

Low Density Lipoprotein Receptor-Related Proteins (LRPs), Alzheimer's and Cognition

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Abstract: This review will focus primarily on the role of the low density receptor-related protein (LRP-1) in neuronal synapse formation and function in Alzheimer's Disease (AD). We review the role that its ligands may have in cognition or AD: apolipoprotein E (ApoE), α 2-macroglobulin, Transforming Growth Factor-Beta (TGF β), Tissue Plasminogen Activator (tPA), insulin growth factor binding protein-3 (IGFBP-3), which all bind LRP-1 and apolipoprotein J (ApoJ), which is a ligand for LRP-2. After reviewing its role as a signaling receptor, we discuss the connection between LRP and the NMDA glutamate receptor *via* the post synaptic density 95 (PSD-95) neuronal scaffold protein and the implications it may have for memory and cognition. Finally, we discuss the evidence supporting a role for LRP in AD. Although the evidence for LRP as a genetic risk factor is weak, many of its ligands impose genetic risk, and have been implicated in AD pathogenic cascades. We discuss the role of LRP in amyloid precursor protein (APP) processing and production of beta-amyloid (A β). We identify LRP ligands that accelerate aggregation of toxic A β species. LRP mediates crucial pathways in AD pathogenesis such as A β clearance, A β uptake, intraneuronal A β accumulation and A β -associated neuron death. Interestingly, the TGF β -V receptor is LRP-1. Data show that one critical ligand TGF β 2, associated with neurodegeneration in amyloid diseases, induces LRP expression in PC12 cells. Data from rodent infusion models demonstrate the impact of TGF β 2 in modifying A β -induced Long Term Potentiation (LTP) responses, presynaptic proteins, lipid peroxidation, gliosis and staining for neuronal nuclei. The evidence supports a complex and significant role of LRP in cognition and AD.

Keywords: Synapse, Alzheimer's disease, apolipoprotein E, cognition, signaling, transforming growth factor beta, plasticity, clearance.

INTRODUCTION

The low density lipoprotein receptor-related protein (LRP) is a member of an evolutionarily ancient and highly conserved gene family, the LDL receptor (LDLR) family [1]. The first member of this family, the LDLR, was discovered in 1974 [2], and it has been known for some time that members of this family bind and endocytose numerous structurally diverse ligands with a variety of biological functions. These functions include lipid metabolism, homeostasis of proteinases and proteinase inhibitors, cellular entry of viruses and toxins and activation of lysosomal enzymes. More recently, a role in signal transduction and neurotransmission has been described for this receptor family.

There are seven type-I membrane proteins that form the LDLR gene family [3,4]. The ability to endocytose ligands is a function of all seven members of this family, and all members share five structural elements in common. Extracellular segments contain 1) LDLR ligand-binding domains, 2) epidermal growth factor (EGF)-like cysteine rich repeats and 3) YWTD domains. They also contain 4) a single membrane spanning region and 5) cytoplasmic tails ranging from 50-200 amino acids and containing NPxY motifs, which serve to regulate the endocytic and signaling functions of these receptors.

Classically, the 600 kDa LRP is known as a receptor for chylomicron remnants, taking dietary cholesterol from the gut to the liver *via* lipoproteins. Subsequent studies demonstrating LRP's ability to remove proteinase and proteinase inhibitor complexes raised the notion that LRP may be a multifunctional scavenger receptor. LRP is one of the larger receptors in the LDLR family, recognizing over 40 different ligands [5]. Gene knock out studies have confirmed that LRP is required for embryonic development in the mouse [6], although the exact role of LRP in development remains unclear. Diversity in ligand binding capabilities, an essential role during development and preservation during evolution suggest that LRP is serving a basic and uniquely important role.

LRP is expressed in numerous cell types including fibroblasts, hepatocytes, adipocytes, macrophages and central nervous system (CNS) cells. In the normal human brain, LRP is expressed in neurons, particularly the pyramidal neurons. *In vitro* studies have shown LRP to be expressed in microglia [7] and astrocytes under some pathological conditions [8,9]. Some understanding of the functions of the LDLR family in the brain has begun to emerge in the last five years. Work by several groups [10,11] supports a role for LRP, and other members of the LDLR family, in synaptic transmission in the adult brain. In this review article; we will focus attention on the recently emerging role of LRP in synaptic transmission. We will discuss LRP's endocytic and signaling roles in relation to synaptic plasticity and the neurodegenerative disorder Alzheimer's Disease.

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Table 1. Short List of LRP Ligands Relevant to CNS Functions

CNS LRP-binding ligands	Functions mediated through LRP	References
Transforming Growth Factor-Beta (TGF β)	Increases intraneuronal A β accumulation, toxicity and memory retention Increases LRP expression	[56,160]
Apolipoprotein E (ApoE)	Neurite outgrowth, structural plasticity, dendritic spine morphology. A β clearance	[13,24,30,144,162,163]
α 2-macroglobulin (α 2Mac)	Regulates calcium signaling. Regulates cytokine clearance and catabolism	[51,164,165]
Tissue Plasminogen Activator (tPA)	Extracellular matrix remodeling, increased MMP9 production. Cortical plasticity. Modulates NMDA receptors and excitotoxicity. Regulates vascular permeability.	[76,80,83-85,166]
Apolipoprotein J (Clusterin)	(LRP2) A β oligomer formation and toxicity	[147]
Insulin Growth Factor Binding Protein-3 IGFBP-3	Unknown, possibly insulin signaling and protection from A β toxicity	[62]

LRP LIGANDS

There are well over 40 soluble ligands that bind to LRP [12]. Table 1 contains a short list of ligands that are particularly relevant to CNS function, and as discussed later, relevant to synaptic plasticity and AD.

Apolipoprotein E. Apolipoprotein E (ApoE) is a 299 amino acid, 35 kDa glycoprotein constituent of lipoproteins in the plasma and brain [13]. ApoE binds to and is internalized by many members of the LDLR family including LRP. The major role of ApoE is the delivery and clearance of cholesterol and plasma lipids. Humans have three major isoforms of ApoE that differ at amino acid residues 112 and 158. Functional differences between the isoforms as a result of these single amino acid changes include altered affinities for receptors and lipoprotein subtypes. ApoE2 increases age of onset for macular degeneration [14] and risk for hyperlipidemia and Type III hyperlipoproteinemia (primary dysbetalipoproteinemia) [15]. But ApoE2 is protective against Alzheimer's (AD) [16] and heart disease [17,18]. The LRP connection to these diseases is unknown, but ApoE2 has far lower affinity (only 2%) for the LDLR family (compared to E3 or E4) [19]. A low affinity of LRP for ApoE2 may explain its protective effects in AD.

