible contribution to LOAD pathogenesis is poorly understood and replication studies are needed for many genes, e.g. *PLD3* to establish the association with LOAD. Modified from Karch & Goate 2015.

2.6.3.2 Identified late-onset Alzheimer's disease susceptibility genes

The identified common alleles in genome-wide associated genes associated with AD can be classified in three different pathways, including lipid and cholesterol metabolism (*APOE, CLU, ABCA7* and *SORL1*), synaptic vesicle recycling and endocytosis (*SORL1, BIN1, PICALM, CD2AP, EPHA1* and *FRMD4A*) and immune system function (*CR1, CD33, CLU, ABCA7, EPHA1, BIN1* and *MS4A*-cluster) in which some genes are overlapping between these pathways (table 2.2) (Jones et al., 2010; Bertram et al., 2008; Medway & Morgan 2014; Karch & Goate 2015).

2.6.3.2.1 APOE

APOE gene encodes apolipoprotein E (APOE) and is located on chromosome 19q13.2 (Pericak-Vance et al., 1991; Siest et al., 1995). APOE is a highly pleiotropic glycoprotein comprised of three different isoforms, which are determined by two different variations in amino acid sequence (cysteine-to-arginine substitutions at 112 and 158 positions) and are called APOE2 (C112, C158), APOE3 (C112, R158) and APOE4 (R112, R158) with the corresponding alleles ε2, ε3 (which is the most common form) and ε4, respectively (Zannis et al., 1982; Weisgraber et al., 1981). The principle role of APOE is to participate in transport of cholesterol and other lipids both in the periphery and brain (Mahley 1988). In the brain, APOE is the predominant lipoprotein and functions as a ligand for low density lipoprotein receptors (LDLRs). Additionally, APOE is involved in mediating the binding and internalization of lipoproteins in cells. Besides its crucial role in lipid and lipoprotein metabolism, APOE has numerous other important roles in the CNS, including modulation of neuroinflammation and synaptic plasticity (Kim et al., 2009a).

APOE genotype, especially *APOE* ε4 allele, is the most important genetic risk factor that contributes to the pathogenesis of sporadic, non-familial late-onset form of AD (Strittmatter et al., 1993; Corder et al., 1993). Although the presence of *APOE* ε4 allele increases the risk of AD from three- to even 15-fold compared to non-carriers depending whether the allele is in heterozygosis or in homozygosis, respectively, it is still considered as a risk factor, i.e. *APOE*ε4 alone is not sufficient to cause AD unlike *APP*, *PSEN1* and *-2* mutations (Bertram et al., 2007; Farrer et al., 1997). However, despite of its strong contribution for increasing the risk of AD, the presence of *APOE* ε4 allele is not necessary for developing the disease; approximately half of the patients with AD are *APOE* ε4 non-carriers. In addition, in families with AD, there is a gene dose association between the ε4 allele and the age of AD onset (Corder et al., 1993). On the contrary to ε4, ε2 allele has been reported to decrease the risk of AD and at least delay the age of onset, almost as it has a protective role against developing AD (Corder et al., 1994).

Regardless of more than two decades of intense research the exact pathophysiological and molecular pathways linking APOE to AD remain partly unknown (Chouraki & Seshadri 2014). However, it has been shown that APOE directly binds Aβ and influence its aggregation, and, also facilitates the soluble Aβ clearance most likely by acting as a chaperone protein where ε4 allele has the least efficient role (Castellano et al., 2011; Zhong & Weisgraber 2009). The difference in the efficacy of Aβ clearance is probably due to differences in the amino acid structure resulted by arginine-to-cysteine substitutions (Castellano et al., 2011). Furthermore, Aβ metabolism is indirectly regulated by APOE

Table 2.2. Top susceptibility genes associated with late-onset Alzheimer's disease (LOAD). Characteristics of 11 established common variant LOAD susceptibility genes, *FRMD4A* and rare variant *TREM2* (Cochran et al., 2015; Seshadri et al., 2010; Jonsson et al., 2013; Hollingworth et al., 2011; Naj et al., 2011; Guerreiro et al., 2013a; Guerreiro et al., 2013b; Medway & Morgan 2014; Harold et al., 2009; Calero et al., 2015; Lambert et al., 2013a; Lambert et al., 2013b; Steinberg et al., 2015).

Gene	Chr	Protein	SNPs associated with AD	OR (95% CI)	Possible pathway(s) / Associated function(s)
CR1	1q32	Complement receptor 1	rs6656401	1.18 (1.14-1.22)	Immune and complement systems/inflammatory response; regulation of complement activation
BIN1	2q14	Bridging integrator 1	rs6733839	1.22 (1.18-1.25)	Synaptic vesicle endocytosis Cytoskeletal dynamics
CD2AP	6p12	CD2-associated protein	rs10948363	1.10 (1.07-1.13)	Regulation of actin cytoskeleton reorganiztion Cytokinesis; mediating BBB integrity
EPHA1	7q34	EPH receptor A1	rs11771145	0.9 (0.88-0.93)	Immune and complement systems/inflammatory response; neural development
CLU	8p21-p12	Clusterin (Apolipoprotein J)	rs9331896	0.86 (0.84-0.89)	Immune and complement systems/inflammatory response; cholesterol/lipid metabolism
FRMD4A	10p13	FERM domain containing 4A	rs7081208 rs2446581 rs17314229	1.68§ (1.43-1.96)	Possibly function as a scaffolding protein in cell polarity regulation
MS4A cluster	11q12.2	Membrane-spanning 4-domains subfamily A	rs983392	0.90 (0.87-0.92)	Immune and complement systems/inflammatory response; signal transduction
PICALM	11q14	Phosphatidylinositol-binding clathrin assembly protein	rs10792832	0.87 (0.85-0.89)	Clathrin-mediated endocytosis
SORL1	11q23.2-24.2	Sortilin-related receptor LDLR class A repeats containing	rs11218343	0.77 (0.72-0.82)	Endocytosis; cargo sorting; lipid metabolism
APOE	19q13.2	Apolipoprotein E	rs429358 (C112R)* rs7412 (C158R)*	~2 to 5	Cholesterol/lipid metabolism
ABCA7	19p13.3	ATP-binding cassette transporter A7	rs4147929 Loss-of-function	1.15 (1.11-1.19) 2.03	Cholesterol/lipid metabolism; immune and complement systems/inflammatory response
CD33	19q13.3	CD 33 molecule	rs3865444	0.94 (0.91-0.96)	Immune and complement systems/inflammatory response; cell signaling
TREM2	6p21.1	Triggering receptor expressed on myeloid cells 2	rs75932628 (R47H)[#]	2.26[¶] (1.71-2.98) 4.5[¶] (1.7-11.9)	Immune response; chronic inflammation Rare variant

[§]Combined OR of three indicated SNPs. *Two SNPs resulting in homozygous APOE ε4 genotype. [#]SNP resulting in R47H mutation. [¶]Two different ORs depending on the reference. Chr, chromosome; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidense interval; BBB, blood-brain barrier.

via interaction with receptors such as low-density lipoprotein receptor-related protein 1 (LRP1), i.e. APOE and Aβ compete in LRP1-mediated cellular uptake (Verghese et al., 2013). Also, APOE binds to tau but the functional relevance of this interaction remains unknown (Strittmatter et al., 1994). Interestingly, a recent study showed that a complete absence of APOE in a patient caused by deletion mutation did not result in neurocognitive function defects regardless of the important role of APOE in lipid metabolism (Mak et al., 2014). Hence, there is an implication that APOE function in the brain may not be essential and there may be complementary mechanism(s) to compensate the absence of APOE. Taken together, all the genetic, human, animal and cellular studies truly indicate APOE as a risk factor for sporadic AD by modifying the disease pathogenesis via, at least partly, an APP/Aβ-dependent manner (Karch & Goate 2015).

2.6.3.2.2 Clusterin (APOJ)

Clusterin, another apolipoprotein (also called APOJ), is encoded by *CLU* gene and is located on chromosome 8p21-p12 (Jones & Jomary 2002). Clusterin is a stress-activated glycoprotein mainly secreted in heterodimeric form and is expressed nearly ubiquitously in all tissues and is linked to a wide range of functions, such as lipid transport, apoptosis, intercellular interactions and membrane protection. Multiple SNPs in *CLU*, such as rs7012010, rs1113600, rs9331888, rs7982 and rs2279590 have been identified by GWAS that seem to have a protective role in AD development (Hollingworth et al., 2011; Naj et al., 2011; Harold et al., 2009; Lambert et al., 2009). From these identified SNPs, rs9331888 was associated to AD in mega meta-analyzes with more than 74000 individuals (Lambert et al., 2013b). Interestingly, clusterin was associated with pathogenesis of AD long before the era of GWA studies. It has been shown that clusterin colocalizes with amyloid plaques (May et al., 1990; Choi-Miura et al., 1992; Calero et al., 2000) and that expression levels of clusterin mRNA in AD brains is elevated (Allen et al., 2012; Karch et al., 2012b). Furthermore, the expression of *CLU* exons 3 and 4 were shown to be increased according to increased NFT related AD pathology (i.e. Braak staging) (Martiskainen et al., 2015). Thus, similar with APOE, clusterin may participate in Aβ clearance and amyloid aggregation and also in neurotoxicity. Additionally, clusterin is associated with the complement system by regulating the membrane attack via inhibition of inflammatory responses that are associated with activation of complement system (Jones & Jomary 2002). Because of the crucial importance of neuroinflammation in AD aetiology, SNPs that may alter the expression level of clusterin or its function as a modulator of Aβ metabolism may either directly or indirectly modulate AD pathogenesis and have an influence on downstream effects of the disease progression (Karch & Goate 2015).

2.6.3.2.3 ABCA7

ATP-binding cassette transporter A7 (ABCA7) belongs to the ABC transporter superfamily and is encoded by *ABCA7* located on chromosome 19p13.3 (Ikeda et al., 2003). Alternative splicing can generate two distinct isoforms called ABCA7 I and II, which both are expressed in the brain, mainly in microglia but also in neuronal cells (Kim et al., 2006). Furthermore, subcellular localization of these two isoforms differ within the

cells while type I is highly detectable on the cell surface and intracellular compartments, type II appears to remain merely in the endoplastic reticulum, which may indicate a difference in the function of these two ABCA7 isoforms (Ikeda et al., 2003). ABCA7 is a multispan transmembrane protein with a significant sequence homology to ABCA1 (54%) (Kaminski et al., 2000), suggesting that ABCA7 has a similar role in stimulating the efflux of phospholipids and to lesser extent cholesterol from cells (Wang et al., 2003; Quazi & Molday 2013). Additionally, ABCA7 has been reported to regulate APP processing, which may result in reduced Aβ production (Chan et al., 2008). Furthermore, ABCA7 functions as a stimulator of phagocytosis of apoptotic cells via C1q complement pathway by facilitating the cell surface localization of LRP1 (Jehle et al., 2006).

Several ABCA7 variants that increase the late-onset AD risk have been identified by GWAS, including rs3764650 (Hollingworth et al., 2011; Naj et al., 2011; Lambert et al., 2013b) and rs4147929 (Lambert et al., 2013b). Nonetheless, how these identified SNPs contribute to ABCA7 function, AD pathogenesis and disease risk are yet incompletely characterized (Vasquez et al., 2013; Karch et al., 2012b). Importantly, a recent study reported rare *ABCA7* loss-of-function variants in Icelandic, European and American population cohorts with a combined OR of 2.03 (Steinberg et al., 2015). This strengthens the results from the former studies that *ABCA7* gene is associated to pathogenesis of AD.

2.6.3.2.4 SORL1

Sortilin-related receptor LDLR class A repeats containing (SORL1) is a type I transmembrane protein that is a member of the vacuolar protein sorting 10 (VPS10) domain receptor family and the low-density lipoprotein receptor (LDLR) family (Yin et al., 2015). It is encoded by *SORL1* (also known as *SORLA* or *LR11*) gene located on chromosome 11q23.2-24.2 and is abundant in the brain. Subcellularly, it is predominantly localized in the trans-Golgi network (TGN) and in early endosomes (EEs) (Motoi et al., 1999). SORL1 has multiple physiological functions, including vesicle trafficking, cellular cargo transport, signaling and chaperone-like functions (Yin et al., 2015). Additionally, it functions as a receptor that binds lipoproteins, such as APOE, and further modulates the lipoprotein uptake via endocytic pathways. Furthermore, SORL1 is involved in APP processing by direct trafficking of APP to endocytic and recycling pathways. SORL1 also plays an important role in production of Aβ via directly interacting with APP and BACE (Spoelgen et al., 2006), regulating the Aβ generation via SORL1 expression level (Offe et al., 2006) and via interaction of adaptor proteins GGA and PACS-1, which participate in the antero- and retograde protein transport between TGN and EEs (Schmidt et al., 2007).

The association of *SORL1* as an AD risk gene was first discovered from candidate gene based studies (Lee et al., 2008; Rogaeva et al., 2007). More recently, among other discovered *SORL1* associated SNPs, variant rs11218343 that is located near *SORL1* was identified to decrease the risk of AD (Lambert et al., 2013b). Considering the role of SORL1 in APP metabolism and APOE uptake, it is an important protein in AD pathogenesis and may be a crucial target to novel AD therapies.

2.6.3.2.5 BIN1

Bridging integrator 1 (BIN1, or Amphiphysin 2 (AMPH2)) is a ubiquitous protein that is most abundantly expressed in the brain and muscle (Wechsler-Reya et al., 1997). BIN1 protein is encoded by *BIN1* located on chromosome 2q14.3 and exists in several alternatively spliced variants (Ren et al., 2006). BIN1 directly binds lipid membranes and induces memrane curvature via a highly conserved BAR (Bin/amphiphysin/Rvs) domain (Peter et al., 2004). Additionally, splice variant of BIN1 that includes the central insert domain interacts with clathrin and AP2/α-adaptin (McMahon et al., 1997; Ramjaun & McPherson 1998). BIN1 has multiple cellular functions, including endocytosis regulation, intracellular trafficking, calcium homeostasis, caspase-independent apoptosis, inflammation and interaction with microtubule cytoskeleton (Tan et al., 2013).

Variants of *BIN1* that increase the LOAD risk have been identified by GWAS, from which the most significant SNPs, rs744373 and rs7561528, are located upstream from the coding region of the *BIN1* gene (Naj et al., 2011; Seshadri et al., 2010; Harold et al., 2009). Furthermore, GWAS meta-analysis identified an additional SNP rs6733839 (Lambert et al., 2013b). Additionally, a variant rs59335482, in linkage disequilibrium[2] (LD) with the SNP rs744373, is shown to increase both the mRNA expression of *BIN1* and the risk of LOAD (Chapuis et al., 2013). Conversely, it has been reported in a small case-control study that *BIN1* expression is decreased in sporadic late-onset AD but not in familial AD. Hence, these data requires further confirmation from additional studies.

BIN1 and tau were shown to interact in vitro and in mouse brain (Chapuis et al., 2013). Interestingly, BIN1 also interacts with another microtubule-associated protein called CLIP-170 (Meunier et al., 2009). More importantly, the mentioned linkage disequilibrium of SNPs rs59335482 and rs744373 in near *BIN1* region is associated with tau loads, but not NFTs nor Aβ loads (Chapuis et al., 2013). Moreover, in *Drosophila* AD model, *BIN1* knockdown decreases tau-induced toxicity. These data strongly implicates a possible role of *BIN1* in modulation of tau-related AD pathogenesis.

2.6.3.2.6 PICALM

Phosphatidylinositol binding clathrin assembly lymphoid myeloid leukemia (*PICALM*) gene encodes a protein that is important in clathrin-mediated endocytosis, internalization of cell membrane receptors and mediating intracellular trafficking of endocytic proteins (Miller et al., 2011; Tebar et al., 1999; Dreyling et al., 1996). *PICALM* is located on chromosome 11q14 resulting 23 transcripts via alternative splicing. Although PICALM is widely expressed in neurons (Treusch et al., 2011; Xiao et al., 2012), it is also highly abundant in endothelium of brain capillaries (Baig et al., 2010), i.e. cell population responsible of blood-brain barrier (BBB) function. Additionally, PICALM is a crucial protein in fusion of synaptic vesicles to the presynaptic membrane via VAMP2 (vesicle-associated membrane protein 2, also called as synaptobrevin 2) trafficking (Harel et al.,

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[2] Linkage disequilibrium is the non-random occurrence of some allele combinations in the population, i.e. the presence of associations of various loci alleles that are more often or more less often expected from a random formation based on their individual allele sequences (Tan et al., 2013).

2008) and in recruiting clathrin and adaptor complex 2 (AP2) to the cell membrane (Baig et al., 2010).

SNPs rs3851179, rs541458 and rs10792832 at or near the *PICALM* locus were identified by GWAS and were associated with decreased AD risk (Harold et al., 2009; Lambert et al., 2013b). The possible functional effect of these variants is yet to be elucidated. Interestingly, in vitro and in vivo modulation of PICALM expression has been reported to affect Aβ production, and, APP and PICALM colocalization is shown to occur in encocytic vesicles in neurons (Xiao et al., 2012). Specifically, it has been reported that APP and PICALM are part of a complex with AP2 that could be recognized by autophagosomes and further directing APP-containing vesicles to autophagic degradation pathway (Tian et al., 2013), suggesting a role for PICALM in Aβ clearance and generation. Importantly, a recent study showed that reduced PICALM expression in AD brain endothelium correlated with Aβ pathology and cognitive impairment (Zhao et al., 2015b). Furthermore, in PICALM deficient mice, Aβ clearance across the BBB was decreased resulting in increased brain deposition of Aβ. This process was shown to be reversed by re-expressing endothelial PICALM. In addition, by utilizing in vitro human endothelial monolayer mimicking BBB, PICALM was found to regulate the binding of Aβ to LRP1. This enhanced PICALM binding was then showed to initiate PICALM/clathrin-dependent endocytosis of Aβ-LRP1-PICALM complex, and after internalization, directed Aβ to Rab5- and Rab11-positive endocytic vesicles leading to BBB transcytosis of Aβ (Zhao et al., 2015b). Moreover, the protective SNP rs3851179 expressing iPSCs (inducible pluripotent stem cells) from human endothelial cells showed clearly increased *PICALM* mRNA and protein levels and increased Aβ clearance. Hence, these data reinforces PICALM as a potential target for novel AD therapies.

2.6.3.2.7 CD2AP

CD2-associated protein (CD2AP) is a scaffolding protein interacting directly and with proteins involved in the organization of actin cytoskeleton and affect cytoskeleton polarity (Dustin et al., 1998; Lehtonen et al., 2002). Additionally, CD2AP has a function in synapse formation (Dustin et al., 1998), cell-to-cell interactions (Wolf & Stahl 2003) and role in endocytosis (Cormont et al., 2003). CD2AP protein is encoded by gene *CD2AP* located on chromosome 6p12. Regarding AD, SNPs rs9296559 and rs9349407 in *CD2AP* are associated with increased risk of AD by GWAS (Hollingworth et al., 2011; Naj et al., 2011), from which the latter SNP is linked to increased neuritic plaque burden in AD brain (Shulman et al., 2013), although alterations in *CD2AP* mRNA expression in AD brain have not been observed (Karch et al., 2012b). However, in a functional screen that was performed by using a *Drosophila* model of AD, the knockdown of fly ortholog of *CD2AP* resulted in increased tau-mediated toxicity (Shulman et al., 2014). Recently, CD2AP was associated with maintenance of BBB integrity (Cochran et al., 2015).

2.6.3.2.8 EPHA1

EPHA1 is member of the ephrin receptor subfamily of the receptor tyrosine kinase family. EPHA1 protein binds to membrane-bound ephrin-A ligands, which further lead to contact-dependent signaling to adjacent cells (Yamazaki et al., 2009). Additionally, they

participate in axonal guidance and synaptic plasticity both during development and in adults (Gerlai 2001; Lai & Ip 2009; Martinez et al., 2005). *EPHA1* gene is located on chromosome 7q34 that encodes the protein ephrin type-A receptor 1, which is expressed by CD4-positive T lymphocytes and monocytes (Sakamoto et al., 2011). It is also expressed in epithelial tissues where it regulates cell motility and morphology (Coulthard et al., 2001; Yamazaki et al., 2009). With regards to AD, SNPs rs11767557 and rs11771145 near *EPHA1* locus have been identified by GWAS and both are associated with decreased risk of LOAD (Hollingworth et al., 2011; Naj et al., 2011; Lambert et al., 2013b).

2.6.3.2.9 FRMD4A

FERM domain containing 4A (*FRMD4A*) gene is located on chromosome 10p13. It is a member of FERM superfamily but the function of FRMD4A protein in animal cells is yet unclear (Tepass 2009). FERM super-family proteins include ubiquitous cytocortex components that participate in cellular structure, signaling and transport. Moreover, FRMD4A has been reported to function as a scaffolding protein in a Par-3/FRMD4A/cytohesin-1 complex, which activates Arf6 (Ikenouchi & Umeda 2010). This activation is suggested to regulate epithelial cell polarity. Interestingly, ADP ribosylation factor 6 (Arf6), which is a key molecule in membrane trafficking, actin cytoskeleton dynamics and dendritic branching in neurons (Hernandez-Deviez et al., 2002), was shown to control APP processing via endosomal sorting of BACE1 (Sannerud et al., 2011). Thus, these data may implicate a similar role for FRMD4A in APP metabolism. According to AD, multiple SNPs at the *FRMD4A* locus were idenfied in a study using GWAS complementary method called genome-wide haplotype association (GWHA) approach to correlate with plasma levels of Aβ40 and Aβ42 (Lambert et al., 2013a). Interestingly, *FRMD4A* expression was shown to decrease in relation to increasing AD-related neurofibrillary pathology, i.e. Braak staging (Martiskainen et al., 2015). Additionally, down-regulation of *FRMD4A* functionally affects both the amyloidogenic and tau-regulating pathways in vitro.

2.6.3.2.10 CR1

CR1 gene is located on chromosome 1q32, which is a cluster of complement related proteins and encodes the complement receptor 1 protein (Weis et al., 1987). Four different co-dominant alleles in the gene resulting in four isoforms that differs in size are due to genetic deletions and duplications (Krych-Goldberg et al., 2002). CR1 protein functions as a receptor for complement protein fragments C3b and C4b, and is widely expressed on the surface of different blood cells, including erythrocytes, monocytes, macrophages, B-lymphocytes and neutrophils (Klickstein et al., 1988), and, can also be found in the blood plasma as a dissociated soluble form (Yoon & Fearon 1985).

Multiple SNPs in *CR1* were identified to associate with LOAD by GWAS (Hollingworth et al., 2011; Naj et al., 2011; Lambert et al., 2009; Lambert et al., 2013b), from which the SNP rs6656401 showed the highest association conferring AD risk (Lambert et al., 2009). Another SNP, rs3818361, was shown to associate with LOAD risk only in the APOE ε4 carriers (Lambert et al., 2009). Additionally, variants identified in

the *CR1* locus are associated with neuritic plaque burden (Shulman et al., 2013) and in brain imaging studies in AD patients (Biffi et al., 2010). Furthermore, altered *CR1* mRNA expression in AD brain is associated with AD disease status (Karch et al., 2012b). Interestingly, the expression of complement factors is shown to be upregulated in specific affected regions in AD brain (Shen et al., 1997; Eikelenboom & Stam 1982) and both glial cells and neurons are reported to express proteins of classical complement pathway (Gasque et al., 1993; Terai et al., 1997). Moreover, the isolated material from NFTs and Aβ plaques is shown to lead to activation of the complement system (Shen et al., 2001; McGeer et al., 1989). Finally, an association with CR1 and Aβ clearance has been proposed to function via binding of C3b and C4b to Aβ, which is likely mediated by the copy number variation of two CR1 isoforms (CR1-F and CR1-S) (Rogers et al., 2006; Hazrati et al., 2012; Brouwers et al., 2012).

2.6.3.2.11 CD33

CD33 is a type I transmembrane protein, which belongs to the sialic acid-binding immunoglobulin-like lectin (Siglec) family of cell surface receptors (Crocker et al., 1998; Jiang et al., 2014). *CD33* gene is located on chromosome19q13.33 and in mammals CD33 protein is expressed on the surface of phagocytic and hematopoietic cells, such as hematopoietic progenitors, myelomonocytic precursors, monocytes, dendritic cells, macrophages and microglia (Crocker et al., 2007; Griciuc et al., 2013). CD33 participates in adhesion processes of primary immune cells in humans by binding to sialoglycans on target cells thereby mediating interaction between the cells (Jandus et al., 2011). In addition, CD33 is an important molecule in inhibiting immune cell functions, for example in inhibition of pro-inflammatory cytokine production in human monocytes (Crocker & Redelinghuys 2008). Specifically, CD33 activation by binding of sialic acid further leads to monocyte inhibition via cytosolic immunoreceptor tyrosine-based inhibitory motifs (ITIMs) (Jiang et al., 2014). Furthermore, a role of CD33 in clathrin-independent receptor mediated endocytosis has been reported (Walter et al., 2008).

GWAS identified SNPs near *CD33* are associated with reduced LOAD risk (Bertram et al., 2008; Hollingworth et al., 2011; Naj et al., 2011). The SNP rs3865444 (located in the *CD33* promoter region) is associated with increased expression of *CD33* that lacks exon 2, which encodes the V-set domain that mediates sialic acid binding (Malik et al., 2013). Moreover, rs3865444 is reported to associate with CD33 surface expression on monocytes (Bradshaw et al., 2013), larger expression of the V-set domain containing CD33 isoform (Raj et al., 2014; Malik et al., 2013) and reduced levels of both *CD33* mRNA expression and insoluble Aβ42 in AD brain (Griciuc et al., 2013). Another SNP, rs12459419, which is in strong LD with rs3865444, modulates the splicing efficiency of exon 2 (Malik et al., 2013). Overall, splicing modulates the ability of *CD33* to regulate the inhibition of microglial activation. In addition, elevated CD33 expression is associated with AD status and more severe clinical dementia (Griciuc et al., 2013; Karch et al., 2012b). Although SNP rs3865444 failed to reach genome-wide significance in large meta-analyzes, the additional data highly support its role in AD pathogenesis (Lambert et al., 2013b).

2.6.3.2.12 MS4A -cluster

Membrane-spanning 4-domains subfamily A (*MS4A*) gene cluster, a locus containing several genes that are implicated in the inflammatory response, such as *MS4A4A*, *MS4A4E*, *MS4A6A* and *MS4A6E*, is located on chromosome 11q12 and consists of at least 16 genes in human and mouse with variable expression (Liang & Tedder 2001; Liang et al., 2001; Ishibashi et al., 2001). Overall, *MS4A* gene cluster is poorly characterized but it is known to have structural similarity with CD20 protein, which is calcium influx regulator in B-cell antigen receptor activation (Bubien et al., 1993). *MS4A* gene cluster genes are widely expressed in hematopoietic cells (Liang et al., 2001).

Variants near *MS4A4E* (rs670139) and *MS4A6A* (rs983392) were identified by GWAS associated to increased and decreased LOAD risk, respectively (Hollingworth et al., 2011; Naj et al., 2011; Lambert et al., 2013b). Importantly, altered expression levels of *MS4A6A* in AD brain and blood have been reported (Proitsi et al., 2014; Karch et al., 2012b; Martiskainen et al., 2015). Furthermore, SNPs rs2304933 and rs2304935 in the *MS4A4A* locus are also associated with altered *MS4A4A* expression in LOAD brain (Allen et al., 2012).

2.6.3.2.13 Other known susceptibility genes

Very little is known about the function of the novel 11 AD susceptibility loci (*CASS4, CELF1, DSG2, FERMT2, HLA-DRB5/DRB1, INPP5D, MEF2C, NME8, PTK2B, SLC24H4-RIN3, SORL1*, and *ZCWPW1)* that were identified in the largest AD GWAS study to date (Lambert et al., 2013b). Nonetheless, some of these genes are known to participate in pathways related to AD aetiology, including immune response (*HLA-DRB5/DRB1, INPP5D* and *MEF2C*), synaptic function (*PTK2B* and *MEF2C*), axonal transport and function of cytoskeleton (*CASS4, CELF1* and *NME8*) and implicated in tau (*CASS4* and *FERMT2*) and APP (*CASS4*) metabolism. Importantly, the association of these loci with AD risk needs further verification in future studies.

Taken together, most of the identified AD associated loci have been discovered by using statistical methodology to test the association between a set of SNPs, which are usually proxies, i.e. located in the proximity of the gene of interest, and disease risk (Chouraki & Seshadri 2014). In a general praxis where genetic variants that reach genome-wide significance are linked to the closest gene at a specific locus may complicate the data interpretation because several genes of interest are usually located near or at the same locus. Hence, the next objective after identification of a novel candidate locus is to try to search the functional variant(s) within that locus and study their effects on a gene or a set of genes. Finally, it is of importance to study how these effects might be related to known pathways associated with the pathophysiology and pathogenesis of LOAD.

2.6.3.3 Rare variants in Alzheimer's disease

The design of GWAS typically aims to identify frequent variants associated with complex disease, with a minor allele frequency (MAF) more than 5% (Chouraki & Seshadri 2014). Different techniques, such as next generation whole exome and whole genome sequencing

are utilized in order to identify less frequent (1-5% MAF) or even rare (<1% MAF) variants. With constantly decreasing costs and increasing speed of these emerging sequencing techniques, attempts to characterize every single base pair of an individual's genome has become more and more efficient. Conversely to common frequency low penetrance variants, rare variants usually have larger effect size on disease pathogenesis and are predominantly located in exons, i.e. coding regions of genes.

2.6.3.3.1 TREM2

TREM2 gene was among the first rare AD associated variants reported simultaneously by two distinct studies that used advanced sequencing technologies (Jonsson et al., 2013) and candidate gene approach (Guerreiro et al., 2013a). The identified rare missense variant in *TREM2*, rs75932628, results in an arginine-to-histidine substitution at position 47 (R47H) and confers an elevated risk and earlier age of disease onset (Jonsson et al., 2013; Guerreiro et al., 2013a). After these initial reports on Icelandic and American populations with odds ratios (OR) of 2.92 and 4.5, respectively, the role of R47H involvement in AD risk has been replicated and verified in numerous population cohorts, including a French population (Pottier et al., 2013), an American Spanish population (Benitez et al., 2013), a Spanish/Catalan study (Ruiz et al., 2014a), the Cache County study (Gonzalez Murcia et al., 2013), a Belgian study (Cuyvers et al., 2014), an African-American sample cohort (Reitz & Mayeux 2013) and a Columbian family study with frontotemporal and AD dementia (Giraldo et al., 2013). However, in two Han Chinese populations (Yu et al., 2014; Jiao et al., 2014) and in one Japanese population (Miyashita et al., 2014) the R47H was not present or no association with AD was detected, respectively. In a recent study, the R47H mutation was connected to impairment of TREM2 to recognize lipid ligands in vitro (Wang et al., 2015). Interestingly, it remains yet to be seen whether there are other *TREM2* variants in these populations that may affect the risk of AD.

The triggering receptor expressed on myeloid cells 2 (TREM2) is mainly expressed in migroglial cells and functions in stimulation of phagocytosis and in suppression of inflammation (Rohn 2013). *TREM2* gene is located on chromosome 6q21.1 and exists in three alternatively spliced transcripts, from which the longest form encodes a transmembrane protein that is further trafficked to membrane surface where it binds to several ligands and interacts with DAP12 (also known as *TYROPB*) (Colonna 2003). DAP12 is a key signaling molecule for TREM2 and is a crucial modulator in a gene module involved in phagocytosis of pathogens (Zhang et al., 2013). The two shorter transcripts of TREM2 lack the transmembrane domain yet still they are predicted to secrete from the cell (Colonna 2003). Moreover, after being trafficked to cell surface, TREM2 is further cleaved by γ-secretase hereby linking two AD associated molecules in a single pathway (Wunderlich et al., 2013).

AD patients who carry a *TREM2* mutation are reported to have more extensive and more rapidly progressing brain atrophy compared to non-carrier AD patients (Rajagopalan et al., 2013). Interestingly, SNPs in the *TREM2* gene cluster are associated with increased CSF levels of both phosphorylated and total tau levels (Cruchaga et al., 2013). Moreover, increased expression level of *TREM2* exons 3 and 4 in AD brain has been observed in accordance to more advanced Braak staging (Martiskainen et al., 2015). Recently, it was reported that *TREM2* deficiency increases accumulation of Aβ and loss of neurons in a

mouse model of AD via dysfunctional response of microglia to senile plaques (Wang et al., 2015). However, another study showed that inflammation and both tau and Aβ pathologies were reduced in AD mouse model (APPPS1) with *TREM2* deficiency occurring most likely via elimination of TREM2 positive inflammatory macrophages (Jay et al., 2015).

In addition to AD risk, homozygous *TREM2* mutations are associated with Nasu-Hakola disease, a recessive early onset form of dementia with bone cysts and fractures (Paloneva et al., 2002). Furthermore, autosomal recessive *TREM2* mutations have been identified in a family with FTD-like syndrome that lacked the bone derived aspects (Guerreiro et al., 2013b). Generally, these data suggest that TREM2 probably plays an important role in modulation of neurodegeneration in general via its role in clearance of extracellular debris and protein aggregates, and, by modulating neuroinflammation.

2.6.3.3.2 PLD3

Another rare coding variant associated with AD risk was recently reported. Phospholipase D3 (PLD3) was identified in a family-based study design with additional genotyping as a risk factor for increased AD incidence (Cruchaga et al., 2014). Identified SNP, rs145999145, which results in a valine-to-methionine substitution at location 232 (V232M), has been suggested to increase the AD risk 2- to 3-fold. However, this finding has proved to be really difficult to replicate although there has been a lot of effort from various groups with different experimental settings and population cohorts (van der Lee et al., 2015; Heilmann et al., 2015; Lambert et al., 2015; Jiao et al., 2014; Hooli et al., 2015). Hence, additional experimental data will be required to further verify if rare variants in *PLD3* have any role in AD risk.

PLD3 gene is located on chromosome 19q13.2 and is expressed in as many as 25 alternatively spliced transcripts (Karch & Goate 2015). PLD3 is a non-classical member of a superfamily of PLD proteins with a poorly characterized function(s) (Cruchaga et al., 2014). PLD1 and PLD2, which are the classical phospholipases, catalyze the hydrolysis of phosphodiester bond of a glycerolipid phosphatidylcholine resulting in phosphatidic acid and free choline (Jenkins & Frohman 2005). Furthermore, the activity of phospholipase enzymes regulates the organization of actin cytoskeleton, modulate vesicle trafficking for endocytosis and secretion and participate in receptor signaling. Intriguingly, these proteins have been previously associated with APP-trafficking thereby playing a possible role in AD pathogenesis (Oliveira et al., 2010; Jin et al., 2007; Cai et al., 2006a; Cai et al., 2006b).

2.6.3.3.3 Other rare variants

Other AD-associated rare variants have been proposed with either protective or disease driving roles. These include e.g. genes *ADAM10* (an α-secretase) (Cai et al., 2012; Kim et al., 2009b), *ABCA1* (ATP-binding cassette transporter 1) (Lupton et al., 2014) and a protective variant of *APP* (aa substitution A637T; so called arctic mutation) (Jonsson et al., 2012). These recently discovered AD associated rare variants are currently undergoing replication studies in order to establish the accuracy of the findings. Altogether, the studies on rare AD-associated variants underline the importance of population

stratification because of its possible impact on initial and replication results (e.g. Lord et al., 2014). Moreover, to distinguish if a failure of replication is caused by a lack of statistical power or a lack of association *per se* large cohort sample size is a prerequisite. Additionally, the differences in the disease aetiology between the discovery and replication cohorts emphasize the replication phases to be executed both in the same and in alternative populations at the gene level.

2.6.3.4 Missing heritability

The disease-causing *APP*, *PSEN1* and *PSEN2* mutations in EOAD and the association of *APOE* with AD that were all identified more than two decades ago initiated an era of pursuing to understand AD through genetic perspective. These discoveries built the foundation for drug development and multiple clinical trials driven by the amyloid cascade -hypothesis, which, unfortunately, have not resulted in any improvement in the treatment of AD (Bettens et al., 2013). Furthermore, in the advent of GWASs, this new approach was anticipated to elaborate the highly complex aetiology of LOAD, in which it at least partly has succeeded. However, the functional relevance of these newly identified susceptibility genes to LOAD pathogenesis and pathophysiology yet remains largely unknown. Indeed, within the past few years GWASs have resulted in more novel genetic information than probably could have been imagined regardless of the mostly subtle effects of individual genes conferring to the overall risk of LOAD. In particular, after the semi-stagnated period in LOAD genetics that was between the discovery of *APOE*-association and before the beginning of GWASs, these novel data has presented numerous previously uncertain or unknown common genetic variants in LOAD. Besides causative-mutations and GWAS susceptibility genes, however, there is still a substantial proportion of the genetic disease risk yet to be explained. It has been estimated that all the genetic factors combined accounts for approximately 61% of the population attributable risk (PAR) of LOAD (Medway & Morgan 2014). Hence, there is a missing heritability - component (Manolio et al., 2009), which needs to be uncovered in order to gain more general view on the LOAD aetiology.

