Published in final edited form as:

J Neuroophthalmol. 2011 June ; 31(2): 139–146. doi:10.1097/WNO.0b013e31821b602c.

Low Density Lipoprotein Receptor-Related Protein (LRP) is Decreased in Optic Neuropathy of Alzheimer's Disease

Lloyd M. Cuzzo, BA¹, Fred N. Ross-Cisneros, BA¹, Kenneth M. Yee, BS^{1,2}, Michelle Y. Wang, MD¹, and Alfredo A. Sadun, MD, PhD¹

¹ Doheny Eye Institute, Dept of Ophthalmology; Keck School of Medicine at the University of Southern California, Los Angeles, CA

² VMR Institute, Huntington Beach, CA

Abstract

Objective—Alzheimer's disease (AD) is associated with optic nerve degeneration yet the underlying pathophysiology of this disease and the optic nerve disorder remains poorly understood. Low density lipoprotein receptor-related protein (LRP) is implicated in the pathogenesis of AD by mediating the transport of amyloid- β (A β) out of the brain into the systemic circulation. As a key player in the reaction to central nervous system (CNS) injury, astrocytes associate with LRP in AD. This study investigates the role of LRP and astrocytes in the pathogenesis of AD optic neuropathy.

Methods—To investigate the role of LRP and astrocytes in the pathogenesis of AD optic neuropathy, we conducted immunohistochemical (IHC) studies on postmortem optic nerves in AD patients ($n = 11$) and age-matched controls ($n = 10$) to examine the presence of LRP. Quantitative analyses using imaging software were used to document the extent of LRP in neural tissues. Axonal integrity was assessed by performing IHC on the subjects' optic nerves with an antibody to neurofilament (NF) protein. Double-immunofluorescence labeling was performed to investigate whether LRP colocalized with astrocytes expressing glial fibrillary acidic protein (GFAP).

Results—LRP expression was decreased in AD optic nerves compared to controls ($p < 0.001$). LRP immunoreactivity was observed in the microvasculature and perivascularly in close proximity to astrocytic processes. Colocalization of LRP in astrocytes of optic nerves was also demonstrated. The presence of optic neuropathy was confirmed in the AD optic nerves by demonstrating greatly reduced immunostaining for NF protein as compared to controls.

Conclusion—The reduction of LRP in the AD degenerative optic nerves supports the hypothesis that LRP may play a role in the pathophysiology of AD optic neuropathy.

Keywords

Alzheimer's disease; Alzheimer's optic neuropathy; astrocytes; low density lipoprotein receptor-related protein; microvasculature; optic nerve

Address correspondence to: Alfredo A. Sadun, MD, PhD, Flora Thornton Chair of Vision Research, Professor of Ophthalmology and Neurological Surgery, Doheny Eye Institute, USC-Keck School of Medicine, 1450 San Pablo St., Los Angeles, CA 90089-0228, asadun@usc.edu, Tel: 323 442 6417, Fax: 323 442 6407.

Conflict of interest statement: The authors declare no conflict of interest.

Background

Alzheimer's disease (AD) is the leading cause of dementia in the elderly, affecting 5.3 million people in the US, and 40% of people over 85 (1,2). AD may also manifest as optic nerve degeneration (3–7). This presents clinically in patients with mild to moderate AD as abnormal visual evoked responses, poor contrast sensitivity, and a reduction of retinal nerve fiber layer (RNFL) on OCT; in severe AD, there may be impaired visual acuity, visual fields, and color vision (4,8–11). Visual complaints in AD are often attributed to impaired cognition and may be overshadowed by higher cortical visual dysfunction which occurs frequently in AD patients.

The major pathological hallmark of AD is the accumulation of amyloid-beta protein ($A\beta$), a neurotoxic peptide centrally involved in the pathogenesis of AD (12–16). This may be due to faulty clearance of $A\beta$ from the CNS (15, 17–24). The blood-brain barrier (BBB), largely maintained by tight junctions between cerebrovascular endothelial cells (25), limits the transport of polar solutes such as $A\beta$. Receptor mediated-transport accounts for most $A\beta$ transported across the BBB (17, 19, 24, 26–28). LRP is the major receptor at the BBB responsible for clearing $A\beta$ from the CNS (19, 29, 30). A decreased amount of LRP is found in the cerebral microvasculature in both human AD brains and transgenic AD animal models and is associated with regional accumulation of $A\beta$ as compared to controls (23, 24, 30–32).

