J Inherit Metab Dis
DOI 10.1007/s10545-017-0048-0

REVIEW

Linking mitochondrial dysfunction to neurodegeneration in lysosomal storage diseases

Afshin Saffari[1] · **Stefan Kölker**[1] · **Georg F. Hoffmann**[1] · **Darius Ebrahimi-Fakhari**[2]

Received: 16 January 2017 / Revised: 1 April 2017 / Accepted: 5 April 2017

Abstract Lysosomal storage diseases (LSD) are inborn errors of metabolism resulting in multisystem disease. Central nervous system involvement, often with progressive neurodegeneration, accounts for a large portion of the morbidity and mortality seen in many LSD. Available treatments fail to prevent or correct neurologic symptoms and decline. Emerging evidence points to an important role for mitochondrial dysfunction in the pathogenesis and progression of LSD-associated neurodegeneration. Mitochondrial dysfunction in LSD is characterized by alterations in mitochondrial mass, morphology and function. Disturbed mitochondrial metabolism in the CNS may lead to excessive production of mitochondrial reactive oxygen species and dysregulated calcium homeostasis. These metabolic disturbances ultimately result in mitochondria-induced apoptosis and neuronal degeneration. Here, we review the current evidence for mitochondrial dysfunction in neuronal models of seven LSD, including GM1-gangliosidosis, mucopolysaccharidosis IIIC, multiple sulfatase deficiency, Krabbe disease, Gaucher disease, Niemann Pick disease type C and the neural ceroid lipofuscinoses and outline current experimental therapies aimed at restoring mitochondrial function and neuroprotection in LSD.

Responsible editor: William Ross Wilcox

✉ Darius Ebrahimi-Fakhari
darius.ebrahimi-fakhari@childrens.harvard.edu

[1] Division of Pediatric Neurology and Metabolic Medicine, Center for Child and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany

[2] Department of Neurology, Harvard Medical School, Boston Children's Hospital, 3 Blackfan Circle, CLS 14060, Boston, MA 02115, USA

Introduction

Lysosomal storage diseases (LSD) are a heterogeneous group of about 60 distinct monogenic disorders characterized by defects in lysosomal structure and metabolism that result in the accumulation of undigested macromolecules. Loss-of-function mutations in LSD map to lysosomal enzymes, membrane proteins or non-enzymatic soluble lysosomal proteins. Clinically, LSD often present as multisystem disorders, frequently with prominent central nervous system involvement and progressive neurodegeneration. Enzyme replacement therapy is available for a small number of LSD and patients benefit from early intervention. Yet, available treatments fail to halt or prevent neurodegeneration and the morbidity and mortality associated with progressive neurological decline remains high.

Although LSD primarily involve deficits in lysosomal metabolism and associated pathways such as autophagy or the endosomal pathway, emerging evidence points to a role of mitochondrial dysfunction in disease onset and progression. Mitochondria are essential organelles for brain development and synaptic function and their importance has been delineated in many neurodevelopmental and neurodegenerative diseases (Nunnari and Suomalainen 2012). Since mitochondrial homeostasis critically depends on intact autophagy and lysosomal degradation, secondary deficits in mitochondrial function and turnover are common pathologic findings in LSD. However, many studies report impaired mitochondrial homeostasis that cannot be explained by defective autophagy alone, but rather suggests interference of disease-specific processes such as pathologic interactions of accumulating macromolecules with mitochondrial dynamics or dysregulation of important cellular pathways (Sanz-Blasco et al 2008; Sano et al 2009; Cantuti Castelvetri et al 2013; Voccoli et al 2014). These disease-specific deficits in lysosomal function

Published online: 05 May 2017

activate common downstream mitochondrial pathways that ultimately converge on mitochondrial membrane permeabilization and mitochondria-induced apoptosis. This seems particularly important to neurons as post-mitotic cells that almost exclusively depend on mitochondrial respiration. Mitochondrial dysfunction thus likely contributes to the progressive neurodegeneration seen in many LSD (also see this recent review by Plotegher and Duchen 2017).

Understanding the mechanisms of mitochondrial dysfunction in LSD holds the potential to identify novel therapeutic targets that might complement current treatments. Here, we summarize the emerging evidence for mitochondrial dysfunction in neuronal models of seven LSD: GM1-gangliosidosis, mucopolysaccharidosis (MPS) IIIC, multiple sulfatase deficiency (MSD), Krabbe disease, Gaucher disease, Niemann Pick disease type C (NPC) and the neural ceroid lipofuscinoses (NCL). Experimental therapies aimed at alleviating mitochondrial dysfunction in neuronal models of LSD are discussed.

The role of mitochondria in neuronal metabolism

Mitochondrial ATP production is essential to neuronal metabolism and serves as the major energy supply for synaptic function. Generation and maintenance of electrochemical gradients, neurotransmitter synthesis and vesicle release strictly depend on the presence of intact mitochondria. For instance, correct glutamatergic neurotransmission is ensured by perisynaptic astrocytes, which remove glutamate from the synaptic cleft and recycle it back to the pre-synaptic terminal via the glutamate-glutamine cycle. This process is highly dependent on mitochondrial ATP supply and even small disturbances in mitochondrial metabolism can lead to excitotoxicity and neuronal death. It is thus not surprising that mitochondrial dysfunction is implicated in many neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis. In children, LSD represent a major cause of neurodegeneration (Verity et al 2010) and Gaucher disease, the most frequent LSD, caused by homozygous and compound heterozygous mutations in the *GBA* gene, is increasingly linked to Parkinson's disease and other synucleinopathies. Importantly, heterozygous mutations in the *GBA* gene were identified as the most frequent genetic risk factor for Parkinson's disease (Sidransky et al 2009), exemplifying the link between lysosomal impairment, mitochondrial dysfunction and neurodegeneration.

Despite emerging evidence from the literature, involvement of mitochondrial pathways in LSD-associated neurodegeneration remains largely unappreciated in clinical practice and available therapies do not target mitochondria-dependent mechanisms of neuronal loss.

