We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Free Cholesterol — A Double-Edge Sword in Alzheimer Disease

José C. Fernández-Checa

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/59935>

---

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 ($A\beta$) 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 worldwide, whose incidence is expected to triple 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 majority 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 $\epsilon 4$ 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 A β 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 amyloidogenic processing of APP and the generation of A β peptide, but most importantly, recent findings indicate that cholesterol trafficking to mitochondria stands as a novel critical factor that sensitizes to A β -induced neuroinflammation and neurotoxicity, emerging as a potential target for intervention.

2. The involvement of A β and cholesterol in AD

2.1. The role of A β in AD

Since A β was first identified as a component of amyloid plaques, increasing evidence has suggested that A β is a major player in AD pathogenesis. According to the amyloid cascade hypothesis, the dysregulation of APP metabolism and A β deposition are primary events in the onset of the disease [17, 18]. The A β 1–42 and A β 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 A β 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 A β peptides are key to amyloid aggregation, which contributes to the development of NFTs and neurodegeneration in AD pathology. Intraneuronal A β can derive by either APP cleavage within neuronal endocytic compartments, as well as by A β internalization from the extracellular space. Both sources are of relevance in the formation of the pool of A β involved in neurodegeneration. Intraneuronal A β 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 A β and neuronal

loss have been reported [22-26]. Furthermore, A β plaques could generate from the death of neurons that contained elevated amounts of A β and this release can account for the loss of intraneuronal A β immunoreactivity in areas of plaque formation [24, 27, 28]. Moreover, recent findings indicated that internalized A β 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 A β to contribute to the extracellular A β pool [29, 30]. The contribution of intracellular A β to neuronal death has been well documented in cortical neurons from brains of AD and Down syndrome patients that undergo apoptosis after accumulation of A β 42 [31, 32]. In addition, microinjection of A β 1-42 or cDNA-expressing cytosolic A β 1-42 induces cell death in primary human neurons, while neuronal loss associated with intracellular accumulation of A β 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 A β accumulation has been associated with neuritic and synaptic pathology and transgenic mice harboring constructs that target A β intracellularly developed neurodegeneration [35-37]. Furthermore, antibodies against A β reduced intraneuronal A β 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 A β may be a rationale approach to prevent or treat AD progression, recent findings have unfolded the diversity of A β structures revealed by the immune response to fibrillar A β [40]. This outcome may account for the failure of single therapeutic monoclonal antibodies against A β in the treatment of AD.

2.2. Amyloidogenic processing of APP and A β generation

Amyloidogenic processing of APP yields toxic A β peptides (Fig 1). In this pathway, the β - and γ -secretases cleave APP at the N- and C-termini of the A β peptide, respectively. APP, β -secretase, PS1 and A β 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 rafts 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-1 α 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 A β peptide generation, whereas genetic silencing of Arrb2 (encoding β -arrestin 2) reduces generation of A β in cell cultures and in Arrb2 $^{-/-}$ mice. In addition to its amyloidogenic processing by β - and γ -secretases, APP can be cleaved within the A β domain by α -secretase. This non-amyloidogenic processing prevents the deposition of intact A β peptide and results

in the release of a large soluble ectodomain, sAPP α , 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 A β peptide, respectively. APP, β -secretase, PS1 and A β 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 rafts is processed via the α -secretase pathway. In the former, β -secretase-mediated A β 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 A β to intracellular sites, including mitochondria [44]. Moreover, levels of mitochondrial APP are higher in affected brain areas and in subjects with advanced disease symptoms [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 structural and functional properties and, hence, a pleiotropic number of cell functions and in the intracellular

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 A β fragments is known to occur in cholesterol-enriched membrane domains of the plasma membrane, called lipid rafts. The first evidence that cholesterol may impact A β 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 A β demonstrated that a cholesterol-enriched diet resulted in increased A β 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 A β 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 A β 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 cholesterol 24-hydroxylase (CYP46A1), the acyl-coenzyme A:cholesterol acyltransferase (ACAT), the cholesterol efflux transporters ABCA1 and ABCA7, and the lipoprotein 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 A β 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 A β 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 A β 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 A β reciprocally regulate each other and that A β impacts negatively in cholesterol synthesis, in part, by inhibiting sterol-regulated element binding proteins-2 (SREBP-2) cleavage [82, 83]. Interestingly, the consequent decrease in protein prenylation contributes to A β -induced neuronal death, which is reversed by exogenous supply of isoprenoids. Further work is needed to ascertain whether the intracellular 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.

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 LXR β 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.

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-hydroxycholesterol—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-hydroxycholesterol) 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, LXR α and LXR β . LXR α expression is mainly limited to the liver, adrenals, intestine and spleen, while LXR β 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 A β levels. However, results with LXR agonists have been inconsistent. For instance, T0901317 has been shown in some studies to decrease A β , while others reported an increase in A β 42 without changing A β 40 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 intracellular 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 A β accumulation. Previous findings have shown that mutated NPC1 in mice causes the accumulation of A β 40 and A β 42, which coincided with accumulation of presenilins in early endocytic compartment [103]. Similar findings have been reported in human NPC1 brain, with accumulation of A β occurring in early endosomes [104]. In CHO cells deficient in NPC1 protein and in cells treated with U18666A, which inhibits NPC1/2, A β 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 A β in mouse models and AD patients. Indeed, transgenic mice overexpressing SORLA exhibit decreased A β concentrations in brain [107]. Mechanistically, A β 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 A β 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 A β accumulation [109]. The intracellular accumulation of S1P interferes with the maturation of cathepsin D and degrada-

