REVIEW

The Role of Cholesterol in α-Synuclein and Lewy Body Pathology in *GBA1* Parkinson's Disease

Patricia García-Sanz, PhD,[1,2*] Johannes M.F.G. Aerts, PhD,[3] and Rosario Moratalla, PhD[1,2]

[1]*Instituto Cajal, CSIC, Madrid, Spain*
[2]*Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas, Instituto de Salud Carlos III, Madrid, Spain*
[3]*Medical Biochemistry, Leiden Institute of Chemistry, Leiden University, Faculty of Science, Leiden, the Netherlands*

ABSTRACT: Parkinson's disease (PD) is a progressive neurodegenerative disease where dopaminergic neurons in the substantia nigra are lost, resulting in a decrease in striatal dopamine and, consequently, motor control. Dopaminergic degeneration is associated with the appearance of Lewy bodies, which contain membrane structures and proteins, including α-synuclein (α-Syn), in surviving neurons. PD displays a multifactorial pathology and develops from interactions between multiple elements, such as age, environmental conditions, and genetics. Mutations in the *GBA1* gene represent one of the major genetic risk factors for PD. This gene encodes an essential lysosomal enzyme called β-glucocerebrosidase (GCase), which is responsible for degrading the glycolipid glucocerebroside into glucose and ceramide. GCase can generate glucosylated cholesterol via transglucosylation and can also degrade the sterol glucoside. Although the molecular mechanisms that predispose an individual to neurodegeneration remain unknown, the role of cholesterol in PD pathology deserves consideration. Disturbed cellular cholesterol metabolism, as reflected by accumulation of lysosomal cholesterol in *GBA1*-associated PD cellular models, could contribute to changes in lipid rafts, which are necessary for synaptic localization and vesicle cycling and modulation of synaptic integrity. α-Syn has been implicated in the regulation of neuronal cholesterol, and cholesterol facilitates interactions between α-Syn oligomers. In this review, we integrate the results of previous studies and describe the cholesterol landscape in cellular homeostasis and neuronal function. We discuss its implication in α-Syn and Lewy body pathophysiological mechanisms underlying PD, focusing on the role of GCase and cholesterol. © 2020 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

Key Words: autophagy; glycosphingolipid; lipid storage diseases; lysosomes; multilamellar bodies; neurodegeneration

*__Correspondence to:__ Dr. Patricia García-Sanz, Cajal Institute, CSIC, Av. Dr. Arce 37,28002 Madrid, Spain; E-mail: pgarcia@cajal.csic.es

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Brain Cholesterol Homeostasis and Trafficking

Mammalian cells require cholesterol for membrane integrity and fluidity, and for regulation of cell membrane organization and biophysical properties.[1,2] Cholesterol intercalates with phospholipids in the membrane, preventing their clustering and stabilizing the membrane.[3] Cholesterol also intercalates with sphingolipids, other membrane anchor proteins and receptors, forming dynamic lipid rafts in the Golgi apparatus (GA) and plasma membrane.[4,5]

In neurons, lipid rafts are specialized, semiordered membrane domains where vesicle trafficking and signal transduction are triggered by neurotrophic factors.[6-9] Neuronal lipid rafts are abundant at the synapse, where

cholesterol directly interacts with neurotransmitter receptors. Cholesterol is required for the correct functioning of the synaptic transmission and synaptogenesis. Hence cholesterol homeostasis is carefully heterogeneously regulated in different brain cells.[10-13] In other tissues, cholesterol is synthesized within the cells and acquired from lipoproteins in the blood; however, in the brain, it must be synthesized *de novo* because plasma lipoproteins cannot cross the blood–brain barrier (BBB).[14-16]

Cholesterol is primarily synthesized in astrocytes within the endoplasmic reticulum (ER) in a multistep process in which the rate-limiting step is catalyzed by 3-hydroxy-3-methyglutaryl-coenzyme-A reductase (HMGR). This synthesis is regulated by a transcription factor called sterol-regulatory element-binding protein-2 (SREBP-2), which enters the nucleus and binds sterol-regulatory element-1 (SRE-1) in the *HMGR* gene,[17] inducing its expression and, consequently, cholesterol production. SREBP-2 also induces genes of lipid/cholesterol uptake, such as *LDLR*.[18] This process is regulated by SREBP cleavage activating protein (SCAP), an ER membrane protein that acts as a cholesterol sensor. When cellular cholesterol levels are high, the SREBP-2/SCAP complex is kept in the ER. However, when cholesterol levels decline, this complex is delivered to the GA, where SCAP discharges the N terminus of SREBP-2 by proteolytic cleavage. SREBP-2 then enters the nucleus, where it binds to SRE-1[18] (Fig. 1). Once synthesized, cholesterol is distributed among cell membranes and organelles, and its levels vary among them; thus, intracellular cholesterol trafficking is a dynamic process, important for understanding neural function.[19,20]

To deliver cholesterol to neurons, astrocytes synthesize apolipoproteins (Apos), proteins that bind lipids forming lipoproteins.[21,22] The most abundant Apos in the brain are ApoE, ApoJ (both bind cholesterol), and ApoA1. In response to increased cholesterol, astrocytes express ApoE and specific lipid transporters on their membranes, such as the ATP-binding cassette transporter protein A1 (ABCA1). The ApoE–cholesterol complex exits the astrocyte via ABCA1, then is internalized into the neuron by low-density lipoprotein (LDL) receptors (LDLR) and the LDLR-related protein 1 (LRP1)[1,21-24] (Fig. 1). This complex is hydrolyzed within the late endosome/lysosome, resulting in free cholesterol. Subsequent trafficking into subcellular membrane compartments is facilitated by the Niemann–Pick type C1 (NPC1) and C2 (NPC2) proteins[25] (Fig. 1). Neurons use this cholesterol for building their extensive membranes of axons, dendrites, synapses, and synaptic vesicles.[1,2,21]

Neurons have various mechanisms to manage excess cholesterol, including cholesterol esterification and storage in intracellular lipid droplets and secretion through the ABCA1 transporter. The main pathway to eliminate excess intracellular cholesterol is mediated by cholesterol 24-hydroxylase (CYP46A1), which converts cholesterol to 24S-hydroxycholesterol (24-OHC).[26] Activated liver X receptors (LXRs) translocate 24-OHC to the nucleus, where it inhibits cholesterol synthesis, induces the expression of *ABCA1* and *APOE* genes, and activates cholesterol efflux to astrocytes.[27] 24-OHC and other oxysterols can cross the BBB or be delivered to the plasma via cerebrospinal fluid (Fig. 1). Oxysterol homeostasis is tightly controlled in the brain to maintain specific levels in each region. The role of these cholesterol oxidation products in neurodegeneration remains unclear; more research is needed to reveal their exact function.

Parkinson's Disease and *GBA1* Mutations

Alterations in cholesterol homeostasis, biosynthesis, transport, and lipid raft organization lead to structural and functional central nervous system (CNS) neurodegenerative diseases.[9,28] In Parkinson's disease (PD), the relationship between abnormal cholesterol and neurodegeneration remains unclear, and present literature suggests that either an increase or decrease in brain cholesterol is associated with this disease.