Evidence for mitochondrial dysfunction in neuronal models of LSD

Healthy mitochondria form communicating networks, an indicator of high metabolic activity. Upon damage, mitochondria lose their mitochondrial membrane potential ($\Delta\Psi_m$) and become fragmented, which serves as a strong stimulus for mitophagy. Mitophagy comprises several selective cellular degradation pathways. The best-described mechanism involves the accumulation of the proteins PINK1 and Parkin on the mitochondrial surface and subsequent polyubiquitination of damaged mitochondria (Pickrell and Youle 2015). However, other mechanisms exist, involving different mitophagy receptor systems and lipid-mediated mitophagy (reviewed by Hamacher-Brady and Brady 2016). Ultimately, mitochondrial damage leads to the recruitment of the autophagy machinery and lysosomal degradation of damaged mitochondria. In LSD, mitochondrial dysfunction, possibly secondary to impaired lysosomal degradation of damaged mitochondria, appears to be a common theme (Table 1). Pathologic phenotypes in neuronal models of LSD include altered mitochondrial mass, morphologic abnormalities and functional deficits. In the majority of studies mitochondrial mass has been reported to be increased likely secondary to a block in lysosome-dependent degradation (Settembre et al 2008; Osellame et al 2013; Martins et al 2015). Accumulating mitochondria consistently show morphologic abnormalities such as fragmentation and swelling (Sano et al 2009; de Pablo-Latorre et al 2012; Osellame et al 2013; Martins et al 2015), indicative of on-going mitochondrial damage. Interestingly, electron microscopic examinations of iPSC-derived neurons from a patient diagnosed with juvenile NCL (CLN3) detailed further morphologic abnormalities such as reduced mitochondrial cristae and inner-mitochondrial vacuolation. These observations are somewhat reminiscent of histopathological findings in amyotrophic lateral sclerosis (Lojewski et al 2014), highlighting the shared presence of mitochondrial dysfunction both in childhood-onset and adult-onset neurodegenerative diseases. The morphologic abnormalities reported in the literature are complemented by evidence for functional deficits in mitochondrial respiration (Fossale et al 2004; Kennedy et al 2013; Xu et al 2014; Dasgupta et al 2015). Mitochondrial membrane potential was found to be decreased in several models of LSD including a MO3.13 human oligodendrocytic cell model of Krabbe disease (Haq et al 2003; Voccoli et al 2014), $Gba^{-/-}$ murine neurons, a model for Gaucher disease (Osellame et al 2013) and a $\beta\text{-}gal^{-/-}$ mouse model of GM1-gangliosidosis (Takamura et al 2008; Sano et al 2009). Interestingly, mild reduction in $\Delta\Psi_m$ in primary murine $Gba^{-/-}$ neurons was not sufficient to initiate recruitment of Parkin, thus preventing the induction of mitophagy and leading to further mitochondrial

Table 1 Domains of mitochondrial dysfunction in neuronal models of seven LSD. $\Delta\Psi_m$ mitochondrial membrane potential, *APP* amyloid precursor protein, *ATP* adenosine triphosphate, Ca^{2+} calcium, *GD* Gaucher disease, *GM1* GM1-gangliosidosis, *GSH* glutathione, *KD* Krabbe disease, *MPS IIIC* mucopolysaccharidosis IIIC, *MSD* multiple sulfatase deficiency, *NCL* neural ceroid lipofuscinosis, *NPC* Niemann Pick disease type C, *OXPHOS* oxidative phosphorylation, *PGC1α* peroxisome proliferator-activated receptor gamma coactivator 1α, *ROS* reactive oxygen species, *SMAC* second mitochondria-derived activator of caspase

Disease	Disease model	Domains of mitochondrial dysfunction	Reference
GM1	*β-gal*$^{-/-}$ mouse	• Fragmentation • Decreased $\Delta\Psi_m$ • Selective reduction of OXPHOS complexes • Increased vulnerability to oxidative stress • Ca^{2+} overload • Cytochrome *c* release	(Sano et al 2009) (Takamura et al 2008)
MPS IIIC	*Hgsnat-Geo* mouse	• Accumulation • Fragmentation/swelling • Selective reduction of OXPHOS complexes • Decreased coenzyme Q10	(Martins et al 2015)
MSD	*Sumf1*$^{-/-}$ mouse	• Accumulation • Fragmentation • Decreased ATP content	(Settembre et al 2008) (de Pablo-Latorre et al 2012)
KD	MO3.13 (human oligodendrocytic cell line)	• Decreased $\Delta\Psi_m$ • Elevated ROS/reduced GSH • Ca^{2+} overload • Cytochrome *c* release	(Voccoli et al 2014) (Haq et al 2003)
	twitcher mouse	• Impaired axonal transport	(Cantuti Castelvetri et al 2013)
GD	*4 L;C** mouse	• Selective reduction of OXPHOS complexes • Reduced O_2 consumption/reduced ATP levels	(Dasgupta et al 2015)
	4 L/PS-NA/9H/PS-NA mouse	• Swelling • Accumulation of APP and α-synuclein • Reduced O_2 consumption/reduced ATP levels	(Xu et al 2014)
	Gba$^{-/-}$ neurons	• Accumulation • Fragmentation • Decreased $\Delta\Psi_m$ • Selective reduction of OXPHOS complexes • Deficits in Parkin recruitment	(Osellame et al 2013)
NPC	*Npc1*$^{-/-}$ mouse	• Loss of healthy mitochondria • Mitochondrial cholesterol accumulation • Decreased oxidative respiration/reduced ATP levels • Increased susceptibility to oxidative stress • Decrease in mitochondrial GSH • Cytochrome *c* release • SMAC activation	(Kennedy et al 2013) (Fernandez et al 2009) (Huang et al 2006) (Yu et al 2005)
NCL	*CatD*$^{-/-}$ mouse	• Oxidative damage	(Okada et al 2015)
	iPSC-derived *CLN2/CLN3* neurons	• Swelling • Reduced mitochondrial cristae • Mitochondrial vacuolation	(Lojewski et al 2014)
	Ppt1$^{-/-}$ mouse	• Decreased density • Decreased PGC1α-levels	(Wei et al 2011)
	Cln8^{mnd} mouse	• Deficient mitochondrial Ca^{2+} buffering	(Kolikova et al 2011)
	CbCln3$^{\Delta ex7/8}$ and *CbCln6*^{nclf} mice	• Reduced ATP levels	(Cao et al 2011) (Fossale et al 2004)
	Cln3$^{-/-}$ mouse	• Swelling • Decrease in OXPHOS complexes • Impaired axonal transport • Ca^{2+} overload	(Luiro et al 2006)

