Receptor-Associated Protein (RAP) Plays a Central Role in Modulating Aβ Deposition in APP/PS1 Transgenic Mice

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Abstract

Background: Receptor associated protein (RAP) functions in the endoplasmic reticulum (ER) to assist in the maturation of several membrane receptor proteins, including low density lipoprotein receptor-related protein (LRP) and lipoprotein receptor 11 (SorLA/LR11). Previous studies in cell and mouse model systems have demonstrated that these proteins play roles in the metabolism of the amyloid precursor protein (APP), including processes involved in the generation, catabolism and deposition of β-amyloid (Aβ) peptides.

Methodology/Principal Findings: Mice transgenic for mutant APPswe and mutant presenilin 1 (PS1dE9) were mated to mice with homozygous deletion of RAP. Unexpectedly, mice that were homozygous null for RAP and transgenic for APPswe/PS1dE9 showed high post-natal mortality, necessitating a shift in focus to examine the levels of amyloid deposition in APPswe/PS1dE9 mice that were hemizygous null for RAP. Immunoblot analysis confirmed 50% reductions in the levels of RAP with modest reductions in the levels of proteins dependent upon RAP for maturation (LRP trend towards a 20% reduction; SorLA/LR11 statistically significant 15% reduction (p<0.05)). Changes in the levels of these proteins in the brains of APPswe/PS1dE9(+/−)/RAP(+/−) mice correlated with 30–40% increases in amyloid deposition by 9 months of age.

Conclusions/Significance: Partial reductions in the ER chaperone RAP enhance amyloid deposition in the APPswe/PS1dE9 model of Alzheimer amyloidosis. Partial reductions in RAP also affect the maturation of LRP and SorLA/LR11, which are each involved in several different aspects of APP processing and Aβ catabolism. Together, these findings suggest a central role for RAP in Alzheimer amyloidogenesis.

Introduction

The genetic alterations in APP and PS1 (and PS2) that cause early onset familial Alzheimer’s disease (AD) are well characterized, as are the consequences that disease-linked mutations in these proteins have on the endoproteolytic processing of APP (for review see [1]). However, the cause of sporadic AD is less well defined. The major genetic risk factor for sporadic AD is the presence of the E4 allele of the apolipoprotein E (Apo E) gene [2]. Polymorphisms in the LRP and SorLA/LR11 genes have also been associated with increased risk for Alzheimer’s disease [3,4]. However, there are studies that refute the LRP finding [5–8].

LRP, a member of the low density lipoprotein (LDL) receptor family, is a large, multifunctional endocytic receptor, highly expressed in hepatocytes, fibroblasts, activated astrocytes, and neurons (reviewed by Willnow [9]). Mature LRP is composed of two subunits, 515 kDa (α-chain) and 85 kDa (β-chain), which are produced by proteolytic cleavage from a single polypeptide precursor of 600 kDa in the trans-Golgi network [10]. Maturation of LRP to the trans-Golgi network is partially dependent upon the presence of LRP-receptor-associated protein (RAP), which binds to LRP at multiple sites to block the receptor’s ability to interact with its ligands [11]. Premature binding of LRP ligands to the receptor interferes with maturation of LRP, and similar receptors, and with translocation to the plasma membrane (reviewed by Willnow [12]).

LRP is a potentially important etiological agent for AD because three proteins clearly involved in AD - APP, APOE, and α2-macroglobulin (α2M), are ligands of LRP [for review see [13]]. Importantly, each of these proteins plays significant roles in the production and metabolism of Aβ peptide, the principal component of amyloid plaques that characterize AD. LRP has been found to bind to the amyloid precursor protein (APP) in a manner that alters its trafficking and processing [14–18]. LRP-deficient cells secrete less Aβ and restoring LRP function substantially increases Aβ production [15]. One study has reported reduced levels of LRP in the brains of AD patients [19], but this finding has not been confirmed by others [20]. LRP also appears to mediate the clearance of Aβ that is bound to α2M or ApoE[19,21–23]. It is, therefore, important to examine the role of LRP in the pathogenesis of Alzheimer-type amyloidosis in...
transgenic mouse models of this pathology. Unfortunately, the large size of the LRP gene (>10 kb) makes production of transgenic animals that express the whole protein difficult by standard cDNA approaches. Zerbinatti et al. [24] reported that overexpression of an LRP minireceptor in the PDAPP mouse model of AD, resulted in increased levels of soluble Aβ but did not impact amyloid burden. Similarly, it is not possible to examine adult animals that lack LRP because targeted deletion of the LRP gene in mice leads to death of the embryo at day 13.5 [25]. However, the levels of functional LRP in the brain are regulated by RAP. Unlike LRP, mice deficient in RAP are viable and have normal lifespans because the maturation of LRP is only partially dependent upon RAP; neurons of RAP KO mice show 75% reductions in the levels of endoproteolytically processed, mature, LRP [26]. It is noteworthy that expression of PS1 variants M146L or L286V, which are linked to familial AD, also cause 40% reductions in the levels of mature LRP in brain [27].

