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Review

Oxysterols as a biomarker in diseases

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ABSTRACT

Cholesterol is one of the most important chemical substances as a structural element in human cells, and it is very susceptible to oxidation reactions that form oxysterol. Oxysterols exhibit almost the exact structure as cholesterol and a cholesterol precursor (7-dehydrocholesterol) with an additional hydroxyl, epoxy or ketone moiety. The oxidation reaction is performed via an enzymatic or non-enzymatic mechanism. The wide array of enzymatic oxysterols encountered in the human body varies in origin and function. Oxysterols establish a concentration equilibrium in human body fluids. Disease may alter the equilibrium, and oxysterols may be used as a diagnostic tool. The current review presents the possibility of using non-enzymatic oxysterols and disturbances in enzymatic oxysterol equilibrium in the human body as a potential biomarker for diagnosing and/or monitoring of the progression of various diseases.

1. Introduction

Cholesterol is one of the most important chemical compounds in the human body. It is present in all cells primarily as a structural element of lipid bilayers, where it regulates membrane fluidity. Cholesterol is also an essential substrate for the biosynthesis of bile acids, steroid hormones, vitamin D and oxysterols [1–3]. Most cholesterol is biosynthesized in cells, despite its relatively high uptake in the intestines from food. Brain cells must synthesize cholesterol *de novo* from acetyl-CoA because the blood-brain barrier (BBB) efficiently prevents cholesterol uptake from the circulation into the brain. The average content of unesterified cholesterol in the central nervous system (CNS) accounts for 25% of the sterol present in the body, which is the highest content compared to any other tissue. A total of 70% of the cholesterol in the brain is localized in myelin, which acts as a discontinuous electrical insulator. The rest of the cholesterol pool is localized in glial cells (primarily astrocytes and microglia) and neurons, which contribute to 20% and 10%, respectively. The high content of cholesterol supports its involvement in essential processes in the brain, like CNS maturation, participation in signal transduction, neurotransmitter release, synaptogenesis and membrane trafficking [4].

The cholesterol molecule is relatively easily oxidized, and it may be transformed into numerous oxidation products, including oxysterols. Sterol oxidation products include various chemical compounds that contain additional oxygen moieties, such as alcohol, ketone, epoxy or carboxyl (Fig. 1). These compounds are formed via different mechanisms, such as cholesterol auto-oxidation [1], as a result of lipid peroxidation [2] or due to enzymatic cholesterol metabolism [3]. Structures of the most abundant oxysterols and their end products with the routes of synthesis are presented in Fig. 1.

One of the primary sterol autoxidation products is 7-hydroperoxidecholesterol, which is rapidly transformed into the major oxidized oxysterols: 7-ketocholesterol (7-kCh), 7α-hydroxycholesterol (7α-hCh), and 7β-hydroxycholesterol (7β-hCh) [5]. The most abundant oxysterols in human sera are generated from the enzymatic reaction of mitochondrial or endoplasmic reticulum cholesterol hydroxylases, which are part of the cytochrome P450 family, and include 4β-hydroxycholesterol (4β-hCh), 7α-hydrocholesterol (7α-hCh), 24(*S*)-hydroxycholesterol (24(*S*)-hCh), and 27-hydroxycholesterol (27-hCh) [6]. Therefore, oxysterols are compounds that constantly exist in the human

Abbreviations: CSF, cerebrospinal fluid; BBB, blood-brain barrier; CNS, central nervous system; CAD, coronary artery disease; LXR, liver X receptor; ERα, estrogen receptor alpha; 4β-hCh, 4β-hydroxycholesterol; 7α-hCh, 7α-hydroksycholesterol; 7β-hCh, 7β-hydroksycholesterol; 7-kCh, 7-ketocholesterol; α-epoxCh, cholesterol-5α,6α-epoxide; β-epoxCh, cholesterol-5β,6β-epoxide; triolCh, 5α-cholestane-3β,5,6β-triol; 22(*R*)-hCh, 22-hydroxycholesterol; 24(*S*)-hCh, 24-hydroxycholesterol, cerebrosterol; 25-hCh, 25-hydroxycholesterol; 27-hCh, 27-hydroxycholesterol; 7αh-3o-4chol acid, 7α-hydroxy-3-oxo-4-cholestenoic acid; 7-dCh, 7-dehydrocholesterol; 8-dCh, 8-dehydrocholesterol; dhCeo, 3β,5α-dihydroxycholest-7-en-6-one; MCI, mild cognitive impairment; AD, Alzheimer's disease; MS, multiple sclerosis; HD, Huntington Disease; NPC, Niemann-Pick C Disease; PD, Parkinson's disease; ALS, Amyotrophic Lateral Sclerosis; SLOS, Smith-Lemli-Opitz Syndrome; ASD, Autism Spectrum Disorders; SPG5, Spastic paraplegia type 5; GC–MS, gas chromatography-mass spectrometry; LC-MS, liquid chromatography-mass spectrometry

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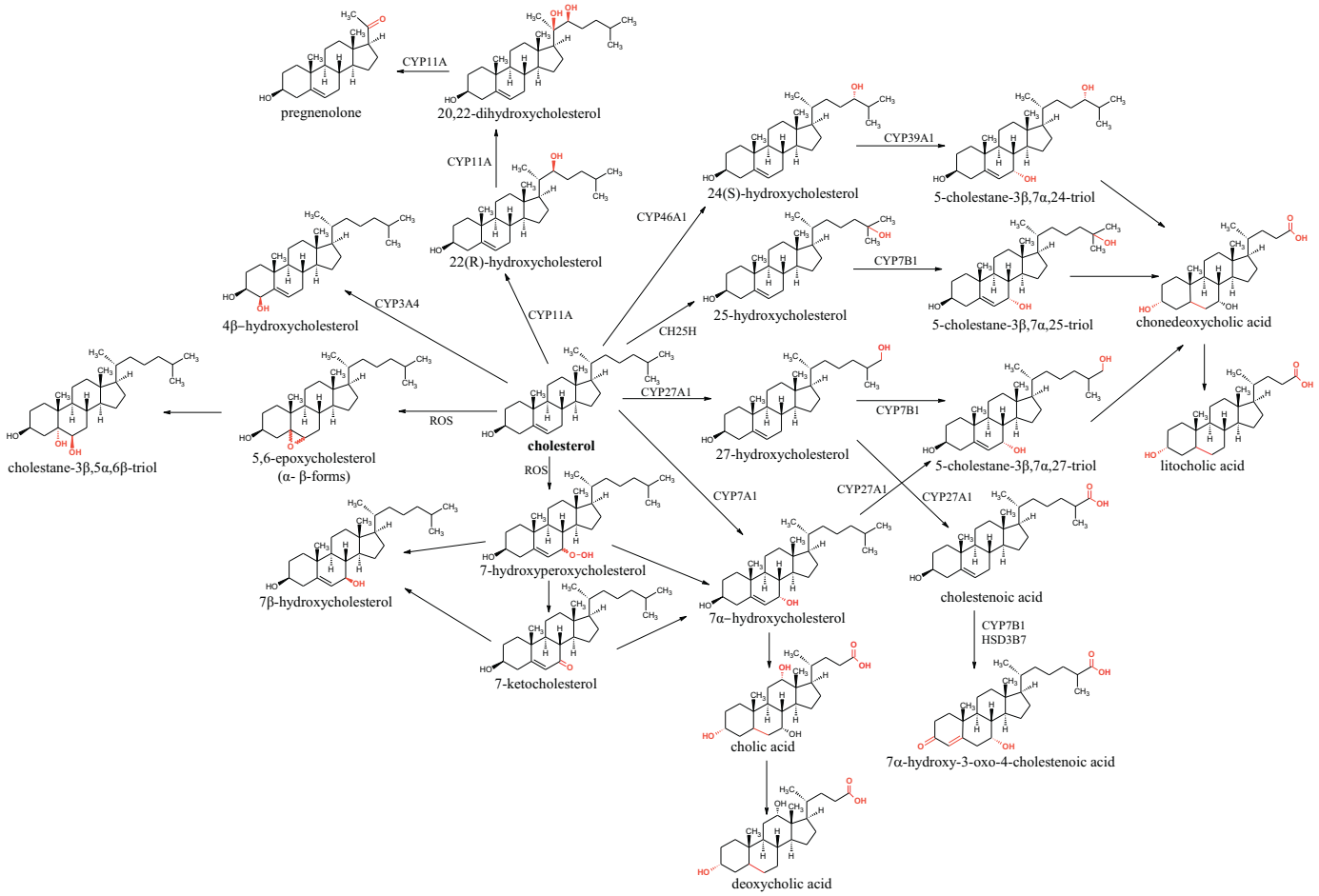

Fig. 1. Main products of enzymatic or auto-oxidation of cholesterol found in humans [2,6–8].

body as a result of cholesterol reaction with radical forms of oxygen or cholesterol hydroxylases in the cytochrome P450 family [6]. Oxysterols formed via non-enzymatic or enzymatic reactions establish an equilibrium in the human body. Notably, oxysterol equilibrium in human bodily fluids are disturbed during certain diseases and may be used as a biomarker for diagnosing and/or monitoring of the progression of these diseases.

1.1. Coronary artery disease; atherosclerosis

Atherosclerosis is a disease that affects the multifocal repetitive regions of the arterial tree. It is not a disease strictly connected with the ageing process, but rather a chronic inflammatory condition that may lead to intimal destruction, arterial thrombosis and end-organ ischaemia [9]. Pro-atherogenic properties of oxidized forms of cholesterol were postulated because of their presence in atherosclerotic lesions [5]. The influence of oxysterols on the initiation and progression of atherosclerotic plagues has been exhaustively reviewed previously [9]. Numerous researchers demonstrated that oxysterols from auto-oxidized cholesterol induced significant apoptosis or necrosis in vascular cells [10–12]. Reported auto-oxidated sterol composition in atherosclerotic lesions or plasma primarily includes six oxysterols: 7-kCh, 7α-hCh, 7β-hCh, 5,6α-epoxycholesterol (α-epoxCh), 5,6β-epoxycholesterol (β-epoxCh) and cholestane-3β,5,6β-triol (triolCh) [13]. Further, oxysterols exhibit at least 100-fold higher concentrations in atherosclerotic plaques than human plasma [5]. Several reports indicated that plasma concentrations of 7β-hCh are related to atherosclerotic disease progression. The presence of the physiologically occurring oxysterol 7β-hCh suggests an increase in lipid peroxidation *in vivo*. Salonen et al. [14] demonstrated that increased plasma concentrations of 7β-hCh are associated with the risk of developing coronary atherosclerotic plaques. Increased serum 7β-hCH concentration is one of the strongest single predictors of progression in carotid atherosclerosis and carotid wall thickening. Increased levels of 7β-hCh are also associated with a high risk of many cardiovascular diseases [15]. Rimner et al. [16] measured, using GC-FID, free plasma oxysterol levels of 42 patients with an atherogenic risk profile and symptoms of coronary artery disease (CAD). Total free plasma oxysterols were elevated more than 2-fold in patients (232.6 ± 49.1 ng/mL, $p < 0.05$) with stable CAD compared to a control group (107.9 ± 18.6 ng/mL) with a similar atherogenic risk profile and angiographically normal coronary arteries. Oxysterols 7-kCh, β-epoxCh (25.7 ± 10.0 ng/mL vs 7.3 ± 1.4 ng/mL, $p = 0.07$) and 7β-hCh (65.1 ± 15.7 ng/mL vs 19.4 ± 8.9 ng/mL, $p < 0.01$) were primarily responsible for this increase. Yasunobu et al. [17] investigated the relevance of oxysterols to CAD in 183 patients undergoing coronary angiography. Serum concentrations of 7β-hCh (98.9 ± 8.7 nmol/L vs. 67.9 ± 6.8 nmol/L, $p < 0.05$), 25-hCh (28.2 ± 5.0 nmol/L vs. 13.1 ± 2.3 nmol/L, $p < 0.05$), and 27-hCh (47.7 ± 4.8 nmol/L vs. 30.4 ± 4.3 nmol/L, $p < 0.05$) were significantly higher in the stenotic group than the normal group, which was measured using high-performance liquid chromatography method. Khatib et al. [18] concluded that plasma 7β-hCh level was useful as a biomarker for CAD. Umetani et al. [19] found that 27-hCh promoted atherosclerosis via proinflammatory processes mediated by estrogen receptor alpha (ERα), and this oxysterol attenuated estrogen-related atheroprotection. Additionally, proinflammatory responses were observed, such as increased leukocyte-endothelial adhesion and upregulation of NF-κB, which are associated

with atherogenesis. Fuhrmann et al. [20] recently investigated 376 patients admitted for elective coronary angiography, who were not on lipid-lowering drugs. The oxysterol contents were measured by isotope dilution gas chromatography–mass spectrometry (GC–MS). Eighty-two of these patients suffered cardiovascular events, and these patients had significantly higher 7α-hydroxycampesterol (7α-hCamp) plasma levels (0,51 ± 0,29 nmol/L vs 0.39 ± 0.22 nmol/L, $p = 0.003$) and 7α-hCamp-to-cholesterol ratios than controls without events (0.08 vs. 0.05 nmol/mmol; $p = 0.005$). They concluded that 7α-hCamp and its cholesterol-corrected ratio were associated with cardiovascular events based on the results [20].