ApoE is highly expressed in the brain and cerebrospinal fluid (CSF). Unlike peripheral ApoE pathways, relatively little is known about ApoE functions in the brain. The ApoE component of CSF lipoproteins appears to be produced locally [20] and ApoE is expressed predominantly by microglia and astrocytes [21]. Cholesterol homeostasis in the brain is maintained by a balance between cholesterol influx/efflux and biosynthesis, and is important for many biochemical processes including synapse formation. There is considerable evidence supporting a neurotrophic role for ApoE. ApoE particles released from astrocytes can interact with LRP on neurons and facilitate neurite outgrowth [22-26]. Experiments to determine the isoform-specific effects of ApoE on neurite extension have been carried out. Holtzman et. al., [27] show that ApoE2- and E3-, but not E4-, containing β -

VLDL particles effect a 1.5-2 fold increase in neurite extension in a neuronal cell line. A similar study showed that plasma HDL and CSF lipoproteins containing ApoE3, but not ApoE4, significantly increased neurite outgrowth similar to that seen with ApoE3 enriched β -VLDL [24]. These effects were antagonized by the receptor associated protein (RAP) and/or anti-LRP antibody. This strongly suggests that one normal function of LRP in the CNS is mediation of neurotrophic support. Nevertheless, a second pathological role for this pathway may contribute to pathogenesis in AD and will also be explored in this review.

ApoE might play an important role in maintaining the stability of the synapto-dendritic complex. ApoE is central to maintaining cholesterol and phospholipid homeostasis; the integrity of synapses and dendrites may very well rely on the presence of ApoE. Neurons favor cholesterol internalization through the ApoE/LDLR family pathway during dendritic remodeling [28]. In ApoE-deficient mice, there is an age-dependent (15 to 40%) loss of synaptophysin-immunoreactive nerve terminals and microtubule-associated protein 2-immunoreactive dendrites in the cortex and hippocampus, when compared to controls [29]. Human ApoE3 (hApoE3) transgenic and wild-type mice had a higher dendritic spine density than ApoE deficient or human ApoE4 (hApoE4) transgenic mice at 1-2 years of age [30]. hApoE3 mice, but not hApoE4 mice, had more pre-synaptic boutons following environmental enrichment [31].

Changes in neurotransmission and cognition are seen in mice deficient in ApoE. Morris water maze testing of ApoE deficient mice shows deficiencies in working memory by 6 months of age [32] and performance impairment was associated with decreased synaptic excitability [33]. Reports suggest that ApoE plays a role in LTP, but the precise function of ApoE is unclear [34-38]. Long term potentiation (LTP) is an experimentally induced increase in synaptic efficacy that models memory. One recent study showed that LTP was greater in wild-type and hApoE3 transgenic mice compared to ApoE deficient or hApoE4 transgenic mice [39]. ApoE may regulate synaptic function by elevation of neuronal calcium levels through release of intracellular calcium stores and influx of calcium [40]. hApoE4 enhanced the calcium response to

NMDA and increased resting calcium levels, and this effect was blocked by two LRP ligands, RAP and α 2-macroglobulin (α 2Mac) [41], raising the question as to how LRP-NMDA receptor interaction (linked by the scaffold protein, postsynaptic density 95 (PSD95) [42]) may contribute to memory.

Although the LDLR families relatively low affinity for ApoE2 may relate to its protective effects in AD and heart disease and its deleterious effects in other disorders, functional differences between ApoE3 and ApoE4 may relate to an isoform-specific lipid efflux from astrocytes and neurons *via* cell surface HSPGs and/or HSPG-LRP pathway [43]. That is, adverse effects of ApoE4 relative to ApoE3 for neuritic outgrowth and LTP may be explained by differential affinity of the isoforms for cell surface HSPGs ($E2 < E3 < E4$). HSPGs modulate ApoE isoform promotion of cholesterol efflux (ApoE4 is less effective in promotion of cholesterol efflux because of high HSPG affinity) [43,44]. ApoE4 has the lowest affinity for A β , which may render it more permissive for uptake (*via* an A β receptor such as LRP). After uptake, the fact that both ApoE4 and A β have the highest affinities for HSPGs may interfere with export trafficking (not only of cholesterol but also A β), leading to accumulation of toxic substrates (see section on intra-neuronal accumulation).

α 2-macroglobulin. LRP is also referred to as the α 2Mac receptor. LRP binds α 2Mac and α 2Mac-proteinase complexes and clears them from the extracellular space. The formation of α 2Mac-protease complexes results in the transformation of the native 'slow' form to the 'fast' (activated) form, which is recognized by LRP [45]. α 2Mac is a broad spectrum protease inhibitor induced with inflammation and carrier protein for many cytokines including transforming growth factor-betas (TGF β s), interleukins, nerve growth factor, platelet-derived growth factor as well as endogenous, soluble beta-amyloid protein [46-49].

In addition to α 2Mac's binding and clearance functions, activated α 2Mac can affect neurite outgrowth of neurons. This effect may be mediated by α 2Mac's binding to neurotrophic factors and alterations of neurotrophic factor receptor signal transduction. α 2Mac can inhibit the development and maintenance of LTP in a concentration and time-dependent manner [50]. The accumulation of activated α 2Mac in association with inflammatory neuropathology could inhibit synaptic plasticity.

α 2Mac has also been shown to control intracellular calcium levels. Calcium signaling plays important roles in both normal physiological and pathological neuronal functions. Calcium is an important second messenger but can be neurotoxic at high concentrations. Activated α 2Mac can alter the neuronal calcium response to NMDA by down regulating the NMDA receptor 1 [51]. This effect can be inhibited by RAP, suggesting α 2Mac mediated this effect through the LRP.

Although it is not clear that serum α 2Mac levels are elevated in AD [52], levels appear to be increased in CSF [53]. Further, genetic variants may be linked to late onset AD, and increased α 2Mac is found in a subgroup of

cortical and hippocampal neuritic plaques of AD patients and in large hippocampal neurons [54].

Transforming Growth Factor-Beta (TGF β) and its V receptor, LRP-1. TGF β s are part of a large superfamily of growth factors that fulfill key functions during development and in maintaining tissue homeostasis [55]. There are three mammalian isoforms of TGF β , TGF- β 1, - β 2 and - β 3. For many years it was thought that the diverse effects of TGF β s were mediated through the same type-I and type-II heterodimeric transmembrane serine/threonine kinase receptors. Activation of the type II receptor and subsequent activation of the type I receptor leads to phosphorylation of the Smad protein family and downstream signaling events. In addition to this "classical" type I/II/Smad signaling cascade, recent studies indicate that other signaling cascades utilizing TGF β receptor V (T β R-V) are involved in mediating TGF β 's actions [56]. Recently, T β R-V was found to be identical to LRP-1 [57]. This finding has revealed a novel growth regulatory function of LRP and may explain the embryonic lethal phenotype of LRP knockouts.

