Mitochondrial Dysfunction and Neurodegeneration in Lysosomal Storage Disorders

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Abstract

Lysosomal storage disorders (LSDs) are rare inherited debilitating and often fatal disorders. Caused by mutations of lysosomal proteins, they are characterized by the accumulation of undegraded material in lysosomes and by lysosomal dysfunction. While the LSDs are multisystemic diseases, the majority present neurologic symptoms and neurodegeneration. Only very recently has a role emerged for mitochondrial dysfunction in the pathophysiology of some LSDs, suggesting a direct or indirect impact of lysosomal dysfunction on mitochondria. Moreover, mitochondrial damage may also cause lysosomal dysfunction, further supporting the activity of common signaling pathways and cross-talk between the two organelles. Thus, the interface between lysosomal and mitochondrial (dys)function is emerging as an important contributor to the pathogenesis of the LSDs and other neurodegenerative disorders. In this review, we explore the mechanisms linking lysosomal and mitochondrial dysfunction, in order to assess whether specific mitochondrial pathways represent a new therapeutic frontier in the management of the LSDs.

Etiopathogenesis of Lysosomal Storage Disorders

Lysosomal storage disorders (LSD) are a class of more than 50 rare inherited metabolic diseases caused by genetic mutations in either lysosomal enzymes, non-enzymatic lysosomal proteins or non-lysosomal proteins, affecting lysosomal function. The loss of function of these proteins leads to accumulation of storage material in the endosomal, autophagic and/or lysosomal systems, influencing a number of cell signaling pathways and reducing cell survival [1].

The combined frequency of all LSDs is estimated to be between 1 in 5000 and 1 in 7500 live births in many population studies [2,3], but may be higher, as a number of children are likely misdiagnosed or undiagnosed. A number of different clinical presentations have been described for each LSD, with variable symptoms, severity, age of onset, as well as prognosis.

Different LSDs, such as Gaucher's disease (GD), Niemann Pick disease C (NPC), Neuronal Ceroid Lipofuscinosis (NCL) or Mucopolysaccharidosis (MPS), tend to affect different organ systems. The majority of LSDs --but not all -- have a profound impact on the central nervous system (CNS) and can affect multiple brain regions leading to neurodegeneration (see Box 1). In fact, LSDs are the major cause of neurodegeneration in childhood at least in the United Kingdom [4]. As current treatments are broadly ineffective for various neurologic symptoms [5], there is an urgent need for new therapeutic strategies and for the development of drugs that can effectively target affected brain regions (see Box 2).

Since LSDs are individually rare disorders, the idea that common underlying mechanisms may help to define common clinical approaches to treat different LSDs is particularly appealing. Moreover, interest in these diseases has recently grown, as new studies have highlighted shared signaling pathways that seem to underlie neurodegeneration in both the LSDs and more common and more extensively studied neurodegenerative disorders, including Alzheimer's and Parkinson's Diseases [6–9]. For example, Parkinson's disease (PD) has strong links with GD (Box 1). Indeed, heterozygous mutations in the lysosomal enzyme glucocerebrosidase (GCase, encoded by the gene *GBA1*), -- otherwise associated with GD-- are the major known genetic risk factor for PD [10]. Moreover, (ii) the protein **alpha-synuclein (aS),** strongly associated with Parkinson's Disease, also accumulates and is aggregated in GD animal models and in GD patients [11–14]. And lastly, for reasons that remain obscure, GCase activity is reduced in the brains of patients with sporadic PD [15]. Common genetic deficiencies between LSDs and neurdegenerative disorders [8,10], involvement of proteins associated with PD and AD etiopathogenesis [6,9,12,14] and common impaired mechanisms as autophagy-lysosomal pathway or mitochondrial dysfunction [7,9,16] are new lines of research that

deserve special attention. In fact, comparing the similarities and differences between LSDs and other neurodegenerative disease groups may help elucidate further the molecular mechanisms underlying the pathogenesis of the LSDs and consequently, facilitate the development of novel therapeutic approaches to treat these debilitating conditions. In this review, we will therefore compare mitochondrial dysfunctions in different LSDs models and discuss their causes and consequences ranging from mitophagy impairment, increased ROS production, calcium dysregulation, with a special mention to the contribution of aS aggregation to mitochondrial damage.

The Dialogue between Mitochondrial and Lysosomal Function

The functional integrity of lysosomes and mitochondria is critically important for their roles in cell physiology and for the maintenance of cell 'health' (see Box 3 and 4, respectively). In non-mitotic cells, degradation of material by lysosomes is especially crucial, because the lack of cell division prevents the dilution of molecules that accumulate in the cytoplasm. Mitochondrial function is also particularly important in neurons which have a limited capacity to upregulate **glycolytic ATP generation** and are therefore dependent on **oxidative phosphorylation** for their energy supply [17]. Strong links between mitochondria and lysosomal biology are emerging. Lysosomes play a very important role in most of the processes that allow removal of dysfunctional mitochondria (mitophagy). Moreover, **Mitochondrial Derived Vesicles** (MDVs) are vesicular carriers in the cell cytoplasm which move mitochondrial components to other organelles and constitute an integral part of the mitochondrial quality control systems which remove from the mitochondrial pool those that are damaged, even carrying selected mitochondrial cargoes to lysosomes for degradation in HeLa cells [18].

Activation of the lysosomal **transcription factor EB** (TFEB) (promoting lysosomal biogenesis – see text Box 3) proved sufficient to restore **mitochondrial membrane potential** and ATP production in human induced Pluripotent Stem Cell (iPSC) derived neurons in which mitochondrial function was compromised following long term treatment with rotenone, (which inhibits **mitochondrial complex I)** [19]. TFEB overexpression by adenovirus injection lead also to a significant upregulation of **peroxisome proliferator-activated receptor γ coactivator 1 α (PGC1α)** in mice liver during starvation [20]. Since PGC1α is known to cooperate with Peroxisome Proliferator-Activated Receptor α in the regulation of mitochondrial biogenesis [21,22], this further tighten the link between mitochondria and lysosomes, with different pathways and molecular mechanisms that regulate the relationship between the two.

Interestingly, two recent studies also showed that primary mitochondrial dysfunction could induce lysosomal dysfunction [23,24]. Indeed, T cells from mice deficient in **mitochondrial transcription factor A (*TFAM*)**, were found to present mitochondrial defects and decreased respiration; this was associated with increased nuclear translocation of TFEB, weaker lysosomal calcium mobilization, reduced lysosomal enzyme activity and **sphingomyelin** accumulation, which are all relevant parameters for the evaluation of lysosomal function [23]. Similarly, abnormal lysosomes were observed in neurons and brains from mice lacking the mitochondrial protein **apoptosis inducing factor (AIF)** [24]. Moreover, in mouse embryonic fibroblasts (MEFs) from **Optic Atrophy Protein 1 (OPA1)** knockout mice for (a protein involved in the regulation of **mitochondrial cristae** morphology), accumulation of lysosomes, dysregulation of lysosomal pH, as well as decreased in the activity of the lysosomal enzyme **cathepsin B** were observed [24]. The lysosomal defects were at least partially rescued using antioxidant naturally present in cells like N-acetylcysteine or Coenzyme Q_{10} to treat cells in culture, suggesting that increased free radical generation from damaged mitochondria might mediate altered lysosomal biology [24]. Collectively, these studies have suggested a link between mitochondrial and lysosomal function, but whether this represents a reciprocal crosstalk remains to be determined.

