Chapter 6

Free Cholesterol — A Double-Edge Sword in Alzheimer Disease

José C. Fernández-Checa

Additional information is available at the end of the chapter

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1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that accounts for most of dementia cases in elder people. The main features of AD include a progressive deterioration of intellectual functions, most prominently memory impairment, loss of language ability, and cognitive deficits. Motor defects appear in the late phases of the disease and basic activities of daily living are gradually compromised as the pathology progresses to advanced phases, and are often accompanied by psychosis and agitation [1, 2]. The hallmarks of the disease include the accumulation of amyloid-β peptide (Aβ) inside neurons and in the extracellular brain space, and the intracellular formation of neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau protein, loss of synapses at specific brain sites as well as the degeneration of cholinergic neurons from the basal forebrain [3]. The prevalence of AD is about 8–10 % of the population over 65 years of age, which increases 2-fold every 5 years afterwards [4, 5]. This high rate prevalence together with the increase in life expectancy, point to AD as one of the most serious health concerns wordlwide, whose incidence is expected to tiple in the next 2-3 decades unless more effective therapies are available [6].

The identification of new targets for the development of more effective therapeutic approaches requires a better understanding of the molecular pathways leading to AD. In this regard, both genetic and environmental factors are increasingly recognized to contribute to the development of AD, which occurs in two forms. The sporadic form of the disease, which affects people over 65 years of age and accounts for the vast mayority of AD cases. In a small proportion (6–8 %), the disease is inherited as an autosomal dominant trait and appears as an early onset in people younger than 65 years of age. Mutations within three genes, the amyloid precursor protein (APP) gene on chromosome 21, the presenilin 1 (PSEN1) gene on chromosome 14, and the presenilin 2 (PSEN2) gene on chromosome 1, have been identified as the main cause of

early onset familial AD [7-10]. While these findings are key for our understanding of the pathogenesis of familial AD, these mutations account for 30–50% of all autosomal dominant early onset cases.

In the last years, it has become increasingly recognized that cholesterol plays a significant role in AD. The first evidence of the importance of cholesterol was the discovery that the ε4 allele of the cholesterol transport protein apolipoprotein E (ApoE) is associated with a higher risk of developing both familial and sporadic AD and modulates the age of AD onset [11-14]. Despite these findings, the impact and causal role of cholesterol in AD remains controversial (see below), as exemplified by the inconsistent outcome of statins in modulating AD risk and progression, which calls for the need for large-scale trials to document whether statins and cholesterol modification regulate or modify the course of AD [15, 16]. Emerging data, however, position the small pool of cholesterol in mitochondria as a key player in AD by determining the susceptibility of neurons to Aβ neuroinflammation, synaptotoxicity and neurotoxicity and as a culprit of cognitive decline by depleting specific mitochondrial antioxidant defense mechanisms. In this review, we will briefly summarize the role of cholesterol in AD, focusing not only in the evidence that cholesterol may foster the amiloidogenic processing of APP and the generation of Aβ peptide, but most importantly, recent findings indicate that cholesterol trafficking to mitochondria stands as a novel critical factor that sensitizes to Aβ-induced neuroinflammation and neurotoxicity, emerging as a potential target for intervention.

2. The involvement of Aβ and cholesterol in AD

2.1. The role of Aβ in AD

Since Aβ was first identified as a component of amyloid plaques, increasing evidence has suggested that Aβ is a major player in AD pathogenesis. According to the amyloid cascade hypothesis, the dysregulation of APP metabolism and Aβ deposition are primary events in the onset of the disease [17, 18]. The Aβ 1–42 and Aβ 1–40 peptides are the major forms found in amyloid plaques, which are generated by proteolytic cleavage of APP. These plaques are predominantly found in areas affected by neurodegeneration, such as entorhinal cortex, amygdala, neocortex, and particularly, hippocampus [19, 20]. While the number of plaques usually does not correlate with the severity of dementia, clinical correlation between elevated Aβ deposition in the brain and cognitive decline has been reported [21]. As described below, several lines of evidence suggest that overproduction and/or reduced clearance of Aβ peptides are key to amyloid aggregation, which contributes to the development of NFTs and neurodegeneration in AD pathology. Intraneuronal Aβ can derive by either APP cleavage within neuronal endocytic compartments, as well as by Aβ internalization from the extracellular space. Both sources are of relevance in the formation of the pool of Aβ involved in neurodegeneration. Intraneuronal Aβ accumulation is one of the earliest pathological events in humans and in animal models of AD, which correlates with early abnormalities in long term potentiation (LTP), cognitive dysfunctions and precedes the formation of amyloid plaques and NFTs formation and the neurodegeneration in animal models in which intracellular Aβ and neuronal

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loss have been reported [22-26]. Furthermore, Aβ plaques could generate from the death of neurons that contained elevated amounts of Aβ and this release can account for the loss of intraneuronal Aβ immunoreactivity in areas of plaque formation [24, 27, 28]. Moreover, recent findings indicated that internalized Aβ elicits fibrillization in the multivesicular bodies (MVBs), which after reaching the plasma membrane, cause cell death and the release of amyloid structures into the extracellular space, indicating that exosomes derived from MVBs could release part of the intracellular pool of Aβ to contribute to the extracellular Aβ pool [29, 30]. The contribution of intracellular Aβ to neuronal death has been well documented in cortical neurons from brains of AD and Down syndrome patients that undergo apoptosis after accumulation of Aβ42 [31, 32]. In addition, microinjection of Aβ1-42 or cDNA-expressing cytosolic Aβ1-42 induces cell death in primary human neurons, while neuronal loss associated with intracellular accumulation of Aβ has been described in a transgenic APP(SL)PS1KI mouse that closely mimics the development of AD-related neuropathological features of AD [33, 34]. Intracellular Aβ accumulation has been associated with neuritic and synaptic pathology and transgenic mice harboring constructs that target Aβ intracellularly developed neurodegeneration [35-37]. Furthermore, antibodies against Aβ reduced intraneuronal Aβ accumulation prevented synaptotoxicity and reversed cognitive impairment in triple transgenic mice [22, 38]. Finally, a coding mutation (A673TT) in APP has been recently shown to protect against AD and age-related cognitive decline in elderly Icelanders [39]. This mutation that affects the aspartyl protease β-site in APP significantly reduces the formation of amyloidogenic peptides, strongly indicating that reducing the β-cleavage of APP protect against the disease. Thus, although targeting Aβ may be a rationale approach to prevent or treat AD progression, recent findings have unfolded the diversity of Aβ structures revealed by the immune response to fibrillar Aβ [40]. This outcome may account for the failure of single therapeutic monoclonal antibodies against Aβ in the treatment of AD.