At this time it is not clear how TGF β s regulate LRP function or signaling pathways or if there is a difference between the isoforms of TGF β . Recent studies in our lab demonstrate that TGF β 2 can increase LRP protein and mRNA levels in PC12 (Fig. 1). PC12 cells do not contain the "classical" type I/II receptors [58], suggesting that this effect is mediated through LRP/ T β R-V. In addition to direct binding of TGF β s to LRP, TGF β s can bind other ligands of LRP such as α 2Mac. TGF β s 1 and 2 both bind α 2Mac but TGF β 2 has a particularly high affinity for α 2Mac, in both its native and activated forms [59]. Hypothetically, this binding interaction could modulate the actions of both α 2Mac and TGF β at the LRP. Work by Zhu *et al.*, [60,61] focusing on TGF β 1 demonstrates that the phosphatidylinositol-3-OH kinase (PI3K/Akt) and mitogen-activated protein kinase/extracellular-signal regulated kinase (MAPK/Erk1,2) pathways and nuclear factor kappa B (NF κ B) activity are necessary for the anti-apoptotic activity of TGF β 1. Although signaling through the type I/II TGF β pathway has been known for some time, it is now becoming apparent that LRP/ T β R-V signaling also occurs.

The T β R-V is also identical to the insulin-like growth factor binding protein-3 receptor (IGFBP-3r) [62]. IGFBPs determine the relatively complex mechanisms regulating the bioavailability of the IGFs in circulating fluids and cellular surroundings. IGFBP-3 regulates IGF-dependent and IGF-independent (e.g., TGF β antagonist sensitive growth inhibition) processes and may render protection from type I diabetes [63]. Apart from their roles as IGF carriers, IGFBPs also possess intrinsic activities that are divorced from their association with IGFs. These so-called IGF-independent activities modulate numerous cellular processes, including cell growth, differentiation and apoptosis. Very little is known about IGFBP-3/LRP-1 interactions in AD, but IGFBP may be involved in protection against A β toxicity [64]. It remains controversial how the role of LRP-1 in early stage AD, or its loss later in the disease, contributes to vulnerability of A β toxicity, cell death pathways, or altered insulin signaling. It is not known whether the LRP ligand IGFBP-3 is altered in AD, and it is unclear what role IGFBP-3 might play in the brain. It

may increase insulin signaling as it does in cancer cells [65,66] or, in contrast, may sequester factors controlling insulin signaling. Clarification of this issue may provide insight into the importance of LRP in altering insulin signaling in AD, since defects in insulin signaling are likely playing an important role in the disease process [67].

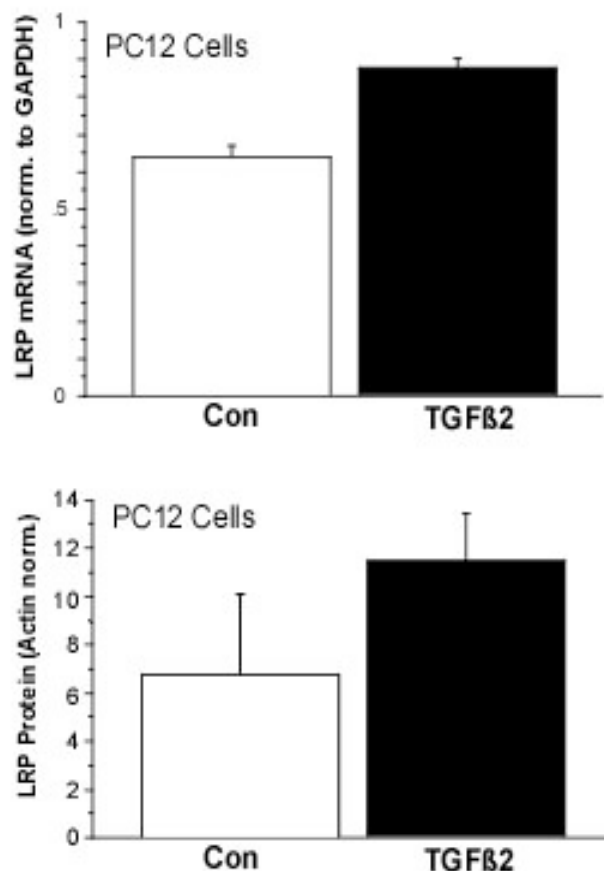


Fig. (1). TGFβ2 increases LRP protein and mRNA. PC12 cells were treated for 3 days with 0.5 ng/ml TGFβ2 (R & D Systems) and assessed for LRP protein and mRNA levels. Following SDS-PAGE of cell homogenate preparations, the 85 kDa chain of LRP was detected using monoclonal 5A6 antibody and detected using an enhanced chemiluminescent system. LRP protein was quantified and normalized to actin staining. RT-PCR was used to determine mRNA levels and mRNA values were normalized to the housekeeping gene, GAPDH.

Tissue Plasminogen Activator (tPA). tPA is a ligand for and is regulated by LRP [77,78], and synthesis of tPA is increased by activation of the cyclic AMP-Protein Kinase A pathway [79]. Outside of the CNS, tPA is found in the blood where it functions as a thrombolytic enzyme cleaving plasminogen [68]. However, tPA is thought to have a very different function inside the CNS. tPA is one of the immediate early genes induced by LTP; it is induced during learning of a complex motor task [69] and is widely expressed within the CNS [68]. Neuronal activity increases mRNA for tPA in pyramidal neurons [68] and LTP is decreased in mice lacking the tPA gene [70-72]. tPA activity in the CNS is correlated with regeneration [73], migration [74], neurite outgrowth [75] and visual cortex plasticity [76].

Some studies indicate an important interaction between tPA and LRP in synaptic plasticity and memory development. LRP is abundantly expressed in hippocampal neurons and has a role in hippocampal LTP. RAP can block the LTP-enhancing effects of tPA in hippocampal slice prepared from tPA knockout mice [80].

