Cellular and Molecular Life Sciences
https://doi.org/10.1007/s00018-018-2825-9

REVIEW

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

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Received: 14 November 2017 / Revised: 4 April 2018 / Accepted: 23 April 2018

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

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Published online: 27 April 2018

in endosomes. Aβ42 accumulates intracellularly in multivesicular 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 transmembrane 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

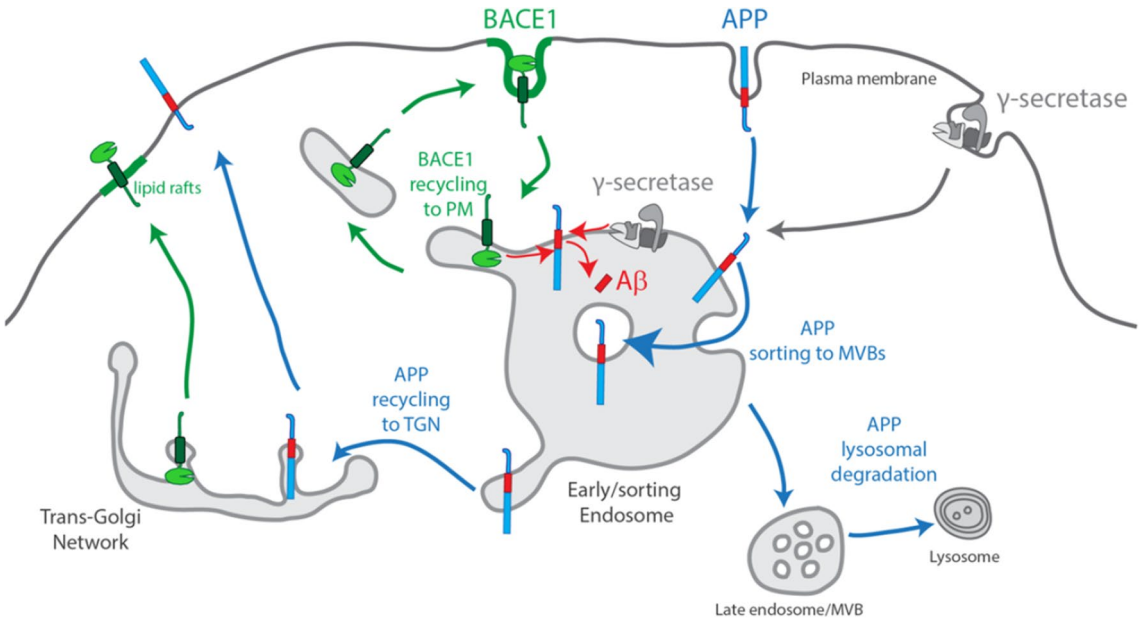

Fig. 1 Scheme of normal endocytic production of Aβ. APP and BACE1 exit the Trans-Golgi Network (TGN) to the plasma membrane in separate post-Golgi secretory vesicles. At the plasma membrane, BACE1 prefers lipid rafts, and is endocytosed independently of APP. Less clear is γ-secretase complex assembly 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.

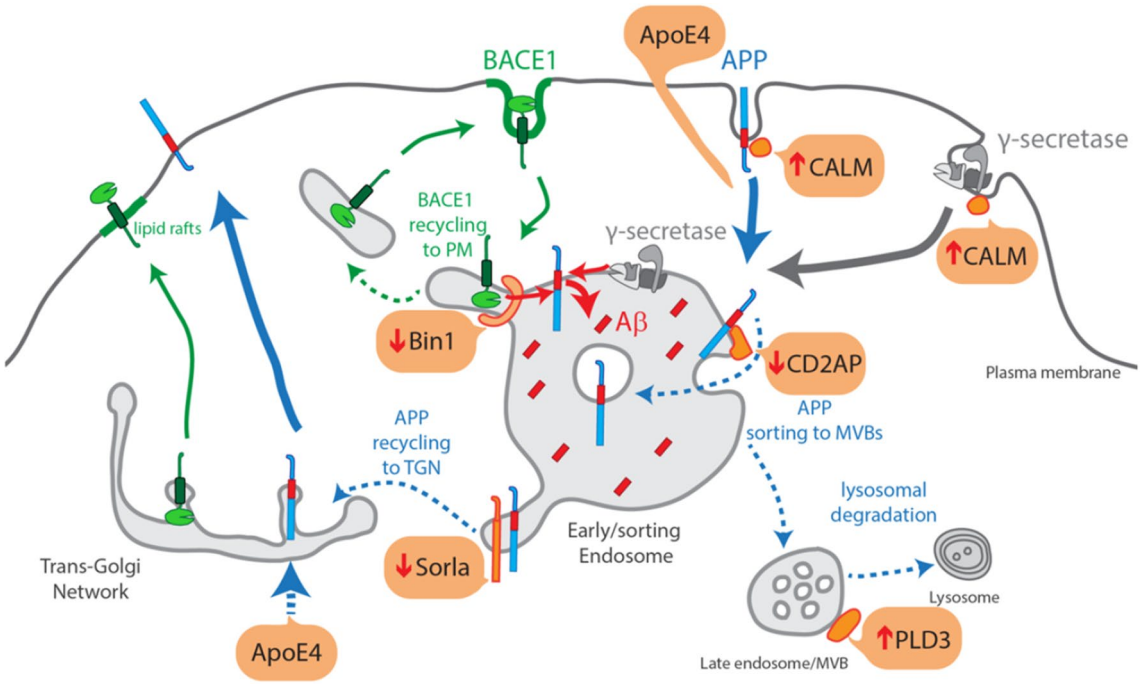

Fig. 2 Scheme of the increased endocytic production of Aβ due to LOAD genetic risk factors. ApoE4-mediated increase in APP endocytosis and/or via increased APP in the secretory pathway due to increased APP transcription; Bin1 loss-of-function impedes BACE1 to recycle out of sorting endosomes; Sorla loss-of-function 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.

Acknowledgements We thank Inês Figueira for revising the manuscript. The Almeida lab has been supported by a Marie Curie Integration Grant (334366 TrafficInAD FP7-PEOPLE-2012-CIG; Marie Curie Actions, EC). iNOVA4Health—UID/Multi/04462/2013, a program financially supported by Fundação para a Ciência e Tecnologia (FCT)/Ministério da Educação e Ciência, through national funds and co-funded by FEDER under the PT2020 Partnership Agreement is acknowledged. CGA is funded by Investigator FCT (IF/00998/2012, FCT). FM has been the recipient of an FCT doctoral fellowship (PD/BD/128344/2017), CP has been the recipient of an FCT doctoral fellowship (SFRH/BD/128374/2017) and TB has been the recipient of an FCT doctoral fellowship (SFRH/BD/131513/2017).

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