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Presenilin 1 mutations influence processing and trafficking of the ApoE receptor apoER2

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Abstract

Presenilin (PS)-1 is an intramembrane protease serving as the catalytic component of γ -secretase. Mutations in the PS1 gene are the most common cause of familial Alzheimer's disease (FAD). The LDL-receptor family member apoER2 is a γ -secretase substrate that has been associated with AD in several ways, including acting as a receptor for apolipoprotein E. ApoER2 is processed by γ -secretase into a C-terminal fragment (γ -CTF) that appears to regulate gene expression. FAD PS1 mutations were tested for effects on apoER2. PS1 mutation R278I showed impaired γ -secretase activity for apoER2 in the basal state or after exposure to Reelin. PS1 M146V mutation permitted accumulation of apoER2 CTFs after Reelin treatment, while no difference was seen between wild-type (WT) and M146V in the basal state. PS1 L282V mutation, combined with the γ -secretase inhibitor DAPT, greatly reduced the cell-surface levels of apoER2 without affecting total apoER2 levels, suggesting a defect in receptor trafficking. These findings indicate that impaired processing or localization of apoER2 may contribute to the pathogenic effects of FAD mutations in PS1.

Schematic illustration of apoER2 processing at the membrane

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ApoER2 is a type I transmembrane protein. Cleavage by sheddase produces secreted apoER2 ectodomain and C-terminal fragment designated as “SR” (shedase remnant). The latter is further processed within the transmembrane region by γ -secretase (presenilin 1, PS1), releasing C-terminal fragment (γ -CTF). DAPT, a γ -secretase inhibitor blocks the intramembrane cleavage. ApoER2 is bound by ligands such as ApoE and Reelin, the latter of which enhances proteolytic processing at both sites of apoER2 upon binding. We find that mutations in PS1 associated with inheritance of Alzheimer’s disease attenuate the cleavage of SR and the formation of γ -CTF.

Keywords

Alzheimer’s disease; apoER2; γ -secretase; lipoprotein receptor; presenilin; protein processing; protein trafficking

1. INTRODUCTION

Several aspects of Alzheimer’s disease (AD) pathology and genetics are associated with the LDL receptor family of lipoprotein receptors. An important link is conferred by apolipoprotein E (ApoE), which binds LDL receptors alone or as a component of lipoprotein complexes; polymorphisms in the *APOE* gene are responsible for the most prevalent genetic risk factor for late-onset AD. In the LDL receptor family, two members have garnered considerable attention from neuroscientists: apoER2 (a.k.a. LRP8) and VLDLR. Unlike LDL receptor-related protein 1 (LRP1) and most other members of the family, these are signal-transducing receptors. Binding of Reelin, a potent developmental paracrine factor, to apoER2 and VLDL activates a well-established signal transduction pathway (Ballif et al., 2003). It can lead to inhibition of the activity of glycogen synthase kinase 3 β (GSK3 β), preventing hyperphosphorylation of tau and stabilizing microtubules (Beffert et al., 2002). In addition, the interaction of apoER2 and postsynaptic density protein 95 (PSD-95) couples Reelin signals to the N-methyl-D-aspartate receptor (NMDAR) (Chen et al., 2005), which is critical for long-term potentiation (LTP) and synaptic plasticity required for memory formation and maintenance. Neuronal apoER2 and VLDLR participate in critical regulation of neuroblast migration and the neurophysiology of intact adult circuits (Reddy et al., 2011). Genetic ablation of either produces a diminution of LTP and disrupts migration of new

dentate granule cells into their proper position during adult neurogenesis, resulting in seizures (Forster et al., 2006). These critical roles in LTP and dentate maintenance provide additional links between the LRP6 and AD; LTP is a biochemical component of memory, and emerging data is extending the proposed role for epileptiform activity in AD (Vossel et al., 2013).

The γ -secretase is an integral membrane protein complex which has been chiefly characterized as a complex of four subunits: nicastrin, Pen-2, Aph-1, and presenilin (PS) 1 or 2. PS is the catalytic core of γ -secretase, and it may be sufficient (Ahn et al., 2010). The PSs are nonhelical transmembrane proteins with two aspartyl residues that are critical for proteolysis (Edbauer et al., 2003). Mutations in the genes for either PS1 or PS2 can convey autosomal-dominant inheritance of FAD. The mechanistic explanation of PS's genetic contribution to AD has focused primarily on the tendency of FAD mutations to elevate the ratio of A β ₄₂:A β ₄₀ produced, and Takami et al. (Takami et al., 2009) have proposed that this reflects a consistent loss of processivity in PSs bearing FAD mutations. However, it is dissatisfying that certain FAD mutations do not manifest this shift and others do so within the context of a diminished overall production of total A β (Page et al., 2008; Shioi et al., 2007; Walker et al., 2005; Xia et al., 2016). In addition, some PS1 mutations result in γ -secretase processivity that is higher than wild type (Quintero-Monzon et al., 2011). As the most aggressive PS1 mutations result in loss of function toward several substrates, Chávez-Gutiérrez et al. (Chavez-Gutierrez et al., 2012) concluded that "it is not unlikely that 'partial loss' of γ -secretase function at Notch or other γ -secretase substrates acts as an aggravating factor in FAD."

ApoER2 has been identified as one of the substrates of γ -secretase (Hoe Rebeck, 2005; May et al., 2003), but how FAD-linked mutations in PS1 impact its processing is heretofore unknown. Moreover, some PS mutations have been connected to Reelin signaling elements such as PI3-kinase and Crk/Dock1/Rac (Chen et al., 2004; Parent Thinakaran, 2010; Qiu et al., 2006), which suggests that part of the effect of PS mutation on synaptic processes could be related to Reelin receptors such as apoER2. We have initiated studies of the impact of FAD *PSEN1* mutations on the processing and trafficking of apoER2. These have utilized primary embryonic fibroblasts and astrocytes from mice "knocked in" with two different FAD PS1s, as well as mouse embryonic fibroblasts (MEF) from mice genetically ablated of both *Psen1* and -2. Our results indicate differential effects of wild-type and mutated PS1s on apoER2 γ -cleavage and intracellular trafficking, suggesting the possibility that this receptor and its coupled Reelin signaling pathway could contribute to the pathological mechanism of the development of AD conferred by PS1 mutations.

2. MATERIALS AND METHODS

2.1. Plasmid constructs

Mouse apoER2 cDNA with deletion of exon 5 (apoER2- EX5) was subcloned into pcDNA3.1 (Invitrogen), which was then digested with XhoI and KpnI. The excised fragment of DNA was ligated into the corresponding sites of the p1005+ expression vector with eGFP (generously provided by Yuzhi Chen, University of Arkansas for Medical Sciences). Human PS1 wild-type (WT) and L282V open reading frames were subcloned into pcDNA3.1. All

plasmid constructs were made using standard techniques and were verified by sequence analysis.

