Metabolism of Cholesterol in Brain and Its Association with Parkinson's disease.

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Abstract

Parkinson's disease (PD) is the second most progressive neurodegenerative disorder of the aging population after Alzheimer's disease (AD). Defects in the lysosomal systems and mitochondria have been suspected to cause the pathogenesis of PD. Nevertheless, the pathogenesis of PD remains obscure. Abnormal cholesterol metabolism is linked to numerous disorders, including atherosclerosis. The brain contains the highest level of cholesterol in the body and abnormal cholesterol metabolism links also many neurodegenerative disorders such as AD, PD, Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). The blood brain barrier effectively prevents uptake of lipoprotein-bound cholesterol from blood circulation. Accordingly, cholesterol level in the brain is independent from that in peripheral tissues. Because cholesterol metabolism in both peripheral tissue and the brain are quite different, cholesterol metabolism associated with neurodegeneration should be examined separately from that in peripheral tissues.

Keywords: Parkinson's disease, Brain cholesterol, Metabolism, Neurodegeneration

Introduction

Parkinson's disease (PD) is the second most progressive neurodegenerative disorder of the aging population after Alzheimer's disease (AD). It is characterized clinically by movement symptoms, including resting tremor, rigidity, bradykinesia , and postural instability, as well as various non-motor symptoms. Pathologically, it is characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta and the presence of intracellular protein inclusions called Lewy body or Lewy neurites [1]. Cholesterol is an essential constituent of eukaryotic membranes. Different concentrations of cholesterol regulate membrane fluidity, and thereby the structural integrity and functional specificity of various cellular locations, including membrane trafficking and trans-membrane signaling [2]. Especially, membrane cholesterol levels play a key factor in determining the stability and organization of microdomain termed lipid rafts. Cholesterol is also the precursor of all steroid hormones and bile acids [3]. Abnormal cholesterol metabolism is linked to numerous disorders, including atherosclerosis. In humans, the brain contains the highest level of cholesterol in the body; it contains approximately 20% of whole body cholesterol [4]. Abnormal cholesterol metabolism in the brain is also associated with many neurodegenerative disorders, such as AD, PD, Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) [5]. In addition, cholesterol lowering drugs, statins have been reported to exert beneficial effects in many neurodegenerative diseases. However, it is not

fully known whether the underlying mechanism of statins mediated neuroprotection is associated with lowering cholesterol level due to their pleiotrophic effects such as anti-inflammatory, anti-oxidant effects. Here, we review and compare cholesterol metabolism in the brain and peripheral tissues. Furthermore, the relationship between alterations in cholesterol metabolism and PD pathogenesis is reviewed.

Metabolism of Cholesterol

The pool of cholesterol is 2.2 mg/g body weight in the whole human body. Cholesterol is supplied by dietary intake. The liver accounts for only 15% of de novo cholesterol synthesis, and up to 85% of cholesterol synthesis occurs in organs other than the liver [6]. Most organs and tissues meet their cholesterol requirements through endogenous cholesterol biosynthesis. However, many cell types have mechanisms to absorb exogenous cholesterol sources in the form of plasma-derived lipoproteins [7]. All nucleated cells in the body can synthesize cholesterol from acetyl-CoA. It consists of two paths: the Bloch pathway and the Kandutsch-Russell (K-R) pathway. These pathways are the two major post-squalene cholesterol biosynthetic pathways. The structure of cholesterol biosynthesis pathways is significantly different between tissues. The reason why cells synthesize cholesterol through different routes is not well understood; some hypotheses have been proposed. In an experiment of genetically and environmentally controlled cholesterol formation, the flux of the K-R pathway remains relatively constant, but that of the Bloch pathway changes [8]. These results showed that, unlike the Bloch pathway, the K-R pathway can ensure a certain percentage of cholesterol synthesis with a subtle change in cholesterol requirements. The Bloch pathway can be a method for the production of cholesterol depending on the surrounding environment. There have been some cases of cell type-specific implementation of these pathways. The skin, muscles, and