In addition to the synapse-promoting effects of tPA, there are numerous studies demonstrating detrimental effects of tPA. tPA appears to be involved in the neuron death induced by oxygen deprivation, focal cerebral ischemia and excitotoxicity [81-83]. Neuron death from oxygen deprivation [82], focal ischemia [81] and excitotoxins [83] was reduced in tPA knockout mice. A recent study by Wang *et al.*, [84] suggests that tPA induces matrix metalloproteinase 9 (MMP9) through the signaling component of the LRP. MMP9 can modify the extracellular matrix (ECM) and reduce neurovascular matrix integrity. The authors suggest a complex interaction between LRP's signaling and endocytic functions to be involved in regulating MMP9 and the ECM. tPA also increases opening of the blood brain barrier (BBB) after cerebral ischemia [85]. RAP and anti-LRP antibodies attenuated the increase in vascular permeability suggesting an LRP-mediated process. However, this process was not dependent on MMP9 or plasminogen.

In summary, several well-known LRP ligands including ApoE, alpha 2 macroglobulin, TGFβ and tPA can be modulators of neuronal function, synaptic plasticity and potentially cognitive function.

LRP AS A SIGNALING RECEPTOR

LRP is well-established as an endocytic receptor but relatively little is known about its signaling function. LRP can indirectly regulate cell signaling by controlling the concentrations and activity of membrane proteins [86]. Early work on LRP signaling cascades demonstrated that direct ligation of LRP's 85 kDa light chain with monoclonal antibody induces a conformational change that activates signal transduction [87]. Furthermore, this study showed that LRP is coupled to a pertussis toxin-sensitive G-protein, and antibody binding increased protein kinase C (PKC) activity and IP₃ and Ca²⁺ levels.

LRP binds a wide range of ligands and many of these ligands appear to modulate neuronal activity or synaptic plasticity. However, there are also many differences in function between these ligands, suggesting a variety of mechanisms to direct LRP signaling or endocytic activities after ligand binding. These mechanisms are relatively unknown, but recent work suggests the involvement of specific intracellular adaptor or scaffold proteins that bind to the cytoplasmic tail of LRP (See Table 2). One common characteristic of LDLR family members is that they have at least one copy of the NPxY sequence in their cytoplasmic tail. For the LDLR, binding to the NPxY motif serves as the signal for rapid endocytosis [88], and it had been assumed that the NPxY sequences are the predominate endocytosis signal for all LDLR family receptors. It was subsequently demonstrated that two mammalian adaptor proteins, Disabled-1 (Dab1) and Fe65, interact with the NPxY sequence in the cytoplasmic tail of LRP, LDLR, ApoER2 and VLDLR [89-91]. In the case of ApoER2 and VLDLR, this binding of the NPxY motif

Table 2. LRP Cytoplasmic Tail Binding Proteins Relevant to CNS Functions

Intracellular adaptor protein involved in downstream LRP signaling	Function	Reference
JNK	Activation of c-Jun and Elk1 transcription factors. Effector of Rac and Cdc42 GTPases	[99,100]
JIP	Cytosolic scaffold protein that binds JNK	[100]
MEKK-1	JNK activation pathway	[100]
MKK4 (MAPKK4)	JNK activation pathway	[100]
Dab1	PI3K pathway	[90]
Fe65	Bridge between LRP and Amyloid Precursor Protein (APP)	[90]
Shc	Ras and c-myc pathways	[96]
CED-6/GULP	Regulates engulfment of apoptotic cells	[97]
PSD-95	Links NMDA and LRP and organizes active components of the postsynaptic neurotransmission machinery such as glutamate receptors, potassium channels, kinases, and neuronal nitric oxide synthase into functional microdomains	[42]

activates the Reelin/Dab-mediated neuronal migration pathway [92,93]. Therefore, in addition to regulating endocytosis, binding of the NPxY motif within the cytoplasmic tails of LDLR members may function as a binding element for cellular adaptor proteins involved in signal transduction. LRP has a cytoplasmic tail 100 amino acids long and containing five potential binding sites to regulate endocytosis and signaling. There are two NPxY sequences, two di-leucine sequences and one YXXL motif. For LRP, the YXXL motif, and not the NPxY motifs, serves as the dominant endocytosis signal [94].

Phosphorylation of LRP's cytoplasmic tail represents a mechanism to switch LRP function from endocytosis to signaling by modulating the class and type of adaptor proteins that interact with LRP. Phosphoamino acid analysis of metabolically labeled LRP demonstrated that LRP is phosphorylated at serine, threonine and tyrosine residues, and PKC has been identified as a kinase capable of phosphorylating LRP [95]. Serine and threonine phosphorylation modifies phosphorylation at tyrosine [80] and appears necessary for interaction of Shc with LRP. Shc is an adaptor protein that interfaces LRP with the Ras-extracellular signal-regulated kinase (ERK)-MAPK and c-myc pathways. LRP association with Dab1 (part of the PI3K pathway) [96] and Ced-6/GULP (involved in engulfment of apoptotic cells) [97] is modulated by serine/threonine, but not tyrosine, phosphorylation of LRP. LRP can also interact directly with other extracellular signaling molecules. Platelet-derived growth factor (PDGF) binding protein binding to LRP has been reported [98]. PDGF-dependent tyrosine phosphorylation of LRP within the second NPxY motif, which requires PDGF receptor β and involves Src-family tyrosine kinases, allows LRP to bind to Shc and possibly other adaptor/scaffold proteins that connect to signaling pathways.

LRP may play a large role in modulation of the Jun N-terminal kinase (JNK) pathway. The JNK pathway is activated in response to cytokines and stress with the activation of JNK contributing to apoptosis and inflammation. JNK is also a downstream effector of Rac

and Cdc42 GTPases involved in actin reorganization [99]. Using an LRP receptor fusion construct bearing the cytoplasmic COOH-terminal tail of human LRP, LRP bound to JIP-1 suppressed signal transduction by the JNK pathway [100]. JNK pathway is implicated in AD, cell death and neurofibrillary tangle formation, through its impact on tau phosphorylation [101-103]. It has also been hypothesized that LRP/JIP-1 may target JNK to specific substrates *via* microdomains at the plasma membrane. LRP/JIP/JNK would then be poised to actively regulate the cytoskeleton, potentially regulating vesicle transport, axon guidance and synaptic plasticity [100].

