Biochemical and Biophysical Research Communications 446 (2014) 697–701

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Review

Study of cholesterol metabolism in Huntington's disease

Valerio Leoni *, Claudio Caccia

Laboratory of Clinical Pathology and Medical Genetics, Foundation IRCCS Institute of Neurology Carlo Besta, Milano, Italy

ARTICLE INFO

Article history:
Available online 10 February 2014

Keywords:
Oxysterols
Neurodegeneration
Mass spectrometry
Lipids
Biomarker

ABSTRACT

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by an abnormal expansion of a CAG repeat in the huntingtin gene. Neurodegeneration of striatum and cortex with a severe atrophy at MRI are common findings in HD.

The expression of genes involved in the cholesterol biosynthetic pathway such as HMG-CoA reductase and the levels of cholesterol, lanosterol, lathosterol and 24S-hydroxycholesterol are reduced in the brain, striatum and cortex in several HD mouse models. Mutant huntingtin affects the maturation and translocation of SREBP and cannot up-regulate LXR. There is a lower synthesis and transport of cholesterol from astrocytes to neurons via ApoE. In primary oligodendrocytes, mutant huntingtin inhibits the regulatory effect of PGC1α on cholesterol metabolism and the expression of Myelin Basic Protein. In humans the decrease of plasma 24S-hydroxycholesterol follows disease progression proportionally to motor and neuropsychiatric dysfunctions and MRI brain atrophy. Huntingtin seems to play a regulatory role in lipid metabolism. Dysregulation of PGC1α and mitochondrial dysfunction may reduce synthesis of Acetyl-CoA and ATP contributing to the cerebral and whole body impairment of cholesterol metabolism.

Contents

1. Brain cholesterol metabolism

Brain cholesterol represents about 25% of the total body cholesterol and is distributed between myelin (70%), glial cells (20%) and neurons (10%), mainly in unesterified form (>99.5%). It is a structural element of cellular membranes and it is involved in the formation, turnover and stabilisation of synapses, as well as outgrowth of dendrites, vesicle transport and exocytosis at synaptic levels [1,2]. It is organised in microdomains named lipid rafts and involved in the three-dimensional conformation of membrane proteins. Local de novo synthesis covers >95% of requirements [3].

Cholesterol synthesis is an endoplasmic reticulum (ER) multistep pathway starting with condensation of acetyl-coenzymeA (CoA), proceeding to the formation of mevalonate and then further to lanosterol, the first cyclic sterol. The tissue levels of intermediate sterols (lanosterol, lathosterol and desmosterol) reflect the rate of cholesterol synthesis [4]. The expression of hydroxy-methyl-glutaryl-Coenzyme A reductase (HMGCR), the rate limiting enzyme of the pathway, is regulated by feedback inhibition via the sterol-regulated element binding protein (SREBP) that binds to the sterol-regulated element (SRE-1) in the HMGCR gene [5]. In the adult brain, for an efficient formation and maintenance of synapses, neurons totally relay on the cholesterol synthesised by astrocytes and delivered via Apolipoprotein E (ApoE), the main lipid transporter in the central nervous system (CNS) (the so called "outsourcing hypothesis" of cholesterol metabolism) [2]. The cholesterol synthesised in astrocytes is loaded on ApoE-particles by the ATP

* Corresponding author. Address: Laboratory of Clinical Pathology and Medical Genetics, R17, Foundation IRCCS Institute of Neurology Carlo Besta, Via Celoria 11, 20133 Milano (MI), Italy.
E-mail address: valerioleoni@hotmail.com (V. Leoni).