2.2. Amiloidogenic processing of APP and Aβ generation

Amiloidogenic processing of APP yields toxic Aβ peptides (Fig 1). In this pathway, the β- and γ-secretases cleave APP at the N- and C-termini of the Aβ peptide, respectively. APP, β -secretase, PS1 and Aβ are all present in lipid rafts, which are enriched in cholesterol and glycosphingolipids. This led to the suggestion that APP in lipid rafts is primarily processed via the β-secretase, and APP outside of ratfs is processed via the α-secretase pathway. β-secretase has been characterized as a membrane-bound aspartic protease termed beta-site APP-cleaving enzyme 1 (BACE1), while γ-secretase is a complex comprised of presenilin-1 or -2, nicastrin, anterior pharynx-defective 1 (Aph-1) and presenilin enhancer 2 (Pen-2) [41]. β-arrestin 2, is a novel member of the γ-secretase complex that physically associates with the Aph-1α subunit of the γ-secretase complex and redistributes the complex toward detergent-resistant membranes, increasing the catalytic activity of the complex [42]. Moreover, β-arrestin 2 expression is elevated in individuals with AD and its overexpression leads to an increase in Aβ peptide generation, whereas genetic silencing of Arrb2 (encoding β-arrestin 2) reduces generation of Aβ in cell cultures and in Arrb2-/- mice. In addition to its amyloidogenic processing by β- and γ-secretases, APP can be cleaved within the Aβ domain by α-secretase. This non-amyloidogenic processing prevents the deposition of intact Aβ peptide and results

in the release of a large soluble ectodomain, sAPPα, from the cell, which has neuroprotective and memory-enhancing effects. Members of the ADAMs, a disintegrin and metalloprotease family of proteases, have been shown to possess α-secretase activity [43].

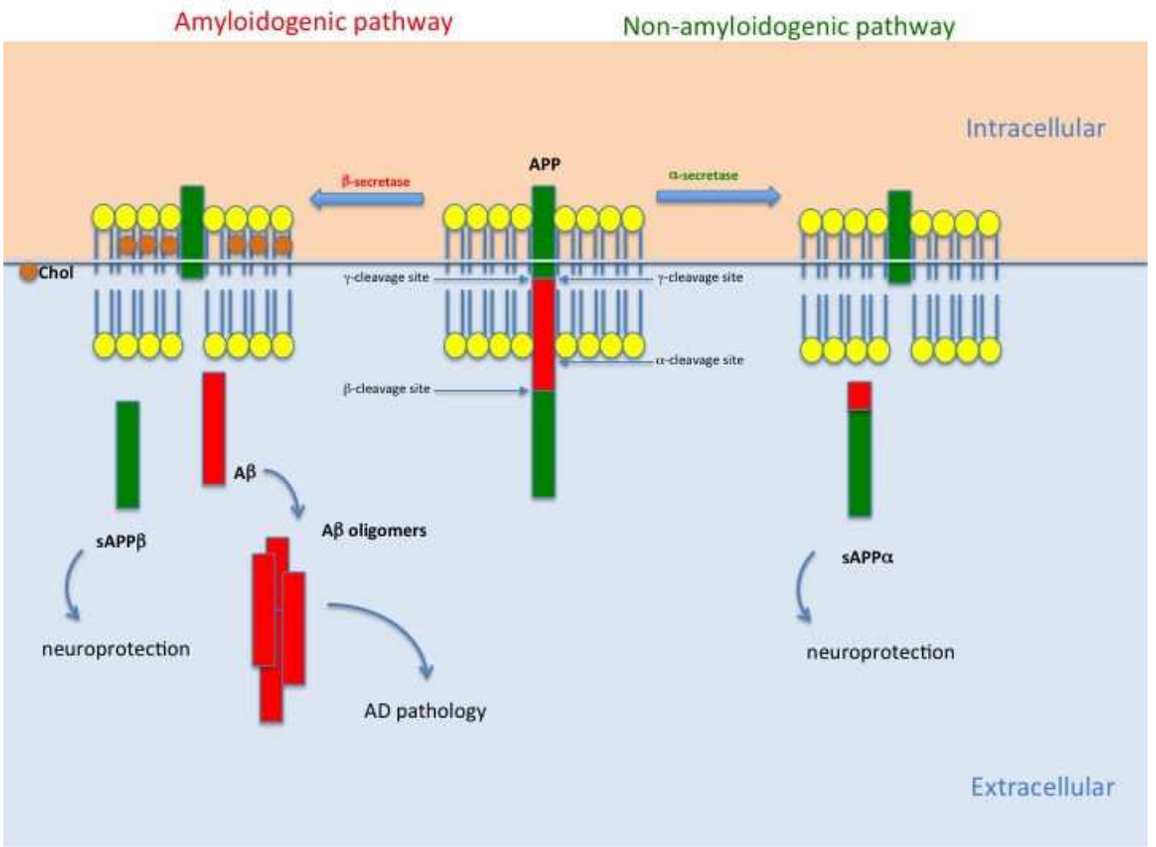

Figure 1. Role of membrane cholesterol in amyloidogenesis. β- and γ-secretases cleave APP at the N- and C-termini of the Aβ peptide, respectively. APP, β-secretase, PS1 and Aβ are all present in lipid rafts, which are enriched in cholesterol and glycosphingolipids. Therefore, APP in lipid rafts is primarily processed via the β-secretase, and APP outside of ratfs is processed via the α-secretase pathway. In the former, β-secretase-mediated Aβ peptides oligomerize and accumulate in plaques contributing to the neurotoxicity of AD.

