

Cholesterol metabolism in spinocerebellar ataxias: trying to unravel the underlying mechanism in the neurodegenerative disorders



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Abstract Autosomal Dominant Spinocerebellar Ataxia's (ADCAs) are a subset of hereditary spinocerebellar ataxias showing atrophy of the cerebellum and loss of Purkinje cells (PCs) leading to uncoordinated movement and gait. Most SCA subtypes can be attributed to CAG trinucleotide repeat expansions with several lengths, leading to protein misfolding and aggregation mainly in the cerebellum. Defects in cholesterol metabolism have been observed in SCA and it has already been shown in other neurological diseases such as Alzheimer's disease, Huntington disease and Niemann-Pick type C that it contributes to neurodegeneration. A defective clearance of cholesterol might be the cause of cholesterol accumulation observed in SCA. CYP46A1 is responsible for the turnover of cholesterol to 24-OHC and be excreted from the brain. In SCA, CYP46A1 levels are decreased, leading to cholesterol accumulation in the brain. In addition, CYP46A1 mediates autophagy in the brain. When CYP46A1 levels are decreased, autophagy is attenuated due to defects in late-endosomes and leads to aggregation of the misfolded proteins seen in SCA. The aggregated proteins cause Endoplasmic reticulum (ER) stress. ER stress might be the start of a vicious cycle of cholesterol synthesis and defective clearance, leading to cholesterol accumulation and contributes to pathology.

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Introduction

Ataxia refers to clumsiness or a loss of balance and coordination that is not due to muscle weakness. Cerebellar damage is a main cause of ataxia, but can also result from a dysfunction of pathways that are associated with the cerebellum. There are several causes that can result in either acquired or hereditary ataxia, such as drugs and genetic disorders [1][2]. Autosomal dominant Spinocerebellar ataxia's (ADCAs) are a subset of hereditary spinocerebellar ataxias (SCAs) resulting in uncoordinated movement, gait and balance problems amongst the symptoms caused by SCA [3][4]. The prevalence of SCAs varies based on geography and ethnicity. In Europeans, it is estimated to be 1-3 per 100,000[5]. SCAs are a late onset, slowly progressive disease which manifest generally around middle age [6]. Cerebellar cell loss is a common feature seen in spinocerebellar ataxias and can be detected by MRI [7]. Cerebellar atrophy varies between SCA types and correlate with the CAG repeat expansion, however if this is truly the case remains debatable [8][9]. Several mutations are identified that alter Purkinje cell properties and are associated with ataxia and loss of PC is often seen in SCA patients [10]. ADCAs result from genetic variations such as point mutations or expansion of non-coding repeats. Over 40 SCA types have been identified, resulting from these mutations [3], which can be classified in polyglutamine (PolyQ) expansions, non-coding expansions, and conventional mutations [11]. Most of the SCA subtypes genes (SCA1, SCA2, SCA3, SCA6, SCA7, and SCA17) are caused by a CAG trinucleotide repeat expansions, which can differ in length, in the coding region of the respective gene [6][12] and can lead to a toxic gain of function[13][14]. Polyglutamine expansion SCAs have a diffuse neurological dysfunction. SCA3 is the most common polyglutamine expansion SCA type worldwide and in the Netherlands [12]. Some SCA types are caused by non-coding expansions or conventional mutations in associated genes [11]. However, SCAs caused by conventional mutations are less frequent than polyglutamine expansion SCAs. In addition, the phenotype differs between these SCAs. Lifespan in conventional SCAs is normal, where in SCA caused by CAG expansions suffer from neurological damage resulting from atrophy of pons, brainstem or cerebellum in different CAG SCA types [11]. Based on the genes identified so far [3], several underlying mechanisms have been identified that play a role in SCA, including protein aggregation and clearance, autophagy, mitochondrial defects and toxic gain-of-function mechanisms leading to neuronal and Purkinje Cell deficits [15]. Recently several shared mechanisms have been identified that are associated with SCA [16]. Accumulating evidence shows that alterations in metabolic pathways including lipid and cholesterol homeostasis contribute to SCA pathology. In this review, cholesterol metabolism will briefly be discussed. A link will be made between alterations and neurodegenerative diseases to identify the effect of impaired cholesterol metabolism in the brain and determine similarities between pathways in neurodegenerative diseases and SCA. Furthermore, we will discuss the altered cholesterol metabolism in SCA and try to identify the underlying mechanism that contribute to impaired cholesterol metabolism in SCA.

Cholesterol metabolism in the brain

Cholesterol is a common lipid in the human body and is particularly important for brain functioning. Cholesterol is one of the main brain lipids important for brain functioning. Glycerophospholipids and sphingolipids are two other brain lipids important for brain functioning. During brain development over the years, the total cholesterol content in the brain increases to a constant value in adult life [17]. The brain contains about 20% of all cholesterol in the human body, making it the most cholesterol-rich organ [18]. Most of the brain's cholesterol is present in the unesterified form of cholesterol and mainly present in myelin sheaths, astrocyte plasma membranes and nerve cell membranes [18] [19][20]. Cell cholesterol homeostasis is heavily regulated and maintained by the balance between cholesterol synthesis and efflux, secretion of cholesterol to the liver [21]. Circulating lipoproteins, particles carrying cholesterol and triglycerides, are unable to cross the blood-brain-barrier (BBB), so brain cholesterol is provided by *de novo* synthesis which primarily takes place in the endoplasmic reticulum (ER) of neurons and astrocytes and is then transferred to the plasma membrane [18] [20]. This process is ATP dependent. The brain relies on endogenous cholesterol synthesis and in most cells, the cholesterol is

