The FEBS Journal

FEBS PRESS science publishing by scientists

STATE-OF-THE-ART REVIEW

Expanding our understanding of synucleinopathies: proteinopathy, proteinopenia, and lipidopathy

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Keywords
alpha-synuclein; lipids; Parkinson's disease; protein aggregation; synucleinopathy; transcription

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(Received 7 June 2024, revised 3 December 2024, accepted 30 January 2025)

doi:10.1111/febs.70011

A possible consequence of the process of protein aggregation in neurodegenerative diseases is the depletion of soluble protein species (proteinopenia), which may, at least in some cases, reduce protein function/activity. This concept, which is often overlooked, may play a role in synucleinopathies such as Parkinson's disease (PD), and dementia with Lewy bodies (DLB), where the protein α-synuclein (aSyn) is known to accumulate in insoluble inclusions. aSyn is at the crossroads between cellular proteostasis and lipidostasis networks and, therefore, we must be aware of the complexity we face when we try to understand the molecular basis of synucleinopathies. Importantly, aSyn and β-glucocerebrosidase (GCase), a sphingolipid hydrolase also strongly implicated in PD and DLB, are connected to lipid biology and to protein quality control function. Thus, changes in the normal relationship between these two proteins may shift the balance in the cell and lead to proteinopathy and/or proteinopenia, while also affecting lipidostasis of cells in the brain. Thus, pathological mechanisms that are a consequence of (a) loss-of-function, (b) gain-of-toxic function, and (c) alterations in lipidostasis need to be carefully analyzed and integrated in our study of the molecular underpinnings of neurodegenerative mechanisms. Here, we highlight implications of the depletion of the soluble form of aSyn, and of GCase, and discuss how state-of-the-art 'omics technologies' could be deployed to assist in the clinical assessment of synucleinopathies.

Introduction

Neurodegenerative diseases, such as Parkinson's disease (PD), and dementia with Lewy bodies (DLB), are known as synucleinopathies due to the accumulation of the protein α-synuclein (aSyn) in intraneuronal protein aggregates known as Lewy bodies (LB) and Lewy neurites (LN) [1–3]. However, the precise role of these

Abbreviations
ApoE, apolipoprotein E; aSyn, α-synuclein; CBE, conduritol-β-epoxide; CMA, chaperon-mediated autophagy; DETs, differentially expressed transcripts; DLB, dementia with Lewy bodies; ER, endoplasmic reticulum; GCase, β-glucocerebrosidase; GoT, gain-of-toxic function; GWAS, genome wide association studies; KO, knock-out; LB, Lewy bodies; LLPS, liquid–liquid phase separation; LN, Lewy neurites; LoF, loss-of-function; LRRK2, leucine-rich repeat kinase 2; LUHMES, Lund human mesencephalic neuronal cell line; PD, Parkinson's disease; PRS, polygenic risk score; RNAseq, RNA sequencing; TDP-43, transactive response DNA binding protein of 43 kDa; TWAS, transcriptomic-wide association studies; UPR, unfolded protein response; VLDL, very low-density lipoprotein.

protein aggregates in neurodegeneration is still elusive. In addition to aSyn, other proteins have emerged as important players in PD, such as the Leucine-rich repeat kinase 2 (LRRK2) [4,5], β-glucocerebrosidase (GCase) [6,7] and, more recently, the transactive response DNA binding protein of 43 kDa (TDP-43) [8,9]. One of the consequences of protein aggregation, which is often overlooked, is the depletion of soluble (and likely functional) species of the same protein, leading to proteinopenia [10–12].

Recently, LBs were shown to have a complex composition that includes proteins, lipids, fragmented organelles, and nucleic acids [3,13–16], and although more evidence is needed regarding the role of these biomolecules and specificity in PD mechanisms, it is likely that nucleic acids and lipids are also important players in the processes that lead to neurodegeneration. Proteins like aSyn and GCase are connected to lipid biology and to protein quality control function. Even though a mechanistic link has been established between them, individual changes in the quantity of these proteins may shift the balance in the cell and lead to proteinopathy and/or proteinopenia, while also affecting lipidostasis of different cells in the brain.

Protein aggregation can lead to a gain-of-toxic function (GoF) of the protein aggregates, and protein depletion due to aggregation can lead to a loss-of-function (LoF) of the soluble species. Protein aggregation and changes in proteostasis are hallmarks of normal aging [17,18]. Interestingly, there are cases where neurologically 'normal' patients show an accumulation of protein aggregates in the brain [19,20]. This opens the possibility to explore other mechanisms such as proteinopenia rather than only focusing on the predominant view of GoF and proteinopathy and its role in pathological mechanisms that may lead to neurodegeneration.