http://dx.doi.org/10.1016/j.bbrc.2014.01.188
0006-291X/

binding cassette (ABC) transporter A1 (ABC-A1) [6]. Once internalised in neurons, the ApoE-cholesterol-LDL-receptor (LDLR) complexes are hydrolysed and the free cholesterol can be distributed to the plasma membrane as well as to the ER, which serves as a negative feedback sensor for the cholesterol genes. The excess cholesterol in ER is esterified into cholesteryl esters (CEs) by Acyl-CoA:cholesterol acyltransferase (ACAT)-1 and stored in cytoplasm lipid droplets. This intracellular pool of cholesterol is involved in the synaptic and dendritic formation and in the membrane remodelling [3]. A part of the cholesterol is converted by neurons into the more polar 24S-hydroxycholesterol (24OHC) via the cholesterol 24-hydroxylase (CYP46A1) to maintain the homeostasis [7]. 24OHC and other oxysterols are important ligands of the liver X-activated receptor (LXR), potentially able to regulate the expression of both the APOE and the ABCA1 genes in astrocytes [8]. Cholesterol and 24OHC are excreted from neurons via ABCG1/-G4 to ApoE particles into the interstitial and the cerebrospinal fluid (CSF). Through the blood–brain barrier, 24OHC passes into plasma where it is esterified in lipoproteins (LDL and HDL) for further elimination by liver [7].

The rate of synthesis of cholesterol is high in oligodendrocytes to form myelin sheets, which are sections of cellular membranes wrapped around axons to enable the saltatory conduction of the action potential. Expression of cholesterol synthesis genes and myelin basic protein (MBP) is under control of peroxisome-proliferator-activated receptor gamma co-activator 1 alpha (PGC-1α) [9] (Fig. 1).

Neurodegenerative diseases are associated with disturbances in cholesterol metabolism.

2. Brain cholesterol in Huntington's disease

Huntington's disease (HD) (MIM #143100) is an autosomal dominant neurodegenerative disorder characterized by motor impairment, cognitive decline, psychiatric manifestation and progression to death 15–20 years from the time of onset. HD is caused by an abnormal expansion of CAG repeats (>36) in the 5′ end of the huntingtin (*HTT*) gene (chromosome 4p16.3). MRI investigation shows a progressive neurodegeneration resulting in a severe striatal and cortical atrophy [10].

HTT is widely expressed in all tissues and has been ascribed to numerous roles in intracellular functions including protein trafficking, vesicle transport, endocytosis, postsynaptic signalling, transcriptional regulation and an anti-apoptotic function [11].

The expression of some genes involved in the cholesterol biosynthesis, HMGCR, cytochrome P450 lanosterol 14α-demethylase (CYP51) and 7-dehydrocholesterol 7-reductase (DHCR7) was found to be reduced in inducible mutant HTT cell lines, in striatum and cortex of transgenic R6/2 mice over-expressing exon-1 fragment of the mutant human *HTT* gene and in post mortem cortical tissue from HD patients [12,13]. The rate of cholesterol synthesis was reduced in human derived fibroblasts exposed to delipidated

