



**UNIVERSIDAD
DE GRANADA**



Universidad de Granada

Programa de Doctorado: Biomedicina

Centro de Investigaciones Biomédicas (CIBM)

Instituto de Neurociencias

Instituto de Investigación Biosanitaria (IBS)

Implicación de las enzimas lisosomales en la etiología de la enfermedad de Parkinson

Clara Ruz Illescas

Granada, 2022



UNIVERSIDAD
DE GRANADA



Involvement of lysosomal enzymes in the aetiology of Parkinson's disease

Implicación de las enzimas lisosomales en la etiología de la enfermedad de Parkinson

Tesis doctoral presentada por
Clara Ruz Illescas
Para optar al grado de
Doctora con Mención Internacional

Dirigida por los doctores
Francisco Vives Montero
Raquel Durán Ogalla
Departamento de Fisiología
Instituto de neurociencias
Universidad de Granada

Editor: Universidad de Granada. Tesis Doctorales
Autor: Clara Ruz Illescas
ISBN: 978-84-1117-785-6
URI: <https://hdl.handle.net/10481/81244>

La doctoranda/The doctoral candidate **Clara Ruz Illescas** y los directores de la tesis/and the thesis supervisors **Francisco Vives Montero** y **Raquel Durán Ogalla**:

Garantizamos que el trabajo ha sido realizado por la doctoranda bajo nuestra dirección y hasta donde nuestro conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores a ser citados, cuando se han utilizado sus resultados o publicaciones.

/

Guarantee, by signing this doctoral thesis, that the work has been done by the doctoral candidate under the direction of the thesis supervisors and, as far as our knowledge reaches, in the performance of the work, the rights of others authors to be cited (when their results or publications have been used) have been respected.

Lugar y fecha/ Place and date: Granada a 19 de Diciembre de 2022

Directores de la Tesis/Thesis supervisors:

Doctoranda/ Doctoral candidate:

Fdo.: Francisco Vives Montero

Fdo.: Clara Ruz Illescas

Fdo.: Raquel Durán Ogalla

A mis padres. Jamás podré agradecerlos tanto, esta tesis es una pequeña muestra de ello.

Para Jonathan y Martina. Empecé la tesis por voluntad propia y la acabo por una promesa contigo antes de que nos dejaras. Dónde quiera que estés, espero que estés orgulloso de mi trabajo. Martina, espero que algún día te sientas tan orgullosa de tus padres, como nosotros de tí.

ÍNDICE

FINANCIAL SOURCES	I
PUBLICATIONS	III
ABBREVIATIONS	VII
LIST OF FIGURES	XI
LIST OF TABLES	XIII
RESUMEN	XV
ABSTRACT	XVII
I. INTRODUCTION	1
Chapter 1. Introducing Parkinson's disease	3
1.1. Descriptive epidemiology: Prevalence and incidence of PD	5
1.2. Clinical aspects. Motor symptoms and non-motor symptoms.	7
1.3. Etiology	9
1.4. Pathological mechanisms in PD	11
1.5. Diagnosis and treatment	17
Chapter 2. Proteotoxicity in Parkinson Disease	20
2.1. α -Syn aggregation in PD: general aspects, structure and function	22
2.2. Pathologic mechanisms of α -Syn aggregation	23
Chapter 3. Autophagy: lysosomal degradation pathways and PD	27
3.1. Autophagy process and genetic alterations linked to PD	28
3.2. Implications of lysosomal dysfunction as a pathological mechanism of sporadic PD.	33
3.3. Cofactors or activating proteins of sphingolipids necessary for enzymatic hydrolysis: Saposins	36
II. HYPOTHESIS AND OBJECTIVES	41
III. ANALYSIS OF LYSOSOMAL ENZYME ACTIVITY IN PATIENTS WITH PD	45
3.1. Material and methods	47
3.1.1. Study Population and Blood Sample Collection	47
3.1.2. White Blood Cells Extraction	47
3.1.3. Enzymatic Assays	48

	49
3.1.4 Antibodies	50
3.1.5. Western Blot Analysis	50
3.1.6. Statistical Analysis	51
3.2. Results	52
3.2.1. Evaluation of the Lysosomal Enzymatic Activities	52
3.2.2. Determination of GBA, BGAL, HEXB, CTSD, and α -Syn Protein Levels	53
3.3. Discussion	61
VI. ANALYSIS OF THE INHIBITION OF LRRK2 KINASE ACTIVITY ON GCASE ACTIVITY IN HUMAN NEUROGLIOMA CELL LINES	65
4.1. Material and methods	67
4.1.1. Inhibitors	67
4.1.2. Antibodies	67
4.1.3. Cell culture and pharmacological treatments	67
4.1.4. β -Glucocerebrosidase enzymatic assay	69
4.1.5. Endo-H treatment	69
4.1.6. SDS-PAGE and western blot analysis	69
4.1.7. Statistical analyses	70
4.2. Results	71
4.3. Discussion	77
V. GENERAL DISCUSSION	81
VI. CONCLUSIONS	89
VII. ACKNOWLEDGMENTS/AGRADECIMIENTOS	93
VIII. REFERENCES	101

FINANCIAL SOURCES

This doctoral thesis has been carried out financed by the following sources:

- **FPU 14/03473:** Predoctoral fellowship for University Teacher Training (FPU) resolved on May 14, 2015, granted to doctoral student Clara Ruz Illescas. Motivated by a change of project, director and program approved by the doctoral commission and the Ministry of Education, this scholarship is developed in the Department of Physiology, Faculty of Medicine and Institute of Neurosciences Federico-Olóriz, of the University of Granada.
- **EST16/00809:** Research stay financed within the FPU program, this grant was directed to the analysis of the interaction between GBA and LRRK2 in the autophagy-lysosome pathway in a cell model of human neuroglioma. Centre: Institute of Neurology. Department: Molecular Neuroscience, University College London (London, UK). Duration: 3 months.

In addition, this thesis has been cofinancially supported by the following sources:

- Foundation “Progreso y Salud”, Junta de Andalucía (grant PI-0424-2014).
- Programa Operativo FEDER de Andalucía (B-CTS-702-UGR20).

PUBLICATIONS

The work derived from this doctoral thesis has been published in the following sources:

- **List of papers:**

Ruz C, Alcantud JL, Vives Montero F, Duran R, Bandres-Ciga S. Proteotoxicity and Neurodegenerative Diseases. *Int J Mol Sci.* 2020 Aug 6;21(16):5646. doi: 10.3390/ijms21165646. PMID: 32781742; PMCID: PMC7460676.

Ruz C, Alcantud JL, Vives F, Arrebola F, Hardy J, Lewis PA, Manzoni C, Duran R. Seventy-Two-Hour LRRK2 Kinase Activity Inhibition Increases Lysosomal GBA Expression in H4, a Human Neuroglioma Cell Line. *Int J Mol Sci.* 2022 Jun 22; 23(13):6935. doi: 10.3390/ijms23136935. PMID: 35805938; PMCID: PMC9266636.

Ruz C, Barrero FJ, Pelegrina J, Bandrés-Ciga S, Vives F, Duran R. Saposin C, Key Regulator in the Alpha-Synuclein Degradation Mediated by Lysosome. *Int J Mol Sci.* 2022 Oct 9; 23(19):12004. doi: 10.3390/ijms231912004. PMID: 36233303; PMCID: PMC9569857.

- **Book chapters:**

Bandrés-Ciga S, Durán R, Barrero FJ, **Ruz C**, Vives F. Estrategias metodológicas para la investigación en genética: Una aproximación al conocimiento de las enfermedades complejas. *Encuentros en Neurociencias*, 2017 Vol IV. pp. 53 - 68.

