Emerging roles for cholesterol in Huntington’s disease

Marta Valenza and Elena Cattaneo

Department of Pharmacological Sciences and Centre for Stem Cell Research, Università degli Studi di Milano, Milan, Italy

Recent findings suggest that alterations in cholesterol homeostasis might be associated with Huntington’s disease (HD). Although alterations in cholesterol content have been detected in cell models and several rodent models of HD, it is currently unclear what the potential mechanisms underlying cholesterol perturbations are. Furthermore, it remains to be determined whether these observed changes in cholesterol homeostasis are involved in the pathogenesis of HD or occur as a secondary event. In this review, we provide an overview of current studies that have begun to address these issues and discuss recent findings suggesting that normal huntingtin protein might participate in regulating cholesterol biosynthesis. A better understanding of how alterations in cholesterol levels contribute to the pathophysiology of HD is likely to have significant therapeutic implications for the treatment of this debilitating neurodegenerative disorder.

The importance of cholesterol for brain function

Nearly a quarter of the cholesterol present in humans is found in the brain; it is produced locally because the blood–brain barrier (BBB) effectively prevents its uptake from circulation [1]. The half-life of cholesterol in the brain is extremely long, estimated at 4–6 months in rodents and 5 years in humans [2]. Brain cholesterol plays a role in myelin formation and a large majority (>70% of brain cholesterol mass) is present in myelin sheaths [3]. Indeed, the rate of cholesterol synthesis decreases sharply after myelination [3], which suggests that most of the cholesterol produced by oligodendrocytes during development is used to build myelin scaffolding.

Cholesterol is also a structural component of glial and neuronal membranes and by reducing membrane fluidity it contributes to define the membrane properties [4]. In neuronal membranes, cholesterol is concentrated in detergent-resistant microdomains or lipid rafts [5]. These rafts define the functional properties of ion channels, neurotrophic factors and transmitter receptors, as well as their localization within specific membrane sites, thus creating a platform for the initiation, propagation and maintenance of signal transduction events [6]. Finally, cholesterol plays a role in promoting neurite outgrowth [6] and synaptogenesis [7–10] and in maintaining the integrity of synapses and in neurotransmitter release [11,12] (Figure 1). In addition to these brain-specific activities, cholesterol serves as an obligatory precursor for the production of steroid hormones (Figure 1) [13]. Steroids made locally in the brain are called neurosteroids and are multifunctional lipids that play roles in regulating receptor activity, myelination, neuroprotection and neurite growth [13]. Cholesterol synthesis also generates intermediate products, such as isoprenoids (important precursors for molecules involved in cell signaling and inflammatory responses [14]) and ubiquinone (coenzyme Q10, a component of the electron transport chain involved in generating energy in the form of ATP) (Figure 1).

The relevance of cholesterol synthesis in mammals is highlighted by the fact that breakdown due to genetic defects in its effectors or pharmacological interference causes severe developmental abnormalities and intellectual disabilities [13]. One of these disorders is Smith–Lemli–Opitz syndrome (SLOS), an autosomal recessive syndrome caused by mutations in the gene coding for the enzyme 7-dehydrocholesterol reductase (7DHCR). This enzyme is responsible for reducing the Δ7 double bond in 7-dehydrocholesterol (7DHC) to yield cholesterol in the final step of cholesterol synthesis (Figure 1). Both cholesterol deficiency and the toxic effects of elevated 7DHC levels contribute to the SLOS phenotype [15], a spectrum that ranges from a mild disorder with behavioral and learning problems to a lethal malformation syndrome [16]. Perturbed signaling of Sonic hedgehog (SHH), a cholesterol-binding morphogen [17] during embryonic patterning, might also underlie some of the developmental malformations found in severe cases of SLOS and other disorders of cholesterol biosynthesis [18].

Abnormalities in brain cholesterol content have also been observed in a number of common neurodegenerative disorders. In this review, we discuss recent findings suggesting that perturbations in cholesterol homeostasis and cholesterol levels might be associated with Huntington’s disease (HD). We also discuss possible mechanisms that might underlie these alterations and whether they are involved in the pathogenesis of HD. Furthermore, we briefly mention two other neurodegenerative diseases, Alzheimer’s disease (AD) and Parkinson’s disease (PD), for which evidence of alterations in cholesterol levels has recently been suggested.

