Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Ahmad R. Sylvester Date

Characterization of LR11's Relationship to Tau Pathology

By

Ahmad R. Sylvester
Doctor of Philosophy

Graduate Division of Biological and Biomedical Sciences
Neuroscience

James J. Lah, M.D., Ph.D.
Advisor

Allan I. Levey, M.D., Ph.D.
Co-Advisor

Marla Gearing, Ph.D.
Committee Member

Randy Hall, Ph.D.
Committee Member

Elliott J. Mufson, Ph.D.
Committee Member

Richard Kahn, Ph.D.
Committee Member

Accepted:

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

Date

Characterization of LR11's Relationship to Tau Pathology

By

Ahmad R. Sylvester
M.A., State University of New York at Buffalo, 2005

Advisor: James J. Lah, M.D., Ph.D.

An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

in

Graduate Division of Biomedical and Biological Sciences
Neuroscience
2014

Abstract

Characterization of LR11's Relationship to Tau Pathology

By

Ahmad R. Sylvester

Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by amyloid plaques and neurofibrillary tangles (NFTs). Projected estimates of elderly population growth suggest that the already substantial societal and economic burdens imposed by AD will surge in coming years. Current treatments provide transient relief of select symptoms but fail to slow disease progression. Understanding the pathophysiology of AD may uncover pathological processes amenable to pharmacological manipulation, allowing interruption of disease progression.

Recently, lower neuronal expression of LR11/SorLA was observed in cortex from cases with sporadic but not in familial AD (FAD). However, a subset of brains from individuals with mild cognitive impairment, a prodromal stage of AD, displayed reduced LR11/SorLA. Intronic polymorphisms in the LR11 gene, *SORL1*, correlated with increased risk of sporadic AD, suggesting altered protein expression but not function, confers increased risk of disease. *In vitro* and *in vivo* studies revealed an inverse relationship between LR11 expression and amyloid burden, presumably mediated by LR11 negatively regulating amyloidogenic processing of amyloid precursor protein via an intracellular sorting function. These data suggested that reduction of neuronal LR11 is an incipient event in AD that facilitates development of amyloid pathology.

In this dissertation, I report a novel association between LR11 and NFTs in sporadic AD and FAD brain, with a preferential association between LR11 and early-stage NFTs. Differences in antibody use, disease severity, and brain region examined are shown to underlie the discrepancy between current and previous findings. I also demonstrate that LR11 associates with tau pathology in progressive supranuclear palsy (PSP) but not corticobasal degeneration (CBD) or Pick's disease (PiD) brain, indicating a preferential association with the 4 repeat straight filaments that characterize PSP. Evidence that 4 repeat aggregates and straight filaments are early forms of tau pathology is discussed and I propose two complimentary mechanisms by which LR11 may regulate development of tau pathology.

Characterization of LR11's Relationship to Tau Pathology

By

Ahmad R. Sylvester
M.A., State University of New York at Buffalo, 2005

Advisor: James J. Lah, M.D., Ph.D.

A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

in

Graduate Division of Biomedical and Biological Sciences
Neuroscience
2014

Table of Contents

General Introduction 1
- **Discovery 1**
- **Current and Impending Societal Impact 3**
- **Neuropathological Hallmarks of Alzheimer's Disease 4**
  - Amyloid Plaques 4
  - Neurofibrillary Tangles 15
- **Genetic Risk Factors Associated with Development of AD 18**
  - Down Syndrome 18
  - Familial AD 19
  - Sporadic AD 21
- **LR11/SorLA 23**
  - Discovery 23
  - The VPS10 Receptor Family 24
  - The Low-Density Receptor Family 25
  - Structure of LR11/SorLA 26
  - LR11/SorLA's Relationship to AD 28
- **Proposed Research 33**

Materials and Methods 34
- LR11 Antibodies 34
- Tau Antibodies 36
- Post-Mortem Human Brain Tissue 37
- Human Embryonic Kidney (HEK) 293 Cells 39
- **Chromagen Immunohistochemistry (IHC) 39**
  - Single-Chromagen IHC 39
  - Dual-Chromagen IHC 40
- **Modified Braak Staging Using LR11 CT-Positive Neurofibrillary Tangles 41**
- **Dual-Fluorescence Immunohistochemistry 43**
- **Western Blotting 43**
- **Statistical Analyses 45**

LR11 Associates with Early-Stage Neurofibrillary Tangles in Alzheimer's Disease 46
- **Introduction 46**
- **Results 48**
  - Identification of NFT-Like Structures with LR11 CT Antibody 48
  - LR11 CT Antibody Labeling of NFTs Confirmed 50
  - LR11 CT but Not LR11 3850 Labels NFTs in Sporadic AD 55
  - Case Selection and Regional Differences: LR11 CT-Positive NFTs and Braak Staging 55
  - Revisiting FAD (and DS): LR11 CT Antibody Labels NFTs in FAD and DS Brain 58
  - LR11 Preferentially Associates with Early-Stage NFTs 61
- **Discussion 64**

LR11 Associates with Tau Pathology in Progressive Supranuclear Palsy but Not Corticobasal Degeneration or Pick's Disease 68
- **Introduction 68**
  - Alzheimer's Disease (AD) 68

Progressive Supranuclear Palsy (PSP) 69
Corticobasal Degeneration (CBD) 70
Pick's Disease (PiD) 71
Results 73
LR11 CT Antibody Labels NFTs in PSP Brains 73
LR11 CT Antibody Does Not Label CBD Pathology 73
LR11 CT Labels Isolated Pick Bodies in PiD Brains 76
Discussion 76
General Discussion 80
LR11/SorLA 80
Tau Pathology 82
Summary and Interpretation of Results 85
LR11 in AD Brain 85
LR11 in Non-AD Tauopathies 89
Conclusion 96
References 99

List of Figures

Figure 1.1. Neuropathological Hallmarks of Alzheimer's Disease..... 2

Figure 1.2. Amyloid Precursor Protein (APP) Processing.....10

Figure 1.3. Structural Relationship Between LR11 and the Low Density Lipoprotein (LDL) and VPS10 Receptor Families.....27

Figure 1.4. LR11 Staining in Human Control and Alzheimer's Disease Frontal Cortex.....30

Figure 1.5. Quantitative Analysis of LR11 Immunoreactivity in Cortical Neurons of Individuals with No Cognitive Impairment (NCI), Mild Cognitive Impairment (MCI), and Alzheimer's Disease (AD) 31

Figure 3.1. Photomicrographs of LR11 CT Immunostaining in Human Frontal Cortex.....49

Figure 3.2. Photomicrographs of LR11 CT Peptide Competition in Human Frontal Cortex.....51

Figure 3.3. LR11 CT Staining of 250 kDa Band in Human Brain Homogenate but No Staining of Tau Bands.....52

Figure 3.4. Dual-Immunofluorescent Immunohistochemistry in Advanced Sporadic Alzheimer's Disease (Braak V-VI) Frontal Cortex.....54

Figure 3.5. LR11 3850 and LR11 CT Immunostaining in Human Frontal Cortex 56

Figure 3.6. Regional Distribution of LR11 CT-Positive Neurofibrillary Tangles (NFTs) is Consistent with Braak Staging.....60

Figure 3.7. LR11 CT Immunostaining in Familial Alzheimer's Disease (FAD) and Down's Syndrome (DS) Frontal Cortex 62

Figure 3.8. Dual-Chromagen Immunohistochemistry (IHC) in Advanced Sporadic Alzheimer's Disease (Braak V-VI) Frontal Cortex.....63

Figure 4.1. LR11 CT and AT8 Immunostaining in Progressive Supranuclear Palsy (PSP) Basal Ganglia, Coticobasal Degeneration (CBD) Frontal Cortex, and Pick's Disease (PiD) Dentate Gyrus.....75

List of Tables

Table 2.1. Antibodies 35
Table 2.2. Human Cases Used 38
Table 3.1. Control and Sporadic AD Cases 53
Table 3.2. Braak-Staged Cases Stained with LR11 CT 59
Table 4.1. Tauopathies 74
Table 2.1. Semiquantitative Analysis of Tauopathies 77

Chapter 1

General Introduction

Discovery

In 1901, Auguste D. (May 16, 1849 – June 8, 1906) was admitted to the Hospital for the Mentally Ill and Epileptics, Frankfurt am Main, presenting with a host of cognitive deficits, including memory loss, disorientation, aphasia, auditory hallucinations, paranoid delusions, and psycho-social incompetence. Under the intermittent care of Dr. Alois Alzheimer (June 14, 1864 – December 19, 1915), a senior physician and psychiatrist, Auguste D.'s cognitive decline was monitored until her death, at which point her brain was neuropathologically evaluated. Using the recently-developed Bielschowsky silver staining technique (Maurer, Volk, and Gerbaldo 1997), Alzheimer discovered two prominent lesions: amyloid plaques and neurofibrillary tangles (NFTs) (Figure 1.1) (Alzheimer et al. 1995).

At the time, Auguste D.'s clinico-pathological profile was thought to be indicative of "pre-senile dementia," an antiquated diagnosis used to distinguish rare, early-onset dementia from the more common, late-onset type (referred to as senile dementia) (Selkoe 2001, Goedert et al. 1995). However, extensive correlational analyses of cognitive ability and lesion frequency identified similar clinico-pathological profiles in individuals with both "pre-senile" and senile dementia

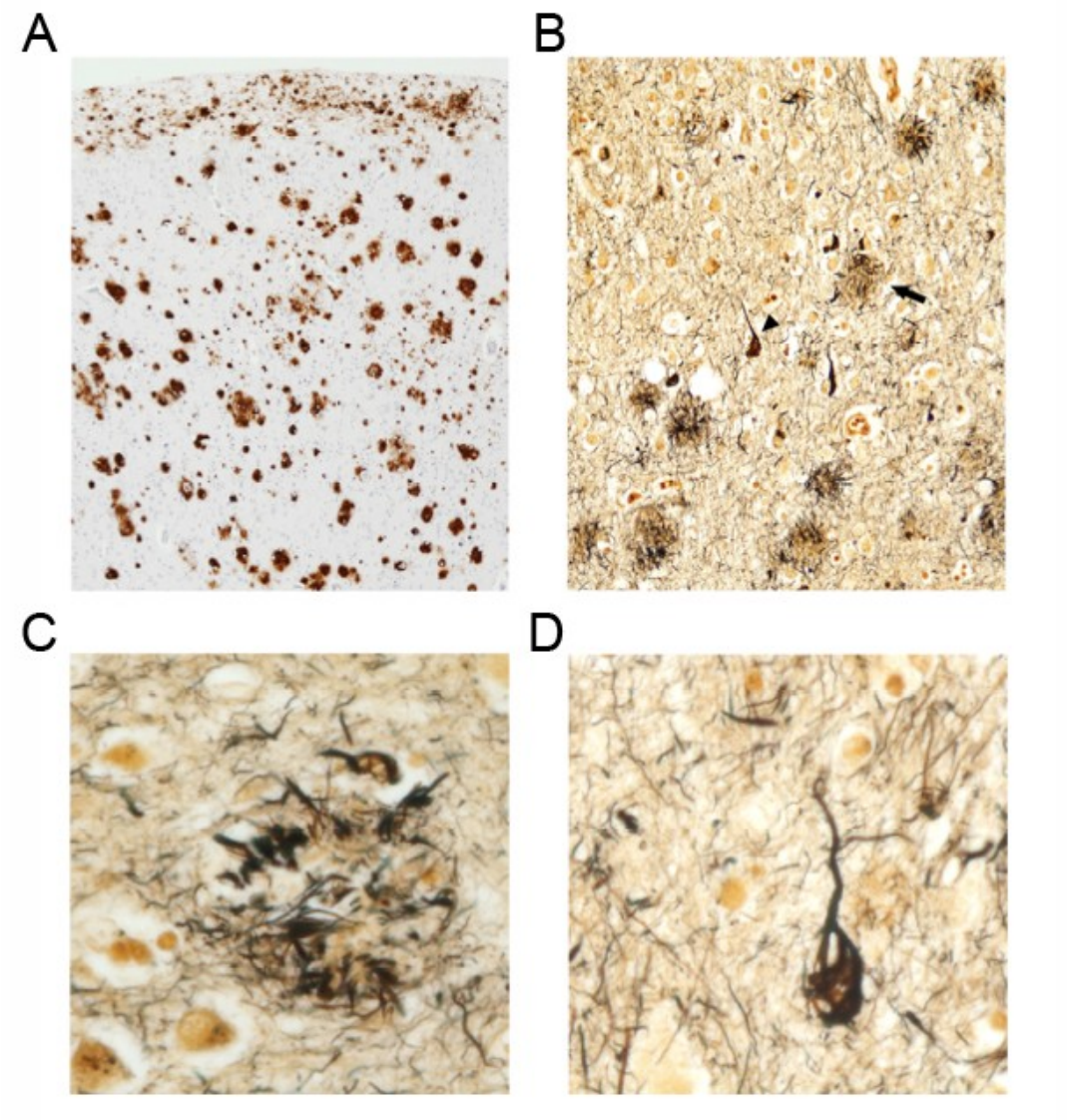


Figure 1.1. Neuropathological Hallmarks of Alzheimer's Disease. (A) Low magnification image of immunolabeled amyloid plaques. (B) High magnification image of argyrophilic amyloid plaques (arrow) and neurofibrillary tangles (arrowhead). (C) High magnification image of a neuritic plaque. (D) High magnification image of a neurofibrillary tangle.

Images courtesy of the Levey/Lah laboratory, Emory University

(Blessed, Tomlinson, and Roth 1968), strongly suggesting that the two diagnoses identified the same disease, with age of onset being the only distinguishing characteristic (Roth, Tomlinson, and Blessed 1967, 1966, Tomlinson, Blessed, and Roth 1968). These results led to the eventual unification of presenile and senile dementia into the single diagnosis – Alzheimer's disease (AD) – and brought about the realization that AD is the most common cause of dementia among the elderly (Roth, Tomlinson, and Blessed 1967, 1966, Tomlinson, Blessed, and Roth 1968).

Current and Impending Societal Impact

Currently, the prevalence of AD is estimated to double every five years beyond the age of 65, approaching 50% in individuals aged 85 or older (Evans et al. 1989). In the United States, alone, that translates into an estimated 5.4 million individuals afflicted with AD (2012). Treatment costs associated with individual care exceed $80-100 billion per year, but the greater societal and economic impact is felt when the relatives of AD patients are called upon to act as caretakers – a tremendous task that often results in their withdrawal from the workforce. Their absence results in over $200 billion in lost productivity (2012). With medical advances driving increases in life expectancy, it is projected that by 2050, there will be 13.2 million Americans with AD and disease related costs are expected to reach $1.2 trillion (2012).

Acetylcholinesterase inhibitors and the N-methyl-D-aspartate receptor blocker, memantine, are currently the sole forms of treatment for AD (Lleo, Greenberg, and Growdon 2006). Although these drugs provide transient relief to

some individuals in the early to mild stages of disease, they do nothing to alter disease progression (Lleo, Greenberg, and Growdon 2006). Effective treatment will require the identification of early pathological processes that are amenable to pharmacological manipulation, allowing the disease progression to be slowed or stopped. As such, considerable effort is being exerted to expand our understanding of early pathological events associated with the development of plaques and NFTs, as well as identify novel pathological events linked to the early stages of AD.

Neuropathological Hallmarks of Alzheimer's Disease

Amyloid Plaques

Amyloid Precursor Protein

In the mid 1980s, the amyloid beta (Aβ) peptide was purified from AD brain homogenates and identified as the primary component of plaques (Glenner and Wong 1984, Masters et al. 1985a). Shortly thereafter, the amyloid precursor protein (APP) was identified as the holoprotein from which Aβ is derived (Kang et al. 1987, Tanzi et al. 1987), raising questions about the normal function of APP including its role in the generation of Aβ and its pathological functions.

APP is a type I transmembrane protein that belongs to a protein family known as the amyloid-precursor like proteins (APLPs). Contained within this protein family are three isoforms of APP and two other proteins: APLP1 and APLP2. The APP isoforms vary in length, based largely on the inclusion or exclusion of the Kunitz protease inhibitor (KPI) domain. They also display differences in cell type

expression. The two longer isoforms, APP-751 and APP-770, are preferentially expressed in non-neuronal cells, while the shortest isoform, APP-695, is the dominant isoform expressed in neurons (Holtzman, Morris, and Goate 2011). However, comparisons of control and AD brain have revealed increased neuronal levels of APP-751 and APP-770 in AD brain, suggesting that the KPI domain plays a role in the disease process (Moir et al. 1998). It has been suggested that the KPI domain interferes with lipoprotein receptor-mediated clearance of Aβ (Moir and Tanzi 2005), thereby facilitating plaque deposition.

Unlike the APP isoforms, APLP1 and APLP2 do not contain the Aβ sequence (Holtzman, Morris, and Goate 2011), the absence of which is also noted in APP homologs found in other species, including *Drosophila melanogaster* (*Appl*), *Caenorhabditis elegans* (*apl-1*), and other mammals (Holtzman, Morris, and Goate 2011, Selkoe 2001). This suggests that the primary function of APP, while important enough to be conserved across species, is not related to the production of Aβ. Elucidating the specific function of APP, however, has been confounded by: (1) an incomplete understanding of the functional overlap between APP, APLP1, and APLP2; (2) protein interactors that associate with APP, serving to greatly expand its functional potential but also masking its independent effects; and (3) functional overlap between APP and proteolytically-derived metabolites.

APLP Family Function

Loss of function studies are the standard approach for deducing the physiological role of a gene, and the APLP family has been studied extensively with

regard to gene inactivation and knockout in *C. elegans* (worm), *D. melanogaster* (fly), and *Mus musculus* (mouse). In *C. elegans*, *apl-1* null mutants displayed a developmentally lethal phenotype as a result of molting defects. In addition, hypersensitivity to the acetylcholinesterase inhibitor, aldicarb, was observed, suggesting a role for APL-1 protein at the synaptic junction (Hornsten et al. 2007, Wiese, Antebi, and Zheng 2010). In *D. melanogaster*, *Appl* null mutants were viable and fertile, but had a reduced number of buttons at the neuromuscular junction. Conversely, larvae overexpressing APPL had an increased number of buttons (Torroja et al. 1999). APPL has also been implicated in the regulation of neurite arborization (Leyssen et al. 2005). Both the facilitation of synaptic formation and neurite arborization were shown to be dependent upon the C-terminal YENPTY motif, suggesting the potential for modulation by protein interactors, such as Fasciclin II and dX11/Mint (Ashley et al. 2005). Overall, these studies suggest that the ancestral *App*-like gene evolved to promote synapse formation and neurite arborization in the nervous system.

The role of the APLP family in mammals is much more complex. In *M. musculus*, *App* null mutants are viable and reproduce, but display impaired forelimb strength, locomotion, and long-term potentiation (Zheng et al. 1995). Mixed results were obtained when analyzing dendrite spine formation, but most studies found that loss of APP resulted in a decreased number of dendritic spines (Shariati and De Strooper 2013), suggesting a mechanism of action similar to that underlying APPL's regulation of neurite arborization in *D. melanogaster*. *Aplp* null mutants have not been extensively studied, but if there was functional redundancy between APP,

APLP1, and APLP2, perhaps the loss of function of APLP1 or APLP2 would result in a phenotype similar to *App* null mutants (Shariati and De Strooper 2013). However, *Aplp1* knock out mice show no evidence of impaired forelimb strength or locomotion. Likewise, no alterations in dendritic spines were observed in *Aplp2* null mutants (Shariati and De Strooper 2013). These findings suggest that APP is specialized in its functions at the synaptic junction, which are not likely compensated for by APLP1 and APLP2.

Double knock out of *APP* and *Aplp1* studies revealed expression of APLP2 alone is sufficient for survival, but in the absence of APLP2, mice survive for only a few weeks beyond birth when expressing both APP and APLP1 (Heber et al. 2000). This suggests that APP and APLP1 act together to compensate for the loss of APLP2. Alternatively, unrelated factors may compensate for the loss of APLP2. In addition, impairments at the neuromuscular junction (NMJ) have been noted in *App*/*Aplp2* double knockouts. Specifically, reduced vesicle density in the presynaptic active zone, excessive nerve terminal sprouting, and aberrant apposition of presynaptic markers have been noted, suggesting a key role for APP in the proper formation of synaptic structures at the NMJ (Weyer et al. 2011). Interestingly, expression of soluble APP α (sAPPα), but not soluble APP β (sAPPβ) (discussed below), was able to rescue the lethality. However, they still displayed impaired neuromuscular transmitter release and structural abnormalities at the NMJ that correlated with reduced forelimb strength, as well as impaired LTP (Weyer et al. 2011). These results suggest that minor differences in the amino acid sequences of sAPPα and sAPPβ are critical for their biological function and that full length APP may be

needed to rescue the *App*/*Aplp2* double knock out phenotype. The function of APP at the NMJ may be mediated via the C-terminal YENPTY motif. APP knock in mice with a Y682G mutation in an *Aplp2* null background display lethality and neuromuscular impairments similar to *App*/*Aplp2* double knockout mice (Barbagallo et al. 2011). Again, this indicates the potential for functional modulation by protein interactors.

In addition to playing a role in the proper development of the NMJ, the APLP family also effects cortical development. During cortical development, glia guided migration of post-mitotic neurons from the ventricular zone to the cortical plate is halted by Cajal-Retzius (CR) cells expressing Reelin. Triple deletion of *App*, *Aplp1*, and *Aplp2* resulted in a reduced number of CR cells and the accumulation of neurons, that migrated beyond the cortical plate, in the marginal zone (Herms et al. 2004). However, conflicting results have been observed in *App* knock down and *App* overexpressing mice, with *App* knock down inhibiting migration of cortical neurons and *App* overexpression promoting migration (Young-Pearse et al. 2007). Interestingly, endogenous APLP1 and APLP2 were not able to compensate for the loss of APP in *App* knock down mice, but co-electroporation of *Aplp1* or *Aplp2* rescued the impaired migration phenotype, indicating that, when overexpressed, the proteins can be redundant (Young-Pearse et al. 2007). Therefore, the regulation of expression levels of APP and the APLPs is critical for their biological function.

APP Protein Interactors

APP protein interactors can be divided into two groups: those that bind the extracellular domain and possibly behave as ligands and those that bind the

intracellular domain and possibly influence intracellular signaling. F-spondin, Tag1, Reelin, Nterin, Lingo-1, and Pancortins are examples of extracellular protein interactors and Fe65, JIP, JNK, Mint/X11, Dab1, and LR11 are examples of intracellular protein interactors (Rice et al. 2012, Reinhard, Hebert, and De Strooper 2005, Zheng and Koo 2011, Herskowitz et al. 2012b). These protein interactors also have the potential to influence the biological functions of APP metabolites.

APP Processing (Figure 1.2)

APP metabolites are the byproduct of enzymatic processing by α-, β-, and γ-secretases (Selkoe et al. 1996). Different sets of metabolites are generated based on which of two mutually exclusive metabolic pathways APP is processed along. These pathways are referred to as the non-amyloidogenic and amyloidogenic pathways and, as their names suggest, preclude or potentiate the generation of Aβ, respectively. Non-amyloidogenic processing is initiated by α-secretase cleavage at a site 12 amino acids N-terminal to the transmembrane domain of APP. This cleavage site is located within the Aβ sequence, which precludes generation of the Aβ peptide (Esch et al. 1990) and results, instead, in the generation of a soluble APP fragment (sAPPα), reported to stimulate cell growth and increase synaptic density (Thinakaran and Koo 2008), and an 83 amino acid, membrane-bound carboxyl-terminal fragment (CTFα). CTFα is subsequently cleaved by the intramembranous enzyme complex, γ-secretase, generating the p3 fragment and a small peptide called the APP intracellular domain (AICD), which may be involved in intracellular signaling and/or transcriptional regulation

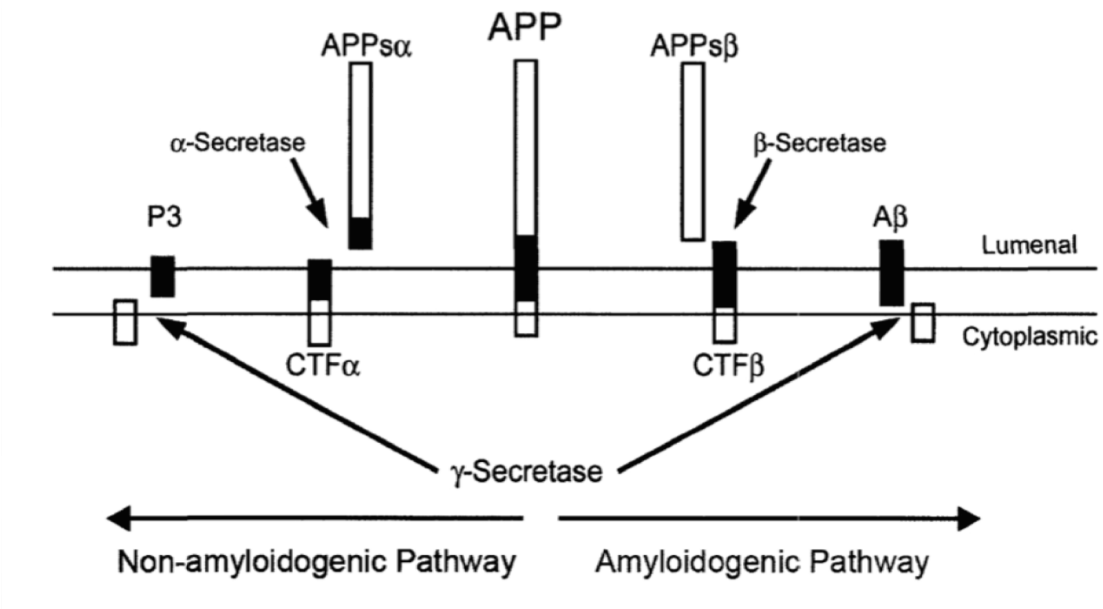


Figure 1.2. Amyloid Precursor Protein (APP) Processing. Proteolytic processing of APP can occur along one of two pathways. In the amyloidogenic pathway (right), APP is first cleaved by β-secretase within the extracellular domain at the N-terminal of the Aβ peptide sequence, releasing the APPsβ fragment and leaving the CTFβ fragment remaining within the membrane. This fragment is then cleaved at the C-terminal of the Aβ peptide sequence, releasing Aβ from the membrane and generating the APP intracellular domain fragment (AICD). Alternatively, APP can be processed along the non-amyloidogenic pathway (left). In non-amyloidogenic processing, full-length APP is first cleaved by α-secretase within the Aβ fragment itself, releasing the APPsα fragment and precluding the formation of Aβ. The remaining CTFα fragment is then cleaved by γ-secretase to generate the p3 fragment and the APP AICD.

Image courtesy of the Levey/Lah Lab, Emory University

(Thinakaran and Koo 2008, Selkoe 2001).

Amyloidogenic processing is initiated by β-secretase cleavage at a site located 16 amino acids N-terminal to the α-secretase cleavage site, resulting in the generation of a soluble APP fragment (sAPPβ) and a 99 amino acid, membrane-bound carboxyl-terminal fragment (CTFβ) (Haass et al. 1992, Thinakaran and Koo 2008). Subsequent cleavage of CTFβ by γ-secretase results in the generation of the Aβ peptide and the same AICD fragment resulting from non-amyloidogenic processing (Seubert et al. 1992). γ-secretase cleaves at multiple sites within the transmembrane domain of APP, resulting in Aβ peptides ranging in length from 37 to 43 amino acids (Iwatsubo 2004, Morgan et al. 2004, Steiner, Fluhrer, and Haass 2008). Aβ40 is the most abundant species, accounting for 90% of amyloid in human brain, but the longer, more fibrillogenic Aβ42 is the dominant species in plaques (Iwatsubo 2004, Morgan et al. 2004, Steiner, Fluhrer, and Haass 2008), suggesting that Aβ42 transitions from a soluble monomeric state into the insoluble fibrils found in plaques (discussed below).

APP's exposure to the necessary compliment of secretases to promote either amyloidogenic or non-amyloidogenic processing is dependent upon its intracellular localization. Following synthesis in the endoplasmic reticulum, APP is post-translationally modified in the trans-Golgi network (TGN), traffics to the cell surface, and is subsequently internalized to early endosomes, at which point it is either recycled to the TGN or targeted for degradation (Caporaso et al. 1994, Selkoe et al. 1996, Bayer et al. 2001). Under normal conditions, the majority of APP is destined for non-amyloidogenic processing (Sisodia 1992, Lammich et al. 1999), following α-

secretase cleavage by a host of metalloproteases (including tumor necrosis factor converting enzyme (TACE), members of the A Disintegrin and Metalloprotease (ADAM) family, such as ADAM17, ADAM9, and ADAM10) located at the cell surface (Sisodia 1992, Allinson et al. 2003, Lammich et al. 1999, Buxbaum et al. 1998). The uncleaved portion of APP internalizes to early endosomes where it is subject to β-secretase cleavage by beta-site APP cleaving enzyme 1 (BACE-1), a transmembrane aspartyl protease (Walter et al. 2001, Shiba et al. 2004, Cai et al. 2001). CTFβ can then be cleaved by γ-secretase, releasing the Aβ peptide.

The Amyloid Cascade Hypothesis

Early studies found that aggregation of Aβ peptides is essential for them to exert a toxic effect (Katzman 1986, Terry et al. 1991, Dickson et al. 1995). These aggregates appear in the form of soluble, low molecular weight oligomers and insoluble, larger molecular weight fibrils that adopt a β-pleated sheet conformation and coalesce into diffuse and neuritic plaques (Selkoe 2001, Morgan et al. 2004). It is neuritic plaques (NPs) that were originally identified by Dr. Alzheimer in the brain of Auguste D. These argyrophilic structures are spherical in appearance and possess a dense amyloid core, containing both Aβ40 and Aβ42. Surrounding the amyloid core are dystrophic neurites, astrocytes, and activated microglia (Selkoe 2001, Morgan et al. 2004). NPs are often found in limbic and association cortices in AD brain. Diffuse plaques (DPs), on the other hand, have a more widespread distribution and often appear in the brains of healthy individuals (Morgan et al. 2004). Structurally, DPs differ from NPs as the former tend to be amorphous, as

opposed to the dense nature of NPs. In addition, DP are composed solely of Aβ42, and are not associated with dystrophic neurites (Morgan et al. 2004). This has led to the widely-held belief that DPs are an immature form of plaques that will develop into mature NPs containing neurites and surrounded by extensive inflammatory microglial activation (Selkoe 2001).

The conspicuous presence of amyloid plaques in AD brain, localization of the *APP* gene to chromosome 21, the development of AD pathology in Down's syndrome (DS)/trisomy 21 cases that live beyond the age of 40, and the discovery of mutations in the genes for APP and presenilin 1 and 2 that cause AD (see *Genetic Factors* section for detailed discussion) provide the basis for the amyloid cascade hypothesis. This hypothesis states that fibrillar Aβ is the causative agent underlying the full spectrum of pathological changes seen in AD brain (Hardy and Allsop 1991), including hyperphosphorylation of tau leading to the development of neurofibrillary tangles (discussed below), reduction of neurotransmitters (e.g., basal forebrain cholinergic and locus coeruleus norepinephrine neurons), and other long projection perikarya (Mufson et al. 2008, Weinshenker 2008), impaired synaptic function (which correlates strongly with cognitive impairment) (Terry et al. 1991), mitochondrial dysfunction and oxidative stress (which are linked to impaired synaptic function) (Mungarro-Menchaca et al. 2002), aberrant activation of inflammatory pathways (Schultzberg et al. 2007), enlargement of early endosomes (Nixon, Cataldo, and Mathews 2000), and altered cholesterol metabolism (Ricciarelli et al. 2012).

Despite the prominence of the amyloid cascade hypothesis, it has long been

recognized that plaque burden does not correlate well with cognitive impairment (Terry et al. 1991, Dickson et al. 1995, Katzman 1986). This has been considered the greatest weakness of the amyloid cascade hypothesis, but current research has demonstrated that Aβ oligomers correlate well with synaptic dysfunction and cognitive impairment (Lue et al. 1999, Wang et al. 1999), suggesting that Aβ oligomers, and not fibrillar Aβ, is the primary toxic species of Aβ. Support for this idea comes from transgenic mouse models, cell culture paradigms, and studies with synthetic Aβ.

Injection of an anti-Aβ antibody into a mouse model of AD that displayed impairment in an object recognition task reversed the impairment without decreasing plaque burden (Dodart et al. 2002), suggesting that the antibody may have acted on a soluble species of Aβ. In a separate mouse model of AD, injection of a purified preparation of Aβ oligomers resulted in impairment of spatial memory, supporting a role of Aβ oligomers in impairing cognitive ability. Additional experiments, in which wild type mice and rats were injected with conditioned media (CM) from cells overexpressing APP, have also been performed (Cleary et al. 2005). To assess the specific effects of Aβ oligomers, the CM was biochemically manipulated to remove Aβ oligomers or other APP metabolites. These studies demonstrated that CM containing Aβ oligomers inhibited LTP (Cleary et al. 2005) and reversibly decreased dendritic spine number and impaired short-term memory (Shankar et al. 2007). In studies using synthetic Aβ preparations, low micromolar concentration of oligomers have been shown to cause widespread neuronal death, within 24 hours, while fourfold higher concentrations of fibrillar Aβ are necessary to

cause only modest neuronal death, after 10 days (Deshpande et al. 2006). Together, these in vivo and in vitro studies provide support for a role of Aβ oligomers in initiating the pathological cascade seen in AD.