PD is the most common neurodegenerative movement disorder, affecting 1.5% of people aged 65 years or older.[29] PD is characterized by the loss of dopaminergic neurons in the substantia nigra (SN), and the resulting dopamine depletion in the striatum triggers the characteristic motor symptoms. Lewy bodies (LBs), the neuropathological hallmark of PD, are composed of α-synuclein (α-Syn) aggregates[30] surrounded by proteins involved in ubiquitin-proteasome degradation or in the autophagy process[31] that accumulated with aging.[32]

Although the etiology of PD is unknown, many epidemiological studies indicate that the disease develops from complex interactions among age, environmental factors, and susceptibility genes that affect numerous cellular processes.[33,34] Evidence indicates that changes in lipid homeostasis occur in the CNS during physiological aging; this alteration is potentiated in neurodegenerative diseases, such as PD and AD. Understanding the role of cholesterol in PD will identify new targets to treat PD.

Over the past 15 years, PD research has focused on the link among various lysosomal storage diseases, α-Syn aggregation, and dopamine loss. *GBA1* encodes a lysosomal hydrolase called β-glucocerebrosidase (GCase), which cleaves glucose moieties from the common glycosphingolipid (GSL) glucocerebroside (glucosylceramide [GlcCer]).[35] GSLs are glycolipids that

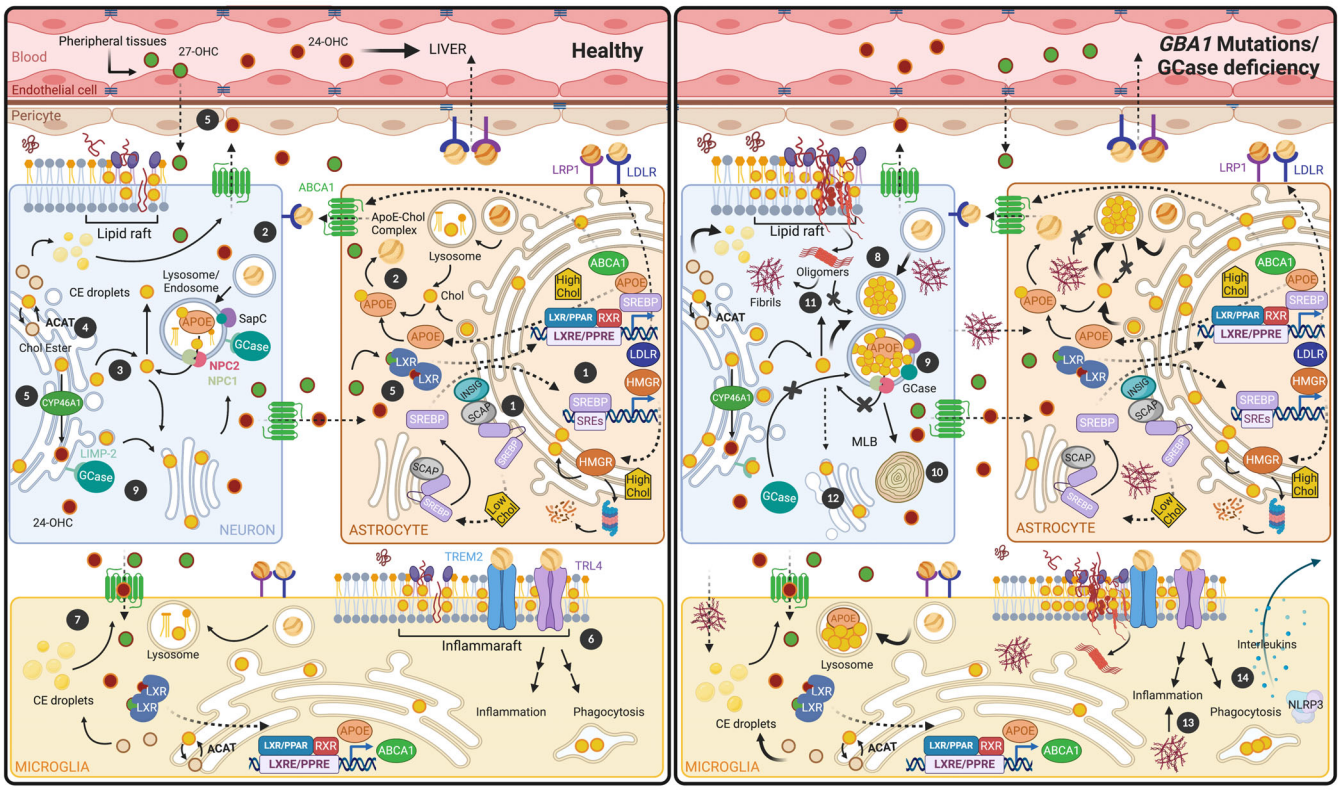

FIG. 1. Impaired brain cholesterol trafficking induced by β-glucocerebrosidase (GCase) deficiency. Cholesterol is primarily synthesized in astrocytes. (1) Under high cholesterol conditions, astrocytes express specific lipid transporter proteins on their membranes (ATP-binding cassette transporter protein A1 [ABCA1]) and apolipoprotein E (ApoE) to decrease cholesterol content. In addition, sterol-regulatory element-binding protein (SREBP) binds to SREBP cleavage activating protein (SCAP), and both are retained in the endoplasmic reticulum (ER) bound to insulin induced gene (INSIG) to inhibit cholesterol synthesis. When cholesterol content decreases, SREBP-SCAP is transported to the GA, where SREBP is cleaved. Then SREBP is translocated to the nucleus to activate genes required for its synthesis (3-hydroxy-3-methyglutaryl-coenzyme-A reductase [HMGR]) and uptake (low-density lipoprotein receptor [LDLR]) through its binding to sterol-regulatory element-1 (SRE-1). HMGR can be degraded by proteasome when ER-cholesterol is accumulated. (2) Synthesized cholesterol binds ApoE, forming an ApoE–cholesterol complex that exits the astrocyte via ABCA1, and it is internalized into the neuron by LDLR and LDLR-related protein 1. (3) In neurons, LDLR complexes are hydrolyzed, and free cholesterol is disseminated among the plasma membrane and other organelles. (4) The excess of neuronal cholesterol is modulated by cholesterol esterification through ACAT (acetyl-coenzyme A acetyltransferase 1) enzyme and storage in lipid droplets that are secreted through the ABCA1. (5) In the ER, the excess cholesterol is converted into 24S-hydroxycholesterol (24-OHC). In astrocytes, 24-OHC and other oxysterols (such as 27-OHC) bind LXR(Liver X Receptor) to induce the expression of *APOE* and *ABCA1* genes. Moreover, 24-OHC can cross the BBB. (6) ApoE–cholesterol complex from astrocyte could bind to Triggering Receptor Expressed on Myeloid Cells 2 (TREM2), Toll-Like Receptor 4 (TLR4), and LDLR on the inflammaraft of the microglia surface to trigger inflammation and phagocytosis. (7) Oxysterols (24-OCH and 27-OCH) can bind LXRs to activate microglia. (8) In a pathological state of *GBA1*-PD or GCase deficiency, we proposed that cholesterol is accumulating in the lysosomes independently of the cell type. (9) Cholesterol could disturb the interaction between GCase1 and its transporter (LIMP-2) impairing GCase activity, and it also might disrupt the contact between GCase and its coactivator SapC, favoring lysosomal cholesterol buildup. (10) Cholesterol accumulation appears to lead to lysosome degeneration called multilamellar bodies (MLBs). (11) Lysosomal cholesterol accumulation could affect cholesterol pools in the rest of membrane organelles, which in turn could alter the α-synuclein (α-Syn) interaction with lipid rafts and contribute to α-Syn oligomerization. Ultimately, these α-Syn oligomers cannot be degraded by lysosomes; as a consequence, they lead to α-Syn fibrils. (12) The α-Syn overburden appears to affect GA tubulation and fragmentation. (13) Aberrant α-Syn is released from neurons and transferred to microglia and astrocytes, and triggers the inflammatory response. (14) We hypothesized that *GBA1* mutations increase interleukins and NLRP3 inflammasome activating the inflammatory response. Peroxisome Proliferator-Activated Receptor (PPAR); Retinoid X Receptor (RXR); Peroxisome Proliferator Response Elements (PPRE); Liver X Receptor Response Elements (LXRE).

