

UPDATE**Is Parkinson's disease a lysosomal disorder?****Andrés D. Klein^{1,*} and Joseph R. Mazzulli^{2,*}**

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Common forms of Parkinson's disease have long been described as idiopathic, with no single penetrant genetic factor capable of influencing disease aetiology. Recent genetic studies indicate a clear association of variants within several lysosomal genes as risk factors for idiopathic Parkinson's disease. The emergence of novel variants suggest that the aetiology of idiopathic Parkinson's disease may be explained by the interaction of several partially penetrant mutations that, while seemingly complex, all appear to converge on cellular clearance pathways. These newly evolving data are consistent with mechanistic studies linking α -synuclein toxicity to lysosomal abnormalities, and indicate that idiopathic Parkinson's disease resembles features of Mendelian lysosomal storage disorders at a genetic and biochemical level. These findings offer novel pathways to exploit for the development of disease-altering therapies for idiopathic Parkinson's disease that target specific components of the lysosomal system.

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Abbreviations: GlcCerase = β -glucocerebrosidase; LSD = lysosomal storage disorder

Introduction

Lysosomal storage disorders (LSDs) are caused by loss-of-function variants in genes that encode lysosomal proteins, leading to lysosomal dysfunction and intra-lysosomal build-up (so-called 'storage') of non-degraded metabolites. The LSD family is composed of over 55 distinct diseases with an incidence of \sim 1:7000 live births. The majority of LSDs are autosomal recessive and are caused by severe loss-of-function mutations in hydrolytic enzymes, trafficking

components, or integral membrane transporters (Futerman and van Meer, 2004). Although clinical symptoms are usually manifested in childhood, adult-onset forms also occur, which are commonly undiagnosed (Wassif *et al.*, 2016). Neurodegeneration is a prominent phenotype in nearly all LSDs, emphasizing the importance of lysosomal degradation in maintaining neuronal health (Fraldi *et al.*, 2016).

Parkinson's disease is the second most common neurodegenerative disorder of ageing, affecting \sim 1% of the population over 65 years old. Parkinson's disease symptoms and

signs can include tremor, bradykinesia, muscular rigidity, speech difficulties and can occasionally present with cognitive disturbances, but clinical features can be variable. The histopathological hallmark of Parkinson's disease is the intracellular accumulation of α -synuclein in the form of Lewy body inclusions, and death of dopaminergic neurons in the substantia nigra as well as other circumscribed regions of the nervous system (Braak and Del Tredici, 2017). Accumulation of α -synuclein is a common feature observed in many LSDs (Shachar *et al.*, 2011).

Most individuals with early onset Parkinson's disease, which correspond to ~10% of all cases, show Mendelian inheritance. Familial cases of Parkinson's disease can be due to variants in several genes, including *LRRK2*, *PARK7*, *PINK1*, *PRKN*, *ATP13A2* (which encodes for a component of the lysosomal acidification machinery) and *SNCA* (which encodes for α -synuclein protein). However, most Parkinson's disease cases are idiopathic without a single clear genetic link. Early studies describing anecdotal Parkinson's disease cases bearing variants in other lysosomal genes in addition to *ATP13A2*, including variants in the *GBA1* gene were reported (Goker-Alpan *et al.*, 2004). The *GBA1* gene encodes for the lysosomal β -glucocerebrosidase (GlcCerase), an enzyme responsible for degrading the lipid glucosylceramide into ceramide and glucose. Originally it was believed that the *GBA1*–Parkinson's disease association was not causative. However, a multi-centre analysis of idiopathic Parkinson's disease patients demonstrated that loss-of-function mutations in one allele of *GBA1* is a significant risk factor for developing disease (Sidransky *et al.*, 2009).