Neurofibrillary Tangles

In the late 1980s, a major breakthrough was made in our understanding of NFT pathology when NFTs were found to be primarily composed of the microtubule-associated protein tau (Wischik, Novak, Edwards, et al. 1988, Wischik, Novak, Thogersen, et al. 1988, Goedert et al. 1988, Grundke-Iqbal et al. 1986, Kosik, Joachim, and Selkoe 1986, Wood et al. 1986). Tau is a microtubule-associated phosphoprotein that is most abundant in neuronal axons (Trojanowski et al. 1989) where it regulates microtubule assembly and stabilization (Weingarten et al. 1975, Witman et al. 1976). Tau's ability to regulate microtubule assembly and stabilization is dependent on structural elements within tau as well as its phosphorylation state. Structurally, tau can be divided into four regions: (1) an N-terminal projection region, (2) a proline-rich domain, (3) a microtubule binding domain (MBD), and (4) a C-terminal region (Mandelkow et al. 1996). Alternative splicing of the tau gene, *MAPT*, produces six isoforms that differ based on the inclusion or exclusion of exons 2, 3, and 10. Exons 2 and 3 correspond to 29 amino acid N-terminal inserts and exon 10 corresponds to a 31 amino acid MBD repeat (Goedert, Spillantini, Jakes, et al. 1989). Isoforms are named based on a combination of their number of N-terminal inserts (termed N) and MBD repeats (termed R). 0N tau includes no N-terminal inserts, 1N tau includes one N-terminal

insert (exon 2), and 2N tau includes both N-terminal inserts (exons 2 and 3). Likewise, 3R tau has three MBDs (excludes exon 10) and 4R tau has four MBDs (includes exon 10) (Lee, Goedert, and Trojanowski 2001).

Tau's ability to bind microtubules is dependent on the MBD and adjacent regions (Gustke et al. 1994). Mutations in exon 10 have been shown to cause FTDP-17 by reducing microtubule binding affinity (i.e., P301L mutation) or decreasing the 3R:4R tau ratio (Ingram and Spillantini 2002, Fischer et al. 2007). The disease-related effect of decreasing the 3R:4R tau ratio correlates with findings in transgenic mice and AD brain. Tau knockout mice are viable and do not display major neurological deficits, which is likely due to the redundant nature of other MAPs, like MAP1A and MAP1B (Harada et al. 1994, Dawson et al. 2001, Ikegami, Harada, and Hirokawa 2000). However, mice overexpressing tau develop severe neuropathies or gliopathies (Gotz 2001). This suggests that one mechanism underlying the development of tau pathology is related to the overproduction of tau, in general (similar to APP overproduction in DS), and 4R tau, specifically. The pathological effect of increasing 4R tau expression is highlighted by *in vitro* studies showing that heparin-induced fibrilization occurs more rapidly with 4R tau than 3R tau, and that this effect is inhibited by maintaining comparable levels of the two isoform types (Adams et al. 2010). In AD and MCI, cholinergic basal forebrain neurons within the nucleus basalis and hippocampal (CA1) neurons display a decreased 3R:4R tau ratio at both the mRNA and protein level, when compared to age-matched controls (Ginsberg et al. 2006). This suggests that a decrease in the 3R:4R tau ratio may underlie the development of NFTs during the progression of

AD, as well as other tau-based pathologies, due to the increased fibrillogenic potential of 4R tau. The reason behind 4R tau's increased fibrillogenicity, however, remains unclear.

Phosphorylation, particularly near or within the MBD, also plays a prominent role in the development of NFTs. MBD repeats possess a positive net charge that facilitates binding to the negatively charged residues in tubulin, and phosphorylation may neutralize the positive charge of MBD repeats (Jho et al. 2010) or alter their conformation (Fischer et al. 2009) in such a way as to promote detachment from microtubules. Like Aβ, detached tau aggregates into increasingly higher order structures, including oligomers, fibrils, straight filaments (SFs), and paired helical filaments (PHFs) (Lee, Goedert, and Trojanowski 2001, von Bergen et al. 2005). Accumulation of PHFs in neuronal cell bodies gives rise to the NFTs that characterize AD (Wischik, Novak, Edwards, et al. 1988, Wischik, Novak, Thogersen, et al. 1988, Goedert et al. 1988, Grundke-Iqbal et al. 1986, Kosik, Joachim, and Selkoe 1986, Wood et al. 1986). Additional neurofibrillary lesions found in AD brain include neuropil threads (NTs) and dystrophic neurites, both of which reflect aggregation of PHF tau in neuronal processes; but dystrophic neurites can be distinguished based on their association with neuritic plaques (Buee et al. 2000, Goedert et al. 1995).

Regional distribution of NFTs and NTs follows a predictable ascension within AD brain, with their frequency increasing in earlier-impacted brain regions, as the disease progresses. Beginning in entorhinal cortex, NFTs and NTs progress to limbic structures before appearing in cortical brain regions (Braak and Braak

1991) (see Chapter 3 for greater detail). This progression is mirrored, in subsequent years, by ascending cortical atrophy (Frisoni et al. 2009) and correlates with severity of cognitive impairment better than the regional distribution or frequency of either diffuse or neuritic plaques (Bierer et al. 1995, Holzer et al. 1994). It has long been thought that NFTs cause a disruption in axonal transport (Kanaan et al. 2011, Spittaels et al. 1999) that impedes neurotransmission and gives rise to cognitive impairment. However, a growing body of literature suggests that soluble tau oligomers are the primary toxic species of aggregated tau (Santacruz et al. 2005, O'Leary et al. 2010, Mocanu et al. 2008, Sydow et al. 2011, Berger et al. 2007).

Genetic Risk Factors Associated with Development of AD

Down Syndrome

DS/trisomy 21 is a genetic disorder associated with the overproduction of APP and accelerated development of plaques and NFTs (Mann 1988a, b). By 40 years of age, virtually all individuals with DS develop characteristic AD pathology (Wisniewski, Wisniewski, and Wen 1985, Heston 1984) in the same brain regions affected in AD (Mann et al. 1986). As in AD, plaques and NFTs in DS brain are comprised of Aβ and tau (Masters et al. 1985b, Joachim et al. 1987, Heston 1984). In 1984, the realization that plaques in AD and DS brain were both comprised of Aβ led Glenner and Wong (Glenner and Wong 1984) to predict that the gene encoding the Aβ sequence would be located on chromosome 21. Their prediction was confirmed,

in 1987, when the gene encoding APP, was mapped to chromosome 21 (Goldgaber et al. 1987, Kang et al. 1987). Combined with subsequent identification of several cohorts with early-onset, familial AD (FAD) that results from small duplications of chromosome 21 loci that contain the *APP* gene and a case report describing an individual with DS, but no AD pathology, whose trisomy 21 did not include duplication of the *APP* gene (Prasher et al. 1998, Rovelet-Lecrux et al. 2006), overproduction of APP, alone, was determined to be sufficient to trigger AD pathogenesis in DS individuals.

Familial AD

In the years that followed, over 27 *APP* mutations were discovered that cause FAD (Ertekin-Taner 2007), but none have been associated with the development of DS (further supporting the independent role of increased APP production in triggering AD pathogenesis). All of these *APP* mutations cluster around one of the secretase cleavage sites (Ertekin-Taner 2007, Selkoe 2001), with mutations near the α- or β-secretase cleavage sites (i.e., the APP-SWE mutations that occur in the two amino acids immediately preceding the β-secretase cleavage site), resulting in increased production of total Aβ (Selkoe 2001). Mutations near the γ-secretase cleavage site, however, leave total Aβ levels unchanged but cause a shift in the Aβ42:Aβ40 ratio such that production of the more fibrillogenic Aβ42 is increased (Holtzman, Morris, and Goate 2011, Hardy 2006).

Shortly after the discovery of *APP* mutations, FAD cases attributed to mutations in *PSEN1* and *PSEN2*, the genes that code for presenilin 1 (PS1) and

presenilin 2 (PS2), were discovered (Schellenberg et al. 1992, Levy-Lahad et al. 1995, Rogaev et al. 1995). PS1 or PS2 combine with, at least, three other proteins – nicastrin, APH-1, and PEN2 – to form the γ-secretase complex (Iwatsubo 2004, Edbauer et al. 2003, Serneels et al. 2009, Yu et al. 2000), but it is the presenilins that are thought to facilitate cleavage of γ-secretase's many substrates, which include Notch, ErbB4, the epidermal growth factor receptor (EGFR), LR11/SorLA, the p75NTR low affinity neurotrophin receptor, and APP (Kimberly et al. 2003, Bohm et al. 2006b, Fluhrer et al. 2008, Wolfe 2008). Accordingly, studies have shown that APP co-immunoprecipitates presenilin (Weidemann et al. 1997, Xia et al. 1997) and mutation of two aspartates within the transmembrane domain of presenilin results in a significant reduction in Aβ production (Wolfe et al. 1999). In addition, presenilin knock out mice display normal levels of APP holoprotein, sAPPα, and sAPPβ, but have increased levels of C99 and C83 (γ-secretase substrates), and reduced levels of Aβ (γ-secretase metabolite) (De Strooper et al. 1998). Lastly, pharmacological compounds known to inhibit γ-secretase cleavage show selective binding to presenilins (Esler et al. 2000, Li et al. 2000). Together, these data support a direct role for presenilin in executing the final cleavage event in amyloidogenic processing of APP.

The early-onset FAD caused by mutations in APP, PSEN1, and PSEN2 account for approximately 5% of the total number of AD cases (Ertekin-Taner 2007). The remaining 95% of AD cases are considered to be sporadic and usually occur in the 7th decade of life, or later. Currently, allelic variation in *APOE*, the gene encoding ApoE, stands as the greatest genetic risk factor for developing late-onset, sporadic

AD (Kim, Basak, and Holtzman 2009).

Sporadic AD

ApoE is a 299 amino acid apolipoprotein that mediates the internalization of lipids via receptor-mediated endocytosis (Pfrieger 2003), with low-density lipoprotein receptors (LDLRs) acting as the primary ApoE receptor in human brain. There are three alleles of *APOE* – ε2, ε3, and ε4 – that result from missense mutations at amino acids 112 and 158; the resulting ApoE isoforms contain either cysteine or arginine at these sites. ApoE ε2 has cysteines at both sites, while ApoE ε3 (the most common isoform) has cysteine at 112 and an arginine at 158. ApoE ε4 has arginines at both sites (Dong et al. 1994, Mahley, Weisgraber, and Huang 2006). ApoE ε2 has been associated with decreased risk of developing sporadic AD (Farrer et al. 1997), while ApoE ε4 has been associated with an increased risk of developing sporadic AD such that heterozygosity for ε4 confers a 2-3 fold increase in lifetime risk and homozygosity for ε4 confers a 12 fold increase in lifetime risk (Strittmatter et al. 1993). This increased risk is also associated with a decrease in the age of disease onset, with 55% of ε4/ε4 carriers developing AD by the age of 80 as opposed to 3.1% of ε3/ε3 carriers (Myers et al. 1996).

The prevailing hypothesis regarding ApoE ε4's association with increased disease susceptibility suggests a toxic loss of function. ApoE ε4 has demonstrated the weakest affinity for Aβ, suggesting that it prevents glial-mediated clearance of ApoE-bound Aβ from the extracellular space (Gearing, Mori, and Mirra 1996, Beffert et al. 1998, Strittmatter et al. 1993). Studies showing that there is no difference in

Aβ production between ApoE alleles but a dose-dependent increase in plaque deposition associated with ApoE ε4 supports this idea (Polvikoski et al. 1995, Rebeck et al. 1993, Schmechel et al. 1993). In addition, ApoE's role in lipid internalization is thought to be critical for promoting neurite outgrowth, synaptogenesis, and membrane maintenance, and it has been suggested that ApoE ε4 has a reduced affinity for LDLRs. Therefore, ApoE ε4 carriers may have a reduced ability to repair membrane damage following an insult, such as that exerted by neurotoxic Aβ oligomers (Bellosta et al. 1995, DeMattos, Curtiss, and Williams 1998, Teter et al. 1999, Narita, Bu, et al. 1997, Nathan et al. 1994, Puttfarcken et al. 1997). Alternative hypotheses suggest that ApoE ε4 may confer a toxic gain of function. For example, a strong association between ApoE and Aβ40 in the neuritic plaques of ApoE ε4 carriers has led some to speculate that binding of ApoE to Aβ may induce structural changes that promote fibrillogensis (Gearing, Mori, and Mirra 1996, Mann et al. 1997, Castano et al. 1995, Ma et al. 1994, Wisniewski and Frangione 1992). There is also evidence to suggest that ApoE ε4 promotes neuroinflammation (Barger and Harmon 1997, Colton et al. 2004, Guo, LaDu, and Van Eldik 2004, LaDu et al. 2001, Lynch et al. 2003, Vitek, Brown, and Colton 2009).

Unlike the causative FAD mutations, inheritance of ApoE ε4 does not guarantee the development of AD; it simply increases the its likelihood. Nevertheless, ApoE ε4 is thought to contribute to approximately 50% of sporadic AD cases (Herz and Beffert 2000). The remaining cases have no clear genetic association, which is likely due to the complex, multifactorial nature of AD pathogenesis. However, detailed family histories reported by clinicians have

revealed a substantial genetic component to sporadic AD (Rosen et al. 2007), suggesting that continued investigation of disease-linked polymorphisms will uncover novel contributors to the disease process along with targets for therapeutic intervention. As such, a comprehensive online database has been established to organize studies of AD genetics and facilitate meaningful interpretation via meta-analysis (Bertram et al. 2007), http://www.alzgene.org.

In contrast, no mutations in *MAPT*, the gene that encodes tau, are associated with AD. Instead, *MAPT* mutations give rise to frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (Lee, Goedert, and Trojanowski 2001), one of several neurodegenerative tauopathies (see Chapter 4 for greater detail).

LR11/SorLA

Discovery

In 1996, two groups set out to identify novel low-density lipoprotein receptor (LDLR) family members. Using a cDNA screen for rabbit mRNA with sequence homology to the characteristic LDLR type A ligand binding repeats, the LA cluster, Yamazaki et al. (Yamazaki et al. 1996) identified a protein with 11 such domains, which was named LR11. In a separate set of experiments, Jacobsen et al. (Jacobsen et al. 1996b) used an affinity column coated with receptor-associated protein (RAP), known to bind the LA cluster of LDLRs, to isolate LDLR relatives from human brain extracts. SorLA was the name given to the isolated protein, due to its

classification within the vacuolar protein sorting 10 (VPS10) family of sorting receptors and possession of the LDLR family's characteristic LA cluster. The cDNA sequences reported in both studies were virtually identical, which served to (1) confirm that both studies identified the same protein, (2) validate the method of protein identification used in each study, and (3) demonstrate the highly conserved nature of LR11 in mammalian species. Similar expression patterns were also reported, with noted enrichment of LR11 in brain, when compared to peripheral tissues, such as the liver, thyroid, adrenal glands, and testes. Within the brain, expression was higher in the hippocampus, basal forebrain, cerebral cortex, and cerebellum, when compared to the thalamus, hypothalamus, and basal ganglia, in human and rodent brain (Yamazaki et al. 1996, Motoi et al. 1999).

The VPS10 Receptor Family

The VPS10 family of sorting receptors includes LR11, sortilin, three highly homologous proteins: SorC1, SorC2, and SorC3 (Jacobsen et al. 1996a, Hampe et al. 2000a, Hermey et al. 1999, Petersen et al. 1997, Rezgaoui et al. 2001). A generalized function of VPS10 receptors is the regulation of intracellular sorting from the trans-Golgi network (TGN) to endosomal and/or lysosomal compartments (Willnow, Petersen, and Nykjaer 2008). Sortilin, closest VPS10 family member to LR11, has been implicated in numerous cellular pathways associated with neurodegeneration. In cholinergic cells of the basal forebrain, sortilin binds/acts as a co-receptor for the neurotrophin receptor, p75NTR, and promotes apoptotic signaling (Teng et al. 2005, Al-Shawi et al. 2007, Volosin et al. 2006). Sortilin has also been suspected to be able

to facilitate BACE trafficking from endosomes to the TGN via an interaction with GGAs (He et al. 2005, Wahle et al. 2005).

The Low-Density Lipoprotein Receptor Family

The LDLR family is a collection of proteins that contain variable numbers of cysteine-rich, complement type A ligand binding repeats in their extracellular domains. Members of this family include: (1) the low-density lipoprotein receptor (LDLR), for which this protein family is named; (2) the very low-density lipoprotein receptor (VLDLR); (3) apolipoprotein receptor 2 (ApoER2); (4) the low-density lipoprotein receptor related protein 1 (LRP1) and its homologs LRP1B, megalin (LRP2), LRP5, and LRP6; (5) MegF7; and (6) LR11 (Herz and Bock 2002, Wagner and Pietrzik 2012). Functionally, all LDLR family members are able to bind and internalize low-density lipoproteins (including ApoE), contribute to the regulation of cholesterol homeostasis (Herz and Bock 2002), and participate in clathrin-mediated endocytosis of extracellular or membrane-bound ligands (Jaeger and Pietrzik 2008). The number of known LDLR ligands, as well as intracellular adaptor proteins, is continuing to grow, increasing the functional potential of LDLRs considerably. Among the putative functions assigned to LDLRs are regulation of protease activity at the cell surface, activation of intracellular signaling pathways, regulation of Ca^{2+} homeostasis, transport and activation of steroid hormones, and intracellular sorting (Herz 2001, Herz and Bock 2002, Willnow, Nykjaer, and Herz 1999, Nykjaer and Willnow 2002).

LDLRs are also associated with AD. LRP1, the most abundant ApoE receptor

in human brain, is thought to facilitate clearance of extracellular deposits of Aβ (Kounnas et al. 1995, Narita, Holtzman, et al. 1997, Kang et al. 2000) and possibly regulate APP processing into Aβ via direct interactions with the N-terminal region of APP and indirect interactions with the C-terminal mediated by the cytosolic adaptor proteins Fe65 and DAB1 at the NPXY internalization motif (Kinoshita et al. 2001, Rebeck et al. 2001, Pietrzik et al. 2002). VLDLR and ApoER2 have indirect interactions with APP that are mediated by DAB1, a gene that encodes a key regulator of Reelin signaling (Homayouni et al. 1999, Trommsdorff et al. 1999, Gotthardt et al. 2000). ApoER2 has been also suggested to regulate APP processing via interactions with the extracellular matrix protein, f-spondin (Hoe et al. 2005). LRP1B is also capable of interacting with APP and has demonstrated an ability to regulate the rate of APP endocytosis from the cell surface (Cam et al. 2004). Polymorphisms in LRP1 have also been associated with increased risk of developing AD, but other reports refute these findings (Hollenbach, Schulz, and Lehnert 1998, Sanchez et al. 2001, Pritchard et al. 2005). LR11 may also regulate APP processing into Aβ in AD (discussed below).

Structure of LR11/SorLA

LR11 is a 250 kDa type 1 transmembrane receptor composed of functional domains that link it to the low-density lipoprotein and VPS10 receptor families (Figure 1.3). The ectodomain of LR11 contains 6 functional domains (Yamazaki et al. 1996, Jacobsen et al. 1996b):

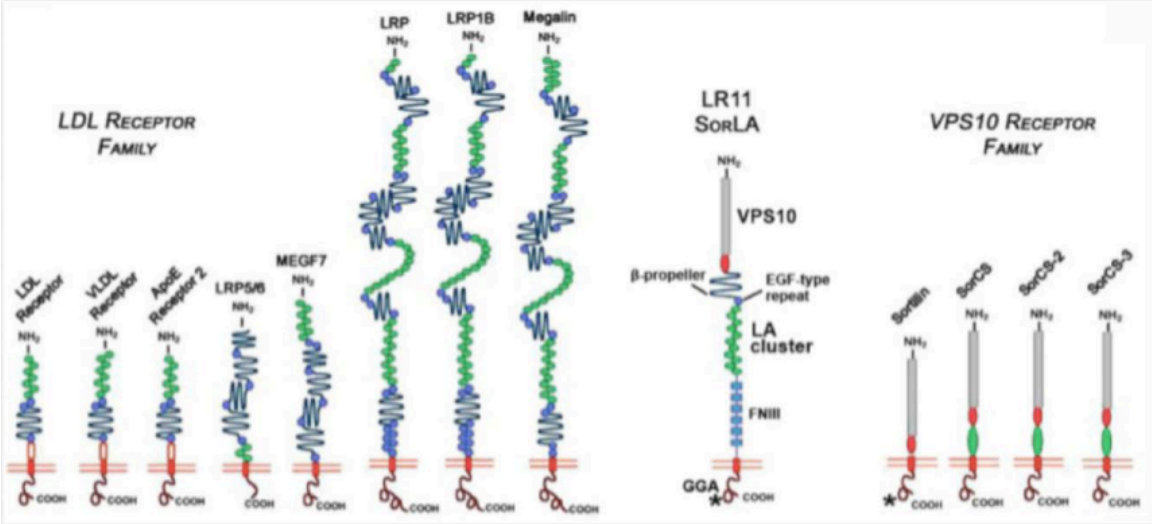


Figure 1.3. Structural Relationship Between LR11 and the Low Density Lipoprotein (LDL) and VPS10 Receptor Families. The domain structure of LR11 places it in both the LDL receptor and VPS10 receptor families. The large N-terminal ectodomain of LR11 contains a VPS10p homology domain, a β-propeller domain, five tandem LDL receptor consensus sequences founf in the EGF precursor (labeled EG-type repeate in the image), 11 LDL receptor type A ligand repeats (labeled LA cluster in the image) and six fibronectin type III (labeled FNIII). The short cytoplasmic tail of LR11 also harbors a GGA-binding domain, which can also be found in sortilin.

1. RAP, a short, N-terminal propeptide.
2. A large VPS10 homology domain. The VPS10 domain was first described in yeast and functionally mediates the sorting of carboxypeptidase Y from the Golgi to the vacuole, the yeast correlate of mammalian lysosomes (Marcusson et al. 1994).
3. A β-propeller domain.
4. 5 tandem LDLR consensus sequences found in an epidermal growth factor (EGF) precursor domain (also known as EGF-type repeats).
5. 11 LA clusters
6. 6 copies of fibronectin type II repeats, which are common to neural adhesion molecules.

Within the cytoplasmic tail, LR11 contains a seventh functional domain:

7. An adaptor protein binding domain that facilitates interaction with Golgi-localizing, γ-adaptin ear homology domain, ADP ribosylation factor (ARF) interacting proteins (GGAs) (Jacobsen et al. 2002)

LR11/SorLA's Relationship to AD

In 2004, LR11 was identified as a down-regulated transcript in immortalized lymphoblasts from individuals with probable and pathologically confirmed AD, when compared to non-disease, age-matched controls (Scherzer et al. 2004). Immunohistochemical analysis of LR11 in control and AD hippocampus, frontal cortex, and cerebellum revealed lower levels of neuronal LR11 in pyramidal cells of the hippocampus and frontal cortex, but not in purkinje cells of the cerebellum;

there were no differences in glial LR11 levels (Figure 1.4) (Scherzer et al. 2004, Offe et al. 2006a, Andersen et al. 2005b, Rogaeva et al. 2007). Interestingly, lower levels of neuronal LR11 were not found in cortical tissue from FAD (APP, FAD PS1 or FAD PS2) cases (Dodson et al. 2006), but were evident in mild cognitive impairment (MCI) brain (Figure 1.5) (Sager et al. 2007), suggesting that reduced neuronal LR11 is an early phenomenon that coincides with a transition from normal cognitive function to AD. Single nucleotide polymorphisms (SNPs) and SNP haplotypes of *SORL1*, the gene encoding LR11, have also been associated with increased risk of developing sporadic AD, as well as a reduced age of symptogenesis (Rogaeva et al. 2007, Lee, Barral, and Reitz 2008, Lee et al. 2008, Lee et al. 2007, Bettens et al. 2008, Kolsch et al. 2009, Meng et al. 2007). The presence of SNPs in intronic regions of *SORL1* lead the authors to speculate that the effects were likely reflected in protein expression levels but not protein structure or function. Additionally, *SORL1* variants have been associated with cognitive ability and MRI measure of cortical atrophy (Cuenco et al. 2008, Seshadri et al. 2007, Houlihan et al. 2009). It is important to note, that there is controversy surrounding which *SORL1* mutations, if any, are linked to increased risk of developing sporadic AD, and whether detected associations are specific to certain ethnic groups (Kauwe et al. 2010, Schjeide et al. 2009, Li et al. 2008, Liu et al. 2009, Minster, DeKosky, and Kamboh 2008). Taken together, these data support a proximal role for the reduction of neuronal LR11 in AD pathogenesis, with an unconfirmed genetic linkage.

In vitro and *in vivo* studies have demonstrated that LR11 expression is inversely related to Aβ levels. Cell culture studies overexpressing LR11 produced

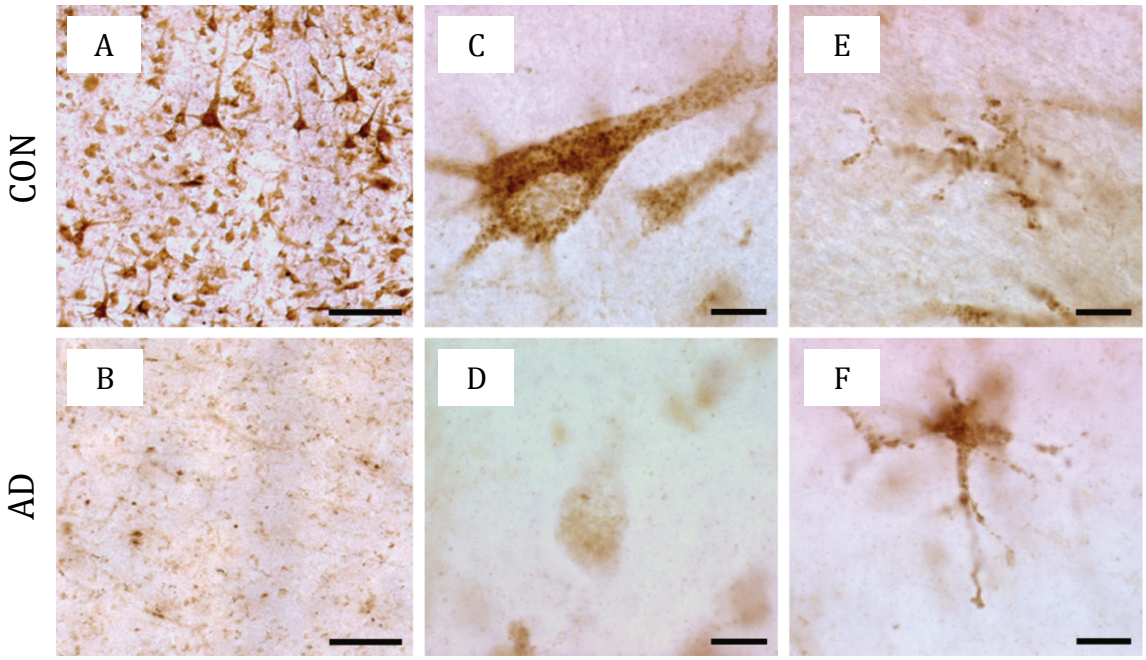


Figure 1.4. LR11 Staining in Human Control and Alzheimer's Disease Frontal Cortex. Low magnification image of pyramidal neurons immunoreactive for LR11 in control cortex (A). Low magnification image of neuronal LR11 staining in Alzheimer's disease cortex (B). High magnification image of a pyramidal neuron immunoreactive for LR11 in control cortex (C). High magnification image of a neuronal LR11 staining in Alzheimer's disease cortex (D). LR11 immunoreactivity in glial cells in control (E) and Alzheimer's disease (F) cortex.

Image reproduced from Scherzer CR et al (2004) (Scherzer et al. 2004)

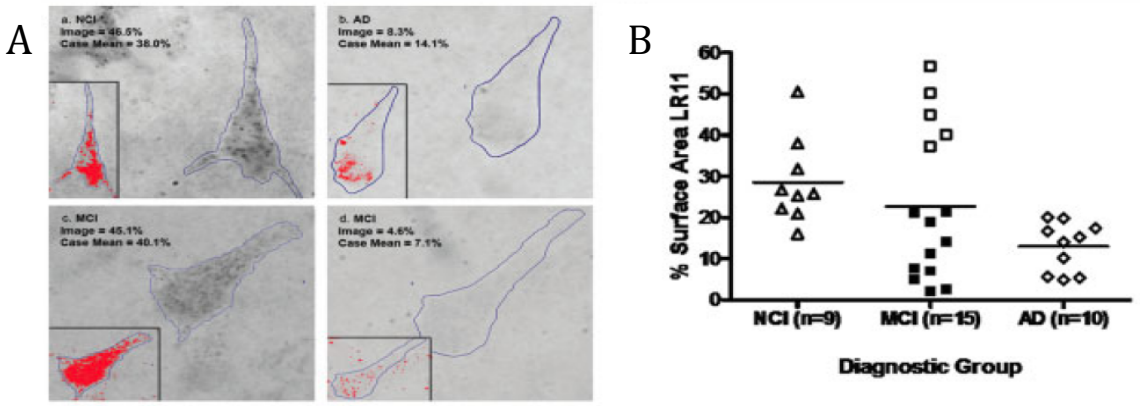


Figure 1.5. Quantitative Analysis of LR11 Immunoreactivity in Cortical Neurons of Individuals with No Cognitive Impairment (NCI), Mild Cognitive Impairment (MCI), and Alzheimer's Disease (AD). Pictorial representation of the quantitative method (A). Neuronal LR11 expression in the NCI group was significantly higher than neuronal LR11 expression in the AD group, but the MCI group displayed a bimodal distribution with two distinct subsets – one resembling the NCI group and the other resembling the AD group (B).

Image reproduced from Sager KL et al (2007) (Sager et al. 2007)

reductions in Aβ that correlated with the level of LR11 expression in the system (Andersen et al. 2005a, Offe et al. 2006b). Additionally, transgenic mice deficient in LR11 (LR11 -/-) had increased levels of soluble Aβ40 and Aβ42, but the inability of murine Aβ to aggregate prevented the development of plaques (Andersen et al. 2005a). Crossing the LR11 -/- mice with a well-established mouse model of AD expressing human transgenes with mutations in APP and PS1 (that promote aggressive amyloidosis) allowed the effects of LR11 expression on plaque accumulation to be tested. A dose-dependent relationship between LR11 expression and plaque burden was observed such that LR11 -/- mice had the greatest number of plaques, LR11 +/+ had the lowest number of plaques, and LR11 +/- mice had an intermediate number of plaques (Dodson et al. 2008b). Neither system detected a change in total levels of APP based on LR11 expression, suggesting that differences in Aβ deposition were a result of altered APP processing.

Biochemical analyses support a role for LR11 in negatively regulating Aβ production via intracellular sorting of APP to compartments without strong BACE-1 expression and also through competitive binding. LR11 has been shown to interact with APP and co-localize at the cell surface and within intracellular vesicular compartments (Spoelgen et al. 2006, Andersen et al. 2005a, Offe et al. 2006b). Overexpression of LR11 is associated with accumulation of APP in the endocytic recycling compartment, limiting its exposure to BACE-1 and prohibiting production of Aβ (Offe et al. 2006b, Schmidt et al. 2007, Andersen et al. 2005a, Herskowitz et al. 2012b). In the absence of LR11, APP missorts to alternative compartments, increasing its exposure to BACE-1 and initiating amyloidogenic processing (Offe et al.

2006b, Schmidt et al. 2007, Andersen et al. 2005a). In addition to binding APP, LR11 has been shown to bind to BACE-1 as well (Andersen et al. 2005a, Spoelgen et al. 2006). Overexpression of LR11 and BACE-1 blocks the increase in Aβ production that is seen with overexpression of BACE-1 alone (Andersen et al. 2005a, Spoelgen et al. 2006).

Proposed Research

The goal of this dissertation is to characterize the relationship between LR11 and tau pathology. To address this goal, I elected to immunohistochemically label post-mortem human brain tissue from individuals diagnosed with Alzheimer's disease, progressive supranuclear palsy, corticobasal degeneration, and Pick's disease for LR11. The tau pathology in each diagnostic group contains unique combinations of pathological epitopes that correspond to pathological tau aggregates in various stages of maturation and with differing structural characteristics. Understanding the relationship between LR11 and these various forms of tau pathology will provide insight into whether LR11 preferentially associates with a specific species of pathological tau. I hypothesized that LR11 preferentially associates with early-stage tau pathology, allowing for a functional relationship that may be manipulated to therapeutic advantage.

Chapter 2

Materials and Methods

Antibodies (Table 2.1)

LR11 Antibodies

Three LR11 antibodies were used throughout this dissertation; of these, two (LR11 CT and LR11 3850) were rabbit polyclonals generated against amino acids 2198-2215 of human LR11 protein, a sequence corresponding to the last 18 amino acids of the C-terminal of LR11. LR11 CT, an in-house antibody, was prepared and characterized as previously described (Ciliax et al. 1995, Ciliax et al. 2000, Hersch et al. 1994, Levey et al. 1991). Briefly, amino acids 2198-2215 of human LR11 protein were linked to thyroglobulin for use as an immunogen. A New Zealand white rabbit was immunized (Covance Research Products, Inc.; Denver, PA) and sera were purified on an LR11 CT affinity column. This antibody previously demonstrated specificity via its ability to immunoprecipitate LR11 from human brain homogenate (Herskowitz et al. 2011a). Antibody specificity was further demonstrated, in Chapter 3, via peptide competition in an immunohistochemical assay. LR11 3850

was kindly provided by Dr. C. Schaller (Zentrum fur Molekulare Neurobiologie, Universitat Hamburg, Hamburg, Germany) (Hampe et al. 2000b). The third LR11 antibody was a commercially available mouse monoclonal generated against amino acids 1220-1337 of human LR11 protein; this sequence corresponds to the LDLR ligand binding repeats located in the ectodomain/N-terminal (BD Bioscience, Franklin Lakes, NJ) of LR11 (Jacobsen et al. 1996b, Yamazaki et al. 1996, Posse De Chaves et al. 2000, Herskowitz et al. 2012a, Herskowitz et al. 2011b).

