BIOCELL

Tech Science Press

http://doi.org/10.32604/biocell.2026.079585

ARTICLE

LRRK2 Inhibition Differently Affects Lysosomal Hydrolase Activity and Autophagy-Related Protein Levels in PBMC-Derived Macrophages from Patients with Different Synucleinopathies

Katerina Basharova[1,2,*,#], Anastasia Bezrukova[1,2,#], Alena Kopytova[1,2], Anna Lavrinova[1,2], Galina Baydakova[3], Irina Miliukhina[1,4], Ekaterina Zakharova[1,3], Anton Emelyanov[1,2], Sofya Pchelina[1,2] and Tatiana Usenko[1,2,*]

[1]Petersburg Nuclear Physics Institute Named by B.P. Konstantinov of National Research Centre «Kurchatov Institute», Gatchina, Russia
[2]Department of Molecular Genetic and Nanobiological Technologies, Pavlov First Saint Petersburg State Medical University, St. Petersburg, Russia
[3]Research Center for Medical Genetics, Moscow, Russia
[4]N.P. Bechtereva Institute of the Human Brain of Russian Academy of Sciences, St. Petersburg, Russia
*Corresponding Authors: Katerina Basharova. Email: basharova_ks@pnpi.nrcki.ru; Tatiana Usenko. Email: usenko_ts@pnpi.nrcki.ru
#These authors contributed equally to this work

Received: 24 January 2026; Accepted: 21 May 2026; Published: 27 July 2026

ABSTRACT: Objectives: Synucleinopathies—Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA)—involve alpha-synuclein aggregation and lysosomal dysfunction. We conducted a longitudinal study of lysosomal hydrolase activities and lysosphingolipid levels in blood and PBMC-derived macrophages from patients, and assessed the effects of LRRK2 inhibition (MLi-2). **Methods:** Blood and PBMC-derived macrophages were collected from patients with idiopathic PD (iPD), DLB, MSA, and controls. Enzyme activities (GCase, ASMase, GLA, GALC) and lysosphingolipids (HexSph, LysoGb3, LysoSM) were measured. Autophagy markers (p62, LC3B-II) and cathepsin D (CTSD) were analyzed by western blot. Effects of MLi-2 were evaluated in macrophages. **Results:** All synucleinopathy groups showed reduced blood GCase activity and elevated HexSph levels, with the highest HexSph levels observed in MSA ($p < 0.05$). DLB exhibited reduced ASMase, whereas MSA showed broader sphingolipid disruption (reduced ASMase activity, increased GLA and GALC activities, elevated LysoGb3 levels ($p < 0.05$)). In iPD, LysoGb3 levels increased, and LysoSM levels decreased. In PBMC-derived macrophages, MSA showed reduced GCase, GALC, and ASMase activities. HexSph levels were elevated in all groups, while LysoGb3 levels were increased in iPD and MSA ($p < 0.05$). DLB macrophages showed increased mature CTSD, whereas MSA cells exhibited reduced CTSD and p62 ($p < 0.05$). MLi-2 had minimal effects on enzyme activities and lipid levels. It increased p62 in iPD, reduced CTSD in DLB ($p < 0.05$), and had no effect in MSA ($p > 0.05$). **Conclusion:** HexSph may represent a common marker of synucleinopathies, while MSA demonstrates the most pronounced lysosomal dysfunction. Limited effects of LRRK2 inhibition suggest a minor role for this pathway in sporadic synucleinopathies.

KEYWORDS: Synucleinopathies; lysosomal hydrolases; leucine-rich repeat kinase 2; MLi-2; autophagy

1 Introduction

Synucleinopathies comprise a heterogeneous group of neurodegenerative disorders characterized by the progressive accumulation of misfolded alpha-synuclein in neurons and glial cells. Their clinical

manifestations range from predominant motor dysfunction in Parkinson's disease (PD) to early cognitive impairment in dementia with Lewy bodies (DLB) and rapid multisystem neurodegeneration in multiple system atrophy (MSA) [1–3]. These phenotypic differences are thought to reflect disease-specific patterns of alpha-synuclein distribution and cellular vulnerability. Despite substantial advances in clinical characterization, the molecular mechanisms underlying synucleinopathies remain incompletely understood, and reliable biomarkers for early differential diagnosis are still lacking.

Accumulating evidence implicates lysosomal dysfunction and disturbed sphingolipid metabolism as central contributors to the pathogenesis of synucleinopathies [4]. Beyond their classical definition as proteinopathies, these disorders are increasingly recognized as lipid-associated diseases, characterized by sphingolipid dyshomeostasis [5]. Impaired activity of lysosomal hydrolases leads to the accumulation of bioactive lipid species, such as ceramides and glycosphingolipids, which may promote alpha-synuclein misfolding and aggregation [6,7]. In line with this, genetic variants in genes encoding lysosomal enzymes, including *GBA1* and *SMPD1*, significantly modify disease risk [8,9]. At the same time, we and others reported significant alterations in autophagy-related protein levels across different forms of PD, including idiopathic PD (iPD) and forms with known genetic etiology, as well as in DLB and MSA [10–12].

Evidence for impairment of lysosomal hydrolase activities has been obtained across multiple biological matrices, including postmortem brain tissue, cerebrospinal fluid, and peripheral blood from patients with synucleinopathies [13–15]. In this context, we recently performed analyses of lysosomal hydrolase activities in blood from patients with synucleinopathies, demonstrating systemic alterations across PD, DLB, and MSA [16]. While these findings support the concept of systemic impairment of lysosomal hydrolase activity, they do not provide insight into cell-specific mechanisms. To address this, in the current study, we chose to obtain peripheral blood mononuclear cell (PBMC)-derived macrophages, which represent a more homogeneous and biologically relevant model to investigate lysosomal function in a patient-specific context. Previously, PBMC-derived macrophages have been successfully employed by us and others to investigate lysosomal dysfunction, particularly the consequences of diminished glucocerebrosidase (GCase) activity in PD [17–19]. These cells also serve as a reliable peripheral model to explore autophagy impairment [12].

One of the key regulators of lysosomal function is leucine-rich repeat kinase 2 (LRRK2), mutations in which are a common cause of familial PD and lead to increased kinase activity. Pharmacological inhibition of LRRK2 has been shown to enhance lysosomal hydrolase activity, reduce alpha-synuclein accumulation, and modulate autophagy in cellular and animal models [20–22]. Several LRRK2 inhibitors are currently being evaluated in clinical trials for PD patients with and without *LRRK2* mutations (NCT06680830, NCT06602193, NCT05348785). The effect of LRRK2 inhibition on autophagy was also shown *in vivo* models for PD [23]. However, despite ongoing clinical trials of LRRK2 inhibitors for PD, it is still unknown how LRRK2 inhibition will affect lysosomal hydrolase activities and autophagy in iPD and other synucleinopathies.

In the present study, we integrated longitudinal blood-based analyses with functional investigations in PBMC-derived macrophages obtained from patients with iPD, DLB, and MSA, as well as neurologically healthy controls. We assessed the activities of key lysosomal hydrolases involved in sphingolipid metabolism and quantified corresponding lipid substrates. Furthermore, we investigated the effects of LRRK2 inhibition using MLi-2 on lysosomal hydrolases activity in PBMC-derived macrophages from patients with synucleinopathies. In addition, we assessed the effects of LRRK2 inhibition on steady-state autophagy by analyzing two key autophagy-related proteins, phosphatidylethanolamine-conjugated microtubule-associated protein 1 light chain 3 (LC3B-II) and sequestosome 1 (p62), together with cathepsin D (CTSD), a major protease involved in α-synuclein degradation. By combining systemic and cell-based

approaches, our study provides new insights into disease-specific lysosomal alterations and explores their potential as biomarkers and therapeutic targets in synucleinopathies.