accumulation (Osellame et al 2013). Consistent with deficits in mitochondrial polarization, oxidative phosphorylation driven by $\Delta\Psi_m$ was deficient in many LSD (Table 1). Providing interesting mechanistic insights, Yu et al demonstrated that in the *Npc*$^{-/-}$ mouse brain an increase in mitochondrial membrane cholesterol inhibited ATP synthase activity resulting in suppressed neuronal outgrowth and increased susceptibility to cellular stress, thus linking disease-specific alterations in mitochondrial function to neurodegeneration in NPC (Yu et al 2005). In contrast, different groups have shown that

ectopic dendritic outgrowth and formation of so-called meganeurites, likely due to aberrant sphingolipid accumulation, appears as a common finding in many LSD, including NPC (Futerman et al 1999; Walkley 2004). Thus, it remains uncertain which pathogenic mechanisms explain the neuronal phenotype reported by Yu et al. However, there seems to be consensus that primary defects in lysosomal metabolism lead to some kind of aberrant neuronal outgrowth. Along these lines, reduced ATP availability due to a lack of mitochondria at synaptic sites might contribute to impaired neurotransmission and neuronal death. Indeed, mitochondrial transportation along axons was impaired in the Krabbe disease *twitcher* mouse and a *Cln3*$^{-/-}$ mouse model, suggesting a depletion of axonal mitochondria and therefore reduced ATP-availability at synaptic sites. This axonal transport phenotype is thought to be secondary to an abnormal interaction of GSK3β with kinesin motors acting through kinesin light chain phosphorylation, which leads to premature cargo release (Cantuti Castelvetri et al 2013). In addition down-regulation of the dynein/dynactin motor protein complex has been reported (Luiro et al 2006).

Taken together, findings in multiple neuronal models of LSD provide evidence for mitochondrial dysfunction.

Common downstream signaling pathways

Mitochondrial dysfunction ultimately leads to two key events: excessive production of mitochondrial reactive oxygen species (ROS) and dysregulation of mitochondrial calcium homeostasis.

Mitochondrial ROS, the major source of cellular ROS, is usually generated by complex I and to a lesser degree by complex III of the mitochondrial respiratory chain. The major primary type of mitochondrial ROS, the superoxide anion radical, forms when leaking electrons from the respiratory chain react with molecular oxygen. A subsequent spontaneous or enzyme-catalyzed reaction with molecular water forms hydrogen peroxide. The most potent type of ROS is the hydroxyl radical, which results from the iron-catalyzed reaction of superoxide anion radicals with hydrogen peroxide (Fenton reaction) and is thought to be the major cause for oxidative damage to DNA bases. Besides this, a number of other oxygen-containing free radicals exist, such as nitric oxide, peroxinitrite and others, that can potently attack different cellular components. Excessive ROS is usually detoxified by cellular antioxidants such as superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase or coenzyme Q_{10}. Elevated levels of mitochondrial ROS production were reported in galactosylsphingosine treated MO3.13 human oligodendrocytes, mimicking Krabbe disease (Voccoli et al 2014). Along these lines, studies in *Npc*$^{-/-}$ mouse brains (Torres et al 2016) and MO3.13 human oligodendrocytes (Haq et al 2003) report depletion in mitochondrial glutathione (GSH) impairing mitochondrial ROS-buffering capacities and rendering neurons more susceptible to oxidative stress. Interestingly, a recent review proposed the involvement of reactive astrocytes and microglia in response to mitochondrial dysfunction and oxidative stress in the pathogenesis of LSD-associated neurodegeneration (Rama Rao and Kielian 2016). Along the same lines, a mouse model of infantile NCL (*Ppt1*$^{-/-}$ mouse) described by Wei et al shows neuronal loss and astrogliosis following oxidative stress. Here mitochondrial biogenesis was decreased due to reduced SIRT1 levels, which exemplifies the role of sirtuins in mitochondrial homeostasis (Tang 2016). Moreover mTOR signaling was up-regulated and inversely correlated with survival (Wei et al 2011). The role of mTOR signaling in other LSD remains unexplored. However, findings by Pena-Llopis et al postulate a role for mTOR in lysosomal biogenesis by preventing the nuclear translocation of transcription factor EB (TFEB), a master transcriptional regulator of lysosomal biogenesis and autophagy (Pena-Llopis et al 2011). mTOR modulation could thus hold great potential for LSD.

Altered mitochondrial calcium metabolism appears to be a second pathologic pattern in neuronal models of LSD. Neuronal survival and synaptic activity critically depend on precise temporal and spatial calcium regulation. During glutamatergic neurotransmission synaptic calcium levels dramatically rise due to NMDA (N-methyl-D-aspartate) receptor mediated calcium influx as well as calcium release from intracellular stores. In this context of high neuronal calcium transients, mitochondria hold an essential role in calcium buffering. In addition, mitochondrial ATP production is highly calcium-dependent since important enzymes of the tricarboxylic acid cycle are stimulated by calcium. Therefore, mitochondria are located in close proximity to the endoplasmic reticulum (ER), the major cellular calcium store. Together these two organelles form communicating membranes, so-called mitochondria-associated membranes (MAMs). MAMs are rich in IP_3 receptors and ryanodine receptors that allow high calcium transients from the ER to directly enter the mitochondrial matrix. Thus, neuronal mitochondria are constantly exposed to high calcium concentrations, which in turn lead to transient mitochondrial depolarization. Calcium overload and subsequently prolonged mitochondrial depolarization, however, result in the breakdown of $\Delta\Psi_m$ and mitochondria-induced neuronal death (Luiro et al 2006; Sano et al 2009; Voccoli et al 2014). Interestingly, in a human oligodendrocytic cell line addition of galactosylsphingosine, mimicking Krabbe disease pathology, acted as an agonist for the ryanodine receptor leading to an increase in mitochondrial calcium (Voccoli et al 2014). Similarly, in the *β-gal*$^{-/-}$ mouse, an animal model of GM1-gangliosidosis, accumulation of GM1-ganglioside in MAMs increased the opening of IP_3 receptors leading to mitochondrial calcium overload (Sano et al 2009). Conversely, reduced mitochondrial calcium buffering exposing the cell to the pro-

apoptotic effects of elevated cytoplasmic calcium has also been reported in a number of LSD (Kiselyov and Muallem 2008). For instance, an age-dependent reduction of mitochondrial calcium buffering capacity that increases the vulnerability of neurons to excitatory glutamate signals has been reported in *Cln8*^{mnd} neurons (Kolikova et al 2011). Thus, mitochondrial calcium homeostasis involves complex regulatory mechanisms and both mitochondrial calcium overload and deficient cytoplasmic calcium buffering may act as coexisting pathomechanisms in LSD.