mainly derived from endogenous synthesis from acetyl-CoA, which is processed in multiple steps to the final cholesterol product [19] [22]. This cholesterol synthesis correlates with myelination of nerve cells during brain development, when the myelination process occurs a peak in cholesterol turnover occurs and vice versa when cholesterol levels are decreased, the myelination process slows [23]. When cholesterol synthesis is higher than the usage which is often the case in adult neurons, cholesterol accumulates in cells as a result of aging [24]. Several pathways have been described to decrease cholesterol accumulation by enhancing the cholesterol efflux out of the brain. Cholesterol may be acylated by acyl-coenzyme A-cholesterol:acyltransferases (ACAT) 1 and 2 and stored in cholesterol droplets in a form known as esterified cholesterol [18] [21]. This stored cholesterol can be mobilized by apolipoprotein A1 (apoA1) and is important for cholesterol excretion out of the brain (efflux) (Figure 1) [21]. However, cholesterol cannot cross the blood brain barrier (BBB), preventing cholesterol uptake from and excretion into the circulation [25].

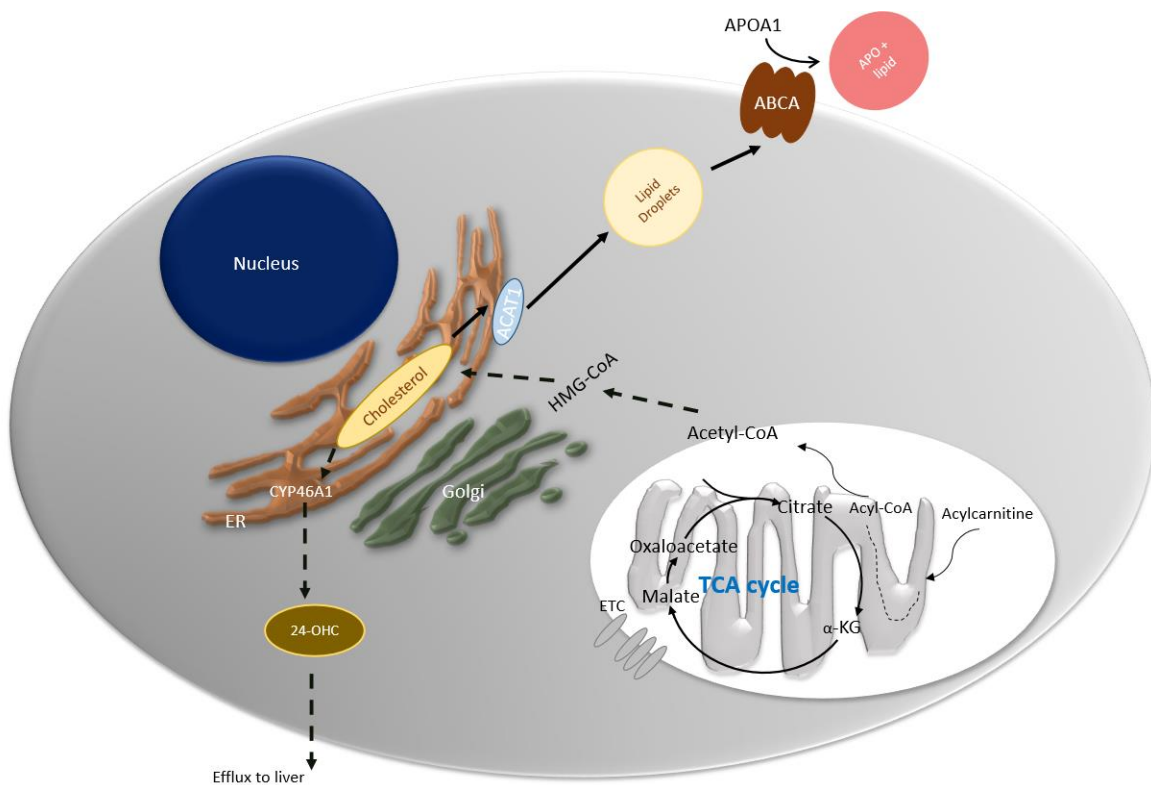


Figure 1. Cholesterol in the brain. Cholesterol is synthesised from Acetyl-CoA in the ER. Cholesterol excretion is facilitated by conversion to 24-OHC by CYP46A1. 24-OHC can pass the BBB and can be secreted from to brain towards the liver. Cholesterol can also be stored in lipid droplets, facilitated by the ACAT enzyme. Lipid droplets can be secreted from the cell by ABCA, it associates with APOA1 and forms APO enriched lipid vesicles.

Cholesterol degradation in the brain

Within the brain, the major cholesterol excretion pathway in neurons is the hydroxylation of cholesterol to 24-hydroxycholesterol (24-OHC) (Figure 1) [26]. This conversion is catalysed by the 24-droxyase, also known as CYP46A1 which is a member of the cytochrome p450 family[18][17] [26]. CYP46A1 is responsible for the cholesterol elimination from the brain. In addition, CYP46A1 also controls the rate of cerebral cholesterol production and turnover [27]. CYP46A1 resides in the ER (Figure 1) [27]. The BBB forms a protective layer to prevent substances to enter the brain. 24-OHC is able to cross the BBB in a fast rate. It is transferred over the lipophilic BBB membrane and enters the systemic circulation [17][26].