Interestingly, one of the signaling pathways involved in the activation of autophagy – the mechanism that allow cells to degrade and recycle dysfunctional and unneeded cellular components -- (see Box 4), AMP-activated protein kinase (AMPK) pathway, is regulated by the AMP/ATP ratio and by calcium levels [25]. In particular, ATP depletion or increased cytosolic calcium concentration can both activate AMPKα. This in turn inhibits **mechanisticTarget Of Rapamycin (mTOR)**, activating the lysosomal and autophagy master regulator TFEB, and inducing autophagy in human immortalized cell lines and in mouse models [26–28]. These findings lend further support to the tightly integrated connection between mitochondrial and lysosomal function. The functional interplay between these organelles might also be facilitated by a spatial dialogue, coordinating the localization and trafficking of these structures. In fact, it was recently shown that the changse in the expression level of the protein RAB26 are responsible for the relocalization of lysosomes in a secretory pancreatic cell lines that in turn leads to changes in mitochondria localization[29].

Studies of lysosomal and mitochondrial biology support the hypothesis that lysosomal dysfunction negatively affects mitochondrial function, and *vice versa*. However, even if a strong reciprocal interconnection between lysosomal and mitochondrial function exists, dissecting the specific mechanisms by which these affect each other remains challenging.

Mitochondrial Impairment in LSDs

All the LSDs described in Box 1 present some form of mitochondrial dysfunction associated with a primary lysosomal impairment, (summarized in Table 1), affecting the CNS and other tissues, suggesting that impaired mitochondrial function may be a common pathogenic feature of these disorders. However, the degree and nature of the mitochondrial damage reported vary considerably depending on experimental models used.

Morphological changes have been the easiest to detect, mainly through transmission electron microscopy (TEM) and fluorescence imaging microscopy (see examples in ref. [11] and [30]), which mostly reveal an abnormal mitochondrial network, i.e. mitochondrial fragmentation or elongation. These abnormalities are in most cases associated with mitochondrial bioenergetic dysfunction, quantified as a reduced mitochondrial membrane potential and/or impaired mitochondrial respiration.

In neurons and astrocytes from a mouse model for neuropathic GD [11] and in fibroblasts from GD patients [31], mitochondria have been shown to be small and fragmented, exhibiting reduced membrane potential , mitochondrial complex levels and activity, as well as impaired respiration.

Experimental models of Niemann Pick disease C (NPC) also present alterations in mitochondrial morphology and function (Box 1). In yeast cells lacking Ncr1p, an orthologue of the mammalian protein Niemann Pick C1 (NPC1, an intracellular cholesterol transporter whose mutations cause NPC), mitochondria are fragmented, and both mitochondrial membrane potential and oxygen consumption rate are reduced [32]. Two independent studies of fibroblasts from patients carrying mutations in NPC1 gave conflicting results: one described disruption of the mitochondrial network, but increased mitochondrial mass corresponding to increased oxygen consumption [33]. The other study reported reduced mitochondrial membrane potential but no effect on mitochondrial mass or morphology [34]. Neurons derived from NPC1 knockdown human Embryonic Stem Cells (hESCs) showed accumulated fragmented mitochondria and mitochondrial proteins, without any measurable change in mitochondrial membrane potential [35]. These latest findings were in agreement with those from NPC1 knockout (KO) mice, in which neurons presented smaller rounded mitochondria with reduced membrane potential, decreased ATP synthase activity and reduced ATP generation [36].

A mouse model of Multiple Sulfatase Deficiency (MSD) generated by knocking out *Sumf1,* which encodes for the protein Sulfatase-modifying factor 1, involved in the pathway of sulfatase

oxidation, was again associated with mitochondrial fragmentation, accumulation of dysfunctional mitochondria and impaired ATP production in brain and liver[37,38] (Box 1). Fragmented and circular mitochondria with reduced membrane potential were also observed in neurons and astrocytes cultured from a GM1-gangliosidosis mouse model (Box 1) [39]. Degenerating and electron dense mitochondria were observed using TEM in brain tissues from $Hexb^{-/-}$ mice, a model of GM2-gangliosidosis (Box 1) through knock-out the beta-hexosaminidase subunit beta (responsible for the degradation of GM2 gangliosides) [30]. Moreover, in neurons from a mouse model for Mucopolysaccharidosis III (MPS-III) type C (Box 1),mitochondria appeared swollen, and activity of the respiratory **complexes II and IV** were reduced, while expression levels of the Coenzyme Q protein, a component of the electron transport chain, were also reduced [40].

Earlier reports suggested that mitochondrial morphology and function are damaged in animal models for Neuronal Ceroid Lipofuscinosis (NCL) [41,42]. More recently, it was shown that mitochondrial cristae were reduced and disorganized in human iPSC-derived neurons for NCL induced by CLN3 mutations [43], were elongated in cerebellar precursor cells in a *Cln3Δex7/8 knock-in mice* [44], and showed no structural alterations (apart from some smaller mitochondria) in primary cultures from *Cln3-/-* mouse neurons, in which oxygen consumption was nevertheless decreased [45]. Moreover, mitochondria were depolarized in a lymphoblast model of CLN3 NCL [46]. When considering fibroblasts from NCL patients carrying *CLN1* and *CLN6* mutations, mitochondria were fragmented and redistributed at the cell periphery, with increased expression levels of the voltage-dependent anion channel (VDAC) and complex IV, consistent with accumulation of mitochondria [47].

Progressive neuronal injury (especially in motor neurons) was found in Pompe disease (Box 1) patients and models [48,49]. This evidence, the fact that other tissues (such as muscles) in Pompe disease present abnormal mitochondrial cristae and glycogen accumulation in the organelle [50], and that mitochondrial membrane potential, mitochondrial respiration and ATP production are reduced in muscle cells from an acid α-glucosidase (*Gaa*) knockout mouse model [51], suggests that mitochondrial abnormalities may be also present and contribute to neuronal damage.

Some of the published work exploring mitochondrial function in relation to the LSDs remains contradictory, with conflicting measurements of mitochondrial membrane potential, mitochondrial mass and morphology and mitochondrial respiration. The variations in findings may depend on the methods or models used, and greater consistency may be achieved through standardization of methodology for the systematic characterization of mitochondrial function [52]. Thus, while

mitochondrial involvement in the pathophysiology of the LSDs is increasingly clear, mechanistic details still need to be clarified. Ideally, appropriate models for the different diseases should be selected to consider the specific tissues that are affected in the disease, and in particular classes of neurons when trying to explain neurological and neurodegenerative symptoms. Live cell imaging systems should be used to characterize mitochondrial membrane potential (using mainly TMRM or Rhod123 dyes), whilst also providing quantifiable data on mitochondrial morphology and mass. Otherwise, immunocytochemical methods to label cells using antibody against mitochondrial protein (such as Stress-70 protein - grp75 – or mitochondrial import receptor subunit TOM20) could be used to assess mitochondrial morphology and mass. In both cases, a relatively inert cytosolic dye such as calcein (in living cells) or an antibody against a cytosolic protein (for immunofluorescence of fixed material) should be used to evaluate the cell volume, allowing expression of mitochondrial mass as a relative volume fraction.

Mitochondrial oxygen consumption should be measured, using the fluorescence-based 'Seahorse' or MitoXpress (Luxcel) systems [53] or a more classical platinum based Clark electrode (e.g. the Oxygraph 2K, Oroboros) instruments, either using purified mitochondria or intact cells. Indirect measurement of mitochondrial respiration level can be obtained by evaluating the expression levels or the activity of the mitochondrial respiratory chain complexes.

Systematic studies of mitochondrial fission and fusion in LSDs, are few and far between, despite the widely described profound changes in mitochondrial morphology. Indeed, the functional significance of altered mitochondrial morphology is not yet clear. When mitochondrial fragmentation in primary neurons from a GD mouse model was rescued by overexpressing the dominant-negative fission mediator GFP-Drp1K38A. This is a mutant of the GTPase Dynamin-Related Protein 1 (Drp1) which is defective in GTP binding, causing reduced mitochondrial fission and therefore mitochondrial elongation. Mitochondrial function was unaffected by this change in morphology, [11] showing that mitochondrial fragmentation was a consequence and not a cause of mitochondrial dysfunction. It would be interesting to determine whether the same phenomenon applies to other LSDs.