Besides the low increment of the each locus that confer to disease risk identified by GWAS, additional limiting factor is the invariably increasing sample size needed to detect the increasingly minute impact on LOAD risk that is provided by each GWAS loci (Lord et al., 2014). Consequently, in the case of highly complex disease, such as AD, this missing heritability is very unlikely to be resolved with GWAS or any single methodology but will require a combination of complementary approaches. Interestingly, possible suggestions and models that might explain the missing heritability have been proposed including (i) the possibility of vast number of variants with smaller effect size is still undiscovered, (ii) identification of additional rare variants with moderate to high effect size (which are difficult to detect with methods like GWAS but may become less arduous and cost-effective upon the improvement of next generation sequencing (NGS) methods) and (iii) the poor existing capacity to detect genotype-by-genotype interactions by existing arrays and (iiii) genotype-by-environment interactions (Gibson 2011; Manolio et al., 2009). Noteworthy, particularly in the case of large amount of small effect variant - methods such as GWAS, the term 'missing' heritability may be misleading since the heritability is rather hidden than missing (Gibson 2010), which is a concept that can be

generally extrapolated to the entire field of natural sciences: nothing is missing *per se* but (almost) all the components are already there just waiting to be discovered.

In addition to the proposed explanations for missing heritability, epigenetic modifications, i.e. alterations in gene regulation by modification of DNA or chromatin-associated proteins without any changes in the DNA sequence, or more specifically a novel field of epigenome-wide association studies (EWAS) have been suggested to have an effect on the risk of LOAD. Recently, two distinct studies showed overlapping alteration in DNA methylation in various loci in AD brain (De Jager et al., 2014; Lunnon et al., 2014). Furthermore, the methylation changes in different regions were observed in the brain areas that are usually affected in AD according to Braak staging with both, in samples of cognitively normal and in samples of symptomatic patients. This finding raises the question whether this dysregulation of methylation is caused by early neuropathological changes or if the methylation changes are responsible for triggering neurodegeneration (Lord & Cruchaga 2014). Nevertheless, the novel EWAS approach is an important additional tool to study the epigenetic modifications that may confer the risk for LOAD.

2.7 Protein spreading in neurodegenerative diseases

2.7.1 Prion-like spreading – common hypothesis

Neurodegenerative diseases, as a group of CNS disorders without any disease-modifying treatment currently available, present remarkable variety both in clinical and neuropathological phenotypes (Brettschneider et al., 2015; Guo & Lee 2014; Jucker & Walker 2013). However, majority of NDDs share a common hallmark feature, which is the cerebral intracellular or extracellular accumulation of disease-specific proteins into insoluble aggregates. These characteristic accumulating proteins include Aβ in senile plaques of AD (Glenner & Wong 1984b), tau in NFTs and neuropil threads of AD and other tauopathies (Kosik et al., 1986; Lee et al., 2001), α-synuclein (α-syn) in Lewy bodies and in Lewy neurites of idiopathic PD and PD with dementia and Lewy body dementia (Spillantini et al., 1998a), TDP-43 aggregates in FLTD-TDP43 and amyotrophic lateral sclerosis (ALS) (Neumann et al., 2006), prion plaque formations in Creutzfeldt-Jacob disease (CJD) (Bolton et al., 1982) and inclusions of polyglutamine-rich huntingtin protein in Huntington's disease (DiFiglia et al., 1997) (table 2.3).

The transition of the disease-related proteins from their normal functional conformation into oligomeric or multimeric fibrillar species typically results in decreased clearance and degradation leading to aggregation (Jucker & Walker 2013). The aggregates that are composed of misfolded proteins are usually referred to as amyloid, which are thermodynamically highly stable structures. Each twisted unbranched amyloid filament is composed of β-sheets (cross-β quaternary structure), which in turn is composed of β-strands. In all amyloidoses, such as tauopathies, aggregates of misfolded protein can be pathogenic by either directly causing damage within residing cell, tissue or organs, or, the aggregated protein in amyloid structures loses their ability to maintain its physiological function (Eisenberg & Jucker 2012; Blancas-Mejia & Ramirez-Alvarado 2013). In the process of amyloidogenesis, smaller intermediate assemblies, i.e. protein species known as soluble protofibrils and oligomers exist and appear to be even more toxic than mature

Table 2.3. Examples of neurodegenerative diseases involving protein misfolding. Letter "F" (familial) indicates that the disease is caused by an inherited dominant mutation(s) in the particular gene encoding the protein or gene multiplications. Combination "F/S" indicates that in some cases the disease is inherited and caused by mutation(s) or multiplications of the gene encoding the misfolded protein. "S" (sporadic) indicates that known cases of the disease are not caused by any dominant mutation(s) in the gene or multiplications of the gene that encodes the misfolded protein. * formerly called also as tangle-only dementia. Modified from Goedert at al., 2010.

Misfolded protein	Human disease	Familial / Sporadic
Prion protein	Kuru	S
	Creutzfeldt-Jakob disease (different variants)	F/S
	Gerstmann–Sträussler–Scheinker disease	F
	Fatal familial insomnia	F/S
Tau	Alzheimer's disease	S
	Gerstmann–Sträussler–Scheinker disease	S
	British dementia	S
	Danish dementia	S
	Pick's disease	F/S
	Progressive supranuclear palsy	F/S
	Corticobasal degeneration	F/S
	Argyrophilic grain disease	F/S
	Guam Parkinsonism-dementia complex	S
	Primary age-related tauopathy*	S
	Globular glial tauopathy	F/S
	Frontotemporal dementia and Parkinsonism linked to chromosome 17	F
	Progressive subcortical gliosis	F/S
β-Amyloid	Alzheimer's disease	F/S
BRI2	British dementia	F
	Danish dementia	F
α-Synuclein	Parkinson's disease	F/S
	Dementia with Lewy bodies	F/S
	Multiple system atrophy	S
	Pure autonomic failure	S
	Lewy body dysphagia	S
Superoxide dismutase 1	Amyotrophic lateral sclerosis	F/S
TAR DNA-binding protein 43	Amyotrophic lateral sclerosis	F/S
	Frontotemporal dementia	F/S
Fused in sarcoma	Amyotrophic lateral sclerosis	F/S
	Frontotemporal dementia	S
Huntingtin	Huntington's disease	F

fibril formations (Mucke & Selkoe 2012; Haass & Selkoe 2007). Although various disease-related oligomers and protofibrils have been isolated in vitro, it is very difficult to observe or isolate these protein species in human brains, which hinder the establishment of a direct connection of the function of protofibrils and oligomers in disease pathogenesis (Guo & Lee 2014).

Molecular mechanism in prion diseases (also known as transmissible spongiform encephalopathies) is defined by conversion of normal cellular prion protein (PrP^C) into conformationally altered, misfolded prion protein (PrP^{Sc}), which is an unconvential infectious agent with ability to recruit and induce ("agitate") the conformational switch in their normal counterparts (PrP^C) leading to aggregation of pathological PrP^{Sc} (Prusiner 1982; Aguzzi 2009; Aguzzi et al., 2008). Thus, PrP^{Sc} acts as a corruptive template, or seed, thereby initiating a process that resembles a chain-reaction resulting in further misfolding of PrP^C and progressive aggregation. Regardless that the trigger responsible for initiation of soluble native protein into misfolded conformation is yet unknown, it is suggested that once seeded aggregation is started it will progress persistently. As prion aggregates grow, they also fragment and spread thereby producing new seeds subsequently resulting in additional aggregate formation by newly generated seeds. Although this self-perpetuating process was thought to be specific only for prions, in the past few years there have been numerous convincing studies presenting evidence that the 'prion-like' self-propagating mechanism may also be plausible for non-prion NDD-specific proteins (Brettschneider et al., 2015; Guo & Lee 2014). Indeed, lysates that contain aggregates and/or fibrils assembled from recombinant proteins of amyloidogenic proteins Aβ, tau, α-syn, huntingtin, superoxidase dismutase 1 and TDP-43, have been reported to act as seeds capable of recruiting and inducing the soluble form of same protein into progressively growing fibrils both in vitro and in vivo, in some studies even without overexpression of protein in question in recipient cells or animals (Luk et al., 2012a; Volpicelli-Daley et al., 2011). Of note, TDP-43 differs from other amyloidogenic proteins by presenting mainly as inclusions comprised predominantly from granular non-amyloid fibrils (Thorpe et al., 2008). Importantly, upon in vivo administration of aggregate-containing inoculums, the resulted pathology was not restricted to the site or its close proximity but additionally lead to an invariable spreading of the specific pathology to synaptically interconnected brain areas in a time-dependent manner (Stohr et al., 2012; Masuda-Suzukake et al., 2013; Luk et al., 2012b; Luk et al., 2012a; Volpicelli-Daley et al., 2011; Iba et al., 2013; Clavaguera et al., 2009).

Besides prion-like spreading, another feature shared by amyloidogenic proteins is their ability to form fibrils that are structurally diverse protein aggregates that are conformationally distinct from each other collectively referred as strains (Aguzzi et al., 2007). The most studied example of conformational variants, or strains, is PrP^{Sc}, which is found to exhibit different strains characterized by specific clinical phenotypes and pathological lesion patterns. Importantly, the phenotypic variations are able to propagate in vivo without any alterations in phenotype in subsequently infected animals after serial passage. Similarly to prion strains, assembly of polymorphic fibrils of other pathological protein aggregates of the same protein with single amino acid substitutions have been reported. Additionally, this has been also shown for proteins having identical primary structures under altered fibrillization conditions. Nonetheless, regardless of the structure, these aggregates are able to propagate through seeding fibrillization both in vitro and in

vivo (Heilbronner et al., 2013; Nekooki-Machida et al., 2009; Petkova et al., 2005; Furukawa et al., 2010; Yonetani et al., 2009; Aoyagi et al., 2007; Guo et al., 2013). Interestingly, different strains of a single protein may display different biological activities.

Like prion diseases, each disease class of non-prion proteinopathies exhibits wide range of heterogeneity (Brettschneider et al., 2015). Concomitantly, significant overlap is present between distinct categories of proteinopathies, in both clinical manifestations (Morris et al., 1989; Mayeux et al., 1992) and neuropathologies, in which aggregates consisting of different pathological proteins such as NFTs and Lewy bodies, are not uncommon co-inclusions in the brains of disease-affected individuals (Galpern & Lang 2006). Actually, mixed disease pathologies are fairly common among NDDs and only rarely cases with stringently "pure" AD or PD neuropathology is observed. Given the aspects of prion diseases and non-prion proteinopathies, it has been suggested that various protein aggregate strains may possibly explain the differences and similarities of NDDs (Brettschneider et al., 2015). Specifically, transmission of fibrillar protein aggregates composed of different conformational strains may present significantly differential kinetic properties in seeded fibrillization subsequently leading to variable rates of progression of specific diseases. Consequently, the development of pathology and progression into various brain regions in addition to their ability to induce cross-seeding of other amyloidogenic proteins may vary substantially. Hence, besides the potential effect of strains on remarkable heterogeneity among NDDs with the same predominant protein depositions, they could offer an explanation to rather frequent occurrence of coincidental deposition of different protein inclusion. Notably, upon propagation from one cell to another with an involvement of templated fibrillization, the strain may convert into different conformational strain, which has been shown to occur at least for synthetic fibrils of α-syn (Guo et al., 2013).

The concept of prion-like spreading of non-prion proteins is rather novel and although it could probably deliver a unifying pathophysiological model of NDDs, there are some critical aspects yet to be elucidated. Among others, one highly important aspect according to describing the various processes of prion-like spreading is semantics; it is essential to establish uniform and consistent nomenclature, i.e. to clearly distinguish which biological phenomena are referred to various terms. For instance, the crucial differences in terms cell-to-cell/intercellular transmission or propagation compared to transcellular propagation where the first process merely includes the cellular release and subsequent cellular uptake of a protein whereas transcellular propagation in addition to former includes the passage of the protein or aggregate throughout the cell (i.e. release-uptake-intracellular propagation-release). Moreover, terms like transmission or transmissable and spreading of proteins should be used in their correct context. Furthermore, nomenclature is not a specific problem for terminology used in the literature but also concerns laboratory practices, for example when using synthetic fibrils or seeds, it is essential for the repeatability and confirmation of the previous results to specifically describe the exact nature of the fibrils used. Notably, synthetic fibril variants may not be identical, or could even display quite significant differences to those formed in the human brain (Brettschneider et al., 2015). However, human brain is highly complex related to artificial fibrillization conditions and the probability to generate diverse strains in disease-affected brain is therefore high. Importantly, more studies are required to convincingly confirm the

existence of various strains in non-prion disease groups and their possible impact on phenotypic manifestation of the disease. Few recent studies, however, have presented some evidence on the existence of pathological strains of tau, α-syn and Aβ in the human brains (Clavaguera et al., 2013a; Lu et al., 2013; Guo et al., 2013).

The most important aspect, however, is the proper definition regarding prions and pathological non-prion proteins. By definition, prions are 'proteinacious infectious particles' (Prusiner 1982), that are capable of infecting other organisms upon the transfer from one to another. Owing to this, infectivity is one of the most important features in the characterization of prions and distinguishing them from other amyloidogenic proteins. Accordingly, the term prionoids has been coined to specifically differentiate bona fide prions from other disease-associated proteins that seemingly are able to spread from one cell to another to emphasize the similarities of prions with other amylodogenic proteins the term prion-like protein is suitable, whereas if pointing out the differences of these two group of proteins the term non-prion protein is considered more descriptive (Aguzzi & Rajendran 2009). Hence, until now, only bona fide prions have been referred as prions and other pathological proteins with variable nomenclature. Unexpectedly, a very recent study suggested that α-syn fibrils are true prions in multiple system atrophy (MSA), which is a NDD defined by progressive deterioration of autonomic nervous system function with usual signs of parkinsonism and neuropathological hallmark presenting as cytoplasmic glial α-syn filament inclusions (Prusiner et al., 2015). Intriguingly, the α-syn isolated from the brains of MSA patients but not from PD patients induced aggregation of α-syn in cells overexpressing mutant A53T α-syn and in TgM83$^{+/-}$ mice heterozygous for the same mutant suggesting that α-syn strain causing MSA is different from the α-syn in PD. Importantly, these findings have been supported with additional evidence provided by a follow-up study presenting similar data using partially enriched α-syn strains by selective precipitation and reported the existence of at least three different α-syn strains capable of causing MSA (Woerman et al., 2015) Hence, the MSA α-syn strain is now suggested to be the first transmissible human prion since the discovery of CJD. However, of now, there is no clinical evidence of transmission of MSA from one person to another. Additionally, recent autopsy study revealed the co-existence of Aβ pathology in the brain of patients deceased for iatrogenic CJD (Jaunmuktane et al., 2015), which is a form of CJD caused by transmission via contaminated surgical equipment, medical procedures or ritualistic cannibalism in Kuru disease (Collinge et al., 2006; Collinge 2001). Specifically, in four out of eight individuals (aged 36-51 years) for all of whom had received prion-contaminated human cadaveric pituitary-derived growth hormone (Brown et al., 2012; Swerdlow et al., 2003), showed moderate to severe vascular and grey matter Aβ pathology, which were typical to ones seen in the blood vessel walls of cerebral amyloid angiopathy and in grey matter of AD, respectively. Moreover, another recent study provided results on the persistence of Aβ in the brain (Ye et al., 2015). Interestingly, it was reported that Aβ seeds inoculated into APP null mice did not induce further β-amyloidosis unlike in the APP transgenic mice. However, in subsequent secondary transmission, intracerebral injections containing the brain extracts of Aβ seed inoculated APP null mice induced β-amyloidosis in APP transgenic mice. Hence, these data suggests that Aβ pathology may be transmissible e.g. by iatrogenic routes and underline the longevity and existence of Aβ seeds in the brain that could at least partially explain the long preclinical phase and delay before the onset of dementia in AD (Jaunmuktane et al.,

2015; Ye et al., 2015). However, it should be emphasized that this evidence of spreading of Aβ pathology by no means equals contagiousness of AD; the presence of NFTs have not been reported to co-deposit with Aβ in any of these cases with iatrogenic CJD (Jaunmuktane et al., 2015).

2.7.2 Propagation of tau pathology

The observed spreading of tau pathology in various tauopathies, as shown by Braak staging in AD for example, strongly implicates that cell-to-cell transfer of tau aggregates occurs during the clinical progression of the disease (Braak & Braak 1991). The mounting experimental evidence within a past few years have strongly supported the theory of cell-to-cell transfer of tau aggregates both in vitro and in vivo building a foundation for a novel 'prion paradigm' in NDDs. Indeed, results provided by studies conducted on other amyloidogenic proteins support the suggested prion paradigm for NDDs.

Induction and propagation of tau pathology was first experimentally demonstrated by using transgenic mice (ALZ17; human wild-type tau and P301S mutant) and intracerebral injections of aggregate-containing P301S mouse brain homogenates into hippocampus and cerebral cortex of ALZ17 mice, which resulted in filamentous formations of wild-type tau in neurons presenting as NFTs and neuropil threads and oligodendrocytic coiled bodies (Clavaguera et al., 2009). Moreover, tau filament formation was observed first to spread to areas at close proximity to the site of injection and further into more distant brain regions that are synaptically connected to each other. Additionally, this time-dependent progression of pathology was shown to completely depend on tau. These results were confirmed by showing that intracerebral injection of tau oligomers and PHFs extracted from human AD brains into hippocampus of wild-type mice induce filamentous tau pathology that spread over time into other brain areas apart from hippocampus such as cerebral cortex, corpus callosum and hypothalamus (Lasagna-Reeves et al., 2012). Furthermore, two independent studies replicated the results by using mice overexpressing human P301L mutant tau, in which the transgene is restricted to entorhinal cortex for specific examination of tau spreading between entorhinal cortex and hippocampus (Liu et al., 2012; de Calignon et al., 2012). In both studies, time-dependent spreading of tau accumulation was observed in the hippocampus besides the initial formation of filamentous tau pathology in transgene-containing neurons in the entorhinal cortex, strongly implicating neuron-to-neuron propagation of aggregated tau.

Intracerebral injections consisting of pure synthetic filaments assembled from human mutant recombinant tau protein into presymptomatic mice expressing human P301S mutant tau induce the formation of NFT-like tau inclusions in a dose- and time-dependent manner (Iba et al., 2013). Consistent with observations from earlier studies, tau pathology spread through synaptically interconnected areas; from injected hippocampus to contralateral hippocampus and entorhinal cortex. These results are consistent with the previous finding demonstrating that the induction and propagation of tau pathology in ALZ17 mice inoculated with brain homogenates of P301S mice is predominantly responsible by the insoluble fractions of the extracted homogenates (Clavaguera et al., 2009). Similar findings were presented upon injecting hippocampus and the overlying cerebral cortex of young (3 months old) homozygous human P301S mice with heparin-containing tau filaments assembled from recombinant human P301S mutant tau

(Clavaguera et al., 2013b). Notably, tau depositions at the injection sites were apparent only 4 weeks post-injection. Comparable results were obtained in an additional independent study, in which young presymptomatic P301S mice were injected with brain extracts of P301S mice (Ahmed et al., 2014). Specifically, two weeks after unilateral injection at different regions, the NFT pathology was visible and was further spreading in time-dependent and stereotypical manner, i.e. consistent with the previous findings regarding the spreading pattern of tau pathology. Subsequently, contralateral and anterior/posterior propagation of tau pathology became evident in various nuclei that were connected synaptically to the site of injection. Hence, these results further underline the notion that it is not the spatial location or proximity but anatomically connected regions that are strongly synaptically interconnected that serve as a tract for spreading of tau pathology. Moreover, transsynaptic spreading of tau pathology has also been demonstrated by using lentivirus-mediated rat model (Dujardin et al., 2014b). After 5 months of lentiviral injection into hippocampus, human wild-type tau was detectable in all synaptically interconnected brain regions related to injection site, whereas injection with lentiviral human P301L mutant tau resulted in strong induction of tau aggregation, which, however, mostly remained restricted close to the injection sites. Thus, these data suggest the more prone nature of wild-type tau to synaptically spread as compared to mutant tau implicating that transgenic mice with tau mutations may not serve as an optimal model of sporadic tauopathies. Interestingly, it was recently reported that upon intraperitoneal injection of presymptomatic P301S mice with brain extracts isolated from the same transgenic mice presenting symptoms, formation of tau inclusions in the brain is increased through a yet unknown mechanism (Clavaguera et al., 2014). Expectedly, intraperitoneal injection induces less effective tau deposition in the brain than intracerebral injection nonetheless providing preliminary evidence that peripherally administered tau aggregates are capable of initiating cerebral tau seeding.

The formation of both neuronal and glial tau filaments have been shown to occur after intracerebral injection with brain homogenates of pathologically confirmed subjects with human tauopathies into the ALZ17 mice expressing single isoform of wild-type 4R human tau (Clavaguera et al., 2013a). More specifically, tau inclusions formed after injection of all cases of various tauopathies (AD, PART, PiD, AGD, PSP and CBD), although disease-specific lesions similar to those observed in human cases were detected following the homogenate injections of AGD (astrocytic tau pathology), PSP (tufted astrocyte - reminiscent aggregates) and CBD (astrocytic plaque -resembling structures). Moreover, time-dependent stereotypical spreading of filamentous tau pathology was observed in all cases except after injecting PiD homogenates, where visible filaments were restricted only at the injection sites. Interestingly, similar filamentous tau deposits were present after identical injections with homogenates of different tauopathies in wild-type mice. Additionally, it was reported that brain homogenates from ALZ17 mice, that were injected 18 months earlier bilaterally with P301S brain homogenates, injected into young (3 months of age) ALZ17 mice induced tau filament pathology into neurons and oligodendroglial cells at the site of injection 12 months later. Similarly, brain homogenates of wild-type mice that were injected with brain homogenates of human PART and AGD (bilaterally, 18 months earlier), were injected into ALZ17 mice. One year following the injections, neuronal tau aggregates and neuropil threads together were present at the sites of injections. Importantly, these results have been confirmed and

further extended in an independent study, in which conformationally distinct tau strains were assembled of tau containing only 4R repeats in HEK293 cells (Sanders et al., 2014). Specifically, two different clones (namely clone 9 and clone 10) isolated from cells had distinct properties: clone 9 generated small intranuclear aggregates with highly efficient seeding property whereas clone 10 resulted in somewhat larger juxtanuclear depositions. Through serial passages, these clones maintained the identical conformational strains when transferred into naïve cells. Interestingly, cell lysate injection of different clones of tau strains into hippocampus of young transgenic human P301S tau mice induced pathology identical to the original presentation after serial passage of injections. Strikingly, when tau aggregates isolated from the hippocampi of mice after third round of injection were introduced to HEK293 cells stably expressing 4R-tau, the seeding of aggregates formed inclusions similar to those displayed in the original cells.

Taken together, these data collectively and strongly indicate the existence of various tau strains, which may be able to induce distinct human tauopathies. Given that multiple tau strains capable of self-propagation that is seemingly indefinite and the persistence of conformational strains through serial passage it has been suggested that tau would be considered as a prion. In fact, data from a very recent study further substantiates the notion of tau strains (Woerman et al., 2015). It was reported that tau strains isolated from brains of two individuals with pathologically confirmed PSP induce rapid tau aggregation in a cell line stably expressing tau 4R-repeats (Woerman et al., 2015). However, regardless of the advances in cell-to-cell propagation of tau pathology, the specific molecular mechanism underlying cellular tau release and uptake are both poorly understood processes.

2.7.2.1 Cellular tau release

A prerequisite for subsequent internalization by adjacent or connected neurons or glial cells, tau must be first translocated from cytoplasm to extracellular space. Previously, it was thought that tau found in the cerebrospinal fluid (CSF) was only a result of passive release from degenerated axons and dead neurons (Blennow et al., 1995). However, besides patients with AD where the levels of tau in the CSF are increased, tau is also found in the CSF of healthy individuals (Vigo-Pelfrey et al., 1995; Vandermeeren et al., 1993; Blennow et al., 1995). Moreover, tau is found to be present in the brain interstitial fluid (ISF) of both healthy wild-type mice and in transgenic human tau P301S mutant mice (Yamada et al., 2011). It was reported that the total level of ISF-tau was higher than CSF-tau, and, that ISF-tau in P301S mice was five fold higher compared to endogenous tau. Interestingly, it was shown that there was a significant reduction in the level of monomeric ISF-tau upon the initiation of tau aggregation. Thus, there is an inverse correlation between the decrease of soluble tau and the increase of insoluble tau in the mouse brain homogenates. Hence, these data strongly implicate that tau is secreted from cells in an activity-dependent manner without the presence of any signal peptide sequence or protein lipidation thereby diminishing the possibility of conventional secretion via endoplasmic reticulum-Golgi-pathway (Gendreau & Hall 2013).

Induction of neuronal activity and calcium-mediated AMPA-receptor activation are both shown to promote tau release from cells in vitro (Pooler et al., 2013). These results have been confirmed and partly extended by using in vivo microdialysis showing that

induction of neuronal activity increase the steady-state level of ISF tau in both wild-type mice and transgenic human tau P301S mutant mice, and that presynaptic glutamate release increases the level of ISF-tau, implicating a connection between presynaptic excitatory neuronal activity and tau release (Yamada et al., 2014). Recently, it was reported that synaptic connections significantly enhance the cell-to-cell propagation of tau pathology in co-cultures in artificial neuronal networks created in microfluidic devices (Calafate et al., 2015). Also, decrease in tau propagation between neurons due to either suppression of the activity in the synapse or reduction of synaptic density was demonstrated. In addition, nutrient deprivation and lysosomal inhibition or dysfunction, which is an established feature of many NDDs, was reported to increase cellular tau release from primary cortical neurons (Mohamed et al., 2014). Interestingly, the level of extracellular tau is altered relative to changes in tau protein structure (Karch et al., 2012a). More specifically, the ratio of extracellular/intracellular tau of 3R- and 4R-tau isoforms lacking N-terminal inserts (exons 2 and 3; 0N/3R and 0N/4R) is highly similar when compared to each other whereas it is remarkably higher than 2N/4R and significantly lower than ratio of 2N/3R isoforms. These results implicate that rate of tau secretion is dependent on tau isoforms and that 3R-tau isoforms are more abundant in the extracellular space compared to 4R-tau in vitro. Moreover, the impact of different tau mutations to cellular tau release was shown; all the mutations tested (P301L, P301S and R406W) in both isoforms 2N/3R (R406W) and 2N/4R (P301L, P301S and R406W) significantly reduced the level of extracellular tau as compared to wild-type tau without altering intracellular total tau levels suggesting that FTDP-17 *MAPT* mutations regulate the rate of tau secretion in vitro. Another study according to structure of tau relative to its cellular release reported that upon C-terminal cleavage of tau at D421, which is prefenrential site for caspase 3 -mediated cleavage, tau secretion is enhanced in vitro (Plouffe et al., 2012). Furthermore, similar results regarding to secretion of C-terminal truncated tau into CSF have been proposed also to occur in vivo (Barten et al., 2011). These results have been replicated and extended in a recent study suggesting that extracellular tau is predominantly cleaved at the C-terminus (Kanmert et al., 2015). Particularly, it was proposed that majority of extracellular tau lacks the MBRD and that the minority of extracellular tau with MBRD is originated from dead cells. Due to the suggested necessity of MBRD involvement in aggregate formation, it was proposed that induction of cell death mechanism is required for initiating extracellular tau propagation in vitro.

Tau is secreted from cells in a vesicle-free form (Kim et al., 2010a; Chai et al., 2012) and by exocytic mechanism either inside or associated with vesicles (Simon et al., 2012; Saman et al., 2012). Recently, it was reported that besides vesicle-free and exosome-associated tau, active tau release is predominantly mediated by ectosomes (Dujardin et al., 2014a). Ectosomes are plasma membrane-originated vesicles that are larger in size compared to exosomes (Dujardin et al., 2014a), which are vesicles released into extracellular space when multivesicular endosomes fuse with plasma membrane at cell surface (Fevrier & Raposo 2004). Specifically, it was demonstrated by both in vitro and in vivo models that in normal physiological conditions tau is secreted by ectosomes and upon over-accumulation of tau in cells there is a shift of mechanism toward exosome release of tau functioning in concert with ectosomal release. Additionally, by using an alternative in situ lamprey model to study tau secretion it was shown that N-terminus of tau, which is known to interact with plasma membrane and other membrane-associated

proteins, is required for cellular tau release (Kim et al., 2010b). In the lamprey model, tau secretion is dependent on whether MBRD is present or not thus representing in two distinct patterns; secretion is 'diffuse-like' and mostly occurring in soma when lacking the MBRD whereas full-length tau is predominantly secreted from dendrites in a more focal manner (Kim et al., 2010b). Hence, these patterns suggest an interaction between tau MBRD and elements of extracellular matrix that may have an effect on inclusion spreading and possible extracellular tau-induced toxicity (Lee et al., 2012). Finally, taken together, it is currently not fully understood by which mechanisms under physiological and pathophysiological conditions tau is secreted from cells, and, how the altered conditions affect the ratio between extracellular vesicle-free and microvesicle-associated tau. Accordingly, the excess of vesicle-free tau in extracellular space may also result from post-secretional release by microvesicles. Moreover, whether the majority of secreted tau is in aggregated (insoluble) or in monomeric or oligomeric (soluble) form relative to differential conditions is yet to be elucidated.

2.7.2.2 Cellular tau uptake

Uptake of tau into cells has been currently characterized even in less detailed manner than secretion. Fibrils assembled of recombinant tau, but not monomers, are reported to be uptaken by cells (Frost et al., 2009a). Subsequently, internalized aggregates are capable of inducing fibrillization of intracellular full-length tau in recipient cells. Upon internalization, tau aggregates were shown to co-localize with a marker of fluid-phase endocytosis (dextran) rather than lipid raft marker (cholera toxin B), strongly implicating the involvement of endocytic processes and not mere penetration of cell membrane. Interestingly, the fibrillization of intracellular tau induced by extracellular-originated aggregates was transferred to co-cultured cells and also seeded further fibrillization of recombinant monomer of tau in vitro. Similar results have been reported when tau seeds are introduced to cells with a transfection reagent in vitro (Nonaka et al., 2010). It was also reported that protein aggregation is nucleation-dependent as well as isoform- and protein-specific since 4R-tau did not induce fibrillization in 3R-tau expressing cells and vice versa. Additionally, a distinct study reported the templated transmission of the conformational features of fibrils assembled of recombinant tau (Frost et al., 2009b). Specifically, fibrils assembled of mutated tau (P301L/V337M) were reported to induce fibrillization of wild-type tau monomer, of which both were shown to have distinct secondary structures, leading to formation of novel fibrillar tau conformation, which was shown to be maintained over multiple rounds of seeding.

Before fibril formation, tau misfolds into dimers followed by formation of PHFs, which are established as a critical intermediates in aggregate formation, and low molecular weight oligomers in a so called nucleation process (Friedhoff et al., 1998; Wu et al., 2013). These extracellular low molecular weight oligomers and short fibrils, but not long fibrils or monomers, are uptaken by neurons (Wu et al., 2013). More specifically, internalization was mediated by bulk-endocytosis and it was shown to be dependent on the size and conformation of tau aggregates. Previously, results supporting endocytosis-mediated tau internalization have been presented (Guo & Lee 2011). Transduction of filaments generated from either full-length human tau or truncated tau (containing only MBRD) into cells transfected with 2N/4R tau, induced fibrillization and formation of

tangle-like aggregates. Interestingly, when cells were incubated in 4°C to block endocytosis, the amount of cells containing aggregates was clearly reduced whereas upon incubation in normal culture temperature (37°C) aggregate-containing cells were increased in number.

Macropinocytosis, which is a subtype of bulk endocytosis specialized in internalization of fluids and macromolecular structures, has been implicated to be involved in cellular uptake of tau fibrils (Holmes et al., 2013). It was demonstrated that extracellular tau fibrils promoted the dynamic rearrangement of the plasma membrane inducing the formation of endocytic vesicles that were consistent to macropinosomes and further stimulated macropinocytosis in a dose-dependent manner. Heparan sulfate proteoglycans (HSPGs) on the cell surface participate in a variety of signaling and endocytic functions in cells (Bishop et al., 2007). Similar to infectious prion proteins, which have been shown to bind HSPGs on the cell surface plasma membrane for propagation of pathological strains (Horonchik et al., 2005; Schonberger et al., 2003), binding of tau aggregates to HSPGs was shown to be required for macropinocytosis-mediated cellular uptake and subsequent seeding of intracellular aggregates (Holmes et al., 2013). HSPG-mediated uptake of tau fibrils was reported to significantly decrease in the presence of HSPG inhibitors such as soluble heparin (to compete for the binding) in vitro and in vivo (synthetic heparin mimic F6). However, it currently remains unclear how tau fibrils or aggregates escape from macropinosomes to the cytosol to interact and transduce endogenous tau to pathological form.

Internalization of PHFs purified from AD-brains have been reported to be mediated by an endocytic process and result in formation of aggresome-like bodies in vitro (Santa-Maria et al., 2012). Aggresomes are perinuclear MT-dependent inclusions containing misfolded proteins that have an important function in disposing misfolded proteins upon overload of various degradative systems such as ubiquitin-proteosome pathway (Kopito 2000; Johnston et al., 1998). Indeed, perinuclear aggregates that form following the uptake of AD-brain derived PHFs were shown to have aggresome-like resemblence (Santa-Maria et al., 2012). In another study, presynaptically released tau dimers were shown to be increased in synaptosomes isolated from cryopreserved human *post mortem* AD-brains as compared to healthy controls (Sokolow et al., 2015). Moreover, it was shown that quarter or less of tau localized in the synaptosomes contained the C-terminus, which consistent with previous results reporting the C-terminally truncated tau being the major tau species released from cells. However, recent study reported that tau trimer, but not dimer, is the minimum unit capable of propagating and induce subsequent aggregate seeding in recipient cells (Mirbaha et al., 2015). Additionally, it was shown that both synthetic fibrils of various sizes and aggregates isolated from AD-brains with a minimum unit of tau trimer were internalized in an endocytic process mediated by HSPGs.

Overall, similar to tau secretion, very little is currently known about the exact mechanisms or their resiprocal relations of coincidental in different conditions according to cellular uptake of tau. Furthermore, the molecular structure or identity of tau species that are predominantly transferring between cells remains unclear. Hence, the identification of cellular mechanisms of tau release and uptake and the ratios of vesicle-free and microvesicle associated tau secretion and internalization under normal and pathophysiological conditions is critical to understand the cell-to-cell propagation of tau in tauopathies (figure 2.9). Additional mechanism on how tau may transfer from one cell to

another could also be discovered beside the pathways that have been already more or less established. For example, tunneling nanotubes that connect adjacent cells have been proposed to be involved in the cell-to-cell tau propagation although very little is known about their functions in disease pathogenesis (Guo & Lee 2014; Clavaguera et al., 2015).
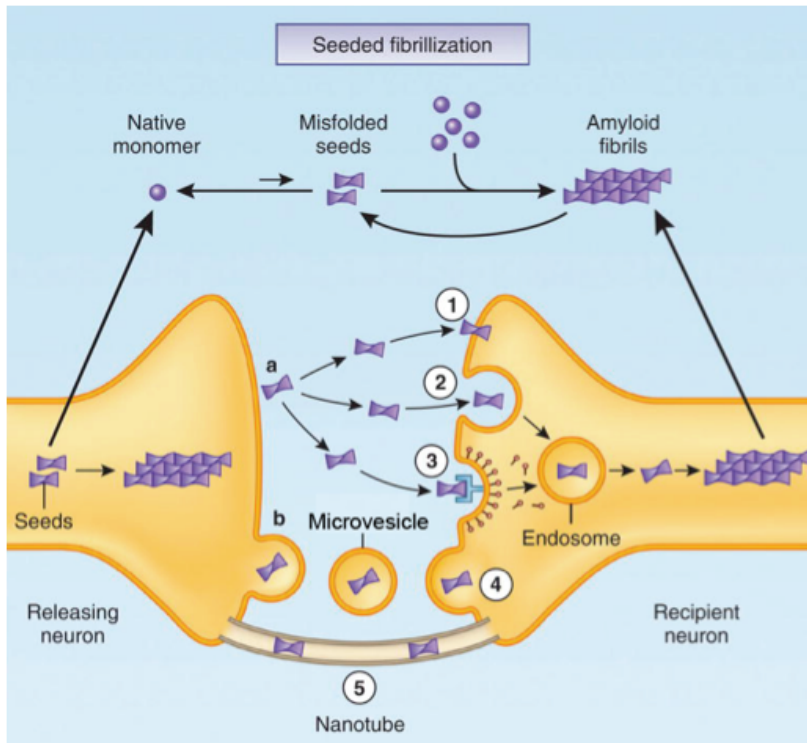

Figure 2.9. Putative mechanisms of cell-to-cell transmission of cytosolic tau aggregates. Seeding of misfolded tau protein first occurs in the cytoplasm of a releasing or 'donor' cell. In seeding fibrillization, soluble tau monomers are recruited or sequestered into growing intracellular aggregates and subsequent fragmentation or secondary nucleation may initiate indefinite prion-like replication of amyloid fibrils. Tau can be released from the donor cell into extracellular space either in an unbound vesicle-free form (a) or via membrane-bound microvesicles such as exosomes or other microvesicles (b). Unbound extracellular tau aggregates may either directly translocate across the plasma membrane of a recipient cell via unknown mechanism (1), bind to surface of the plasma membrane e.g. via heparan sulfate proteoglycans, which induce macropinocytosis (a fluid-phase endocytic process) (2) or enter into recipient cell via receptor-mediated endocytosis (3). Extracellular aggregate-containing microvesicles may fuse with the plasma membrane of a recipient cell (4). However, the mechanism(s) of tau aggregates escaping from the lumen of endosomes/macropinosomes is currently unknown. Additionally, tau seeds may be transferred from one cell to another via nanotubes that directly connect the cytoplasm of two adjacent cells (5). Modified from Guo & Lee 2014.

