REVIEW

CrossMark

Impact of late-onset Alzheimer's genetic risk factors on beta-amyloid endocytic production

Cláudia Guimas Almeida[1] · Farzaneh Sadat Mirfakhar[1] · Catarina Perdigão[1] · Tatiana Burrinha[1]

Abstract

The increased production of the 42 aminoacids long beta-amyloid (Aβ42) peptide has been established as a causal mechanism of the familial early onset Alzheimer's disease (AD). In contrast, the causal mechanisms of the late-onset AD (LOAD), that affects most AD patients, remain to be established. Indeed, Aβ42 accumulation has been detected more than 30 years before diagnosis. Thus, the mechanisms that control Aβ accumulation in LOAD likely go awry long before pathogenesis becomes detectable. Early on, APOE4 was identified as the biggest genetic risk factor for LOAD. However, since APOE4 is not present in all LOAD patients, genome-wide association studies of thousands of LOAD patients were undertaken to identify other genetic variants that could explain the development of LOAD. *PICALM, BIN1, CD2AP, SORL1,* and *PLD3* are now with *APOE4* among the identified genes at highest risk in LOAD that have been implicated in Aβ42 production. Recent evidence indicates that the regulation of the endocytic trafficking of the amyloid precursor protein (APP) and/or its secretases to and from sorting endosomes is determinant for Aβ42 production. Thus, here, we will review the described mechanisms, whereby these genetic risk factors can contribute to the enhanced endocytic production of Aβ42. Dissecting causal LOAD mechanisms of Aβ42 accumulation, underlying the contribution of each genetic risk factor, will be required to identify therapeutic targets for novel personalized preventive strategies.

Keywords Late-onset Alzheimer's disease · Trafficking · Endocytosis · APOE4 · PICALM · BIN1 · CD2AP · SORL1 · PLD3

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that impairs memory, behavior and the ability to be independent. It is an overwhelming disease not only for patients but also for their caregivers and families. AD can be familial and rare with an early onset (eFAD) starting in the thirties; or very common affecting 1 in 10 elderlies with more than 65 years old, a late-onset AD (LOAD). The lack of an effective treatment and the increasing aging of the population has transformed LOAD into a health and socio-economic problem.

Pathologically, AD is characterized by progressive accumulation of amyloid plaques and tau neurofibrillary tangles. However, it is the progressive synapse loss which better predicts cognitive decline with aging [1].

eFAD is caused by inheritance of familial mutations in amyloid precursor protein (APP) or presenilins 1 and 2 (PSEN1, PSEN2; γ-cleavage of APP), that lead to the excessive neuronal production of the longest form of beta-amyloid (Aβ42) or an increased ratio of Aβ42 over Aβ40. Aβ42 is more prone to oligomerize and the oligomers have been established as the most toxic species in AD [2]. Mice carrying eFAD mutations recapitulate cognitive memory deficits and develop amyloid plaques and tau neurofibrillary tangles, modeling essential AD features. In eFAD, synapses progressively become dysfunctional, lost and eventually, neurons degenerate due to progressive accumulation and aggregation of Aβ42 with aging [3–5]. At synapses, Aβ42 accumulates both extra- and intracellularly [6–13]. Aβ42 is generated by intracellular processing of APP in endosomes [14–18]. Upon production, Aβ is either secreted or retained

✉ Cláudia Guimas Almeida
  Claudia.almeida@nms.unl.pt

[1] Neuronal Trafficking in Aging Lab, CEDOC, Chronic
  Diseases Research Centre, NOVA Medical School|Faculdade
  de Ciências Médicas, Universidade NOVA de Lisboa,
  Campo Mártires da Pátria, 130, 1169-056 Lisbon, Portugal

in endosomes. Aβ42 accumulates intracellularly in multi-vesicular endosomes, altering the sorting and lysosomal degradation of endocytosed membrane receptors [10, 19]. Indeed, intracellular Aβ accumulation precedes extracellular Aβ deposits and abnormal tau phosphorylation and aggregation [9].

AD silent cellular mechanisms that lead to Aβ42 accumulation and synaptic dysfunction are predicted to begin more than 30 years before diagnosis. LOAD is a multifactorial disease, caused by a combination of genetic and lifestyle risk factors. The most important genetic risk factor is APOE4, identified in 1993 [20–23]. However, APOE4 is not present in all cases of LOAD and this prompted geneticists to search for other genetic risk factors. Genome-wide association studies (GWAS) of thousands of LOAD patients were undertaken to identify genetic variants (or single nucleotide polymorphisms, SNP) that could explain the development of LOAD [24]. Among the first identified genes at the highest risk in AD, were PICALM, BIN1, and CD2AP [25–28]. SORL1 was later found by meta-analysis of the large LOAD patients' consortiums [28]. Genome sequencing of smaller cohorts identified rare variants in PLD3 associated with AD [29], although this association has yet to be confirmed in larger cohorts; for further details on the genetic associations, see a recent review by R. Guerreiro [30]. All together with APOE4 have been implicated in Aβ production and linked to endosomal trafficking. Here, we will review their impact on Aβ production at endosomes. Dissecting causal LOAD mechanisms of Aβ42 accumulation and synaptic dysfunction will be required to identify therapeutic targets and novel personalized preventive and curative strategies.

Endocytic production of Aβ

Normal Aβ production only occurs to a small extent, because the neuronal trafficking pathways of APP and BACE1 are largely segregated (Fig. 1). APP and BACE1, both trans-membrane proteins, initiate their secretory pathway to the plasma membrane with their exit from the trans-Golgi network (TGN) in distinct post-Golgi carriers [31]. At the plasma membrane, evidence supports a segregation of APP from BACE1, with BACE1 being more present in membrane microdomains rich in cholesterol and flotillin (lipid rafts) than APP [31–33]. BACE1 and APP undergo endocytosis through different internalization mechanisms.

APP endocytosis is mostly clathrin-mediated [34]. The YENPTY motif in APP C-terminus is the sorting signal for endocytosis [35], and it is involved in the interaction of APP with auxiliary proteins [36]. There is evidence that a cholesterol/flotillin-dependent clustering of APP may stimulate the internalization via clathrin-dependent endocytosis to promote Aβ production [37]. Indeed, altering the lipid membrane composition in cholesterol, flotillin and caveolin-1 levels influenced the rate of APP processing and Aβ
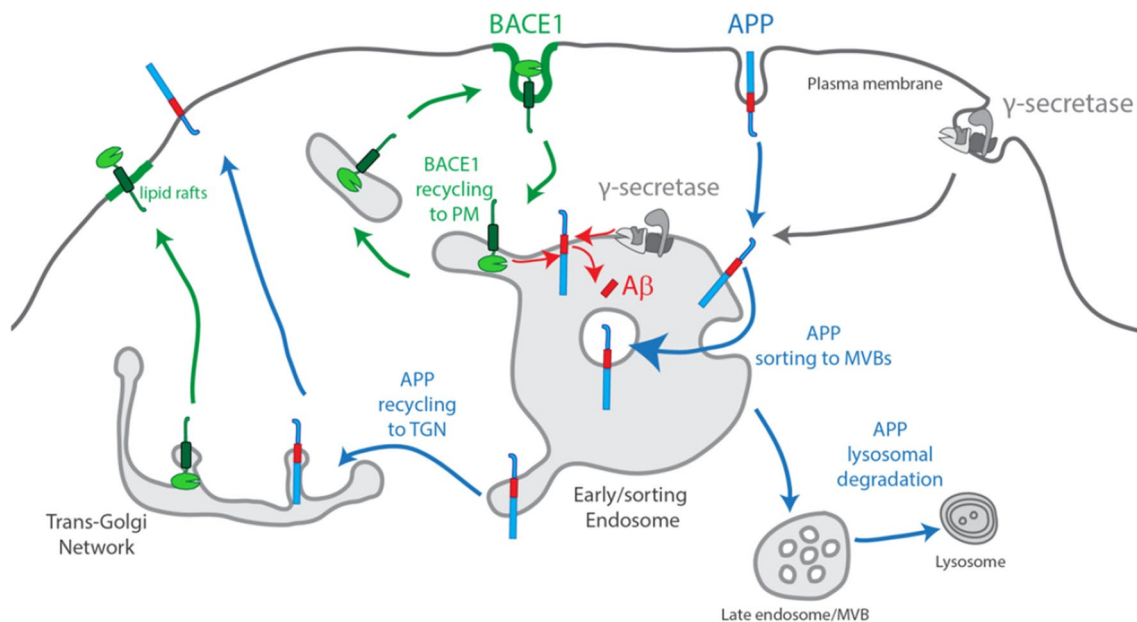

Fig. 1 Scheme of normal endocytic production of Aβ. APP and BACE1 exit the Trans-Golgi Network (TGN) to the plasma membrane in separate post-Golgi secretory vesicles. At the plasma membrane, BACE1 prefers lipid rafts, and is endocytosed independently of APP. Less clear is γ-secretase complex assembly and endocytic trafficking. Upon endocytosis, APP, BACE1 and γ-secretase reach early/sorting endosomes. BACE1 recycles fast out of sorting endosomes to the plasma membrane, while APP is sorted into inner luminal vesicles during MVB biogenesis. Aβ production occurs upon acidification of sorting endosomes which favors BACE1 activity and APP processing at the endosomal limiting membrane. APP degradation occurs upon fusion with the lysosome

production [37–39]. Lipids can potentially alter APP endocytosis. APP endocytosis may also be regulated by protein interaction, such as with ApoE receptors [40–42].

BACE1 endocytosis occurs by a less defined mechanism, independently of clathrin, regulated by Arf6 [43] or by clathrin-mediated endocytosis [44, 45]. A dileucine acidic motif in BACE1 C-terminus is the sorting signal for endocytosis and endosomal trafficking [44, 46, 47].