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 A β 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 die within 10 days due to adrenocortical lipid 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, parallel 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 A β generation from APP, recent data has provided evidence that mitochondrial cholesterol accumulation sensitizes neurons to A β -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 A β *via* ER stress, confirming previous findings in hepatocytes [121]. Consistent with 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 depletion 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 A β accumulation and tau pathology [122]. This triple transgenic model exhibited increased mitochondrial cholesterol loading and mGSH depletion and accelerated A β 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 A β 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 in 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,

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 A₂C 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. A β 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 A β -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) 1 α , PKR-like ER kinase (PERK), and activating transcription factor (ATF) 6 α , 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 A β . 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 A β 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 A β induces ER stress. As ER Ca²⁺ homeostasis is a key housekeeping mechanism to maintain ER function, it is conceivable that A β may disrupt ER Ca²⁺, thereby causing ER stress. Whether A β modulates the activity of the ER Ca²⁺ 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 worldwide. 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 A β 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 mitochondrial 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 possibility in the future. This approach requires a better understanding of the cell biology of StARD1 and in the identification of BBB permeable specific StARD1 inhibitors. We are looking forward to these and other more exciting discoveries to start controlling the onset and progression of this devastating disease.

Acknowledgements

This chapter is dedicated to the many patients, past and present that suffer AD, and very especially to my mother.

The work was supported by CIBEREHD, Fundació la Marató de TV3 and Grants PI11/0325 (META) from the Instituto de Salud Carlos III and Grant SAF2012-34831 from Plan Nacional de I+D, Spain; and the Center Grant P50-AA-11999 (Research Center for Liver and Pancreatic Diseases) funded by NIAAA/NIH. I want to thank the people in my lab who have made major

contributions to unveil the role of mitochondrial cholesterol in Alzheimer, in particular Drs. Anna Fernandez, Carmen Garcia-Ruiz, Elisabet Barbero-Camps and Anna Colell.

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

References

- [1] Katzov H, Chalmers K, Palmgren J, Andreassen N, Johansson B, Cairns NJ et al (2004) Genetic variants of ABCA1 modify Alzheimer disease risk and quantitative traits related to beta-amyloid metabolism. *Hum Mutat* 23(4):358–367
- [2] Whitehouse PJ (1997) Genesis of Alzheimer's disease. *Neurology* 48(5 Suppl 7):S2–S7
- [3] Selkoe, D.J. 2001. Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.* 81(2): 741–766.
- [4] Cummings JL (2004) Alzheimer's disease. *N Engl J Med* 351 (1):56–67
- [5] Bertram L, Tanzi RE (2005) The genetic epidemiology of neurodegenerative disease. *J Clin Invest* 115(6):1449–1457
- [6] Minati, L., Edginton, T., Grazia Bruzzone, M., and Giaccone, G. 2009. Current concepts in Alzheimer's disease: a multidisciplinary review. *Am. J. Alzheimers Dis. Other Dement.* 24(2): 95–121.
- [7] Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE (2007) Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. *Nat Genet* 39(1):17–23
- [8] Holmes C (2002) Genotype and phenotype in Alzheimer's disease. *Br J Psychiatry* 180:131–134

- [9] St George-Hyslop PH, Petit A (2005) Molecular biology and genetics of Alzheimer's disease. *C R Biol* 328(2):119–130
- [10] Tanzi RE, Bertram L (2005) Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. *Cell* 120 (4):545–555
- [11] Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., et al. 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science*, 261(5123): 921–923.
- [12] Strittmatter, W.J., Saunders, A.M., Schmechel, D., Pericak-Vance, M., Enghild, J., Salvesen, G.S., and Roses, A.D. 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. U.S.A.* 90(5): 1977–1981.
- [13] Poirier, J., Bertrand, P., Poirier, J., Kogan, S., Gauthier, S., Poirier, J., et al. 1993. Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet*, 342(8873): 697–699.
- [14] Chartier-Harlin, M.-C., Parfitt, M., Legrain, S., Pérez-Tur, J., Brousseau, T., Evans, A., et al. 1994. Apolipoprotein E, 3 4 allele as a major risk factor for sporadic early and late-onset forms of Alzheimer's disease: analysis of the 19q13.2 chromosomal region. *Hum. Mol. Genet.* 3(4): 569–574.
- [15] Mendoza-Oliva A, Zepeda A, Arias C. The Complex Actions of Statins in Brain and their Relevance for Alzheimer's Disease Treatment: an Analytical Review. *Curr Alzheimer Res.* 2014 Oct 1
- [16] Opie L.H. Can dementia be lessened by statins. *The Lancet* 384: 953, 2014.
- [17] Hardy, J., and Allsop, D. 1991. Amyloid deposition as the central event in the aetiology of Alzheimer's disease. *Trends Pharmacol. Sci.* 12(10): 383–388.
- [18] Hardy, J.A., and Higgins, G.A. 1992. Alzheimer's disease: the amyloid cascade hypothesis. *Science*, 256(5054): 184–185.
- [19] Clippingdale AB, Wade JD, Barrow CJ (2001) The amyloid-beta peptide and its role in Alzheimer's disease. *J Pept Sci* 7(5):227–249
- [20] Dickson DW (1997) The pathogenesis of senile plaques. *J Neuro- pathol Exp Neurol* 56(4):321–339
- [21] Naslund J, Haroutunian V, Mohs R, Davis KL, Davies P, Greengard P et al (2000) Correlation between elevated levels of amyloid beta- peptide in the brain and cognitive decline. *JAMA* 283(12):1571– 1577
- [22] Billings, L.M., Oddo, S., Green, K.N., McGaugh, J.L., and LaFerla, F.M. 2005. Intraneuronal Ab causes the onset of early Alzheimer's disease-related cognitive deficits in transgenic mice. *Neuron*, 45 (5): 675–688.