LRP also exists in a soluble form (sLRP) in plasma and has been shown to bind to 70–90% of plasma $A\beta$ preventing its access to the CNS (33,34). In AD individuals, levels of sLRP in plasma are reduced, thereby allowing free $A\beta$ in plasma to enter the CNS (29).

LRP is also found in astrocytes (35–37). In response to injury, astrocytes undergo both hypertrophy and hyperplasia displaying prominent fibrous ramifying processes, enhanced immunoreactivity for glial fibrillary acidic protein (GFAP), and increased release of bioactive molecules. LRP is expressed by astrocytes in normal human brains, but expression is increased in AD (35,37).

Since the optic nerve is part of the CNS, we hypothesized that LRP was decreased in optic nerves of AD patients possibly contributing to $A\beta$ accumulation as part of the pathogenesis of AD optic neuropathy. We conducted an immunohistochemical study, first to histologically verify the presence of AD optic neuropathy, and second to characterize the presence of LRP and the extent of its association with the microvasculature and astrocytes in AD optic nerves.

Methods

Human autopsy specimens

Postmortem retrobulbar optic nerves were obtained from 11 AD patients (81.0 ± 12.0) and 10 control subjects (72.8 ± 13.9) (mean $\pm$ standard deviation). AD tissues were provided by the Alzheimer's Disease Research Center at the University of Southern California and controls were obtained from the Lions Eye Bank of Oregon. The diagnosis of AD was confirmed clinicopathologically (38–40). Control and AD optic nerves were from subjects with no history of neurodegenerative disorders. Patient data is summarized in Table 1.

Tissue processing

Nerves were immersion-fixed in 10% neutral buffered formalin immediately following enucleation of eyes with optic nerves attached. Dissections of the optic nerves into longitudinal profiles 5 mm in length were performed approximately 7–10 mm behind the globe. Tissues were dehydrated in ethanol and processed for paraffin embedding. The

paraffin tissue blocks were cut at 5 μ m on a retractable microtome and the tissue sections were placed on electrostatically charged glass microscope slides for immunohistochemistry.

Immunohistochemistry: immunoperoxidase labeling

Tissue sections were deparaffinized, rehydrated and antigen retrieval was performed in a 1 $\times$ citrate buffer, pH 6.2 (BioGenex, San Ramon, CA) within a steamer bath. The bath was microwaved at 480 W for 10 minutes. The sections were rinsed with tris-buffered saline and endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide. Tissue sections were incubated with a monoclonal mouse anti-human LRP primary antibody (EMD Chemicals Inc., Gibbstown, NJ) at a dilution of 1:1000 in a humidity chamber for 1 hour. Negative control sections were incubated in antibody diluent (Dako North America, Inc., Carpinteria, CA) in the absence of primary antibody. Tissue sections were next incubated in a goat anti-mouse secondary antibody conjugated to horseradish peroxidase (Dako) for 30 minutes. The substrate 3,3'-diaminobenzidine (Dako) was added to produce a brown reaction product (chromagen). All AD and control tissue sections were either counterstained with Mayer's hematoxylin (Dako) for general nuclear morphology or immunostained for LRP without counterstain for densitometry analysis. Finally, the sections were dehydrated in alcohol, cleared in xylene, and cover-slipped. The stained nerves were observed on a Zeiss Axioskop light microscope and images were captured with a Spot II digital camera.

To examine the axonal integrity in both control and AD optic nerve samples, immunoperoxidase staining was performed with a monoclonal mouse anti-human neurofilament protein primary antibody (Dako) at a dilution of 1:500 and counterstained with hematoxylin utilizing the methodology above.

Immunohistochemistry: double-immunofluorescence labeling

Tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval as described previously. Sections were washed with phosphate-buffered saline (PBS) and incubated with 1% BSA with 0.1% Triton X-100 in PBS for 15 minutes. Tissues were incubated with a monoclonal mouse anti-human LRP primary antibody (EMD Chemicals), as used previously for immunoperoxidase staining, at a dilution of 1:1000 at 37°C for 1 hour in a humidity chamber. Goat anti-mouse secondary antibody conjugated to Fluorescein Iso-Thiocyanate (FITC) (Dako) was added at a dilution of 1:20 for 45 minutes. To determine the association of LRP with astrocytes, tissue sections were incubated with a second primary antibody, a polyclonal rabbit anti-human glial fibrillary acidic protein (GFAP) antibody (Dako) at a dilution of 1:500 at 37°C for 1 hour. A swine anti-rabbit secondary antibody conjugated to Tetramethyl Rhodamine Iso-Thiocyanate (TRITC) (Dako) was added at a dilution of 1:60 for 45 minutes. Tissue sections were mounted with Vectashield containing DAPI (4',6-diamidino-2-phenylindole) for nuclear staining. Images were captured on a Zeiss LSM 510 confocal microscope.