Besides its extracellular deposition, current evidence indicates the processing and targeting of APP and Aβ to intracellular sites, including mitochondria [44]. Moreover, levels of mitochondrial APP are higher in affected brain areas and in subjects with advanced disease symptons [45]. Immunoelectron microscopy analyses indicated the association of APP with mitochondrial protein translocation components, TOM40 and TIM23, which correlated with decreased import of respiratory chain subunits *in vitro*, decreased cytochrome oxidase activity, increased ROS generation and impaired mitochondrial reducing capacity [45].

2.3. The role of cholesterol in AD: facts and controversies

Cholesterol is an essential component of membrane bilayers that regulate strucutral and functional properties and, hence, a pleiotropic number of cell functions and in the intracellular

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trafficking of proteins [46]. Cholesterol is required for synapse formation, biogenesis of synaptic vesicles and regulation of neurotransmitter release and the precursor of steroid hormones and oxysterols, which are critical intermediates in many metabolic pathways [47-49]. Cholesterol homeostasis is altered in AD; however, whether cholesterol levels are upregulated or downregulated in AD remains to be established. The pathogenic processing of APP into toxic Aβ fragments is known to occur in cholesterol-enriched membrane domains of the plasma membrane, called lipid rafts. The first evidence that cholesterol may impact Aβ production in the brain came from observations that dietary cholesterol increases amyloid production in rabbit hippocampal neurons [50]. Work in mice genetically modified to deposit cerebral Aβ demonstrated that a cholesterol-enriched diet resulted in increased Aβ deposition and increased amyloid plaque formation [51], and these observations were confirmed in subsequent studies in mice fed diets enriched in cholesterol [52-55]. As discussed below, very little cholesterol is transferred from the periphery to the brain due to the impermeability of the blood brain barrier (BBB), so the observations of diet-induced cholesterol-mediated Aβ deposition in neurons are puzzling. A potential explanation for these findings is the fact that BBB permeability is impaired in AD [56, 57]. Exploiting the relative detergent insolubility of lipid rafts, there has been evidence indicating the localization of APP, the α-, β- and γ-secretases in rafts [58, 59]. In addition, the activities of BACE1 and γ-secretase are stimulated by lipid components of rafts, in particular glycosphingolipids and cholesterol. Consistent with these findings supporting a role for cholesterol in AD pathogenesis, high cholesterol levels have been shown to correlate with Aβ deposition and the risk of developing AD [60-62]. Patients taking the cholesterol-lowering drug statins have a lower incidence of the disease [62, 63]. Besides ApoE, other genes encoding proteins involved in cholesterol homeostasis, including cholesteol 24-hydroxylase (CYP46A1), the acyl-coenzyme A:cholesterol acyltransferase (ACAT), the cholesterol efflux transporters ABCA1 and ABCA7, and the lipopotrein-receptor-related protein (LRP) have been linked to the risk, development or progression of AD [64-68]. Despite this experimental and epidemiological evidence, the role of increased cholesterol in AD is controversial with findings showing the opposite. For instance, earlier studies indicated that hippocampal membranes of AD brains showed a reduced fluidity in the hydrocarbon core region compared to control subjects that correlated with the cholesterol content in AD samples [69]. Moreover, reduced cholesterol levels and cholesterol/phospholipids mole ratio have been reported in the temporal gyrus but unaffected in the cerebellum of AD patients with respect to controls [70]. Decreased 24-hydroxycholesterol levels that correlated with lower lathosterol content was reported in the frontal and occipital cortex of patients with AD compared to subjects controls [71]. In addition, cholesterol levels were slightly increased in frontal cortex gray matter in AD patients with the ApoE4 genotype compared with ApoE4 control subjects [72]. Finally, neuronal membrane cholesterol loss has been shown to enhance Aβ generation in hippocampal membranes from AD patients exhibiting increased colocalization of BACE1 and APP [73], and hippocampal membranes from the brain of AD patients contain less membrane cholesterol than control [74]. Furthermore, it has been reported that inhibition of the mevalonate pathway increased production of Aβ and amyloid plaques [75]. Prospective cohort studies have failed to demonstrate the protective effect of statins on dementia, while others reports did not replicate the Aβ lowering effect of

statins in the cerebrospinal fluid [76-81]. In addition to these findings arguing against the correlation between increased brain cholesterol and AD, there is evidence that both cholesterol and Aβ reciprocally regulate each other and that Aβ impacts negatively in cholesterol synthesis, in part, by inhibiting stero-regulated element binding proteins-2 (SREBP-2) cleavage [82, 83]. Interestingly, the consequent decrease in protein prenylation contributes to Aβ-induced neuronal death, which is reversed by exogenous supply of isoprenoids. Further work is needed to ascertain whether the intracelular distribution rather than the levels of brain cholesterol levels may correlate with disease severity.