Thus, for our understanding of the molecular underpinnings of neurodegeneration, it is essential to (a) explore how proteinopenia affects neuronal homeostasis and brain function, (b) address the possibility that, in the long run, the balance between LoF and GoF may play a role in neurodegeneration, and (c) determine the implications of these processes on lipidostasis. In the present review, we highlight the individual implications of the depletion of the soluble form of the major component of pathological protein inclusions, aSyn, and the important genetic risk factor GCase. Additionally, we discuss how 'omics' technologies may be integrated to characterize the molecular fingerprints of disease and help in the clinical assessment in synucleinopathies.

Implications of aSyn depletion in neuronal survival and neuroinflammation

aSyn is a 140 amino acid protein encoded by the *SNCA* gene and is highly abundant in the brain [21–24]. aSyn comprises an N-terminal domain that adopts α-helical structure upon interactions with membranes, a hydrophobic middle region, and a disordered C-terminal domain [25–27]. aSyn has been reported to exist in different states, ranging from monomers to tetramers, oligomers, protofibrils, and fibrils [28,29]. Monomers are assumed to be the most common form found in the presynapses and in the nucleus [30,31], while oligomers and fibrils are thought to be associated with pathological states of the protein [28,29]. Presumably, the normal aSyn function is lost from the moment when the protein changes its conformation and starts to polymerize [11]. Although the levels of aSyn oligomers and aggregates may increase during normal aging, and in neurodegenerative diseases [32,33], these assemblies, in particular the oligomers, are thought to be dynamic, and in equilibrium with aSyn monomers.

Although the physiological function(s) of aSyn continue under research, it has been mainly characterized as a key player in neurotransmitter release (vesicle trafficking and recycling) and, in some cases, regulating the expression of dopamine-synthesis related genes maybe through histone binding, or by activating nuclear receptors [34–37].

Silencing aSyn expression in experimental models has shown, as expected, that the protein plays a role neuronal physiology. In a study performed with two shRNAs that modulated aSyn expression at different levels in the adult rodent midbrain caused degeneration of the nigral neurons [38]. The degree of neurodegeneration of each shRNA was tightly associated with their capability to downregulate aSyn. Furthermore, this neuronal loss could be prevented if endogenous rat aSyn is supplemented to the neurons [38]. In a similar study, shRNAs targeting aSyn were injected in the *substantia nigra* of nonhuman primates (St.Kitts green monkeys). After 3 months, aSyn downregulation reproduced, in region-specific and titer-related manner, the degeneration of tyrosine hydroxylase (TH) neurons seen in the study with rats. Interestingly, the observed pattern of nigrostriatal degeneration of the nonhuman primates was similar to the one found in PD patients [39]. Other studies associated the presence of aSyn as a modulator of gene expression. Here, CRISPR-Cas9 was used to delete aSyn in the Lund Human Mesencephalic (LUHMES) neuronal cell line to evaluate altered physiological cellular functions that might be

associated with neuronal activity and ultimately neurodegeneration. This knock-out (KO) besides leading to a decrease in the expression of cell cycle and differentiation genes, it also shows a downregulation of genes associated with synaptic activity and mitochondria-mediated apoptosis [40]. This highlights the role of the presence of aSyn for neuronal function and survival [38,39]. Overall, these findings suggest that the neuronal toxicity observed may be associated with the deletion of aSyn through different pathways that need further confirmation and experimentation.

Aging studies demonstrated that the levels of aSyn remain unchanged but its phosphorylation in serine 129 is increased affecting processes, such as dopamine uptake [41]. Furthermore, there is a decrease in the locomotor skills and anxiety-like behavior when analyzing and comparing the results of an open field test of 18- and 4-month-old *SNCA*−/− mice [42]. Additionally, when comparing the TH+ neurons in the *substantia nigra* of these aged mice, there is a tendency of a diminished content, associated with dopaminergic loss. Also, there is an inflammatory response, shown by an increase in GFAP, Iba1, and IL-1β [42]. This suggests that molecular mechanisms underlying the pathology are not completely related to aSyn increased levels as it has been strongly established.

Intriguingly, overexpression of aSyn in the SH-SY5Y cell line, even to what might be considered pathological levels, leads to improved viability and proliferation [43]. Additionally, several studies revealed an association of increased levels of aSyn with different types of cancer, like hepatomas and melanoma [44–47], suggesting a role in proliferation. In general, the findings with KO or overexpression of aSyn suggest that the balance in the levels of soluble aSyn protein is important in cell survival, although the precise mechanisms involved are still unclear.

One of the proposed mechanisms that might be behind these effects is the activation of inflammatory pathways, as nigrostriatal neuronal KO of aSyn upregulates inflammatory components, such as the histocompatibility complex class 1, and induces the recruitment of activated microglia, finally leading to cell death [48]. This suggests that aSyn LoF, thus proteinopenia, might be associated with the initial neuroinflammatory response seen in synucleinopathies, preceding the GoF associated with aSyn aggregates in later stages of disease.