2.2. Packaging of replication-defective HSV-1 virus

All HSV-1 viruses were packaged using the 2-2 cell line (generously provided by Yuzhi Chen, University of Arkansas for Medical Sciences) according to Neve et al. (Neve et al., 2005). Briefly, 2-2 cells were transfected with empty p1005+ vector or p1005-apoER2-EX5. The cells were superinfected with helper virus 27 h after transfection. Virus was amplified by three passages of cells and purified from crude cell lysate by sucrose-gradient centrifugation. The infectious units (IU) of virus stock was determined by infecting NTERA2 human neuronal cells for 12 h with serial dilutions. After fixation with 4% paraformaldehyde, eGFP-positive cells were counted with fluorescence microscopy. All the experimental cell cultures were infected with the appropriate viruses at a multiplicity of infection of 1.

2.3. Production of Reelin and ApoE

Reelin was partially purified from serum-free medium (50/50 MEM/F12) conditioned by a HEK293 line stably transfected with Reelin expression construct pCrl (D'Arcangelo et al., 1997) (courtesy of Tom Curran; Children's Hospital of Philadelphia Research Inst.) or, as a control, conditioned medium from untransfected HEK293 cells.

Phenylmethylsulfonylfluoride (PMSF) was added to the conditioned medium to a final concentration of 0.5 mM, and the medium was chilled to 4 °C. A saturated solution of (NH₄)₂SO₄ was then added gradually, with continuous stirring, to a concentration of 45%. After stirring ~18 h at 4 °C, the suspension was subjected to centrifugation at 20,000 × *g* for 1 h at 4 °C. Pellets were dissolved in Dulbecco's phosphate-buffered saline (PBS), pH 7.4, 1 mM CaCl₂, 0.5 mM MgSO₄. This protein preparation was dialyzed against the above solution for 1 h at 4 °C to remove excess ammonium sulfate and then subjected to sterile filtration (0.2 μm). Glycerol was then added to 25% before storage at -80 °C. Reelin concentrations were determined by densitometric comparison of the major bands to those produced by known quantities of bovine serum albumin in Coomassie-stained SDS-PAGE. The preparations of conditioned medium from untransfected HEK293 cells were tested in pilot experiments for stimulation of Dab1 phosphorylation and other indices of apoER2 activity; no activity was noted, thus this preparation was omitted in subsequent experiments.

ApoE proteins were expressed in clones of the T98G human astrogloma cell line stably transfected with expression plasmids encoding ApoE3 and ApoE4. Conditioned medium was collected from these lines as described above. Rather than (NH₄)₂SO₄ precipitation, centrifugal filtration (Amicon filters, 10-kDa cutoff) was employed to concentrate the lipoprotein content of the conditioned medium. This concentrated fraction was then loaded onto a Pharmacia Superdex 200 (10/300) gel-filtration column resolved with phosphate buffer (20 mM NaPO₄, pH 7.5, 50 mM NaCl, 1 mM EDTA) at a flow rate of 0.5 ml/min. Elution fractions were monitored at A₂₈₀, and those with elution times shorter than that of free ApoE were subjected to detection of ApoE by western blot analysis. Pools that contained high concentrations of ApoE within an elution time consistent with lipoprotein particles were pooled and sterilized by filtration. Serial dilutions of these samples were run

on western blots alongside dilutions of purified ApoE of known concentration; the antibody used for detection was verified to have equal reactivity with ApoE3 and ApoE4. Digital images of the western blot results were scanned and compared for the determination of ApoE content within the lipoprotein preparations.

2.4. Cell culture and treatments

The primary glial cells generated from heterozygous *Psen1* knock-in mice carrying the M146V mutation were generously provided by Mark P. Mattson (National Institute on Aging); primary MEF generated from homozygous *Psen1* knock-in mice carrying the R278I mutation were generously provided by Takaomi C. Saido (RIKEN Brain Science Institute); the *Psen1*^{-/-}; *Psen2*^{-/-} double-knockout (DKO) MEF cell line was generously provided by Dr. Bart De Strooper (Katholieke Universiteit Leuven) (Herreman et al., 2000). All cells were maintained in minimal essential medium with Earle's salts (MEM, Invitrogen) plus 10% fetal bovine serum (FBS, Invitrogen).

Primary glial cells and primary MEF cells were washed with PBS and then treated with 10 nM Reelin, 20 nM ApoE3, or 20 nM ApoE4 combined with 30 μ M γ -secretase inhibitor DAPT (EMD Millipore) or vehicle (DMSO) in serum-free MEM. Three hours later, cells were infected with an HSV-1 vector or the vector containing sequences encoding apoER2-EX5. After 16 h, cells were lysed in SDS lysis buffer (2.5% SDS, 78 mM Tris, pH 6.8).

Psen1^{-/-}; *Psen2*^{-/-} DKO MEF cells were transiently co-transfected with empty pcDNA3.1 or pcDNA3.1-apoER2-EX5 and pcDNA3.1-PS1 WT or L282V [identical mutation to L286V but in the context of a shorter splice variant (Vidal et al., 1996)] with Lipofectamine 2000 (Invitrogen). At 24 h after transfection, cells were washed with PBS and treated with 50 μ M DAPT and/or 10 nM Reelin in serum-free medium for 16 h. Cells were then harvested with SDS lysis buffer as above.

2.5. SDS-PAGE and western blot

Cell extracts adjusted to equivalent total protein content were resolved by 7.5% Tris-glycine SDS-PAGE for full-length apoER2 and 12% bis-Tris SDS-PAGE for apoER2 CTF with 2.5% β -mercaptoethanol in the sample buffer. Protein was electrophoretically transferred at 250 mA for 2 h onto PVDF membranes, which were then blocked for 1 h at room temperature with 0.2% I-Block (Applied Biosystems). Blots were probed overnight at 4 °C with primary antibody and incubated with alkaline phosphatase-conjugated secondary antibody (Santa Cruz, 1:5000) at room temperature for 1 h. Then blots were developed with a chemiluminescence system (Western-Light; Applied Biosystems). The density of protein bands were quantified with ImageJ.

Rabbit anti-apoER2 (Novus) recognizing the apoER2 C-terminus was used to detect both the full-length and CTF of apoER2 at a final dilution of 1:600. Rabbit anti-GFP (Sigma) was used to detect GFP expression in HSV-1 infected cell samples at a final dilution of 1:2000. Mouse anti- β -tubulin (Sigma) was used to detect β -tubulin levels in PS1^{-/-}/PS2^{-/-} DKO MEF cells at a final dilution of 1:20,000. Rabbit anti-PS1 (Novus) was used to detect PS1 levels in transfected PS1^{-/-}/PS2^{-/-} DKO MEF cells at a final dilution of 1:500. A goat anti-ApoE (Panvera) was used for quantification of recombinant preparations.