While heterozygote *GBA1* mutations increase risk for developing Parkinson's disease, homozygous variants cause Gaucher disease with an incidence of 1:50 000 births in the general population and rising up to 1:800 in Ashkenazi Jews (Stirnemann *et al.*, 2017). Clinically, Gaucher disease is a heterogeneous syndrome and is divided into three subtypes: type 1, non-neuropathic; and types 2 and 3, the neuropathic forms. These distinctions are not absolute, and currently Gaucher disease is largely recognized as continuum spectrum of phenotypes (Sidransky, 2012). Gaucher disease-causing *GBA1* mutations result in an accumulation of glucosylceramide within lysosomes. The mechanisms that lead to cell death in Gaucher disease are still under investigation; however, disruptions in autophagic-lysosomal pathway (Sun and Grabowski, 2010; Schöndorf *et al.*, 2014), mitophagy (Osellame *et al.*, 2013), necroptosis (Vitner *et al.*, 2014) and enhanced inflammation through activation of complement C5a receptor 1 (Pandey *et al.*, 2017) are thought to play a role.

Genetics implicates multiple lysosomal variants in Parkinson's disease aetiology

Newly emerging data have now indicated that the lysosomal connection in Parkinson's disease likely extends far beyond

GBA1. Using an unbiased approach, a recent genome wide association study (GWAS) encompassing the largest cohort to date (26 035 idiopathic Parkinson's disease cases and 403 190 controls) showed that the majority of variants identified were components of the autophagic-lysosomal pathway (Chang *et al.*, 2017). Of the loci identified, novel genes included *CTSB* (cathepsin B), *ATP6V0A1* (ATPase H+ transporting V0 subunit A1), and *GALC* (galactocerebrosidase) of the lysosomal pathway, and *KAT8* (lysine acetyltransferase), which has been shown to alter autophagic flux (Füllgrabe *et al.*, 2013). An independent study recently published in *Brain* also demonstrated an excessive burden of rare, likely damaging LSD gene variants in association with Parkinson's disease risk. In this study, a cohort of 1156 patients with Parkinson's disease and 1679 control subjects was analysed for variants within 54 lysosomal genes known to cause paediatric LSDs, and novel variants within *CTSD* (cathepsin D), *SLC17A5* (sialin), and *ASAH1* (acid ceramidase) were identified. Most (56%) of the Parkinson's disease patients presented with at least one putative damaging variant in a LSD gene, and 21% carried multiple alleles (Robak *et al.*, 2017). Consistent with a focus on lysosomal-related proteins, previous studies have shown an association of Parkinson's disease with variants in *NPC1*, a lysosomal cholesterol transporter that regulates vesicular trafficking and causes Niemann-Pick type C disease (Josephs *et al.*, 2004; Klunemann *et al.*, 2013), and α -N-acetylglucosaminidase (*NAGLU*), the gene responsible for Sanfilippo syndrome (Winder-Rhodes *et al.*, 2012). Independent of direct mutations in lysosomal components, variants in vesicular trafficking machinery have been associated with Parkinson's disease including Rab GTPases (MacLeod *et al.*, 2013; Lesage *et al.*, 2015), retromer components such as *VPS35* (Zimprich *et al.*, 2011), *LIMP2*, which is essential for the targeting of GlcCerase to lysosomes (Do *et al.*, 2011), and *LRRK2*, which can phosphorylate and deregulate Rab GTPases to influence vesicular trafficking (Satake *et al.*, 2009; Steger *et al.*, 2017). These mutations likely lead to cellular dysfunction by affecting multiple pathways, possibly by disrupting protein secretion or targeting of essential proteins in the synapse such as dopamine transporter (DAT), which may lead to aberrant dopamine metabolism and oxidation (Oaks *et al.*, 2013). However, disruptions in the early secretory pathway strongly affects the maturation and targeting of lysosomal hydrolases, which may particularly influence protein accumulation and aggregation. Since efficient cellular clearance involves the concerted action of proper enzyme targeting, vesicular trafficking or secretion, and cargo delivery processes, a combination of damaging variants in one or both of these pathways are likely to amplify the perturbation, culminating in protein aggregation. Indeed, recent mechanistic studies have demonstrated the interaction of different Parkinson's disease-linked variants including Rab7L1/*LRRK2*, which contribute to endosomal-lysosomal dysfunction (MacLeod *et al.*, 2013). Together, these studies suggest that not a single variant, but a combination of many