Cholesterol biosynthesis and cholesterol content in HD rodent models and HD patients

HD is an inherited, autosomal dominant neurodegenerative disorder characterized by motor and psychiatric disturbances, as well as dementia. It is caused by a CAG
repeat expansion in the gene coding for huntingtin (HTT). Widespread expression of mutant HTT throughout the brain is observed, although neurons in the striatum and cortex seem to be selectively vulnerable. Pathological pathways and mechanisms underlying neurodegeneration in HD are beginning to be unraveled. Previous reviews have summarized the various different cellular and synaptic disturbances observed in HD [19,20] and in this review we restrict our discussion to findings suggesting that cholesterol alterations might be associated with HD.

The possibility that changes in brain cholesterol homeostasis occur in HD has attracted increasing attention over the past several years [21]. A few studies conducted in the 1970s and 1980s (before the discovery of the HD gene) first proposed a lipid imbalance in primary fibroblasts taken from HD patients [22,23], although others did not confirm this finding [24]. A subsequent microarray study performed in 2002, using immortalized cell lines from fetal rodent striatum with inducible expression of the N-terminal fragment of mutant HTT, found that expression of mutant HTT [25]. Further studies demonstrated transcriptional deregulation of cholesterogenic genes in the striatum and..
The current findings on changes in brain cholesterol content emerged from an independent study using the YAC72 HD mouse model, in which filipin staining and thin layer chromatography (TLC) revealed cholesterol accumulation in mutant compared to wild-type mouse brain. An increase in cholesterol content, as measured by an enzyme method based on cholesterol esterase and oxidase, was found in brain of HD HdhQ111 knock-in mice (Table 1) [28]. However, a further study that used mass spectrometry to assess absolute levels of cholesterol and its metabolites in brain revealed the opposite result, with a decrease in cholesterol content in YAC72 mice, HdhQ111 knock-in mice and other HD animal models (Table 1) [29].

Besides changes in total cholesterol content (albeit with discrepancies among studies), reduced cholesterol biosynthesis has also been observed in the presence of the HD mutation. This is implied by the demonstration of reduced activity of the synthetic enzyme hydroxy-methylglutaryl-coenzyme A reductase (HMGC-CoAR) [30,31] and by reduced levels of cholesterol precursors (such as lanosterol and lathosterol; Figure 1) in adult brain from four different HD rodent models (R6/2 mice, YAC mice, HdhQ111 knock-in mice and transgenic HD rats) (Table 1) [29,31]. Notably, these molecular abnormalities worsened in YAC mice carrying CAG repeats of progressively increasing length in the HTT protein [29], whereas mice overexpressing normal HTT (the YAC18 model) displayed increased HMGC-CoAR activity and increased levels of cholesterol precursors and cholesterol in brain [29,31]. The occurrence of this dysfunction across multiple HD animal models that recapitulate different aspects of the chronic human disease process reinforces its potential relevance to human pathology. In addition, in R6/2 and YAC128 HD mouse models, HMGC-CoAR activity and cholesterol precursors in brain were significantly reduced before the onset of motor and cognitive symptoms, whereas changes (reductions in this study) in cholesterol content were detected only in advanced symptomatic stages [29–31]. This evidence suggests that decreased cholesterol biosynthesis, in terms of HMGC-CoAR activity and cholesterol precursor levels, might be involved in HD pathogenesis.

The current findings on changes in brain cholesterol-related genes, cholesterol metabolites and cholesterol itself...
Cholesterol synthesis and degradation in the brain are balanced through the oxidation of cholesterol to 24S-hydroxycholesterol (24OHC), which is catalyzed by CYP46A1. CYP46A1 is considered to be a neuron-specific enzyme that is particularly prevalent in the striatum and cortex [127]. Unlike cholesterol, 24OHC can cross the blood–brain barrier and enter the circulation [128]. 24OHC efflux is believed to be the main mechanism by which the brain removes daily excesses of cholesterol [129]; therefore, plasma 24OHC levels are considered an index of brain cholesterol elimination (and, indirectly, of cholesterol biosynthesis).

With respect to HD, lower brain and plasma 24OHC levels were observed in several rodent models of the disease compared with control mice [29,31]. Similarly, plasma 24OHC levels were substantially lower in HD patients compared with healthy subjects [32,36]. Intriguingly, a study conducted in a small cohort of pre-manifest HD patients revealed that subjects who were closer to disease onset exhibited lower 24OHC levels compared to healthy subjects and pre-manifest HD subjects who were further from disease onset [32]. Further studies to evaluate whether peripheral 24OHC levels might be a reliable and valid biomarker for the early detection of disease onset in HD are necessary [32].