Another important aspect of mitochondrial biology is mitochondrial trafficking, especially in the CNS. Mitochondrial transport to regions of the cell where ATP demand is high needs to be finely tuned, especially in large cells with long processes such as neurons, where energy consumption may be highest at remote outposts [54,55]. The Ca^{2+} buffering capacity of mitochondria in specific cellular regions also has important implications for neuronal function by controlling the

spatiotemporal patterning of intracellular Ca^{2+} signaling [54]. Mitochondrial transport mechanisms are understudied in the LSDs, but a recent study reported a significant reduction in the number of moving mitochondria and in their speed *in vitro* in axons treated with psychosine, a lipid that accumulates in Krabbe disease [56].

Causes and Consequences of Mitochondrial Dysfunction in LSDs

The specific molecular mechanisms that lead to neurodegeneration in LSDs remain poorly understood. Here, we consider candidate causes and consequences of mitochondrial damage associated with primary lysosomal dysfunction (see Figure 1, Key Figure). A schematic representation of the interplay between lysosomal dysfunction and mitochondrial dysfunction is shown in Figure 1, where the contribution of autophagy impairment, ROS production and calcium homeostasis dysregulation is also presented.

Ultimately, it seems critical to establish i) whether there is a common molecular mechanism that culminates in mitochondrial dysfunction in LSDs; ii) the extent to which mitochondrial dysfunction contributes to the disease phenotype and iii) whether the pathways leading to mitochondrial dysfunction represent viable potential therapeutic targets.

Impaired Autophagy and Mitophagy

Dysregulation of autophagic lysosomal pathways has been described in most of the lysosomal storage disorders we consider here [11,31,35,37–40,50,57–60,51]. Most of these LSDs also show fragmentation and a loss of mitochondrial membrane potential (as previously described), which is a prerequisite for mitochondrial removal through PINK1/Parkin-mediated mitophagy [61,62] (Box 5). The most extensively described pathway for mitophagy is triggered by the reduction of mitochondrial membrane potential below a threshold which favors the accumulation of the protein PTEN Induced Putative Kinase 1 (PINK1) at the outer membrane of damaged mitochondria. This recruits the E3 ubiquitin-protein ligase, Parkin, which ubiquitinates proteins at the outer mitochondrial membrane. Parkin-ubiquitinated mitochondria recruit optineurin which can bind Microtubule-associated protein 1A/1B-light chain 3 (LC3), triggering autophagosome formation around mitochondria, and promoting the removal of dysfunctional organelles [63–65]. However, other mitophagy pathways based on Outer Mitochondrial Membrane localized mitophagy receptors whose regulation occurs through their phosphorylation were recently characterized, as well as lipid-mediated mitophagy [66], while autophagy can also be triggered by increased free radical

generation [67]. No information on these alternate mitophagy pathways in LSDs are available at the moment and more studies will be needed to clarify their relevance in the etiopathogenesis and progression of these diseases.

The PINK1/Parkin pathway has been most intensively studied in relation to PD, as mutations of *PINK1 or PARK2*, which encodes for parkin, are genetic causes of early onset PD. Specific impairment of mitophagy through impaired Parkin/Pink1 recruitment or function has also been investigated in relation to GD and MSD in mouse models. In primary neurons cultured from a GD mouse model, colocalization of autophagosomes and mitochondria was reduced, suggesting reduced recycling of damaged mitochondria [11]. However, mitochondria in GD neurons retained the capacity to recruit Parkin following treatment by a mitochondrial uncoupler -- carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone, FCCP --, which transports hydrogen ions through mitochondrial membranes leading to collapse of the mitochondrial membrane potential (ucoupling respiration from oxidative phosphorylation), suggesting that the Parkin-mediated mitophagy pathway was functional. On the other hand, in GD neurons, under basal conditions, depolarized mitochondria did not recruit Parkin, suggesting that mitochondrial membrane potential was not sufficiently compromised to trigger mitophagy through PINK1 accumulation. Conversely, a study of GD human fibroblasts showed increased co-localization between mitochondria and autophagosomes [31], suggesting that differences may arise from the use of different experimental models, not only because mouse neurons and human fibroblasts were used, respectively, but also because in the first case the cells completely lack the enzyme, while in the second case fibroblasts carry single point mutations that cause a loss of enzyme folding, expression and activity, but not its complete depletion. In MSD mouse liver (but not brain), the expression level of Parkin was reduced, relative to control tissue. However, its recruitment to mitochondria upon depolarization to trigger mitophagy was effective, pointing toward a tissue-specific defect in Parkin turnover which was not seen in the brain, rather than toward an impairment of this specific mitophagy pathway [38].

When mitophagy is disrupted, either because of a negative feedback mechanism that inhibits the autophagic pathway upon lysosomal impairment, or through dysfunctional regulation of autophagy and autophagolysosome formation, it seems plausible that impaired/defective mitochondria could accumulate within a cell. Dysfunctional mitochondria may not have the capacity to support normal bioenergetic demands, and consequently, cell function might suffer. Furthermore, a damaged respiratory chain will tend to generate reactive oxygen species (ROS) at an increased rate, which

results in oxidative damage to proteins, lipids and DNA, a common feature of several LSDs [30,32,68–71].

Macromolecules and protein aggregates can also accumulate when autophagy is impaired and this may be of further detriment to mitochondrial function, as discussed below.

Oxidative Stress

ROS are highly reactive oxygen radical species that can cause oxidative damage to proteins, lipids and DNA. As an engine driven by redox and electron transfer reactions, the mitochondrial respiratory chain is one of the major mechanisms generating ROS within most cell types, often viewed as an inevitable by-product of oxidative phosphorylation. ROS are scavenged by antioxidant defences which limit net ROS generation. In healthy cells, low levels of ROS generation may be required for various signaling pathways [72], and therefore, the balance between ROS production and ROS scavenging must be finely tuned. An increase in net ROS generation exceeding the antioxidant scavenging capacity is defined as oxidative stress, leading to lipid peroxidation, oxidation of proteins and DNA damage.

Altered rates of ROS generation have been reported in a number of experimental models for the LSDs. For instance, in patient derived NPC fibroblasts, ROS generation has been shown to be decreased [33], while for NCL human fibroblasts -- associated with *CLN2* and *CLN3* [58] -- and GD human fibroblasts [31], production of superoxide and hydrogen peroxide were found to be increased. In different studies using NCL human lymphoblasts [46], a yeast NPC model [32] or NPC human fibroblasts[69], a human oligodendryocyte cell line exposed to psychosine to model Krabbe disease [73], or mytoubes derived from a knockout mice model for Pompe disease [51], basal ROS and/or H_2O_2 production were also increased. Of note, increased protein oxidation and lipid peroxidation, as well as DNA damage due to oxidative stress, were reported in the urine, plasma and leukocytes of MPS IV patients [68], in a yeast NPC model, in NPC human fibroblasts and in different NPC mice tissues [32,69,70], as well as in brain tissues of GM2 gangliosidosis mice [30] and in NCL patients [71].

Other models also showed increased vulnerability to H_2O_2-induced oxidative stress. Cerebellar neuronal precursor cells derived from the Cln3Δex7/8 knock-in mouse model showed a reduced survival compared to control when treated with H_2O_2 in concentration ranging from 50 to 200 μM . An NPC yeast model treated with 1.5 mM H_2O_2 not only showed decreased cell viability, but also increased ROS production, protein carbonylation and lipid peroxidation compared to control cells

[32]. Pompe disease myotubes derived from a *Gaa* knockout mice model showed a decreased viability compared to control when treated with H_2O_2 up to 2 mM [51].