Tau Antibodies

Four mouse monoclonal tau antibodies were used throughout this dissertation. Three of the antibodies were generated against pathological tau epitopes corresponding to various phases of NFT maturation. AT8, whose phospho-specific epitope (pSer202/Thr205) originates in the "pre-tangle" stage of NFT maturation (Braak, Braak, and Mandelkow 1994a, Goedert, Jakes, and Vanmechelen 1995, Porzig, Singer, and Hoffmann 2007), was used to label early-stage NFTs; Alz50, which identifies a conformational change in tau such that the N-terminus folds and contacts one of the microtubule binding repeats (Carmel et al. 1996), was used to label NFTs in the middle stages of maturation; and MN423, which recognizes tau truncated at glutamic acid 391, labeled NFTs in the late stages of maturation (Khuebachova et al. 2002) (for a review, see (Guillozet-Bongaarts et al. 2005b). Dr. L. I. Binder (Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, IL) generously provided the Alz50

TABLE 2.1. Antibodies

Antibody	Epitope	Host/Type	Application(s)	Working Concentration/Dilution	Source
Primary Antibodies					
LR11 CT	Human LR11 aa2198-2215	Rabbit/Polyclonal	IHC WB	0.05 µg/mL 0.1 µg/mL	Levey-Lah Lab (Emory University)
LR11 3850	Human LR11 aa2198-2215	Rabbit/Polyclonal	IHC	1:1000	Dr. C. Schaller (Universitat Hamburg*)
LR11 NT	Human LR11 aa1220-1337	Mouse/Monoclonal	WB	1:2000	BD Bioscience (Franklin Lakes, NJ)
AT8	Human PHF-Tau pSer202/Thr205	Mouse/Monoclonal	IHC	1:5000** 1:10000**	Thermo Scientific (Waltham, MA)
Alz50	5-15/312-322	Mouse/Monoclonal	IHC	1:250	Dr. L. I. Binder (Northwestern University)
MN423	Tau1-391	Mouse/Monoclonal	IHC	1:500	Dr. L. I. Binder (Northwestern University)
Tau	Human Tau (352) aa23-179	Mouse/Monoclonal	WB	1:1000	BD Bioscience (Franklin Lakes, NJ)
Secondary Antibodies					
Biotinylated Goat Anti-Rabbit	Rabbit IgG (H+L)	Goat/Polyclonal	IHC	1:200	Vector Labs (Burlingame, CA)
Biotinylated Goat Anti-Mouse	Mouse IgG (H+L)	Goat/Polyclonal	IHC	1:200	Vector Labs (Burlingame, CA)
FITC Goat Anti-Mouse	Mouse IgG (H+L)	Goat/Polyclonal	IHC	1:100	Jackson Labs (Bar Harbor, MA)
Alexa Fluor 680 Goat Anti-Rabbit	Rabbit IgG (H+L)	Goat/Polyclonal	WB	1:25000	Invitrogen (Grand Island, NY)
IR Dye 800 CW Goat Anti-Mouse	Mouse IgG (H+L)	Goat/Polyclonal	WB	1:25000	Li-Cor Biosciences (Lincoln, NE)

*Universitat Hamburg is in Hamburg, Germany. **AT8 was used at 1:5000 in dual-fluorescence IHC and 1:10000 in dual-chromagen IHC. FITC = Fluorescein Isothiocyanate. IgG = Immunoglobulin G. H+L = Heavy and Light Chain.

and MN423 antibodies. The fourth antibody used was generated against amino acids 23-179 of human tau (352) (BD Bioscience, Franklin Lakes, NJ) (Alonso, Grundke-Iqbal, and Iqbal 1996, Goedert, Spillantini, Potier, et al. 1989, Kanai and Hirokawa 1995, Takeda et al. 2000, Wilson and Binder 1997).

Post-Mortem Human Brain Tissue

Free-floating and paraffin-embedded sections from cognitively normal controls (n=13), sporadic AD (n=19), FAD (n=14; 2 APP, 6 presenilin 1, and 6 presenilin 2), DS (n=12), and tauopathy cases (n=12; 4 progressive supranuclear palsy, 4 corticobasal degeneration, and 4 Pick's disease) were obtained from the Emory University Alzheimer's Disease Research Center (ADRC) and Center for Neurodegenerative Disease (CND), Rush University ADRC, and University of Washington ADRC (Table 2). Assessment of all tissues was performed by an experienced neuropathologist. Neuropathological assessment of AD was made according to the Consortium to Establish a Registry for Alzheimer's Disease (Mirra et al. 1991), as well as the National Institute on Aging (NIA)-Reagan criteria (1997, Hyman and Trojanowski 1997). Tauopathy diagnoses were also made based on established criteria (Hauw et al. 1994, Dickson 2001, Dickson, Bergeron, Chin, Duyckaerts, Horoupian, Ikeda, Jellinger, Lantos, Lippa, Mirra, Tabaton, Vonsattel, Wakabayashi, and Litvan 2002, Zhukareva et al. 2002). Control cases had no neuropathological diagnosis of AD or other neuropathological diseases or stroke. Each brain was fixed in 4% paraformaldehyde for 24-48 hours and cryoprotected in

Table 2.2. Human Cases Used

Neuropathological Diagnosis	Source	Number of Cases	Tissue Preparation
Control	Emory University	13*	Free Floating/Paraffin Embedded
Sporadic Alzheimer's Disease	Emory University	19**	Free Floating/ Paraffin Embedded
FAD APP	U. Washington	2	Paraffin Embedded
FAD PS1	U. Washington	6	Paraffin Embedded
FAD PS2	U. Washington	6	Paraffin Embedded
Down's Syndrome	Rush University U. Washington	6 6	Free Floating Paraffin Embedded
Progressive Supranuclear Palsy	Emory University	4	Paraffin Embedded
Corticobasal Degeneration	Emory University	4	Paraffin Embedded
Pick's Disease	Emory University	4	Paraffin Embedded

* Free floating only in 3 cases, paraffin embedded only in 3 cases, and free floating and paraffin embedded in 7 cases

** Free floating only in 7 cases, paraffin embedded only in 6 cases, and free floating and paraffin embedded in 5 cases

FAD APP = Familial Alzheimer's disease (AD) with an amyloid precursor protein mutation; FAD PS1 = Familial AD with a presenilin 1 mutation; and FAD PS2 = Familial AD with a presenilin 2 mutation.

30% sucrose (Emory University) or a glycerol solution (Rush University) (Gilmor et al. 1999, Mufson et al. 1999) prior to sectioning. Blocks designated for free-floating sectioning were sectioned on a freezing, sliding microtome (Microm, Heidelberg, Germany) at 40 μm thickness and cryoprotected in 30% sucrose prior to immunostaining (Emory University, Rush University, and University of Washington). Paraffin-embedded blocks were cut into 8 μm thick sections (Emory University and University of Washington).

Human Embryonic Kidney (HEK) 293 Cells

HEK293 cells were either unmodified or transiently transfected with V5-tagged human LR11 cDNA in pcDNA3.1 (Invitrogen, Carlsbad, CA), as previously described (Herskowitz et al. 2012a). All cells were maintained in DMEM with high glucose and glutamine (Lonza, Walkersville, MD), 10% fetal bovine serum (Invitrogen, Grand Island, NY) and 1% penicillin/streptomycin (Cellgro/Mediatech Inc., Herndon, VA) at 37°C and 5% CO_2.

Chromagen Immunohistochemistry (IHC)

Single-Chromagen IHC

Single-chromagen IHC was performed as previously described (Offe et al. 2006a, Ciliax et al. 1995). Briefly, 40 μm-thick free-floating sections were prepared with a freezing, sliding microtome (Microm, Heidelberg, Germany), treated with 3%

H_2O_2, to quench endogenous peroxidase activity, blocked with normal goat serum, and incubated overnight at 4°C with LR11 CT antibody, at 0.05 µg/mL. For paraffin-embedded tissue, 8 µm-thick sections were de-waxed, and microwaved in citrate buffer (0.01 M, pH 6). After cooling to room temperature, for 1 hour, sections were rinsed, treated with 3% H_2O_2, blocked with normal goat serum, and incubated overnight at 4°C with LR11 CT antibody, at 5 µg/mL. On day 2, sections were incubated with a biotinylated, goat anti-rabbit secondary antibody (1:200; Vector Laboratories, Burlingame, CA) followed by avidin-biotin-peroxidase complex (Elite ABC kit; Vector Laboratories). Immunoreactivity was visualized by light microscopy using 3,3'-diaminobenzidine (DAB) as a chromagen. Paraffin-embedded sections were counterstained with hematoxylin. Peptide preadsorption of the LR11 CT antibody was performed by simultaneously incubating tissue sections with equal concentrations of the LR11 CT antibody and its peptide immunogen. Additional immunocontrol sections were processed without exposure to the primary antibody. Peptide preadsorption and omission of primary antibody resulted in no detectable immunoreactivity, indicating that the LR11 CT antibody specifically identified its target antigen and that there was no non-specific, cross reactivity associated with other reagents (Figure 3.2A).

Dual-Chromagen IHC

For dual-chromagen IHC, free-floating sections were first processed using the single-chromagen IHC protocol described above. After development of the DAB reaction product from the first antibody (LR11 CT), any remaining peroxidase

activity was quenched with 3% H_2O_2 for 30 minutes at room temperature. Blocking buffer containing avidin, at 10 μg/mL, was reapplied for 1 hour at room temperature, and the tissue was incubated overnight at 4°C with the second primary antibody (AT8 at 1:10000, Alz50 at 1:250, or MN423 at 1:500) in buffer containing biotin, at 50 μg/mL. The following day, sections were incubated with a biotinylated, goat anti-mouse secondary antibody (Vector Laboratories, Burlingame, CA) followed by avidin-biotin-peroxidase complex (Elite ABC kit; Vector Laboratories, Burlingame, CA). Immunoreactivity was visualized using Vector SG Substrate Kit (blue reaction product; Vector Laboratories, Burlingame, CA). The following sets of immunohistochemical controls were performed to rule out any cross reactivity or non-specific staining: 1) omission of the first primary antibody or 2) omission of the second primary antibody. These controls ensured that the quenching (with 3% H_2O_2) and blocking (with avidin and biotin) were effective and that there was no cross-reactivity of the primary or secondary antibodies, indicating that each antibody specifically identified its target antigen and that there was no non-specific cross reactivity associated with other reagents (data not shown). Images were captured using an Olympus BX51 microscope and Olympus software.

Modified Braak Staging Using LR11 CT-Positive Neurofibrillary Tangles

Braak staging refers to a classification scheme derived via systematic evaluation of neurofibrillary pathology spreading from the medial temporal lobe to

more isocortical regions. Briefly, Braak and Braak (Braak and Braak 1991) classified the progression of neurofibrillary pathology into six categories, using a qualitative assessment of NFT and NT frequency across numerous brain regions. Categories I and II are referred to as the transentorhinal stage, at which point NFTs and NTs are primarily found in the transentorhinal cortex, with limited expression in hippocampus (CA1); categories III and IV are referred to as the limbic stage, the key feature of which is conspicuous expression of NFTs and NTs in the entorhinal and transentorhinal cortices and mild-moderate expression in the hippocampus; and categories V and VI are referred to as the isocortical stage, due to the widespread and frequent presence of NFTs and NTs throughout the isocortex. To assess the relationship between LR11 CT-positive NFTs and Braak staging, paraffin-embedded sections of hippocampus, temporal cortex, and frontal cortex from twenty one Emory University cases, with Braak scores ranging from 0-VI, were immunohistochemically labeled for LR11, as described above. While blinded to the diagnosis, all three brain regions, from each case, were semi-quantitatively assessed by three independent raters, using a modified version of the Braak scoring criteria (Braak and Braak 1991, Harding, Kril, and Halliday 2000): zero (0) was used to indicate absence of, or isolated, NFTs; (S) was used to indicate some NFTs; (M) was used to indicate moderate NFTs; and (F) was used to indicate frequent NFTs. Each case was then assigned to one of three categories: transentorhinal (Braak 0-II), limbic (Braak III-IV), or isocortical (Braak V-VI). The accuracy of these assignments was determined via comparison with the Braak score assigned by an experienced neuropathologist, during neuropathological assessment of tau staining.

Dual-Fluorescence Immunohistochemistry

Free-floating sections were treated with 3% H_2O_2 for 30 min at room temperature, blocked with normal goat serum for 1 hour at 4°C, and incubated overnight at 4°C with both the LR11 CT antibody, at 0.05 µg/ml, and mouse monoclonal AT8 (Thermo Scientific, Waltham, MA) antibody, at a dilution of 1:5,000. The following day, sections were incubated with a biotinylated, goat anti-rabbit secondary antibody (1:200; Vector Laboratories, Burlingame, CA) and a fluorescein isothiocyanate-conjugated, goat anti-mouse secondary antibody (1:100; Jackson Laboratories, Bar Harbor, MA) for 1 hour at 4°C. LR11 was then visualized by incubating with avidin-biotin-peroxidase complex for 1 hour at 4°C followed by a 10-minute incubation in a solution containing a tyramide plus cyanine 3 reagent (Molecular Probes, Eugene, OR). Sections were then mounted on glass slides, counterstained with Hoechst (bis-benzamide), and treated with Auto-fluorescence Eliminator according to manufacturer instructions (Chemicon, Temecula, CA). Immunocontrol sections incubated with only one primary antibody and both secondary antibodies demonstrated the specificity of the secondary antibodies for their respective antigens.

Western Blotting

Unmodified human embryonic kidney 293 (HEK293) cells and HEK293 cells transduced with a human LR11 lentiviral vector were harvested in phosphate-

buffered saline plus protease inhibitor cocktail (PIC) (Roche Diagnostics) and centrifuged at 2,300 rpm for 5 minutes at 4°C. The supernatant was discarded and the pellet was resuspended in lysis buffer (0.5% NP-40, 0.5% deoxycholate, 150 mM sodium chloride, and 50 mM Tris base) plus PIC for 1 hour at 4°C. Samples were then centrifuged at 2,300 rpm for 10 minutes at 4°C, and the pellet was discarded. Fresh-frozen blocks of human frontal cortex, from control and advanced sporadic AD brains (Braak V-VI), were also homogenized in lysis buffer plus PIC. Protein concentrations in all samples were determined and 2x Laemmli sample buffer was added. Thirty μg of cell lysate or 100 μg of brain homogenate was loaded onto 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and run at 200 V for 60 minutes. Gels were electrophoretically transferred to polyvinylidene diflouride membranes (Millipore, Billerica, MA) at 100 mA for 16 hours. Membranes were then blocked with tris-buffered saline (TBS) plus blocking buffer (USB Corporation, Cleveland, OH) for 1 hour at room temperature and probed with the LR11 CT antibody, at 0.1 μg/mL, and an anti-tau antibody (BD Biosciences, Franklin Lakes, NJ), at 1:1,000, in buffer containing TBS, 0.1% Tween-20, and blocking buffer overnight at 4°C. The following day, the membranes were rinsed in TBS and simultaneously incubated with an Alexa Fluor 680 goat anti-rabbit secondary antibody (Invitrogen, Grand Island, NY) and an IRDye 800 goat anti-mouse secondary antibody (Li-Cor Biosciences, Lincoln, NE) for 1 hour at room temperature; both secondary antibodies were used at a dilution of 1:25,000. The membranes were rinsed with TBS, dried, and visualized on an Odyssey Infrared Imaging System (Li-Cor Biosciences, Lincoln, NE). All experiments were performed

in triplicate.

Statistical Analyses

Demographic characteristics were compared across neuropathological diagnostic groups by Mann-Whitney U test, Fisher's exact test, Chi-Square, and Kruskal-Wallis test, with Dunn's multiple comparisons test used for post hoc comparison ($p = 0.05$). To assess the reliability with which LR11-positive NFTs accurately reflected Braak stage per individual cases, the Braak stages assigned by three independent raters were compared to the Braak stages assigned during neuropathological assessment; inter-rater reliability was measured using the multi-rater kappa (Fleiss) index.

Chapter 3

LR11 Associates with Early-Stage Neurofibrillary Tangles in Alzheimer's Disease

Introduction

Shortly after being identified as an essential factor in microtubule assembly and stabilization (Weingarten et al. 1975, Witman et al. 1976), tau was found to be the primary component of NFTs in AD brain, but unlike the soluble tau monomers in neurons of control brain, tangle-bearing neurons in AD contained insoluble tau filaments (Wischik, Novak, Edwards, et al. 1988, Wischik, Novak, Thogersen, et al. 1988, Goedert et al. 1988, Grundke-Iqbal et al. 1986, Kosik, Joachim, and Selkoe 1986, Wood et al. 1986). Tau's transition from soluble monomers to insoluble filaments is not fully understood, but immunohistochemical studies analyzing the appearance of pathological tau epitopes in AD brain suggest that this process involves a succession of molecular alterations that begins with hyperphosphorylation, progresses through a stage of misfolding, and ends with extensive protein cleavage (Augustinack et al. 2002b, Garcia-Sierra et al. 2003, Guillozet-Bongaarts et al. 2005b, Guillozet-Bongaarts et al. 2006, Horowitz et al. 2004). Identifying these early, middle, and late phases of NFT maturation allows a temporal relationship to be established between NFTs and related pathological changes. Associations with the early phase of NFT development indicate potential

mechanistic relationships that may be manipulated to regulate NFT development.

Initial immunohistochemical studies in postmortem human brain, revealed prominent LR11 immunoreactivity in the somatodendritic endosomes of neurons in frontal cortex and hippocampus harvested from individuals with no cognitive impairment (Offe et al. 2006a, Scherzer et al. 2004). In sporadic AD, significantly lower levels of neuronal LR11 were observed in frontal cortex and hippocampus but not occipital cortex (Scherzer et al. 2004), suggesting that LR11 was lost in brain regions vulnerable to AD pathology. Reduced neuronal LR11 was also evident in a subset of individuals with mild cognitive impairment (MCI), suggesting that a reduction in neuronal LR11 is an early pathological change associated with the transition from normal cognitive functioning to AD (Sager et al. 2007). Interestingly, neuronal LR11 levels in the frontal cortex of individuals with familial AD (FAD) linked to mutations in presenilin 1 (FAD PS1) and presenilin 2 (FAD PS2) were comparable to levels observed in brain from cases with no cognitive impairment control brain, suggesting that changes in neuronal LR11 levels are specific to sporadic AD and not simply a downstream consequence of accumulated AD pathology (Dodson et al. 2006). Recent studies, however, revealed a range of neuronal LR11 levels in the precuneus, frontal cortex, and occipital cortex of individuals with clinical diagnoses of no cognitive impairment, mild cognitive impairment, and early AD (Sager et al. 2012). There was no relationship between LR11 and AD pathology reported.

The impetus for the current study came from a preliminary investigation of LR11 in temporal cortex (a brain region vulnerable to AD-related

neurodegeneration) of sporadic AD brain in which LR11-positive structures that resembled NFTs were identified. Surprisingly, sporadic AD frontal cortex included as an experimental control also contained NFT-like structures immunoreactive for LR11. These results suggested the possibility of a functional relationship between LR11 and tau but raised questions as to why this relationship was not observed in previous studies. Possible sources of the discrepancy may relate to differences in antibodies, disease severity, and brain regions examined.

The current study confirmed the existence of a relationship between LR11 and NFTs in sporadic AD brain and then examined whether differences in antibodies, disease severity, or brain region played a role in the discrepancy reported in previous investigation lacking a relationship between these markers. The functional implications of LR11 associating with NFTs was assessed using dual immunolabeling for LR11 and tau epitopes associated with early, middle, and late stages of NFT maturation.

Results

Identification of NFT-Like Structures with LR11 CT Antibody

Frontal cortex sections from control and sporadic AD cases matched for age, gender, and postmortem interval (PMI) (Table 3.1) were stained using the LR11 CT antibody. Control brains contained punctate staining in somatodendritic compartments of pyramidal neurons (Figure 3.1A, 3.1B), similar to previous reports (Scherzer et al. 2004, Offe et al. 2006a). AD brains contained neuronal cell bodies

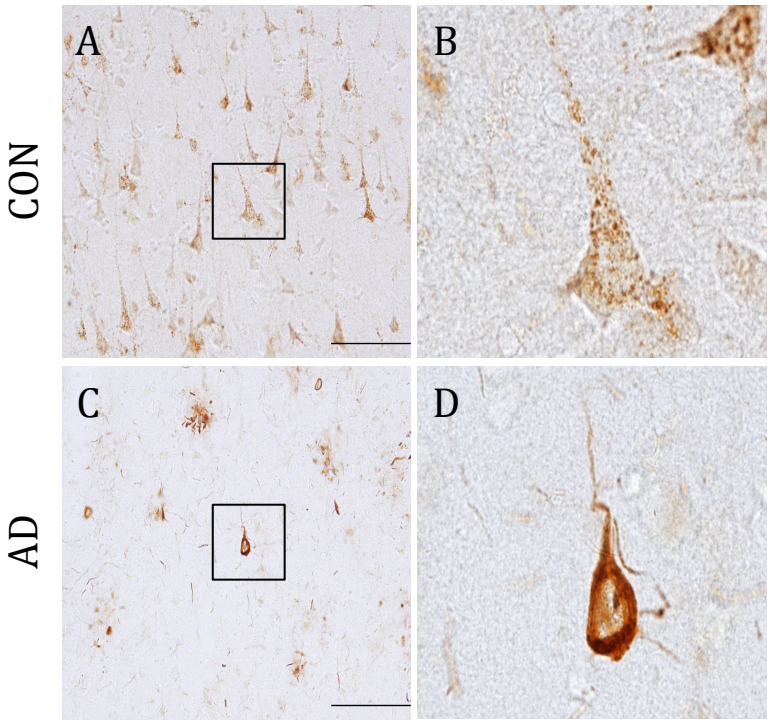


Figure 3.1. Photomicrographs of LR11 CT Immunostaining in Human Frontal Cortex. (A) Somatodendritic punctate staining in aged control frontal cortex. (B) Higher magnification image of boxed neuron shown in panel a. (C) LR11 CT-positive structure with a neurofibrillary tangle-like appearace in sporadic Alzheimer's disease frontal cortex. (D) Higher magnification image of boxed in structure in panel c. Scale bar = 100 μm.

that displayed dense, fibrillar LR11 immunoreactivity (Figure 3.1C, 3.1D). Since aggregated proteins, in neuropathological lesions, are prone to non-specific staining, peptide preadsorption controls were performed in immunohistochemical and western blotting assays to demonstrate antibody specificity. Competition with the specific immunogen eliminated immunoreactivity of somatodendritic punctain control brains (Figure 3.2B) and the structures presumed to be NFTs in sporadic AD brains (Figure 3.2D). Additionally, western blots showed no cross-reactivity of the LR11 CT antibody with bands associated with tau protein (Figure 3.3).

LR11 CT Antibody Labeling of NFTs Confirmed

To determine whether neuronal LR11 immunoreactivity associated with NFTs containing hyperphosphorylated tau, sections were dual stained for LR11 CT and AT8 antibody, which recognizes tau phosphorylated at serine 202 and threonine 205, in AD frontal cortex tissue. Qualitative microscopic analysis revealed strong co-labeling of LR11 and AT8 immunoreactivity within pyramidal neurons of layers 3 and 5 (Figure 3.4).

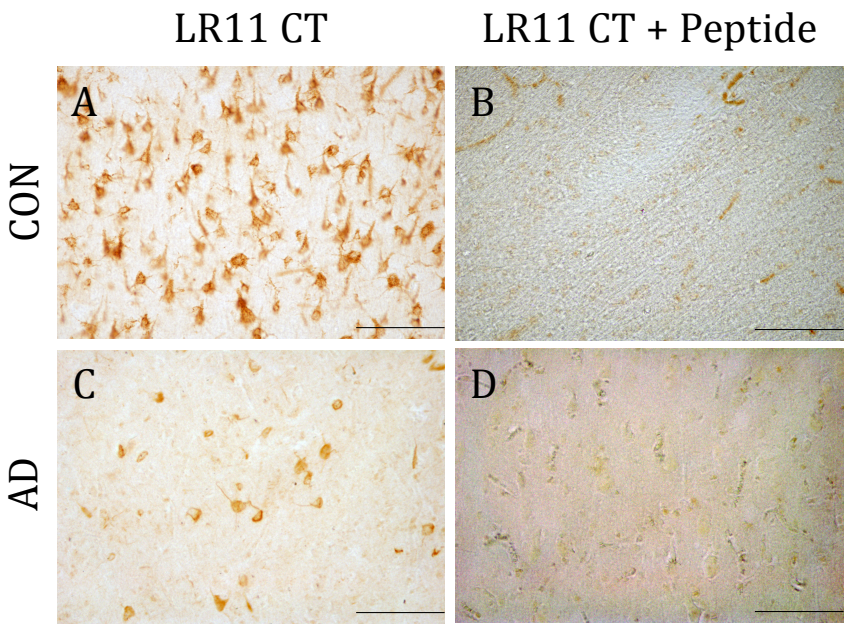


Figure 3.2. Photomicrographs of LR11 CT Peptide Competition in Human Frontal Cortex. (A) Somatodendritic punctate staining in aged control frontal cortex. (B) Preadsorption of LR11 CT staining in control frontal cortex. (C) LR11 CT-positive structure with a neurofibrillary tangle-like appearace in sporadic Alzheimer's disease frontal cortex. (D) Preadsorption of LR11 CT immunoreactivity in NFT-like structures in sporadic AD frontal cortex. Scale bar = 100 μm.

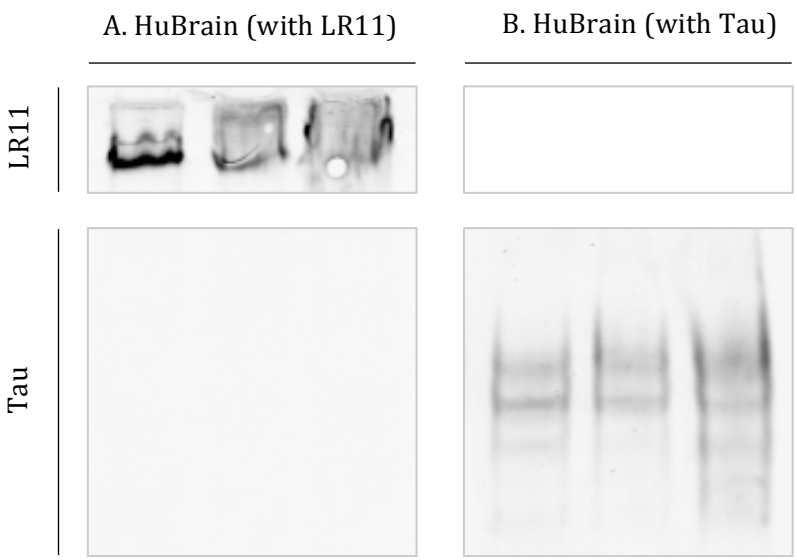


Figure 3.3. LR11 CT Staining of 250 kDa Band in Human Brain Homogenate but No Staining of Tau Bands. (A) Upper: LR11 CT stained the 250 kDa band that corresponds to the molecular weight of LR11. Lower: LR11 CT does not detect tau protein. (B) Upper: Anti-tau antibody does not detect 250 kDa LR11 band. Lower: Anti-tau antibody detects bands between 45-70 kDa, which correspond to the molecular weights of the six tau isoforms.

Table 3.1. Control and Sporadic AD Cases

	Diagnosis		Comparison by Diagnostic Group (P value)
	Control (N = 8)	**Sporadic Alzheimer's Disease** (N = 12)	
Age at Death (years) Mean ± SD (Range)	71.4 ± 17.3 (46 – 92)	73.2 ± 10.1 (58 – 91)	0.9544*
No. (%) of Males	4 (50%)	5 (42%)	1.0000**
Postmortem Interval (hours) Mean ± SD (Range)	14.2 ± 10.3 (3.0 – 33.5)	10.0 ± 5.3 (3.5 – 22.0)	0.5326*

* Mann-Whitney U test

* * Fisher's exact test

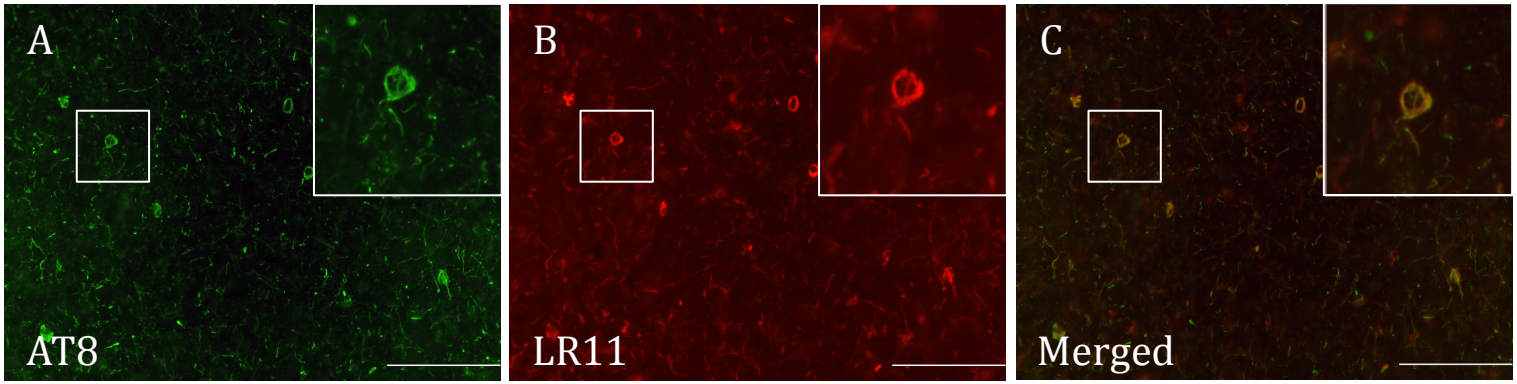


Figure 3.4. Dual-Immunofluorescent Immunohistochemistry in Advanced Sporadic Alzheimer's Disease (Braak V-VI) Frontal Cortex. (A) Neurofibrillary tangles (NFTs) stained with AT8 (green). (B) Structures immunoreactive for LR11 CT (red). (C) Co-expression of AT8 and LR11 CT immunoreactivity (yellow). Scale bar = 100 μm.

LR11 CT but Not LR11 3850 Labels NFTs in Sporadic AD

Initial immunohistochemical studies of LR11 in postmortem human brain, where no association between LR11 and NFTs were noted, were performed using the LR11 3850 antibody, which was generated against the same immunogen as the LR11 CT antibody (amino acids 2198-2215 of human LR11). Despite this similarity, I hypothesized that differences in antibody specificity might underlie differences in staining patterns. To test this hypothesis, frontal cortex from control and sporadic AD brain was stained with both the LR11 3850 and LR11 CT antibody (Figure 3.5), allowing each case to serve as its own control. Both LR11 3850 and LR11 CT effectively labeled the somatodendritic puncta in pyramidal neurons in control brain (Figure 3.5A, 3.5B, 3.5E, 3.5F); however, only LR11 CT labeled NFTs in AD brain (Figure 3.5G, 3.5H).

Case Selection and Regional Differences: LR11 CT-Positive NFTs and Braak Staging

In addition to earlier studies using LR11 3850 (Dodson et al. 2008a, Dodson et al. 2006, Offe et al. 2006a, Scherzer et al. 2004), recent studies (Sager et al. 2012, Sager et al. 2007), using LR11 CT serum (from which the LR11 CT antibody was purified) also failed to identify a relationship between LR11 and NFTs. Comparing these earlier studies with the current investigation revealed differences in brain regions and extent of clinical evaluation. For example, in the earlier studies (Sager et al. 2012, Sager et al. 2007) cases were selected based on cognitive test scores and

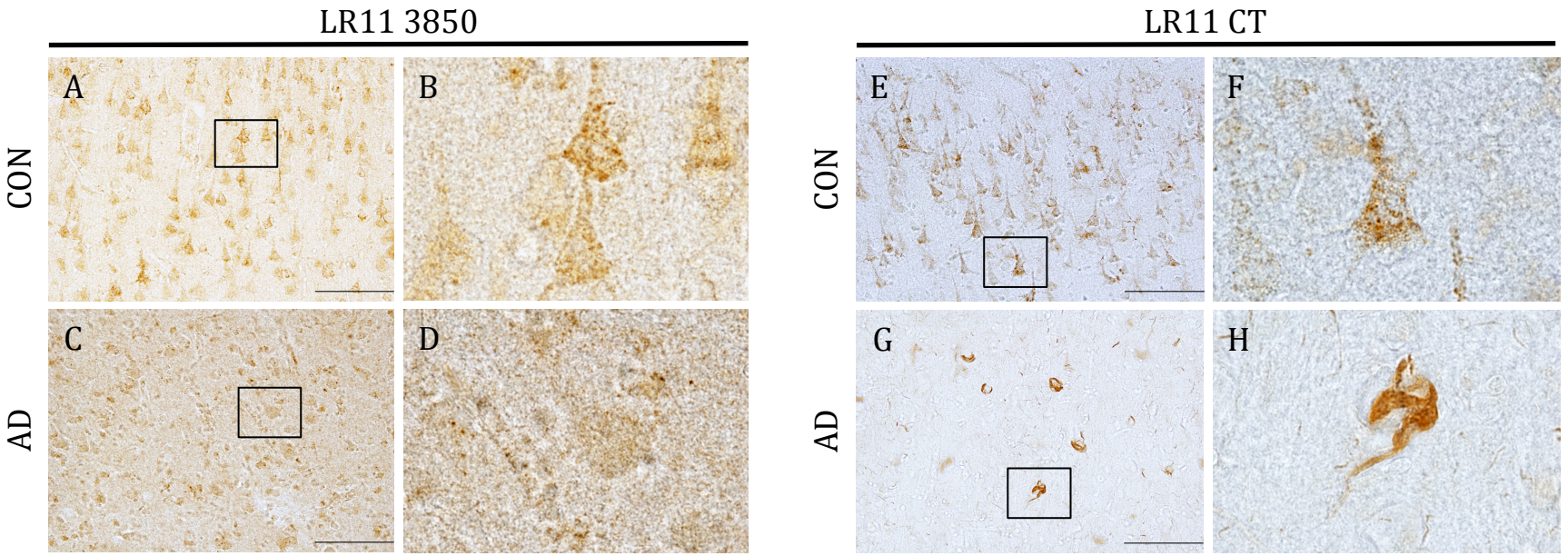


Figure 3.5. LR11 3850 and LR11 CT Immunostaining in Human Frontal Cortex. Aged control brains stained with LR11 3850 and LR11 CT antibodies contained pyramidal neurons with a somatodendritic, punctate staining pattern (A, B, E, F). Alzheimer's disease brains stained with LR11 3850 antibody exhibited negligible immunoreactivity (C, D). Alzheimer's disease brains stained with LR11 CT antibody contained frequent LR11-positive NFTs (G, H). Scale bar = 100 μm.

neuropathological AD criteria, whereas clinical tests data were not available for all of the cases used in the current study. Therefore, a neuropathological assessment of disease progression, referred to as Braak staging was used identifying differences in disease progression of cases used between studies.