Potential mechanisms underlying cholesterol alterations in HD

Sterol regulatory element binding proteins (SREBPs) as global lipid regulators and more

Nearly all genes involved in cholesterol biosynthesis are transcriptionally regulated by SREBPs, a subfamily of basic helix–loop–helix leucine zipper (bHLH-LZ) transcription factors that are conserved from fungi to humans [38]. In humans, the family consists of three different SREBP proteins: SREBP1A, SREBP1C and SREBP2. The SREBPs are synthesized as large, transcriptionally inactive precursors and are localized in the endoplasmic reticulum (ER) with two membrane-spanning domains. When cholesterol levels are low, the SREBPs translocate from the ER to the Golgi apparatus after activation of SREBP cleavage activation protein (SCAP), a process that is blocked under cholesterol excess by insulin-induced gene 1 (INSIG1) and INSIG2 proteins [39,40]. Once in the Golgi, the SREBPs are cleaved by two membrane-associated proteases, the site 1 (S1P) and site 2 (S2P) proteases, which yields an active 68-kDa N-terminal fragment that translocates to the nucleus, binds the sterol response element (SRE) in the promoters of target cholesterogenic genes (Figure 2) and activates their transcription. Binding sites for nuclear transcription factor Y (NFY), specificity protein 1 (SP1) and cAMP response-element-binding protein (CREB), all common transcriptional coactivators for a variety of genes, are usually found in the sequence adjacent to the SRE. Accordingly, NFY, SP1 and CREB participate with SREBPs in activating SRE-dependent gene transcription [41–43]. Notably, mice lacking CREB in forebrain exhibit greatly reduced brain cholesterol biosynthesis [44]. In addition, CREB-binding protein (CBP), another ubiquitous factor involved in transcriptional coactivation of many different transcription factors in several metabolic tissues [45], can be recruited by SREBPs to maximize SRE-dependent gene transcription [46].

There might be SREBP functions beyond their involvement in sterol metabolism. Genome-wide and model organism approaches indicate that SREBPs coordinate cellular lipid metabolism with other cellular physiological processes that are broadly related to each other, such as cellular adaptations to environmental changes ranging
from nutrient fluctuations to toxin exposure [38]. In mammalian cells, SREBP can also be cleaved during apoptosis by caspase-3 and caspase-7, and the domains released by caspase cleavage can activate a reporter gene under control of a synthetic SREBP target promoter [47]. However, the physiological significance of SREBP cleavage during apoptosis is currently unknown.

Brain SREBP expression has been only partly described. Srebp1 mRNA levels increase with age in mouse brain [48], whereas Srebp2 mRNA and protein levels are high during active myelination but are downregulated thereafter [49]. In 5-week-old rats, the active nuclear form of SREBP2 is highly expressed in the hippocampus, cerebral cortex and striatum [50]. However, much remains to be investigated regarding SREBPs in different brain regions and cells under both physiological and pathological conditions.

SREBP activity was reduced in an inducible cell model of HD and in brain from a HD mouse model [26]; in another study that used a high-throughput yeast two-hybrid screen, SREBP2 interacted with HTT fragments [51]. Intriguingly, mutant HTT interacted with and negatively regulated the function of GP78 [52], an ER membrane-anchored ubiquitin ligase (E3) required for ubiquitination and degradation of INSIG1 in sterol-depleted cells [53]. One possibility is that cholesterol may prevent competition between GP78 and SCAP for INSIG1 binding, and inhibits INSIG degradation via the ubiquitin–proteasome system (grey cylinder) and hence blocks SREBP activation. In feedback regulation of cholesterol biosynthesis, GP78 is also responsible for ubiquitination and degradation of HMGCoAR (yellow) under sterol-loaded conditions [53]. Mutant HTT interacts with and negatively regulates the function of GP78 [52] and thus probably contributes to a decrease in INSIG1 and HMGCoAR degradation under different sterol conditions.

Can perturbations in cholesterol synthesis damage HD neurons?
Neurons have a high cholesterol demand for the formation and maintenance of axons, dendrites and synaptic connections [10], but are mostly dependent on cholesterol
supplied by astrocytes [8,54–56]. Notably, most synapses in the developing brain are formed after astrocyte differentiation [57] and neurons exhibited very low levels of lanosterol-converting enzymes and produced cholesterol less efficiently compared to glia [58]. Thus, it is likely that glia and neurons undergo different metabolic specializations in vivo, which results in specific cholesterol biosynthesis and homeostasis functions.