heart contain more K-R pathway components than Bloch pathway components [9]. On the contrary, in the testis and adrenal gland, which require large amounts of cholesterol, the Bloch pathway is markedly active. Cholesterol transfer Cholesterol is transferred into cells in the form of lipoproteins such as chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Lipoproteins contain apolipoproteins. Apolipoproteins have several important functions, such as structural functions and roles as ligands of lipoprotein receptors, directors of the formation of lipid protein, and enzymes involved in lipoprotein metabolism [10]. The low-density lipoprotein receptor (LDLR) is a cell surface receptor that mediates the uptake and catabolism of ApoB or ApoE-containing plasma lipoproteins [11]. The main function of this receptor is to remove highly atherogenic LDL from blood circulation. Because the liver contains 70% of the total LDLR in the body, liver LDLR activity plays an important role in regulating plasma LDL cholesterol levels. Cholesterol turnover Cellular cholesterol is not generally degraded. Rather, it is stored as cholesterol ester droplets, exported to plasma lipoproteins, or converted to oxysterol, bile acids, and functionally important derivatives, such as steroids and vitamin D3 [12]. The liver is the sole organ that can remove cholesterol by secreting it into bile or converting it to bile acids. Hepatic receptors, such as LDLR, LRP1, and SR-BI bind plasma lipoproteins, eliminating excess cholesterol in the blood. Cholesterol is removed from the liver by two mechanisms. Cholesterol is removed directly through the ATPbinding cassette (ABC) subfamily G5/G8 (ABCG5/G8) receptor and then excreted into the gall bladder [13]. Cholesterol is also converted to 7α-hydroxycholesterol by cholesterol 7α-hydroxylase, which is expressed only in the liver; this is the major mechanism of bile acid synthesis. Another crucial mechanism of

cholesterol turnover is cellular efflux of oxysterols derived from cholesterol [14]. Cholesterol 24-hydroxylase is expressed mainly in neurons and expressed at far lower levels in the liver. Cholesterol 25-hydroxylase is present at a high level in the lung, but is also expressed at low levels in most tissues. Cholesterol 25-hydroxylase may have a role in cholesterol catabolism in a tissue-specific manner, as does cholesterol 24-hydroxylase. 27-HC is the most abundant cholesterol-derived oxysterol in blood circulation [15].

Metabolism of Cholesterol in Brain

The brain contains the highest level of cholesterol in the body; it contains approximately 20% of whole body cholesterol. Cholesterol in the brain is present mostly in the unesterified form, and the concentration of unesterified cholesterol in the brain is higher than that in any other tissues (~23 mg/g). The blood brain barrier (BBB) effectively prevents the uptake of lipoprotein-bound cholesterol from blood circulation. Accordingly, cholesterol level in the brain is independent from that in peripheral tissues, and thus de novo synthesis is considered responsible for practically all cholesterol in the brain [16]. Cholesterol is mainly observed in glial cells and is produced at higher rates in astrocytes than in neurons. During embryogenesis, both neurons and glia actively synthesize cholesterol for myelinogenesis. However, in adults, differentiated neurons gradually lose their de novo synthetic ability and rely on lipoprotein-conjugated cholesterol produced by glia [17]. However, the amounts of cholesterol produced by glia and taken up by neurons increase significantly, supporting the dependency of neurons on cholesterol produced by astrocytes. Some studies showed elevated transcript level of cholesterol synthesis enzymes in neurons compared to that in astrocytes [18].

Especially, when neurons are stimulated, such as by brain-derived neurotropic factor (BDNF), de novo synthesis of cholesterol in neurons recover to some extent. In studies using radioactive labels, glia synthesize cholesterol through the Bloch pathway, as do other cholesterol forming peripheral tissues, whereas neurons synthesize cholesterol mainly through the K-R pathway, indicating that the K-R pathway is activated in neurons to maintain homeostasis. In addition, cholesterols over than this essential cholesterol may be produced through the Bloch pathway in glia. Although cholesterol cannot cross the BBB, some cholesterols are absorbed into the brain in the form of plasma lipoprotein-bound cholesterol [19]. SR-BI, which plays an important role in the selective absorption of HDL cholesterol in hepatocytes, is also present in brain capillary endothelial cells, thereby mediating the uptake of cholesterol from plasma HDL and LDL. Surprisingly, it has been shown that brain endothelial cells have the potential to take up LDL cholesterol through luminal LDLR and translocate this LDL across the cells. However, as judged from this isotope experiments, receptor-mediated uptake of cholesterol from blood circulation yields low amount of cholesterol; thus, it is not considered important in normal conditions. Cholesterol transfer Most lipoproteins in plasma are not found in the brain owing to BBB [20]. Instead, a discoidal protein containing phospholipid, cholesterol, and apolipoprotein sized approximately 8~12 nm is identified. This lipoprotein is called 'HDL-like particle' because it is similar in size and density to plasma HDL. Astrocytes are suspected to be mainly responsible for most of lipoprotein production in the brain. Most of the lipoproteins found in cerebrospinal fluid (CSF) are spherical in form and different in size from nascent poorly-lipidated HDL secreted from astrocytes, suggesting that the lipoproteins secreted from astrocytes are modified as in plasma HDL maturation [21].