LRP, THE NMDA RECEPTOR AND COGNITION

Interestingly, the NMDA receptor and LRP appear to be linked by the scaffold protein, postsynaptic density 95 (PSD95), and proteins such as PSD95 may facilitate signal transduction between LRP and NMDA receptors [42]. Therefore, LRP ligands are likely to influence NMDA receptor processes and neuronal function related to memory in the CNS. It is clear that LRP has effects on the synapto-dendritic complex, migration, plasticity, regeneration, and LTP, and these effects will undoubtedly impact learning and memory and cognition. LDLR is the first described receptor for ApoE. Like ApoE deficient mice [33,35,36], LDLR deficient mice display impaired learning and memory functions [104] that may be attributable to loss of synaptic boutons. In the liver, LRP takes over binding of chylomicrons in the absence of LDLR [105], and LRP may also compensate for LDLR deficiency in the brain. However, LRP knockouts are embryonic lethal, making it difficult to perform learning and memory studies identical to those performed in LDLR knockout mice. Clearly, LRP is not simply a redundant LDLR system and the complexity of LRP makes it difficult to be studied. One solution is to perform studies using RAP knockout mice. These mice have reduced expression of mature LRP [106] and are cognitively impaired relative to controls in the Morris Water Maze [107]. Cre/loxP-mediated conditional LRP- deficient mice (MX1cre(+)/LRP(flox/flox)) may also be used in the future to study the role of LRP and cognition [108].

ALZHEIMER'S DISEASE AND LRP

Alzheimer's Disease: Many genetic risk factors interact with LRP. LRP appears to be the common link among multiple proteins relevant to AD. Three of the genes where mutations impart increased susceptibility to AD: APP, ApoE and $\alpha 2$ Mac, encode proteins that are ligands for LRP [48,109-114].

Could LRP itself be a genetic risk factor for AD? Although unconfirmed [115-117], a Chinese study has demonstrated a correlation between LRP C/C genotype and AD [118]. Patients with both the C/C genotype of LRP and the G allele of MAPK8IP1 gene show generalized increased transcriptional activity [119]. The MAPK8IP1 gene produces JIP1, a neuronal scaffold protein previously discussed. Patients bearing the C/C LRP genotype may be more susceptible to neuronal death *via* the JNK pathway in AD, due to enhanced binding of G-allele JIP1 to C/C-allele LRP.

Impact of LRP on APP processing and A β production. Evidence suggests that LRP affects the processing of APP and amyloid β protein (A β) production. In the absence of LRP, A β production, APP secretion and internalization, turnover of full-length APP and APP C-terminal fragment stability are affected, and this effect is not APP isoform dependent [120]. In this same study, the cytoplasmic tail and the second NPxY motif were shown to be critical for modulating APP processing. Furthermore, interactions between APP and LRP are facilitated by Fe65, a neuronal adaptor protein containing two distinct protein interaction domains that interact with LRP and APP, respectively [90]. Fe65 is capable of linking LRP and APP in a functional complex and modulating the intracellular trafficking of APP. Interestingly, the LRP ligand TGF β 2 is a binding protein for soluble APP derivatives [121], suggesting a mechanistic link between APP and TGF β 2 in the disease process, that may result from the impact of LRP on APP processing.

BACE is a transmembrane protease with β -secretase activity that cleaves APP. After BACE cleavage, APP is a substrate for γ -secretase leading to production of A β . BACE and the light chain of LRP interact at the cell surface in association with lipid rafts, and this interaction leads to secretion of the C-terminal fragment of LRP [122]. LRP itself also undergoes a presenilin-dependent γ -secretase proteolysis in the cell membrane, releasing a transcriptionally active fragment similar to APP and Notch [123,124]. APP and LRP both undergo regulated intramembranous cleavage by γ -secretase and the released cytoplasmic tails translocate to the nucleus and interact with Fe65 and Tip60. The LRP fragment has a novel signaling activity, opposing the transcriptional activity of the APP/Fe65/Tip60 complex. The physiological target genes regulated by LRP and APP fragments are currently unknown. Interestingly, when a soluble LRP cytoplasmic tail was overexpressed in CHO cells, an increase in APP and A β production through the BACE pathway was observed [125]. The effect was not dependent on membrane-anchored LRP or membrane-anchored LRP cytoplasmic tails, indicating that there are cellular

pathways involving soluble LRP fragments that can be augmented to change A β production.

Impact of the LRP ligand TGF β on oligomerization of amyloid proteins. Oligomerization is a process occurring in many amyloid producing diseases, including AD and prion neurodegenerative diseases, which coincide with early neuronal loading of the LRP ligand TGF β 2 [134]. Although extracellular deposition of A β (neuritic or senile plaques) may play an important pathogenic role in aberrant or misdirected sprouting and some synaptic changes [126], extracellular amyloid is insufficient to cause neuron loss. Further learning impairment and/or synapse loss frequently precede plaque formation in most APP overexpressing mouse models of AD [127]. This has led to more attention on soluble and oligomeric species of A β in AD pathogenesis. Both the LRP ligands, TGF β 1 and 2 can bind A β and accelerate oligomerization [133].

Impact of LRP on A β clearance. LRP can mediate the export of A β from the brain [90,109,110,128,129]. Bigenic mice overexpressing LRP and APP show increased levels of soluble Ab oligomers that correlate with memory loss [161]. In this system, increased clearance of amyloid mediated by LRP minigene overexpression leads to release of toxic oligomers. The accumulation of A β is a key pathogenic event in AD. Previous studies have demonstrated a negative impact of A β on CNS cells including oxidative stress [130-133], rises in intracellular calcium and excitotoxicity [134], inflammation [135] and activation of the JNK pathway [136]. It has been demonstrated *in vitro* that LRP clears A β 40 and A β 42 [137] and confers protection against A β toxicity in the presence of $\alpha 2$ Mac, a carrier for A β [138]. Further support for a protective role of LRP comes from experiments demonstrating increased A β toxicity when LRP is downregulated by RAP or presenilin 1 [138]. *In vivo*, it has been suggested that LRP on endothelial cells, in concert with RAGE, work to control A β levels and loss of LRP in the brain [139]. Besides a role for LRP in mediating A β degradation at the level of the blood brain barrier, LRP may also mediate neuronal degradation of A β . On neurons, LRP may endocytose A β , through binding of the LRP ligands, $\alpha 2$ Mac or ApoE, and degrade A β through the endosomal-lysosomal system. A role for LRP in A β clearance is also strengthened by the observation that over co-expression of the LRP ligand TGF β in astrocytes and APP in a bigenic mouse led to reduction in plaques [140]. Uptake through the LRP/ApoE pathway may indeed be a normal pathway for clearance of A β in human brain [137], and its loss would contribute to increased A β burden. For example, LRP ligands such as TGF β s have also been reported to cause A β accumulation in vasculature and neuropil [141,142], which may relate to LRP loss or down regulation. Because of its beneficial role in export of A β out of the brain, the known LRP loss in AD likely contributes to moderate and late stage disease (progression and A β accumulation).