When considering the HD condition, immortalized striatal cell lines with inducible expression of the first 548 amino acids of human mutant HTT showed a progressive reduction in total sterol content after 96- and 120-h exposure to delipidated medium compared to non-induced cells [26], whereas addition of increasing cholesterol concentrations (from 0.3 to 10 μM) promoted cell survival in a dose-dependent manner in primary rat striatal neurons undergoing cell death after transfection of the N terminus of mutant HTT [26]. Conversely, in striatal primary neurons in which the first 171 amino acids of human mutant HTT containing a stretch of 82Q (Htt171-82Q) were expressed by lentiviral transduction, sterols accumulated and contributed to higher levels of cell death compared to neurons overexpressing non-pathological human HTT (Htt171-18Q) [59]. Indeed, in this case, genetic and pharmacological reduction of sterol biosynthesis led to increased neuroprotection in Htt171-82Q-expressing neuronal cultures, which suggests that strategies aimed at decreasing sterol accumulation in HD might curtail mutant HTT toxicity [59], although this result is debated [60,61]. In agreement with findings described in [59], accumulation and impaired distribution of cholesterol were observed in other immortalized striatal cells and primary striatal neurons from homozygous HdhQ111 knock-in mice compared to cell lines and neurons from HdhQ7/7 knock-in mice [28]. These changes were associated with increases in highly ordered domains in plasma membrane and in NMDA-medicated excitotoxicity [28]. The cholesterol-lowering drug simvastatin restored lipid balance and protected both wild-type and HD cells against excitotoxicity, although this treatment had no effect on total cholesterol content in both cell types [28]. In the same study, accumulation of caveolin-1 (CAV1), a cholesterol-binding integral membrane protein implicated in cholesterol transport and homeostasis [13], was also found in immortalized striatal cells and in primary striatal neurons from HdhQ111 knock-in mice compared to controls [28], which suggests that such accumulation, along with cholesterol accumulation, might increase highly ordered domains in the plasma membrane [28]. Impaired intracellular trafficking of cholesterol was also observed in primary striatal neurons from a YAC72 HD mouse model [27]. This process was associated with inhibition of endocytosis through a pathway involving CAV1 [27], although in this study (unlike results in [28]), protein levels of CAV1 were similar in primary striatal neurons and brain tissue from YAC72 and control mice.

The reasons for opposing results with regard to cholesterol alterations in various different studies are currently unknown. However, the sterol accumulation observed in these particular studies and models [28,59] suggests that the neuronal cytochrome P450, family 46, subfamily A, polypeptide 1 (CYP46A1) enzyme (Box 1) might be less able to process excess cholesterol in HD neurons. Alternatively, cholesterol accumulation might be the consequence of an impaired autophagic process. Autophagy regulates lipid metabolism [62] and altered recognition of autophagic cargo (i.e. altered organelles) has been demonstrated in several HD cells (embryonic fibroblasts, immortalized striatal cells and primary striatal neurons from homozygous HdhQ111 knock-in mice compared with HdhQ18 knock-in mice and in lymphoblasts from two HD patients), which led to a marked increase in the content of lipid droplets, cellular organelles whose task is to store triglycerides and esters of cholesterol [63] (Figure 3). Such defects might be even more evident in neurons, because they probably have

Figure 3. Possible links between autophagy and lipid metabolism in HD. Lipid droplets are cellular organelles that specialize in the storage of neutral lipids, such as sterol esters and triglycerides. Thus, they provide the main cellular reservoir for lipid energy storage and membrane synthesis, and protect cells from the lipotoxic effects of nonesterified lipids. Autophagy also regulates lipid metabolism by removing intracellular lipid droplets [62]. Under conditions of nutrient deprivation, lipid droplets and autophagic components associate to promote lipid hydrolysis and free fatty acid generation by releasing the contents of lipid droplets to lysosomes for degradation. In different HD cell models (striatal cells and primary neurons from HdhQ111 knock-in mice and in primary lymphoblasts from HD patients) autophagosomes show reduced ability to recognize organelles, which leads to accumulation of lipid droplets and altered mitochondria [63] in the presence of mutant HTT (Red). However, it is not known whether this increase in lipid droplets results in an increase in cholesterol accumulation in different HD cell models (as shown by other studies).
low or no capacity to store lipid droplets under physiological conditions, because most of the cholesterol in brain is nonesterified and is predominantly localized in myelin and plasma membranes [64]. At present it is not possible to establish a direct link between cholesterol accumulation and increased lipid droplets in HD cells and tissues in the different studies. However, the evidence that 24OHC levels (Box 1) are decreased in brain and plasma from HD rodent models and in blood from patients [29,31,32] suggests that there is generally less cholesterol available for catabolism in the HD brain.