2.6. Cell surface protein labelling

PS1^{-/-}/PS2^{-/-} DKO MEF cells were cotransfected transiently with apoER2- EX5 and PS1 WT or L282V for 40 h. At 24 h after transfection, cells were washed with PBS and treated with 50 μ M DAPT or vehicle (DMSO) in serum-free medium for 16 h. Cells were then washed twice with ice-cold PBS and cell-surface proteins were biotinylated with 0.25 mg/ml Sulfo-NHS-SS-Biotin (Pierce) for 30 min at 4 °C. After quenching unreacted biotin, total protein was extracted in lysis buffer (1X PBS, pH7.4, 1% NP-40, 1% sodium deoxycholate, 2 mM EDTA, 0.2 mM PMSF, 10 μ g/ml aprotinin, and 10 μ g/ml leupeptin). Supernatants with equal amounts of protein were incubated with NeutrAvidin agarose beads (Pierce) at room temperature for 1 h with rocking. Biotinylated proteins were recovered by boiling in 1X SDS sample buffer with 2.2 mM DTT. Eluted protein samples were then resolved by 7.5% tris-glycine SDS-PAGE and probed by western blot using an antibody against the apoER2 C-terminus (Novus).

2.7. Metabolic labelling and immunoprecipitation of apoER2

Psen1^{-/-}; *Psen2*^{-/-} DKO MEF cells were co-transfected transiently with apoER2- EX5 and PS1 WT or L282V for 36 h. At 20 h after transfection, cells were treated with 50 μ M DAPT or vehicle (DMSO) in DMEM plus 0.5% FBS for 16 h. After a wash with methionine-free DMEM, cells were pre-incubated in the same plus 0.5% FBS for 1 h, followed by a 30-min pulse with 65 μ Ci/ml [³⁵S]methionine at 37 °C. Cells were lysed immediately after the pulse (time 0), or chased for 3–24 h in regular DMEM plus 0.5% FBS with 50 μ M DAPT or vehicle (DMSO). Cells were then washed with cold PBS and lysed in lysis buffer (1X PBS, pH7.4, 1% NP-40, 1% sodium deoxycholate, 2 mM EDTA, 0.2 mM PMSF, 10 μ g/ml aprotinin, 10 μ g/ml leupeptin). Cell lysates were equilibrated by cpm and incubated overnight at 4 °C with anti-apoER2 antibody (Novus). Immunoprecipitates were collected by Protein-A/G beads and resolved by 7.5% tris-glycine SDS-PAGE. The gels were soaked in EN³HANCE (Perkin-Elmer), dried, and visualized by autoradiography on X-ray film at –80 °C for 10 days.

2.8. Statistics

Values are expressed as mean $\pm$ standard deviation (S.D.). Pairwise comparisons were made by Student's *t*-test. Values of *p* less than or equal to 0.05 were taken to be significant. The half-life of apoER2 in pulse-chase experiments was determined by curve fit (one-phase exponential decay) in GraphPad Prism 3.03.

3. RESULTS

3.1. R278I mutation in PS1 dramatically impairs γ -secretase activity towards apoER2 in primary MEF

To avoid potential artifacts arising from over-expression of exogenous PS1 without the other components of γ -secretase, we procured mouse cell lines carrying two “knock-in” FAD mutations, as well as *Psen1* and -2 null mutant MEF lines. Primary MEF have undetectable levels of apoER2, so we introduced exogenous receptor by a high-efficiency viral transduction system into cell cultures. This system exhibits the characteristics of endogenous

apoER2 expression, including the fact that two C-terminal fragments (CTFs) of the receptor are often detected: *i)* the larger resulting from sheddase activity (sheddase remnant, “SR”) and *ii)* the smaller resulting from γ -secretase processing of the SR (γ -CTF). Under basal conditions, PS1-R278I knock-in MEFs had increased accumulation of the SR fragment of apoER2 compared to WT MEFs (Fig. 1). Treatment with γ -secretase inhibitor DAPT elevated the SR in WT MEFs to a level similar to that seen in R278I knock-in cells. (Because the SR was so abundant and obscured the γ -CTF, we could not reliably quantify the γ -CTF in these experiments.) DAPT had little or no effect on the SR in PS1-R278I knock-in MEFs. These data suggest that R278I mutation essentially abolishes the γ -secretase activity PS1 exhibits toward apoER2. Also, DAPT treatment diminished the difference in the SR fragment between the two cell types, which indicates sheddase cleavage was not affected appreciably by the R278I mutation. In addition to the proteolytic products of apoER2, two full-length apoER2 bands were detected (Fig. 1A). Based on prior studies, the upper band is presumed to be the mature form with complete glycosylation and the lower band the immature form. PS1-R278I mutation had no effect on either form of full-length apoER2 in these whole-cell lysates.

Binding of apoER2 by ligands stimulates proteolytic processing of the receptor (Hoe Rebeck, 2005). To determine the effect of PS1 mutation on this phenomenon, Reelin was applied to PS1-R278I knock-in MEFs and to WT MEFs. Reelin treatment decreased the full-length apoER2 equally in WT and PS1-R278I knock-in MEF cells; DAPT did not affect this phenomenon (Fig. 2). There was a mutation-dependent difference, however, in the accumulation of SR after Reelin application. PS1-R278I knock-in cells exhibited an increase in SR that was not detected in WT MEFs (Fig. 3). In WT cells, it is likely that processing of the SR by γ -secretase, as well as subsequent proteasomal degradation of the resulting γ -CTF, is not rate-limiting and efficiently removes any enhancement of SR production resulting from ligand binding.

We also tested whether human ApoE proteins affect apoER2 processing equally. ApoE3 and ApoE4 expressed in an astroglial cell line elute from a size-exclusion chromatography column in a manner consistent with lipoprotein particles. Biological activity of the ApoE via apoER2 was confirmed through enhancement of NMDA receptor responses in neuronal cells (Supplemental Fig. 1). These preparations, containing 20 nM of either ApoE3 or ApoE4 were applied to MEFs. Again, PS1-R278I knock-in cells accumulated higher levels of apoER2 SR than seen in WT cells (Fig. 4). However, unlike Reelin treatment, ApoE failed to elevate apoER2 SR in either cell type. ApoE also failed to alter apoER2 full-length protein levels.