Ruz, C., Bandrés-Ciga, S., Barrero, F.J.; Pelegrina, J.; Gutiérrez, J.; Minguez, A.; Gallo, C.; Luna, M.; Vives, F.; Duran, R. Analysis of the b-glucocerebrosidase and b-galactosidase lysosomal enzymes activity in the pathogenesis of Parkinson disease. *Ars Pharmaceutica*, 2018 vol. 59 - 1, pp. 113 - 113. ISSN: 2340-9894

Ruz, C., Bandrés-Ciga, S., Barrero, F.J., Pelegrina, J.; Vives, F.; Durán, R. Alteraciones genéticas, autofagia y disfunción lisosomal en la enfermedad de Parkinson. Encuentros en Neurociencias, Ediciones Sider, 2018 vol. 5, pp. 65 - 81. ISBN: 978-84-943468-0-4

Ruz C., Bandrés-Ciga, S., Alcantud, J.L., Vives, F., Durán, R. Saposina C en la Enfermedad de Parkinson: estructura y funciones. Encuentros en Neurociencias, Editorial Universidad de Granada, 2021 Vol. 6, pp.151-160. ISBN978-84-338-6904-3

Alcantud, J. L, **Ruz, C.,** Pelegrina, J., Bandrés-Ciga, S., Barrero, F. J., Vives, F., Durán, R. Evaluación de la contribución genética a la enfermedad de Parkinson. Encuentros en Neurociencias, Editorial Universidad de Granada, 2021 Vol. 6, pp.127-150. ISBN 978-84-338-6904-3

- **Additional publications during this thesis:**

Bandrés-Ciga, S; **Ruz,C;** Barrero, F J; Escamilla-Sevilla, F; Pelegrina, J; Ramirez, M; Vives, F; Durán, R. Exploring the genetic architecture of Parkinson's disease in a Southern Spanish population. Exploring the genetic architecture of Parkinson's disease in a Southern Spanish population. Journal of Alzheimer's Disease & Parkinsonism, 2017 7 - 4, pp. 1 - 2. DOI: [10.4172/2161-0460.1000351](https://doi.org/10.4172/2161-0460.1000351)

Bandrés-Ciga, S., **Ruz, C.,** Barrero, F. J., Escamilla-Sevilla, F., Pelegrina, J., Vives, F., & Duran, R. Structural genomic variations and Parkinson's disease. Minerva Medica, 2017 108(5), 438–447. <https://doi.org/10.23736/S0026-4806.17.05246-6>

Bandres-Ciga, S., Ahmed, S., Sabir, M. S., Blauwendraat, C., Adarmes-Gómez, A. D., Bernal-Bernal, I., Bonilla-Toribio, M., Buiza-Rueda, D., [...] **Ruz, C.,** ... The Genetic Architecture of Parkinson Disease in Spain: Characterizing Population-Specific Risk,

Differential Haplotype Structures, and Providing Etiologic Insight. *Mov Disord*. 2019 Dec; 34(12):1851-1863. doi: 10.1002/mds.27864. Epub 2019 Oct 29. PMID: 31660654; PMCID: PMC8393828.

Saini P, Bandres-Ciga S, Alcantud JL, **Ruz C**, Postuma RB, Gan-Or Z; International Parkinson's disease Genomics Consortium. Common and rare variants in HFE are not associated with Parkinson's disease in Europeans. *Neurobiol Aging*. 2021 Nov; 107:174-177. doi: 10.1016/j.neurobiolaging.2021.05.019. Epub 2021 Jun 8. PMID: 34215448; PMCID: PMC8595489.

ABBREVIATIONS

AD: Alzheimer disease

ALP: Autophagy-lysosome pathway

ALS: Amyotrophic lateral sclerosis

ALS: Autophagy-lysosome system

approx.: Approximately

ATP: Adenosine triphosphate

BCA: Bicinchoninic acid protein assay

BGAL: β -galactosidase

CatD: Cathepsin D

CMA: Chaperone-mediated autophagy

CNS: Central nervous system

CTSD: Cathepsin D

DA: Dopaminergic

DMEM: Dulbecco's Modified Eagle Medium

DUB: Deubiquitinating enzyme

EDTA: Ethylenediaminetetraacetic acid

Endo-H: Endoglycosidase H

ER: Endoplasmic reticulum

FBS: Fetal bovine serum

FTD: Frontotemporal dementia

GA: Golgi apparatus

GBA: β -glucocerebrosidase

GD: Gaucher disease

GWAS: Genome-wide Association Studies

H4: Human neuroglioma cell line

HD: Huntington's disease

Hex B: β -hexosaminidase

HEXB: β -hexosaminidase

HLA: Human leukocyte antigen

HSP: Mediatory heat shock proteins

HY: Hoehn and Yahr scale, 2001

IDRs: Intrinsically disordered regions

LAMP1: Lysosome-associated membrane protein

LB: Lewy bodies

LC3: LC3-phosphatidylethanolamine conjugate (LC3-II), recruited to autophagosomal membranes

L-DOPA: Levodopa

LN: Lewy neurites

LRRK2: Leucine rich repeat kinase 2

MAPT: Tau protein gene

MDS: Movement Disorders Society

min: Minute

ml: Millilitre

mm: Millimetre

MPTP: 1 -methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine

MS: Motor symptoms

NAC: Non-amyloid β -component domain

NaCl: Sodium Chloride

ND: Neurodegenerative diseases

nm: Nanometres

NMS: Non-motor symptoms

PBS: Phosphate-buffered saline

PD: Parkinson disease

PQC: Protein quality control

PSAP: Prosaposin

PVDF: Polyvinylidene difluoride

RBC: Red blood cells lysis solution

RFU: Relative fluorescence units

RIPA: Cell lysis buffer

ROS: Reactive oxygen species

SAPs: Sphingolipid activator proteins

TNF α : Tumour necrosis factor α

UK: United Kingdom

UN: United Nations

UPS: Ubiquitin-proteasome system

WBC: White blood cells/leukocytes

α -Syn: α -synuclein

B-gal: β -galactosidase

β -GCase or GCase: β -glucocerebrosidase

LIST OF FIGURES

Figure 1. Represented images of the nigrostriatal pathway under normal conditions (A), in PD under degenerated conditions (B) and inclusions or LB (C).	12
Figure 2 Markers associated with neuronal loss in PD.	18
Figure 3. The imbalance of cellular homeostasis in PD mediated by the failure of protein degradation pathways seems to be a key factor in the pathological cascade of the disease..	33
Figure 4. Structure of the PSAP gene.....	38
Figure 5. Lysosomal enzymatic activity of β -GCase (A), Hex B (B), β -gal (C), and CatD (D) in WBC from PD patients compared to healthy controls.....	56
Figure 6. Immunoblot analysis of GBA, BGAL, HEXB, and CTSD proteins from the WBC lysates of PD patients and healthy controls	58
Figure 7. Immunoblot analysis of PSAP, Saposin C (A), and α -Syn (B) protein levels from the WBC lysates of PD patients and healthy controls.....	60
Figure 8. Correlation analysis of PSAP and α -Syn (A) and CTSD and α -Syn.....	60
Figure 9. Data obtained from enzymatic activities and protein expression levels assessed 24 and 72 hours post treatments in H4 cells.	73
Figure 10. Phosphorylation status of Rab10 as a direct readout for LRRK2 kinase activity..	74
Figure 11. GCase activity/GBA estimate ratio of cell lysates treated with LRRK2in1, MLi2 and CBE.....	75
Figure 12. Endo-H resistance of GBA protein in cell lysates.....	76

LIST OF TABLES

Table 1. Misfolded proteins and principal toxic effects in PD.	26
Table 2. Specific details of every enzymatic assay.	49
Table 3. Demographic and clinical data of the study cohort..	52
Table 4. Inhibitor Concentrations for the 24 h and 72 h treatments.....	68

RESUMEN

Las enfermedades neurodegenerativas afectan a millones de personas a nivel mundial, lo que supone un problema sanitario y económico muy importante, debido especialmente al aumento de la esperanza de vida en nuestra sociedad. Uno de los aspectos menos estudiados en relación a este tema, son los mecanismos de proteotoxicidad, a pesar de ser procesos comunes entre estas enfermedades incurables y debilitantes. Comprender mejor cómo la proteotoxicidad afecta la etiología y progresión de las enfermedades neurodegenerativas proporcionaría una visión que permitiría mejorar el tratamiento terapéutico. En relación a los factores proteotóxicos que influyen en la etiología de una de las enfermedades neurodegenerativas más frecuentes, la enfermedad de Parkinson (EP), se ha propuesto la disfunción lisosomal como uno de los mecanismos moleculares más importantes. Esta asociación se consolida desde que las mutaciones en heterocigosis para el gen *GBA*, que codifica para la enzima lisosomal β -glucocerebrosidasa (GCase), se consideran el principal factor de riesgo genético para la EP esporádica. Por estas razones, analizamos un grupo de enzimas lisosomales en pacientes con EP y sujetos sanos, y empleamos un modelo celular de neuroglioma humano H4 a través de la inducción del proceso de autofagia con inhibidores específicos para estudiar la contribución de la disfunción lisosomal en la etiología de la EP.