In human GD fibroblasts and *gba1-/-* mice neurons, Increased ROS production or mitochondrial dysfunction could be rescued when cells in culture were treated with mitochondria-targeted antioxidants like Coenzyme Q or MitoQ, respectively [11,31], consistent with a mitochondrial origin for the generation of ROS. This suggests that enhanced oxidative stress or increased vulnerability to it may be a feature of LSDs, and that scavenging mitochondrial ROS may be a possible therapeutic approach to consider.

More in general, these various findings suggest that the balance between ROS generation and antioxidant defence, such as superoxide dismutases, catalase and glutathione, may be disrupted in several LSDs and that in some models, antioxidant defences may be defective or inadequate to scavenge increased ROS generation. In NPC human fibroblasts, exhibiting decreased ROS production, decreased superoxide dysmutase 1 and 2 (SOD1 and SOD2, responsible for the dismutation of superoxide in cell cytoplasm or in mitochondria, respectively) protein expression was observed [33]. In contrast, in all other models showing increased ROS production, the levels of antioxidant defences varied considerably. For instance, In MPS IV patient erythrocytes, the antioxidant glutathione, a tripeptide that reduces disulfide bonds formed within cytoplasmic proteins and is therefore oxidized to glutathione disulfide, was decreased, while SOD activity was increased [68]. In NCL human fibroblasts, the activity of the enzyme catalase, responsible for catalyzing the formation of water and oxygen from H_2O_2, was decreased [58], and in a NPC yeast model and in NPC human fibroblasts, SOD2 and/or catalase activity and glutathione levels were decreased [32,69], while SOD2 mRNA and protein levels were reduced in GM2 gangliosidosis mouse brains [30]. Furthermore, extensive studies of oxidative pathways in the cerebellum of NPC mouse models [74] as well as from post mortem material from NPC patients [75], further suggest increased oxidative stress in NPC.

From the available evidence in the literature, it seems clear that oxidative stress is a common feature of different LSDs which is also a feature of other neurodegenerative disorders, and a possible therapeutic target. However, at this stage the contribution of mitochondrial ROS or of other ROS sources is still unclear, as well as the degree of dysregulation of the different antioxidant defences within mitochondria and in the cell cytoplasm. All these pathways deserve further systematic investigations in LSDs.

Mitochondrial Calcium Dysregulation

Considering the severe mitochondrial damage observed in many LSDs, it is not surprising that defects in Ca^{2+} homeostasis have also been reported, as mitochondria are intimately involved in calcium signaling, both directly participating and also providing the energy required for calcium signals to operate [76,77]. Moreover, lysosomal Ca^{2+} is emerging as a significant contributor to global cellular Ca^{2+} signaling [78]. In addition, lysosomes can regulate their own refilling via ER Ca^{2+} stores [79], which can also be affected in LSDs [80–82]. Lysosomal calcium stores may affect global calcium homeostasis, further impacting on mitochondrial calcium handling. Moreover, blockers of inositol triphosphate receptors (IP3R), such as 2-Aminoethoxydiphenyl borate and Xestospongin-C, can induce the accumulation of Lysosomal-associated membrane protein 1 (LAMP1), which is a lysosomal protein, similarly to the accumulation seen in LSDs, further linking dysregulated calcium homeostasis to pathogenic mechanisms in these diseases [79].

For example, mitochondrial Ca^{2+} buffering was clearly disrupted in neurons from ***Cln8^{mnd}* mice** (NCL model) [83]. In this study, cellular Ca^{2+} clearance was impaired because of decreased Ca^{2+} uptake by mitochondria through the Mitochondrial Calcium Uniporter (MCU), while the plasma membrane ATPase (PMCA) and the sarco/endoplasmic Ca^{2+} ATPase (SERCA) were properly functioning. Moreover, neurons showed increased vulnerability to excitotoxicity. In another study, increased Ca^{2+} responses to glutamate or caffeine-- likely due to increased Ca^{2+} release from the ER-- were observed in a pharmacological rat neuronal model of GD, leading to glutamate-induced excitotoxicity [84]. The machinery that allows Ca^{2+} to be taken up by mitochondria, i.e. the MCU complex, has not been studied at all in the LSDs. Interestingly, MCU expression controls excitotoxicity [85], further highlighting a need for studies focused on the MCU complex in these diseases.

With regard to apoptotic processes, the application of **psychosine** to neurons in vitro was found to raise cytosolic calcium concentration, which was associated with transient mitochondrial membrane hyperpolarization followed by depolarization; this eventually led to apoptosis [73]. The accumulation of GM1 in mouse embryonic fibroblasts (MEFs) from *beta-gal^{/-}* mice (a model of GM1 gangliosidosis - Box 1) also showed a significant increase in mitochondrial Ca^{2+} concentration following histamine treatment compared to control MEFs [86]; this increase was sufficient to cause opening of the **mitochondrial permeability transition pore,** a mitochondrial catastrophe that leads to cell death.

Conversely, mitochondrial Ca^{2+} uptake was reduced in fibroblasts from patients with ML-IV [59]; moreover ML-IV fibroblasts treated with the Ca^{2+}-mobilizing agonist, bradykinin, demonstrated an increased apoptotic rate relative to controls [59]. ML-IV is caused by mutations in the TRPML1 channel which is involved in controlling lysosomal pH [87], regulating autophagy by sensing mitochondrial ROS production at the lysosomal membrane [88] in lysosomal adaptation upon starvation, which causes a rapid change in TRPML1 activity [89] as well as regulating ER Ca^{2+} release and Ca^{2+} influx [90]. Consequently, unlike GM1 gangliosidosis models, decreases in mitochondrial calcium might constitute an important contribution to ML-IV pathogenesis, and potentially to that of other LSDs. This is very interesting, because it clearly suggests that even if mitochondrial dysfunction is a common feature of LSDs, the contribution of this damage to the overall Ca^{2+} signaling regulation can be different: mitochondrial calcium overload on one side, and reduced mitochondrial calcium uptake on the other. Further studies will be needed to clarify the mechanisms that lead to one or to the other.

At least in NPC models, calcium dyshomeostasis seems to be primarily related to lysosomal Ca^{2+} dysregulation. Lysosomal Ca^{2+} stores can be assessed by tracking either Gly-Phe b-naphthylamide (GPN) levels -- a compound which is degraded by cathepsin C and causes a leak of Ca^{2+} stores from lysosomes-- or bafilomycin A1-- an inhibitor of the vacuolar-ATPase. Using Fura-2 dye and fluorescence imaging, lysosomal stored Ca^{2+} was found to be decreased in lymphoblasts from NPC patients [91] and in human fibroblasts carrying a mutation in NPC1 [34]. However, ER and mitochondrial calcium content remained unaffected in NPC lymphoblasts [34]. It was also recently shown, by using a caged-sphingosine that can be activated by light exposure, that sphingosine can trigger Ca^{2+} release from lysosomes in human immortalized cell lines and this leads to TFEB activation [92]. Interestingly, in NPC human fibroblasts sphingosine is accumulated and when uncaged by light trigger a smaller release of calcium from lysosomes, further suggesting that lysosomal Ca^{2+} handling is crucial for the etiopathogenesis of this disease [92]. Consequently, it is clear that more work is needed to understand the effect of altered lysosomal Ca^{2+} signaling on global cellular Ca^{2+} homeostasis not only in NPC but also in LSDs.