3. Regulation of cholesterol metabolism in the brain

3.1. De novo cholesterol synthesis

Compared with other organs, the brain is the highest cholesterol-containing organ, which is present mainly in unsterified form. Most of the free cholesterol pool is localized predominantly in specialized membranes (myelin) and to a lesser extent in neurons and glial cells. Experimental evidence indicates that brain cholesterol is independent of serum cholesterol levels, as the BBB is impermeable to circulating cholesterol, which determines that both neurons and glial cells synthesize cholesterol *de novo*. Oligodendrocytes control the synthesis of myelin and therefore have the highest capacity to synthesize cholesterol, followed by astrocytes [84, 85]. Cholesterol is synthesized from acetate in a multistep cascade that requires oxygen and energy. The precursor acetyl-CoA is first converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) and then to mevalonate (Fig. 2). The phosphorylation of mevalonate yields 5-pyrophosphomevalonate, which is converted to isopentenyl pyrophosphate (IPP). IPP can be reversibly transformed to dimethylallyl pyrophosphate (DMAPP), and the combination of both IPP and DMAPP yields the 10-carbon isoprenoid geranyl pyrophosphate (GPP). The sequential addition of 1 or 2 more IPP units to GPP generates the 15-carbon and the 20-carbon isoprenoids farnesyl pyrophosphate (FPP) and the geranylgeranyl pyrophosphate (GGPP), respectively. FPP branches into the non-sterol pathways, which contributes to the generation of other derivatives such as ubiquinol, dolichol, and the sterol pathway via conversion into squalene by squalene synthase, which catalyzes the first committed step in cholesterol synthesis. The rate-limiting step of cholesterol biosynthesis is the conversion of HMG-CoA to mevalonate catalyzed by the HMG-CoA reductase (HMGCR), which is bound to endoplasmic reticulum (ER). Cholesterol levels control HMGCR through several mechanisms. First, high cholesterol exerts a feedback inhibition by activating HMGCR ubiquitination and subsequent proteasomal degradation. Moreover, HMGCR expression is regulated by ER-bound transcription factor SREBP-2, which in turn is controlled by a sterol-sensitive SREBP cleavage-activating protein (SCAP) [86]. In the presence of sterols, full-length SREBP-2 is restricted to the ER. Upon sterol depletion, SREBP-2 interacts with SCAP and is transported from the ER to the Golgi apparatus, where SREBP-2 is cleaved by two proteases and the released N terminus domain acts as a transcription factor to subsequently enhance the levels of HMGCR [86, 87]. Thus, ER plays a key role in the supply of endogenous cholesterol synthesis, which operates to meet demand for cell cholesterol.

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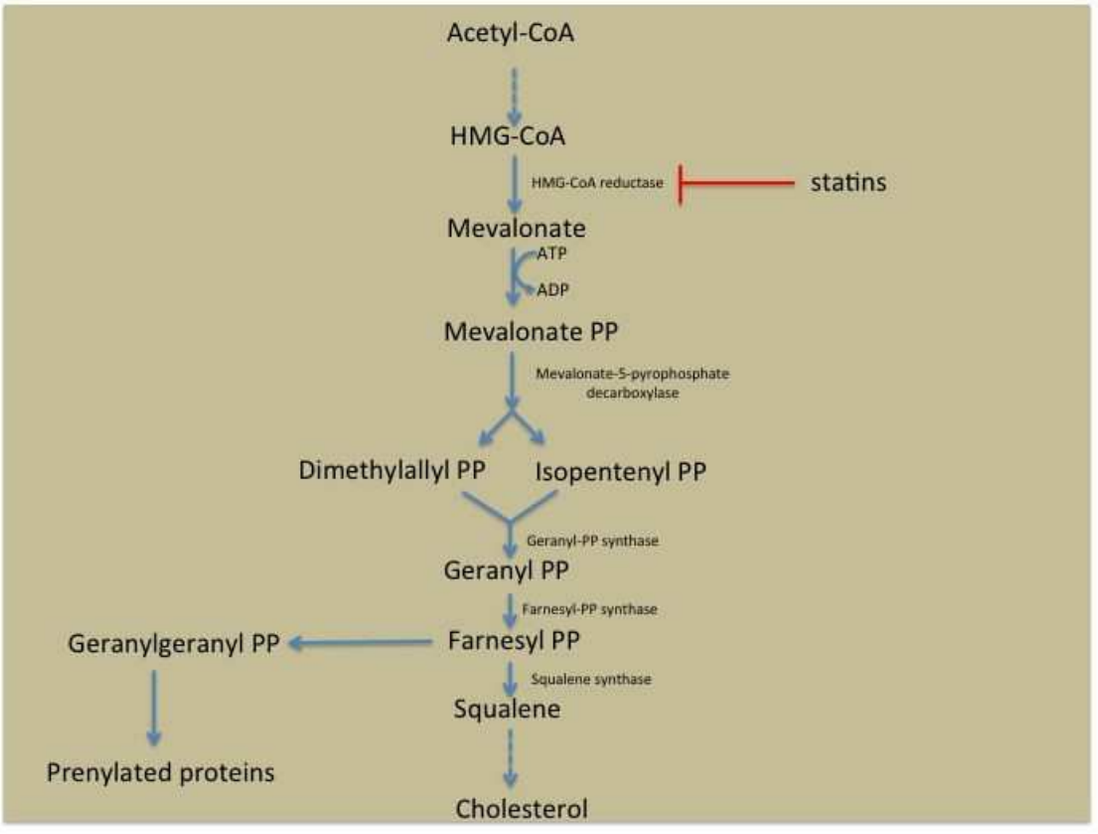

Figure 2. Cholesterol synthesis in the mevalonate pathway. Cholesterol is synthesized from acetate in a multistep cascade that requires oxygen and energy. The precursor acetyl-CoA is first converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) and then to mevalonate. This pathway also generates isoprenoids. Farnesyl pyrophosphate (FPP) branches into the non-sterol pathways, which contributes to the generation of other derivatives such as ubiquinol, dolichol, and the sterol pathway via conversion into squalene by squalene synthase, which catalyzes the first committed step in cholesterol synthesis. The rate-limiting step of cholesterol biosynthesis is the conversion of HMG-CoA to mevalonate catalyzed by the HMG-CoA reductase (HMGCR), which is the target of statins. Statins hence will not only block cholesterol synthesis but also isoprenoids and other non-sterols, which may account for the pleiotropic effects of statins.