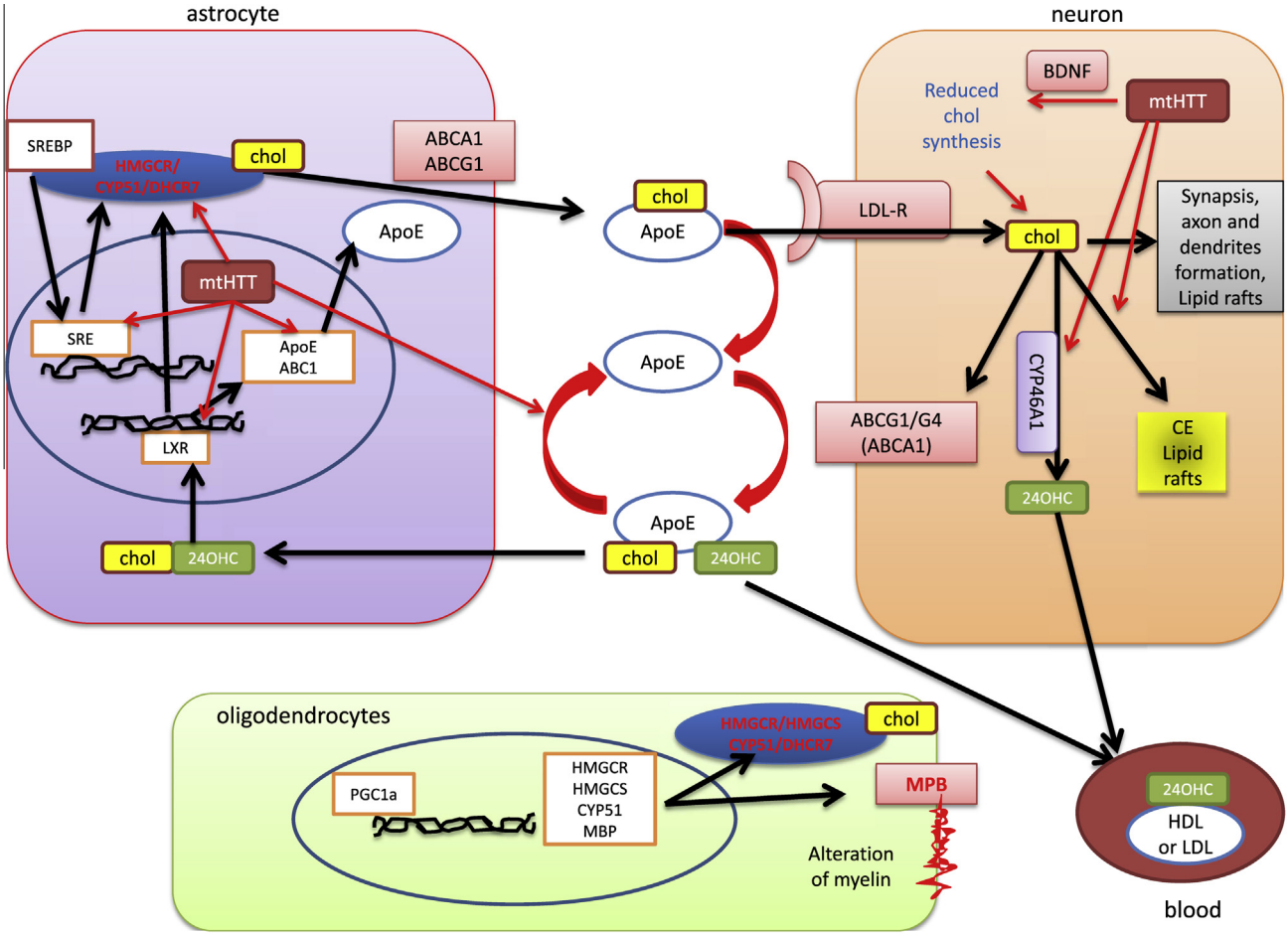

Fig. 1. Hypothetic model of brain cholesterol metabolism in Huntington's disease. In adult brain, neurons relay on the cholesterol (chol) synthesised in astrocytes and transported by ApoE (see text for details). Mutant huntingtin (mtHtt) interferes (red arrows) with the SREBP maturation and LXR regulation via expression of genes regulating synthesis (HMGCR, HMGCS, and CYP51) and transport (ABCA1, ABCG1) of cholesterol in astrocytes. As a consequence, the flux of ApoE bound cholesterol to the neuronal cells is reduced. In the latter cells cholesterol synthesis is already low due to a direct inhibition by mtHtt or due to a lower stimulation by BDNF. Less ApoE in the interstitial fluid may also impair cholesterol removal from neurons with a transitory accumulation of free or esterified cholesterol (CEs). A reduced expression of PGC-1α due to mtHtt interferes with MBP expression and cholesterol synthesis in oligodendrocytes ending up in an alteration of myelin structure [19,20].