- [23] D'Andrea, M.R., Nagele, R.G., Wang, H.Y., Peterson, P.A., and Lee, D.H. 2001. Evidence that neurones accumulating amyloid can undergo lysis to form amyloid plaques in Alzheimer's disease. *Histopathology*, 38(2): 120–134.
- [24] Gouras, G.K., Tsai, J., Naslund, J., Vincent, B., Edgar, M., Checler, F., et al. 2000. Intraneuronal Ab42 accumulation in human brain. *Am. J. Pathol.* 156(1): 15–20.
- [25] Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kaye, R., et al. 2003. Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Ab and synaptic dysfunction. *Neuron*, 39(3): 409–421.
- [26] Wirths, O., Multhaup, G., and Bayer, T.A. 2004. A modified beta-amyloid hypothesis: intraneuronal accumulation of the beta-amyloid peptide—the first step of a fatal cascade. *J. Neurochem.* 91(3): 513–520.
- [27] Glabe, C. 2001. Intracellular mechanisms of amyloid accumulation and pathogenesis in Alzheimer's disease. *J. Mol. Neurosci.* 17(2):137–145.
- [28] Bahr, B.A., Hoffman, K.B., Yang, A.J., Hess, U.S., Glabe, C.G., and Lynch, G. 1998. Amyloid beta protein is internalized selectively by hippocampal field CA1 and causes neurons to accumulate amyloidogenic carboxyterminal fragments of the amyloid precursor protein. *J. Comp. Neurol.* 397(1): 139–147.
- [29] Friedrich, R.P., Tepper, K., Ronicke, R., Soom, M., Westermann, M., Reymann, K., et al. 2010. Mechanism of amyloid plaque formation suggests an intracellular basis of Ab pathogenicity. *Proc. Natl. Acad. Sci. U.S.A.* 107(5): 1942–1947.
- [30] Rajendran, L., Honsho, M., Zahn, T.R., Keller, P., Geiger, K.D., Verkade, P., and Simons, K. 2006. Alzheimer's disease beta-amyloid peptides are released in association with exosomes. *Proc. Natl. Acad. Sci. U.S.A.* 103(30): 11172–11177.
- [31] Busciglio, J., Pelsman, A., Wong, C., Pigino, G., Yuan, M., Mori, H., and Yankner, B.A. 2002. Altered metabolism of the amyloid beta precursor protein is associated with mitochondrial dysfunction in Down's syndrome. *Neuron*, 33(5): 677–688.
- [32] Chui, D.H., Dobo, E., Makifuchi, T., Akiyama, H., Kawakatsu, S., Petit, A., et al. 2001. Apoptotic neurons in Alzheimer's disease frequently show intracellular Ab42 labeling. *J. Alzheimers Dis.* 3 (2): 231–239.
- [33] Zhang, Y., McLaughlin, R., Goodyer, C., and LeBlanc, A. 2002. Selective cytotoxicity of intracellular amyloid beta peptide 1–42 through p53 and Bax in cultured primary human neurons. *J. Cell Biol.* 156(3): 519–529.
- [34] Casas, C., Sergeant, N., Itier, J.M., Blanchard, V., Wirths, O., van der Kolk, N., et al. 2004. Massive CA1/2 neuronal loss with intraneuronal and N-terminal truncated Ab42 accumulation in a novel Alzheimer transgenic model. *Am. J. Pathol.* 165(4): 1289–1300.