APP and BACE1 endocytic vesicles are delivered to a common early endosome. Indeed, endocytosis has been shown to be required for the sequential processing of APP by BACE1 and by γ-secretase specifically in neurons, producing mainly Aβ40 and Aβ42 [48–51]. In non-neuronal cells, evidence indicates that the TGN is a preferential site for APP processing upon APP endocytosis [52]. Normally, APP processing is likely avoided by BACE1 sorting into endosomal tubules for the recycling pathway, whereas APP is sorted into intraluminal vesicles for the degradative pathway in a process termed multivesicular endosome (MVB) biogenesis [45, 53, 54]. Since endosomal acidification is required for optimal BACE1 activity [31], and γ-secretase is active at late-endosomes [55], it is likely that APP processing occurs during early endosome maturation [31, 43]. Indeed, APP processing and Aβ production increase by blocking APP sorting to MVBs intraluminal vesicles [54, 56] and by blocking BACE1 recycling [57]. Aβ can be secreted or retained within neurons in MVBs [7, 58].

In the past few years, the mechanisms whereby several LOAD genetic risk factors contribute to Aβ accumulation have started to be uncovered. As such, their impact deregulating the neuronal endosomal trafficking of APP and its secretases will be reviewed in the next section.

Regulators of endosomal trafficking identified as risk factors for AD

Apolipoprotein E4

APOE4 was identified associated with AD in 1993, and it remains the strongest genetic risk factor for LOAD [20–23]. APOE4 is one of the three polymorphic alleles of the APOE gene. The other alleles are *APOE2* and *APOE3*. Apolipoprotein E (ApoE) is highly expressed in the brain, mainly by astrocytes [59]. Upon secretion, ApoE binds cholesterol and other lipids enabling their endocytosis, via ApoE receptors [60]. The three different protein isoforms, ApoE2, ApoE3, and ApoE4, have a different effect on AD pathogenesis. ApoE4 is pathological, while ApoE2 and ApoE3 are neuroprotective or neutral, respectively [61].

The underlying mechanisms of action of ApoE4 in AD are still poorly understood [20, 22]. ApoE4 contribution to Aβ accumulation likely includes multiple mechanisms.

Upregulation of Aβ production by ApoE4 is supported by several findings. Namely, exogenous ApoE4 increases Aβ accumulation by stimulation of APP endocytosis and processing, via ApoE receptor 2 (ApoER2) or lipoprotein receptor-related protein (LRP) but not low-density lipoprotein receptor (LDLR) [42, 62]. Importantly, ApoE3 and ApoE4 injection in hippocampus also increased APP processing [63]. In contrast, exogenous ApoE2, 3, and 4 were described to reduce extracellular Aβ, while APP processing increased [64]. Interestingly, the interaction between APP and γ-secretase complex can be up-regulated by expression of an ApoE interacting protein, TMC22, thus increasing Aβ production [65]. Remarkably, abnormal endosomes were described in the brain of AD patients with the APOE4 genotype [66] as well as in the aging brain of APOE4-humanized mice [67]. The mechanism underlying the increase of APP endocytosis in the presence of ApoE could be linked to alterations in the lipid membrane composition given ApoE function in lipid transport. However, experimental evidence suggests that the effect of ApoE4 on APP endocytosis/Aβ production is independent of its lipidation [42, 68].

Unexpectedly, a recent study from the Sudhof lab identified an ApoE4 mechanism independent of APP endocytosis. Instead, ApoE4 was found to boost APP transcription and thus Aβ production by activating a signal transduction pathway [69].

Aβ accumulation may also result from decreased clearance, since ApoE4 binds secreted Aβ less efficiently than ApoE3 and ApoE2, compromising Aβ uptake and lysosomal degradation [70–72]. Otherwise, ApoE4 may compete with Aβ for the same degradation pathways, without binding Aβ [73].

Alternative ApoE4 mechanisms, independent of Aβ, may exist as indicated by an ApoE4-dependent impairment of synaptic plasticity due to trapping of AMPA and NMDA receptors in intracellular compartments [74]. The uptake of cholesterol itself is compromised, since ApoE4 is lipidated less efficiently, which could, in turn, affect membrane trafficking [68].

Alternative ApoE4 mechanisms, independent of Aβ, may exist as indicated by an ApoE4-dependent impairment of synaptic plasticity due to trapping of AMPA and NMDA receptors in intracellular compartments [74]. The uptake of cholesterol itself is compromised, since ApoE4 is lipidated less efficiently, which could, in turn, affect membrane trafficking [68].

Taken together, as illustrated in Fig. 2, ApoE4 could mediate an increase in APP endocytosis via alterations in lipid membrane composition or via the increased APP in the secretory pathway due to ApoE4-dependent upregulation of APP transcription.
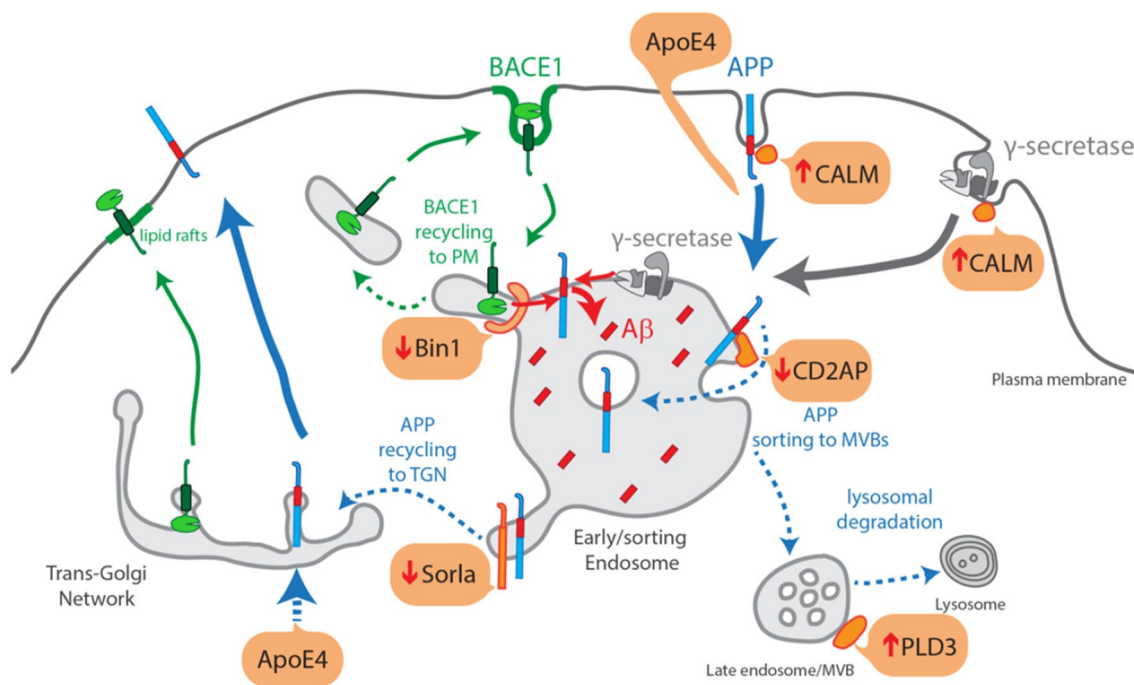
Fig. 2 Scheme of the increased endocytic production of Aβ due to LOAD genetic risk factors. ApoE4-mediated increase in APP endocytosis and/or via increased APP in the secretory pathway due to increased APP transcription; Bin1 loss-of-function impedes BACE1 to recycle out of sorting endosomes; Sorla loss-of-function decreases APP recycling out of endosomes to the TGN; CD2AP loss-of-function decreases APP sorting into MVBs and lysosomal degradation; CALM loss-of-function increases APP and γ-secretase endocytosis and delivery to sorting endosomes; PLD3 loss-of-function affects lysosome morphology and perhaps APP processing at endosomes

PICALM

PICALM genetic variants were associated with LOAD by GWAS [25, 75–80]. A recent GWAS meta-analysis confirmed the association of one of the *PICALM* variants with higher risk of AD [81].

Correlative studies between *PICALM* variants and cognitive reserve, assessed based on brain volume and thickness, suggest that *PICALM* variants confer protection [82–84].

PICALM encodes for CALM (Clathrin Assembly Lymphoid Myeloid leukemia) [85], a cytosolic clathrin–endocytic adaptor [86]. CALM is ubiquitously expressed and is detected pre- and post-synaptically, while its neuron-specific homolog, AP180, is predominantly in presynaptic compartments [87, 88]. Despite their similarity, CALM and AP180 are not functionally redundant [86]. CALM interacts with clathrin and membrane lipids to promote the formation of endocytic vesicles [89, 90], while AP180 is more specifically implicated in synaptic vesicle retrieval [88]. CALM also functions in the retrieval of VAMP proteins, SNAREs that mediate fusion of exocytic vesicles from the plasma membrane [86, 90, 91].

Thus far, the data on the expression of CALM in AD are inconsistent, since it has been found decreased in the AD brain due to abnormal cleavage [92], but increased in the cortex of an eFAD mice model (Tg2576) [93]. Unpredictably, a modest increase in *PICALM* mRNA correlated with a protective genotype [94]. Moreover, *PICALM* depletion decreased amyloid plaques in the hippocampus of an eFAD mouse model (APP/PS1 mice) [95]. More research will be necessary to establish how CALM expression is altered in AD.