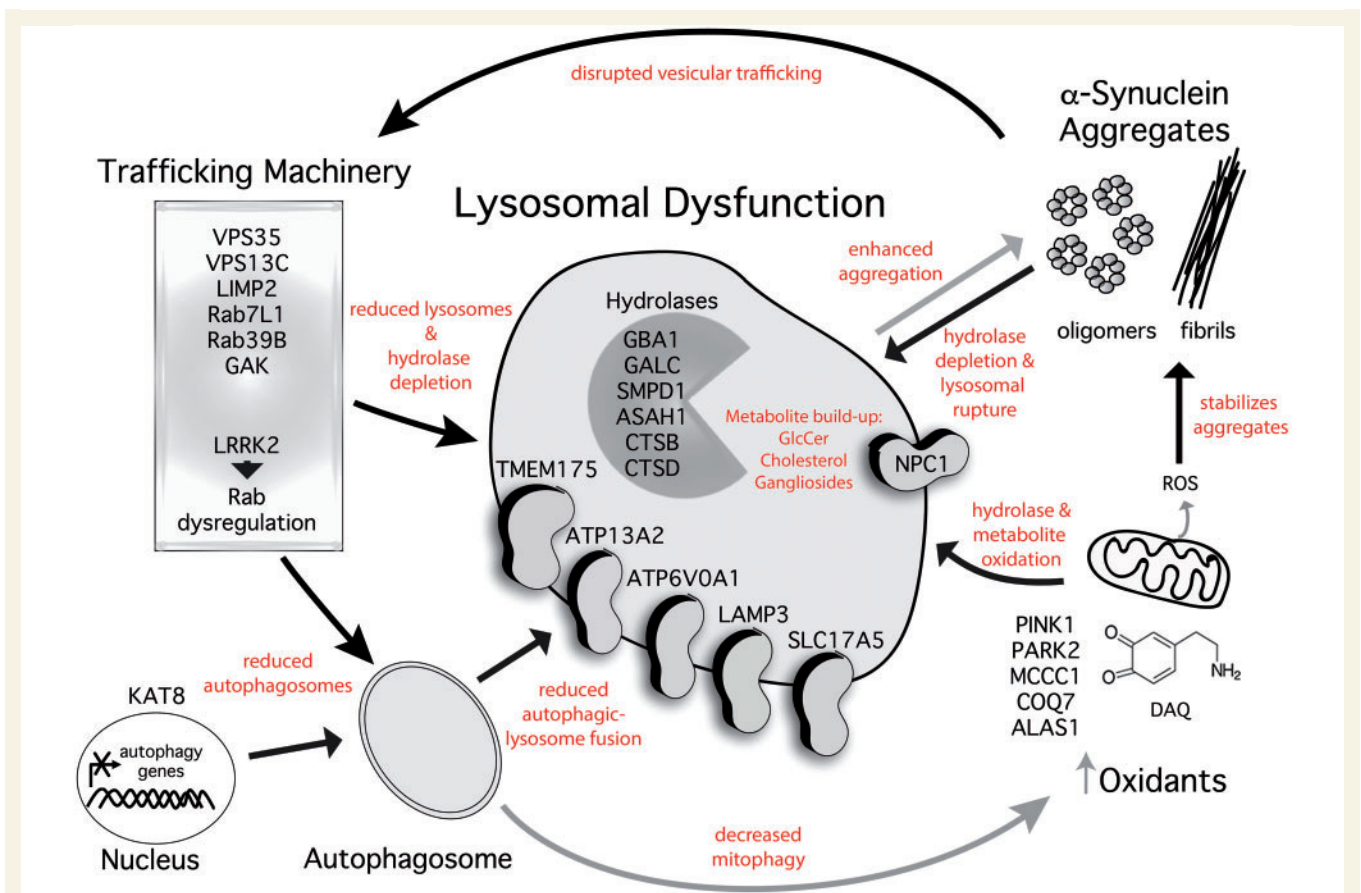


Figure 1 Parkinson's disease genes reveal an association of multiple variants that converge on deficiencies in cellular clearance. The emergence of several genes associated with Parkinson's disease have highlighted essential cellular pathways key to pathogenesis. Genetic variants associated with idiopathic or familial Parkinson's disease are listed above organized by their normal cellular pathway and function. Lysosomes are critical for the degradation of α -synuclein, and disruptive lysosomal gene variants are expected to enhance the formation of pathogenic α -synuclein oligomers and fibrils directly and indirectly through the interaction of lysosomal membrane lipids. Once formed, these aggregates actively disrupt trafficking and lysosomal clearance pathways, which permits their persistence in neurons and ultimately results in cellular self-destruction. Other Parkinson's disease genes, such as LRRK2, can influence trafficking and autophagic-lysosomal function through phosphorylating Rab proteins. Parkinson's disease genes that disrupt mitochondrial function can lead to reactive oxygen species (ROS) or dopamine-o-quinone (DAQ) that can damage lysosomal machinery, leading to α -synuclein aggregate formation. Black arrows indicate an inhibitory process and grey arrows indicate enhancement of potential pathogenic processes described in red text. GlcCer = glucosylceramide.

common variants converge in the same pathway ultimately leading to dysfunction, consistent with the notion of idiopathic Parkinson's disease as a complex genetic disorder that converges on lysosomal dysfunction (Fig. 1). The identification of non-Mendelian variants that are not individually causative, but rather predispose one to disease, have been notoriously difficult to identify due to difficulties in achieving adequate statistical power. These recent large-scale studies bring us one step closer to identifying critical pathogenic pathways to prioritize for therapeutic development centred on the lysosomal system.

Lysosomal dysfunction can influence α -synuclein aggregation

Mechanistic studies have begun to illuminate the relationship between lysosomal dysfunction and α -synuclein aggregation.