En esta tesis, encontramos una reducida actividad de las enzimas lisosomales β -GCase y β -gal en pacientes con EP comparado con los sujetos sanos. Los niveles de expresión de cofactores PSAP y Saposina C estaban disminuidos en pacientes y correlacionaba con unos niveles aumentados de proteína α -sinucleína (α -Syn). Sin embargo, los niveles de actividad y expresión de la enzima CatD se encontraban aumentados en el grupo de pacientes, lo cual se correlacionó con unos niveles aumentados de α -Syn en este grupo. En el modelo celular

H4 inducido con autofagia mediante el uso de inhibidores específicos de LRRK2, encontramos unos niveles de expresión de proteína GBA aumentados y una disminución de la actividad específica de la enzima 72 h. post-tratamiento con el inhibidor LRRK2in1. Sin embargo, este efecto no fue causado por alteración del transporte de la proteína desde el aparato de Golgi al lisosoma.

Nuestros hallazgos sugieren que la disfunción lisosomal en la EP esporádica se debe, al menos en parte, a una alteración en la Saposina C derivada de niveles reducidos de PSAP. Eso llevaría a una disminución significativa en la actividad de β -GCasa, lo que resulta en la acumulación de α -Syn. Debido a esta disfunción enzimática, la acumulación de sustratos como las monohexosilceramidas podría actuar a favor de la activación de CTSD y, por tanto, aumentar su actividad enzimática de forma puntual. Además, observamos una posible regulación de la función lisosomal a través del dominio quinasa LRRK2, lo cual sugiere una interacción entre la actividad de la quinasa LRRK2 y GBA.

Aunque son necesarios más estudios sobre este tema, la mejora de la actividad de GCasa podría restaurar el metabolismo proteico defectuoso observado en la EP. De acuerdo con nuestros resultados, la evaluación de la actividad lisosomal en sangre periférica de pacientes con EP se presenta como un enfoque prometedor para investigar mecanismos patológicos. Nuevas terapias destinadas a restaurar la función lisosomal, permitirían mejorar el tratamiento de la EP esporádica.

ABSTRACT

Neurodegenerative diseases affect millions of people worldwide, which is a very important health and economic problem, especially due to the increase in life expectancy in our society. One of the most important aspects, and relatively less studied in relation to this subject, are the mechanisms of proteotoxicity, despite being common processes among these incurable and debilitating diseases. A better understanding of how proteotoxicity affects the etiology and progression of neurodegenerative diseases would provide insight for improved therapeutic treatment. In relation to the proteotoxic factors that influence the etiology of one of the most frequent neurodegenerative diseases, Parkinson's disease (PD), lysosomal dysfunction has been proposed as one of the most important molecular mechanisms. This association has been consolidated since heterozygous mutations for the GBA gene, which codes for the lysosomal enzyme β -glucocerebrosidase (GCase), are considered the main genetic risk factor for sporadic PD. Therefore, we analyzed a group of lysosomal enzymes in patients with PD and healthy subjects, as well as a cell model of human H4 neuroglioma through the induction of the autophagy process with specific inhibitors, to study the contribution of lysosomal dysfunction in the aetiology of PD.

In this thesis, we found a reduced activity of the lysosomal enzymes β -GCase and β -gal in patients compared to healthy subjects. Expression levels of PSAP and Saposin C cofactors were decreased in patients and correlated with increased levels of α -Syn protein. However, the activity and expression levels of CatD enzyme were increased in patients, which correlated with increased levels of α -Syn in this group. In the H4 cell model induced with autophagy through the use of specific LRRK2 inhibitors, we found increased levels of GBA protein expression and a decrease in the specific activity of the enzyme 72 h. post-treatment

with the LRRK2in1 inhibitor. However, this effect was not caused by an altered transport of the protein from the Golgi apparatus to the lysosome.

Our findings suggest that the lysosomal dysfunction in sporadic PD is due, at least in part, to an alteration in Saposin C resulting from reduced levels of PSAP. That would lead to a significant decrease in β -GCase activity, resulting in the accumulation of α -Syn. Due to this enzymatic dysfunction, the accumulation of substrates such as monohexosylceramides could act in favor of CTSD activation and, therefore, increase its enzymatic activity in a timely manner. In addition, we observed a possible regulation of lysosomal function through the LRRK2 kinase domain, suggesting an interaction between LRRK2 kinase activity and GBA.

Although more studies are needed on this topic, enhancement of GCase activity could restore the defective protein metabolism observed in PD. According to our results, the evaluation of lysosomal activity in peripheral blood of patients with PD appears to be a promising approach to investigate pathological mechanisms. New therapies aimed at restoring lysosomal function would make it possible to improve the treatment of sporadic PD.

I. INTRODUCTION

Chapter 1. Introducing Parkinson's disease

Aging is a natural process to which all living beings are subjected. This process entails a series of gradual biological changes that compromise the correct functioning of the organism at both physical and cognitive levels.

According to the United Nations (UN, Department of Economic and Social Affairs Population Division) report in 2013, the population over 60 years of age is expected to increase by more than 2 billion in 2050. This increase in the life expectancy of the population has resulted in an improvement in the quality of life, but it has subjected us to certain pathologies associated with age, such as neurodegenerative diseases (ND), with age being the main risk factor for its development (Johnson, 2015).

ND are characterised by the selective loss of populations of neurons. In all of them, typical cellular processes occur in a common way, such as the abnormal accumulation of toxic protein species, oxidative stress, alteration of the ubiquitin-proteasome system and autophagy, neuroinflammation and finally cell death. The most common neurodegenerative disorder is Alzheimer's disease (AD), followed by Parkinson's disease (PD) (Kritsilis et al., 2018).

PD was first described in 1817 by James Parkinson (1755-1824) in his essay "Assay on the Shaking Palsy", in which different aspects of the clinical symptomatology were recognized, which he called *paralysis agitans* (shaking paralysis). Later, Jean-Martin Charcot, named the first neurologist of the 19th century, contributed to the knowledge of the disease by identifying new clinical aspects and proposed the name by which it is currently known in deference to the work of Parkinson (Obeso et al., 2017).

The development of PD causes motor (MS) and non-motor (NMS) symptoms that result in a significant decrease in the quality of life of patients (Barone et al., 2009). This

affectation worsens with the progression of the disease, promoting dependency and care of the patient by a caregiver (Aarsland et al., 2000), which entails physical and psychological wear and tear for the health professional and/or caregiver and an economic expense for public administration. Due to the heterogeneous nature of symptoms, not all patients respond equally to treatment with dopaminergic drugs, and their continued use has been associated with long-term disabling motor disturbances (Poewe, 2009). Furthermore, the lack of an early diagnosis makes the symptoms worse and therapeutic strategies less effective.

From its discovery to the present day, identification and diagnosis continue to be mostly clinical. Very little is still known about the origin and progression of the neurodegenerative process, being one of the main objectives to be achieved in the study of PD. This chapter aims to briefly introduce the general aspects of PD at the epidemiological and clinical levels.

1.1. Descriptive epidemiology: Prevalence and incidence of PD

Descriptive epidemiology is the part of epidemiology that describes a phenomenon in time, place and person, through its frequency and distribution, using measures of prevalence, incidence and mortality. Thus, the percentage of individuals in a population that present the phenomenon or condition under study at a given time is known as prevalence. The prevalence depends on many factors, such as the standard of living of the population at a social or economic level, which makes it difficult to compare PD or any other disease between large populations. On the other hand, the incidence of a disease is the number of new cases in a given period, being a good indicator of the risk of developing said disease (Benito-Leon, 2018). In general, it is estimated that in industrialised countries the prevalence of PD usually

affects 1-2% of the population over 60 years of age and 3-4% of those over 80. While the incidence rate is between 0.8 -0.18 per 1,000 people/year (De Lau et al., 2006; Lee et al., 2016).

As mentioned above, the socioeconomic, health and survival levels, different between populations, makes the prevalence rate vary throughout the world. The prevalence of PD is considered higher in Europe and America, compared to other continents such as Africa, Asia and Latin America; data that is also maintained with respect to incidence (Benito-Leon, 2018). These a priori differences could be explained by the different genetic or environmental factors in the different geographical areas or by the lower mortality rate and higher life expectancy in industrialised countries.

Several studies have found differences regarding the prevalence and incidence of PD based on sex, being 1.5-2 times higher for men (Li et al., 1985; de Lau et al., 2004; Benito-Leon et al., 2004; Wooten et al., 2004; Haaxma et al., 2007). Although the cause is unknown, there are certain studies that suggest different hypotheses. Exposure to toxic substances and head injuries have been shown to be risk factors for PD (Tanner et al., 1996). This type of behaviour has been associated with certain sexual roles mostly assumed by men, which could explain the greater incidence in them. Other authors suggest the relevant role of estrogens as a neuroprotector (Inestrosa et al., 1998); even genetic studies defend the susceptibility of the X chromosome to PD (Pankratz et al., 2002).