Braak staging is a neuropathological schema, which marks the stage of AD based upon systematic evaluation of the regional distribution spread of NFTs throughout the brain (Braak and Braak 1991) that can be divided into six stages. Stages I and II are referred to as the transentorhinal stage, where NFTs are primarily found in the transentorhinal cortex with limited expression in hippocampus (CA1); stages III and IV compose the limbic stage, the key feature of which is the conspicuous expression of NFTs in the entorhinal and transentorhinal cortices and mild-moderate expression of NFTs in the hippocampus; and stages V and VI are referred to as the isocortical stage due to the widespread and frequent presence of NFTs throughout the isocortex.

The studies of Sager et al. (Sager et al. 2012, Sager et al. 2007) used precuneus, frontal cortex, and occipital cortex from brains with a Braak stage no greater than V, suggesting that NFTs were not likely to be present in the frontal or occipital cortices but may have been present in the precuneus. Moderate amounts of LR11-positive NFTs were detected in the precuneus and none or limited numbers appeared in the frontal and occipital cortices examined in the previous studies (Sager, personal communication), presenting a pattern of distribution similar to that observed by Braak and Braak (1991).

We, therefore, hypothesized that LR11-positive NFTs follow a Braak-staged

pattern of distribution. To test this hypothesis, three independent raters performed blind assessments of LR11 staining in the hippocampus, temporal cortex, and frontal cortex of twenty-one aged control and sporadic AD cases (Table 3.2) with Braak scores ranging from 0 to VI (Figure 3.6). In each brain region, the abundance of LR11-positive NFTs was rated, using a modified version of the Braak criteria (Braak and Braak 1991, Harding, Kril, and Halliday 2000) (described in Materials and Methods). Raters 1 and 2 accurately categorized 18/20 cases (90.00%) and rater 3 accurately categorized 17/20 cases (85.00%). Staging by all three raters agreed in 14/20 cases (70.00%). Fleiss' kappa yielded a $\kappa = 0.670$, indicating substantial inter-rater agreement. These data support the adherence of LR11-positive NFTs to a Braak-staged pattern of distribution.

Revisiting FAD (and DS): LR11 CT Antibody Labels NFTs in FAD and DS Brain

Previously, immunostaining of frontal cortex of FAD PS1 and FAD PS2 with LR11 3850 revealed no reduction in neuronal LR11, when compared to frontal cortex of control brain (Dodson et al. 2006), nor was there a relationship between LR11 and NFTs observed. All cases used in this experiment were Braak stage V or VI, suggesting that the failure to detect the relationship between LR11 and NFTs was not due to the absence of NFTs in the frontal cortex, but likely a consequence of using the LR11 3850 antibody. I re-examined these cases using the LR11 CT antibody. In addition to PS1 and PS2 brains, tissue from individuals with other genetic forms of

Table 3.2. Braak-Staged Cases Stained with LR11 CT

	Braak Stage				Comparison by Diagnostic Group (P value)
	0 (N = 2)	**I-II** (N = 6)	**III-IV** (N = 5)	**V-VI** (N = 8)	
Neuropathological Diagnosis (No. of Cases)	Control (2)	Control (5) Early AD (1)	Control (3) AD (2)	AD (8)	
Age at Death (years) Mean ± SD (Range)	66.0 ± 19.8 (52 – 80)	75.3 ± 12.1 (57 – 87)	79.6 ± 8.0 (71 – 92)	80.6 ± 11.4 (62 – 94)	0.5529*
No. (%) of Males	0 (0%)	3 (50%)	3 (60%)	6 (75%)	0.6219**
Postmortem Interval (hours) Mean ± SD (Range)	3.75 ± 1.1 (3 – 4.5)	14.7 ± 5.1 (9 – 20.5)	36.0 ± 25.7 (15 – 78)	20.4 ± 11.3 (6.5 – 40)	0.0427*

Hippocampus, Temporal Cortex, Basal Forebrain, and Frontal Cortex, from each case, were stained. Missing 1 PMI for Braak Stage V-VI.

* Kruskal-Wallis test. Dunn's multiple comparisons test revealed a significant difference between Braak 0 and Braak III-IV PMI was detected (alpha 0.05).
** Chi-Square (Braak Stage 0 was omitted from this analysis because the test could not produce valid results with this group included).

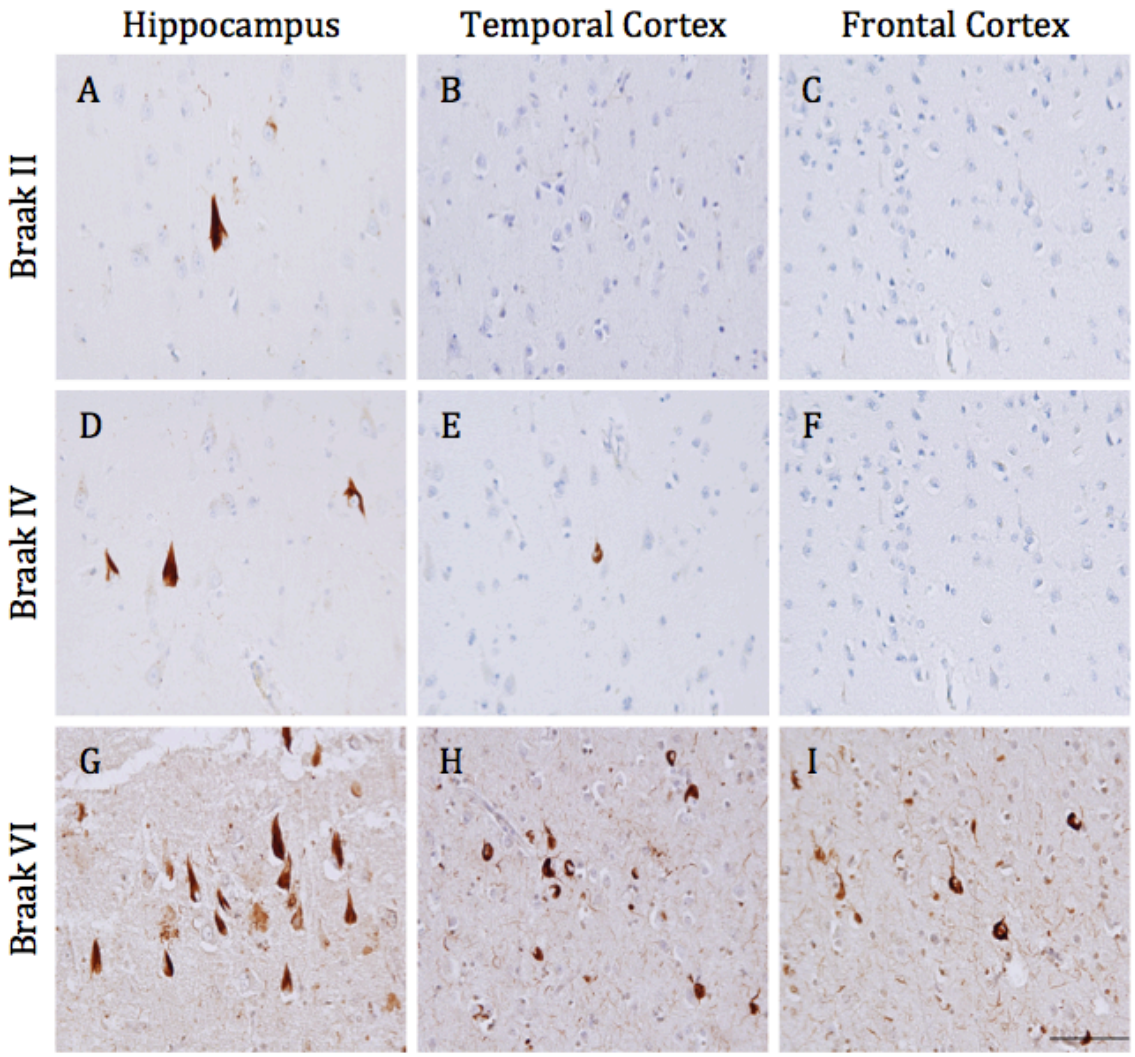


Figure 3.6. Regional Distribution of LR11 CT-Positive Neurofibrillary Tangles (NFTs) is Consistent with Braak Staging. Brains from individuals at the transentorhinal stage (Braak I-II) contained a limited number of NFTs in the hippocampus (A) and no NFTs in the temporal cortex (B) or frontal cortex (C). Brains from individuals at the limbic stage (Braak III-IV) contained a moderate amount of NFTs in the hippocampus (D), a limited amount of NFTs in the temporal cortex (E), and no NFTs in the frontal cortex (F). Brains from individuals at the isocortical stage (Braak V-VI) contained frequent NFTs in the hippocampus (G), temporal cortex (H), and frontal cortex (I). Scale bar = 100 µm.

AD, including an APP mutation and Down's syndrome (DS) were also examined. Staining revealed that all cases contained LR11 CT-positive NFTs in similar proportions to what was observed in Braak-staged V or VI sporadic AD frontal cortex (Figure 3.7). As in sporadic AD, these NFTs were found in pyramidal neurons of layers 3 and 5.

LR11 Preferentially Associates with Early-Stage NFTs

To explore the relationship LR11 and NFT maturation, dual-chromagen labeling was performed in frontal cortex sections from advanced sporadic AD brains (Braak V-VI), and the relationships between LR11 and epitopes of early-, middle-, and late-stage NFTs were assessed. The phospho-specific antibody, AT8, was used to label early NFTs; Alz50, which identifies a conformational change in tau, in which the N-terminal folds and contacts one of the microtubule binding repeats (Carmel et al. 1996), labels NFTs in the middle stages of maturation; and MN423, which recognizes tau truncated at glutamic acid 391, labels NFTs in the late stages of maturation (for a review, see (Guillozet-Bongaarts et al. 2005b). As expected, there was strong co-expression of LR11 immunoreactivity in all AT8-positive NFTs and all LR11-positive NFTs were immunoreactive for AT8 (Figure 3.8A, 3.8B). Similarly, all Alz50-positive NFTs were also LR11immunoreactive; however, there were many LR11-positive NFTs that were not Alz50 immunoreactive (Figure 3.8C, 3.8D). There was no observed overlap in LR11 and MN423 immunoreactivity despite numerous NFTs being immunoreactive for either LR11 or MN423 (Figure 3.8E, 3.8F).

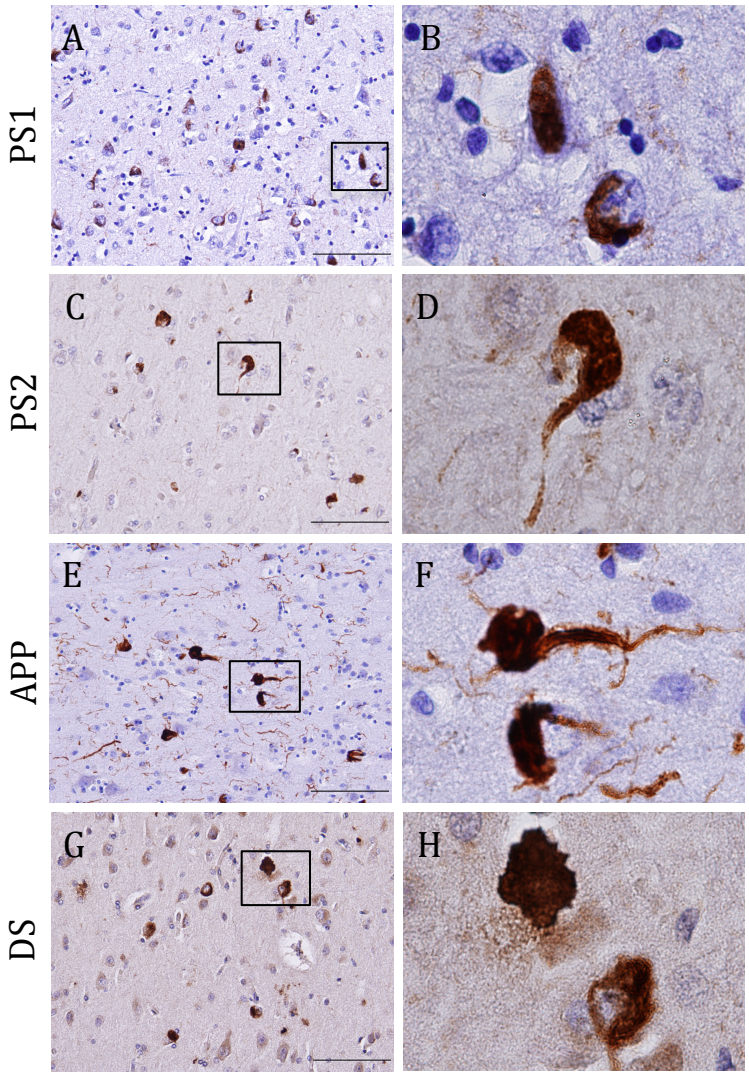


Figure 3.7. LR11 CT Immunostaining in Familial Alzheime's Disease (FAD) and Down's Syndrome (DS) Frontal Cortex. Low- and High-magnification images of LR11 CT-positive neurofibrillary tangles in frontal cortex of individuals with mutations in presenilin 1 (PS1) (A, B), presenilin 2 (PS2) (C, D), and the amyloid precursor protein (APP) (E, F), as well as individuals with Down's syndrome (DS) (G, H). Scale bar = 100 μm.

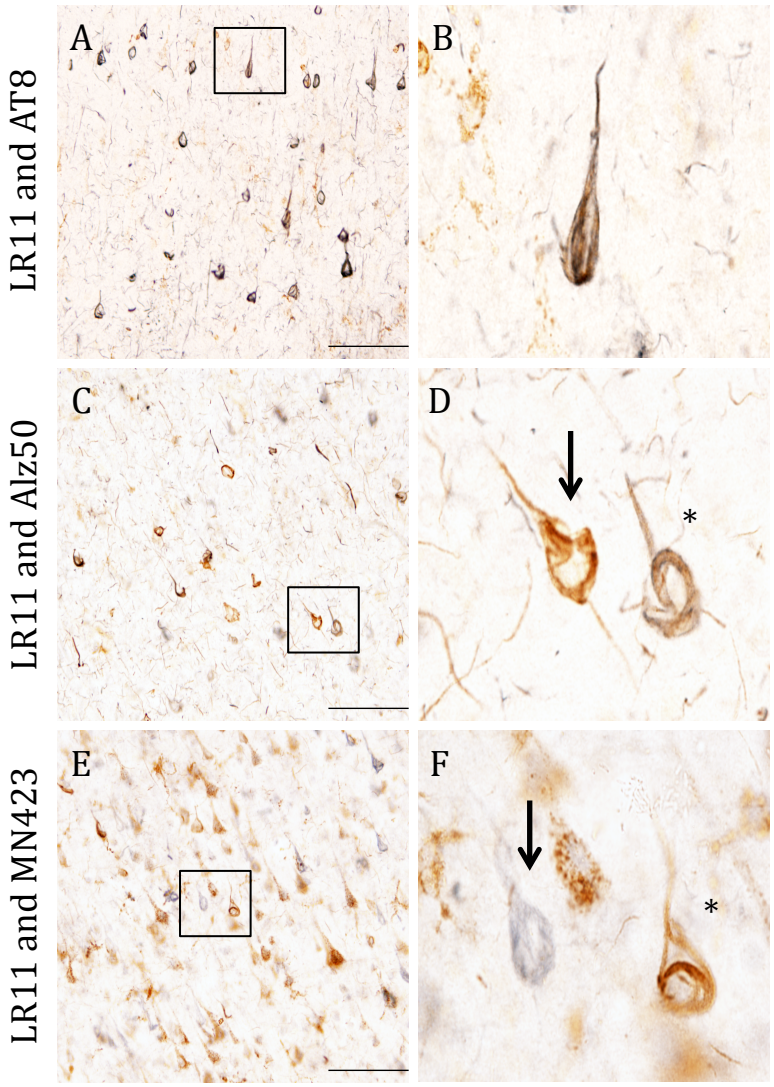


Figure 3.8. Dual-Chromagen Immunohistochemistry (IHC) in Advanced Sporadic Alzheimer's Disease (Braak V-VI) Frontal Cortex. (A) Neurofibrillary tangles (NFTs) immunostained with LR11 CT (brown) and AT8 (blue) were indistinguishable. (B) Higher magnification image of boxed NFT in panel a. (C) NFTs immunostained with LR11 CT (brown) and Alz50 (blue) and NFTs immunoreactive for LR11 CT only. (D) Higher magnification image of boxed NFTs in panel c, showing a NFT immunoreactive for LR11 CT and Alz50 (asterisk) and LR11 CT only (arrow). (E) NFTs immunoreactive for LR11 (brown) and MN423 (blue) reflected separate populations of NFTs. (F) Higher magnification image of boxed NFTs in panel e, showing a NFT immunoreactive for LR11 (asterisk) and a NFT immunoreactive for MN423 (arrow). Scale bar = 100 µm.

Discussion

Previous immunohistochemical studies of LR11 in postmortem human brain reported significantly lower levels of neuronal LR11 in MCI and sporadic AD, when compared to aged controls (Scherzer et al. 2004, Offe et al. 2006a, Sager et al. 2007). Although autosomal-dominant FAD cases generally display neuropathology similar to that seen in sporadic AD, persistent LR11 immunoreactivity was observed in FAD PS1 and FAD PS2 frontal cortex (Dodson et al. 2006), suggesting that a reduction in neuronal LR11 is an early pathological change denoting the transition from normal cognitive function to AD. Recent studies, however, revealed a range of neuronal LR11 levels in the precuneus, frontal cortex, and occipital cortex of individuals with a premortem clinical diagnoses of no cognitive impairment, mild cognitive impairment, and early AD (Sager et al. 2012), pointing at a more complex relationship between LR11 and AD than initially thought (see Future Directions of the Discussion section). However, none of these earlier studies reported a relationship between LR11 and NFT pathology. In the current study, I observed an association of LR11 with NFTs (Figure 3.4) in individuals with sporadic AD (Figure 3.1C, 3.1D), FAD PS1 and FAD PS2, as well as AD associated with an APP mutation and DS (Figure 3.7).

Three factors were found to underlie the discrepancy between the previous and current study's ability to identify a relationship between LR11 and NFTs. Earlier studies (Dodson et al. 2006, Scherzer et al. 2004) used the LR11 3850 antibody, which (despite being generated against the same immunogen as the LR11 CT antibody used here) was shown to be ineffective at identifying NFTs, but capable

of labeling LR11 in control and AD brain (Figure 3.5). An explanation for this difference in staining can be extrapolated from the polyclonal nature of both antibody preparations. Polyclonal antibodies are composites of multiple immunoglobulin Gs, each produced by a different B cell and binding to only several amino acids within a given immunogen. Thus, the 18 amino acid human LR11 immunogen used to generate LR11 3850 and LR11 CT likely resulted in two antibody preparations with compositions of IgGs that recognized different sites on LR11. LR11 3850 could, therefore, contain IgGs that recognize LR11 in control brain but not LR11 associated with NFTs. If a direct molecular interaction exists between LR11 and tau, the steric hindrance caused by such an interaction could interfere with the ability of LR11 3850's IgGs to bind LR11. Alternatively, LR11 may undergo molecular alterations, similar to tau, that result in steric hindrance or otherwise serve to make the necessary epitope inaccessible.

In addition to differences in antibodies, the severity of disease and the brain regions examined were also found to contribute to the discrepancy in the ability to identify the relationship between LR11 and NFTs. Capitalizing on the well-characterized progression of NFTs throughout the AD brain (Braak and Braak 1991), I was able to demonstrate that LR11 CT-positive NFTs follow a similar cortical transition, making the combination of disease severity and brain region critical for detecting the LR11/NFT association.

After resolving the discrepancies between current and previous studies, the functional implications of LR11 associating with NFTs was addressed by evaluating the relationship between LR11 and maturing NFTs. Dual labeling with the LR11 CT

antibody and AT8, Alz50, or MN423 (as markers of early-, middle-, and late-stage NFT epitopes, respectively) revealed complete overlap between LR11 and AT8 immunoreactivity (Figure 3.8A, 3.8B), indicating that LR11 associates with NFTs early in the maturation process. Similar to AT8-positive NFTs, all Alz50-immunostained NFTs were also LR11 positive (Figure 3.bC, 3.8D). Unlike AT8, however, Alz50 failed to label all LR11-positive NFTs (Figure 3.8C, 3.8D) suggesting that LR11-positive/Alz50-negative NFTs are in an earlier stage of NFT maturation and have yet to develop the Alz50 epitope. Distinct from both AT8 and Alz50, MN423 containing neurons failed to display LR11 despite the presence of numerous NFTs immunoreactive for one or the other markers (Figure 3.8E, 3.8F). Since MN423 is marker of late-stage NFTs, LR11-positive/MN423-negative NFTs are likely still in the early stage of the maturation and have yet to progress through the latest stages of tau evolution. LR11-negative/MN423-positive NFTs likely reflect NFTs in which LR11 has been lost or the epitope has become inaccessible.

An alternative explanation for the absence of LR11 immunoreactivity in MN423-positive NFTs is that the LR11 CT epitope has been lost due to proteolytic processing, or other modifications of LR11. Full-length LR11 undergoes metalloprotease-mediated cleavage and shedding of its large ectodomain (Guo et al. 2002, Hampe et al. 2000b) and the remaining membrane-associated fragment is a substrate for intramembranous cleavage by the γ-secretase complex (Bohm et al. 2006a). Since the LR11 CT antibody recognizes the extreme C-terminal portion of LR11, its staining of tau pathology implies the presence of either the full-length receptor, the membrane-bound stub, or the free intracellular domain. NFTs that do

not show LR11 CT immunoreactivity presumably lack this epitope, but I cannot exclude the presence of the LR11 ectodomain. Interpretation of these observations will require dual labeling experiments using epitope-specific reagents that are currently unavailable. Taken together, these results suggest that LR11 preferentially associates with early-stage NFT maturation, positioning it as a potential regulator of NFT development.

Chapter 4

LR11 Associates with Tau Pathology in Progressive Supranuclear Palsy but Not Corticobasal Degeneration or Pick's Disease

Introduction

In addition to being a defining feature of AD, tau inclusions are also a neuropathological hallmark of a class of age-related, neurodegenerative diseases devoid of amyloid pathology referred to as non-AD tauopathies, the most common of which are progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and Pick's disease (PiD). Comparing antigenic profiles of the tau inclusions contained in AD, PSP, CBD, and PiD revealed marked similarity (Schmidt et al. 1996, Bancher et al. 1987, Guillozet-Bongaarts et al. 2007), suggesting that tau protein undergoes similar pathological alterations, across these disease states. However, differences in age of symptogenesis, clinical presentation, regional pathology, ultrastructural characteristics, and biochemical profiles of tau inclusions distinguish the various diseases.

Alzheimer's Disease (AD)

AD is the most common form of dementia, with prevalence estimated at 790/100,000, in the United States (2012). Clinical diagnosis of AD requires a

demonstrated impairment in memory as well as impairment in, at least, one other cognitive domain (visuospatial ability, language, and executive function) (McKhann et al. 1984, Dickson, Bergeron, Chin, Duyckaerts, Horoupian, Ikeda, Jellinger, Lantos, Lippa, Mirra, Tabaton, Vonsattel, Wakabayashi, and Litvan 2002). As stated above, the neuropathological hallmarks of AD are senile plaques and NFTs, with NFTs (but not senile plaques) ascending in a predictable fashion through transentorhinal, limbic, and isocortical brain regions, as the disease progresses (Braak and Braak 1991). Ultrastructurally, NFTs display classic paired helical filaments (PHFs) with diameters between 8 – 24 nm and periodicities of 70-80 nm in AD brain (Crowther 1991, Arima 2006). Less abundant 15 nm straight filaments (SFs) are also present and appear in the same population of NFTs (Perry et al. 1987). Western blot analysis of insoluble, hyperphosphorylated tau, from AD brain, identified three major bands at 68, 64, and 60 kDa, as well as a minor band at 72 kDa (Goedert et al. 1992, Greenberg and Davies 1990). Dephosphorylation led to resolution of six bands that correspond to the six tau isoforms found in normal adult human brain; band intensity was similar in both groups (Goedert et al. 1992, Greenberg and Davies 1990).

Progressive Supranuclear Palsy (PSP)

Currently, an estimated 5/100,000 individuals, in the United States, have PSP (Williams 2006). Clinically, these individuals are typified by supranuclear gaze palsy, pseudobulbar palsy, postural instability, progressive axial rigidity, and mild dementia of the frontal dysexecutive type (Steele, Richardson, and Olszewski 1964).

Symptoms typically appear during the 5th decade of life and persist for 6 – 10 years (Colosimo et al. 2013). Neuropathological identifiers of PSP include a high density of NFTs (typically round or globose) in the basal ganglia, subthalamic nucleus, periaqueductal gray, and substantia nigra, as well as glial inclusions in both astrocytes (tufted astrocytes) and oligodendrocytes (coiled bodies) (Hauw et al. 1994). Electron microscopy has revealed that both the neuronal and glial inclusions, in PSP brain, are predominantly composed of 13 – 18 nm straight filaments (Arima 2006, Tellez-Nagel and Wisniewski 1973). Immunohistochemical analysis of PSP brain identified 4R tau isoforms as the primary constituent of inclusions (Chambers et al. 1999, Lee, Goedert, and Trojanowski 2001), a finding supported by western blot analysis of insoluble, hyperphosphorylated tau from PSP brain, which identified prominent bands at 64 and 68 kDa and a minor 72 kDa band (that was variably detected). Following dephosphorylation, bands displayed electrophoretic mobility similar to bands associated with recombinant 4R tau isoforms (Sergeant, Wattez, and Delacourte 1999, Lee, Goedert, and Trojanowski 2001, Buee and Delacourte 1999).

Corticobasal Degeneration (CBD)

CBD has a prevalence of 1 per 100, 000, in the United States (Williams 2006). Clinical similarities between PSP and CBD are commonly cited, with CBD patients displaying progressive axial rigidity and dementia of the frontal dysexecutive type; however, CBD can be distinguished by a progressive non-fluent aphasia and apraxia (Litvan, Grimes, and Lang 2000). CBD is considered to be a late-onset disease, with

symptoms typically manifesting in the 6th or 7th decade of life and persisting for less than 10 years (Williams 2006). Neuropathological characteristics of CBD include achromatic, balloon-shaped neurons, prominent NTs, coiled bodies, and astrocytic plaques (distinct from tufted astrocytes), in the substantia nigra and frontoparietal cortex (Rebeiz, Kolodny, and Richardson 1967, Dickson, Bergeron, Chin, Duyckaerts, Horoupian, Ikeda, Jellinger, Lantos, Lippa, Mirra, Tabaton, Vonsattel, Wakabayashi, Litvan, et al. 2002). Additionally, an abundance of NTs throughout the white matter is one of the most striking features of CBD (Dickson, Bergeron, Chin, Duyckaerts, Horoupian, Ikeda, Jellinger, Lantos, Lippa, Mirra, Tabaton, Vonsattel, Wakabayashi, Litvan, et al. 2002). Ultrastructural analysis of CBD brain suggests that both neuronal and glial inclusions contain 13 – 28 nm twisted filaments with a 120 – 202 nm periodicity, but neuronal inclusions also contain 15 nm straight filaments (Arima 2006). The biochemical profile of insoluble tau inclusions, in CBD brain, is similar to that of PSP, indicating the dominant presence of 4R tau isoforms (Lee, Goedert, and Trojanowski 2001, Sergeant, Wattez, and Delacourte 1999, Buee and Delacourte 1999).

Pick's Disease (PiD)

PiD is a rare form of frontal lobe dementia, afflicting less than 1/100,000 Americans aged 60 and over (Williams 2006). Similar to other frontal lobe dementias, PiD displays a constellation of clinical symptoms, with progressive non-fluent aphasia and dementia of the semantic and frontal dysexecutive types being the most consistent (Williams 2006). Neuropathologically, PiD is denoted by

conspicuous frontotemporal lobar and limbic atrophy accompanied by severe neuronal loss, spongiosis, gliosis, ballooned neurons, and Pick bodies (Dickson 1998). Pick bodies are most prominent in granule cells of the dentate gyrus, pyramidal cells of the hippocampus, and entorhinal cortex (Iwatsubo, Hasegawa, and Ihara 1994, Dickson 1998). Ultrastructural assessment of Pick bodies has identified 10 – 18 nm straight filaments and 15 – 28 nm twisted filaments with a 120 – 160 nm periodicity (Arima 2006, Dickson 1998). The biochemical profile of PiD is distinct from PSP and CBD in that western blot analysis of insoluble tau, from PiD brain, identified prominent bands at 60 and 64 kDa and a minor band at 68 kDa. Following dephosphorylation, bands displayed electrophoretic mobility similar to bands associated with recombinant 3R tau isoforms (Lee, Goedert, and Trojanowski 2001, Buee and Delacourte 1999).

Aim of Current Study

The aim of the current study was to assess whether the LR11 CT antibody labels tau pathology in PSP, CBD, and/or PiD. Anticipated outcomes include: 1) Lack of labeling in non-AD tauopathies, indicating that LR11 associates with a pathological tau variant unique to AD; 2) Indiscriminant labeling, suggesting that LR11 associates with a pathological tau variant common to all tauopathies; and 3) Labeling in select tauopathies, the latter provides the greatest insight into the dominant isoform and ultrastructural makeup of the pathological tau variant(s) that LR11 may be associating with.

Results

LR11 CT Antibody Labels NFTs in PSP Brains

Adjacent, paraffin-embedded tissue sections of the basal ganglia and subthalamic nucleus, from four PSP brains (Table 4.2), were immunohistochemically processed with the LR11 CT and AT8 antibodies, separately. Microscopic evaluation of LR11 CT immunoreactivity revealed NFTs with a granular staining pattern, indicative of an early-stage of development, as well as NTs and tufted astrocytes, in all four cases (Figure 4.1). Two cases also contained fibrillar NFTs staining, indicative of late-stage/mature NFTs. AT8 also stained NFTs with a granular and fibrillar appearance, as well as NTs and tufted astrocytes (Figure 4.1). Semiquantitative analysis suggested that both antibodies detected a comparable (moderate) amount of pathology (Table 4.2).

LR11 CT Antibody Does Not Label CBD Pathology

Adjacent, paraffin-embedded tissue sections of basal ganglia, from four CBD brains (Table 4.2) were immunohistochemically processed, separately. Microscopic evaluation revealed no LR11 CT immunoreactivity in any of the CBD cases (Figure 4.1), but AT8 detected numerous astrocytic plaques, coiled bodies, and NTs (in both gray and white matter) (Figure 4.1). Semiquantitative analysis indicated an absence LR11 CT-positive pathology, but a high level of AT8 immunoreactivity (Table 4.2).