- [35] LaFerla, F.M., Tinkle, B.T., Bieberich, C.J., Haudenschild, C.C., and Jay, G. 1995. The Alzheimer's Ab peptide induces neurodegeneration and apoptotic cell death in transgenic mice. *Nat. Genet.* 9(1): 21–30.
- [36] Takahashi, R.H., Milner, T.A., Li, F., Nam, E.E., Edgar, M.A., Yamaguchi, H., et al. 2002. Intraneuronal Alzheimer Ab42 accumulates in multivesicular bodies and is associated with synaptic pathology. *Am. J. Pathol.* 161(5): 1869–1879.
- [37] Almeida, C.G., Tampellini, D., Takahashi, R.H., Greengard, P., Lin, M.T., Snyder, E.M., and Gouras, G.K. 2005. Beta-amyloid accumulation in APP mutant neurons reduces PSD-95 and GluR1 in synapses. *Neurobiol. Dis.* 20(2): 187–198.
- [38] Tampellini, D., Magrane, J., Takahashi, R.H., Li, F., Lin, M.T., Almeida, C.G., and Gouras, G.K. 2007. Internalized antibodies to the Ab domain of APP reduce neuronal Ab and protect against synaptic alterations. *J. Biol. Chem.* 282(26): 18895–18906.
- [39] Jonsson, T., Atwal, J. K., Steinberg, S., Snaedal, J., Jonsson, P. V., Bjornsson, S., et al. (2012). A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* 488, 96–99.
- [40] Hatami A, Albay R 3rd, Monjazebe S, Milton S, Glabe C., Monoclonal Antibodies Against A β 42 Fibrils Distinguish Multiple Aggregation State Polymorphisms in vitro and in Alzheimer's Disease Brain. *J.Biol. Chem.*, October 3 2014
- [41] Haass, C. (2004). Take five – BACE and the gamma-secretase quartet conduct Alzheimer's amyloid beta-peptide generation. *EMBO J.* 23, 483–488.
- [42] Thathiah, A., Horre, K., Snellinx, A., Vandeweyer, E., Huang, Y., Ciesielska, M., et al. (2013). beta-arrestin 2 regulates Abeta generation and gamma-secretase activity in Alzheimer's disease. *Nat. Med.* 19, 43–49.
- [43] Hooper, N. M., and Turner, A. J. (2002). The search for alpha-secretase and its potential as a therapeutic approach to Alzheimer's disease. *Curr. Med. Chem.* 9, 1107–1119.
- [44] Lin, M. T., and Beal, M. F. (2006). Alzheimer's APP mangles mitochondria. *Nat. Med.* 12, 1241–1243.
- [45] Devi, L., Prabhu, B. M., Galati, D. F., Avadhani, N. G., and Anandatheerthavarada, H. K. (2006). Accumulation of amyloid precursor protein in the mitochondrial import channels of human Alzheimer's disease brain is associated with mitochondrial dysfunction. *J. Neurosci.* 26, 9057–9068.
- [46] Maxfield FR, Tabas I. Role of cholesterol and lipid organization in disease. *Nature* 438:612-621, 2005.
- [47] Goritz, C., Mauch, D.H., and Pfrieder, F.W. 2005. Multiple mechanisms mediate cholesterol-induced synaptogenesis in a CNS neuron. *Mol. Cell. Neurosci.* 29(2): 190–201.

- [48] Pfrieder, F.W. 2003. Cholesterol homeostasis and function in neurons of the central nervous system. *Cell. Mol. Life Sci.* 60(6): 1158– 1171.
- [49] Thiele, C., Hannah, M.J., Fahrenholz, F., and Huttner, W.B.2000. Cholesterol binds to synaptophysin and is required for biogenesis of synaptic vesicles. *Nat. Cell Biol.*2(1): 42–49.
- [50] Sparks DL, Scheff SW, Hunsaker JC 3rd, Liu H, Landers T, Gross DR. Induction of Alzheimer-like beta-amyloid immunoreactivity in the brains of rabbits with dietary cholesterol. *Exp Neurol.* 1994 Mar;126(1):88-94
- [51] Refolo LM, Malester B, LaFrancois J, Bryant-Thomas T, Wang R, Tint GS, Sambamurthi K, Duff K, Pappolla MA. Hypercholesterolemia accelerates the Alzheimer's amyloid pathology in a transgenic mouse model. *Neurobiol Dis.* 2000 Aug;7(4):321-31
- [52] Thirumangalakudi L, Prakasam A, Zhang R, Bimonte-Nelson H, Sambamurthi K, Kindy MS, Bhat NR. High cholesterol-induced neuroinflammation and amyloid precursor protein processing correlate with loss of working memory in mice. *J Neurochem.* 2008 Jul;106(1):475-85.
- [53] Oksman M, Iivonen H, Högges E, Amtul Z, Penke B, Leenders I, Broersen L, Lütjohann D, Hartmann T, Tanila H. Impact of different saturated fatty acid, polyunsaturated fatty acid and cholesterol containing diets on beta-amyloid accumulation in APP/PS1 transgenic mice. *Neurobiol Dis.* 2006 Sep;23(3):563-72.
- [54] Shie FS, Jin LW, Cook DG, Leverenz JB, LeBoeuf RC. Diet-induced hypercholesterolemia enhances brain A beta accumulation in transgenic mice. *Neuroreport.* 2002 Mar 25;13(4):455-9
- [55] Levin-Allerhand JA, Lominska CE, Smith JD. Increased amyloid- levels in APPSWE transgenic mice treated chronically with a physiological high-fat high-cholesterol diet. *J Nutr Health Aging.* 2002;6(5):315-9.
- [56] Ujiie M, Dickstein DL, Carlow DA, Jefferies WA. Blood-brain barrier permeability precedes senile plaque formation in an Alzheimer disease model. *Microcirculation.* 2003 Dec;10(6):463-70.
- [57] Winkler EA, Sagare AP, Zlokovic BV. The pericyte: a forgotten cell type with important implications for Alzheimer's disease? *Brain Pathol.* 2014 Jul;24(4):371-86
- [58] Wahrle, S., Das, P., Nyborg, A.C., McLendon, C., Shoji, M., Kawarabayashi, T., et al. 2002. Cholesterol-dependent gamma- secretase activity in buoyant cholesterol-rich membrane micro- domains. *Neurobiol. Dis.* 9(1): 11–23.
- [59] Vetrivel, K.S., and Thinakaran, G. 2010. Membrane rafts in Alzheimer's disease beta-amyloid production. *Biochim. Biophys. Acta*, 1801(8): 860–867.