In fact age, being the main risk factor for the development of neurodegenerative diseases, also acts increasing the prevalence and incidence rate in PD.

The differences regarding the epidemiology of PD are a key resource for obtaining new clues about the aetiology of the disease. However, given the differences in the unification and uniformity of the methodology for obtaining these data and the problems in diagnosis, the variation in the prevalence and incidence of PD still yields inconclusive results.

1.2. Clinical aspects. Motor symptoms and non-motor symptoms.

PD is a complex and heterogeneous disorder, characterised by cardinal motor signs that classically comprise: bradykinesia/akinesia; tremor at rest, rigidity and postural instability (mainly in the extremities, neck or trunk). Currently, gait disturbance and the "freezing" phenomenon have also been included as classic features of PD (Jankovic, 2008).

The phenomenon of bradykinesia or akinesia is the most characteristic in patients with PD and refers to the slowness or absence of movement. (Berardelli et al., 2001). This symptom is distinctive of a failure in the basal ganglia circuitry, which makes it difficult to plan, initiate and execute movement, and to perform sequential and simultaneous tasks as simple as buttoning a shirt (Cooper et al., 1994). Akinesia is also manifested in other signs such as dysarthria (difficulty making sounds), facial hypomimia (loss of facial expression), blink disturbance, even drooling or excessive salivation (Bagheri et al., 1999).

Resting tremor is the most common and visible manifestation in PD, usually unilaterally in the distal part of an extremity (usually the dominant hand) and with a frequency of 4-6 Hz (Martin et al., 1973; Rajput et al., 1991). This sign can also manifest itself in other areas such as the mouth, lips, jaw and chin or the legs; but it rarely affects the head, neck, and voice, which helps to distinguish it from essential tremor not associated with PD. The tremor disappears during action and sleep, but worsens in situations of stress and patient fatigue.

Some studies report a significant atrophy of a group of neurons of the midbrain reticular formation (A8) in patients with PD and marked tremor, while it is not affected in those patients without tremor (Jankovic, 2008).

Stiffness is one of the initial symptoms in PD characterised by increased resistance to movement due to elevated muscle tone to passive movement of a joint (flexion, extension and/or rotation of the joint). In this sense, the extremities of the distal area (such as wrists or ankles) and the proximal area (neck, hips and shoulders) are affected. Stiffness is usually accompanied in most cases by pain and postural instability, including limb deformities.

The freezing phenomenon is also known as motor block, a very disabling form of akinesia. It most frequently affects the legs while the patient is walking, although it can affect other structures, spontaneously and acutely, producing an inability to move the feet or to initiate movement, which usually causes the patient to fall. Although it is very common in PD, it does not affect all cases equally. Considering as risk factors for the development of the freezing phenomenon: the presence of akinesia, rigidity, postural instability and a longer duration of the disease (Giladi et al., 2001); early tremor, on the other hand, seems to reduce the presence of frostbite symptoms in patients. This usually occurs in patients with ON, that is, when they are under the effects of dopaminergic therapy, to which other symptoms do not seem to respond as well (Giladi et al., 1997).

Ocular alterations such as the lack of blinking, or oropharyngeal alterations such as speech alteration, sialorrhea or dysphagia, are some of the symptoms derived from motor alterations and that affect the PD patient. Another common symptom is anosmia (lack of

smell), or hyposmia (reduced ability to smell), distinctive in early stages of the disease, usually before the PD diagnosis (Stern et al., 1994).

The Hoehn and Yahr scale is the most widely used to assess disease progression based on symptoms. It is comprised of stages from 1 (mild, non-disabling symptoms) to 5 (severe muscle atrophy or disabling cachexia) (Hoehn et al., 2001). The development of the disease is slow and progressive, and it is estimated that at the time of onset of motor manifestations there is approximately a 50% loss of dopaminergic (DA) neurons or even greater (Cheng et al., 2010).

NMS are very common in PD and usually appear even before symptoms or motor dysfunction are visible. So much so that the preclinical or prodromal phase is usually characterized by the presence of these symptoms, such as anosmia, constipation, depression or sleep disorders (Kalia et al., 2015). The clinical spectrum is broader and includes neuropsychological manifestations such as cognitive impairment, depression, apathy, anxiety; sleep disorders and autonomic dysfunction (constipation, urinary and sexual dysfunction), in addition to other symptoms such as fatigue, which affect the quality of life of patients from early stages (Bostantjopoulou et al., 2013). Most of them precede MS and pass unnoticed by patients and health personnel, increasing the time for early diagnosis.

1.3. Aetiology

Due to the heterogeneity of PD (clinical and pathological), the identification of a cause or pathogenic mechanism of the disease is still ambiguous. To date, there is clear agreement on the main factors involved in the pathogenesis of PD: age, environmental factors and genetic

factors. However, how they trigger the disease process or how these factors interfere with each other is unclear.

1.3.1. Environmental factors

The literature shows the influence of certain environmental factors such as exposure to certain toxins (pesticides, heavy metals, contamination, etc.) as potential triggers of PD. The first association was made with the compound 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), a neurotoxin capable of inhibiting mitochondrial complex I and selectively damaging the DA neurons of the substantia nigra (Langston et al., 1983). This fact, in addition to the use of MPTP in PD animal models, led to the idea of PD (Xu et al., 2006), as a cause of exposure to toxins. This association yielded new compounds related to PD, such as the pesticides and herbicides Paraquat and rotenone (Tanner et al., 2011).

In addition to exposure to these toxins, certain environments (rural living, drinking well water, etc.) or exposure to some heavy metals such as iron, aluminium and zinc have also been considered as risk factors for the development of PD (reviewed in Bjorklund et al., 2018).

1.3.2. Genetic factors

In a greater percentage of cases, PD is an idiopathic or sporadic disease, of spontaneous or unknown origin. However, around 10-15% of cases are derived from a clear genetic or family inheritance (Deng et al., 2018). Additionally, genetic factors have been found to be involved in a common way in both forms of the disease (Chai & Lim, 2013), highlighting certain common aspects in the pathogenesis of both, as well as the importance of knowing how both factors (genetic and environmental) interact with each other in the development of PD.

The genes involved in familial PD are named as PARK genes. Mutations in some of these genes follow an autosomal dominant genetic inheritance pattern, such as *SNCA*, *LRRK2*,

and *VPS32*; or recessive, for mutated *PINK1*, *DJ-1*, or *PRKN* genes (reviewed in Schulte et al., 2011). In addition to the mutations involved, others have recently been associated as the main genetic risk factors for the development of PD in its sporadic form, being *GBA* the most closely related (Nichols et al., 2009; Sidransky et al., 2012), among others (*MAPT*, *BST1*, *HLA*, *PARK16*, *GAK*).

One of the main aspects to be discussed in this thesis is related to *GBA* and its involvement with PD, so the main genes and proteins involved, and the interaction between them, will be detailed later.

1.4. Pathological mechanisms in PD

Regardless of the type of PD form (familial or sporadic), both share the main pathological hallmark, the selective loss of DA neurons in the *substantia nigra*, more severely affecting groups of ventrolateral cells (*pars compacta*, starting point of nigrostriatal pathway) which are those neurons that project from the basal ganglia to the striatum (Dickson, 2018). At the cellular level, the presence of abnormal cytoplasmic deposits or aggregates is observed in these neurons, commonly called Lewy bodies (LB). These cytoplasmic inclusions are mainly composed α -Syn protein, the one codified by *SNCA* gene (Figure 1). The aggregation of this protein in its neurotoxic form is the central pathological event characteristic of PD, however, another number of events are involved in the pathological process, among them: the failure of the protein degradation or proteotoxicity systems including lysosomal dysfunction, mitochondrial dysregulation, altered vesicle trafficking, neuroinflammation and ultimately cell death. Some of those events will be described in this section.

Given that α -Syn proteotoxicity and defective protein degradation system are two of the main issues to be addressed in this work, an independent section for each process will be destined in subsequent chapters of the introduction.