3.2. Cholesterol storage and transport

In contrast to developing neurons, which synthesize most of the cholesterol required for growth and synaptogenesis, mature neurons depend on the availability of exogenous cholesterol derived from astrocytes (Figure 3). This process not only ensures steady supply of cholesterol to neurons but also spares energy, as ATP hydrolysis is required to synthesize cholesterol de novo [88]. Besides de novo synthesis, astrocytes can also internalize and recycle the cholesterol released from degenerating nerve terminals and deliver it back to neurons [89]. The transport of cholesterol from astrocytes to neurons requires binding to one of the variants of ApoE, the most prevalent lipoprotein in the central nervous system. In this process, cholesterol first forms a complex with ApoE, which is then secreted in a process involving ABCA1 and ABCG1 transporters [90, 91]. Using cultured cerebellar murine astroglia cells, it has been shown that partially lipidated apoE, secreted directly by glia, is likely to be the major

extracellular acceptor of cholesterol released from glia in a process mediated by ABCG1 rather than ABCA1 [91]. The secreted ApoE–cholesterol complex is then internalized into neurons predominantly via the LDL receptor (LDLR) as well as LRP, and to a minor extent by very-low-density lipoprotein receptor (VLDL), ApoE receptor 2, and megalin [92]. The specific contribution of these receptors in the uptake of ApoE-cholesterol complex and hence in the maintenance of neuronal cholesterol homeostasis remains to be established. Once internalized the receptor-bound ApoE–cholesterol complex is delivered to the late endosomes/lysosomes where acid lipase hydrolyses the cholesterol esters within the lipoprotein complex, resulting in the release of intracellular free cholesterol. This unesterified cholesterol subsequently exits the late endosomes/lysosomes via Niemann–Pick type C (NPC) 1 and 2 protein-dependent mechanism and is distributed primarily to the plasma membrane as well as to the ER, which serves as a negative feedback sensor for the cholesterol homeostasis genes such as HMGCR and LDLR. Excess cholesterol, on the other hand, is esterified in the ER by ACAT and stored in cytoplasmic lipid droplets, which serves as reserve source of cholesterol needed for synaptic and dendritic formation and remodeling [93, 94].

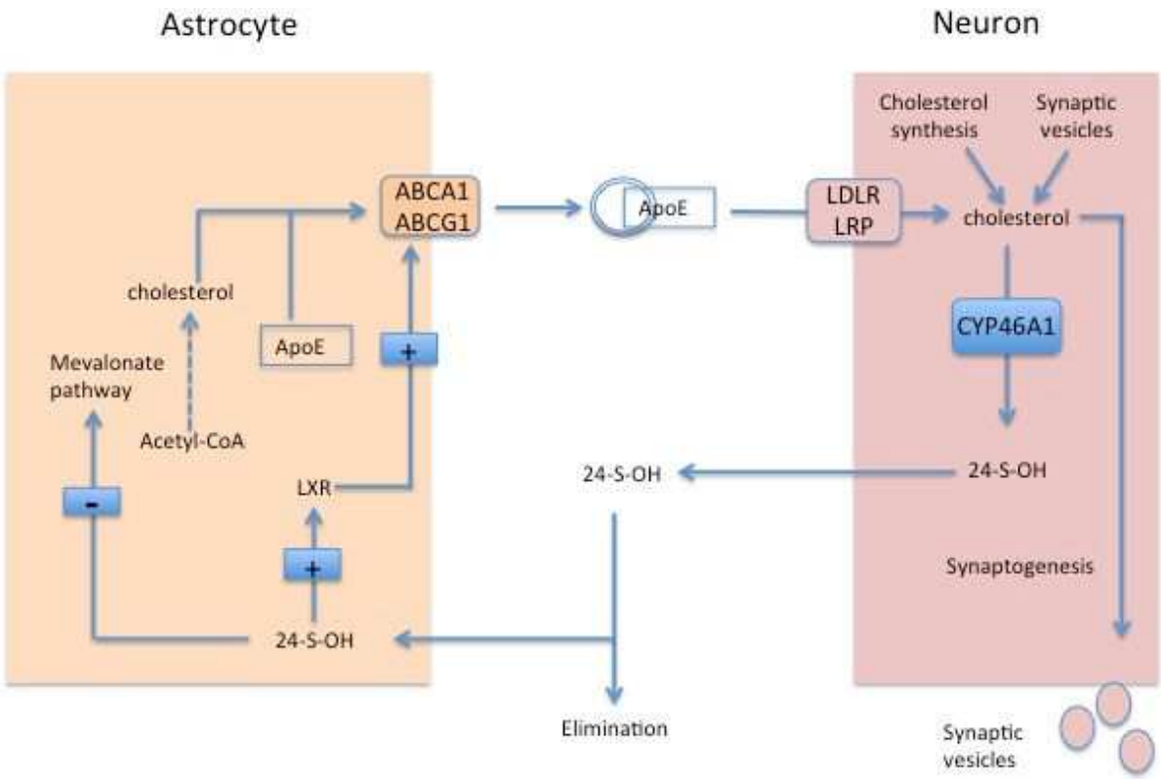

Figure 3. Cross talk between astrocytes and neurons in cholesterol homeostasis. Although neurons can synthesize cholesterol de novo in the adult state neurons rely on the delivery of cholesterol from astrocytes, which exhibit a significantly higher rate of de novo cholesterol activity than neurons. Cholesterol packed in ApoE particles assembled in astrocytes are delivered via ABCA1 carrier to neurons. Excess neuronal cholesterol is transformed into the oxysterol 24S-hydroxysterol (24S-OH) by the CYP46A1 which represents the major mechanism for the elimination of brain cholesterol, as it crosses the BBB to the periphery for disposal. Oxysterols activate transcription factor LXRβ isoform, which in turn induces the activation of carriers such as ABCA1 to stimulate trafficking of cholesterol in the form of ApoE to neurons. 24S-OH inhibits the novo cholesterol synthesis in astrocytes.