Nevertheless, it is clear that lysosomal Ca^{2+} signaling, ER Ca^{2+} stores and mitochondrial Ca^{2+} buffering capacity are crucial pathways in the etiopathogenesis of LSDs. This is likely due to the interplay in term of signaling and also in term of physical contact sites among mitochondria, lysosomes and ER. Ca^{2+} chelator, agonist/antagonist of Ca^{2+} channels and other molecules able to regulate Ca^{2+}

concentration in these organelles and in the cytoplasm and Ca^{2+} release or uptake from them may be beneficial in LSDs models and used as therapeutic approaches.

The presence of storage material in lysosomes, the alterations in membranes composition due to changes in the overall lipid contents and mitochondrial damage may all be contributing to alteration in the Ca^{2+} handling, but further causes are probably still unknown. Once elucidated, these mechanisms may also be target for designing new therapies.

Accumulation of molecules and macromolecules in LSDs

The most obvious impact of lysosomal impairment in LSDs is the accumulation of macromolecules in the endo-lysosomal compartment and cytoplasm.

Amongst various macromolecules that can accumulate, the **lipofuscins** and lipofuscin-like aggregates are particularly interesting and important for mitochondrial biology. Lipofuscins are yellow brown aggregates made up of oxidized cross-linked proteins and lipids that cannot be digested by cell degradation systems; they also fluoresce green when illuminated with blue light [93]. Lipofuscins can aggregate under physiological and pathological conditions in different tissues; for example, they are found in ageing brains but their accumulation is also a major histopathological feature of a number of LSDs [94].

Lipofuscin granules are not inert: they can be chemically reactive at the cell surface, binding metals, and driving further protein and lipid oxidation that can affect lysosomal and proteasome function [93]. Moreover, remarkably, one of the main constituents of lipofuscin granules is the **ATP-synthase subunit c**, especially in NCL across different species [43,44,57,95]. This suggests a role for mitochondrial damage in the formation of lipofuscins although the mechanism by which the protein is singled out to accumulate in these structures remains obscure.

Accumulation of alpha-synuclein (aS) and aS aggregates is another interesting and potentially important feature of several LSDs. aS is a protein strongly implicated in sporadic and genetic forms of PD that normally localizes primarily at presynaptic terminals in the mammalian brain [96]. In pathological conditions, aS tends to aggregate and form oligomers and **amyloid fibrils** that eventually deposit in **Lewy Bodies** which are protein and lipid aggregates found in PD and in other synucleinopathies [97]. How different aS species contribute to the disease etiopathogenesis is still under debate, but interestingly aS accumulation and aggregation has been associated with mitochondrial dysfunction and fragmentation in several cellular models of PD [98–100]. However, it is currently unclear whether aS accumulation is a primary cause of mitochondrial damage in those

models or has a secondary effect of generating ROS production and/or impaired autophagy. Possible mechanisms previously reported include direct damage by aS oligomers at the mitochondrial membrane in cells and neurons overexpressing the protein [99,101] or secondary effects due to altered calcium signaling in the presence of aS oligomers [102] or to autophagy inhibition caused by aS accumulation in the cytoplasm [103,104]. Although speculative, it is possible that a mechanism such as this might also occur in certain LSDs, feeding into a vicious cycle to cause further accumulation of dysfunctional mitochondria when quality control pathways become impaired.

Indeed, aS aggregates have been documented in a number of LSDs -- in post mortem material from brain samples of patients with GD [105], and GM2 gangliosidosis [106]. Lewy Bodies were also noted in the brains of patients with NPC [107] while neurons in the brains of MPS III subjects were reactive for phosphorylated aS [108]. aS oligomers were also found in the plasma of patients with different LSDs, including GD, NPC and Krabbe disease [109] and in NPC lymphoblasts [110]. Many LSD rodent models (for GD, NCL, GM2 gangliosidosis, MPS III and MSD) that recapitulate the features of these diseases to a certain degree, also show aS accumulation, aggregation or overexpression [11,30,37,45].

For PD and for all other neurodegenerative disorders presenting aS accumulation, it is difficult to define whether aS accumulation is a cause or an effect of mitochondrial dysfunction, or both. However, in a $Hexb^{-/-}$ mouse model for GM2 gangliosidosis, silencing aS by generating double knock out mice $Hexb^{-/-}$ $SNCA^{-/-}$ was found to ameliorate mitochondrial function, suggesting that aS might contribute to mitochondrial pathology, at least in this model [30].

Metabolite and lipid accumulation are a common feature of LSDs and lipids seem to be the most interesting in relation to mitochondrial deficiencies because of their role as structural molecules and as signaling molecules as well. Dysregulation of sphingolipid or cholesterol metabolism are features of many LSDs, including GD, Krabbe, NPC and NCL, because of the direct impact of gene mutations on constituents of lipid metabolism pathways in those diseases. With regard to mitochondrial dysfunction, cholesterol has been demonstrated to accumulate in the mitochondria of NPC human fibroblasts [33]. Accordingly, the concentration of cholesterol in mitochondrial inner or outer membranes isolated from brain tissues of $npc1^{-/-}$ mice was increased compared to $npc1^{+/-}$ and $npc1^{+/+}$ mice [36]. The activity and assembly of the electron transport complexes is critically dependent on the lipid environment in the membrane, but the specific consequences of altered cholesterol content are not well characterised.

In an MPS III mouse model, GM2 and GM3 gangliosides were increased in total brain extracts and their colocalization to mitochondria was verified by immunocytochemistry in brain slices using specific antibodies against GM2-GM3 and against the mitochondrial marker cytochrome c oxidase (complex IV) [40]. In these models, mitochondrial dysfunction was also observed, suggesting a mechanistic link between lipid accumulation in mitochondria and impaired bioenergetic function. More work is required to understand how the accumulation occurs, how it is responsible for mitochondrial damage and how it can be prevented. Moreover, further research is warranted to determine whether the accumulation of lipids observed in other LSDs especially occurs in mitochondria.

Concluding Remarks

The fact that most of the neurological symptoms in the LSDs cannot be treated with available therapies (see Box 2 and Box 5) is of great concern. Given the fundamental importance of mitochondrial function in all tissues, but especially in neurons, the mechanisms that lead to mitochondrial deficiencies in LSDs deserve more attention. Mitochondrial fragmentation, reduction in mitochondrial respiration and membrane potential, increased oxidative stress, as well as decreased calcium handling capacity in several different LSDs suggest that mitochondrial impairment is a common feature of LSDs. It is of special interest that similar impairments are also features of other major neurodegenerative disorders, suggesting common mechanistic links in neurodegenerative pathways. Similarities and differences between these groups of diseases need to be properly characterized with the final aim of understanding their specific and/or common features, in order to pave the way to novel putative therapeutic strategies (see Outstanding questions and Box 2,5).

Multiple cellular pathways are impaired in the LSDs, and these may require multiple therapeutic approaches. Among several possibilities, targeting different regulatory pathways and employing strategies aiming to improve mitochondrial function in combination with approaches to enhance autophagy may prove to be beneficial for many LSDs. Indeed, it will be quite exciting to follow how future mechanistic insight and drug development unfold in the fields of mitochondrial biology and LSDs.

Box 1 – Neurodegeneration in Lysosomal Storage Disorders

Gaucher's disease (GD): The most common LSD is GD, caused by mutations in the *GBA* gene, which encodes the lysosomal enzyme glucocerebrosidase (GCase), responsible for the cleavage of glucosylceramides. GD causes a broad spectrum of phenotypes: while systemic symptoms such as hepatosplenomegaly and bone abnormalities are usual and can be treated, no treatments are available for the neurological symptoms, which include seizures and brain damage, present in a subset of patients. The most severely affected children survive for only a few years.

Niemann Pick disease C (NPC): is caused by mutations in *NPC1*, a gene encoding the membrane protein NPC1 that mediates intracellular cholesterol trafficking. Similarly to GD, patients show accumulation of harmful lipids in different tissues. The disease primarily affects liver, spleen and brain. The first symptoms appear in early childhood and the patients survive for just a few years after diagnosis.