Interaction of aSyn with lipids

Given the amino acid composition of aSyn in the N-terminal and in the middle hydrophobic regions (amphipathic region), aSyn binds glycosphingolipids that contain sulfate, phosphate, or sialic acid in membranes and in synaptic vesicles (Fig. 1A) [49–52]. Interestingly, most of the PD-associated mutations in the *SNCA* gene directly modify the properties of the amphipathic region, thereby affecting aSyn-lipid interactions [49,53,54]. For example, the A53T and A30P mutants appear to have altered membrane-binding affinities when compared to WT aSyn (Fig. 1B) [53,55–58].

Interestingly, glycosphingolipids, and specifically gangliosides, are reduced by up to 20% in PD [59], which might further contribute to reducing the interaction of aSyn with membranes, increasing the pool of soluble species and, possibly, making it easier for aSyn to aggregate [60]. Particularly, this is relevant in the context of endosomes and lysosomes where the lipid content is affected and an acidic environment is normally found [61,62]. *In vitro* experiments, such as single-molecule fluorescence tethered approach for probing of intermolecular interaction (TAPIN) [63] and aggregation assays using preformed seed fibrils (PFFs) [64], demonstrate that the stability of aSyn dimers is more than 3 times higher in an acidic (pH = 5–6) environment and that the rate of secondary nucleation increases. Thus, if lipid interactions and composition are altered in the endolysosomal system, where there is an acidic pH, then an increased aSyn aggregation rate might be plausible. Therefore, this mechanism might contribute to the uptake and aggregation of aSyn through an impaired endolysosomal system [65].

The N-terminal aSyn domain can also interact with apolipoproteins, such as apolipoprotein E (ApoE). Strikingly, the *APOE4* allele has been identified as one of the strongest genetic risk factors for PD and DLB [66–68]. ApoE is involved in lipid exchange between neurons and glial cells [60–64] playing an important role in brain lipidostasis [69–73]. Even though, astrocytes are the main producers of ApoE in physiological conditions [71,72], during inflammation and neuronal damage, microglia and neurons are also able to produce it [74–76]. *In vitro* and *in vivo* experimental models demonstrate that aSyn has a higher propensity to aggregate when the APOE4 variant is present, when compared to the APOE3 and APOE2 variants (Fig. 1) [77,78]. Furthermore, the presence of ApoE4 exacerbates the aggregation of aSyn, alongside with an increase in astrogliosis and neuronal loss [78,79]. Interestingly, transcriptomic profiling of a mouse model of synucleinopathy (based on the overexpression of aSyn via adeno-associated viral injections into both lateral ventricles) carrying the ApoE4 variant shows alterations in lipid and energy metabolism pathways [79].