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3.3. Cholesterol efflux from the brain

Unlike other organs and epithelial cells, neurons and glial cells do not degrade cholesterol, therefore in order to maintain homeostasis they export cholesterol to the circulation for its disposal by peripheral organs. Two different mechanisms are involved in the elimination of cholesterol from the brain. The major mechanism by which cholesterol is excreted from the brain is by its conversion to 24S-hydroxylcholesterol—an oxidized lipophilic metabolite that can freely cross the BBB [95]. The conversion of free cholesterol to 24S-hydroxycholesterol is mediated by the cytochrome P450-containing enzyme cholesterol 24-hydroxylase, encoded by the Cyp46A1 gene, which is expressed selectively in the brain [96]. High levels of this enzyme are found in certain neuronal cells such as pyramidal neurons of the hippocampus and cortex, Purkinje cells of the cerebellum, thalamic neurons, and in hippocampal and cerebellar interneurons. Interestingly, a minor fraction of cholesterol 24-hydroxylase immunoreactivity has also been detected in glial cells from the brains of AD patients [95]. It is estimated that about 40% of the total cholesterol turnover is mediated by cholesterol 24-hydroxylase [97]. Indeed, deletion of the Cyp46A1 gene encoding cholesterol 24-hydroxylase leads to about 50% reduction in brain cholesterol excretion. This decrease, however, is compensated by the reduction in de novo synthesis, thus suggesting a close relationship between synthesis and metabolism of cholesterol in the brain. The other mechanism of cholesterol elimination, called reverse cholesterol transport pathway, involves translocation of a fraction of brain cholesterol to the blood by membrane transport protein such as ABCA1 [98]. The level of ABCA1 is partly regulated by cholesterol-derived ligand oxysterols (e.g., 24S-hydroxylcholesterol) of the liver X receptor (LXR), which has been shown to influence the transcription of multiple genes involved in cholesterol metabolism [99-101]. There are two known LXR isoforms, LXRα and LHRβ. LXRα expression is mainly limited to the liver, adrenals, intestine and spleen, while LXRβ is expressed in all tissue types, including the brain. In vivo induction of LXR results in increased expression of ABCA1 and ABCG1, increased cholesterol efflux and a reduction in synaptosomal plasma membrane cholesterol [101]. As such, it is expected that LXR agonists (e.g. T0901317) should lower Aβ levels. However, results with LXR agonists have been inconsistent. For instance, T0901317 has been shown in some studies to decrease Aβ, while others reported an increase in Aβ42 without chaning Aβ40 levels [102]. Hence, it is conceivable that both synthesis and elimination of cholesterol, especially in the adult brain, are not only tightly regulated but also compartmentalized. Astrocytes are responsible for the majority of cholesterol synthesis but contribute relatively little to its elimination, whereas neurons with reduced synthetic ability can eliminate about two thirds of the cholesterol from the brain.

4. Intracellular cholesterol and AD

As a key component of membrane bilayers, intracellular cholesterol traffics to different compartments to maintain physical and functional membrane properties. Cholesterol that enters the cell via the endocytic pathway is transported to the ER for processing, while cholesterol synthesized in the ER de novo it is transported to the plasma membrane within a short time frame. Of relevance to AD pathogenesis, in the following sections we will focus on

the endo-lysosomal cholesterol and in the small pool of cholesterol in mitochondria and their contribution to AD.

4.1. Endo-lysosomal cholesterol

The supply of cholesterol from astrocytes to neurons via receptor-bound ApoE-cholesterol complex relies on the trafficking and subsequent hydrolysis of these complexes in endo/lysosomes. The generated free cholesterol exits lysosomes via NPC1/2 proteins to be distributed to other membrane bilayers. The impact of NPC proteins in intracelular cholesterol homeostasis has been best characterized in the NPC disease, a neurological disorders caused by mutations in NPC1/2 proteins characterized by increased accumulation of cholesterol and other lipids (e.g. glycosphingolipids) in lysosomes in the affected organs, predominantly brain and liver. NPC knockout mice, which mimic the pathology of NPC patients, exhibit increased lysosomal cholesterol in cerebellum, mainly in Purkinje cells, and suffer from progressive motor deterioration and a short life span (typically 8-10 weeks). NPC disease and AD share many parallels including endo/lysosomal abnormalities and APP processing and Aβ accumulation. Previous findings have shown that mutated NPC1 in mice causes the accumulation of Aβ40 and Aβ42, which coincided with accumulation of presenilins in early endocytic compartment [103]. Similar findings have been reported in human NPC1 brain, with accumulation of Aβ ocurring in early endosomes [104]. In CHO cells deficient in NPC1 protein and in cells treated with U18666A, which inhibits NPC1/2, Aβ and presenilin accumulation were found in late endosomes [105]. However, the expression of NPC1 in AD has been poorly characterized. Quite intriguingly, recent findings have reported increased expression of the lysosomal cholesterol transporter NPC1 in AD [106]. NPC1 expression was described to be upregulated at both mRNA and protein levels in the hippocampus and frontal cortex of AD patients compared to controls subjects. However, no difference in NPC1 expression was detected in the cerebellum, a brain region that is relatively spared in AD. Moreover, murine NPC1 mRNA levels increased in the hippocampus of 12-month-old APP/PS1 mice compared to wild type mice. While these findings strongly suggest the lack of lysosomal cholesterol accumulation in AD, endosomal abnormalities have been found in AD that precede amyloid and tau pathology in the neocortex. In addition to the proteolytic processing by secretases, APP and its corresponding C-terminal fragments are also metabolized by lysosomal proteases. SORLA/SORL1 is a unique neuronal sorting receptor for APP that has been causally implicated in sporadic and autosomal dominant familial AD. Brain concentrations of SORLA are inversely correlated with Aβ in mouse models and AD patients. Indeed, transgenic mice overexpressing SORLA exhibit decreased Aβ concentrations in brain [107]. Mechanistically, Aβ binds to the amino-terminal VP10P domain of SORLA and this binding is impaired by a familial AD mutation in SORL1. Although previous studies have shown that lysosomal cholesterol accumulation impairs autophagy by disrupting lysosomal function [108], the lysosomal impairment and subsequent contribution to decreased Aβ degradation in AD may occur through mechanisms independent of cholesterol accumulation in lysosomes. Moreover, sphingosine-1-phosphate (S1P) accumulation by S1P lyase deficiency has recently been shown to impair lysosomal APP metabolism, resulting in increased Aβ accumulation [109]. The intracellular accumulation of S1P interferes with the maturation of cathepsin D and degrada-

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tion of Lamp2, suggesting a general impairment of lysosomal function and autophagy. Sphingolipids have strong affinity to bind cholesterol and play a role in Alzheimer disease [110]. However, it remains to be established whether increased lysosomal cholesterol may contribute to impaired lysosomal Aβ degradation and if sphingolipids accumulate in lysosomes in AD.