Physiological α -synuclein can be degraded through the lysosomal system (Cuervo *et al.*, 2004) and mutations that perturb lysosomal function are expected to affect α -synuclein levels. As α -synuclein is an abundant protein in physiological conditions, even a subtle elevation in protein concentration may drive the protein towards pathological aggregation since the polymerization process is highly concentration dependent (Giasson *et al.*, 1999). Generalized lysosomal dysfunction may be expected to induce widespread protein aggregation; however, several factors may explain the relative selectivity of α -synuclein aggregation. The amino acid sequence of α -synuclein contains a region in the middle of the protein with an abundance of hydrophobic residues that renders the protein susceptible to polymerization (Giasson *et al.*, 2001). Multiple studies have shown that sphingolipid metabolites that accumulate in LSDs, such as glucosylceramide, psychosine, glucosylsphingosine, and gangliosides can specifically interact and induce α -synuclein aggregation (Mazzulli *et al.*, 2011; Smith *et al.*,

2014; Suzuki *et al.*, 2015; Taguchi *et al.*, 2017). This process is thought to initiate through a conversion of physiological α -synuclein conformers into an alternate, assembly-state oligomer that can subsequently seed the formation of amyloidogenic fibrils (Zunke *et al.*, 2017). Cholesterol may also be an important player in Parkinson's disease pathogenesis. In addition to the genetic association with *NPC1* with Parkinson's disease, accumulated oxidized metabolites of cholesterol have been identified in synucleinopathy brain and can directly induce α -synuclein fibrilization (Bosco *et al.*, 2006). A recent study has shown that fibroblasts derived from Parkinson's disease patients bearing the *N370S-GBA1* variant accumulate lysosomal cholesterol and present multilamellar bodies, analogous to what occurs in the lysosomal cholesterol storage disorder Niemann-Pick type C (García-Sanz *et al.*, 2017). Related to Gaucher disease pathogenesis, increased intracellular cholesterol can modify GlcCerase processing by inducing its degradation through endoplasmic reticulum (ER)-associated degradation (ERAD) in proteasomes, an activity that is associated with disease severity (Ron and Horowitz, 2008). This, in turn, may lower lysosomal GlcCerase activity and alter glucosylceramide and α -synuclein levels. However, whether this occurs in the context of Parkinson's disease requires further investigation. Together, this indicates that lysosomal storage of certain toxic metabolites can influence the structural state of α -synuclein, converting it into a pathogenic conformation capable of inducing neurodegeneration. This may explain the selectivity and relationship between certain lysosomal mutations and synucleinopathies.