- [60] Anstey, K. J., Lipnicki, D. M., and Low, L. F. (2008). Cholesterol as a risk factor for dementia and cognitive decline: a systematic review of prospective studies with meta-analysis. *Am. J. Geriatr. Psychiatry* 16, 343–354.
- [61] Notkola, I. L., Sulkava, R., Pekkanen, J., Erkinjuntti, T., Ehnholm, C., Kivinen, P., et al. (1998). Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease. *Neuroepidemiology* 17, 14–20.
- [62] Wolozin, B., Kellman, W., Ruosseau, P., Celesia, G. G., and Siegel, G. (2000). Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Arch. Neurol.* 57, 1439–1443.
- [63] Martins, I.J., Berger, T., Sharman, M.J., Verdile, G., Fuller, S.J., and Martins, R.N. 2009. Cholesterol metabolism and transport in the pathogenesis of Alzheimer's disease. *J. Neurochem.* 111(6): 1275– 1308.
- [64] Burns MP, Igbavboa U, Wang L, Wood WG, Duff K. Cholesterol distribution, not total levels, correlate with altered amyloid precursor protein processing in statin-treated mice. *Neuromolecular Med.* 2006;8(3):319-28
- [65] Chan SL, Kim WS, Kwok JB, Hill AF, Cappai R, Rye KA, Garner B. ATP-binding cassette transporter A7 regulates processing of amyloid precursor protein in vitro. *J Neurochem.* 2008 Jul;106(2):793-804.
- [66] Kim WS, Weickert CS, Garner B. Role of ATP-binding cassette transporters in brain lipid transport and neurological disease. *J Neurochem.* 2008 Mar;104(5):1145-66.
- [67] DiPaolo G, Kim TW. Linking lipids to Alzheimer's disease: cholesterol and beyond. *Nat Rev Neurosci.* 2011 May;12(5):284-96
- [68] Urano Y, Ochiai S, Noguchi N. Suppression of amyloid- β production by 24S-hydroxycholesterol via inhibition of intracellular amyloid precursor protein trafficking. *FASEB J.* 2013 Oct;27(10):4305-15.
- [69] Eckert GP, Cairns NJ, Maras A, Gattaz WF, Müller WE. Cholesterol modulates the membrane-disordering effects of beta-amyloid peptides in the hippocampus: specific changes in Alzheimer's disease. *Dement Geriatr Cogn Disord.* 2000 Jul-Aug;11(4): 181-6.
- [70] Mason RP, Shoemaker WJ, Shajenko L, Chambers TE, Herbette LG. Evidence for changes in the Alzheimer's disease brain cortical membrane structure mediated by cholesterol. *Neurobiol Aging.* 1992 May-Jun;13(3):413-9.
- [71] Heverin M, Bogdanovic N, Lütjohann D, Bayer T, Pikuleva I, Bretillon L, Diczfalusy U, Winblad B, Björkhem I. Changes in the levels of cerebral and extracerebral sterols in the brain of patients with Alzheimer's disease. *J Lipid Res* 2004 Jan;45(1):186-93.
- [72] Sparks DL. Coronary artery disease, hypertension, ApoE, and cholesterol: a link to Alzheimer's disease? *Ann N Y Acad Sci.* 1997 Sep 26;826:128-46.

- [73] Abad-Rodriguez J, Ledesma MD, Craessaerts K, Perga S, Medina M, Delacourte A, Dingwall C, De Strooper B, Dotti CG. Neuronal membrane cholesterol loss enhances amyloid peptide generation. *J Cell Biol.* 2004 Dec 6;167(5):953-60.
- [74] Ledesma MD, Abad-Rodriguez J, Galvan C, Biondi E, Navarro P, Delacourte A, Dingwall C, Dotti CG. Raft disorganization leads to reduced plasmin activity in Alzheimer's disease brains. *EMBO Rep.* 2003 Dec;4(12):1190-6.
- [75] Cole SL, Grudzien A, Manhart IO, Kelly BL, Oakley H, Vassar R. Statins cause intracellular accumulation of amyloid precursor protein, beta-secretase-cleaved fragments, and amyloid beta-peptide via an isoprenoid-dependent mechanism. *J Biol Chem.* 2005 May 13;280(19):18755-70.
- [76] Li G, Higdon R, Kukull WA, Peskind E, Van Valen Moore K, Tsuang D, van Belle G, McCormick W, Bowen JD, Teri L, Schellenberg GD, Larson EB. Statin therapy and risk of dementia in the elderly: a community-based prospective cohort study. *Neurology.* 2004 Nov 9;63(9):1624-8.
- [77] Rea TD, Breitner JC, Psaty BM, Fitzpatrick AL, Lopez OL, Newman AB, Hazzard WR, Zandi PP, Burke GL, Lyketsos CG, Bernick C, Kuller LH. Statin use and the risk of incident dementia: the Cardiovascular Health Study. *Arch Neurol.* 2005 Jul;62(7):1047-51.
- [78] Zandi PP, Sparks DL, Khachaturian AS, Tschanz J, Norton M, Steinberg M, Welsh-Bohmer KA, Breitner JC; Cache County Study investigators. Do statins reduce risk of incident dementia and Alzheimer disease? The Cache County Study. *Arch Gen Psychiatry.* 2005 Feb;62(2):217-24.
- [79] Carlsson CM, Gleason CE, Hess TM, Moreland KA, Blazel HM, Kosciuk RL, Schreiber NT, Johnson SC, Atwood CS, Puglielli L, Hermann BP, McBride PE, Stein JH, Sager MA, Asthana S. Effects of simvastatin on cerebrospinal fluid biomarkers and cognition in middle-aged adults at risk for Alzheimer's disease. *J Alzheimers Dis.* 2008 Mar;13(2):187-97.
- [80] Hoglund K, Thelen KM, Syversen S, Sjogren M, von Bergmann K, Wallin A, Vanmechelen E, Vanderstichele H, Lutjohann D, Blennow K. The effect of simvastatin treatment on the amyloid precursor protein and brain cholesterol metabolism in patients with Alzheimer's disease. *Dement Geriatr Cogn Disord.* 2005;19(5-6):256-65.
- [81] Riekse RG, Li G, Petrie EC, Leverenz JB, Vavrek D, Vuletic S, Albers JJ, Montine TJ, Lee VM, Lee M, Seubert P, Galasko D, Schellenberg GD, Hazzard WR, Peskind ER. Effect of statins on Alzheimer's disease biomarkers in cerebrospinal fluid. *J Alzheimers Dis.* 2006 Dec;10(4):399-406.
- [82] Posse de Chaves E. Reciprocal regulation of cholesterol and beta amyloid at the sub-cellular level in Alzheimer's disease. *Can J Physiol Pharmacol* 90: 753-764, 2012.