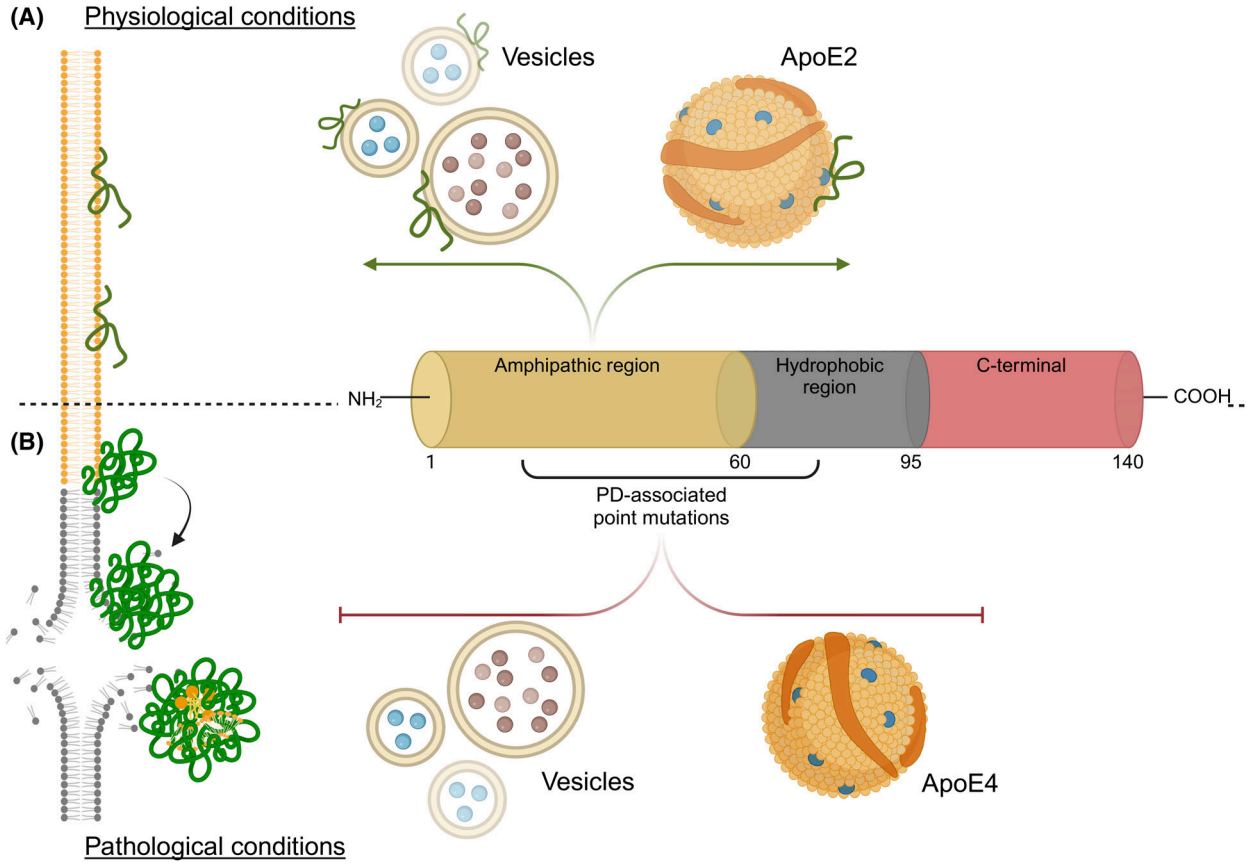

Fig. 1. aSyn and its interactions with lipids. (A) In physiological conditions, aSyn contributes to neuronal lipidostasis through the N-terminal amphipathic region. This domain interacts with the membranes of vesicles containing neurotransmitters, with organelle membranes rich in specific lipid species, and even with apolipoproteins, such as ApoE2. (B) In pathological conditions where lipidostasis might be disrupted, or in the presence of aSyn mutations, the interaction of aSyn with vesicles and apolipoproteins is compromised due to changes in its structure. This raises the pool of soluble aSyn, increasing the chance that it might interact with other lipids in the membranes, and also favoring nucleation, aggregation, and eventually the capture of lipid species and membrane fragments in aggregates that may, ultimately lead to the formation of Lewy bodies.

Conversely, this same synucleinopathy mouse model had reduced aSyn aggregation when carrying the ApoE2 variant [80], which might reflect the protective role of this allele. Furthermore, studies in human *post-mortem* samples revealed that the APOE4 variant carriers had an increased LB pathology in DLB cases [81,82], highlighting the importance of the ApoE variants in disease. This was further confirmed in another set of human samples were a high quantity of LBs contained fragments of APOE and the CSF of PD patients was enriched with APOE along with aSyn [83,84].

Even though APOE has been established as a risk factor for synucleinopathies, there are still inconclusive studies suggesting that the different alleles are more related to the decrease of cognitive function rather than motor symptoms [85–88]. Thus, APOE variants are currently proposed to be a risk factor for the progression of synucleinopathies with just LB pathology, like PD, to dementia, such as DLB or Parkinson's Disease Dementia (PDD). It is interesting to point out that APOE variants are also considered as a risk factor for other neurodegenerative diseases, such as Alzheimer's disease [89–91], and they are also related to inflammation [92,93], a common process found in neurodegeneration. Given this and how sometimes synucleinopathies and amyloidopathies can coexist in the brain during neurodegeneration, it might be plausible to think that APOE variants are related to a more general neurodegenerative context. Nevertheless, it is important to continue the efforts in dissecting the mechanisms that are affected by APOE variants in the presence and/or absence of aSyn and in other neurodegenerative diseases.

Interestingly, the interaction of aSyn with lipids and membranes raises the question of whether this is related to the presence of lipids and shattered organelle membranes in some LBs [14,16]. aSyn nucleation appears to start on the surface of membranes, and as the oligomers grow, some of them adopt a spherical shape on the edges (Fig. 1B) [94,95], possibly related to a liquid–liquid phase separation (LLPS) phenomenon that was recently shown to be promoted by lipids. This mechanism might explain the complex structure of LBs, with a lipidic core derived from organelle-membrane fragments that were captured, possibly by aSyn, into LBs [14,16,94]; nevertheless, further research on the specificity of these interactions and their contribution to aSyn pathology needs to be performed.