Table 4.1. Tauopathies

Tauopathy	Dominant Tau Isoforms	Ultrastructural Characteristics of Straight Filaments	Ultrastructural Characteristics of Twisted Filaments	Affected Brain Regions	Symptogenesis (Decade of Life)	Clinical Presentation	Prevalence (Per 100,000)	Prognosis (Years)
Progressive Supranuclear Palsy	4R	13 – 18 nm diameter		Pallidum Subthalamic Nucleus Substantia Nigra Basal Ganglia Periaqueductal Gray	> 5th	Supranuclear Gaze Palsy Pseudobulbar Palsy Postural Instability Progressive Axial Rigidity Frontal Dysexecutive Dementia	5	6 – 10
Corticobasal Degeneration	4R	15 nm diameter	13 – 28 nm diameter 120 – 202 nm periodicity	Frontoparietal Cortex Substantia Nigra	6th – 7th	Progressive Axial Rigidity Apraxia Progressive Non-Fluent Aphasia Frontal Dysexecutive Dementia	< 1	< 10
Alzheimer's Disease	3R/4R	15 nm diameter	8 – 28 nm diameter 80 nm periodicity	Frontal Cortex Temporal Cortex Basal Forebrain Hippocampus Entorhinal Cortex	7th	Amnestic Dementia *Accompanied by Impairment in At Least One Other Cognitive Domain*	790	4 – 8
Pick's Disease	3R	10 – 18 nm diameter	15 – 28 nm diameter 120 – 160 nm periodicity	Frontal Cortex Temporal Cortex Dentate Gyrus Hippocampus Entorhinal Cortex	6th – 7th	Frontal Dysexecutive Dementia Semantic Dementia Progressive Non-Fluent Aphasia	< 1	10 – 15

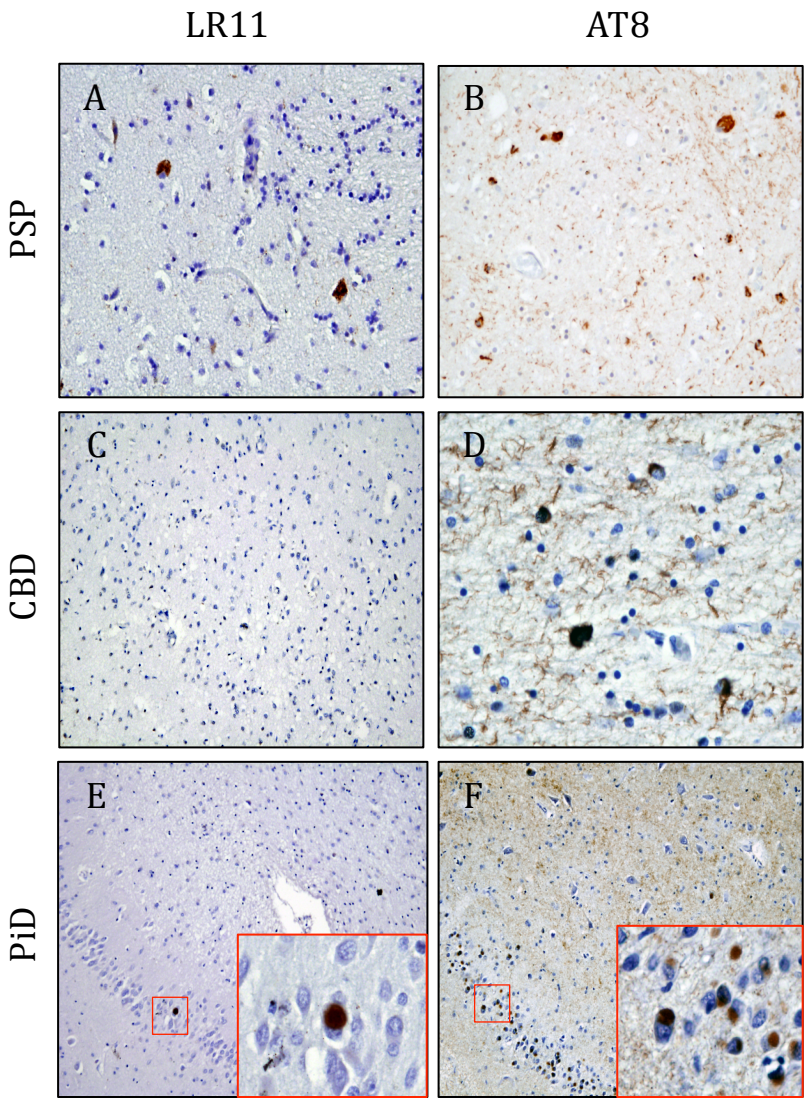


Figure 4.1. LR11 CT and AT8 Immunostaining in Progressive Supranuclear Palsy (PSP) Basal Ganglia, Coticobasal Degeneration (CBD) Frontal Cortex, and Pick's Disease (PiD) Dentate Gyrus. PSP basal ganglia contained moderate levels of LR11 CT (A) and AT8 (B) immunoreactive pathology. CBD frontal cortex contained no LR11 CT immunoreactivity (C) but contained a high level of AT8 immunoreactive pathology (D). PiD dentate gyrus was devoid of LR11 CT immunoreactivity, except for one case that contained a single immunoreactive pick body (E). A high level of pick bodies were immunoreactive for AT8 in PiD dentate gyrus (F).

LR11 CT Labels Isolated Pick Bodies in PiD Brains

Adjacent, paraffin-embedded tissue sections of the hippocampal formation, from four PiD brains (Table 4.2), were immunohistochemically processed with the LR11 CT and AT8 antibodies, separately. Microscopic evaluation revealed no LR11 CT immunoreactivity throughout the hippocampal formation in three of the cases examined. A single LR11-immunoreactive Pick body was found in one case, despite the detection of numerous immunonegative inclusions in hemotoxylin counterstain sections (Figure 4.1). AT8 stained numerous Pick bodies throughout the dentate gyrus (Figure 4.1). Semiquantitative analysis indicated an absence LR11 CT-positive pathology (except for the isolated Pick body mentioned above), but a high level of pathology immunoreactive for AT8 (Table 4.2).

Discussion

AD-related tau pathology is restricted to neuronal cell bodies (NFTs) or neurites (NTs and dystrophic neurites) and contains a mixture of 3R and 4R tau isoforms with SFs and twisted filaments in the form of PHFs. However, tauopathies contain neuronal and glial inclusions that are dominated by either 3R or 4R tau isoforms with a SF or twisted filament structure (Crowther 1991, Perry et al. 1987, Goedert et al. 1992, Greenberg and Davies 1990). PSP's pathological profile includes neuronal inclusions (globose tangles) and glial inclusions (tufted astrocytes and coiled bodies) that are dominated by 4R tau isoforms with a SF structure (Hauw et al. 1994, Arima 2006, Chambers et al. 1999, Sergeant, Wattez, and Delacourte 1999, Lee, Goedert, and Trojanowski 2001). These lesions appear in the basal ganglia,

Table 4.2. Semiquantitative analysis of Tauopathies

Case	Neuropathological Diagnosis	Age	Race/Sex	PMI	Brain Region	LR11+ Pathology	AT8+ Pathology
1	PSP	82	w/f	11.5	Basal Ganglia	++	++
2	PSP	83	w/m	23	Basal Ganglia	++	++
3	PSP	60	w/m	63	Basal Ganglia	++	++
4	PSP	70	w/f	15	Basal Ganglia	++	++
5	CBD	68	w/f	3	Frontal Cortex	0	+++
6	CBD	86	w/f	4	Frontal Cortex	0	+++
7	CBD	44	w/m	24	Basal Ganglia	0	+++
8	CBD	77	w/m	---	Basal Ganglia	0	+++
9	PiD	65	w/m	---	Hippocampus	0	+++
10	PiD	59	w/m	---	Hippocampus	0	+++
11	PiD	71	w/f	4	Hippocampus	0	+++
12	PiD	69	w/m	9	Hippocampus	0*	+++

0 = absence of LR11+ pathology; + = low level of LR11+ pathology; ++ = moderate level of LR11+ pathology; +++ = high level of LR11+ pathology; * = this case only had one LR11+ Pick body in the entire dentate gyrus, amidst numerous non-reactive Pick bodies. PMI was not available for 1 CBD case and 2 PiD cases.

subthalamic nucleus, periaqueductal gray, and substantia nigra (Hauw et al. 1994). CBD is also a 4R tauopathy with a pathological profile that includes achromatic, balloon-shaped neurons in the substantia nigra and prominent NTs, coiled bodies, and astrocytic plaques in the basal ganglia and frontoparietal cortex (Rebeiz, Kolodny, and Richardson 1967, Dickson, Bergeron, Chin, Duyckaerts, Horoupian, Ikeda, Jellinger, Lantos, Lippa, Mirra, Tabaton, Vonsattel, Wakabayashi, Litvan, et al. 2002). CBD brain also contains abundant NTs throughout the white matter (Dickson, Bergeron, Chin, Duyckaerts, Horoupian, Ikeda, Jellinger, Lantos, Lippa, Mirra, Tabaton, Vonsattel, Wakabayashi, Litvan, et al. 2002). PiD pathology, on the other hand, is dominated by 3R tau isoforms with a twisted filament structure (Lee, Goedert, and Trojanowski 2001, Arima 2006, Dickson 1998). Pick bodies, found in granule cells of the dentate gyrus, are the most conspicuous neuropathological feature of PiD (Dickson 1998, Iwatsubo, Hasegawa, and Ihara 1994).

In the current study, immunohistochemical labeling of PSP basal ganglia, CBD basal ganglia and frontal cortex, and PiD dentate gyrus with LR11 CT antibody revealed a moderate level of LR11 CT-positive pathology in PSP basal ganglia, when compared to near adjacent sections labeled with AT8 antibody. CBD basal ganglia and frontal cortex and PiD dentate gyrus were essentially devoid of LR11 CT immunoreactivity with the exception of an isolated Pick body. There were numerous non-reactive Pick bodies in the vicinity of the LR11 immunoreactive Pick body visualized in a hematoxylin counterstained section. A near adjacent section labeled with AT8 revealed frequent Pick bodies throughout the dentate gyrus. These data suggest that LR11 preferentially associates with 4R tau isoforms with SF

structure. This is in accord with the results from Chapter 3 in which dual labeling revealed strong co-labeling with LR11 CT and AT8. AT8 is widely acknowledged for its ability to label pretangles – neurons that are morphologically indistinguishable from healthy neurons but contain accumulations of pathological tau. Interestingly, Uchihara et al. (2012) dual labeled AD hippocampal sections with antibodies specific to 3R and 4R tau isoforms and examined the relationship between tau isoform expression and morphological alterations in NFTs, demonstrating that pretangles were 4R-selective (Uchihara et al. 2012).

In addition, I reported a complete divergence in NFTs immunoreactive for LR11 CT and MN423, widely held as a marker of late-stage NFTs/ghost tangles. Uchihara et al. (2012) found that late-stage NFTs/ghost tangles were 3R-selective. Our dual labeling with LR11 CT and Alz50, as a marker of NFTs at an intermediate stage, revealed that Alz50 only labeled a subset of LR11 CT-positive NFTs. Uchihara et al. (2012) found that NFTs at an intermediate stage contained both 3R and 4R tau isoforms. This suggests that LR11 CT-positive/Alz50-negative NFTs contained aggregates of 4R tau isoforms but had not begun to accumulate aggregates of 3R tau isoforms. NFTs immunoreactive for both LR11 CT and Alz50, however, may have contained aggregates of 4R and 3R tau isoforms, reflecting a more advanced stage of NFT maturation.

The current findings expand upon the hypothesis that LR11 preferentially associates with early-stage NFTs by demonstrating a preferential association with the 4R tau aggregates found in PSP brain.

Chapter 5

General Discussion

LR11/SorLA

LR11/SorLA is a type 1 transmembrane protein with structural elements linking it to both the VPS10 family of sorting receptors and the LDLR family of endocytic receptors (Yamazaki et al. 1996). LR11 has been found in peripheral tissues such as liver, thyroid, adrenal glands, and testes, but its most abundant expression has been noted in the hippocampus, frontal cortex, and cerebellum (Yamazaki et al. 1996, Motoi et al. 1999). Approximately 10% of neuronal LR11 is found at the plasma membrane where it is rapidly internalized to early endosomes (Jacobsen et al. 2001, Nielsen et al. 2007). From early endosomes, LR11 is either recycled to the plasma membrane, traffics retrogradely to the TGN, or traffics to the lysosome, where it is degraded (Nielsen et al. 2007, Schmidt et al. 2007). Associations between LR11 and ApoE (Taira et al. 2001), lipoprotein lipase (Jacobsen et al. 2001, Klinger et al. 2011a), glia cell line-derived neurotrophic factor (Westergaard et al. 2004), platelet-derived growth factor (Gliemann et al. 2004), and APP (Andersen et al. 2006, Schmidt et al. 2007, Spoelgen et al. 2006) have been noted, suggesting that LR11 influences a wide range of cellular processes.

LR11 was linked to AD following observations of reduced neuronal expression in brain regions vulnerable to AD pathology (Scherzer et al. 2004). These

results were later confirmed in MCI and AD frontal cortex (Sager et al. 2007), but a recent study reported variable neuronal LR11 expression in the frontal cortex, precuneus, and occipital cortex of no cognitive impairment (NCI), MCI, and AD brain (Sager et al. 2012), suggesting that the relationship between neuronal LR11 expression and AD pathology is not straight forward. Nevertheless, there is considerable evidence that LR11 plays a role in AD pathogenesis. Single nucleotide polymorphisms in non-coding regions of *SORL1*, the gene that encodes for LR11, have been suggested to influence LR11 expression levels and increase risk of developing sporadic AD (Rogaeva et al. 2007). In addition, transgenic mouse models of AD deficient in LR11 display accelerated deposition of plaques throughout the cortex (Dodson et al. 2008b). These effects are mediated via the ability of LR11 to interact with and negatively regulate processing of APP into Aβ (Offe et al. 2006b, Andersen et al. 2005a, Spoelgen et al. 2006, Dodson et al. 2008b, Herskowitz et al. 2012b). Our recent studies suggest that interactions with golgi-localized, gamma adaptin ear-containing, ARF-binding 1 (GGA1) protein regulate sorting of both BACE1 and LR11, with LR11 trafficking to endosomal recycling compartments (ERC) and BACE1 trafficking to the late endocytic compartments or the TGN (Herskowitz et al. 2012b). This model posits that the shunting of APP away from BACE1 precludes processing of APP along the amyloidogenic pathway. Together, these data indicate a strong link between lower neuronal LR11 and the development of amyloid plaques in AD brain.

The data presented in this dissertation provides additional evidence of a relationship between LR11 and AD by linking it to the other neuropathological

hallmark of the disease: NFTs.

Tau Pathology

In addition to amyloid plaques, AD brain is characterized by tau pathology in the form of NFTs, NT, and dystrophic neurites. The development and propagation of NFTs has been shown to correlate with the degree of cognitive decline (Augustinack et al. 2002a, Ghoshal et al. 2002, Holzer et al. 1994), suggesting that factors involved in NFT development and propagation may serve as therapeutic targets.

The initiation of NFT development is not fully understood, but a decrease in the ratio of 3R:4R tau isoforms has been associated with increased NFT development (Ingram and Spillantini 2002, Fischer et al. 2007). This is attributed to the increased propensity of 4R tau isoforms to dissociate from microtubules and aggregate – a tendency that is mitigated by 3R tau isoforms – into increasingly higher ordered structures, including oligomers, SFs, and PHFs (Adams et al. 2010). Although SFs and PHFs are often thought of as distinctly separate structures, research suggests that SFs may be immature forms of PHFs. Crowther (1991) compared the antigen profile and structural subunits of SFs and PHFs in AD brain and found that both were labeled with an antibody raised against the core of PHFs (a MBD fragment), suggesting that they share a common subunit (Crowther 1991). Ultrastructural analysis confirmed that SFs and PHFs are both composed of two C-shaped morphological subunits that display three peaks of density (that the author designated A, B, C, and A', B', and C'), but the orientation of these subunits within SFs and PHFs differ such that they are arranged "back-to-back" (AB | A'B') in SFs and

"base-to-base" (BC | B'C') in PHFs (Figure 5.1) (Crowther 1991). Therefore, a clockwise rotation of each subunit within SFs would achieve a base-to-base orientation, suggesting that SFs are simply immature PHFs. This idea was supported by the discovery of hybrid filaments in which a portion of the filament has a SF arrangement and a portion has a PHF arrangement (Crowther 1991). The author also reported that a subset of SFs were composed of subunits that were arranged B | B' (Crowther 1991). This suggests that rearrangement of SF subunits can occur within a limited portion of the SF or the entire length of the SF. Crowther and Wischik (1985) also found that, although most twisted filaments found in AD brain have the 70-80 nm periodicity characteristic of PHFs, periodicities ranged from 65 nm to >100 nm. Together, these findings suggest that filaments in AD brain transition from SFs to twisted filaments whose periodicities progressively decrease until reaching the 70-80 nm periodicity of PHFs, which may represent the most stable arrangement of filaments.

In addition to a decrease in the 3R:4R tau isoform ratio, hyperphosphorylation of tau is also thought to play a primary role in facilitating dissociation from microtubules by neutralizing the positive charge of the MBDs on tau that associate with the negatively-charge microtubule (Jho et al. 2010) or altering tau's conformation (Fischer et al. 2009). In addition, tau undergoes subsequent posttranslational modifications that have been shown to occur in a sequence that can be used to determine the degree of NFT maturation (Guillozet-Bongaarts et al. 2005a, Binder et al. 2005). Following hyperphosphorylation, tau is misfolded, and eventually undergoes cleavage (Binder et al. 2005, Guillozet-

Bongaarts et al. 2005a), effectively creating early (hyperphosphorylation), middle (misfolded), and late (cleaved) stages of NFT maturation. The progression of tau through these pathological stages is loosely correlated with changes to cell morphology, which is attributable to the breakdown of the cytoarchitectural structure provided by microtubules.

As with the pathological posttranslational modifications that tau undergoes, changes in cell morphology have been categorized in such a way as to reflect NFT maturation. This morphology-based classification scheme was devised by Braak et al. (1994) (Braak, Braak, and Mandelkow 1994b) and classified neurons into five categories based on the extent of their morphological abnormalities: group 1 includes pre-tangles, neurons that are immunoreactive for early-stage pathological tau epitopes or show tau fibrils at the electron microscopy level, but retain their normal cell morphology; group 2 and 3 neurons have altered cell morphology due to the accumulation of increasing amounts of tau pathology and are often referred to as intracellular NFTs (iNFTs); and group 4 (early) and 5 (late) ghost tangles refer to NFTs that have a gnarled appearance and have lost the majority or all of their cell membrane, resulting in them being called extracellular NFTs (eNFTs).

The propagation of maturing NFTs throughout AD brain (described above) follows a predictable ascension through transentorhinal, limbic, and isocortical regions (Braak, Braak, and Mandelkow 1994b). Classification of NFT propagation is referred to as Braak staging. The transentorhinal phase is associated with Braak stages I and II; the limbic phase is associated with Braak stages III and IV; and the isocortical phase is associated with Braak stages V and VI (Braak, Braak, and

Mandelkow 1994b).

Together, the staging of NFT maturation and propagation provides a strong temporal backdrop against which other pathological changes can be assessed. Pathological changes that associate with early stages of NFT maturation and propagation may indicate processes that can be manipulated to therapeutic advantage.

Summary and Interpretation of Results

LR11 in AD Brain

In Chapter 3, I identified a previously unreported association between LR11 and NFTs in sporadic and familial AD brain, as well as DS brain. It was determined that antibody differences were responsible for the discrepancy between these findings and the initial studies investigating the relationship of LR11 to AD pathogenesis (Scherzer et al. 2004, Offe et al. 2006a, Dodson et al. 2006). I also demonstrated that LR11-positive NFTs follow a cortical progression that mirrors Braak staging, thus highlighting the combined influence of pathological disease severity and the brain region examined in detecting LR11-positive NFTs. Perhaps this underlies the inconsistent appearance of LR11-positive NFTs in the frontal cortex, precuneus, and occipital cortex of cases with Braak scores ranging from 0 to V previously examined (Sager et al. 2012, Sager et al. 2007).

To examine the relationship between LR11 and the development of NFTs, dual labeling with LR11 CT antibody and antibodies to pathological tau epitopes

associated with various stages of maturation was performed. The phospho-specific, anti-tau antibody, AT8 (noted for its ability to label pretangles) was used as a marker of early-stage NFT maturation; the Alz50 antibody, which recognizes misfolded tau was used as a marker of middle-stage NFT maturation; and MN423, which recognizes a cleaved form of tau (often associated with ghost tangles) was used as a marker of late-stage NFT maturation. I observed co-labeling of early-stage NFTs stained with LR11 CT and AT8 antibodies. Middle-stage NFTs labeled with Alz50 also co-labeled with LR11 CT, but not all LR11 CT-positive NFTs co-labeled with Alz50. The LR11 CT-positive/Alz50-negative NFTs were interpreted to represent early-stage NFTs that had not yet developed the expression of the Alz50 epitope. MN423-positive NFTs were devoid of LR11 CT immunoreactivity and vice versa. This temporal staging is consistent with LR11 playing a role in the development of NFTs.

Although most studies relating LR11 to AD have focused on its ability to mitigate amyloidogenic processing of APP, recent studies have noted an association between LR11 and tau pathology. A recent study provides evidence that sAPPα negatively regulates the expression and activity of cyclin-dependent kinase 5 (CDK5), a kinase involved in the hyperphosphorylation of tau, and that this effect is directly related to the cellular expression of LR11 (Hartl et al. 2013). In addition, transgenic mice deficient for LR11 display significantly higher numbers of AT8-positive neurons in the hippocampus and entorhinal cortex, when compared to wild type controls (Capsoni et al. 2013). *In vitro* (Imahori and Uchida 1997) and *in vivo* (Otth et al. 2002) studies have associated CDK5 activity with phosphorylation of tau

at serine 202 and threonine 205 (the AT8 epitope). Moreover, *SORL1* polymorphisms have been associated with a significant increase in soluble tau in the cerebrospinal fluid of AD patients (Guo et al. 2012). Together, these data suggest that low neuronal LR11 results in decreased uptake of sAPPα and increased expression and activity of CDK5, which triggers hyperphosphorylation of tau. Sequestration of LR11 in NFTs could, therefore, be part of a positive feedback loop in which LR11 sequestration leads to increased CDK activity and more tau aggregation, which leads to further sequestration of LR11. However, the mechanism underlying the recruitment of LR11 into NFTs is unclear.

LR11 may accumulate in NFTs as a response to neuronal injury. ApoE expression has been found to be increased following neuronal damage (Danik and Poirier 1998, Hall et al. 1995, Horsburgh et al. 1999) and its role in lipoprotein metabolism and lipid homeostasis suggests that it may be involved in mobilization and redistribution of lipids to facilitate the repair of axonal membranes. Through its role as an ApoE receptor, LR11 may serve as a substrate by which ApoE is taken up into injured neurons.

Like LR11, ApoE has been found to associate with NFTs in the AD brain (Benzing and Mufson 1995, Gomez-Ramos, Mufson, and Moran 2001, Han, Einstein, et al. 1994, Han, Hulette, et al. 1994, Namba et al. 1991a, Richey et al. 1995, Wang et al. 2005, Yamaguchi et al. 1994). ApoE's association with NFTs was confirmed via dual labeling with antibodies to pathological tau epitopes, including AT8, PHF-1, and Tau2 (Binder, Frankfurter, and Rebhun 1985). However, the temporal relationship between ApoE and NFTs, assessed via changes in cell morphology, is contentious.

Some studies report that ApoE associates with neurons that do not contain neurofibrillary pathology (Han, Einstein, et al. 1994, Han, Hulette, et al. 1994), as well as iNFTs and eNFTs (Namba et al. 1991a, Richey et al. 1995, Yamaguchi et al. 1994), while other studies conclude that ApoE almost exclusively associates with eNFTs and has minimal association with neurons devoid of neurofibrillary pathology (Benzing and Mufson 1995, Gomez-Ramos, Mufson, and Moran 2001). Despite these inconsistencies, these results suggest interactions between LR11, ApoE and NFT formation.

The appearance of ApoE in neurons without neurofibrillary pathology could suggest that these cells have suffered damage and respond by up regulation of ApoE, perhaps in conjunction with LR11. Alternatively, since ApoE is a heparin binding protein (Weisgraber et al. 1986) and heparin sulfate proteoglycans (HSPGs) are reported to be associated with iNFTs and eNFTs (Perry et al. 1992), the association between ApoE and NFTs may be due to ApoE binding HSPGs in tangles, suggesting the possibility of an indirect role in NFT development. Another possibility is that ApoE accumulates in NFTs because its clearance/degradation is inhibited due to cytoskeletal disruption. Lastly, Strittmatter et al. (1994) demonstrated that ApoE has a high binding affinity for tau, suggesting that ApoE's association with both iNFTs and eNFTs could be due to a direct molecular interaction that may or may not have functional significance.

Recently, a comprehensive assessment of NFT protein constituents was performed using laser capture microdissection of NFTs, followed by liquid chromatography-mass spectrometry (MS)/MS in AD brain (Wang et al. 2005).

Although the complete list of 72 proteins found was not provided, tau, α-synuclein, and ApoE were reported as well as glyceryldehyde-3-phosphate dehydrogenase (GAPDH). GAPDH IHC resulted in the selective labeling of NFTs and neuropil threads (as well as plaques) and, although dual labeling for GAPDH and a NFT marker was not performed, GAPDH was successfully co-immunoprecipitated from homogenates of AD hippocampus, using PHF-1. However, without the establishment a temporal relationship, caution must be taken when considering the functional implications of such an interaction. Nevertheless, as the complete list of proteins becomes available, it will provide a more complete context in which to place the association between LR11 and NFTs.

LR11 in Non-AD Tauopathies

In Chapter 4, I investigated the relationship between LR11 and tau pathology in a category of neurodegenerative diseases referred to, collectively, as tauopathies. Specifically, PSP, CBD, and PiD (the most common tauopathies) were examined. LR11 CT antibody labeled a moderate amount of pathology in PSP brain, which was comparable to the amount of pathology seen in adjacent sections stained with AT8. On the other hand, CBD and PiD, were virtually devoid of LR11 CT immunoreactivity, except for a single Pick body in the dentate gyrus of one PiD case. These results suggest a preferential relationship between LR11 and NFTs in PSP brain. Differences in the dominant tau isoform and ultrastructural characteristics of lesions in PSP, CBD, and PiD provide insight into the species of pathological tau that LR11 may associate with. While PiD is considered to be a 3R tauopathy, both PSP

and CBD are 4R tauopathies (Buee and Delacourte 1999). Ultrastructurally, PSP is distinguishable from both CBD and PiD in that NFTs in PSP brain are almost exclusively composed of SFs, while CBD and PiD tau pathology is composed of a mixture of SFs and twisted filaments (Arima 2006, Tellez-Nagel and Wisniewski 1973). This suggests that LR11 preferentially associates with 4R tau isoforms in a SF conformation. This idea is in accord with the results from Chapter 3, as NFTs in AD brain have been shown to be composed of both 3R and 4R tau isoforms (Goedert et al. 1992, Greenberg and Davies 1990) that take on SF and PHF conformations (Crowther 1991, Arima 2006). Given the proposed relationship between SFs and twisted filaments (discussed above), LR11 associating with the NFTs in PSP suggests a relationship with an early species of pathological tau.

I interpreted Chapter 3's results in two ways. First, reduced neuronal LR11 may indirectly trigger hyperphosphorylation of tau via reduced uptake of sAPPα, which leads to increased expression and activity of CDK5. In this scenario, accumulation of LR11 in NFTs is thought to reflect impaired degradation, due to disrupted cytoskeletal trafficking to lysosomes. Applying this interpretation to the results obtained in tauopathy brains suggests a similar pathological process at work in PSP brain but not in CBD or PiD brain. This may indicate differences in the regional expression of LR11 across disease states. The relative abundance of tau pathology in PSP, CBD, and PiD varies based on brain region, with pathology most prevalent in the basal ganglia, frontal and parietal cortices, and dentate gyrus of PSP, CBD, and PiD brain, respectively (Hauw et al. 1994, Rebeiz, Kolodny, and Richardson 1967, Dickson, Bergeron, Chin, Duyckaerts, Horoupian, Ikeda, Jellinger, Lantos,

Lippa, Mirra, Tabaton, Vonsattel, Wakabayashi, and Litvan 2002, Iwatsubo, Hasegawa, and Ihara 1994, Dickson 1998). Neuronal expression of LR11 has been found to be lower in the basal ganglia when compared to the cortex and hippocampal formation (Motoi et al. 1999, Yamazaki et al. 1996), suggesting that this neuronal population may be particularly susceptible to the development of neurofibrillary pathology.

Second, LR11 associating with NFTs in AD brain might reflect a compensatory mechanism used to increase uptake of ApoE to facilitate cellular repair. A complete assessment of this idea in tauopathy brain could not be done, since a comprehensive immunohistochemical assessment of ApoE has not been performed in tauopathy brain. PiD brain, however, has been labeled with an antibody to an 18 kDa truncated form of ApoE, termed the N-terminal ApoE cleaved fragment (nApoECF), which has been shown to label NFTs in AD brain (Rohn et al. 2012, Rohn et al. 2013). Interestingly, nApoECF labeled Pick bodies in CA1 but not the dentate gyrus (Rohn et al. 2013), a pattern reminiscent of a regional distribution documented for caspase-cleaved tar DNA-binding protein-43 (Rohn and Kokoulina 2009). These results prompted Rohn et al. (2013) to perform dual-fluorescent labeling with TauC3, an antibody that recognizes tau cleaved at aspartic acid 421 by caspase 3, and nApoECF. They found 75% co-localization between TauC3 and nApoECF in CA1 neurons. These findings suggest that the same processes that lead to aberrant cleavage of tau may also facilitate ApoE cleavage, but this process was not caspase 3-mediated (Rohn et al. 2012, Rohn et al. 2013).

In a separate study, Huang et al. (2001) also labeled NFTs in AD brain with an

anti-N-termimal ApoE antibody. Comparison of nondemented control and AD brain homogenate revealed two N-terminal ApoE fragments: one fragment was ~ 14-20 kDa and was found only in AD brain homogenate, while the other fragment, ~ 30 kDa, was found in both AD and nondemented control homogenate albeit in greater amounts in AD brain homogenate (Huang et al. 2001). In addition, transfection of N-terminal ApoE3 and ApoE4 constructs facilitated the development of neurofibrillary pathology in neuronal but not nonneuronal cells; this effect was more pronounced in cells transfected with the ApoE4 construct (Huang et al. 2001). Moreover, a ~30 kDa N-terminal ApoE fragment was detected in neurons exposed to extracellular ApoE and transfected neurons that synthesized and secreted ApoE; here, ApoE4 was found to be more susceptible to cleavage (Huang et al. 2001).

Together, these studies suggest that proteolytic processing of ApoE occurs in healthy brain but this process is altered in AD brain and may trigger the development of neurofibrillary pathology in neurons. The enzyme responsible for ApoE cleavage, however, has yet to be discovered, but the staining pattern of nApoECF, in PiD brain, suggests that enzyme responsible is absent in the dentate gyrus (Rohn et al. 2013). Interestingly, lipoprotein lipase (LPL) has been shown to be specifically decreased in the dentate gyrus of AD brain (Gong et al. 2013). LPL has typically been studied in peripheral tissue, where it facilitates receptor-mediated uptake of lipoprotein particles, lipoprotein-associated lipids, and lipophilic vitamins (Mead, Irvine, and Ramji 2002). Thus, the full scope of LPL's function in brain remains unclear, but *in vitro* studies suggest that LPL may serve in a protective capacity to prevent neurotoxicity in neurons exposed to oxidized lipoproteins

(Wang and Eckel 2012). Additionally, mice undergoing middle cerebral occlusion, to induce cerebral ischemia, displayed decreased LPL levels 2 hours post occlusion, but LPL levels were elevated 4-6 hours post occlusion (Wang and Eckel 2012). In a separate mouse model of hippocampal deafferentiation that mimics pathological changes found in AD brain, LPL mRNA and protein levels were upregulated during the degenerative phase, but returned to baseline levels during the reinnervation phase (Blain and Poirier 2004a). These data suggest that LPL increases in response to neuronal injury, and although LPL has been shown to interact with various LDLRs (Wang and Eckel 2012, Blain and Poirier 2004b), the increased expression has been found to coincide with heparan sulfate proteoglycans (HSPGs) (Blain and Poirier 2004a). LPL has also been shown to bind ApoE (Wang and Eckel 2012). Together, this suggests that LPL expression increases in response to neuronal injury and facilitates uptake of ApoE-rich lipids via interactions with HSPGs or LDLRs.

A relationship between LPL and AD is also beginning to emerge, with intronic polymorphisms in LPL being associated with increased numbers of plaques and NFTs in AD brain (Blain et al. 2006). In addition, immunohistochemical analysis has shown that LPL associates with plaques and neurite pathology (Blain and Poirier 2004b, Gong et al. 2013), suggesting that LPL plays a role in the development of plaques and neurofibrillary pathology in AD brain.

Given these findings, it is tempting to propose a role for LR11 in LPL-mediated uptake of ApoE following neuronal injury. To test the effect(s) of LR11 on LPL, Klinger et al. (2011) conducted a series of cell culture experiments in HEK293 cells transfected with LPL, LR11, or both. Substantial co-localization in vesicular

structures was observed in cells co-transfected with LR11 and LPL. Subsequent manipulation of protein synthesis, lysosomal degradation, and LPL endocytosis via treating cells with cyclohexamide, leupeptin and pepstatin, and heparin, respectively, revealed that LR11-transfected cells can endocytose exogenous LPL and that LR11 traffics LPL directly from the TGN to endosomal compartments (Klinger et al. 2011b). Measurements of LPL activity in cells co-transfected with LR11 and LPL revealed reduced intracellular (83%) and extracellular (67%) activity of LPL, when compared to cells transfected with LPL alone. This effect coincided with increased expression of LPL in lysosomes (Klinger et al. 2011b), suggesting that LR11 mitigates LPL activity by trafficking LPL to lysosomes for degradation. This was corroborated by pulse/chase experiments in which the amount of non-degraded LPL from cells transfected with LPL or co-transfected with LR11 and LPL was measured at 2 and 4 hours; non-degraded LPL levels were significantly higher at both time points in cells transfected with LPL alone (Klinger et al. 2011b). Immunocytochemical analysis of primary cultures of hippocampal neurons from LR11-knocout mice, revealed diffuse cytoplasmic staining of LPL, when compared to wild type cultures (Klinger et al. 2011b), highlighting the importance of LR11 in trafficking of LPL to endosomal compartments.