Excessive mitochondrial ROS production and disturbed mitochondrial calcium homeostasis are two closely connected phenomena that heavily influence each other. For instance, ROS possess the ability to oxidize cysteine residues of IP_3 receptors and ryanodine receptors creating disulfide bonds that change channel conformation and lead to calcium release into the mitochondrial matrix (Gorlach et al 2015). Conversely, elevated mitochondrial calcium levels activate ROS-generating enzymes. In a Gaucher disease mouse model, amyloid precursor protein (APP) and α-synuclein influx into the mitochondrial matrix led to impaired mitochondrial ATP production and oxygen consumption (Xu et al 2014). The link between disturbed calcium homeostasis and protein aggregates is well described in classic neurodegenerative diseases such as Parkinson's disease and other synucleinopathies. Overexpression of α-synuclein was shown to increase mitochondrial calcium influx by enhancing calcium release from the ER directly into mitochondria (Cali et al 2012). Further studies in human brain samples of Parkinson's disease patients revealed that elevated levels of the calcium buffering protein calbindin protected dopaminergic midbrain neurons from degeneration (Yamada et al 1990). Conversely, calcium overload was shown to propagate α-synuclein aggregation, a mechanism that might promote the occurrence of parkinsonism in Gaucher disease (Nath et al 2011).

Ultimately, dysregulated mitochondrial calcium homeostasis and excessive mitochondrial ROS levels lead to cell death through mitochondrial membrane permeabilization (MMP) and activation of caspase-9 dependent apoptosis (Fig. 1). MMP can be subdivided into mitochondrial outer membrane permeabilization (MOMP) and mitochondrial inner membrane permeabilization (MIMP). MOMP occurs through activation of the pro-apoptotic proteins Bax and Bak, members of the Bcl-2 family. Bax and Bak form homo- and hetero-oligomeric pores enabling the release of intermembranous mitochondrial proteins such as second mitochondria-derived activator of caspase (SMAC), high temperature requirement protein A2 (HTRA2) and cytochrome *c* into the cytosol. SMAC and HTRA2 exert their pro-apoptotic function by inhibiting another set of Bcl-2 family proteins, the inhibitors of apoptosis proteins (IAPs) Bcl-2 and Bcl-X_L. Cytochrome *c*, by contrast, acts by activating apoptotic protease activating factor 1 (Apaf-1), which forms the apoptosome and initiates caspase-9 dependent apoptosis. Evidence for the release of cytochrome *c* and SMAC from neuronal mitochondria has been found in a number of LSD (Haq et al 2003; Fernandez et al 2009; Sano et al 2009). MIMP is characterized by formation of the permeability transition pore (PTP), a direct connection between the mitochondrial matrix and the cytosol. This pore allows H_2O and ions to enter the mitochondrial matrix leading to swelling and rupture of the mitochondrial membrane with release of intermembranous mitochondrial proteins into the cytosol. The molecular composition of the PTP remains controversial. Supported by several studies, the c subunit of the F_0F_1-ATP synthase has been proposed as a promising candidate (Giorgio et al 2013; Alavian et al 2014; Carraro et al 2014). Interestingly the c subunit of the F_0F_1-ATP synthase was shown to accumulate in particular in the NCL (Cao et al 2011; Lojewski et al 2014; Okada et al 2015). Recently, a mouse model of *PARK9*-linked early-onset parkinsonism showed neuronal accumulation of the c subunit of the F_0F_1-ATP synthase, suggesting common lysosome-dependent mechanisms with NCL (Sato et al 2016).

Together these reports demonstrate that mitochondrial dysfunction contributes to neuronal death in LSD. Targeting mitochondrial dysfunction upstream of neurodegeneration thus holds great potential.

Therapeutic approaches to alleviate mitochondrial dysfunction in LSD

The importance of mitochondrial dysfunction in LSD is increasingly appreciated and therapies aimed at reversing mitochondrial dysfunction have been explored (Table 2, Fig. 1). The pharmacologic reduction of mitochondrial ROS appears to be a promising target. For instance in *Gba*$^{-/-}$ midbrain neurons, a model for Gaucher disease, the antioxidant $MitoQ_{10}$ reduced mitochondrial ROS production and restored $\Delta\Psi_m$ (Osellame et al 2013). Similarly, antioxidative treatment with GSH ethyl-ester in a NPC mouse model restored mitochondrial function and prolonged survival (Torres et al 2016). Along these lines, N-acetylcysteine (NAC) prevented cell death in an oligodendrocytitic cell model of Krabbe disease (Haq et al 2003; Voccoli et al 2014). NAC is a potent thiol antioxidant, which, in the CNS, showed neuroprotective properties in in vitro and in vivo models of Parkinson's disease (Bahat-Stroomza et al 2005) and could thus be of interest in the treatment of LSD. A study by Fu et al showed NAC efficacy in rodent NPC models, however failed to demonstrate clinically significant effects in NPC patients (Fu et al 2013), underscoring that antioxidant therapy is yet at an early stage and novel approaches are needed.