To date, there are an arsenal of diagnostic tests and tools to assess risk or confirm the presence of atherosclerosis – blood morphology including lipid profile with total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein (LDL); an exercise stress test (cardiac stress test), assessment of the ratio of ankle-brachial index (ABI) and imaging tests: angiogram (arteriogram), carotid duplex ultrasound, computed tomography (CT) scan, an echocardiogram, an electrocardiogram (ECG or EKG), an intravascular ultrasound, magnetic resonance imaging scan (MRI) and positron emission tomography scan (PET) [21]. Identifying atherosclerotic disease, with those techniques rely heavily on the evaluation of the arterial lumen or obstruction of blood flow [22]. However, a significant portion of the arterial wall can be involved with an atherosclerotic plague before the lumen is compromised [23]. Further, techniques such as carotid ultrasound and CT scan are only moderate at best for detection of subclinical atherosclerosis [24]. Therefore, it is necessary to find an appropriate biomarker that will allow the diagnosis of atherosclerosis at the earliest possible stage. Available studies suggest that increased concentrations of oxysterols in the circulation are important in the diagnosis of CAD. These various data strongly suggest the potential use of auto-oxidation oxysterols as biomarkers of atherosclerosis. Further studies are needed to assess whether blood may be sufficient to measure this type of biomarker in the diagnosis of CAD.

2. Neurological diseases

2.1. Alzheimer's disease

Alzheimer's disease (AD) is the most common neurodegenerative disorder resulting in cognitive impairment. AD is a gradually debilitating disease that leads to dementia. Approximately 11% of people over the age of 65 years and 32% over the age of 85 years are estimated to suffer from AD [25]. The molecular mechanisms underlying AD are still not clear, and no reliable biomarkers are available for early diagnosis. An analysis of three biomarkers in cerebrospinal (CSF) fluid is used in the laboratory diagnosis of AD: $A\beta_{42}$, total tau, and phospho-tau [26,27].

However, epidemiological and molecular studies indicate a link between cholesterol and neurodegenerative diseases. One of the important risk factors of the AD is hypercholesterolemia because cholesterol influences various processes involved in the generation of the neurotic plaques and neurofibrillary tangles [28,29]. Cholesterol undergoes continuous biosynthesis in brain cells, and the concentration of cholesterol must be maintained in homeostasis with excretion of excess cholesterol (See Fig. 2). The BBB efficiently prevents cholesterol uptake from the circulation into the brain and the release of cholesterol from brain to the circulation. Therefore, excess cholesterol is converted into 24(*S*)-hCh (also known as cerebrosterol) via the neuron-specific enzyme CYP46A1, which exhibits more hydrophilic properties and penetrates the BBB [30]. Greater than 98% of the 24(*S*)-hCh produced in brain fluxes directly into the bloodstream, which is approximately 6–8 mg daily [31]. The concentration of 24(*S*)-hCh in blood strictly depends on the number of metabolically active neurons in the grey matter of the brain. Therefore, neurodegeneration significantly reduces the concentrations of this oxysterol. Less than 1% of 24(*S*)-hCh, which is approximately 1–2 mg/day, goes to the CSF in ApoE-bound form [31,32], and this result likely reflects the rate of neuronal degeneration rather than the total number of metabolically active neuronal cells. Patients with neurodegenerative disorders exhibit increased concentrations of 24(*S*)-hCh in CSF in parallel with decreased concentrations in the circulation. The oxysterol 24(*S*)-hCh maintains cholesterol homeostasis and influences neuron signalling via activation of tyrosine hydroxylase expression, which is involved in converting tyrosine into a substrate for dopamine biosynthesis, L-DOPA. 24(*S*)-hCh also exhibits an anti-amyloidogenic character via increasing α-secretase activity and the α/β-secretase activity ratio [33].

Another side-chain oxysterol found in the brain is 27-hCh. In contrast to 24(*S*)-hCh, 27-hCh is formed in most body cells by sterol 27-hydroxylase (CYP27A1), and there is a constant flux of this oxysterol to the liver [34]. 27-hCh has no flux into the brain, and it can pass the BBB, similarly to 24(*S*)-hCh. Most of the 27-hCh present in the human CSF is of vascular origin [35]. However, the levels of 27-hCh in this organ are approximately 10-fold lower than 24(*S*)-hCh [31]. Any increase in 27-hCh concentrations in the brain may be related to a defect in the BBB. 27-hCh blocks postsynaptic signalling and causes apoptosis and neurodegeneration. 27-hCh also increases the formation of β-amyloid via antagonizing the suppressive effect of 24(*S*)-hCh [33], which increases tau phosphorylation and the aggregation of α-synuclein (via liver X receptor (LXR)) and induces oxidative stress, which are hallmarks of AD and Parkinson's disease (PD). 27-hCh also affects *N*-methyl-D-aspartate receptors and inhibits the activity of regulated cytoskeleton-associated protein formation, which likely interferes with memory consolidation [36]. Therefore, 27-hCh is highly efficiently metabolized into 7α-hydroxy-3-oxo-4-cholestenoic acid (7αh-3o-4chol acid), which may be very efficiently eliminated from the brain into the bloodstream.

Decreased levels of $A\beta_{42}$ or a reduced $A\beta_{42}$:$A\beta_{40}$ ratio in combination with elevated levels of total tau and phospho-tau in CSF were consistently confirmed as biomarkers in patients with different stages of AD, including mild cognitive impairment (MCI) [37]. Several reviews and studies concluded that changes in 24(*S*)-hCh and 27-hCh concentrations may also be appropriate biomarkers for AD, besides $A\beta_{42}$, total tau and phospho-tau [38–42]. Leoni et al. demonstrated that 24(*S*)-hCh was the most sensitive marker in MCI patients [43]. The balance between 24(*S*)-hCh and 27-hCh levels is important, and an increased ratio of 27-hCh to 24(*S*)-hCh in AD brains is consistent with AD pathogenesis. Reduced levels of 24(*S*)-hCh may accelerate disease progression, and the shift to an imbalance between the two oxysterols may lead to increased generation and accumulation of Aβ and neurofibrillary tangles with consequent neurodegeneration [44–46]. Notably, the small fraction of 24(*S*)-hCh entering the CSF seems to reflect the rate of neuronal degeneration rather than the mass of metabolically active neuronal cells [47]. This characteristic means that plasma levels of this 24(*S*)-hCh are reduced in patients with the advanced AD, and the levels in CSF are increased [47,48]. The changes in the CSF were more marked than the circulation and may be more important from a diagnostic point of view in AD and other neurological diseases. The brains of patients with AD who died exhibited reduced levels of CYP7B1, which is the enzyme responsible for the metabolism of 27-hCh [49]. This reduction likely underlies the findings of Leoni and others of elevated CSF 27-hCh levels in patients with neurodegeneration [47]. An impaired BBB functionality would also allow a higher passage of 27-hCh from the periphery into the brain. BBB dysfunction is key in the development and progression of AD [50,51], which allows a higher passage of 27-hCh from the periphery into the brain. Therefore, BBB functional deficiency may be identified using the concentration of a metabolite of 27-hCh, 7αh-3o-4chol acid, in CSF [52]. A dysfunctional BBB increases the concentration of this metabolite in CSF. There was a high correlation between the levels of 7αh-3o-4chol acid in CSF and the CSF/serum albumin ratio.

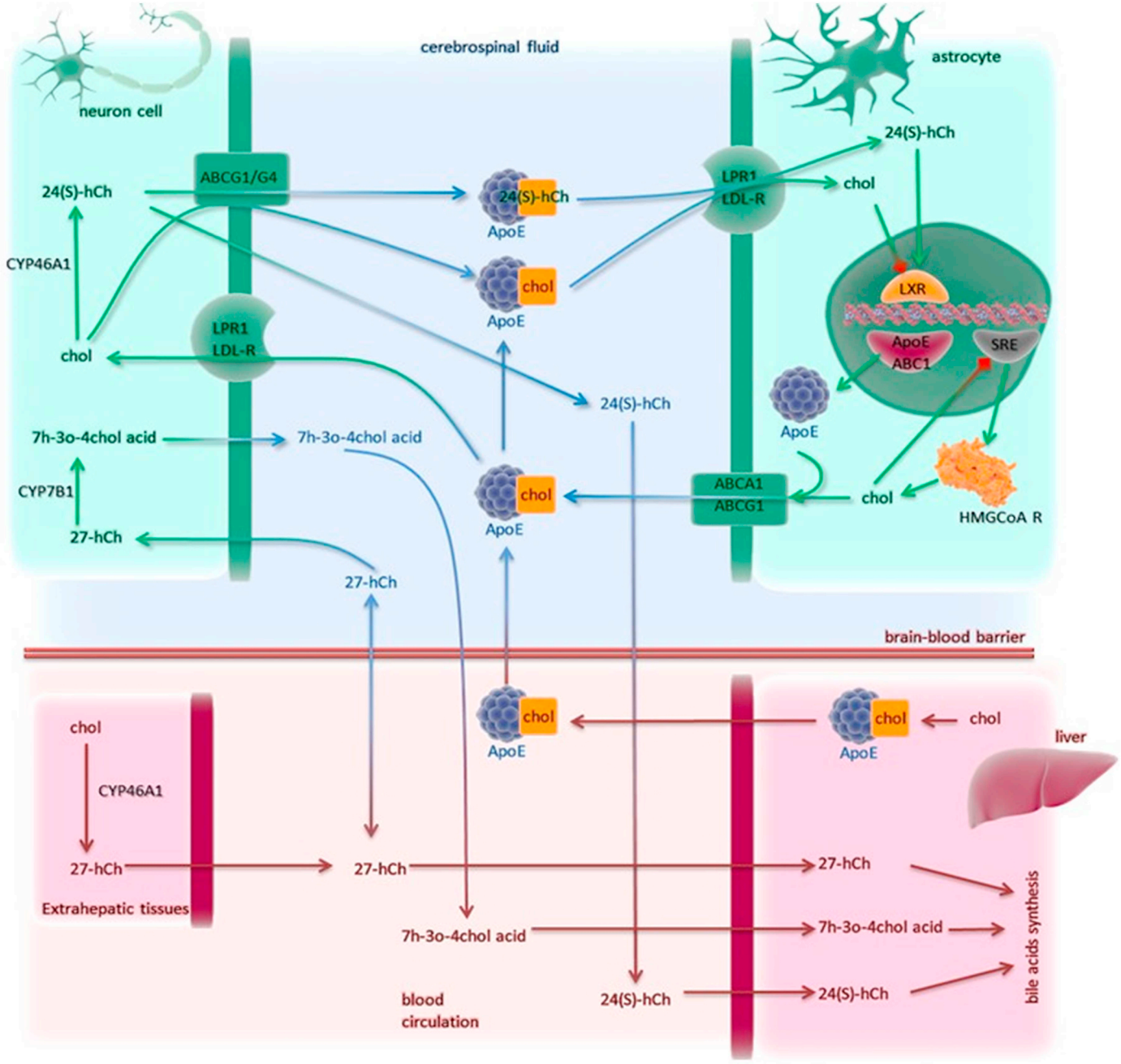

Fig. 2. Cholesterol and oxysterol distribution in human circulation. Cholesterol biosynthesis in the brain occurs in astrocytes via SRE activation and catalysis by HMG-CoA reductase. Cholesterol is produced and assembled with ApoE lipoproteins. ABCA1 plays a key role in cholesterol-ApoE efflux from astrocytes. Neurons acquire ApoE-containing cholesterol via the highly expressed ApoE receptor LRP1. Cholesterol accumulation may be very dangerous to neurons, and any excess must be eliminated from the brain. However, the BBB prevents the release of excess of cholesterol. Homeostasis of this sterol is maintained via conversion to 24(*S*)-hCh, which may cross the BBB and enter the blood circulation. Additionally, 24(*S*)-hCh activates LXRs, which induces ApoE and ABCA1 expression to promote cholesterol efflux and regulate cholesterol biosynthesis. Therefore, cholesterol synthesis and elimination are constantly adjusted and homeostatically regulated in cells. Another oxysterol, 27-hCh, which is generated by body cells, is found in the brain after penetrating the BBB from blood circulation. 27-hCh blocks postsynaptic signalling and causes apoptosis and neurodegeneration. Therefore, 27-hCh is highly efficiently metabolized by CYP7B1 into 7α-hydroxy-3-oxo-4-cholestenoic acid (7αh-3o-4chol acid). This steroid is an acid that passes the BBB to bloodstream much more efficiently than its precursor 27-hCh because of its higher polarity, and it is also very efficiently taken up by the liver and metabolized into bile acids. Ultimately, 24(*S*)-hCh and 27-hCh are also taken up by the liver from the circulation and metabolized into bile acids.