3.2. PS1 M146V mutation leads to reduced γ -secretase activity towards apoER2 in primary glial cultures

We also tested the impact of another PS1 mutation using mixed-glial cultures derived from WT and PS1-M146V knock-in mice. These cultures overwhelmingly comprise astrocytes, but low levels of microglia and oligodendroglia cannot be excluded. Unlike results with R278I in primary MEF cells, no differences in apoER2 SR levels were apparent between glia with WT or M146V PS1 under normal conditions (Fig. 5). However, treatment with 30

μM DAPT elevated SR levels in PS1-M146V knock-in glia without affecting its levels in WT cultures (Fig. 5), suggesting a vulnerability resulting from the mutation that is only revealed after a marginal additional inhibition. Reelin binding elevated SR levels in M146V-expressing cells compared to WT cells (Fig. 5), suggesting that the PS1-M146V mutation impairs γ -secretase activity such that it cannot process the additional apoER SR burden created by Reelin treatment as efficiently as WT PS1 can. Combined Reelin and DAPT treatment greatly increased the SR amount in both cell populations, but glia with the M146V mutation had a somewhat higher level; this further confirms that 30 μM DAPT is a subthreshold concentration for WT PS1. These results indicate that the M146V mutation does not completely abolish γ -secretase activity of PS1 like the R278I mutation does, so alterations in apoER2 SR processing in M146V knock-in glia are only apparent when the system is further taxed by ligand and/or marginal inhibition.

3.3. PS1 L282V mutation affects cell-surface levels of apoER2 without altering its processing in *Psen1*^{-/-}; *Psen2*^{-/-} DKO MEF cells

Two highly homologous presenilin genes encoding PS1 and PS2 have been identified (Cruts et al., 1996). Both gene products are capable of serving as the catalytic core of γ -secretase, and PS2 may have contributed to the DAPT-sensitive γ -secretase activity retained in the context of the PS1 M146V mutation in astrocytes. To investigate the effects of FAD mutations in PS1 without PS2 interference, *Psen1*^{-/-}; *Psen2*^{-/-} DKO MEF cells were utilized. First, plasmids expressing WT or L282V PS1 were transfected into cells. A similar amount of PS1 N-terminal fragment was detected in both transfectants (Fig. 6A), indicating that the L282V mutation does not affect the stability of PS1. As would be expected, a large accumulation of SR fragment was apparent in *Psen1*^{-/-}; *Psen2*^{-/-} DKO MEF cells. Introduction of exogenous PS1 dramatically decreased the SR level equally, regardless of whether the PS1 supplied was WT and L282V. Nor was any difference detected between the WT or L286V PS1 transfectants after application of Reelin (Fig. 6B and 6C). Together, these data suggest that the L282V mutation does not affect γ -secretase activity toward apoER2.

Presenilins appear to influence intracellular protein trafficking independently of γ -secretase activity (L'Hernault-Arduengo, 1992; Naruse et al., 1998; Nishimura et al., 1999). Therefore, we assessed PS1 L282V for effects on apoER trafficking by assaying cell-surface levels of apoER2 protein with a biotinylation approach. DKO cells were cotransfected with apoER2 and WT or L286V PS1, surface proteins were biotinylated, then biotinylated proteins were precipitated and subjected to immunoblotting for apoER2. The resulting immunoblots revealed almost exclusively mature full-length apoER2, validating the technique; no SR nor γ -CTF was detected. The expression of L282V PS1 resulted in steady-state levels of apoER2 at the cell surface that appeared lower than in WT-transfectants, but the difference did not reach statistical significance (Fig. 7A and 7B). DAPT treatment greatly decreased cell-surface apoER2 only in PS1 L282V transfected MEFs without affecting the total amount of apoER2 (Fig. 7C).

It is possible that a change in subcellular trafficking of apoER2 might reflect impacts on the protein's stability. To test for effects on apoER2 half-life, we conducted pulse-chase experiments with [³⁵S]methionine metabolic labeling. Results from these experiments

revealed no evidence of an effect on half-life in the L282V-transfected cells with or without DPAT treatment (Supplemental Fig. 2). The half-life of apoER2 in each treatment condition was as follows (h): PS1 WT: 3.83; L282V: 3.97; L282V+DAPT: 3.75. This is consistent with the lack of effect on steady-state levels of apoER2.

4. DISCUSSION

APOE makes the most demographically significant genetic impact on the risk for developing AD. Roles in relevant CNS processes may be mediated by two lipoprotein receptors that are shared with the ligand Reelin, one of which is apoER2. Because apoER2 is a substrate for PS/ γ -secretase and mutations in the PS1 gene account for the majority of FAD cases, we have begun to examine the effects that mutations in PS1 have on processing of the receptor. Surveying three different mutations of PS1 we find evidence that FAD mutations can inhibit the γ -secretase processing or intracellular trafficking of apoER2. To the extent that apoER2 contributes to normal neurophysiology of learning and memory, this line of investigation justifies further examination of the role such deficits may play in the detrimental effects of *PSEN1* mutations.

Our survey of several *PSEN1* mutations in various cell systems indicated a range of effects. One objective of our study was to include approaches that would avoid artifacts potentially from overexpression of PS1. MEF cells and primary astrocytes from mice carrying knock-in mutations of the *Psen1* gene were useful in this regard. Final processing of the apoER2 sheddase remnant to γ CTF was almost nonexistent in MEFs carrying the R278I mutation, leading to a large accumulation of the sheddase remnant after ligand-evoked processing triggered by Reelin. The M146V mutation produced similar effects if γ -secretase activity was additionally compromised by a subthreshold concentration of DAPT. Even subtler effects on apoER2 were unmasked by DAPT in cells expressing the L282V mutation, where cell-surface levels of the receptor were markedly suppressed without detectable differences in processing.

In recent years, there has been broad-ranging speculation over the biochemical and genetic mode(s) by which FAD mutations in *PSEN1* and -2 cause disease. The discussion has revolved around whether clinical PS1 mutations induce FAD through loss-of-function or gain-of-function. Biochemically many of these mutations result in a loss-of-function in γ -secretase activity, while genetically they give rise to a gain of pathogenicity. The processivity hypothesis (Chandu et al., 2006; Takami et al., 2009) seeks to resolve this issue by invoking two activities that γ -secretase has toward some substrates: an initial endoproteolysis (ϵ cleavage), followed by C-terminal exoproteolysis that proceeds in three-amino-acid steps. The processivity of the latter reaction may be compromised by FAD mutations in PSs without affecting the ϵ cleavage, resulting in a loss of function that creates a gain in toxic products (longer A β fragments). The separation of these activities is consistent with the fact that FAD mutations in PSs generally have preserved Notch processing. With regard the impact on APP, M146V has been reported to elevate the A β ₄₂:A β ₄₀ ratio 20–50% (Duff et al., 1996; Murayama et al., 1999), perhaps by engaging ϵ cleavage at a different position than wild-type PS1 (Li et al., 2016); L286V (the same mutation, in context, as L282V) has also been reported to raise the A β ₄₂:A β ₄₀ ratio, but

more modestly (Annaert et al., 1999; Kulic et al., 2000). R278I, by contrast, seems very deficient in processivity and yields almost no A β ₄₀ (Nakaya et al., 2005)