2 Materials and Methods

2.1 Characteristic of Study Participants

The study was conducted according to the guidelines of the Declaration of Helsinki. The biomaterial was collected at the Pavlov First Saint Petersburg State Medical University with the permission of the ethics committee (Protocol number: 307, Approval Date: 20 October 2025). Written informed consent or assent was obtained from patients to collect samples. All research is conducted anonymously. The study consisted of a primary cohort used for longitudinal blood-based analyses of lysosomal hydrolase activities and a secondary cohort used for cellular experiments. Peripheral blood mononuclear cell (PBMC)-derived macrophages were obtained from a subset of participants and used for functional and molecular analyses. Within this macrophage cohort, two partially overlapping subgroups were analyzed: one for the assessment of lysosomal hydrolase activities (macrophage cohort a) and another for the evaluation of autophagy-related proteins and pharmacological treatment with the LRRK2 inhibitor MLi-2 (macrophage cohort b). All patients were followed up by a neurologist at the N.P. Bechtereva Institute of the Human Brain, RAS of St. Petersburg, as well as 43 individuals without neurological disorders (controls), were enrolled in the current study (Table 1). The control group included in the current study consisted of individuals who were observed in the consultative and diagnostic center of Pavlov First Saint Petersburg State Medical University. In order to exclude the diagnosis of synucleinopathies and other neurodegenerative diseases, all individuals in the control group were examined by a neurologist. The diagnosis of PD was established in accordance with the Movement Disorder Society Clinical Diagnostic Criteria for PD [24]. DLB and MSA were diagnosed according to consortium criteria [25,26]. To avoid biases and exclude carriers of major mutations in the *GBA1* and *LRRK2* genes, all participants were screened for the major mutations in the *GBA1* gene (p.L444P, p.N370S) and in the *LRRK2* gene (p.G2019S) as previously described [27,28].

Table 1: The demographics and clinical characteristics of the studied groups.

Group	Cohort	N	Sex (M:F)	Age at Exam (Years, Mean ± SD)	Age at Onset (Years, Mean ± SD)
iPD	Blood	248	98:150	65.3 ± 8.3	58.4 ± 10.7
	Macrophages (a)	22	11:11	65.8 ± 10.0	54.9 ± 10.5
	Macrophages (b)	10	6:4	61.2 ± 9.9	54.1 ± 9.7
DLB	Blood	55	21:34	71.6 ± 8.7	66.2 ± 9.6
	Macrophages (a, b)	6	4:2	65.3 ± 9.7	59.8 ± 11.5
MSA	Blood	118	44:74	61.5 ± 7.0	57.9 ± 6.7
	Macrophages (a, b)	10	5:5	62.6 ± 5.9	58.7 ± 6.0
Controls	Blood	228	93:135	62.5 ± 7.9	-
	Macrophages (a)	43	24:19	64.9 ± 9.0	-
	Macrophages (b)	12	2:6	62.9 ± 6.4	-

Note: Macrophages (a), for the assessment of lysosomal hydrolase activities; Macrophages (b), for the evaluation of autophagy-related proteins and pharmacological treatment with the LRRK2 inhibitor MLi-2; iPD, idiopathic Parkinson's disease; DLB, dementia with Lewy bodies; MSA, multiple system atrophy; N, number of individuals in group; SD, standard deviation; "-", not applicable.

2.2 Primary Peripheral Blood Macrophages Culture and Treatment with MLi-2

PBMCs were isolated from the peripheral blood of all participants by density gradient centrifugation in Ficoll (Biolot, St. Petersburg, Russia).

PBMCs were differentiated into macrophages by adding colony-stimulating factor (M-CSF) (10 ng/mL) (574806, Biolegend, San Diego, CA, USA) to the RPMI-1640 medium (Biolot, Russia) supplemented with 10% fetal bovine serum (FBS) (FBS-11A, Capricorn, Ebsdorfergrund, Germany). Cells were incubated at 37°C in a 5% CO_2 atmosphere for 5 days prior to treatment. After 5 days, PBMC-derived macrophages were treated with a 100 nM selective inhibitor of LRRK2 kinase activity, MLi-2 (ab254528, Abcam, Cambridge, UK), once for 4 days without replacement of medium. Phenotypical maturation of monocyte-derived macrophages was confirmed by light microscopy (TSA-9, LOMO, St. Petersburg, Russia) and flow cytometry with specific antibodies to $CD14^+$ cat. no. 130-050-201, Miltenyi Biotec, USA) and $CD68^+$ (eBioscience, San Diego, CA, USA), as described earlier [18,19]. Flow cytometry was performed using a Navios (Beckman Coulter, USA). For each participant, PBMC-derived macrophages were obtained in triplicate.

2.3 Enzyme Activity and Sphingolipid Concentrations in PBMC-Derived Macrophages

Dry blood spot (DBS) cards (WHA1053461, Millipore Sigma, Burlington, MA, USA) were prepared by pipetting 20 µL of PBMC-derived macrophages at a concentration of 2×10^6 cells/mL onto each spot. The DBS cards were then allowed to dry in open air at room temperature for 2 h and subsequently stored at −20°C until extraction. The enzyme activities of glucocerebrosidase (GCase, EC 3.2.1.45, deficient in Gaucher disease), alpha-galactosidase A (GLA, EC 3.2.1.22 deficient in Fabry disease), acid sphingomyelinase (ASMase, EC 3.1.4.12, deficient in Neimann-Pick disease types A and B), galactosylceramidase (GALC, EC 3.2.1.46, deficient in Krabbe disease) and concentration of corresponding sphingolipids (hexosylsphingosine (HexSph) (glucosylsphingosine (GlcSph) + galactosylsphingosine (GalSph)), globotriaosylsphingosine (LysoGb3), lysosphingomyelin (LysoSM)) were estimated by high performance liquid chromatography tandem-mass spectrometry (HPLC-MS/MS) in triplicate as described earlier [18,19]. The HPLC-MS/MS system for assessment of lysosomal activities consisted of a LC-20 Prominence HPLC (Shimadzu, Japan) and an API 3200 QTrap (SCIEX, Marlborough, MA, USA). The HPLC-MS/MS system for measuring lysosphingolipid concentrations consisted of a Nexera HPLC (Shimadzu, Japan) and an API-5500 QTrap mass spectrometer (SCIEX, MA, USA).

2.4 Western Blot Analysis

For cell lysis, PBMC-derived macrophages were lysed on ice in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% (w/v) SDS, 0.5% (w/v) sodium deoxycholate, 1% Triton X-100) supplemented with protease/phosphatase inhibitor cocktail (Merck, Darmstadt, Germany). The protein concentration was determined using the Pierce BCA Protein Assay kit (#A55865, Thermo Scientific, Rockford, IL, USA). Following protein quantification, the lysates were denatured at 95°C for 5 min in 2× Laemmli sample buffer (BioRad, Hercules, CA, USA; 2× Laemmli Sample Buffer #1610737) with 2-mercaptoethanol addition in a 1:19 ratio. Equal amounts of protein were separated by electrophoresis in polyacrylamide gel (20% SDS-PAGE—for LC3B-II protein, 12% SDS-PAGE—for pThr73-Rab10, CTSD, and p62 proteins). The separated proteins were transferred onto a polyvinylidene fluoride membrane (Millipore, MA, USA) using a Mini Gel Tank system (A25977, Invitrogen, Carlsbad, CA, USA). Blots used to detect the proteins of interest were blocked with 5% milk in tris-buffered saline with 0.1% Tween-20. Subsequently, the membranes were incubated with primary antibodies overnight at 4°C (Table 2). After washing, the membranes were incubated with anti-rabbit secondary antibodies (Abcam, UK) for 1 h at room temperature (Table 2). Chemiluminescence signals were assessed using Clarity™ Western ECL Substrate (#1705060, BioRad, Hercules, CA, USA), and the protein bands were imaged using a ChemiDoc MP Imaging System (BioRad,

Hercules, CA, USA). Quantification of the protein bands was performed using ImageJ software [29]. Normalization was carried out using the GAPDH protein signal.

Table 2: Antibodies used for Western blot analysis.

Antigen	Host Species	Dilution	Source
SQSTM1 (p62)	Rabbit polyclonal	1:1000	Cloud Clone Corp. (PAD198Hu01)
LC3B	Rabbit monoclonal	1:1000	ABclonal (A19665)
CTSD	Rabbit polyclonal	1:1000	Cloud Clone Corp. (PAB280Hu01)
GAPDH	Rabbit monoclonal	1:15,000	ABclonal (AC036)
pThr73-Rab10	Rabbit monoclonal	1:1000	Abcam (ab230261)
Goat anti-rabbit HRP conjugate	Goat polyclonal	1:5000	Abcam (ab6721)

2.5 Statistical Analysis

Statistical data processing was conducted using pre-installed R packages (version 4.3.2) (https://cran.r-project.org/bin/windows/base/). Conformity of findings to normal distribution was tested using the Shapiro–Wilk test. Datasets were compared between the studied groups using the Wilcoxon test due to non-normally distributed data. Clinical characteristics of the study participants are expressed as mean ± standard deviation of the mean; experimental values are presented as median (min-max). Results were considered statistically significant at $p < 0.05$.