Mechanistically, evidence supports that CALM is required for clathrin-mediated endocytosis of APP and thus Aβ endocytic production [95–99]. Additional mechanisms include increased sorting of APP/APPCTFs for lysosomal degradation upon CALM overexpression [100]. Moreover, CALM may also be required for γ-secretase endocytosis, since CALM depletion increased nicastrin, a γ-secretase component, at the plasma membrane [98]. Alternatively, CALM deficiency decreases Aβ clearance across the murine blood–brain barrier (BBB) [101]. The decreased Aβ clearance could be due to a reduced endocytosis and recycling of Aβ bound to LRP1, impeding Aβ clearance by transcytosis across the microvessels epithelium [101]. CALM has also been shown to function at synapses, mediating the reclustering of synaptic vesicles proteins after exocytosis [102]. A role for CALM in cholesterol uptake has also been suggested, since CALM depletion alters LDL receptor endocytosis [103].

BIN1

BIN1 was first associated with LOAD by GWAS performed by Seshadri et al., 2010, which identified the most common SNP rs744373 in a locus within 30 kb of the gene *BIN1* [104]. BIN1 association to LOAD was further confirmed in other large family-based GWAS [105], in candidate gene studies with independent cohorts [28, 80], and meta-analysis of multicenter datasets [28, 106, 107]. Subsequent analysis of GWAS patients found *BIN1* associated with alterations in cortical thickness, lower scores on episodic memory and an earlier AD onset [79, 84, 108]. *BIN1* sequencing identified rare coding variants associated with LOAD [109, 110].

BIN1 encodes for Bin1 (bridging integrator 1) first identified as an interactor of MYC, the oncoprotein [111]. BIN1 undergoes alternative splicing originating at least ten isoforms. All Bin1 isoforms are membrane-associated and share an N-terminal BAR domain, thus belonging to the BAR (Bin–Amphiphysin–Rvsp) family proteins. Through its BAR domain, Bin1 confers curvature to membranes, critical for its function in membrane tubulation and vesicle formation. The C-terminal SH3 domain mediates Bin1 interaction with proteins involved in endocytosis, such as dynamin [112, 113] and endophilin [114] that regulate membrane dynamics.

Importantly, in brain mainly the Bin1 neuronal-specific isoform (isoform 1) and at least one ubiquitous isoform (isoform 9) are expressed [115]. Neuronal *BIN1* was almost simultaneously identified by different groups [113, 116–118]. Neuronal Bin1 was initially found enriched in brain synaptosomes and localizes to axon initial segments and nodes of Ranvier [116–118]. It is the longest isoform and contains a clathrin-associated protein-binding region (CLAP domain) [119]. Bin1 is very similar to amphiphysin, and dimerization with amphiphysin can enhance clathrin-mediated endocytosis [118]. Amphiphysin knockout mice present reduced levels of Bin1 and exhibit synaptic vesicle recycling defects [120]. *BIN1* knockout mice die after birth, due to muscle defects, but embryonic primary neuronal cultures showed unaffected synapse morphology [121]. Ubiquitous *BIN1* knockdown did not alter significantly endocytosis, instead increased defects in the recycling of transferrin receptor, in fibroblasts or HeLa cells [121, 122].

BIN1 mRNA transcripts were found increased in AD brains, maybe due to the augmented transcriptional activity of LOAD variants [123]. Higher *BIN1* gene expression was found correlated with later onset and shorter disease duration [124]. Interestingly, the expression of *BIN1* was highly correlated with that of *CD2AP* and *PICALM* [124]. Neuronal Bin1, but not ubiquitous Bin1, has been found decreased in LOAD [125–127].

We and Tomita's lab found Bin1 depletion to increase Aβ production due to the accumulation of BACE1 in endosomes [128, 129]. A differential impact on the secretion of Aβ was observed. While in the Miyagawa study secreted Aβ42 and Aβ40 increased, we found a decrease in Aβ40, but not in Aβ42 secretion [128, 129]. More consensual was the increase in intracellular Aβ42 upon Bin1 depletion [128, 129]. Surprisingly, we found by subcellular analysis of Aβ42 accumulation that Aβ42 increased mainly in axons [129]. Both groups found BACE1 accumulating in early endosomes, suggesting that Bin1 controls Aβ production by regulating BACE1 trafficking [128, 129]. In the Miyagawa study, BACE1 levels increased in neurons depleted for Bin1 pointing to a function for Bin1 in controlling BACE1 degradation; however, the exact mechanism involved has yet to be investigated [128]. We found that Bin1 depletion led to an impaired BACE1 recycling, specifically in axons [129]. Mechanistically, Bin1 was found required for scission of BACE1 tubules from early endosomes enabling BACE1 recycling [129]. How this Bin1 role in BACE1 recycling affects BACE1 degradation needs to be investigated. Bin1 could also contribute to AD by playing a role in disease propagation, since Bin1 depletion increased tau propagation via an endosomal route [130].

CD2AP

CD2AP genetic variants were associated with LOAD by several GWAS [26, 79, 107]. *CD2AP* susceptibility loci correlate with AD progression [131]. Meta-analysis of GWAS studies confirmed *CD2AP* association and identified the non-coding variant, rs9346407, as the most frequent in LOAD patients [28, 132]. *CD2AP* sequencing identified rare coding variants in LOAD [110].

CD2AP encodes for CD2-associated protein (CD2AP), a membrane-associated scaffolding protein, first identified as a T cells adaptor protein [133]. CD2AP is an endocytic [134–137] and an actin cytoskeleton regulator [135, 138–140]. CD2AP may control endosome maturation and protein sorting for degradation via its actin regulation [135].

CD2AP is most expressed in kidney podocytes [141], where it anchors important adaptors of the slit diaphragm to the actin cytoskeleton [142]. Interestingly, podocytes, like neurons, have actin-rich protrusions and share actin regulators such as synaptopodin and drebrin [143, 144].

CD2AP is less expressed in the brain [141]; nevertheless, in situ hybridization clearly shows CD2AP mRNA expression in cortical and hippocampal neurons (Allen brain Atlas; ID 12488). CD2AP is detected in primary cortical neurons especially in dendrites, where it localizes to endosomes [129]. CD2AP expression in the LOAD brain has not been investigated, but there is evidence that it could be reduced as in the peripheral blood lymphocytes of a Chinese LOAD cohort [145].

The decreased CD2AP expression can increase intracellular exogenous Aβ40 and Aβ42 levels without increasing extracellular Aβ levels in neuroblastoma cells overexpressing APP [146]. We found that decreased CD2AP expression increased intracellular endogenous Aβ42 in wild-type neuroblastoma cells and particularly in dendrites of primary cortical neurons [129]. Importantly, the decreased function of CD2AP at dendritic endosomes was found responsible for an accumulation of APP at early endosomes limiting membrane [129]. The impaired sorting into multivesicular endosomes likely precluded an efficient degradation of APP by the lysosome and favored APP processing and Aβ production [129]. In young eAD transgenic mice (PS1/APP), *CD2AP* knockout did not alter Aβ accumulation nor amyloid plaques load [146]. Thus, the impact of *CD2AP* variants on the development of LOAD pathology needs to be assessed in a LOAD mouse model or in human neurons derived from fibroblasts of patients carrying CD2AP variants. Alternatively, CD2AP loss-of-function could have an impact on Aβ clearance, since it is detected in brain endothelial cells and *CD2AP* knockout mice have reduced blood–brain integrity [141, 147].

SORL1

SORL1 was initially associated with LOAD in candidate gene approaches and later in GWAS studies [28, 81, 148–153]. Subsequent sequencing studies identified rare missense variants in *SORL1* both in eAD and LOAD [81, 152, 154–156].

SORL1 encodes for sortilin-related receptor with A-type repeats (Sorla), that belongs to the family of low-density lipoprotein receptors, as well as to the family of vacuolar protein sorting ten domain receptors (VPS10p) [156]. Sorla is a neuronal sorting receptor mainly found in sorting endosomes in the somatodendritic domain [157].

Sorla levels are decreased in AD [158, 159] and several underlying mechanisms have been identified: increased methylation of *SORL1* in AD repressing gene expression [160]; the presence of shorter *SORL1* splice variants in AD reducing full-length Sorla expression [161]; and the presence of SORL1 variants limiting the increase in Sorla expression upon brain-derived neurotrophic factor (BDNF) stimulation [154].

Sorla binds directly to APP, via an extracellular domain and via a motif in the cytosolic tail [162]. Sorla binding selects endocytosed APP to be retrogradely transported back to the TGN, reducing APP processing at endosomes and Aβ production [156, 157]. Evidence supports an important role for Sorla in removing APP from endosomes. Depletion of Sorla increases Aβ production and amyloid plaques load [163].

Human neurons carrying *SORL1* AD variants showed decreased APP processing upon BDNF stimulation [154]. Some rare variants, such as p.Asn2174Ser, have been shown to decrease Sorla capacity to retrieve APP back to the TGN, increasing APP at endosomes and Aβ production [152]. The mechanism by which Sorla sorts APP back to the TGN has been shown to be dependent on the retromer. The retromer is a protein complex responsible for the formation of endosomal tubules enriched in APP and Sorla that upon scission will be transported back to the TGN [164, 165]. APP phosphorylation and dimerization have been shown to regulate APP trafficking dependent on Sorla [166, 167].

Alternatively, Sorla loss-of-function could decrease Aβ clearance, since Sorla binds to Aβ promoting its delivery to lysosome and degradation [158]. Interestingly, Sorla mediates the cellular uptake of cholesterol-loaded APOE, with a preference for APOE4 [168]. It is important to note that protective variants have also been identified, although their mechanism remains to be investigated [81].

Increasing Sorla could be a therapeutic approach, since it reduces Aβ concentration in mouse brain [158]. A promising study identified a Sorla activator, 6-shogoal, with therapeutic potential against AD [169].