However, in early stage AD, LRP may exacerbate the problem. Our data suggest that signaling interactions between the LRP and TGF β pathways not only alter normal A β clearance pathways, but also neuronal function. We showed important functional differences among the TGF β isoforms in their ability to alter cellular distribution and degradation of A β [143]. Alterations in LRP ligands (ApoE, TGF β s, etc) or LRP

receptor function/number may mediate early death pathways, LRP loss, and failed clearance. As discussed in the next sections, early in the disease, LRP appears to play a pivotal role in mediation of both the early neuron loss that occurs (and LRP loss).

Impact of LRP on uptake and intraneuronal accumulation of A β . Uptake of soluble, ApoE-bound A β occurs through LRP receptors and results in the accumulation of A β in nerve terminals [144]. LaFerla *et al.*, [145] observed co-uptake of both ApoE and A β into the same cytoplasmic granules, and suggested that lipids may stabilize the hydrophobic A β protein within the cell. The ApoE-containing neurons also exhibit high expression of a cell surface receptor, LRP2 (or glycoprotein 330), which is known to bind ApoE (as well as to bind Apolipoprotein J (ApoJ) [146]. Although there is no known genetic association of ApoJ with AD, ApoJ is implicated in soluble oligomer formation and toxicity [147]. LRP2 is expressed in neurons and may have similar functions as LRP2. Although there is no clear relationship between cognition and intraneuronal accumulation in humans, A β 42 immunostaining in hippocampal neurons from cognitively impaired patients and Down's patients indicate that intraneuronal A β accumulation is also an early event in AD [148-150]. In a triple transgenic (3x Tg-AD) model, intraneuronal A β accumulation has been linked to the onset of cognitive dysfunction [151]. In this study, mice transgenic for Swedish mutation APP (KM670/671NL), human four-repeat tau harboring the P301L mutation and PS1 (M146V mutation) were used.

These triple transgenic mice (3xTg-AD) exhibit intraneuronal accumulation of A β 42, correlating with memory retention deficits at only four months of age. The mechanism of intraneuronal loading of A β in the 3xTg-AD mice is currently unknown. Given that these mice carry a PS1 mutation known to increase A β levels and that PS1 activity can also affect LRP processing and signaling, a role for LRP in intraneuronal A β accumulation can be speculated. Changes in A β and/or LRP signaling could have important implications for signaling involved in establishment of learning and memory.

Impact of LRP and LRP ligands on A β -associated neuron death. Evidence is emerging that prior to loss of LRP, LRP may mediate A β -associated neuron loss. The expression of LRP ligands such as TGF β are increased in AD [152-155], particularly the isoform TGF β 2. TGF β 2 is localized in plaque neurites and neurofibrillary tangle (NFT)-bearing neurons which correlate [153] well with neuron loss [156].

TGF β exacerbation of A β neurotoxicity has been previously reported to occur in the absence of "classical" type I/II TGF β receptors [157]. Recent studies have revealed a potential link between LRP and TGF β s [158,159], and LRP1 has been shown recently to be identical to TGF β receptor V [57]. Recently published data from our laboratory demonstrate a crucial role for TGF β 2 in driving neuronal A β uptake and targeting and increasing neurodegeneration of A β both *in vitro* and *in vivo* [160]. These data support the hypothesis that TGF β 2 promotes uptake of extracellular A β thus increasing intracellular A β . A β targeting and neurotoxicity are blocked by RAP, demonstrating mediation of this effect through an

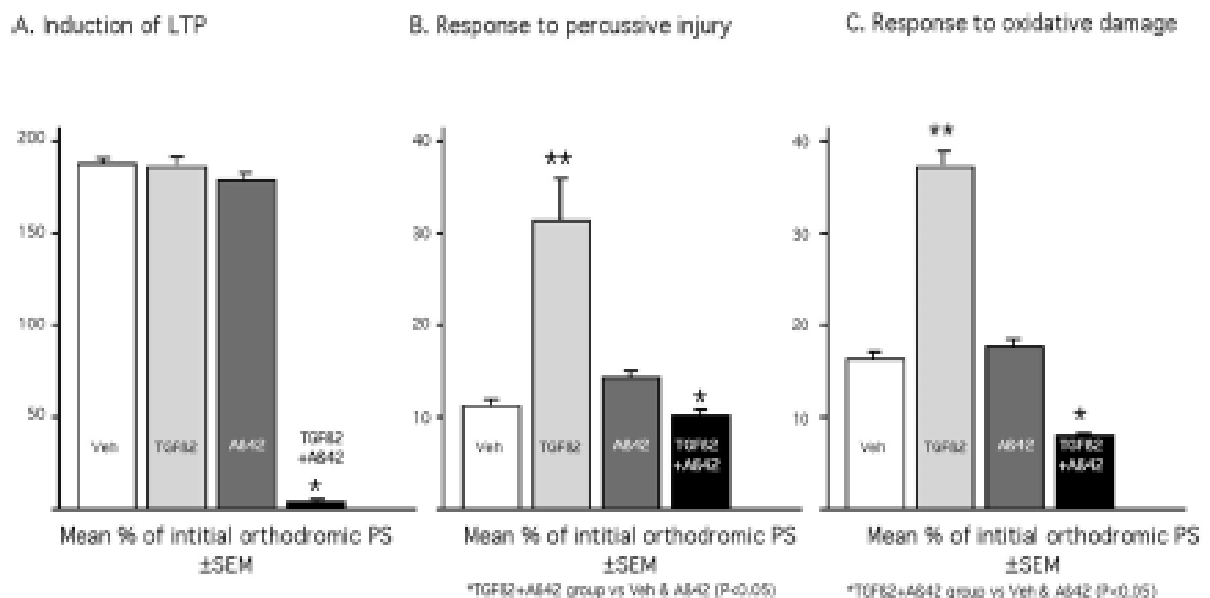


Fig. (2). Impact of TGF β 2 and/or A β 42 intracerebroventricular infusion into the mouse brain on LTP. 1 μ l volume of drug was injected into the third ventricle of the mouse brain for all four treatments using the following drug regimens (4 mM HCL vehicle, 10 ng TGF β 2, 2.5 μ g of A β 42 or A β 42+TGF β 2 at the midline -0.94 mm posterior to Bregma and 1.6 mm ventral to dura.) Mice were sacrificed 7 days after infusion. Normal LTP was observed in vehicle-treated mice, in TGF β 2 alone treated mice and in A β treated mice. Despite a normal initial orthodromic spike, TGF β 2 + A β 42 abolished LTP in all mice tested (n=8, $p < 0.001$). Orthodromic stimulation is shown. Antidromic stimulation showed similar trends (not shown), demonstrating the effect was not from synaptic depression. TGF β 2 appeared to exacerbate damage after traumatic or oxidative injury compared to the combined A β and vehicle groups ($p < 0.05$). Consistent with the hypothesis that TGF β 2 is exerting neurotrophic effects, TGF β 2 alone appeared to protect neurons from trauma and oxidation ($p < 0.01$). TGF β 2-induced loss of LTP may relate to an actual LTP defect, or else due to an excitotoxic mechanism. These data demonstrate TGF β 2 exacerbation of A β induced neuron damage in adult mice *in vivo*.