Quantitative and qualitative analyses

LRP immunolabeling was quantitatively graded by scanning slides with a light microscope at a magnification of 1000 $\times$. Twenty images were captured from central to peripheral regions of each nerve section in a systematic, linear, non-overlapping fashion, and analyzed with AnalySIS image software. The average immunopositive area for every slide and average ratio of specific immunolabeled area to total optic nerve area was recorded.

Slides immunohistochemically stained for neurofilament (NF) were viewed under a light microscope at 200 $\times$ and 1000 $\times$. The intensity of immunoreactivity for NF staining was evaluated qualitatively and AD optic nerve slides were compared to controls.

Results

Immunoperoxidase localization of LRP in AD and control optic nerves

Immunoperoxidase staining demonstrated that LRP was decreased in AD optic nerves, especially within the vasculature, as compared to controls (Fig. 1). We observed increased perivascular LRP staining within AD astrocytes in a punctate, perinuclear pattern (Fig. 2B & D). Our quantitative analysis using densitometry showed that total expression of LRP was significantly reduced in the AD group as compared to controls (Fig. 3 & 4). The extent of LRP immunolabeling was $121.5 \pm 13.3 \mu\text{m}^2$ (mean $\pm$ standard error) in the control optic nerves and $18.9 \pm 3.5 \mu\text{m}^2$ in the AD optic nerves ($p < 0.0001$, Fig. 4). The 95% confidence interval of the mean quantity of LRP immunostaining was $91.4 \mu\text{m}^2 - 151.6 \mu\text{m}^2$ in controls vs. $11.2 \mu\text{m}^2 - 26.6 \mu\text{m}^2$ in AD optic nerves ($p < 0.0001$).

Immunoperoxidase labeling of NF in AD and control optic nerves

Immunoperoxidase staining of NF, as a marker of axonal integrity, demonstrated clear degeneration of AD optic nerves compared to control tissues (Fig. 5). This example is representative of the study population. The vast majority of the AD optic nerves from the Alzheimer's Disease Research Center came from patients with severe AD (Braak stage V–VI, Table 1) and were all severely affected as evidenced by greatly decreased NF staining.

Double-immunofluorescence labeling for LRP and astrocytes

Double-immunofluorescence labeling revealed colocalization of LRP within GFAP-positive astrocytes of optic nerves in both age-matched controls (Fig. 2A & C) and AD nerves (Fig. 2B & D), but to a greater degree in the AD tissues.

Discussion

We demonstrated a decrease in the expression of LRP in AD optic nerves compared to age-matched controls; this corroborates with other studies showing decreased LRP in AD patients (24, 30). We presented histochemical evidence of AD optic nerve degeneration by showing a large decrease in NF staining, which is interpreted as a large loss of axons. This is consistent with previous reports identifying optic neuropathy in AD patients (3–7).

In humans and in animal models LRP has been shown to be responsible for clearing A β out of the CNS via transport across the BBB (19, 29, 30). Reduced LRP has been identified in the cerebral vasculature of AD patients and appears to be associated with the accumulation of A β in the brain believed to initiate pathogenic cascades seen in AD. (17). Furthermore, the accumulation of A β within cerebral blood vessels in AD, known as cerebral amyloid angiopathy (CAA), is associated with the cognitive decline of this disease. Decreased clearance of A β across the BBB may contribute to CAA and parenchymal A β deposits (17). This is supported by studies that have demonstrated that impairment in the clearance of CNS beta-amyloid may be fundamental to the pathophysiology of Alzheimer's Disease (41). These findings suggest that the decreased expression of LRP found in our study could, by reducing the efflux of A β out of the optic nerve, play an important role in the pathogenesis of AD optic neuropathy.