One recent study supported a more universal loss-of-function hypothesis with data from knock-in mouse models bearing rarely studied FAD-associated mutations of *PSEN1* (Xia et al., 2015). The L435F and C410Y mutations were reported to abolish γ -secretase activity towards APP and Notch. In the homozygous state, each of these knock-ins displayed a phenotype nearly identical to *Psen1*-null mice; mice heterozygous for the L435F mutation exhibited typical features of AD, including impaired hippocampal memory and cerebral neurodegeneration. It has been speculated that attenuated production of the APP intracellular domain (AICD) or the equivalent Notch fragment (NICD) by a mutated PS1 would be pathogenic through compromised gene regulation by these products (Shen Kelleher, 2007). The apoER2 γ -CTF also translocates to the nucleus where it inhibits Reelin transcription, creating a negative feedback loop for regulating Reelin levels; indeed, PS1 depletion elevates mRNA and protein levels of both Reelin and apoER2 in mouse brain (Balmaceda et al., 2014). More recently, Telese et al (Telese et al., 2015) showed that nuclear translocation of the apoER2 γ -CTF stimulates specific cohorts of enhancers, which they dubbed LRP8-Reelin neuronal (LRN) enhancers, critically involved in hippocampus-dependent learning and memory. This implies that Reelin can impact genes related to learning and memory via the apoER2 γ -CTF and that this process might be attenuated by PS1 mutations.

Our data indicate that FAD mutations in *PSEN1* inefficiently cleave apoER2 to varying extents, consistent with the possibility that attenuated processing of apoER2 contributes to the varying malignancy of different *PSEN1* mutations; each mutation may target a specific spectrum of substrates, resulting in a distinctive constellation of effects on biological functions. Interestingly, the onset of the symptoms generally happens earlier in the FAD patients with *PSEN1* mutations compared to *APP* mutations (Ryman et al., 2014), which may indicate that PS1 mutations cause broader effects than do *APP* mutations. Emerging evidence indicates that at least some cases of AD are multifactorial in origin (Rollo et al., 2016; Schneider et al., 2007). In alignment with this, the findings presented here augment the evidence that FAD-linked mutations in PS proteins affect physiological processes beyond the production of fibrillogenic A β _{42/43} peptides. In addition, it implies that effects on apoER2 should be taken into account for the development of γ -secretase modulators for therapeutic application.

We found that the differences between R278I and WT PS1 were exaggerated by the pressure put on the system via ligand-stimulated proteolysis of apoER2. An important neurodevelopmental paracrine factor, Reelin has been documented to have key roles in adult neurophysiology and neuroblast migration, as well; interference with Reelin expression or its intracellular signaling pathway has effects on LTP and seizure potential that are partially replicated in apoER2 knockouts (Fatemi et al., 2001; Fatemi et al., 2005; Heinrich et al., 2006; Herz Chen, 2006). Here, we found that Reelin caused a reduction of full-length receptor, likely due to enhanced processing by a sheddase after ligand-stimulated internalization. More importantly, the SR fragment produced by this process accumulated to a greater extent in cells expressing the R278I. No enhancement of apoER2 processing was detected under our conditions after application of ApoE. This is consistent with previous

reports of the fates of apoER2 upon binding by Reelin and ApoE. According to Duit et al. (2010), Reelin binding to apoER2 causes clustering of the receptor, which triggers endocytosis via clathrin-coated vesicles and degradation in the lysosome. By contrast, apoER2 bound by lipoproteins is induced to undergo surface recycling whereby the receptor is internalized, separated from its ligand within peripheral (surface-proximal) endosomes, and then returned to the cell surface for additional shuttling of lipoproteins (Chen et al., 2010; Heeren et al., 2004). This difference between the ligands has implication for the gene-regulatory function of the γ -CTF (Hoe Rebeck, 2005; May et al., 2003). Balmaceda et al. (Balmaceda et al., 2014) demonstrated that ApoE, by contrast, may impact this process indirectly by reducing the amount of apoER2 available on the cell surface for stimulation by Reelin. Chen et al. (Chen et al., 2010) reported that ApoE4 has a greater inhibitory impact on apoER2 activation than does ApoE3 because it causes a prolonged retention of the receptor in the cell's interior. Therefore, ApoE4 should impact the gene-regulatory activity of apoER2 in a similar direction (i.e., diminution) to that effected by PS1 mutations. These impacts of ApoE4 and PS1 mutation may have more significant consequences when Reelin levels are reduced, as is evident in the entorhinal cortex and hippocampus in mouse models of AD and frontal cortex in patients with preclinical AD (Chin et al., 2007; Herring et al., 2012).

Our results with the L282V mutant add to existing data indicating that PS1 can impact trafficking of proteins (Naruse et al., 1998; Nishimura et al., 1999) as well as membrane-bound vesicles (Stenovec et al., 2016). It is not clear that this effect is dependent on γ -secretase, because expression of WT PS1 in *Psen1*^{-/-}/*Psen2*^{-/-} DKO MEF cells did not diminish cell-surface levels of apoER2; however, the trafficking defect was accentuated by simultaneous inhibition of γ -secretase with DAPT. One possible explanation is that DAPT binding changes the conformation of PS1 or the γ -secretase complex, which may interfere the role of PS1 in protein trafficking. Another interesting finding is that PS1 depletion had a different effect on apoER2 trafficking than that of FAD-linked PS1 mutation. Such differences are also seen in APP delivery to the cell membrane. Cai et al. (Cai et al., 2003) found that loss-of-function mutations in *PSEN1* caused elevations in transport of APP from ER to Golgi and Golgi to cell surface, whereas FAD-linked *PSEN1* mutations delayed APP transport in both respects. This may suggest a toxic gain-of-function of FAD mutations in PS1 with regard to subcellular trafficking roles. More in-depth studies are needed to understand the mechanism by which PS1 regulates the membrane protein trafficking and how its mutations contribute to AD pathogenesis.