Krabbe disease (also called globoid cell leukodystrophy): results from autosomal recessive mutations in galactocerebrosidase gene (*GALC)* cause, encoding for the lysosomal enzyme galactosylceramidase, responsible for the degradation of galacto-lipids such as galactosyl-ceramide and galactosyl-sphingosine (also known as psychosine). GALC enzyme deficiencies lead to psychosine accumulation and progressive demyelination of cells in the nervous system. The symptoms vary but include deterioration of cognitive and motor skills. The early onset forms are fatal before age 2, while the later onset forms are usually milder and associated with a longer life expectancy.

Mucopolysaccharidosis III (MPS-III, or Sanfilippo syndrome) is an autosomal recessive disease caused by deficiencies in different enzymes involved in heparan sulfate metabolism, leading to accumulation of the latter in lysosomes. There are four different subtypes (A, B, C and D) caused by mutations in different proteins (heparan N-sulfatase, N-acetyl-alpha-D-glucosaminidase, acetyl-CoA:alpha-glucosaminide acetyltransferase and N-acetylglucosamine-G-sulfate sulfatase, respectively) but characterized by similar severe symptoms. MPS-III mainly affects the brain and the spinal cord. Patients are asymptomatic at birth but develop neurological problems in early childhood. In particular, behavioral abnormalities are followed by intellectual deterioration and dementia, and life expectancy is severely reduced.

GM1 and GM2 gangliosidoses are major LSDs, caused by deficiencies in the enzymes beta-galactosidase and beta-hexosaminidase, respectively, and characterized by the accumulation of gangliosides. GM1 gangliosidoses are classified depending on severity and age of onset (infantile, junvenile and adult forms). Infantile GM1 gangliosidoses are the most severe and show very severe neurological symptoms including developmental regression, seizures and profound intellectual disability. GM2 gangliosidoses (also called Tay-Sachs and Sandhoff diseases, depending on the dysfunctional enzyme – i.e. hexosaminidase A or B) present similar characteristics; affected children lose motor skills, vision and hearing, develop seizures, and present intellectual disability with disease progression. For both infantile GM1 gangliosidoses and GM2 gangliosidoses, life expectancy does not extend beyond early childhood.

Neuronal Ceroid Lipofuscinosis (NCL), or Batten disease, is often defined as a non-classical lysosomal storage disorder. NCL can be caused by mutations in 12 different genes which encode proteins with different localizations and functions. Each one of these NCL forms is characterized by lipofuscin accumulation and symptoms which include seizures, visual loss and progressive mental impairment. Classification depends on disease onset, i.e. infantile, late infantile, juvenile and adult NCL, and life expectancy varies with the NCL form and symptoms.

Other LSDs, that impact the nervous system and present neurological symptoms include **multiple sulfatase deficiency (MSD),** caused by dysfunctions in the protein SUMF1, located in the ER lumen, which catalyzes the hydrolysis of sulfate esters. The severity of MSD can vary, and it is classified depending on the age of onset as neonatal, late-infantile, and juvenile. Neurological symptoms include movement problems, seizures and developmental delay (at least for the most severe types), while other symptoms are heart problems, skeletal abnormalities and hepatosplenomegaly. The age of onset and how quickly the neurological symptoms worsen determine life expectancy.

Mucolipidosis type IV is caused by mutations in the *MCOLN1* gene, encoding a lysosomal membrane cation channel involved in calcium signaling. Symptoms include delayed development of mental and motor skills, hypotonia, spasticity and visual impairment. A minority of the affected individuals has an atypical form of the disease with milder psychomotor delay.

Pompe disease is caused by mutations in the gene encoding the lysosomal enzyme acid α-glucosidase (GAA), which converts glycogen to glucose, and leads to glycogen accumulation. The main symptoms are cardiomyopathy and muscle weakness, which can vary depending on severity, and in most cases lead to respiratory failure and death. It was also shown a relevant damage to central nervous system and in particular to motor neurons.

Box 2 – Therapeutic Approaches: Present and Future

The main available treatments for LSDs rely on replacing the dysfunctional enzyme (enzyme replacement therapy, ERT) or restoring enzyme activity [5]. However, these approaches do not help with the neurological symptoms because most of these molecules cannot cross the blood brain barrier. Recent advances in ERT for the treatment of neuropathic LSDs were achieved by fusing the enzyme to peptides or proteins that increase brain delivery. Other options are intracerebroventricular injection or nanoparticle-mediated delivery of the enzymes. In vivo (systemic or CNS-directed) or ex vivo gene therapy are also under evaluation [111].

Given the profound mitochondrial dysfunction described in some LSDs, treatments that affect mitochondrial function may prove helpful especially in neurons. In particular, it seems clear that interventions that reduce oxidative stress through delivery of antioxidants such as MitoQ may be beneficial [11,31]. Other approaches might potentially target mitochondria, impacting mitochondrial morphology, improving mitochondrial respiration to restore ATP levels or modulating calcium levels or handling.

Accumulation of damaged mitochondria can also be harmful. Thus, strategies that enhance autophagy might prove valuable, bearing in mind that lysosomal overload is not desirable. This could be achieved by inhibiting mTOR, which successfully restored ATP levels in iPSC-derived neurons generated from a patient with an ATP synthase deficiency [112], or by acting on other upstream pathways. Another strategy may involve direct manipulation of TFEB activity to promote autophagy and lysosomal biogenesis. Interestingly, TFEB was found to be predominantly localized in the nucleus in MEFs from MPS-II, MPS-III and MSD, suggesting that the TFEB signaling pathway is activated in these diseases, probably in response to lysosomal stress [113]. It remains to be determined whether further activation may be effective in removing dysfunctional mitochondria.

Another approach that may be beneficial concerns the targeted removal of aS aggregates, which are toxic and can damage mitochondria [99]; this might be potentially achieved either by enhancing autophagy [114,115] or via immunotherapy, an approach that has been effective in some PD models [116,117]. Nonetheless, further preclinical studies are warranted to validate these possibilities.

Box 3 Lysosome Biology

Lysosomes are organelles enclosed by a single membrane in which more than 50 hydrolytic enzymes break down macromolecules and organelles that cells target for degradation. Lysosomal degradation is essential to avoid the accumulation of damaged or misfolded proteins or lipids, damaged organelles as well as pathogens.

The optimal pH for most lysosomal enzymes is acidic [7]. An acidic pH between 4.5 and 5 [87], is achieved within lysosomes by the proton-pumping V-type adenosine triphosphatase (ATPase) [118], in concert with the chloride channel 7 (ClC-7), the nonselective cation channel mucolipin 1 (TRPML1), the two-pore calcium channels (TPC1 and TPC2) and the potassium channel TMEM175 [119–123].

Hydrolytic enzymes are synthesized in the ER and Golgi and transferred to lysosomes through different mechanisms [124]. Substrates destined for degradationd within lysosomes are delivered to these organelles by endocytosis -- in the case of extracellular material-- and by autophagy -- for disposal of intracellular waste [125].

It was long thought that the role of lysosomes was limited to the degradation of substrates, however this view has now changed and it is clear that these organelles are involved in several other crucial processes which include regulation of signaling pathways, calcium signaling as well as energy and nutrient metabolism [125,126].

Lysosome biogenesis is regulated by the transcription factor EB (TFEB). TFEB activates the coordinated lysosomal expression and regulation (CLEAR) network, which enhances the expression of lysosomal genes, increasing the number of lysosomes, but also increasing the expression of other proteins involved in lysosomal cellular clearance mechanisms such as autophagy [26,113]. TFEB is activated by Ca^{2+} release from lysosomes, further emphasising a central role of lysosomes in cell signaling [126].