consist of ceramide (Cer) and oligosaccharides.[36] GSLs and cholesterol are components of lipid rafts in membranes.[37] *GBA1* mutations with prominent deficient lysosomal GCase activity cause Gaucher disease (GD), a lysosomal storage disorder. *GBA1* mutations are risk factors for neuronal synucleinopathies (PD, PD dementia, or dementia with LBs).[38] These mutations are present in 7%–12% of PD cases, increasing PD risk by 20- to 30-fold.[39-42] However, PD develops in only 10%–30% of these monoallelic or biallelic *GBA1* mutant carriers by the age of 80 years.[43-45] Gene dose poorly correlates with PD risk; that is, there is no significantly higher incidence of PD among patients with GD and *GBA1* carriers. There seems to be a link between severity of *GBA1* mutations and PD phenotype and earlier disease onset.[46,47] Compared with patients with idiopathic PD, patients with *GBA1*-associated-PD (*GBA1*-PD) show earlier disease onset and faster progression, more severe symptoms, and greater incidence of non-motor symptoms, such as rapid eye movement, sleep

disorder, hallucinations, depression and anxiety, and often, cognitive impairment and dementia.[41,48-50] A clear relationship between residual GCase activity of particular *GBA1* mutations and PD risk has not been unequivocally documented. The actual lysosomal environment in aging brain might conceivably have a major impact on the catalytic capacity of a mutant enzyme.

Notably, the phenotypic disparity among patients with GD[51] or PD[52] with the same *GBA1* genotype might indicate the existence of environmental or genetic modifiers.[53,54] These modifiers could be lysosomal genes, for example, *CTSB* (cathepsin B)[55] or *GBA2* (discussed later). Moreover, mutations in *LRRK2* (leucine-rich repeat kinase 2), which participates in lysosomal function and inflammatory response, seem to alleviate *GBA1* mutations effects in human induced pluripotent stem cell (iPSC)-derived neurons[56] or astrocytes.[57]

Currently, how *GBA1* mutations increase the PD risk is unknown. *GBA1* mutations decrease lysosomal GCase levels and activity because GCase is partially retained in the ER, triggering stress and leading to impaired autophagy and apoptosis.[58-60] *GBA1* mutations cause GlcCer accumulation, which in turn causes insoluble α-Syn oligomers to polymerize into fibrils in patient iPSC-derived dopamine neurons.[61,62] This α-Syn aggregation reduces lysosomal degradation, causing neurotoxicity. It also impairs GCase movement from the ER to the GA, decreasing its presence in lysosomes, further promoting GlcCer accumulation and producing a positive feedback loop.[62-64] *GBA1* overexpression decreases α-Syn aggregation in PD models,[65] indicating that increased GCase activity slows down the degenerative process.[66,67] *In vitro* models demonstrated that GCase interacts closely with the C terminus of α-Syn.[68] Remarkably, reduced GCase activity is not limited to *GBA*1 carriers but is also found in the SN and putamen of idiopathic PD[69] and is found to decrease with aging even in individuals with normal *GBA1*.[65] Low GCase activity and high levels of the corresponding glycolipid substrates are found in postmortem brains of aged control subjects[65,70,71] and in patients with sporadic PD,[65,69] as well as in *Gba1*$^{+/-}$ mice.[72]

Cellular and animal PD models revealed that reducing GCase activity in early stages can potentiate preexisting α-Syn pathology independently of brain cell type,[73] disturb physiological α-Syn tetramers/multimers,[74] and contribute to α-Syn spreading.[75] In turn, progressive toxic α-Syn accumulation in lysosomes decreases GCase activity.[75]

Cholesterol and α-Syn

α-Syn is a 140-residue lipid-binding protein with a typical Apo structure.[23] α-Syn is abundantly and ubiquitously present in the brain, located predominantly in presynaptic terminals inside membranes, such as synaptic vesicles, mitochondria, and the ER.[76,77] The amphipathic helices of α-Syn allow its insertion into the cell membrane, changing its curvature while maintaining its integrity.[23,78] Multiple steps of synaptic activity are regulated by α-Syn, participating in synaptic vesicle cycle[79] and neurotransmitter release.[80,81] These α-Syn-mediated actions take place through regulation of the soluble NSF attachment proteins repector (SNARE) complex (proteins involved in membrane fusion)[82] and its interaction with cholesterol in the lipid rafts.[83] In dopaminergic neurons, α-Syn is involved in regulating dopamine release through direct[84,85] and indirect[86] interactions with tyrosine hydroxylase, which result in reduced tyrosine hydroxylase activity and dopamine levels.

In vitro cholesterol also modulates α-Syn expression and aggregation, enabling α-Syn oligomers to interact with neutral charged membranes, leading to membrane disruption and cell death.[87] In intracellular membranes, α-Syn interacts with the isooctyl chain of cholesterol through its binding domain called tilted peptide[88] (Fig. 2A). This interaction is modulated by fatty acids, GSLs, phospholipids, and gangliosides, and its alteration favors α-Syn oligomerization inside cells or the synaptic membrane, contributing to dysfunctional neurotransmitter release.[91] In *in vitro* models, cholesterol-rich regions, such as lipid rafts, can act as aggregation sites for α-Syn,[92] and cholesterol also regulates α-Syn binding to synaptic-like vesicles, triggering their clustering.[93] Likewise, α-Syn can potentially stimulate cholesterol efflux in neuronal cells,[94] creating a regulatory feedback loop between cholesterol and α-Syn. Moreover, it is reported that extracellular α-Syn may bind to the neuronal and glial membranes, and thus α-Syn moves from these membranes to the inside of these cells, where in turn they can spread in a prion-like fashion.[95]

α-Syn phosphorylation at serine 129 is the most significant posttranslational change related to LB pathology and is involved in α-Syn aggregation and toxicity.[30,96,97] This phosphorylation changes the structure and its protein-lipid binding[98] and inhibits α-Syn–cholesterol membrane interactions, impairing its synaptic function.[99] α-Syn also interacts with some Apos.[23] ApoE4 contributes to α-Syn aggregation in *A53T* α-Syn-transgenic-*APOEε4* mice and accelerates cognitive impairment in patients with PD.[100] Interestingly, both *APOEε4*[100] and *GBA1* mutations[101] are risk factors for dementia with LBs.