Lysosomal and ER alterations due to changes in lipidostasis

Mutations in *GBA1*, the gene encoding for GCase, have been identified as risk variants in PD and DLB patients through genome wide association studies (GWAS) [96–100]. GCase is a lysosomal enzyme that regulates sphingolipid metabolism by transforming glucosylceramide into ceramide and glucose [101,102], but the precise mechanisms by which diseases are triggered are still under research.

One of the cellular processes affected by mutations is the folding time of GCase by the resident chaperones in the endoplasmic reticulum (ER). A longer retention time in the ER signals a stress response [103–105], and this activates de Unfolded Protein Response (UPR) that, ultimately, leads to a decrease in the levels of GCase in neuronal lysosomes (Fig. 2A) [106–108]. This can be straight forward interpreted as a LoF mechanism, given that there is not enough enzyme to fulfill the lysosomal duties regarding sphingolipid metabolism [109,110]. Therefore, strategies have been investigated to overcome the LoF in homozygous carriers, such as the use of pharmacological chaperones that work along with the endogenous chaperone system to aid in the folding of the mutant GCase, and delivery to lysosomes [108]. Nevertheless, results regarding the accumulation of sphingolipid species, the activation of the UPR, and the degree of lysosomal dysfunction in heterozygous conditions are still actively investigated and debated, suggesting other mechanisms may also contribute to neuronal dysfunction.

One of the hypotheses is that GCase deficiency in the lysosomes has an impact in sphingolipid metabolism, leading to the accumulation of glucosylceramide and glucosylsphingosine [111–113]. This, in turn, is associated with reduced lysosomal activity [114–116] and, therefore, with reduced degradation of proteins such as aSyn through chaperone-mediated autophagy (CMA) [116]. Furthermore, GCase mutants are not only retained in the ER, but also adhere to the outer lysosomal membrane (Fig. 2B) [109]. This affects the translocation system of proteins to the lumen of the lysosome, further contributing to the aggregation of proteins in the cytosol and to lysosomal dysfunction.

The ER is a cellular compartment where most lipids are produced [61,117] and, importantly, proteins involved in ER stress responses are associated with lipid homeostasis. For example, Ire1α plays an important role in the assembly of very low-density lipoprotein (VLDL) in the ER, and reduced levels of this protein reduce the export of triglycerides, possibly leading to an intracellular increase of lipids [118]. XBP, another key player in ER stress responses, is involved in lipogenesis by regulating the transcription of several genes associated with lipid metabolism [119]. Furthermore, the overactivation of the PERK pathway is associated with an increase in the activity of the transcription factors SREBP and ATF4, upregulating genes associated with lipogenesis and cholesterol synthesis (Fig. 2D) [120,121]. Together, these data suggest that when GCase mutants are retained for longer in the ER, elements of the ER stress responses may also affect lipid metabolism. This further supports a close relationship between proteostasis and lipidostasis (Fig. 2C), given that any fluctuation and impairment in one of these two processes will also likely impact the other [122].

Moreover, the exchange of lipids between glia and neurons is an important mechanism to reduce the accumulation of toxic lipid species [123,124]. GCase inhibition in the mouse brain recapitulates the redistribution of neutral lipids between neurons and glia observed in PD patients [125]. Furthermore, genetic analyses and clinical evidence show that cognitive decline is accelerated in patients carrying both *GBA1* mutations and the *APOE4* variant, when compared to patients carrying only one of them [126]. Thus, it is important to consider the role that alterations in two key proteins in lipid metabolism might have for neurodegeneration.

Experiments in APOE−/− cerebral organoids showed reduced levels of GCase alongside a reduction in ceramide intermediates [78]. Interestingly, the excess of some sphingolipids, such as glucosylceramide, promotes the formation of aSyn oligomers in acidic environments such as the lysosomes (Fig. 2E) [116]. One hypothesis is that the absence of APOE impacts