4.2. Mitochondrial cholesterol

Unlike plasma membrane, mitochondria are cholesterol-poor organelles. The limited pool of mitochondrial cholesterol plays important physiological roles, such as in the synthesis of steroids in specialized tissues and bile acids in the liver. However, under pathological conditions the unphysiological accumulation of cholesterol in mitochondrial membranes have profound effects in mitochondrial function and antioxidant defense and has emerged as an important factor in liver diseases and neurodegeneration [111-114]. In particular, the transport of cholesterol from the outer to the inner mitochondria is essential for the generation of steroid precursor pregnenolone upon metabolism of cholesterol by the P540 side-chain cleavage enzyme CYP11A1. Availability of cholesterol in mitochondria inner membrane is rate-limiting step in steroidogenesis and is a highly regulated process.

StARD1 is the founding member of a family of lipid transporting proteins that contain StAR-related lipid transfer (START) domains. StARD1 is an outer mitochondrial membrane protein which was first described and best characterized in steroidogenic cells where it plays an essential role in cholesterol transfer to the mitochondrial inner membrane for metabolism by CYP11A1 to generate pregnenolone. Despite similar features with StARD1, other START members cannot replace StARD1 deficiency as global StARD1 knockout mice dye within 10 days due to adrenocortical lipoid hyperplasia [115]. These findings imply that other members of the family cannot functionally replace StARD1, indicating the key role of this member in the regulation of cholesterol trafficking to the mitochondrial inner membrane. Recent findings in APP/PS1 models of AD have indicated the expression of StARD1 in neurons, which correlate with the age-dependent increase in mitochondrial cholesterol [114, 116]. Consistent with these findings in experimental models, enhanced immunocytochemical localization of StARD1 has been described in the pyramidal hippocampal neurons of AD-affected patients [117]. Given the role of StARD1 in the mitochondrial transport of cholesterol and hence in the modulation of mitochondrial cholesterol levels, the increased expression of StARD1 in AD patients, would strongly suggest that mitochondrial cholesterol accumulation may actually occur in patients with AD. Furthermore, the increase in mitochondrial cholesterol in brain mitochondria of Alzheimer's disease was not accompanied by a selective increase in mitochondrial-associated membranes (MAM), a specific membrane domain made of ER and mitochondria bilayers thought to be of relevance in the traffic of lipids, suggesting that StARD1-mediated cholesterol trafficking to mitochondria is independent of MAM. TSPO, a protein particularly abundant in steroidogenic tissues and primarily localized in the mitochondrial outer membrane, has been suggested to play an important role in steroidogenesis via the transport of cholesterol to the mitochondrial inner membrane (IMM) [118, 119]. However, quite interestingly, recent studies using tissue-specific genetic deletion of TSPO demonstrated that TSPO is dispensable for

steroidogenesis in Leydig cells [120], questioning the relevance of previous findings on TSPO using pharmacological ligands and inhibitors. These data underscore that TSPO does not play a significant role in the trafficking of cholesterol to IMM, and highlights the relevance of StARD1 in this process. Overall, these findings underscore the accumulation of cholesterol in mitochondrial membranes in patients and models of AD, and quite interestingly, paralell the increase in mitochondrial cholesterol observed in brain and liver mitochondria in NPC, arguing that this pool of cholesterol may be a common nexus in both AD and NPC.

5. Mitochondrial cholesterol promotes AD by depleting GSH

In addition to the amyloidogenic effect of cholesterol by fostering Aβ generation from APP, recent data has provided evidence that mitochondrial cholesterol accumulation sensitizes neurons to Aβ-induced neuroinflammation and neurotoxicity by depleting mGSH, effects that are prevented by mGSH replenishment [114, 116]. The mechanism of mitochondrial cholesterol accumulation involves the upregulation of StARD1 induced by Aβ *via* ER stress, confirming previous findings in hepatocytes [121]. Consistent wiht the reported increased expression of StARD1 in pyramidal hippocampal neurons of AD-affected patients, it is likely that this outcome may be accompanied by increased accumulation of cholesterol in mitochondria and subsequent depeltion of mGSH levels [117]. Moreover, a novel mouse model engineered to have enhanced cholesterol synthesis by SREBP-2 overexpression superimposed to APP/PS1 mutations triggered Aβ accumulation and tau pathology [122]. This triple transgenic model exhibited increased mitochondrial cholesterol loading and mGSH depletion and accelerated Aβ generation by β-secretase activation compared to APP/PS1 mice. Moreover, SREBP-2/APP/PS1 mice displayed synaptotoxicity, cognitive decline, tau hyperphosphorylation and neurofibrillary tangle formation in the absence of mutated tau, indicating that cholesterol, particularly mitochondrial cholesterol, can precipitate Aβ accumulation and tau pathology. Importantly, *in vivo* replenishment of mGSH with cell-permeable GSH monoethyl ester (GSH-EE) attenuated neuropathological features of AD in SREBP-2/APP/PS1 mice. These findings established that mitochondrial cholesterol promotes AD by selective depletion of mGSH stores. Therefore, understanding the molecular mechanisms on this cause-and-effect relationship may be of interest in AD.