In cells outside the CNS, the cholesterol moves from the endoplasmic reticulum through the Golgi apparatus and glycosphingolipid-enriched complexes to sterol-rich microdomains in the plasma membrane. These microdomains can cross the cell membrane by the adenosine triphosphate binding cassette transporter (ABC) A1 transporter. It associates with apoA1 lipoproteins and esterified by lecithin cholesterol acyltransferase. The cholesterol is processed through clathrin-coated pit pathway to late endosomes and lysosomes, is esterified by acyl coenzyme A cholesterol transferase and the cholesteryl ester is incorporated into the very low density protein (VLDL) and secreted into the plasma [20]. A small part of the cholesteryl esters are incorporated into the low density proteins (LDL). This LDL can be hydrolysed in lysosomes and the cholesterol is transferred by the Niemann-Pick type C protein 1 (NPC1) to the metabolic pathway of newly synthesized cholesterol moving to the plasma membrane [20].

Similar to cholesterol efflux outside the CNS, neurons contain ABC transporters that allow efflux of cholesterol over the cell membrane. The cholesterol then associates with APOE proteins, which are removed from the brain through LDL-receptor related protein1 (LRP1) or scavenger receptor class B1[18]. Several alterations have been determined in SCA subtypes that contribute to PC degeneration and SCA pathology. Many of these alterations are implicated in sterol synthesis. Cholesterol accumulation is associated with several other neurodegenerative diseases and links with SCA have been made as well. A common finding is that cholesterol accumulation is, for at least partly, the result of defective cholesterol turnover to 24-OHC. Alterations in CYP46A1 lies at the heart of this defective turnover. CYP46A1 mediates activation of the autophagy pathway. Autophagy is the main clearance pathway and plays an important role in the degradation of proteins and are dependent on the formation of autophagolysosomes by the fusing of autophagosomes with lysosomes to degrade proteins [28]. Autophagy commences upon fusion of autophagosomes with lysosomes. LC3 is directly involved with autophagy. LC3B is the most studied and essential for the execution of autophagy (reviewed in [29]). Overexpression of CYP46A1 showed an increase in autophagy, measured by the turnover of LC3B-1 to LC3B-2. In addition, a significant increase in the number of autophagosomes and autolysosomes were determined upon CYP46A1 overexpression [30].

Purkinje cells

Cholesterol metabolism has been linked to the cerebellum and specifically to the functioning of PCs [31]. As stated earlier, PCs are affected in SCA. The PCs are a distinct cell type present in the cerebellum. PCs have an elaborate branched dendritic tree and a single axon. The dendritic tree lies perpendicular to the folds in the cerebellar cortex. The parallel fibers pass through the distal dendritic tree and forms weak synapses [78]. The PC processes input of incoming synaptic signals and generates output to the cerebellum via the mossy fiber-granule cell-parallel fiber (PF) pathway and the climbing fibers (CFs). These are in direct contact with PCs. PCs can regulate movement indirectly by receiving excitatory input from outside the cerebellum and inhibitory signals via the Golgi, stellate and basket cells and in this way control the information flow through the cerebellar cortex. The cerebellar output also occurs via the Purkinje Cells [79]. PC synapse formation is dependent on several molecules including GluD2 and neurexin [32]. PCs are the most important cells in the brain because they provide output of the cortex[33]. CYP46A1 is highly expressed in cerebellar PCs, mainly in the ER. CYP46A1 is only expressed in certain types of neurons in the brain such as pyramidal cells of the cortex and PCs of the cerebellum and might have an effect on synaptic activity by modulating membrane cholesterol content [31][34]. Cholesterol is present in the dendritic tree of PCs. Depletion of cholesterol resulted in the absence of cholesterol in the dendritic trees [34]. In contrast, cholesterol accumulation of cholesterol in the neuronal cell body was seen in the cerebellum was associated with PC cell death [35]. These results suggest that cholesterol is important for PC signalling and defects might affect PC signalling. PCs synthesize neurosteroids such as progesterone and its precursor pregnenolone *de novo* from cholesterol by cytochrome P450 [36]. It plays a crucial role in PC dendritic growth and spine

development [36][37]. In addition, it protects PCs from developmental cell death [37]. Refined cholesterol metabolism is therefore extremely important in PCs. Defects in cholesterol metabolism may result in decreased functioning of PCs and may eventually result in PC loss in the brain.

Long chain fatty acids in the brain

Not only cholesterol is important for brain functioning. Recent studies revealed a function of very long chain fatty acids (VLCFA) in neural signalling [38]. Long chain fatty acids have to be obtained from diet and are most commonly ingested in the forms of polyunsaturated omega-3 or omega-6 fatty acids [39]. These polyunsaturated fatty acids are claimed to give health benefits in disease, cancer and neurological disorders amongst others [39]. Short- and middle chain fatty acids are able to cross the BBB, most probably due to their lipophilic properties [39]. However, LCFA are less soluble, making it harder to cross the BBB. The passage of LCFA is most likely aided by fatty acid transport proteins. Fatty acids are important for cerebral energy metabolism. The brain is a highly active organ, taking up about 20% of the body's daily energy intake [40]. To meet its high energy demand, the brain uses a lot of oxygen for β -oxidation [40], a pathway that breaks down fatty acids in the mitochondria to generate acetyl-CoA (Figure 2). This process generates energy in the form of NADH. The acetyl-CoA can then be further processed in the citric acid cycle. Neurons have the highest energy demand in the brain for neurotransmission [40]. LCFAs are able to cross the BBB, but it is also believed that crossing of LCFA over the BBB is facilitated by transport proteins [63][40]. Defect in LCFA homeostasis are associated with neurological diseases including defective peroxisomal degradation of very long-chain fatty acids (VLCFAs) [40]. The Elongation of Very Long chain fatty acids (ELOVL) family is responsible for the elongation of saturated and unsaturated fatty acids and reside in the ER [41]. They are responsible for