3 Results

3.1 Lysosomal Hydrolase Activities and Lysosphingolipid Concentrations in Peripheral Blood

In this longitudinal study, conducted with an expanded cohort of patients with iPD, DLB, and MSA, we demonstrated that all patients exhibit decreased GCase activity and increased HexSph concentration in whole blood compared to controls (GCase: $p = 0.0045$, $p = 0.015$, $p = 0.0029$; HexSph: $p = 0.048$, $p = 0.0056$, $p < 0.0001$, respectively) (Fig. 1a,e). In addition, patients with MSA were characterized by increased HexSph concentration in the whole blood compared to iPD ($p = 0.00058$).

ASMase activity was markedly decreased in the blood of MSA patients compared to controls, iPD, and DLB ($p < 0.0001$, $p < 0.0001$, $p = 0.015$, respectively) and in DLB patients compared only to controls ($p = 0.043$) (Fig. 1c). At the same time, GLA activity was elevated in MSA compared to controls, iPD, and DLB ($p = 0.021$, $p = 0.016$, $p = 0.032$, respectively) (Fig. 1b), whereas GALC activity was increased in MSA compared to DLB ($p = 0.034$) (Fig. 1d). Patients with MSA also demonstrated increased LysoGb3 levels, substrate of GLA, in blood compared to controls, iPD and DLB patients ($p = 0.000049$, $p = 0.034$, $p = 0.0092$, respectively), while patients with iPD exhibited elevated concentration of LysoGb3 and reduced concentration of LysoSM, substrate of ASMase, compared to controls ($p = 0.025$, $p = 0.004$, respectively), with no difference in GLA and ASMase activities compared to controls ($p > 0.05$) (Fig. 1f,g).

3.2 Lysosomal Hydrolase Activities and Lysosphingolipid Concentrations in PBMC-Derived Macrophages

To overcome the heterogeneity inherent to the whole blood and to assess lysosomal dysfunction at the level of a defined cell population, we next analyzed lysosomal hydrolase activities and lysosphingolipid concentrations in PBMC-derived macrophages. GCase and GALC activities were significantly lower in PBMC-derived macrophages from patients with MSA compared to iPD patients and controls (GCase: $p = 0.00086$, $p = 0.013$; GALC: $p = 0.0076$, $p = 0.0068$; respectively) (Fig. 2a,d). ASMase activity was decreased in MSA and iPD macrophages compared to controls ($p < 0.0001$, $p = 0.014$, respectively),

as well as in MSA compared to iPD ($p = 0.022$) (Fig. 2c). No significant differences in GLA activity were observed across the studied groups (Fig. 2b).

Elevated HexSph levels were observed in PBMC-derived macrophages from patients with iPD, DLB, and MSA compared to controls ($p < 0.0001$, $p = 0.044$, $p = 0.0028$, respectively) (Fig. 2e). Patients with iPD also were characterized by increased HexSph concentration in macrophages compared to MSA ($p = 0.022$) (Fig. 2e). Notably, LysoGb3 concentration was significantly increased in iPD compared to MSA, DLB and controls ($p = 0.0024$, $p = 0.02$, $p < 0.0001$, respectively) and also in MSA compared to controls ($p = 0.041$) (Fig. 2f). No significant differences in LysoSM concentration were observed between the studied groups (Fig. 2g).

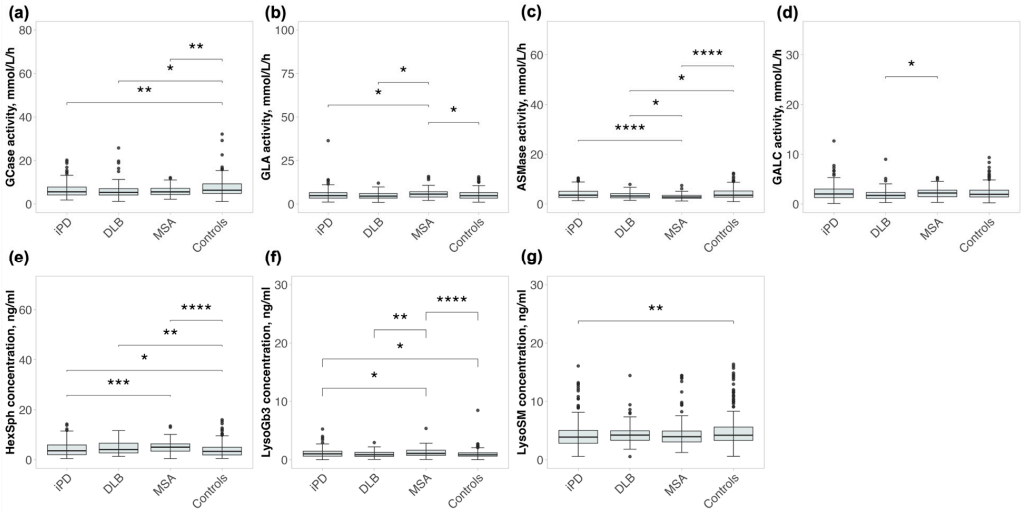

Figure 1: Activities of lysosomal hydrolases and lysosphingolipids concentration in blood from patients with synucleinopathies (iPD, DLB, MSA). (**a**) GCase activity, (**b**) GLA activity, (**c**) ASMase activity, (**d**) GALC activity, (**e**) HexSph concentration, (**f**) LysoGb3 concentration, (**g**) LysoSM concentration. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

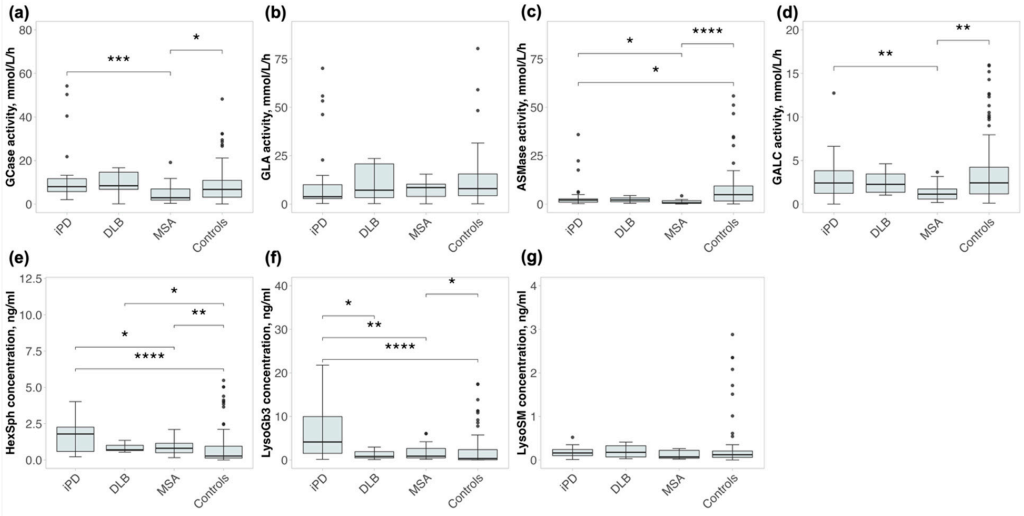

Figure 2: Activities of lysosomal hydrolases and lysosphingolipids concentration in PBMC-derived macrophages from patients with synucleinopathies (iPD, DLB, MSA). (**a**) GCase activity, (**b**) GLA activity, (**c**) ASMase activity, (**d**) GALC activity, (**e**) HexSph concentration, (**f**) LysoGb3 concentration, (**g**) LysoSM concentration. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

3.3 Lysosomal Alterations in PBMC-Derived Macrophages

In the present study, we investigate the effects of LRRK2 inhibition using the selective inhibitor MLi-2 on the activities of lysosomal hydrolases. Interestingly, inhibition of LRRK2 kinase activity by MLi-2 did not affect GCase, GLA, ASMase, or GALC activities, nor HexSph, LysoGb3, or LysoSM concentrations ($p > 0.05$) (Fig. 3).