- [83] Mohamed A, Saavedra L, Di Pardo A, Sipione S, Posse de Chaves E. β -amyloid inhibits protein prenylation and induces cholesterol sequestration by impairing SREBP-2 cleavage. *J Neurosci*. 2012 May 9;32(19):6490-500.
- [84] Bjorkhem I, Meaney S (2004) Brain cholesterol: long secret life behind a barrier. *Arterioscler Thromb Vasc Biol* 24(5):806–815
- [85] Dietschy JM, Turley SD (2004) Thematic review series: brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *J Lipid Res* 45(8):1375–1397
- [86] Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. *Cell*. 2006 Jan 13;124(1):35-46
- [87] Jo Y, DeBose-Boyd RA. Control of cholesterol synthesis through regulated ER-associated degradation of HMG CoA reductase. *Crit Rev Biochem Mol Biol*. 2010 Jun;45(3):185-98.
- [88] Pfrieger FW. Cholesterol homeostasis and function in neurons of the central nervous system. *Cell Mol Life Sci*. 2003 Jun;60(6):1158-71.
- [89] Jurevics H, Morell P. Cholesterol for synthesis of myelin is made locally, not imported into brain. *J Neurochem* 64: 895-901, 1995.
- [90] Wahrle SE, Jiang H, Parsadanian M, Legleiter J, Han X, Fryer JD, Kowalewski T, Holtzman DM. ABCA1 is required for normal central nervous system ApoE levels and for lipidation of astrocyte-secreted apoE. *J Biol Chem*. 2004 Sep 24;279(39):40987-93.
- [91] Karten B, Campenot RB, Vance DE, Vance JE. Expression of ABCG1, but not ABCA1, correlates with cholesterol release by cerebellar astroglia. *J Biol Chem*. 2006 Feb 17;281(7):4049-57
- [92] Herz J. Apolipoprotein E receptors in the nervous system. *Curr Opin Lipidol*. 2009 Jun;20(3):190-6.
- [93] Poirier J. Apolipoprotein E and cholesterol metabolism in the pathogenesis and treatment of Alzheimer's disease. *Trends Mol Med*. 2003 Mar;9(3):94-101.
- [94] Puglielli L, Konopka G, Pack-Chung E, Ingano LA, Berezovska O, Hyman BT, Chang TY, Tanzi RE, Kovacs DM. Acyl-coenzyme A: cholesterol acyltransferase modulates the generation of the amyloid beta-peptide. *Nat Cell Biol*. 2001 Oct;3(10):905-12.
- [95] Bogdanovic N, Bretillon L, Lund EG, Diczfalusy U, Lannfelt L, Winblad B, Russell DW, Björkhem I. On the turnover of brain cholesterol in patients with Alzheimer's disease. Abnormal induction of the cholesterol-catabolic enzyme CYP46 in glial cells. *Neurosci Lett*. 2001 Nov 13;314(1-2):45-8.