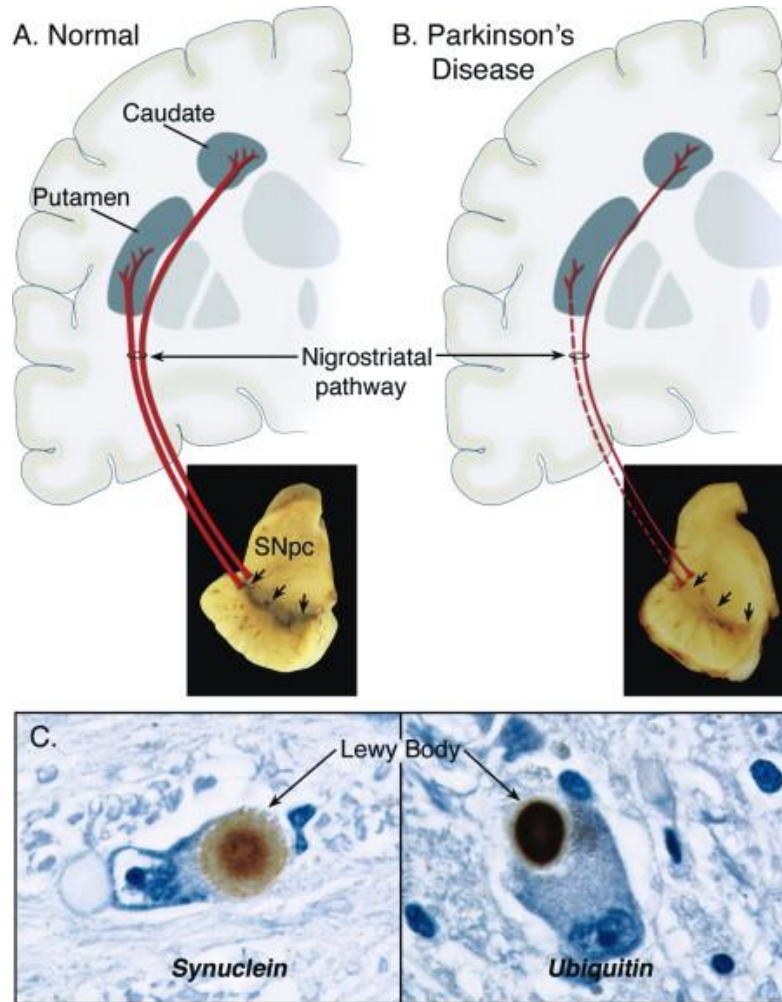


Figure 1. Represented images of the nigrostriatal pathway under normal conditions (A), in PD under degenerated conditions (B) and inclusions or LB (C).

- **Mitochondrial dysfunction**

The idea that defective mitochondrial activity is found in PD arises from the use of models exposed to MPTP, capable of blocking mitochondrial complex I. The inhibition of this mitochondrial complex not only produces a loss of energy due to defective oxidative phosphorylation, but it increases the production of superoxide radicals (ROS) capable of increasing cell damage (reviewed in Moon & Sun Ha Paek, 2015). This failure in mitochondrial complex I appears to be visible beyond brain tissue in PD, such as in patient platelets, platelet-derived cell models, or skeletal muscle (Bindhoff et al., 1991; Kridge et al., 1992; Swedlow et al., 1996). The chain-like response of this process drags other proteins involved in mitochondrial dysfunction such as chaperones, enzymes responsible for metabolism, ubiquitin-proteasome system (UPS), calcium and dopamine signalling, ROS and apoptosis (Burté et al., 2011; Zhanh et al., 2010).

Another point in favour of the mitochondria role in the pathology of PD is related to genetic factors. An example is the involvement of some genes such as *PARK2* and *PARK6*, which encode for PINK1 and Parkin proteins, respectively. These proteins are closely related to the regulation and recycling of defective mitochondria, called mitophagy. Mutations in any of these genes are considered the cause of familial forms of PD, where mitochondrial control processes and mitophagy are altered (Pickrell & Youle, 2015).

Other pathological aspects of the disease can interfere with an appropriate mitochondrial function in PD. The α -Syn protein is capable of penetrating from the cytosol to organelles as mitochondria, inhibiting complex I, increasing oxidative stress and interfering with the synthesis of energy (adenosine triphosphate, ATP). This fact also interferes with the correct function of the protein control systems, the UPS and the autophagy-lysosome system

or pathway (ALS/ALP), breaking cellular homeostasis and contributing to neuronal death (reviewed in Moon & Paek, 2015).

1.4.2. Vesicle trafficking

It is assumed that DA neurons require a large cell traffic machinery, due to the multiple synapses with other neurons and the length and complexity of their arborizations (Bolam & Pissadaki, 2012).

Physiologically, α -Syn acts at the presynaptic level as a chaperone on SNARE complexes (soluble N-ethylmaleimide-sensitive factor attachment protein receptor). SNARE complexes facilitate fusion between membranes to allow neurotransmitter exocytosis at the synapse. If they are dissociated, they tend to misfold or generate unspecific unions, for which chaperones such as α -Syn act preventing and regulating these proteins in the synapse (Chandra et al., 2005). However, the oligomeric or toxic form of α -Syn seems to alter this process. Studies in a transgenic mouse model for mutated *SNCA* show alterations in the distribution of synaptic vesicles in DA neurons, followed by the accumulation of α -Syn, motor alteration and neuronal death (Janezic et al., 2013). In addition, the α -Syn protein seems to alter the presynaptic space, promoting clusters of vesicles, blocking their mobility, increasing the number of vesicles to be recycled, altering the function of SNARE proteins and the correct release of dopamine (Nemani et al., 2010; Garcia-Reitböck, 2010; Scott & Roy, 2012). Not only at the synapse level, but some studies show an impairment in vesicle formation. The accumulation of α -Syn seems to block/disrupt the ER-Golgi system, where this process takes place, through the inhibition of SNARE complexes. (Cooper et al., 2006; Thayanidhi et al., 2010).

Thanks to genome-wide association studies (GWAS), new genetic regions associated with an increased risk of sporadic PD have been identified. In line with vesicular trafficking,

a region has been identified on chromosome 17q21 whose H1 haplotype appears to contribute to an increased risk of the disease. The tau protein gene (*MAPT*) associated with the H1 haplotype is the gene with the most evidence (Nalls et al., 2014). This protein is involved in the stabilisation and elongation of microtubules as well as in axonal transport (Wade-Martins et al., 2012). Chu et al. (2012) observed a decrease in protein transport through the axon, mediated by kinesin, in a mouse model with α -Syn alteration as example of sporadic PD. In addition, both tau and α -synuclein proteins seem to interact with each other, altering the organization of the cytoskeleton, axonal transport and promoting synapse dystrophy. All these would ultimately affect the correct transport of the cytotoxic material or aggregates for their degradation through the degradation systems (ALP) (Roy & Jackson, 2014; Maday et al., 2012).

Another affected protein in PD with involvement in vesicular trafficking is the leucine-rich repeat kinase 2 protein (LRRK2). This protein, encoded by the *LRRK2* gene, has kinase and GTPase activities, and although its function is not entirely clear, there is increasingly evidence of its involvement in vesicular trafficking, recycling, and autophagy process (Piccoli et al., 2011; Matta et al., 2012). Protein association studies indicate that LRRK2 interacts with other PD-risk candidate proteins: RAB7L1/2AB29 and GAK (Beilina et al., 2014; MacLeod et al., 2013). This protein complex acts in the elimination of Golgi-derived vesicles through ALP, showing its potential role in regulating these processes (Alegre-Abbarrategui et al., 2009).

Finally, it is mentioned the existence of other genes associated with familiar forms of PD, also involved in the protein cellular trafficking such as: PARK2, PINK1, VPS35, SYNJ1, DNAJC6 (reviewed in Hunn et al., 2015), which together with the aforementioned, support the relevance of vesicular trafficking processes in the pathology of PD.

1.4.3. Neuroinflammation and cell death

Neuroinflammation and death of DA neurons is another of the main pathological processes in PD. Studies in human post-mortem brain tissue and in patients through neuroimaging techniques (as PET), show an increase in the microglia activation, presence of T lymphocytes and a higher concentration of proinflammatory cytokines in affected areas (striatum and substantia nigra) compared to controls (McGeer et al., 1988; Gerhard et al., 2006; Edison et al., 2013). Physiologically, programmed cell death is a process initiated by cells to maintain homeostasis, however in pathological conditions, it is found to be altered, contributing to neurodegeneration. In addition to the factors above mentioned, capable of inducing cell death, specific apoptotic markers such as certain caspases and apoptotic regulators are altered in PD (reviewed in Dauer and Przedborski, 2003).