Box 4 Mitochondrial biology

Mitochondria are organelles bound by a double membrane that provide energy to eukaryotic cells through oxidative phosphorylation. Mitochondrial respiratory complexes are redox proteins constituted by multiple subunits that generate and maintain a proton gradient across the inner mitochondrial membrane through proton pumping across the inner membrane, and coupled to electron transfer. This process is driven from Complex I through the oxidation of NADH and at Complex 2, by the oxidation of $FADH_2$, ultimately transferring electrons to molecular oxygen – the basis for breathing itself. The chemiosmotic potential generated drives the ATP synthase (complex V) to phosphorylate ADP, generating ATP. A constitutive electron leak is always present during respiration and may lead to the formation of the free radical superoxide, which can chemically interact with proteins and lipids if not catabolized by enzymes or antioxidant scavenging molecules, such as superoxide dismutase (SOD) or glutathione.

Mitochondria are also involved in other processes including the regulation of apoptosis and calcium homeostasis. This is particularly important for neuronal function as calcium signaling plays a central role in neuronal signaling. Mitochondrial Ca^{2+} uptake shapes calcium signals in the cytoplasm through a transmembrane protein, a Ca^{2+} selective channel localized at the inner mitochondrial membrane, termed the Mitochondrial Calcium Uniporter (MCU) [127]. The MCU mediates calcium uptake into mitochondria, which regulates not only cell signaling but also aerobic metabolism and cell death [128]. MCU activity is tightly regulated by different interactors that form the MCU complex, including the Essential MCU Regulator (EMRE) and MICU1/MICU2 proteins [129]. The activity of the complex is regulated directly by the concentration of cytosolic calcium to which it is exposed. Calcium uptake is also dependent on the mitochondrial membrane potential, i.e. calcium accumulation is reduced in depolarized mitochondria.

Given the importance of mitochondrial function in all cell types, a variety of mitochondrial quality control mechanisms have evolved that serve to remove damaged mitochondrial proteins or even entire organelles through the ubiquitin proteasome system and via autophagic degradation [130,131].

Box 4. Autophagic Mechanisms

Autophagy is a mechanism that allows the controlled and orderly removal of cellular waste, including damaged organelles and aggregated proteins, and recycling of useful cellular components.

Autophagy can be distinguished as macroautophagy, microautophagy and chaperone-mediated autophagy. During macroautophagy (referred to as 'autophagy' in this review), cytoplasmic material is sequestered into vesicles enclosed by a double membrane, termed autophagosomes, which then fuse with lysosomes. After formation of the autophagolysosomes, lysosomal enzymes degrade and recycle autophagosome cargo.

Mitophagy constitutes a type of autophagy specific to the removal of dysfunctional mitochondria by defined pathways (mitochondrial autophagy). It is widely regarded as especially important in maintaining cell health, since for example, a damaged mitochondrial electron transport chain is more likely to generate excessive free radical species leading to oxidative damage in a host cell.

Microautophagy is a special type of autophagy in which cytoplasmic material is engulfed into lysosomes/vacuoles by membrane invagination and then degraded.
Chaperone-mediated autophagy is a process of degradation of soluble cytosolic protein through their targeting to lysosomes by a chaperone-mediated selection.

Box 5. The Clinician's Corner
- LSDs account for most neurodegenerative disorders in children but no effective treatment for the neurological symptoms associated are available. The major therapeutic strategies for systemic symptoms are based on restoring or replacing the activity of a given dysfunctional enzyme, by using molecular chaperones, by providing the enzyme or by gene therapy. Inhibition of substrate synthesis is also possible.
- Interesting similarities with other neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease are emerging: in particular, similar mechanisms cause mitochondrial dysfunction. Moreover, calcium dyshomeostasis and substrate/macromolecule accumulation may also be common pathways associated with the etiopathogenesis of LSDs and other prevalent neurodegenerative disorders.

- LSDs are rare diseases each of which presents a different phenotype for reasons that are still unclear. Therefore, the identification of common underlying targets as mitochondrial pathways may signpost a path to new putative therapeutic approaches.

Figure legends

Key figure. Figure 1. Mechanisms of Mitochondrial Dysfunction in LSDs.

In healthy conditions, mitochondria are responsible for producing ATP through mitochondrial respiration , and oxidative phosphorylation, a process that leads to ROS production. ROS levels are controlled by antioxidant defences and are crucial to mantaining cell health. Mitochondria also manage calcium homeostasis by buffering calcium when needed.

In LSDs, dysfunctional lysosomes are associated with impaired autophagy, and therefore impaired mitophagy. This can lead to accumulation of dysfunctional mitochondria which can be further damaged by accumulated aggregates or macromolecules such as alpha synuclein. Dysfunctional mitochondria produce ATP less efficiently and an impaired respiratory chain may generate more ROS, while damaged mitochondria have a reduced capacity to buffer calcium. Impaired calcium handling may also be associated also with altered ER and lysosomal calcium signaling.

Aside from impaired mitophagy, other molecular mechanisms leading to mitochondrial dysfunction in LSDs, might potentially be linked to the relationship between mitochondria and lysosomes.

Table 1. LSDs and Mitochondrial Dysfunction at a Glance.

The table summarises published studies on mitochondria biology in LSDs, grouped according to the experimental approach used to examine changes in mitochondrial function and the models in which these features were studied. Findings vary depending on the models and on the methods used, suggesting that standardization would be helpful in clarifying the role of mitochondrial dysfunction in LSDs and the molecular mechanisms involved.

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Acknowledgments:

Resources

The Online Metabolic and Molecular Basis of Inherited Disease (OMMIB) is available online for a more detailed description of the different LSDs described in this review (website: http://ommbid.mhmedical.com/book.aspx?bookid=971).

Trends

- Most LSDs show profound mitochondrial dysfunction. This is particularly relevant in neurons which have a strong dependence on oxidative phosphorylation for ATP production and on fine-tuning intracellular calcium levels governing neuronal signaling.

- Mitochondrial defects impact lysosomal function, suggesting a functional relationship – potentially reciprocal -- between these two organelles.

- Lysosomal dysfunction in LSDs is associated with impaired autophagy, dysregulation of mitochondrial quality control pathways as well as accumulation of damaged mitochondria.

- Other new causes and/or consequences of mitochondrial dysfunction reported in LSDs include increased ROS production and oxidative stress, calcium dyshomeostasis.

- Damage mitochondria, calcium signaling and ROS unbalances may all be targets for the development of new therapeutic strategies.