Cholesterol in PD

Aging adults are more susceptible to changes in lipid or cholesterol levels and to age-related progressive

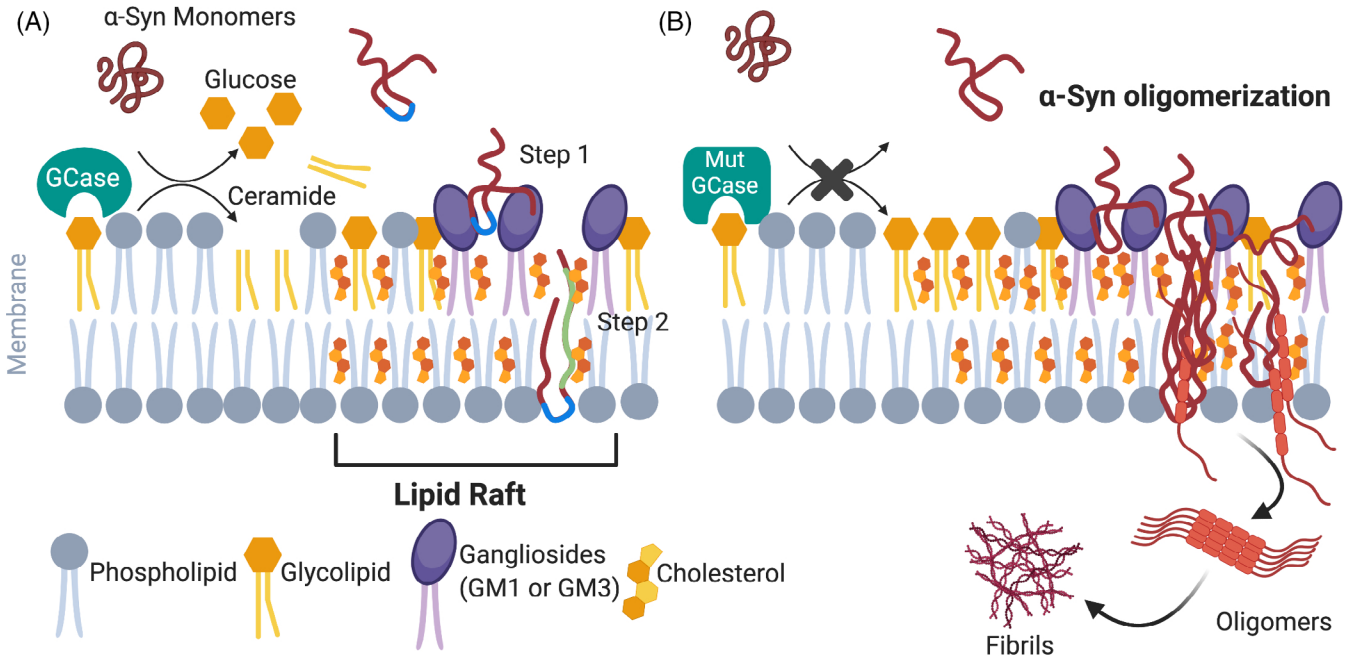

FIG. 2. A schematic model illustrating the potential α-synuclein (α-Syn) interaction with lipid rafts through cholesterol in PD. Lipid rafts are enriched in cholesterol and other membrane glycolipids, such as sphingolipids. (**A**) Under physiological conditions, the extracellular domain (blue) of α-Syn harbors polar amino acid residues that allow its interaction with lipid raft gangliosides, such as ganglioside 1 (GM1) expressed by neurons or GM3 expressed by astrocytes. Thus, GMs allow the attachment of α-Syn to the surface of plasma membrane (step 2). Then α-Syn is transferred through the membrane by forming a complex with cholesterol and its tilted peptide domain (green) (step 1).[88,89] (**B**) In the pathological state of *GBA1*-PD or β-glucocerebrosidase (GCase) deficiency, we proposed that cholesterol accumulation in the lysosomes could affect cholesterol levels in the lipid raft that in turn alter α-Syn interactions with cholesterol. Ultimately, this alteration could favor α-Syn oligomerization inside the cells or in the synaptic membrane, contributing to PD pathology. Noticeably, gangliosides content in lipid raft is increased in PD.[90]

diseases, including PD. Although a plethora of *in vitro* and *in vivo* studies associate cholesterol to genetically linked PD, the involvement of cholesterol remains controversial. Specifically, mutations in various PD-related genes, such as *GBA1*, *LRRK2*, *SNCA*, *DJ-1*, or *PRKN* (among others), change cholesterol levels.[58,64,102] In a PD mouse model, the cholesterol precursor lanosterol is decreased in dopaminergic neurons,[103] and cholesterol biosynthesis is reduced in PD fibroblasts,[104] suggesting possible cholesterol biosynthesis alteration in PD. In rodents, hypercholesterolemia is involved in nigral dopaminergic neurodegeneration[105,106] similar to other studies demonstrating that a high-fat diet exacerbates parkinsonian pathologies.[107,108]

Several clinical studies reported that PD is linked to hypercholesterolemia and hyperlipidemia, but these findings are controversial.[109] Various reports showed a heightened PD risk in individuals with high cholesterol,[110,111] whereas other studies reported a decreased PD risk.[112-116] Moreover, a link was reported between low cholesterol and high PD risk,[117-120] along with reports of a nonassociation between cholesterol levels and PD.[121-123] These varying data might be caused by differences in subject age and sex or other factors. Furthermore, most clinical studies determine blood cholesterol levels; however, these do not necessarily reflect cholesterol in tissues or cells (see the following section). A rigorous analysis of cholesterol impact in PD will conclusively determine which specific changes contribute to PD pathology.