In summary, LR11 was found to associate with tau pathology in the basal ganglia of PSP brain but not in frontal cortex of CBD brain or dentate gyrus of PiD brain. However, there was, a single PiD case that contained a single Pick body in the dentate gyrus that was immunoreactive for LR11. Given that cellular expression of LR11 is positively correlated with intracellular levels of sAPPα, which negatively

regulates CDK5-mediated phosphorylation of tau (Hartl et al. 2013), this disease-specific association of LR11 with PSP tau pathology may reflect regional differences in neuronal expression of LR11, with neuronal populations with low LR11 levels, such as the basal ganglia (Motoi et al. 1999), being more susceptible to the development of neurofibrillary pathology. In addition, aberrant proteolysis of ApoE produces N-terminal fragments that are increased in AD brain, when compared to nondemented control brain, or are specific to AD brain (Rohn et al. 2012, Huang et al. 2001). These N-terminal ApoE fragments have been associated with NFTs in AD brain (Rohn et al. 2012) and have been shown to induce the development of neurofibrillary pathology in neuronal cultures (Huang et al. 2001). The enzyme responsible for the proteolysis of ApoE, however, is unknown. Interestingly, PiD brain revealed regional differences in the expression of an N-terminal ApoE fragment, with pyramidal neurons in the hippocampus containing the fragment, but granule cells in the dentate gyrus lacking the fragment (Rohn et al. 2013). This coincides with the reduced expression of LPL seen in the dentate gyrus of AD brain (Gong et al. 2013) – also devoid of LR11-positive pathology (data not shown) – suggesting that LPL may be involved in the proteolysis of ApoE and that its effects may be mediated via LR11. In the peripheral nervous system, LPL is known to facilitate uptake of lipoprotein particles, lipoprotein-associated lipids, and lipophilic vitamins via interactions with ApoE and HSPGs or LDLRs (Mead, Irvine, and Ramji 2002). In brain, oxidative and mechanical damage of neurons (Wang and Eckel 2012, Blain and Poirier 2004a) produces an increase in LPL expression that coincides with increased expression of HSPGs, suggesting that LPL may facilitate

uptake of ApoE-rich lipids for use in cellular repair. LPL has been found to associate with plaques and neurites (Blain and Poirier 2004b, Gong et al. 2013), and polymorphisms in LPL have been associated with increased numbers of plaques and NFTs in AD brain (Blain et al. 2006), suggesting that disrupting LPL function contributes to the development of AD pathology. Recently, LR11 has been suggested to play an important role in LPL function, with reduction of LR11 expression leading to aberrant cellular localization and increased enzymatic activity of LPL (Klinger et al. 2011b). Together, this suggests that low neuronal LR11 levels may contribute to the development of neurofibrillary pathology by facilitating the increased and aberrant proteolysis of ApoE by LPL.

Conclusion

In this dissertation, I report the novel association of LR11 with NFTs in sporadic and familial AD brain, as well as DS brain. Examination of the relationship between LR11 and NFTs at various stages of maturation indicated a preferential association with early-stage NFTs, suggesting that LR11 may play a role in the development of NFTs in AD brain. Interestingly, LR11 displayed disease-specific differences in its association with tau pathology in tauopathy brains. NFTs in the basal ganglia of PSP brain were immunoreactive for LR11, but CBD frontal cortex and PiD dentate gyrus were absent of, or contained negligible, LR11 immunoreactivity. I propose that the disease-specific between LR11 and tau pathology may reflect differences in regional expression of neuronal LR11. Despite a recent report to the contrary (Sager et al. 2012), studies examining the relationship

between LR11 and AD have noted decreased neuronal expression in vulnerable AD brain regions (Scherzer et al. 2004, Offe et al. 2006a, Sager et al. 2007, Dodson et al. 2006). In addition, intronic polymorphisms of *SORL1*, which may affect protein expression, have been associated with an increase in tau in the CSF of AD patients (Guo et al. 2012). This supports a relationship between low neuronal LR11 and NFTs in AD brain. Mechanistically, reduced cellular expression of LR11 has been associated with decreased intracellular levels of sAPPα resulting in increased expression and activity of CDK5, a kinase associated with the pathological hyperphosphorylation of tau (Hartl et al. 2013). LR11 has also been demonstrated to regulate cellular localization and activity of LPL, which may be involved in the aberrant proteolysis of ApoE, generating fragments that can induce neurofibrillary pathology (Huang et al. 2001). The involvement of LPL in ApoE proteolysis, however, remains to be determined.

A number of proteins have been identified as components of amyloid and tau pathology in AD, including, Aβ, ApoE, LRP, and presenilins, (Burns et al. 2003, Levey et al. 1997, Namba et al. 1991b). Reports of a genetic association between the LR11 gene (*SORL1*) and AD risk supports a proximal role for LR11 in the disease process (Rogaeva et al. 2007); and we, and others, have shown that LR11 can directly interact with APP, alter APP intracellular sorting, and influence Aβ production and the development of amyloid pathology *in vivo* (Andersen et al. 2005b, Andersen et al. 2006, Dodson et al. 2006, Offe et al. 2006a). The findings presented in this dissertation provide the first link between LR11 and tau pathology in human brain, and suggest that low neuronal LR11 expression may contribute to the development

of amyloid and tau pathology. Future studies are needed to clarify the intriguing role that LR11 plays in the evolution of amyloid and tau pathologies in AD.

References

1997. "Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease." *Neurobiology of Aging* 18 (4 Suppl):S1-2.

2012. "2012 Alzheimer's disease facts and figures." *Alzheimer's & dementia : the journal of the Alzheimer's Association* 8 (2):131-68. doi: 10.1016/j.jalz.2012.02.001.

Adams, S. J., M. A. DeTure, M. McBride, D. W. Dickson, and L. Petrucelli. 2010. "Three repeat isoforms of tau inhibit assembly of four repeat tau filaments." *PLoS One* 5 (5):e10810. doi: 10.1371/journal.pone.0010810.

Al-Shawi, R., A. Hafner, S. Chun, S. Raza, K. Crutcher, C. Thrasivoulou, P. Simons, and T. Cowen. 2007. "ProNGF, sortilin, and age-related neurodegeneration." *Ann N Y Acad Sci* 1119:208-15. doi: 10.1196/annals.1404.024.

Allinson, T. M., E. T. Parkin, A. J. Turner, and N. M. Hooper. 2003. "ADAMs family members as amyloid precursor protein alpha-secretases." *J Neurosci Res* 74 (3):342-52. doi: 10.1002/jnr.10737.

Alonso, A. C., I. Grundke-Iqbal, and K. Iqbal. 1996. "Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules." *Nat Med* 2 (7):783-7.

Alzheimer, A., R. A. Stelzmann, H. N. Schnitzlein, and F. R. Murtagh. 1995. "An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde"." *Clin Anat* 8 (6):429-31. doi: 10.1002/ca.980080612.

Andersen, O. M., J. Reiche, V. Schmidt, M. Gotthardt, R. Spoelgen, J. Behlke, C. A. von Arnim, T. Breiderhoff, P. Jansen, X. Wu, K. R. Bales, R. Cappai, C. L. Masters, J. Gliemann, E. J. Mufson, B. T. Hyman, S. M. Paul, A. Nykjaer, and T. E. Willnow. 2005a. "Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein." *Proceedings of the National Academy of Sciences of the United States of America* 102 (38):13461-6. doi: 10.1073/pnas.0503689102.

Andersen, O. M., V. Schmidt, R. Spoelgen, J. Gliemann, J. Behlke, D. Galatis, W. J. McKinstry, M. W. Parker, C. L. Masters, B. T. Hyman, R. Cappai, and T. E. Willnow. 2006. "Molecular dissection of the interaction between amyloid precursor protein and its neuronal trafficking receptor SorLA/LR11." *Biochemistry* 45 (8):2618-28. doi: 10.1021/bi052120v.

Andersen, Olav M., Juliane Reiche, Vanessa Schmidt, Michael Gotthardt, Robert Spoelgen, Joachim Behlke, Christine A. F. von Arnim, Tilman Breiderhoff, Pernille Jansen, Xin Wu, Kelly R. Bales, Roberto Cappai, Colin L. Masters, Jorgen Gliemann, Elliott J. Mufson, Bradley T. Hyman, Steven M. Paul, Anders Nykjaer, and Thomas E. Willnow. 2005b. "Neuronal sorting protein-related receptor sorLA/LR11 regulates processing of the amyloid precursor protein." *Proceedings of the National Academy of Sciences of the United States of America* 102 (38):13461-6.

Arima, K. 2006. "Ultrastructural characteristics of tau filaments in tauopathies: immuno-electron microscopic demonstration of tau filaments in tauopathies." *Neuropathology* 26 (5):475-83.

Ashley, J., M. Packard, B. Ataman, and V. Budnik. 2005. "Fasciclin II signals new synapse formation through amyloid precursor protein and the scaffolding protein dX11/Mint." *J Neurosci* 25 (25):5943-55. doi: 10.1523/JNEUROSCI.1144-05.2005.

Augustinack, J. C., A. Schneider, E. M. Mandelkow, and B. T. Hyman. 2002a. "Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease." *Acta Neuropathol* 103 (1):26-35.

Augustinack, J. C., A. Schneider, E. M. Mandelkow, and B. T. Hyman. 2002b. "Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease." *Acta Neuropathologica* 103 (1):26-35.

Bancher, C., H. Lassmann, H. Budka, I. Grundke-Iqbal, K. Iqbal, G. Wiche, F. Seitelberger, and H. M. Wisniewski. 1987. "Neurofibrillary tangles in Alzheimer's disease and progressive supranuclear palsy: antigenic similarities and differences. Microtubule-associated protein tau antigenicity is prominent in all types of tangles." *Acta neuropathologica* 74 (1):39-46.

Barbagallo, A. P., Z. Wang, H. Zheng, and L. D'Adamio. 2011. "A single tyrosine residue in the amyloid precursor protein intracellular domain is essential for developmental function." *J Biol Chem* 286 (11):8717-21. doi: 10.1074/jbc.C111.219873.

Barger, S. W., and A. D. Harmon. 1997. "Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E." *Nature* 388 (6645):878-81. doi: 10.1038/42257.

Bayer, T. A., O. Wirths, K. Majtenyi, T. Hartmann, G. Multhaup, K. Beyreuther, and C. Czech. 2001. "Key factors in Alzheimer's disease: beta-amyloid precursor protein processing, metabolism and intraneuronal transport." *Brain Pathol* 11 (1):1-11.

Beffert, U., M. Danik, P. Krzywkowski, C. Ramassamy, F. Berrada, and J. Poirier. 1998. "The neurobiology of apolipoproteins and their receptors in the CNS and Alzheimer's disease." *Brain Res Brain Res Rev* 27 (2):119-42.

Bellosta, S., B. P. Nathan, M. Orth, L. M. Dong, R. W. Mahley, and R. E. Pitas. 1995. "Stable expression and secretion of apolipoproteins E3 and E4 in mouse neuroblastoma cells produces differential effects on neurite outgrowth." *J Biol Chem* 270 (45):27063-71.

Benzing, W. C., and E. J. Mufson. 1995. "Apolipoprotein E immunoreactivity within neurofibrillary tangles: relationship to Tau and PHF in Alzheimer's disease." *Exp Neurol* 132 (2):162-71.

Berger, Z., H. Roder, A. Hanna, A. Carlson, V. Rangachari, M. Yue, Z. Wszolek, K. Ashe, J. Knight, D. Dickson, C. Andorfer, T. L. Rosenberry, J. Lewis, M. Hutton, and C. Janus. 2007. "Accumulation of pathological tau species and memory loss in a conditional model of tauopathy." *J Neurosci* 27 (14):3650-62. doi: 10.1523/JNEUROSCI.0587-07.2007.

Bertram, L., M. B. McQueen, K. Mullin, D. Blacker, and R. E. Tanzi. 2007. "Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database." *Nat Genet* 39 (1):17-23. doi: 10.1038/ng1934.

Bettens, K., N. Brouwers, S. Engelborghs, P. P. De Deyn, C. Van Broeckhoven, and K. Sleegers. 2008. "SORL1 is genetically associated with increased risk for late-onset Alzheimer disease in the Belgian population." *Hum Mutat* 29 (5):769-70. doi: 10.1002/humu.20725.

Bierer, L. M., P. R. Hof, D. P. Purohit, L. Carlin, J. Schmeidler, K. L. Davis, and D. P. Perl. 1995. "Neocortical neurofibrillary tangles correlate with dementia severity in Alzheimer's disease." *Arch Neurol* 52 (1):81-8.

Binder, L. I., A. Frankfurter, and L. I. Rebhun. 1985. "The distribution of tau in the mammalian central nervous system." *J Cell Biol* 101 (4):1371-8.

Binder, L. I., A. L. Guillozet-Bongaarts, F. Garcia-Sierra, and R. W. Berry. 2005. "Tau, tangles, and Alzheimer's disease." *Biochim Biophys Acta* 1739 (2-3):216-23. doi: 10.1016/j.bbadis.2004.08.014.

Blain, J. F., N. Aumont, L. Theroux, D. Dea, and J. Poirier. 2006. "A polymorphism in lipoprotein lipase affects the severity of Alzheimer's disease pathophysiology." *Eur J Neurosci* 24 (5):1245-51. doi: 10.1111/j.1460-9568.2006.05007.x.

Blain, J. F., and J. Poirier. 2004a. "Cholesterol homeostasis and the pathophysiology of Alzheimer's disease." *Expert Rev Neurother* 4 (5):823-9. doi: 10.1586/14737175.4.5.823.

Blain, J. F., and J. Poirier. 2004b. "Could lipoprotein lipase play a role in Alzheimer's disease?" *ScientificWorldJournal* 4:531-5. doi: 10.1100/tsw.2004.111.

Blessed, G., B. E. Tomlinson, and M. Roth. 1968. "The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects." *Br J Psychiatry* 114 (512):797-811.

Bohm, C., N. M. Seibel, B. Henkel, H. Steiner, C. Haass, and W. Hampe. 2006a. "SorLA signaling by regulated intramembrane proteolysis." *Journal of Biological Chemistry* 281 (21):14547-53.

Bohm, C., N. M. Seibel, B. Henkel, H. Steiner, C. Haass, and W. Hampe. 2006b. "SorLA signaling by regulated intramembrane proteolysis." *The Journal of biological chemistry* 281 (21):14547-53. doi: 10.1074/jbc.M601660200.

Braak, E., H. Braak, and E. M. Mandelkow. 1994a. "A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads." *Acta Neuropathologica* 87 (6):554-67.

Braak, E., H. Braak, and E. M. Mandelkow. 1994b. "A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads." *Acta Neuropathol* 87 (6):554-67.

Braak, H., and E. Braak. 1991. "Neuropathological stageing of Alzheimer-related changes." *Acta Neuropathologica* 82 (4):239-59.

Buee, L., T. Bussiere, V. Buee-Scherrer, A. Delacourte, and P. R. Hof. 2000. "Tau protein isoforms, phosphorylation and role in neurodegenerative disorders." *Brain Res Brain Res Rev* 33 (1):95-130.

Buee, L., and A. Delacourte. 1999. "Comparative biochemistry of tau in progressive supranuclear palsy, corticobasal degeneration, FTDP-17 and Pick's disease." *Brain Pathol* 9 (4):681-93.

Burns, M. P., W. J. Noble, V. Olm, K. Gaynor, E. Casey, J. LaFrancois, L. Wang, and K. Duff. 2003. "Co-localization of cholesterol, apolipoprotein E and fibrillar Abeta in amyloid plaques." *Brain Research. Molecular Brain Research* 110 (1):119-25.

Buxbaum, J. D., K. N. Liu, Y. Luo, J. L. Slack, K. L. Stocking, J. J. Peschon, R. S. Johnson, B. J. Castner, D. P. Cerretti, and R. A. Black. 1998. "Evidence that tumor necrosis factor alpha converting enzyme is involved in regulated alpha-secretase cleavage of the Alzheimer amyloid protein precursor." *J Biol Chem* 273 (43):27765-7.

Cai, H., Y. Wang, D. McCarthy, H. Wen, D. R. Borchelt, D. L. Price, and P. C. Wong. 2001. "BACE1 is the major beta-secretase for generation of Abeta peptides by neurons." *Nat Neurosci* 4 (3):233-4. doi: 10.1038/85064.

Cam, J. A., C. V. Zerbinatti, J. M. Knisely, S. Hecimovic, Y. Li, and G. Bu. 2004. "The low density lipoprotein receptor-related protein 1B retains beta-amyloid precursor protein at the cell surface and reduces amyloid-beta peptide production." *J Biol Chem* 279 (28):29639-46. doi: 10.1074/jbc.M313893200.

Caporaso, G. L., K. Takei, S. E. Gandy, M. Matteoli, O. Mundigl, P. Greengard, and P. De Camilli. 1994. "Morphologic and biochemical analysis of the intracellular trafficking of the Alzheimer beta/A4 amyloid precursor protein." *J Neurosci* 14 (5 Pt 2):3122-38.

Capsoni, S., A. S. Carlo, D. Vignone, G. Amato, C. Criscuolo, T. E. Willnow, and A. Cattaneo. 2013. "SorLA deficiency dissects amyloid pathology from tau and cholinergic neurodegeneration in a mouse model of Alzheimer's disease." *Journal of Alzheimer's disease : JAD* 33 (2):357-71. doi: 10.3233/JAD-2012-121399.

Carmel, G., E. M. Mager, L. I. Binder, and J. Kuret. 1996. "The structural basis of monoclonal antibody Alz50's selectivity for Alzheimer's disease pathology." *The Journal of biological chemistry* 271 (51):32789-95.

Castano, E. M., F. Prelli, T. Wisniewski, A. Golabek, R. A. Kumar, C. Soto, and B. Frangione. 1995. "Fibrillogenesis in Alzheimer's disease of amyloid beta peptides and apolipoprotein E." *Biochem J* 306 (Pt 2):599-604.

Chambers, C. B., J. M. Lee, J. C. Troncoso, S. Reich, and N. A. Muma. 1999. "Overexpression of four-repeat tau mRNA isoforms in progressive supranuclear palsy but not in Alzheimer's disease." *Ann Neurol* 46 (3):325-32.

Ciliax, B. J., N. Nash, C. Heilman, R. Sunahara, A. Hartney, M. Tiberi, D. B. Rye, M. G. Caron, H. B. Niznik, and A. I. Levey. 2000. "Dopamine D(5) receptor immunolocalization in rat and monkey brain." *Synapse* 37 (2):125-45.

Ciliax, Brian J., Craig Heilman, Sharon Edmunds, Steven M. Hersch, and Allan I. Levey. 1995. "Anti-fusion protein antibodies specific for receptor subtypes." In *Methods in Neurosciences*, edited by C. Sealfon Stuart, 431-454. Academic Press.

Cleary, J. P., D. M. Walsh, J. J. Hofmeister, G. M. Shankar, M. A. Kuskowski, D. J. Selkoe, and K. H. Ashe. 2005. "Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function." *Nat Neurosci* 8 (1):79-84. doi: 10.1038/nn1372.

Colosimo, C., T. H. Bak, M. Bologna, and A. Berardelli. 2013. "Fifty years of progressive supranuclear palsy." *J Neurol Neurosurg Psychiatry*. doi: 10.1136/jnnp-2013-305740.

Colton, C. A., L. K. Needham, C. Brown, D. Cook, K. Rasheed, J. R. Burke, W. J. Strittmatter, D. E. Schmechel, and M. P. Vitek. 2004. "APOE genotype-specific differences in human and mouse macrophage nitric oxide production." *J Neuroimmunol* 147 (1-2):62-7.

Crowther, R. A. 1991. "Straight and paired helical filaments in Alzheimer disease have a common structural unit." *Proc Natl Acad Sci U S A* 88 (6):2288-92.

Cuenco, K. T., R. C. Green, J. Zhang, K. Lunetta, P. M. Erlich, L. A. Cupples, L. A. Farrer, C. DeCarli, and Mirage Study Group. 2008. "Magnetic resonance imaging traits in siblings discordant for Alzheimer disease." *J Neuroimaging* 18 (3):268-75. doi: 10.1111/j.1552-6569.2007.00191.x.

Danik, M., and J. Poirier. 1998. "Apolipoprotein E and neuronal plasticity following experimental de-afferentation and in Alzheimer's disease." *Biochem Soc Trans* 26 (2):262-6.

Dawson, H. N., A. Ferreira, M. V. Eyster, N. Ghoshal, L. I. Binder, and M. P. Vitek. 2001. "Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice." *J Cell Sci* 114 (Pt 6):1179-87.

De Strooper, B., P. Saftig, K. Craessaerts, H. Vanderstichele, G. Guhde, W. Annaert, K. Von Figura, and F. Van Leuven. 1998. "Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein." *Nature* 391 (6665):387-90. doi: 10.1038/34910.

DeMattos, R. B., L. K. Curtiss, and D. L. Williams. 1998. "A minimally lipidated form of cell-derived apolipoprotein E exhibits isoform-specific stimulation of neurite outgrowth in the absence of exogenous lipids or lipoproteins." *J Biol Chem* 273 (7):4206-12.

Deshpande, A., E. Mina, C. Glabe, and J. Busciglio. 2006. "Different conformations of amyloid beta induce neurotoxicity by distinct mechanisms in human cortical neurons." *J Neurosci* 26 (22):6011-8. doi: 10.1523/JNEUROSCI.1189-06.2006.

Dickson, D. W. 1998. "Pick's disease: a modern approach." *Brain Pathol* 8 (2):339-54.

Dickson, D. W. 2001. "Neuropathology of Pick's disease." *Neurology* 56 (11 Suppl 4):S16-20.

Dickson, D. W., C. Bergeron, S. S. Chin, C. Duyckaerts, D. Horoupian, K. Ikeda, K. Jellinger, P. L. Lantos, C. F. Lippa, S. S. Mirra, M. Tabaton, J. P. Vonsattel, K. Wakabayashi, and I. Litvan. 2002. "Office of Rare Diseases neuropathologic criteria for corticobasal degeneration." *Journal of Neuropathology and Experimental Neurology* 61 (11):935-46.

Dickson, D. W., C. Bergeron, S. S. Chin, C. Duyckaerts, D. Horoupian, K. Ikeda, K. Jellinger, P. L. Lantos, C. F. Lippa, S. S. Mirra, M. Tabaton, J. P. Vonsattel, K. Wakabayashi, I. Litvan, and Health Office of Rare Diseases of the National

Institutes of. 2002. "Office of Rare Diseases neuropathologic criteria for corticobasal degeneration." *J Neuropathol Exp Neurol* 61 (11):935-46.

Dickson, D. W., H. A. Crystal, C. Bevona, W. Honer, I. Vincent, and P. Davies. 1995. "Correlations of synaptic and pathological markers with cognition of the elderly." *Neurobiol Aging* 16 (3):285-98; discussion 298-304.

Dodart, J. C., K. R. Bales, K. S. Gannon, S. J. Greene, R. B. DeMattos, C. Mathis, C. A. DeLong, S. Wu, X. Wu, D. M. Holtzman, and S. M. Paul. 2002. "Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model." *Nat Neurosci* 5 (5):452-7. doi: 10.1038/nn842.

Dodson, S. E., O. M. Andersen, V. Karmali, J. J. Fritz, D. Cheng, J. Peng, A. I. Levey, T. E. Willnow, and J. J. Lah. 2008a. "Loss of LR11/SORLA enhances early pathology in a mouse model of amyloidosis: evidence for a proximal role in Alzheimer's disease." *Journal of Neuroscience* 28 (48):12877-86. doi: 28/48/12877 [pii]
10.1523/JNEUROSCI.4582-08.2008.

Dodson, S. E., O. M. Andersen, V. Karmali, J. J. Fritz, D. Cheng, J. Peng, A. I. Levey, T. E. Willnow, and J. J. Lah. 2008b. "Loss of LR11/SORLA enhances early pathology in a mouse model of amyloidosis: evidence for a proximal role in Alzheimer's disease." *The Journal of neuroscience : the official journal of the Society for Neuroscience* 28 (48):12877-86. doi: 10.1523/JNEUROSCI.4582-08.2008.

Dodson, S. E., M. Gearing, C. F. Lippa, T. J. Montine, A. I. Levey, and J. J. Lah. 2006. "LR11/SorLA expression is reduced in sporadic Alzheimer disease but not in familial Alzheimer disease." *Journal of neuropathology and experimental neurology* 65 (9):866-72. doi: 10.1097/01.jnen.0000228205.19915.20.

Dong, L. M., C. Wilson, M. R. Wardell, T. Simmons, R. W. Mahley, K. H. Weisgraber, and D. A. Agard. 1994. "Human apolipoprotein E. Role of arginine 61 in mediating the lipoprotein preferences of the E3 and E4 isoforms." *J Biol Chem* 269 (35):22358-65.

Edbauer, D., E. Winkler, J. T. Regula, B. Pesold, H. Steiner, and C. Haass. 2003. "Reconstitution of gamma-secretase activity." *Nat Cell Biol* 5 (5):486-8. doi: 10.1038/ncb960.

Ertekin-Taner, N. 2007. "Genetics of Alzheimer's disease: a centennial review." *Neurol Clin* 25 (3):611-67, v. doi: 10.1016/j.ncl.2007.03.009.

Esch, F. S., P. S. Keim, E. C. Beattie, R. W. Blacher, A. R. Culwell, T. Oltersdorf, D. McClure, and P. J. Ward. 1990. "Cleavage of amyloid beta peptide during constitutive processing of its precursor." *Science* 248 (4959):1122-4.

Esler, W. P., W. T. Kimberly, B. L. Ostaszewski, T. S. Diehl, C. L. Moore, J. Y. Tsai, T. Rahmati, W. Xia, D. J. Selkoe, and M. S. Wolfe. 2000. "Transition-state analogue inhibitors of gamma-secretase bind directly to presenilin-1." *Nat Cell Biol* 2 (7):428-34. doi: 10.1038/35017062.

Evans, D. A., H. H. Funkenstein, M. S. Albert, P. A. Scherr, N. R. Cook, M. J. Chown, L. E. Hebert, C. H. Hennekens, and J. O. Taylor. 1989. "Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported." *JAMA* 262 (18):2551-6.

Farrer, L. A., L. A. Cupples, J. L. Haines, B. Hyman, W. A. Kukull, R. Mayeux, R. H. Myers, M. A. Pericak-Vance, N. Risch, and C. M. van Duijn. 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 (16):1349-56.

Fischer, D., M. D. Mukrasch, J. Biernat, S. Bibow, M. Blackledge, C. Griesinger, E. Mandelkow, and M. Zweckstetter. 2009. "Conformational changes specific for pseudophosphorylation at serine 262 selectively impair binding of tau to microtubules." *Biochemistry* 48 (42):10047-55. doi: 10.1021/bi901090m.

Fischer, D., M. D. Mukrasch, M. von Bergen, A. Klos-Witkowska, J. Biernat, C. Griesinger, E. Mandelkow, and M. Zweckstetter. 2007. "Structural and microtubule binding properties of tau mutants of frontotemporal dementias." *Biochemistry* 46 (10):2574-82. doi: 10.1021/bi061318s.

Fluhrer, R., A. Fukumori, L. Martin, G. Grammer, M. Haug-Kroper, B. Klier, E. Winkler, E. Kremmer, M. M. Condron, D. B. Teplow, H. Steiner, and C. Haass. 2008. "Intramembrane proteolysis of GXGD-type aspartyl proteases is slowed by a familial Alzheimer disease-like mutation." *J Biol Chem* 283 (44):30121-8. doi: 10.1074/jbc.M806092200.

Frisoni, G. B., A. Prestia, P. E. Rasser, M. Bonetti, and P. M. Thompson. 2009. "In vivo mapping of incremental cortical atrophy from incipient to overt Alzheimer's disease." *J Neurol* 256 (6):916-24. doi: 10.1007/s00415-009-5040-7.

Garcia-Sierra, F., N. Ghoshal, B. Quinn, R. W. Berry, and L. I. Binder. 2003. "Conformational changes and truncation of tau protein during tangle evolution in Alzheimer's disease." *Journal of Alzheimer's Disease* 5 (2):65-77.

Gearing, M., H. Mori, and S. S. Mirra. 1996. "Abeta-peptide length and apolipoprotein E genotype in Alzheimer's disease." *Ann Neurol* 39 (3):395-9. doi: 10.1002/ana.410390320.

Ghoshal, N., F. Garcia-Sierra, J. Wuu, S. Leurgans, D. A. Bennett, R. W. Berry, and L. I. Binder. 2002. "Tau conformational changes correspond to impairments of episodic memory in mild cognitive impairment and Alzheimer's disease." *Exp Neurol* 177 (2):475-93.

Gilmor, M. L., J. D. Erickson, H. Varoqui, L. B. Hersh, D. A. Bennett, E. J. Cochran, E. J. Mufson, and A. I. Levey. 1999. "Preservation of nucleus basalis neurons containing choline acetyltransferase and the vesicular acetylcholine transporter in the elderly with mild cognitive impairment and early Alzheimer's disease." *J Comp Neurol* 411 (4):693-704.

Ginsberg, S. D., S. Che, S. E. Counts, and E. J. Mufson. 2006. "Shift in the ratio of three-repeat tau and four-repeat tau mRNAs in individual cholinergic basal forebrain neurons in mild cognitive impairment and Alzheimer's disease." *J Neurochem* 96 (5):1401-8. doi: 10.1111/j.1471-4159.2005.03641.x.

Glenner, G. G., and C. W. Wong. 1984. "Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein." *Biochem Biophys Res Commun* 120 (3):885-90.

Gliemann, J., G. Hermey, A. Nykjaer, C. M. Petersen, C. Jacobsen, and P. A. Andreasen. 2004. "The mosaic receptor sorLA/LR11 binds components of the plasminogen-activating system and platelet-derived growth factor-BB similarly to LRP1 (low-density lipoprotein receptor-related protein), but mediates slow internalization of bound ligand." *The Biochemical journal* 381 (Pt 1):203-12. doi: 10.1042/BJ20040149.

Goedert, M., R. Jakes, and E. Vanmechelen. 1995. "Monoclonal antibody AT8 recognises tau protein phosphorylated at both serine 202 and threonine 205." *Neurosci Lett* 189 (3):167-9.

Goedert, M., M. G. Spillantini, N. J. Cairns, and R. A. Crowther. 1992. "Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms." *Neuron* 8 (1):159-68.

Goedert, M., M. G. Spillantini, R. Jakes, R. A. Crowther, E. Vanmechelen, A. Probst, J. Gotz, K. Burki, and P. Cohen. 1995. "Molecular dissection of the paired helical filament." *Neurobiol Aging* 16 (3):325-34.

Goedert, M., M. G. Spillantini, R. Jakes, D. Rutherford, and R. A. Crowther. 1989. "Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease." *Neuron* 3 (4):519-26.

Goedert, M., M. G. Spillantini, M. C. Potier, J. Ulrich, and R. A. Crowther. 1989. "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 (2):393-9.

Goedert, M., C. M. Wischik, R. A. Crowther, J. E. Walker, and A. Klug. 1988. "Cloning and sequencing of the cDNA encoding a core protein of the paired helical filament of Alzheimer disease: identification as the microtubule-associated protein tau." *Proceedings of the National Academy of Sciences of the United States of America* 85 (11):4051-5.

Goldgaber, D., M. I. Lerman, O. W. McBride, U. Saffiotti, and D. C. Gajdusek. 1987. "Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease." *Science* 235 (4791):877-80.

Gomez-Ramos, P., E. J. Mufson, and M. A. Moran. 2001. "Apolipoprotein E immunoreactivity in neurons and neurofibrillary degeneration of aged non-demented and Alzheimer's disease patients." *Microsc Res Tech* 55 (1):48-58. doi: 10.1002/jemt.1155.

Gong, H., W. Dong, S. W. Rostad, S. M. Marcovina, J. J. Albers, J. D. Brunzell, and S. Vuletic. 2013. "Lipoprotein Lipase (LPL) is Associated with Neurite Pathology and Its Levels Are Markedly Reduced in the Dentate Gyrus of Alzheimer's Disease Brains." *J Histochem Cytochem* 61 (12):857-68. doi: 10.1369/0022155413505601.

Gotthardt, M., M. Trommsdorff, M. F. Nevitt, J. Shelton, J. A. Richardson, W. Stockinger, J. Nimpf, and J. Herz. 2000. "Interactions of the low density lipoprotein receptor gene family with cytosolic adaptor and scaffold proteins suggest diverse biological functions in cellular communication and signal transduction." *J Biol Chem* 275 (33):25616-24. doi: 10.1074/jbc.M000955200.

Gotz, J. 2001. "Tau and transgenic animal models." *Brain Res Brain Res Rev* 35 (3):266-86.

Greenberg, S. G., and P. Davies. 1990. "A preparation of Alzheimer paired helical filaments that displays distinct tau proteins by polyacrylamide gel electrophoresis." *Proc Natl Acad Sci U S A* 87 (15):5827-31.

Grundke-Iqbal, I., K. Iqbal, Y. C. Tung, M. Quinlan, H. M. Wisniewski, and L. I. Binder. 1986. "Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology." *Proceedings of the National Academy of Sciences of the United States of America* 83 (13):4913-7.

Guillozet-Bongaarts, A. L., M. E. Cahill, V. L. Cryns, M. R. Reynolds, R. W. Berry, and L. I. Binder. 2006. "Pseudophosphorylation of tau at serine 422 inhibits caspase cleavage: in vitro evidence and implications for tangle formation in vivo." *Journal of Neurochemistry* 97 (4):1005-14.

Guillozet-Bongaarts, A. L., F. Garcia-Sierra, M. R. Reynolds, P. M. Horowitz, Y. Fu, T. Wang, M. E. Cahill, E. H. Bigio, R. W. Berry, and L. I. Binder. 2005a. "Tau truncation during neurofibrillary tangle evolution in Alzheimer's disease." *Neurobiol Aging* 26 (7):1015-22. doi: 10.1016/j.neurobiolaging.2004.09.019.

Guillozet-Bongaarts, A. L., F. Garcia-Sierra, M. R. Reynolds, P. M. Horowitz, Y. Fu, T. Wang, M. E. Cahill, E. H. Bigio, R. W. Berry, and L. I. Binder. 2005b. "Tau truncation during neurofibrillary tangle evolution in Alzheimer's disease." *Neurobiology of Aging* 26 (7):1015-22.

Guillozet-Bongaarts, A. L., K. E. Glajch, E. G. Libson, M. E. Cahill, E. Bigio, R. W. Berry, and L. I. Binder. 2007. "Phosphorylation and cleavage of tau in non-AD tauopathies." *Acta neuropathologica* 113 (5):513-20. doi: 10.1007/s00401-007-0209-6.

Guo, L., J. R. Eisenman, R. M. Mahimkar, J. J. Peschon, R. J. Paxton, R. A. Black, and R. S. Johnson. 2002. "A proteomic approach for the identification of cell-surface proteins shed by metalloproteases." *Molecular & Cellular Proteomics* 1 (1):30-6.