- Alpha-synuclein aggregation, which is involved in neurodegeneration in Parkinson's disease, may be associated with mitochondrial dysfunction and neurons death in LSDs
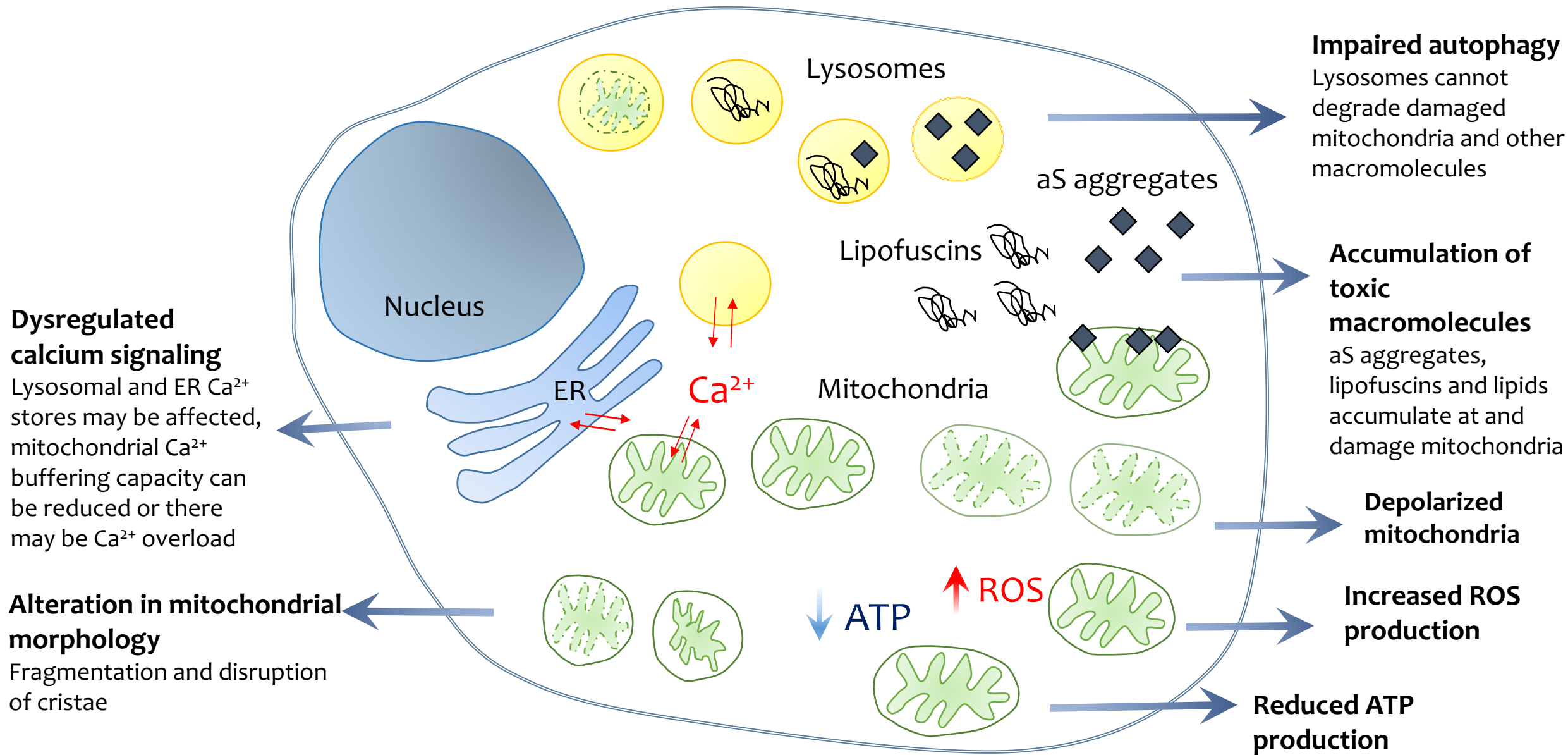
Outstanding questions

- **How does a primary lysosomal dysfunction impair mitochondrial function?**
Most LSDs are caused by mutations in lysosomal proteins which lead to dysfunction of the autophagy /lysosomal pathway. Mitochondrial dysfunction has been identified, but the molecular mechanisms by which lysosome dysregulation lead to mitochondrial damage are still unclear and may vary according to the specific disease.

- **how does a primary mitochondrial deficiency impair lysosomal function?**
It was recently shown that deficiencies in *TFAM* or mitochondrial complexes can lead to lysosomal dysfunction resembling those present in LSDs, suggesting a link, perhaps reciprocal, between the two organelles.

- **Are defects in lysosomal calcium signaling responsible for altered mitochondrial calcium handling?**

- **Are mitochondrial defects in LSDs primarily due to downstream deficiencies in mitochondrial quality control pathways or to a direct effect of lysosomal dysfunction affecting mitochondria?**

- **To what extent do lysosome storage materials in different LSDs contribute to mitochondrial dysfunction?**

- **Does the loss of products normally generated by lysosomal degradation, e.g. sphingolipids, affect mitochondrial function?**

- **What are the common or specific mechanisms that lead to mitochondrial dysfunction in different LSDs and other neurodegenerative disorders?** LSDs present multiple forms of mitochondrial

dysfunction: some are shared by different disorders while others seem to be more specific. However, the reason for the similarities and differences have not been explored and deserve careful consideration.

- **could mitochondria be effective therapeutic targets to treat neurodegeneration in LSDs?**
  Mitochondrial fragmentation, decreased mitochondrial membrane potential, calcium dyshomeostasis and increased ROS production are features of different LSDs. Consequently, thesemay be represent putative therapeutic targets to treat these disoders.

Impaired autophagy
Lysosomes cannot degrade damaged mitochondria and other macromolecules

Accumulation of toxic macromolecules
aS aggregates, lipofuscins and lipids accumulate at and damage mitochondria

Depolarized mitochondria

Increased ROS production

Reduced ATP production

Dysregulated calcium signaling
Lysosomal and ER Ca^{2+} stores may be affected, mitochondrial Ca^{2+} buffering capacity can be reduced or there may be Ca^{2+} overload

Alteration in mitochondrial morphology
Fragmentation and disruption of cristae

Lysosomes

aS aggregates

Lipofuscins

Nucleus

ER

Ca^{2+}

Mitochondria

↓ ATP

↑ ROS

LSDs	Mito morphology, accumulation and trafficking	Mito respiration	Calcium	Oxidative stress	Macromolecules accumulation at/in mitochondria
Gaucher's disease	In neurons and astrocytes from mice model [9] and in fibroblasts [24]	In neurons and astrocytes from mice model [9] and in fibroblasts [24]	In a pharmocological model [66]	In neurons from mice model [9] and in fibroblasts [24]	aS aggregates in brains from mice model [9] and from patients [82]; aS oligomers in patients' plasma [86]
Niemann-Pick C disease	In yeasts [25], in fibroblasts [26,27], in hESCs-derived neurons [28], in brains and neurons from a mice model [29]	In yeasts [25], in fibroblasts [26,27], in brains and neurons from a mice model [29]	In fibroblasts [27] and in lymphoblasts [60]	In yeasts [25], in fibroblasts [26], in cellular model [53], in mice liver and cerebellum [56], in murine brain [58] and in cerebellum from patient [59]	Lewy bodies in brains from patients [84]; aS oligomers in patients' plasma [86] and in lymphoblasts [87]; cholesterol increased in fibroblasts and in a KO mice brain [29]
Krabbe disease	In psychosine-treated neurons [44]		In psychosine-treated oligodendryocytes [54]	In psychosine-treated oligodendryocytes [54]	aS oligomers in patients' plasma [86]
Mucopolysaccharidosis III-IV	In brains from a mice model [34]	In brains from a mice model [34]		In urine, plasma and leukocytes from patients [55]	Phosphylated aS excess in brain from patients [85] and aS aggregates in a cell model [30];GM2 and GM3 gangliosides in brain from a mice model [34]
GM1 gangliosidosis	In brain and astrocytes from mice model [32]	In brain and astrocytes from mice model [32]	In MEFs from a mice model [68]		
GM2 gangliosidosis	In CNS from a mice model [33]			In CNS from a mice model [33]	aS aggregates in brain from a mice model [33] and from patients [83]
Neuronal Ceroid Lipofuscinosis	In animal model [35,36], in human iPSC-derived neurons [37], in KI mice model [38], in fibroblasts [41]	In animal model [35,36], in KO mice model [39], in lymphoblasts [40]	In neurons from a mice model [65]	In lymphoblasts [40], in a KI mice model [38], in fibroblasts [46], in brain from patients [57]	Upregulated aS-encoding gene in neurons from a KO mice model [39]; ATPase synthase containing lipofuscins in iPSC-derived models [37], in cerebellar cell models [38], in KI mice model [45] and in animal models [74]
Multiple sulfatase deficiency	In a mice model [30,31]	In a mice model [30,31]			aS aggregates in a cell model [30]

Mucolipidosis IV			In fibroblasts [47,71]		