Consequences of changes in membrane cholesterol levels in HD
In plasma membranes, cholesterol is concentrated in lipid rafts that define the functional properties of membrane-resident proteins, including ion channels and receptors, which creates a platform for the initiation, propagation and maintenance of signal transduction events. It has been demonstrated that HTT is associated with lipid rafts obtained from mouse neurons and this association was stronger for mutant than for wild-type HTT [65]. Changes (either increases or decreases) in membrane cholesterol might influence the organization and properties of lipid rafts and their interaction with mutant HTT and could thus contribute to impaired neuronal signaling in HD [19,66]. For example, a relationship between BDNF signaling in neurons and cholesterol metabolism has been proposed. BDNF stimulates the transcriptional activation of cholesterol synthesis enzymes in cultured neurons and increases the cholesterol content in lipid rafts [67]. In addition, BDNF-dependent cholesterol biosynthesis plays an important role in the development of a readily releasable pool of presynaptic vesicles [67]. BDNF controls cannabinoid receptor 1 (CNR1) activity in striatum, mainly by regulating cholesterol metabolism and membrane lipid raft function [68]. BDNF levels [69] and CNR1 activity are both reduced in HD [70,71], which suggests a potential interconnection between dysfunctions involving BDNF, cholesterol and synaptic transmission alterations in HD.

Relevance of cholesterol efflux in crosstalk between neurons and astrocytes in HD
Cholesterol supply from astrocytes is important for synaptogenesis and synapse maturation and maintenance [72,73]. Among the proteins involved in cholesterol efflux from astrocytes, ATP-binding cassette transporter 1 (ABCA1) plays a crucial role in facilitating cholesterol efflux via apolipoprotein E (APOE), which provides neurons with the cholesterol required for their functions (Figure 4) [13]. mRNA levels of cholesterol biosynthesis genes were reduced in primary astrocytes cultured from two HD mouse models (R6/2 and YAC128) compared to control littermates [29]. Primary astrocytes from YAC128 mice also displayed reduced mRNA levels of Abca1, ATP-binding cassette sub-family G member 4 (another ATP-binding cassette transporter highly expressed in the brain) and Apoe compared with controls, which led to reduced production and secretion of APOE (Figure 4) [29]. However, cholesterol content in HD astrocytes was similar to that in controls [29], which suggests that cholesterol biosynthesis and efflux genes in HD astrocytes are in equilibrium to maintain a constant intracellular cholesterol level. Furthermore, APOE was predominantly associated with smaller lipoprotein particles in the cerebrospinal fluid of HD mice, which indicates that reduced cholesterol transport occurs on APOE-containing lipoproteins circulating in HD brain [29]. Taken together, these findings suggest that reduced cholesterol biosynthesis and efflux occur in HD astrocytes, along with reduced transport in the HD brain [29].

The expression of Abca1 and ApoE (and other lipid metabolism genes) is induced by liver X receptors (LXRs) [74]. LXRs are ligand-activated transcription factors that serve as cellular cholesterol sensors and are activated by oxysterols and some cholesterol precursors (Figure 4) [75,76]. Although it is possible to speculate that the reduced levels of brain cholesterol precursors (due to reduced mRNA levels of cholesterologenic genes) and 24OHC observed in some studies [29,31] might lead to reduced LXR activation and affect LXR-mediated transcription of ABCA1 and APOE, and thus ultimately contribute to reduced cholesterol biosynthesis, the temporal nature of these changes observed in HD remains unknown. Thus, it is also possible that primary changes in LXR activity in HD cells might result in increased cholesterol content (as observed in other studies) and, as a negative feedback mechanism resulting from cholesterol accumulation, to a decrease in mRNA levels of cholesterologenic genes.

Is myelin formation impaired in HD?
Other types of cells besides astrocytes might contribute to the cholesterol alterations observed in HD. Oligodendrocytes produce myelin in the brain, a process that is highly dependent on cholesterol biosynthesis (Figure 4). Expression of SREBP2 and cholesterologenic enzymes is high during active myelination and is downregulated soon after [49]. Accordingly, mice that are unable to synthesize cholesterol in oligodendrocytes develop ataxia and tremor [77] and high cholesterol levels are required for correct myelin compaction [78]. In addition, deletion of SCAP in Schwann cells, the glia cells that form myelin sheaths in the peripheral nervous system, led to a loss of SREBP-mediated gene expression and hypomyelination in mutant mice, which suggests that SCAP is required for timely and proper myelin membrane synthesis [79].