medium. Cellular survival of rat primary striatal neurons transfected with human mutated HTT was promoted adding hexogen cholesterol to culture medium in a dose dependent manner [12]. Several HD mouse models exhibited reduced amounts of lanosterol, lathosterol, cholesterol and 24OHC in cortex, striatum and whole brain. In case of transgenic mice created with yeast artificial chromosome (YAC) technology over-expressing full-length human HTT with 18-46-72-128 glutamine residues, the reduction was proportional to the length of the CAG expansion (YAC18>wt>YAC46>YAC72>YAC128) [14]. With the brain maturation, cholesterol synthesis is progressively reduced [3] with a proportional reduction of brain lathosterol and lanosterol. In the R6/2 mice, the reduction observed at any time point was proportionally larger compared to wild-type mice [13,15]. Finally, sterols were reduced in knock-in mice carrying the CAG expansion within the murine *HTT* gene homolog ($Hdh^{Q111/111}$), proportionally to the load of mutant HTT (wt > $Hdh^{Q7/111}$ > $Hdh^{Q111/111}$) [14]. Metabolomic markers of cholesterol synthesis, accumulation, turnover and expression of genes involved in cholesterol synthesis, were reduced proportionally to the length of the CAG repeats and to the load of mutant HTT (heterozygous > homozygous) [13–15].

Total cholesterol was significantly lower in primary neurons from $Hdh^{(Q140/140)}$ knock-in HD mice over-expressing full-length endogenous mutant HTT compared to wild-type, $Hdh^{(Q7/7)}$. At an early differentiation stage, $Hdh^{(Q140/140)}$ neuronal stem (NS) cells had reduced cholesterol compared to $Hdh^{(Q7/7)}$; no reduction was found in NS from heterozygous knock-in mice $Hdh^{(Q140/7)}$ while NS cells from knock-out mouse, $Hdh^{(-/-)}$, showed elevated cholesterol compared to $Hdh^{(Q7/7)}$ [16]. Reduced cholesterol synthesis was observed in immortalised knock-in cells derived from the embryonic striatum of $Hdh^{(Q109/109)}$ mouse [17], in astrocytes from R6/2 and YAC 128 mice [14] and in primary mouse oligodendrocytes transduced with lenti-mutant HTT exon 1 (Q72) [17,18].

A proper cholesterol supply is critical for neurite outgrowth, for synapses and dendrites formation and for axonal guidance. Cholesterol depletion leads to synaptic and dendritic spine degeneration, failing neurotransmission and decreased synaptic plasticity. Such changes are observed at an early stage in several neurodegenerative disorders including HD [1]. In primary astrocytes from R6/2 and YAC 128 mice compared to wild-type and YAC18, the mRNA levels of genes involved in cholesterol biosynthesis (HMGCR, CYP51, DHCR7) and in cholesterol efflux (ABCA1, ABCG4 and ApoE) were found to be significantly reduced. The astrocytes with mutant HTT synthesize and secrete less ApoE than wild-type cells and the ApoE-lipoproteins present in CSF collected from YAC128 mice were smaller and less lipidated [15]. All together these observations suggest that in HD there is a reduced supply of cholesterol from astrocytes to neurons due to reduced synthesis and less efficient transport via ApoE.

PGC-1α plays a role in the transcriptional regulation of energy metabolism and has been implicated in several neurodegenerative disorders, including HD. PGC1α is highly expressed in all highly oxidative tissues (as CNS) and is involved in mitochondrial biogenesis, fatty acid oxidation and oxidative metabolism regulating also the expression of cytochrome C, component of respiratory chain complexes I–V [9,19,20].

In primary oligodendrocytes, mutant HTT inhibited the regulatory effect of PGC-1α on HMG-CoA synthetase (HMGCS) and HMGCR, the expression of MBP and the cholesterol metabolism. Brains from R6/2 and bacterial artificial chromosome (BAC) HD mice, over-expressing full-length human mutant *HTT* transgene, had abnormal myelination, reduced expression of MBP and PGC-1α: similar findings were also found in a PGC-1α knock-out mouse model [18]. PGC-1α knockout mice presented evidence of degeneration in cortex, thalamus, basal ganglia, and hippocampus, with the most pronounced degeneration in the striatum and vacuolar abnormalities that were primarily associated with the white matter [21]. It is likely that PGC-1α affects myelination because of its involvement in the expression of MBP and its potential dysregulation of cholesterol synthesis by direct or indirect interaction with SREBP and LXR.