Although cholesterol cannot cross the BBB, elevation of dietary cholesterol increases its metabolite, 27-OHC, which is able to do so (Fig. 1). In human dopaminergic neuronal precursor cells, 27-OHC is reported to regulate α-Syn expression,[124] possibly by binding to LXR, which in turn binds the LXR response element in the α-Syn promotor, increasing α-Syn expression.[125,126] Thus, it is possible that the conflicting results of previous PD and cholesterol studies are related to oxysterols such as 27-OHC hitherto not considered as PD risk factors. Notably, oxysterol 24-OHC is reduced in plasma but elevated in the brains of patients with neurodegenerative diseases, including PD.[127]

Cholesterol derivatives β-sitosterol or β-D-glucoside can induce α-Syn aggregation in mice[128,129] and *in vitro* reactive oxygen species (ROS) production, oxidative damage, and ultimately, neuronal death.[130,131] Inversely, *SNCA* overexpression in patient iPSC-derived dopaminergic neurons impairs cellular cholesterol homeostasis.[132]

Intracellular Cholesterol in *GBA1*-PD

Because the role of intracellular cholesterol abnormalities in PD induction is tentative, it is worth

considering whether the exact site of cholesterol accumulation protects cells[133,134] or renders them more sensitive to cell death.[135] Studies in PD mouse models indicate a dual role of intracellular cholesterol, protecting against lysosomal membrane permeabilization but also stimulating α-Syn accumulation.[136]

Research in *GBA1* knockdown SH-SY5Y cells and embryonic fibroblasts from *Gba1*$^{-/-}$ or *Gba1*$^{+/-}$ mice indicates increased cholesterol accumulation; this was also observed in mouse primary cortical cells treated with Conduritol B Epoxide (CBE), a GCase inhibitor.[137] In addition, fibroblasts from *Gba1*$^{-/-}$ mice show augmented levels of cholesterol and cholesteryl esters.[137] We demonstrated in *N370S*-PD fibroblasts that lysosomal cholesterol accumulation appears to lead to lysosome degeneration and increases vulnerability to cytotoxic stimuli.[58,59,138] Hence we hypothesize that higher GlcCer and cholesterol levels caused by diminished GCase activity might indirectly increase α-Syn levels by decreasing lysosomal maturation and function. Recently, an altered lysosomal GlcCer export seems to increase risk for PD in individuals with mutations in the *ATP10B* gene.[139] More research is needed to elucidate whether other mechanisms exist that explain the lysosomal cholesterol accumulation in the presence of *GBA1* mutations. A cholesterol binding site has been found on the GCase1-specific transporter, lysosomal integral membrane protein-2 (LIMP-2)[140,141] (Fig. 1). This suggests that the cholesterol trafficking impairment detected in *GBA1*-PD models could also interfere with the GCase transport to lysosomes. Indeed, some cholesterol accumulation occurs almost in any type of lysosomal storage disease; likely cholesterol gets stuck in dysfunctional lysosomes. Although it is known that mammalian (or mechanistic) target of rapamycin complex 1 (mTORC1) is involved in regulating cholesterol trafficking to lysosomes,[142,143] the exact role of mTORC1 in GCase dysfunction and autophagy is still under debate.

Lysosomal cholesterol accumulation could affect cholesterol pools in the rest of the membrane organelles; thus, it could alter how α-Syn interacts with lipid rafts (Fig. 2B) and further downstream interactions, which are required for synaptic localization, integrity, and vesicle cycling described in mouse models.[144] The pathological accumulation of intracellular cholesterol could also affect the role of α-Syn in modulating the SNARE complex, preventing lysosomes from fusing with autophagosomes as we previously suggested.[58] Moreover, α-Syn overexpression appears to affect GA tubulation through Rab and SNARE proteins; this mechanism is involved in the GA fragmentation that occurs in PD and *GBA1*-PD.[58,59,63,145] Because cholesterol could ultimately control endolysosomal membrane organization, its storage could modulate GCase activity by disrupting the contact between GCase and its coactivator SapC[146,147] (Fig. 1). Therefore, in *GBA1*-PD models, alterations caused by cholesterol or GCase perturbation can synergize, producing a deleterious vicious cycle.

It was proposed that the accumulation of degenerating lysosomes in dopaminergic neurons, which contain α-Syn aggregates, could form future LBs that are eventually secreted. Because of their membranous nature, these degenerating lysosomes could easily bind other neuronal membranes, thereby transmitting α-Syn aggregates between neurons. Once inside the healthy neuron, these α-Syn aggregates could seed the formation of new toxic aggregates in a prion-like manner.[148] We propose that the MLBs caused by *GBA1* mutations, which appear to be degenerating lysosomes full of cholesterol and other lipids, favor the aberrant interaction of cholesterol and α-Syn. Ultimately, these MLBs could facilitate the prion-like and transneuronal propagation of α-Syn in *GBA1*-PD (Fig. 3).

Mitochondrial dysfunction is also associated with *GBA1* heterozygous mutations in cellular PD models through impaired mitophagy.[59,149] Because mitochondrial cholesterol is reported to be involved in mitophagy,[150] we speculate that cholesterol accumulation in *GBA1*-PD models could cause mitochondrial dysfunction. Mitochondrial DNA disorganization is observed in NPC1 disease human fibroblasts and in control fibroblasts treated with pravastatin or U18666A, inhibitors of cholesterol synthesis or trafficking, respectively.[151] Therefore, this might also occur in *GBA1*-PD models.

GBA2 and Cholesterol Metabolism

Cells contain, besides the lysosomal GCase, another beta-glucosidase named GBA2.[152] GBA2 is located in the ER and endosomes, with its catalytic pocket facing the cytosol.[153,154] GlcCer is formed from Cer and UDP (Uridine DiPhosphate) -glucose by the enzyme GlcCer synthase in the cytosolic leaflet of the GA. Most of the generated GlcCer is transferred to the luminal membrane leaflet for further processing into complex GSLs, but some of the lipid remains at the cytosolic leaflet, where it is subject to metabolism by GBA2.[155] Interestingly, GBA2 does not only cleave GlcCer to Cer and glucose but also acts as transglucosidase, transferring the glucose from GlcCer to cholesterol.[156] Thus, GBA2 can generate from GlcCer and glucosylated cholesterol (GlcChol).[156] Importantly, GBA2 directly links in this manner GlcCer to cholesterol metabolism.

The presence of GlcChol was demonstrated in various tissues.[156,157] Under normal conditions, GCase degrades GlcChol in lysosomes; however, when lysosomes excessively accumulate cholesterol, as is the case in NPC disease in which cholesterol egress from lysosomes is impaired because of mutated variants of NPC1 or NPC2 protein, GCase inside lysosomes also performs transglucosylation using the abundant cholesterol