3   Aims of the study

The general aim of this study was to develop a novel live-cell platform method to investigate cellular protein interactions of tau and to further study the effects of genetic and pharmacological modulation of its protein-protein interactions, phosphorylation and cellular secretion and uptake.

The specific aims of the study were to:

1. Set up and validate a method to study protein-protein interactions of tau in live cells and use this assay to perform proof-of-concept screening with a focused library of chemical compounds.

2. Explore the functional association of late-onset Alzheimer's disease susceptibility genes with known AD-related pathways by investigating the effect of RNAi-based gene silencing on protein-protein interactions, phosphorylation of tau and amyloidogenic interactions and proteolytic processing of APP.

3. Study the impact of late-onset Alzheimer's disease susceptibility gene silencing on intracellular dimerization/oligomerization, cellular secretion and uptake of tau in live cells.

4   Materials and methods

More detailed descriptions of used methodology are in the materials and methods/experimental procedures -sections of adjoining original publications (I-III).

4.1   Cell culture and transfections (I, II, III)

Cells were cultured in full Dulbecco's Modified Eagle Medium (DMEM with 10% (v/v) FBS (Gibco, Invitrogen), 1% (v/v) L-Glutamine-Penicillin-Streptomycin solution (Lonza) (Neuro2A, HEK293T and CHME-5) or in neurobasal medium containing 2% B27 supplement, 1% penicillin/streptomycin, and 1% glutamine (primary neurons) at 37°C, 5% CO_2 and water saturated air. Transfection of plasmid DNA and siRNA-DNA cotransfection were done using JetPei and Jetprime reagents (Polyplus) according to manufacturer's instructions, respectively. The effect of gene silencing was assessed using real time quantative polymerase chain reaction (qPCR, comparative Ct method) to measure the relative level of mRNA (table 4.1).

4.2   Protein-fragment complementation assay (I, II, III)

Protein-fragment complementation assay (PCA) is a method to study protein-protein interactions in live cells in their native environment. PCA is based on a reconstitution of enzymatic activity of reporter protein fragments (Michnick 2001). In PCA, two complementary fragments of reporter protein are fused with proteins of interest. When the proteins of interest interact, the reporter fragments fold into their active native conformation thereby restoring the enzymatic activity, which can be measured as a bioluminescence in the presence of substrate (Michnick et al., 2007). In this study, the reporter protein used was split humanized *Gaussia princeps* luciferase (GLuc, 19.9 kDa, 185 aa) and native coelenterazine (nCol, NanoLight Technology) was used as a substrate.

Briefly, cells were transiently transfected with proteins of interests fused with reporter protein fragments (GLuc1 and GLuc2) 24 h postplating on 96-well plates (table 4.2) or cotransfected with selected LOAD susceptibility gene siRNAs (table 4.3). Next, cells were washed with PBS prior to starting possible treatments and media was changed to phenol red-free DMEM (Gibco, Invitrogen) without serum (PRF-DMEM). In case of chemical treatment of cells, the chemicals were diluted in phenol red-free DMEM. In all experiments, comparisons were done to control conditions, e.g. mock transfection, vehicle treatment and positive and negative controls. Bioluminescence was measured 48 h posttransfection in the presence of nCol (final concentration of 20 µM) regardless of performed treatments/treatment time by using either Victor[3] 1420 Multilabel counter (PerkinElmer) or Varioskan Flash multiplate reader (Thermo Scientific).

Table 4.1. Primers used for qPCR.

Gene	Forward / Reverse	Sequence 5' → 3'
BIN1	Forward	AGACCTTCCCAGCAACTGTG
	Reverse	GATCACCAGCACCACATCAC
APOE	Forward	GGTCGCTTTTGGGATTACCT
	Reverse	TTCCTCCAGTTCCGATTTGT
CLU	Forward	CCAGTGGAAGATGCTCAACA
	Reverse	AGCTTCACGACCACCTCAGT
ABCA7	Forward	AGCCCGCAACATCTCAAG
	Reverse	CTCACGGAAAAGTCCTCCAC
CR1	Forward	GCCAGGCCTACCAACCTAAC
	Reverse	GCCATTCACAGGATCTGGAG
PICALM	Forward	ACCCCCTGTAATGGCCTATC
	Reverse	CTCCTGATACAGGGCCAAAG
MS4A6A	Forward	GCTCTCTATCAATCGCCACAG
	Reverse	ACTGCAGTGAGGCAGGATTT
CD33	Forward	GACCAGAGCAGGAGTGGTTC
	Reverse	GTGGTAGGGTGGGTGTCATT
MS4A4E	Forward	AACCATGCAAGGAATGGAAC
	Reverse	TTCCCATGCTAAGGCTCATC
CD2AP	Forward	GGTGGCTGGAAGGAGAACTA
	Reverse	GTGGATGTGGCTGAATTCCT
FRMD4A	Forward	ATCAAGCCCAAAATGTGGAG
	Reverse	CTGCAAGGAGTTGCTTCCTC
TREM2	Forward	TGGCACTCTCACCATTACGC
	Reverse	GAGGCTCCTGGAGATGCTGTG

Table 4.2. GLuc reporter proteins used in the study.

Name	Insert / Gene	Accession number	Source of cDNA	Studies used
tau-GLuc1	Human MAPT (0N4R)	BC114948	OpenBioSystems	I, II, III
tau-GLuc2	Human MAPT (0N4R)	BC114948	OpenBioSystems	I, II, III
Pin1-GLuc1	Human PIN1	NM_006221	OpenBioSystems	I, II, III
PP2A-GLuc1	Human PPP2R2A (Bα)	BC041071	OpenBioSystems	I
GSK-3β-GLuc1	Human GSK3B	BC000251	OpenBioSystems	II
BACE1-GLuc1	Human BACE1	NM_012104.4	Dr. Oksana Berezovska, Harvard Medical School	II
APP-GLuc2	Human APP (695)	NM_201414.2	Dr. Dora Kovacs, Harvard Medical School	II

Table 4.3 List of used human siRNAs (Silencer Select, Ambion, Life Technologies) for LOAD risk gene knockdown.

Gene	siRNA ID #	Sense sequence (5' > 3')
BIN1	s1340	AGGUUUCAUGUUCAAGGUAtt
BIN1	s1341	GGAGGUGUAUGAGCCCGAUtt
BIN1	s1342	AGAUCGCAGAGAACAACGAtt
APOE	s1496	CUAGUUUAAUAAAGAUUCAtt
APOE	s1495	GGAGUUGAAGGCCUACAAAtt
APOE	s194291	GACAAUCACUGAACGCCGAtt
CLU	s3156	GGAAGUAAGUACGUCAAUAtt
CLU	s3157	AGAUAAAGACUCUCAUAGAtt
CLU	s3158	GCUGAGAGGUUGACCAGGAtt
ABCA7	s20241	CCUUUUACCCGGAAGCUCAtt
ABCA7	s20239	GAUCUGGUUCAACAACAAAtt
ABCA7	s20240	CUACAGCGGAGGGAACAAAtt
CR1	s3471	GUCCUACGAUCCCAAUUAAtt
CR1	s3472	GGACAUCUUUAAAGUACGAtt
CR1	s3473	CGAUGAAGGGUUCCGAUUAtt
PICALM	s15799	GGAUAUGACAUGUCUACAUtt
PICALM	s15800	GGCAAGCACUGGUCUAUCUtt
PICALM	s15801	CCACCUAGCAAGUUAGUAUtt
MS4A6A	s224607	GCAUGAUGGUAUUGAGCUUtt
MS4A6A	s34589	CUCUCCAAAUUUUACCCAAtt
MS4A6A	s34587	CUGCUUCCUUCUCUCCAAAtt
CD33	s2637	CAACUUUCAUGGGAUGAAUtt
CD33	s2638	CCCAACAACUGGUAUCUUUtt
CD33	s2636	GCACCAACCUGACCUGUCAtt
MS4A4E	s55398	UGAUGUAUCCUUAUCAGUUtt
MS4A4E	s55399	AUUUAGUUCUUAUGAAGAAtt
MS4A4E	s55397	GACAUAAGUUUUCAACUCAtt
CD2AP	s24192	CUAUGAAGGUACUAAUGAAtt
CD2AP	s24193	CUGGAACAGUGUACCCAAAtt
CD2AP	s24191	CGAACUUAAUGGUAAAGAAtt
FRMD4A	s31260	GGGCUUCGCUGAUCAUAGAtt
FRMD4A	s31261	GGGAGAUUUUUCUAGCAAUtt
FRMD4A	s31262	GGAGUACUUUGGAAUAGCAtt
TREM2	s28905	GAGGUGGAAUGGGAGCACAtt
TREM2	s28906	GGAGCCUCUUGGAAGGAGAtt
TREM2	s28907	CCAUUACGCUGCGGAAUCUtt
Negative ctrl #1		
Negative ctrl #2		

4.2.1 High throughput screening (I)

High-throughput screening (HTS) was carried out in cooperation with Dr. Päivi Tammela from Centre of Drug Research (CDR, University of Helsinki, Helsinki, Finland) using CDR's libraries of pharmaceutical compounds (PC) and natural compounds (NC), which were pre-diluted in dimethyl sulfoxide (DMSO) on 96-well master plates (10 mM and 20 mM, respectively). The interaction pair selected and used in the HTS was tau-Pin1 and the screen was performed using Neuro2A cells.

The sample plates were prepared manually from master plates by diluting the pre-diluted stock solutions to desired concentrations in PRF-DMEM. At the primary screening round the concentration used for compounds was 50 μM and controls (untransfected, 5 μM Juglone and 25 mM KCl), 4 replicate wells each. The first screening round included the whole PC/NC library containing altogether 355 compounds (240 and 115, respectively). On the secondary screening round, 5 different concentrations (50 μM, 25 μM, 12,5 μM, 6,25 μM and 3,125 μM, 4 replicate wells each) were used for selected compounds (15 PCs, freshly dissolved) to verify results from the first round. Based on the observations from the second round, the third screening round was performed for further selected compounds.

The plating of cells (10 000 per well) on 96-well plates and transfections for HTS were performed as described. 46 h posttransfection the assay plates were washed once with warm PBS followed by addition of compounds from pre-warmed sample plates to assay plates. The measurements were performed using Varioskan Flash 48 h posttransfection. The luminescence was measured well-by-well immediately after the injection of the substrate (final concentration of 20 μM).

4.2.2 Tau secretion assay (III)

Coating, plating, transfection of cells and PCA measurement were done as described above. In addition to previous protocol, 16 h before measurement, cells were washed once with PBS and changed to PRF-DMEM (140 μl per well). 30 minutes before measurement, plate was span at 200-300 × g for 3-5 minutes using a swing bucket rotor and conditioned PRF-DMEM was recollected. 75 μl of this conditioned media was used for measuring PCA signal (secreted tau dimers), and 50 μl was used for lactate dehydrogenase (LDH) release measurement (Promega CytoTox 96® Non-Radioactive Cytotoxicity Assay, G1781) according to manufacturer's instructions. After collection of conditioned media, the cells were changed to 75 μl of fresh PRF-DMEM and PCA signal was detected as described for a standard PCA (intracellular tau dimers).

For RNAi screen of tau secretion, tau-GLuc1, tau-GLuc2 and LOAD risk gene siRNAs (at 5 nM) were cotransfected to HEK293T cells as described in the PCA section and further followed the tau secretion assay protocol as described above.

4.2.3 Assessment of tau uptake (III)

4.2.3.1 Production of conditioned media

HEK293T cells were plated into poly-L-lysine-coated 100-mm plates at density of 2.0-2.5 million cells per plate and transiently transfected with tau-GLuc1 and tau-GLuc2 plasmids 24 h post-plating. 24 h after transfection, cells were washed once with PBS and media replaces to PRF-DMEM. Media was conditioned for 24 h, collected and cleared by centrifugation at 3,000 × g for 30 min for removal of cell debris. The level of Tau-GLuc1/2 dimers in the media was determined by PCA. Additional tau-conditioned media characterization was performed using total human Tau ELISA kit (#KHB0041, Novex/Life technologies).

4.2.3.2 Tau uptake assay

Naïve HEK293T cells on 96-well plates were washed once with PBS and changed to conditioned media containing Tau-Gluc1/2. After 4 h incubation, media was completely removed by gently pipetting followed by a wash with PBS. Cells were then incubated with 20 µg/ml heparin (Sigma) diluted in PRF-DMEM for 5 min to remove cell surface-associated Tau. Heparin solution was removed, cells were washed with PBS and 75 µl PRF-DMEM was added to the wells and cellular PCA signal was measured as described above.

For RNAi screen of tau uptake, LOAD risk gene siRNAs were transfected to HEK293T cells using JetPrime, and after 24 h cells were changed to fully supplemented DMEM. Next, 44 h post-plating the media was replaced with tau-GLuc1/2 conditioned media and incubated for 4 h. Cells were washed and processed for PCA detection as described above.

4.3   Western blotting (I, II, III)

Cells were washed twice with ice-cold PBS 48 h posttransfection, and extracted with buffer (10 mM Tris-HCl, 1 mM EDTA, pH 6.8, 150 mM NaCl, 1% Triton X-100, 0.25% Nonidet P-40, Protease and Phosphatase Inhibitor cocktail tablets (Roche Molecular Biochemicals), 1 µM NaF) and incubated on ice for additional 30 minutes. Cell debris was removed by centrifuging total lysates at 16,000 × g, and protein concentration was determined by BCA protein assay kit (Thermo). Equal amounts of cell lysates were loaded per lane on 4-12% gradient Bis-Tris gels (Novex, Invitrogen) and resolved under reducing conditions. Proteins were transferred to PVDF membranes (GE Healthcare) by using semidry blotting (Bio-Rad). Various antibodies were used for detection of different proteins and posttranslational modifications (table 4.4).

Table 4.4. List of antibodies used for Western blotting analyses.

Antibody	Detected epitope	Manufacturer	Catalog #
Primary antibodies			
Tau-5	Total	Thermo scientific	MA5-12808
AT8	pSer202/pThe205	Thermo scientific	MN1020
PHF13	pSer396	Cell signaling	9632
PHF-6	pThr231	Millipore	MAB5450
AT100	pThr212/pSer214	Thermo scientific	MN1060
TG3	pThr231/Ser235	gift from Dr. Jing-Jing Pei*	
GSK-3β	Total	Cell signaling	9315
phospho-GSK3β	pSer9	Cell signaling	9336
Pin1	Total	Santa Cruz	Sc-46660
GAPDH	Total	Millipore	MAB347
Secondary antibodies			
HRP-anti-mouse		GE Healthcare	NA931V
HRP-anti-rabbit		GE Healthcare	NA934V

4.4 Phosphatase activity assay (I)

The phosphatase activity assay was performed according to manufacturer's protocol, provided in the Serine/Threonine Phosphatase Assay System (V2460, Promega). Briefly, cell lysates were prepared from mature (21 days in vitro) rat cortical neurons (2 × 6-well plates, 400 000 cells/well). After the cells were washed once with ice-cold phosphatase storage buffer (PhSB, 50 mM Tris-HCl pH 7.4, 0.25 M sucrose, 0.1 M EDTA) cells were lysed in 75 µl of ice-cold full PhSB (PhSB including 0.1% β-mercaptoethanol, protease inhibitor cocktail) and collected. Cells from 6-wells were pooled into one sample tube, incubated on ice for 20 min and were pulled 10 times through a needle. Pooled samples were centrifuged for 1 h at 100 000 × g at +4°C (Beckman TL-100 ultracentrifuge, TLA-55 rotor) and supernatants were then transferred into fresh tubes on ice. Following the stabilization of spin columns, the cell lysates were transferred into the spin columns, centrifuged for 5 min at 600 × g at +4°C to remove endogenous free phosphate and pooled the lysates resulting one lysate sample. Protein concentration of the cell lysate was further determined.

The PP2A assay was performed according to manufacturer's protocol (Phosphatase Assay protocol, Promega). The 5 × PPase buffer used was 5 × PP2A buffer (250 mM imidazole, 1 mM EGTA, 0.1% β-mercaptoethanol, 0.5 mg/ml bovine serum albumin). All the reactions and proper controls were done in duplicates/triplicates and were incubated at 37°C for 30 min before stopping the reactions by adding the Molybdate dye/additive mixture. After 15 min of incubation in room temperature the PP2A, activity was determined by measuring the absorbance (620 nm) of released phosphate-molybdate-malachite green complex.

4.5 Statistical analysis

A minimum of three independent repetitions were used for each experiment and four independent wells was considered as one datapoint in PCA (I, II, III). GraphPad Prism and Microsoft Excel software were used for statistical analyses and generation of graphs. Statistical significance was evaluated with two-tailed Student's t-test and two-way ANOVA, where appropriate, with the significance threshold set at $p < 0.05$ (*), and ** = $p < 0.01$ and *** = $p < 0.001$ (I, III). Detailed statistical methods used in study II are in the Methods/statistical analyses -section of original publication II.

5 Results

5.1 Assay development and HTS to identify novel modulators of tau phosphorylation (I)

5.1.1 Assay development and high-throughput screening

Tau phosphorylation is a highly important regulator of its normal functions. In AD and related tauopathies, tau phosphorylation is increased resulting in a decrease in its ability to bind and stabilize MTs. In order to study protein-protein interactions of tau in live cells we developed a protein-fragment complementation assay (PCA) based on split humanized *Gaussia princeps* luciferase (GLuc) (Remy & Michnick 2006). PCA-based assay allows detection of direct interaction of proteins of interest fused with complementary reporter fragments. Importantly, PCA allows studying protein-protein interactions in their native cellular environment without need to lyse or extract cells for analysis.

Peptidyl-prolyl isomerase Pin1, which recognizes and catalyzes the *cis/trans* isomerization of phosphorylated serine/threonine-proline sequences, facilitates the PP2A-mediated dephosphorylation of tau. Hence, as a critical regulator of tau dephosphorylation, Pin1 was selected as an interaction partner for development and validation of luminescence-based PCA to investigate dynamic protein-protein interaction of tau that may serve as a readout of tau phosphostatus in live cells. In addition to Pin1, multiple constructs were generated and tested during the set up and validation of PCA platform for tau protein-protein interactions (table 5.1). The identity, expression and detection of specific interaction of generated tau and Pin1 constructs were assessed (I / figures 1A, 1C and 2). Furthermore, localization of tau-GLuc2 construct to neurites and cytoskeletal structures suggested that the hGLuc fusion tag did not alter subcellular localization and functions of tau (I / figure 1C). Subsequently, the validated Pin1-tau PCA was optimized for various parameters in order to perform a screen of a focused library of pharmaceutical and natural compounds that may modulate Pin1-tau interaction.

The primary screen of Pin1-tau interaction pair resulted in 25 initial hits as detected either in increase (21 hits) or in decrease (4 hits) of protein-protein interaction signal (I / table 1 and supplemental table 1). From these hit compounds 14 were further selected based on their biological relevance for secondary screening and were tested in five different concentrations (I / supplemental table 2). Interestingly, sedative-hypnotic compounds, including barbiturates and benzodiazepines, were strongly presented among the hits of the secondary screening round. The hit compounds butethal and desalkylflurazepam, which both are known $GABA_A$ receptor activators, from secondary round were further selected and confirmed (I / figure 3A). Since $GABA_A$ receptor activity was not directly linked to tau phosphorylation based on literature, we used muscimol (a selective $GABA_A$ receptor agonist) and picrotoxin (a noncompetitive $GABA_A$ receptor antagonist and channel blocker) to confirm that the sedative-induced alteration of Pin1-tau interaction is most likely $GABA_A$ receptor-mediated (I / figure 3B).

Table 5.1. Assay validation criteria used in this study.

Test	Acceptance criteria	Notes and corrective actions
Intra-plate tests	$Z' \geq 0.2$, CV% ≤ 20%	For a transient transfection-based assay Z' ≥ 0.2 was considered acceptable (≥ 0.5 would be optimal), four replicate wells used per data point, comparisons made to reference samples per each plate
Inter-day tests	Normalized average mid-signal should not translate into a fold shift within > 2 experiments	Observed variations (day-to-day) caused by cell number and transfection efficiency differences, comparisons made to reference samples per each plate
Material plate tests	Edge or drift effects not accepted	Due to observed minor edge effects, empty wells left on all edges of the 96-well plate
DMSO compatibility	> 0.1% (v/v)	0.1-1.0% (v/v) tolerated well; maximum of 1.0% (v/v) DMSO to be used

5.1.2 GABA$_A$ receptor modulation promotes tau phosphorylation in neurons

Given that in the brain of Pin1 deficient mice tau phosphorylation is significantly increased (Liou et al., 2003), we tested whether our screen-identified sedatives affect the phosphorylation of tau in mature (21 days in vitro) primary cortical rat neurons. After 6 h treatment with either butethal or desalkylflurazepam, there was a dose-dependent increase in tau phosphorylation at the AT8, a phosphoepitope with pathophysiological importance (I / figures 4A and B). Furthermore, to assess the stability of observed increase in tau phosphorylation, which is naturally transient, induced by GABA$_A$ receptor activators, a washout experiment was performed. Primary rat cortical neurons were incubated for 24 h with either butethal or desalkylflurazepam, which was followed by a wash-out period of 24 h, showed that tau phosphorylation remained increased at an elevated level after 24 h washout period as compared to vehicle treated control (I / figure 4C). These data suggest that pharmacological GABA$_A$ receptor activation enhances tau phosphorylation in a way that is rather persistent in vitro at least for 24 h after washout of sedatives.

5.1.3 Pharmacological modulators of GABA$_A$ receptor reduce tau-PP2A interaction

Increased tau phosphorylation is typically a result from increased protein kinase activity and/or decreased protein phosphatase activity. Moreover, barbiturates have been shown to directly inhibit phosphatases such as PP2B/calcineurin (Humar et al., 2004) and there are reports indicating an association between anesthesia and increased tau hyperphosphorylation mediated by decreased PP2A activity (Planel et al., 2007). Since PP2A is known to be the phosphatase that predominantly dephosphorylate tau, we tested whether butethal and desalkylflurazepam directly alter the activity of PP2A in a cell-free PP2A assay in vitro. No effect was detected with either sedative whereas significant calyculin A (PP2A inhibitor) -mediated decrease in PP2A activity was observed (I / figure 5A).

To investigate the effect of sedatives on direct protein-protein interaction of tau-PP2A, GLuc-PP2A construct was generated using the regulatory Bα subunit of PP2A. Pharmacological validation of this interaction by using Juglone (Pin1 inhibitor) resulted in dose-dependent decrease in tau-PP2A interaction (I / figure 5B), which was expected as reduced Pin1 activity was previously reported to decrease tau-PP2A interaction in cells (Galas et al., 2006; Landrieu et al., 2011; Zhou et al., 2000). Butethal and desalkylflurazepam treatment of cells expressing tau and PP2A PCA reporters significantly decreased (approximately 50%) tau-PP2A interaction (I / figure 5C). Hence, despite the unaltered activity of PP2A in cell-free system by these sedatives, tau-PP2A protein-protein interaction is reduced upon butethal and desalkylflurazepam treatments suggesting that increased tau phosphorylation is a resulted by decreased tau-PP2A interaction.

5.1.4 GABA$_A$ receptor activity-induced tau phosphorylation is mediated by CDK5

Multiple kinases contribute to tau phosphorylation by either directly phosphorylating tau or acting as a priming kinase. Of tau kinases, GSK-3β and CDK5 are considered as the main kinases that phosphorylate tau at multiple disease-associated proline-directed serine/threonine sites. To test whether GABA$_A$ receptor activity-induced aberrant tau phosphorylation was associated with GSK-3β or CDK5, we used kinase inhibitors SB216763 and roscovitine, respectively, together with desalkylflurazepam on primary rat cortical neurons. SB216763 had no effect while roscovitine effectively decreased desalkylflurazepam-induced abnormal tau phosphorylation at AT8 epitope (I / figure 6A). This indicates a role for CDK5 but not GSK-3β activity in GABA$_A$ receptor activity-mediated tau phosphorylation.

In order to confirm the contribution of CDK5 in GABA$_A$ receptor activity-induced tau phosphorylation, we modulated CDK5 activity by overexpressing p35, which is a regulatory subunit of CDK5 kinase strongly expressed in neurons (Tsai et al., 1994; Spillantini et al., 1998b). Expectedly, functional validation performed by simultaneous expression of tau and Pin1 PCA reporters and p35 resulted in increased Pin1-tau interaction, which was significantly inhibited upon treatment with roscovitine (I / figure 6B). Interestingly, when p35 was overexpressed with Pin1 and tau PCA reporters, treatment of desalkylflurazepam increased Pin1-tau interaction approximately 3-fold compared to mock/Pin1-tau PCA reporter-transfected cells (I / figure 6C).

Taken together, these data suggest that GLuc-based PCA is a sensitive and dynamic assay to measure protein-protein interactions of tau in live cells and to identify novel modulators of tau phosphorylation by using screening methodology. Moreover, our chemical biology approach identified a novel connection between GABA$_A$ receptor activity and tau phosphorylation, likely mediated by CDK5 and PP2A.

5.2 Expression profiles and functional association of LOAD susceptibility genes (II)

5.2.1 Characterization of sample cohort

Since the advent of genome-wide association studies (GWAS) in late-onset Alzheimer's disease (LOAD) several novel susceptibility genes with rather small effect size have been identified. Although many of the identified genes pinpoints them into different subgroups regarding to their suggested functions on a more general level, such as endocytic trafficking, cholesterol metabolism and inflammatory pathway, the exact mechanism of these genes to underlying disease pathogenesis and progression remain largely unknown. Accordingly, to assess possible pathophysiological roles of LOAD-associated susceptibility genes, two distinct approaches were utilized: analysis of the expression and splicing status of these genes in *post mortem* AD brain, and, a functional study of the effect of these genes in previously established AD pathogenesis-related pathways.

Thorough characterization of *post mortem* brain sample set of 60 individuals that were previously assessed at the memory clinic was performed. 41 individuals of this cohort were diagnosed as probable AD. The brain sample set was classified according to Braak staging (I-VI), which is based on the anatomical distribution of neurofibrillary pathology in AD brain (chapter 2.5.1.1) (Braak & Braak 1991), and further subdivided into 3 distinct groups: Braak 0-II, Braak III-IV and Braak V-VI (II / table 1). Results from the biochemical assessment of the extracts of the brain sample set of inferior temporal cortex showed significantly increased level of soluble Aβ42 in the Braak V-VI group as compared to Braak 0-II (II / figure 1A), and increased β-secretase activity in both Braak III-IV and V-VI groups as compared to Braak 0-II group (II / figure 1B), which also had strong correlation with γ-secretase activity (II / figure 1C). Subsequently, CSF measurements of Aβ42, total tau and phopsho tau (p-tau) were performed. CSF levels of Aβ42 were significantly decreased in Braak groups III-IV and V-VI as compared to Braak 0-II (Aβ42 CSF samples available form 22 individuals) (II / figure 1D) and both total- and p-tau CSF levels were both increased in Braak V-VI group as compared to Braak 0-II (tau CSF samples available form 21 individuals) (II / figure 1E and F). These biochemical assessments, additional characterization of the sample set (II / table 2 and 3) and Braak staging confirmed this as an AD cohort that is applicable for further examination. Notably, no change in *MAPT* expression and splicing status were observed in relation to increasing neurofibrillary pathology in this cohort.

5.2.2 Expression of LOAD susceptibility genes in relation to Braak staging in AD

Before assessing the expression level of LOAD susceptibility genes *APOE*, *BIN1*, *CLU*, *ABCA7*, *CR1*, *PICALM*, *MS4A6A*, *CD33*, *MS4A4E*, *CD2AP*, *FRMD4A* and *TREM2*, the single-nucleotide polymorphisms (SNPs) associated with AD risk of our sample cohort were genotyped (II / table 2). Expression analysis revealed a linear increase in *MS4A6A* expression and decrease in *FRMD4A* expression in relation to increasing AD-related neurofibrillary pathology (both normalized to Braak 0-II) (II / figure 2A and B). Additionally, normalization of the expression of both *MS4A6A* and *FRMD4A* to the expression of neuronal, astrocytic and microglial genes was performed. There was a significant reduction in *FRMD4A* expression when normalized to astrocytic marker for

glial fibrillary acidic protein gene *GFAP* expression and positive correlation between *MS4A6A* expression normalized to microglial marker for allograft inflammatory factor 1 gene *AIF-1* (or *Iba-1*) expression. This positive correlation may implicate a role for *MS4A6A* in microglial function. The overall expression of neuronal marker genes was decreased and astrocytic marker gene expression was increased in our neuropathological cohort related to increasing neurofibrillary pathology (II / supplemental figure 3). Furthermore, exon level linear analysis resulted in significant increase in expression of exon 3 and exon 4 of both *CLU* and *TREM2* genes according to increased severity of Braak staging (II / figure 2C and D).

5.2.3 Expression of LOAD susceptibility genes in normal pressure hydrocephalus

In addition to our neuropathologically confirmed AD cohort, biopsies were obtained from 22 individuals with shunt-responding idiopathic normal pressure hydrocephalus (NPH) during life. The right frontal cortical samples from individuals with NPH were obtained during cranial shunt implantation followed by histological analyzes.

In some NPH cases pathogenesis may share similar features as AD and some individuals with NPH are prone to develop AD during life (Leinonen et al., 2012). Since Aβ-pathology is present in 22%-42% of NPH cases samples were divided into Aβ-negative and Aβ-positive subgroups (II / supplemental table 1). The expression and splicing status of LOAD susceptibility genes were examined in NPH cohort to assess whether these genes are associated in NPH pathogenesis. Both the expression and splicing status of studied genes were not significantly altered neither in Aβ-negative nor Aβ-positive subgroups of NPH patients, suggesting that investigated LOAD-related genes are not associated with NPH pathogenesis but are specifically associated with development of LOAD pathology.

5.2.4 Functional association of FRMD4A with APP and tau pathways

Potential functional association of LOAD susceptibility genes with established major cellular pathways involved in AD pathogenesis were assessed utilizing PCA methodology combined with siRNA-mediated gene silencing. Knockdown efficiency of 3 separate siRNAs per gene was first performed (II / figure 3A). Due to the low level of expression of both *MS4A4E* and *MS4A6A* in HEK293T cells, these genes were excluded from subsequent experiments. Three distinct protein-protein interaction pairs were used in PCA-gene knockdown experiments: BACE1-GLuc1 / APP-GLuc2, Pin1-GLuc1 / tau-GLuc2 and GSK-3β-GLuc1 / tau-GLuc2. One by one silencing of LOAD genes upon studying BACE1-APP interaction revealed that knockdown of *CLU*, *ABCA7*, *CD2AP* and *FRMD4A* significantly increased this protein-protein interaction (II /figure 3B and 4A). Moreover, when *ABCA7* and *FRMD4A* were silenced a corresponding increase in Aβ$_{40}$ secretion was observed (II / figure 4B). By monitoring the changes in intracellular Pin1-tau interaction, siRNA knockdown of *ABCA7* and *FRMD4A* resulted in enhanced interaction (II / figure 3C and 4C). Furthermore, an increase in GSK-3β-tau interaction resulted from reduced expression of *ABCA7*, *CD2AP* and *FRMD4A* genes (II / figure 3D and 4D).

To examine the possible effect of LOAD susceptibility genes on tau phosphorylation the assessment of phosphorylation status of 4 specific disease-associated phosphoepitopes (AT8, AT100, PHF-6 and PHF13) were performed using Western blot analysis. These experiments showed an altered phosphorylation status of tau at phosphoepitopes PHF-6 and PHF13 when *FRMD4A* expression was reduced (II / figure 4E, F, G and H).

Taken together, the assessment of the expression and splicing status of LOAD susceptibility genes in AD brain according to increasing severity of AD-related neurofibrillary pathology revealed increased expression of *MS4A6A* and reduction in *FRMD4A* expression. Additionally, the results from exon-level linear analysis showed increased expression of exons 3 and 4 of both *CLU* and *TREM2* genes in our neuropathologically confirmed AD cohort. The expression and splicing status of LOAD genes examined in patients with NPH did not show any significant changes suggesting that these genes are specifically associated with development of LOAD pathology. Furthermore, functional association studies using a PCA-based in vitro pathway analysis platform showed that altered expression of *FRMD4A* is involved in both amyloidogenic APP processing and tau-regulation pathways.

5.3 Impact of LOAD susceptibility genes to cell-to-cell propagation of tau (III)

5.3.1 Studying cellular tau secretion and uptake in live cells

The concept of prion-like cell-to-cell propagation of tau and other accumulating amyloidogenic proteins in neurodegenerative diseases (NDDs) has evolved during the past few years as a 'prion-paradigm', which could possibly deliver a common pathophysiological model for spreading of pathology in NDDs. Despite of emerging evidence on cell-to-cell spreading of tau pathology, the exact molecular mechanism on cellular secretion and uptake of tau remains poorly characterized.

In order to investigate cellular release and uptake of tau, we utilized our PCA-based in vitro platform system. To study dimerization of tau, which is known to be the first step in rate-limiting nucleation process before the formation of higher order oligomers and aggregates (Friedhoff et al., 1998), PCA reporter constructs tau-GLuc1 and tau-GLuc2 were generated (III / figure 1A). Expectedly, there was a dose-dependent correlation in both intracellular tau dimerization and level of secreted tau in conditioned media relative to the level of tau-Gluc1/2 expression (the amount of reporter constructs used in transfection), which was independent from lactate dehydrogenase (LDH) release (III / figure 1B and C). This observation suggests that tau is secreted effectively via physiological pathways and is not due to passive leakage or release of tau from apoptotic cells. Kinetics of tau secretion into media showed almost a linear increase in the observation period of 24 h (III / figure 1D). To study whether the increase of cellular tau secretion in relation to intracellular tau dimerization is entirely due to overexpression of tau-GLuc1/2 plasmids in HEK293T cells, Pin1-tau interaction was tested. No alteration was detected in the ratio of intracellular and secreted Pin1-tau interaction (III / figure 1E), i.e. intracellular interaction was highly similar to the signal measured from conditioned media suggesting that Pin1-tau complex is not actively secreted from cells.

Although PCA is not capable of distinguishing protein complexes larger than dimer, i.e. the minimum signal generating unit is dimer, it does not exclude the possibility that

detected signal is generated in higher order tau species. Western blot analysis of cell lysates and conditioned media (with or without 3-fold concentaration of conditioned media and chemical crosslinking) implicated that majority of secreted tau in our cellular system is in the form of low molecular weight oligomers or small fibrils, whereas tau in cell lysates is predominantly in monomeric form (III / figure 1F). To elucidate the proportion of intracellular tau compared to secreted tau, total tau ELISA was used to determine tau levels in both conditioned media and cell lysates resulting in a ratio of approximately 1:200, respectively, suggesting that less than 0.5% of tau is secreted (III / figure 2A). Furthermore, given that tau is suggested to be secreted from the cells in exosomes (Saman et al., 2012) and other microvescles such as ectosomes (Dujardin et al., 2014a), exosome biogenesis was inhibited using GW4869 showing a reduction of more than 30% in tau secretion from the cells (III / figure 1G). GW4869 is an inhibitor of neutral sphingomyelinase (nSMase) acting via ceramide generation that regulates exocytosis (Rohrbough et al., 2004). To further characterize whether tau is secreted predominantly in association with microvesicles (either inside or outside of vesicles) or as a vesicle-free form, conditioned media was incubated with either trypsin (0.005% v/v) or saponin (0,005% v/v) or both. Results from these experiments indicate that vesicle-free tau dimers accounts for 99.8%-99.9% of all secreted tau with less than 0.5% being inside vesicles (III / figure 2B) in this HEH293T cell-based assay system. Fractionation of tau-GLuc1/2 conditioned media further revealed that, in our cellular system, the vast majority of secreted tau in conditioned media is in vesicle-free form (99.7%), whereas ectosomal fraction (0.22%) and exosomal fraction (0.05%) represent only minor portions from the extracellular tau (III / figure 2C, D and E). Similar results were obtained from PCA analysis performed with fractionated and unfractioned conditioned media (III / figure 2F).

To study cellular uptake of tau in our PCA platform, conditioned media containing tau-GLuc1 and tau-GLuc2 reporter plasmids was produced by conditioning media with cells overexpressing the reporter plasmids for 24 h. Immunostaining of naïve cells after incubation with tau-conditioned media for 4 h resulted in punctate patterns of tau staining, whereas no immunoreactivity for tau was observed when recipient cells were exposed to conditioned media generated by mock-transfected cells (III / figure 3A), suggesting cellular uptake of tau reporters. Different washes of cells were tested in PCA-uptake experiments after incubation with tau-GLuc1/2 containing conditioned media (III / figure 3B). Consequently, to confirm that the measured signal is generated by internalized tau, additional washing step with heparin was included into uptake protocol to remove cell surface-bound tau, which significantly reduced the signal as compared to unwashed and PBS washed cells. To determine the most optimal time to incubate cells with conditioned media to allow the internalization of tau into cells, the rate of uptake were observed for 24 h. The rate of uptake started to slow down after 4 h incubation suggesting a saturable mechanism of tau internalization (III / figure 3C). Furthermore, when GW4896 was added to cells 16 h prior to addition of conditioned media (with or without GW4869), the detected signal from internalized tau was significantly increased from cells treated with GW4869 as compared to control. Hence, there may be rapid resecretion mechanism of internalized tau dimers, a process that can be at least partially inhibited by GW4869. Additionally, fractionated conditioned media was used to assess whether vesicle-associated and vesicle-free tau are internalized with varying efficiency by cells. Due to a relatively low secretion level of vesicle-associated tau and rather low overall uptake

activity of tau, 100-fold concentrated media-resuspended batches of exosome and ectosome fractions were used. The comparison of unconcentrated vesicle-free conditioned media and concentrated vesicle-associated fractions showed that vesicle-associated fractions are internalized relatively more actively (III / figure 3E). However, due to the majority of secreted tau being in vesicle-free form, unconcentrated and unfractioned conditioned media was used in further experiments.