It is not yet clear whether neuroinflammation is the cause or consequence of other neuropathological events. In previous studies with PD animal models induced by toxins, the inhibition of microglia (pre and post exposure to the neurotoxin) produced an attenuation of the death of DA neurons, in both experimental conditions (He et al. 2001; Wu et al., 2002). On the contrary, the α -Syn protein itself is capable of activating the function of microglia and triggering inflammation (Su et al., 2008).

Both, inflammatory and degenerative responses also extend over other types of cells such as astrocytes. Models of these cells with the mutation A53T, which overexpresses α -Syn also activates microglial inflammatory response and loss of DA neurons in the nervous system (Gu et al., 2010). This fact has been corroborated in post-mortem tissue from PD patients, where an accumulation of α -Syn in astrocytes in the striatum and neocortex was observed (Braak et al., 2007).

Other events related to neuroinflammation compromise the Ca^{2+} ion, since altered levels of it trigger various cellular inflammatory processes, release of cytokines and activation of inflammatory mediators (Cam, PKC, NF-KappaB). Additionally, there is evidence of alteration in the blood-brain barrier permeability of PD patients (reviewed in Belarbi et al., 2020).

Interestingly, GWAS-derived studies have found robust associations between human leukocyte antigens (HLA), molecules with a key role in the immune system, and PD risk (Nalls et al., 2014). *HLA* codes for a set of proteins closely related to the immune response, which are found in those cells that present antigens, such as microglia, inducing the response of T cells (Zhang et al., 2017; Greenbaum et al., 2011). Thereby, pro or anti-inflammatory markers seem to play an important role in the possible diagnosis or development of the disease. While PD patients with higher levels of proinflammatory markers in serum have more aggressive progression of symptoms (Williams-Gray et al., 2016); other studies ensure lower incidence of PD cases in people who usually take anti-inflammatory drugs (Gao et al., 2011).

Taken all together, these studies highlight the contribution of the neuroinflammation to the pathology of PD; however, it remains to be elucidated if neuroinflammation is a primary event or is promoted by the rest of pathological events above mentioned.

1.5. Diagnosis and treatment

Although recent advances in genetics have contributed to a better understanding of the different aspects of PD, to date there is no specific test or markers for its diagnosis, being essentially clinical and definitive only after post-mortem analysis of the tissue. The clinic features of PD are very extensive, making diagnosis even more difficult. The most widely used clinical criteria for PD diagnosis are those collected from the UK Parkinson's Disease

Society Brain Bank, where the presence of MS prevails. Recently, new criteria for the PD diagnosis have been published by the International Parkinson's and Movement Disorders Society (MDS), which does not include dementia as an exclusion criterion and incorporates a greater number of non-motor manifestations in the diagnosis (Postuma et al., 2015). Although diagnosis criteria has improved, the current clinical practice is strongly based on the manifestation of the cardinal MS (bradykinesia or akinesia, in combination with tremor at rest, rigidity, or both). At this point, the need for early biomarkers of PD is visibly increasing, through simple, inexpensive, non-invasive and reproducible methods that can facilitate the diagnosis (DeKosky & Marek, 2003; Scherzer, 2009). They need to be detected before the appearance of MS, because when these manifestations occur, there is already a considerable loss of neuron cells (Figure 2).

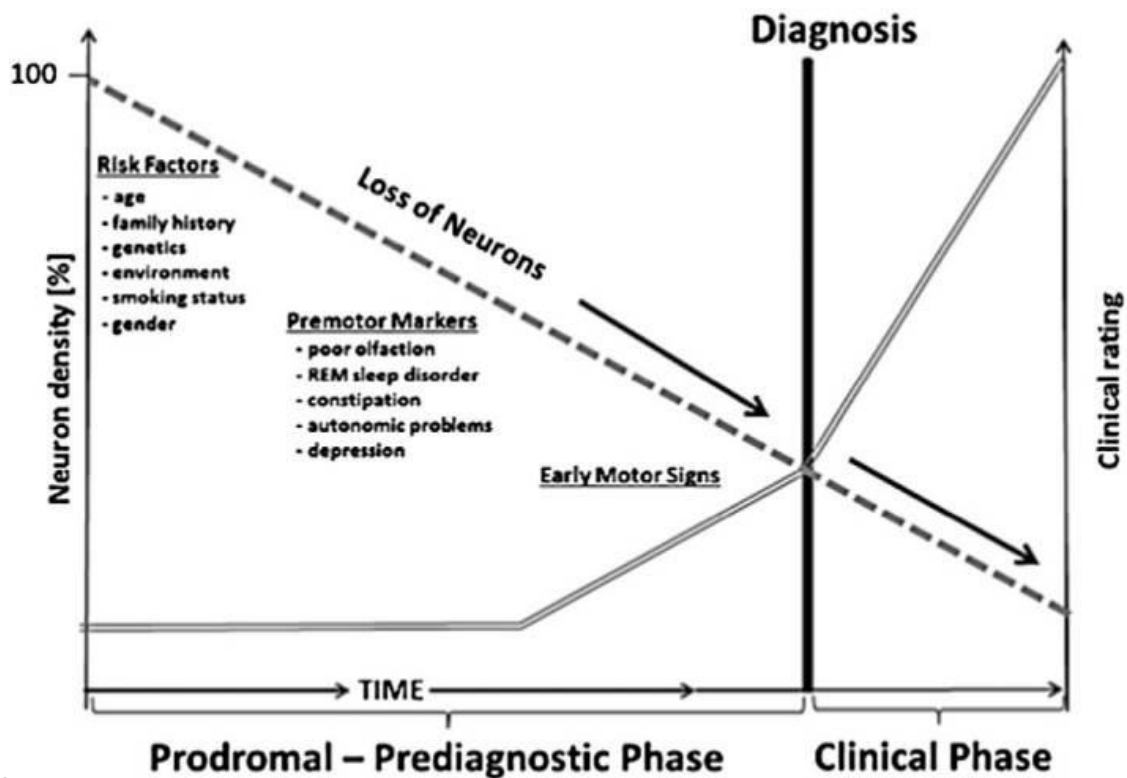


Figure 2 Markers associated with neuronal loss in PD. (----) Loss of neurons; (====) Motor signs and diagnosis. Extracted from Miller & O'Callaghan, 2015.

From a therapeutic point of view, pharmacological treatment with levodopa (L-DOPA) continues to be the most widely used and most effective treatment to palliate the symptoms of patients with PD (Katzenschlager & Lees, 2002). Pharmacological practice is currently nourished by the advances derived from pharmacogenetics, optimizing the treatment according to the specific profile of every patient, what has led to new pharmacological approaches (Cacabelos et al., 2017).

In addition to pharmacological therapy, there are several therapeutic approaches directed to the different pathological mechanisms involved. Recently in 2021, Vijiaratnam et al. reviewed therapeutic approaches based on the main pathological mechanisms of PD. Basically they are summarized in: production of α -Syn, as well as its transmission or dispersion; impaired mitochondrial function; degradation mechanisms, through the involvement of LRRK2 and lysosomal dysfunction; and those related to neuronal survival. In this sense, there are a large number of approaches, including the use of drugs, targeted monoclonal antibodies, drug reuse, immunotherapy, cell and/or stem cell therapy, use of neurotrophic factors, deep brain stimulation, optogenetic therapy, etc. (reviewed in Elkouzi et al., 2020).

Chapter 2. Proteotoxicity in Parkinson Disease

Proteotoxicity, meaning impairment of cellular function as a result of protein misfolding or aggregation, is a major hallmark of a broad range of ND, including AD, PD, amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and Huntington's disease (HD) among others. A common denominator in the aetiology of these diseases is that neuronal damage is frequently caused by abnormal aggregation and deposition of proteins in which altered specific molecular mechanisms lead to cell toxicity and degeneration.

As we age, the ability of our organism to maintain protein homeostasis substantially decreases. Several studies have proven that the aging process actively suppresses our capacity to clear toxic protein aggregates, promoting the accumulation of these aggregates and initiating neurodegeneration late in life (Sweeney et al., 2017). In general, aggregates of toxic proteins originate when soluble proteins gradually become insoluble filamentary polymers. These filamentous structures accumulate in the form of fibrils and end up depositing and aggregating in the nucleus, cytoplasm, or extracellular space of affected brain cells (Kumar et al., 2016). Eukaryotic cells have essential protein quality control (PQC) systems including chaperones that safeguard proteins against aggregation, the UPS and the ALS, that act in the degradation of misfolded proteins. A decrease in activity or saturation of these proteolytic systems, results in neuronal impairment and cell death.