In the present study, we have utilized RAP KO mice crossed with mice that co-express mutant APP (APPswe) and PS1 (PS1dE9) to examine how loss of RAP influences amyloid deposition. This work, in part, is a repetition of a previous effort by van Uden and colleagues in which RAP knockout mice were crossed to the PDAPP model of AD amyloidosis [29]. However, the present study differs from this previous work in a few important ways, providing new insights into the mechanisms involved. First, we determine the effects of partial loss of RAP on amyloid deposition by examining mice with heterozygous deletion of RAP. Second, we use mice that co-express mutant presenilin, which has been shown in other studies to reduce the maturation of LRP [27]. Third, we study mice that express the 695 isoform of APP, which lacks a domain critical for interaction with LRP. Also, we noted that methods described in the van Uden study indicated that mice heterozygous for the APP transgene and heterozygous for RAP [APP (+/−)/RAP(+−)] were intercrossed to produce the animals ultimately analyzed: APP transgene positive and RAP null. It is noteworthy that expression of PS1 variants M146L or L286V, which are linked to familial AD, also cause 40% reductions in the levels of mature LRP in brain [27].

Estimation of amyloid plaque loads was performed by counting amyloid plaques in 6 sagittal sections through the hippocampus of 7 male [APPswe/PS1dE9(+/−)/RAP(+/−)] mice and the 6 parental APPswe/PS1dE9-Line 85 male mice that were used in the initial cross to RAP(−/−) females. Images of hippocampus were captured by digital photography. The hippocampus was defined and the amyloid deposits contained within were counted separately by two people blinded to the genotype. The number of plaques in each section were summed and then averaged for each animal. Statistical analyses (2-tailed t-Test) were performed using the average number of deposits in hippocampus for each animal as a single data point.

**Methods**

**Transgenic mice**

The AD mouse model used in this study (APPswe/PS1dE9-Line 85) co-expresses a chimeric mouse/human APP695 harboring the Swedish K670M/N671L mutations (Mo/HuAPPswe) and human PS1 with the exon-9 deletion mutation (PS1dE9). This model was generated by co-injection of MoPrP.Xho expression plasmids for each gene; the two transgenes co-integrated and segregate as a single locus [29].

Mice with targeted-deletions of RAP (Strain B6, 129S-Lrpap(+/Her______)) were purchased from the Jackson Laboratories (stock # 002987, Bar Harbor, ME). These RAP knockout mice [RAP(−/−)], which were congenic in the C57BL/6J strain, were crossed to APPswe/PS1dE9 mice (Line 05), which were F2 hybrids of C57BL/6J and C3H/HeJ. Progeny that were [APPswe/PS1dE9(+/−)/RAP(+/−)] were backcrossed to the congenic RAP(−/−) mice to hasten the production of mice that were APPswe/PS1dE9 transgene positive and RAP null.

**Tissue preparation, histology and amyloid burden measurements**

All procedures involving animals were approved by the Johns Hopkins Institutional Animal Care and Use Committee. At the specified age, animals were euthanized by overdose with ethyl ether before the brains were removed and bisected sagittally. One hemibrain was frozen on dry ice and stored at −80°C for biochemical studies. The other hemibrain was immersion-fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS pH 7.4). Later, the fixed tissues were embedded in paraffin for silver staining according to the Bielschowsky method [30] modified from the Hirano method (detailed description in [31]).