PLD3

Rare variants in *PLD3* were associated with increased LOAD risk [29, 170]. However, the association has not yet been replicated in AD [171] neither in eFAD [172]. *PLD3* variants were weakly associated with cognitive decline and not with amyloid pathology [173, 174].

PLD3 encodes for phospholipase D3, a membrane-associated protein of the PLD family, which includes phospholipases D1 and D2, both involved in endocytic trafficking [175, 176]. Less studied, PLD3 does not have the PX and PH domains that localize PLD1 and 2 to membranes. While PLD1 and PLD2 produce phosphatidic acid, PLD3 has a conserved substitution in the lipase domain PLD3 that likely prevents its activity as a classical PLD [176]. PLD3 is a transmembrane glycoprotein associated with the endoplasmic reticulum, involved in its reorganization during myotube formation [177].

Importantly, PLD3 is highly expressed in hippocampus and cortex, regions more vulnerable to AD pathology [29, 178]. *PLD3* mRNA and protein expression are decreased in LOAD patients brain [29, 179]. Notably, PLD3 accumulates in neuritic plaques [179]. Interestingly, depletion of PLD3 increased resistance to oxidative stress-dependent loss of cell viability [180].

PLD3 loss-of-function increased secretion of Aβ42 and Aβ40 [29]; however, recently, this result was not replicated in similar experimental conditions [181]. Instead, PLD3 was found enriched in lysosomes which became morphologically

abnormal upon PLD3 loss-of-function [181]. Whether the lysosomal degradative activity is affected and whether it contributes to Aβ42 clearance instead of Aβ42 production will need to be further investigated.

Outlook

The studies of ApoE4, CALM, Bin1, CD2AP, Sorla, and PLD3 encoded by LOAD genetic risk factors reviewed here support that increased production of Aβ42 is a mechanism of LOAD. ApoE4 and loss-of-function of Bin1, CD2AP, CALM, Sorla, and PLD3 lead, by different mechanisms, to deregulation in intracellular trafficking of APP and/or of its secretases, to an increase in the retention of APP and/or its secretases in sorting endosomes, potentiating Aβ42 endocytic production (Fig. 2). However, this may not be the only causal mechanism of Aβ42 accumulation in LOAD, since at least two other mechanisms have been identified to be impaired by loss-of-function of the genetic risk factors: 1. defective clearance of Aβ42 through the BBB due to impaired endocytosis/transcytosis via sorting endosomes for ApoE4, CD2AP, CALM, and Sorla and 2. defective lysosomal clearance of Aβ42 for ApoE4, Sorla, PLD3 by neurons, and other brain cells. Additional mechanisms independent of Aβ may also occur in parallel, reflected by defects in glutamate receptors, cholesterol, and tau trafficking due to ApoE4, Sorla, and CALM. More research will be necessary to integrate the multiple ways by which the endocytic genetic risk factors contribute to AD development.

Most of the studies reviewed here used a knockdown or overexpression approach to study the role of the endocytic genetic risk factors in AD. The only variant associated with AD for which the impact on Aβ production has been determined is APOE4. It is critical in the future to identify functional variants for *PICALM*, *BIN1*, *CD2AP*, *SORL1*, and *PLD3* to enable research aimed at validating or identifying the underlying mechanisms. Sequencing of such genetic risk factors has started identifying rare but predicted to be deleterious variants; however, the number of studies and patients sequenced is still very small. Moreover, given that AD is a human-specific disease, future research should consider using human neurons derived from patients or even genetically edited with patients' mutations to dissect the causal mechanisms of LOAD.

Another aspect of major importance that should be addressed in the future is to determine whether the increase in Aβ42 triggered by the endocytic risk factors is sufficient to cause synaptic dysfunction, an earlier and functionally more relevant disease phenotype than amyloid plaques. Importantly, it is possible that aging together with the Aβ42 accumulation-triggered by genetic risk factors, will be sufficient to lead to the deposition of amyloid plaques, tangles

formation and ultimately full-blown neurodegeneration. It is worthwhile mentioning the Model-AD initiative (https://model-ad.org/) which, by generating knock-in mice with the most promising genetic variants, may help to prove causality between endocytic deregulation and the development of LOAD.

Bibliography

1. Tampellini D, Gouras GK (2010) Synapses, synaptic activity and intraneuronal abeta in Alzheimer's disease. Front Aging Neurosci. https://doi.org/10.3389/fnagi.2010.00013

2. Benilova I, Karran E, De Strooper B (2012) The toxic Aβ oligomer and Alzheimer's disease: an emperor in need of clothes. Nat Neurosci 15:349–357. https://doi.org/10.1038/nn.3028

3. Almeida CG, Tampellini D, Takahashi RH et al (2005) Beta-amyloid accumulation in APP mutant neurons reduces PSD-95 and GluR1 in synapses. Neurobiol Dis 20:187–198. https://doi.org/10.1016/j.nbd.2005.02.008

4. Snyder EM, Nong Y, Almeida CG et al (2005) Regulation of NMDA receptor trafficking by amyloid-beta. Nat Neurosci 8:1051–1058. https://doi.org/10.1038/nn1503

5. Mucke L, Selkoe DJ (2012) Neurotoxicity of amyloid β-protein: synaptic and network dysfunction. Cold Spring Harb Perspect Med 2:a006338. https://doi.org/10.1101/cshperspect.a006338

6. Takahashi RH, Nagao T, Gouras GK (2017) Plaque formation and the intraneuronal accumulation of β-amyloid in Alzheimer's disease. Pathol Int 67:185–193. https://doi.org/10.1111/pin.12520

7. Takahashi RH, Milner TA, Li F et al (2002) Intraneuronal Alzheimer abeta42 accumulates in multivesicular bodies and is associated with synaptic pathology. Am J Pathol 161:1869–1879

8. Pensalfini A, Albay R, Rasool S et al (2014) Intracellular amyloid and the neuronal origin of Alzheimer neuritic plaques. Neurobiol Dis 71:53–61. https://doi.org/10.1016/j.nbd.2014.07.011

9. Gouras GK, Almeida CG, Takahashi RH (2005) Intraneuronal Abeta accumulation and origin of plaques in Alzheimer's disease. Neurobiol Aging 26:1235–1244. https://doi.org/10.1016/j.neurobiolaging.2005.05.022

10. Almeida CG, Takahashi RH, Gouras GK (2006) Beta-amyloid accumulation impairs multivesicular body sorting by inhibiting the ubiquitin-proteasome system. J Neurosci 26:4277–4288. https://doi.org/10.1523/JNEUROSCI.5078-05.2006

11. Sahlin C, Lord A, Magnusson K et al (2007) The Arctic Alzheimer mutation favors intracellular amyloid-beta production by making amyloid precursor protein less available to alpha-secretase. J Neurochem 101:854–862. https://doi.org/10.1111/j.1471-4159.2006.04443.x

12. Norvin D, Kim G, Baker-Nigh A, Geula C (2015) Accumulation and age-related elevation of amyloid-β within basal forebrain cholinergic neurons in the rhesus monkey. Neuroscience 298:102–111. https://doi.org/10.1016/j.neuroscience.2015.04.011

13. LaFerla FM, Green KN, Oddo S (2007) Intracellular amyloid-beta in Alzheimer's disease. Nat Rev Neurosci 8:499–509. https://doi.org/10.1038/nrn2168

14. Mecozzi VJ, Berman DE, Simoes S et al (2014) Pharmacological chaperones stabilize retromer to limit APP processing. Nat Chem Biol 10:443–449. https://doi.org/10.1038/nchembio.1508

15. Matsuda S, Matsuda Y, Snapp EL, D'Adamio L (2011) Maturation of BRI2 generates a specific inhibitor that reduces APP processing at the plasma membrane and in endocytic vesicles. Neurobiol Aging 32:1400–1408. https://doi.org/10.1016/j.neurobiolaging.2009.08.005

16. Sun M, Asghar SZ, Zhang H (2016) The polarity protein Par3 regulates APP trafficking and processing through the endocytic adaptor protein Numb. Neurobiol Dis 93:1–11. https://doi.org/10.1016/j.nbd.2016.03.022

17. Zhang X, Song W (2013) The role of APP and BACE1 trafficking in APP processing and amyloid-β generation. Alzheimers Res Ther 5:46. https://doi.org/10.1186/alzrt211

18. Takahashi K, Niidome T, Akaike A et al (2008) Phosphorylation of amyloid precursor protein (APP) at Tyr687 regulates APP processing by alpha- and gamma-secretase. Biochem Biophys Res Commun 377:544–549. https://doi.org/10.1016/j.bbrc.2008.10.013

19. Tammineni P, Jeong YY, Feng T et al (2017) Impaired axonal retrograde trafficking of the retromer complex augments lysosomal deficits in Alzheimer's disease neurons. Hum Mol Genet 26:4352–4366. https://doi.org/10.1093/hmg/ddx321

20. Saunders AM, Strittmatter WJ, Schmechel D et al (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 43:1467–1472

21. Corder EH, Saunders AM, Strittmatter WJ et al (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261:921–923. https://doi.org/10.1126/science.8346443

22. Strittmatter WJ, Saunders AM, Schmechel D et al (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 90:1977–1981

23. Rebeck GW, Reiter JS, Strickland DK, Hyman BT (1993) Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. Neuron 11:575–580. https://doi.org/10.1016/0896-6273(93)90070-8

24. Chouraki V, Seshadri S, Theodore Friedmann JCD and SFG (2014) Chapter five—genetics of Alzheimer's disease. Advances in Genetics. Academic Press, Cambridge, pp 245–294

25. Harold D, Abraham R, Hollingworth P et al (2009) Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 41:1088–1093. https://doi.org/10.1038/ng.440