Disturbed calcium homeostasis appears as a second potential therapeutic target. Lysosomal calcium signaling is emerging as a novel target for the treatment of neurodegenerative

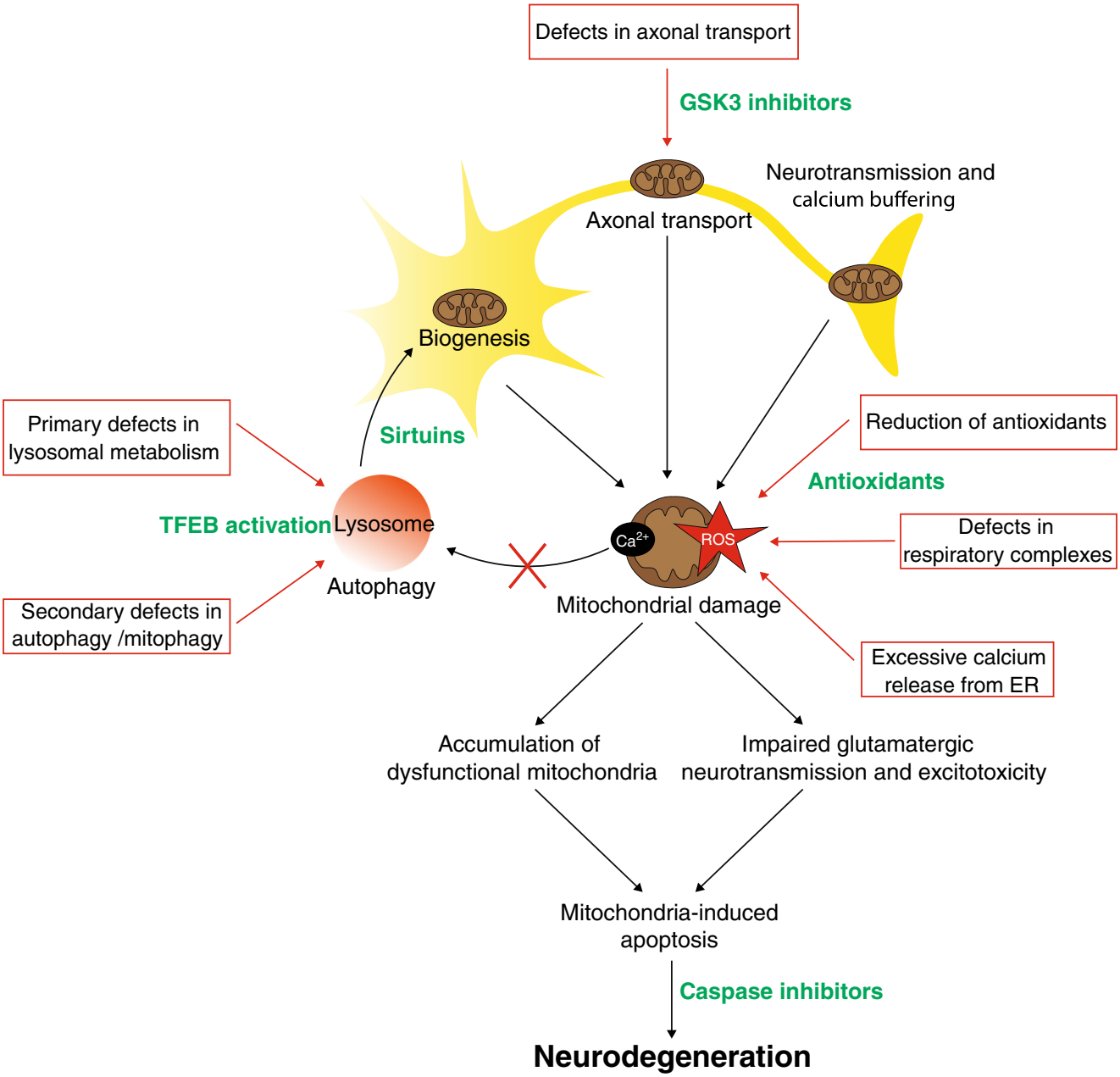

Fig. 1 Mitochondrial dysfunction in LSD-associated neurodegeneration and experimental therapeutic approaches. Mitochondria are clustered in the neuronal cell body and transported along the axon to remote synaptic compartments where they support neurotransmission and calcium buffering. Mitochondrial damage may occur at all steps of this pathway. Damaged mitochondria are usually degraded via autophagy and basic building blocks are recycled back to the cell body. In LSD, disease-specific interactions with mitochondrial pathways, such as reduction of antioxidants, defects in respiratory complexes and excessive calcium release from the ER, add further damage to neuronal mitochondria. In addition, lysosomal degradation is defective due to primary defects in lysosomal metabolism or secondary defect in autophagy/mitophagy. The resulting accumulation of dysfunctional mitochondria leads to excessive ROS production and impaired calcium buffering capacity. Furthermore, damaged mitochondria fail to uphold ATP supply at synaptic sites leading to impaired glutamatergic neurotransmission and excitotoxicity. Ultimately, these events lead to mitochondria-induced apoptosis and neuronal death. Several experimental therapies target aberrant pathways in LSD, including reduction of ROS with antioxidants, modulation of lysosomal pathways via TFEB activation, direct inhibition of apoptosis with caspase inhibitors, restoration of axonal transport with GSK3 inhibitors and enhancing mitochondrial biogenesis and turnover with sirtuins. Ca^{2+} calcium, *ER* endoplasmic reticulum, *GSK* glycogen synthase kinase, *ROS* reactive oxygen species, *TFEB* transcription factor EB

diseases. Interesting insights come form Raffaelo et al who propose the molecule nicotinic acid adenine dinucleotide phosphate (NAADP) as a possible activator of calcium release from lysosomal stores. By acting at lysosomal two-pore channels (TPC) and TRPML (mucolipin family of transient receptor potential) channels, NAADP was shown to induce local

calcium peaks at lysosome-ER contact sides and trigger calcium-induced calcium release from the ER (Raffaello et al 2016) possibly contributing to cytoplasmic and mitochondrial calcium overload. Furthermore, local lysosomal calcium release was shown to regulate autophagy through calcineurin-dependent dephosphorylation and subsequent nuclear translocation of TFEB (Medina et al 2015). Thus, a novel role for the lysosome as a central signaling hub for calcium homeostasis and autophagy is emerging. Several studies in LSD support the role of the lysosome in disturbed calcium signaling and neurodegeneration. Indeed, in NPC patient fibroblasts a depletion of lysosomal calcium stores was shown, along with reduced lysosomal calcium release, likely disrupting TFEB-mediated autophagy (Lloyd-Evans et al 2008; Hoglinger et al 2015). In this scenario, TFEB appears as a promising therapeutic target. Overexpression of TFEB in neuronal stem cells of MSD mice led to striking reduction of glycosaminoglycans and restoration of normal cellular morphology (Medina et al 2011). In addition, in a Parkinson's disease rat model, activation of TFEB through rapamycin, a mTOR inhibitor, blocked α-synuclein induced neurodegeneration and disease progression (Decressac et al 2013). mTOR acts by phosphorylating TFEB at the lysosomal surface and preventing its translocation to the nucleus (Pena-Llopis et al 2011). This link may put mTOR modulation in LSD in the focus of future research. mTOR inhibition has already been successfully applied in experimental models of mitochondrial disorders like Leigh syndrome (Johnson et al 2013) and in neurodegenerative diseases (Bove et al 2011). Further promising developments come from a recent study in a mouse model of juvenile NCL ($Cln3^{\Delta ex7-8}$) that successfully used trehalose as a mTOR-independent activator of TFEB signaling to attenuate the neuropathology and prolong survival (Palmieri et al 2017).