2.2. Multiple sclerosis

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the CNS that causes cognitive and physical disability [53]. The pathological hallmark of MS is inflammation and demyelinated plaques, which are well-demarcated hypocellular areas characterized by myelin loss, the relative preservation of axons in the early phase, oedema, gliosis, formation of astrocytic scars and BBB dysfunction [54,55]. The disease in most patients begins with a relapsing-remitting (RR-MS) form, and 50–60% of these patients progress to secondary progressive disease. Only approximately 15% of patients develop progressive disease from the beginning, and these patients are referred to as primary progressive multiple sclerosis (PP-MS) [56].

Plasma levels of 24(*S*)-hCh were significantly reduced in RR-MS and

PP-MS patients [57,58]. However, patients with an early stage of the disease presented normal or even increased levels of 24(*S*)-hCh. Increased levels of 24(*S*)-hCh seem to occur at an early stage likely due to the loss of BBB integrity [9]. Decreased levels of 24(*S*)-hCh are common in severely affected patients with a long disease duration, which is caused by neurodegeneration. However, Mukhopadhyay et al. found, using liquid chromatography–mass spectroscopy (LC-MS) method, that 27-hCh (210 ± 114 ng/mL vs. 251 ± 117 ng/mL., $p = 0.004$) and 7α-hCh (69.9 ± 74 ng/mL vs. 118 ± 18 ng/mL, $p < 0.001$) also exhibited lower concentrations in MS patients compared to healthy controls [59]. These results demonstrate that the observed reductions in 27-hCh and 7α-hCh are likely a reflection of changes in the cholesterol metabolism pathways in the periphery, which may be responsible for the pro-inflammatory changes that allow MS disease progression. In contrast, higher 7-kCh levels are observed in PP-MS (18.1 ± 17 ng/mL) compared to RR-MS patients (13.5 ± 8 ng/mL), which may indicate that the oxysterol network is disrupted in MS. The observed increase in 7-kCh in PP-MS compared to RR-MS reflects higher oxidative stress, which is also conducive to neurodegeneration in PP-MS [57–59]. The available studies suggest the use of oxysterols as a potential biomarker for MS diagnosis, and the levels of 24(*S*)-hCh may also be used as a marker for different phases in the progression of MS.

2.3. Huntington's disease

Huntington's Disease (HD) is an autosomal dominant, progressive neurodegenerative disorder that is characterized by expanded CAG repeats, which lead to a polyglutamine expansion of variable length within the N-terminus of the huntingtin protein (HTT) [60]. HTT is widely expressed throughout the body, and it was assigned numerous roles in various intracellular functions, including protein trafficking, vesicle transport, endocytosis, postsynaptic signalling, transcriptional regulation and an anti-apoptotic function [61]. The typical onset of symptoms occurs in middle-age and consists of chorea and dystonia, uncoordination, cognitive decline, and behavioural difficulties. One of the causes of these symptoms is widespread neurodegeneration over the entire cerebral cortex. The loss of brain mass is quite substantial in HD during the 15–20 years of disease from symptom onset to death, and 25–30% of the brain mass may be lost in advanced HD [62]. This massive neurodegeneration in HD primarily occurs as striatal and cortical atrophy, which may be measured using MRI. The precise pathophysiological mechanisms of HD are still poorly understood, but research using transgenic animal models of the disorder is providing valuable insight into causative factors, possible biomarkers, and potential treatments.

HD significantly affects brain cells, and changes in cholesterol levels and oxysterol concentrations are expected. HTT decreases brain cholesterol by inducing inhibition of a series of essential genes that are responsible for cholesterol biosynthesis (HMG–CoA and 7-dehydroxycholesterol reductase), which are decreased at the mRNA level [63,64]. A deficiency of 7-dehydroxycholesterol reductase, as in Smith-Lemli-Opitz Syndrome (SLOS), impairs brain development [65]. Reduced levels of mRNA of cholesterol biosynthesis and efflux genes (SRE- and LXR-regulated genes) were reduced in primary astrocytes from a mouse model of HD. This reduction caused a subsequent decrease in the production and secretion of cholesterol-ApoE [66]. However, other studies demonstrated increased cholesterol accumulation [67,68] and lipid droplets [69] in HD neurons. 24(*S*)-hCh formation would also be expected to decrease because of the reduced 24-hydroxylase levels in the reduced numbers of active neurons, which also leads to a lower efflux from the brain to the circulation. Lower brain and plasma 24(*S*)-hCh levels were reduced in several rodent models of HD compared to control mice [66,70]. Similarly, plasma 24(*S*)-hCh levels were significantly reduced in HD patients compared to healthy subjects [71–73]. All studies by Leoni et al. (2008, 2011, 2013) used isotope dilution GC–MS method for 24(*S*)-hCh levels analysis. The first study analyses the patient's plasma levels of both Italian and British origin, which have been divided according to the stage from early (HD1), to moderate (HD2) and to advanced disease (HD3) – Italian subjects: control–57.9 ± 9.9 μg/L; HD1–45.8 ± 7.9 μg/L; HD2–44.6 ± 15 μg/L; HD3–42.3 ± 5.8 μg/L, $p < 0.001$); UK subjects: control–56.7 ± 13 HD1–47.3 ± 12; HD2–47.5 ± 15; HD3–48.2 ± 12, $p < 0.05$. In the second study done in 2011, Italian origin patients were divided in the same way as it has been done in the first study and the result are comparable: control–58.3 ± 10.7 μg/L, HD1–46.6 ± 8.8 μg/L; HD2–43.3 ± 9.2 μg/L; HD3–42.7 ± 9.7 μg/L, $p < 0.001$. In the third study, HD patients were divided into 3 groups: Low, Medium, High. The descriptors of Low, Medium, and High refer to the level of cumulative toxicity of mutant huntingtin at study entry. Group differences for 24(*S*)-hCh concentration showed a progression gradient, decreasing in mean value as progression group increased. The highest progression group (47.83 ± 13.30 ng/mL, $p < 0.001$) had the most substantial difference relative to the controls (58.95 ± 8.26 ng/mL) and lower progression group (Low–64.57 ± 20.13 ng/mL; Medium–52.17 ± 9.84 ng/mL, both groups significant different $p < 0.001$ vs. control). The decrease in plasma 24(*S*)-hCh occurred in parallel with the degree of atrophy as measured using morphometric MRI, which suggests that the observed reduction of plasma 24(*S*)-hCh also reflects the progressive neuronal loss in the grey matter [73]. Overall, all of the results obtained from available studies in animal models and patients suggest that 24(*S*)-hCh is a viable candidate as a biomarker of HD diagnosis and progression.

2.4. Niemann-Pick type C disease

Niemann-Pick type C (NPC) disease is a rare neurovisceral disorder that is characterized by progressive hepatosplenomegaly and CNS neurodegeneration [74]. The estimated incidence is 1 in 120,000 to 150,000 people [74,75]. Most NPC patients (~95%) suffer from a disease caused by mutation of the NPC1 gene located on chromosome 18q11 [76]. The remaining cases are caused by mutations in the NPC2 gene, which is located on chromosome 14q24.3 [77]. Mutations in the NPC1 and NPC2 genes influence the intracellular trafficking of cholesterol, which leads to an accumulation of free cholesterol in late endosomal/lysosomal structures [78,79]. The lysosomal lipid accumulation is accompanied by an increased production of reactive oxygen species and oxidative damage, which subjects cholesterol to oxidation reaction mediated via enzymatic or non-enzymatic reactions [80]. Formed oxysterols, specifically triolCh and 7-kCh, are significantly increased in the plasma of NPC patients and in tissues and the blood plasma of $NPC1^{-/-}$ mice [81,82]. These results suggest that triolCh and 7-kCh are NPC1 disease-specific biochemical or therapeutic evaluation markers of NPC1 disease [83]. *In vivo* studies found elevated oxysterols in patients with NPC1 [83–90] or NPC2 mutations [85,87,91] (Table 1).

A very strong correlation was demonstrated between triolCh and 7-kCh, and a relatively weak correlation was reported with disease severity and age of onset [84,92]. However, triolCh appears to exhibit greater discriminatory power than 7-kCh for patients with NPC versus normal subjects [83–85,87,92]. Ultimately, the high sensitivity and selectivity of triolCh and 7-kCh in the diagnosis of patients with NPC was positively confirmed. The possibility of triolCh measurement as a biomarker was recently demonstrated in the identification of 72 new patients with NPC1 or NPC2 over the course of 3 years [87]. Further tests are needed to confirm the usefulness of triolCh and 7-kch in diagnosis, but the non-invasive nature, low cost, short turnaround time and good sensitivity of testing changes in oxysterol levels supports the use as a primary test in confirming suspicions of NPC [93].

2.5. Parkinson's disease

Parkinson's disease (PD) is a sporadic progressive

Table 1
Measured levels of triolCh and 7-kCh in patients with NPC1/NPC2 and control groups.

Reference	Concentration of oxysterol				Method of oxysterol analysis
	TriolCh		7-kCh		
	Control	NPC1	Control	NPC1	
Jiang et al. (2011) [83]	Mean: 14.6 ng/mL range: 7.42–21.2 ng/mL	Mean: 80.3 ng/mL; range 15.1–201 ng/mL; $p < 0.001$	Mean: 29.0 ng/mL, range: 11.4–44.4 ng/mL;	Mean: 229 ng/mL, range 24.7–489 ng/mL; $p < 0.001$	LC-MS
Porter et al. (2010) [84]	Mean: 20.1 ng/mL; range: 7.9–42.9 ng/mL	Mean: 193.6 ng/mL; range: 82.9–328.8 ng/mL $p < 0.001$	Mean: 77.4 ng/mL; range: 39.4–338.8 ng/mL	Mean: 725.9 ng/mL; range: 311.6–1294.1 ng/mL; $p < 0.001$	GC–MS
Boenzi et al. (2014) [85]	Median: 6.4 ng/mL range: 3.7–21.8 ng/mL	Median: 55.5 ng/mL; range: 33.4–184.3 ng/mL $p < 0.001$	Median: 26.6 ng/mL range: 12.8–38.8 ng/mL	Median: 120.0 ng/mL range: 33.3–253.3 ng/mL $p < 0.001$	LC-MS
Zhang et al. (2014) [86]	–	–	5.5 ± 5.2 ng/mL mean ± SD	20.9 ± 17.2 ng/mL mean ± SD	LC-MS
Reunert et al. (2015) [87]	< 50 ng/mL	Range: 50–840 ng/mL	–	–	GC–MS
Romanello et al. (2016) [90]	Median: 9.03 ng/mL range: 5.28–16.98 ng/mL	Median: 48.44 ng/mL; range: 8.13–483.52 ng/mL, $p < 0.001$	Median: 27.08 ng/mL; range: 13.76–40.32 ng/mL	Median: 137.95 ng/mL; range: 31.41–949.91 ng/mL, $p < 0.001$	LS-MS
Reference	TriolCh		7-kCh		Method of oxysterol analysis
	Control	NPC2	Control	NPC2	
Boenzi et al. (2014) [85]	Median: 6.4 ng/mL range: 3.7–21.8 ng/mL	162,9 ng/mL	Median: 26.6 ng/mL range: 12.8–38.8 ng/mL	217,4 ng/mL	LC-MS
Reunert et al. (2015) [87,91]	< 50 ng/mL	150 ng/mL; 226 ng/mL; 515 ng/mL	–	–	LC-MS; GC–MS

neurodegenerative disease that is characterized by the loss of dopaminergic neurons in the midbrain and the accumulation of α-synuclein (Lewy bodies) [94]. PD is the second most common neurodegenerative disorder in elderly populations, and it affects 5 million people. The prevalence of PD is expected to double within the next 20 years because of the ageing of the population [95].