26. Hollingworth P, Harold D, Sims R et al (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet 43:429–435. https://doi.org/10.1038/ng.803

27. Naj AC, Jun G, Beecham GW et al (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. Nat Genet 43:436–441. https://doi.org/10.1038/ng.801

28. Lambert JC, Ibrahim-Verbaas CA, Harold D et al (2013) Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. Nat Genet 45:1452–1458. https://doi.org/10.1038/ng.2802

29. Cruchaga C, Karch CM, Jin SC et al (2014) Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease. Nature 505:550–554. https://doi.org/10.1038/nature12825

30. Carmona S, Hardy J, Guerreiro R (2018) The genetic landscape of Alzheimer disease. Handb Clin Neurol 148:395–408. https://doi.org/10.1016/B978-0-444-64076-5.00026-0

31. Das U, Scott DA, Ganguly A et al (2013) Activity-induced convergence of APP and BACE-1 in acidic microdomains via an endocytosis-dependent pathway. Neuron 79:447–460. https://doi.org/10.1016/j.neuron.2013.05.035

32. Ehehalt R, Keller P, Haass C et al (2003) Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. J Cell Biol 160:113–123. https://doi.org/10.1083/jcb.200207113

33. Kalvodova L, Kahya N, Schwille P et al (2005) Lipids as modulators of proteolytic activity of BACE: involvement of cholesterol, glycosphingolipids, and anionic phospholipids in vitro. J Biol Chem 280:36815–36823. https://doi.org/10.1074/jbc.M504484200

34. Koo EH, Squazzo SL (1994) Evidence that production and release of amyloid beta-protein involves the endocytic pathway. J Biol Chem 269:17386–17389

35. Lai A, Sisodia SS, Trowbridge IS (1995) Characterization of sorting signals in the beta-amyloid precursor protein cytoplasmic domain. J Biol Chem 270:3565–3573

36. Van der Kant R, Goldstein LSB (2015) Cellular functions of the amyloid precursor protein from development to dementia. Dev Cell 32:502–515. https://doi.org/10.1016/j.devcel.2015.01.022

37. Schneider A, Rajendran L, Honsho M et al (2008) Flotillin-dependent clustering of the amyloid precursor protein regulates its endocytosis and amyloidogenic processing in neurons. J Neurosci 28:2874–2882. https://doi.org/10.1523/JNEUROSCI.5345-07.2008

38. Marquer C, Devauges V, Cossec J-C et al (2011) Local cholesterol increase triggers amyloid precursor protein-Bace1 clustering in lipid rafts and rapid endocytosis. FASEB J 25:1295–1305. https://doi.org/10.1096/fj.10-168633

39. Kang MJ, Chung YH, Hwang CI et al (2006) Caveolin-1 upregulation in senescent neurons alters amyloid precursor protein processing. Exp Mol Med 38:126–133. https://doi.org/10.1038/emm.2006.16

40. Zerbinatti CV, Bu G (2005) LRP and Alzheimer's disease. Rev Neurosci 16:123–135

41. Pietrzik CU, Busse T, Merriam DE et al (2002) The cytoplasmic domain of the LDL receptor-related protein regulates multiple steps in APP processing. EMBO J 21:5691–5700

42. Ye S, Huang Y, Müllendorff K et al (2005) Apolipoprotein (apo) E4 enhances amyloid beta peptide production in cultured neuronal cells: apoE structure as a potential therapeutic target. Proc Natl Acad Sci USA 102:18700–18705. https://doi.org/10.1073/pnas.0508693102

43. Sannerud R, Declerck I, Peric A et al (2011) ADP ribosylation factor 6 (ARF6) controls amyloid precursor protein (APP) processing by mediating the endosomal sorting of BACE1. Proc Natl Acad Sci USA 108:E559–E568. https://doi.org/10.1073/pnas.1100745108

44. Prabhu Y, Burgos PV, Schindler C et al (2012) Adaptor protein 2-mediated endocytosis of the β-secretase BACE1 is dispensable for amyloid precursor protein processing. Mol Biol Cell 23:2339–2351. https://doi.org/10.1091/mbc.E11-11-0944

45. Chia PZC, Toh WH, Sharples R et al (2013) Intracellular itinerary of internalised β-secretase, BACE1, and its potential impact on β-amyloid peptide biogenesis. Traffic 14:997–1013. https://doi.org/10.1111/tra.12088

46. Pastorino L, Ikin AF, Nairn AC et al (2002) The carboxyl-terminus of BACE contains a sorting signal that regulates BACE

trafficking but not the formation of total A(beta). Mol Cell Neurosci 19:175–185. https://doi.org/10.1006/mcne.2001.1065

47. He X, Zhu G, Koelsch G et al (2003) Biochemical and structural characterization of the interaction of memapsin 2 (beta-secretase) cytosolic domain with the VHS domain of GGA proteins. Biochemistry 42:12174–12180. https://doi.org/10.1021/bi035199h

48. Cirrito JR, Kang J-E, Lee J et al (2008) Endocytosis is required for synaptic activity-dependent release of amyloid-beta in vivo. Neuron 58:42–51. https://doi.org/10.1016/j.neuron.2008.02.003

49. Zou L, Wang Z, Shen L et al (2007) Receptor tyrosine kinases positively regulate BACE activity and Amyloid-beta production through enhancing BACE internalization. Cell Res 17:389–401. https://doi.org/10.1038/cr.2007.5

50. Yan R, Vassar R (2014) Targeting the β secretase BACE1 for Alzheimer's disease therapy. Lancet Neurol 13:319–329. https://doi.org/10.1016/S1474-4422(13)70276-X

51. Rajendran L, Schneider A, Schlechtingen G et al (2008) Efficient inhibition of the Alzheimer's disease beta-secretase by membrane targeting. Science 320:520–523. https://doi.org/10.1126/science.1156609

52. Choy RW-Y, Cheng Z, Schekman R (2012) Amyloid precursor protein (APP) traffics from the cell surface via endosomes for amyloid β (Aβ) production in the trans-Golgi network. Proc Natl Acad Sci USA 109:E2077–E2082. https://doi.org/10.1073/pnas.1208635109

53. Buggia-Prévot V, Fernandez CG, Udayar V et al (2013) A function for EHD family proteins in unidirectional retrograde dendritic transport of BACE1 and Alzheimer's disease Aβ production. Cell Rep 5:1552–1563. https://doi.org/10.1016/j.celrep.2013.12.006

54. Morel E, Chamoun Z, Lasiecka ZM et al (2013) Phosphatidylinositol-3-phosphate regulates sorting and processing of amyloid precursor protein through the endosomal system. Nat Commun 4:2250. https://doi.org/10.1038/ncomms3250

55. Sannerud R, Esselens C, Ejsmont P et al (2016) Restricted Location of PSEN2/γ-Secretase Determines Substrate Specificity and Generates an Intracellular Aβ Pool. Cell 166:193–208. https://doi.org/10.1016/j.cell.2016.05.020

56. Edgar JR, Willén K, Gouras GK, Futter CE (2015) ESCRTs regulate amyloid precursor protein sorting in multivesicular bodies and intracellular amyloid-β accumulation. J Cell Sci 128:2520–2528. https://doi.org/10.1242/jcs.170233

57. Udayar V, Buggia-Prévot V, Guerreiro RL et al (2013) A paired RNAi and RabGAP overexpression screen identifies Rab11 as a regulator of β-amyloid production. Cell Rep 5:1536–1551. https://doi.org/10.1016/j.celrep.2013.12.005

58. Takahashi RH, Almeida CG, Kearney PF et al (2004) Oligomerization of Alzheimer's beta-amyloid within processes and synapses of cultured neurons and brain. J Neurosci 24:3592–3599. https://doi.org/10.1523/JNEUROSCI.5167-03.2004

59. Oropeza RL, Wekerle H, Werb Z (1987) Expression of apolipoprotein E by mouse brain astrocytes and its modulation by interferon-gamma. Brain Res 410:45–51

60. Bu G (2009) Apolipoprotein E and its receptors in Alzheimer's disease: pathways, pathogenesis and therapy. Nat Rev Neurosci 10:333–344. https://doi.org/10.1038/nrn2620

61. Conejero-Goldberg C, Gomar JJ, Bobes-Bascaran T et al (2014) APOE2 enhances neuroprotection against Alzheimer's disease through multiple molecular mechanisms. Mol Psychiatry 19:1243–1250. https://doi.org/10.1038/mp.2013.194

62. He X, Cooley K, Chung CHY et al (2007) Apolipoprotein receptor 2 and X11 alpha/beta mediate apolipoprotein E-induced endocytosis of amyloid-beta precursor protein and beta-secretase, leading to amyloid-beta production. J Neurosci 27:4052–4060. https://doi.org/10.1523/JNEUROSCI.3993-06.2007

63. Hoe H-S, Pocivavsek A, Dai H et al (2006) Effects of apoE on neuronal signaling and APP processing in rodent brain. Brain Res 1112:70–79. https://doi.org/10.1016/j.brainres.2006.07.035

64. Irizarry MC, Deng A, Lleo A et al (2004) Apolipoprotein E modulates gamma-secretase cleavage of the amyloid precursor protein. J Neurochem 90:1132–1143. https://doi.org/10.1111/j.1471-4159.2004.02581.x

65. Hopkins PCR, Sáinz-Fuertes R, Lovestone S (2011) The impact of a novel apolipoprotein E and amyloid-β protein precursor-interacting protein on the production of amyloid-β. J Alzheimers Dis 26:239–253. https://doi.org/10.3233/JAD-2011-102115