Further approaches targeting downstream apoptosis pathways include the use of either the caspase-9 inhibitor taurine or the caspase-3 inhibitor Z-DEVD-FMK in a neuronal model of NPC (Huang et al 2006) as well as the pan-caspase inhibitor Z-VAD-FMK in $\beta\text{-}gal^{-/-}$ mouse astrocytes (Takamura et al 2008). Interestingly, the compound Z-DEVD-FMK was successfully delivered across the blood-brain barrier of mouse brains using nanoparticle technology, making the use of this compound a viable therapeutic option (Karatas et al 2009).

Another interesting therapeutic approach is the modulation of sirtuins, a group of proteins with important roles in neuronal physiology. SIRT1, the best-described member of the family, is a predominantly nuclear protein that has been involved in longevity and neuroprotection, likely due to its ability to increase mitochondrial biogenesis through activation of the transcription factor PGC1α (Tang 2016). Indeed, in a mouse model of infantile NCL, resveratrol, a SIRT1-activator, was able to raise ATP and mitochondrial mRNA levels and to increase lifespan (Wei et al 2011). In addition, a role for sirtuins

Table 2 Experimental therapies aimed at restoring mitochondrial dysfunction in neuronal models of 5 LSD. *GD* Gaucher disease, *GM1-GD* GM1-gangliosidosis, *KD* Krabbe disease, *NCL* neural ceroid lipofuscinosis, *NPC* Niemann Pick disease type C

Drug	Mode of action	Disease model	Outcome	Reference
$MitoQ_{10}$	Antioxidant	GD: $Gba^{-/-}$ mouse primary neurons	Reduction of mitochondrial ROS and restoration of $\Delta\Psi_m$in vitro	(Osellame et al 2013)
NAC	Antioxidant	KD: MO3.13 human oligodendrocytic cell line	Reduction of caspase 3 activity, reduction of mitochondrial ROS and prolonged survival in vitro	(Haq et al 2003; Voccoli et al 2014)
GSH ethyl ester	Antioxidant	NPC: *Npc1−/−* mouse	Increase in mitochondrial GSH and prolonged survival in vivo	(Torres et al 2016)
Trehalose	Akt-inhibition	NCL: $Cln3^{\Delta ex7-8}$ mouse	Increase of lysosomal degradation and prolonged survival in vivo	(Palmieri et al 2017)
Taurine	Caspase-9 inhibitor	NPC: progesterone treated mouse primary neurons	Reduced cell death in vitro	(Huang et al 2006)
Z-DEVD-FMK	Caspase-3 inhibitor	NPC: progesterone treated mouse primary neurons	Reduced cell death in vitro	(Huang et al 2006)
Z-VAD-FMK	Pan-caspase inhibitor	GM1-GD: $\beta\text{-}gal^{-/-}$ mouse	Reduced cell death in vitro	(Takamura et al 2008)
Resveratrol	SIRT1 activator	Infantile NCL: *Ppt1−/−* mouse	Increase of ATP and mitochondrial biogenesis, reduction of mTOR-signaling and prolonged survival in vivo	(Wei et al 2011)
L803	GSK3β inhibitor	KD: NSC34 motoneuron-like cells, *twitcher* mouse	Restoration of mitochondrial transport in vitro and in vivo	(Cantuti Castelvetri et al 2013)

in mitophagy has been evoked. A recent study found that primary *Sirt2*$^{-/-}$ mouse hippocampal neurons showed baseline accumulation of PINK1/Parkin and the adaptor protein p62 indicating mitochondrial damage. Further, upon mitophagy stimulation these damaged mitochondria failed to undergo autophagic degradation, suggesting a role of SIRT2 in mitochondrial quality control (Liu et al 2016). The fact that sirtuins seem to promote both mitochondrial biogenesis and mitophagy suggests an attractive role of sirtuins in mitochondrial maintenance by replenishing the cell's mitochondrial pool. Thus, pharmacologic modulation of sirtuins, particularly the thus far poorly-characterized mitochondrial sirtuins SIRT3, SIRT4 and SIRT5 could be a promising future avenue in the management of mitochondrial dysfunction.

Finally, aberrant GSK3-signaling has emerged as an important mechanism in neurodegenerative diseases. GSK3 is a serine/threonine kinase that plays a particular role in familial and sporadic Alzheimer's disease (Hooper et al 2008). GSK3 inhibition was shown to promote mitochondrial transport along axons (Chen et al 2007). Driven by these findings, a number of GSK3-inhibitors have been developed in recent years, many of which show good bioavailability in the brain (Eldar-Finkelman and Martinez 2011). One of these compounds is L803-mts, which was able to restore mitochondrial trafficking along axons in a NSC34 motoneuron-like cell model of Krabbe disease and further showed restoration of mitochondrial transport in sciatic nerves of the *twitcher* mouse (Cantuti Castelvetri et al 2013).

In summary, early experimental therapies targeting mitochondrial pathways show promising results in preventing mitochondria-induced apoptosis in neuronal models of LSD.

Conclusions

Alterations in mitochondrial mass, morphology and function have been reported in many neuronal models of LSD (Table 1). Proposed mechanisms for the accumulation of dysfunctional mitochondria are primary defects in lysosomal metabolism, secondary defects in the autophagy pathway and interference of accumulating disease-specific molecules with mitochondrial dynamics (Fig. 1). Mitochondrial dysfunction leads to excessive mitochondrial ROS production and disturbed calcium homeostasis, ultimately resulting in mitochondrial membrane permeabilization and the activation of caspase-9 dependent apoptosis. Experimental therapeutic approaches in neuronal models of LSD target mitochondrial pathways to alleviate mitochondria-induced neuronal death. Promising approaches include the reduction of ROS levels by antioxidant treatment, modulation of lysosomal pathways via TFEB activation, direct caspase inhibition, as well as modulation of sirtuin and GSK3 signaling pathways (Table 2, Fig. 1). Further research is needed to optimize therapeutic approaches in in vivo models.