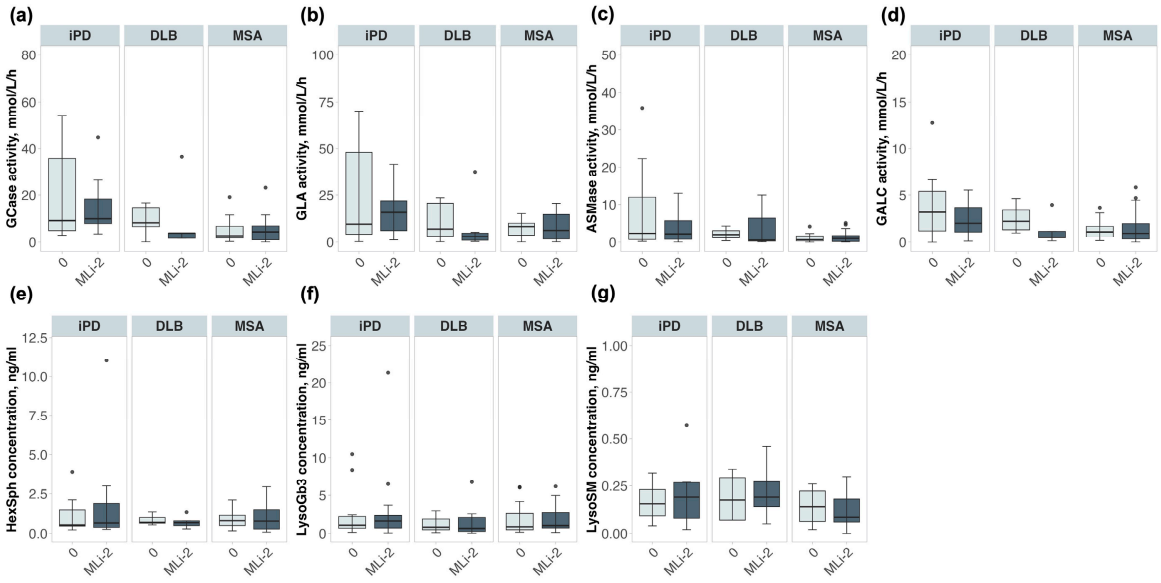

Figure 3: Activities of lysosomal hydrolases and lysosphingolipids concentration in MLi-2-treated PBMC-derived macrophages from patients with synucleinopathies (iPD, DLB, MSA). (**a**) GCase activity, (**b**) GLA activity, (**c**) ASMase activity, (**d**) GALC activity, (**e**) HexSph concentration, (**f**) LysoGb3 concentration, (**g**) LysoSM concentration.

Autophagy is a highly conserved and complex cellular process essential for the degradation and recycling of cellular components. LC3 and p62 are frequently used markers to assess autophagy, as they are integral to autophagosome formation and cargo delivery [30]. In the current study, we assessed levels of p62 and LC3B-II, as well as two forms of lysosomal protease CTSD, one of the major lysosomal proteases responsible for the degradation of proteins within lysosomes (the precursor (pro-CTSD) and the double-chain mature form of CTSD) [31].

Patients with iPD and MSA showed decreased relative level of p62 protein in macrophages compared to controls. This decrease was previously reported for iPD patients [12] ($p = 0.00041$) and is now demonstrated for the first time in MSA ($p = 0.037$) (Fig. 4a). Similarly, LC3B-II levels in PBMC-derived macrophages were previously shown to be elevated in iPD [12] and are now reported for iPD compared to MSA ($p = 0.027$) (Fig. 4b).

We found that PBMC-derived macrophages from patients with DLB were characterized by increased relative levels of pro-CTSD compared to controls ($p = 0.0037$) and mature CTSD protein compared to iPD patients and controls ($p = 0.0096$, $p = 0.025$, respectively) (Fig. 4c,d). In contrast, PBMC-derived macrophages from patients with MSA were characterized by decreased relative level of mature CTSD protein compared to controls ($p = 0.0054$). iPD patients did not differ from controls (Fig. 4d). iPD patients exhibited decreased relative levels of pro-CTSD, as was shown by us earlier [12].

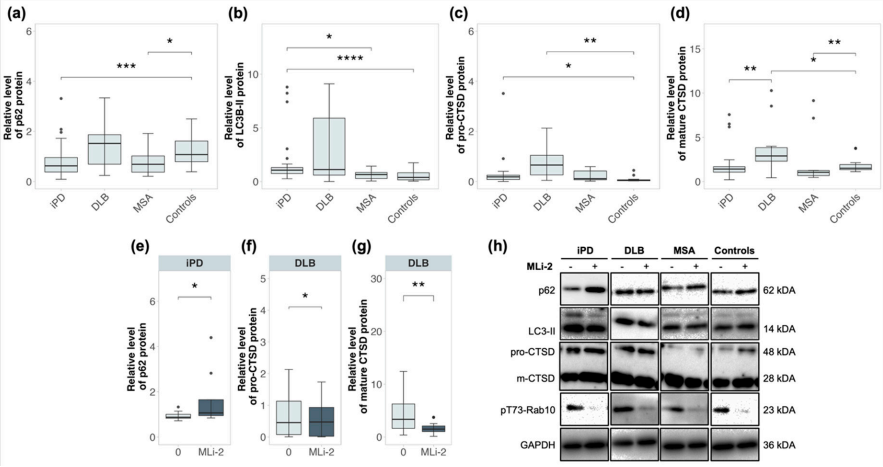

Figure 4: Levels of p62, LC3B-II, pro- and mature CTSD proteins in treated and untreated with MLi-2 PBMC-derived macrophages from patients with synucleinopathies (iPD, DLB, MSA) and controls (**a**) Relative level of p62 protein, (**b**) Relative level of LC3B-II protein, (**c**) Relative level of pro-CTSD protein, (**d**) Relative level of mature CTSD protein, (**e**) Effect of LRRK2 inhibition by MLi-2 on relative level of p62 protein in PBMC-derived macrophages of iPD patients, (**f**) Effect of LRRK2 inhibition by MLi-2 on relative level of pro-CTSD protein in PBMC-derived macrophages of DLB patients, (**g**) Effect of LRRK2 inhibition by MLi-2 on relative level of mature CTSD protein in PBMC-derived macrophages of DLB patients, (**h**) Western-blot analysis for all studied proteins. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$.

To assess the efficacy of LRRK2 kinase inhibitor MLi-2, we assessed the level of phosphorylated LRRK2 substrate, pThr73-Rab10. Consistent with expectations, MLi-2 treatment effectively reduced LRRK2 activity in PBMC-derived macrophages (Fig. 4h) [18,21,32].

MLi-2 treatment elevated levels of p62 protein in PBMC-derived macrophages from patients with iPD ($p = 0.031$) (Fig. 4e,h). At the same time, inhibition of LRRK2 kinase activity by MLi-2 led to a decrease in relative levels of pro-CTSD and mature CTSD in PBMC-derived macrophages from patients with DLB ($p = 0.012$, $p = 0.0049$, respectively) (Fig. 4f–h).

4 Discussion

In the present study, we expanded our previous blood-based analysis of lysosomal hydrolase activities in synucleinopathies (iPD, DLB, MSA) by increasing cohort sizes and incorporating PBMC-derived macrophage analyses to better capture disease-specific lysosomal dysfunction. As well, we evaluated the effects of pharmacological inhibition of LRRK2 with MLi-2 on lysosomal function in synucleinopathies, focusing on lysosomal hydrolase activities (GCase, GALC, ASMase, and GLA), lysosphingolipid concentrations (HexSph, LysoGb3, and LysoSM), and major protein markers of autophagy. This approach was motivated by our previous findings demonstrating that the selective LRRK2 inhibitor MLi-2 modulates lysosomal hydrolase activities in inherited forms of PD, including LRRK2-PD and GBA1-PD [18].

Here, we evaluated lysosomal hydrolase activities, including GCase, GLA, GALC, and ASMase, whose encoding genes (*GBA1*, *GLA*, *GALC* and *SMPD1*, respectively) have been implicated in PD risk [8,33,34]. Functional impairment of these enzymes may contribute to the accumulation of bioactive lipid substrates, promoting alpha-synuclein aggregation [6,35]. Notably, *GBA1* variants are well-established genetic risk factors for PD and DLB, highlighting shared lysosomal vulnerabilities across synucleinopathies [34,36]. In contrast, the genetic contribution of lysosomal storage disorder–related genes to MSA appears to be less pronounced [37]. Nevertheless, converging evidence from biochemical, cellular, and lipidomic studies

indicates that dysregulation of lipid and sphingolipid metabolism is a shared pathological feature across synucleinopathies, including sporadic PD, DLB, and MSA [38–40].