66. Cataldo AM, Peterhoff CM, Troncoso JC et al (2000) Endocytic pathway abnormalities precede amyloid β deposition in sporadic Alzheimer's disease and Down syndrome: differential effects of APOE genotype and presenilin mutations. Am J Pathol 157:277–286

67. Zhao N, Liu C-C, Van Ingelgom AJ et al (2017) Apolipoprotein E4 impairs neuronal insulin signaling by trapping insulin receptor in the endosomes. Neuron 96(115–129):e5. https://doi.org/10.1016/j.neuron.2017.09.003

68. Rapp A, Gmeiner B, Hüttinger M (2006) Implication of apoE isoforms in cholesterol metabolism by primary rat hippocampal neurons and astrocytes. Biochimie 88:473–483. https://doi.org/10.1016/j.biochi.2005.10.007

69. Huang Y-WA, Zhou B, Wernig M, Südhof TC (2017) Apoe2, apoe3, and apoe4 differentially stimulate APP transcription and aβ secretion. Cell 168(427–441):e21. https://doi.org/10.1016/j.cell.2016.12.044

70. Castellano JM, Kim J, Stewart FR et al (2011) Human apoE isoforms differentially regulate brain amyloid-β peptide clearance. Sci Transl Med 3:89ra57. https://doi.org/10.1126/scitranslmed.3002156

71. Fryer JD, Simmons K, Parsadanian M et al (2005) Human apolipoprotein E4 alters the amyloid-beta 40:42 ratio and promotes the formation of cerebral amyloid angiopathy in an amyloid precursor protein transgenic model. J Neurosci 25:2803–2810. https://doi.org/10.1523/JNEUROSCI.5170-04.2005

72. Wildsmith KR, Holley M, Savage JC et al (2013) Evidence for impaired amyloid β clearance in Alzheimer's disease. Alzheimers Res Ther 5:33. https://doi.org/10.1186/alzrt187

73. Verghese PB, Castellano JM, Garai K et al (2013) ApoE influences amyloid-β (Aβ) clearance despite minimal apoE/Aβ association in physiological conditions. Proc Natl Acad Sci USA 110:E1807–E1816. https://doi.org/10.1073/pnas.1220484110

74. Chen Y, Durakoglugil MS, Xian X, Herz J (2010) ApoE4 reduces glutamate receptor function and synaptic plasticity by selectively impairing ApoE receptor recycling. Proc Natl Acad Sci USA 107:12011–12016. https://doi.org/10.1073/pnas.0914984107

75. Jun G, Naj AC, Beecham GW et al (2010) Meta-analysis confirms CR1, CLU, and PICALM as alzheimer disease risk loci and reveals interactions with APOE genotypes. Arch Neurol 67:1473–1484. https://doi.org/10.1001/archneurol.2010.201

76. Carrasquillo MM, Belbin O, Hunter TA et al (2010) Replication of CLU, CR1, and PICALM associations with alzheimer disease. Arch Neurol 67:961–964. https://doi.org/10.1001/archneurol.2010.147

77. Ferrari R, Moreno JH, Minhajuddin AT et al (2012) Implication of common and disease specific variants in CLU, CR1, and PICALM. Neurobiol Aging 33(1846):e7–e18. https://doi.org/10.1016/j.neurobiolaging.2012.01.110

78. Moreno DJ, Ruiz S, Ríos Á et al (2017) Association of GWAS top genes with late-onset Alzheimer's disease in Colombian population. Am J Alzheimers Dis Other Demen 32:27–35. https://doi.org/10.1177/1533317516679303

79. Naj AC, Jun G, Reitz C et al (2014) Effects of multiple genetic loci on age at onset in late-onset Alzheimer disease: a

genome-wide association study. JAMA Neurol 71:1394–1404. https://doi.org/10.1001/jamaneurol.2014.1491

80. Lee JH, Cheng R, Barral S et al (2011) Identification of novel loci for Alzheimer disease and replication of CLU, PICALM, and BIN1 in Caribbean Hispanic individuals. Arch Neurol 68:320–328. https://doi.org/10.1001/archneurol.2010.292

81. Wang Z, Lei H, Zheng M et al (2016) Meta-analysis of the Association between Alzheimer Disease and Variants in GAB2, PICALM, and SORL1. Mol Neurobiol 53:6501–6510. https://doi.org/10.1007/s12035-015-9546-y

82. Xu W, Wang H-F, Tan L et al (2016) The impact of PICALM genetic variations on reserve capacity of posterior cingulate in AD continuum. Sci Rep 6:24480. https://doi.org/10.1038/srep24480

83. Mengel-From J, Christensen K, McGue M, Christiansen L (2011) Genetic variations in the CLU and PICALM genes are associated with cognitive function in the oldest old. Neurobiol Aging 32(554):e7–e11. https://doi.org/10.1016/j.neurobiolaging.2010.07.016

84. Biffi A, Anderson CD, Desikan RS et al (2010) Genetic variation and neuroimaging measures in Alzheimer disease. Arch Neurol 67:677–685. https://doi.org/10.1001/archneurol.2010.108

85. Dreyling MH, Martinez-Climent JA, Zheng M et al (1996) The t(10;11)(p13;q14) in the U937 cell line results in the fusion of the AF10 gene and CALM, encoding a new member of the AP-3 clathrin assembly protein family. Proc Natl Acad Sci USA 93:4804–4809

86. Miller SE, Sahlender DA, Graham SC et al (2011) The molecular basis for the endocytosis of small R-SNAREs by the clathrin adaptor CALM. Cell 147:1118–1131. https://doi.org/10.1016/j.cell.2011.10.038

87. Yao PJ, Petralia RS, Bushlin I et al (2005) Synaptic distribution of the endocytic accessory proteins AP180 and CALM. J Comp Neurol 481:58–69. https://doi.org/10.1002/cne.20362

88. Vanlandingham PA, Barmchi MP, Royer S et al (2014) AP180 couples protein retrieval to clathrin-mediated endocytosis of synaptic vesicles. Traffic 15:433–450. https://doi.org/10.1111/tra.12153

89. Meyerholz A, Hinrichsen L, Groos S et al (2005) Effect of clathrin assembly lymphoid myeloid leukemia protein depletion on clathrin coat formation. Traffic 6:1225–1234. https://doi.org/10.1111/j.1600-0854.2005.00355.x

90. Sahlender DA, Kozik P, Miller SE et al (2013) Uncoupling the functions of CALM in VAMP sorting and clathrin-coated pit formation. PLoS One 8:e64514. https://doi.org/10.1371/journal.pone.0064514

91. Koo SJ, Markovic S, Puchkov D et al (2011) SNARE motif-mediated sorting of synaptobrevin by the endocytic adaptors clathrin assembly lymphoid myeloid leukemia (CALM) and AP180 at synapses. Proc Natl Acad Sci USA 108:13540–13545. https://doi.org/10.1073/pnas.1107067108

92. Ando K, Brion J-P, Stygelbout V et al (2013) Clathrin adaptor CALM/PICALM is associated with neurofibrillary tangles and is cleaved in Alzheimer's brains. Acta Neuropathol 125:861–878. https://doi.org/10.1007/s00401-013-1111-z

93. Thomas RS, Lelos MJ, Good MA, Kidd EJ (2011) Clathrin-mediated endocytic proteins are upregulated in the cortex of the Tg2576 mouse model of Alzheimer's disease-like amyloid pathology. Biochem Biophys Res Commun 415:656–661. https://doi.org/10.1016/j.bbrc.2011.10.131

94. Parikh I, Fardo DW, Estus S (2014) Genetics of PICALM expression and Alzheimer's disease. PLoS One 9:e91242. https://doi.org/10.1371/journal.pone.0091242

95. Xiao Q, Gil S-C, Yan P et al (2012) Role of phosphatidylinositol clathrin assembly lymphoid-myeloid leukemia (PICALM) in intracellular amyloid precursor protein (APP) processing

96. Boehm C, Kaden D, St. George-Hyslop P (2012) Picalm but not bin1 alters the secretion of beta-amyloid peptide. Alzheimers Dement 8:P652. https://doi.org/10.1016/j.jalz.2012.05.2175

97. Thomas RS, Henson A, Gerrish A et al (2016) Decreasing the expression of PICALM reduces endocytosis and the activity of β-secretase: implications for Alzheimer's disease. BMC Neurosci 17:50. https://doi.org/10.1186/s12868-016-0288-1

98. Kanatsu K, Morohashi Y, Suzuki M et al (2014) Decreased CALM expression reduces Aβ42 to total Aβ ratio through clathrin-mediated endocytosis of γ-secretase. Nat Commun 5:3386. https://doi.org/10.1038/ncomms4386

99. Kanatsu K, Hori Y, Takatori S et al (2016) Partial loss of CALM function reduces Aβ42 production and amyloid deposition in vivo. Hum Mol Genet 25:3988–3997. https://doi.org/10.1093/hmg/ddw239

100. Tian Y, Chang JC, Fan EY et al (2013) Adaptor complex AP2/PICALM, through interaction with LC3, targets Alzheimer's APP-CTF for terminal degradation via autophagy. Proc Natl Acad Sci USA 110:17071–17076. https://doi.org/10.1073/pnas.1315110110

101. Zhao Z, Sagare AP, Ma Q et al (2015) Central role for PICALM in amyloid-β blood-brain barrier transcytosis and clearance. Nat Neurosci 18:978–987. https://doi.org/10.1038/nn.4025

102. Gimber N, Tadeus G, Maritzen T et al (2015) Diffusional spread and confinement of newly exocytosed synaptic vesicle proteins. Nat Commun 6:8392. https://doi.org/10.1038/ncomms9392