5.3.2 Effect of LOAD susceptibility genes on cellular secretion and uptake of tau

To investigate whether LOAD susceptibility genes are functionally associated to either tau secretion or uptake, we performed a screen based on RNAi. The siRNAs used for knocking down the genes (*APOE*, *BIN1*, *CLU*, *ABCA7*, *CR1*, *PICALM*, *CD33*, *CD2AP*, *FRMD4A* and *TREM2*) in the screen were the same utilized in study II, in which the knockdown efficiencies were determined (II / figure 3A and III / figure 4A). Cotransfection of PCA reporter constructs tau-GLuc1/2 and siRNAs did not significantly alter the intracellular level of tau dimerization in HEK293T cells (III / figure 4B).

For studying the impact of RNAi knockdown to tau secretion, conditioned media were collected from reporter plasmid-siRNA transfected cells and tau dimer content was measured by PCA. Reduction of LDH-normalized tau dimer signal in the conditioned media was shown in cells upon knockdown of *TREM2* (55%), *CD33* (27%), *CD2AP* (23%) and *FRMD4A* (19%) (III / figure 4C). Values are average of cells transfected with two independent siRNAs per target gene. Since *TREM2* and *CD33* are predominantly expressed in myelomonocytic cells and their expression level in HEK293T cells is relatively low, the effect of gene knockdown to secretion level of tau was tested in more physiological context. In fetal human microglial CHME-5 cells, cotransfection of tau-GLuc1/2 and *TREM2*/*CD33* siRNAs did not result in changes of either intracellular tau dimerization or the signal level of secreted tau dimer in conditioned media (III / figure 4E).

For investigating internalization of tau, recipient cells were transfected with the panel of LOAD susceptibility siRNAs followed by an exposure of tau-GLuc1/2 conditioned media for 4h. Knockdown of *APOE* was the only gene to significantly affect the uptake of tau, which was observed as a 29% increase in intracellular tau dimer signal as compared to control (III / figure 4D).

5.3.3 Regulation of cellular tau secretion by FRMD4A-cytohesin signaling

Our previous results from study II showed decreased *FRMD4A* expression in the brain of LOAD patients and its functional association with tau. Since the current data implicated an involvement of *FRMD4A* in cellular tau secretion, we studied the possible mechanism of this phenomenon. Immunostaining of cells expressing FRMD4A-GFP showed cytosolic localization of FRMD4A in structures resembling vesicles (III / figure 5A, left) suggesting a functional interaction with vesicle membrane-associated proteins. Coexpression of FRMD4A-GFP and tau-GLuc2 did not show significant colocalization although they both occasionally localized in same regions close to plasma membrane in HEK293T cells (III / figure 5A, right). PCA measurements of cells coexpressing tau-GLuc1/2 reporter plasmids and FRMD4A showed a significant dose-dependent increase

in tau secretion in conditioned media relative to FRMD4A expression, whereas intracellular tau dimerization remained almost unchanged (III / figure 5B). Moreover, FRMD4A-induced increase in tau secretion seems to be at least partially dependent on ceramide signaling, since GW4869 decreased the tau secretion signal by nearly 30% (III / figure 5C). However, the effect of GW4869 on tau secretion may be more general and not necessarily specific to effect of FRMD4A-related secretion.

Although very little is known about FRMD4A function in mammalian cells, in epithelial cells it has been shown to function as a scaffolding protein in the interplay of Par3/Par6 cell polarity complex and Arf6 signaling via cytohesin-1, which is a guanine-nucleotide exchange factor (GEF) (Ikenouchi & Umeda 2010). FRMD4A overexpression-induced tau secretion was decreased and intracellular tau dimerization was increased when cells were treated with SecinH3 (III / figure 5D), a small molecule cytohesin/Sec-7 GEF activity antagonist (Hafner et al., 2006). Moreover, in cells expressing only endogenous FRMD4A intracellular tau dimerization was increased whereas level of tau secretion to media was almost unchanged (III / figure 5E). Given that FRMD4A-cytohesin signaling is known to induce translocation of active Arf6 to the plasma membrane (Ashery et al., 1999; Hafner et al., 2006; Ikenouchi & Umeda 2010), we co-overexpressed Arf6 and tau-GLuc1/2 in cells to study whether it produces a similar response as FRMD4A to level of tau secretion. Overexpression of wild-type Arf6 induced 34-fold increase in tau secretion as compared to endogenous Arf6 levels, which was almost completely suppressed by Arf6 siRNA coexpression (III / figure 5F). However, SecinH3 did not have any effect on Arf6-induced tau secretion suggesting that Arf6 overexpression robustly increases the activity of Arf6, which seems to be independent of cytohesin GEF activity.

To further elucidate the mechanism involved in tau secretion, the co-overexpression of tau-GLuc1/2 and atypical protein kinase C subtype ζ (aPKCζ) was studied using PCA approach. As a part of Par polarity signaling complex, ceramide-binding protein aPKCζ regulates multiple membrane trafficking events and is also associated with exocytosis (Wang et al., 2009; Horikoshi et al., 2009; Joberty et al., 2000). The resulted significant increase in tau secretion upon aPKCζ overexpression was comparable to FRMD4A-induced effect (III / figure 6A). No significant effect was observed when cells were overexpressed with C-terminal ceramide-binding region of aPKCζ (C20ζ) (Wang et al., 2009) instead of full aPKCζ. Interestingly, coexpression of aPKCζ and C20ζ decreased aPKCζ-induced tau secretion suggesting that ceramide-binding is important for this activity of aPKCζ. Since Par6 is known to connect aPKCζ to Par3 (Joberty et al., 2000), which further activates Arf6 via FRMD4A (Ikenouchi & Umeda 2010), we explored the impact of Par6 to tau secretion. Wild-type Par6 expression enhanced tau secretion 7-fold as compared to control, whereas expression of Par6(S345A), an inactive mutant that is not aPKCζ-phosphorylated (Gunaratne et al., 2013), had only subtle effect on tau secretion (III / figure 6C). Neither wild-type Par6 nor Par6(S345A) mutant altered intracellular tau dimerization. Finally, SecinH3 had no effect on aPKCζ and Par6 expression-induced tau secretion (III / figure 6B and D) implicating that aPKCζ/Par6 complex functions independently of cytohesin GEF activity, possibly as a downstream component in the FRMD4A/cytohesin-signaling pathway.

Taken together, according to these data, PCA-based assay is a sensitive, cost-effective and dynamic method to investigate cellular tau uptake and secretion in live cells. Furthermore, PCA method combined with siRNA-mediated knockdown of LOAD

susceptibility genes is an effective approach to study the association of these genes to known pathophysiological pathways connected to cell-to-cell propagation of tau in AD and related tauopathies. We identified a novel pathway related to the LOAD susceptibility gene *FRMD4A*, that regulates tau secretion. Specifically, aPKCζ/Par6-FRMD4A-cytohesin-Arf6 signaling pathway is connected to cellular secretion of tau.

6 Discussion

6.1 Cellular physiology and cell-to-cell propagation of tau (I, III)

Tau undergoes various posttranslational modifications (PTMs) in normal physiological conditions to maintain and regulate its functions. The impact of tightly controlled regulation of tau phosphorylation and dephosphorylation alone, which is by far the most widely studied PTM, is essential for its major cellular function in stabilizing and promoting assembly and disassembly of MTs. The central role of phosphorylation in regulation of MT interaction highlights the importance of tau PTMs in its protein-protein interactions and cellular functions. The amount of putative serine and threonine phosphorylation sites (approximately 80 Ser/Thr sites) in relation to total number of amino acids in the longest human tau isoform (441 aa) provides a vast variety in combinations and patterns of tau phosphorylation. Although all of the nearly 80 sites have not been shown to be phosphorylated in vivo, proper regulation of phosphorylation equilibrium of tau is critical even without the involvement other PTMs.

Relatively recent discoveries of novel tau PTMs, such as methylation (Thomas et al., 2012; Funk et al., 2014), as well as confirmation and possible exclusion or critical reviewing of previously suggested PTMs have been established mainly by using mass spectrometry approach (Morris et al., 2015). Nonetheless, as additional PTMs are emerging, their effect on tau function in both physiological and pathophysological context remains to be defined. Since a wide number of PTMs may affect practically all six splicing isoforms of tau, the pool of differentially modified tau species in cells is enormously diverse. Hence, despite of rigorous studies conducted over the past few decades, the complexity of physiological tau pool may at least partly explain the observed difficulties in examining the role of pathophysiological tau in disease pathogenesis of AD and other tauopathies, in which there is typically a disequilibrium in normally highly regulated PTMs.

Recently, however, a study showed that tau acetylated at lysine 174 (K174) is an early change in both human AD brain and in a mouse model of FTD (PS19) (Min et al., 2015). Furthermore, it was reported that inhibition of acyltransferase p300-induced acetylation of tau by salsalate, a prodrug of salicylate that has been used in treatment of rheumatoid arthritis, significantly reduced the level of both total and acetylated tau at K174 in PS19 mouse. Interestingly, administration of salsalate after disease onset in PS19 mice was also shown to ameliorate the memory deficits and rescue from hippocampal atrophy as compared to control-treated mice thereby providing a novel candidate for neurodegeneration therapeutics. Moreover, considering the reported substantial overlap of various competitive PTMs toward lysine residues including methylation, acetylation and ubiquitination, it is an intriguing question how these PTMs affect normal conventional tau functions or e.g. the recently suggested tau (mis)localization to dendritic postsynapse contributing to Aβ-NMDA receptor-mediated excitotoxicity (Ittner et al., 2010). Accordingly, the interactions of tau with proteins regulating its PTMs collectively determine not only the physiological or pathophysiological nature of a single tau molecule but may affect the entire pool of tau in specific cellular compartments owing to its ability to sequester additional physiological tau.

Photoprotein-based protein-fragment complementation assays (PCA) are highly useful and widely utilized for studying protein-protein interactions (Tannous et al., 2005). Diverse properties of various photoproteins such as fluorescent (e.g. green fluorescent protein, GFP) and bioluminescent proteins (various luciferases) allow detection of differential phenomena in live cells. In fluorescent proteins the refolding of split reporter protein is an irreversible process enabling the examination of more rarely occurring protein interactions or complex formations, and generally localize cellular protein interactions and relocalization of possible complexes using small molecules that may disturb their normal balance (Hu et al., 2002; Michnick et al., 2007). Conversely, bioluminescence-based PCAs are suitable for studying dynamics and kinetics of protein-protein interactions due to the mostly reversible nature of reporter protein refolding preventing the trapping of proteins in a complex (Remy & Michnick 2006). Multiple beneficial properties including reversibility, sensitive and real-time quantification and investigation of dynamics of protein-protein interactions and modulation of interactions by pharmacological and genetic methods, underline the suitability of luminescence-based PCA for high-throughput screening (HTS) purposes (Michnick et al., 2007). However, overexpression of reporter protein constructs and addition of substrate are required to obtain an active luminescence generating reaction in luminescence-PCA methods. Our assay validation work and proof-of-concept small molecule library screen using split *Gaussia princeps* luciferase (GLuc) PCA with a focus on Pin1-tau interaction pair revealed the suitability of this approach for studying tau protein interactions in live cells. Interestingly, the screen identified several sedative-hypnotic class compounds (benzodiazepines and barbiturates) commonly considered to have positive allosteric modulator activity on γ-aminobutyric acid type A (GABA$_A$) receptor, as regulators of Pin1-tau interaction. Furthermore, an association of GABA$_A$ receptor modulation with increased tau phosphorylation was found in primary rat cortical neurons.

GABA$_A$ receptors belong to the superfamily of ligand-gated ion-channels and mediate most of the fast inhibitory neurotransmission in the mammalian brain (Jacob et al., 2008; Luscher et al., 2011). Moreover, GABA$_A$ receptors are clinically significant targets for multiple CNS active drugs such as anxiolytics, anti-convulsants and sedative-hypnotics. Beside the abundant expression in CNS, GABA$_A$ receptors are functionally expressed also in Neuro2A cells (Baraldi et al., 1979) that were utilized in our screen and further mechanistic studies. Different subunit composition of pentameric GABA$_A$ receptors result in varying expression, cellular localization and sensitivity of pharmacological and physiological properties, for example benzodiazepine-sensitive GABA$_A$ receptors are mostly located in the synapse while other types, such as GABA$_A$ receptors mediating tonic inhibition, are predominantly extrasynaptic, i.e. not located at synaptic sites (Brunig et al., 2002). Hence, according to our data, benzodiazepine-induced increase was observed in tau phosphorylation at the AT8 phosphoepitope. Observed increase of tau phosphorylation remained at elevated level 24 h after washout of the drug in mature synaptically connected cortical neurons, an effect which appears to be predominantly mediated by synaptic GABA$_A$ receptors. Furhermore, the major inhibitory synapse scaffolding protein gephyrin is known to associate with synaptic GABA$_A$ receptors and microtubules (Prior et al., 1992; Essrich et al., 1998). Since stabilizing and regulating MT dynamics is a predominant tau function, this may implicate a function for tau in dynamic regulation of clustering or trafficking of GABA$_A$ receptors and could be also a modulation target of GABAergic

synaptic signaling. Interestingly, it has been reported that GSK-3β-mediated regulation of GABAergic synapse formation occurs via gephyrin phosphorylation (Tyagarajan et al., 2011). Importantly, a role for CDK5 in gephyrin-mediated stabilization of GABA$_A$ receptor clustering was recently reported (Kalbouneh et al., 2014). In hippocampal neurons, it was shown that shRNA-mediated knockdown and pharmacological inhibition of CDK5 significantly decreased phosphorylated gephyrin clusters and postsynaptic GABA$_A$ receptors. Also, site-directed mutagenesis and dephosphorylation by PP1/PP2A of gephyrin at Ser270 residue reduced CDK5-induced phosphorylation implicating Ser270 as target site for CDK5 phosphorylation (Kalbouneh et al., 2014), which is the same site suggested to be phosphorylated by GSK-3β (Tyagarajan et al., 2011). In further examination of the effects of screen-identified GABA$_A$ receptor activators, our data suggest a mechanism involving CDK5 kinase but not GSK-3β in observed increase of tau phosphorylation.

Phosphorylation of GABA$_A$ receptor subunit β3 is reported to play an important role in the regulation of GABA$_A$ receptor function, and, β3 associates and is dephosphorylated by PP2A (Jovanovic et al., 2004). Our data suggest that increased GABA$_A$ receptor activity decreases PP2A-tau interaction without reduction of PP2A enzyme activity. Decrease in PP2A-tau interaction upon treatment of GABA$_A$-active sedatives may result from compartment-specific expression variations of PP2A. Subsequently, a possible recruitment of PP2A for GABA$_A$ receptor β3 subunit dephosphorylation and desensitization induced by persistent and strong GABA$_A$ receptor activation may further reduce the availability of PP2A to interact and dephosphorylate tau. Additionally, association of synaptic activity and PP2A with tau phosphorylation has been reported (Sun et al., 2012). Specifically, zinc released from synaptic terminal was suggested to result in tau hyperphosphorylation via inhibition of PP2A, which was reversed by zinc chelators. However, whether the level of synaptic activity contributes to regulation of tau phosphorylation remains to be elucidated.

Phosphorylation of multiple proline-directed serine and threonine residues located in the proline-rich region flanking the MBRD is a central mechanism regulating tau functions. Pin1 binds and facilitates dephosphorylation of tau by PP2A (Liou et al., 2003). Specifically, Pin1 acts on phosphorylated Thr231-Pro-motif of tau and catalyzes the *cis/trans* isomerization of peptidyl-prolyl bond (Lu et al., 1999). This isomerization facilitates the binding of PP2A to tau since PP2A is Pro-directed conformation-specific phosphatase (Zhou et al., 2000). Importantly, PP2A interacts with and deposphorylates *trans* isoforms of its target proteins, such as tau, which emphasizes the importance of Pin1-mediated *cis/trans* conversion of tau and subsequent restoration of its cellular functions via PP2A dephosphorylation. Additionally, Pin1 acts on phosphorylated Pro-directed gephyrin sites and isomerizes its *cis/trans* conversion, which is suggested as an important mechanism of gephyrin-mediated regulation of glycine receptor function (Zita et al., 2007). Among the numerous cellular functions of Pin1, it has been suggested to act as a molecular timer in order to modulate the duration and amplitude of different cellular processes including e.g. cell signaling and neuronal function (Lu et al., 2007). Based on these observations and our data, Pin1-mediated prolyl isomerization of both tau and the scaffolding protein gephyrin may together regulate the duration and timing of GABA$_A$ receptor trafficking signals in the inhibitory synapse. Overall, GSK-3β, CDK5 and PP2A all have an essential role in both regulation of tau phosphorylation and GABA$_A$ receptor

clustering and trafficking. Both tau and gephyrin are tightly connected to MTs, which raises intriguing questions whether also tau has a role in GABA$_A$ receptor modulation or if regulation of tau phosphorylation is affected by activation and trafficking of GABA$_A$ receptors or MT-mediated cytoskeletal changes. Hence, studying the interaction of tau and gephyrin could provide novel insights on clustering and trafficking of GABA$_A$ receptors. However, whether there is a direct or indirect interaction between these proteins is yet to be established.

Tau phosphorylated at Thr231 has been described to appear early in human AD, particularly in cells containing pre-tangles (Luna-Munoz et al., 2007), and its CSF levels was reported to correlate with memory deficits in mild cognitive impairment (MCI) and AD (Hampel et al., 2010). Moreover, by using a specific antibody that is able to distinguish between *cis* and *trans* isoforms of Thr231 phosphorylated tau, *cis* isomer was observed early in human MCI and reported to accumulate in degenerating neurons and localize into dystrophic neurites (Nakamura et al., 2012). Additionally, tau in *cis* conformation at Thr231 site is unable to regulate MT dynamics and becomes increasingly resistant to dephosphorylation and protein degradation, which, in turn, leads to cellular conditions in which tau is more prone to aggregate. Interestingly, a recent study showed that initiation and possible progression of AD-like tau pathology could be prevented after traumatic brain injury (TBI) using a monoclonal antibody specific for *cis*-isoform, Thr231 phosphorylated tau (*cis* mAb) (Kondo et al., 2015). TBI may cause acute neurological dysfunction and occasionally lead to development of more long-lasting events, such as chronic traumatic encephalopathy (CTE) (e.g. Blennow et al., 2012; Smith et al., 2013; McKee et al., 2013) and is also considered as risk factor for AD (e.g. Guo et al., 2000; Mortimer et al., 1991). Acute production of Thr231 phosphorylated *cis* tau in neurons after TBI in mice and after stress in vitro was shown to result in disrupted MT networks, altered mitochondrial transport, interneuronal spreading and eventual apoptosis, a process termed 'cistauosis' that precede other markers of tauopathy (Kondo et al., 2015). Importantly, treatment of TBI mice with *cis* mAb inhibited cistauosis and halted the development and spreading of further tauopathy, and, also rescued multiple functional and structural TBI-related dysfunctions. These data strongly suggest cistauosis as an early disease process of TBI, CTE and AD, and that *cis* mAb may be an effective tool to diagnose and halt the spreading of tau pathology in the early stage of disease pathogenesis. The Pin1-tau PCA tool developed in this study provides an effective in vitro tool for studying cellular regulation and mechanisms that underlie accumulation of *cis*-tau in cells.

Emerging evidence of prion-like spreading of conformationally distinct protein aggregates, or 'strains', in various NDDs suggest a common mechanism for spreading of non-prion amyloidogenic proteins such as tau. Regardless of the mounting evidence of transcellular tau propagation in mouse models and human brain in anatomically interconnected areas, several questions according to exact mechanisms of cell-to-cell propagation still remain unanswered. Particularly, the molecular mechanisms of cellular tau secretion and uptake are poorly understood. Although a few intriguing mechanistic studies have reported how tau is translocated from intracellular to extracellular compartment by associating with various microvesicles and how tau is internalized into cells, the tau species predominantly involved in these processes still remains poorly characterized. Moreover, whether cell-to-cell propagation of tau in physiological and

pathophysiological conditions occurs via differential mechanisms is yet to be clarified. A novel PCA-based assay system for investigation of cellular secretion and uptake of soluble tau was developed in this study. The assay allows detection of secreted and internalized tau dimers. Dimerization is a fundamental step for further formation of higher order fibrils and aggregates (Friedhoff et al., 1998), and likely oligomers. Accordingly, in our split GLuc-based assay tau dimer is the minimal signal generating unit due to a refolding of complementary reporter protein fragments. However, PCA is incapable of distinguishing whether the detected signal is generated by tau dimers or higher order oligomers. Regardless of common limitations of the assay set up in relation to generally utilized cellular models, such as requirement of overexpression of reporter plasmids and C-terminally tagged tau, thorough assay validation for multiple critical parameters resulted in reliable approach to study cellular secretion and uptake of tau in live cells. Furthermore, Western blot analysis suggested spontaneous assembly of reporter-tagged tau into higher molecular weight and/or oligomeric species implicating that detected luminescence signal may be also originated from tau species larger than dimer.

Cellular secretion of tau to extracellular space is suggested to occur in association with microvesicles such as exosomes and ectosomes and in vesicle-free form (Barten et al., 2011; Kim et al., 2010b; Chai et al., 2012; Dujardin et al., 2014a; Saman et al., 2012). In our system the portion of vesicle-associated tau secretion was less than 1% and more than 99% of secreted tau was in vesicle-free form. Although it is possible that post-secretional release of tau from the microvesicles via unknown mechanism contributes to this balance, it seems that vast majority of tau released in our system is vesicle-free. Notably though, the internalization of vesicle-associated tau was slightly more active as compared to vesicle-free tau. Binding of extracellular, vesicle-free tau with cell surface HSPGs is reported to mediate tau internalization most like occurring via macropinocytosis (Holmes et al., 2013), a bulk fluid-phase endocytic mechanism. Additionally, it has been recently reported that trimer is the minimal propagating tau unit that is spontaneously uptaken into cells and capable to induce further seeding (Mirbaha et al., 2015). Internalization of different tau species via different mechanisms may result in various outcomes according to compartmentalization and cellular fate, such as degradation or rapid re-secretion of uptaken tau. The levels of tau uptake in our assay system were relatively low, which could be at least partially explained by re-secretion of tau following the uptake, a process typical to macropinosomes that undergo a rapid exocytosis resulting in bidirectional trafficking regulated by collective endocytic and exocytic coordination (Falcone et al., 2006). Based on our data, inhibition of ceramide generation by neutral sphingomyelinase, a main regulator of exosome biogenesis (Trajkovic et al., 2008), increased cellular levels of uptaken tau while it decreased the secretion of tau.

We performed an siRNA-based screen using 12 LOAD associated risk genes to study their potential functional link to cellular tau secretion and uptake. Knockdown of genes *TREM2*, *CD33*, *CD2AP* and *FRMD4A* showed significant decrease in tau secretion whereas only *APOE* gene affected tau uptake by increasing the signal in recipient cells. Since *TREM2* and *CD33* are predominantly expressed in microglial cells, we tested the knockdown effect of these genes on tau secretion in human fetal microglial cell line. The decrease upon siRNA-mediated gene knockdown observed in HEK293T cells was not recapitulated in microglial cells suggesting an off-target effect of used siRNAs rather than an effect targeted on TREM2 and CD33 expression and function. Interestingly, a recent

study reported that depletion of microglia significantly reduce tau propagation in vivo (Asai et al., 2015). Exosome-mediated tau spreading by microglia was suppressed by genetic and pharmacological inhibition of exosome synthesis both in vitro and in vivo suggesting a new role for microglia in propagation of tau pathology. These data strongly emphasize the importance of studying non-transsynaptic tau propagation mechanisms in addition those focusing on transsynaptic propagation. The finding that neither of the genes with proven functional roles in endocytosis, *PICALM* (Miller et al., 2011; Tebar et al., 1999) and *BIN1* (McMahon et al., 1997; Tan et al., 2013), had any effect on tau uptake was surprising and may indicate that cellular uptake of tau does not rely on receptor-mediated endocytic mechanisms, such as clathrin-mediated endocytosis, but may be related to fluid-phase endocytosis mechanisms, specifically macropinocytosis, as has been suggested recently (Holmes et al., 2013). It should also be noted that different cell types may internalize tau using different mechanisms and at specific times. For example, due to extensive membrane turnover associated with macropinocytosis, neurons may perform macropinocytosis only during axonal development and remodeling

Based on the observations that *FRMD4A* expression is decreased in AD brain in relation to increasing disease severity according to increasing neurofibrillary degeneration and functional association to tau phosphorylation (II), we further examined the mechanistic basis of the *FRMD4A*-tau secretion finding. Our results clearly indicate that decreased expression level of *FRMD4A* reduce cellular tau secretion whereas overexpression significantly increase tau release.

Although very little is known about its function in mammalian cells, FRMD4A is involved in regulation of epithelial cell polarity in association with the Par polarity complex (Ikenouchi & Umeda 2010). FRMD4A connects Par3/Par6/aPKCζ complex to Arf6 activation via cytohesins, a family of Arf6 guanine-nucleotide exchange factors (GEF). Signaling of Par polarity complex is essential in neuronal polarization (Insolera et al., 2011) and in various cellular membrane trafficking events such as vesicular secretion (Balklava et al., 2007). Recently, tau release from neurons was suggested to associate with plasma membrane fusion of presynaptic vesicles regulated by neuronal activity (Pooler et al., 2013; Yamada et al., 2014). Hence, considering that facilitation of synaptic transmission by direct interaction of cytohesin-1/mSec7 and Munc13-1, which is most likely resulting in increasing reserve of presynaptic vesicles ready to fuse with plasma membrane (Neeb et al., 1999; Ashery et al., 1999), the levels and activity of FRMD4A and cytohesins may correlate with the level of tau secretion. Interestingly, cellular tau secretion is suggested to increase Aβ generation in a process most likely regulated by neuronal hyperactivity (Bright et al., 2015). Additionally, since increase in extracellular level of Aβ is known to promote neuronal hyperactivity (Fogel et al., 2014; Busche et al., 2008) and enhanced glutamatergic synaptic activity is reported to promote tau secretion from neurons (Yamada et al., 2014), these observation collectively suggest an existence of a feedback loop between Aβ production and tau secretion in pathogenesis and progression of AD. Also, considering our results suggesting an increase in tau phosphorylation by GABA$_A$ receptor activation, it remains to be seen whether GABA$_A$ receptor activation has an impact on tau secretion and uptake.

Extensive amount of evidence has been presented to support the prion-like propagation of amyloidogenic proteins in NDDs. Nonetheless, various discrepancies are observed when comparing data from mouse models of non-prion protein transmission and human

brain such as different time course of spreading and onset of pathology (Guo & Lee 2014). Some of the inconsistencies could be explained for example by the size difference between mouse and human brain, the rate-limiting step of protein nucleation phase (Friedhoff et al., 1998) and utilization of overexpression mouse models (Guo & Lee 2014). Hence, multiple concerns still exist before the suggested prion paradigm can be fully established. Nevertheless, strong evidence point toward a paradigm shift in neurodegenerative non-prion proteinopathies, such as co-transmission of Aβ-pathology after iatrogenic CJD-contaminated human growth hormone treatment (Jaunmuktane et al., 2015) and the propagation of specific α-syn prion strains from human MSA brain into transgenic mouse causing the disease (Woerman et al., 2015; Prusiner et al., 2015).

Taken together, our results identify a novel connection between GABA$_A$ receptor activation and tau phosphorylation, and, cell polarity complex signaling and tau secretion. Given the importance of polarized protein delivery into various plasma membrane subdomains for physiological functioning of cells, it is fundamental to further establish the exact molecular mechanisms of how synaptic activity and cell polarity complex may be involved in cell-to-cell propagation of tau in AD and related tauopathies.

6.2 New insights into functional association of GWAS identified LOAD susceptibility genes with disease pathogenesis (II, III)

The association of *APOE* with AD was discovered nearly three decades ago and was further confirmed as a genetic risk factor using candidate gene approach (Saunders et al., 1993; Ignatius et al., 1987; Ignatius et al., 1986; Strittmatter et al., 1993; Corder et al., 1993). Currently, besides aging, *APOE* is the strongest risk factor contributing to onset and pathogenesis of LOAD (Bettens et al., 2013). In the last ten years, a major technological advances such as large-scale genome-wide association studies (GWAS) allowing identification of millions of genetic variations covering the whole genome in a largely hypothesis-free experimental approach has produced tremendous amount of data improving knowledge of the genetic landscape of LOAD. Since there are currently approximately 700 AD-associated susceptibility genes, we focused on the top 10 genes presented in AlzGene database compiled from meta-analyses of published AD GWASs (Bertram et al., 2007) and two additional recently identified genes *FRMD4A* (Lambert et al., 2013a) and *TREM2* (Jonsson et al., 2013; Guerreiro et al., 2013a).

Regardless of persistently growing number of possible novel LOAD-related susceptibility genes, the functional association and expression in relation to disease pathogenesis and progression are poorly understood. Although the exact cellular functions and direct association to established pathophysiological AD pathways of identified LOAD susceptibility genes are yet to be clarified, these genes can be classified in three distinct pathways, including lipid and cholesterol metabolism, immune system function and vesicle recycling and endocytosis with some overlap of genes between the classes (Jones et al., 2010; Bertram et al., 2008; Medway & Morgan 2014; Karch & Goate 2015). Of the 10 LOAD genes studied, APOE and CLU (APOJ) are brain lipoproteins, BIN1 and PICALM are intracellular proteins associated with endocytic machinery and CD33, CR1 and MS4A proteins are associated with immune and complement systems. Also, ABCA7 is suggested to function in cholesterol and lipid metabolism, and, CD2AP in regulation of actin cytoskeleton reorganization. In our neuropathologically validated AD cohort the

expression of *FRMD4A* was decreased and the expression of *MS4A6A* was increased according to increasing AD-related neurofibrillary pathology.

FRMD4A has been associated as a susceptibility gene for LOAD (Lambert et al., 2013a). The function and expression of *FRMD4A* in mammalian cells is in general poorly understood and its connection to AD pathogenesis is currently unknown. Interestingly, a homozygous coding region *FRMD4A* mutation was recently associated with autosomal recessive congenital microcephaly and intellectual disability, disturbed genesis of corpus callosum and dysmorphism in a Bedouin kindred (Fine et al., 2014). Specifically, the frame shift mutation resulting in truncation is located in the domain of *FRMD4A* that interact with Par3 (Fine et al., 2014), which is essential for regulation of cell polarity (Ikenouchi & Umeda 2010). We showed that siRNA-mediated genetic knockdown of *FRMD4A* is functionally associated with increased interaction of APP and BACE1 and corresponding increase of secreted $A\beta_{40}$. Furthermore, decreased expression of *FRMD4A* increased the interaction of tau with both Pin1 and GSK-3β and was associated with altered phosphorylation status of tau. By studying the effect of LOAD susceptibility genes on cell-to-cell propagation of tau, we showed significant changes in tau secretion in relation to expression level of *FRMD4A*. Further mechanistic experiments confirmed the association of tau secretion with cell polarity complex signaling pathway. Activity of cytohesins, which play a crucial part in cell polarity signaling (Ikenouchi & Umeda 2010), and are also involved in synaptic transmission (Neeb et al., 1999; Ashery et al., 1999), were recently shown to be associated with ALS (Zhai et al., 2015). More specifically, pharmacological and genetic inhibition of cytohesins was shown to protect motor neurons and rescue locomotor defects in vitro and in *C. elegans* model of ALS, respectively. Complex formation of mutant superoxidase dismutase 1 (SOD1), a causal factor of familial ALS, and cytohesins was not associated with alterations in either activity of GEF or ARF activation. However, mutant SOD1-induced ER stress was mitigated upon decreased cytohesin activity resulting in reduced mutant SOD1-driven protein toxicity and enhanced autophagic flux. These observations in concert with our data suggest that FRMD4A-cytohesin-ARF signaling may have a significant role in neuronal protein homeostasis in general and possibly contribute to pathogenesis of several NDDs.

The amount of information regarding to impact of LOAD susceptibility genes to cellular secretion and uptake of tau is limited. Since tau is known to interact with some of the proteins encoded by LOAD susceptibility genes (APOE, BIN1, CLU and PICALM), it has been suggested that these genes may have a potential effect on cell-to-cell propagation of tau (Avila et al., 2015). Based on our data, gene silencing of *APOE* increased the internalization of tau, which may relate to previously reported property of tau to bind APOE (Fleming et al., 1996; Strittmatter et al., 1994). In addition to *FRMD4A*, our results showed that knockdown of *CD2AP* decreased cellular tau secretion and was associated with increased BACE1-APP and GSK-3β-tau interactions in the PCA-based in vitro platform. Also, reduced level of *CD2AP* expression altered tau phosphorylation. Interestingly, novel function for CD2AP in maintaining blood-brain barrier (BBB) integrity was recently reported suggesting a cerebrovascular role for CD2AP (Cochran et al., 2015) in addition to its roles in signaling between T cells and antigen presenting cells (Dustin et al., 1998) and in cell junction signaling in the kidney slit diaphragm (Shih et al., 1999). Intriguingly, a recent study showed that depletion of tau in a tauopathy mouse model prevents progressive BBB damage (Blair et al., 2015). Given that tau-mediated

toxicity is reported to exacerbate upon CD2AP deficiency in *Drosophila* model of AD (Shulman et al., 2014), these data collectively implicate the involvement of both tau and CD2AP in regulation of BBB integrity, which may affect the late-stage AD-related vascular changes contributing to cognitive impairment and dementia. However, whether CD2AP-mediated BBB dysregulation is a cause or a consequence of pathological tau or how CD2AP deficiency may affect cell-to-cell propagation of tau is a subject of future studies.

Conversely to common identified variants, novel emerging sequencing techniques such as whole exome and whole genome sequencing have facilitated the discovery of additional rare coding mutations, e.g. in genes *CD2AP*, *EPHA1*, *ABCA7* and *BIN1* (Vardarajan et al., 2015; Steinberg et al., 2015). Moreover, whether these novel and previously identified rare AD risk increasing variants such as *TREM2* (Jonsson et al., 2013; Guerreiro et al., 2013a) are connected to tau propagation is currently unknown. In our AD cohort, the expression of exons 3 and 4 of both genes *TREM2* and *CLU* were increased related to increasing AD severity according to Braak staging. It was recently reported in a large meta-analysis that carriers of *TREM2* SNP rs75932628 present significant increase in the level of CSF total-tau as compared to non-carriers suggesting that role of *TREM2* in AD could be related to tau dysfunction (Lill et al., 2015). Furthermore, it was recently reported that microglial TREM2 is upregulated during disease progression in P301S mice and gene silencing of *TREM2* in P301S mice brain exacerbated tau pathology (Jiang et al., 2015). More specifically, it was suggested that *TREM2* silencing-induced increase of tau pathology and learning deficits may result from loss of ability of TREM2 to attenuate activity of tau related kinases. Additionally, a novel locus near *MAPT* gene encoding tau protein was identified to associate with LOAD particularly in patients lacking *APOE* ε4 allele (Jun et al., 2015). Hence, it will be interesting to see whether *TREM2* is functionally connected to tau-related pathways in AD and if this is possibly mediated by differential *APOE* genotype. We observed an increase in expression level of *MS4A6A* in relation to increasing Braak stage severity in AD. In particular, there was a significant positive correlation between *MS4A6A* expression and microglial *AIF-1* (encoding microglial marker Iba-1) gene expression, which is consistent with the previous finding (Karch et al., 2012b) further corroborating the suggestion that genes of *MS4A* -cluster are involved in immune system function (Ishibashi et al., 2001). Recently, *MS4A* gene cluster has been specifically associated with LOAD patients who are *APOE* ε4 non-carriers (Jun et al., 2015). In our *APOE* ε4-stratified cohort (36 carriers; 24 non-carriers), we were unable to show this since majority of the patients in Braak group V-VI were *APOE* ε4 carriers whereas in Braak group 0-II, in which the expression level was normalized to, the majority were non-carriers. This inconsistency may be at least partially due to a small sample size of our cohort (n=60).

Level of *ABCA7* expression was clearly indicated to associate with established pathophysiological pathways related to AD in our in vitro platform. Specifically, siRNA-mediated gene silencing of *ABCA7* resulted in increased BACE1-APP interaction and corresponding elevation of $A\beta_{40}$ level. Furthermore, down regulation of *ABCA7* was associated with increased tau interaction with both GSK-3β and Pin1. Interestingly, rare *ABCA7* loss-of-function mutations were shown increase the risk of AD (Steinberg et al., 2015). Recently, it was shown that *ABCA7* loss-of-function mutations and decreased expression promote amyloidogenic APP processing in vitro and in vivo (Satoh et al.,

2015). This is consistent with a previous study reporting that deletion of *ABCA7* significantly increased the amount of cerebral accumulation of Aβ in a mouse model of AD (Kim et al., 2013). These data strongly implicate an important role for ABCA7 in Aβ production. However, whether *ABCA7* loss-of-function or depletion contributes to Aβ clearance remains to be elucidated.