In one HD mouse model (R6/2), atrophy and degeneration of the sciatic nerve were observed in a pre-symptomatic stage [80] and myelin isolated from brain of this model showed low sterol content in symptomatic stages [29]. Decreased expression of myelin basic protein (MBP), a major constituent of myelin sheaths, and deficient myelination were recently found in postnatal and adult R6/2 mice [81]. Electron microscopy also revealed thinner myelin sheaths and increased myelin periodicity in another HD mouse model expressing full-length human mutant HTT with 97 glutamine repeats under the control of an endogenous regulatory region in a bacterial artificial chromosome (BAC; BACHD mice) [81]. This study also found that peroxisome-proliferator-activated receptor γ coactivator 1α (PGC1α), a transcription factor involved in regulating
energy metabolism and that is reduced in HD cell and animal models and in HD patients [82], affected the expression of MBP and cholesterol synthesis, whereas oligodendrocytes carrying mutant HTT showed decreased expression levels of PGC1α and of its targets, MBP, HMGCoA-synthase and HMGCoAR [81].

Although the exact trigger that initiates myelin breakdown remains unknown, current evidence suggests that it is more likely to be an early event in HD rather than occurring in the later stages of disease progression. Imaging and biochemical studies have revealed progressive loss of white matter in HD patients 10 years before the onset of
overt cognitive and motor symptoms, as defined using the UHDRS scale [83]. If cholesterol dysfunction plays a role in HD pathogenesis and the greatest cholesterol production in brain is during development, why does the onset of HD occur so late in life? Myelination is considered a key developmental phase in the human brain. Many years are required before the structural maturation of neural pathways is complete and the process continues into adolescence [84]. One possibility is that alterations in cholesterol biosynthesis in HD lead to slower myelination of some specific brain structures that might not be detectable at an early age. Slower myelination might be partly masked by compensatory mechanisms for years, and thus would only affect the stability and activity of neuronal networks in later HD stages. However, it remains to be determined how alterations in myelination might contribute to neuronal dysfunction and subsequent neurodegeneration. Many other changes are known to occur in HD, besides cholesterol changes, and the reasons why neural functions deteriorate only in later stages of life remain a central question.

**Does HTT play a physiological role in cholesterol biosynthesis and/or trafficking?**

It is curious that YAC18 mice overexpressing normal HTT display increased HMGCGR activity and increased levels of cholesterol precursors and cholesterol in brain [31]. In addition, heterozygous knock-in mice that express one copy of the expanded polyQ repeat region within the *Htt* gene (*Hdh*Q111/111) exhibit intermediate levels of brain cholesterol (Table 1) and cholesterol precursors compared with littermates and homozygous *Hdh*Q111/111 knock-in mice [29]. These results suggest that, at least in adult brain, levels of HTT protein might influence cholesterol biosynthesis and/or overall levels of cholesterol.

Observations have also revealed that HTT plays a role in regulating LXR activity. LXR-mediated transcription was activated in human embryonic kidney (HEK) cells overexpressing HTT, and primary astrocytes from mice overexpressing normal HTT (YAC18) showed increased mRNA levels of *Abca1* and *Apoe* compared to primary astrocytes from littermate mice and YAC128 HD mice [29]. Notably, the expression of LXR-regulated genes was reduced in embryonic stem cells from *Htt* knockout mice and after knockdown of *htt* in developing zebrafish [85]. Moreover, *htt* knockdown in zebrafish resulted in impaired neuronal development (caused by reduced BDNF levels) [86] and severe reduction of cartilage in the lower jaw (probably because of a HTT role in neural crest cell differentiation) [87]. Notably, fish mutant for the SREBP-processing protease SIP also displayed defects in cartilage formation, which suggests that both SIP and HTT are required for cartilage development in zebrafish [88]. An LXR agonist increased the expression of LXR target genes and partially rescued the cartilage-loss phenotype during early development in HTT-deficient fish [85]. Although this study did not investigate the effect of LXR agonists on neuronal development, the observations open new questions regarding whether and how HTT participates in regulating cholesterol biosynthesis and efflux in different tissues, and to what extent mutant HTT might fulfill these functions during early stages of human life.

Other studies have also shown functional roles for HTT during embryonic development [19]. *Htt* homozygous knockout mice die by embryonic day 7.5–8.5 [90,91], which demonstrates that HTT is necessary for embryogenesis. The demand for cholesterol is particularly high during this stage of embryogenesis [92] and mice lacking SREBP2 die by embryonic day 8 [93]. Furthermore, mice lacking squalone synthase, a key enzyme in cholesterol biosynthesis, exhibit embryonic lethality and defective neural tube closure [94]. Although these separate observations do not causally link HTT function to cholesterol biosynthesis early in development, this is an area that deserves further investigation and studies.