Transcriptional dysregulation could explain the observed impairment of cholesterol metabolism at the molecular level. In presence of low intracellular cholesterol, SREBP translocates from the cytosol to the nucleus where it activates the transcription of SRE-controlled genes [5]. In cellular models of HD and in brain striatum collected from R6/2 mice, a decrease of active SREBP translocation was observed resulting into decreased cholesterol synthesis [12]. Wild-type HTT is able to bind to some nuclear receptors involved in lipid metabolism: LXR, peroxisome-proliferator-activated receptor γ (PPARγ) and vitamin D receptor [22]. Overexpression of HTT was shown to activate LXR, while a lack of HTT led to an inhibition of LXR-mediated transcription. The mutant HTT may have less capacity to up-regulate LXR and LXR-targeted genes, including SREBP [22]. Finally, brain-derived neurotrophic factor (BDNF) is released by cortical neurons projecting into striatum and is involved in synaptic plasticity and neuronal survival. BDNF induces cholesterol synthesis in postsynaptic neurons. Mutant HTT affects the BDNF transport and release, presumably resulting in a further inhibition of the already inefficient cholesterol synthesis in neurons [19,20].

In contrast to studies based on isotope dilution-gas chromatography–mass spectrometry (ID-GC–MS), the studies based on filipin staining and thin layer chromatography (TLC) reported high levels of cholesterol in the striatum of YAC72 compared to wild-type mice [23]. Its accumulation was measured with a cholesterol oxidase assay on protein extracts in striatum and neurons of $Hdh^{(Q111/111)}$ mice [24].

Cholesterol oxidase method, ID-GC–MS for sterols and oxysterols and filipin staining were all able to detect either cholesterol accumulation or depletion in immortalized knock-in cells derived from the embryonic striatum of $Hdh^{(Q7/7)}$ or $Hdh^{(Q109/109)}$ mice. Filipin staining showed high variability of results and could not detect small amounts of intracellular cholesterol accumulation. In normal and mutant stem cells cultured in a lipid-containing medium, there was no accumulation of lipids, cholesterol or cholesterol esters. In sub-confluent cultures with delipidated medium, a significant reduction in cholesterol synthesis and total cholesterol was observed by ID-GC–MS, but no differences were observed by filipin staining. No differences were detected in over-confluent cultures. It is evident that the sample preparation, the degree of confluence and clone properties as well as the composition of the culture medium (with or without extracellular source of lipids) affect the level of total or intracellular cholesterol detected. ID-GC–MS should be the most accurate and reproducible technique to adopt. In similar cells, an accumulation of cholesterol was observed in lipid rafts (as suggested by increased amount of GM1), and caveolae [24]. Lower expression of ApoE can be expected to reduce the cholesterol efflux from neurons to ApoE-lipoproteins. In the presence of reduced activity of CYP46A1, it can be expected to an accumulation of CEs in neurons. In theory, reduced levels of 24OHC may impair astrocytic cholesterol metabolism due to reduced activation of LXR and reduced SREBP-activation.

3. Sterols and oxysterols in patients affected by HD

The cholesterol synthesis (as evaluated by incorporation of ^{14}C acetate into cholesterol) in fibroblasts from HD patients was found to be less than 50% compared to controls [12]. Compared to wild-type, cholesterol precursors were reduced about 25% in plasma from 10-month-old YAC128 and increased about 25% in YAC18

mice. The plasma concentrations of 24OHC were reduced ~15% in YAC128 and increased about 12% in YAC18 [15]. Cholesterol, lanosterol, lathosterol and 27OHC were reduced proportionally to the disease stage: the whole-body cholesterol homeostasis was found impaired in HD patients [25,26].