SLRP under normal conditions is the major endogenous A β chaperone protein in plasma, acting as a peripheral “sink” pulling A β from the brain to the blood, preventing A β entrance into the CNS and facilitating its clearance in the liver (29, 34). However, there is decreased sLRP in AD, most of which may become impaired by oxidation initiated by reactive oxygen species generated by the receptor for advanced glycation end products (RAGE) proinflammatory cascade (17). RAGE receptors in the BBB are responsible for the influx of

A β into the CNS and have been shown to be upregulated in AD optic neuropathy (42). This suggests that the sLRP “peripheral sink” for A β is compromised in AD, leading to elevated free A β levels in plasma that exacerbate the increased A β levels in the CNS via RAGE-mediated transport across the BBB (29).

We also found a relative increase in LRP staining in AD astrocytes (Fig. 2). While quantification of the increased LRP staining in AD astrocytes was not performed, the example shown in Fig. 2 is representative of the nerves sampled, as supported by other studies (35,43,44). We demonstrated colocalization of LRP within astrocytes in optic nerves, usually in a perinuclear fashion (Fig. 2), which is also supported by previous studies (44). The increased perivascular LRP staining observed in astrocytes in AD nerves likely represents astrocytic foot processes abutting against the vasculature (44). The expression of LRP in reactive astrocytes, coupled with the perinuclear Golgi network staining, suggests these cells were actively manufacturing LRP. LRP production by these reactive astrocytes may have been a compensatory response for decreased LRP in the neighboring vasculature. This could also be part of a process of monocyte recruitment to the area of injury, as LRP binds ligands involved in cell migration in response to injury or injection such as C3 and uPA (16,45). Increasing LRP might also restore A β homeostasis and contribute to the development of neural networks (16, 46,47).

Astrocytes are normally neuroprotective, functioning to monitor the surrounding tissue environment, supply nutrients, support BBB functions, and repair by way of gliosis (48). When chronically activated, they release neurotoxic cytokines and activate destructive pathways (49,50). Cellular LRP not only clears A β but also has the potential to produce A β . LRP can internalize amyloid precursor protein (APP) and deliver it to the endosomal compartment where it can undergo amyloidogenic processing by β -secretase. This is followed by γ -secretase action to produce A β . As a consequence of increased APP internalization, LRP can enhance A β secretion (31,51). However, LRP has been shown to modulate APP trafficking between the cell surface and compartments of the endocytic pathway by interacting with APP in the endoplasmic reticulum (ER) (52). By doing so, LRP has been shown to retain APP in the ER, reducing the levels of APP that reach the plasma membrane (53). Thus, LRP is intricately involved with cellular pathways that suppress A β generation but that can be altered to facilitate production (54). While we were able to show colocalization of LRP within astrocytes, future studies examining LRP immunolabeling with microglial markers and neurofilament protein may further uncover the complicated role of LRP in the pathogenesis of AD optic neuropathy.

It should be noted that while the AD subjects were slightly older than the controls, this was not statistically significant ($p = 0.13$). LRP has been shown to decrease with normal aging in rodents, non-human primates, and in AD patients, but it has not been shown to decrease with age in normal humans (29).

A potential therapeutic approach to AD optic neuropathy might be to target the interruption of LRP mediated internalization of APP, a necessary step for LRP-facilitated production of A β . LRP and APP must form a complex with the adaptor protein FE65 to internalize APP from the plasma membrane in order to process it into A β (55). Interrupting this process could harness the protective effects of LRP effluxing A β from the CNS without the downside of producing more A β . This is supported by studies which suggest that LRP can be protective against AD (56). Another potential avenue of treatment would be to develop a synthetic sLRP which could be administered intravenously that would bind to A β with high affinity and minimal toxicity. This could shift the A β transport equilibrium towards the plasma (21). Furthermore, other studies have shown that administering recombinant LRP clusters can effectively sequester plasma A β in human AD plasma and in AD mice (18). In

mice, sequestration resulted in reductions of A β accumulation in the brain parenchyma and vasculature. This resulted in improvements in memory, learning, and cerebral blood flow responses (57).

In conclusion, our findings demonstrate a significantly reduced expression of LRP in AD optic nerves as compared to age matched controls. This result, along with the LRP expression in the AD microvasculature, is concordant with other investigators' work and supports the hypothesis that decreased LRP may play a role in the underlying pathophysiology of AD optic neuropathy by reduced efflux of A β out of the optic nerve into the systemic circulation.