Compliance with ethics standards This article does not contain any studies with human or animal subjects performed by the author.

Conflict of interest None.

Funding Darius Ebrahimi-Fakhari has received funding from the Daimler and Benz Foundation (Daimler und Benz Stiftung, Ladenburg, Germany) and the Reinhard-Frank Foundation (Reinhard-Frank-Stiftung, Hamburg, Germany) and awards from the Child Neurology Society and the International Parkinson and Movement Disorder Society.

References

Alavian KN, Beutner G, Lazrove E et al (2014) An uncoupling channel within the c-subunit ring of the F1FO ATP synthase is the mitochondrial permeability transition pore. Proc Natl Acad Sci U S A 111: 10580–10585

Bahat-Stroomza M, Gilgun-Sherki Y, Offen D et al (2005) A novel thiol antioxidant that crosses the blood brain barrier protects dopaminergic neurons in experimental models of Parkinson's disease. Eur J Neurosci 21:637–646

Bove J, Martinez-Vicente M, Vila M (2011) Fighting neurodegeneration with rapamycin: mechanistic insights. Nat Rev Neurosci 12:437–452

Cali T, Ottolini D, Negro A, Brini M (2012) Alpha-Synuclein controls mitochondrial calcium homeostasis by enhancing endoplasmic reticulum-mitochondria interactions. J Biol Chem 287:17914–17929

Cantuti Castelvetri L, Givogri MI, Hebert A et al (2013) The sphingolipid psychosine inhibits fast axonal transport in Krabbe disease by activation of GSK3beta and deregulation of molecular motors. J Neurosci 33:10048–10056

Cao Y, Staropoli JF, Biswas S et al (2011) Distinct early molecular responses to mutations causing vLINCL and JNCL presage ATP synthase subunit C accumulation in cerebellar cells. PLoS One 6: e17118

Carraro M, Giorgio V, Sileikyte J et al (2014) Channel formation by yeast F-ATP synthase and the role of dimerization in the mitochondrial permeability transition. J Biol Chem 289:15980–15985

Chen S, Owens GC, Crossin KL, Edelman DB (2007) Serotonin stimulates mitochondrial transport in hippocampal neurons. Mol Cell Neurosci 36:472–483

Dasgupta N, Xu YH, Li R et al (2015) Neuronopathic Gaucher disease: dysregulated mRNAs and miRNAs in brain pathogenesis and effects of pharmacologic chaperone treatment in a mouse model. Hum Mol Genet 24:7031–7048

Decressac M, Mattsson B, Weikop P, Lundblad M, Jakobsson J, Bjorklund A (2013) TFEB-mediated autophagy rescues midbrain dopamine neurons from alpha-synuclein toxicity. Proc Natl Acad Sci U S A 110:E1817–E1826

Eldar-Finkelman H, Martinez A (2011) GSK-3 inhibitors: preclinical and clinical focus on CNS. Front Mol Neurosci 4:32

Fernandez A, Llacuna L, Fernandez-Checa JC, Colell A (2009) Mitochondrial cholesterol loading exacerbates amyloid beta peptide-induced inflammation and neurotoxicity. J Neurosci 29: 6394–6405

Fossale E, Wolf P, Espinola JA et al (2004) Membrane trafficking and mitochondrial abnormalities precede subunit c deposition in a

cerebellar cell model of juvenile neuronal ceroid lipofuscinosis. BMC Neurosci 5:57

Fu R, Wassif CA, Yanjanin NM et al (2013) Efficacy of N-acetylcysteine in phenotypic suppression of mouse models of Niemann-Pick disease, type C1. Hum Mol Genet 22:3508–3523

Futerman AH, Boldin SA, Brann AB, Pelled D, Meivar-Levy I, Zisling R (1999) Regulation of sphingolipid and glycosphingolipid metabolism during neuronal growth and development. Biochem Soc Trans 27:432–437

Giorgio V, von Stockum S, Antoniel M et al (2013) Dimers of mitochondrial ATP synthase form the permeability transition pore. Proc Natl Acad Sci U S A 110:5887–5892

Gorlach A, Bertram K, Hudecova S, Krizanova O (2015) Calcium and ROS: a mutual interplay. Redox Biol 6:260–271

Hamacher-Brady A, Brady NR (2016) Mitophagy programs: mechanisms and physiological implications of mitochondrial targeting by autophagy. Cell Mol Life Sci 73:775–795

Haq E, Giri S, Singh I, Singh AK (2003) Molecular mechanism of psychosine-induced cell death in human oligodendrocyte cell line. J Neurochem 86:1428–1440

Hoglinger D, Haberkant P, Aguilera-Romero A et al (2015) Intracellular sphingosine releases calcium from lysosomes. elife 4:e10616

Hooper C, Killick R, Lovestone S (2008) The GSK3 hypothesis of Alzheimer's disease. J Neurochem 104:1433–1439

Huang Z, Hou Q, Cheung NS, Li QT (2006) Neuronal cell death caused by inhibition of intracellular cholesterol trafficking is caspase dependent and associated with activation of the mitochondrial apoptosis pathway. J Neurochem 97:280–291

Johnson SC, Yanos ME, Kayser EB et al (2013) mTOR inhibition alleviates mitochondrial disease in a mouse model of Leigh syndrome. Science 342:1524–1528

Karatas H, Aktas Y, Gursoy-Ozdemir Y et al (2009) A nanomedicine transports a peptide caspase-3 inhibitor across the blood-brain barrier and provides neuroprotection. J Neurosci 29:13761–13769

Kennedy BE, LeBlanc VG, Mailman TM et al (2013) Pre-symptomatic activation of antioxidant responses and alterations in glucose and pyruvate metabolism in Niemann-Pick type C1-deficient murine brain. PLoS One 8:e82685

Kiselyov K, Muallem S (2008) Mitochondrial Ca2+ homeostasis in lysosomal storage diseases. Cell Calcium 44:103–111

Kolikova J, Afzalov R, Surin A, Lehesjoki AE, Khiroug L (2011) Deficient mitochondrial ca(2+) buffering in the Cln8(mnd) mouse model of neuronal ceroid lipofuscinosis. Cell Calcium 50:491–501