There is no specific test to diagnose PD. The current diagnosis of PD is based primarily on neurological examination in search of motor impairment, known as Parkinsonism (akinesia, rest tremor, stiffness and gait disturbances), with many additional non-motor symptoms, such as hyposuria, sleep disorders, psychiatric disorders or functional disorders, behavioural problems and dysautonomics. Imaging tests, such as MRI, CT, ultrasound of the brain, and PET scans, may also be used to help exclude other Parkinsonian syndromes with focal atrophies. However, imaging tests are not helpful for the diagnosing PD. The only useful imaging tests in PD diagnosis are PET and single-photon emission computerized tomography using specific dopaminergic tracers. However, only autopsy can definitely confirm the disease. Therefore, there is a great need to identify an appropriate biomarker for PD.

One consistent observation in PD brains is an accumulation of α-synuclein protein, which is the major constituent of Lewy bodies, with a simultaneous reduction in levels of tyrosine hydroxylase (TH), which is responsible for the rate of dopamine synthesis. A link between oxysterols, TH and α-synuclein protein was also demonstrated [96,97]. Rantham Prabhakara et al. evaluated the effects of 27-hCh on the expression levels of α-synuclein and TH and demonstrated that the oxysterol 27(*S*)-hCh reduced TH expression and increased α-synuclein levels in human neuroblastoma cells via the estrogen receptors (ER) and LXR [96]. The results of Marwarha et al. are consistent with Rantham Prabhakara et al. 24-hCh also increased TH levels in cells [97].

PD patients exhibited significantly higher 7β-hCh, 7-kCh, and 27-hCh, but low 24(*S*)-hCh levels, in plasma compared to healthy controls. No difference in cholesterol level was found between PD patients and healthy controls [98]. Björkhem et al. revealed that 24(*S*)-hCh levels were increased in CSF (2.0 ± 0.2 ng/mL vs. control 1.4 ± 0.5 ng/mL), and its concentration significantly correlated with disease duration [99]. The method used for 24(*S*)-hCh levels analysis was isotope dilution GC–MS. The level of 24(*S*)-hCh in plasma was not altered, which is not consistent with the previous study [98]. The CSF level of 27-hCh was also increased (1.0 ± 0.3 ng/mL vs. control 0.5 ± 0.1 ng/mL), but no correlation between 27-hCh levels in CSF and disease duration was found. Björkhem et al. indicated that oxysterol levels in CSF may be a biomarkers to follow the disease progression [99].

2.6. Amyotrophic lateral sclerosis

Amyotrophic Lateral Sclerosis (ALS), also known as motor neuron disease or Lou Gehrig's disease, is a late-onset fatal neurodegenerative disease that affects motor neurons with an incidence of approximately 1 to 100,000 people. Most ALS cases are sporadic, but 5–10% of the cases are familial ALS (FALS). Sporadic and FALS are associated with the degeneration of cortical and spinal motor neurons [100]. The neurodegeneration of corticosteroid-spinal neurons causes hyperreflexia, reversal of Babinski's sign and spasticity, and degeneration of spinal and bulbar motor neurons, which causes progressive muscle weakness, hyporeflexia, cramps, and fasciculations. ALS often leads to frontotemporal dementia characterized by behavioural disorders because of the substantial neuronal loss [101].

Kim et al., measured by LC-MS method, 24(*S*)-hCh, 27-hCh, 25-hCh levels in the plasma and CSF of non-riluzole ALS patients, riluzole-treated ALS patients, and healthy subjects [102]. The levels of 24(*S*)-hCh and 25-hCh in CSF (2.03 ± 0.63 ng/mL and 0.14 ± 0.06 ng/mL, respectively) were significantly higher in the non-riluzole group compared to the riluzole group (1.33 ± 0.46 ng/mL, $p = 0.006$ and 0.07 ± 0.03 ng/mL, $p = 0.001$) and controls (1.59 ± 0.05 ng/mL, $p = 0.018$ and 0.09 ± 0.04 ng/mL, $p = 0.012$). The levels of 27-hCh

(CSF) and 25-hCh (plasma) were also higher (1.05 ± 0.39 ng/mL and 5.39 ± 1.94, ng/mL, respectively) in non-riluzole ALS patients than controls (0.77 ± 0.32 ng/mL, $p = 0.014$ and 4.27 ± 1.18 ng/mL, $p = 0.017$). Disease severity and progression were also significantly associated with 25-hCh levels in plasma [102]. However, another study investigating the levels of 24(*S*)-hCh (63.4 ± 15.1 vs. control 61.3 ± 12.6 ng/mL), 27-hCh (0.16 ± 0.05 vs. control 0.2 ± 0.05 ng/mL) and 25-hCh (8.2 ± 2.8 vs. control 7.5 ± 2.6 ng/mL) in the plasma of ALS patients, analysed by isotope dilution GC–MS, found no statistically significant correlation between the levels of these oxysterols and the presence of ALS, despite levels that tended to be higher in ALS patients [103]. La Marca et al. [104] demonstrated that 24(*S*)-hCh levels were significantly higher in controls compared to ALS patients in plasma (15.98 ± 1.43 vs. control 20.55 ± 1.89 ng/mL, $p < 0.0001$) and CSF (2.48 ± 0.31 vs control 3.26 ± 0.28 ng/mL, $p < 0.001$). Further, levels of 24(*S*)-hCh esterified were lower in ALS patients than controls in plasma (25.79 ± 3.89 vs. control 68.38 ± 2.68 ng/mL, $p < 0.0001$) and CSF (2.96 ± 0.32 vs. control 6.92 ± 0.48 ng/mL, $p < 0.0001$). These results may be due to oxidative stress, which may be a major cause of limited esterification of 24(*S*)-hCh from ALS patients [104]. These data suggest the use of oxysterols as potential biomarkers in ALS. However, further studies of larger populations are needed.

2.7. Smith-Lemli- Opitz syndrome

SLOS was first described in 1964 by Drs. Smith, Lemli and Opitz. SLOS is a severe autosomal recessive, malformation syndrome that is characterized by intellectual disability and behavioural problems, which result from blockade of the last enzymatic step in cholesterol biosynthesis [105–107]. A defect in the enzyme 7-dehydrocholesterol reductase causes a build-up of the cholesterol precursor 7-dehydrocholesterol (7-dCh) and its isomer 8-dehydrocholesterol (8-dCh) in tissues and blood plasma [65,108]. The BBB prevents the transfer of cholesterol, and the cholesterol in the brain is formed by local synthesis. Therefore, the accumulation of 7-dCh is higher in the brain than elsewhere in SLOS patients [107].

The molecular mechanisms of the SLOS disease suggest that an obvious biomarker for the diagnosis this disease is an increased concentration of 7-dCh with 8-dCh in the body or an increased ratio of the 7-dCh and 8-hCh to cholesterol [109]. However, the metabolism of this sterol is also disturbed because of the dysfunctional cholesterol synthesis. Plasma levels of 24(*S*)-hCh are reduced in SLOS infants (by approximately 50%). The levels of 27-hCh in the circulation are also significantly increased, which was most likely due to the reduced metabolism of this oxysterol [110].

The accumulation of high levels of 7-dCh results in enzymatic and free radical oxidation of 7-dCh, which leads to the formation of new oxysterols that are not found in other diseases. 7-dehydrocholesterol-derived oxysterols were found in a Dhcr7-null mouse model (rodent model of SLOS) in the brain, liver, CNS and serum: 3β,5α-dihydroxycholest-7-en-6-one (dhCeo), 4α-hydroxy-7-dehydrocholesterol (4αh7dCh), 4β-hydroxy-7-dehydrocholesterol (4βh7dCh), 24-hydroxy-7-dehydrocholesterol and 7-kCh [111–114]. Griffins et al. recently found additional oxysterol metabolites of 8-dCh in the plasma of SLOS patients, including hydroxy-8-dehydrocholesterol, which is 24- or 25-hydroxy-8-dehydrocholesterol and 26-hydroxy-8-dehydrocholesterol, 4αh7dCh, dhCeo and 7α,8α-epoxycholesterol (Fig. 3). None of these metabolites were detected in control plasma [115]. The concentrations of 7β-hCh and 7-kCh in plasma SLOS patients were increased, and the levels of 7-kCh positively correlated with the severity scores, which suggests that this oxysterol plays some important roles in the pathogenesis of SLOS and may be used as a diagnosis tool for disease progression [116]. More research must be performed to identify unique oxysterols that may be used as diagnosis/progression biomarkers in SLOS patients.

2.8. Autism spectrum disorders

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder that occurs within the first 3 years of life, and it is characterized by pervasive difficulties that being in early childhood [117,118]. ASD includes a wide spectrum of clinically and biologically heterogeneous disorders that present with various types of symptoms: problems in social communication and social interaction, impaired speech and nonverbal communication, and restricted, repetitive patterns of behaviour, interests or activities [119].

A study of 100 samples from the Autism Genetic Resource Exchange on cholesterol levels in serum was performed. Approximately 20% of children with ASD exhibited substantial hypocholesterolemia [120], which interfered with three mechanisms during brain development: impaired sonic hedgehog patterning, alterations in membrane lipid raft structure and protein function resulting in abnormal synaptic plasticity, and impaired neurosteroids synthesis [121]. The disorders in cholesterol homeostasis or metabolism suggest that the profiles and levels of oxysterols are also altered.

Measured by GC–MS method significantly higher concentrations of 7α-hCh, 7β-hCh, 7-kCh, 4α-hCh, α-epoxyCh, β-epoxyCh, triolCh, 5α-hydroxy-6-oxocholesterol, 4β-hCh, 25-hCh, 22-hydroxycholesterol (22(*R*)-hCh), 24(*S*)-hCh and 27-hCh – 24(*S*)-hCh were found in human children plasma of ASD patients (128.13 ± 29,93 ng/mL, $p < 0.001$) compared to a control group (93.96 ± 20.13 ng/mL) [122]. Greyaa et al. provided the first evidence of a relationship between autism and oxysterol levels and suggested a potential role of 24(*S*)-hCh as a diagnostic marker for ASD. However, more research in this area must be performed [122].

2.9. Spastic paraplegia type 5

Spastic paraplegia type 5 (SPG5) is an autosomal recessive subtype of hereditary spastic paraplegia and a neurodegenerative disorder that is defined by a progressive neurodegeneration of the corticospinal tract motor neurons due to mutations in CYP7B1, which encodes oxysterol 7α-hydroxylase [123]. Oxysterol 7α-hydroxylase is one of the enzymes in the synthesis of bile acids from cholesterol.

SPG5 is easily diagnosed thanks to the significant elevation of two plasma oxysterols, 25-hCh and 27-hCh. Schüle et al. investigated four patients with the SPG5 disease and found 6- to 9-fold increased plasma levels of 27-hCh and 30- to 50-fold in the CSF. The plasma levels of 25-hCh were also increased approximately 100-fold [124]. Increases in 25-hCh (285.3 ± 249.3 nmol/L vs. control 15.3 ± 17.5 nmol/L; $p < 0.001$) and 27-hCh (1580 ± 498 nmol/L vs. control 139.3 ± 76.3 nmol/L; $p < 0.001$) were also demonstrated in SPG5 patients in a study by Marelli et al., who found, using LC-MS method, an average 19-fold and 11-fold increase in plasma levels compared to control, respectively [125]. These data indicate that increases in 25-hCh and 27-hCh oxysterols should be included in the screening of SPG5. However, further studies on larger population are needed to acquire confirmation.