Acknowledgments

Funding: This work was supported by Research to Prevent Blindness Institutional Grant and Medical Student Eye Research Fellowship, NIA Grant # P50-AG05142, and NIH Grant # EY03040.

The authors express their gratitude to the USC Alzheimer's Disease Research Center and Lions Eye Bank of Oregon for supplying the tissues. This work was supported by a Research to Prevent Blindness Institutional Grant and Medical Student Eye Research Fellowship, NIA Grant # P50-AG05142, NIH Grant # EY03040, and VMR Consulting, Inc., Huntington Beach, CA. Additionally, the authors thank Laurie Dustin for her assistance with the statistics and Ernesto and Eric Barron for facilitating the use of the confocal microscope and formatting the figures.

References

1. Alzheimer's Association. 2010 Alzheimer's disease facts and figures. *Alzheimers Dement*. 2010; 6:158–194. [PubMed: 20298981]
2. Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch Neurol*. 2003; 60:1119–1122. [PubMed: 12925369]
3. Hinton DR, Sadun AA, Blanks JC, Miller CA. Optic-Nerve Degeneration in Alzheimer's Disease. *NEJM*. 1986; 315:485–487. [PubMed: 3736630]
4. Sadun AA. The optic neuropathy of Alzheimer's disease. *Metab Pediatr Syst Ophthalmol*. 1989; 12:64–68. [PubMed: 2770524]
5. Sadun AA, Bassi CJ. Optic nerve damage in Alzheimer's disease. *Ophthalmology*. 1990; 97:9–17. [PubMed: 2314849]
6. Danesh-Meyer HV, Birch H, Ku JY, Carroll S, Gamble G. Reduction of optic nerve fibers in patients with Alzheimer disease identified by laser imaging. *Neurology*. 2006; 67:1852–1854. [PubMed: 17130422]
7. Syed AB, Armstrong RA, Smith CU. A quantitative analysis of optic nerve axons in elderly control subjects and patients with Alzheimer's disease. *Folia Neuropathol*. 2005; 43:1–6. [PubMed: 15827884]
8. Sadun AA, Borchert M, DeVita E, Hinton DR, Bassi CJ. Assessment of visual impairment in patients with Alzheimer's disease. *Am J Ophthalmol*. 1987; 104:113–120. [PubMed: 3618708]
9. Katz B, Rimmer S. Ophthalmologic manifestations of Alzheimer's disease. *Surv Ophthalmol*. 1989; 34:31–43. [PubMed: 2678551]
10. Iseri PK, Altinas O, Tokay T, Yuksel N. Relationship between cognitive impairment and retinal morphological and visual functional abnormalities in Alzheimer disease. *J Neuroophthalmol*. 2006; 26:18–24. [PubMed: 16518161]
11. Parisi V. Correlation between morphological and functional retinal impairment in patients affected by ocular hypertension, glaucoma, demyelinating optic neuritis and Alzheimer's disease. *Semin Ophthalmol*. 2003; 18:50–57. [PubMed: 14566623]
12. Selkoe DJ. Deciphering the genesis and fate of amyloid beta-protein yields novel therapies for Alzheimer disease. *J Clin Invest*. 2002; 110:1375–1381. [PubMed: 12438432]
13. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*. 2002; 297:353–356. [PubMed: 12130773]