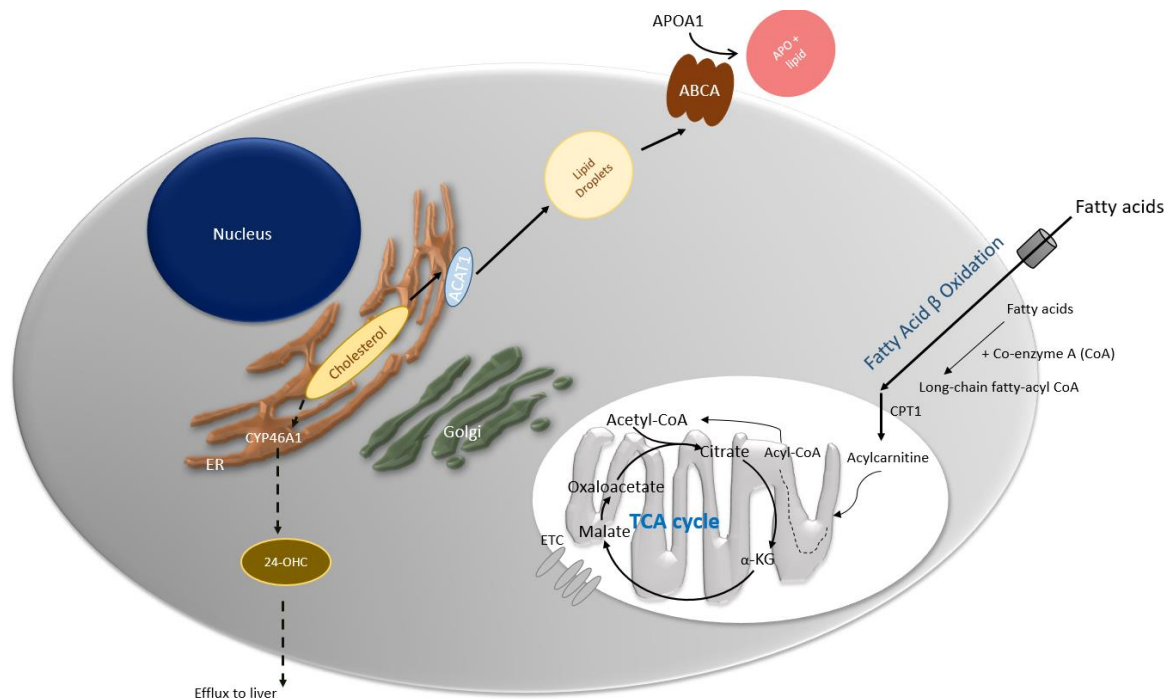


Figure 2. Fatty acid β -oxidation fuels the TCA cycle. Fatty acids are broken down during fatty acid β -oxidation. Fatty acids enter the cell and are converted to long-chain fatty acid-acyl CoA by CoA. The long chain fatty acid acyl CoA enters the mitochondria through CPT1 and is converted to acylcarnitine. Acylcarnitine is then converted to acyl-CoA and eventually acetyl-CoA. Acetyl CoA is able to enter the TCA cycle, providing energy.

the rate-controlling condensation reaction [42]. ELOVLs are essential for the biosynthesis of very long chain saturated fatty acids (VLC-SFA). ELOVL4 and ELOVL5 are selective for very long chain polyunsaturated fatty acids (CLV-PUFA)[43]. Cerebellar PCs expresses ELOVL5, where it is localized in the soma and proximal dendrites [43]. ELOVL5 is involved in the synthesis of PUFAs. ELOVL5 is localized in PCs to the soma and the proximal portion of the dendritic tree [41]. ELOVL4 is mostly expressed in the brain and expression varies in a region- and cell type specific manner. Expression of ELOVL4 is also prominent in the cerebellum, a region often linked to neurodegenerative diseases, where it is highly expressed in granule cells and low in PCs. Mutant ELOVL4 causes misallocation away from the ER. Mutations in the ELOVL family (subclasses ELOVL 1, 4, 5 and 7) are associated neurological disease [38].

Alterations in cholesterol metabolism is associated with neurodegenerative diseases

Defects in cholesterol metabolism have been linked to central nervous system diseases such as Huntington's Disease (HD), Alzheimer Disease (AD) and Niemann-Pick Type C (NPC) (Table 1). In AD, an increase in circulating cholesterol levels, hypercholesterolemia, resulted in abnormal cholesterol metabolism in the brain [44]. In the case of an increase in circulating cholesterol, the BBB permeability increases and affects BBB function which is a key contributor to AD pathology [44]. In AD patients, beta-amyloid accumulates in AD. The amyloid protein precursor (APP) shows to be affected by CYP46A1. CYP46A1 showed to significantly reduce the generation of amyloid beta. In brains of AD patients, a decrease of 24-OHC is observed. CYP46A1 is responsible for the breakdown of cholesterol to 24-OHC. The decrease

in 24-OHC in the brain is most likely a result of a decrease in CYP46A1. This decrease in CYP46A1 might be attributed to neuronal loss in the brain [44][30]. The loss of CYP46A1 in turn results in a deficient amyloid-beta reduction, contributing to neurodegeneration.