**Detection of high-molecular-weight aggregates of Aβ**

Amyloid burden was estimated biochemically by filter assay as previously described [32]. Briefly, mouse hemi-forebrains (cerebellum and brain stem removed) were homogenized by probe sonication in 10 volumes of Tris-HCl buffered saline (20 mM Tris-HCl, pH 8.0, 150 mM NaCl) with protease inhibitor. Six parental Line 85 mice (all male) and 7 [APPswe/PS1dE9(+/−)/RAP(+/−)] mice (F1 and F2 offspring—all male) were used in this study. Homogenates were centrifuged at 800 x g for 5 min in a microcentrifuge. The supernatant was adjusted to a final SDS concentration of 1% and then passed through cellulose acetate membranes, 0.2-μm pore size (OE66, Schleicher & Schuell, Keene, NH), using a 96-well dot-blott apparatus under vacuum. Proteins trapped by the filter were detected by immunostaining following protocols used in immunoblotting with a rabbit polyclonal antibody from Zymed, CA (Cat. #71–5800). Enhanced chemiluminescence (ECL) signal was digitally captured with LAS-3000 imaging system, and the intensity of the dots was quantified using Multi Gauge software (Fujifilm, Japan). Two-tailed student t-test with equal variance was used to estimate the difference between two groups.

**Western blot analysis**

Levels of RAP, LRP, SorLA/LR11 and APP were assessed by western blot, using standard methods, as previously described [33]. Briefly, brains were homogenized as described above and then
centrifuged at 800 x g for 5 minutes. Portions of the supernatant, containing 100 µg or 50 µg of total protein, were separated by SDS-PAGE and transferred to nitrocellulose membranes. Blots were incubated with rabbit polyclonal antibody 32LRP (1:1000, gift of Dr. J. Herz, University of Texas Southwestern Medical Center, Dallas, Texas, USA), rabbit polyclonal antibody 4109 zRAP (1:1000, also a gift of Dr. J. Herz), SorLA/LR11 antibody (1:1000; monoclonal, BD Biosciences, San Jose, CA) or 6E10 Aβ (1:5000, monoclonal, Signet Laboratories, Dedham, MA, USA). Proteins bound to antibodies were revealed by incubation with HRP conjugated secondary antibodies (KPL, Gaithersburg, Maryland) and chemiluminescence. The signal was captured and quantified using the LAS-3000 imaging system as described above. The statistical methods of analysis are described in the Figure Legends.

**Results**

RAP(−/−) mice, first described by Willimow et al. [26], were obtained from the Jackson laboratories as congenic on the C57BL/6j background. Animals were mated to APPswe/PS1dE9- Line 85 mice (B6C3F2/P) background to produce a F1 generation of mice that were [APP/PS1][+/−]/RAP(+/−), which we then backcrossed to RAP(−/−) mice in an attempt to produce mice that were [APP/PS1][+/−]/RAP(+/−). However, we unexpectedly failed to obtain weaning age mice that were [APP/PS1][+/−]/RAP(+/−) (Table 1). The mating scheme used was expected to yield litters in which 50% of the offspring were RAP(−/−), with roughly half of these offspring also harboring the APPswe/PS1dE9 transgene. Similarly 50% of the offspring should be RAP(+/−), with half of these offspring also harboring the APPswe/PS1dE9 transgene. However, weaning age offspring of the [APPswe/PS1dE9][+/−]/RAP(+/−) genotype were present at far lower percentages than expected; only 2 animals of this genotype reached weaning age to be identified and both of these died (of unknown causes) before reaching 3 months of age. These results suggested that some type of interaction between an activity of APPswe or PS1dE9 and the absence of RAP leads to diminished survival of these animals. Presently, we don’t understand the basis for this outcome.