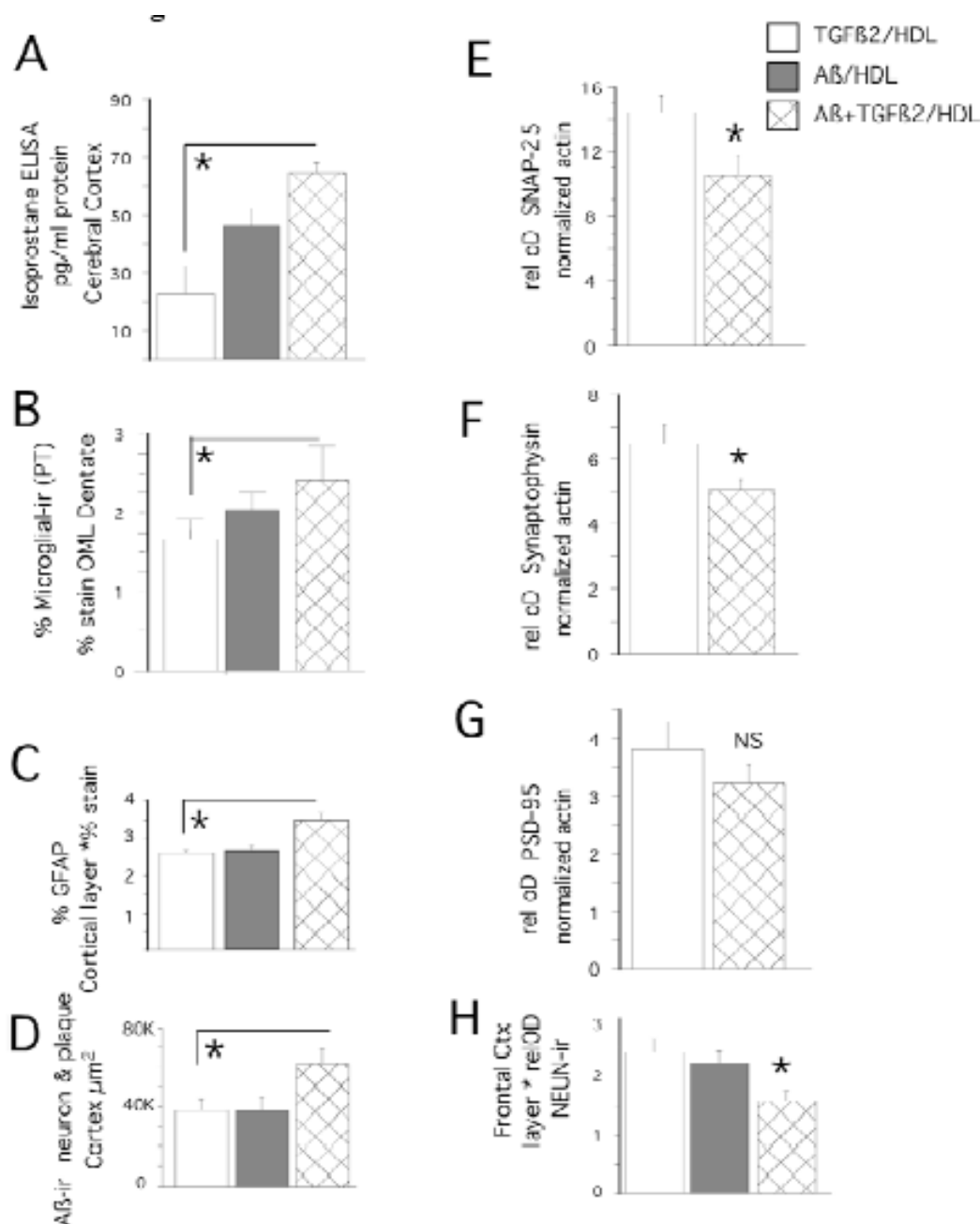


Fig. (3). TGFβ2 modulates Aβ effects on Oxidation and Presynaptic loss. Rats were anesthetized and infused with Aβ oligomers (42 and 40, 30 μg each) and TGFβ2 (20 ng) in 200 μl volume over 2 months (6.25 μl/day), HDL (200 μg) was used to reduce aggregation in the pump and as a vehicle peptide control. A) Compared to vehicle HDL, Aβ increased lipid peroxidation, which was further increased by co-infusing TGFβ2. Similarly, TGFβ2 increased the B) astroglial responses to Aβ as well as the C) inflammatory microglial response (phosphotyrosine, PT). D) Aβ Oligomer infusion did not result in significant Aβ deposition; however, Aβ co-infusion with TGFβ2 did increase Aβ immunoreactivity, which included both increased neuronal and plaque like staining. Presynaptic proteins E) synaptosomal-associated protein 25 (SNAP-25) and F) synaptophysin were measured by Western blot and shown to be preferentially reduced by co-infusion of TGFβ2 and Aβ, compared to Aβ alone or vehicle compared to post-synaptic proteins such as PSD-95 (G). Aβ infusion alone preferentially reduces post-synaptic proteins such as PSD-95, drebrin and NR2b, but not presynaptic loss (not shown), while presynaptic loss in AD is more closely related to neuron loss. H). Neuronal nuclei staining was preferentially reduced in the frontal cortex by coinfusion of TGFβ2 and Aβ compared to vehicle.

LRP-like receptor. Retention of spatial learning was impaired in mice intracerebroventricularly infused with Aβ and TGFβ2 without impairment of acquisition, suggesting that TGFβ2 alters Aβ's effects on the processing of spatial

memory learning. We present data supporting a role for TGFβ2 in modulating Aβ induced LTP deficits (Fig. 2) as well as lipid peroxidation, pre-synaptic protein loss and reductions in neuronal nuclei staining (Fig. 3).