HD is a dominantly inherited genetic disorder caused by a coding CAG trinucleotide repeat expansion leading to an expanded polyglutamine (polyQ) tract in the Huntingtin protein [19]. The view that in HD pathology, disturbed cholesterol synthesis might play a key role and that cholesterol homeostasis is impaired in HD is now more accepted. Expression of HMG-CoA and 7-DHC reductase have been reported to be reduced in a mutant huntingtin expressing cell line. This decrease resulted in a reduced ability to stimulate *de novo* cholesterol synthesis [45], which is important for the brain to properly function. Also in HD, the reduced synthesis is associated with a reduction in cholesterol conversion to 24S-OHC, which results in cholesterol accumulation in the brain [45]. Also increased plasma cholesterol uptake has been proposed as a causing factor of cholesterol accumulation. Due to the lack of *de novo de novo* cholesterol synthesis, cholesterol present in the plasma cell membranes might be taken up by neurons [45]. The huntingtin protein affected in HD is expressed in several compartments that are of importance in cholesterol metabolism, including the ER, Golgi apparatus and mitochondria. Huntingtin also associates with clathrin-coated vesicles [45], important for cholesterol processing to early and late endosomes.

In AD and HD, CYP46A1 defects are associated with disease. CYP46A1 levels are decreased in affected brain regions of patients of HD and AD [30]. In HD, the brain-specific rate-limiting enzyme CYP46A1 that is essential in cholesterol degradation is decreased in the putamen of patients with HD [46]. The decrease in cholesterol conversion lead to a cholesterol accumulation in the brain. A knockdown of CYP46A1 confirmed the involvement of cholesterol conversion in HD [46]. The mouse model showed a HD-disease like phenotype, including a reduction of CYP46A1 in the striatum and an increase in neuronal death associated with it [46]. These results were also observed in a mouse model for AD. Downregulation of CYP46A1 showed an increase in cholesterol accumulation and an increased production of amyloid- β was found, probably as a result of the downregulation of CYP46A1[47]. In an AD mouse model with silenced CYP46A1, enlarged endosomes were found [47]. Enlarged endosomes are caused by defects in endosome maturation and result in a defect in trafficking.). Decreased 24-

OHC levels observed in both AD and HD might be a compensatory mechanism to compensate for the decrease in *de novo* cholesterol metabolism. The decrease in cholesterol synthesis might decrease CYP46A1 activity, thereby preventing cholesterol efflux from the brain, resulting in cholesterol accumulation in the brain.

Niemann-Pick type C disease (NPC) is an autosomal recessive lysosomal lipid storage disease caused by pathogenic variants in the NPC1 or NPC2 genes and lead to lysosomal storage dysfunction, resulting in deregulated cholesterol storage in the brain [48][49]. NPC is characterized by abnormal intracellular trafficking of endocytosed cholesterol and in particular unesterified cholesterol in lysosomes and late endosomes. The NPC1 and NPC2 genes might be involved in the cellular postlysosomal/late endosomal transport of cholesterol and cholesterol and glycosphingolipid accumulation[48], [50]. An NPC1 KO mouse model showed that the autophagy-lysosome system was altered. In PCs, one of the main cells that are affected in NPC, cholesterol clusters were observed that were colocalized with aggregated autophagosomes. This cholesterol accumulation might impair autophagosome fusion with lysosomes, inhibiting autophagy [51]. In these diseases, involvement of cholesterol have been well established and might be an underlying mechanism in disease. The altered cholesterol metabolism might also play a role in other neurodegenerative diseases.

Table 1. Cholesterol defect and possible mechanism in neurodegenerative disorders

<i>Disease</i>	<i>Cholesterol defects</i>	<i>Mechanism</i>
Alzheimer's disease	Increased plasma cholesterol levels, Amyloid- β accumulation, decreased 24-OHC levels[44][30][52]	Loss of CYP46A1 in cerebellum [44][30].
Huntington's disease	Increased cholesterol content by decreased conversion of cholesterol to 24-OHC.[45]	Possible decreased CYP46A1 activity, deregulated HMG-COA reductase and 7-DHC reductase. [45]
Niemann-Pick type C	Deregulated cholesterol storage[48][49]. Cholesterol accumulation [48], [50].	Impaired autophagosome/lysosome trafficking of cholesterol, inhibiting autophagy[48], [50] [51]

Cholesterol metabolism in SCA

Altered cholesterol metabolism has been linked to SCA. These changes in cholesterol metabolism can lead to cholesterol accumulation within the cerebellum of SCA patients. Several causes have been identified that decrease brain cholesterol levels in SCA that are associated with cholesterol processing and homeostasis in the brain. Cholesterol metabolism is affected in several SCA subtypes and has been associated with several abnormalities. In SCA3, an indirect effect has been observed between SCA pathology and cholesterol metabolism. Brain cholesterol metabolism dysfunction is found in SCA3 patients with a reduction of CYP46A1 in post-mortem patient cerebellar samples and cerebellar extracts from transgenic mouse expressing human mutant ATX3 [30]. The CYP46A1 deficiency in this mouse model was accompanied by a decrease in the levels of 24-OHC. CYP46A1 upregulation improved the neuropathology in SCA3 transgenic mice [30]. A knockdown of CYP46A1 in wildtype mice resulted in a SCA3 similar phenotype, with decreased levels of 24OHC [30]. These results show that cholesterol metabolism is impaired in SCA3 and contributes to pathology.