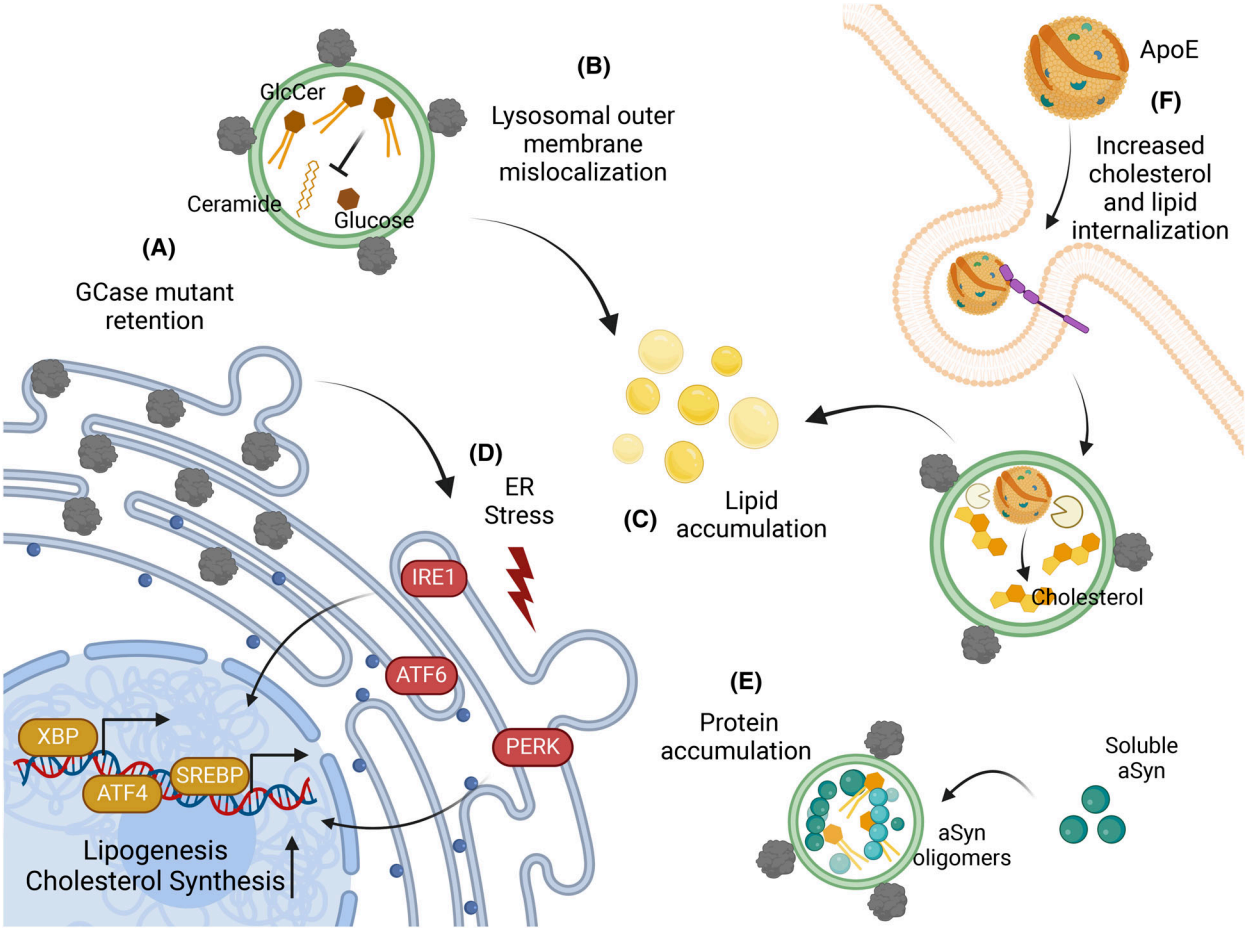

Fig. 2. Relationship between β-glucocerebrosidase (GCase) mutants and lipid and protein accumulation. (A) GCase mutants retained in the endoplasmic reticulum (ER) are recognized as 'misfolded' proteins, triggering ER stress and activating the unfolded protein response (UPR). (B) GCase mutants are not only retained in the ER, but they can be mislocalized onto the outer lysosomal membrane. This deficiency and mislocalization in lysosomes result in the accumulation of glucosylceramide (GlcCer), decreasing lysosomal activity. This is associated with lipid (C) and protein accumulation, such as aSyn (E). (D) Components of the ER stress response pathways, such as XBP and PERK, are also involved in lipogenesis and cholesterol synthesis by regulating the transcription of several genes. (E) Sphingolipid excess in lysosomes can promote the formation of aSyn oligomers, contributing to the aggregation process. (F) Lipoproteins containing ApoE can be internalized into lysosomes, where the lipids are hydrolyzed to free fatty acids and cholesterol. When GCase is not present/functional, cholesterol accumulates inside lysosomes, contributing to alterations in lipidostasis.

membrane lipid composition, leading to endolysosomal dysfunction, lipid droplet accumulation, and GCase LoF [78]. Accordingly, GCase inhibition in mice using conduritol-β-epoxide (CBE) elevates the levels of LAMP1. This can be further exacerbated when reducing the activity of GCase in ApoE−/− mice [127]. Additionally, ApoE content in the cortex, hippocampus, and substantia nigra is increased when GCase is inhibited [127]. These data suggest (a) that alterations in lipid species deregulate other lipid components leading to their accumulation, and (b) that lysosomal activity might increase in response to the alterations in lipidostasis (Fig. 2F), thereby affecting proteostasis.

In summary, lipid metabolism alterations and lipid accumulation compromise cellular functions and might alter interactions with lipid-binding proteins, such as aSyn, promoting their accumulation.