The decrease of plasma 24OHC was proportional to the degree of caudate atrophy (measured as reduction of caudate volume at MRI) and to the motor impairment [27]. In three progression groups (Low, Medium, High, evaluated by the CAG-Age product or CAP score, there was a progressive reduction of plasma 24OHC in relation to the HD progression. The highest progression group had the most substantial difference relative to the controls and to the lowest progression group [26]. The progression gradient was consistent with the findings in other studies using several psychological and neurological tests or MRI measurements of structural atrophy. Plasma levels of 24OHC were significantly correlated to the latter changes [28]. The reduction of 24OHC was found to mirror the progression of striatum atrophy and was positively correlated with striatal volumes. The plasma levels of 24OHC in the Low group patients was higher than in the controls, Medium and High groups. It is possible that at an early stage of disease a higher cholesterol turnover might ensue from cyto-architectural and synaptic rearrangements in the HD brain [28].

Almost all the 24OHC in human plasma has cerebral origin and its concentration depends on the cholesterol turnover in brain neuronal cells, the blood–brain barrier integrity, the metabolism of plasma lipoproteins and the liver clearance [29]. Plasma 24OHC was found to be decreased in Parkinson's and Alzheimer's disease, in Vascular Dementia, in Multiple Sclerosis and in HD proportionally to the disease burden, the functional impairment and the MRI atrophy of grey structures. Thus plasma 24OHC was proposed as surrogate marker for the number of metabolically active neurons [7]. Along the course of a neurodegenerative disease, a higher membrane turnover associated with cellular damage may result in a larger amount of cholesterol converted by CYP46A1, with a higher efflux of 24OHC into plasma. At an advanced stage this transitory increase may be followed by a progressive decrease of cholesterol turnover and reduced plasma 24OHC. These two phases may largely overlap. The progression through this time course is related to the individual progression of disease [29].

The different processes involved in pathogenesis of HD (toxic gain of function and loss of function of the HTT, mitochondria dysfunction, bioenergetic defect, and transcriptional dysregulation), the activation of glia with extraneuronal expression of CYP46A1, the inflammatory response and the blood–brain barrier dysfunction may all have an effect on brain cholesterol metabolism.

Age, body mass index, diet, liver diseases, common forms of dyslipidemia (like familial combined hypercholesterolemia or polygenic hypercholesterolemia), metabolic syndrome and diabetes, use of inhibitors of cholesterol synthesis (i.e. statins) all have significant effects on the plasma oxysterol concentrations [28–30]. In addition, the time of sampling (after overnight fasting vs random sampling) significantly affects plasma concentrations of sterols and oxysterols [29]. All these factors should be considered in studies of cholesterol metabolism based on plasma analysis of sterols and oxysterols. Despite the data supporting 24OHC as plasma marker of disease progression, its power is limited by the effect of extracerebral factors on its plasma levels.

HTT mutation seems to have a general global effect on cholesterol synthesis. Both in the brain of several rodent models and in HD patients there is a reduced expression of key regulatory genes in parallel with a metabolic impairment of synthesis and turnover in neurons, astrocytes and oligodendrocytes. The transcriptional dysregulation reduces the nuclear translocation of SREBP, the activation of LXR and LXR-dependent genes and the PGC-1α function. The PGC-1α transcriptional disturbance is a central feature of HD pathogenesis. Microarray and RT-PCR studies on human striatal transcripts revealed that the vast majority of PGC-1α target genes were down-regulated in asymptomatic and pre-symptomatic HD patients [9]. PGC-1α expression was reduced in medium spiny neurons from HD patients, and PGC-1α transcriptional activity was markedly decreased in striatal HD-like cells, in medium spiny neurons from a knock-in HD mouse model and in post-mortem human striatum. The observed dysfunction of mitochondria energy supply to metabolic pathways, the dysfunction of respiratory chain and oxidative phosphorylation and the reduced biosynthesis of intermediates in HD, could, in part, be related to PGC-1α dysfunction [9]. Cholesterol synthesis depends on the amount of Acetyl-CoA and the ATP originating from mitochondria. Thus, it may be speculated that the transcriptional alterations of cholesterol genes are associated with an inefficient production of metabolic products from mitochondria. At an early stage of the disease, there may be a neuronal accumulation of cholesterol in esterified form due to reduced CYP46A1 activity. Alternatively, and more likely, a reduced removal of cholesterol via ApoE may contribute to an alteration of the membrane structures and cellular homeostasis. Since less than 10% of the total cholesterol in the brain is present in the neuronal cells, the local neuronal process should however not affect the whole brain cholesterol metabolism.