CYP46A1 also plays a role in NPC, a lysosomal trafficking disorder, where it is responsible for cholesterol accumulation. This contributes to neurodegeneration. NPC is associated with cerebellar

ataxia mainly in the patients with neurological adult onset. NPC is associated with cerebellar ataxia mainly in the patients with neurological adult onset. A recently new mouse model with slower progression of NPC, which can be translated to late-onset NPC, have been developed to determine the effects of an NPC1 mutation on cholesterol changes and CYP46A1 [53]. This mutated NPC1 resulted in an enhanced degradation of the mutant NPC by the proteasome and was associated with a decrease of synapses in the hippocampal CA1 region which resulted in a decrease in spatial learning [53]. Long term potentiation triggered cholesterol redistribution and reduction is necessary for synaptic delivery of AMPA receptors, which in turn allows LTP progression. It is believed that NPC mediates LTP-triggered cholesterol changes. An increase of LTP indeed showed reduced cholesterol levels, which indicates that high cholesterol levels are associated with a decrease in LTP progression. However, in mutated NPC1, no decrease in cholesterol was observed which in turn decreases LTP. CYP46A1 regulates cholesterol reduction in LTP and in accordance with the previous finding, CYP46A1 expression was not increased after LTP stimulation. These results suggests that mutations in NPC1 are associated with decreased learning which are possibly caused by a decrease in cholesterol regulation due to decreased expression of CYP46A1, resulting in cholesterol accumulation [53].

CYP46A1 is associated with endosomal trafficking dysfunction [54]. Lysosomes are important in protein degradation and play a key role in autophagy. Autophagy is the main clearance pathway and plays an important role in the degradation of proteins and are dependent on the formation of autophagolysosomes by the fusing of autophagosomes with lysosomes to degrade proteins [28]. In NPC, endosomal trafficking was disturbed as a results of enlarged endosomes caused by decreased CYP46A1 levels. Lysosomal morphology was altered, suggesting that altered CYP46A1 levels results in defective endosomal trafficking and cholesterol accumulates in late endosomes [55]. The endosomal-lysosomal trafficking is impaired in a SCA3 mouse model. Lysosomal morphology was altered and an increase in lysosomes was detected reflected in an increase in lysosomal-associated late endosomes (LAMP-1) [30], which can be attributed to the decreased CYP46A1 caused by the mutation in ataxin-3. Due to defects in the endosomal-lysosomal pathway, accumulation of the ataxin-3 aggregates are observed in a cell line expression mutant ataxin-3 [30].

To prevent the accumulation of the mutant ataxin, protein degradation is crucial. If the degradation mechanism is altered, protein degradation does not occur and misfolded protein accumulates in the brain [28]. In SCA-3, the clearance of aggregated proteins is impaired due to defective autophagy [56]. SCA7 is also associated with defects in the endosomal-lysosomal pathway. SCA7 is one of the CAG trinucleotide repeat disorders and results in misfolded proteins. Mutant ataxin-7 accumulates in neurons and forms insoluble neuronal intranuclear inclusion. In a SCA7 knock-in mice, ataxin-7 accumulation is especially seen in PCs. Autophagy initiators were increased in the cytoplasm of PCs in the brains of SCA7 patients. The autophagosome marker LC33 was strongly increased in the PCs. In addition, accumulation of autophagosome/lysosome markers rab7 LAMP-1 and LAMP-2 were found to be increased in PCs [28]. These results show accumulating evidence that a defect in autophagy might be the cause of the clinical features observed in SCAs. CYP46A1 is also involved in removal of mutant ataxin-2 [30].

Alterations in cholesterol metabolism is accompanied by changes in lipid metabolism in SCA3 patients. The metabolic profile determined in SCA3 patients significantly varied from the metabolic profile seen in to healthy controls. These changes were mostly associated with amino acid and fatty acid metabolism [57]. β -oxidation is the catabolic process in which fatty acids are broken down into acetyl-CoA, which can be used in the electron transport chain to generate ATP. In SCA3 patients, the level of unsaturated fatty acids were decreased in serum. In addition, polyunsaturated fatty acids (PUFAs) were increased and this increase was accompanied by an increase in SCD, the key enzyme that catalyses the conversion of saturated fatty acids to unsaturated fatty acids [57]. Several mutations in ELOVL's have been identified that are to be the cause of two SCA types. Four different heterozygous mutations in ELOLV4 has been associated with ataxia type 34 (SCA34). The authors suggest that the

mutations in ELOVL4 affect synthesis of VLC-SFA more than VLC-PUFA [38]. Mutations in ELOVL5 has been directly linked to SCA38 [41]. The mutated ELOVL5 was increased in the Golgi apparatus. The authors suggests that misfolded ELOVL5 might accumulate in the Golgi apparatus, leading to an activation of the unfolded protein response (UPR) [41]. Motor deficits and cerebellar atrophy were observed in a EVOVL5 knockout mouse model, with a markedly decreased PC layer. PCs highly express ELOVL5 transcripts and mutations in EVOLV5 showed an alteration of the distribution of the distal dendrites [43]. Very long chain fatty acids play an important role in a wide variety of physiologic function, including peroxisome β -oxidation known to be involved in neurodevelopmental disorders [58]. Peroxisome β -oxidation is essential for the degradation of LCFAs in the brain [59]. ELOVLs are also associated with the ER. Deletion of ELOVL5 in a mouse model showed that sterol regulatory element-binding proteins (SREBPs), located in the ER, was increased. SREBPB is important for cholesterol synthesis. This result suggests that ELOVL5 is involved in cholesterol synthesis. Based on this result, the mutated ELOVL5 in SCA38 might increase cholesterol synthesis and contributes to cholesterol accumulation in the brain.