Some cholesterol remodeling enzymes, such as lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester transfer protein (CETP), and phospholipid transfer protein (PLTP), which are known to promote spherical HDL formation from nascent HDL, were found in the brain; these proteins may play a role in brain lipoprotein maturation, although this role is yet to be clearly elucidated [22]. These apolipoproteins form HDL-like particles, and undergo cell membrane repair and remodeling through rearrangement of cholesterol and phospholipids in differentiated adult neurons. ApoE is synthesized in the brain, which is the second largest producer of ApoE after the liver. ApoE is mainly produced in astrocytes, followed by oligodendrocytes, microglia, and ependymal layer cells. In neurons, ApoE is produced when a specific stress condition or damage occurs [23]. The main function of ApoE is to regulate lipid transport between neurons and glia, as well as metabolism of lipoprotein-bound cholesterol. ApoA-I is not synthesized in the brain, but most may be transported from plasma HDL through SR-BI-mediated uptake via the choroid plexus; however, this transport mechanism is not fully understood [24]. ApoE acts as a ligand for these LDLR family members expressed in the brain. Among these receptors, LRP1 and LDLR are the major receptors related to ApoE-containing lipoproteins carrying cholesterol between neurons and glia. LRP1 is a large endocytic receptor with various functions. In the brain, LRP1 is expressed by both neurons and glia, but predominantly by neurons. LDLR is also present in both neurons and glia, however, contrary to LRP1, LDLR is more expressed in glia than in neurons. Cholesterol turnover Brain cholesterol has an extremely long half-life. In humans, the half-life of cholesterol in brain has been estimated to be between 6 months and 5 years, whereas that in plasma is only a few days [25].

Surplus cholesterol is stored in the esterified form, corresponding to 1% of total cholesterol content in the brain. The level of this esterification enzyme is higher in neurons than in glia . Cholesterol can be hydroxylated to 24-HC by cholesterol 24-hydroxylase and this form of oxysterol is the main form of excreted cholesterol in the brain. The expression level of cholesterol 24-hydroxylase is much higher in neurons than in glia This oxysterol can pass lipophilic membranes, such as BBB. Therefore, most 24-HC in plasma are released from the brain. Therefore, there are many attempts to use 24-HC as a marker of the aging process or neurodegenerative disease, but this use is still debated. Moreover, 27-HC, which is similar in character to 24-HC, is also present in the brain; however, 27-HC is present in low amounts and most are of extracerebral origin owing to the characteristics of this oxysterol that can cross the BBB [26]. Cholesterol is also excreted from neurons through ABC transporters, such as ABCA1, ABCG1, and ABCG4. These ABC transporters are involved in the transport of various substances beyond the membrane, between the cells in the central nervous system (CNS) express these transporters. Generally, neurons express more ABC transporters than astrocytes. The cholesterols released via ABC transporters connect to the ApoAI-containing lipoproteins present in the CSF, and then removed through LRP1 or SR-BI, which is expressed in brain capillary endothelial cells.

Cholesterol and Parkinsons

The association of cholesterol biosynthesis with the pathogenesis of PD has been rarely studied. It has been reported that cholesterol biosynthesis is decreased in fibroblasts from patients with PD owing to reduced β-Hydroxy β-methylglutaryl-CoA (HMGCoA) reductase activity [27].