Genetic variants may modify the development of Gaucher and Parkinson's disease

Important information pertaining to the genetic variants that modify the severity of Gaucher disease has also been obtained through the analysis of mouse models. The *in vivo* use of a GlcCerase inhibitor in many pure inbred strains has led to the identification of candidate modifier genes of Gaucher disease severity. These findings may also relate to modifiers of idiopathic Parkinson's disease through the study of long-lived Gaucher disease mouse models where age-related Parkinsonism can be studied (Klein *et al.*, 2016). This work has identified NMDA receptor signalling as an important modifier of disease onset, indicating a possible novel therapeutic pathway in Gaucher disease and Parkinson's disease. Importantly, this may provide clues not only into the heterogeneity that underlies Gaucher disease, but may also identify novel genetic variants that predispose one to the development of neurological disease.

A bidirectional relationship between α -synuclein and lysosomes

A critical pathogenic factor that could combine with predisposing genetic variants involves the accumulation of

α -synuclein. In a remarkable connection to the genetic studies, α -synuclein aggregates have been shown to inhibit autophagic-lysosomal pathways either through direct disruption of lysosomal components (Cuervo *et al.*, 2004; Martínez-Vicente *et al.*, 2008; Yap *et al.*, 2011; Freeman *et al.*, 2013), or through inhibiting trafficking events (Cooper *et al.*, 2006; Mazzulli *et al.*, 2011, 2016a; Chung *et al.*, 2013). Previous work demonstrated that the build-up of α -synuclein blocks the trafficking of newly synthesized GlcCerase to the lysosome and thus amplifies glucosylceramide accumulation, creating a bidirectional pathogenic loop (Mazzulli *et al.*, 2011, 2016a). In addition to GlcCerase, recent studies have found that the enzyme deficient in Fabry's disease, alpha-galactosidase A, is also deficient in idiopathic Parkinson's disease brain and correlates with pathological α -synuclein accumulation (Nelson *et al.*, 2018). Loss of NPC1 function, which affects vesicular trafficking and lysosomal function, can also impede the clearance of α -synuclein (Ko *et al.*, 2001; Liao *et al.*, 2007; Eriksson *et al.*, 2017). Since build-up of α -synuclein has been documented in many lysosomal disorders (Shachar *et al.*, 2011), LSD patients may develop α -synuclein deposits by mechanisms that are similar to idiopathic Parkinson's disease. Although the precise function of α -synuclein is not completely understood, it plays a role in synaptic vesicle recycling and transmission at presynaptic terminals (Bendor *et al.*, 2013). Specifically, α -synuclein can interact with membrane lipids, such as acidic phospholipids, cholesterol, gangliosides, glucosylceramide and others that accumulate in many LSDs and may regulate its function (Perrin *et al.*, 2000; Galvagnion, 2017). Previous work showed that α -synuclein may work as a chaperone that aids the function of synaptic SNARE proteins, regulating the activity of synaptic-vesicle fusion machinery at nerve terminals (Burré *et al.*, 2010; Garcia-Reitböck *et al.*, 2010). Additional studies suggested that multiple copies of α -synuclein coalesce on synaptic vesicles, possibly forming multimeric structures (Burré *et al.*, 2014). It is possible that pathology is initiated at the synapse, since elevated local concentrations will potentiate its aggregation propensity. Autophagic delivery of substrates, such as α -synuclein, from distal axons into lysosomes at the cell body for degradation require intact retrograde axonal transport machinery. Therefore, subtle perturbations in axonal trafficking or lysosomal function, which may occur through heterozygote mutations in LSD genes, may influence the transport of α -synuclein from the presynaptic terminal into the cell body for degradation (Maday and Holzbaur, 2016). Consistent with this, impairments in retrograde axonal transport have been documented in Krabbes disease mouse models with *GALC* deficiency that also accumulate pathological α -synuclein, as well as in Niemann-Pick type C mice (Ohara *et al.*, 2004; Smith *et al.*, 2014; Teixeira *et al.*, 2014). Aggregation of α -synuclein in neurites or cell bodies may contribute to neurodegeneration by impeding axonal transport (Volpicelli-Daley *et al.*, 2014), or through reducing its putative physiological

chaperoning activity at synapses with consequent presynaptic failure (Burgoyne and Morgan, 2011). The relationship between α -synuclein accumulation, lysosomal hydrolase dysfunction, and build-up of lysosomal membrane lipids indicates that any synucleinopathy including Parkinson's disease exhibits similar but more mild features compared to paediatric LSDs, such as neuronopathic Gaucher disease or other storage diseases characterized by a multiple hydrolase deficiency.