Acknowledgment

Financial support: Italian Minister of Health, GR-2008-1145270 to V. Leoni.

References

[1] A.R. Koudinov, N.V. Koudinova, Cholesterol homeostasis failure as a unifying cause of synaptic degeneration, J. Neurol. Sci. 229–230 (2005) 233–240.
[2] F.W. Pfrieger, N. Ungerer, Cholesterol metabolism in neurons and astrocytes, Prog. Lipid Res. 50 (2011) 357–371.
[3] J.M. Dietschy, S.D. Turley, Thematic review series, brain lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal, J. Lipid Res. 45 (2004) 1375–1397.
[4] N.R. Matthan, M. Raeini-Sarjaz, A.H. Lichtenstein, et al., Deuterium uptake and plasma cholesterol precursor levels correspond as methods for measurement of endogenous cholesterol synthesis in hypercholesterolemic women, Lipids 35 (2000) 1037–1044.
[5] J.D. Horton, J.L. Goldstein, M.S. Brown, SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver, J. Clin. Invest. 109 (2002) 1125–1131.
[6] G. Bu, Apolipoprotein E and its receptors in Alzheimer's disease, pathways, pathogenesis and therapy, Nat. Rev. Neurosci. 10 (2009) 333–344.
[7] I. Björkhem, A. Cedazo-Minguez, V. Leoni, et al., Oxysterols and neurodegenerative diseases, Mol. Aspects Med. 30 (2009) 171–179.
[8] K. Abildayeva, P.J. Jansen, V. Hirsch-Reinshagen, et al., 24(S)-hydroxycholesterol participates in a liver X receptor-controlled pathway in astrocytes that regulates apolipoprotein E-mediated cholesterol efflux, J. Biol. Chem. 281 (2006) 12799–12808.
[9] T. Tsunemi, A.R. La Spada, PGC-1α at the intersection of bioenergetics regulation and neuron function: from Huntington's disease to Parkinson's disease and beyond, Prog. Neurobiol. 97 (2012) 142–151.
[10] E.H. Aylward, Change in MRI striatal volumes as a biomarker in preclinical Huntington's disease, Brain Res. Bull. 72 (2007) 152–158.
[11] J.M. Gil, A.C. Rego, Mechanisms of neurodegeneration in Huntington's disease, Eur. J. Neurosci. 27 (2008) 2803–2820.
[12] M. Valenza, D. Rigamonti, D. Goffredo, et al., Dysfunction of the cholesterol biosynthetic pathway in Huntington's disease, J. Neurosci. 25 (2005) 9932–9939.
[13] M. Valenza, V. Leoni, A. Tarditi, C. Mariotti, I. Bjorkhem, S. Di Donato, E. Cattaneo, Progressive dysfunction of the cholesterol biosynthesis pathway in the R6/2 mouse model of Huntington's disease, Neurobiol. Dis. 28 (2007) 133–142.
[14] M. Valenza, V. Leoni, J.M. Karasinska, et al., Cholesterol defect is marked across multiple rodent models of Huntington's disease and is manifest in astrocytes, J. Neurosci. 30 (2010) 10844–10850.
[15] M. Valenza, J.B. Carroll, V. Leoni, et al., Cholesterol biosynthesis pathway is disturbed in YAC128 mice and is modulated by huntingtin mutation, Hum. Mol. Genet. 16 (2007) 2187–2198.
[16] J.J. Ritch, A. Valencia, J. Alexander, et al., Multiple phenotypes in Huntington disease mouse neural stem cells, Mol. Cell Neurosci. 50 (2012) 70–81.