3. Conclusion

The elevated levels of oxysterols have shown promise for assessing risk or diagnosis of CAD and atherosclerosis. The potential usage of appropriate oxysterol could provide fast, simple and non-invasive diagnostic method even at an early stage when it is not yet possible to diagnosis it with currently used tests. The available data also suggest that oxysterols are particularly useful as a tool for diagnosing and/or monitoring of the progression of neurodegenerative diseases. This characteristic is extremely important for the diagnosis of PD, which lacks specific tests for diagnosis.

In most of presented studies, oxysterols contents have been measured using GC–MS or LC-MS, due to highly specific and more sensitive

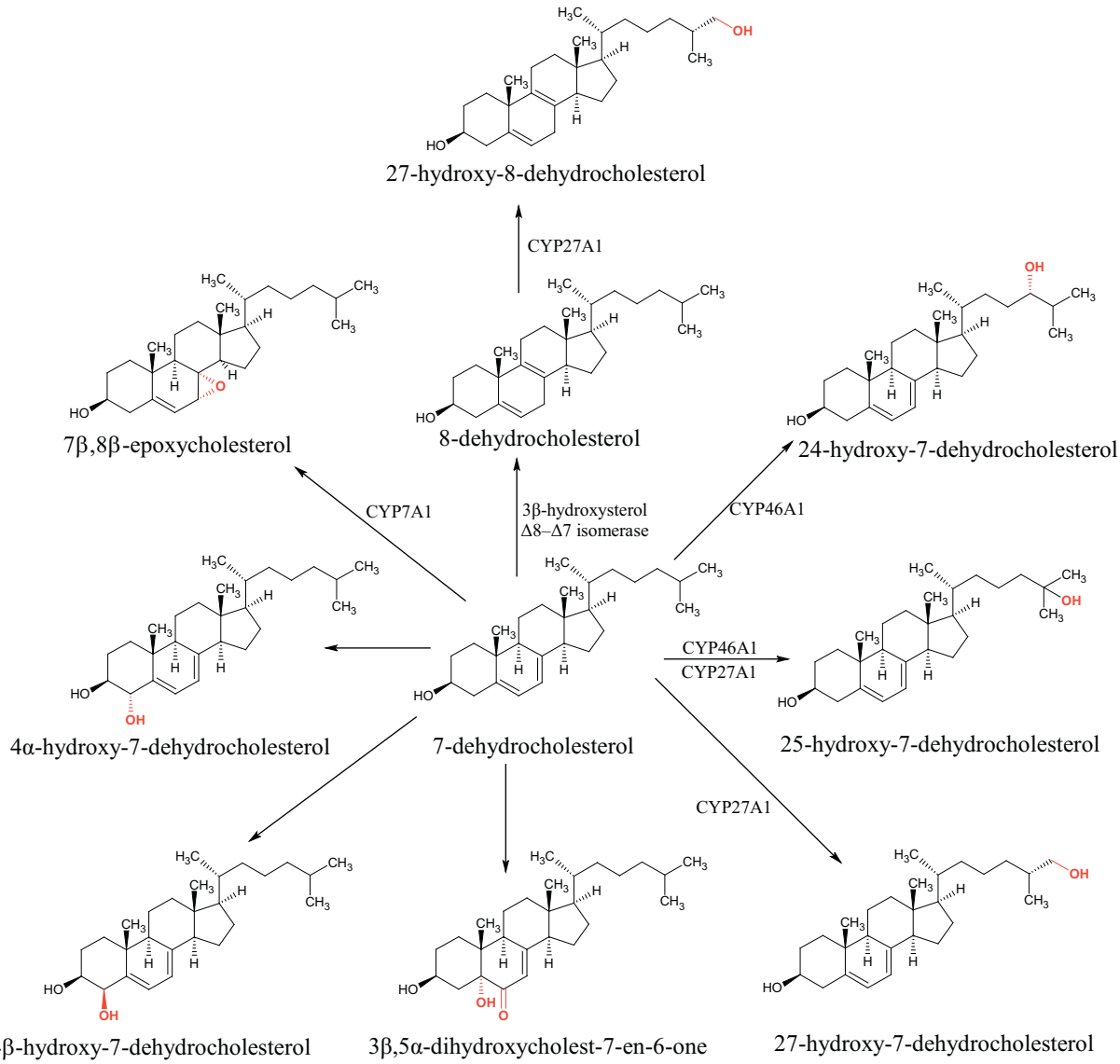

Fig. 3. Oxysterols derived from 7-dehydrocholesterol, which are found in SLOS patients.

detection compared to other detectors. Each year more reliable mass spectrometers are available with higher sensitivity and reproducible results. The mass spectrometry is not generally cost-effective and it requires a high level of operator expertise, and therefore is not well suited for routine clinical laboratories, however, the ongoing technological advancements are reducing the cost of detectors, which overall may improve their clinical utility in the future.

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References

[1] T. Li, J.Y.L. Chiang, Regulation of bile acid and cholesterol metabolism by PPARs, PPAR Res. 2009 (2009), https://doi.org/10.1155/2009/501739.
[2] A.H. Payne, D.B. Hales, Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones, Endocr. Rev. 25 (2004) 947–970, https://doi.org/10.1210/er.2003-0030.
[3] H.F. DeLuca, Overview of general physiologic features and functions of vitamin D, Am. J. Clin. Nutr. 80 (2004) 1689S–1696S, https://doi.org/10.1093/ajcn/80.6.1689S.
[4] 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, https://doi.org/10.1194/jlr.R400004-JLR200.
[5] A.J. Brown, W. Jessup, Oxysterols and atherosclerosis, Atherosclerosis 142 (1999) 1–28.
[6] D.W. Russell, Oxysterol biosynthetic enzymes, Biochimica et Biophysica Acta (BBA) - Mol. Cell Biol. Lipids 1529 (2000) 126–135, https://doi.org/10.1016/S1388-1981(00)00142-6.
[7] A.J. Brown, W. Jessup, Oxysterols: sources, cellular storage and metabolism, and new insights into their roles in cholesterol homeostasis, Mol. Asp. Med. 30 (2009) 111–122, https://doi.org/10.1016/j.mam.2009.02.005.
[8] S. Meaney, M. Heverin, U. Panzenboeck, L. Ekström, M. Axelsson, U. Andersson, U. Diczfalusy, I. Pikuleva, J. Wahren, W. Sattler, I. Björkhem, Novel route for elimination of brain oxysterols across the blood-brain barrier: conversion into 7alpha-hydroxy-3-oxo-4-cholestenoic acid, J. Lipid Res. 48 (2007) 944–951, https://doi.org/10.1194/jlr.M600529-JLR200.
[9] A. Zmysłowski, A. Szterk, Current knowledge on the mechanism of atherosclerosis and pro-atherosclerotic properties of oxysterols, Lipids Health Dis. 16 (2017) 188, https://doi.org/10.1186/s12944-017-0579-2.
[10] G. Lizard, V. Deckert, L. Dubrez, M. Moisant, P. Gambert, L. Lagrost, Induction of apoptosis in endothelial cells treated with cholesterol oxides, Am. J. Pathol. 148 (1996) 1625–1638.
[11] X.M. Yuan, W. Li, U.T. Brunk, H. Dalen, Y.H. Chang, A. Sevanian, Lysosomal