In addition, isopentenyl diphosphate isomerase, a cholesterol-synthesizing enzyme, is localized in Lewy bodies, proposing that cholesterol metabolites may play a role in the aggregation of α-synuclein, enhancing Lewy body formation. Several studies of serum lipid profiles in PD patients showed the inverse correlation between cholesterol level and PD. The serum levels of total cholesterol, LDL-cholesterol, VLDL-cholesterol, and triglyceride are significantly reduced in patients with PD, compared with those in the controls, which are correlated with previous study showing decrease in cholesterol biosynthesis in fibroblast from patients with PD. A case control study by Huang et al. also demonstrated that low serum LDL-cholesterol level is associated with higher occurrence of PD. A population based cohort study indicated that higher levels of serum total and LDL cholesterol among men over time are associated with a lower PD risk [28]. Cross-sectional study by Cassani et al. demonstrated that HDL levels are favorably associated with duration of PD. Given that cholesterol metabolism in the CNS is independent on that in the periphery, measurement of cholesterol in the brain may be more important. Nevertheless, no studies measuring cholesterol level in the brain with PD have been reported. Instead, it has been reported that the level of 24-HC, which possibly reflects brain cholesterol metabolism, is decreased in the plasma of patients with PD. In contrast, one study showed that 24-HC level is increased in the CSF of patient with PD, whereas there is no difference in plasma 24-HC level between the control and patients with PD. In addition, there was a significant correlation between the level of 24-HC in the CSF and the duration of the disease, which may reflect the release of 24-HC from dying neuronal cells [29]. Alterations in the lipid composition of lipid rafts, cholesterol-rich microdomains of the plasma membrane, have been reported in the autopsied brains of PD patients.

The proportion of phospholipids to cholesterol in lipid rafts in the frontal cortex of PD brains appeared to be increased compared with that in control subjects, indicating a net reduction in the proportion of cholesterol in membrane rafts. In addition, careful examination of neuropathology shows that AD and non-AD type proteinopathies frequently co-occur in the brains of both cognitively unimpaired and demented, aged individuals, whereas the presence of a single disease is rather the exception than the rule [30]. Given that AD has a strong association with cholesterol metabolism, attention is required in interpreting the data on cholesterol metabolism in patients with PD, especially in cases that are not confirmed by pathology. It has been reported that high cholesterol diet increases brain cholesterol level and exacerbates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced reduction of striatal dopamine and dopaminergic neurons in the substantia nigra with motor behavioral depreciation in mice [31]. In addition, the high cholesterol level incorporated into differentiated SH-SY5Y cells, a human neuroblastoma cell line, worsens dopaminergic neuronal survivability through increased depolarization of mitochondrial membrane potential [32]. In addition, many genetic factors associated with familial PD have been reported to be involved in cholesterol metabolism. Especially, accumulating evidence indicates that α-synuclein, a major player in the pathogenesis of PD, seems to be strongly involved in cholesterol metabolism. α-Synuclein shares striking structural similarities with ApoE and contains two cholesterol-binding domains. α-Synuclein has a strong propensity to bind to lipid membranes, particularly the regions enriched in cholesterol. α-Synuclein has been reported to form structures that are similar to nascent lipoproteins, which are the premature forms of the larger spherical lipoproteins (i.e., HDL), and interact with lipoproteins in human plasma.

Cholesterol also mediates the interaction of oligomeric α-synuclein with the cell membrane, which leads to membrane disruption and cell death [33]. Elevated levels of oxidized cholesterol metabolites in Lewy body disease brains accelerate α-synuclein fibrilization. α-Synuclein aggregation increases at low concentrations of ApoE and decreases at high concentrations of ApoE. 27-HC increases α-synuclein protein levels through proteasomal inhibition in human dopaminergic neurons, suggesting that dysfunctions of cholesterol metabolism may also induce the aggregation of α-synuclein, thus causing PD. These studies suggested that α-synuclein may influence cholesterol metabolism and cholesterol may also contribute to the aggregation of α-synuclein. A E3 ubiquitin ligase, parkin, of which genetic mutations were considered a common cause of early onset PD, is also associated with cholesterol metabolism. It has been reported that the total cellular cholesterol level is increased and the membrane fluidity is decreased in parkin deficient mouse embryonic fibroblast (MEF) cells, causing dysregulation of lipid rafts-dependent endocytosis [34]. Parkinson has also been reported to be a lipid-responsive regulator of fat uptake, and the increase in serum cholesterol level by high fat diet is less pronounced in parkin KO mice. DJ-1, a multifunctional protein and a causative gene product as sociated with autosomal recessive familial PD, has also been reported to be associated with cholesterol metabolism. DJ-1 deficiency in astrocytes causes decrease in cellular cholesterol level, increase in membrane fluidity, and decrease in lipid rafts-dependent endocytosis. In addition, cholesterol supplementation rescues the synaptic endocytic defects observed in DJ-1-deficient neurons [35]. Reduced expression of LDLR mRNA and protein was observed in DJ-1-knockdown cells and DJ-1 KO mice.