Guo, L. H., C. Westerteicher, X. H. Wang, M. Kratzer, A. Tsolakidou, M. Jiang, T. Grimmer, S. M. Laws, P. Alexopoulos, H. Bujo, A. Kurz, and R. Perneczky. 2012. "SORL1 genetic variants and cerebrospinal fluid biomarkers of Alzheimer's disease." *Eur Arch Psychiatry Clin Neurosci* 262 (6):529-34. doi: 10.1007/s00406-012-0295-x.

Guo, L., M. J. LaDu, and L. J. Van Eldik. 2004. "A dual role for apolipoprotein e in neuroinflammation: anti- and pro-inflammatory activity." *J Mol Neurosci* 23 (3):205-12. doi: 10.1385/JMN:23:3:205.

Gustke, N., B. Trinczek, J. Biernat, E. M. Mandelkow, and E. Mandelkow. 1994. "Domains of tau protein and interactions with microtubules." *Biochemistry* 33 (32):9511-22.

Haass, C., M. G. Schlossmacher, A. Y. Hung, C. Vigo-Pelfrey, A. Mellon, B. L. Ostaszewski, I. Lieberburg, E. H. Koo, D. Schenk, D. B. Teplow, and et al. 1992. "Amyloid beta-peptide is produced by cultured cells during normal metabolism." *Nature* 359 (6393):322-5. doi: 10.1038/359322a0.

Hall, E. D., J. A. Oostveen, E. Dunn, and D. B. Carter. 1995. "Increased amyloid protein precursor and apolipoprotein E immunoreactivity in the selectively vulnerable hippocampus following transient forebrain ischemia in gerbils." *Exp Neurol* 135 (1):17-27. doi: 10.1006/exnr.1995.1062.

Hampe, W., I. B. Riedel, J. Lintzel, C. O. Bader, I. Franke, and H. C. Schaller. 2000a. "Ectodomain shedding, translocation and synthesis of SorLA are stimulated by its ligand head activator." *Journal of cell science* 113 Pt 24:4475-85.

Hampe, W., I. B. Riedel, J. Lintzel, C. O. Bader, I. Franke, and H. C. Schaller. 2000b. "Ectodomain shedding, translocation and synthesis of SorLA are stimulated by its ligand head activator." *Journal of Cell Science* 113 (Pt 24):4475-85.

Han, S. H., G. Einstein, K. H. Weisgraber, W. J. Strittmatter, A. M. Saunders, M. Pericak-Vance, A. D. Roses, and D. E. Schmechel. 1994. "Apolipoprotein E is localized to the cytoplasm of human cortical neurons: a light and electron microscopic study." *J Neuropathol Exp Neurol* 53 (5):535-44.

Han, S. H., C. Hulette, A. M. Saunders, G. Einstein, M. Pericak-Vance, W. J. Strittmatter, A. D. Roses, and D. E. Schmechel. 1994. "Apolipoprotein E is present in hippocampal neurons without neurofibrillary tangles in Alzheimer's disease and in age-matched controls." *Exp Neurol* 128 (1):13-26. doi: 10.1006/exnr.1994.1108.

Harada, A., K. Oguchi, S. Okabe, J. Kuno, S. Terada, T. Ohshima, R. Sato-Yoshitake, Y. Takei, T. Noda, and N. Hirokawa. 1994. "Altered microtubule organization in small-calibre axons of mice lacking tau protein." *Nature* 369 (6480):488-91. doi: 10.1038/369488a0.

Harding, A. J., J. J. Kril, and G. M. Halliday. 2000. "Practical measures to simplify the Braak tangle staging method for routine pathological screening." *Acta Neuropathologica* 99 (2):199-208.

Hardy, J. 2006. "Has the amyloid cascade hypothesis for Alzheimer's disease been proved?" *Curr Alzheimer Res* 3 (1):71-3.

Hardy, J., and D. Allsop. 1991. "Amyloid deposition as the central event in the aetiology of Alzheimer's disease." *Trends Pharmacol Sci* 12 (10):383-8.

Hartl, D., S. Klatt, M. Roch, Z. Konthur, J. Klose, T. E. Willnow, and M. Rohe. 2013. "Soluble alpha-APP (sAPPalpha) regulates CDK5 expression and activity in neurons." *PLoS One* 8 (6):e65920. doi: 10.1371/journal.pone.0065920.

Hauw, J. J., S. E. Daniel, D. Dickson, D. S. Horoupian, K. Jellinger, P. L. Lantos, A. McKee, M. Tabaton, and I. Litvan. 1994. "Preliminary NINDS neuropathologic criteria for Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy)." *Neurology* 44 (11):2015-9.

He, X., F. Li, W. P. Chang, and J. Tang. 2005. "GGA proteins mediate the recycling pathway of memapsin 2 (BACE)." *J Biol Chem* 280 (12):11696-703. doi: 10.1074/jbc.M411296200.

Heber, S., J. Herms, V. Gajic, J. Hainfellner, A. Aguzzi, T. Rulicke, H. von Kretzschmar, C. von Koch, S. Sisodia, P. Tremml, H. P. Lipp, D. P. Wolfer, and U. Muller. 2000. "Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members." *J Neurosci* 20 (21):7951-63.

Hermey, G., I. B. Riedel, W. Hampe, H. C. Schaller, and I. Hermans-Borgmeyer. 1999. "Identification and characterization of SorCS, a third member of a novel receptor family." *Biochem Biophys Res Commun* 266 (2):347-51. doi: 10.1006/bbrc.1999.1822.

Herms, J., B. Anliker, S. Heber, S. Ring, M. Fuhrmann, H. Kretzschmar, S. Sisodia, and U. Muller. 2004. "Cortical dysplasia resembling human type 2 lissencephaly in mice lacking all three APP family members." *EMBO J* 23 (20):4106-15. doi: 10.1038/sj.emboj.7600390.

Hersch, S. M., C. A. Gutekunst, H. D. Rees, C. J. Heilman, and A. I. Levey. 1994. "Distribution of m1-m4 muscarinic receptor proteins in the rat striatum: light and electron microscopic immunocytochemistry using subtype-specific antibodies." *Journal of Neuroscience* 14 (5 Pt 2):3351-63.

Herskowitz, J. H., K. Offe, A. Deshpande, R. A. Kahn, A. I. Levey, and J. J. Lah. 2012a. "GGA1-mediated endocytic traffic of LR11/SorLA alters APP intracellular distribution and amyloid-beta production." *Mol Biol Cell* 23 (14):2645-57. doi: 10.1091/mbc.E12-01-0014.

Herskowitz, J. H., K. Offe, A. Deshpande, R. A. Kahn, A. I. Levey, and J. J. Lah. 2012b. "GGA1-mediated endocytic traffic of LR11/SorLA alters APP intracellular distribution and amyloid-beta production." *Molecular biology of the cell* 23 (14):2645-57. doi: 10.1091/mbc.E12-01-0014.

Herskowitz, J. H., N. T. Seyfried, M. Gearing, R. A. Kahn, J. Peng, A. I. Levey, and J. J. Lah. 2011a. "Rho kinase II phosphorylation of the lipoprotein receptor LR11/SORLA alters amyloid-beta production." *The Journal of biological chemistry* 286 (8):6117-27. doi: 10.1074/jbc.M110.167239.

Herskowitz, J. H., N. T. Seyfried, M. Gearing, R. A. Kahn, J. Peng, A. I. Levey, and J. J. Lah. 2011b. "Rho kinase II phosphorylation of the lipoprotein receptor LR11/SORLA alters amyloid-beta production." *J Biol Chem* 286 (8):6117-27. doi: 10.1074/jbc.M110.167239.

Herz, J. 2001. "The LDL receptor gene family: (un)expected signal transducers in the brain." *Neuron* 29 (3):571-81.

Herz, J., and U. Beffert. 2000. "Apolipoprotein E receptors: linking brain development and Alzheimer's disease." *Nat Rev Neurosci* 1 (1):51-8. doi: 10.1038/35036221.

Herz, J., and H. H. Bock. 2002. "Lipoprotein receptors in the nervous system." *Annu Rev Biochem* 71:405-34. doi: 10.1146/annurev.biochem.71.110601.135342.

Heston, L. L. 1984. "Down's syndrome and Alzheimer's dementia: defining an association." *Psychiatr Dev* 2 (4):287-94.

Hoe, H. S., D. Wessner, U. Beffert, A. G. Becker, Y. Matsuoka, and G. W. Rebeck. 2005. "F-spondin interaction with the apolipoprotein E receptor ApoEr2 affects processing of amyloid precursor protein." *Mol Cell Biol* 25 (21):9259-68. doi: 10.1128/MCB.25.21.9259-9268.2005.

Hollenbach, E., C. Schulz, and H. Lehnert. 1998. "Rapid and sensitive determination of catecholamines and the metabolite 3-methoxy-4-hydroxyphen-ethyleneglycol using HPLC following novel extraction procedures." *Life Sci* 63 (9):737-50.

Holtzman, D. M., J. C. Morris, and A. M. Goate. 2011. "Alzheimer's disease: the challenge of the second century." *Sci Transl Med* 3 (77):77sr1. doi: 10.1126/scitranslmed.3002369.

Holzer, M., H. P. Holzapfel, D. Zedlick, M. K. Bruckner, and T. Arendt. 1994. "Abnormally phosphorylated tau protein in Alzheimer's disease: heterogeneity of individual regional distribution and relationship to clinical severity." *Neuroscience* 63 (2):499-516.

Homayouni, R., D. S. Rice, M. Sheldon, and T. Curran. 1999. "Disabled-1 binds to the cytoplasmic domain of amyloid precursor-like protein 1." *J Neurosci* 19 (17):7507-15.

Hornsten, A., J. Lieberthal, S. Fadia, R. Malins, L. Ha, X. Xu, I. Daigle, M. Markowitz, G. O'Connor, R. Plasterk, and C. Li. 2007. "APL-1, a Caenorhabditis elegans protein related to the human beta-amyloid precursor protein, is essential for viability." *Proc Natl Acad Sci U S A* 104 (6):1971-6. doi: 10.1073/pnas.0603997104.

Horowitz, P. M., K. R. Patterson, A. L. Guillozet-Bongaarts, M. R. Reynolds, C. A. Carroll, S. T. Weintraub, D. A. Bennett, V. L. Cryns, R. W. Berry, and L. I. Binder. 2004. "Early N-terminal changes and caspase-6 cleavage of tau in Alzheimer's disease." *Journal of Neuroscience* 24 (36):7895-902.

Horsburgh, K., D. I. Graham, J. Stewart, and J. A. Nicoll. 1999. "Influence of apolipoprotein E genotype on neuronal damage and apoE immunoreactivity in human hippocampus following global ischemia." *J Neuropathol Exp Neurol* 58 (3):227-34.

Houlihan, L. M., S. E. Harris, M. Luciano, A. J. Gow, J. M. Starr, P. M. Visscher, and I. J. Deary. 2009. "Replication study of candidate genes for cognitive abilities: the Lothian Birth Cohort 1936." *Genes Brain Behav* 8 (2):238-47. doi: 10.1111/j.1601-183X.2008.00470.x.

Huang, Y., X. Q. Liu, T. Wyss-Coray, W. J. Brecht, D. A. Sanan, and R. W. Mahley. 2001. "Apolipoprotein E fragments present in Alzheimer's disease brains induce neurofibrillary tangle-like intracellular inclusions in neurons." *Proc Natl Acad Sci U S A* 98 (15):8838-43. doi: 10.1073/pnas.151254698.

Hyman, B. T., and J. Q. Trojanowski. 1997. "Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease." *Journal of Neuropathology and Experimental Neurology* 56 (10):1095-7.

Ikegami, S., A. Harada, and N. Hirokawa. 2000. "Muscle weakness, hyperactivity, and impairment in fear conditioning in tau-deficient mice." *Neurosci Lett* 279 (3):129-32.

Imahori, K., and T. Uchida. 1997. "Physiology and pathology of tau protein kinases in relation to Alzheimer's disease." *J Biochem* 121 (2):179-88.

Ingram, E. M., and M. G. Spillantini. 2002. "Tau gene mutations: dissecting the pathogenesis of FTDP-17." *Trends Mol Med* 8 (12):555-62.

Iwatsubo, T. 2004. "The gamma-secretase complex: machinery for intramembrane proteolysis." *Curr Opin Neurobiol* 14 (3):379-83. doi: 10.1016/j.conb.2004.05.010.

Iwatsubo, T., M. Hasegawa, and Y. Ihara. 1994. "Neuronal and glial tau-positive inclusions in diverse neurologic diseases share common phosphorylation characteristics." *Acta Neuropathol* 88 (2):129-36.

Jacobsen, L., P. Madsen, C. Jacobsen, M. S. Nielsen, J. Gliemann, and C. M. Petersen. 2001. "Activation and functional characterization of the mosaic receptor SorLA/LR11." *The Journal of biological chemistry* 276 (25):22788-96. doi: 10.1074/jbc.M100857200.

Jacobsen, L., P. Madsen, S. K. Moestrup, A. H. Lund, N. Tommerup, A. Nykjaer, L. Sottrup-Jensen, J. Gliemann, and C. M. Petersen. 1996a. "Molecular characterization of a novel human hybrid-type receptor that binds the alpha2-macroglobulin receptor-associated protein." *The Journal of biological chemistry* 271 (49):31379-83.

Jacobsen, L., P. Madsen, S. K. Moestrup, A. H. Lund, N. Tommerup, A. Nykjaer, L. Sottrup-Jensen, J. Gliemann, and C. M. Petersen. 1996b. "Molecular characterization of a novel human hybrid-type receptor that binds the alpha2-macroglobulin receptor-associated protein." *J Biol Chem* 271 (49):31379-83.

Jacobsen, L., P. Madsen, M. S. Nielsen, W. P. Geraerts, J. Gliemann, A. B. Smit, and C. M. Petersen. 2002. "The sorLA cytoplasmic domain interacts with GGA1 and -2 and defines minimum requirements for GGA binding." *FEBS Lett* 511 (1-3):155-8.

Jaeger, S., and C. U. Pietrzik. 2008. "Functional role of lipoprotein receptors in Alzheimer's disease." *Curr Alzheimer Res* 5 (1):15-25.

Jho, Y. S., E. B. Zhulina, M. W. Kim, and P. A. Pincus. 2010. "Monte carlo simulations of tau proteins: effect of phosphorylation." *Biophys J* 99 (8):2387-97. doi: 10.1016/j.bpj.2010.06.056.

Joachim, C. L., J. H. Morris, K. S. Kosik, and D. J. Selkoe. 1987. "Tau antisera recognize neurofibrillary tangles in a range of neurodegenerative disorders." *Annals of Neurology* 22 (4):514-20.

Kanaan, N. M., G. A. Morfini, N. E. LaPointe, G. F. Pigino, K. R. Patterson, Y. Song, A. Andreadis, Y. Fu, S. T. Brady, and L. I. Binder. 2011. "Pathogenic forms of tau inhibit kinesin-dependent axonal transport through a mechanism involving activation of axonal phosphotransferases." *J Neurosci* 31 (27):9858-68. doi: 10.1523/JNEUROSCI.0560-11.2011.

Kanai, Y., and N. Hirokawa. 1995. "Sorting mechanisms of tau and MAP2 in neurons: suppressed axonal transit of MAP2 and locally regulated microtubule binding." *Neuron* 14 (2):421-32.

Kang, D. E., C. U. Pietrzik, L. Baum, N. Chevallier, D. E. Merriam, M. Z. Kounnas, S. L. Wagner, J. C. Troncoso, C. H. Kawas, R. Katzman, and E. H. Koo. 2000. "Modulation of amyloid beta-protein clearance and Alzheimer's disease susceptibility by the LDL receptor-related protein pathway." *J Clin Invest* 106 (9):1159-66. doi: 10.1172/JCI11013.

Kang, J., H. G. Lemaire, A. Unterbeck, J. M. Salbaum, C. L. Masters, K. H. Grzeschik, G. Multhaup, K. Beyreuther, and B. Muller-Hill. 1987. "The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor." *Nature* 325 (6106):733-6. doi: 10.1038/325733a0.

Katzman, R. 1986. "Alzheimer's disease." *N Engl J Med* 314 (15):964-73. doi: 10.1056/NEJM198604103141506.

Kauwe, J. S., C. Cruchaga, S. Bertelsen, K. Mayo, W. Latu, P. Nowotny, A. L. Hinrichs, A. M. Fagan, D. M. Holtzman, Initiative Alzheimer's Disease Neuroimaging, and A. M. Goate. 2010. "Validating predicted biological effects of Alzheimer's disease associated SNPs using CSF biomarker levels." *J Alzheimers Dis* 21 (3):833-42. doi: 10.3233/JAD-2010-091711.

Khuebachova, M., V. Verzillo, R. Skrabana, M. Ovecka, P. Vaccaro, S. Panni, A. Bradbury, and M. Novak. 2002. "Mapping the C terminal epitope of the Alzheimer's disease specific antibody MN423." *J Immunol Methods* 262 (1-2):205-15.

Kim, J., J. M. Basak, and D. M. Holtzman. 2009. "The role of apolipoprotein E in Alzheimer's disease." *Neuron* 63 (3):287-303. doi: 10.1016/j.neuron.2009.06.026.

Kimberly, W. T., M. J. LaVoie, B. L. Ostaszewski, W. Ye, M. S. Wolfe, and D. J. Selkoe. 2003. "Gamma-secretase is a membrane protein complex comprised of presenilin, nicastrin, Aph-1, and Pen-2." *Proc Natl Acad Sci U S A* 100 (11):6382-7. doi: 10.1073/pnas.1037392100.

Kinoshita, A., C. M. Whelan, C. J. Smith, I. Mikhailenko, G. W. Rebeck, D. K. Strickland, and B. T. Hyman. 2001. "Demonstration by fluorescence resonance energy transfer of two sites of interaction between the low-density lipoprotein receptor-related protein and the amyloid precursor protein: role of the intracellular adapter protein Fe65." *J Neurosci* 21 (21):8354-61.

Klinger, S. C., S. Glerup, M. K. Raarup, M. C. Mari, M. Nyegaard, G. Koster, T. Prabakaran, S. K. Nilsson, M. M. Kjaergaard, O. Bakke, A. Nykjaer, G. Olivecrona, C. M. Petersen, and M. S. Nielsen. 2011a. "SorLA regulates the activity of lipoprotein lipase by intracellular trafficking." *Journal of cell science* 124 (Pt 7):1095-105. doi: 10.1242/jcs.072538.

Klinger, S. C., S. Glerup, M. K. Raarup, M. C. Mari, M. Nyegaard, G. Koster, T. Prabakaran, S. K. Nilsson, M. M. Kjaergaard, O. Bakke, A. Nykjaer, G. Olivecrona, C. M. Petersen, and M. S. Nielsen. 2011b. "SorLA regulates the activity of lipoprotein lipase by intracellular trafficking." *J Cell Sci* 124 (Pt 7):1095-105. doi: 10.1242/jcs.072538.

Kolsch, H., F. Jessen, J. Wiltfang, P. Lewczuk, M. Dichgans, S. J. Teipel, J. Kornhuber, L. Frolich, I. Heuser, O. Peters, B. Wiese, H. Kaduszkiewicz, H. van den Bussche, M. Hull, A. Kurz, E. Ruther, F. A. Henn, and W. Maier. 2009. "Association of SORL1 gene variants with Alzheimer's disease." *Brain Res* 1264:1-6. doi: 10.1016/j.brainres.2009.01.044.

Kosik, K. S., C. L. Joachim, and D. J. Selkoe. 1986. "Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease." *Proceedings of the National Academy of Sciences of the United States of America* 83 (11):4044-8.

Kounnas, M. Z., R. D. Moir, G. W. Rebeck, A. I. Bush, W. S. Argraves, R. E. Tanzi, B. T. Hyman, and D. K. Strickland. 1995. "LDL receptor-related protein, a multifunctional ApoE receptor, binds secreted beta-amyloid precursor protein and mediates its degradation." *Cell* 82 (2):331-40.

LaDu, M. J., J. A. Shah, C. A. Reardon, G. S. Getz, G. Bu, J. Hu, L. Guo, and L. J. Van Eldik. 2001. "Apolipoprotein E and apolipoprotein E receptors modulate A beta-induced glial neuroinflammatory responses." *Neurochem Int* 39 (5-6):427-34.

Lammich, S., E. Kojro, R. Postina, S. Gilbert, R. Pfeiffer, M. Jasionowski, C. Haass, and F. Fahrenholz. 1999. "Constitutive and regulated alpha-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease." *Proc Natl Acad Sci U S A* 96 (7):3922-7.

Lee, J. H., S. Barral, and C. Reitz. 2008. "The neuronal sortilin-related receptor gene SORL1 and late-onset Alzheimer's disease." *Curr Neurol Neurosci Rep* 8 (5):384-91.

Lee, J. H., R. Cheng, L. S. Honig, J. P. Vonsattel, L. Clark, and R. Mayeux. 2008. "Association between genetic variants in SORL1 and autopsy-confirmed Alzheimer disease." *Neurology* 70 (11):887-9. doi: 10.1212/01.wnl.0000280581.39755.89.

Lee, J. H., R. Cheng, N. Schupf, J. Manly, R. Lantigua, Y. Stern, E. Rogaeva, Y. Wakutani, L. Farrer, P. St George-Hyslop, and R. Mayeux. 2007. "The association between genetic variants in SORL1 and Alzheimer disease in an urban, multiethnic, community-based cohort." *Arch Neurol* 64 (4):501-6. doi: 10.1001/archneur.64.4.501.

Lee, V. M., M. Goedert, and J. Q. Trojanowski. 2001. "Neurodegenerative tauopathies." *Annu Rev Neurosci* 24:1121-59. doi: 10.1146/annurev.neuro.24.1.1121.

Levey, A. I., C. J. Heilman, J. J. Lah, N. R. Nash, H. D. Rees, M. Wakai, S. S. Mirra, D. B. Rye, D. Nochlin, T. D. Bird, and E. J. Mufson. 1997. "Presenilin-1 protein expression in familial and sporadic Alzheimer's disease." *Annals of Neurology* 41 (6):742-53. doi: 10.1002/ana.410410610.

Levey, A. I., C. A. Kitt, W. F. Simonds, D. L. Price, and M. R. Brann. 1991. "Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies." *Journal of Neuroscience* 11 (10):3218-26.

Levy-Lahad, E., W. Wasco, P. Poorkaj, D. M. Romano, J. Oshima, W. H. Pettingell, C. E. Yu, P. D. Jondro, S. D. Schmidt, K. Wang, and et al. 1995. "Candidate gene for the chromosome 1 familial Alzheimer's disease locus." *Science* 269 (5226):973-7.

Leyssen, M., D. Ayaz, S. S. Hebert, S. Reeve, B. De Strooper, and B. A. Hassan. 2005. "Amyloid precursor protein promotes post-developmental neurite arborization in the Drosophila brain." *EMBO J* 24 (16):2944-55. doi: 10.1038/sj.emboj.7600757.

Li, Y. M., M. Xu, M. T. Lai, Q. Huang, J. L. Castro, J. DiMuzio-Mower, T. Harrison, C. Lellis, A. Nadin, J. G. Neduvelil, R. B. Register, M. K. Sardana, M. S. Shearman, A. L. Smith, X. P. Shi, K. C. Yin, J. A. Shafer, and S. J. Gardell. 2000. "Photoactivated gamma-secretase inhibitors directed to the active site covalently label presenilin 1." *Nature* 405 (6787):689-94. doi: 10.1038/35015085.

Li, Y., C. Rowland, J. Catanese, J. Morris, S. Lovestone, M. C. O'Donovan, A. Goate, M. Owen, J. Williams, and A. Grupe. 2008. "SORL1 variants and risk of late-onset Alzheimer's disease." *Neurobiol Dis* 29 (2):293-6. doi: 10.1016/j.nbd.2007.09.001.

Litvan, I., D. A. Grimes, and A. E. Lang. 2000. "Phenotypes and prognosis: clinicopathologic studies of corticobasal degeneration." *Adv Neurol* 82:183-96.

Liu, F., M. A. Ikram, A. C. Janssens, M. Schuur, I. de Koning, A. Isaacs, M. Struchalin, A. G. Uitterlinden, J. T. den Dunnen, K. Sleegers, K. Bettens, C. Van Broeckhoven,

J. van Swieten, A. Hofman, B. A. Oostra, Y. S. Aulchenko, M. M. Breteler, and C. M. van Duijn. 2009. "A study of the SORL1 gene in Alzheimer's disease and cognitive function." *J Alzheimers Dis* 18 (1):51-64. doi: 10.3233/JAD-2009-1137.

Lleo, A., S. M. Greenberg, and J. H. Growdon. 2006. "Current pharmacotherapy for Alzheimer's disease." *Annu Rev Med* 57:513-33. doi: 10.1146/annurev.med.57.121304.131442.

Lue, L. F., Y. M. Kuo, A. E. Roher, L. Brachova, Y. Shen, L. Sue, T. Beach, J. H. Kurth, R. E. Rydel, and J. Rogers. 1999. "Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease." *Am J Pathol* 155 (3):853-62.

Lynch, J. R., W. Tang, H. Wang, M. P. Vitek, E. R. Bennett, P. M. Sullivan, D. S. Warner, and D. T. Laskowitz. 2003. "APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response." *J Biol Chem* 278 (49):48529-33. doi: 10.1074/jbc.M306923200.

Ma, J., A. Yee, H. B. Brewer, Jr., S. Das, and H. Potter. 1994. "Amyloid-associated proteins alpha 1-antichymotrypsin and apolipoprotein E promote assembly of Alzheimer beta-protein into filaments." *Nature* 372 (6501):92-4. doi: 10.1038/372092a0.

Mahley, R. W., K. H. Weisgraber, and Y. Huang. 2006. "Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease." *Proc Natl Acad Sci U S A* 103 (15):5644-51. doi: 10.1073/pnas.0600549103.

Mandelkow, E. M., O. Schweers, G. Drewes, J. Biernat, N. Gustke, B. Trinczek, and E. Mandelkow. 1996. "Structure, microtubule interactions, and phosphorylation of tau protein." *Ann N Y Acad Sci* 777:96-106.

Mann, D. M. 1988a. "Alzheimer's disease and Down's syndrome." *Histopathology* 13 (2):125-37.

Mann, D. M. 1988b. "The pathological association between Down syndrome and Alzheimer disease." *Mech Ageing Dev* 43 (2):99-136.

Mann, D. M., T. Iwatsubo, S. M. Pickering-Brown, F. Owen, T. C. Saido, and R. H. Perry. 1997. "Preferential deposition of amyloid beta protein (Abeta) in the form Abeta40 in Alzheimer's disease is associated with a gene dosage effect of the apolipoprotein E E4 allele." *Neurosci Lett* 221 (2-3):81-4.

Mann, D. M., P. O. Yates, B. Marcyniuk, and C. R. Ravindra. 1986. "The topography of plaques and tangles in Down's syndrome patients of different ages." *Neuropathology and Applied Neurobiology* 12 (5):447-57.

Marcusson, E. G., B. F. Horazdovsky, J. L. Cereghino, E. Gharakhanian, and S. D. Emr. 1994. "The sorting receptor for yeast vacuolar carboxypeptidase Y is encoded by the VPS10 gene." *Cell* 77 (4):579-86.

Masters, C. L., G. Simms, N. A. Weinman, G. Multhaup, B. L. McDonald, and K. Beyreuther. 1985a. "Amyloid plaque core protein in Alzheimer disease and Down syndrome." *Proc Natl Acad Sci U S A* 82 (12):4245-9.

Masters, C. L., G. Simms, N. A. Weinman, G. Multhaup, B. L. McDonald, and K. Beyreuther. 1985b. "Amyloid Plaque Core Protein in Alzheimer-Disease and

Down Syndrome." *Proceedings of the National Academy of Sciences of the United States of America* 82 (12):4245-4249.
Maurer, K., S. Volk, and H. Gerbaldo. 1997. "Auguste D and Alzheimer's disease." *Lancet* 349 (9064):1546-9. doi: 10.1016/S0140-6736(96)10203-8.
McKhann, G., D. Drachman, M. Folstein, R. Katzman, D. Price, and E. M. Stadlan. 1984. "Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease." *Neurology* 34 (7):939-44.
Mead, J. R., S. A. Irvine, and D. P. Ramji. 2002. "Lipoprotein lipase: structure, function, regulation, and role in disease." *J Mol Med (Berl)* 80 (12):753-69. doi: 10.1007/s00109-002-0384-9.
Meng, Y., J. H. Lee, R. Cheng, P. St George-Hyslop, R. Mayeux, and L. A. Farrer. 2007. "Association between SORL1 and Alzheimer's disease in a genome-wide study." *Neuroreport* 18 (17):1761-4. doi: 10.1097/WNR.0b013e3282f13e7a.
Minster, R. L., S. T. DeKosky, and M. I. Kamboh. 2008. "No association of SORL1 SNPs with Alzheimer's disease." *Neurosci Lett* 440 (2):190-2. doi: 10.1016/j.neulet.2008.05.082.
Mirra, S. S., A. Heyman, D. McKeel, S. M. Sumi, B. J. Crain, L. M. Brownlee, F. S. Vogel, J. P. Hughes, G. van Belle, and L. Berg. 1991. "The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease." *Neurology* 41 (4):479-86.
Mocanu, M. M., A. Nissen, K. Eckermann, I. Khlistunova, J. Biernat, D. Drexler, O. Petrova, K. Schonig, H. Bujard, E. Mandelkow, L. Zhou, G. Rune, and E. M. Mandelkow. 2008. "The potential for beta-structure in the repeat domain of tau protein determines aggregation, synaptic decay, neuronal loss, and coassembly with endogenous Tau in inducible mouse models of tauopathy." *J Neurosci* 28 (3):737-48. doi: 10.1523/JNEUROSCI.2824-07.2008.
Moir, R. D., T. Lynch, A. I. Bush, S. Whyte, A. Henry, S. Portbury, G. Multhaup, D. H. Small, R. E. Tanzi, K. Beyreuther, and C. L. Masters. 1998. "Relative increase in Alzheimer's disease of soluble forms of cerebral Abeta amyloid protein precursor containing the Kunitz protease inhibitory domain." *J Biol Chem* 273 (9):5013-9.
Moir, R. D., and R. E. Tanzi. 2005. "LRP-mediated clearance of Abeta is inhibited by KPI-containing isoforms of APP." *Curr Alzheimer Res* 2 (2):269-73.
Morgan, C., M. Colombres, M. T. Nunez, and N. C. Inestrosa. 2004. "Structure and function of amyloid in Alzheimer's disease." *Prog Neurobiol* 74 (6):323-49. doi: 10.1016/j.pneurobio.2004.10.004.
Motoi, Y., T. Aizawa, S. Haga, S. Nakamura, Y. Namba, and K. Ikeda. 1999. "Neuronal localization of a novel mosaic apolipoprotein E receptor, LR11, in rat and human brain." *Brain Res* 833 (2):209-15.
Mufson, E. J., E. Y. Chen, E. J. Cochran, L. A. Beckett, D. A. Bennett, and J. H. Kordower. 1999. "Entorhinal cortex beta-amyloid load in individuals with mild cognitive impairment." *Exp Neurol* 158 (2):469-90. doi: 10.1006/exnr.1999.7086.

Mufson, E. J., S. E. Counts, S. E. Perez, and S. D. Ginsberg. 2008. "Cholinergic system during the progression of Alzheimer's disease: therapeutic implications." *Expert Rev Neurother* 8 (11):1703-18. doi: 10.1586/14737175.8.11.1703.

Mungarro-Menchaca, X., P. Ferrera, J. Moran, and C. Arias. 2002. "beta-Amyloid peptide induces ultrastructural changes in synaptosomes and potentiates mitochondrial dysfunction in the presence of ryanodine." *J Neurosci Res* 68 (1):89-96.

Myers, R. H., E. J. Schaefer, P. W. Wilson, R. D'Agostino, J. M. Ordovas, A. Espino, R. Au, R. F. White, J. E. Knoefel, J. L. Cobb, K. A. McNulty, A. Beiser, and P. A. Wolf. 1996. "Apolipoprotein E epsilon4 association with dementia in a population-based study: The Framingham study." *Neurology* 46 (3):673-7.

Namba, Y., M. Tomonaga, H. Kawasaki, E. Otomo, and K. Ikeda. 1991a. "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 (1):163-6.

Namba, Y., M. Tomonaga, H. Kawasaki, E. Otomo, and K. Ikeda. 1991b. "Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease." *Brain Research* 541 (1):163-6.

Narita, M., G. Bu, D. M. Holtzman, and A. L. Schwartz. 1997. "The low-density lipoprotein receptor-related protein, a multifunctional apolipoprotein E receptor, modulates hippocampal neurite development." *J Neurochem* 68 (2):587-95.

Narita, M., D. M. Holtzman, A. L. Schwartz, and G. Bu. 1997. "Alpha2-macroglobulin complexes with and mediates the endocytosis of beta-amyloid peptide via cell surface low-density lipoprotein receptor-related protein." *J Neurochem* 69 (5):1904-11.

Nathan, B. P., S. Bellosta, D. A. Sanan, K. H. Weisgraber, R. W. Mahley, and R. E. Pitas. 1994. "Differential effects of apolipoproteins E3 and E4 on neuronal growth in vitro." *Science* 264 (5160):850-2.

Nielsen, M. S., C. Gustafsen, P. Madsen, J. R. Nyengaard, G. Hermey, O. Bakke, M. Mari, P. Schu, R. Pohlmann, A. Dennes, and C. M. Petersen. 2007. "Sorting by the cytoplasmic domain of the amyloid precursor protein binding receptor SorLA." *Molecular and cellular biology* 27 (19):6842-51. doi: 10.1128/MCB.00815-07.

Nixon, R. A., A. M. Cataldo, and P. M. Mathews. 2000. "The endosomal-lysosomal system of neurons in Alzheimer's disease pathogenesis: a review." *Neurochem Res* 25 (9-10):1161-72.