'Omics' approaches to decipher the 'puzzle' of synucleinopathies

Currently, clinical diagnosis of PD and other synucleinopathies is achieved upon the onset of the typical features of the diseases. Diagnosing the diseases earlier will be essential for future clinical trials and for personalized medicine, but this requires the use of

different levels of biological information for enabling patient stratification [128,129]. Therefore, other novel strategies should be considered for diagnosing, for identifying patients, for following disease progression, and for defining therapeutic strategies. In this context, basic science approaches using 'omics' analyses are highly suited to provide insight into the molecular basis of disease and for identifying targets for therapeutic intervention [130].

Proteomics is one of the most used approaches, not only for dissecting disease mechanisms, but also for measuring molecules of interest in a wide variety of human samples. In this context, different techniques can be exploited, such as mass spectrometry, multiplex immunoassays, or proximity extension assays [131–135]. Although these experimental strategies are very specific at a molecular level, they are still not widely implemented due to limited access to expensive instrumentation and to technical issues that require unique expertise [132,134]. Nevertheless, technological advances in the field of proteomics, such as tremendous increases in sensitivity, make these technologies powerful instruments for dissecting molecular mechanisms associated with neurodegeneration.

Next generation sequencing technologies have also been important approaches in the field. Genomics enabled various GWAS that resulted in the identification of genetic variants associated with low, middle, or high risk of developing the disease. Likewise, transcriptomic studies are bringing tremendous information into the molecular mechanisms of neurodegeneration, by providing unparalleled information about coding and noncoding molecules, including chromatin and RNA modifications [115,136,137]. In particular, the advent of single-cell/single-nucleus transcriptomics is providing mechanistic insight into disease etiology and may also aid in diagnosis and in delineating treatment strategies. For this, identifying differentially expressed transcripts (DETs) due to alternative splicing is also a priority. Characterization of spliced variants might shed light into cell-autonomous pathways and into genetic interactions leading to neurodegeneration. In synucleinopathies, such studies are still scarce, but this strategy has revealed novel transcriptomic signatures in other dementias such as AD [138,139]. In DLB, RNA sequencing (RNAseq) and single-cell RNAseq revealed a clear alteration in transcript ratios across cell types [136]. These sorts of analyses can lead to transcriptome-wide association studies (TWAS), which might help further interpret disease risk arising from GWAS. Although additional detailed studies are needed to support this strategy, this clearly suggests that already identified genetic variants may harbor additional secrets that reflect the complexity of the neurodegenerative landscape.

Supervised machine learning and AI-assisted models are starting to prove all their power for predicting and assessing pathology risk and outcome. Until recently, the most common computational technique using genomic information was the polygenic risk score (PRS) [140]. This score aims to provide a predictive metric of an individual's predisposition to develop a certain pathology based on his/her genetic landscape, using the cumulative data usually found through GWAS data [141]. The main goal is to consider as much variance as possible in order to assess the risk of pathology. Nevertheless, this approach does not explain causality, meaning that it does not take into account other risk factors that may be key players in pathology. Nevertheless, owing to their sensitivity for unraveling 'hidden' data within a complex dataset [142], novel supervised machine learning algorithms hold great promise for integrating information from different 'omic' technologies in order to improve the accuracy of genomic prediction [142,143]. However, a major problem in most of the datasets is that they focus on particular populations, primarily, white caucasians, that are not representative of the risk that certain factors might have on other populations. In this context, it is imperative that we are aware of this bias, and that we make all efforts to study diverse populations that represent the human species as a whole, to ensure that AI and supervised machine learning approaches are not leaving any population 'behind'.

We should also keep in mind that the brain is extremely rich in lipids, and that various lipid species have been implicated in neurodegenerative conditions, including synucleinopathies [144]. Thus, lipidomics is also an important field in the study of disease-associated mechanisms and for the development of putative therapeutic strategies. In this context, methods such as mass spectrometry and ion chromatography are extremely important for assessing lipid composition in various types of biological samples (including biopsies and body fluids) [145].

Several lipidomic studies show that various lipid species are altered in the brains of PD and DLB patients [146,147]. Particularly, species, such as cholesterol, and sphingolipids are detected in different levels in PD patients [148,149]. Additionally, studies performed in the cerebrospinal fluid and plasma of PD patients identified different lipidomic signatures (increased monohexosylceramides, ceramides, and decreased sphingomyelin) compared to healthy controls [150,151]. Although lipidomic profiles of PD and DLB patients have been reported, it is still challenging