Elevated plasma cholesterol level in leucine-rich repeat kinase 2 (LRRK2) KO rats has also been reported. In addition, parkin, PINK1, DJ-1, LRRK2, and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) have been reported to be associated with lipid rafts in in vitro model. The molecular pathways of neurodegeneration triggered by each mutation may be shared by several genetic forms of PD, and may also play a common role in its pathogenesis. Accordingly, given the fact that each mutation of genetic factors alters cholesterol metabolism, alterations in cholesterol metabolism can contribute to the pathogenesis of PD.

Conclusions

The brain is the most cholesterol-rich organ, and owing to the BBB, cholesterol metabolism in the brain is independent on that in peripheral tissues. Nevertheless, detailed knowledge on cholesterol metabolism in the brain remains incomplete, and it should be considered that altered cholesterol metabolism in the periphery does not represent that in the CNS. In addition, most studies have analyzed the features of CSF lipoprotein. Although the brain and CSF are interconnected, the features of lipoproteins in CSF are not the same as those in the brain. Not much is known about the composition of lipoproteins in brain and how CSF lipoproteins communicate with lipoproteins in the brain. It may cause the discrepancy between human studies and model studies, in addition to several limitations of human studies including measuring cholesterol level in the plasma, not in the brain with PD patients as described above. Although the relationship between cholesterol metabolism in the CNS and pathogenesis of PD has not been wellstudied, given that many PD-related gene products are involved in cholesterol metabolism, further studies of cholesterol metabolism in PD may provide clues to elucidate unsolved pathogenesis of PD.

References

1. Kalia LV, Lang AE (2015) Parkinson's disease. Lancet 386:896- 912.

2. Karimi-Moghadam A, Charsouei S, Bell B, Jabalameli MR (2018) Parkinson disease from mendelian forms to genetic susceptibility: new molecular insights into the neurodegeneration process. Cell Mol Neurobiol 38:1153-1178.

3. Plotegher N, Duchen MR (2017) Crosstalk between lysosomes and mitochondria in Parkinson's disease. Front Cell Dev Biol 5:110.

4. Zhang J, Culp ML, Craver JG, Darley-Usmar V (2018) Mitochondrial function and autophagy: integrating proteotoxic, redox, and metabolic stress in Parkinson's disease. J Neurochem 144:691-709.

5. Yeagle PL (1991) Modulation of membrane function by cholesterol. Biochimie 73:1303-1310.

6. Schroeder F, Jefferson JR, Kier AB, Knittel J, Scallen TJ, Wood WG, Hapala I (1991) Membrane cholesterol dynamics: cholesterol domains and kinetic pools. Proc Soc Exp Biol Med 196:235-252.

7. Korade Z, Kenworthy AK (2008) Lipid rafts, cholesterol, and the brain. Neuropharmacology 55:1265-1273.

8. Mahley RW (2016) Central nervous system lipoproteins: ApoE and regulation of cholesterol metabolism. Arterioscler Thromb Vasc Biol 36:1305-1315.

9. Martín MG, Pfrieger F, Dotti CG (2014) Cholesterol in brain disease: sometimes determinant and frequently implicated. EMBO Rep 15:1036-1052.

10. Saeedi Saravi SS, Saeedi Saravi SS, Arefidoust A, Dehpour AR (2017) The beneficial effects of HMG-CoA reductase inhibitors in the processes of neurodegeneration. Metab Brain Dis 32:949-965.