Partial reduction in RAP levels increases amyloid burden

Because we were unable to produce [APPswe/PS1dE9][+/−]/RAP(+/−) mice, we chose to compare the parental lines of APPswe/PS1dE9 animals, which were wild-type with respect to RAP, to F1 and F2 [APPswe/PS1dE9][+/−]/RAP(+/−) mice. By multiple measures, we found that partial loss of RAP was associated with increased levels of amyloid deposition. Histologically, we found that partial deletion of RAP increased the number of amyloid deposits 1.5 fold in 9 month old mice (Figs. 1A and B). The average number of silver stained plaques in the hippocampus of [APPswe/PS1dE9][+/−]/RAP(+/−) mice was 13.5 as compared to 8.1 in the parental line 85 mice. Statistical analyses of the data estimated the difference in amyloid burden between the two genotypes has a low probability of resulting from random chance (<0.05). To further quantify the levels of aggregated β-amyloid in these mice, we used a filter-trap assay [32] to demonstrate that the brains of mice expressing APPswe/PS1dE9 with a partial loss of RAP contained about twice as much high-molecular-weight Aβ as controls (Fig. 2). Homogenates of forebrain were serially diluted (2-fold) and filtered through 0.2 µm cellulose acetate membrane, then immunoblotted as described in Methods. We determined that the forebrain of [APPswe/PS1dE9][+/−]/RAP(+/−) mice, as compared to [APPswe/PS1dE9][+/−]/RAP(+/+) mice, contained about twice as much Aβ immunoreactivity that was retained by the filter (p<0.025). Together, these findings provide evidence for higher levels of amyloid in [APPswe/PS1dE9][+/−]/RAP(+/−) mice.

Table 1. Genotypes of offsprings between crosses of APPswe/PS1dE9+[+/−] and mice and RAP(−/−) mice.

| Genotype                  | Expected Frequency | Number of Offspring | Observed Frequency |
|---------------------------|--------------------|---------------------|--------------------|
| APPswe/PS1dE9+[+/−]/RAP(+/−) | 25%                | 23                  | 41.0%              |
| APPswe/PS1dE9+[+/−]/RAP(+/−) | 25%                | 2*                  | 3.5%               |
| RAP(+/−)                  | 25%                | 21                  | 37.5%              |
| RAP(−/−)                  | 25%                | 10                  | 17.8%              |

*Both animals died before 3 months of age.

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**Partial reduction in RAP lowers the levels of SorLA/LR11 and LRP**

Immunoblot analysis of tissue homogenates from the forebrain of mice from the different genotypes was used to analyze the levels of RAP, LRP, and SorLA/LR11 (Figs. 3A and B). As expected, mice harboring one disabled RAP allele produced about 50% less RAP protein (Figs. 3A and B). By contrast, the levels of LRP and SorLA/LR11 did not show the same level of reduction (Figs. 3A and B). On average, the levels of LRP were reduced 20%, but variability between animals reduced statistical significance to only a trend (p<0.20). The levels of SorLA/LR11, however, were less variable and were measured as reduced on average by 15% (p<0.05). Thus, the levels of both of these RAP-dependent receptors were modestly reduced in mice partially deficient in RAP. As previously reported in the literature [20], the brains of mice lacking the expression of RAP showed marked reductions in the levels of mature endoproteolytically processed LRP (85 KDa) with higher levels of immature LRP (Fig. 4, lanes 6 and 7). Surprisingly, the co-expression of PS1dE9 with partial reductions in RAP did not lead to more robust reductions in the levels of mature LRP (Fig. 4, lanes 2, 4, 5, 8, and 9 compared to lanes 1 and 3). Immunoblot analyses were also used to examine whether reducing RAP levels had an effect on the levels of full-length APPswe protein. Immunoblots with antibody 6E10, which specifically recognizes the human Aβ domain of the transgene product, revealed similar amounts of full length APP in the brains of mice with the two RAP genotypes (+/+ and +/−) (Fig. 5). Together, we find that the partial loss of RAP modestly lowers the levels of mature LRP and SorLA/LR11 in the brains of mice that co-express APPswe/PS1dE9.

**Discussion**

The goal of the present investigation was to examine the roles of RAP, and indirectly of LRP, on amyloidogenesis by reducing the
levels of RAP. As a result of unexpected lethality in mice that expressed APPswe/PS1dE9 in a RAP null background, we focused our analysis on mice with only partial reductions of RAP, finding that partial reduction increased the rate of $\beta$ deposition by 1.5 to 2-fold. By our estimates, 50% reductions in the level of RAP cause 15–20% reductions in the levels of mature LRP and SorLA/LR11. We expected that mice co-expressing mutant PS1dE9 with partial reductions of RAP would exhibit larger effects on LRP maturation because mice that are wild-type for RAP and transgenic for PS1-M146L or L286V show 40% reductions in the levels of mature LRP [27]. However, we did not observe an obvious effect of PS1dE9 expression on LRP processing. Thus, the strongest correlate to increased $\beta$ deposition in this experiment is the 50% reduction in RAP levels.