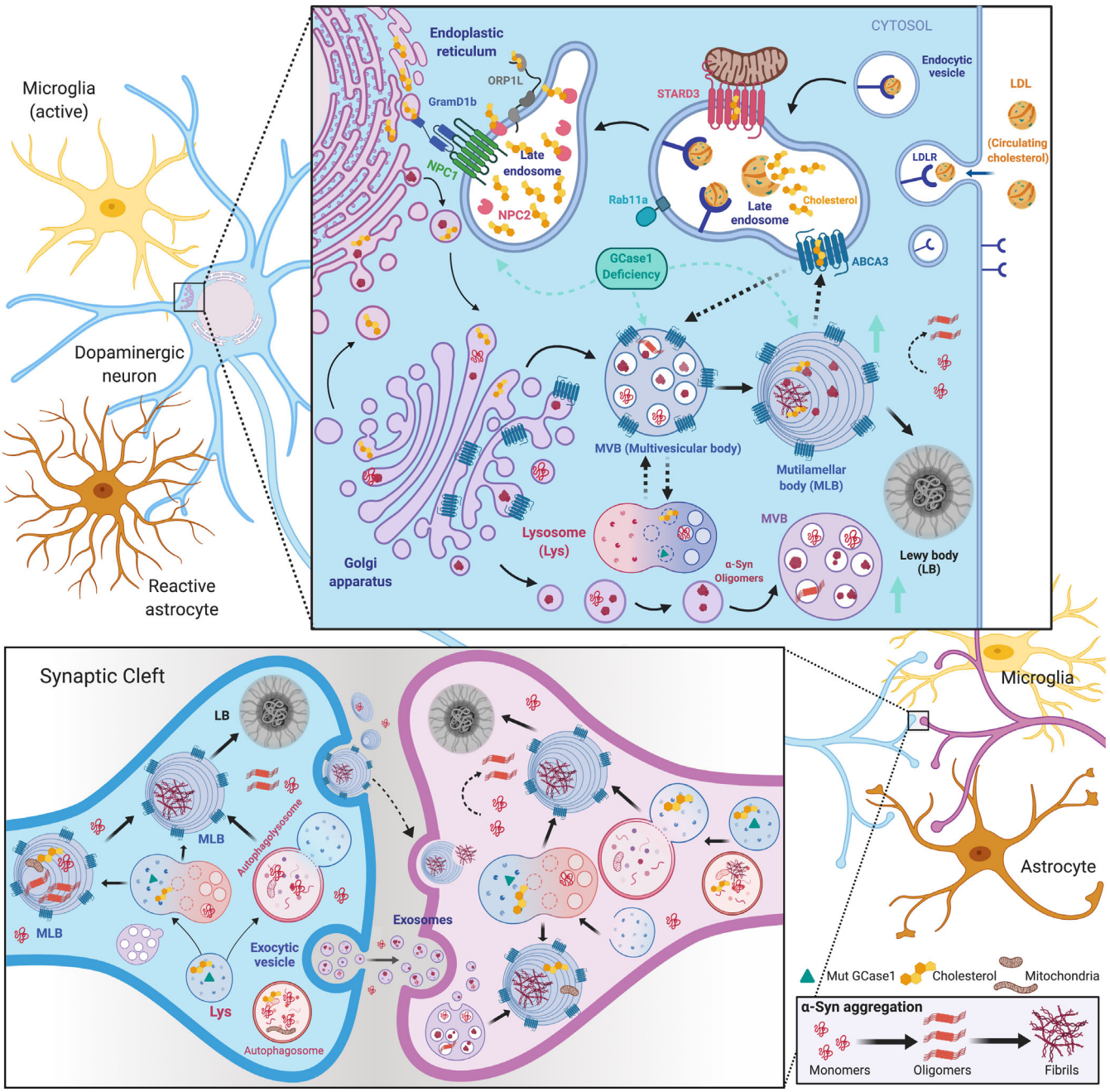

FIG. 3. Transneuronal propagation of pathogenic α-synuclein (α-Syn) through multilamellar bodies (MLBs) in Parkinson's disease (PD). First, a schematic illustration representing the role of intracellular cholesterol trafficking in hypothetical MLB biogenesis is zoom visualized. The MLB biogenesis, a pathological type of lamellar body found in *GBA1*-PD fibroblasts, is completely unknown. We hypothesize that Rab11a and ABCA-like transporters could be involved. ABCA3 could ingress cholesterol or other lipids from other donor organelles into MLB for its biogenesis and, in turn, from MLBs into the endosomes. Overall, ABCA3 play a critical role in the maturation of the multivesicular bodies (MVBs) into the MLBs through fusion with the autophagolysosome by helping these vesicles become filled with lipids and cholesterol. Second, a diagram illustrates how *GBA1* mutations alter the dynamics of autophagosome–lysosomes and endosomes. Accumulating cholesterol generates MVBs and MLBs containing pathogenic α-Syn that could degenerate into Lewy bodies (LBs). Multilamellar (ML) or multivesicular (MV) organelles, as well as naked α-Syn, could be secreted from dying neurons (blue) and, in turn, captured by healthy neurons (pink) or glial cells (yellow and orange) propagating α-Syn pathology. Once inside the healthy neuron, α-Syn fibrils break the ML membrane to reach the cytosol, where they seed new aggregates by recruiting monomeric α-Syn. Notably, GCase lysosomal dysfunction increases the amount of MV endosomes that secrete α-Syn by exosomes. Bottom right panel shows the α-Syn aggregation process.

as acceptor.[156] Consequently, GlcChol is markedly increased in patients with NPC and mouse models.[156] In the lipid-laden lysosomes of NPC cells and tissues, GCase activity tends to be partly reduced, which is accompanied by increased activity of GBA2.[158] This disbalance further favors GlcChol levels. Pharmacological inhibition or genetic ablation of GBA2 in $Npc^{-/-}$ mice significantly increases life span and delays

onset of motor impairment.[158] Based on this, excessive GBA2 activity and the related formation of toxic GlcChol might be hypothesized to contribute to neuropathology. Notably, glucosylated sitosterol, a very close analogue of GlcChol, induced PD symptoms in rodent models.[128] Recently, GBA2 was found to also form galactosylated cholesterol, a lipid that accumulates in the brain.[159]

The precise role of GBA2 in neuropathology in PD-*GBA1* is far from clear. In GD, GBA2 activity is found to be increased in response to reduced GCase.[154,160,161] However, in cellular PD models[59,162] and SN of patients with PD[69] reduced GCase and GBA2 activities were observed. It should be kept in mind that the actual ratio of degradative lysosomal GCase and synthetic GBA2, as well as the availability of cholesterol as acceptor, will determine GlcChol levels. A careful analysis of GlcChol levels in PD-*GBA1* brain seems warranted. The various aspects of GlcChol generation have recently been reviewed.[163-165]

Cholesterol in Neuroinflammation

Neuroinflammation and gliosis, relevant hallmarks of neurodegeneration in PD,[166] have emerged during the past few years as one of the causes of neuronal death rather than the consequence. Chronic microglial activation is detected in striatal and cortical regions of postmortem brains from patients with PD and from animal models.[167] Due to the role of microglia in tissue debris clearance, a proper lysosomal function is critical.[168] In cellular/neuronal models, PD-*GBA1* mutations impair lysosomal function promoting ROS,[59] which might lead to proinflammatory factors release from activated microglia and/or astrocytes. Ultimately, activated microglia might induce neurotoxic reactive astrocytes, promoting a faster dopaminergic neuronal death in PD.[169] In addition, aberrant α-Syn is transferred from neurons to microglia and astrocytes, triggering a proinflammatory response.[170,171]

α-Syn accumulation by GCase inhibition in mice induces complement C1q[172] expression required for microglial phagocytosis. Intriguingly, genetic *Gba1* ablation in dopaminergic mouse neurons activates microglia without neurodegeneration.[173] Furthermore, GlcCer accumulation in experimental and clinical GD induces complement C5a modulating inflammation.[174] Increased inflammation plasma markers are well established for GD[175] and now for patients with *GBA1*-PD.[176] *N370S*-GD macrophages show increased interleukins and NLRP3 (nucleotide-binding domain, leucine-rich repeat family pyrin domain containing 3) inflammasome hypersensitivity as a result of autophagy–lysosomal failure[177] as it occurs in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-PD mice.[178] Notably, *GBA1*-mutant mouse astrocytes exhibited dysfunction in inflammatory responses not directly associated with α-Syn lysosomal degradation.[57]