[17] M. Marullo, M. Valenza, V. Leoni, et al., Pitfalls in the detection of cholesterol in Huntington's disease models, PLoS Curr. 4 (2012), http://dx.doi.org/10.1371/505886e9a1968. e505886e9a1968.

[18] Z. Xiang, M. Valenza, L. Cui, V. Leoni, H.K. Jeong, E. Brilli, J. Zhang, Q. Peng, W. Duan, S.A. Reeves, E. Cattaneo, D. Krainc, Peroxisome-proliferator-activated receptor gamma coactivator 1 á contributes to dysmyelination in experimental models of Huntington's disease, J. Neurosci. 31 (2011) 9544–9553.

[19] M. Valenza, E. Cattaneo, Emerging roles for cholesterol in Huntington's disease, Trends Neurosci. 34 (2011) 474–486.

[20] J.M. Karasinska, M.R. Hayden, Cholesterol metabolism in Huntington disease, Nat. Rev. Neurol. 7 (2011) 561–572. Review.

[21] T.C. Leone, J.J. Lehman, B.N. Finck, et al., PGC-1alpha deficiency causes multi-system energy metabolic derangements: muscle dysfunction, abnormal weight control and hepatic steatosis, PloS Biol. 3 (2005) e101.

[22] M. Futter, H. Schoenmakers, O. Sadiq, et al., Wild-type but not mutant huntingtin modulates the transcriptional activity of liver X receptors, J. Med. Genet. 46 (2009) 438–446.

[23] E. Trushina, R.D. Singh, R.B. Dyer, S. Cao, V.H. Shah, R.G. Parton, R.E. Pagano, C.T. McMurray, Mutant huntingtin inhibits clathrin-independent endocytosis and causes accumulation of cholesterol in vitro and in vivo, Hum. Mol. Genet. 15 (2006) 3578–3591.

[24] D. del Toro, X. Xifró, A. Pol, et al., Altered cholesterol homeostasis contributes to enhanced excitotoxicity in Huntington's disease, J. Neurochem. 115 (2010) 153–167.

[25] M. Markianos, M. Panas, N. Kalfakis, et al., Low plasma total cholesterol in patients with Huntington's disease and first-degree relatives, Mol. Genet. Metab. 93 (2008) 341–346.

[26] V. Leoni, C. Mariotti, L. Nanetti, et al., Whole body cholesterol metabolism is impaired in Huntington's disease, Neurosci. Lett. 494 (2011) 245–249.

[27] V. Leoni, C. Mariotti, S.J. Tabrizi, et al., Plasma 24S-hydroxycholesterol and caudate MRI in pre-manifest and early Huntington's disease, Brain 131 (2008) 2851–2859.

[28] V. Leoni, J.D. Long, J.A. Mills, et al., Plasma 24S-hydroxycholesterol correlation with markers of Huntington disease progression, Neurobiol. Dis. 55 (2013) 37–43.

[29] V. Leoni, C. Caccia, 24S-hydroxycholesterol in plasma: a marker of cholesterol turnover in neurodegenerative diseases, Biochimie 95 (2013) 595–612.

[30] G. Lupattelli, S. De Vuono, E. Mandarino, Patterns of cholesterol metabolism: pathophysiological and therapeutic implications for dyslipidemias and the metabolic syndrome, Nutr. Metab. Cardiovasc. Dis. 21 (2011) 620–627.