destabilization during macrophage damage induced by cholesterol oxidation products, Free Radic. Biol. Med. 28 (2000) 208–218, https://doi.org/10.1016/S0891-5849(99)00220-8.
[12] M.P.S. Ares, M.I. Pörn-Ares, S. Moses, J. Thyberg, L. Juntti-Berggren, P.-O. Berggren, A. Hultgårdh-Nilsson, B. Kallin, J. Nilsson, 7β-Hydroxycholesterol induces Ca2+ oscillations, MAP kinase activation and apoptosis in human aortic smooth muscle cells, Atherosclerosis 153 (2000) 23–35, https://doi.org/10.1016/S0021-9150(00)00380-4.
[13] W.J. Griffiths, J. Abdel-Khalik, P.J. Crick, E. Yutuc, Y. Wang, New methods for analysis of oxysterols and related compounds by LC–MS, J. Steroid Biochem. Mol. Biol. 162 (2016) 4–26, https://doi.org/10.1016/j.jsbmb.2015.11.017.
[14] J.T. Salonen, K. Nyysso¨nen, R. Salonen, E. Porkkala-Sarataho, T.-P. Tuomainen, U. Diczfalusy, I. Bjo¨rkhem, Lipoprotein oxidation and progression of carotid atherosclerosis, Circulation 95 (1997) 840–845, https://doi.org/10.1161/01.CIR.95.4.840.
[15] B. Ziedén, A. Kaminskas, M. Kristenson, Z. Kucinskienê, B. Vessby, A.G. Olsson, U. Diczfalusy, Increased plasma 7 beta-hydroxycholesterol concentrations in a population with a high risk for cardiovascular disease, Arterioscler. Thromb. Vasc. Biol. 19 (1999) 967–971.
[16] A. Rimner, S. Al Makdessi, H. Sweidan, J. Wischhusen, B. Rabenstein, K. Shatat, P. Mayer, I. Spyridopoulos, Relevance and mechanism of oxysterol stereospecifity in coronary artery disease, Free Radic. Biol. Med. 38 (2005) 535–544, https://doi.org/10.1016/j.freeradbiomed.2004.11.016.
[17] Y. Yasunobu, K. Hayashi, T. Shingu, T. Yamagata, G. Kajiyama, M. Kambe, Coronary atherosclerosis and oxidative stress as reflected by autoantibodies against oxidized low-density lipoprotein and oxysterols, Atherosclerosis 155 (2001) 445–453, https://doi.org/10.1016/S0021-9150(00)00581-5.
[18] S. Khatib, J. Vaya, Oxysterols and symptomatic versus asymptomatic human atherosclerotic plaque, Biochem. Biophys. Res. Commun. 446 (2014) 709–713, https://doi.org/10.1016/j.bbrc.2013.12.116.
[19] M. Umetani, P. Ghosh, T. Ishikawa, J. Umetani, M. Ahmed, C. Mineo, P.W. Shaul, The cholesterol metabolite 27-hydroxycholesterol promotes atherosclerosis via proinflammatory processes mediated by estrogen receptor alpha, Cell Metab. 20 (2014) 172–182, https://doi.org/10.1016/j.cmet.2014.05.013.
[20] A. Fuhrmann, O. Weingärtner, S. Meyer, B. Cremers, S. Seiler-Mußler, H.-F. Schött, A. Kerksiek, S. Friedrichs, U. Ulbricht, A.M. Zawada, U. Laufs, B. Scheller, D. Fliser, P.C. Schulze, M. Böhm, G.H. Heine, D. Lütjohann, Plasma levels of the oxyphytosterol 7α-hydroxycampesterol are associated with cardiovascular events, Atherosclerosis (2018), https://doi.org/10.1016/j.atherosclerosis.2018.10.010.
[21] V. Aboyans, J.-B. Ricco, M.-L.E.L. Bartelink, M. Björck, M. Brodmann, T. Cohnert, J.-P. Collet, M. Czerny, M. De Carlo, S. Debus, C. Espinola-Klein, T. Kahan, S. Kownator, L. Mazzolai, A.R. Naylor, M. Roffi, J. Röther, M. Sprynger, M. Tendera, G. Tepe, M. Venermo, C. Vlachopoulos, I. Desormais, ESC Scientific Document Group, 2017 ESC guidelines on the diagnosis and treatment of peripheral arterial diseases, in collaboration with the European Society for Vascular Surgery (ESVS): Document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteriesEndorsed by: the European Stroke Organization (ESO) the task force for the diagnosis and treatment of peripheral arterial diseases of the European Society of Cardiology (ESC) and of the European Society for Vascular Surgery (ESVS), Eur. Heart J. 39 (2018) 763–816, https://doi.org/10.1093/eurheartj/ehx095.
[22] M.R. Dweck, M.K. Doris, M. Motwani, P.D. Adamson, P. Slomka, D. Dey, Z.A. Fayad, D.E. Newby, D. Berman, Imaging of coronary atherosclerosis — evolution towards new treatment strategies, Nat. Rev. Cardiol. 13 (2016) 533–548, https://doi.org/10.1038/nrcardio.2016.79.
[23] J.D. Anderson, C.M. Kramer, MRI of atherosclerosis: diagnosis and monitoring therapy, Expert. Rev. Cardiovasc. Ther. 5 (2007) 69–80, https://doi.org/10.1586/14779072.5.1.69.
[24] B. Schroeder, G. Francis, J. Leipsic, B. Heilbron, G.B. John Mancini, C.M. Taylor, Early atherosclerosis detection in asymptomatic patients: a comparison of carotid ultrasound, coronary artery calcium score, and coronary computed tomography angiography, Can. J. Cardiol. 29 (2013) 1687–1694, https://doi.org/10.1016/j.cjca.2013.10.003.
[25] Alzheimer's Association, Alzheimer's disease facts and figures, Alzheimers Dement. 12 (2016) (2016) 459–509.
[26] K. Blennow, H. Zetterberg, Cerebrospinal fluid biomarkers for Alzheimer's disease, J. Alzheimers Dis. 18 (2009) 413–417, https://doi.org/10.3233/JAD-2009-1177.
[27] M. Ewers, N. Mattsson, L. Minthon, J.L. Molinuevo, A. Antonell, J. Popp, F. Jessen, S.-K. Herukka, H. Soininen, W. Maetzler, T. Leyhe, K. Bürger, M. Taniguchi, K. Urakami, S. Lista, B. Dubois, K. Blennow, H. Hampel, CSF biomarkers for the differential diagnosis of Alzheimer's disease: a large-scale international multicenter study, Alzheimers Dement. 11 (2015) 1306–1315, https://doi.org/10.1016/j.jalz.2014.12.006.
[28] L. Puglielli, R.E. Tanzi, D.M. Kovacs, Alzheimer's disease: the cholesterol connection, Nat. Neurosci. 6 (2003) 345–351, https://doi.org/10.1038/nn0403-345.
[29] A.R. Koudinov, N.V. Koudinova, Essential role for cholesterol in synaptic plasticity and neuronal degeneration, FASEB J. 15 (2001) 1858–1860.
[30] D. Lütjohann, S. Meichsner, H. Pettersson, Lipids in Alzheimer's disease and their potential for therapy, Clin. Lipidol. 7 (2012) 65–78, https://doi.org/10.2217/clp.11.74.
[31] D. Lütjohann, O. Breuer, G. Ahlborg, I. Nennesmo, A. Sidén, U. Diczfalusy, I. Björkhem, Cholesterol homeostasis in human brain: evidence for an age-dependent flux of 24S-hydroxycholesterol from the brain into the circulation, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 9799–9804.
[32] C. Xie, E.G. Lund, S.D. Turley, D.W. Russell, J.M. Dietschy, Quantitation of two pathways for cholesterol excretion from the brain in normal mice and mice with neurodegeneration, J. Lipid Res. 44 (2003) 1780–1789, https://doi.org/10.1194/jlr.M300164-JLR200.
[33] D. Famer, S. Meaney, M. Mousavi, A. Nordberg, I. Björkhem, M. Crisby, Regulation of alpha- and beta-secretase activity by oxysterols: cerebrosterol stimulates processing of APP via the alpha-secretase pathway, Biochem. Biophys. Res. Commun. 359 (2007) 46–50, https://doi.org/10.1016/j.bbrc.2007.05.033.
[34] M. Heverin, N. Bogdanovic, D. Lütjohann, T. Bayer, I. Pikuleva, L. Bretillon, U. Diczfalusy, B. Winblad, I. Björkhem, Changes in the levels of cerebral and extracerebral sterols in the brain of patients with Alzheimer's disease, J. Lipid Res. 45 (2004) 186–193, https://doi.org/10.1194/jlr.M300320-JLR200.
[35] V. Leoni, T. Masterman, P. Patel, S. Meaney, U. Diczfalusy, I. Björkhem, Side chain oxidized oxysterols in cerebrospinal fluid and the integrity of blood-brain and blood-cerebrospinal fluid barriers, J. Lipid Res. 44 (2003) 793–799, https://doi.org/10.1194/jlr.M200434-JLR200.
[36] L. Mateos, S. Akterin, F.-J. Gil-Bea, S. Spulber, A. Rahman, I. Björkhem, M. Schultzberg, A. Flores-Morales, A. Cedazo-Mínguez, Activity-regulated cytoskeleton-associated protein in rodent brain is down-regulated by high fat diet in vivo and by 27-hydroxycholesterol in vitro, Brain Pathol. 19 (2009) 69–80, https://doi.org/10.1111/j.1750-3639.2008.00174.x.
[37] K. Blennow, H. Hampel, CSF markers for incipient Alzheimer's disease, Lancet Neurol. 2 (2003) 605–613.
[38] H.-L. Wang, Y.-Y. Wang, X.-G. Liu, S.-H. Kuo, N. Liu, Q.-Y. Song, M.-W. Wang, Cholesterol, 24-hydroxycholesterol, and 27-hydroxycholesterol as surrogate biomarkers in cerebrospinal fluid in mild cognitive impairment and Alzheimer's disease: a meta-analysis, J. Alzheimers Dis. 51 (2016) 45–55, https://doi.org/10.3233/JAD-150734.
[39] J. Popp, P. Lewczuk, H. Kölsch, S. Meichsner, W. Maier, J. Kornhuber, F. Jessen, D. Lütjohann, Cholesterol metabolism is associated with soluble amyloid precursor protein production in Alzheimer's disease, J. Neurochem. 123 (2012) 310–316, https://doi.org/10.1111/j.1471-4159.2012.07893.x.
[40] V. Leoni, A. Solomon, A. Lövgren-Sandblom, L. Minthon, K. Blennow, O. Hansson, L.-O. Wahlund, M. Kivipelto, I. Björkhem, Diagnostic power of 24S-hydroxycholesterol in cerebrospinal fluid: candidate marker of brain health, J. Alzheimers Dis. 36 (2013) 739–747, https://doi.org/10.3233/JAD-130035.
[41] T.M. Hughes, C. Rosano, R.W. Evans, L.H. Kuller, Brain cholesterol metabolism, oxysterols, and dementia, J. Alzheimers Dis. 33 (2013) 891–911, https://doi.org/10.3233/JAD-2012-121585.
[42] V. Leoni, Oxysterols as markers of neurological disease—a review, Scand. J. Clin. Lab. Invest. 69 (2009) 22–25, https://doi.org/10.1080/00365510802651858.
[43] V. Leoni, M. Shafaati, A. Salomon, M. Kivipelto, I. Björkhem, L.O. Wahlund, Are the CSF levels of 24S-hydroxycholesterol a sensitive biomarker for mild cognitive impairment? Neurosci. Lett. 397 (2006) 83–87, https://doi.org/10.1016/j.neulet.2005.11.046.
[44] I. Björkhem, A. Cedazo-Minguez, V. Leoni, S. Meaney, Oxysterols and neurodegenerative diseases, Mol. Asp. Med. 30 (2009) 171–179, https://doi.org/10.1016/j.mam.2009.02.001.
[45] I. Björkhem, Crossing the barrier: oxysterols as cholesterol transporters and metabolic modulators in the brain, J. Intern. Med. 260 (2006) 493–508, https://doi.org/10.1111/j.1365-2796.2006.01725.x.
[46] F. Glöckner, V. Meske, D. Lütjohann, T.G. Ohm, Dietary cholesterol and its effect on tau protein: a study in apolipoprotein E-deficient and P301L human tau mice, J. Neuropathol. Exp. Neurol. 70 (2011) 292–301, https://doi.org/10.1097/NEN.0b013e318212f185.
[47] V. Leoni, T. Masterman, F.S. Mousavi, B. Wretlind, L.-O. Wahlund, U. Diczfalusy, J. Hillert, I. Björkhem, Diagnostic use of cerebral and extracerebral oxysterols, Clin. Chem. Lab. Med. 42 (2004) 186–191, https://doi.org/10.1515/CCLM.2004.034.
[48] A. Papassotiropoulos, D. Lütjohann, M. Bagli, S. Locatelli, F. Jessen, R. Buschfort, U. Ptok, I. Björkhem, K. von Bergmann, R. Heun, 24S-hydroxycholesterol in cerebrospinal fluid is elevated in early stages of dementia, J. Psychiatr. Res. 36 (2002) 27–32.
[49] J.L.W. Yau, S. Rasmuson, R. Andrew, M. Graham, J. Noble, T. Olsson, E. Fuchs, R. Lathe, J.R. Seckl, Dehydroepiandrosterone 7-hydroxylase CYP7B: predominant expression in primate hippocampus and reduced expression in Alzheimer's disease, Neuroscience 121 (2003) 307–314.
[50] X. Jiang, M. Guo, J. Su, B. Lu, D. Ma, R. Zhang, L. Yang, Q. Wang, Y. Ma, Y. Fan, Simvastatin blocks blood-brain barrier disruptions induced by elevated cholesterol both in vivo and in vitro, Int. J. Alzheimers Dis. 2012 (2012) 109324, https://doi.org/10.1155/2012/109324.
[51] R. Deane, B.V. Zlokovic, Role of the blood-brain barrier in the pathogenesis of Alzheimer's disease, Curr. Alzheimer Res. 4 (2007) 191–197.
[52] A. Saeed, F. Floris, U. Andersson, I. Pikuleva, A. Lövgren-Sandblom, M. Bjerke, M. Paucar, A. Wallin, P. Svenningsson, I. Björkhem, 7α-hydroxy-3-oxo-4-cholestenoic acid in cerebrospinal fluid reflects the integrity of the blood-brain barrier, J. Lipid Res. 55 (2014) 313–318, https://doi.org/10.1194/jlr.P044982.
[53] W.-J. Huang, W.-W. Chen, X. Zhang, Multiple sclerosis: pathology, diagnosis and treatments, Exp. Ther. Med. 13 (2017) 3163–3166, https://doi.org/10.3892/etm.2017.4410.
[54] J.H. Noseworthy, C. Lucchinetti, M. Rodriguez, B.G. Weinshenker, Multiple sclerosis, N. Engl. J. Med. 343 (2000) 938–952, https://doi.org/10.1056/NEJM200009283431307.
[55] B.F.G. Popescu, I. Pirko, C.F. Lucchinetti, Pathology of multiple sclerosis: where do we stand? Continuum (Minneap Minn) 19 (2013) 901–921, https://doi.org/10.1212/01.CON.0000433291.23091.65.
[56] J. Antel, S. Antel, Z. Caramanos, D.L. Arnold, T. Kuhlmann, Primary progressive multiple sclerosis: part of the MS disease spectrum or separate disease entity? Acta