Each individual common LOAD susceptibility gene identified to date has only a minor addition on conferring the overall risk of disease incidence strongly implicating the existence of missing, or more precisely hidden heriditary component (Lord et al., 2014). According to highly complex disease aetiologies of NDDs, such as LOAD, it is unlikely that this hidden genetic component will be discovered using a single methodology such as GWAS but requires combinations of various approaches complementing each other. Hence, epigenetics and epigenome-wide association studies (EWAS) among others could provide an additional tool to accompany the more established methodologies in order to reveal possible genotype-by-environment interactions, such as DNA methylation (Gibson 2011; De Jager et al., 2014; Lunnon et al., 2014; Lord et al., 2014). Furthermore, large portion of the known LOAD associated SNPs are located in the non-coding regions of genes, which is significantly complicating functional studies of the impact of these variants to disease pathogenesis and pathophysiology. Moreover, considering the non-coding region located SNPs and the subtle effect of each gene to disease onset, functional association studies are highly challenging and novel methodology is sorely needed to address this issue. Moreover, single gene silencing approach may not be able to detect epistatic interactions between LOAD genes. Indeed, one possible approach could be targeting the LOAD susceptibility genes in clusters, i.e. since some identified genes can be classified for e.g. involving endocytosis and vesicle transport, it may prove efficient to investigate a cluster of genes associated to particular cellular phenomenon with varying endpoint measurements.

Overall, in our study combining the expression and splicing status of LOAD susceptibility genes in AD brain and functional association of disease pathogenesis in established pathways in vitro, we show the differential expression of *MS4A6A*, *FRMD4A*, *TREM2* and *CLU* during progression of AD-related neurofibrillary pathology. Furthermore, *FRMD4A*, *CD2AP* and *ABCA7* are associated with both amyloidogenic APP processing and altered tau phosphorylation in vitro. Moreover, the expression level of *FRMD4A* and *CD2AP* modulates the cellular secretion of tau. Finally, our mechanistic studies on FRMD4A suggest that tau secretion may be affected more generally by cell polarity complex Par3/Par6/aPKCζ signaling via cytohesin-mediated Arf6 activation.

7 Concluding remarks and future prospects

Microtubule-associated protein tau is an essential regulator of cellular and particularly neuronal function via modulation of microtubule integrity and dynamics. Abnormal tau function upon disequilibrium in various posttranslational modifications such as phosphorylation, contribute to detachment from MTs, aggregation and to cell-to-cell spreading of tau pathology and subsequent neurofibrillary degeneration in numerous neurodegenerative diseases (NDDs), including Alzheimer's disease (AD) and other tauopathies. This thesis provides new insights into cellular tau physiology and suggests a novel mechanism regulating cellular secretion of tau.

Firstly, we have developed and validated a novel method based on protein-fragment complementation assay (PCA) to study protein-protein interactions of tau in live cells (I, II, III). Moreover, PCA-based in vitro platform is suitable for investigating dynamic changes upon pharmacological and genetic modulation of protein-protein interactions of tau and other established pathogenic protein pathways in AD (I, II). Additionally, we have further validated these assays to effectively examine cellular secretion and internalization of tau in live cells (III).

Secondly, our proof-of-concept chemical library screen identified GABA$_A$ receptor modulators, such as benzodiazepines, as novel modulators of tau phosphorylation (I). Accordingly, the increased tau phosphorylation at the AT8 phosphoepitope, which is aberrantly phosphorylated in AD and related tauopathies, is most likely a result of reduced tau binding with protein phosphatase 2A and dependent on CDK5 protein kinase.

Thirdly, we show that the expression of LOAD susceptibility genes *MS4A6A* is increased and the expression of *FRMD4A* is decreased in regards to increasing AD-related neurofibrillary pathology in temporal cortex of *post mortem* patient samples of our neuropathologically validated AD cohort (II). Based on our in vitro data, *FRMD4A* is functionally associated with both established pathogenic AD-pathways: amyloidogenic APP processing and increased tau phosphorylation.

Finally, we showed that the expression level of *FRMD4A* modulates cellular tau secretion (III). Based on our mechanistic studies, we revealed a novel connection between cell polarity complex Par6/aPKCζ-FRMD4A-cytohesin-Arf6 signaling pathway and cellular tau secretion hereby for the first time, implicating a functional association between a LOAD susceptibility gene and a central pathological spreading mechanism of the disease.

The constantly growing list of disappointing outcomes of clinical trials assessing potential therapies for AD and other NDDs strongly emphasizes the urgent need for better understanding of the risk factors and aetiology of these disease, hopefully leading to development of disease-modufying treatments and more effective preventive measures in the future. Owing to this, it is initially crucial to investigate and establish the exact molecular mechanism(s) how amyloidogenic proteins such as tau are posttranslationally modified and how these modifications further affect the secretion and internalization of misfolded and aggregated proteins by cells. Answering these fundamental mechanistical questions regarding NDD pathogenesis and pathophysiology is expected to provide a major leap toward the development of strategies to halt the progression of disease-specific pathology.

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-niko-

References

Abraham, R., Moskvina, V., Sims, R., Hollingworth, P., Morgan, A., Georgieva, L. et al. (2008). A genome-wide association study for late-onset Alzheimer's disease using DNA pooling. BMC Med Genomics 1: 44.

Adams, S.J., DeTure, M.A., McBride, M., Dickson, D.W. & Petrucelli, L. (2010). Three repeat isoforms of tau inhibit assembly of four repeat tau filaments. PLoS One 5: e10810.

Agarwal-Mawal, A., Qureshi, H.Y., Cafferty, P.W., Yuan, Z., Han, D., Lin, R. et al. (2003). 14-3-3 connects glycogen synthase kinase-3 beta to tau within a brain microtubule-associated tau phosphorylation complex. J Biol Chem 278: 12722-12728.

Aguzzi, A. (2009). Cell biology: Beyond the prion principle. Nature 459: 924-925.

Aguzzi, A., Heikenwalder, M. & Polymenidou, M. (2007). Insights into prion strains and neurotoxicity. Nat Rev Mol Cell Biol 8: 552-561.

Aguzzi, A. & Rajendran, L. (2009). The transcellular spread of cytosolic amyloids, prions, and prionoids. Neuron 64: 783-790.

Aguzzi, A., Sigurdson, C. & Heikenwaelder, M. (2008). Molecular mechanisms of prion pathogenesis. Annu Rev Pathol 3: 11-40.

Ahmed, Z., Bigio, E.H., Budka, H., Dickson, D.W., Ferrer, I., Ghetti, B. et al. (2013). Globular glial tauopathies (GGT): consensus recommendations. Acta Neuropathol 126: 537-544.

Ahmed, Z., Cooper, J., Murray, T.K., Garn, K., McNaughton, E., Clarke, H. et al. (2014). A novel in vivo model of tau propagation with rapid and progressive neurofibrillary tangle pathology: the pattern of spread is determined by connectivity, not proximity. Acta Neuropathol 127: 667-683.

Ahmed, Z., Doherty, K.M., Silveira-Moriyama, L., Bandopadhyay, R., Lashley, T., Mamais, A. et al. (2011). Globular glial tauopathies (GGT) presenting with motor neuron disease or frontotemporal dementia: an emerging group of 4-repeat tauopathies. Acta Neuropathol 122: 415-428.

Ahn, K.J., Jeong, H.K., Choi, H.S., Ryoo, S.R., Kim, Y.J., Goo, J.S. et al. (2006). DYRK1A BAC transgenic mice show altered synaptic plasticity with learning and memory defects. Neurobiol Dis 22: 463-472.

Allen, M., Zou, F., Chai, H.S., Younkin, C.S., Crook, J., Pankratz, V.S. et al. (2012). Novel late-onset Alzheimer disease loci variants associate with brain gene expression. Neurology 79: 221-228.

Alonso Adel, C., Mederlyova, A., Novak, M., Grundke-Iqbal, I. & Iqbal, K. (2004). Promotion of hyperphosphorylation by frontotemporal dementia tau mutations. J Biol Chem 279: 34873-34881.

Alonso, A., Zaidi, T., Novak, M., Grundke-Iqbal, I. & Iqbal, K. (2001a). Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. Proc Natl Acad Sci U S A 98: 6923-6928.

Alonso, A.C., Zaidi, T., Grundke-Iqbal, I. & Iqbal, K. (1994). Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer disease. Proc Natl Acad Sci U S A 91: 5562-5566.

Alonso, A.D., Di Clerico, J., Li, B., Corbo, C.P., Alaniz, M.E., Grundke-Iqbal, I. et al. (2010). Phosphorylation of tau at Thr212, Thr231, and Ser262 combined causes neurodegeneration. J Biol Chem 285: 30851-30860.

Alonso, A.D., Grundke-Iqbal, I., Barra, H.S. & Iqbal, K. (1997). Abnormal phosphorylation of tau and the mechanism of Alzheimer neurofibrillary degeneration: sequestration of microtubule-associated proteins 1 and 2 and the disassembly of microtubules by the abnormal tau. Proc Natl Acad Sci U S A 94: 298-303.

Alonso, A.D., Zaidi, T., Novak, M., Barra, H.S., Grundke-Iqbal, I. & Iqbal, K. (2001b). Interaction of tau isoforms with Alzheimer's disease abnormally hyperphosphorylated tau and in vitro phosphorylation into the disease-like protein. J Biol Chem 276: 37967-37973.

Amos, L.A. (2004). Microtubule structure and its stabilisation. Org Biomol Chem 2: 2153-2160.

Andreadis, A., Broderick, J.A. & Kosik, K.S. (1995). Relative exon affinities and suboptimal splice site signals lead to non-equivalence of two cassette exons. Nucleic Acids Res 23: 3585-3593.

Andreadis, A., Brown, W.M. & Kosik, K.S. (1992). Structure and novel exons of the human tau gene. Biochemistry 31: 10626-10633.

Aoyagi, H., Hasegawa, M. & Tamaoka, A. (2007). Fibrillogenic nuclei composed of P301L mutant tau induce elongation of P301L tau but not wild-type tau. J Biol Chem 282: 20309-20318.

Arkin, M.R. & Wells, J.A. (2004). Small-molecule inhibitors of protein-protein interactions: progressing towards the dream. Nat Rev Drug Discov 3: 301-317.

Arnold, C.S., Johnson, G.V., Cole, R.N., Dong, D.L., Lee, M. & Hart, G.W. (1996). The microtubule-associated protein tau is extensively modified with O-linked N-acetylglucosamine. J Biol Chem 271: 28741-28744.

Arnold, S.E., Hyman, B.T., Flory, J., Damasio, A.R. & Van Hoesen, G.W. (1991). The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. Cereb Cortex 1: 103-116.

Asai, H., Ikezu, S., Tsunoda, S., Medalla, M., Luebke, J., Haydar, T. et al. (2015). Depletion of microglia and inhibition of exosome synthesis halt tau propagation. Nat Neurosci

Ashery, U., Koch, H., Scheuss, V., Brose, N. & Rettig, J. (1999). A presynaptic role for the ADP ribosylation factor (ARF)-specific GDP/GTP exchange factor msec7-1. Proc Natl Acad Sci U S A 96: 1094-1099.

Avila, J., Gomez-Ramos, A. & Bolos, M. (2015). AD genetic risk factors and tau spreading. Front Aging Neurosci 7: 99.

Avila, J., Wandosell, F. & Hernandez, F. (2010). Role of glycogen synthase kinase-3 in Alzheimer's disease pathogenesis and glycogen synthase kinase-3 inhibitors. Expert Rev Neurother 10: 703-710.

Bai, X.C., Yan, C., Yang, G., Lu, P., Ma, D., Sun, L. et al. (2015). An atomic structure of human gamma-secretase. Nature 525: 212-217.

Baig, S., Joseph, S.A., Tayler, H., Abraham, R., Owen, M.J., Williams, J. et al. (2010). Distribution and expression of picalm in Alzheimer disease. J Neuropathol Exp Neurol 69: 1071-1077.

Balklava, Z., Pant, S., Fares, H. & Grant, B.D. (2007). Genome-wide analysis identifies a general requirement for polarity proteins in endocytic traffic. Nat Cell Biol 9: 1066-1073.

Ballatore, C., Lee, V.M. & Trojanowski, J.Q. (2007). Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat Rev Neurosci 8: 663-672.

Bancher, C., Brunner, C., Lassmann, H., Budka, H., Jellinger, K., Wiche, G. et al. (1989). Accumulation of abnormally phosphorylated tau precedes the formation of neurofibrillary tangles in Alzheimer's disease. Brain Res 477: 90-99.

Baraldi, M., Guidotti, A., Schwartz, J.P. & Costa, E. (1979). GABA receptors in clonal cell lines: a model for study of benzodiazepine action at molecular level. Science 205: 821-823.

Barten, D.M., Cadelina, G.W., Hoque, N., DeCarr, L.B., Guss, V.L., Yang, L. et al. (2011). Tau transgenic mice as models for cerebrospinal fluid tau biomarkers. J Alzheimers Dis 24 Suppl 2: 127-141.

Beecham, G.W., Naj, A.C., Gilbert, J.R., Haines, J.L., Buxbaum, J.D. & Pericak-Vance, M.A. (2010). PCDH11X variation is not associated with late-onset Alzheimer disease susceptibility. Psychiatr Genet 20: 321-324.

Benilova, I., Karran, E. & De Strooper, B. (2012). The toxic Abeta oligomer and Alzheimer's disease: an emperor in need of clothes. Nat Neurosci 15: 349-357.

Benitez, B.A., Cooper, B., Pastor, P., Jin, S.C., Lorenzo, E., Cervantes, S. et al. (2013). TREM2 is associated with the risk of Alzheimer's disease in Spanish population. Neurobiol Aging 34: 1711.e15-1711.e17.

Bergeron, C., Davis, A. & Lang, A.E. (1998). Corticobasal ganglionic degeneration and progressive supranuclear palsy presenting with cognitive decline. Brain Pathol 8: 355-365.

Bergeron, C., Pollanen, M.S., Weyer, L., Black, S.E. & Lang, A.E. (1996). Unusual clinical presentations of cortical-basal ganglionic degeneration. Ann Neurol 40: 893-900.

Bertram, L., Lange, C., Mullin, K., Parkinson, M., Hsiao, M., Hogan, M.F. et al. (2008). Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. Am J Hum Genet 83: 623-632.

Bertram, L., Lill, C.M. & Tanzi, R.E. (2010). The genetics of Alzheimer disease: back to the future. Neuron 68: 270-281.

Bertram, L., McQueen, M.B., Mullin, K., Blacker, D. & Tanzi, R.E. (2007). Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nat Genet 39: 17-23.

Bertrand, J., Plouffe, V., Senechal, P. & Leclerc, N. (2010). The pattern of human tau phosphorylation is the result of priming and feedback events in primary hippocampal neurons. Neuroscience 168: 323-334.

Bettens, K., Sleegers, K. & Van Broeckhoven, C. (2013). Genetic insights in Alzheimer's disease. Lancet Neurol 12: 92-104.

Biffi, A., Anderson, C.D., Desikan, R.S., Sabuncu, M., Cortellini, L., Schmansky, N. et al. (2010). Genetic variation and neuroimaging measures in Alzheimer disease. Arch Neurol 67: 677-685.

Birdi, S., Rajput, A.H., Fenton, M., Donat, J.R., Rozdilsky, B., Robinson, C. et al. (2002). Progressive supranuclear palsy diagnosis and confounding features: report on 16 autopsied cases. Mov Disord 17: 1255-1264.

Bishop, J.R., Schuksz, M. & Esko, J.D. (2007). Heparan sulphate proteoglycans fine-tune mammalian physiology. Nature 446: 1030-1037.

Blair, L.J., Frauen, H.D., Zhang, B., Nordhues, B.A., Bijan, S., Lin, Y.C. et al. (2015). Tau depletion prevents progressive blood-brain barrier damage in a mouse model of tauopathy. Acta Neuropathol Commun 3: 8.

Blancas-Mejia, L.M. & Ramirez-Alvarado, M. (2013). Systemic amyloidoses. Annu Rev Biochem 82: 745-774.

Blennow, K., Hardy, J. & Zetterberg, H. (2012). The neuropathology and neurobiology of traumatic brain injury. Neuron 76: 886-899.

Blennow, K., Wallin, A., Agren, H., Spenger, C., Siegfried, J. & Vanmechelen, E. (1995). Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer disease? Mol Chem Neuropathol 26: 231-245.

Boada, M., Antunez, C., Ramirez-Lorca, R., DeStefano, A.L., Gonzalez-Perez, A., Gayan, J. et al. (2014). ATP5H/KCTD2 locus is associated with Alzheimer's disease risk. Mol Psychiatry 19: 682-687.

Boeve, B.F. & Hutton, M. (2008). Refining frontotemporal dementia with parkinsonism linked to chromosome 17: introducing FTDP-17 (MAPT) and FTDP-17 (PGRN). Arch Neurol 65: 460-464.

Bolton, D.C., McKinley, M.P. & Prusiner, S.B. (1982). Identification of a protein that purifies with the scrapie prion. Science 218: 1309-1311.

Boutillier, S., Lannes, B., Buee, L., Delacourte, A., Rouaux, C., Mohr, M. et al. (2007). Sp3 and sp4 transcription factor levels are increased in brains of patients with Alzheimer's disease. Neurodegener Dis 4: 413-423.

Braak, E., Braak, H. & Mandelkow, E.M. (1994). A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads. Acta Neuropathol 87: 554-567.

Braak, H., Alafuzoff, I., Arzberger, T., Kretzschmar, H. & Del Tredici, K. (2006). Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol 112: 389-404.

Braak, H. & Braak, E. (1991). Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 82: 239-259.

Braak, H. & Braak, E. (1998). Argyrophilic grain disease: frequency of occurrence in different age categories and neuropathological diagnostic criteria. J Neural Transm 105: 801-819.

Braak, H. & Del Tredici, K. (2011). The pathological process underlying Alzheimer's disease in individuals under thirty. Acta Neuropathol 121: 171-181.

Bradshaw, E.M., Chibnik, L.B., Keenan, B.T., Ottoboni, L., Raj, T., Tang, A. et al. (2013). CD33 Alzheimer's disease locus: altered monocyte function and amyloid biology. Nat Neurosci 16: 848-850.

Brady, R.M., Zinkowski, R.P. & Binder, L.I. (1995). Presence of tau in isolated nuclei from human brain. Neurobiol Aging 16: 479-486.

Brandt, R., Leger, J. & Lee, G. (1995). Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain. J Cell Biol 131: 1327-1340.

Brettschneider, J., Del Tredici, K., Lee, V.M. & Trojanowski, J.Q. (2015). Spreading of pathology in neurodegenerative diseases: a focus on human studies. Nat Rev Neurosci 16: 109-120.

Bright, J., Hussain, S., Dang, V., Wright, S., Cooper, B., Byun, T. et al. (2015). Human secreted tau increases amyloid-beta production. Neurobiol Aging 36: 693-709.

Brouwers, N., Van Cauwenberghe, C., Engelborghs, S., Lambert, J.C., Bettens, K., Le Bastard, N. et al. (2012). Alzheimer risk associated with a copy number variation in the complement receptor 1 increasing C3b/C4b binding sites. Mol Psychiatry 17: 223-233.

Brown, P., Brandel, J.P., Sato, T., Nakamura, Y., MacKenzie, J., Will, R.G. et al. (2012). Iatrogenic Creutzfeldt-Jakob disease, final assessment. Emerg Infect Dis 18: 901-907.

Brunig, I., Scotti, E., Sidler, C. & Fritschy, J.M. (2002). Intact sorting, targeting, and clustering of gamma-aminobutyric acid A receptor subtypes in hippocampal neurons in vitro. J Comp Neurol 443: 43-55.

Bubien, J.K., Zhou, L.J., Bell, P.D., Frizzell, R.A. & Tedder, T.F. (1993). Transfection of the CD20 cell surface molecule into ectopic cell types generates a Ca2+ conductance found constitutively in B lymphocytes. J Cell Biol 121: 1121-1132.

Buee, L., Bussiere, T., Buee-Scherrer, V., Delacourte, A. & Hof, P.R. (2000). Tau protein isoforms, phosphorylation and role in neurodegenerative disorders. Brain Res Brain Res Rev 33: 95-130.

Bugiani, O., Murrell, J.R., Giaccone, G., Hasegawa, M., Ghigo, G., Tabaton, M. et al. (1999). Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in tau. J Neuropathol Exp Neurol 58: 667-677.

Bulbarelli, A., Lonati, E., Cazzaniga, E., Gregori, M. & Masserini, M. (2009). Pin1 affects Tau phosphorylation in response to Abeta oligomers. Mol Cell Neurosci 42: 75-80.

Busche, M.A., Eichhoff, G., Adelsberger, H., Abramowski, D., Wiederhold, K.H., Haass, C. et al. (2008). Clusters of hyperactive neurons near amyloid plaques in a mouse model of Alzheimer's disease. Science 321: 1686-1689.

Cai, D., Netzer, W.J., Zhong, M., Lin, Y., Du, G., Frohman, M. et al. (2006a). Presenilin-1 uses phospholipase D1 as a negative regulator of beta-amyloid formation. Proc Natl Acad Sci U S A 103: 1941-1946.

Cai, D., Zhong, M., Wang, R., Netzer, W.J., Shields, D., Zheng, H. et al. (2006b). Phospholipase D1 corrects impaired betaAPP trafficking and neurite outgrowth in familial Alzheimer's disease-linked presenilin-1 mutant neurons. Proc Natl Acad Sci U S A 103: 1936-1940.

Cai, G., Atzmon, G., Naj, A.C., Beecham, G.W., Barzilai, N., Haines, J.L. et al. (2012). Evidence against a role for rare ADAM10 mutations in sporadic Alzheimer disease. Neurobiol Aging 33: 416-417.e3.

Cairns, N.J., Bigio, E.H., Mackenzie, I.R., Neumann, M., Lee, V.M., Hatanpaa, K.J. et al. (2007). Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar

degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. Acta Neuropathol 114: 5-22.

Calafate, S., Buist, A., Miskiewicz, K., Vijayan, V., Daneels, G., de Strooper, B. et al. (2015). Synaptic Contacts Enhance Cell-to-Cell Tau Pathology Propagation. Cell Rep

Calero, M., Gomez-Ramos, A., Calero, O., Soriano, E., Avila, J. & Medina, M. (2015). Additional mechanisms conferring genetic susceptibility to Alzheimer's disease. Front Cell Neurosci 9: 138.

Calero, M., Rostagno, A., Matsubara, E., Zlokovic, B., Frangione, B. & Ghiso, J. (2000). Apolipoprotein J (clusterin) and Alzheimer's disease. Microsc Res Tech 50: 305-315.

Carrasquillo, M.M., Belbin, O., Hunter, T.A., Ma, L., Bisceglio, G.D., Zou, F. et al. (2010). Replication of CLU, CR1, and PICALM associations with alzheimer disease. Arch Neurol 67: 961-964.

Carrasquillo, M.M., Zou, F., Pankratz, V.S., Wilcox, S.L., Ma, L., Walker, L.P. et al. (2009). Genetic variation in PCDH11X is associated with susceptibility to late-onset Alzheimer's disease. Nat Genet 41: 192-198.

Castellano, J.M., Kim, J., Stewart, F.R., Jiang, H., DeMattos, R.B., Patterson, B.W. et al. (2011). Human apoE isoforms differentially regulate brain amyloid-beta peptide clearance. Sci Transl Med 3: 89ra57.

Chai, X., Dage, J.L. & Citron, M. (2012). Constitutive secretion of tau protein by an unconventional mechanism. Neurobiol Dis 48: 356-366.

Chan, S.L., Kim, W.S., Kwok, J.B., Hill, A.F., Cappai, R., Rye, K.A. et al. (2008). ATP-binding cassette transporter A7 regulates processing of amyloid precursor protein in vitro. J Neurochem 106: 793-804.

Chapuis, J., Hansmannel, F., Gistelinck, M., Mounier, A., Van Cauwenberghe, C., Kolen, K.V. et al. (2013). Increased expression of BIN1 mediates Alzheimer genetic risk by modulating tau pathology. Mol Psychiatry 18: 1225-1234.

Chartier-Harlin, M.C., Crawford, F., Houlden, H., Warren, A., Hughes, D., Fidani, L. et al. (1991). Early-onset Alzheimer's disease caused by mutations at codon 717 of the beta-amyloid precursor protein gene. Nature 353: 844-846.

Chau, M.F., Radeke, M.J., de Ines, C., Barasoain, I., Kohlstaedt, L.A. & Feinstein, S.C. (1998). The microtubule-associated protein tau cross-links to two distinct sites on each alpha and beta tubulin monomer via separate domains. Biochemistry 37: 17692-17703.

Cho, J.H. & Johnson, G.V. (2003). Glycogen synthase kinase 3beta phosphorylates tau at both primed and unprimed sites. Differential impact on microtubule binding. J Biol Chem 278: 187-193.

Choi-Miura, N.H., Ihara, Y., Fukuchi, K., Takeda, M., Nakano, Y., Tobe, T. et al. (1992). SP-40,40 is a constituent of Alzheimer's amyloid. Acta Neuropathol 83: 260-264.

Chouraki, V. & Seshadri, S. (2014). Genetics of Alzheimer's disease. Adv Genet 87: 245-294.

Chow, H.M., Guo, D., Zhou, J.C., Zhang, G.Y., Li, H.F., Herrup, K. et al. (2014). CDK5 activator protein p25 preferentially binds and activates GSK3beta. Proc Natl Acad Sci U S A 111: E4887-95.

Chuang, Y.-F., An, Y., Bilgel, M., Wong, D.F., Troncoso, J.C., O'Brien, R.J. et al. (2015). Midlife adiposity predicts earlier onset of Alzheimer's dementia, neuropathology and presymptomatic cerebral amyloid accumulation. Mol Psychiatry

Churcher, I. (2006). Tau therapeutic strategies for the treatment of Alzheimer's disease. Curr Top Med Chem 6: 579-595.

Citron, M., Oltersdorf, T., Haass, C., McConlogue, L., Hung, A.Y., Seubert, P. et al. (1992). Mutation of the beta-amyloid precursor protein in familial Alzheimer's disease increases beta-protein production. Nature 360: 672-674.

Clark, L.N., Poorkaj, P., Wszolek, Z., Geschwind, D.H., Nasreddine, Z.S., Miller, B. et al. (1998). Pathogenic implications of mutations in the tau gene in pallido-ponto-nigral degeneration and related neurodegenerative disorders linked to chromosome 17. Proc Natl Acad Sci U S A 95: 13103-13107.

Clavaguera, F., Akatsu, H., Fraser, G., Crowther, R.A., Frank, S., Hench, J. et al. (2013a). Brain homogenates from human tauopathies induce tau inclusions in mouse brain. Proc Natl Acad Sci U S A 110: 9535-9540.

Clavaguera, F., Bolmont, T., Crowther, R.A., Abramowski, D., Frank, S., Probst, A. et al. (2009). Transmission and spreading of tauopathy in transgenic mouse brain. Nat Cell Biol 11: 909-913.

Clavaguera, F., Hench, J., Goedert, M. & Tolnay, M. (2015). Invited review: Prion-like transmission and spreading of tau pathology. Neuropathol Appl Neurobiol 41: 47-58.

Clavaguera, F., Hench, J., Lavenir, I., Schweighauser, G., Frank, S., Goedert, M. et al. (2014). Peripheral administration of tau aggregates triggers intracerebral tauopathy in transgenic mice. Acta Neuropathol 127: 299-301.

Clavaguera, F., Lavenir, I., Falcon, B., Frank, S., Goedert, M. & Tolnay, M. (2013b). "Prion-like" templated misfolding in tauopathies. Brain Pathol 23: 342-349.

Cochran, J.N., Rush, T., Buckingham, S.C. & Roberson, E.D. (2015). The Alzheimer's disease risk factor CD2AP maintains blood-brain barrier integrity. Hum Mol Genet

Cohen, T.J., Friedmann, D., Hwang, A.W., Marmorstein, R. & Lee, V.M. (2013). The microtubule-associated tau protein has intrinsic acetyltransferase activity. Nat Struct Mol Biol 20: 756-762.

Cohen, T.J., Guo, J.L., Hurtado, D.E., Kwong, L.K., Mills, I.P., Trojanowski, J.Q. et al. (2011). The acetylation of tau inhibits its function and promotes pathological tau aggregation. Nat Commun 2: 252.

Collinge, J. (2001). Prion diseases of humans and animals: their causes and molecular basis. Annu Rev Neurosci 24: 519-550.

Collinge, J., Whitfield, J., McKintosh, E., Beck, J., Mead, S., Thomas, D.J. et al. (2006). Kuru in the 21st century--an acquired human prion disease with very long incubation periods. Lancet 367: 2068-2074.

Colonna, M. (2003). TREMs in the immune system and beyond. Nat Rev Immunol 3: 445-453.

Cook, C., Carlomagno, Y., Gendron, T.F., Dunmore, J., Scheffel, K., Stetler, C. et al. (2014). Acetylation of the KXGS motifs in tau is a critical determinant in modulation of tau aggregation and clearance. Hum Mol Genet 23: 104-116.

Cook, C., Gendron, T.F., Scheffel, K., Carlomagno, Y., Dunmore, J., DeTure, M. et al. (2012). Loss of HDAC6, a novel CHIP substrate, alleviates abnormal tau accumulation. Hum Mol Genet 21: 2936-2945.

Cook, L.J., Ho, L.W., Taylor, A.E., Brayne, C., Evans, J.G., Xuereb, J. et al. (2004). Candidate gene association studies of the alpha 4 (CHRNA4) and beta 2 (CHRNB2) neuronal nicotinic acetylcholine receptor subunit genes in Alzheimer's disease. Neurosci Lett 358: 142-146.

Coon, K.D., Myers, A.J., Craig, D.W., Webster, J.A., Pearson, J.V., Lince, D.H. et al. (2007). A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease. J Clin Psychiatry 68: 613-618.

Corcoran, N.M., Martin, D., Hutter-Paier, B., Windisch, M., Nguyen, T., Nheu, L. et al. (2010). Sodium selenate specifically activates PP2A phosphatase, dephosphorylates tau and reverses memory deficits in an Alzheimer's disease model. J Clin Neurosci 17: 1025-1033.

Corder, E.H., Saunders, A.M., Risch, N.J., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C.J. et al. (1994). Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. Nat Genet 7: 180-184.

Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W. et al. (1993). Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261: 921-923.

Cormont, M., Meton, I., Mari, M., Monzo, P., Keslair, F., Gaskin, C. et al. (2003). CD2AP/CMS regulates endosome morphology and traffic to the degradative pathway through its interaction with Rab4 and c-Cbl. Traffic 4: 97-112.

Coulthard, M.G., Lickliter, J.D., Subanesan, N., Chen, K., Webb, G.C., Lowry, A.J. et al. (2001). Characterization of the Epha1 receptor tyrosine kinase: expression in epithelial tissues. Growth Factors 18: 303-317.

Crary, J.F., Trojanowski, J.Q., Schneider, J.A., Abisambra, J.F., Abner, E.L., Alafuzoff, I. et al. (2014). Primary age-related tauopathy (PART): a common pathology associated with human aging. Acta Neuropathol

Cripps, D., Thomas, S.N., Jeng, Y., Yang, F., Davies, P. & Yang, A.J. (2006). Alzheimer disease-specific conformation of hyperphosphorylated paired helical filament-Tau is polyubiquitinated through Lys-48, Lys-11, and Lys-6 ubiquitin conjugation. J Biol Chem 281: 10825-10838.

Crocker, P.R., Clark, E.A., Filbin, M., Gordon, S., Jones, Y., Kehrl, J.H. et al. (1998). Siglecs: a family of sialic-acid binding lectins. Glycobiology 8(2): v.

Crocker, P.R., Paulson, J.C. & Varki, A. (2007). Siglecs and their roles in the immune system. Nat Rev Immunol 7: 255-266.

Crocker, P.R. & Redelinghuys, P. (2008). Siglecs as positive and negative regulators of the immune system. Biochem Soc Trans 36: 1467-1471.

Cross, D.A., Alessi, D.R., Cohen, P., Andjelkovich, M. & Hemmings, B.A. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 378: 785-789.

Cruchaga, C., Karch, C.M., Jin, S.C., Benitez, B.A., Cai, Y., Guerreiro, R. et al. (2014). Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease. Nature 505: 550-554.

Cruchaga, C., Kauwe, J.S., Harari, O., Jin, S.C., Cai, Y., Karch, C.M. et al. (2013). GWAS of cerebrospinal fluid tau levels identifies risk variants for Alzheimer's disease. Neuron 78: 256-268.

Cruz, J.C. & Tsai, L.H. (2004a). A Jekyll and Hyde kinase: roles for Cdk5 in brain development and disease. Curr Opin Neurobiol 14: 390-394.

Cruz, J.C. & Tsai, L.H. (2004b). Cdk5 deregulation in the pathogenesis of Alzheimer's disease. Trends Mol Med 10: 452-458.

Cuyvers, E., Bettens, K., Philtjens, S., Van Langenhove, T., Gijselinck, I., van der Zee, J. et al. (2014). Investigating the role of rare heterozygous TREM2 variants in Alzheimer's disease and frontotemporal dementia. Neurobiol Aging 35: 726.e11-726.e19.

D'Souza, I., Poorkaj, P., Hong, M., Nochlin, D., Lee, V.M., Bird, T.D. et al. (1999). Missense and silent tau gene mutations cause frontotemporal dementia with parkinsonism-chromosome 17 type, by affecting multiple alternative RNA splicing regulatory elements. Proc Natl Acad Sci U S A 96: 5598-5603.

D'Souza, I. & Schellenberg, G.D. (2002). tau Exon 10 expression involves a bipartite intron 10 regulatory sequence and weak 5' and 3' splice sites. J Biol Chem 277: 26587-26599.

de Calignon, A., Polydoro, M., Suarez-Calvet, M., William, C., Adamowicz, D.H., Kopeikina, K.J. et al. (2012). Propagation of tau pathology in a model of early Alzheimer's disease. Neuron 73: 685-697.

De Jager, P.L., Srivastava, G., Lunnon, K., Burgess, J., Schalkwyk, L.C., Yu, L. et al. (2014). Alzheimer's disease: early alterations in brain DNA methylation at ANK1, BIN1, RHBDF2 and other loci. Nat Neurosci 17: 1156-1163.

Delacourte, A., Robitaille, Y., Sergeant, N., Buee, L., Hof, P.R., Wattez, A. et al. (1996). Specific pathological Tau protein variants characterize Pick's disease. J Neuropathol Exp Neurol 55: 159-168.

Delobel, P., Flament, S., Hamdane, M., Mailliot, C., Sambo, A.V., Begard, S. et al. (2002). Abnormal Tau phosphorylation of the Alzheimer-type also occurs during mitosis. J Neurochem 83: 412-420.

Di Fede, G., Catania, M., Morbin, M., Rossi, G., Suardi, S., Mazzoleni, G. et al. (2009). A recessive mutation in the APP gene with dominant-negative effect on amyloidogenesis. Science 323: 1473-1477.

Dickson, D.W. (1999). Neuropathologic differentiation of progressive supranuclear palsy and corticobasal degeneration. J Neurol 246 Suppl 2: II6-15.

Dickson, D.W., Bergeron, C., Chin, S.S., Duyckaerts, C., Horoupian, D., Ikeda, K. et al. (2002). Office of Rare Diseases neuropathologic criteria for corticobasal degeneration. J Neuropathol Exp Neurol 61: 935-946.

Dickson, D.W., Rademakers, R. & Hutton, M.L. (2007). Progressive supranuclear palsy: pathology and genetics. Brain Pathol 17: 74-82.

Diedrich, J.F., Minnigan, H., Carp, R.I., Whitaker, J.N., Race, R., Frey, W.n. et al. (1991). Neuropathological changes in scrapie and Alzheimer's disease are associated with increased expression of apolipoprotein E and cathepsin D in astrocytes. J Virol 65: 4759-4768.

DiFiglia, M., Sapp, E., Chase, K.O., Davies, S.W., Bates, G.P., Vonsattel, J.P. et al. (1997). Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277: 1990-1993.

Donahue, C.P., Muratore, C., Wu, J.Y., Kosik, K.S. & Wolfe, M.S. (2006). Stabilization of the tau exon 10 stem loop alters pre-mRNA splicing. J Biol Chem 281: 23302-23306.

Dreyling, M.H., Martinez-Climent, J.A., Zheng, M., Mao, J., Rowley, J.D. & Bohlander, S.K. (1996). The t(10;11)(p13;q14) in the U937 cell line results in the fusion of the AF10 gene and CALM, encoding a new member of the AP-3 clathrin assembly protein family. Proc Natl Acad Sci U S A 93: 4804-4809.

Dujardin, S., Begard, S., Caillierez, R., Lachaud, C., Delattre, L., Carrier, S. et al. (2014a). Ectosomes: a new mechanism for non-exosomal secretion of tau protein. PLoS One 9: e100760.

Dujardin, S., Lecolle, K., Caillierez, R., Begard, S., Zommer, N., Lachaud, C. et al. (2014b). Neuron-to-neuron wild-type Tau protein transfer through a trans-synaptic mechanism: relevance to sporadic tauopathies. Acta Neuropathol Commun 2: 14.

Dustin, M.L., Olszowy, M.W., Holdorf, A.D., Li, J., Bromley, S., Desai, N. et al. (1998). A novel adaptor protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. Cell 94: 667-677.

Edbauer, D., Winkler, E., Regula, J.T., Pesold, B., Steiner, H. & Haass, C. (2003). Reconstitution of gamma-secretase activity. Nat Cell Biol 5: 486-488.

Eikelenboom, P. & Stam, F.C. (1982). Immunoglobulins and complement factors in senile plaques. An immunoperoxidase study. Acta Neuropathol 57: 239-242.

Eisenberg, D. & Jucker, M. (2012). The amyloid state of proteins in human diseases. Cell 148: 1188-1203.