Nykjaer, A., and T. E. Willnow. 2002. "The low-density lipoprotein receptor gene family: a cellular Swiss army knife?" *Trends Cell Biol* 12 (6):273-80.

O'Leary, J. C., 3rd, Q. Li, P. Marinec, L. J. Blair, E. E. Congdon, A. G. Johnson, U. K. Jinwal, J. Koren, 3rd, J. R. Jones, C. Kraft, M. Peters, J. F. Abisambra, K. E. Duff, E. J. Weeber, J. E. Gestwicki, and C. A. Dickey. 2010. "Phenothiazine-mediated rescue of cognition in tau transgenic mice requires neuroprotection and reduced soluble tau burden." *Mol Neurodegener* 5:45. doi: 10.1186/1750-1326-5-45.

Offe, K., S. E. Dodson, J. T. Shoemaker, J. J. Fritz, M. Gearing, A. I. Levey, and J. J. Lah. 2006a. "The lipoprotein receptor LR11 regulates amyloid beta production and amyloid precursor protein traffic in endosomal compartments." *Journal of Neuroscience* 26 (5):1596-603. doi: 26/5/1596 [pii]
10.1523/JNEUROSCI.4946-05.2006.

Offe, K., S. E. Dodson, J. T. Shoemaker, J. J. Fritz, M. Gearing, A. I. Levey, and J. J. Lah. 2006b. "The lipoprotein receptor LR11 regulates amyloid beta production and amyloid precursor protein traffic in endosomal compartments." *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26 (5):1596-603. doi: 10.1523/JNEUROSCI.4946-05.2006.

Otth, C., Concha, II, T. Arendt, J. Stieler, R. Schliebs, C. Gonzalez-Billault, and R. B. Maccioni. 2002. "AbetaPP induces cdk5-dependent tau hyperphosphorylation in transgenic mice Tg2576." *J Alzheimers Dis* 4 (5):417-30.

Perry, G., P. Mulvihill, V. Manetto, L. Autilio-Gambetti, and P. Gambetti. 1987. "Immunocytochemical properties of Alzheimer straight filaments." *J Neurosci* 7 (11):3736-8.

Perry, G., P. Richey, S. L. Siedlak, P. Galloway, M. Kawai, and P. Cras. 1992. "Basic fibroblast growth factor binds to filamentous inclusions of neurodegenerative diseases." *Brain Res* 579 (2):350-2.

Petersen, C. M., M. S. Nielsen, A. Nykjaer, L. Jacobsen, N. Tommerup, H. H. Rasmussen, H. Roigaard, J. Gliemann, P. Madsen, and S. K. Moestrup. 1997. "Molecular identification of a novel candidate sorting receptor purified from human brain by receptor-associated protein affinity chromatography." *J Biol Chem* 272 (6):3599-605.

Pfrieger, F. W. 2003. "Cholesterol homeostasis and function in neurons of the central nervous system." *Cell Mol Life Sci* 60 (6):1158-71. doi: 10.1007/s00018-003-3018-7.

Pietrzik, C. U., T. Busse, D. E. Merriam, S. Weggen, and E. H. Koo. 2002. "The cytoplasmic domain of the LDL receptor-related protein regulates multiple steps in APP processing." *EMBO J* 21 (21):5691-700.

Polvikoski, T., R. Sulkava, M. Haltia, K. Kainulainen, A. Vuorio, A. Verkkoniemi, L. Niinisto, P. Halonen, and K. Kontula. 1995. "Apolipoprotein E, dementia, and cortical deposition of beta-amyloid protein." *N Engl J Med* 333 (19):1242-7. doi: 10.1056/NEJM199511093331902.

Porzig, R., D. Singer, and R. Hoffmann. 2007. "Epitope mapping of mAbs AT8 and Tau5 directed against hyperphosphorylated regions of the human tau protein." *Biochem Biophys Res Commun* 358 (2):644-9. doi: 10.1016/j.bbrc.2007.04.187.

Posse De Chaves, E. I., D. E. Vance, R. B. Campenot, R. S. Kiss, and J. E. Vance. 2000. "Uptake of lipoproteins for axonal growth of sympathetic neurons." *J Biol Chem* 275 (26):19883-90.

Prasher, V. P., M. J. Farrer, A. M. Kessling, E. M. Fisher, R. J. West, P. C. Barber, and A. C. Butler. 1998. "Molecular mapping of Alzheimer-type dementia in Down's syndrome." *Ann Neurol* 43 (3):380-3. doi: 10.1002/ana.410430316.

Pritchard, A., J. Harris, C. W. Pritchard, D. St Clair, H. Lemmon, J. C. Lambert, M. C. Chartier-Harlin, A. Hayes, U. Thaker, T. Iwatsubo, D. M. Mann, and C. Lendon. 2005. "Association study and meta-analysis of low-density lipoprotein receptor related protein in Alzheimer's disease." *Neurosci Lett* 382 (3):221-6. doi: 10.1016/j.neulet.2005.03.016.

Puttfarcken, P. S., A. M. Manelli, M. T. Falduto, G. S. Getz, and M. J. LaDu. 1997. "Effect of apolipoprotein E on neurite outgrowth and beta-amyloid-induced toxicity in developing rat primary hippocampal cultures." *J Neurochem* 68 (2):760-9.

Rebeck, G. W., R. D. Moir, S. Mui, D. K. Strickland, R. E. Tanzi, and B. T. Hyman. 2001. "Association of membrane-bound amyloid precursor protein APP with the apolipoprotein E receptor LRP." *Brain Res Mol Brain Res* 87 (2):238-45.

Rebeck, G. W., J. S. Reiter, D. K. Strickland, and B. T. Hyman. 1993. "Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions." *Neuron* 11 (4):575-80.

Rebeiz, J. J., E. H. Kolodny, and E. P. Richardson, Jr. 1967. "Corticodentatonigral degeneration with neuronal achromasia: a progressive disorder of late adult life." *Trans Am Neurol Assoc* 92:23-6.

Reinhard, C., S. S. Hebert, and B. De Strooper. 2005. "The amyloid-beta precursor protein: integrating structure with biological function." *EMBO J* 24 (23):3996-4006. doi: 10.1038/sj.emboj.7600860.

Rezgaoui, M., G. Hermey, I. B. Riedel, W. Hampe, H. C. Schaller, and I. Hermans-Borgmeyer. 2001. "Identification of SorCS2, a novel member of the VPS10 domain containing receptor family, prominently expressed in the developing mouse brain." *Mech Dev* 100 (2):335-8.

Ricciarelli, R., E. Canepa, B. Marengo, U. M. Marinari, G. Poli, M. A. Pronzato, and C. Domenicotti. 2012. "Cholesterol and Alzheimer's disease: a still poorly understood correlation." *IUBMB Life* 64 (12):931-5. doi: 10.1002/iub.1091.

Rice, H. C., M. Townsend, J. Bai, S. Suth, W. Cavanaugh, D. J. Selkoe, and T. L. Young-Pearse. 2012. "Pancortins interact with amyloid precursor protein and modulate cortical cell migration." *Development* 139 (21):3986-96. doi: 10.1242/dev.082909.

Richey, P. L., S. L. Siedlak, M. A. Smith, and G. Perry. 1995. "Apolipoprotein E interaction with the neurofibrillary tangles and senile plaques in Alzheimer disease: implications for disease pathogenesis." *Biochem Biophys Res Commun* 208 (2):657-63. doi: 10.1006/bbrc.1995.1389.

Rogaev, E. I., R. Sherrington, E. A. Rogaeva, G. Levesque, M. Ikeda, Y. Liang, H. Chi, C. Lin, K. Holman, T. Tsuda, and et al. 1995. "Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene." *Nature* 376 (6543):775-8. doi: 10.1038/376775a0.

Rogaeva, E., Y. Meng, J. H. Lee, Y. Gu, T. Kawarai, F. Zou, T. Katayama, C. T. Baldwin, R. Cheng, H. Hasegawa, F. Chen, N. Shibata, K. L. Lunetta, R. Pardossi-Piquard, C. Bohm, Y. Wakutani, L. A. Cupples, K. T. Cuenco, R. C. Green, L. Pinessi, I. Rainero, S. Sorbi, A. Bruni, R. Duara, R. P. Friedland, R. Inzelberg, W. Hampe, H. Bujo, Y. Q. Song, O. M. Andersen, T. E. Willnow, N. Graff-Radford, R. C. Petersen, D. Dickson, S. D. Der, P. E. Fraser, G. Schmitt-Ulms, S. Younkin, R.

Mayeux, L. A. Farrer, and P. St George-Hyslop. 2007. "The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease." *Nature genetics* 39 (2):168-77. doi: 10.1038/ng1943.

Rohn, T. T., L. W. Catlin, K. G. Coonse, and J. W. Habig. 2012. "Identification of an amino-terminal fragment of apolipoprotein E4 that localizes to neurofibrillary tangles of the Alzheimer's disease brain." *Brain Res* 1475:106-15. doi: 10.1016/j.brainres.2012.08.003.

Rohn, T. T., R. J. Day, L. W. Catlin, R. J. Brown, A. J. Rajic, and W. W. Poon. 2013. "Immunolocalization of an Amino-Terminal Fragment of Apolipoprotein E in the Pick's Disease Brain." *PLoS One* 8 (12):e80180. doi: 10.1371/journal.pone.0080180.

Rohn, T. T., and P. Kokoulina. 2009. "Caspase-cleaved TAR DNA-binding protein-43 in Pick's disease." *Int J Physiol Pathophysiol Pharmacol* 1 (1):25-32.

Rosen, A. R., N. K. Steenland, J. Hanfelt, S. A. Factor, J. J. Lah, and A. I. Levey. 2007. "Evidence of shared risk for Alzheimer's disease and Parkinson's disease using family history." *Neurogenetics* 8 (4):263-70. doi: 10.1007/s10048-007-0100-6.

Roth, M., B. E. Tomlinson, and G. Blessed. 1966. "Correlation between scores for dementia and counts of 'senile plaques' in cerebral grey matter of elderly subjects." *Nature* 209 (5018):109-10.

Roth, M., B. E. Tomlinson, and G. Blessed. 1967. "The relationship between quantitative measures of dementia and of degenerative changes in the cerebral grey matter of elderly subjects." *Proc R Soc Med* 60 (3):254-60.

Rovelet-Lecrux, A., D. Hannequin, G. Raux, N. Le Meur, A. Laquerriere, A. Vital, C. Dumanchin, S. Feuillette, A. Brice, M. Vercelletto, F. Dubas, T. Frebourg, and D. Campion. 2006. "APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy." *Nat Genet* 38 (1):24-6. doi: 10.1038/ng1718.

Sager, K. L., J. Wuu, J. H. Herskowitz, E. J. Mufson, A. I. Levey, and J. J. Lah. 2012. "Neuronal LR11 expression does not differentiate between clinically-defined Alzheimer's disease and control brains." *PloS one* 7 (8):e40527. doi: 10.1371/journal.pone.0040527.

Sager, K. L., J. Wuu, S. E. Leurgans, H. D. Rees, M. Gearing, E. J. Mufson, A. I. Levey, and J. J. Lah. 2007. "Neuronal LR11/sorLA expression is reduced in mild cognitive impairment." *Annals of neurology* 62 (6):640-7. doi: 10.1002/ana.21190.

Sanchez, L., V. Alvarez, P. Gonzalez, I. Gonzalez, R. Alvarez, and E. Coto. 2001. "Variation in the LRP-associated protein gene (LRPAP1) is associated with late-onset Alzheimer disease." *Am J Med Genet* 105 (1):76-8.

Santacruz, K., J. Lewis, T. Spires, J. Paulson, L. Kotilinek, M. Ingelsson, A. Guimaraes, M. DeTure, M. Ramsden, E. McGowan, C. Forster, M. Yue, J. Orne, C. Janus, A. Mariash, M. Kuskowski, B. Hyman, M. Hutton, and K. H. Ashe. 2005. "Tau suppression in a neurodegenerative mouse model improves memory function." *Science* 309 (5733):476-81. doi: 10.1126/science.1113694.

Schellenberg, G. D., T. D. Bird, E. M. Wijsman, H. T. Orr, L. Anderson, E. Nemens, J. A. White, L. Bonnycastle, J. L. Weber, M. E. Alonso, and et al. 1992. "Genetic

linkage evidence for a familial Alzheimer's disease locus on chromosome 14." *Science* 258 (5082):668-71.

Scherzer, C. R., K. Offe, M. Gearing, H. D. Rees, G. Fang, C. J. Heilman, C. Schaller, H. Bujo, A. I. Levey, and J. J. Lah. 2004. "Loss of apolipoprotein E receptor LR11 in Alzheimer disease." *Archives of neurology* 61 (8):1200-5. doi: 10.1001/archneur.61.8.1200.

Schjeide, B. M., M. B. McQueen, K. Mullin, J. DiVito, M. F. Hogan, M. Parkinson, B. Hooli, C. Lange, D. Blacker, R. E. Tanzi, and L. Bertram. 2009. "Assessment of Alzheimer's disease case-control associations using family-based methods." *Neurogenetics* 10 (1):19-25. doi: 10.1007/s10048-008-0151-3.

Schmechel, D. E., A. M. Saunders, W. J. Strittmatter, B. J. Crain, C. M. Hulette, S. H. Joo, M. A. Pericak-Vance, D. Goldgaber, and A. D. Roses. 1993. "Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease." *Proc Natl Acad Sci U S A* 90 (20):9649-53.

Schmidt, M. L., R. Huang, J. A. Martin, J. Henley, M. Mawal-Dewan, H. I. Hurtig, V. M. Lee, and J. Q. Trojanowski. 1996. "Neurofibrillary tangles in progressive supranuclear palsy contain the same tau epitopes identified in Alzheimer's disease PHFtau." *Journal of neuropathology and experimental neurology* 55 (5):534-9.

Schmidt, V., A. Sporbert, M. Rohe, T. Reimer, A. Rehm, O. M. Andersen, and T. E. Willnow. 2007. "SorLA/LR11 regulates processing of amyloid precursor protein via interaction with adaptors GGA and PACS-1." *The Journal of biological chemistry* 282 (45):32956-64. doi: 10.1074/jbc.M705073200.

Schultzberg, M., C. Lindberg, A. F. Aronsson, E. Hjorth, S. D. Spulber, and M. Oprica. 2007. "Inflammation in the nervous system--physiological and pathophysiological aspects." *Physiol Behav* 92 (1-2):121-8. doi: 10.1016/j.physbeh.2007.05.050.

Selkoe, D. J. 2001. "Alzheimer's disease: genes, proteins, and therapy." *Physiol Rev* 81 (2):741-66.

Selkoe, D. J., T. Yamazaki, M. Citron, M. B. Podlisny, E. H. Koo, D. B. Teplow, and C. Haass. 1996. "The role of APP processing and trafficking pathways in the formation of amyloid beta-protein." *Ann N Y Acad Sci* 777:57-64.

Sergeant, N., A. Wattez, and A. Delacourte. 1999. "Neurofibrillary degeneration in progressive supranuclear palsy and corticobasal degeneration: tau pathologies with exclusively "exon 10" isoforms." *J Neurochem* 72 (3):1243-9.

Serneels, L., J. Van Biervliet, K. Craessaerts, T. Dejaegere, K. Horre, T. Van Houtvin, H. Esselmann, S. Paul, M. K. Schafer, O. Berezovska, B. T. Hyman, B. Sprangers, R. Sciot, L. Moons, M. Jucker, Z. Yang, P. C. May, E. Karran, J. Wiltfang, R. D'Hooge, and B. De Strooper. 2009. "gamma-Secretase heterogeneity in the Aph1 subunit: relevance for Alzheimer's disease." *Science* 324 (5927):639-42. doi: 10.1126/science.1171176.

Seshadri, S., A. L. DeStefano, R. Au, J. M. Massaro, A. S. Beiser, M. Kelly-Hayes, C. S. Kase, R. B. D'Agostino, Sr., C. Decarli, L. D. Atwood, and P. A. Wolf. 2007. "Genetic correlates of brain aging on MRI and cognitive test measures: a

genome-wide association and linkage analysis in the Framingham Study." *BMC Med Genet* 8 Suppl 1:S15. doi: 10.1186/1471-2350-8-S1-S15.

Seubert, P., C. Vigo-Pelfrey, F. Esch, M. Lee, H. Dovey, D. Davis, S. Sinha, M. Schlossmacher, J. Whaley, C. Swindlehurst, and et al. 1992. "Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids." *Nature* 359 (6393):325-7. doi: 10.1038/359325a0.

Shankar, G. M., B. L. Bloodgood, M. Townsend, D. M. Walsh, D. J. Selkoe, and B. L. Sabatini. 2007. "Natural oligomers of the Alzheimer amyloid-beta protein induce reversible synapse loss by modulating an NMDA-type glutamate receptor-dependent signaling pathway." *J Neurosci* 27 (11):2866-75. doi: 10.1523/JNEUROSCI.4970-06.2007.

Shariati, S. A., and B. De Strooper. 2013. "Redundancy and divergence in the amyloid precursor protein family." *FEBS Lett* 587 (13):2036-45. doi: 10.1016/j.febslet.2013.05.026.

Shiba, T., S. Kametaka, M. Kawasaki, M. Shibata, S. Waguri, Y. Uchiyama, and S. Wakatsuki. 2004. "Insights into the phosphoregulation of beta-secretase sorting signal by the VHS domain of GGA1." *Traffic* 5 (6):437-48. doi: 10.1111/j.1600-0854.2004.00188.x.

Sisodia, S. S. 1992. "Beta-amyloid precursor protein cleavage by a membrane-bound protease." *Proc Natl Acad Sci U S A* 89 (13):6075-9.

Spittaels, K., C. Van den Haute, J. Van Dorpe, K. Bruynseels, K. Vandezande, I. Laenen, H. Geerts, M. Mercken, R. Sciot, A. Van Lommel, R. Loos, and F. Van Leuven. 1999. "Prominent axonopathy in the brain and spinal cord of transgenic mice overexpressing four-repeat human tau protein." *Am J Pathol* 155 (6):2153-65. doi: 10.1016/S0002-9440(10)65533-2.

Spoelgen, R., C. A. von Arnim, A. V. Thomas, I. D. Peltan, M. Koker, A. Deng, M. C. Irizarry, O. M. Andersen, T. E. Willnow, and B. T. Hyman. 2006. "Interaction of the cytosolic domains of sorLA/LR11 with the amyloid precursor protein (APP) and beta-secretase beta-site APP-cleaving enzyme." *The Journal of neuroscience : the official journal of the Society for Neuroscience* 26 (2):418-28. doi: 10.1523/JNEUROSCI.3882-05.2006.

Steele, J. C., J. C. Richardson, and J. Olszewski. 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* 10:333-59.

Steiner, H., R. Fluhrer, and C. Haass. 2008. "Intramembrane proteolysis by gamma-secretase." *J Biol Chem* 283 (44):29627-31. doi: 10.1074/jbc.R800010200.

Strittmatter, W. J., K. H. Weisgraber, D. Y. Huang, L. M. Dong, G. S. Salvesen, M. Pericak-Vance, D. Schmechel, A. M. Saunders, D. Goldgaber, and A. D. Roses. 1993. "Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease." *Proc Natl Acad Sci U S A* 90 (17):8098-102.

Sydow, A., A. Van der Jeugd, F. Zheng, T. Ahmed, D. Balschun, O. Petrova, D. Drexler, L. Zhou, G. Rune, E. Mandelkow, R. D'Hooge, C. Alzheimer, and E. M. Mandelkow. 2011. "Tau-induced defects in synaptic plasticity, learning, and

memory are reversible in transgenic mice after switching off the toxic Tau mutant." *J Neurosci* 31 (7):2511-25. doi: 10.1523/JNEUROSCI.5245-10.2011.

Taira, K., H. Bujo, S. Hirayama, H. Yamazaki, T. Kanaki, K. Takahashi, I. Ishii, T. Miida, W. J. Schneider, and Y. Saito. 2001. "LR11, a mosaic LDL receptor family member, mediates the uptake of ApoE-rich lipoproteins in vitro." *Arteriosclerosis, thrombosis, and vascular biology* 21 (9):1501-6.

Takeda, K., T. Hatai, T. S. Hamazaki, H. Nishitoh, M. Saitoh, and H. Ichijo. 2000. "Apoptosis signal-regulating kinase 1 (ASK1) induces neuronal differentiation and survival of PC12 cells." *J Biol Chem* 275 (13):9805-13.

Tanzi, R. E., J. F. Gusella, P. C. Watkins, G. A. Bruns, P. St George-Hyslop, M. L. Van Keuren, D. Patterson, S. Pagan, D. M. Kurnit, and R. L. Neve. 1987. "Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus." *Science* 235 (4791):880-4.

Tellez-Nagel, I., and H. M. Wisniewski. 1973. "Ultrastructure of neurofibrillary tangles in Steele-Richardson-Olszewski syndrome." *Arch Neurol* 29 (5):324-7.

Teng, H. K., K. K. Teng, R. Lee, S. Wright, S. Tevar, R. D. Almeida, P. Kermani, R. Torkin, Z. Y. Chen, F. S. Lee, R. T. Kraemer, A. Nykjaer, and B. L. Hempstead. 2005. "ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin." *J Neurosci* 25 (22):5455-63. doi: 10.1523/JNEUROSCI.5123-04.2005.

Terry, R. D., E. Masliah, D. P. Salmon, N. Butters, R. DeTeresa, R. Hill, L. A. Hansen, and R. Katzman. 1991. "Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment." *Ann Neurol* 30 (4):572-80. doi: 10.1002/ana.410300410.

Teter, B., P. T. Xu, J. R. Gilbert, A. D. Roses, D. Galasko, and G. M. Cole. 1999. "Human apolipoprotein E isoform-specific differences in neuronal sprouting in organotypic hippocampal culture." *J Neurochem* 73 (6):2613-6.

Thinakaran, G., and E. H. Koo. 2008. "Amyloid precursor protein trafficking, processing, and function." *J Biol Chem* 283 (44):29615-9. doi: 10.1074/jbc.R800019200.

Tomlinson, B. E., G. Blessed, and M. Roth. 1968. "Observations on the brains of non-demented old people." *J Neurol Sci* 7 (2):331-56.

Torroja, L., M. Packard, M. Gorczyca, K. White, and V. Budnik. 1999. "The Drosophila beta-amyloid precursor protein homolog promotes synapse differentiation at the neuromuscular junction." *J Neurosci* 19 (18):7793-803.

Trojanowski, J. Q., T. Schuck, M. L. Schmidt, and V. M. Lee. 1989. "Distribution of tau proteins in the normal human central and peripheral nervous system." *J Histochem Cytochem* 37 (2):209-15.

Trommsdorff, M., M. Gotthardt, T. Hiesberger, J. Shelton, W. Stockinger, J. Nimpf, R. E. Hammer, J. A. Richardson, and J. Herz. 1999. "Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2." *Cell* 97 (6):689-701.

Uchihara, T., M. Hara, A. Nakamura, and K. Hirokawa. 2012. "Tangle evolution linked to differential 3- and 4-repeat tau isoform deposition: a double

immunofluorolabeling study using two monoclonal antibodies." *Histochem Cell Biol* 137 (2):261-7. doi: 10.1007/s00418-011-0891-2.

Vitek, M. P., C. M. Brown, and C. A. Colton. 2009. "APOE genotype-specific differences in the innate immune response." *Neurobiol Aging* 30 (9):1350-60. doi: 10.1016/j.neurobiolaging.2007.11.014.

Volosin, M., W. Song, R. D. Almeida, D. R. Kaplan, B. L. Hempstead, and W. J. Friedman. 2006. "Interaction of survival and death signaling in basal forebrain neurons: roles of neurotrophins and proneurotrophins." *J Neurosci* 26 (29):7756-66. doi: 10.1523/JNEUROSCI.1560-06.2006.

von Bergen, M., S. Barghorn, J. Biernat, E. M. Mandelkow, and E. Mandelkow. 2005. "Tau aggregation is driven by a transition from random coil to beta sheet structure." *Biochim Biophys Acta* 1739 (2-3):158-66. doi: 10.1016/j.bbadis.2004.09.010.

Wagner, T., and C. U. Pietrzik. 2012. "The role of lipoprotein receptors on the physiological function of APP." *Exp Brain Res* 217 (3-4):377-87. doi: 10.1007/s00221-011-2876-8.

Wahle, T., K. Prager, N. Raffler, C. Haass, M. Famulok, and J. Walter. 2005. "GGA proteins regulate retrograde transport of BACE1 from endosomes to the trans-Golgi network." *Mol Cell Neurosci* 29 (3):453-61. doi: 10.1016/j.mcn.2005.03.014.

Walter, J., R. Fluhrer, B. Hartung, M. Willem, C. Kaether, A. Capell, S. Lammich, G. Multhaup, and C. Haass. 2001. "Phosphorylation regulates intracellular trafficking of beta-secretase." *J Biol Chem* 276 (18):14634-41. doi: 10.1074/jbc.M011116200.

Wang, H., and R. H. Eckel. 2012. "Lipoprotein lipase in the brain and nervous system." *Annu Rev Nutr* 32:147-60. doi: 10.1146/annurev-nutr-071811-150703.

Wang, J., D. W. Dickson, J. Q. Trojanowski, and V. M. Lee. 1999. "The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging." *Exp Neurol* 158 (2):328-37. doi: 10.1006/exnr.1999.7085.

Wang, Q., R. L. Woltjer, P. J. Cimino, C. Pan, K. S. Montine, J. Zhang, and T. J. Montine. 2005. "Proteomic analysis of neurofibrillary tangles in Alzheimer disease identifies GAPDH as a detergent-insoluble paired helical filament tau binding protein." *FASEB J* 19 (7):869-71. doi: 10.1096/fj.04-3210fje.

Weidemann, A., K. Paliga, U. Durrwang, C. Czech, G. Evin, C. L. Masters, and K. Beyreuther. 1997. "Formation of stable complexes between two Alzheimer's disease gene products: presenilin-2 and beta-amyloid precursor protein." *Nat Med* 3 (3):328-32.

Weingarten, M. D., A. H. Lockwood, S. Y. Hwo, and M. W. Kirschner. 1975. "A protein factor essential for microtubule assembly." *Proc Natl Acad Sci U S A* 72 (5):1858-62.

Weinshenker, D. 2008. "Functional consequences of locus coeruleus degeneration in Alzheimer's disease." *Curr Alzheimer Res* 5 (3):342-5.

Weisgraber, K. H., S. C. Rall, Jr., R. W. Mahley, R. W. Milne, Y. L. Marcel, and J. T. Sparrow. 1986. "Human apolipoprotein E. Determination of the heparin binding sites of apolipoprotein E3." *J Biol Chem* 261 (5):2068-76.

Westergaard, U. B., E. S. Sorensen, G. Hermey, M. S. Nielsen, A. Nykjaer, K. Kirkegaard, C. Jacobsen, J. Gliemann, P. Madsen, and C. M. Petersen. 2004. "Functional organization of the sortilin Vps10p domain." *J Biol Chem* 279 (48):50221-9. doi: 10.1074/jbc.M408873200.

Weyer, S. W., M. Klevanski, A. Delekate, V. Voikar, D. Aydin, M. Hick, M. Filippov, N. Drost, K. L. Schaller, M. Saar, M. A. Vogt, P. Gass, A. Samanta, A. Jaschke, M. Korte, D. P. Wolfer, J. H. Caldwell, and U. C. Muller. 2011. "APP and APLP2 are essential at PNS and CNS synapses for transmission, spatial learning and LTP." *EMBO J* 30 (11):2266-80. doi: 10.1038/emboj.2011.119.

Wiese, M., A. Antebi, and H. Zheng. 2010. "Intracellular trafficking and synaptic function of APL-1 in Caenorhabditis elegans." *PLoS One* 5 (9). doi: 10.1371/journal.pone.0012790.

Williams, D. R. 2006. "Tauopathies: classification and clinical update on neurodegenerative diseases associated with microtubule-associated protein tau." *Intern Med J* 36 (10):652-60. doi: 10.1111/j.1445-5994.2006.01153.x.

Willnow, T. E., A. Nykjaer, and J. Herz. 1999. "Lipoprotein receptors: new roles for ancient proteins." *Nat Cell Biol* 1 (6):E157-62. doi: 10.1038/14109.

Willnow, T. E., C. M. Petersen, and A. Nykjaer. 2008. "VPS10P-domain receptors - regulators of neuronal viability and function." *Nat Rev Neurosci* 9 (12):899-909. doi: 10.1038/nrn2516.

Wilson, D. M., and L. I. Binder. 1997. "Free fatty acids stimulate the polymerization of tau and amyloid beta peptides. In vitro evidence for a common effector of pathogenesis in Alzheimer's disease." *Am J Pathol* 150 (6):2181-95.

Wischik, C. M., M. Novak, P. C. Edwards, A. Klug, W. Tichelaar, and R. A. Crowther. 1988. "Structural characterization of the core of the paired helical filament of Alzheimer disease." *Proceedings of the National Academy of Sciences of the United States of America* 85 (13):4884-8.

Wischik, C. M., M. Novak, H. C. Thogersen, P. C. Edwards, M. J. Runswick, R. Jakes, J. E. Walker, C. Milstein, M. Roth, and A. Klug. 1988. "Isolation of a fragment of tau derived from the core of the paired helical filament of Alzheimer disease." *Proceedings of the National Academy of Sciences of the United States of America* 85 (12):4506-10.

Wisniewski, K. E., H. M. Wisniewski, and G. Y. Wen. 1985. "Occurrence of neuropathological changes and dementia of Alzheimer's disease in Down's syndrome." *Annals of Neurology* 17 (3):278-82.

Wisniewski, T., and B. Frangione. 1992. "Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid." *Neurosci Lett* 135 (2):235-8.

Witman, G. B., D. W. Cleveland, M. D. Weingarten, and M. W. Kirschner. 1976. "Tubulin requires tau for growth onto microtubule initiating sites." *Proc Natl Acad Sci U S A* 73 (11):4070-4.

Wolfe, M. S. 2008. "Gamma-secretase: structure, function, and modulation for Alzheimer's disease." *Curr Top Med Chem* 8 (1):2-8.

Wolfe, M. S., W. Xia, B. L. Ostaszewski, T. S. Diehl, W. T. Kimberly, and D. J. Selkoe. 1999. "Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity." *Nature* 398 (6727):513-7. doi: 10.1038/19077.

Wood, J. G., S. S. Mirra, N. J. Pollock, and L. I. Binder. 1986. "Neurofibrillary tangles of Alzheimer disease share antigenic determinants with the axonal microtubule-associated protein tau (tau)." *Proceedings of the National Academy of Sciences of the United States of America* 83 (11):4040-3.

Xia, W., J. Zhang, R. Perez, E. H. Koo, and D. J. Selkoe. 1997. "Interaction between amyloid precursor protein and presenilins in mammalian cells: implications for the pathogenesis of Alzheimer disease." *Proc Natl Acad Sci U S A* 94 (15):8208-13.

Yamaguchi, H., K. Ishiguro, S. Sugihara, Y. Nakazato, T. Kawarabayashi, X. Sun, and S. Hirai. 1994. "Presence of apolipoprotein E on extracellular neurofibrillary tangles and on meningeal blood vessels precedes the Alzheimer beta-amyloid deposition." *Acta Neuropathol* 88 (5):413-9.

Yamazaki, H., H. Bujo, J. Kusunoki, K. Seimiya, T. Kanaki, N. Morisaki, W. J. Schneider, and Y. Saito. 1996. "Elements of neural adhesion molecules and a yeast vacuolar protein sorting receptor are present in a novel mammalian low density lipoprotein receptor family member." *J Biol Chem* 271 (40):24761-8.

Young-Pearse, T. L., J. Bai, R. Chang, J. B. Zheng, J. J. LoTurco, and D. J. Selkoe. 2007. "A critical function for beta-amyloid precursor protein in neuronal migration revealed by in utero RNA interference." *J Neurosci* 27 (52):14459-69. doi: 10.1523/JNEUROSCI.4701-07.2007.

Yu, G., M. Nishimura, S. Arawaka, D. Levitan, L. Zhang, A. Tandon, Y. Q. Song, E. Rogaeva, F. Chen, T. Kawarai, A. Supala, L. Levesque, H. Yu, D. S. Yang, E. Holmes, P. Milman, Y. Liang, D. M. Zhang, D. H. Xu, C. Sato, E. Rogaev, M. Smith, C. Janus, Y. Zhang, R. Aebersold, L. S. Farrer, S. Sorbi, A. Bruni, P. Fraser, and P. St George-Hyslop. 2000. "Nicastrin modulates presenilin-mediated notch/glp-1 signal transduction and betaAPP processing." *Nature* 407 (6800):48-54. doi: 10.1038/35024009.

Zheng, H., M. Jiang, M. E. Trumbauer, D. J. Sirinathsinghji, R. Hopkins, D. W. Smith, R. P. Heavens, G. R. Dawson, S. Boyce, M. W. Conner, K. A. Stevens, H. H. Slunt, S. S. Sisoda, H. Y. Chen, and L. H. Van der Ploeg. 1995. "beta-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity." *Cell* 81 (4):525-31.

Zheng, H., and E. H. Koo. 2011. "Biology and pathophysiology of the amyloid precursor protein." *Mol Neurodegener* 6 (1):27. doi: 10.1186/1750-1326-6-27.

Zhukareva, V., D. Mann, S. Pickering-Brown, K. Uryu, T. Shuck, K. Shah, M. Grossman, B. L. Miller, C. M. Hulette, S. C. Feinstein, J. Q. Trojanowski, and V. M. Lee. 2002. "Sporadic Pick's disease: a tauopathy characterized by a spectrum of pathological tau isoforms in gray and white matter." *Annals of Neurology* 51 (6):730-9. doi: 10.1002/ana.10222.