Neuropathol. 123 (2012) 627–638, https://doi.org/10.1007/s00401-012-0953-0.
[57] V. Leoni, T. Masterman, U. Diczfalusy, G. De Luca, J. Hillert, I. Björkhem, Changes in human plasma levels of the brain specific oxysterol 24S-hydroxycholesterol during progression of multiple sclerosis, Neurosci. Lett. 331 (2002) 163–166.
[58] C.E. Teunissen, C.D. Dijkstra, C.H. Polman, E.L.J. Hoogervorst, K. von Bergmann, D. Lütjohann, Decreased levels of the brain specific 24S-hydroxycholesterol and cholesterol precursors in serum of multiple sclerosis patients, Neurosci. Lett. 347 (2003) 159–162.
[59] S. Mukhopadhyay, K. Fellows, R.W. Browne, P. Khare, S. Krishnan Radhakrishnan, J. Hagemeier, B. Weinstock-Guttman, R. Zivadinov, M. Ramanathan, Interdependence of oxysterols with cholesterol profiles in multiple sclerosis, Mult. Scler. 23 (2017) 792–801, https://doi.org/10.1177/1352458516666187.
[60] F.O. Walker, Huntington's disease, Lancet 369 (2007) 218–228, https://doi.org/10.1016/S0140-6736(07)60111-1.
[61] J.M. Gil, A.C. Rego, Mechanisms of neurodegeneration in Huntington's disease, Eur. J. Neurosci. 27 (2008) 2803–2820, https://doi.org/10.1111/j.1460-9568.2008.06310.x.
[62] G.M. Halliday, D.A. McRitchie, V. Macdonald, K.L. Double, R.J. Trent, E. McCusker, Regional specificity of brain atrophy in Huntington's disease, Exp. Neurol. 154 (1998) 663–672, https://doi.org/10.1006/exnr.1998.6919.
[63] M. Katsuno, H. Adachi, G. Sobue, Getting a handle on Huntington's disease: the case for cholesterol, Nat. Med. 15 (2009) 253–254, https://doi.org/10.1038/nm0309-253.
[64] S. Sipione, D. Rigamonti, M. Valenza, C. Zuccato, L. Conti, J. Pritchard, C. Kooperberg, J.M. Olson, E. Cattaneo, Early transcriptional profiles in huntingtin-inducible striatal cells by microarray analyses, Hum. Mol. Genet. 11 (2002) 1953–1965, https://doi.org/10.1093/hmg/11.17.1953.
[65] H.R. Waterham, R.J. Wanders, Biochemical and genetic aspects of 7-dehydrocholesterol reductase and Smith-Lemli-Opitz syndrome, Biochim. Biophys. Acta 1529 (2000) 340–356.
[66] M. Valenza, V. Leoni, J.M. Karasinska, L. Petricca, J. Fan, J. Carroll, M.A. Pouladi, E. Fossale, H.P. Nguyen, O. Riess, M. MacDonald, C. Wellington, S. DiDonato, M. Hayden, E. Cattaneo, Cholesterol defect is marked across multiple rodent models of Huntington's disease and is manifest in astrocytes, J. Neurosci. 30 (2010) 10844–10850, https://doi.org/10.1523/JNEUROSCI.0917-10.2010.
[67] D. del Toro, X. Xifró, A. Pol, S. Humbert, F. Saudou, J.M. Canals, J. Alberch, Altered cholesterol homeostasis contributes to enhanced excitotoxicity in Huntington's disease, J. Neurochem. 115 (2010) 153–167, https://doi.org/10.1111/j.1471-4159.2010.06912.x.
[68] 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, https://doi.org/10.1093/hmg/ddl434.
[69] M. Martinez-Vicente, Z. Talloczy, E. Wong, G. Tang, H. Koga, S. Kaushik, R. de Vries, E. Arias, S. Harris, D. Sulzer, A.M. Cuervo, Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease, Nat. Neurosci. 13 (2010) 567–576, https://doi.org/10.1038/nn.2528.
[70] M. Valenza, J.B. Carroll, V. Leoni, L.N. Bertram, I. Björkhem, R.R. Singaraja, S. Di Donato, D. Lutjohann, M.R. Hayden, E. Cattaneo, Cholesterol biosynthesis pathway is disturbed in YAC128 mice and is modulated by huntingtin mutation, Hum. Mol. Genet. 16 (2007) 2187–2198, https://doi.org/10.1093/hmg/ddm170.
[71] V. Leoni, C. Mariotti, S.J. Tabrizi, M. Valenza, E.J. Wild, S.M.D. Henley, N.Z. Hobbs, M.L. Mandelli, M. Grisoli, I. Björkhem, E. Cattaneo, S. Di Donato, Plasma 24S-hydroxycholesterol and caudate MRI in pre-manifest and early Huntington's disease, Brain 131 (2008) 2851–2859, https://doi.org/10.1093/brain/awn212.
[72] V. Leoni, C. Mariotti, L. Nanetti, E. Salvatore, F. Squitieri, A.R. Bentivoglio, M. Bandettini di Poggio, M. Bandettini Del Poggio, S. Piacentini, D. Monza, M. Valenza, E. Cattaneo, S. Di Donato, Whole body cholesterol metabolism is impaired in Huntington's disease, Neurosci. Lett. 494 (2011) 245–249, https://doi.org/10.1016/j.neulet.2011.03.025.
[73] V. Leoni, J.D. Long, J.A. Mills, S. Di Donato, J.S. Paulsen, Plasma 24S-hydroxycholesterol correlation with markers of Huntington disease progression, Neurobiol. Dis. 55 (2013) 37–43, https://doi.org/10.1016/j.nbd.2013.03.013.
[74] T.-Y. Chang, P.C. Reid, S. Sugii, N. Ohgami, J.C. Cruz, C.C.Y. Chang, Niemann-Pick Type C disease and intracellular cholesterol trafficking, J. Biol. Chem. 280 (2005) 20917–20920, https://doi.org/10.1074/jbc.R400040200.
[75] M.T. Vanier, G. Millat, Niemann-Pick disease type C, Clin. Genet. 64 (2003) 269–281.
[76] E.D. Carstea, J.A. Morris, K.G. Coleman, S.K. Loftus, D. Zhang, C. Cummings, J. Gu, M.A. Rosenfeld, W.J. Pavan, D.B. Krizman, J. Nagle, M.H. Polymeropoulos, S.L. Sturley, Y.A. Ioannou, M.E. Higgins, M. Comly, A. Cooney, A. Brown, C.R. Kaneski, E.J. Blanchette-Mackie, N.K. Dwyer, E.B. Neufeld, T.Y. Chang, L. Liscum, J.F. Strauss, K. Ohno, M. Zeigler, R. Carmi, J. Sokol, D. Markie, R.R. O'Neill, O.P. Vvan Diggelen, M. Elleder, M.C. Patterson, R.O. Brady, M.T. Vanier, P.G. Pentchev, D.A. Tagle, Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis, Science 277 (1997) 228–231.
[77] S. Naureckiene, D.E. Sleat, H. Lackland, A. Fensom, M.T. Vanier, R. Wattiaux, M. Jadot, P. Lobel, Identification of HE1 as the second gene of Niemann-Pick C disease, Science 290 (2000) 2298–2301, https://doi.org/10.1126/science.290.5500.2298.
[78] D.S. Ory, Niemann-Pick type C: a disorder of cellular cholesterol trafficking, Biochim. Biophys. Acta 1529 (2000) 331–339.
[79] S.L. Sturley, M.C. Patterson, P. Pentchev, Unraveling the sterol-trafficking defect in Niemann-Pick C disease, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 2093–2094, https://doi.org/10.1073/pnas.0812934106.
[80] G.S. Ribas, R. Pires, J.C. Coelho, D. Rodrigues, C.P. Mescka, C.S. Vanzin, G.B. Biancini, G. Negretto, C.A.Y. Wayhs, M. Wajner, C.R. Vargas, Oxidative stress in Niemann-Pick type C patients: a protective role of N-butyl-deoxynojirimycin therapy, Int. J. Dev. Neurosci. 30 (2012) 439–444, https://doi.org/10.1016/j.ijdevneu.2012.07.002.
[81] G.S. Tint, P. Pentchev, G. Xu, A.K. Batta, S. Shefer, G. Salen, A. Honda, Cholesterol and oxygenated cholesterol concentrations are markedly elevated in peripheral tissue but not in brain from mice with the Niemann-Pick type C phenotype, J. Inherit. Metab. Dis. 21 (1998) 853–863.
[82] J.R. Zhang, T. Coleman, S.J. Langmade, D.E. Scherrer, L. Lane, M.H. Lanier, C. Feng, M.S. Sands, J.E. Schaffer, C.F. Semenkovich, D.S. Ory, Niemann-Pick C1 protects against atherosclerosis in mice via regulation of macrophage intracellular cholesterol trafficking, J. Clin. Invest. 118 (2008) 2281–2290, https://doi.org/10.1172/JCI32561.
[83] X. Jiang, R. Sidhu, F.D. Porter, N.M. Yanjanin, A.O. Speak, D.T. te Vruchte, F.M. Platt, H. Fujiwara, D.E. Scherrer, J. Zhang, D.J. Dietzen, J.E. Schaffer, D.S. Ory, A sensitive and specific LC-MS/MS method for rapid diagnosis of Niemann-Pick C1 disease from human plasma, J. Lipid Res. 52 (2011) 1435–1445, https://doi.org/10.1194/jlr.D015735.
[84] F.D. Porter, D.E. Scherrer, M.H. Lanier, S.J. Langmade, V. Molugu, S.E. Gale, D. Olzeski, R. Sidhu, D.J. Dietzen, R. Fu, C.A. Wassif, N.M. Yanjanin, S.P. Marso, J. House, C. Vite, J.E. Schaffer, D.S. Ory, Cholesterol oxidation products are sensitive and specific blood-based biomarkers for Niemann-Pick C1 disease, Sci. Transl. Med. 2 (2010) 56ra81, https://doi.org/10.1126/scitranslmed.3001417.
[85] S. Boenzi, F. Deodato, R. Taurisano, D. Martinelli, D. Verrigni, R. Carrozzo, E. Bertini, A. Pastore, C. Dionisi-Vici, D.W. Johnson, A new simple and rapid LC–ESI-MS/MS method for quantification of plasma oxysterols as dimethylaminobutyrate esters. Its successful use for the diagnosis of Niemann–Pick type C disease, Clin. Chim. Acta 437 (2014) 93–100, https://doi.org/10.1016/j.cca.2014.07.010.
[86] H. Zhang, Y. Wang, N. Lin, R. Yang, W. Qiu, L. Han, J. Ye, X. Gu, Diagnosis of Niemann-Pick disease type C with 7-ketocholesterol screening followed by NPC1/NPC2 gene mutation confirmation in Chinese patients, Orphanet J. Rare Dis. 9 (2014) 82, https://doi.org/10.1186/1750-1172-9-82.
[87] J. Reunert, M. Fobker, F. Kannenberg, I. Du Chesne, M. Plate, J. Wellhausen, S. Rust, T. Marquardt, Rapid diagnosis of 83 patients with Niemann Pick Type C disease and related cholesterol transport disorders by cholestantriol screening, EBioMedicine 4 (2015) 170–175, https://doi.org/10.1016/j.ebiom.2015.12.018.
[88] J. Reunert, F. Kannenberg, M. Fobker, T. Marquardt, Improved Diagnostics of Niemann–Pick Disease Type C by the Analysis of Plasma Oxysterols, Vol. 114 MGM, 2015, p. S98, https://doi.org/10.1016/j.ymgme.2014.12.219.
[89] H.J. Church, H. Wu, J. Cooper, K.L. Tylee, L. Heptinstall, C. Hartley, S. Phillippo, E. Jameson, A. Broomfield, C. Hendriksz, S. Jones, Successful Implementation of Plasma Oxysterol for Screening of Niemann-Pick Disease Type C in Manchester, UK, MGM, 117 (2016), p. S35, https://doi.org/10.1016/j.ymgme.2015.12.224.
[90] M. Romanello, S. Zampieri, N. Bortolotti, L. Deroma, A. Sechi, A. Fiumara, R. Parini, B. Borroni, F. Brancati, A. Bruni, C.V. Russo, A. Bordugo, B. Bembi, A. Dardis, Comprehensive evaluation of plasma 7-ketocholesterol and cholestan-3β,5α,6β-triol in an Italian cohort of patients affected by Niemann-Pick disease due to NPC1 and SMPD1 mutations, Clin. Chim. Acta 455 (2016) 39–45, https://doi.org/10.1016/j.cca.2016.01.003.
[91] J. Reunert, A.S. Lotz-Havla, G. Polo, F. Kannenberg, M. Fobker, M. Griese, E. Mengel, A.C. Muntau, P. Schnabel, O. Sommerburg, I. Borggraefe, A. Dardis, A.P. Burlina, M.A. Mall, G. Ciana, B. Bembi, A.B. Burlina, T. Marquardt, Niemann-Pick Type C-2 disease: identification by analysis of plasma cholestane-3β,5α,6β-triol and further insight into the clinical phenotype, JIMD Rep. 23 (2015) 17–26, https://doi.org/10.1007/8904_2015_423.
[92] S. Pajares, A. Arias, J. García-Villoria, J. Macías-Vidal, E. Ros, J. de las Heras, M. Girós, M.J. Coll, A. Ribes, Cholestane-3β,5α,6β-triol: high levels in Niemann-Pick type C, cerebrotendinous xanthomatosis, and lysosomal acid lipase deficiency, J. Lipid Res. 56 (2015) 1926–1935, https://doi.org/10.1194/jlr.M060343.
[93] M.T. Vanier, P. Gissen, P. Bauer, M.J. Coll, A. Burlina, C.J. Hendriksz, P. Latour, C. Goizet, R.W.D. Welford, T. Marquardt, S.A. Kolb, Diagnostic tests for Niemann-Pick disease type C (NP-C): a critical review, Mol. Genet. Metab. 118 (2016) 244–254, https://doi.org/10.1016/j.ymgme.2016.06.004.
[94] M. Doria, L. Maugest, T. Moreau, G. Lizard, A. Vejux, Contribution of cholesterol and oxysterols to the pathophysiology of Parkinson's disease, Free Radic. Biol. Med. 101 (2016) 393–400, https://doi.org/10.1016/j.freeradbiomed.2016.10.008.
[95] K. Natasa, M. Franca, M. Cosentino, Peripheral immunity, immunoaging and neuroinflammation in Parkinson's disease, Curr. Med. Chem. (2018), https://doi.org/10.2174/0929867325666181009161048.
[96] J.P. Rantham Prabhakara, G. Feist, S. Thomasson, A. Thompson, E. Schommer, O. Ghribi, Differential effects of 24-hydroxycholesterol and 27-hydroxycholesterol on tyrosine hydroxylase and alpha-synuclein in human neuroblastoma SH-SY5Y cells, J. Neurochem. 107 (2008) 1722–1729, https://doi.org/10.1111/j.1471-4159.2008.05736.x.
[97] G. Marwarha, T. Rhen, T. Schommer, O. Ghribi, The oxysterol 27-hydroxycholesterol regulates α-synuclein and tyrosine hydroxylase expression levels in human neuroblastoma cells through modulation of liver X receptors and estrogen receptors-Relevance to Parkinson's disease, J. Neurochem. 107 (2008) 1722–1729, https://doi.org/10.1111/j.1471-4159.2008.05736.x.
[98] C.-Y.J. Lee, R.C.S. Seet, S.H. Huang, L.H. Long, B. Halliwell, Different patterns of oxidized lipid products in plasma and urine of dengue fever, stroke, and Parkinson's disease patients: cautions in the use of biomarkers of oxidative stress, Antioxid. Redox Signal. 11 (2009) 407–420, https://doi.org/10.1089/ars.2008.