- [96] Lung EG, Guileyardo JM, Russell DW. cDNA cloning of cholesterol 24-hydroxylase, a mediator of cholesterol homeostasis in the brain. *Proc Natl Acad Sci. USA* 96: 7238-43, 1999.
- [97] Lund EG, Xie C, Kotti T, Turley SD, Dietschy JM, Russell DW. Knockout of the cholesterol 24-hydroxylase gene in mice reveals a brain-specific mechanism of cholesterol turnover. *J Biol Chem.* 2003 Jun 20;278(25):22980-8.
- [98] Kim WS, Rahmanto AS, Kamili A, Rye KA, Guillemin GJ, Gelissen IC, Jessup W, Hill AF, Garner B. Role of ABCG1 and ABCA1 in regulation of neuronal cholesterol efflux to apolipoprotein E discs and suppression of amyloid-beta peptide generation. *J Biol Chem* 282: 2851-2861, 2007.
- [99] Muscat GE, Wagner BL, Hou J, Tangirala RK, Bischoff ED, Rohde P, Petrowski M, Li J, Shao G, Macondray G, Schulman IG. Regulation of cholesterol homeostasis and lipid metabolism in skeletal muscle by liver X receptors. *J Biol Chem.* 2002 Oct 25;277(43):40722-8.
- [100] Whitney KD, Watson MA, Collins JL, Benson WG, Stone TM, Numerick MJ, Tippin TK, Wilson JG, Winegar DA, Klierer SA. Regulation of cholesterol homeostasis by the liver X receptors in the central nervous system. *Mol Endocrinol.* 2002 Jun;16(6): 1378-85.
- [101] Eckert GP, Vardanian L, Rebeck GW, Burns MP. Regulation of central nervous system cholesterol homeostasis by the liver X receptor agonist TO-901317. *Neurosci Lett.* 2007 Aug 9;423(1):47-52.
- [102] Burns MP, Rebeck GW. Intracellular cholesterol homeostasis and amyloid precursor protein processing. *Biochim Biophys Acta* 1801: 853-859, 2010.
- [103] Burns M, Gaynor K, Olm V, Mercken M, LaFrancois J, Wang L, Mathews PM, Noble W, Matsuoka Y, Duff K. Presenilin redistribution associated with aberrant cholesterol transport enhances beta-amyloid production in vivo. *J Neurosci.* 2003 Jul 2;23(13): 5645-9.
- [104] Jin LW, Shie FS, Maezawa I, Vincent I, Bird T. Intracellular accumulation of amyloidogenic fragments of amyloid-beta precursor protein in neurons with Niemann-Pick type C defects is associated with endosomal abnormalities. *Am J Pathol.* 2004 Mar; 164(3):975-85.
- [105] Runz H, Rietdorf J, Tomic I, M Bernard, Byereuthe K, Pepperkok R, Hartmann T. Inhibition of intracellular cholesterol transport alters presenilin localization and amyloid precursor protein processing in neuronal cells. *J Neurosci* 22: 1679-1689.
- [106] Kågedal K, Kim WS, Appelqvist H, Chan S, Cheng D, Agholme L, Barnham K, McCann H, Halliday G, Garner B. Increased expression of the lysosomal cholesterol transporter NPC1 in Alzheimer's disease. *Biochim Biophys Acta.* 2010 Aug;1801(8): 831-8.

- [107] Caglayan S, Takagi-Niidome S, Liao F, Carlo AS, Schmidt V, Burgert T, Kitago Y, Füchtbauer EM, Füchtbauer A, Holtzman DM, Takagi J, Willnow TE. Lysosomal sorting of amyloid- β by the SORLA receptor is impaired by a familial Alzheimer's disease mutation. *Sci Transl Med*. 2014 Feb 12;6(223):223ra20.
- [108] Fucho R, Martínez L, Baulies A, Torres S, Tarrats N, Fernandez A, Ribas V, Astudillo AM, Balsinde J, Garcia-Rovés P, Elena M, Bergheim I, Lotersztajn S, Trautwein C, Appelqvist H, Paton AW, Paton JC, Czaja MJ, Kaplowitz N, Fernandez-Checa JC, García-Ruiz C. ASMase regulates autophagy and lysosomal membrane permeabilization and its inhibition prevents early stage non-alcoholic steatohepatitis. *J Hepatol*. 2014 Nov;61(5):1126-34.
- [109] Karaca I, Tamboli IY, Glebov K, Richter J, Fell LH, Grimm MO, Haupenthal VJ, Hartmann T, Gräler MH, van Echten-Deckert G, Walter J. Deficiency of sphingosine-1-phosphate lyase impairs lysosomal metabolism of the amyloid precursor protein. *J Biol Chem*. 2014 Jun 13;289(24):16761-72.
- [110] Van Echten-Deckert G, Walter J. Sphingolipids: critical players in Alzheimer's disease. *Prog Lipid Res* 51: 378-93, 2012.
- [111] Garcia-Ruiz C, Mari M, Colell A, Morales A, Montero J, Terrones O, Basañez G, Fernandez-Checa JC Mitochondrial cholesterol in health and disease. *Histol Histopathol* 24: 117-32, 2009
- [112] Bosch M, Marí M, Herms A, Fernández A, Fajardo A, Kassan A, Giralt A, Colell A, Balgoma D, Barbero E, González-Moreno E, Matias N, Tebar F, Balsinde J, Camps M, Enrich C, Gross SP, García-Ruiz C, Pérez-Navarro E, Fernández-Checa JC, Pol A. Caveolin-1 deficiency causes cholesterol-dependent mitochondrial dysfunction and apoptotic susceptibility. *Curr Biol*. 2011 Apr 26;21(8):681-6.
- [113] Colell A, Fernández A, Fernández-Checa JC. Mitochondria, cholesterol and amyloid beta peptide: a dangerous trio in Alzheimer disease. *J Bioenerg Biomembr*. 2009 Oct; 41(5):417-23.
- [114] Fernandez A, Llacuna L, Fernandez-Checa JC, Colell A. Mitochondrial cholesterol loading exacerbates amyloid beta peptide-induced inflammation and neurotoxicity. *J Neurosci* 2009; 29: 6394-405.
- [115] Caron KM, Soo SC, Wetsel WC, Stocco DM, Clark BJ, Parker KL. targeted disruption of the mouse gene encoding steroidogenic acute regulatory protein provides insights into congenital lipid adrenal hyperplasia. *Proc Natl Acad Sci U S A*. 1997 Oct 14;94(21):11540-5.
- [116] Barbero-Camps E, Fernández A, Baulies A, Martinez L, Fernández-Checa JC, Colell A. Endoplasmic reticulum stress mediates amyloid β neurotoxicity via mitochondrial cholesterol trafficking. *Am J Pathol*. 2014 Jul;184(7):2066-81.
- [117] Webber KM, Stocco DM, Casadesus G, Bowen RL, Atwood CS, Preville LA, Harris PL, Zhu X, Perry G, Smith MA. Steroidogenic acute regulatory protein (StAR): evidence

of gonadotropin-induced steroidogenesis in Alzheimer disease. *Mol Neurodegener* 1:14, 2006.