The properties of GSH transport in isolated rat brain mitochondria appear to differ from those reported previously other tissues such as liver and kidney, as they were influenced most by inhibitors of the tricarboxylate carrier, citrate, isocitrate, and benzenyl-1,2,3-tricarboxylate [123] Moreover, in mouse brain mitochondria it has been shown that 2-oxoglutarate (OGC) and dicarboxylate (DIC) are both expressed in cortical neurons and astrocytes [124]. In addition, butylmalonate, an inhibitor of DIC, significantly decreased mGSH, suggesting DIC as an important GSH transporter in mouse cerebral cortical mitochondria. Interestingly, a role for UCP2 in the transport of mGSH has been described in neurons, suggesting that the transport of protons back into the matrix by UCP2 may favor the movement of GSH [125]. These studies suggest that multiple IMM anion transporters might be involved in mGSH transport and that they might differ in different cell populations within the brain. However,

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the fact that mitochondrial cholesterol loading selectively depleted mGSH indicated that this transport function is sensitive to cholesterol-mediated changes in membrane dynamics, similar to what has been reported in liver mitochondria. Indeed, the effect of cholesterol in the regulation of mGSH is mediated by the susceptibility of the OGC to perturbations in membrane dynamics. Functional expression analyses in Xenopus laevis oocytes microinjected with OGC cRNA showed enhanced transport of GSH in isolated mitochondria [126]. Moreover, cholesterol enrichment impairs the transport kinetics of 2-oxoglutarate via the OGC by decreasing mitochondrial membrane fluidity. Restoration of membrane dynamics by the fatty acid analog A_2C improves the activity of OGC and mGSH transport despite cholesterol enrichment. Therefore, strategies aimed to replenish mitochondrial membrane physical properties may be of relevance to AD by replenishing mGSH.

6. Regulation of mitochondrial cholesterol

The ER plays an essential role in the integration of multiple metabolic signals and the maintenance of cell homeostasis, particularly protein synthesis and folding. Under stress conditions induced by protein misfolding, the ER triggers an adaptive response called uncoupled protein response (UPR). To resolve ER stress, UPR promotes a decrease in protein synthesis, and an increase in protein degradation and chaperone production for protein folding. Aβ is well known to induce ER stress, which is believed to mediate in part the pathogenesis of AD [127]. Moreover, tauroursodeoxycholic acid (TUDCA), a chemical chaperone that prevents ER stress, has been shown to restore the mGSH pool in alcohol fed rats [128] and ameliorates alcohol-induced ER stress [121]. In line with these findings in liver, we have recently reported that TUDCA and PBA abolish Aβ-induced hepatic ER stress, mitochondrial cholesterol loading and subsequent mGSH depletion [116]. Emerging evidence has demonstrated that StARD1 is a previously unrecognized target of the UPR and ER stress signaling. Indeed, tunicamycin, an ER stress trigger, induces the expression of StARD1 in isolated hepatocytes and this effect is prevented by TUDCA treatment [121]. Moreover, mice fed a high cholesterol diet (HC) exhibited increased expression of StARD1. However, HC feeding downregulates the expression of SREBP-2-regulated target genes, including hydroxymethylglutaryl Co-A reductase, demonstrating that StARD1 is and an ER stress but not SREBP-2 regulated gene. In contrast to StARD1, the role of ER stress in the regulation of StART family members has been limited to StARD5 [129, 130], with conflicting results reported for StARD4 [130, 131]. As the UPR comprises three transducers, namely inositol requiring (IRE) 1α, PKR-like ER kinase (PERK), and activating transcription factor (ATF) 6α, which are controlled by the master regulator glucose-regulated protein 78 (GRP78 also known as BiP), further work is needed to examine the relative contribution of the involved arms of the UPR in the regulation of StARD1 by Aβ. Besides ER stress, StARD1 activation is regulated at the transcriptional and post-translational levels. In murine steroidogenic cells StARD1 activity and subsequent steroidogenesis increases upon StARD1 phosphorylation at serine residues [132, 133]. Whether or not StARD1 phosphorylation by Aβ regulates mitochondrial cholesterol homeostasis remains to be explored. If so, then the identification of putative kinases that phosphorylate and activate StARD1 may be

of potential relevance in AD. The other unresolved question relates as to the mechanism whereby Aβ induces ER stress. As ER Ca^{2+} homeostasis is a key housekeeping mechanism to maintain ER function, it is conceivable that Aβ may disrupt ER Ca^{2+}, thereby causing ER stress. Whether Aβ modulates the activity of the ER Ca^{2+} pump SERCA, whose disruption is known to trigger ER stress remains to be investigated.

7. Concluding remarks

With an expected increase in cases, AD may represent one of the most important health burdens in the near future worlwide. Therefore the identification of effective therapeutic treatments for AD is of priority for health authorities around the world. Unfortunately our limited understanding of the molecular pathways underlying AD has curved the possibilities to have effective treatments at hand. While cholesterol and in particular hypercholesterolemia has been identified as a risk factor for AD development, the causal effect of cholesterol and the impact of cholesterol-lowering approach in AD still remains controversial. Unexpectedly, evidence in the last five years has indicated that the small pool of cholesterol in mitochondria plays an important role in AD, as its accumulation in mitochondria causes mGSH depletion amplifying the neurotoxic effects of Aβ peptides. Therefore, targeting mGSH may be of therapeutic relevance in AD. However, mGSH is regulated by its transport through mitochondrial inner membrane via specific carriers that are sensitive to changes in mitochondrial membrane properties. Hence the mere increase in cytosolic GSH by GSH prodrugs, such as N-acetylcysteine, may not be effective in restoring mGSH as cytosolic GSH would not be transported into mitochndrial matrix due to cholesterol-mediated disruption in mitochondrial membrane dynamics. Thus, more specific approaches would imply the use of membrane-permeable GSH prodrugs such as GSH ethyl ester, which has been shown to protect against AD in experimental models and is known to cross the BBB. Alternatively, targeting the increase in mitochondrial cholesterol by antagonizing StARD1 may arise as another attractive possiblity in the future. This approach requires a better understanding of the cell biology of StARD1 and in the identification of BBB permeable specific StARD1inhibitors. We are looking forward to these and other more exciting discoveries to start controlling the onset and progression of this devastating disease.

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Author details

José C. Fernández-Checa[1,2,3]

Address all correspondence to: checa229@yahoo.com

1 Department of Cell Death and Proliferation, Instituto Investigaciones Biomedicas de Barcelona, CSIC, Barcelona, and Liver Unit-Hospital Clinic-IDIBAPS, Spain

2 Centro de Investigación Biomédica en Red (CIBERehd), Barcelona, Spain

3 University of Southern California Research Center for Alcohol Liver and Pancreatic Diseases and Cirrhosis, Keck School of Medicine, USC, Los Angeles, CA, USA

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