14. Tanzi RE, Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. *Cell*. 2005; 120:545–555. [PubMed: 15734686]
15. Rosenberg RN. The molecular and genetic basis of AD: the end of the beginning: the 2000 Wartenberg lecture. *Neurology*. 2000; 54:2045–2054. [PubMed: 10851361]
16. Herz J, Strickland DK. LRP: a multifunctional scavenger and signaling receptor. *J Clin Invest*. 2001; 108:779–784. [PubMed: 11560943]
17. Bell RD, Zlokovic BV. Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. *Acta Neuropathol*. 2009; 118:103–113. [PubMed: 19319544]
18. Zlokovic BV. New therapeutic targets in the neurovascular pathway in Alzheimer's disease. *Neurotherapeutics*. 2008; 5:409–414. [PubMed: 18625452]
19. Deane R, Zlokovic BV. Role of the blood-brain barrier in the pathogenesis of Alzheimer's disease. *Curr Alzheimer Res*. 2007; 4:191–197. [PubMed: 17430246]
20. Deane R, Wu Z, Zlokovic BV. RAGE (yin) versus LRP (yang) balance regulates alzheimer amyloid beta-peptide clearance through transport across the blood-brain barrier. *Stroke*. 2004; 35:2628–2631. [PubMed: 15459432]
21. Zlokovic BV. Clearing amyloid through the blood-brain barrier. *J Neurochem*. 2004; 89:807–811. [PubMed: 15140180]
22. Hyman BT, Strickland D, Rebeck GW. Role of the low-density lipoprotein receptor-related protein in beta-amyloid metabolism and Alzheimer disease. *Arch Neurol*. 2000; 57:646–650. [PubMed: 10815129]
23. Wang YJ, Zhou HD, Zhou XF. Clearance of amyloid-beta in Alzheimer's disease: progress, problems and perspectives. *Drug Discov Today*. 2006; 11:931–938. [PubMed: 16997144]
24. Deane R, Bell RD, Sagare A, Zlokovic BV. Clearance of amyloid-beta peptide across the blood-brain barrier: implication for therapies in Alzheimer's disease. *CNS Neurol Disord Drug Targets*. 2009; 8:16–30. [PubMed: 19275634]
25. Begley DJ, Brightman MW. Structural and functional aspects of the blood-brain barrier. *Prog Drug Res*. 2003; 61:39–78. [PubMed: 14674608]
26. DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM. Brain to plasma amyloid-beta efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science*. 2002; 295:2264–2267. [PubMed: 11910111]
27. Tanzi RE, Moir RD, Wagner SL. Clearance of Alzheimer's Aβ peptide: the many roads to perdition. *Neuron*. 2004; 43:605–608. [PubMed: 15339642]
28. Donahue JE, Flaherty SL, Johanson CE, Duncan JA 3rd, Silverberg GD, Miller MC, Tavares R, Yang W, Wu Q, Sabo E, Hovanesian V, Stopa EG. RAGE, LRP-1, and amyloid-beta protein in Alzheimer's disease. *Acta Neuropathol*. 2006; 112:405–415. [PubMed: 16865397]
29. Deane R, Sagare A, Zlokovic BV. The role of the cell surface LRP and soluble LRP in blood-brain barrier Aβ clearance in Alzheimer's disease. *Curr Pharm Des*. 2008; 14:1601–1605. [PubMed: 18673201]
30. Shibata M, Yamada S, Kumar SR, Calero M, Bading J, Frangione B, Holtzman DM, Miller CA, Strickland DK, Ghiso J, Zlokovic BV. Clearance of Alzheimer's amyloid-ss(1–40) peptide from brain by LDL receptor-related protein-1 at the blood-brain barrier. *J Clin Invest*. 2000; 106:1489–1499. [PubMed: 11120756]
31. Waldron E, Jaeger S, Pietrzik CU. Functional role of the low-density lipoprotein receptor-related protein in Alzheimer's disease. *Neurodegener Dis*. 2006; 3:233–238. [PubMed: 17047362]
32. Van Uden E, Mallory M, Veinbergs I, Alford M, Rockenstein E, Masliah E. Increased extracellular amyloid deposition and neurodegeneration in human amyloid precursor protein transgenic mice deficient in receptor-associated protein. *J Neurosci*. 2002; 22:9298–9304. [PubMed: 12417655]
33. Quinn KA, Pye VJ, Dai YP, Chesterman CN, Owensby DA. Characterization of the soluble form of the low density lipoprotein receptor-related protein (LRP). *Exp Cell Res*. 1999; 251:433–441. [PubMed: 10471328]
34. Quinn KA, Grimsley PG, Dai YP, Tapner M, Chesterman CN, Owensby DA. Soluble low density lipoprotein receptor-related protein (LRP) circulates in human plasma. *J Biol Chem*. 1997; 272:23946–23951. [PubMed: 9295345]