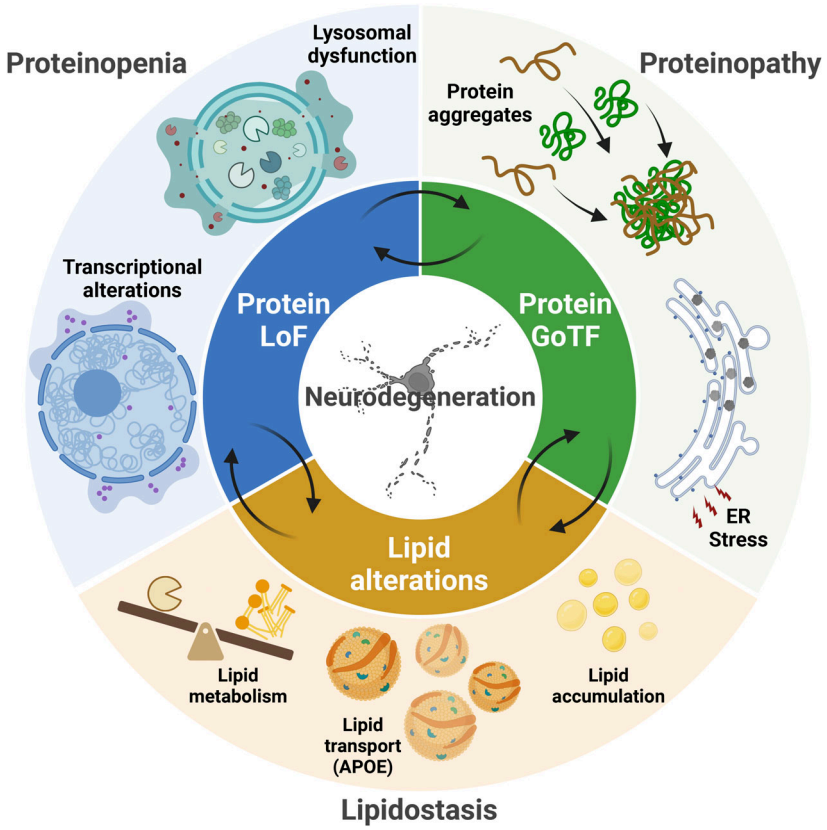

Fig. 3. Proteinopenia, proteinopathy, and lipidostasis as key players in synucleinopathies. Different mechanisms have been implicated in neurodegenerative diseases such as synucleinopathies. Proteinopenia can lead to transcriptional alterations and lysosomal dysfunction, but at the same time these proteins can mislocalize and aggregate in the cytoplasm, or can be retained in the endoplasmic reticulum (ER), triggering the ER stress response. In turn, a reduction in the levels of functional proteins, due to mislocalization and aggregation, may lead to altered lipid metabolism, transport, and accumulation, affecting the overall neuronal lipidostasis. Most likely, all of these mechanisms coexist and contribute to disease. Thus, the interactions between loss-of-function (LoF), gain-of-toxic function (GoF), and alterations in lipidostasis need to be carefully analyzed and integrated in our quest to diagnose and treat synucleinopathies and other neurodegenerative disorders.

to compare them due to the variability in the methods and sampling used in the different studies [152].

In summary, a combination of 'omics' approaches, such as the ones highlighted here, will be essential to generate molecular fingerprints that can help us uncover the biological basis of disease and, thereby, to enable precision medicine even in complex neurodegenerative diseases such as synucleinopathies.

Concluding remarks

Synucleinopathies are highly complex, multi-factorial, and still untreatable neurodegenerative diseases. It is a fact that, despite tremendous effort and progress in our understanding of the biology involved in synucleinopathies, successes have been very limited. Scientific advances come, often, from breaking boundaries and from challenging dogmas. In this review, we discussed the need for considering alternative, but not necessarily mutually exclusive perspectives, such as proteinopenia and lipidostasis dysfunction, in our list of synucleinopathy-associated pathological mechanisms (Fig. 3).

Nowadays, state-of-the-art 'omics' approaches, such as proteomics, genomics, and lipidomics, provide molecular fingerprints of cells, organs, or organisms. The challenge will be to exploit the large datasets generated by these approaches, using machine learning and AI tools, in order to understand cell physiology as a whole. This will enable us not only to formulate and test more unbiased hypothesis, but also to foster the development of biomarkers for early diagnosis, and of personalized medicine strategies for treating complex neurodegenerative diseases.

Acknowledgements

TFO is supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC 2067/1-390729940. MF-L is supported by the Secretaría de Educación, Ciencia, Tecnología e Innovación de la Ciudad de México (SECTEI) under the Fellowship number SECTEI/137/2023. All illustrations were created with BioRender.com. Open Access funding enabled and organized by Projekt DEAL.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

MF-L designed the review outline, did the literature search, wrote the manuscript, designed, and prepared illustrations. TFO designed the review outline, performed literature research, wrote, and proofread the manuscript. All authors read and approved the final manuscript.

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