In the current longitudinal study involving an extended cohort, all patients with synucleinopathies exhibited reduced GCase activity compared to healthy donors. HexSph concentrations, which were previously increased in DLB and MSA but not in iPD [16], were also significantly elevated in iPD patients in the expanded cohort. Similarly, LysoGb3 levels were increased in both iPD and MSA, whereas no significant changes were detected in our earlier analysis [16]. The results on GCase activity are contradictory, which may be due to differences in measurement methods, cohort size and composition, disease stage, and variability in patient populations across studies of synucleinopathies. Post-mortem studies of the substantia nigra report decreased GCase activity in PD, with a similar trend in DLB [13,41], suggesting tissue-specific regulation.

In addition, we identified a pronounced reduction of ASMase activity in MSA compared to controls, iPD, and DLB, consistent with our previous observations [17]. We also report novel findings that MSA patients were characterized by increased GLA activity in the whole blood compared to all studied groups.

These discrepancies compared to our previous study are most likely attributable to the substantially increased cohort size and the exclusion of younger patients aged ≤45 years [17].

As the whole blood represents a heterogeneous mixture of cell types, which may mask disease-related alterations occurring in specific immune or metabolic compartments [42]. To overcome this limitation, we analyzed lysosomal enzyme activities and lysosphingolipid concentrations in PBMC-derived macrophages, a defined cell population with a central role in cellular clearance, lipid metabolism, and immune responses. Macrophages have previously been shown to recapitulate biochemical abnormalities observed in the blood and brain in PD, including GBA1-PD and LRRK2-PD, and to serve as a valuable model for mechanistic studies and therapeutic screening [17,18,43].

The most pronounced alterations in lysosomal hydrolase activities were observed in PBMC-derived macrophages from MSA patients, who showed reduced GCase, GALC, and ASMase activities. Consistent with blood-based results, HexSph concentrations were significantly increased in PBMC-derived macrophages from patients with iPD, DLB, and MSA. These findings are in agreement with recent plasma lipidomics studies demonstrating increased monohexylceramides and decreased sphingosine-1-phosphate levels across multiple neurodegenerative diseases, including iPD, DLB, and MSA, highlighting a common disturbance of ceramide-derived sphingolipid metabolism in neurodegeneration [44]. Additionally, earlier we observed HexSph accumulation in PBMC-derived macrophages of patients with GBA1-PD and LRRK2-PD [18,43].

Previously, we have shown that LRRK2 inhibition enhances GCase trafficking to lysosomes in patient-specific macrophages from individuals with GBA1- and LRRK2-associated PD, consistent with observations in iPSC-derived dopaminergic neurons, highlighting the suitability of macrophages as a model for investigating alterations in lysosomal homeostasis in PD [18] and possibly in other synucleinopathies. Moreover, LRRK2 has previously been shown to be recruited to lysosomes of specialized cell types, including macrophages, which allows the use of macrophages as a relevant model assessing the consequences of LRRK2 inhibition [45,46].

Therefore, we evaluated the effects of the selective LRRK2 inhibitor, MLi-2, on the activities of lysosomal hydrolases in PBMC-derived macrophages from patients with synucleinopathies. LRRK2 inhibition with MLi-2 did not significantly alter the activity of lysosomal hydrolases or lysosphingolipid concentrations in PBMC-derived macrophages across all patient groups with synucleinopathies. These results suggest that while LRRK2 can modulate lysosomal enzyme trafficking in certain forms of PD, associated with mutations in genes involved in the maintenance of lysosomes [18,21]. Its impact may be limited in iPD and other synucleinopathies due to disease-specific alterations. Notably, a similarly limited effect has been reported

in the context of biallelic *GBA1* mutations, where modulation of LRRK2 activity did not significantly rescue GCase activity [47]. Apparently, in GBA1- and LRRK2-associated PD, dysregulation of lysosomal enzyme trafficking regulated by LRRK2 may contribute more substantially to the underlying pathology, whereas in iPD, DLB, and MSA, similar alterations are potentially influenced by mechanisms that may be independent of LRRK2.

Given the emerging role of autophagy impairment in synucleinopathies, we estimated levels of p62 and LC3B-II, which are widely used markers of autophagy [30]. We confirmed our previous findings that patients with iPD exhibit decreased p62 and increased LC3B-II levels in PBMC-derived macrophages [12]. In DLB, p62 and LC3B-II levels remain unchanged, consistent with normal autophagosome formation. MSA patients also showed decreased levels of p62 but, in contrast to iPD, displayed reduced LC3B-II levels in macrophages. Previous studies have shown accumulation of LC3B-II in PD in PBMC and postmortem brain samples [48,49]. Interestingly, post-mortem studies of brain samples from MSA patients, as well as studies using iPSC-derived neurons from MSA patients and transgenic MSA mouse models overexpressing human alpha-synuclein under the oligodendroglial proteolipid protein promoter (PLP), reported increased LC3 levels [10,50,51], indicating that autophagy alterations in MSA may be cell type-dependent. The reduced p62 in MSA macrophages may also reflect alternative degradation mechanisms, such as increased proteasomal activity or impaired p62 synthesis, potentially compromising the clearance of ubiquitinated proteins. These findings align with reports that alpha-synuclein fibrils may directly bind LC3B and inhibit p62-mediated selective autophagy, providing a potential mechanism for impaired substrate clearance and alpha-synuclein accumulation [52].

CTSD is considered to be the major lysosomal protease involved in alpha-synuclein degradation [53]. CTSD is synthesized as an inactive precursor (pro-CTSD), which undergoes proteolytic maturation within the acidic lysosomal compartment to generate the catalytically active mature enzyme responsible for protein degradation, including alpha-synuclein. Here, as was shown in our previous study, macrophages from iPD patients were characterized by increased pro-CTSD levels and no differences in levels of mature CTSD [12]. Surprisingly, DLB patients demonstrated elevated levels of pro-CTSD levels compared to controls and mature CTSD levels compared to iPD patients and controls in PBMC-derived macrophages, whereas macrophages from MSA patients were characterized by decreased levels of mature CTSD compared to controls. No significant differences were obtained when comparing the relative levels of pro-CTSD among all studied groups. Studies on post-mortem brain samples of PD and DLB patients report a decrease in CTSD activity in the frontal cortex, with more pronounced activity in DLB [13]. Interestingly, several studies have associated higher CTSD levels with dementia-related pathology. Elevated CTSD levels have been found in the post-mortem neocortex of DLB and Alzheimer's disease (AD) patients [54]. Additionally, higher serum CTSD concentration has been linked to AD diagnosis, cortical atrophy, and global cognitive and functional decline [55]. While increased CTSD may represent a compensatory response to protein aggregation, it could also reflect activation of peripheral immune and systemic inflammation, both of which may contribute to neurodegeneration [56].

In PBMC-derived macrophages from iPD patients, MLi-2 treatment increased p62 while LC3B-II remained unchanged, consistent with our and others' previous findings [18,57]. LRRK2 inhibition also did not affect levels of LC3B-II and p62 in macrophages from DLB and MSA. Notably, LRRK2 phosphorylates p62 on Thr138 [58]. Although p62 is a substrate of LRRK2, MLi-2 increased its levels only in iPD, possibly because LRRK2-dependent regulation of p62 is more pronounced in iPD and may be masked or overridden by different autophagic changes in DLB and MSA.

In macrophages from iPD and MSA patients, MLi-2 failed to modify pro- or mature CTSD levels. Remarkably, inhibition of LRRK2 reduced both pro- and mature CTSD levels in PBMC-derived macrophages from DLB patients. As it was mentioned earlier, previously, plasma CTSD levels were positively associated with the proinflammatory cytokines [56]. Speculatively, the observed effect may be linked to the previously demonstrated anti-inflammatory effects of LRRK2 kinase inhibition; however, further research is needed [59]. Nevertheless, further studies are needed to clarify the relationship between CTSD levels, enzymatic activity, and systemic inflammation in DLB.