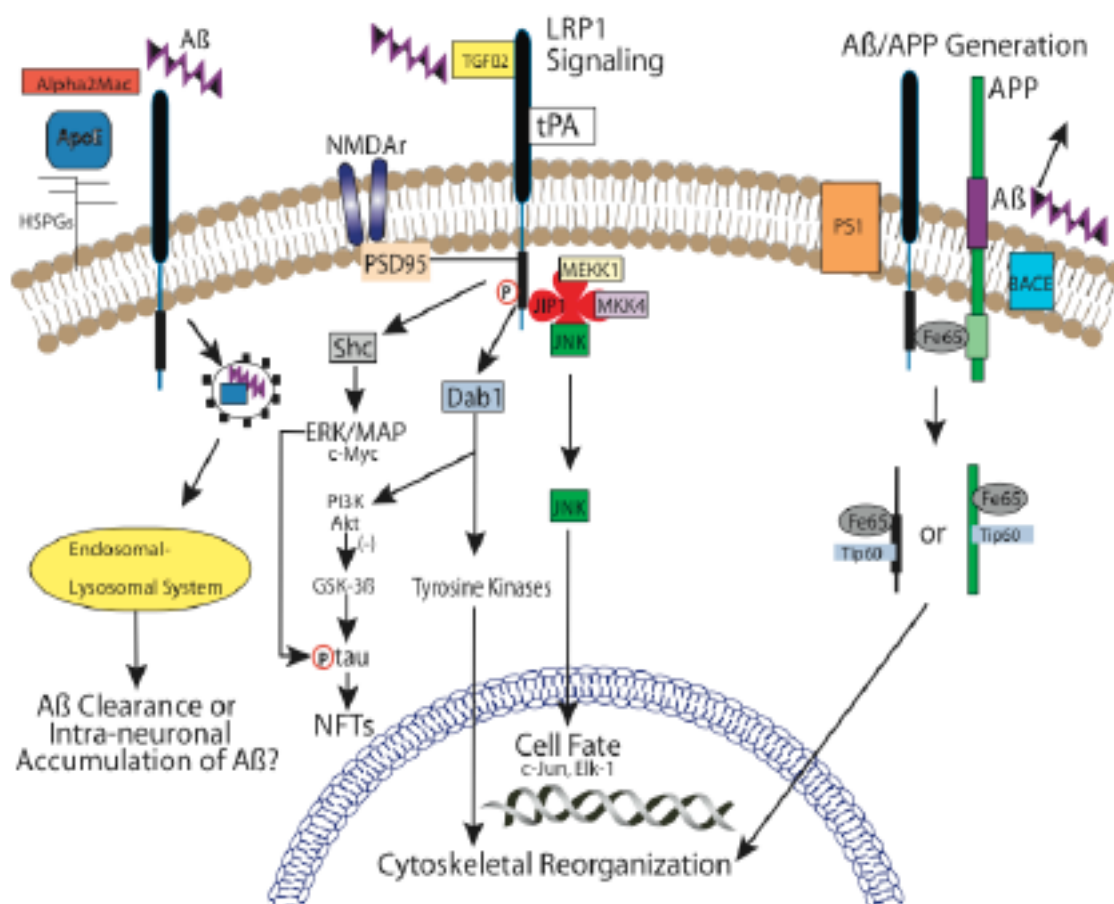


Fig. (4). Schematic of LRP signaling relevant to AD and Cognition.

A pathogenic role of LRP is also supported by data from an APP and LRP minigene overexpressing transgenic mouse model, showing an increase in the pool of small, soluble form of A β that correlates with impairments in spatial learning & memory [161]. Increased soluble A β could occur if there was increased clearance of A β or increased A β production.

SUMMARY

In summary, LRP plays an important role in neuronal function, and is intricately involved in modulation NMDA receptor function, supporting an important role in cognition. Many LRP ligands are genetically associated with Alzheimer's disease and implicated in its pathogenesis. Figure 4 depicts a schematic diagram showing interaction of A β with the LRP receptor. LRP on the endothelial cells of the blood brain barrier as well as on neurons, can mediate clearance of the toxic amyloid species but ultimately leads to their demise. LRP mediated endothelial cell death leads to reduced A β efflux out of the brain, while LRP-mediated neuronal death may be an early trigger in the Alzheimer pathogenic cascade.

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ABBREVIATIONS

AD	= Alzheimer's disease
Akt	= Serine/threonine kinase (also known as protein kinase B)
α 2 Mac	= Alpha 2 macroglobulin
ApoE	= Apolipoprotein E
ApoJ	= Apolipoprotein J (also clusterin)
APP	= Amyloid precursor protein
A β	= Beta-amyloid
BACE	= β secretase
Ced-6/GULP	= Caenorhabditis elegans cell death adaptor protein -6/human analog
CNS	= Central nervous system
CSF	= Cerebrospinal fluid
Dab-1	= Disabled 1
ECM	= Extracellular matrix
ERK	= Extracellular regulated kinase
γ -sec	= γ -secretase
HDL	= High density lipoprotein

HSPG	=	Heparan sulfate proteoglycan
IGFBP-3	=	Insulin growth factor binding protein-3
IGFBP-3r	=	Insulin growth factor binding protein-3 receptor
IP3	=	Inositol triphosphate
JIP-1	=	JNK interactive protein -1
JNK	=	cJun N-terminal kinase
LDLR	=	Low density lipoprotein receptor
LRP	=	Low density lipoprotein receptor-related protein
LTP	=	Long term potentiation
MAPKKK	=	(MEKK 1 and 4) -mitogen-activated protein kinase kinase kinase
MAPK	=	Mitogen activated protein kinase
MMP	=	Matrix metalloproteases
Neun	=	Neuronal nuclear protein
NMDA	=	N methyl D aspartate (glutamate)
NPXY	=	Sequence in LDLR that interacts with adaptor proteins Fe65 and Dab-1
NR2b	=	NMDA receptor subunit 2b
PDGF	=	Platelet-derived growth factor
PI3K	=	Phosphatidylinositol 3-kinase
PSD-95	=	Post-synaptic density protein -95
RAP	=	Receptor associated protein used as LRP and LDLR antagonist
Ras	=	Rat sarcome
Shc	=	SH2-containing $\alpha 2$ collagen-related proteins
SNAP-25	=	Synaptosomal-associated protein 25
Src	=	Sarcoma-related tyrosine kinase
TGF β	=	Transforming growth factor beta
Tg	=	Transgene
TIP60	=	Tat interactive protein -60
TPA	=	Tissue plasminogen activator
T β R-V	=	Transforming growth factor β receptor-V
VLDL	=	Very low density lipoprotein

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