103. Mercer JL, Argus JP, Crabtree DM et al (2015) Modulation of PICALM levels perturbs cellular cholesterol homeostasis. PLoS One 10:e0129776. https://doi.org/10.1371/journal.pone.0129776

104. Seshadri S, Fitzpatrick AL, Ikram MA et al (2010) Genome-wide analysis of genetic loci associated with Alzheimer disease. JAMA 303:1832–1840. https://doi.org/10.1001/jama.2010.574

105. Wijsman EM, Pankratz ND, Choi Y et al (2011) Genome-wide association of familial late-onset Alzheimer's disease replicates BIN1 and CLU and nominates CUGBP2 in interaction with APOE. PLoS Genet 7:e1001308. https://doi.org/10.1371/journal.pgen.1001308

106. Hu X, Pickering E, Liu YC et al (2011) Meta-analysis for genome-wide association study identifies multiple variants at the BIN1 locus associated with late-onset Alzheimer's disease. PLoS One 6:e16616. https://doi.org/10.1371/journal.pone.0016616

107. Kamboh MI, Demirci FY, Wang X et al (2012) Genome-wide association study of Alzheimer's disease. Transl Psychiatry 2:e117. https://doi.org/10.1038/tp.2012.45

108. Barral S, Bird T, Goate A et al (2012) Genotype patterns at PICALM, CR1, BIN1, CLU, and APOE genes are associated with episodic memory. Neurology 78:1464–1471. https://doi.org/10.1212/WNL.0b013e3182553c48

109. Tan M-S, Yu J-T, Jiang T et al (2014) Genetic variation in BIN1 gene and Alzheimer's disease risk in Han Chinese individuals. Neurobiol Aging 35(1781):e1–e8. https://doi.org/10.1016/j.neurobiolaging.2014.01.151

110. Vardarajan BN, Ghani M, Kahn A et al (2015) Rare coding mutations identified by sequencing of Alzheimer disease genome-wide association studies loci. Ann Neurol 78:487–498. https://doi.org/10.1002/ana.24466

111. Sakamuro D, Elliott KJ, Wechsler-Reya R, Prendergast GC (1996) BIN1 is a novel MYC-interacting protein with features of a tumour suppressor. Nat Genet 14:69–77. https://doi.org/10.1038/ng0996-69

112. David C, McPherson PS, Mundigl O, de Camilli P (1996) A role of amphiphysin in synaptic vesicle endocytosis suggested by its

binding to dynamin in nerve terminals. Proc Natl Acad Sci USA 93:331–335

113. Leprince C, Romero F, Cussac D et al (1997) A new member of the amphiphysin family connecting endocytosis and signal transduction pathways. J Biol Chem 272:15101–15105. https://doi.org/10.1074/jbc.272.24.15101

114. Micheva KD, Kay BK, McPherson PS (1997) Synaptojanin forms two separate complexes in the nerve terminal. Interactions with endophilin and amphiphysin. J Biol Chem 272:27239–27245

115. Prokic I, Cowling BS, Laporte J (2014) Amphiphysin 2 (BIN1) in physiology and diseases. J Mol Med 92:453–463. https://doi.org/10.1007/s00109-014-1138-1

116. Ramjaun AR, Micheva KD, Bouchelet I, McPherson PS (1997) Identification and characterization of a nerve terminal-enriched amphiphysin isoform. J Biol Chem 272:16700–16706. https://doi.org/10.1074/jbc.272.26.16700

117. Butler MH, David C, Ochoa GC et al (1997) Amphiphysin II (SH3P9; BIN1), a member of the amphiphysin/Rvs family, is concentrated in the cortical cytomatrix of axon initial segments and nodes of ranvier in brain and around T tubules in skeletal muscle. J Cell Biol 137:1355–1367

118. Wigge P, Köhler K, Vallis Y et al (1997) Amphiphysin heterodimers: potential role in clathrin-mediated endocytosis. Mol Biol Cell 8:2003–2015

119. Ramjaun AR, McPherson PS (1998) Multiple amphiphysin II splice variants display differential clathrin binding: identification of two distinct clathrin-binding sites. J Neurochem 70:2369–2376

120. Di Paolo G, Sankaranarayanan S, Wenk MR et al (2002) Decreased synaptic vesicle recycling efficiency and cognitive deficits in amphiphysin 1 knockout mice. Neuron 33:789–804

121. Muller AJ, Baker JF, DuHadaway JB et al (2003) Targeted disruption of the murine Bin1/Amphiphysin II gene does not disable endocytosis but results in embryonic cardiomyopathy with aberrant myofibril formation. Mol Cell Biol 23:4295–4306

122. Pant S, Sharma M, Patel K et al (2009) AMPH-1/Amphiphysin/Bin1 functions with RME-1/Ehd1 in endocytic recycling. Nat Cell Biol 11:1399–1410. https://doi.org/10.1038/ncb1986

123. Chapuis J, Hansmannel F, Gistelinck M et al (2013) Increased expression of BIN1 mediates Alzheimer genetic risk by modulating tau pathology. Mol Psychiatry 18:1225–1234. https://doi.org/10.1038/mp.2013.1

124. Karch CM, Jeng AT, Nowotny P et al (2012) Expression of novel Alzheimer's disease risk genes in control and Alzheimer's disease brains. PLoS One 7:e50976. https://doi.org/10.1371/journal.pone.0050976

125. Glennon EBC, Whitehouse IJ, Miners JS et al (2013) BIN1 is decreased in sporadic but not familial Alzheimer's disease or in aging. PLoS One 8:e78806. https://doi.org/10.1371/journal.pone.0078806

126. Holler CJ, Davis PR, Beckett TL et al (2014) Bridging integrator 1 (BIN1) protein expression increases in the Alzheimer's disease brain and correlates with neurofibrillary tangle pathology. J Alzheimers Dis 42:1221–1227. https://doi.org/10.3233/JAD-132450

127. De Rossi P, Buggia-Prévot V, Clayton BLL et al (2016) Predominant expression of Alzheimer's disease-associated BIN1 in mature oligodendrocytes and localization to white matter tracts. Mol Neurodegener 11:59. https://doi.org/10.1186/s13024-016-0124-1

128. Miyagawa T, Ebinuma I, Morohashi Y et al (2016) BIN1 regulates BACE1 intracellular trafficking and amyloid-β production. Hum Mol Genet 25:2948–2958. https://doi.org/10.1093/hmg/ddw146

129. Ubelmann F, Burrinha T, Salavessa L et al (2017) Bin1 and CD2AP polarise the endocytic generation of beta-amyloid. EMBO Rep 18:102–122. https://doi.org/10.15252/embr.201642738

130. Calafate S, Flavin W, Verstreken P, Moechars D (2016) Loss of bin1 promotes the propagation of tau pathology. Cell Rep 17:931–940. https://doi.org/10.1016/j.celrep.2016.09.063

131. Shulman JM, Chen K, Keenan BT et al (2013) Genetic susceptibility for Alzheimer disease neuritic plaque pathology. JAMA Neurol 70:1150–1157. https://doi.org/10.1001/jamaneurol.2013.2815

132. Chen H, Wu G, Jiang Y et al (2015) Analyzing 54,936 samples supports the association between CD2AP rs9349407 polymorphism and Alzheimer's disease susceptibility. Mol Neurobiol 52:1–7. https://doi.org/10.1007/s12035-014-8834-2

133. Dustin ML, Olszowy MW, Holdorf AD et al (1998) A novel adaptor protein orchestrates receptor patterning and cytoskeletal polarity in T-cell contacts. Cell 94:667–677. https://doi.org/10.1016/S0092-8674(00)81608-6

134. Cormont M, Metón I, Mari M et al (2003) CD2AP/CMS regulates endosome morphology and traffic to the degradative pathway through its interaction with Rab4 and c-Cbl. Traffic 4:97–112

135. Gauthier NC, Monzo P, Gonzalez T et al (2007) Early endosomes associated with dynamic F-actin structures are required for late trafficking of *H. pylori* VacA toxin. J Cell Biol 177:343–354. https://doi.org/10.1083/jcb.200609061

136. Monzo P, Mari M, Kaddai V, et al (2005) CD2AP, Rabip4, and Rabip4′: Analysis of Interaction with Rab4a and Regulation of Endosomes Morphology. Meth Enzymol. Academic Press, Cambridge, pp 107–118

137. Kobayashi S, Sawano A, Nojima Y et al (2004) The c-Cbl/CD2AP complex regulates VEGF-induced endocytosis and degradation of Flt-1 (VEGFR-1). FASEB J 18:929–931. https://doi.org/10.1096/fj.03-0767fje

138. Lynch DK, Winata SC, Lyons RJ et al (2003) A Cortactin-CD2-associated protein (CD2AP) complex provides a novel link between epidermal growth factor receptor endocytosis and the actin cytoskeleton. J Biol Chem 278:21805–21813. https://doi.org/10.1074/jbc.M211407200

139. Tang VW, Brieher WM (2013) FSGS3/CD2AP is a barbed-end capping protein that stabilizes actin and strengthens adherens junctions. J Cell Biol 203:815–833. https://doi.org/10.1083/jcb.201304143

140. Zhao J, Bruck S, Cemerski S et al (2013) CD2AP links cortactin and capping protein at the cell periphery to facilitate formation of lamellipodia. Mol Cell Biol 33:38–47. https://doi.org/10.1128/MCB.00734-12

141. Li C, Ruotsalainen V, Tryggvason K et al (2000) CD2AP is expressed with nephrin in developing podocytes and is found widely in mature kidney and elsewhere. Am J Physiol Renal Physiol 279:F785–F792. https://doi.org/10.1152/ajprenal.2000.279.4.F785

142. Wolf G, Stahl RAK (2003) CD2-associated protein and glomerular disease. The Lancet 362:1746–1748. https://doi.org/10.1016/S0140-6736(03)14856-8