The severe and widespread lysosomal abnormalities observed in MSA suggest that targeting a single molecular pathway may be insufficient for this patient cohort. Indeed, treatments that are beneficial in PD, such as mTOR inhibition, have failed to translate to MSA [60]. Small pharmacological molecules that enhance lysosomal function, such as ambroxol, have shown promise in iPD and DLB by increasing glucocerebrosidase activity and improving lysosomal clearance [61]. However, to date, there are no clinical trials evaluating ambroxol in MSA, highlighting the lack of effective therapeutic options for this disease. These observations emphasize the necessity for integrated therapeutic strategies that simultaneously target multiple components of the autophagy–lysosomal pathway. An integrated summary of these findings in PBMC-derived macrophages is presented in Fig. 5.

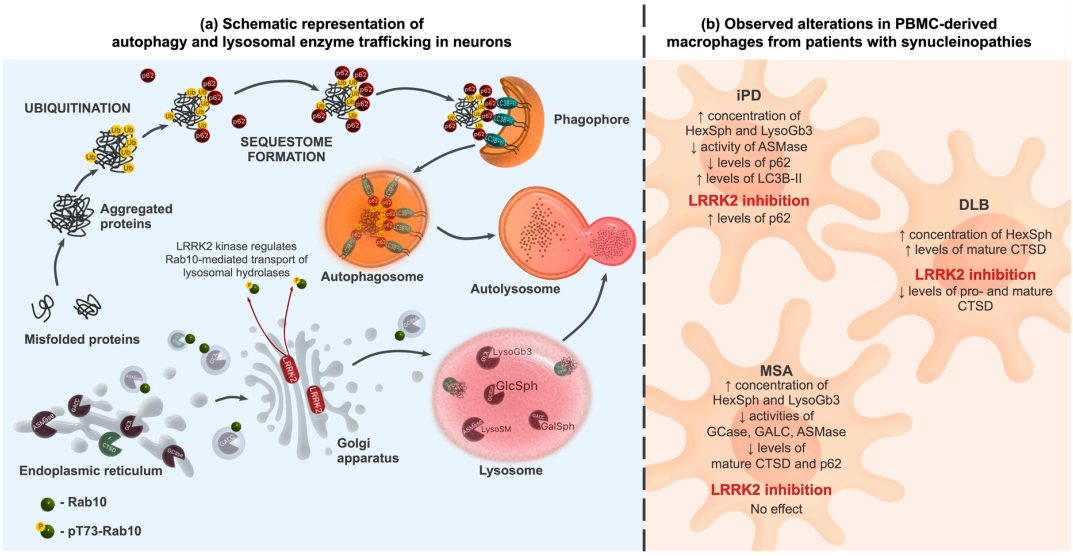

Figure 5: Possible molecular mechanism of lysosomal dysfunction in synucleinopathies. (**a**) Schematic representation showing how misfolded and aggregated proteins are processed via the autophagy–lysosomal pathway in neurons. LRRK2 kinase regulates the transport of lysosomal hydrolases from the Golgi apparatus to the lysosomes through Rab10 (shown as green balls). (**b**) Observed alterations in PBMC-derived macrophages from patients with iPD, DLB, and MSA. Patients with iPD exhibit decreased p62 levels and elevated LC3B-II levels. In DLB, elevated levels of mature CTSD are observed. MSA is characterized by decreased levels of mature CTSD and p62. These lysosomal alterations appear to be disease-specific, indicating distinct molecular pathways underlying lysosomal dysfunction in different synucleinopathies. Inhibition of LRRK2 kinase activity affects lysosomal parameters in a disease-dependent manner: increases p62 levels in iPD, decreases pro- and mature CTSD levels in DLB, and shows no effect in MSA, indicating that LRRK2-dependent mechanisms contribute differently to lysosomal impairment across synucleinopathies. Created in Inkscape, version 1.3.2.

Several limitations of the present study should be acknowledged. First, the relatively small sample sizes in part of the study on PBMC-derived macrophages of patients from all studied groups may limit

the generalizability of the findings and the statistical power to detect subtle effects. Second, we did not use bafilomycin A1 or chloroquine to block autophagosome degradation and assess autophagic flux, which limits our ability to determine whether the observed changes in LC3B-II levels reflect increased autophagosome formation or impaired degradation. In our study, all experiments were conducted under non-stimulating conditions. Previous work has shown that stimulating cells with interferon gamma, which strongly induces expression of the LRRK2 protein, led to an increase in cathepsin activity and had monocyte subset-dependent effects on lysosomal GCase activity [62]. In macrophage lineage cells, lysosomal stress induced by chloroquine enhances recruitment of LRRK2 and Rab10 to damaged lysosomes and drives release of α synuclein aggregates via the LRRK2–Rab10 pathway, indicating that disease relevant stimuli can modify LRRK2-dependent processes in immune cells [63]. Taken together, these observations suggest that experiments under stimulating conditions may reveal additional disease-specific effects of LRRK2 activity on autophagy and lysosomal pathways, and this point warrants further investigation. And last, while PBMC-derived macrophages provide an accessible and relevant model to study lysosomal and autophagic dysfunction, they do not exactly match the brain environment. Therefore, further studies in neuronal and glial cell models, as well as in relevant animal models, are needed to validate our findings.

5 Conclusions

Our study highlights distinct lysosomal and autophagic alterations in synucleinopathies despite shared alpha-synuclein pathology. HexSph levels were consistently elevated across all synucleinopathies in both the whole blood and PBMC-derived macrophages, suggesting a common lipid dysregulation signature independent of clinical phenotype. Notably, MSA exhibited the most extensive impairment of lysosomal hydrolase activities.

At the protein level, MSA showed reduced levels of p62 and mature CTSD, iPD demonstrated alteration in levels of lysosomal markers, p62 and LC3B-II, and DLB was characterized by elevated levels of mature CTSD in PBMC-derived macrophages. Importantly, pharmacological inhibition of LRRK2 with the selective inhibitor MLi-2 did not affect lysosomal hydrolase activities or lysosphingolipid concentrations across synucleinopathies, suggesting that LRRK2-dependent pathways are not major drivers of lysosomal dysfunction in sporadic disease forms. However, pharmacological inhibition of LRRK2 with MLi-2 modulated autophagy- and lysosome-related protein levels in a disease-specific manner, increasing p62 levels in iPD, reducing pro- and mature CTSD levels in DLB, and having minimal effect in MSA, proposing that LRRK2-dependent mechanisms play a limited role in lysosomal dysfunction in MSA.

Overall, our results highlight the molecular heterogeneity of synucleinopathies and suggest that peripheral lysosomal signatures may be useful for disease stratification. Combined enzymatic and protein-level alterations may allow partial discrimination between iPD, DLB, and MSA, with MSA showing the most pronounced multisystem lysosomal dysfunction. In addition, the limited effects of LRRK2 inhibition suggest a minor role of LRRK2-dependent mechanisms in sporadic synucleinopathies, supporting the need for disease-specific therapeutic approaches.

Acknowledgement: The research was performed using the facilities and equipment of the “Metabolome” Shared Resource Centre at the Research Centre for Medical Genetics (Moscow, Russia).

Funding Statement: This research was funded by the Russian Science Foundation Grant No. 24-15-00177.

Author Contributions: The authors confirm contribution to the paper as follows: conceptualization, Tatiana Usenko and Sofya Pchelina; investigation, Katerina Basharova, Anastasia Bezrukova, Alena Kopytova, Anna Lavrinova, Galina Baydakova, Irina Miliukhina, Ekaterina Zakharova, Anton Emelyanov and Tatiana Usenko; formal analysis,

Katerina Basharova, Anastasia Bezrukova, Anna Lavrinova and Tatiana Usenko; visualization, Katerina Basharova and Anastasia Bezrukova; writing—original draft preparation, Katerina Basharova and Tatiana Usenko; writing—review and editing, Ekaterina Zakharova, Anton Emelyanov, Sofya Pchelina and Tatiana Usenko; supervision, Tatiana Usenko and Sofya Pchelina; funding acquisition, Sofya Pchelina. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials: The authors confirm that the data supporting the findings of this study are available within the article.