Liu G, Park SH, Imbesi M et al (2016) Loss of NAD-dependent protein deacetylase Sirtuin-2 alters mitochondrial protein acetylation and dysregulates Mitophagy. Antioxid Redox Signal. doi:10.1089/ars.2016.6662

Lloyd-Evans E, Morgan AJ, He X et al (2008) Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium. Nat Med 14:1247–1255

Lojewski X, Staropoli JF, Biswas-Legrand S et al (2014) Human iPSC models of neuronal ceroid lipofuscinosis capture distinct effects of TPP1 and CLN3 mutations on the endocytic pathway. Hum Mol Genet 23:2005–2022

Luiro K, Kopra O, Blom T et al (2006) Batten disease (JNCL) is linked to disturbances in mitochondrial, cytoskeletal, and synaptic compartments. J Neurosci Res 84:1124–1138

Martins C, Hulkova H, Dridi L et al (2015) Neuroinflammation, mitochondrial defects and neurodegeneration in mucopolysaccharidosis III type C mouse model. Brain 138:336–355

Medina DL, Fraldi A, Bouche V et al (2011) Transcriptional activation of lysosomal exocytosis promotes cellular clearance. Dev Cell 21:421–430

Medina DL, Di Paola S, Peluso I et al (2015) Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. Nat Cell Biol 17:288–299

Nath S, Goodwin J, Engelborghs Y, Pountney DL (2011) Raised calcium promotes alpha-synuclein aggregate formation. Mol Cell Neurosci 46:516–526

Nunnari J, Suomalainen A (2012) Mitochondria: in sickness and in health. Cell 148:1145–1159

Okada R, Wu Z, Zhu A et al (2015) Cathepsin D deficiency induces oxidative damage in brain pericytes and impairs the blood-brain barrier. Mol Cell Neurosci 64:51–60

Osellame LD, Rahim AA, Hargreaves IP et al (2013) Mitochondria and quality control defects in a mouse model of Gaucher disease–links to Parkinson's disease. Cell Metab 17:941–953

de Pablo-Latorre R, Saide A, Polishhuck EV, Nusco E, Fraldi A, Ballabio A (2012) Impaired parkin-mediated mitochondrial targeting to autophagosomes differentially contributes to tissue pathology in lysosomal storage diseases. Hum Mol Genet 21:1770–1781

Palmieri M, Pal R, Nelvagal HR et al (2017) mTORC1-independent TFEB activation via Akt inhibition promotes cellular clearance in neurodegenerative storage diseases. Nat Commun 8:14338

Pena-Llopis S, Vega-Rubin-de-Celis S, Schwartz JC et al (2011) Regulation of TFEB and V-ATPases by mTORC1. EMBO J 30:3242–3258

Pickrell AM, Youle RJ (2015) The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. Neuron 85:257–273

Plotegher N, Duchen MR (2017) Mitochondrial dysfunction and neurodegeneration in lysosomal storage disorders. Trends Mol Med 23:116–134

Raffaello A, Mammucari C, Gherardi G, Rizzuto R (2016) Calcium at the Center of Cell Signaling: interplay between endoplasmic reticulum, mitochondria, and lysosomes. Trends Biochem Sci 41:1035–1049

Rama Rao KV, Kielian T (2016) Astrocytes and lysosomal storage diseases. Neuroscience 323:195–206

Sano R, Annunziata I, Patterson A et al (2009) GM1-ganglioside accumulation at the mitochondria-associated ER membranes links ER stress to ca(2+)-dependent mitochondrial apoptosis. Mol Cell 36:500–511

Sanz-Blasco S, Valero RA, Rodriguez-Crespo I, Villalobos C, Nunez L (2008) Mitochondrial Ca2+ overload underlies Abeta oligomers neurotoxicity providing an unexpected mechanism of neuroprotection by NSAIDs. PLoS One 3:e2718

Sato S, Koike M, Funayama M et al (2016) Lysosomal storage of subunit c of mitochondrial ATP synthase in brain-specific Atp13a2-deficient mice. Am J Pathol 186(12):3074–3082

Settembre C, Fraldi A, Jahreiss L et al (2008) A block of autophagy in lysosomal storage disorders. Hum Mol Genet 17:119–129

Sidransky E, Nalls MA, Aasly JO et al (2009) Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. N Engl J Med 361:1651–1661

Takamura A, Higaki K, Kajimaki K et al (2008) Enhanced autophagy and mitochondrial aberrations in murine G(M1)-gangliosidosis. Biochem Biophys Res Commun 367:616–622

Tang BL (2016) Sirt1 and the mitochondria. Mol Cells 39:87–95

Torres S, Matias N, Baulies A et al (2016) Mitochondrial GSH replenishment as a potential therapeutic approach for Niemann Pick type C disease. Redox Biol 11:60–72

Verity C, Winstone AM, Stellitano L, Will R, Nicoll A (2010) The epidemiology of progressive intellectual and neurological deterioration in childhood. Arch Dis Child 95:361–364

Voccoli V, Tonazzini I, Signore G, Caleo M, Cecchini M (2014) Role of extracellular calcium and mitochondrial oxygen species in psychosine-induced oligodendrocyte cell death. Cell Death Dis 5:e1529

Walkley SU (2004) Secondary accumulation of gangliosides in lysosomal storage disorders. Semin Cell Dev Biol 15:433–444

Wei H, Zhang Z, Saha A et al (2011) Disruption of adaptive energy metabolism and elevated ribosomal p-S6K1 levels contribute to INCL pathogenesis: partial rescue by resveratrol. Hum Mol Genet 20:1111–1121

Xu YH, Xu K, Sun Y et al (2014) Multiple pathogenic proteins implicated in neuronopathic Gaucher disease mice. Hum Mol Genet 23:3943–3957

Yamada T, McGeer PL, Baimbridge KG, McGeer EG (1990) Relative sparing in Parkinson's disease of substantia nigra dopamine neurons containing calbindin-D28K. Brain Res 526:303–307

Yu W, Gong JS, Ko M, Garver WS, Yanagisawa K, Michikawa M (2005) Altered cholesterol metabolism in Niemann-Pick type C1 mouse brains affects mitochondrial function. J Biol Chem 280:11731–11739