Eperon, L.P., Graham, I.R., Griffiths, A.D. & Eperon, I.C. (1988). Effects of RNA secondary structure on alternative splicing of pre-mRNA: is folding limited to a region behind the transcribing RNA polymerase? Cell 54: 393-401.

Esch, F.S., Keim, P.S., Beattie, E.C., Blacher, R.W., Culwell, A.R., Oltersdorf, T. et al. (1990). Cleavage of amyloid beta peptide during constitutive processing of its precursor. Science 248: 1122-1124.

Essrich, C., Lorez, M., Benson, J.A., Fritschy, J.M. & Luscher, B. (1998). Postsynaptic clustering of major GABAA receptor subtypes requires the gamma 2 subunit and gephyrin. Nat Neurosci 1: 563-571.

Falcone, S., Cocucci, E., Podini, P., Kirchhausen, T., Clementi, E. & Meldolesi, J. (2006). Macropinocytosis: regulated coordination of endocytic and exocytic membrane traffic events. J Cell Sci 119: 4758-4769.

Fang, X., Yu, S.X., Lu, Y., Bast, R.C.J., Woodgett, J.R. & Mills, G.B. (2000). Phosphorylation and inactivation of glycogen synthase kinase 3 by protein kinase A. Proc Natl Acad Sci U S A 97: 11960-11965.

Farrer, L.A., Cupples, L.A., Haines, J.L., Hyman, B., Kukull, W.A., Mayeux, R. et al. (1997). Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 278: 1349-1356.

Feany, M.B. & Dickson, D.W. (1995). Widespread cytoskeletal pathology characterizes corticobasal degeneration. Am J Pathol 146: 1388-1396.

Ferrer, I., Hernandez, I., Boada, M., Llorente, A., Rey, M.J., Cardozo, A. et al. (2003). Primary progressive aphasia as the initial manifestation of corticobasal degeneration and unusual tauopathies. Acta Neuropathol 106: 419-435.

Ferrer, I., Santpere, G. & van Leeuwen, F.W. (2008). Argyrophilic grain disease. Brain 131: 1416-1432.

Fevrier, B. & Raposo, G. (2004). Exosomes: endosomal-derived vesicles shipping extracellular messages. Curr Opin Cell Biol 16: 415-421.

Fine, D., Flusser, H., Markus, B., Shorer, Z., Gradstein, L., Khateeb, S. et al. (2014). A syndrome of congenital microcephaly, intellectual disability and dysmorphism with a homozygous mutation in FRMD4A. Eur J Hum Genet

Fischer, D.F., De Vos, R.A., Van Dijk, R., De Vrij, F.M., Proper, E.A., Sonnemans, M.A. et al. (2003). Disease-specific accumulation of mutant ubiquitin as a marker for proteasomal dysfunction in the brain. FASEB J 17: 2014-2024.

Fleming, L.M., Weisgraber, K.H., Strittmatter, W.J., Troncoso, J.C. & Johnson, G.V. (1996). Differential binding of apolipoprotein E isoforms to tau and other cytoskeletal proteins. Exp Neurol 138: 252-260.

Fogel, H., Frere, S., Segev, O., Bharill, S., Shapira, I., Gazit, N. et al. (2014). APP homodimers transduce an amyloid-beta-mediated increase in release probability at excitatory synapses. Cell Rep 7: 1560-1576.

Forman, M.S. (2004). Genotype-phenotype correlations in FTDP-17: does form follow function? Exp Neurol 187: 229-234.

Francis, R., McGrath, G., Zhang, J., Ruddy, D.A., Sym, M., Apfeld, J. et al. (2002). aph-1 and pen-2 are required for Notch pathway signaling, gamma-secretase cleavage of betaAPP, and presenilin protein accumulation. Dev Cell 3: 85-97.

Friedhoff, P., von Bergen, M., Mandelkow, E.M., Davies, P. & Mandelkow, E. (1998). A nucleated assembly mechanism of Alzheimer paired helical filaments. Proc Natl Acad Sci U S A 95: 15712-15717.

Frost, B., Jacks, R.L. & Diamond, M.I. (2009a). Propagation of tau misfolding from the outside to the inside of a cell. J Biol Chem 284: 12845-12852.

Frost, B., Ollesch, J., Wille, H. & Diamond, M.I. (2009b). Conformational diversity of wild-type Tau fibrils specified by templated conformation change. J Biol Chem 284: 3546-3551.

Fu, H., Subramanian, R.R. & Masters, S.C. (2000). 14-3-3 proteins: structure, function, and regulation. Annu Rev Pharmacol Toxicol 40: 617-647.

Fu, Y.J., Nishihira, Y., Kuroda, S., Toyoshima, Y., Ishihara, T., Shinozaki, M. et al. (2010). Sporadic four-repeat tauopathy with frontotemporal lobar degeneration, Parkinsonism, and motor neuron disease: a distinct clinicopathological and biochemical disease entity. Acta Neuropathol 120: 21-32.

Funk, K.E., Thomas, S.N., Schafer, K.N., Cooper, G.L., Liao, Z., Clark, D.J. et al. (2014). Lysine methylation is an endogenous post-translational modification of tau protein in human brain and a modulator of aggregation propensity. Biochem J 462: 77-88.

Furukawa, Y., Kaneko, K., Yamanaka, K. & Nukina, N. (2010). Mutation-dependent polymorphism of Cu,Zn-superoxide dismutase aggregates in the familial form of amyotrophic lateral sclerosis. J Biol Chem 285: 22221-22231.

Galas, M.C., Dourlen, P., Begard, S., Ando, K., Blum, D., Hamdane, M. et al. (2006). The peptidylprolyl cis/trans-isomerase Pin1 modulates stress-induced dephosphorylation

of Tau in neurons. Implication in a pathological mechanism related to Alzheimer disease. J Biol Chem 281: 19296-19304.

Galpern, W.R. & Lang, A.E. (2006). Interface between tauopathies and synucleinopathies: a tale of two proteins. Ann Neurol 59: 449-458.

Garcia de Ancos, J., Correas, I. & Avila, J. (1993). Differences in microtubule binding and self-association abilities of bovine brain tau isoforms. J Biol Chem 268: 7976-7982.

Gasque, P., Ischenko, A., Legoedec, J., Mauger, C., Schouft, M.T. & Fontaine, M. (1993). Expression of the complement classical pathway by human glioma in culture. A model for complement expression by nerve cells. J Biol Chem 268: 25068-25074.

Gendreau, K.L. & Hall, G.F. (2013). Tangles, Toxicity, and Tau Secretion in AD - New Approaches to a Vexing Problem. Front Neurol 4: 160.

Gendron, T.F. & Petrucelli, L. (2009). The role of tau in neurodegeneration. Mol Neurodegener 4: 13.

Genin, E., Hannequin, D., Wallon, D., Sleegers, K., Hiltunen, M., Combarros, O. et al. (2011). APOE and Alzheimer disease: a major gene with semi-dominant inheritance. Mol Psychiatry 16: 903-907.

Gerlai, R. (2001). Eph receptors and neural plasticity. Nat Rev Neurosci 2: 205-209.

Ghetti, B., Oblak, A.L., Boeve, B.F., Johnson, K.A., Dickerson, B.C. & Goedert, M. (2015). Invited review: Frontotemporal dementia caused by microtubule-associated protein tau gene (MAPT) mutations: a chameleon for neuropathology and neuroimaging. Neuropathol Appl Neurobiol 41: 24-46.

Gibson, G. (2010). Hints of hidden heritability in GWAS. Nat Genet 42: 558-560.

Gibson, G. (2011). Rare and common variants: twenty arguments. Nat Rev Genet 13: 135-145.

Giraldo, M., Lopera, F., Siniard, A.L., Corneveaux, J.J., Schrauwen, I., Carvajal, J. et al. (2013). Variants in triggering receptor expressed on myeloid cells 2 are associated with both behavioral variant frontotemporal lobar degeneration and Alzheimer's disease. Neurobiol Aging 34: 2077.e11-2077.e18.

Glenner, G.G. & Wong, C.W. (1984a). Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. Biochem Biophys Res Commun 122: 1131-1135.

Glenner, G.G. & Wong, C.W. (1984b). Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem Biophys Res Commun 120: 885-890.

Goate, A., Chartier-Harlin, M.C., Mullan, M., Brown, J., Crawford, F., Fidani, L. et al. (1991). Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349: 704-706.

Goedert, M. & Jakes, R. (1990). Expression of separate isoforms of human tau protein: correlation with the tau pattern in brain and effects on tubulin polymerization. EMBO J 9: 4225-4230.

Goedert, M., Jakes, R., Crowther, R.A., Six, J., Lubke, U., Vandermeeren, M. et al. (1993). The abnormal phosphorylation of tau protein at Ser-202 in Alzheimer disease recapitulates phosphorylation during development. Proc Natl Acad Sci U S A 90: 5066-5070.

Goedert, M., Spillantini, M.G., Jakes, R., Rutherford, D. & Crowther, R.A. (1989a). Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. Neuron 3: 519-526.

Goedert, M., Spillantini, M.G., Potier, M.C., Ulrich, J. & Crowther, R.A. (1989b). Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. EMBO J 8: 393-399.

Goldgaber, D., Lerman, M.I., McBride, O.W., Saffiotti, U. & Gajdusek, D.C. (1987). Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. Science 235: 877-880.

Gong, C.X. & Iqbal, K. (2008). Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. Curr Med Chem 15: 2321-2328.

Gong, C.X., Liu, F., Grundke-Iqbal, I. & Iqbal, K. (2005). Post-translational modifications of tau protein in Alzheimer's disease. J Neural Transm 112: 813-838.

Gonzalez Murcia, J.D., Schmutz, C., Munger, C., Perkes, A., Gustin, A., Peterson, M. et al. (2013). Assessment of TREM2 rs75932628 association with Alzheimer's disease in a population-based sample: the Cache County Study. Neurobiol Aging 34: 2889.e11-2889.e13.

Goode, B.L. & Feinstein, S.C. (1994). Identification of a novel microtubule binding and assembly domain in the developmentally regulated inter-repeat region of tau. J Cell Biol 124: 769-782.

Gorno-Tempini, M.L., Murray, R.C., Rankin, K.P., Weiner, M.W. & Miller, B.L. (2004). Clinical, cognitive and anatomical evolution from nonfluent progressive aphasia to corticobasal syndrome: a case report. Neurocase 10: 426-436.

Griciuc, A., Serrano-Pozo, A., Parrado, A.R., Lesinski, A.N., Asselin, C.N., Mullin, K. et al. (2013). Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. Neuron 78: 631-643.

Grimes, C.A. & Jope, R.S. (2001). The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. Prog Neurobiol 65: 391-426.

Grimes, D.A., Lang, A.E. & Bergeron, C.B. (1999). Dementia as the most common presentation of cortical-basal ganglionic degeneration. Neurology 53: 1969-1974.

Grinberg, L.T., Wang, X., Wang, C., Sohn, P.D., Theofilas, P., Sidhu, M. et al. (2013). Argyrophilic grain disease differs from other tauopathies by lacking tau acetylation. Acta Neuropathol 125: 581-593.

Grover, A., Houlden, H., Baker, M., Adamson, J., Lewis, J., Prihar, G. et al. (1999). 5' splice site mutations in tau associated with the inherited dementia FTDP-17 affect a stem-loop structure that regulates alternative splicing of exon 10. J Biol Chem 274: 15134-15143.

Grundke-Iqbal, I., Iqbal, K., Tung, Y.C., Quinlan, M., Wisniewski, H.M. & Binder, L.I. (1986). Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci U S A 83: 4913-4917.

Grupe, A., Abraham, R., Li, Y., Rowland, C., Hollingworth, P., Morgan, A. et al. (2007). Evidence for novel susceptibility genes for late-onset Alzheimer's disease from a genome-wide association study of putative functional variants. Hum Mol Genet 16: 865-873.

Gu, Y., Misonou, H., Sato, T., Dohmae, N., Takio, K. & Ihara, Y. (2001). Distinct intramembrane cleavage of the beta-amyloid precursor protein family resembling gamma-secretase-like cleavage of Notch. J Biol Chem 276: 35235-35238.

Guerreiro, R., Wojtas, A., Bras, J., Carrasquillo, M., Rogaeva, E., Majounie, E. et al. (2013a). TREM2 variants in Alzheimer's disease. N Engl J Med 368: 117-127.

Guerreiro, R.J., Beck, J., Gibbs, J.R., Santana, I., Rossor, M.N., Schott, J.M. et al. (2010). Genetic variability in CLU and its association with Alzheimer's disease. PLoS One 5: e9510.

Guerreiro, R.J., Lohmann, E., Bras, J.M., Gibbs, J.R., Rohrer, J.D., Gurunlian, N. et al. (2013b). Using exome sequencing to reveal mutations in TREM2 presenting as a frontotemporal dementia-like syndrome without bone involvement. JAMA Neurol 70: 78-84.

Gunaratne, A., Thai, B.L. & Di Guglielmo, G.M. (2013). Atypical protein kinase C phosphorylates Par6 and facilitates transforming growth factor beta-induced epithelial-to-mesenchymal transition. Mol Cell Biol 33: 874-886.

Guo, J.L., Covell, D.J., Daniels, J.P., Iba, M., Stieber, A., Zhang, B. et al. (2013). Distinct alpha-Synuclein Strains Differentially Promote Tau Inclusions in Neurons. Cell 154: 103-117.

Guo, J.L. & Lee, V.M. (2011). Seeding of normal Tau by pathological Tau conformers drives pathogenesis of Alzheimer-like tangles. J Biol Chem 286: 15317-15331.

Guo, J.L. & Lee, V.M. (2014). Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. Nat Med 20: 130-138.

Guo, Z., Cupples, L.A., Kurz, A., Auerbach, S.H., Volicer, L., Chui, H. et al. (2000). Head injury and the risk of AD in the MIRAGE study. Neurology 54: 1316-1323.

Haass, C., Hung, A.Y., Schlossmacher, M.G., Teplow, D.B. & Selkoe, D.J. (1993). beta-Amyloid peptide and a 3-kDa fragment are derived by distinct cellular mechanisms. J Biol Chem 268: 3021-3024.

Haass, C., Kaether, C., Thinakaran, G. & Sisodia, S. (2012). Trafficking and proteolytic processing of APP. Cold Spring Harb Perspect Med 2: a006270.

Haass, C. & Selkoe, D.J. (2007). Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide. Nat Rev Mol Cell Biol 8: 101-112.

Hafner, M., Schmitz, A., Grune, I., Srivatsan, S.G., Paul, B., Kolanus, W. et al. (2006). Inhibition of cytohesins by SecinH3 leads to hepatic insulin resistance. Nature 444: 941-944.

Hampel, H., Blennow, K., Shaw, L.M., Hoessler, Y.C., Zetterberg, H. & Trojanowski, J.Q. (2010). Total and phosphorylated tau protein as biological markers of Alzheimer's disease. Exp Gerontol 45: 30-40.

Hardy, J. & Allsop, D. (1991). Amyloid deposition as the central event in the aetiology of Alzheimer's disease. Trends Pharmacol Sci 12: 383-388.

Hardy, J. & Selkoe, D.J. (2002). The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 297: 353-356.

Hardy, J. & Singleton, A. (2009). Genomewide association studies and human disease. N Engl J Med 360: 1759-1768.

Hardy, J.A. & Higgins, G.A. (1992). Alzheimer's disease: the amyloid cascade hypothesis. Science 256: 184-185.

Harel, A., Wu, F., Mattson, M.P., Morris, C.M. & Yao, P.J. (2008). Evidence for CALM in directing VAMP2 trafficking. Traffic 9: 417-429.

Harold, D., Abraham, R., Hollingworth, P., Sims, R., Gerrish, A., Hamshere, M.L. et al. (2009). Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 41: 1088-1093.

Hasegawa, M., Smith, M.J. & Goedert, M. (1998). Tau proteins with FTDP-17 mutations have a reduced ability to promote microtubule assembly. FEBS Lett 437: 207-210.

Hashiguchi, M., Sobue, K. & Paudel, H.K. (2000). 14-3-3zeta is an effector of tau protein phosphorylation. J Biol Chem 275: 25247-25254.

Hazrati, L.N., Van Cauwenberghe, C., Brooks, P.L., Brouwers, N., Ghani, M., Sato, C. et al. (2012). Genetic association of CR1 with Alzheimer's disease: a tentative disease mechanism. Neurobiol Aging 33: 2949.e5-2949.e12.

Heilbronner, G., Eisele, Y.S., Langer, F., Kaeser, S.A., Novotny, R., Nagarathinam, A. et al. (2013). Seeded strain-like transmission of beta-amyloid morphotypes in APP transgenic mice. EMBO Rep 14: 1017-1022.

Heilmann, S., Drichel, D., Clarimon, J., Fernandez, V., Lacour, A., Wagner, H. et al. (2015). PLD3 in non-familial Alzheimer's disease. Nature 520: E3-5.

Hernandez-Deviez, D.J., Casanova, J.E. & Wilson, J.M. (2002). Regulation of dendritic development by the ARF exchange factor ARNO. Nat Neurosci 5: 623-624.

Hernandez, F. & Avila, J. (2007). Tauopathies. Cell Mol Life Sci 64: 2219-2233.

Hernandez, F., Cuadros, R. & Avila, J. (2004). Zeta 14-3-3 protein favours the formation of human tau fibrillar polymers. Neurosci Lett 357: 143-146.

Hershko, A. & Ciechanover, A. (1992). The ubiquitin system for protein degradation. Annu Rev Biochem 61: 761-807.

Himpel, S., Panzer, P., Eirmbter, K., Czajkowska, H., Sayed, M., Packman, L.C. et al. (2001). Identification of the autophosphorylation sites and characterization of their effects in the protein kinase DYRK1A. Biochem J 359: 497-505.

Himpel, S., Tegge, W., Frank, R., Leder, S., Joost, H.G. & Becker, W. (2000). Specificity determinants of substrate recognition by the protein kinase DYRK1A. J Biol Chem 275: 2431-2438.

Hirokawa, N., Shiomura, Y. & Okabe, S. (1988). Tau proteins: the molecular structure and mode of binding on microtubules. J Cell Biol 107: 1449-1459.

Hollingworth, P., Harold, D., Sims, R., Gerrish, A., Lambert, J.C., Carrasquillo, M.M. et al. (2011). Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 43: 429-435.

Holmes, B.B., DeVos, S.L., Kfoury, N., Li, M., Jacks, R., Yanamandra, K. et al. (2013). Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. Proc Natl Acad Sci U S A 110: E3138-47.

Hong, M. (1998). Mutation-Specific Functional Impairments in Distinct Tau Isoforms of Hereditary FTDP-17. Science 282: 1914-1917.

Hooli, B.V., Lill, C.M., Mullin, K., Qiao, D., Lange, C., Bertram, L. et al. (2015). PLD3 gene variants and Alzheimer's disease. Nature 520: E7-8.

Horiguchi, T., Uryu, K., Giasson, B.I., Ischiropoulos, H., LightFoot, R., Bellmann, C. et al. (2003). Nitration of tau protein is linked to neurodegeneration in tauopathies. Am J Pathol 163: 1021-1031.

Horikoshi, Y., Suzuki, A., Yamanaka, T., Sasaki, K., Mizuno, K., Sawada, H. et al. (2009). Interaction between PAR-3 and the aPKC-PAR-6 complex is indispensable for apical domain development of epithelial cells. J Cell Sci 122: 1595-1606.

Horonchik, L., Tzaban, S., Ben-Zaken, O., Yedidia, Y., Rouvinski, A., Papy-Garcia, D. et al. (2005). Heparan sulfate is a cellular receptor for purified infectious prions. J Biol Chem 280: 17062-17067.

Hu, C.D., Chinenov, Y. & Kerppola, T.K. (2002). Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. Mol Cell 9: 789-798.

Humar, M., Pischke, S.E., Loop, T., Hoetzel, A., Schmidt, R., Klaas, C. et al. (2004). Barbiturates directly inhibit the calmodulin/calcineurin complex: a novel mechanism of inhibition of nuclear factor of activated T cells. Mol Pharmacol 65: 350-361.

Hutton, M., Lendon, C.L., Rizzu, P., Baker, M., Froelich, S., Houlden, H. et al. (1998). Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 393: 702-705.

Iba, M., Guo, J.L., McBride, J.D., Zhang, B., Trojanowski, J.Q. & Lee, V.M. (2013). Synthetic tau fibrils mediate transmission of neurofibrillary tangles in a transgenic mouse model of Alzheimer's-like tauopathy. J Neurosci 33: 1024-1037.

Ignatius, M.J., Gebicke-Haerter, P.J., Pitas, R.E. & Shooter, E.M. (1987). Apolipoprotein E in nerve injury and repair. Prog Brain Res 71: 177-184.

Ignatius, M.J., Gebicke-Harter, P.J., Skene, J.H., Schilling, J.W., Weisgraber, K.H., Mahley, R.W. et al. (1986). Expression of apolipoprotein E during nerve degeneration and regeneration. Proc Natl Acad Sci U S A 83: 1125-1129.

Ikeda, K., Akiyama, H., Arai, T., Matsushita, M., Tsuchiya, K. & Miyazaki, H. (2000). Clinical aspects of argyrophilic grain disease. Clin Neuropathol 19: 278-284.

Ikeda, K., Akiyama, H., Arai, T. & Nishimura, T. (1998). Glial tau pathology in neurodegenerative diseases: their nature and comparison with neuronal tangles. Neurobiol Aging 19: S85-91.

Ikeda, K., Akiyama, H., Arai, T., Oda, T., Kato, M., Iseki, E. et al. (1999). Clinical aspects of 'senile dementia of the tangle type'-- a subset of dementia in the senium separable from late-onset Alzheimer's disease. Dement Geriatr Cogn Disord 10: 6-11.

Ikeda, K., Akiyama, H., Kondo, H. & Haga, C. (1995). A study of dementia with argyrophilic grains. Possible cytoskeletal abnormality in dendrospinal portion of neurons and oligodendroglia. Acta Neuropathol 89: 409-414.

Ikeda, Y., Abe-Dohmae, S., Munehira, Y., Aoki, R., Kawamoto, S., Furuya, A. et al. (2003). Posttranscriptional regulation of human ABCA7 and its function for the apoA-I-dependent lipid release. Biochem Biophys Res Commun 311: 313-318.

Ikenouchi, J. & Umeda, M. (2010). FRMD4A regulates epithelial polarity by connecting Arf6 activation with the PAR complex. Proc Natl Acad Sci U S A 107: 748-753.

Insolera, R., Chen, S. & Shi, S.H. (2011). Par proteins and neuronal polarity. Dev Neurobiol 71: 483-494.

Iqbal, K., Alonso Adel, C., Chen, S., Chohan, M.O., El-Akkad, E., Gong, C.X. et al. (2005). Tau pathology in Alzheimer disease and other tauopathies. Biochim Biophys Acta 1739: 198-210.

Iqbal, K., Grundke-Iqbal, I., Smith, A.J., George, L., Tung, Y.C. & Zaidi, T. (1989). Identification and localization of a tau peptide to paired helical filaments of Alzheimer disease. Proc Natl Acad Sci U S A 86: 5646-5650.

Iqbal, K., Liu, F., Gong, C.X., Alonso Adel, C. & Grundke-Iqbal, I. (2009). Mechanisms of tau-induced neurodegeneration. Acta Neuropathol 118: 53-69.

Irwin, D.J., Cohen, T.J., Grossman, M., Arnold, S.E., Xie, S.X., Lee, V.M. et al. (2012). Acetylated tau, a novel pathological signature in Alzheimer's disease and other tauopathies. Brain 135: 807-818.

Ishibashi, K., Suzuki, M., Sasaki, S. & Imai, M. (2001). Identification of a new multigene four-transmembrane family (MS4A) related to CD20, HTm4 and beta subunit of the high-affinity IgE receptor. Gene 264: 87-93.

Ishiguro, K., Shiratsuchi, A., Sato, S., Omori, A., Arioka, M., Kobayashi, S. et al. (1993). Glycogen synthase kinase 3 beta is identical to tau protein kinase I generating several epitopes of paired helical filaments. FEBS Lett 325: 167-172.

Ittner, L.M., Ke, Y.D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J. et al. (2010). Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. Cell 142: 387-397.

Jacob, T.C., Moss, S.J. & Jurd, R. (2008). GABA(A) receptor trafficking and its role in the dynamic modulation of neuronal inhibition. Nat Rev Neurosci 9: 331-343.

Jandus, C., Simon, H.U. & von Gunten, S. (2011). Targeting siglecs--a novel pharmacological strategy for immuno- and glycotherapy. Biochem Pharmacol 82: 323-332.

Janocko, N.J., Brodersen, K.A., Soto-Ortolaza, A.I., Ross, O.A., Liesinger, A.M., Duara, R. et al. (2012). Neuropathologically defined subtypes of Alzheimer's disease differ significantly from neurofibrillary tangle-predominant dementia. Acta Neuropathol 124: 681-692.

Jaunmuktane, Z., Mead, S., Ellis, M., Wadsworth, J.D.F., Nicoll, A.J., Kenny, J. et al. (2015). Evidence for human transmission of amyloid-Î² pathology and cerebral amyloid angiopathy. Nature 525: 247-250.

Jay, T.R., Miller, C.M., Cheng, P.J., Graham, L.C., Bemiller, S., Broihier, M.L. et al. (2015). TREM2 deficiency eliminates TREM2+ inflammatory macrophages and ameliorates pathology in Alzheimer's disease mouse models. J Exp Med 212: 287-295.

Jehle, A.W., Gardai, S.J., Li, S., Linsel-Nitschke, P., Morimoto, K., Janssen, W.J. et al. (2006). ATP-binding cassette transporter A7 enhances phagocytosis of apoptotic cells and associated ERK signaling in macrophages. J Cell Biol 174: 547-556.

Jellinger, K.A. & Attems, J. (2007). Neurofibrillary tangle-predominant dementia: comparison with classical Alzheimer disease. Acta Neuropathol 113: 107-117.

Jenkins, G.M. & Frohman, M.A. (2005). Phospholipase D: a lipid centric review. Cell Mol Life Sci 62: 2305-2316.

Jiang, T., Tan, L., Zhu, X.C., Zhou, J.S., Cao, L., Tan, M.S. et al. (2015). Silencing of TREM2 exacerbates tau pathology, neurodegenerative changes, and spatial learning deficits in P301S tau transgenic mice. Neurobiol Aging 36: 3176-3186.

Jiang, T., Yu, J.T., Hu, N., Tan, M.S., Zhu, X.C. & Tan, L. (2014). CD33 in Alzheimer's disease. Mol Neurobiol 49: 529-535.

Jiang, Z., Cote, J., Kwon, J.M., Goate, A.M. & Wu, J.Y. (2000). Aberrant splicing of tau pre-mRNA caused by intronic mutations associated with the inherited dementia frontotemporal dementia with parkinsonism linked to chromosome 17. Mol Cell Biol 20: 4036-4048.

Jiao, B., Liu, X., Tang, B., Hou, L., Zhou, L., Zhang, F. et al. (2014). Investigation of TREM2, PLD3, and UNC5C variants in patients with Alzheimer's disease from mainland China. Neurobiol Aging 35: 2422.e9-2422.e11.

Jin, J.K., Ahn, B.H., Na, Y.J., Kim, J.I., Kim, Y.S., Choi, E.K. et al. (2007). Phospholipase D1 is associated with amyloid precursor protein in Alzheimer's disease. Neurobiol Aging 28: 1015-1027.

Joberty, G., Petersen, C., Gao, L. & Macara, I.G. (2000). The cell-polarity protein Par6 links Par3 and atypical protein kinase C to Cdc42. Nat Cell Biol 2: 531-539.

Johnston, J.A., Ward, C.L. & Kopito, R.R. (1998). Aggresomes: a cellular response to misfolded proteins. J Cell Biol 143: 1883-1898.

Jones, L., Holmans, P.A., Hamshere, M.L., Harold, D., Moskvina, V., Ivanov, D. et al. (2010). Genetic evidence implicates the immune system and cholesterol metabolism in the aetiology of Alzheimer's disease. PLoS One 5: e13950.

Jones, S.E. & Jomary, C. (2002). Clusterin. Int J Biochem Cell Biol 34: 427-431.

Jonsson, T., Atwal, J.K., Steinberg, S., Snaedal, J., Jonsson, P.V., Bjornsson, S. et al. (2012). A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. Nature 488: 96-99.

Jonsson, T., Stefansson, H., Steinberg, S., Jonsdottir, I., Jonsson, P.V., Snaedal, J. et al. (2013). Variant of TREM2 associated with the risk of Alzheimer's disease. N Engl J Med 368: 107-116.

Jope, R.S. & Johnson, G.V. (2004). The glamour and gloom of glycogen synthase kinase-3. Trends Biochem Sci 29: 95-102.

Josephs, K.A. (2015). Key emerging issues in progressive supranuclear palsy and corticobasal degeneration. J Neurol 262: 783-788.

Josephs, K.A., Duffy, J.R., Strand, E.A., Whitwell, J.L., Layton, K.F., Parisi, J.E. et al. (2006). Clinicopathological and imaging correlates of progressive aphasia and apraxia of speech. Brain 129: 1385-1398.

Josephs, K.A., Whitwell, J.L., Parisi, J.E., Knopman, D.S., Boeve, B.F., Geda, Y.E. et al. (2008). Argyrophilic grains: a distinct disease or an additive pathology? Neurobiol Aging 29: 566-573.

Jovanovic, J.N., Thomas, P., Kittler, J.T., Smart, T.G. & Moss, S.J. (2004). Brain-derived neurotrophic factor modulates fast synaptic inhibition by regulating GABA(A) receptor phosphorylation, activity, and cell-surface stability. J Neurosci 24: 522-530.

Jucker, M. & Walker, L.C. (2011). Pathogenic protein seeding in Alzheimer disease and other neurodegenerative disorders. Ann Neurol 70: 532-540.

Jucker, M. & Walker, L.C. (2013). Self-propagation of pathogenic protein aggregates in neurodegenerative diseases. Nature 501: 45-51.

Julien, C., Tremblay, C., Emond, V., Lebbadi, M., Salem, N.J., Bennett, D.A. et al. (2009). Sirtuin 1 reduction parallels the accumulation of tau in Alzheimer disease. J Neuropathol Exp Neurol 68: 48-58.

Jun, G., Ibrahim-Verbaas, C.A., Vronskaya, M., Lambert, J.C., Chung, J., Naj, A.C. et al. (2015). A novel Alzheimer disease locus located near the gene encoding tau protein. Mol Psychiatry

Kalbouneh, H., Schlicksupp, A., Kirsch, J. & Kuhse, J. (2014). Cyclin-dependent kinase 5 is involved in the phosphorylation of gephyrin and clustering of GABAA receptors at inhibitory synapses of hippocampal neurons. PLoS One 9: e104256.

Kaminski, W.E., Orso, E., Diederich, W., Klucken, J., Drobnik, W. & Schmitz, G. (2000). Identification of a novel human sterol-sensitive ATP-binding cassette transporter (ABCA7). Biochem Biophys Res Commun 273: 532-538.

Kampers, T., Friedhoff, P., Biernat, J., Mandelkow, E.M. & Mandelkow, E. (1996). RNA stimulates aggregation of microtubule-associated protein tau into Alzheimer-like paired helical filaments. FEBS Lett 399: 344-349.

Kang, J., Lemaire, H.G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.H. et al. (1987). The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. Nature 325: 733-736.

Kanmert, D., Cantlon, A., Muratore, C.R., Jin, M., O'Malley, T.T., Lee, G. et al. (2015). C-Terminally Truncated Forms of Tau, But Not Full-Length Tau or Its C-Terminal Fragments, Are Released from Neurons Independently of Cell Death. J Neurosci 35: 10851-10865.

Kar, S., Fan, J., Smith, M.J., Goedert, M. & Amos, L.A. (2003). Repeat motifs of tau bind to the insides of microtubules in the absence of taxol. EMBO J 22: 70-77.

Karch, C.M. & Goate, A.M. (2015). Alzheimer's disease risk genes and mechanisms of disease pathogenesis. Biol Psychiatry 77: 43-51.

Karch, C.M., Jeng, A.T. & Goate, A.M. (2012a). Extracellular Tau levels are influenced by variability in Tau that is associated with tauopathies. J Biol Chem 287: 42751-42762.

Karch, C.M., Jeng, A.T., Nowotny, P., Cady, J., Cruchaga, C. & Goate, A.M. (2012b). Expression of novel Alzheimer's disease risk genes in control and Alzheimer's disease brains. PLoS One 7: e50976.

Kehoe, P.G., Russ, C., McIlory, S., Williams, H., Holmans, P., Holmes, C. et al. (1999). Variation in DCP1, encoding ACE, is associated with susceptibility to Alzheimer disease. Nat Genet 21: 71-72.

Kelleher, I., Garwood, C., Hanger, D.P., Anderton, B.H. & Noble, W. (2007). Kinase activities increase during the development of tauopathy in htau mice. J Neurochem 103: 2256-2267.

Keller, J.N., Hanni, K.B. & Markesbery, W.R. (2000). Impaired proteasome function in Alzheimer's disease. J Neurochem 75: 436-439.

Kertesz, A., Martinez-Lage, P., Davidson, W. & Munoz, D.G. (2000). The corticobasal degeneration syndrome overlaps progressive aphasia and frontotemporal dementia. Neurology 55: 1368-1375.

Kim, D., Won, J., Shin, D.W., Kang, J., Kim, Y.J., Choi, S.Y. et al. (2004). Regulation of Dyrk1A kinase activity by 14-3-3. Biochem Biophys Res Commun 323: 499-504.

Kim, E.K. & Choi, E.J. (2010). Pathological roles of MAPK signaling pathways in human diseases. Biochim Biophys Acta 1802: 396-405.

Kim, J., Basak, J.M. & Holtzman, D.M. (2009a). The role of apolipoprotein E in Alzheimer's disease. Neuron 63: 287-303.

Kim, M., Suh, J., Romano, D., Truong, M.H., Mullin, K., Hooli, B. et al. (2009b). Potential late-onset Alzheimer's disease-associated mutations in the ADAM10 gene attenuate {alpha}-secretase activity. Hum Mol Genet 18: 3987-3996.

Kim, W., Lee, S. & Hall, G.F. (2010a). Secretion of human tau fragments resembling CSF-tau in Alzheimer's disease is modulated by the presence of the exon 2 insert. FEBS Lett 584: 3085-3088.

Kim, W., Lee, S., Jung, C., Ahmed, A., Lee, G. & Hall, G.F. (2010b). Interneuronal transfer of human tau between Lamprey central neurons in situ. J Alzheimers Dis 19: 647-664.

Kim, W.S., Guillemin, G.J., Glaros, E.N., Lim, C.K. & Garner, B. (2006). Quantitation of ATP-binding cassette subfamily-A transporter gene expression in primary human brain cells. Neuroreport 17: 891-896.

Kim, W.S., Li, H., Ruberu, K., Chan, S., Elliott, D.A., Low, J.K. et al. (2013). Deletion of Abca7 increases cerebral amyloid-beta accumulation in the J20 mouse model of Alzheimer's disease. J Neurosci 33: 4387-4394.

Klickstein, L.B., Bartow, T.J., Miletic, V., Rabson, L.D., Smith, J.A. & Fearon, D.T. (1988). Identification of distinct C3b and C4b recognition sites in the human C3b/C4b receptor (CR1, CD35) by deletion mutagenesis. J Exp Med 168: 1699-1717.

Knopman, D.S., Parisi, J.E., Salviati, A., Floriach-Robert, M., Boeve, B.F., Ivnik, R.J. et al. (2003). Neuropathology of cognitively normal elderly. J Neuropathol Exp Neurol 62: 1087-1095.

Komori, T. (1999). Tau-positive glial inclusions in progressive supranuclear palsy, corticobasal degeneration and Pick's disease. Brain Pathol 9: 663-679.

Kondo, A., Shahpasand, K., Mannix, R., Qiu, J., Moncaster, J., Chen, C.H. et al. (2015). Antibody against early driver of neurodegeneration cis P-tau blocks brain injury and tauopathy. Nature 523: 431-436.

Kopito, R.R. (2000). Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 10: 524-530.

Kopke, E., Tung, Y.C., Shaikh, S., Alonso, A.C., Iqbal, K. & Grundke-Iqbal, I. (1993). Microtubule-associated protein tau. Abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. J Biol Chem 268: 24374-24384.

Kosik, K.S., Joachim, C.L. & Selkoe, D.J. (1986). Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease. Proc Natl Acad Sci U S A 83: 4044-4048.

Kosik, K.S., Orecchio, L.D., Bakalis, S. & Neve, R.L. (1989). Developmentally regulated expression of specific tau sequences. Neuron 2: 1389-1397.

Kouri, N., Carlomagno, Y., Baker, M., Liesinger, A.M., Caselli, R.J., Wszolek, Z.K. et al. (2014). Novel mutation in MAPT exon 13 (p.N410H) causes corticobasal degeneration. Acta Neuropathol 127: 271-282.

Kouri, N., Ross, O.A., Dombroski, B., Younkin, C.S., Serie, D.J., Soto-Ortolaza, A. et al. (2015). Genome-wide association study of corticobasal degeneration identifies risk variants shared with progressive supranuclear palsy. Nat Commun 6: 7247.

Kouri, N., Whitwell, J.L., Josephs, K.A., Rademakers, R. & Dickson, D.W. (2011). Corticobasal degeneration: a pathologically distinct 4R tauopathy. Nat Rev Neurol 7: 263-272.

Kovacs, G.G. (2015). Invited review: Neuropathology of tauopathies: principles and practice. Neuropathol Appl Neurobiol 41: 3-23.