Cholesterol homeostasis and neuroinflammatory signaling are connected in neurodegeneration.[179] Indeed, *Npc1*$^{-/-}$ mice displayed dysregulated expression of inflammatory mediators.[180] Hypercholesterolemia produces microglial activation, and high-cholesterol diet promotes inflammatory responses.[181] Furthermore, oxysterols are involved in glial activation,[182] where LXRs can modulate cholesterol and oxysterol metabolisms through repressing neuroinflammation.[183] The inflammarafts are cholesterol-enriched lipid domains that are thought to act as a platform mediating the cellular inflammatory response, conceivably being regulated by cholesterol and sphingolipid metabolism[184] and ApoE[185] (Fig. 1). Hence altered intracellular cholesterol related to GCase deficiency might induce a neuroinflammatory response in PD. This should be further investigated because this neuroinflammatory response may discriminate *GBA1* carriers susceptible to development of clinical PD.

Overall, it is possible that a particular cholesterol or lipid profile could confer susceptibility or resistance to PD, mimicking the altered molecular mechanism of cholesterol homeostasis in patients with *GBA1*-PD. Research to address this hypothesis involves determining the metabolic impairments specific to the disease and investigating interventions that can reestablish cholesterol and lipid homeostasis.

The Autophagy–Lysosomal Pathway and Cholesterol in PD

The autophagy–lysosomal pathway is in charge of bulk degradation of long-lived or damaged proteins and organelles. An imbalance of the neuronal autophagy–lysosomal pathway function leads to excessive protein aggregation in PD.[186] GCase is important for the modulation of these pathways by maintaining effective endosomal recycling and unfolded proteins degradation.

Our studies in human *GBA1*-PD-derived fibroblasts demonstrated decreased GCase activity and changes in the subcellular localization.[58,59] These alterations induce ER stress and disorganization, along with GA fragmentation. Eventually, the ER stress activates the unfolded protein response that primarily leads to an increase in autophagosomes by activated Beclin1 and mTORC1 pathways.[58-60] However, the subsequent autophagy is ineffective due to deficient lysosomal function. This could contribute to general cellular dysfunction because it exacerbates misfolded protein aggregation along with lipid and cholesterol accumulation.[58,59] Future research should determine whether accumulated cholesterol and other lipids are the

primary nondegraded material in *GBA1*-PD or whether they represent secondary abnormalities that may trigger lipid stress and alter lipid trafficking. Moreover, this autophagic flux disruption in cellular *GBA1*-PD models can hamper the degradation of worn-out mitochondria.[149] Consequently, these mitophagy alterations lead to heightened ROS production, similar to what we found in the *N370S*-PD fibroblasts, rendering affected cells more prone to cell death.[58,59]

Oxidative stress could induce lipid peroxidation and oxidized proteins in the lysosomal membrane, reducing lysosomal efficiency to degrade cargo. As mentioned previously, 24S-OHC is generated in the ER of neurons. Although 24S-OHC is important for the maintenance of brain cholesterol homeostasis, it can cause neurotoxicity when it is excessively esterified by acetyl-coenzyme A acetyltransferase 1 in the ER.[187,188] Therefore, further studies are necessary to determine the levels of 24-OHC or 24-OHC esters in *GBA1*-PD models, because accumulation of the latter may disrupt the integrity of the ER membrane triggering secretion of ER luminal proteins, causing ER dysfunction and cell death.[188] Therefore, altered cholesterol homeostasis detected in *N370S*-PD fibroblasts compared with control subjects[59] and *Gba1*-mutant cells[137] could be a primary contributor to ER stress, dysfunction, and unfolded protein response activation instead of a direct consequence of GCase deficiency. Hence disturbance of cholesterol homeostasis may decisively impact the function and viability of neurons and initiate PD development.

Lewy Pathology

The major pathogenic mechanism of PD is presently considered to be aberrant interaction of lipids with α-Syn, promoting its harmful aggregation. However, the relationship between this abnormal lipid–α-Syn interaction and LB formation remains unclear. Indeed, recent studies found that the inner side of most human LBs appears to be filled with lipid-rich structures, such as undigested membrane fragments and damaged organelles, rather than with aggregated proteins,[189] suggesting a widespread cellular dysfunction before protein accumulation. It is not currently known which precise types of α-Syn conformation are buried in these membrane fragments and how they are involved in LB formation.

GBA1-PD Multilamellar Bodies in LB Formation

It was shown that *GBA1* carriers have more LBs that noncarriers.[190,191] Strikingly, altered organelles in the LBs display as packed assemblies of membranes,[189] resembling multivesicular bodies (MVBs) and multilamellar bodies (MLBs) that we observed in *GBA1*-PD fibroblasts.[59] MLBs are a characteristic feature of some lysosomal storage diseases, such as NPC1 disease. Inducing an NPC1 phenotype by treating cells with the intracellular cholesterol transport inhibitor U18666A[192,193] or inducing lysosomal dysfunction with cationic amphiphilic drugs such as chloroquine[194] leads to the generation of numerous MLBs with concentric membrane stacks. These can represent dysfunctional lysosomes and contain undegraded phospholipids and cholesterol. It seems to be a synergistic connection between disturbances in lysosomal and mitochondrial pathways, vesicular transport, and PD pathogenesis (for a review, see Smolders and Van Broeckhoven[195]). Many researchers have pointed out the common neurodegeneration occurrence and α-Syn accumulation in inherited lysosomal sphingolipid disorders. Several genes implicated in inherited deficiencies in lysosomal sphingolipid metabolism have been linked to PD: *GBA1* (discussed earlier), *SMPD1* (sphingomyelin phosphodiesterase 1, which encodes acid sphingomyelinase that degrades sphingomyelin to phosphorylcholine and Cer), and *SCARB2* (Scavenger Receptor Class B Member 2, which encodes LIMP-2, the GCase transporter discussed previously). Less clear yet is the association of PD with mutations in the *NPC1/NPC2*, *GLA* (encoding lysosomal alpha-galactosidase A), and *GALC* (encoding lysosomal galactocerebrosidase) genes.[195]

Preliminary findings indicate that genes encoding other lysosomal proteins not directly involved in GSL metabolism (*NAGLU*, *MCOLN1*, *ARSB*, *GUSB*, *GRN*, and *NEU1*) have an association with PD.[195] Thus, it might be hypothesized that a lysosomal disturbance, particularly when it affects GlcCer metabolism, might increase the PD risk, either by causing secondary abnormalities in prelysosomal compartments (such as accumulation of membranous structures or impaired autophagy completion), and/or by causing formation of toxic metabolites. Regarding the latter, *GBA1* deficiency is known to cause formation of glucosylsphingosine (GlcSph) via diacylation of GlcCer by the enzyme acid ceramidase.[196] GlcSph is a biologically active lipid with apparent toxic features.[165] Indeed, excessive GlcSph[197] and GlcCer[61] levels promote α-Syn aggregation in PD models. Notably, although reduced GCase activity is found in different brain regions of patients with PD (as mentioned earlier), there is not much evidence of GCase substrate accumulation in PD brain.[74,198] Glycolipid buildup is more variable and lower than observed in GD. It was proposed that subtle, prolonged glycolipid anomalies generate neurodegeneration at advance ages.[147,199]