5. CONCLUSIONS

We have determined that the processing of apoER2 into a C-terminal fragment associated with gene regulation is altered by FAD mutations in PS1. This appears to impact the ligand-stimulated accumulation of this γ -CTF in response to Reelin treatment. In at least some settings, PS1 mutation may also impact subcellular trafficking of apoER2. These findings support the hypothesis that at least some *PSEN1* FAD mutations exert stronger pathogenic impacts through alterations in processing of proteins beyond APP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AD	Alzheimer's disease
ApoE	apolipoprotein E
apoER2	ApoE receptor 2
APP	amyloid precursor protein
CTF	carboxyterminal fragment
DAPT	N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester
DKO	double knockout
DMSO	dimethyl sulfoxide
eGFP	eukaryotic-optimized green fluorescent protein
FAD	familial AD
LDL	low-density lipoprotein
LRP1	LDL receptor-related protein 1
LTP	long-term potentiation
MEF	mouse embryonic fibroblast
NMDA	N-methyl D-aspartate
PS1	presenilin 1
SR	shedase remnant

References

- Ahn K, Shelton CC, Tian Y, Zhang X, Gilchrist ML, Sisodia SS, et al. Activation and intrinsic gamma-secretase activity of presenilin 1. *Proc Natl Acad Sci U S A*. 2010; 107:21435–40. [PubMed: 21115843]
- Annaert WG, Levesque L, Craessaerts K, Dierinck I, Snellings G, Westaway D, et al. Presenilin 1 controls gamma-secretase processing of amyloid precursor protein in pre-golgi compartments of hippocampal neurons. *J Cell Biol*. 1999; 147:277–94. [PubMed: 10525535]

- Ballif BA, Arnaud L, Cooper JA. Tyrosine phosphorylation of Disabled-1 is essential for Reelin-stimulated activation of Akt and Src family kinases. *Brain Res Mol Brain Res*. 2003; 117:152–9. [PubMed: 14559149]
- Balmaceda V, Cuchillo-Ibanez I, Pujadas L, Garcia-Ayllon MS, Saura CA, Nimpf J, et al. ApoER2 processing by presenilin-1 modulates reelin expression. *Faseb J*. 2014; 28:1543–54. [PubMed: 24344333]
- Beffert U, Morfini G, Bock HH, Reyna H, Brady ST, Herz J. Reelin-mediated signaling locally regulates protein kinase B/Akt and glycogen synthase kinase 3beta. *J Biol Chem*. 2002; 277:49958–64. [PubMed: 12376533]
- Cai D, Leem JY, Greenfield JP, Wang P, Kim BS, Wang R, et al. Presenilin-1 regulates intracellular trafficking and cell surface delivery of beta-amyloid precursor protein. *J Biol Chem*. 2003; 278:3446–54. [PubMed: 12435726]
- Chandu D, Huppert SS, Kopan R. Analysis of transmembrane domain mutants is consistent with sequential cleavage of Notch by gamma-secretase. *J Neurochem*. 2006; 96:228–35. [PubMed: 16300632]
- Chavez-Gutierrez L, Bammens L, Benilova I, Vandersteen A, Benurwar M, Borgers M, et al. The mechanism of gamma-Secretase dysfunction in familial Alzheimer disease. *Embo J*. 2012; 31:2261–74. [PubMed: 22505025]
- Chen K, Ochalski PG, Tran TS, Sahir N, Schubert M, Pramatarova A, et al. Interaction between Dab1 and CrkII is promoted by Reelin signaling. *J Cell Sci*. 2004; 117:4527–36. [PubMed: 15316068]
- Chen Y, Durakoglugil MS, Xian X, Herz J. ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. *Proc Natl Acad Sci U S A*. 2010; 107:12011–6. [PubMed: 20547867]
- Chen Y, Beffert U, Ertunc M, Tang TS, Kavalali ET, Bezprozvanny I, et al. Reelin modulates NMDA receptor activity in cortical neurons. *J Neurosci*. 2005; 25:8209–16. [PubMed: 16148228]
- Chin J, Massaro CM, Palop JJ, Thwin MT, Yu GQ, Bien-Ly N, et al. Reelin depletion in the entorhinal cortex of human amyloid precursor protein transgenic mice and humans with Alzheimer's disease. *J Neurosci*. 2007; 27:2727–33. [PubMed: 17360894]
- Cruts M, Hendriks L, Van Broeckhoven C. The presenilin genes: a new gene family involved in Alzheimer disease pathology. *Hum Mol Genet*. 1996; 5(Spec No):1449–55. [PubMed: 8875251]
- D'Arcangelo G, Nakajima K, Miyata T, Ogawa M, Mikoshiba K, Curran T. Reelin is a secreted glycoprotein recognized by the CR-50 monoclonal antibody. *J Neurosci*. 1997; 17:23–31. [PubMed: 8987733]
- Duff K, Eckman C, Zehr C, Yu X, Prada CM, Perez-tur J, et al. Increased amyloid-beta42(43) in brains of mice expressing mutant presenilin 1. *Nature*. 1996; 383:710–3. [PubMed: 8878479]
- Duit S, Mayer H, Blake SM, Schneider WJ, Nimpf J. Differential functions of ApoER2 and very low density lipoprotein receptor in Reelin signaling depend on differential sorting of the receptors. *J Biol Chem*. 2010; 285:4896–4908. [PubMed: 19948739]
- Edbauer D, Winkler E, Regula JT, Pesold B, Steiner H, Haass C. Reconstitution of gamma-secretase activity. *Nat Cell Biol*. 2003; 5:486–8. [PubMed: 12679784]
- Fatemi SH, Kroll JL, Stry JM. Altered levels of Reelin and its isoforms in schizophrenia and mood disorders. *Neuroreport*. 2001; 12:3209–15. [PubMed: 11711858]
- Fatemi SH, Snow AV, Stry JM, Araghi-Niknam M, Reutiman TJ, Lee S, et al. Reelin signaling is impaired in autism. *Biol Psychiatry*. 2005; 57:777–87. [PubMed: 15820235]
- Forster E, Jossin Y, Zhao S, Chai X, Frotscher M, Goffinet AM. Recent progress in understanding the role of Reelin in radial neuronal migration, with specific emphasis on the dentate gyrus. *Eur J Neurosci*. 2006; 23:901–9. [PubMed: 16519655]
- Heeren J, Grewal T, Laatsch A, Becker N, Rinninger F, Rye KA, et al. Impaired recycling of apolipoprotein E4 is associated with intracellular cholesterol accumulation. *J Biol Chem*. 2004; 279:55483–92. [PubMed: 15485881]
- Heinrich C, Nitta N, Flubacher A, Muller M, Fahrner A, Kirsch M, et al. Reelin deficiency and displacement of mature neurons, but not neurogenesis, underlie the formation of granule cell dispersion in the epileptic hippocampus. *J Neurosci*. 2006; 26:4701–13. [PubMed: 16641251]