The fact that proteotoxicity is a shared emergent pattern among these diseases, raises the question of whether common therapeutic interventions aimed at targeting dysregulated proteins, by either blocking over aggregation or accelerating degradation, may be applicable. Although it is widely known that similar molecular pathways altering cell viability through protein deposition cause these disorders, the reality is that elimination of proteins in a way that toxicity can be mitigated is more complex than expected.

Since proteotoxicity is a common phenomenon implicated in the majority of ND, this chapter aims to briefly address the pathologic mechanisms of α -Syn aggregation in PD.

2.1. α -Syn aggregation in PD: general aspects, structure and function

Major advancements in genetics and neuropathology led to the identification of α -Syn as the major pathological hallmark of PD. The *SNCA* gene encodes for this presynaptic protein called α -Syn, identified as the main component of amyloidogenic protein deposits known as Lewy bodies (LB) and Lewy neurites (LN) in PD. Deleterious genetic variation in *SNCA* results in the accumulation and aggregation of misfolded α -Syn (Klingelhoef et al., 2017).

Although pathological mechanisms of PD are numerous and diverse, α -Syn proteotoxicity has been hypothesized to be one of the main contributors to the neurodegenerative process, spreading the pathological condition to neighbouring cells like a prion-like disease (Angot et al., 2009). In turn, these α -Syn aggregates can interact to promote disruption of protein homeostasis and thus interfere cell normal topography and physiology including stress formation on the endoplasmic reticulum (ER), mitochondrial dysfunction and defective neurotransmission, which if prolonged, could lead to neuronal death (Hsu et al., 2000, Burré et al., 2010).

α -Syn is a small soluble protein (140 amino acid) highly expressed in the presynaptic zone of neuronal cells (Surguchov, 2008). Unlike its pathological behaviour, the normal or physiological function of this protein remains elusive. Some studies link it with synaptic transmission and vesicle trafficking, mitochondrial homeostasis, gene expression, protein phosphorylation, or even fatty acid binding (Cheng et al., 2011, Benskey et al., 2011).

α -Syn is differentiated into three main domains: the N-terminal domain (amino acids 1–60); an hydrophobic central domain known as non-amyloid β -component domain or NAC

(amino acids 61–95), which is associated to α -Syn aggregation in humans when β -sheet structure is acquired (Uéda et al., 1993); and the C-terminal region (amino acids 96–140) suggested to be involved in multiple functions, including self-protection of α -Syn from aggregation (Park et al., 2002:2004). Except for some cases (Bartels et al., 2011), native α -Syn is commonly found unfolded in a monomeric state (Binolfi et al., 2012; Fauvet et al., 2012). In contrast, there is no controversy under pathological conditions where α -Syn adopts a β -sheet amyloid conformation, promoting proteotoxic deposits.

2.2. Pathologic mechanisms of α -Syn aggregation

The aggregation process of α -Syn in vitro follows a similar pattern to other amyloid diseases.

The oligomerization follows a nucleation-dependent pathway divided in three phases: the lag phase where the monomers form the aggregation nuclei; the elongation phase in which fibrils tend to grow exponentially, and finally the stationary phase where monomers leakage result in a decrease of growing rate (Wood et al., 1999; Lee et al., 2007; Eisele et al., 2015). Oligomers grow faster into the elongation phase, converted into protofibrils due to the monomer addition, which conforms finally to the amyloidogenic mature form. However, although the protein has mechanisms to protect itself from aggregation (Park et al., 2002:2004), the reason why that occurs has not been identified yet.

Thus, potential risk factors have been proposed to initiate/potentiate this procedure, such as genetic mutations (Narhi et al., 1999), environmental factors such as iron exposure and pesticides and dietary aspects (Breydo et al., 2012).

α -Syn toxicity follows the prion-like hypothesis where the misfolded protein spreads cell to cell via endocytosis transmission. Subsequent to α -Syn aggregation, anomalous

oligomers are released from cells. Exogenous α -Syn fibrils can invade inside cells, seeding them into recipient cells, and induce its propagation cell to cell by originating a new cycle of endocytosis. This event would finally end inducing new LB-like inclusions (Desplats et al., 2009). The presence of α -Syn deposits, according to similar mechanisms in other ND, suggests that the main link between α -Syn and PD pathology is the gain of function hypothesis. In this direction, we briefly recap the main proteotoxicity effects of α -Syn in PD pathogenesis.

Post-translational modification including phosphorylation, ubiquitination, and glycosylation, plays an essential role in protein structure, modification, and function. Disruption of these processes has been linked to an intricate cellular proteostasis state within the cell. Accordingly, altered hyper phosphorylation was found in α -Syn site Ser129 in LBs. Therefore, phosphorylation of Ser129 has been evidenced to facilitate cytotoxic transport to the nucleus, being capable of interacting with histones inducing neurotoxicity (Scott et al., 2010). In addition, α -Syn toxicity within the nucleus may implicate the inhibition of histone acetylation, promoting neuronal disruption and cell death (Kontopoulos et al., 2006; Goers et al., 2003).

Furthermore, amyloidogenic α -Syn can modify lipid permeabilization, thus altering the structural components of the cell. One potential hypothesis suggests that these oligomers penetrate the cell membranes and reorganize the lipid environment, stimulating the flux of surrounding molecules (Van Rooijen et al., 2010). An alternative theory supports that oligomers could be fused with the membranes acting as an amyloid pore or channel (Tosatto et al., 2012; Tsigelny et al., 2012). These events have been suggested to enhance extracellular calcium flux within cells so that could be responsible for the cell death of dopaminergic neurons in PD.

In a similar way, α -Syn aggregates disrupt the main protein clearance systems within the cell and therefore mitochondrial function (Lashuel et al., 2013). The ALP and the UPS have been associated with PD pathology. In the last decade, genetic perturbation in several genes including LRRK2, SNCA, LAMP3, GBA, and ATP13A2 affecting lysosomal activity have been elucidated (Gar-Or et al., 2015; Chu et al., 2009). In vitro models showed that pathologic α -Syn blocks macroautophagy by binding key proteins, such as the lysosomal LAMP2 receptor in ALP or the 20S component in UPS (Lindersson et al., 2004; Tanaka et al., 2001). Although further studies are needed, these events suggest that α -Syn drives proteotoxicity by disrupting protein clearance of substrates. Consequently, accumulation of several substrates in addition to toxic oligomers within the cytosol, rise mitochondrial dysregulation associated with complex I activity, which is impaired in the brain tissue of PD patients and in vitro (Liu et al., 2009).

Despite SNCA being the major genetic contributor to PD aetiology, genetic mutations in PINK1 or DJ1 in Mendelian forms of PD result in a mitochondrial perturbation due to a toxic gain of function in the mutated proteins (Devi et al., 2008). PINK1 is a kinase protein involved in several events within the mitochondria, including oxidative phosphorylation or mitophagy. According to the proposed mechanisms above mentioned, α -Syn toxic effects may lead to disrupted mitochondrial dynamics altering electronic potential and facilitating mitochondrial fragmentation, dysfunctional mitophagy or increased mitochondrial ROS levels.

Multiple other cellular pathways are affected by α -Syn, as ER stress promotion, which in turn promotes ER-Golgi transport dysfunction and Golgi fragmentation (Colla et al., 2012; Gosavi et al., 2002).

Taking this together, α -Syn toxicity drives the main pathologic events in PD (Table 1). However, how α -Syn triggers those pathological events in PD, being either cause or consequence of it, remains unclear.

Disease	Protein aggregates	IDRs in proteins structure	Species location	Toxicity	References
Parkinson	α -Synuclein	C-terminal domain	Intracellular LBs formation, extracellular and membrane associated	-Plasma-membrane integrity disruption - Sinapse alteration (including loss of presynaptic proteins, reduced neurotransmitter release, vesicles enlargement, etc.) -Perturbation in calcium homeostasis -Cytoskeleton dynamics altered -Protein degradation system dysfunction -Lysosomal impact -Mitochondrial dysfunction and ROS induction -Endoplasmic reticulum stress	Eftekharzadeh B. et al.2016 Spillantini, 1997 Burré et al. 2014 Pieri et al. 2012 Stefanis L. 2012

Table 1. Misfolded proteins and principal toxic effects in PD. Long disordered regions or intrinsically disordered regions (IDRs) are present in eukaryotic and non-eukaryotic proteins, and have been highly linked to misfolding diseases due to their intrinsic capability to congregate into amyloid-like clusters. LBs: Lewy bodies; ROS: Reactive oxygen species.