Kovacs, G.G., Majtenyi, K., Spina, S., Murrell, J.R., Gelpi, E., Hoftberger, R. et al. (2008). White matter tauopathy with globular glial inclusions: a distinct sporadic frontotemporal lobar degeneration. J Neuropathol Exp Neurol 67: 963-975.

Kovacs, G.G., Rozemuller, A.J., van Swieten, J.C., Gelpi, E., Majtenyi, K., Al-Sarraj, S. et al. (2013). Neuropathology of the hippocampus in FTLD-Tau with Pick bodies: a study of the BrainNet Europe Consortium. Neuropathol Appl Neurobiol 39: 166-178.

Krych-Goldberg, M., Moulds, J.M. & Atkinson, J.P. (2002). Human complement receptor type 1 (CR1) binds to a major malarial adhesin. Trends Mol Med 8: 531-537.

Kuhla, B., Haase, C., Flach, K., Luth, H.J., Arendt, T. & Munch, G. (2007). Effect of pseudophosphorylation and cross-linking by lipid peroxidation and advanced glycation end product precursors on tau aggregation and filament formation. J Biol Chem 282: 6984-6991.

Kuret, J., Johnson, G.S., Cha, D., Christenson, E.R., DeMaggio, A.J. & Hoekstra, M.F. (1997). Casein kinase 1 is tightly associated with paired-helical filaments isolated from Alzheimer's disease brain. J Neurochem 69: 2506-2515.

Kusakawa, G., Saito, T., Onuki, R., Ishiguro, K., Kishimoto, T. & Hisanaga, S. (2000). Calpain-dependent proteolytic cleavage of the p35 cyclin-dependent kinase 5 activator to p25. J Biol Chem 275: 17166-17172.

Lai, K.O. & Ip, N.Y. (2009). Synapse development and plasticity: roles of ephrin/Eph receptor signaling. Curr Opin Neurobiol 19: 275-283.

Lambert, J.C., Grenier-Boley, B., Bellenguez, C., Pasquier, F., Campion, D., Dartigues, J.F. et al. (2015). PLD3 and sporadic Alzheimer's disease risk. Nature 520: E1.

Lambert, J.C., Grenier-Boley, B., Harold, D., Zelenika, D., Chouraki, V., Kamatani, Y. et al. (2013a). Genome-wide haplotype association study identifies the FRMD4A gene as a risk locus for Alzheimer's disease. Mol Psychiatry 18: 461-470.

Lambert, J.C., Heath, S., Even, G., Campion, D., Sleegers, K., Hiltunen, M. et al. (2009). Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 41: 1094-1099.

Lambert, J.C., Ibrahim-Verbaas, C.A., Harold, D., Naj, A.C., Sims, R., Bellenguez, C. et al. (2013b). Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet

Lambert, J.C., Zelenika, D., Hiltunen, M., Chouraki, V., Combarros, O., Bullido, M.J. et al. (2011). Evidence of the association of BIN1 and PICALM with the AD risk in contrasting European populations. Neurobiol Aging 32: 756.e11-756.e15.

Landrieu, I., Smet-Nocca, C., Amniai, L., Louis, J.V., Wieruszeski, J.M., Goris, J. et al. (2011). Molecular implication of PP2A and Pin1 in the Alzheimer's disease specific hyperphosphorylation of Tau. PLoS One 6: e21521.

Langa, K.M. (2015). Is the risk of Alzheimer's disease and dementia declining? Alzheimers Res Ther 7: 34.

Lasagna-Reeves, C.A., Castillo-Carranza, D.L., Sengupta, U., Guerrero-Munoz, M.J., Kiritoshi, T., Neugebauer, V. et al. (2012). Alzheimer brain-derived tau oligomers propagate pathology from endogenous tau. Sci Rep 2: 700.

Lashley, T., Rohrer, J.D., Mead, S. & Revesz, T. (2015). Review: An update on clinical, genetic and pathological aspects of frontotemporal lobar degenerations. Neuropathol Appl Neurobiol

Lee, G., Neve, R.L. & Kosik, K.S. (1989). The microtubule binding domain of tau protein. Neuron 2: 1615-1624.

Lee, G., Thangavel, R., Sharma, V.M., Litersky, J.M., Bhaskar, K., Fang, S.M. et al. (2004). Phosphorylation of tau by fyn: implications for Alzheimer's disease. J Neurosci 24: 2304-2312.

Lee, J.H., Cheng, R., Honig, L.S., Vonsattel, J.P., Clark, L. & Mayeux, R. (2008). Association between genetic variants in SORL1 and autopsy-confirmed Alzheimer disease. Neurology 70: 887-889.

Lee, S., Kim, W., Li, Z. & Hall, G.F. (2012). Accumulation of vesicle-associated human tau in distal dendrites drives degeneration and tau secretion in an in situ cellular tauopathy model. Int J Alzheimers Dis 2012: 172837.

Lee, V.M., Goedert, M. & Trojanowski, J.Q. (2001). Neurodegenerative tauopathies. Annu Rev Neurosci 24: 1121-1159.

Lehtonen, S., Zhao, F. & Lehtonen, E. (2002). CD2-associated protein directly interacts with the actin cytoskeleton. Am J Physiol Renal Physiol 283: F734-43.

Leinonen, V., Koivisto, A.M., Savolainen, S., Rummukainen, J., Sutela, A., Vanninen, R. et al. (2012). Post-mortem findings in 10 patients with presumed normal-pressure hydrocephalus and review of the literature. Neuropathol Appl Neurobiol 38: 72-86.

Levy-Lahad, E., Wasco, W., Poorkaj, P., Romano, D.M., Oshima, J., Pettingell, W.H. et al. (1995a). Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 269: 973-977.

Levy-Lahad, E., Wijsman, E.M., Nemens, E., Anderson, L., Goddard, K.A., Weber, J.L. et al. (1995b). A familial Alzheimer's disease locus on chromosome 1. Science 269: 970-973.

Lew, J., Huang, Q.Q., Qi, Z., Winkfein, R.J., Aebersold, R., Hunt, T. et al. (1994). A brain-specific activator of cyclin-dependent kinase 5. Nature 371: 423-426.

Leyk, J., Goldbaum, O., Noack, M. & Richter-Landsberg, C. (2015). Inhibition of HDAC6 modifies tau inclusion body formation and impairs autophagic clearance. J Mol Neurosci 55: 1031-1046.

Li, H., Wetten, S., Li, L., St Jean, P.L., Upmanyu, R., Surh, L. et al. (2008). Candidate single-nucleotide polymorphisms from a genomewide association study of Alzheimer disease. Arch Neurol 65: 45-53.

Li, H.L., Wang, H.H., Liu, S.J., Deng, Y.Q., Zhang, Y.J., Tian, Q. et al. (2007). Phosphorylation of tau antagonizes apoptosis by stabilizing beta-catenin, a mechanism involved in Alzheimer's neurodegeneration. Proc Natl Acad Sci U S A 104: 3591-3596.

Li, L., Sengupta, A., Haque, N., Grundke-Iqbal, I. & Iqbal, K. (2004). Memantine inhibits and reverses the Alzheimer type abnormal hyperphosphorylation of tau and associated neurodegeneration. FEBS Lett 566: 261-269.

Liang, Y. & Tedder, T.F. (2001). Identification of a CD20-, FcepsilonRIbeta-, and HTm4-related gene family: sixteen new MS4A family members expressed in human and mouse. Genomics 72: 119-127.

Liang, Y., Buckley, T.R., Tu, L., Langdon, S.D. & Tedder, T.F. (2001). Structural organization of the human MS4A gene cluster on Chromosome 11q12. Immunogenetics 53: 357-368.

Lill, C.M., Rengmark, A., Pihlstrom, L., Fogh, I., Shatunov, A., Sleiman, P.M. et al. (2015). The role of TREM2 R47H as a risk factor for Alzheimer's disease, frontotemporal lobar degeneration, amyotrophic lateral sclerosis, and Parkinson's disease. Alzheimers Dement

Liou, Y.C., Ryo, A., Huang, H.K., Lu, P.J., Bronson, R., Fujimori, F. et al. (2002). Loss of Pin1 function in the mouse causes phenotypes resembling cyclin D1-null phenotypes. Proc Natl Acad Sci U S A 99: 1335-1340.

Liou, Y.C., Sun, A., Ryo, A., Zhou, X.Z., Yu, Z.X., Huang, H.K. et al. (2003). Role of the prolyl isomerase Pin1 in protecting against age-dependent neurodegeneration. Nature 424: 556-561.

Litvan, I., Agid, Y., Goetz, C., Jankovic, J., Wenning, G.K., Brandel, J.P. et al. (1997). Accuracy of the clinical diagnosis of corticobasal degeneration: a clinicopathologic study. Neurology 48: 119-125.

Liu, F., Grundke-Iqbal, I., Iqbal, K. & Gong, C.X. (2005). Contributions of protein phosphatases PP1, PP2A, PP2B and PP5 to the regulation of tau phosphorylation. Eur J Neurosci 22: 1942-1950.

Liu, F., Iqbal, K., Grundke-Iqbal, I., Hart, G.W. & Gong, C.X. (2004). O-GlcNAcylation regulates phosphorylation of tau: a mechanism involved in Alzheimer's disease. Proc Natl Acad Sci U S A 101: 10804-10809.

Liu, F., Li, B., Tung, E.J., Grundke-Iqbal, I., Iqbal, K. & Gong, C.X. (2007). Site-specific effects of tau phosphorylation on its microtubule assembly activity and self-aggregation. Eur J Neurosci 26: 3429-3436.

Liu, F., Zaidi, T., Iqbal, K., Grundke-Iqbal, I. & Gong, C.X. (2002a). Aberrant glycosylation modulates phosphorylation of tau by protein kinase A and dephosphorylation of tau by protein phosphatase 2A and 5. Neuroscience 115: 829-837.

Liu, F., Zaidi, T., Iqbal, K., Grundke-Iqbal, I., Merkle, R.K. & Gong, C.X. (2002b). Role of glycosylation in hyperphosphorylation of tau in Alzheimer's disease. FEBS Lett 512: 101-106.

Liu, L., Drouet, V., Wu, J.W., Witter, M.P., Small, S.A., Clelland, C. et al. (2012). Trans-synaptic spread of tau pathology in vivo. PLoS One 7: e31302.

Liu, S.J., Zhang, A.H., Li, H.L., Wang, Q., Deng, H.M., Netzer, W.J. et al. (2003). Overactivation of glycogen synthase kinase-3 by inhibition of phosphoinositol-3 kinase and protein kinase C leads to hyperphosphorylation of tau and impairment of spatial memory. Journal of Neurochemistry 87: 1333-1344.

Liu, S.J., Zhang, J.Y., Li, H.L., Fang, Z.Y., Wang, Q., Deng, H.M. et al. (2004). Tau becomes a more favorable substrate for GSK-3 when it is prephosphorylated by PKA in rat brain. J Biol Chem 279: 50078-50088.

Lord, J. & Cruchaga, C. (2014). The epigenetic landscape of Alzheimer's disease. Nat Neurosci 17: 1138-1140.

Lord, J., Lu, A.J. & Cruchaga, C. (2014). Identification of rare variants in Alzheimer's disease. Front Genet 5: 369.

Lu, J.X., Qiang, W., Yau, W.M., Schwieters, C.D., Meredith, S.C. & Tycko, R. (2013). Molecular structure of beta-amyloid fibrils in Alzheimer's disease brain tissue. Cell 154: 1257-1268.

Lu, K.P. (2004). Pinning down cell signaling, cancer and Alzheimer's disease. Trends Biochem Sci 29: 200-209.

Lu, K.P., Finn, G., Lee, T.H. & Nicholson, L.K. (2007). Prolyl cis-trans isomerization as a molecular timer. Nat Chem Biol 3: 619-629.

Lu, K.P., Hanes, S.D. & Hunter, T. (1996). A human peptidyl-prolyl isomerase essential for regulation of mitosis. Nature 380: 544-547.

Lu, K.P., Liou, Y.C. & Zhou, X.Z. (2002a). Pinning down proline-directed phosphorylation signaling. Trends Cell Biol 12: 164-172.

Lu, M. & Kosik, K.S. (2001). Competition for microtubule-binding with dual expression of tau missense and splice isoforms. Mol Biol Cell 12: 171-184.

Lu, P.J., Wulf, G., Zhou, X.Z., Davies, P. & Lu, K.P. (1999). The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. Nature 399: 784-788.

Lu, P.J., Zhou, X.Z., Liou, Y.C., Noel, J.P. & Lu, K.P. (2002b). Critical role of WW domain phosphorylation in regulating phosphoserine binding activity and Pin1 function. J Biol Chem 277: 2381-2384.

Luk, K.C., Kehm, V., Carroll, J., Zhang, B., O'Brien, P., Trojanowski, J.Q. et al. (2012a). Pathological alpha-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science 338: 949-953.

Luk, K.C., Kehm, V.M., Zhang, B., O'Brien, P., Trojanowski, J.Q. & Lee, V.M. (2012b). Intracerebral inoculation of pathological alpha-synuclein initiates a rapidly progressive neurodegenerative alpha-synucleinopathy in mice. J Exp Med 209: 975-986.

Luna-Munoz, J., Chavez-Macias, L., Garcia-Sierra, F. & Mena, R. (2007). Earliest stages of tau conformational changes are related to the appearance of a sequence of specific phospho-dependent tau epitopes in Alzheimer's disease. J Alzheimers Dis 12: 365-375.

Lunnon, K., Smith, R., Hannon, E., De Jager, P.L., Srivastava, G., Volta, M. et al. (2014). Methylomic profiling implicates cortical deregulation of ANK1 in Alzheimer's disease. Nat Neurosci 17: 1164-1170.

Lupton, M.K., Proitsi, P., Lin, K., Hamilton, G., Daniilidou, M., Tsolaki, M. et al. (2014). The role of ABCA1 gene sequence variants on risk of Alzheimer's disease. J Alzheimers Dis 38: 897-906.

Luscher, B., Fuchs, T. & Kilpatrick, C.L. (2011). GABAA receptor trafficking-mediated plasticity of inhibitory synapses. Neuron 70: 385-409.

Ma, S.L., Pastorino, L., Zhou, X.Z. & Lu, K.P. (2012). Prolyl isomerase Pin1 promotes amyloid precursor protein (APP) turnover by inhibiting glycogen synthase kinase-3beta (GSK3beta) activity: novel mechanism for Pin1 to protect against Alzheimer disease. J Biol Chem 287: 6969-6973.

Mackenzie, I.R., Neumann, M., Bigio, E.H., Cairns, N.J., Alafuzoff, I., Kril, J. et al. (2009). Nomenclature for neuropathologic subtypes of frontotemporal lobar degeneration: consensus recommendations. Acta Neuropathol 117: 15-18.

Mahley, R.W. (1988). Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. Science 240: 622-630.

Mak, A.C., Pullinger, C.R., Tang, L.F., Wong, J.S., Deo, R.C., Schwarz, J.M. et al. (2014). Effects of the absence of apolipoprotein e on lipoproteins, neurocognitive function, and retinal function. JAMA Neurol 71: 1228-1236.

Malik, M., Simpson, J.F., Parikh, I., Wilfred, B.R., Fardo, D.W., Nelson, P.T. et al. (2013). CD33 Alzheimer's risk-altering polymorphism, CD33 expression, and exon 2 splicing. J Neurosci 33: 13320-13325.

Manolio, T.A., Collins, F.S., Cox, N.J., Goldstein, D.B., Hindorff, L.A., Hunter, D.J. et al. (2009). Finding the missing heritability of complex diseases. Nature 461: 747-753.

Marchini, J. & Howie, B. (2010). Genotype imputation for genome-wide association studies. Nat Rev Genet 11: 499-511.

Martinez, A., Otal, R., Sieber, B.A., Ibanez, C. & Soriano, E. (2005). Disruption of ephrin-A/EphA binding alters synaptogenesis and neural connectivity in the hippocampus. Neuroscience 135: 451-461.

Martiskainen, H., Viswanathan, J., Nykanen, N.P., Kurki, M., Helisalmi, S., Natunen, T. et al. (2015). Transcriptomics and mechanistic elucidation of Alzheimer's disease risk genes in the brain and in vitro models. Neurobiol Aging 36: 1221.e15-1221.e28.

Masuda-Suzukake, M., Nonaka, T., Hosokawa, M., Oikawa, T., Arai, T., Akiyama, H. et al. (2013). Prion-like spreading of pathological alpha-synuclein in brain. Brain 136: 1128-1138.

May, P.C., Lampert-Etchells, M., Johnson, S.A., Poirier, J., Masters, J.N. & Finch, C.E. (1990). Dynamics of gene expression for a hippocampal glycoprotein elevated in Alzheimer's disease and in response to experimental lesions in rat. Neuron 5: 831-839.

Mayeux, R., Denaro, J., Hemenegildo, N., Marder, K., Tang, M.X., Cote, L.J. et al. (1992). A population-based investigation of Parkinson's disease with and without dementia. Relationship to age and gender. Arch Neurol 49: 492-497.

McCarthy, M.I., Abecasis, G.R., Cardon, L.R., Goldstein, D.B., Little, J., Ioannidis, J.P. et al. (2008). Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 9: 356-369.

McGeer, P.L., Akiyama, H., Itagaki, S. & McGeer, E.G. (1989). Activation of the classical complement pathway in brain tissue of Alzheimer patients. Neurosci Lett 107: 341-346.

McKee, A.C., Stern, R.A., Nowinski, C.J., Stein, T.D., Alvarez, V.E., Daneshvar, D.H. et al. (2013). The spectrum of disease in chronic traumatic encephalopathy. Brain 136: 43-64.

McMahon, H.T., Wigge, P. & Smith, C. (1997). Clathrin interacts specifically with amphiphysin and is displaced by dynamin. FEBS Lett 413: 319-322.

Medway, C. & Morgan, K. (2014). Review: The genetics of Alzheimer's disease; putting flesh on the bones. Neuropathol Appl Neurobiol 40: 97-105.

Meraz-Rios, M.A., Lira-De Leon, K.I., Campos-Pena, V., De Anda-Hernandez, M.A. & Mena-Lopez, R. (2010). Tau oligomers and aggregation in Alzheimer's disease. J Neurochem 112: 1353-1367.

Mesulam, M.M. (2001). Primary progressive aphasia. Ann Neurol 49: 425-432.

Meunier, B., Quaranta, M., Daviet, L., Hatzoglou, A. & Leprince, C. (2009). The membrane-tubulating potential of amphiphysin 2/BIN1 is dependent on the microtubule-binding cytoplasmic linker protein 170 (CLIP-170). Eur J Cell Biol 88: 91-102.

Miar, A., Alvarez, V., Corao, A.I., Alonso, B., Diaz, M., Menendez, M. et al. (2011). Lack of association between protocadherin 11-X/Y (PCDH11X and PCDH11Y) polymorphisms and late onset Alzheimer's disease. Brain Res 1383: 252-256.

Michnick, S.W. (2001). Exploring protein interactions by interaction-induced folding of proteins from complementary peptide fragments. Curr Opin Struct Biol 11: 472-477.

Michnick, S.W., Ear, P.H., Manderson, E.N., Remy, I. & Stefan, E. (2007). Universal strategies in research and drug discovery based on protein-fragment complementation assays. Nat Rev Drug Discov 6: 569-582.

Miller, S.E., Sahlender, D.A., Graham, S.C., Honing, S., Robinson, M.S., Peden, A.A. et al. (2011). The molecular basis for the endocytosis of small R-SNAREs by the clathrin adaptor CALM. Cell 147: 1118-1131.

Min, S.W., Chen, X., Tracy, T.E., Li, Y., Zhou, Y., Wang, C. et al. (2015). Critical role of acetylation in tau-mediated neurodegeneration and cognitive deficits. Nat Med

Min, S.W., Cho, S.H., Zhou, Y., Schroeder, S., Haroutunian, V., Seeley, W.W. et al. (2010). Acetylation of tau inhibits its degradation and contributes to tauopathy. Neuron 67: 953-966.

Mirbaha, H., Holmes, B.B., Sanders, D.W., Bieschke, J. & Diamond, M.I. (2015). Tau trimers are the minimal propagation unit spontaneously internalized to seed intracellular aggregation. J Biol Chem

Miyashita, A., Koike, A., Jun, G., Wang, L.S., Takahashi, S., Matsubara, E. et al. (2013). SORL1 is genetically associated with late-onset Alzheimer's disease in Japanese, Koreans and Caucasians. PLoS One 8: e58618.

Miyashita, A., Wen, Y., Kitamura, N., Matsubara, E., Kawarabayashi, T., Shoji, M. et al. (2014). Lack of genetic association between TREM2 and late-onset Alzheimer's disease in a Japanese population. J Alzheimers Dis 41: 1031-1038.

Mohamed, N.V., Plouffe, V., Remillard-Labrosse, G., Planel, E. & Leclerc, N. (2014). Starvation and inhibition of lysosomal function increased tau secretion by primary cortical neurons. Sci Rep 4: 5715.

Morris, H.R., Katzenschlager, R., Janssen, J.C., Brown, J.M., Ozansoy, M., Quinn, N. et al. (2002). Sequence analysis of tau in familial and sporadic progressive supranuclear palsy. J Neurol Neurosurg Psychiatry 72: 388-390.

Morris, H.R., Osaki, Y., Holton, J., Lees, A.J., Wood, N.W., Revesz, T. et al. (2003). Tau exon 10 +16 mutation FTDP-17 presenting clinically as sporadic young onset PSP. Neurology 61: 102-104.

Morris, J.C., Drazner, M., Fulling, K., Grant, E.A. & Goldring, J. (1989). Clinical and pathological aspects of parkinsonism in Alzheimer's disease. A role for extranigral factors? Arch Neurol 46: 651-657.

Morris, M., Knudsen, G.M., Maeda, S., Trinidad, J.C., Ioanoviciu, A., Burlingame, A.L. et al. (2015). Tau post-translational modifications in wild-type and human amyloid precursor protein transgenic mice. Nat Neurosci

Mortimer, J.A., van Duijn, C.M., Chandra, V., Fratiglioni, L., Graves, A.B., Heyman, A. et al. (1991). Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group. Int J Epidemiol 20 Suppl 2: S28-35.

Motoi, Y., Aizawa, T., Haga, S., Nakamura, S., Namba, Y. & Ikeda, K. (1999). Neuronal localization of a novel mosaic apolipoprotein E receptor, LR11, in rat and human brain. Brain Res 833: 209-215.

Mucke, L. & Selkoe, D.J. (2012). Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction. Cold Spring Harb Perspect Med 2: a006338.

Mullan, M., Crawford, F., Axelman, K., Houlden, H., Lilius, L., Winblad, B. et al. (1992). A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. Nat Genet 1: 345-347.

Munoz, L. & Ammit, A.J. (2010). Targeting p38 MAPK pathway for the treatment of Alzheimer's disease. Neuropharmacology 58: 561-568.

Murray, R., Neumann, M., Forman, M.S., Farmer, J., Massimo, L., Rice, A. et al. (2007). Cognitive and motor assessment in autopsy-proven corticobasal degeneration. Neurology 68: 1274-1283.

Murrell, J., Farlow, M., Ghetti, B. & Benson, M.D. (1991). A mutation in the amyloid precursor protein associated with hereditary Alzheimer's disease. Science 254: 97-99.

Naj, A.C., Beecham, G.W., Martin, E.R., Gallins, P.J., Powell, E.H., Konidari, I. et al. (2010). Dementia revealed: novel chromosome 6 locus for late-onset Alzheimer disease provides genetic evidence for folate-pathway abnormalities. PLoS Genet 6: e1001130.

Naj, A.C., Jun, G., Beecham, G.W., Wang, L.S., Vardarajan, B.N., Buros, J. et al. (2011). Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet 43: 436-441.

Nakamura, K., Greenwood, A., Binder, L., Bigio, E.H., Denial, S., Nicholson, L. et al. (2012). Proline isomer-specific antibodies reveal the early pathogenic tau conformation in Alzheimer's disease. Cell 149: 232-244.

Namba, Y., Tomonaga, M., Kawasaki, H., Otomo, E. & Ikeda, K. (1991). Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. Brain Res 541: 163-166.

Neary, D., Snowden, J.S., Gustafson, L., Passant, U., Stuss, D., Black, S. et al. (1998). Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 51: 1546-1554.

Neeb, A., Koch, H., Schurmann, A. & Brose, N. (1999). Direct interaction between the ARF-specific guanine nucleotide exchange factor msec7-1 and presynaptic Munc13-1. Eur J Cell Biol 78: 533-538.

Nekooki-Machida, Y., Kurosawa, M., Nukina, N., Ito, K., Oda, T. & Tanaka, M. (2009). Distinct conformations of in vitro and in vivo amyloids of huntingtin-exon1 show different cytotoxicity. Proc Natl Acad Sci U S A 106: 9679-9684.

Nelson, P.T., Abner, E.L., Schmitt, F.A., Kryscio, R.J., Jicha, G.A., Santacruz, K. et al. (2009). Brains with medial temporal lobe neurofibrillary tangles but no neuritic

amyloid plaques are a diagnostic dilemma but may have pathogenetic aspects distinct from Alzheimer disease. J Neuropathol Exp Neurol 68: 774-784.

Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T. et al. (2006). Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314: 130-133.

Neve, R.L., Harris, P., Kosik, K.S., Kurnit, D.M. & Donlon, T.A. (1986). Identification of cDNA clones for the human microtubule-associated protein tau and chromosomal localization of the genes for tau and microtubule-associated protein 2. Brain Res 387: 271-280.

Noack, M., Leyk, J. & Richter-Landsberg, C. (2014). HDAC6 inhibition results in tau acetylation and modulates tau phosphorylation and degradation in oligodendrocytes. Glia 62: 535-547.

Nonaka, T., Watanabe, S.T., Iwatsubo, T. & Hasegawa, M. (2010). Seeded aggregation and toxicity of {alpha}-synuclein and tau: cellular models of neurodegenerative diseases. J Biol Chem 285: 34885-34898.

Offe, K., Dodson, S.E., Shoemaker, J.T., Fritz, J.J., Gearing, M., Levey, A.I. et al. (2006). The lipoprotein receptor LR11 regulates amyloid beta production and amyloid precursor protein traffic in endosomal compartments. J Neurosci 26: 1596-1603.

Oliveira, T.G., Chan, R.B., Tian, H., Laredo, M., Shui, G., Staniszewski, A. et al. (2010). Phospholipase d2 ablation ameliorates Alzheimer's disease-linked synaptic dysfunction and cognitive deficits. J Neurosci 30: 16419-16428.

Onyike, C.U. & Diehl-Schmid, J. (2013). The epidemiology of frontotemporal dementia. Int Rev Psychiatry 25: 130-137.

Paloneva, J., Manninen, T., Christman, G., Hovanes, K., Mandelin, J., Adolfsson, R. et al. (2002). Mutations in two genes encoding different subunits of a receptor signaling complex result in an identical disease phenotype. Am J Hum Genet 71: 656-662.

Park, J., Song, W.J. & Chung, K.C. (2009). Function and regulation of Dyrk1A: towards understanding Down syndrome. Cell Mol Life Sci 66: 3235-3240.

Pastor, P., Pastor, E., Carnero, C., Vela, R., Garcia, T., Amer, G. et al. (2001). Familial atypical progressive supranuclear palsy associated with homozygosity for the delN296 mutation in the tau gene. Ann Neurol 49: 263-267.

Pastorino, L., Ma, S.L., Balastik, M., Huang, P., Pandya, D., Nicholson, L. et al. (2012). Alzheimer's disease-related loss of Pin1 function influences the intracellular localization and the processing of AbetaPP. J Alzheimers Dis 30: 277-297.

Pastorino, L., Sun, A., Lu, P.J., Zhou, X.Z., Balastik, M., Finn, G. et al. (2006). The prolyl isomerase Pin1 regulates amyloid precursor protein processing and amyloid-beta production. Nature 440: 528-534.

Patrick, G.N., Zukerberg, L., Nikolic, M., de la Monte, S., Dikkes, P. & Tsai, L.H. (1999). Conversion of p35 to p25 deregulates Cdk5 activity and promotes neurodegeneration. Nature 402: 615-622.

Patzke, H. & Tsai, L.H. (2002). Calpain-mediated cleavage of the cyclin-dependent kinase-5 activator p39 to p29. J Biol Chem 277: 8054-8060.

Pericak-Vance, M.A., Bebout, J.L., Gaskell, P.C.J., Yamaoka, L.H., Hung, W.Y., Alberts, M.J. et al. (1991). Linkage studies in familial Alzheimer disease: evidence for chromosome 19 linkage. Am J Hum Genet 48: 1034-1050.

Perry, G., Friedman, R., Shaw, G. & Chau, V. (1987). Ubiquitin is detected in neurofibrillary tangles and senile plaque neurites of Alzheimer disease brains. Proc Natl Acad Sci U S A 84: 3033-3036.

Peter, B.J., Kent, H.M., Mills, I.G., Vallis, Y., Butler, P.J., Evans, P.R. et al. (2004). BAR domains as sensors of membrane curvature: the amphiphysin BAR structure. Science 303: 495-499.

Petkova, A.T., Leapman, R.D., Guo, Z., Yau, W.M., Mattson, M.P. & Tycko, R. (2005). Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils. Science 307: 262-265.

Piguet, O., Halliday, G.M., Reid, W.G., Casey, B., Carman, R., Huang, Y. et al. (2011). Clinical phenotypes in autopsy-confirmed Pick disease. Neurology 76: 253-259.

Planel, E., Richter, K.E., Nolan, C.E., Finley, J.E., Liu, L., Wen, Y. et al. (2007). Anesthesia leads to tau hyperphosphorylation through inhibition of phosphatase activity by hypothermia. J Neurosci 27: 3090-3097.

Plouffe, V., Mohamed, N.V., Rivest-McGraw, J., Bertrand, J., Lauzon, M. & Leclerc, N. (2012). Hyperphosphorylation and cleavage at D421 enhance tau secretion. PLoS One 7: e36873.

Poirier, J., Davignon, J., Bouthillier, D., Kogan, S., Bertrand, P. & Gauthier, S. (1993). Apolipoprotein E polymorphism and Alzheimer's disease. Lancet 342: 697-699.

Pooler, A.M., Phillips, E.C., Lau, D.H., Noble, W. & Hanger, D.P. (2013). Physiological release of endogenous tau is stimulated by neuronal activity. EMBO Rep 14: 389-394.

Poorkaj, P., Bird, T.D., Wijsman, E., Nemens, E., Garruto, R.M., Anderson, L. et al. (1998). Tau is a candidate gene for chromosome 17 frontotemporal dementia. Ann Neurol 43: 815-825.

Poorkaj, P., Muma, N.A., Zhukareva, V., Cochran, E.J., Shannon, K.M., Hurtig, H. et al. (2002). An R5L tau mutation in a subject with a progressive supranuclear palsy phenotype. Ann Neurol 52: 511-516.

Pottier, C., Wallon, D., Rousseau, S., Rovelet-Lecrux, A., Richard, A.C., Rollin-Sillaire, A. et al. (2013). TREM2 R47H variant as a risk factor for early-onset Alzheimer's disease. J Alzheimers Dis 35: 45-49.

Powell, D.W., Rane, M.J., Chen, Q., Singh, S. & McLeish, K.R. (2002). Identification of 14-3-3zeta as a protein kinase B/Akt substrate. J Biol Chem 277: 21639-21642.

Prior, P., Schmitt, B., Grenningloh, G., Pribilla, I., Multhaup, G., Beyreuther, K. et al. (1992). Primary structure and alternative splice variants of gephyrin, a putative glycine receptor-tubulin linker protein. Neuron 8: 1161-1170.

Proitsi, P., Lee, S.H., Lunnon, K., Keohane, A., Powell, J., Troakes, C. et al. (2014). Alzheimer's disease susceptibility variants in the MS4A6A gene are associated with altered levels of MS4A6A expression in blood. Neurobiol Aging 35: 279-290.

Prusiner, S.B. (1982). Novel proteinaceous infectious particles cause scrapie. Science 216: 136-144.

Prusiner, S.B., Woerman, A.L., Mordes, D.A., Watts, J.C., Rampersaud, R., Berry, D.B. et al. (2015). Evidence for alpha-synuclein prions causing multiple system atrophy in humans with parkinsonism. Proc Natl Acad Sci U S A

Quazi, F. & Molday, R.S. (2013). Differential phospholipid substrates and directional transport by ATP-binding cassette proteins ABCA1, ABCA7, and ABCA4 and disease-causing mutants. J Biol Chem 288: 34414-34426.

Raggi, A., Marcone, A., Iannaccone, S., Ginex, V., Perani, D. & Cappa, S.F. (2007). The clinical overlap between the corticobasal degeneration syndrome and other diseases of the frontotemporal spectrum: three case reports. Behav Neurol 18: 159-164.

Raj, T., Ryan, K.J., Replogle, J.M., Chibnik, L.B., Rosenkrantz, L., Tang, A. et al. (2014). CD33: increased inclusion of exon 2 implicates the Ig V-set domain in Alzheimer's disease susceptibility. Hum Mol Genet 23: 2729-2736.

Rajagopalan, P., Hibar, D.P. & Thompson, P.M. (2013). TREM2 and neurodegenerative disease. N Engl J Med 369: 1565-1567.

Ramjaun, A.R. & McPherson, P.S. (1998). Multiple amphiphysin II splice variants display differential clathrin binding: identification of two distinct clathrin-binding sites. J Neurochem 70: 2369-2376.

Ranganathan, R., Lu, K.P., Hunter, T. & Noel, J.P. (1997). Structural and functional analysis of the mitotic rotamase Pin1 suggests substrate recognition is phosphorylation dependent. Cell 89: 875-886.

Rebeiz, J.J., Kolodny, E.H. & Richardson, E.P.J. (1968). Corticodentatonigral degeneration with neuronal achromasia. Arch Neurol 18: 20-33.

Reed, L.A., Wszolek, Z.K. & Hutton, M. (2001). Phenotypic correlations in FTDP-17. Neurobiol Aging 22: 89-107.

Reiman, E.M., Webster, J.A., Myers, A.J., Hardy, J., Dunckley, T., Zismann, V.L. et al. (2007). GAB2 alleles modify Alzheimer's risk in APOE epsilon4 carriers. Neuron 54: 713-720.

Reitz, C. & Mayeux, R. (2013). TREM2 and neurodegenerative disease. N Engl J Med 369: 1564-1565.

Remy, I. & Michnick, S.W. (2006). A highly sensitive protein-protein interaction assay based on Gaussia luciferase. Nat Methods 3: 977-979.

Ren, G., Vajjhala, P., Lee, J.S., Winsor, B. & Munn, A.L. (2006). The BAR domain proteins: molding membranes in fission, fusion, and phagy. Microbiol Mol Biol Rev 70: 37-120.

Resh, M.D. (1998). Fyn, a Src family tyrosine kinase. Int J Biochem Cell Biol 30: 1159-1162.

Respondek, G., Stamelou, M., Kurz, C., Ferguson, L.W., Rajput, A., Chiu, W.Z. et al. (2014). The phenotypic spectrum of progressive supranuclear palsy: a retrospective multicenter study of 100 definite cases. Mov Disord 29: 1758-1766.

Reynolds, M.R., Berry, R.W. & Binder, L.I. (2005). Site-specific nitration and oxidative dityrosine bridging of the tau protein by peroxynitrite: implications for Alzheimer's disease. Biochemistry 44: 1690-1700.

Reynolds, M.R., Reyes, J.F., Fu, Y., Bigio, E.H., Guillozet-Bongaarts, A.L., Berry, R.W. et al. (2006). Tau nitration occurs at tyrosine 29 in the fibrillar lesions of Alzheimer's disease and other tauopathies. J Neurosci 26: 10636-10645.

Riley, D.E., Lang, A.E., Lewis, A., Resch, L., Ashby, P., Hornykiewicz, O. et al. (1990). Cortical-basal ganglionic degeneration. Neurology 40: 1203-1212.

Robakis, N.K., Wisniewski, H.M., Jenkins, E.C., Devine-Gage, E.A., Houck, G.E., Yao, X.L. et al. (1987). Chromosome 21q21 sublocalisation of gene encoding beta-

amyloid peptide in cerebral vessels and neuritic (senile) plaques of people with Alzheimer disease and Down syndrome. Lancet 1(8529): 384-385.

Rogaeva, E., Meng, Y., Lee, J.H., Gu, Y., Kawarai, T., Zou, F. et al. (2007). The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat Genet 39: 168-177.

Rogers, J., Li, R., Mastroeni, D., Grover, A., Leonard, B., Ahern, G. et al. (2006). Peripheral clearance of amyloid beta peptide by complement C3-dependent adherence to erythrocytes. Neurobiol Aging 27: 1733-1739.

Rohn, T.T. (2013). The triggering receptor expressed on myeloid cells 2: "TREM-ming" the inflammatory component associated with Alzheimer's disease. Oxid Med Cell Longev 2013: 860959.

Rohrbough, J., Rushton, E., Palanker, L., Woodruff, E., Matthies, H.J., Acharya, U. et al. (2004). Ceramidase regulates synaptic vesicle exocytosis and trafficking. J Neurosci 24: 7789-7803.

Rohrer, J.D., Guerreiro, R., Vandrovcova, J., Uphill, J., Reiman, D., Beck, J. et al. (2009). The heritability and genetics of frontotemporal lobar degeneration. Neurology 73: 1451-1456.

Rohrer, J.D., Nicholas, J.M., Cash, D.M., van Swieten, J., Dopper, E., Jiskoot, L. et al. (2015). Presymptomatic cognitive and neuroanatomical changes in genetic frontotemporal dementia in the Genetic Frontotemporal dementia Initiative (GENFI) study: a cross-sectional analysis. Lancet Neurol 14: 253-262.