Alterations in CYP46A1 and ELOVLs show indirect links with cholesterol metabolism and SCA disease progression. However, cholesterol is also directly linked to cerebellar ataxia. It is reported that statins, a HMG-CoA reductase inhibitor which is important for cholesterol synthesis, used to lower cholesterol levels, induced progressive cerebellar ataxia. Withdrawal of the statins showed to decrease ataxia in this patient [60]. This result shows that the cholesterol pathway is key in inducing cerebellar ataxia. Polymorphisms in apolipoprotein E (APOE) contribute to disease in SCA3. APOE is an important protein involved in lipid metabolism and cholesterol transport from astrocytes to neurons to maintain brain cholesterol levels. Polymorphisms in APOE are associated with the age of onset in SCA3, underlying the fact that cholesterol metabolism is directly linked with disease progression[61][62]. Taken together, alterations in cholesterol metabolism might be the common factor in these SCA types.

Oxidative stress and autophagy defects as underlying mechanisms of SCA pathology

Since defective cholesterol metabolism is observed in these SCA types, they might have an underlying mechanism in common which might act as a cause, or a consequence, of altered cholesterol metabolism. In a mouse model with a CYP46A1 knockdown, ABCA1 and NPC2 genes were upregulated, the gene encoding the LDL receptor expression was decreased[17][47]. Decreased CYP46A1 is accompanied with a decrease in glycerolipids and spingolipids. Cholesterol accumulation due to CYP46A1 silencing was accompanied by an increase in long-chain fatty acid residues. Ceramids and glycoseramid species with long chain fatty acids are increased upon cholesterol accumulation [55]. Ceramids and glycoseramids are associated with oxidative stress. Oxidative stress has gained more interest in neurological diseases the past few years. Oxidative stress showed to be an important factor in neuronal death due to an imbalance in oxidants and anti-oxidants, resulting in production of reactive oxygen species (ROS) production, which is already observed for Multiple Sclerosis (MS), a disease where the central nervous system gets affected. Antioxidant levels were decreased in MS and a demyelination was observed in the cerebellum [63]. Decreased levels of antioxidants suggest an imbalance in oxidants and anti-oxidants resulting in oxidative stress. This imbalance might attribute to demyelination in MS. Oxidative stress might also be important in SCA. Oxidative stress has already been associated with Freidreich's ataxia, an ataxia that is caused by an autosomal recessive mutation that results in a reduced expression of frataxin, a mitochondrial protein [64][65]. In Freidreich's ataxia, an imbalance exists in oxidants and oxidant detoxification by anti-oxidants such as glutathione. This imbalance contributes to disease [66]. In SCA 7, an increase in oxidative environment was found and was associated with mutant ataxin-7 aggregation. This was followed by decrease in cell viability [67]. A decreased clearance of ROS was found, contributing to protein aggregation by ROS accumulation. Anti-oxidant treatment showed to rescue the toxicity found in SCA7 [67]. A recent study shows that oxidative stress is increased in SCA7 and correlates with age of onset and disease severity [68]. Also in

SCA3, oxidative stress is associated with disease. Oxidative stress measured in reactive oxygen species (ROS) were found to be increased in SCA3 patients. The activity of antioxidant enzyme GSH-Px was lower in symptomatic SCA3, related to disease severity. This imbalance in ROS production and clearance contributes to disease severity in SCA3 [69]. SCA20, an autosomal recessive SCA, is caused by mutations in the gene encoding Sorting Nexin 14 (SNX14)[70]. SNX proteins localize to endosomal membranes. Mutations in the SNX14 seen in SCA20 patients results in an accumulation of cytoplasmic vacuoles containing electron dense material. These results suggests that also in SCA, oxidative stress might be a contributor to SCA pathology. Defects in ROS clearance due to protein accumulation leads to decreased cell viability. This decrease in cell viability might be responsible for the atrophy seen in SCA patients [70].

In NPC, cholesterol accumulates in late endosomes [55]. Accumulation of misfolded protein aggregates accumulate in cells due to a defect in autophagy that is observed in several SCA subtypes. Defective ATXN proteins are associated with a reduction of autophagy. Defects in autophagy caused by mutant ATXN3 proteins have been linked to the maturation of autophagosomes, where it interacts with early autophagosomal structures and regulates autophagic flux [56]. ATXN3 binds to the autophagy receptors GABARAP and LC3C and is most probably recruited to autophagosomes [56]. Autophagy is important for metabolic regulation and protein degradation. Proteins for degradation are chaperoned by HSC70 to the lysosomal membrane and are transported into the lysosomes by LAMP-2A [71]. Upregulation of autophagy facilitates the degradation of misfolded proteins. In addition, autophagy is also important for lipid accumulation. Autophagy prevents lipid accumulation in the liver, this prevention of lipid accumulation might also be of interest in SCA, where cholesterol accumulation contributes to pathology [71]. Impaired autophagy increases triglycerids and cholesterol [72]. Inhibition of the autophagic pathway confirmed that lysosomes regulate intracellular lipids, including triglycerids and cholesterol content [72].