Convergence of mitochondrial and lysosomal dysfunction in Parkinson's disease

In addition to lysosomes and trafficking dysfunction, mitochondria dysfunction has been linked to Parkinson's disease both genetically and pathologically. Interestingly, mutations that cause early-onset familial Parkinson's disease have been shown to impede mitophagy, the process of mitochondrial degradation by autophagic-lysosomal system (Pickrell and Youle, 2015). Mechanistic evidence that may explain the convergence of mitochondrial and lysosomal dysfunction in Parkinson's disease has recently emerged. Using midbrain induced pluripotent stem cell (iPSC) neurons and mouse models, a recent study showed that GlcCerase was susceptible to modification and inactivation by oxidized dopamine, which can induce α -synuclein oligomerization directly or by inhibiting lysosomal function (Burbulla *et al.*, 2017; Mor *et al.*, 2017). Using live-cell imaging techniques, direct contacts between lysosomes and mitochondria have been documented, providing the possibility for aberrant contacts of damaged mitochondria that oxidize lysosomal enzymes in the disease state (Wong *et al.*, 2018). This may also lead to oxidation of accumulating lysosomal metabolites, such as cholesterol that occurs in NPC1, to potentiate α -synuclein aggregation (Bosco *et al.*, 2006). Oxidation and mitochondrial dysfunction are well-established pathological features of Parkinson's disease and many LSDs (Takamura *et al.*, 2008; Vázquez *et al.*, 2012; Won *et al.*, 2016), and have been implicated in disease initiation. It is possible that interaction of oxidant stress, α -synuclein, and subtle genetic perturbations that affect the lysosomal system combine to produce prominent deficits in cellular clearance (Fig. 1).

Genetic and pathological similarities of Parkinson's disease and lysosome storage disorders

An additional interesting connection between idiopathic Parkinson's disease-associated genetic variants and mutations that are causative for LSDs is the significant degree of overlap in the cellular pathways involved. Similar to newly discovered factors thought to influence the onset of idiopathic Parkinson's disease, LSDs can be caused by mutations in different components of the lysosomal system

ranging from hydrolases, trafficking machinery, metabolite transporters, or co-activator proteins (Futerman and van Meer, 2004). Mutations that disrupt a key trafficking enzyme located at the Golgi, *N*-acetylglucosamine-1-phosphotransferase, result in near-complete depletion of multiple hydrolases in the lysosomal compartment. These mutations cause a paediatric LSD with severe neurodegeneration called mucopolipidosis type II or inclusion (I)-cell disease, and the multiple hydrolase deficiency results in prominent storage of protein, lipid, and oligosaccharides within lysosomes (Futerman and van Meer, 2004; Klein and Futerman, 2013). Although less severe, the biochemical phenotype of I-cell disease have features that resemble long-lived midbrain cultures of Parkinson's disease patients, which exhibit a deficiency in multiple lysosomal hydrolases and substrate accumulation as a result of α -synuclein-induced disruptions in ER-to-Golgi trafficking (Cooper *et al.*, 2006; Mazzulli *et al.*, 2016a). As ageing is a critical risk factor for synucleinopathies, it is possible that subtle and chronic perturbations in trafficking machinery eventually reach a threshold leading to severe lysosomal dysfunction, substrate accumulation, and neuronal cell death. The study of post-mortem Parkinson's disease brain has shown a deficiency of GlcCerase and other lysosomal components (Chu *et al.*, 2009; Alvarez-Erviti *et al.*, 2010; Murphy *et al.*, 2014). Accumulation of lipids, ubiquitin, and the autophagic marker LC3 has been demonstrated in Parkinson's disease brain (Gai *et al.*, 2000; Dehay *et al.*, 2010), suggestive of autophagic accumulation and general dysfunction in cellular clearance. Studies in transgenic mouse models demonstrate that α -synuclein overexpression induces an LSD-like pathology, as demonstrated by the presence of enlarged lysosomes containing electron-dense inclusion bodies and accumulation of double-membrane vacuoles reminiscent of autophagosomes (Rockenstein *et al.*, 2005).