- [118] Papadopoulos, V., and Miller, W. L. (2012). Role of mitochondria in steroidogenesis. *Best Pract. Res. Clin. Endocrinol. Metab.* 26, 771–790.
- [119] Miller, W. L. (2013). Steroid hormone synthesis in mitochondria. *Mol. Cell. Endocrinol.* 379, 62–73.
- [120] Morohaku, K., Pelton, S. H., Daugherty, D. J., Butler, W. R., Deng, W., and Selvaraj, V. (2014). Translocator protein/peripheral benzodiazepine receptor is not required for steroid hormone biosynthesis. *Endocrinology* 155, 89–97.
- [121] Fernandez A, Matias N, Fucho R, Ribas V, Von Montfort C, Nuño N, Baulies A, Martinez L, Tarrats N, Mari M, Colell A, Morales A, Dubuquoy L, Mathurin P, Bataller R, Caballeria J, Elena M, Balsinde J, Kaplowitz N, Garcia-Ruiz C, Fernandez-Checa JC. ASMase is required for chronic alcohol induced hepatic endoplasmic reticulum stress and mitochondrial cholesterol loading. *J Hepatol.* 2013 Oct;59(4):805-13.
- [122] Barbero-Camps E, Fernández A, Martínez L, Fernández-Checa JC, Colell A. APP/PS1 mice overexpressing SREBP-2 exhibit combined A β accumulation and tau pathology underlying Alzheimer's disease. *Hum Mol Genet.* 2013 Sep 1;22(17):3460-76.
- [123] Wadey, A. L., Muyderman, H., Kwek, P. T., and Sims, N. R. (2009). Mitochondrial glutathione uptake: characterization in isolated brain mitochondria and astrocytes in culture. *J. Neurochem.* 109(Suppl. 1), 101–108.
- [124] Kamga, C. K., Zhang, S. X., and Wang, Y. (2010). Dicarboxylate carrier-mediated glutathione transport is essential for reactive oxygen species homeostasis and normal respiration in rat brain mitochondria. *Am. J. Physiol. Cell Physiol.* 299, C497–C505.
- [125] de Bilbao, F., Arsenijevic, D., Vallet, P., Hjelle, O. P., Ottersen, O. P., Bouras, C., et al. (2004). Resistance to cerebral ischemic injury in UCP2 knockout mice: evidence for a role of UCP2 as a regulator of mitochondrial glutathione levels. *J. Neurochem.* 89, 1283–1292.
- [126] O. Coll, A. Colell, C. García-Ruiz, N. Kaplowitz, J.C. Fernández-Checa. Sensitivity of the 2-oxoglutarate carrier to alcohol intake contributes to mitochondrial glutathione depletion. *Hepatology*, 38 (2003), pp. 692–702
- [127] Ferreiro E, Resende R, Costa R, Oliveira CR, Pereira CM. An endoplasmic-reticulum-specific apoptotic pathway is involved in prion and amyloid-beta peptides neurotoxicity. *Neurobiol Dis.* 2006 Sep;23(3):669-78.
- [128] Colell A, Coll O, García-Ruiz C, París R, Tiribelli C, Kaplowitz N, Fernández-Checa JC. Tauroursodeoxycholic acid protects hepatocytes from ethanol-fed rats against tumor necrosis factor-induced cell death by replenishing mitochondrial glutathione. *Hepatology.* 2001 Nov;34(5):964-71.

- [129] R.E. Soccio, R.M. Adams, K.N. Maxwell, J.L. Breslow, Differential gene regulation of StarD4 and StarD5 cholesterol transfer proteins. Activation of StarD4 by sterol regulatory element-binding protein-2 and StarD5 by endoplasmic reticulum stress, *J. Biol. Chem.* 280 (2005) 19410–19418.
- [130] D. Rodriguez-Agudo, M. Calderon-Dominguez, M.A. Medina, S. Ren, G. Gil, W. M. Pandak, ER stress increases StarD5 expression by stabilizing its mRNA and leads to relocalization of its protein from the nucleus to the membranes, *J. Lipid Res.* 53 (2012) 2708–2715.
- [131] S. Yamada, T. Yamaguchi, A. Hosoda, T. Iwawaki, K. Kohno, Regulation of human STARD4 gene expression under endoplasmic reticulum stress, *Biochem. Biophys. Res. Commun.* 343 (2006) 1079–1085.
- [132] P.R. Manna, J.W. Soh, D.M. Stocco, The involvement of specific PKC iso-enzymes in phorbol ester-mediated regulation of steroidogenic acute regulatory protein expression and steroid synthesis in mouse Leydig cells, *Endocrinology* 152 (2011) 313–325.
- [133] F. Arakane, S.R. King, Y. Du, C.B. Kallen, L.P. Walsh, H. Watari, D.M. Stocco, J. F. Strauss, Phosphorylation of steroidogenic acute regulatory protein (StAR) modulates its steroidogenic activity, *J. Biol. Chem.* 272 (1997) 32656–32662.