SHH and related proteins are essential not only for neural tube patterning during brain formation, but also for controlling axon growth and neurogenesis in postnatal life and adulthood [95]. These proteins undergo post-translational modification by covalent attachment of a cholesterol molecule to their biologically active amino-terminal fragment [96] and responses to SHH signals are compromised in cells from mouse models of SLOS and in normal cells pharmacologically depleted of sterols [18]. In addition, it has been shown that huntingtin-interacting protein 1 (HIP1)–an endocytic protein highly enriched in the brain that interacts with HTT [97]–regulates, together with HIP1 protein interactor (HIPPI), apoptosis and gene transcription, processes that are both implicated in HD [98]. Curiously, HIPPI, whose function is also altered in HD [99], was identified as an essential component of the SHH pathway [100]. Hippi−/− mouse embryos exhibited downregulation of the SHH pathway in the neural tube and failed to establish neural cell fate [100]. Defects in SHH signaling during development have not yet been reported in HD models, but given the observations outlined above, it is a likely possibility.

In addition to possible roles for HTT in cholesterol biosynthesis, an emerging body of evidence indicates an important role for normal HTT in vesicular trafficking and endocytosis [89]. However, there is currently no evidence to suggest that normal HTT plays a role in cholesterol trafficking, although altered intracellularity of cholesterol has been observed in neurons from two different HD models [27,28].

**Cholesterol and other neurodegenerative diseases**

Cholesterol abnormalities have also been highlighted in other neurodegenerative disorders, such as Alzheimer’s disease (AD) and Parkinson’s disease (PD). A main characteristic of AD is accumulation of amyloid β (Aβ) peptide, which is generated by sequential cleavage of the amyloid precursor protein (APP) by γ- and β-secretases. A link between cholesterol and AD pathogenesis originally stemmed from the finding that inheritance of the ApoEε4 allele is an established risk factor, along with aging, for late-onset forms of AD [101]. Statins (well-known HMGCaR inhibitors) can reduce Aβ formation *in vitro* [102] and in some AD mouse models [103–105], although another study revealed that genetic reduction of total cholesterol synthesis in the brain did not affect amyloid formation in transgenic mice overexpressing mutant presenilin-1 and the human mutant APP Swedish isoform [106].
In an attempt to establish a causal link between cholesterol and AD, sterols were measured in AD samples from both patients and mouse models. Levels of cholesterol and its precursors in brain homogenates from a transgenic AD model were similar to those in control mice [107]. However, lower cerebrospinal fluid and plasma levels of cholesterol and its precursors have been observed in AD patients compared to control subjects, which suggests that de novo cholesterol synthesis within the brain of AD patients might be reduced [108]. In line with this interpretation, forebrain neuronal deletion of low-density lipoprotein receptor-related protein 1 (LRP1), the major brain lipoprotein-APOE receptor, led to reduced brain cholesterol and progressive age-dependent synapse loss and neurodegeneration resembling AD [12]. Such in vivo studies have raised doubts about the beneficial effect of cholesterol-lowering agents as a treatment for AD, although it should be noted that a number of epidemiological studies support the hypothesis that statin treatment is associated with reduced AD prevalence [109,110]. It is possible that statins might be neuroprotective through their pleiotropic anti-inflammatory effects. Further studies are needed to clarify the actions of statins in AD and to identify the cellular and molecular mechanisms that link cholesterol and Aβ generation in AD [111–113].

A potential connection between cholesterol and PD has emerged more recently. PD is characterized by accumulation of α-synuclein in Lewy body inclusions in vulnerable neurons, principally dopaminergic neurons of the substantia nigra. Conflicting results have been obtained in retrospective studies in which high dietary intake of cholesterol increased [114,115] or decreased PD risk [116–118]. Epidemiological studies have also produced conflicting results: some support statin treatment for PD [110,119] whereas others do not [120,121]. Similarly to AD, statins reduced neuronal α-synuclein aggregation in vitro in cell models of PD [122] and in vivo [123], and prevented loss of dopaminergic neurons in transgenic mouse models of PD [124]. Statins also reduced the severity of involuntary movements in a 6-hydroxydopamine (6-OHDA) rat model of PD [125]. More recently, lipidomics analysis of select post-mortem PD brain tissues (i.e. anterior cingulate cortex, visual cortex and amygdala) revealed general lipid alterations in PD compared to controls [126]. In particular, cholesterol and 24OHC were increased in the visual cortex of PD patients, but there were no significant increases in several cholesterol precursors [126]. No other studies investigating cholesterol content and biosynthesis in brains from animal models or from PD patients have been performed and the molecular mechanisms involving cholesterol in PD remain poorly understood.