143. Peitsch WK, Hofmann I, Endlich N et al (2003) Cell biological and biochemical characterization of drebrin complexes in mesangial cells and podocytes of renal glomeruli. J Am Soc Nephrol 14:1452–1463

144. Kobayashi N (2002) Mechanism of the process formation; podocytes vs. neurons. Microsc Res Tech 57:217–223. https://doi.org/10.1002/jemt.10077

145. Tao Q-Q, Liu Z-J, Sun Y-M et al (2017) Decreased gene expression of CD2AP in Chinese patients with sporadic Alzheimer's disease. Neurobiol Aging. https://doi.org/10.1016/j.neurobiolaging.2017.03.013

146. Liao F, Jiang H, Srivatsan S et al (2015) Effects of CD2-associated protein deficiency on amyloid-β in neuroblastoma cells

and in an APP transgenic mouse model. Mol Neurodegener 10:12. https://doi.org/10.1186/s13024-015-0006-y

147. Cochran JN, Rush T, Buckingham SC, Roberson ED (2015) The Alzheimer's disease risk factor CD2AP maintains blood-brain barrier integrity. Hum Mol Genet 24:6667–6674. https://doi.org/10.1093/hmg/ddv371

148. Rogaeva E, Meng Y, Lee JH et al (2007) The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat Genet 39:168–177. https://doi.org/10.1038/ng1943

149. Lee JH, Cheng R, Schupf N et al (2007) The association between genetic variants in SORL1 and Alzheimer disease in an urban, multiethnic, community-based cohort. Arch Neurol 64:501–506. https://doi.org/10.1001/archneur.64.4.501

150. Feng X, Hou D, Deng Y et al (2015) SORL1 gene polymorphism association with late-onset Alzheimer's disease. Neurosci Lett 584:382–389. https://doi.org/10.1016/j.neulet.2014.10.055

151. Piscopo P, Tosto G, Belli C et al (2015) SORL1 gene is associated with the conversion from mild cognitive impairment to Alzheimer's disease. J Alzheimers Dis 46:771–776. https://doi.org/10.3233/JAD-141551

152. Verheijen J, Van den Bossche T, van der Zee J et al (2016) A comprehensive study of the genetic impact of rare variants in SORL1 in European early-onset Alzheimer's disease. Acta Neuropathol 132:213–224. https://doi.org/10.1007/s00401-016-1566-9

153. Miyashita A, Koike A, Jun G et al (2013) SORL1 is genetically associated with late-onset Alzheimer's disease in Japanese, Koreans and Caucasians. PLoS One 8:e58618. https://doi.org/10.1371/journal.pone.0058618

154. Young JE, Boulanger-Weill J, Williams DA et al (2015) Elucidating molecular phenotypes caused by the SORL1 Alzheimer's disease genetic risk factor using human induced pluripotent stem cells. Cell Stem Cell 16:373–385. https://doi.org/10.1016/j.stem.2015.02.004

155. Reitz C, Tokuhiro S, Clark LN et al (2011) SORCS1 alters amyloid precursor protein processing and variants may increase Alzheimer's disease risk. Ann Neurol 69:47–64. https://doi.org/10.1002/ana.22308

156. Schmidt V, Subkhangulova A, Willnow TE (2017) Sorting receptor SORLA: cellular mechanisms and implications for disease. Cell Mol Life Sci 74:1475–1483. https://doi.org/10.1007/s00018-016-2410-z

157. Klinger SC, Højland A, Jain S et al (2016) Polarized trafficking of the sorting receptor SorLA in neurons and MDCK cells. FEBS J 283:2476–2493. https://doi.org/10.1111/febs.13758

158. Caglayan S, Takagi-Niidome S, Liao F et al (2014) Lysosomal sorting of amyloid-β by the SORLA receptor is impaired by a familial Alzheimer's disease mutation. Sci Transl Med 6:223ra20. https://doi.org/10.1126/scitranslmed.3007747

159. Andersen OM, Rudolph I-M, Willnow TE (2016) Risk factor SORL1: from genetic association to functional validation in Alzheimer's disease. Acta Neuropathol 132:653–665. https://doi.org/10.1007/s00401-016-1615-4

160. Yu L, Chibnik LB, Srivastava GP et al (2015) Association of Brain DNA methylation in SORL1, ABCA7, HLA-DRB5, SLC24A4, and BIN1 with pathological diagnosis of Alzheimer disease. JAMA Neurol 72:15–24. https://doi.org/10.1001/jamaneurol.2014.3049

161. Grear KE, Ling I-F, Simpson JF et al (2009) Expression of SORL1 and a novel SORL1 splice variant in normal and Alzheimers disease brain. Mol Neurodegener 4:46. https://doi.org/10.1186/1750-1326-4-46

162. Fjorback AW, Seaman M, Gustafsen C et al (2012) Retromer binds the FANSHY sorting motif in SorLA to regulate amyloid precursor protein sorting and processing. J Neurosci 32:1467–1480. https://doi.org/10.1523/JNEUROSCI.2272-11.2012

163. Dodson SE, Andersen OM, Karmali V et al (2008) Loss of LR11/SORLA enhances early pathology in a mouse model of amyloidosis: evidence for a proximal role in Alzheimer's disease. J Neurosci 28:12877–12886. https://doi.org/10.1523/JNEUROSCI.4582-08.2008

164. Bhalla A, Vetanovetz CP, Morel E et al (2012) The location and trafficking routes of the neuronal retromer and its role in amyloid precursor protein transport. Neurobiol Dis 47:126–134. https://doi.org/10.1016/j.nbd.2012.03.030

165. Bonifacino JS, Hurley JH (2008) Retromer. Curr Opin Cell Biol 20:427–436. https://doi.org/10.1016/j.ceb.2008.03.009

166. Eggert S, Gonzalez AC, Thomas C et al (2017) Dimerization leads to changes in APP (amyloid precursor protein) trafficking mediated by LRP1 and SorLA. Cell Mol Life Sci 75:1–22. https://doi.org/10.1007/s00018-017-2625-7

167. Vieira SI, Rebelo S, Esselmann H et al (2010) Retrieval of the Alzheimer's amyloid precursor protein from the endosome to the TGN is S655 phosphorylation state-dependent and retromer-mediated. Mol Neurodegener 5:40. https://doi.org/10.1186/1750-1326-5-40

168. Yajima R, Tokutake T, Koyama A et al (2015) ApoE-isoform-dependent cellular uptake of amyloid-β is mediated by lipoprotein receptor LR11/SorLA. Biochem Biophys Res Commun 456:482–488. https://doi.org/10.1016/j.bbrc.2014.11.111

169. Na J-Y, Song K, Lee J-W et al (2017) Sortilin-related receptor 1 interacts with amyloid precursor protein and is activated by 6-shogaol, leading to inhibition of the amyloidogenic pathway. Biochem Biophys Res Commun 484:890–895. https://doi.org/10.1016/j.bbrc.2017.02.029

170. Schulte EC, Kurz A, Alexopoulos P et al (2015) Excess of rare coding variants in PLD3 in late- but not early-onset Alzheimer's disease. Hum Genome Var 2:14028. https://doi.org/10.1038/hgv.2014.28

171. Van der Lee SJ, Holstege H, Wong TH et al (2015) PLD3 variants in population studies. Nature 520:E2–E3. https://doi.org/10.1038/nature14038

172. Cacace R, Van den Bossche T, Engelborghs S et al (2015) Rare variants in PLD3 do not affect risk for early-onset Alzheimer disease in a European consortium cohort. Hum Mutat 36:1226–1235. https://doi.org/10.1002/humu.22908

173. Wang C, Wang H-F, Tan M-S et al (2016) Impact of common variations in PLD3 on neuroimaging phenotypes in non-demented elders. Mol Neurobiol 53:4343–4351. https://doi.org/10.1007/s12035-015-9370-4

174. Lin E, Tsai S-J, Kuo P-H et al (2017) Association and interaction effects of Alzheimer's disease-associated genes and lifestyle on cognitive aging in older adults in a Taiwanese population. Oncotarget 8:24077–24087. https://doi.org/10.18632/oncotarget.15269

175. Donaldson JG (2009) Phospholipase D in endocytosis and endosomal recycling pathways. Biochim Biophys Acta 1791:845–849. https://doi.org/10.1016/j.bbalip.2009.05.011

176. Jenkins GM, Frohman MA (2005) Phospholipase D: a lipid centric review. Cell Mol Life Sci 62:2305–2316. https://doi.org/10.1007/s00018-005-5195-z

177. Osisami M, Ali W, Frohman MA (2012) A role for phospholipase D3 in myotube formation. PLoS One 7:e33341. https://doi.org/10.1371/journal.pone.0033341

178. Pedersen KM, Finsen B, Celis JE, Jensen NA (1998) Expression of a novel murine phospholipase D homolog coincides with late neuronal development in the forebrain. J Biol Chem 273:31494–31504

179. Satoh J-I, Kino Y, Yamamoto Y et al (2014) PLD3 is accumulated on neuritic plaques in Alzheimer's disease brains. Alzheimers Res Ther 6:70. https://doi.org/10.1186/s13195-014-0070-5

180. Nagaoka-Yasuda R, Matsuo N, Perkins B et al (2007) An RNAi-based genetic screen for oxidative stress resistance reveals retinol saturase as a mediator of stress resistance. Free Radic Biol Med 43:781–788. https://doi.org/10.1016/j.freeradbiomed.2007.05.008

181. Fazzari P, Horre K, Arranz AM et al (2017) PLD3 gene and processing of APP. Nature 541:E1–E2. https://doi.org/10.1038/nature21030