Since both oxidative/ER stress and impaired autophagy are seen in SCA, an interplay between oxidative stress and autophagy might be considered as the underlying mechanism of SCA. Multiple organelles are involved in autophagy, including the ER, mitochondria and the Golgi apparatus. Normally, oxidative stress causes accumulation of (misfolded) proteins. However, in SCA the mutations cause misfolded proteins. These misfolded proteins aggregate, causing ER stress [24]. In addition, mutant ELOVL4 associated with SCA34 may initiate ER stress [73]. This ER stress in turn contributes to mitochondrial stress, leading to oxidative stress, causing a vicious cycle. Misfolded proteins as seen in SCA induce ER stress [24] and ER stress might alter cholesterol synthesis. SREBPs are important regulators in cholesterol metabolism. Low sterol levels activate SREBPs, which are important players in inducing sterol synthesis. SREBP induces cholesterologenic enzymes such as HMG-CoAR. SREBP associates with the SREBP cleavage-activating protein (SCAP) in the ER membrane and translocates to the golgi apparatus under low-cholesterol conditions. HMG-CoAR is activated, resulting in sterol synthesis [45]. and cholesterol sensing and are activated by mTORC1, a protein kinase important in cell growth [74][75]. mTORC1 is activated under low cholesterol conditions, where it activates SREBPs to induce cholesterol synthesis in the ER. mTORC1 activation is then decreased in a negative feedback loop, when cholesterol levels in the ER rise due to the activation of SREBPs, mTORC1 is inhibited to maintain cholesterol homeostasis [76]. When mTORC1 activity is low, cholesterol rich endosomes are rerouted to lysosomes, forming cholesterol-enriched lysosomes to replenish the cholesterol in the ER. When lysosomal trafficking of cholesterol is impaired, SREBP-2 is activated, leading to an increase in cholesterol synthesis and accumulation without mTORc1 activation (Figure 3) [76]. Normally, low mTORC1 triggers autophagy and endosomal-lysosomal trafficking of cholesterol. This results in cholesterol-rich lysosomes and increases ER cholesterol [76]. However, in SCA both lysosomal trafficking and autophagy are impaired. This impairment of cholesterol metabolism might reduce cholesterol-rich lysosomes, which in turn can activate SREBP for cholesterol synthesis in the ER. When cholesterol turnover is impaired due to defects in CYP46A1, cholesterol accumulates in cells. In addition, the autophagic pathway is directly associated with LC3B-2. Increased CYP46A1 showed an

increase in LC3-B2. Defects in CYP46A1 might decrease LC3B-2 and thereby decrease autophagy, resulting in a defective clearance of ataxin aggregates [30].

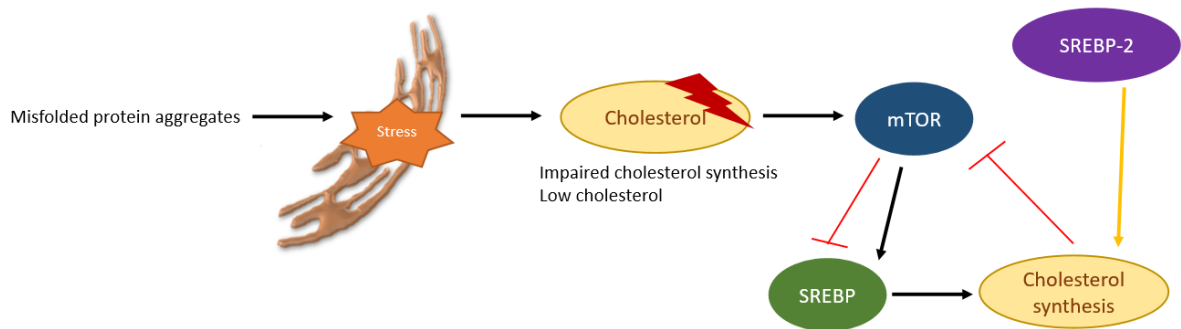


Figure 3. Cholesterol accumulation due to stress and defects in mTOR signalling. Misfolded proteins are able to induce ER stress. The ER stress affects *de novo* cholesterol synthesis, leading to low cholesterol levels. These low cholesterol levels are able to activate mTOR. mTOR activates SREBPs and induces cholesterol synthesis. An increase in cholesterol synthesis than decreases mTOR signalling, SREBP activation and cholesterol synthesis in a negative feedback loop. In case of low mTOR signalling, SREBPs are not activated and cholesterol is not synthesised. When mTOR signalling is defective, SREBP-2 is able to stimulate cholesterol synthesis, independent of mTOR activation.

Conclusions

ADCAs are very complex neurological diseases with several mutations leading to similar disease pathologies. Several underlying mechanisms are identified that contribute to SCA pathology. Here, we determined that cholesterol accumulation in the brain is strongly associated with defects in autophagy and oxidative stress. Oxidative stress has already been identified in Friedreich's ataxia as a mechanism that contributes to disease. However whether it's a primary cause or a secondary effect is yet unclear [65] [77]. In spinocerebellar ataxias, oxidative stress and deregulated autophagy are found to play an important part in disease. Strong evidence suggests that altered autophagosome/lysosomal trafficking contributes to SCA pathology. However the underlying mechanisms is not yet known. A possible underlying mechanism could be the mTORC pathway. The mTORC pathway is able to regulate cholesterol metabolism. However when the mTORC pathway is deregulated, SREBP2 can orchestrate cholesterol metabolism. Together with a decrease of CYP46A1 and autophagosome/lysosomal flux to properly clear cholesterol and protein aggregates from the brain, cholesterol might accumulate as seen in SCA. This in turn leads to disease progression. However whether this is truly the case, and whether these are a the cause or the consequence of mutations have yet to be determined.

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