Collectively, these data suggest that idiopathic Parkinson's disease and other synucleinopathies exhibit remarkably similar features of LSDs at both the genetic and biochemical level, indicating an overlap in the pathogenesis of these disorders. An important distinction between Mendelian LSDs and genetically complex neurodegenerative disorders such as Parkinson's disease is the age-at-onset and rate of disease progression. LSDs can result in a severe neurological phenotype that occurs very early in life, likely due to the severity and penetrance of the mutations involved. For example, severe neuronopathic forms of Gaucher disease are often caused by highly destabilizing mutations (such as L444P) that result in near-complete depletion of GlcCerase with little residual activity. Death can ensue rapidly, within 1 year after birth, which can preclude the diagnosis of Parkinson's disease. As a chronic disease of ageing, lysosomal dysfunction in idiopathic Parkinson's disease likely occurs by mild heterozygote mutations that combine with age-related complications such as oxidant stress. This suggests that LSDs represent a continuum of diseases that span from severe early onset to late-stage neurodegeneration.

Developing lysosomal therapies for Parkinson's disease

The convergence of pathogenic pathways that culminate in lysosomal dysfunction indicate the importance of cellular degradation in Parkinson's disease aetiology, and have identified many targets that may be translated into Parkinson's disease therapies. Several strategies for treating LSDs have been investigated or are under development, and these may provide novel opportunities for treating Parkinson's disease. These include: (i) gene therapy or enzyme replacement; (ii) substrate reduction therapies, aimed at decreasing storage material; (iii) chaperones to rescue misfolded or unstable enzymes, or direct allosteric activators; (iv) cell therapies to replace injured cells; (v) inhibition of pathways that cause cell death; and (vi) stimulation of bypass pathways to compensate for loss of lysosomal proteins (Klein and Futerman, 2013). Among these approaches, small molecule chaperones or activators from studies done in Parkinson's disease iPSC models or mouse models have shown promise. Experimental approaches using iPSC-derived neurons from *SNCA* triplication patients and transgenic mice treated with GlcCerases chaperones or activators, have shown reduced levels of α -synuclein aggregates, opening a new therapeutic strategy for the disease (Mazzulli *et al.*, 2016b; Migdalska-Richards *et al.*, 2016). Brain-penetrant molecules that reduce the synthesis of glucosylceramide can also reduce α -synuclein in iPSC neurons (Zunke *et al.*, 2017; Kim *et al.*, 2018) and improve cognitive symptoms in synucleinopathy mouse models (Sardi *et al.*, 2017). These studies and other work have laid the foundation for clinical trials that will evaluate the efficacy of glucosylceramide reducing agents in patients that carry a *GBA1* mutation. Therapies aimed at lowering cholesterol levels with beta-cyclodextrins, which are currently used for treating Niemann-Pick type C patients, reduced accumulation of α -synuclein in a mouse model of Parkinson's disease (Bar-On *et al.*, 2006). Strategies to increase cellular clearance by overexpressing the transcription factor EB (TFEB), which controls lysosomal biogenesis and autophagy, have therapeutic potential since studies have shown that it can rescue midbrain dopamine neurons in a rat model of Parkinson's disease (Decressac *et al.*, 2013). Interestingly, beta-cyclodextrins activate TFEB nuclear translocation (Kilpatrick *et al.*, 2015), reinforcing the therapeutic potential of these agents for Parkinson's disease.

Concluding remarks

The identification of an overrepresentation of autophagic-lysosomal variants in Parkinson's disease brings us closer to deciphering the aetiology of this complex disorder. Prior to the discovery of *SNCA* mutations as a cause for familial Parkinson's disease more than 20 years ago, Parkinson's disease was classified as strictly idiopathic and considered to be the archetypal non-genetic disease. Recent

advancements have clearly negated this with the discovery of several Mendelian forms of Parkinson's disease, and the identification of risk factors that predispose one to idiopathic Parkinson's disease. Newly emerging genetic evidence is sure to direct the development of future mechanistic studies on the aetiology of idiopathic Parkinson's disease, and personalized therapies centred on correcting specific perturbations in lysosomal components.

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Conflict of interest

J.R.M. is a scientific founder of Lysosomal Therapeutics, Inc. (LTI).

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