Conclusions

Much remains to be understood about how brain cells cope with cholesterol under various conditions, including neurodegenerative disorders. Genetic and pharmacological reduction of cholesterol synthesis has devastating effects on developing neurons, both in vivo and in vitro. Depending on the timing, these effects include neural tube closure defects, and abnormal synaptic maturation and maintenance. In the adult brain, changes in cholesterol content and/or cholesterol biosynthesis might affect brain dysfunction and contribute to neurodegeneration in several neurodegenerative disorders.

Although additional research is needed, molecular and biochemical studies in cellular and animal models of HD and in tissue and fluid samples from HD patients indicate that cholesterol biosynthesis is affected in HD. Data from multiple rodent models support the hypothesis that reduced activity of the cholesterol biosynthetic pathway and lower brain cholesterol levels are associated with HD, which suggests that enhancement of brain cholesterol biosynthesis and/or availability might ameliorate aspects of this disease. However, other studies have found opposite findings, namely that higher cholesterol levels are associated with HD and that reducing cholesterol levels with statins and other cholesterol-lowering drugs could be beneficial for HD treatment. Clearly, further studies are needed to resolve these differences and to establish the underlying molecular and cellular mechanisms that contribute to alterations in cholesterol-related pathways in the HD brain. Furthermore, the question of whether changes in cholesterol homeostasis are involved in HD pathogenesis or occur as a secondary event remains to be definitively addressed. Although many questions remain to be answered (Box 2), a better understanding of cholesterol dysregulation in HD and how to achieve selective modulation of enzymes and proteins important in cholesterol biosynthesis and trafficking in neurons will probably offer novel options for the treatment of this devastating and fatal disorder.

Acknowledgements

Our work described in this review was supported by grants from the Hereditary Disease Foundation (HDF; USA); the Cure Huntington’s Disease Initiative (CHDI; USA), the Huntington’s Disease Society of America (HDSA; USA); NeuroNE (FP6, EU), Stem-HD (FP6, EU), Telethon (Italy), Fondazione Càrpiolo (Italy), Ministero della Salute (Italy) and Ministero dell’Università e della Ricerca Scientific (Italy) to E.C., and by Fondazione Càrpiolo (Italy) and Ministero della Salute (Italy) grants to M.V.

References


Box 2. Outstanding questions

- Are cholesterol levels reduced in HD or does cholesterol aberrantly accumulate in HD neurons?
- Are cholesterol levels differentially affected in different cells and at different time-points of disease progression? Could such variables partly explain why conflicting results have been obtained between different studies investigating HD and cholesterol alterations?
- Is altered cholesterol biosynthesis in HD a primary or secondary event? Is SREBP activity affected in HD?
- Will strategies aimed at normalizing brain cholesterol content in HD be sufficient to protect debilitated neurons?
- Will it be possible to selectively manipulate brain cholesterol levels for therapeutic purposes without adversely affecting serum cholesterol levels to avoid detrimental side effects, such as atherosclerosis?
- Does HTT have a physiological role in cholesterol biosynthesis and/or trafficking of cholesterol?


Yang, T. et al. (2002) Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell* 110, 489–500


Olinger, J.D. et al. (1996) SREBP transcriptional activity is mediated through an interaction with the CREB-binding protein. *Genes Dev.* 10, 2903–2911


Okamoto, K. et al. (2006) Sterol regulatory element binding protein (SREBP)-1 expression in brain is affected by age but not by hormones or metabolic changes. *Brain Res.* 1081, 19–27

Leblanc, S.E. et al. (2005) Regulation of cholesterol/lipid biosynthetic genes by Egr2/Krox20 during peripheral nerve myelination. *J. Neurochem.* 93, 737–748


Yang, H. et al. (2010) Huntington interacts with the cue domain of gp78 and inhibits gp76 binding to ubiquitin and p97/NCP. *PLoS ONE* 5, e99053


Diekmann, H. et al. (2009) Decreased BDNF levels are a major contributor to the embryonic phenotype of huntingtin knockdown zebrafish. "J. Neurosci." 29, 13435–13449


Palma, V. et al. (2005) Sonic hedgehog controls stem cell behavior in the postnatal and adult brain. "Development" 132, 335–344