35. Tooyama I, Kawamata T, Akiyama H, Kimura H, Moestrup SK, Gliemann J, Matsuo A, McGeer PL. Subcellular localization of the low density lipoprotein receptor-related protein (alpha 2-macroglobulin receptor) in human brain. *Brain Res.* 1995; 691:235–238. [PubMed: 8590059]
36. Williams SE, Kounnas MZ, Argraves KM, Argraves WS, Strickland DK. The alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein and the receptor-associated protein. An overview. *Ann N Y Acad Sci.* 1994; 737:1–13. [PubMed: 7524391]
37. Wolf BB, Lopes MB, VandenBerg SR, Gonias SL. Characterization and immunohistochemical localization of alpha 2-macroglobulin receptor (low-density lipoprotein receptor-related protein) in human brain. *Am J Pathol.* 1992; 141:37–42. [PubMed: 1632469]
38. Braak H, Alafuzoff I, Arzberger T, Kretschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol.* 2006; 112:389–404. [PubMed: 16906426]
39. Hyman BT, Trojanowski JQ. 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. *J Neuropathol Exp Neurol.* 1997; 56:1095–1097. [PubMed: 9329452]
40. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology.* 1991; 41:479–486. [PubMed: 2011243]
41. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Bateman RJ. Decreased clearance of CNS beta-amyloid in Alzheimer's disease. *Science.* 330:1774. [PubMed: 21148344]
42. Wang MY, Ross-Cisneros FN, Aggarwal D, Liang CY, Sadun AA. Receptor for advanced glycation end products is upregulated in optic neuropathy of Alzheimer's disease. *Acta Neuropathol.* 2009; 118:381–389. [PubMed: 19277685]
43. Rebeck GW, Harr SD, Strickland DK, Hyman BT. Multiple, diverse senile plaque-associated proteins are ligands of an apolipoprotein E receptor, the alpha 2-macroglobulin receptor/low-density-lipoprotein receptor-related protein. *Ann Neurol.* 1995; 37:211–217. [PubMed: 7531418]
44. Lopes MB, Bogaev CA, Gonias SL, VandenBerg SR. Expression of alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein is increased in reactive and neoplastic glial cells. *FEBS Lett.* 1994; 338:301–305. [PubMed: 8307199]
45. Johnsen M, Lund LR, Romer J, Almholt K, Dano K. Cancer invasion and tissue remodeling: common themes in proteolytic matrix degradation. *Curr Opin Cell Biol.* 1998; 10:667–671. [PubMed: 9818179]
46. May P, Rohlmann A, Bock HH, Zurhove K, Marth JD, Schomburg ED, Noebels JL, Beffert U, Sweatt JD, Weeber EJ, Herz J. Neuronal LRP1 functionally associates with postsynaptic proteins and is required for normal motor function in mice. *Mol Cell Biol.* 2004; 24:8872–8883. [PubMed: 15456862]
47. Herz J, Bock HH. Lipoprotein receptors in the nervous system. *Annu Rev Biochem.* 2002; 71:405–434. [PubMed: 12045102]
48. Frosch, M.; Anthony, DC.; de Girolami, U. Robbins and Cotran pathologic basis of disease. 7. Philadelphia: Elsevier Saunders; 2005.
49. Pike CJ, Cummings BJ, Monzavi R, Cotman CW. Beta-amyloid-induced changes in cultured astrocytes parallel reactive astrogliosis associated with senile plaques in Alzheimer's disease. *Neuroscience.* 1994; 63:517–531. [PubMed: 7891862]
50. Abraham CR. Reactive astrocytes and alpha1-antichymotrypsin in Alzheimer's disease. *Neurobiol Aging.* 2001; 22:931–936. [PubMed: 11755001]
51. Bu G, Cam J, Zerbiniatti C. LRP in amyloid-beta production and metabolism. *Ann N Y Acad Sci.* 2006; 1086:35–53. [PubMed: 17185504]
52. Bell RD, Deane R, Chow N, Long X, Sagare A, Singh I, Streib JW, Guo H, Rubio A, Van Nostrand W, Miano JM, Zlokovic BV. SRF and myocardin regulate LRP-mediated amyloid-beta clearance in brain vascular cells. *Nat Cell Biol.* 2009; 11:143–153. [PubMed: 19098903]