Ethics Approval: The study was conducted according to the guidelines of the Declaration of Helsinki. The biomaterial was collected at the Pavlov First Saint Petersburg State Medical University with the permission of the ethics committee (Protocol number: 307, Approval Date: 20 October 2025). Written informed consent or assent was obtained from patients to collect samples. All research is conducted anonymously.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Coon EA, Singer W. Synucleinopathies. Continuum. 2020;26(1):72–92. [CrossRef].
2. Schneider SA, Alcalay RN. Neuropathology of genetic synucleinopathies with Parkinsonism: review of the literature. Mov Disord. 2017;32(11):1504–23. [CrossRef].
3. Brás IC, Dominguez-Meijide A, Gerhardt E, Koss D, Lázaro DF, Santos PI, et al. Synucleinopathies: where we are and where we need to go. J Neurochem. 2020;153(4):433–54. [CrossRef].
4. Paciotti S, Gatticchi L, Beccari T, Parnetti L. Lysosomal enzyme activities as possible CSF biomarkers of synucleinopathies. Clin Chim Acta. 2019;495:13–24. [CrossRef].
5. Flores-León M, Outeiro TF. Expanding our understanding of synucleinopathies: proteinopathy, proteinopenia, and lipidopathy. FEBS J. 2025;292(18):4774–88. [CrossRef].
6. Kiechle M, Grozdanov V, Danzer KM. The role of lipids in the initiation of α-synuclein misfolding. Front Cell Dev Biol. 2020;8:562241. [CrossRef].
7. Peng YF, Chen SJ, Li JL, Lin CH, Kuo CH. Dysregulated metabolism of ceramides and glycosphingolipids in Parkinson's disease. J Lipid Res. 2026;67(1):100955. [CrossRef].
8. Robak LA, Jansen IE, van Rooij J, Uitterlinden AG, Kraaij R, Jankovic J, et al. Excessive burden of lysosomal storage disorder gene variants in Parkinson's disease. Brain. 2017;140(12):3191–203. [CrossRef].
9. Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, et al. Identification of novel risk loci, causal insights, and heritable risk for Parkinson's disease: a meta-analysis of genome-wide association studies. Lancet Neurol. 2019;18(12):1091–102. [CrossRef].
10. Tanji K, Odagiri S, Maruyama A, Mori F, Kakita A, Takahashi H, et al. Alteration of autophagosomal proteins in the brain of multiple system atrophy. Neurobiol Dis. 2013;49:190–8. [CrossRef].
11. Higashi S, Moore DJ, Minegishi M, Kasanuki K, Fujishiro H, Kabuta T, et al. Localization of MAP1-LC3 in vulnerable neurons and lewy bodies in brains of patients with dementia with lewy bodies. J Neuropathol Exp Neurol. 2011;70(4):264–80. [CrossRef].
12. Bezrukova AI, Basharova KS, Emelyanov AK, Rybakov AV, Miliukhina IV, Pchelina SN, et al. Autophagy process in Parkinson's disease depends on mutations in the GBA1 and LRRK2 genes. Biochem Genet. 2026;64(2):2272–89. [CrossRef].
13. Moors TE, Paciotti S, Ingrassia A, Quadri M, Breedveld G, Tasegian A, et al. Characterization of brain lysosomal activities in GBA-related and sporadic Parkinson's disease and dementia with lewy bodies. Mol Neurobiol. 2019;56(2):1344–55. [CrossRef].
14. Hällqvist J, Toomey CE, Pinto R, Baldwin T, Doykov I, Wernick A, et al. Multi-omic analysis reveals lipid dysregulation associated with mitochondrial dysfunction in Parkinson's disease brain. Nat Commun. 2025;16:10490. [CrossRef].
15. Huebecker M, Moloney EB, van der Spoel AC, Priestman DA, Isacson O, Hallett PJ, et al. Reduced sphingolipid hydrolase activities, substrate accumulation and ganglioside decline in Parkinson's disease. Mol Neurodegener. 2019;14(1):40. [CrossRef].

16. Usenko TS, Senkevich KA, Bezrukova AI, Baydakova GV, Basharova KS, Zhuravlev AS, et al. Impaired sphingolipid hydrolase activities in dementia with lewy bodies and multiple system atrophy. Mol Neurobiol. 2022;59(4):2277–87. [CrossRef].
17. Aflaki E, Stubblefield BK, Maniwang E, Lopez G, Moaven N, Goldin E, et al. Macrophage models of Gaucher disease for evaluating disease pathogenesis and candidate drugs. Sci Transl Med. 2014;6(240):240ra73. [CrossRef].
18. Usenko TS, Basharova KS, Bezrukova AI, Grigor'eva EV, Pavlova SV, Baydakova GV, et al. Restoration of lysosomal hydrolase activities by LRRK2 inhibition in GBA1- and LRRK2-associated Parkinson's disease patient-derived cells. J Neurochem. 2025;169(9):e70214. [CrossRef].
19. Nikolaev MA, Kopytova AE, Baidakova GV, Emel'yanov AK, Salogub GN, Senkevich KA, et al. Human peripheral blood macrophages as a model for studying glucocerebrosidase dysfunction. Cell Tissue Biol. 2019;13(2):100–6. [CrossRef].
20. Kedariti M, Frattini E, Baden P, Cogo S, Civiero L, Ziviani E, et al. LRRK2 kinase activity regulates GCase level and enzymatic activity differently depending on cell type in Parkinson's disease. npj Park Dis. 2022;8:92. [CrossRef].
21. Ysselstein D, Nguyen M, Young TJ, Severino A, Schwake M, Merchant K, et al. LRRK2 kinase activity regulates lysosomal glucocerebrosidase in neurons derived from Parkinson's disease patients. Nat Commun. 2019;10(1):5570. [CrossRef].
22. Sanyal A, Novis HS, Gasser E, Lin S, LaVoie MJ. LRRK2 kinase inhibition rescues deficits in lysosome function due to heterozygous GBA1 expression in human iPSC-derived neurons. Front Neurosci. 2020;14:442. [CrossRef].
23. Albanese F, Mercatelli D, Finetti L, Lamonaca G, Pizzi S, Shimshek DR, et al. Constitutive silencing of LRRK2 kinase activity leads to early glucocerebrosidase deregulation and late impairment of autophagy *in vivo*. Neurobiol Dis. 2021;159:105487. [CrossRef].
24. Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, et al. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 2015;30(12):1591–601. [CrossRef].
25. Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, et al. Second consensus statement on the diagnosis of multiple system atrophy. Neurology. 2008;71(9):670–6. [CrossRef].
26. McKeith IG, Boeve BF, Dickson DW, Halliday G, Taylor JP, Weintraub D, et al. Diagnosis and management of dementia with Lewy bodies: fourth consensus report of the DLB Consortium. Neurology. 2017;89(1):88–100. [CrossRef].
27. Aharon-Peretz J, Badarny S, Rosenbaum H, Gershoni-Baruch R. Mutations in the glucocerebrosidase gene and Parkinson disease: phenotype–genotype correlation. Neurology. 2005;65(9):1460–1. [CrossRef].
28. Hashad DI, Abou-Zeid AA, Achmawy GA, Allah HMOS, Saad MA. G2019S mutation of the leucine-rich repeat kinase 2 gene in a cohort of Egyptian patients with Parkinson's disease. Genet Test Mol Biomark. 2011;15(12):861–6. [CrossRef].
29. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Meth. 2012;9(7):671–5. [CrossRef].
30. Klionsky DJ, Abdelmohsen K, Abe A, Abedin MJ, Abeliovich H, Acevedo Arozena A, et al. Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). Autophagy. 2016;12(1):1–222. [CrossRef].
31. Mijanovic O, Petushkova AI, Brankovic A, Turk B, Solovieva AB, Nikitkina AI, et al. Cathepsin D-managing the delicate balance. Pharmaceutics. 2021;13(6):837. [CrossRef].
32. Steger M, Diez F, Dhekne HS, Lis P, Nirujogi RS, Karayel O, et al. Systematic proteomic analysis of LRRK2-mediated Rab GTPase phosphorylation establishes a connection to ciliogenesis. elife. 2017;6:e31012. [CrossRef].
33. Gan-Or Z, Orr-Urtreger A, Alcalay RN, Bressman S, Giladi N, Rouleau GA. The emerging role of SMPD1 mutations in Parkinson's disease: implications for future studies. Park Relat Disord. 2015;21(10):1294–5. [CrossRef].
34. Emelyanov AK, Usenko TS, Tesson C, Senkevich KA, Nikolaev MA, Miliukhina IV, et al. Mutation analysis of Parkinson's disease genes in a Russian data set. Neurobiol Aging. 2018;71:267.e7–10. [CrossRef].
35. Galvagnion C. The role of lipids interacting with α-synuclein in the pathogenesis of Parkinson's disease. J Parkinsons Dis. 2017;7(3):433–50. [CrossRef].
36. Nalls MA, Duran R, Lopez G, Kurzawa-Akanbi M, McKeith IG, Chinnery PF, et al. A multicenter study of glucocerebrosidase mutations in dementia with Lewy bodies. JAMA Neurol. 2013;70(6):727–35. [CrossRef].
37. Pihlstrøm L, Schottlaender L, Chelban V, Meissner WG, Federoff M, Singleton A, et al. Lysosomal storage disorder gene variants in multiple system atrophy. Brain. 2018;141(7):e53. [CrossRef].