- Herreman A, Serneels L, Annaert W, Collen D, Schoonjans L, De Strooper B. Total inactivation of gamma-secretase activity in presenilin-deficient embryonic stem cells. *Nat Cell Biol.* 2000; 2:461–2. [PubMed: 10878813]
- Herring A, Donath A, Steiner KM, Widera MP, Hamzehian S, Kanakis D, et al. Reelin depletion is an early phenomenon of Alzheimer's pathology. *J Alzheimers Dis.* 2012; 30:963–79. [PubMed: 22495348]
- Herz J, Chen Y. Reelin, lipoprotein receptors and synaptic plasticity. *Nat Rev Neurosci.* 2006; 7:850–9. [PubMed: 17053810]
- Hoe HS, Rebeck GW. Regulation of ApoE receptor proteolysis by ligand binding. *Brain Res Mol Brain Res.* 2005; 137:31–9. [PubMed: 15950758]
- Kulic L, Walter J, Multhaup G, Teplow DB, Baumeister R, Romig H, et al. Separation of presenilin function in amyloid beta-peptide generation and endoproteolysis of Notch. *Proc Natl Acad Sci U S A.* 2000; 97:5913–8. [PubMed: 10811883]
- L'Hernault SW, Arduengo PM. Mutation of a putative sperm membrane protein in *Caenorhabditis elegans* prevents sperm differentiation but not its associated meiotic divisions. *J Cell Biol.* 1992; 119:55–68. [PubMed: 1527173]
- Li N, Liu K, Qiu Y, Ren Z, Dai R, Deng Y, et al. Effect of Presenilin Mutations on APP Cleavage; Insights into the Pathogenesis of FAD. *Front Aging Neurosci.* 2016; 8:51. [PubMed: 27014058]
- May P, Bock HH, Nimpf J, Herz J. Differential glycosylation regulates processing of lipoprotein receptors by gamma-secretase. *J Biol Chem.* 2003; 278:37386–92. [PubMed: 12871934]
- Murayama O, Tomita T, Nihonmatsu N, Murayama M, Sun X, Honda T, et al. Enhancement of amyloid beta 42 secretion by 28 different presenilin 1 mutations of familial Alzheimer's disease. *Neurosci Lett.* 1999; 265:61–3. [PubMed: 10327206]
- Nakaya Y, Yamane T, Shiraishi H, Wang HQ, Matsubara E, Sato T, et al. Random mutagenesis of presenilin-1 identifies novel mutants exclusively generating long amyloid beta-peptides. *J Biol Chem.* 2005; 280:19070–7. [PubMed: 15764596]
- Naruse S, Thinakaran G, Luo JJ, Kusiak JW, Tomita T, Iwatsubo T, et al. Effects of PS1 deficiency on membrane protein trafficking in neurons. *Neuron.* 1998; 21:1213–21. [PubMed: 9856475]
- Neve RL, Neve KA, Nestler EJ, Carlezon WA Jr. Use of herpes virus amplicon vectors to study brain disorders. *Biotechniques.* 2005; 39:381–91. [PubMed: 16206910]
- Nishimura M, Yu G, Levesque G, Zhang DM, Ruel L, Chen F, et al. Presenilin mutations associated with Alzheimer disease cause defective intracellular trafficking of beta-catenin, a component of the presenilin protein complex. *Nat Med.* 1999; 5:164–9. [PubMed: 9930863]
- Page RM, Baumann K, Tomioka M, Perez-Revuelta BI, Fukumori A, Jacobsen H, et al. Generation of Abeta38 and Abeta42 is independently and differentially affected by familial Alzheimer disease-associated presenilin mutations and gamma-secretase modulation. *J Biol Chem.* 2008; 283:677–83. [PubMed: 17962197]
- Parent AT, Thinakaran G. Modeling presenilin-dependent familial Alzheimer's disease: emphasis on presenilin substrate-mediated signaling and synaptic function. *Int J Alzheimers Dis.* 2010:2010.
- Qiu S, Zhao LF, Korwek KM, Weeber EJ. Differential reelin-induced enhancement of NMDA and AMPA receptor activity in the adult hippocampus. *J Neurosci.* 2006; 26:12943–55. [PubMed: 17167084]
- Quintero-Monzon O, Martin MM, Fernandez MA, Cappello CA, Krzysiak AJ, Osenkowski P, et al. Dissociation between the processivity and total activity of gamma-secretase: implications for the mechanism of Alzheimer's disease-causing presenilin mutations. *Biochemistry.* 2011; 50:9023–35. [PubMed: 21919498]
- Reddy SS, Connor TE, Weeber EJ, Rebeck W. Similarities and differences in structure, expression, and functions of VLDLR and ApoER2. *Mol Neurodegener.* 2011; 6:30. [PubMed: 21554715]
- Rollo JL, Banihashemi N, Vafae F, Crawford JW, Kuncic Z, Holsinger RM. Unraveling the mechanistic complexity of Alzheimer's disease through systems biology. *Alzheimers Dement.* 2016; 12:708–18. [PubMed: 26703952]
- Ryman DC, Acosta-Baena N, Aisen PS, Bird T, Danek A, Fox NC, et al. Symptom onset in autosomal dominant Alzheimer disease: a systematic review and meta-analysis. *Neurology.* 2014; 83:253–60. [PubMed: 24928124]

- Schneider JA, Arvanitakis Z, Bang W, Bennett DA. Mixed brain pathologies account for most dementia cases in community-dwelling older persons. *Neurology*. 2007; 69:2197–204. [PubMed: 17568013]
- Shen J, Kelleher RJ 3rd. The presenilin hypothesis of Alzheimer's disease: evidence for a loss-of-function pathogenic mechanism. *Proc Natl Acad Sci U S A*. 2007; 104:403–9. [PubMed: 17197420]
- Shioi J, Georgakopoulos A, Mehta P, Kouchi Z, Litterst CM, Baki L, et al. FAD mutants unable to increase neurotoxic Abeta 42 suggest that mutation effects on neurodegeneration may be independent of effects on Abeta. *J Neurochem*. 2007; 101:674–81. [PubMed: 17254019]
- Stenovec M, Trkov S, Lasic E, Terzieva S, Kreft M, Rodriguez Arellano JJ, et al. Expression of familial Alzheimer disease presenilin 1 gene attenuates vesicle traffic and reduces peptide secretion in cultured astrocytes devoid of pathologic tissue environment. *Glia*. 2016; 64:317–29. [PubMed: 26462451]
- Takami M, Nagashima Y, Sano Y, Ishihara S, Morishima-Kawashima M, Funamoto S, et al. gamma-Secretase: successive tripeptide and tetrapeptide release from the transmembrane domain of beta-carboxyl terminal fragment. *J Neurosci*. 2009; 29:13042–52. [PubMed: 19828817]
- Telese F, Ma Q, Perez PM, Notani D, Oh S, Li W, et al. LRP8-Reelin-Regulated Neuronal Enhancer Signature Underlying Learning and Memory Formation. *Neuron*. 2015; 86:696–710. [PubMed: 25892301]
- Vidal R, Ghiso J, Wisniewski T, Frangione B. Alzheimer's presenilin 1 gene expression in platelets and megakaryocytes. Identification of a novel splice variant. *FEBS Lett*. 1996; 393:19–23. [PubMed: 8804415]
- Vossel KA, Beagle AJ, Rabinovici GD, Shu H, Lee SE, Naasan G, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurol*. 2013; 70:1158–66. [PubMed: 23835471]
- Walker ES, Martinez M, Brunkan AL, Goate A. Presenilin 2 familial Alzheimer's disease mutations result in partial loss of function and dramatic changes in Abeta 42/40 ratios. *J Neurochem*. 2005; 92:294–301. [PubMed: 15663477]
- Xia D, Kelleher RJ 3rd, Shen J. Loss of Abeta43 Production Caused by Presenilin-1 Mutations in the Knockin Mouse Brain. *Neuron*. 2016; 90:417–22. [PubMed: 27100200]
- Xia D, Watanabe H, Wu B, Lee SH, Li Y, Tsvetkov E, et al. Presenilin-1 knockin mice reveal loss-of-function mechanism for familial Alzheimer's disease. *Neuron*. 2015; 85:967–81. [PubMed: 25741723]