A caveat of our study is that the [APPswe/PS1dE9]+−/−/RAP+−− mice and the [APPswe/PS1dE9]+−/+−/RAP+−+ mice they were compared to were of a slightly different genetic background. The RAP knockout mice were congenic on the C57BL/6 background while the parental APPswe/PS1dE9 mice were hybrids of C57BL/6J and C3H/HeJ (maintained by crosses of transgenic males to F1 hybrids of the 2 strains purchased from Jackson Laboratory). In prior studies of mice that harbor the APPswe and mutant PS1 transgene constructs utilized here, we have noted that transfer of the transgene from the hybrid background to C57BL/6J mice and the parental APPswe/PS1dE9 line 85 mice. B). Plot of the results from counting amyloid plaques following procedures described in Methods. Each dot indicates the number of amyloid plaques on a section. Six sections per animal were counted by two independent assessors that were blind to the genotype of the animals. Each section was counted 3 times by each assessor and averaged. Statistical analyses was conducted on the mean number of deposits for each animal (n = 6 for APPswe/PS1dE9 mice and n = 7 for [APPswe/PS1dE9]+−/+/RAP+−− mice). The statistical difference in amyloid burden between animals of the 2 genotypes was estimated by 2-tailed t-test with equal variance (p<0.05).

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Whether modest reductions in LRP or SorLA/LR11 account for the increased amyloid burden we have observed is unclear. In previous studies, complete deficiency in SorLA/LR11 was shown to cause a 30% increase in the secretion of mouse $\beta$40 and 42 [35]. More substantial reductions in SorLA/LR11 levels (80%) in the frontal cortex of AD patients, without changes in LRP levels, has been found to correlate with higher amyloid burden [33]. In the study by Van Uden and colleagues of PDGF-APPind mice mated to RAP KO mice, increases in amyloid burden were correlated with 80% reductions in the levels of mature LRP [27]. Collectively, these studies suggest that reductions in LRP and/or SorLA/LR11 that are of a significantly greater magnitude than found here produce significant changes in amyloidogenesis. We conclude that either RAP possesses an activity that influences amyloidogenesis in the absence of profound effects on LRP and SorLA/LR11 maturation, or relatively modest reductions in the levels of these proteins (separately or in combination) are sufficient to cause significant changes in the rate of amyloid deposition in the APPswe/PS1dE9 model.

The genetic association of LRP and SorLA/LR11, and their ligands, APOE, α2M, and APP, to AD indicates that these membrane receptor proteins could play important roles in the pathogenesis of AD. Previous studies provide evidence that LRP has opposing effects on APP processing and $\beta$ metabolism. LRP can promote $\beta$ production by altering the processing of APP through interactions with the Kuniz protease inhibitor (KPI) domain (APP751 or APP770) [36]. Although the APP695 isoform, which lacks a KPI domain, can weakly bind to LRP through cytoplasmic adaptor proteins, such as FE65 [37], it is not known whether the processing of APP695 can be influenced by LRP. LRP also functions in the catabolism of $\beta$ peptides and it is possible that modest reductions in LRP levels are sufficient to diminish the rate of $\beta$ clearance, which involves the binding of
Aβ to the LRP ligands ApoE or α2M [19,21–23,38,39]. SorLA/LR11 has been shown to promote the trafficking of APP to discrete intracellular compartments that result in decreased Aβ secretion [35]. Thus, reduced levels of SorLA/LR11 could increase Aβ production. Collectively, these studies suggest very complicated inter-connected pathways by which these proteins could influence APP processing and Aβ metabolism. It is possible that the combined effects of modest reductions in both LRP and SorLA/LR11 could, by different mechanisms, alter the balance of Aβ production and clearance to increase the rate of amyloid deposition. However, because the effects of partial RAP deficiency in the levels of these two proteins is so modest, it is possible that RAP may have the capacity to modulate amyloid deposition by other, yet to be defined, pathways.