Lamellar Bodies in Skin and Lung

Membranous structure accumulation in compartments of the endolysosomal apparatus is not unique to

pathological conditions. In specialized cells of the skin and lung, similar membranous organelles called lamellar bodies are actively produced with specific physiological functions. Lamellar bodies are membrane-enclosed compartments that contain well-defined lamellar (membrane-like) lipid structures.[200]

In the skin, keratinocytes produce specific lamellar bodies that contain large amounts of GlcCer molecules, transported by ABCA12 (ATP-binding cassette subfamily A, member 12), with unique Cer moieties, along with GCase.[165] The lamellar bodies are extruded to the outermost layer of skin, the stratum corneum, where GCase converts the GlcCer to Cer molecules, a modification that is essential for the generation of lipid lamellae with appropriate skin barrier features.[165] Defects in ABCA12 and GCase cause marked skin barrier abnormalities; in severe cases, these are incompatible with terrestrial life.[165] It is noteworthy that patients with PD also experience sweating and skin problems; in some cases, their skin become very dry, rough, and wrinkled.[201,202] The possible role of GCase in such skin manifestations warrants further research.

In the lungs, alveolar type II cells also produce specific lamellar bodies. There, the lamellar body accumulates phospholipids in multi-bilayer membranes that form the pulmonary surfactant necessary for the pulmonary innate immune response and to reduce surface tension in the alveolar spaces.[203] The transporter ABCA3, closely related to ABCA12,[204] is localized in the lamellar body membrane[205,206] and is required for the incorporation of lipids in the lamellar bodies of alveolar type II cells. ABCA3 has a crucial role in trafficking phosphatidylcholine, phosphatidylglycerol, sphingomyelin, and cholesterol. ABCA3 is also expressed in the brain, specifically in oligodendrocytes, neurons, astrocytes, and microglia, but its role remains unclear.[207]

Hypothesized Roles for MLBs and MVBs in the Pathogenesis of PD

We would like to put forward the possibility that the pathological MLBs observed in *GBA1*-PD bear some similarity in origin to lamellar bodies. ABCA-like transporters, commonly regulated by cholesterol levels, could play a role in MLB formation, analogous to their role in the formation of lamellar bodies in keratinocytes and alveolar type II cells. It is reported that ABCA subclass proteins might be involved as regulators of cellular lipids in neurodegeneration.[208] In addition, it is known that Rab11a is a critical protein for lamellar body biogenesis in keratinocytes. Rab11a regulates endosomal recycling of extracellular α-Syn,[209] modulating defects in synaptic transmission caused by α-Syn aggregation.[210] Therefore, we suggest that Rab11a participates in the biogenesis of MLBs in *GBA1*-PD.

We also propose that the cholesterol needed for MLB biogenesis originates from two different routes: (1) endogenous cholesterol synthesized within the ER, transported to the cell membrane directly or through the GA; and (2) LDL-containing cholesterol that binds to the LDLR on the cell membrane, where it is engulfed by an endocytic vesicle. These vesicles fuse with primary late endosomes, where LDL is hydrolyzed. Cholesteryl esters are then cleaved by acid lipase, and free cholesterol is released. Cholesterol is further delivered to secondary late endosomes, where NPC2 binds released cholesterol and transfers it onto NPC1, which acts as a hinge between these late endosomes and the ER, flipping the cholesterol across the membrane.[211,212] Finally, cholesterol is distributed via anterograde (ER to GA) and retrograde (GA to ER) trafficking throughout the cell, including all organelles and plasma membrane (Fig. 3). Alterations in these cholesterol trafficking pathways could produce cholesterol delocalization and favor the formation of MLBs. More research is needed to unwrap the participation of ABCA and Rab11a in pathological lipids, particularly those related to *GBA1* mutations and PD.

It would be interesting to determine whether MLBs change in different PD stages. In the case of lysosomal storage diseases, it has been noted that after the formation of storage lysosomes, multiple abnormal vesicular structures develop during the disease course, likely because of different intracellular pathways (autophagy, endocytosis, etc.) that ultimately converge in dysfunctional lysosomes and the secondary disruption of lipid metabolism.[213] In addition, further research is needed to better understand whether MLB formation in *GBA1*-PD is a cell-autonomous process or whether they appear in specific neuronal populations that are more vulnerable. Likewise, MVBs formed by invaginations of endosomes might play a role in the process underlying the formation of the observed membranous bodies in *GBA1*-PD cells.[214] Recently, these MVBs have been identified as the origin of exosomes that allow the release of intracellular cargo. As mentioned previously, it has been speculated that exosomes that contain α-Syn aggregates may spread the disease[214,215]; however, a firm association between exosomal α-Syn and PD severity and/or progression has not yet been established.

Phospholipids appear to contribute to the spreading of α-Syn through exosomes in neuroblastoma cells.[216] Moreover, Cers are thought to be implied in the generation of MVBs, and altered Cer levels are found in the plasma of patients with PD.[217] When GCase activity is reduced, Cer levels decrease in endosomal–lysosomal compartments, contributing to lysosomal failure to degrade α-Syn.[218,219] Indeed, reduction or overexpression of GCase increases or decreases, respectively, exosomal secretion of α-Syn in mice.[220] Exosomes may contribute to α-Syn spreading in cellular

PD models.[75] Likewise, spread of α-Syn aggregates via extracellular vesicles is augmented in *Gba1b*-mutant Drosophila.[221] Along the same line, exocytosis of the lamellated structures with α-Syn aggregates seen in *GBA1*-PD fibroblasts could propagate between neurons.[148] We therefore propose that the MLBs caused by *GBA1* mutations are pathogenic and might facilitate the prion-like and transneuronal propagation of α-Syn in *GBA1*-PD (Fig. 3). We further hypothesize that cholesterol (and other lipids) could participate in the LB formation. Special attention should be given to the potential role of cholesterol in PD pathophysiology and, in particular, its role in the formation of lamellar body-like structures observed in *GBA1*-PD cells.

Conclusion

This review describes the published evidence supporting the role of abnormal cholesterol homeostasis in PD, its link with GCase, whose gene is identified as a major risk factor for PD, and the interaction of cholesterol with α-syn, which can cause the loss of dopaminergic neurons. We consider the role of multilamellar-like bodies found in *GBA1*-PD-derived fibroblasts in the LB pathophysiology. There is an urgent need for more insight in the precise role of cholesterol and sphingolipid metabolism in PD pathogenesis, particularly as related to *GBA1* mutations. Such knowledge might assist development of new therapeutic avenues for patients with *GBA1*-PD. ■

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