HIGHLIGHTS

- ApoE and presenilin are genetic factors in AD as yet unlinked mechanistically.
- Presenilin's γ -secretase activity creates the final C-terminal fragment of apoER2.
- ApoER2, an ApoE receptor, is differently cleaved by wild-type and mutated presenilin.
- Mutations in PS1 may disrupt ligand-evoked production of a transcription factor.

Figure 1. R278I mutation of PS1 results in accumulation of an apoER2 fragment

Primary MEF cells from PS1 R278I knock-in or wild-type mice were infected with HSV-1 vectors expressing apoER2 and GFP or empty vector (GFP alone) for 16 h. Three h prior to infection, indicated cultures were treated with γ -secretase inhibitor DAPT (30 μ M). **A:** Total protein was extracted and analyzed by western blot with an antibody to the apoER2 C-terminus, which detected two CTFs: the product of sheddase (SR) and the product of γ -secretase (γ -CTF). **B:** Densitometric analysis of replicate measures of apoER2 SR normalized to GFP. Data are expressed as mean $\pm$ s.d. (* P <0.03; ** P <0.0003).

Figure 2. Reelin treatment decreases full-length apoER2 in primary MEF

Primary MEF cells from PS1 R278I knock-in and wild-type mice were infected with HSV-1 vectors expressing apoER2 for 16 h. Three h prior to infection, cells were treated with 30 μ M DAPT and/or 10 nM Reelin as indicated. **A:** Total protein was extracted and analyzed by western blot. **B:** Densitometric analysis of replicate measures of full-length apoER2 normalized to GFP. Data are expressed as mean $\pm$ s.d. (* P <0.05 compared to WT control; # P <0.05 compared to R278I knock-in control).

Figure 3. Reelin treatment leads to accumulation of apoER2 SR in PS1 R278I knock-in primary MEF

Primary MEF cells from PS1 R278I knock-in and wild-type mice were infected with HSV-1 vectors expressing apoER2 for 16 h. Three h prior to infection, cells were treated with 30 μ M DAPT and/or 10 nM Reelin as indicated. **A:** Total protein was extracted and analyzed by western blot. **B:** Densitometric analysis of replicate measures of apoER2 CTFs (SR+ γ -CTF) normalized to GFP in Reelin-treated cells. Data are expressed as mean $\pm$ s.d. (* P <0.05).

Figure 4. ApoE treatment leads to accumulation of apoER2 SR in PS1 R278I knock-in primary MEF

Primary MEF cells from PS1 R278I knock-in and wild-type mice were infected with HSV-1 vectors expressing apoER2 for 16 h. Three h prior to infection, cells were treated with 30 μ M DAPT and/or 20 nM ApoE3/4 as indicated. **A:** Total protein was extracted and analyzed by western blot. **B:** Densitometric analysis of replicate measures of apoER2 CTFs (SR+ γ -CTF) normalized to GFP. Data are expressed as mean $\pm$ s.d. (* P <0.05 compared to WT control, # P <0.05 compared to WT ApoE3, ¥ P <0.05 compared to WT ApoE4).

Figure 5. PS1 M146V mutation leads to impaired γ -secretase activity toward apoER2 in primary glial cultures

Primary glia cells from PS1 M146V knock-in and wild-type mice were infected with HSV-1 vectors expressing apoER2 for 16 h. Three h prior to infection, cells were treated with 30 μ M DAPT and/or 10 nM Reelin as indicated. **A:** Total protein was extracted and analyzed by western blot. **B:** Densitometric analysis of replicate measures of apoER2 CTFs (SR+ γ -CTF) normalized to GFP. Data are expressed as mean $\pm$ s.d. (* $P < 0.05$).

Figure 6. No difference in γ -secretase cleavage of apoER2 is detected in PS1 WT and L282V transfected *Psen1*^{-/-}; *Psen2*^{-/-} DKO MEF

Psen1^{-/-}; *Psen2*^{-/-} DKO MEF cells were co-transfected with empty vector or apoER2 and PS1 WT or L282V. Twenty-four h after transfection, cells were treated with 50 μ M DAPT and/or 10 nM Reelin as indicated for 16 h. A: PS1 N-terminal fragments (NTF) in WT and L282V-transfected *Psen1*^{-/-}; *Psen2*^{-/-} DKO MEF cells were analyzed by western blot. B: Total protein was extracted and apoER2 CTFs and β -tubulin were analyzed by western blot. C: Densitometric analysis of duplicate measures of apoER2 CTFs (SR+ γ -CTF) normalized to β -tubulin in transfected cells. Data are expressed as mean $\pm$ s.d.

Figure 7. PS1 L282V mutation attenuates cell-surface levels of apoER2

A: *Psen1*^{-/-}; *Psen2*^{-/-} DKO MEF cells were co-transfected with empty vector or apoER2 and PS1 WT or L282V. Forty h after transfection, cell-surface proteins were labelled using sulfo-NHS-SS-biotin at 4 °C for 30 min. Then cells were lysed and biotinylated proteins were precipitated with NeutroAvidin agarose beads. Total apoER2 (input) and apoER2 expressed on the cell surface were detected by western blot in whole-cell lysates and biotinylated samples, respectively. **B:** Densitometric analysis of replicate cell-surface apoER2 normalized to apoER2 input. Data are expressed as mean ± s.d. **C:** *Psen1*^{-/-}; *Psen2*^{-/-} DKO MEF cells were co-transfected with apoER2 and PS1 WT or L282V. Twenty-four h after transfection, cells were treated with 50 μM DAPT for 16 h as indicated. Cell-surface proteins were labelled using sulfo-NHS-SS-biotin at 4 °C for 30 min. Cells were lysed and biotinylated proteins were precipitated with NeutroAvidin agarose beads. Total apoER2 (input) and apoER2 expressed on the cell surface were detected by western blot in whole-cell lysates and biotinylated samples, respectively.