As outlined in the Introduction, our work replicates and extends a previous study by van Uden and colleagues [28]. Despite the potential caveats to the van Uden study that are described in the Introduction, we seem to produce a similar result, which is increased amyloid burden when RAP levels are decreased. Although we have similar outcomes to the van Uden study, the mechanisms by which these effects occur could be different. The J9 mice used by van Uden were PDGF-hAPPswe/Ind mice, which created by Mucke and colleagues [40]. The J9 model utilizes a mini-APP gene that can produce all three isoforms of APP: APP695, APP751 and APP770 [41]. Thus, based on the discussion above, one prediction might have been for deficiency in RAP to lead to diminished amyloid deposition because the absence of LRP would reduce Aβ production from APP751 and 770 splice variants. However, since amyloid burden increased in RAP deficiency, it is possible that the loss of LRP slowed the clearance of Aβ, resulting in increased amyloid deposition. It is also possible that lowering the levels of SorLA/LR11 by deleting RAP may have contributed to the increase in Aβ deposition.

In a direct test of the role of LRP in amyloidogenesis, transgenic mice that overexpress an LRP-minireceptor, containing the ligand-binding domain II of human LRP and the region representing the transmembrane subunit including the full cytoplasmic tail (mLRP2), were mated to PDAPP mice [24]. The authors reported 3-fold increases in LRP levels and found

**Figure 2. Filter assay of amyloid burden in [APPswe/PS1dE9] (+/-)/RAP (+/-) mice.** A. Filter trap assays of tissue homogenates from 6 parental line 85 mice and 6 offspring that were APPswe/PS1dE9 positive and hemizygous for RAP was performed as described in Methods. Each column contains a serial dilution of the sample. 300 μg total protein was loaded in the first well, followed by serial 2-fold dilutions. The [APPswe/PS1dE9] (+/-)/RAP (+/-) mice have about twice the amount of high molecular weight Aβ burden as the parental line 85 mice at the same age (9 months). B. Quantification of signal intensity was measured as described in Methods across the entire range of dilutions for each group of mice. The amount of high molecular weight “aggregated” Aβ was found to be significantly higher in [APPswe/PS1dE9] (+/-)/RAP (+/-) mice at 9 months as compared to the parental APPswe/PS1dE9 animals of the same age (p values noted on graph).

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Figure 3. Analysis of RAP, LRP and SorLA/LR11 levels. A). Immunoblots were probed with polyclonal antibody 4109 to RAP (1:1000), 377 to LRP (1:1000) and monoclonal antibody anti-LR11 (1:1000; BD Biosciences, San Jose, CA). Protein concentration was determined by BCA. Each lane contains 100 μg total protein. Genotypes of the mice are marked on the figure. B). Quantification of the intensity of the bands in panel A, using a Fuji LAS-3000 imaging device and software provided by the manufacturer. Statistical comparisons of each protein in the two genotypes of interest ([APPswe/PS1dE9] (+/+) /RAP (+/+) and [APPswe/PS1dE9] (+/-) /RAP (+/-) were conducted on the raw quantitative data, which consisted of pixel values for each protein band quantified. Two-tailed student t-Test with equal variance was used to estimate the probability that differences in the levels of each protein, between genotypes, resulted from random chance (p values for each comparison are noted on the figure). Because the levels of each protein were measured as lower in mice lacking one RAP allele, we chose to graph the data by setting the mean value for each protein in the [APPswe/PS1dE9] (+/+) /RAP (+/+) mice to 100, designated controls, and then graphing the values of mice lacking one RAP allele as a percent of the controls. The data represent measures from 6 [APPswe/PS1dE9] (+/-) /RAP (+/+) mice compared to 7 [APPswe/PS1dE9] (+/-) /RAP (+/-) mice (except for measures of LR11 n = 6 as one lane was unmeasurable). The two mice without APPswe/PS1dE9 transgenes were not included in measurements so that the only difference between the two groups of animals analyzed was the number of functional RAP alleles.

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increased soluble Aβ and enhanced memory impairment in PDAPP mice, but did not find evidence of a change in amyloid loads in mice harboring both transgenes as compared to mice harboring only the PDAPP transgene. If LRP directly affects amyloidogenesis by mediating clearance of Aβ peptides, then one would have predicted that the above study would have produced mice with lowered amyloid burden. However, the transgene product was not full-length LRP and the transgene was not expressed by its endogenous promoter. Additional study is clearly required to clarify the role LRP in APP and Aβ metabolism.

Mutant PS1 and LRP maturation

It has been reported that the levels of mature, endoproteolytically cleaved, LRP in the murine nervous system are reduced when AD associated mutants of PS1 (M146L, or L286V, expressed via the hamster prion protein gene promoter) are present [27].

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