2179.
[99] I. Björkhem, A. Lövgren-Sandblom, V. Leoni, S. Meaney, L. Brodin, L. Salveson, K. Winge, S. Pålhagen, P. Svenningsson, Oxysterols and Parkinson's disease: evidence that levels of 24S-hydroxycholesterol in cerebrospinal fluid correlates with the duration of the disease, Neurosci. Lett. 555 (2013) 102–105, https://doi.org/10.1016/j.neulet.2013.09.003.
[100] S. Zarei, K. Carr, L. Reiley, K. Diaz, O. Guerra, P.F. Altamirano, W. Pagani, D. Lodin, G. Orozco, A. Chinea, A comprehensive review of amyotrophic lateral sclerosis, Surg. Neurol. Int. 6 (2015) 171, https://doi.org/10.4103/2152-7806.169561.
[101] A. Vejux, A. Namsi, T. Nury, T. Moreau, G. Lizard, Biomarkers of amyotrophic lateral sclerosis: current status and interest of oxysterols and phytosterols, Front. Mol. Neurosci. 11 (2018) 12, https://doi.org/10.3389/fnmol.2018.00012.
[102] S.-M. Kim, M.-Y. Noh, H. Kim, S.-Y. Cheon, K.M. Lee, J. Lee, E. Cha, K.S. Park, K.-W. Lee, J.-J. Sung, S.H. Kim, 25-Hydroxycholesterol is involved in the pathogenesis of amyotrophic lateral sclerosis, Oncotarget 8 (2017) 11855–11867, https://doi.org/10.18632/oncotarget.14416.
[103] A. Wuolikainen, J. Acimovic, A. Lövgren-Sandblom, P. Parini, P.M. Andersen, I. Björkhem, Cholesterol, oxysterol, triglyceride, and coenzyme Q homeostasis in ALS. Evidence against the hypothesis that elevated 27-hydroxycholesterol is a pathogenic factor, PLoS ONE 9 (2014) e113619, , https://doi.org/10.1371/journal.pone.0113619.
[104] V. La Marca, B. Maresca, M.S. Spagnuolo, L. Cigliano, F. Dal Piaz, G. Di Iorio, P. Abrescia, Lecithin-cholesterol acyltransferase in brain: does oxidative stress influence the 24-hydroxycholesterol esterification? Neurosci. Res. 105 (2016) 19–27, https://doi.org/10.1016/j.neures.2015.09.008.
[105] D.W. Smith, L. Lemli, J.M. Opitz, A newly recognized syndrome of multiple congenital anomalies, J. Pediatr. 64 (1964) 210–217.
[106] G.S. Tint, M. Irons, E.R. Elias, A.K. Batta, R. Frieden, T.S. Chen, G. Salen, Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome, N. Engl. J. Med. 330 (1994) 107–113, https://doi.org/10.1056/NEJM199401133300205.
[107] G.S. Tint, A.K. Batta, G. Xu, S. Shefer, A. Honda, M. Irons, E.R. Elias, G. Salen, The Smith-Lemli-Opitz syndrome: a potentially fatal birth defect caused by a block in the last enzymatic step in cholesterol biosynthesis, Subcell. Biochem. 28 (1997) 117–144.
[108] F.D. Porter, Smith-Lemli-Opitz syndrome: pathogenesis, diagnosis and management, Eur. J. Hum. Genet. 16 (2008) 535–541, https://doi.org/10.1038/ejhg.2008.10.
[109] W.J. Griffiths, Y. Wang, K. Karu, E. Samuel, S. McDonnell, M. Hornshaw, C. Shackleton, Potential of sterol analysis by liquid chromatography - tandem mass spectrometry for the prenatal diagnosis of Smith-Lemli-Opitz syndrome, Clin. Chem. 54 (2008) 1317–1324, https://doi.org/10.1373/clinchem.2007.100644.
[110] I. Björkhem, L. Starck, U. Andersson, D. Lütjohann, S. von Bahr, I. Pikuleva, A. Babiker, U. Diczfalusy, Oxysterols in the circulation of patients with the Smith-Lemli-Opitz syndrome: abnormal levels of 24S- and 27-hydroxycholesterol, J. Lipid Res. 42 (2001) 366–371.
[111] Z. Korade, L. Xu, K. Mirnics, N.A. Porter, Lipid biomarkers of oxidative stress in a genetic mouse model of Smith-Lemli-Opitz syndrome, J. Inherit. Metab. Dis. 36 (2013) 113–122, https://doi.org/10.1007/s10545-012-9504-z.
[112] L. Xu, W. Liu, L.G. Sheflin, S.J. Fliesler, N.A. Porter, Novel oxysterols observed in tissues and fluids of AY9944-treated rats: a model for Smith-Lemli-Opitz syndrome, J. Lipid Res. 52 (2011) 1810–1820, https://doi.org/10.1194/jlr.M018366.
[113] L. Xu, L.G. Sheflin, N.A. Porter, S.J. Fliesler, 7-Dehydrocholesterol-derived oxysterols and retinal degeneration in a rat model of Smith–Lemli–Opitz syndrome, Biochimica et Biophysica Acta (BBA) - Mol. Cell Biol. Lipids 1821 (2012) 877–883, https://doi.org/10.1016/j.bbalip.2012.03.001.
[114] L. Xu, Z. Korade, D.A. Rosado, W. Liu, C.R. Lamberson, N.A. Porter, An oxysterol biomarker for 7-dehydrocholesterol oxidation in cell/mouse models for Smith-Lemli-Opitz syndrome, J. Lipid Res. 52 (2011) 1222–1233, https://doi.org/10.1194/jlr.M014498.
[115] W.J. Griffiths, J. Abdel-Khalik, P.J. Crick, M. Ogundare, C.H. Shackleton, K. Tuschl, M.K. Kwok, B.W. Bigger, A.A. Morris, A. Honda, L. Xu, N.A. Porter, I. Björkhem, P.T. Clayton, Y. Wang, Sterols and oxysterols in plasma from smith-lemi-opitz syndrome patients, J. Steroid Biochem. Mol. Biol. 169 (2017) 77–87, https://doi.org/10.1016/j.jsbmb.2016.03.018.
[116] W. Liu, L. Xu, C.R. Lamberson, L.S. Merkens, R.D. Steiner, E.R. Elias, D. Haas, N.A. Porter, Assays of plasma dehydrocholesteryl esters and oxysterols from Smith-Lemli-Opitz syndrome patients, J. Lipid Res. 54 (2013) 244–253, https://doi.org/10.1194/jlr.M031732.
[117] C.M. Murphy, C.E. Wilson, D.M. Robertson, C. Ecker, E.M. Daly, N. Hammond, A. Galanopoulos, I. Dud, D.G. Murphy, G.M. McAlonan, Autism spectrum disorder in adults: diagnosis, management, and health services development, Neuropsychiatr. Dis. Treat. 12 (2016) 1669–1686, https://doi.org/10.2147/NDT.S65455.
[118] Z. Xiao, T. Qiu, X. Ke, X. Xiao, T. Xiao, F. Liang, B. Zou, H. Huang, H. Fang, K. Chu, J. Zhang, Y. Liu, Autism spectrum disorder as early neurodevelopmental disorder: evidence from the brain imaging abnormalities in 2-3 years old toddlers, J. Autism Dev. Disord. 44 (2014) 1633–1640, https://doi.org/10.1007/s10803-014-2033-x.
[119] M.L. Bauman, Medical comorbidities in autism: challenges to diagnosis and treatment, Neurotherapeutics 7 (2010) 320–327, https://doi.org/10.1016/j.nurt.2010.06.001.
[120] E. Tierney, I. Bukelis, R.E. Thompson, K. Ahmed, A. Aneja, L. Kratz, R.I. Kelley, Abnormalities of cholesterol metabolism in autism spectrum disorders, Am. J. Med. Genet. B Neuropsychiatr. Genet. 141B (2006) 666–668, https://doi.org/10.1002/ajmg.b.30368.
[121] R.W.Y. Lee, E. Tierney, Hypothesis: the role of sterols in autism spectrum disorder, Autism Res. Treat. 2011 (2011), https://doi.org/10.1155/2011/653570.
[122] S. Grayaa, C. Zerbinati, M. Messedi, I. HadjKacem, M. Chtourou, D. Ben Touhemi, M. Naifar, H. Ayadi, F. Ayedi, L. Iuliano, Plasma oxysterol profiling in children reveals 24-hydroxycholesterol as a potential marker for Autism Spectrum Disorders, Biochimie (2018), https://doi.org/10.1016/j.biochi.2018.04.026.
[123] L. Schöls, T.W. Rattay, P. Martus, C. Meisner, J. Baets, I. Fischer, C. Jägle, M.J. Fraidakis, A. Martinuzzi, J.A. Saute, M. Scarlato, A. Antenora, C. Stendel, P. Höflinger, C.M. Lourenco, L. Abreu, K. Smets, M. Paucar, T. Deconinck, D.M. Bis, S. Wiethoff, P. Bauer, A. Arnoldi, W. Marques, L.B. Jardim, S. Hauser, C. Criscuolo, A. Filla, S. Züchner, M.T. Bassi, T. Klopstock, P. De Jonghe, I. Björkhem, R. Schüle, Hereditary spastic paraplegia type 5: natural history, biomarkers and a randomized controlled trial, Brain 140 (2017) 3112–3127, https://doi.org/10.1093/brain/awx273.
[124] R. Schüle, T. Siddique, H.-X. Deng, Y. Yang, S. Donkervoort, M. Hansson, R.E. Madrid, N. Siddique, L. Schöls, I. Björkhem, Marked accumulation of 27-hydroxycholesterol in SPG5 patients with hereditary spastic paresis, J. Lipid Res. 51 (2010) 819–823, https://doi.org/10.1194/jlr.M002543.
[125] C. Marelli, F. Lamari, D. Rainteau, A. Lafourcade, G. Banneau, L. Humbert, M.-L. Monin, E. Petit, R. Debs, G. Castelnovo, E. Ollagnon, J. Lavie, J. Pilliod, I. Coupry, P.J. Babin, C. Guissart, I. Benyounes, U. Ullmann, G. Lesca, C. Thauvin-Robinet, P. Labauge, S. Odent, C. Ewenczyk, C. Wolf, G. Stevanin, D. Hajage, A. Durr, C. Goizet, F. Mochel, Plasma oxysterols: biomarkers for diagnosis and treatment in spastic paraplegia type 5, Brain 141 (2018) 72–84, https://doi.org/10.1093/brain/awx297.