Ros, R., Gomez Garre, P., Hirano, M., Tai, Y.F., Ampuero, I., Vidal, L. et al. (2005a). Genetic linkage of autosomal dominant progressive supranuclear palsy to 1q31.1. Ann Neurol 57: 634-641.

Ros, R., Thobois, S., Streichenberger, N., Kopp, N., Sanchez, M.P., Perez, M. et al. (2005b). A new mutation of the tau gene, G303V, in early-onset familial progressive supranuclear palsy. Arch Neurol 62: 1444-1450.

Ruiz, A., Dols-Icardo, O., Bullido, M.J., Pastor, P., Rodriguez-Rodriguez, E., Lopez de Munain, A. et al. (2014a). Assessing the role of the TREM2 p.R47H variant as a risk factor for Alzheimer's disease and frontotemporal dementia. Neurobiol Aging 35: 444.e1-444.e4.

Ruiz, A., Heilmann, S., Becker, T., Hernandez, I., Wagner, H., Thelen, M. et al. (2014b). Follow-up of loci from the International Genomics of Alzheimer's Disease Project identifies TRIP4 as a novel susceptibility gene. Transl Psychiatry 4: e358.

Ryoo, S.R., Jeong, H.K., Radnaabazar, C., Yoo, J.J., Cho, H.J., Lee, H.W. et al. (2007). DYRK1A-mediated hyperphosphorylation of Tau. A functional link between Down syndrome and Alzheimer disease. J Biol Chem 282: 34850-34857.

Ryoo, S.R., Cho, H.J., Lee, H.W., Jeong, H.K., Radnaabazar, C., Kim, Y.S. et al. (2008). Dual-specificity tyrosine(Y)-phosphorylation regulated kinase 1A-mediated phosphorylation of amyloid precursor protein: evidence for a functional link between Down syndrome and Alzheimer's disease. J Neurochem 104: 1333-1344.

Ryu, Y.S., Park, S.Y., Jung, M.S., Yoon, S.H., Kwen, M.Y., Lee, S.Y. et al. (2010). Dyrk1A-mediated phosphorylation of Presenilin 1: a functional link between Down syndrome and Alzheimer's disease. J Neurochem 115: 574-584.

Sadik, G., Tanaka, T., Kato, K., Yamamori, H., Nessa, B.N., Morihara, T. et al. (2009). Phosphorylation of tau at Ser214 mediates its interaction with 14-3-3 protein: implications for the mechanism of tau aggregation. J Neurochem 108: 33-43.

Saito, Y., Nakahara, K., Yamanouchi, H. & Murayama, S. (2002). Severe involvement of ambient gyrus in dementia with grains. J Neuropathol Exp Neurol 61: 789-796.

Saito, Y., Ruberu, N.N., Sawabe, M., Arai, T., Tanaka, N., Kakuta, Y. et al. (2004). Staging of argyrophilic grains: an age-associated tauopathy. J Neuropathol Exp Neurol 63: 911-918.

Sakamoto, A., Sugamoto, Y., Tokunaga, Y., Yoshimuta, T., Hayashi, K., Konno, T. et al. (2011). Expression profiling of the ephrin (EFN) and Eph receptor (EPH) family of genes in atherosclerosis-related human cells. J Int Med Res 39: 522-527.

Saman, S., Kim, W., Raya, M., Visnick, Y., Miro, S., Saman, S. et al. (2012). Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. J Biol Chem 287: 3842-3849.

Sanders, D.W., Kaufman, S.K., DeVos, S.L., Sharma, A.M., Mirbaha, H., Li, A. et al. (2014). Distinct Tau Prion Strains Propagate in Cells and Mice and Define Different Tauopathies. Neuron

Sannerud, R., Declerck, I., Peric, A., Raemaekers, T., Menendez, G., Zhou, L. et al. (2011). ADP ribosylation factor 6 (ARF6) controls amyloid precursor protein (APP) processing by mediating the endosomal sorting of BACE1. Proc Natl Acad Sci U S A 108: E559-68.

Santa-Maria, I., Varghese, M., Ksiezak-Reding, H., Dzhun, A., Wang, J. & Pasinetti, G.M. (2012). Paired helical filaments from Alzheimer disease brain induce intracellular accumulation of Tau protein in aggresomes. J Biol Chem 287: 20522-20533.

Sasaki, N., Fukatsu, R., Tsuzuki, K., Hayashi, Y., Yoshida, T., Fujii, N. et al. (1998). Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases. Am J Pathol 153: 1149-1155.

Sastre, M., Steiner, H., Fuchs, K., Capell, A., Multhaup, G., Condron, M.M. et al. (2001). Presenilin-dependent gamma-secretase processing of beta-amyloid precursor protein at a site corresponding to the S3 cleavage of Notch. EMBO Rep 2: 835-841.

Satoh, K., Abe-Dohmae, S., Yokoyama, S., St George-Hyslop, P. & Fraser, P.E. (2015). ATP-binding Cassette Transporter A7 (ABCA7) Loss of Function Alters Alzheimer Amyloid Processing. J Biol Chem 290: 24152-24165.

Saunders, A.M., Strittmatter, W.J., Schmechel, D., George-Hyslop, P.H., Pericak-Vance, M.A., Joo, S.H. et al. (1993). Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 43: 1467-1472.

Schaeffer, H.J. & Weber, M.J. (1999). Mitogen-activated protein kinases: specific messages from ubiquitous messengers. Mol Cell Biol 19: 2435-2444.

Schellenberg, G.D., Bird, T.D., Wijsman, E.M., Moore, D.K., Boehnke, M., Bryant, E.M. et al. (1988). Absence of linkage of chromosome 21q21 markers to familial Alzheimer's disease. Science 241: 1507-1510.

Schellenberg, G.D., Bird, T.D., Wijsman, E.M., Orr, H.T., Anderson, L., Nemens, E. et al. (1992). Genetic linkage evidence for a familial Alzheimer's disease locus on chromosome 14. Science 258: 668-671.

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N. et al. (1996). Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat Med 2: 864-870.

Schmidt, V., Sporbert, A., Rohe, M., Reimer, T., Rehm, A., Andersen, O.M. et al. (2007). SorLA/LR11 regulates processing of amyloid precursor protein via interaction with adaptors GGA and PACS-1. J Biol Chem 282: 32956-32964.

Schonberger, O., Horonchik, L., Gabizon, R., Papy-Garcia, D., Barritault, D. & Taraboulos, A. (2003). Novel heparan mimetics potently inhibit the scrapie prion protein and its endocytosis. Biochem Biophys Res Commun 312: 473-479.

Sergeant, N., Delacourte, A. & Buee, L. (2005). Tau protein as a differential biomarker of tauopathies. Biochim Biophys Acta 1739: 179-197.

Seshadri, S., Fitzpatrick, A.L., Ikram, M.A., DeStefano, A.L., Gudnason, V., Boada, M. et al. (2010). Genome-wide analysis of genetic loci associated with Alzheimer disease. JAMA 303: 1832-1840.

Shen, Y., Li, R., McGeer, E.G. & McGeer, P.L. (1997). Neuronal expression of mRNAs for complement proteins of the classical pathway in Alzheimer brain. Brain Res 769: 391-395.

Shen, Y., Lue, L., Yang, L., Roher, A., Kuo, Y., Strohmeyer, R. et al. (2001). Complement activation by neurofibrillary tangles in Alzheimer's disease. Neurosci Lett 305: 165-168.

Sherrington, R., Rogaev, E.I., Liang, Y., Rogaeva, E.A., Levesque, G., Ikeda, M. et al. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 375: 754-760.

Shi, J., Zhang, T., Zhou, C., Chohan, M.O., Gu, X., Wegiel, J. et al. (2008). Increased dosage of Dyrk1A alters alternative splicing factor (ASF)-regulated alternative splicing of tau in Down syndrome. J Biol Chem 283: 28660-28669.

Shih, N.Y., Li, J., Karpitskii, V., Nguyen, A., Dustin, M.L., Kanagawa, O. et al. (1999). Congenital nephrotic syndrome in mice lacking CD2-associated protein. Science 286: 312-315.

Shulman, J.M., Chen, K., Keenan, B.T., Chibnik, L.B., Fleisher, A., Thiyyagura, P. et al. (2013). Genetic susceptibility for Alzheimer disease neuritic plaque pathology. JAMA Neurol 70: 1150-1157.

Shulman, J.M., Imboywa, S., Giagtzoglou, N., Powers, M.P., Hu, Y., Devenport, D. et al. (2014). Functional screening in Drosophila identifies Alzheimer's disease susceptibility genes and implicates Tau-mediated mechanisms. Hum Mol Genet 23: 870-877.

Siest, G., Pillot, T., Regis-Bailly, A., Leininger-Muller, B., Steinmetz, J., Galteau, M.M. et al. (1995). Apolipoprotein E: an important gene and protein to follow in laboratory medicine. Clin Chem 41: 1068-1086.

Simon, D., Garcia-Garcia, E., Royo, F., Falcon-Perez, J.M. & Avila, J. (2012). Proteostasis of tau. Tau overexpression results in its secretion via membrane vesicles. FEBS Lett 586: 47-54.

Sisodia, S.S., Koo, E.H., Beyreuther, K., Unterbeck, A. & Price, D.L. (1990). Evidence that beta-amyloid protein in Alzheimer's disease is not derived by normal processing. Science 248: 492-495.

Smith, D.H., Johnson, V.E. & Stewart, W. (2013). Chronic neuropathologies of single and repetitive TBI: substrates of dementia? Nat Rev Neurol 9: 211-221.

Sokolow, S., Henkins, K.M., Bilousova, T., Gonzalez, B., Vinters, H.V., Miller, C.A. et al. (2015). Pre-synaptic C-terminal truncated tau is released from cortical synapses in Alzheimer's disease. J Neurochem 133: 368-379.

Song, L., Lu, S.X., Ouyang, X., Melchor, J., Lee, J., Terracina, G. et al. (2015). Analysis of tau post-translational modifications in rTg4510 mice, a model of tau pathology. Mol Neurodegeneration 10: a006247.

Spillantini, M.G., Crowther, R.A., Jakes, R., Hasegawa, M. & Goedert, M. (1998a). alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. Proc Natl Acad Sci U S A 95: 6469-6473.

Spillantini, M.G., Murrell, J.R., Goedert, M., Farlow, M.R., Klug, A. & Ghetti, B. (1998b). Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. Proc Natl Acad Sci U S A 95: 7737-7741.

Spoelgen, R., von Arnim, C.A., Thomas, A.V., Peltan, I.D., Koker, M., Deng, A. et al. (2006). Interaction of the cytosolic domains of sorLA/LR11 with the amyloid precursor protein (APP) and beta-secretase beta-site APP-cleaving enzyme. J Neurosci 26: 418-428.

St George-Hyslop, P.H., Haines, J.L., Farrer, L.A., Polinsky, R., Van Broeckhoven, C., Goate, A. et al. (1990). Genetic linkage studies suggest that Alzheimer's disease is not a single homogeneous disorder. Nature 347: 194-197.

St George-Hyslop, P.H., Tanzi, R.E., Polinsky, R.J., Haines, J.L., Nee, L., Watkins, P.C. et al. (1987). The genetic defect causing familial Alzheimer's disease maps on chromosome 21. Science 235: 885-890.

Stanford, P.M., Halliday, G.M., Brooks, W.S., Kwok, J.B., Storey, C.E., Creasey, H. et al. (2000). Progressive supranuclear palsy pathology caused by a novel silent mutation in exon 10 of the tau gene: expansion of the disease phenotype caused by tau gene mutations. Brain 123: 880-893.

Steele, J.C., Richardson, J.C. & Olszewski, J. (1964). Progressive supranuclear palsy. A heterogeneous degeneration involving the brain stem, basal ganglia and cerebellum with vertical gaze and pseudobulbar palsy, nuchal dystonia and dementia. Arch Neurol 10: 333-359.

Steinberg, S., Stefansson, H., Jonsson, T., Johannsdottir, H., Ingason, A., Helgason, H. et al. (2015). Loss-of-function variants in ABCA7 confer risk of Alzheimer's disease. Nat Genet

Stohr, J., Watts, J.C., Mensinger, Z.L., Oehler, A., Grillo, S.K., DeArmond, S.J. et al. (2012). Purified and synthetic Alzheimer's amyloid beta (Abeta) prions. Proc Natl Acad Sci U S A 109: 11025-11030.

Strittmatter, W.J., Saunders, A.M., Goedert, M., Weisgraber, K.H., Dong, L.M., Jakes, R. et al. (1994). Isoform-specific interactions of apolipoprotein E with microtubule-associated protein tau: implications for Alzheimer disease. Proc Natl Acad Sci U S A 91: 11183-11186.

Strittmatter, W.J., Saunders, A.M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G.S. et al. (1993). Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci U S A 90: 1977-1981.

Sun, X.Y., Wei, Y.P., Xiong, Y., Wang, X.C., Xie, A.J., Wang, X.L. et al. (2012). Synaptic released zinc promotes tau hyperphosphorylation by inhibition of protein phosphatase 2A (PP2A). J Biol Chem 287: 11174-11182.

Swerdlow, A.J., Higgins, C.D., Adlard, P., Jones, M.E. & Preece, M.A. (2003). Creutzfeldt-Jakob disease in United Kingdom patients treated with human pituitary growth hormone. Neurology 61: 783-791.

Tacik, P., DeTure, M., Lin, W.L., Sanchez Contreras, M., Wojtas, A., Hinkle, K.M. et al. (2015). A novel tau mutation, p.K317N, causes globular glial tauopathy. Acta Neuropathol 130: 199-214.

Tan, M.S., Yu, J.T. & Tan, L. (2013). Bridging integrator 1 (BIN1): form, function, and Alzheimer's disease. Trends Mol Med 19: 594-603.

Tanimukai, H., Grundke-Iqbal, I. & Iqbal, K. (2005). Up-regulation of inhibitors of protein phosphatase-2A in Alzheimer's disease. Am J Pathol 166: 1761-1771.

Tannous, B.A., Kim, D.E., Fernandez, J.L., Weissleder, R. & Breakefield, X.O. (2005). Codon-optimized Gaussia luciferase cDNA for mammalian gene expression in culture and in vivo. Mol Ther 11: 435-443.

Tanzi, R.E., Gusella, J.F., Watkins, P.C., Bruns, G.A., St George-Hyslop, P., Van Keuren, M.L. et al. (1987). Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. Science 235: 880-884.

Tatebayashi, Y., Haque, N., Tung, Y.C., Iqbal, K. & Grundke-Iqbal, I. (2004). Role of tau phosphorylation by glycogen synthase kinase-3beta in the regulation of organelle transport. J Cell Sci 117: 1653-1663.

Tebar, F., Bohlander, S.K. & Sorkin, A. (1999). Clathrin assembly lymphoid myeloid leukemia (CALM) protein: localization in endocytic-coated pits, interactions with clathrin, and the impact of overexpression on clathrin-mediated traffic. Mol Biol Cell 10: 2687-2702.

Tepass, U. (2009). FERM proteins in animal morphogenesis. Curr Opin Genet Dev 19: 357-367.

Terai, K., Walker, D.G., McGeer, E.G. & McGeer, P.L. (1997). Neurons express proteins of the classical complement pathway in Alzheimer disease. Brain Res 769: 385-390.

Terry, R.D. & Katzman, R. (1983). Senile dementia of the Alzheimer type. Ann Neurol 14: 497-506.

Thomas, S.N., Funk, K.E., Wan, Y., Liao, Z., Davies, P., Kuret, J. et al. (2012). Dual modification of Alzheimer's disease PHF-tau protein by lysine methylation and ubiquitylation: a mass spectrometry approach. Acta Neuropathol 123: 105-117.

Thorpe, J.R., Tang, H., Atherton, J. & Cairns, N.J. (2008). Fine structural analysis of the neuronal inclusions of frontotemporal lobar degeneration with TDP-43 proteinopathy. J Neural Transm 115: 1661-1671.

Tian, Q. & Wang, J. (2002). Role of Serine/Threonine Protein Phosphatase in Alzheimer’s Disease. Neurosignals 11: 262-269.

Tian, Y., Chang, J.C., Fan, E.Y., Flajolet, M. & Greengard, P. (2013). Adaptor complex AP2/PICALM, through interaction with LC3, targets Alzheimer's APP-CTF for terminal degradation via autophagy. Proc Natl Acad Sci U S A 110: 17071-17076.

Togo, T., Cookson, N. & Dickson, D.W. (2002). Argyrophilic grain disease: neuropathology, frequency in a dementia brain bank and lack of relationship with apolipoprotein E. Brain Pathol 12: 45-52.

Togo, T., Isojima, D., Akatsu, H., Suzuki, K., Uchikado, H., Katsuse, O. et al. (2005). Clinical features of argyrophilic grain disease: a retrospective survey of cases with neuropsychiatric symptoms. Am J Geriatr Psychiatry 13: 1083-1091.

Tolnay, M. & Clavaguera, F. (2004). Argyrophilic grain disease: a late-onset dementia with distinctive features among tauopathies. Neuropathology 24: 269-283.

Tolnay, M., Mistl, C., Ipsen, S. & Probst, A. (1998). Argyrophilic grains of Braak: occurrence in dendrites of neurons containing hyperphosphorylated tau protein. Neuropathol Appl Neurobiol 24: 53-59.

Tolnay, M. & Probst, A. (1998). Ballooned neurons expressing alphaB-crystallin as a constant feature of the amygdala in argyrophilic grain disease. Neurosci Lett 246: 165-168.

Trajkovic, K., Hsu, C., Chiantia, S., Rajendran, L., Wenzel, D., Wieland, F. et al. (2008). Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science 319: 1244-1247.

Treusch, S., Hamamichi, S., Goodman, J.L., Matlack, K.E., Chung, C.Y., Baru, V. et al. (2011). Functional links between Abeta toxicity, endocytic trafficking, and Alzheimer's disease risk factors in yeast. Science 334: 1241-1245.

Tsai, L.H., Delalle, I., Caviness, V.S.J., Chae, T. & Harlow, E. (1994). p35 is a neural-specific regulatory subunit of cyclin-dependent kinase 5. Nature 371: 419-423.

Tsuboi, Y., Baker, M., Hutton, M.L., Uitti, R.J., Rascol, O., Delisle, M.B. et al. (2002). Clinical and genetic studies of families with the tau N279K mutation (FTDP-17). Neurology 59: 1791-1793.

Tucholski, J., Kuret, J. & Johnson, G.V. (1999). Tau is modified by tissue transglutaminase in situ: possible functional and metabolic effects of polyamination. J Neurochem 73: 1871-1880.

Tyagarajan, S.K., Ghosh, H., Yevenes, G.E., Nikonenko, I., Ebeling, C., Schwerdel, C. et al. (2011). Regulation of GABAergic synapse formation and plasticity by GSK3beta-dependent phosphorylation of gephyrin. Proc Natl Acad Sci U S A 108: 379-384.

van der Lee, S.J., Holstege, H., Wong, T.H., Jakobsdottir, J., Bis, J.C., Chouraki, V. et al. (2015). PLD3 variants in population studies. Nature 520: E2-3.

van Eersel, J., Ke, Y.D., Liu, X., Delerue, F., Kril, J.J., Gotz, J. et al. (2010). Sodium selenate mitigates tau pathology, neurodegeneration, and functional deficits in Alzheimer's disease models. Proc Natl Acad Sci U S A 107: 13888-13893.

van Hemert, M.J., Steensma, H.Y. & van Heusden, G.P. (2001). 14-3-3 proteins: key regulators of cell division, signalling and apoptosis. Bioessays 23: 936-946.

Vandermeeren, M., Mercken, M., Vanmechelen, E., Six, J., van de Voorde, A., Martin, J.J. et al. (1993). Detection of tau proteins in normal and Alzheimer's disease cerebrospinal fluid with a sensitive sandwich enzyme-linked immunosorbent assay. J Neurochem 61: 1828-1834.

Varani, L., Hasegawa, M., Spillantini, M.G., Smith, M.J., Murrell, J.R., Ghetti, B. et al. (1999). Structure of tau exon 10 splicing regulatory element RNA and destabilization by mutations of frontotemporal dementia and parkinsonism linked to chromosome 17. Proc Natl Acad Sci U S A 96: 8229-8234.

Vardarajan, B.N., Ghani, M., Kahn, A., Sheikh, S., Sato, C., Barral, S. et al. (2015). Rare coding mutations identified by sequencing of Alzheimer disease genome-wide association studies loci. Ann Neurol 78: 487-498.

Vasquez, J.B., Fardo, D.W. & Estus, S. (2013). ABCA7 expression is associated with Alzheimer's disease polymorphism and disease status. Neurosci Lett 556: 58-62.

Vassar, R., Bennett, B.D., Babu-Khan, S., Kahn, S., Mendiaz, E.A., Denis, P. et al. (1999). Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 286: 735-741.

Verghese, P.B., Castellano, J.M., Garai, K., Wang, Y., Jiang, H., Shah, A. et al. (2013). ApoE influences amyloid-beta (Abeta) clearance despite minimal apoE/Abeta association in physiological conditions. Proc Natl Acad Sci U S A 110: E1807-16.

Vigo-Pelfrey, C., Seubert, P., Barbour, R., Blomquist, C., Lee, M., Lee, D. et al. (1995). Elevation of microtubule-associated protein tau in the cerebrospinal fluid of patients with Alzheimer's disease. Neurology 45: 788-793.

Vijayan, S., El-Akkad, E., Grundke-Iqbal, I. & Iqbal, K. (2001). A pool of beta-tubulin is hyperphosphorylated at serine residues in Alzheimer disease brain. FEBS Lett 509: 375-381.

Vogelsberg-Ragaglia, V., Schuck, T., Trojanowski, J.Q. & Lee, V.M. (2001). PP2A mRNA expression is quantitatively decreased in Alzheimer's disease hippocampus. Exp Neurol 168: 402-412.

Volpicelli-Daley, L.A., Luk, K.C., Patel, T.P., Tanik, S.A., Riddle, D.M., Stieber, A. et al. (2011). Exogenous alpha-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. Neuron 72: 57-71.

Walsh, D.A. & Van Patten, S.M. (1994). Multiple pathway signal transduction by the cAMP-dependent protein kinase. FASEB J 8: 1227-1236.

Walter, R.B., Raden, B.W., Zeng, R., Hausermann, P., Bernstein, I.D. & Cooper, J.A. (2008). ITIM-dependent endocytosis of CD33-related Siglecs: role of intracellular domain, tyrosine phosphorylation, and the tyrosine phosphatases, Shp1 and Shp2. J Leukoc Biol 83: 200-211.

Wang, G., Krishnamurthy, K., Umapathy, N.S., Verin, A.D. & Bieberich, E. (2009). The carboxyl-terminal domain of atypical protein kinase Czeta binds to ceramide and regulates junction formation in epithelial cells. J Biol Chem 284: 14469-14475.

Wang, J.Z., Grundke-Iqbal, I. & Iqbal, K. (2007). Kinases and phosphatases and tau sites involved in Alzheimer neurofibrillary degeneration. Eur J Neurosci 25: 59-68.

Wang, J.Z. & Liu, F. (2008). Microtubule-associated protein tau in development, degeneration and protection of neurons. Prog Neurobiol 85: 148-175.

Wang, N., Lan, D., Gerbod-Giannone, M., Linsel-Nitschke, P., Jehle, A.W., Chen, W. et al. (2003). ATP-binding cassette transporter A7 (ABCA7) binds apolipoprotein A-I and mediates cellular phospholipid but not cholesterol efflux. J Biol Chem 278: 42906-42912.

Wang, Y., Cella, M., Mallinson, K., Ulrich, J.D., Young, K.L., Robinette, M.L. et al. (2015). TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. Cell 160: 1061-1071.

Wang, Z., Udeshi, N.D., O'Malley, M., Shabanowitz, J., Hunt, D.F. & Hart, G.W. (2010). Enrichment and site mapping of O-linked N-acetylglucosamine by a combination of

chemical/enzymatic tagging, photochemical cleavage, and electron transfer dissociation mass spectrometry. Mol Cell Proteomics 9: 153-160.

Webster, J.A., Myers, A.J., Pearson, J.V., Craig, D.W., Hu-Lince, D., Coon, K.D. et al. (2008). Sorl1 as an Alzheimer's disease predisposition gene? Neurodegener Dis 5: 60-64.

Wechsler-Reya, R., Sakamuro, D., Zhang, J., Duhadaway, J. & Prendergast, G.C. (1997). Structural analysis of the human BIN1 gene. Evidence for tissue-specific transcriptional regulation and alternate RNA splicing. J Biol Chem 272: 31453-31458.

Wegiel, J., Kaczmarski, W., Barua, M., Kuchna, I., Nowicki, K., Wang, K.C. et al. (2011). Link between DYRK1A overexpression and several-fold enhancement of neurofibrillary degeneration with 3-repeat tau protein in Down syndrome. J Neuropathol Exp Neurol 70: 36-50.

Weingarten, M.D., Lockwood, A.H., Hwo, S.Y. & Kirschner, M.W. (1975). A protein factor essential for microtubule assembly. Proc Natl Acad Sci U S A 72: 1858-1862.

Weis, J.H., Morton, C.C., Bruns, G.A., Weis, J.J., Klickstein, L.B., Wong, W.W. et al. (1987). A complement receptor locus: genes encoding C3b/C4b receptor and C3d/Epstein-Barr virus receptor map to 1q32. J Immunol 138: 312-315.

Weisgraber, K.H., Rall, S.C.J. & Mahley, R.W. (1981). Human E apoprotein heterogeneity. Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. J Biol Chem 256: 9077-9083.

Whalley, L.J., Dick, F.D. & McNeill, G. (2006). A life-course approach to the aetiology of late-onset dementias. Lancet Neurol 5: 87-96.

Willem, M., Tahirovic, S., Busche, M.A., Ovsepian, S.V., Chafai, M., Kootar, S. et al. (2015). eta-Secretase processing of APP inhibits neuronal activity in the hippocampus. Nature

Williams, D.R., de Silva, R., Paviour, D.C., Pittman, A., Watt, H.C., Kilford, L. et al. (2005). Characteristics of two distinct clinical phenotypes in pathologically proven progressive supranuclear palsy: Richardson's syndrome and PSP-parkinsonism. Brain 128: 1247-1258.

Williams, D.R., Holton, J.L., Strand, K., Revesz, T. & Lees, A.J. (2007a). Pure akinesia with gait freezing: a third clinical phenotype of progressive supranuclear palsy. Mov Disord 22: 2235-2241.

Williams, D.R. & Lees, A.J. (2009). Progressive supranuclear palsy: clinicopathological concepts and diagnostic challenges. Lancet Neurol 8: 270-279.

Williams, D.R., Pittman, A.M., Revesz, T., Lees, A.J. & de Silva, R. (2007b). Genetic variation at the tau locus and clinical syndromes associated with progressive supranuclear palsy. Mov Disord 22: 895-897.

Williamson, R., Scales, T., Clark, B.R., Gibb, G., Reynolds, C.H., Kellie, S. et al. (2002). Rapid tyrosine phosphorylation of neuronal proteins including tau and focal adhesion kinase in response to amyloid-beta peptide exposure: involvement of Src family protein kinases. J Neurosci 22: 10-20.

Wiseman, F.K., Alford, K.A., Tybulewicz, V.L. & Fisher, E.M. (2009). Down syndrome-- recent progress and future prospects. Hum Mol Genet 18: R75-83.

Wisniewski, T. & Frangione, B. (1992). Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. Neurosci Lett 135: 235-238.

Woerman, A.L., Stohr, J., Aoyagi, A., Rampersaud, R., Krejciova, Z., Watts, J.C. et al. (2015). Propagation of prions causing synucleinopathies in cultured cells. Proc Natl Acad Sci U S A 112: E4949-58.

Wolf, G. & Stahl, R.A. (2003). CD2-associated protein and glomerular disease. Lancet 362: 1746-1748.

Wolfe, M.S., Xia, W., Ostaszewski, B.L., Diehl, T.S., Kimberly, W.T. & Selkoe, D.J. (1999). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. Nature 398: 513-517.

Woodcock, J.M., Murphy, J., Stomski, F.C., Berndt, M.C. & Lopez, A.F. (2003). The dimeric versus monomeric status of 14-3-3zeta is controlled by phosphorylation of Ser58 at the dimer interface. J Biol Chem 278: 36323-36327.

Woodgett, J.R. (1990). Molecular cloning and expression of glycogen synthase kinase-3/factor A. EMBO J 9: 2431-2438.

Woods, Y.L., Cohen, P., Becker, W., Jakes, R., Goedert, M., Wang, X. et al. (2001). The kinase DYRK phosphorylates protein-synthesis initiation factor eIF2Bepsilon at Ser539 and the microtubule-associated protein tau at Thr212: potential role for DYRK as a glycogen synthase kinase 3-priming kinase. Biochem J 355: 609-615.

World Alzheimer report (2015). The global impact of dementia. Alzheimer's Disease International.

Wszolek, Z.K., Tsuboi, Y., Farrer, M., Uitti, R.J. & Hutton, M.L. (2003). Hereditary tauopathies and parkinsonism. Adv Neurol 91: 153-163.

Wu, J.W., Herman, M., Liu, L., Simoes, S., Acker, C.M., Figueroa, H. et al. (2013). Small misfolded Tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. J Biol Chem 288: 1856-1870.

Wu, Y.T., Fratiglioni, L., Matthews, F.E., Lobo, A., Breteler, M.M., Skoog, I. et al. (2015). Dementia in western Europe: epidemiological evidence and implications for policy making. Lancet Neurol

Wu, Z.C., Yu, J.T., Wang, N.D., Yu, N.N., Zhang, Q., Chen, W. et al. (2010). Lack of association between PCDH11X genetic variation and late-onset Alzheimer's disease in a Han Chinese population. Brain Res 1357: 152-156.

Wunderlich, P., Glebov, K., Kemmerling, N., Tien, N.T., Neumann, H. & Walter, J. (2013). Sequential proteolytic processing of the triggering receptor expressed on myeloid cells-2 (TREM2) protein by ectodomain shedding and gamma-secretase-dependent intramembranous cleavage. J Biol Chem 288: 33027-33036.

Xiao, Q., Gil, S.C., Yan, P., Wang, Y., Han, S., Gonzales, E. et al. (2012). Role of phosphatidylinositol clathrin assembly lymphoid-myeloid leukemia (PICALM) in intracellular amyloid precursor protein (APP) processing and amyloid plaque pathogenesis. J Biol Chem 287: 21279-21289.

Yaffe, M.B. (1997). Sequence-Specific and Phosphorylation-Dependent Proline Isomerization: A Potential Mitotic Regulatory Mechanism. Science 278: 1957-1960.

Yamada, K., Cirrito, J.R., Stewart, F.R., Jiang, H., Finn, M.B., Holmes, B.B. et al. (2011). In vivo microdialysis reveals age-dependent decrease of brain interstitial fluid tau levels in P301S human tau transgenic mice. J Neurosci 31: 13110-13117.

Yamada, K., Holth, J.K., Liao, F., Stewart, F.R., Mahan, T.E., Jiang, H. et al. (2014). Neuronal activity regulates extracellular tau in vivo. J Exp Med

Yamamoto, H., Yamauchi, E., Taniguchi, H., Ono, T. & Miyamoto, E. (2002). Phosphorylation of microtubule-associated protein tau by Ca2+/calmodulin-dependent protein kinase II in its tubulin binding sites. Arch Biochem Biophys 408: 255-262.

Yamazaki, T., Masuda, J., Omori, T., Usui, R., Akiyama, H. & Maru, Y. (2009). EphA1 interacts with integrin-linked kinase and regulates cell morphology and motility. J Cell Sci 122: 243-255.

Yan, S.D., Chen, X., Schmidt, A.M., Brett, J., Godman, G., Zou, Y.S. et al. (1994). Glycated tau protein in Alzheimer disease: a mechanism for induction of oxidant stress. Proc Natl Acad Sci U S A 91: 7787-7791.

Yasojima, K., McGeer, E.G. & McGeer, P.L. (1999). Tangled areas of Alzheimer brain have upregulated levels of exon 10 containing tau mRNA. Brain Res 831: 301-305.

Ye, L., Fritschi, S.K., Schelle, J., Obermuller, U., Degenhardt, K., Kaeser, S.A. et al. (2015). Persistence of Abeta seeds in APP null mouse brain. Nat Neurosci

Yin, H. & Kuret, J. (2006). C-terminal truncation modulates both nucleation and extension phases of tau fibrillization. FEBS Lett 580: 211-215.

Yin, R.H., Yu, J.T. & Tan, L. (2015). The Role of SORL1 in Alzheimer's Disease. Mol Neurobiol 51: 909-918.

Yonetani, M., Nonaka, T., Masuda, M., Inukai, Y., Oikawa, T., Hisanaga, S. et al. (2009). Conversion of wild-type alpha-synuclein into mutant-type fibrils and its propagation in the presence of A30P mutant. J Biol Chem 284: 7940-7950.

Yoon, S.H. & Fearon, D.T. (1985). Characterization of a soluble form of the C3b/C4b receptor (CR1) in human plasma. J Immunol 134: 3332-3338.

Yu, G., Nishimura, M., Arawaka, S., Levitan, D., Zhang, L., Tandon, A. et al. (2000). Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and betaAPP processing. Nature 407: 48-54.

Yu, J.T., Jiang, T., Wang, Y.L., Wang, H.F., Zhang, W., Hu, N. et al. (2014). Triggering receptor expressed on myeloid cells 2 variant is rare in late-onset Alzheimer's disease in Han Chinese individuals. Neurobiol Aging 35: 937.e1-937.e3.

Yuan, Z., Agarwal-Mawal, A. & Paudel, H.K. (2004). 14-3-3 binds to and mediates phosphorylation of microtubule-associated tau protein by Ser9-phosphorylated glycogen synthase kinase 3beta in the brain. J Biol Chem 279: 26105-26114.

Yuzwa, S.A., Shan, X., Macauley, M.S., Clark, T., Skorobogatko, Y., Vosseller, K. et al. (2012). Increasing O-GlcNAc slows neurodegeneration and stabilizes tau against aggregation. Nat Chem Biol 8: 393-399.

Zannis, V.I., Breslow, J.L., Utermann, G., Mahley, R.W., Weisgraber, K.H., Havel, R.J. et al. (1982). Proposed nomenclature of apoE isoproteins, apoE genotypes, and phenotypes. J Lipid Res 23: 911-914.

Zeggini, E. & Ioannidis, J.P. (2009). Meta-analysis in genome-wide association studies. Pharmacogenomics 10: 191-201.

Zhai, J., Zhang, L., Mojsilovic-Petrovic, J., Jian, X., Thomas, J., Homma, K. et al. (2015). Inhibition of Cytohesins Protects against Genetic Models of Motor Neuron Disease. J Neurosci 35: 9088-9105.

Zhang, B., Gaiteri, C., Bodea, L.G., Wang, Z., McElwee, J., Podtelezhnikov, A.A. et al. (2013). Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. Cell 153: 707-720.

Zhang, Y., Li, H.L., Wang, D.L., Liu, S.J. & Wang, J.Z. (2006). A transitory activation of protein kinase-A induces a sustained tau hyperphosphorylation at multiple sites in N2a cells-imply a new mechanism in Alzheimer pathology. J Neural Transm 113: 1487-1497.

Zhao, Y., Tseng, I.C., Heyser, C.J., Rockenstein, E., Mante, M., Adame, A. et al. (2015a). Appoptosin-Mediated Caspase Cleavage of Tau Contributes to Progressive Supranuclear Palsy Pathogenesis. Neuron 87: 963-975.

Zhao, Z., Sagare, A.P., Ma, Q., Halliday, M.R., Kong, P., Kisler, K. et al. (2015b). Central role for PICALM in amyloid-beta blood-brain barrier transcytosis and clearance. Nat Neurosci

Zhong, N. & Weisgraber, K.H. (2009). Understanding the association of apolipoprotein E4 with Alzheimer disease: clues from its structure. J Biol Chem 284: 6027-6031.

Zhou, X.W., Gustafsson, J.A., Tanila, H., Bjorkdahl, C., Liu, R., Winblad, B. et al. (2008). Tau hyperphosphorylation correlates with reduced methylation of protein phosphatase 2A. Neurobiol Dis 31: 386-394.

Zhou, X.Z., Kops, O., Werner, A., Lu, P.J., Shen, M., Stoller, G. et al. (2000). Pin1-dependent prolyl isomerization regulates dephosphorylation of Cdc25C and tau proteins. Mol Cell 6: 873-883.

Zhou, X.Z., Lu, P.J., Wulf, G. & Lu, K.P. (1999). Phosphorylation-dependent prolyl isomerization: a novel signaling regulatory mechanism. Cell Mol Life Sci 56: 788-806.

Zhu, X., Rottkamp, C.A., Boux, H., Takeda, A., Perry, G. & Smith, M.A. (2000). Activation of p38 kinase links tau phosphorylation, oxidative stress, and cell cycle-related events in Alzheimer disease. J Neuropathol Exp Neurol 59: 880-888.

Zhukareva, V., Mann, D., Pickering-Brown, S., Uryu, K., Shuck, T., Shah, K. et al. (2002). Sporadic Pick's disease: a tauopathy characterized by a spectrum of pathological tau isoforms in gray and white matter. Ann Neurol 51: 730-739.

Zita, M.M., Marchionni, I., Bottos, E., Righi, M., Del Sal, G., Cherubini, E. et al. (2007). Post-phosphorylation prolyl isomerisation of gephyrin represents a mechanism to modulate glycine receptors function. EMBO J 26: 1761-1771.

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