38. Don AS, Hsiao JT, Bleasel JM, Couttas TA, Halliday GM, Kim WS. Altered lipid levels provide evidence for myelin dysfunction in multiple system atrophy. Acta Neuropathol Commun. 2014;2(1):150. [CrossRef].
39. Beger AW, Dudzik B, Woltjer RL, Wood PL. Human brain lipidomics: pilot analysis of the basal ganglia sphingolipidome in Parkinson's disease and lewy body disease. Metabolites. 2022;12(2):187. [CrossRef].
40. Bleasel JM, Wong JH, Halliday GM, Kim WS. Lipid dysfunction and pathogenesis of multiple system atrophy. Acta Neuropathol Commun. 2014;2(1):15. [CrossRef].
41. Gegg ME, Burke D, Heales SJR, Cooper JM, Hardy J, Wood NW, et al. Glucocerebrosidase deficiency in substantia nigra of Parkinson disease brains. Ann Neurol. 2012;72(3):455–63. [CrossRef].
42. Ysselstein D, Young TJ, Nguyen M, Padmanabhan S, Hirst WD, Dzamko N, et al. Evaluation of strategies for measuring lysosomal glucocerebrosidase activity. Mov Disord. 2021;36(12):2719–30. [CrossRef].
43. Kopytova AE, Rychkov GN, Nikolaev MA, Baydakova GV, Cheblokov AA, Senkevich KA, et al. Ambroxol increases glucocerebrosidase (GCase) activity and restores GCase translocation in primary patient-derived macrophages in Gaucher disease and Parkinsonism. Park Relat Disord. 2021;84:112–21. [CrossRef].
44. Oizumi H, Sugimura Y, Totsune T, Kawasaki I, Ohshiro S, Baba T, et al. Plasma sphingolipid abnormalities in neurodegenerative diseases. PLoS One. 2022;17(12):e0279315. [CrossRef].
45. Herbst S, Campbell P, Harvey J, Bernard EM, Papayannopoulos V, Wood NW, et al. LRRK2 activation controls the repair of damaged endomembranes in macrophages. EMBO J. 2020;39(18):e104494. [CrossRef].
46. Yadavalli N, Ferguson SM. LRRK2 suppresses lysosome degradative activity in macrophages and microglia through MiT-TFE transcription factor inhibition. Proc Natl Acad Sci U S A. 2023;120(31):e2303789120. [CrossRef].
47. Usenko TS. Evaluation of the effect of inhibition of LRRK2 kinase activity on glucocerebrosidase activity on patient-specific cells from patients with Gaucher disease. Biochemistry. 2025;90(1):107. [CrossRef].
48. Alvarez-Erviti L, Rodriguez-Oroz MC, Cooper JM, Caballero C, Ferrer I, Obeso JA, et al. Chaperone-mediated autophagy markers in Parkinson disease brains. Arch Neurol. 2010;67(12):1464–72. [CrossRef].
49. Wu G, Wang X, Feng X, Zhang A, Li J, Gu K, et al. Altered expression of autophagic genes in the peripheral leukocytes of patients with sporadic Parkinson's disease. Brain Res. 2011;1394:105–11. [CrossRef].
50. Monzio Compagnoni G, Kleiner G, Samarani M, Aureli M, Faustini G, Bellucci A, et al. Mitochondrial dysregulation and impaired autophagy in iPSC-derived dopaminergic neurons of multiple system atrophy. Stem Cell Reports. 2018;11(5):1185–98. [CrossRef].
51. Stefanova N, Kaufmann WA, Humpel C, Poewe W, Wenning GK. Systemic proteasome inhibition triggers neurodegeneration in a transgenic mouse model expressing human α-synuclein under oligodendrocyte promoter: implications for multiple system atrophy. Acta Neuropathol. 2012;124(1):51–65. [CrossRef].
52. Xu Q, Wang H, Yang R, Tao Y, Wang Z, Zhang S, et al. α-Synuclein amyloid fibril directly binds to LC3B and suppresses SQSTM1/p62-mediated selective autophagy. Cell Res. 2025;35(1):72–5. [CrossRef].
53. Qiao L, Hamamichi S, Caldwell KA, Caldwell GA, Yacoubian TA, Wilson S, et al. Lysosomal enzyme cathepsin D protects against alpha-synuclein aggregation and toxicity. Mol Brain. 2008;1(1):17. [CrossRef].
54. Chai YL, Chong JR, Weng J, Howlett D, Halsey A, Lee JH, et al. Lysosomal cathepsin D is upregulated in Alzheimer's disease neocortex and may be a marker for neurofibrillary degeneration. Brain Pathol. 2019;29(1):63–74. [CrossRef].
55. Chai YL, Liang NHP, Chong JR, Venketasubramanian N, Tan BY, Hilal S, et al. Serum cathepsin D is a potential biomarker for Alzheimer's disease dementia and cognitive decline. J Alzheimers Dis. 2023;91(3):989–98. [CrossRef].
56. Ding L, Goossens GH, Oligschlaeger Y, Houben T, Blaak EE, Shiri-Sverdlov R. Plasma cathepsin D activity is negatively associated with hepatic insulin sensitivity in overweight and obese humans. Diabetologia. 2020;63(2):374–84. [CrossRef].
57. Ruz C, Alcantud JL, Vives F, Arrebola F, Hardy J, Lewis PA, et al. Seventy-two-hour LRRK2 kinase activity inhibition increases lysosomal GBA expression in H4, a human neuroglioma cell line. Int J Mol Sci. 2022;23(13):6935. [CrossRef].
58. Kalogeropulou AF, Zhao J, Bolliger MF, Memou A, Narasimha S, Molitor TP, et al. P62/SQSTM1 is a novel leucine-rich repeat kinase 2 (LRRK2) substrate that enhances neuronal toxicity. Biochem J. 2018;475(7):1271–93. [CrossRef].

59. Mutti V, Carini G, Filippini A, Castrezzati S, Giugno L, Gennarelli M, et al. LRRK2 kinase inhibition attenuates neuroinflammation and cytotoxicity in animal models of Alzheimer's and Parkinson's disease-related neuroinflammation. Cells. 2023;12(13):1799. [CrossRef].
60. Palma JA, Martinez J, Millar Vernetti P, Ma T, Perez MA, Zhong J, et al. mTOR inhibition with sirolimus in multiple system atrophy: a randomized, double-blind, placebo-controlled futility trial and 1-year biomarker longitudinal analysis. Mov Disord. 2022;37(4):778–89. [CrossRef].
61. Dreier JE, Stevenson A, Carles E, Schott K, Michaels TCT, Galvagnion C. Ambroxol displaces α-synuclein from the membrane and inhibits the formation of early protein–lipid coaggregates. Chem Sci. 2026;17(1):354–63. [CrossRef].
62. Hughes LP, Wallings RL, Agin-Liebes J, Alcalay RN, Garrido A, Tansey MG, et al. Interferon gamma stimulates coordinated changes in LRRK2, GCase, and cathepsin activities in idiopathic and genetic Parkinson's disease monocytes. npj Park Dis. 2025;11:337. [CrossRef].
63. Abe T, Kuwahara T, Suenaga S, Sakurai M, Takatori S, Iwatsubo T. Lysosomal stress drives the release of pathogenic α-synuclein from macrophage lineage cells via the LRRK2-Rab10 pathway. iScience. 2024;27(2):108893. [CrossRef].