53. Pietrzik CU, Busse T, Merriam DE, Weggen S, Koo EH. The cytoplasmic domain of the LDL receptor-related protein regulates multiple steps in APP processing. *EMBO J.* 2002; 21:5691–5700. [PubMed: 12411487]
54. Yoon IS, Pietrzik CU, Kang DE, Koo EH. Sequences from the low density lipoprotein receptor-related protein (LRP) cytoplasmic domain enhance amyloid beta protein production via the beta-secretase pathway without altering amyloid precursor protein/LRP nuclear signaling. *J Biol Chem.* 2005; 280:20140–20147. [PubMed: 15772078]
55. Pietrzik CU, Yoon IS, Jaeger S, Busse T, Weggen S, Koo EH. FE65 constitutes the functional link between the low-density lipoprotein receptor-related protein and the amyloid precursor protein. *J Neurosci.* 2004; 24:4259–4265. [PubMed: 15115822]
56. Kang DE, Pietrzik CU, Baum L, Chevallier N, Merriam DE, Kounnas MZ, Wagner SL, Troncoso JC, Kawas CH, Katzman R, Koo EH. Modulation of amyloid beta-protein clearance and Alzheimer's disease susceptibility by the LDL receptor-related protein pathway. *J Clin Invest.* 2000; 106:1159–1166. [PubMed: 11067868]
57. Sagare A, Deane R, Bell RD, Johnson B, Hamm K, Pendu R, Marky A, Lenting PJ, Wu Z, Zarcone T, Goate A, Mayo K, Perlmutter D, Coma M, Zhong Z, Zlokovic BV. Clearance of amyloid-beta by circulating lipoprotein receptors. *Nat Med.* 2007; 13:1029–1031. [PubMed: 17694066]

Fig. 1. Immunoperoxidase labeling of vasculature within human optic nerves for LRP. Examples of a capillary from a normal age-matched control (A) and an AD (B) patient. Note increased staining associated with the vessel wall (arrows) and perivascular tissue (arrowheads) of the capillary in control (A) ($\times 1000$).

Fig. 2. Immunoperoxidase and immunofluorescence labeling of LRP among glial cells in human optic nerves. Comparisons of LRP staining in glial cells can be seen in age-matched control (A) and AD (B) (See insets for example). Note increased perinuclear staining (arrows) in the AD glial cells (B). Lower part of figure (C and D) shows double-immunofluorescence labeling of the optic nerves for LRP coupled to FITC (*green*) and GFAP coupled to TRITC (*red*) counterstained with DAPI (*blue*) to label nuclei in control (C) and AD (D). LRP expression was observed in the cytoplasm of the GFAP-positive astrocytes (arrows). LRP expression was also observed in astrocytes mainly in a perinuclear region (arrowheads). Note increased glial staining in AD nerve (D), consistent with immunohistochemistry shown above (B). ($\times 1000$)

Fig. 3.

Examples of micrograph sampling for densitometry analysis in age-matched control and AD optic nerves. Immunoperoxidase labeling for LRP can be observed in a control (A) and an AD (B) optic nerve. In C and D, note corresponding highlighted color for density of the LRP label in control (yellow) and AD (red) optic nerves, as seen in A and B, respectively. ($\times 1000$)

Fig. 4.
The comparison of the mean area of density for LRP immunolabeling in control optic nerves ($121.5 \mu\text{m}^2$) was greater than AD nerves ($18.9\mu\text{m}^2$) ($p<0.0001$).

Fig. 5.

Examples of immunostaining for NF protein in an age-matched control (A and C) and an AD (B and D) optic nerve. Note paucity of axons in the AD optic nerve (B and D) as highlighted by decreased NF immunostaining. This exemplified the optic nerve degeneration observed in the majority of the AD nerves. (A, B $\times 200$; C, D $\times 1000$)

Table 1

Demographic and clinical data of donor optic nerves

Case	Age (years)	Sex	Braak Stage	Duration of disease (years)	Postmortem interval (h)
CONTROLS					
1	89	F	-	-	5.0
2	93	M	-	-	6.5
3	69	F	-	-	5.5
4	52	M	-	-	9.8
5	56	F	-	-	9.3
6	68	M	-	-	8.1
7	80	M	-	-	5.4
8	80	M	-	-	6.3
9	68	F	-	-	4.1
10	64	F	-	-	11.0
AD					
1	83	M	VI	7	13
2	71	M	VI	12	13.7
3	82	M	V	6	5.8
4	79	F	V	9	5
5	87	F	V	N/A	6
6	86	M	V	3	9
7	98	F	V	N/A	7.5
8	62	M	V	3.5	5
9	80	M	V	N/A	6.8
10	64	F	VI	N/A	3.5
11	99	F	III	7	12.3

AD Alzheimer's disease, Braak System of Staging AD³⁸, - none, N/A not available