11. Grundy SM (1983) Absorption and metabolism of dietary cholesterol. Annu Rev Nutr 3:71-96.

12. Dietschy JM (1997) Theoretical considerations of what regulates low-density-lipoprotein and high-density-lipoprotein cholesterol. Am J Clin Nutr 65(5 Suppl):1581S-1589S.

13. Dietschy JM, Turley SD, Spady DK (1993) Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. J Lipid Res 34:1637-1659.

14. Tabas I (2002) Consequences of cellular cholesterol accumu lation: basic concepts and physiological implications. J Clin Invest 110:905-911.

14. Sharpe LJ, Brown AJ (2013) Controlling cholesterol synthesis beyond 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR). J Biol Chem 288:18707-18715.

15. Kandutsch AA, Russell AE (1960) Preputial gland tumor sterols. 3. A metabolic pathway from lanosterol to cholesterol. J Biol Chem 235:2256-2261.

16. Bloch K (1965) The biological synthesis of cholesterol. Science 150:19-28.

17. Mitsche MA, McDonald JG, Hobbs HH, Cohen JC (2015) Flux analysis of cholesterol biosynthesis in vivo reveals multiple tissue and cell-type specific pathways. Elife 4:e07999.

18. Rawson RB, DeBose-Boyd R, Goldstein JL, Brown MS (1999) Failure to cleave sterol regulatory element-binding proteins (SREBPs) causes cholesterol auxotrophy in Chinese hamster ovary cells with genetic absence of SREBP cleavage-activating protein. J Biol Chem 274:28549-28556.

19. Okamoto T, Moroyama T, Morita T, Yoshiga K, Takada K, Okuda K (1984) Differentiation of cultured epidermal keratinocytes related to sterol metabolism and its retardation by chemical carcinogens. Biochim Biophys Acta 805:143-151.

20. Ramasamy I (2014) Recent advances in physiological lipoprotein metabolism. Clin Chem Lab Med 52:1695-1727.

21. Arnold PO, Cholesterol KJ (2003) Absorption, function, and metabolism. In: Encyclopedia of food sciences and nutrition (Caballero B, Trugo L, Finglas M, eds), Academic Press, San Diego.

22. Brown MS, Goldstein JL (1986) A receptor-mediated pathway for cholesterol homeostasis. Science 232:34-47.

23. Bilheimer DW, Goldstein JL, Grundy SM, Starzl TE, Brown MS (1984) Liver transplantation to provide low-densitylipoprotein receptors and lower plasma cholesterol in a child with homozygous familial hypercholesterolemia. N Engl J Med 311:1658-1664.

24. Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M (1996) Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. Science 271:518-520.

25. Landschulz KT, Pathak RK, Rigotti A, Krieger M, Hobbs HH (1996) Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat. J Clin Invest 98:984-995.

26. Steck TL, Lange Y (2010) Cell cholesterol homeostasis: mediation by active cholesterol. Trends Cell Biol 20:680-687.

27. Krieger M (1999) Charting the fate of the "good cholesterol": identification and characterization of the high-density lipoprotein receptor SR-BI. Annu Rev Biochem 68:523-558.

28. Repa JJ, Berge KE, Pomajzl C, Richardson JA, Hobbs H, Mangelsdorf DJ (2002) Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors alpha and beta. J Biol Chem 277:18793-18800.

29. Russell DW (2003) The enzymes, regulation, and genetics of bile acid synthesis. Annu Rev Biochem 72:137-174.

30. Lund EG, Guileyardo JM, Russell DW (1999) cDNA cloning of cholesterol 24-hydroxylase, a mediator of cholesterol homeostasis in the brain. Proc Natl Acad Sci U S A 96:7238- 7243.

31. Lund EG, Kerr TA, Sakai J, Li WP, Russell DW (1998) cDNA cloning of mouse and human cholesterol 25-hydroxylases, polytopic membrane proteins that synthesize a potent oxysterol regulator of lipid metabolism. J Biol Chem 273:34316- 34327.

32. Dzeletovic S, Breuer O, Lund E, Diczfalusy U (1995) Determination of cholesterol oxidation products in human plasma by isotope dilution-mass spectrometry. Anal Biochem 225:73- 80.

33. Björkhem I, Meaney S (2004) Brain cholesterol: long secret life behind a barrier. Arterioscler Thromb Vasc Biol 24:806- 815.