Chapter 3. Autophagy: lysosomal degradation
pathways and PD

As we have previously reviewed, the accumulation of altered protein aggregates is not just a characteristic sign of PD, but also occurs in other ND, such as the so-called synucleinopathies and tauopathies. In others, such as HD, there are also found mutated protein deposits (Bourdenx et al., 2017). The accumulation of proteins in all of them reflects a clear failure in the mechanisms of cell degradation, preventing the correct removal of these aggregates, favouring their accumulation and finally causing neuronal death (Ebrahimi-Fakhari et al., 2012). Many of the PD-related genes in the familiar form, code for proteins involved in proteasomal and lysosomal degradation pathways, including PINK-1, Parkin (ubiquitin ligase), UCHL1 (ubiquitin hydrolase L1), DJ-1 (chaperone activity), and LRRK2 (kinase activity) (Ciechanover & Kwon, 2015; Cook et al., 2012; Moscovitz et al., 2015). Therefore, autophagy, and recently lysosomal dysfunction, have been involved in the pathogenesis of PD and other synucleopathies (Malik et al., 2019).

Given the importance of degradation mechanisms in maintaining cellular homeostasis, the study of them in the genetic and molecular mechanisms implicating the pathogenesis of PD have an important role to play. This chapter aims to address the contribution of mechanisms of protein degradation, focusing on the importance of autophagy and lysosomal dysfunction in PD pathology according to the literature.

3.1. Autophagy process and genetic alterations linked to PD

Through degradation mechanisms, cells are able to recycle and/or remove any susceptible material to be a waste product for itself, such as the derivative of failures in its biogenesis or formation, material from damaged cell organelles, protein aggregates, or invading microorganisms. As a result, these mechanisms contribute to maintaining cellular homeostasis, health and longevity of the organism (Lippai & Szatmári, 2017).

The main mechanism used by cells of the central nervous system (CNS), to maintain homeostasis is the process of autophagy or macro-autophagy via lysosome.

During the process of autophagy via lysosome, the cytoplasmic content where the material to be eliminated is located, is stored in double-membrane vesicles called autophagosomes, which have characteristic proteins associated with the membrane (such as the LC3-II protein) that allow their identification. Subsequently, the traffic of these vesicles takes place, which are directed to the lysosome, the cellular organelle that contains reserves of enzymes such as Cathepsin D, which provide an acidic internal environment necessary for the digestion of material, where the cytoplasmic content is finally integrated by fusion of the membranes of both structures (autophagosome-lysosome) for their degradation (Menzies et al., 2017). As occurs in many ND that range accompanied by the formation and accumulation of protein aggregates in the cells of the CNS, it is feasible to think that there is an affected mechanism or pathway in the metabolic process during autophagy in neuronal and glial cells (Hara et al., 2006; Komatsu et al., 2006:2007).

In the specific case of PD, there is clear evidence that both mechanisms, proteotoxicity and autophagy, are related with the disease whether familial or sporadic.

A large part of the genetic mutations that cause PD, in its familiar form, encode for proteins involved in some way in different degeneration or autophagy processes, including PINK-1, Parkin (a ubiquitin ligase), UCHL1 (ubiquitin hydrolase L1), DJ-1 (chaperone activity), and LRRK2 (kinase activity). Mutations in the **PINK-1** gene cause autosomal recessive forms of early-onset PD. This mutated form of the gene is fundamentally related to mitochondrial regulation (mitochondrial dysfunction, morphological deformation, increased ROS, etc.), thus contributing to neurodegeneration (Wood-Kaczmar et al., 2008; Moiso et al., 2014). Thereby, the role of PINK-1 in the degenerative process is closely linked to

mitophagy, which is the selective degradation of damaged or aged mitochondria, or part of them. Although its relationship with the autophagy process is still unknown, there are studies that indicate that the loss of PINK1 function or its deficit, produces an increase in the formation of autophagosomes (with an increase levels of the membrane protein LC3-II) in cells and animal models, promoting the induction of the autophagy process (Qi et al., 2011; Gómez-Sánchez et al., 2016). The **parkin** protein is found mutated in juvenile autosomal recessive PD. Together with PINK-1, it is closely related to mitochondrial quality control, and is located in the mitochondria, favouring the degradation of those that are damaged. Although normally damaged mitochondria are removed by the UPS, previous studies in mammalian cell models have concluded that depolarized mitochondria are degraded by the autophagy system (Elmore et al., 2001; Twig et al., 2008). **UCHL1** is a deubiquitinating enzyme (DUB) which has been extensively studied for its relationship with familial PD (Leroy et al., 1998). DUBs are a large group of proteases that cleave ubiquitin from proteins. Ubiquitin is attached to proteins in order to regulate the degradation of proteins via the proteasome and lysosome (Glickman & Ciechanover, 2002). There are studies that describe a possible regulation of the autophagic pathway by UCHL1, since it was observed that when inhibited, it produced the activation of the autophagic pathway in various models of α -Syn overexpression (Pukaß et al., 2015; Cartier et al., 2012). In addition, data indicating that UCHL1 interacts with LC3 protein, and inhibits autophagosome formation based on their DUB activity, indicate again a critical role in the regulation of autophagy (Yan et al., 2018). **DJ-1** is involved at a physiological level in various functions, including transcriptional modulation, chaperone-like functions and antioxidant properties. Although the protein is widely distributed throughout various cell compartments, the main physiological function of DJ-1 is contributing to cell protection and homeostasis when it is located at the mitochondria (Blackinton et al., 2009).

In a recent study, using a human cell model where DJ-1 activity was blocked, a decrease in mitochondrial degradation was observed leading to an increase in them and their mass, in addition to an increase in the accumulation of lysosomes (Krebiehl et al., 2010). This represents a clear autophagic response due to neurodegeneration and supports the idea of an axis mitochondria-lysosome and the importance of the autophagic pathway mediated by the lysosome to maintain cellular homeostasis. Several mutations have been identified in the *LRRK2* gene, the most common being G2019S, being responsible for 3-7% of familial cases of PD of European origin, and also found in 1-2% of sporadic cases of PD in Europe and North America (Gilks et al., 2005). Although the LRRK2 protein function is not completely known, there is molecular evidence of its possible involvement in vesicle trafficking and in the lysosome-mediated autophagy pathway. The over-expression of both, the LRRK2 protein and its form mutated (G2019S) produces an inhibitory effect on lysosomal function associated with the accumulation of substrates such as α -Syn protein (Orenstein et al., 2013). Also, in cellular, fly and mouse models it has been found that both, the overexpression such as inhibition or silencing of LRRK2 produces an accumulation of vesicles altering the autophagy process (Dodson et al., 2014; Plowey et al., 2008; Ramonet et al., 2011). In another study, it was found that the expression of the mutated form of the LRRK2 protein, where the kinase activity is altered, caused a decrease in the lysosomal activity, preventing protein degradation and altering the lysosomal pH. With respect to vesicular trafficking, it has been related to other proteins associated with PD such as ATP13A2 and VPS35. The former is an ion transport channel located in the lysosome membrane, while the second is involved in the retrograde transport of proteins in the cell mediated by endosomes towards the Golgi apparatus (trans-Golgi red) (Henry et al., 2015). These studies suggest a possible relationship between altered lysosomal activity in the PD and the LRRK2 protein.

Classically, the sporadic form of the disease has been associated with exposure to environmental factors, without any genetic implication. Nevertheless, GWAS in large populations is proposing that the sporadic variety of PD also has a genetic base, with multiple genes involved. Among them are two of the genes linked to familial PD with an autosomal dominant inheritance, such as *SNCA* and *LRRK2*. This would demonstrate that the pathogenic mechanisms at the molecular level are similar in both forms of the disease (Chai & Lim, 2013). As described in previous chapters of this introduction, the *SNCA* gene encodes for α -Syn protein. Despite the different variations or polymorphisms found for this gene involved in PD, all of them result in the same pathological effect, the accumulation and aggregation of α -Syn in a wrong folded way. Similar deficiencies in the control systems of quality of proteins in the cell, including those that affect the mechanisms of chaperone, UPS and autophagy, can accelerate the accumulation and α -Syn aggregation (Tan et al., 2009). In turn, the accumulation and aggregation of α -Syn may act reciprocally to promote the alteration of homeostasis of proteins and thus perpetuate a vicious circle. The accumulated/aggregated α -Syn that is not efficiently removed by the cell can physically alter the topography cell, function and/or interfere with normal cell physiology, including stress on the ER, mitochondrial dysfunction and defective neurotransmission, that prolonged, could lead to cell death (Smith et al., 2005; Hsu et al., 2000; Burre et al., 2010) (Figure 3).

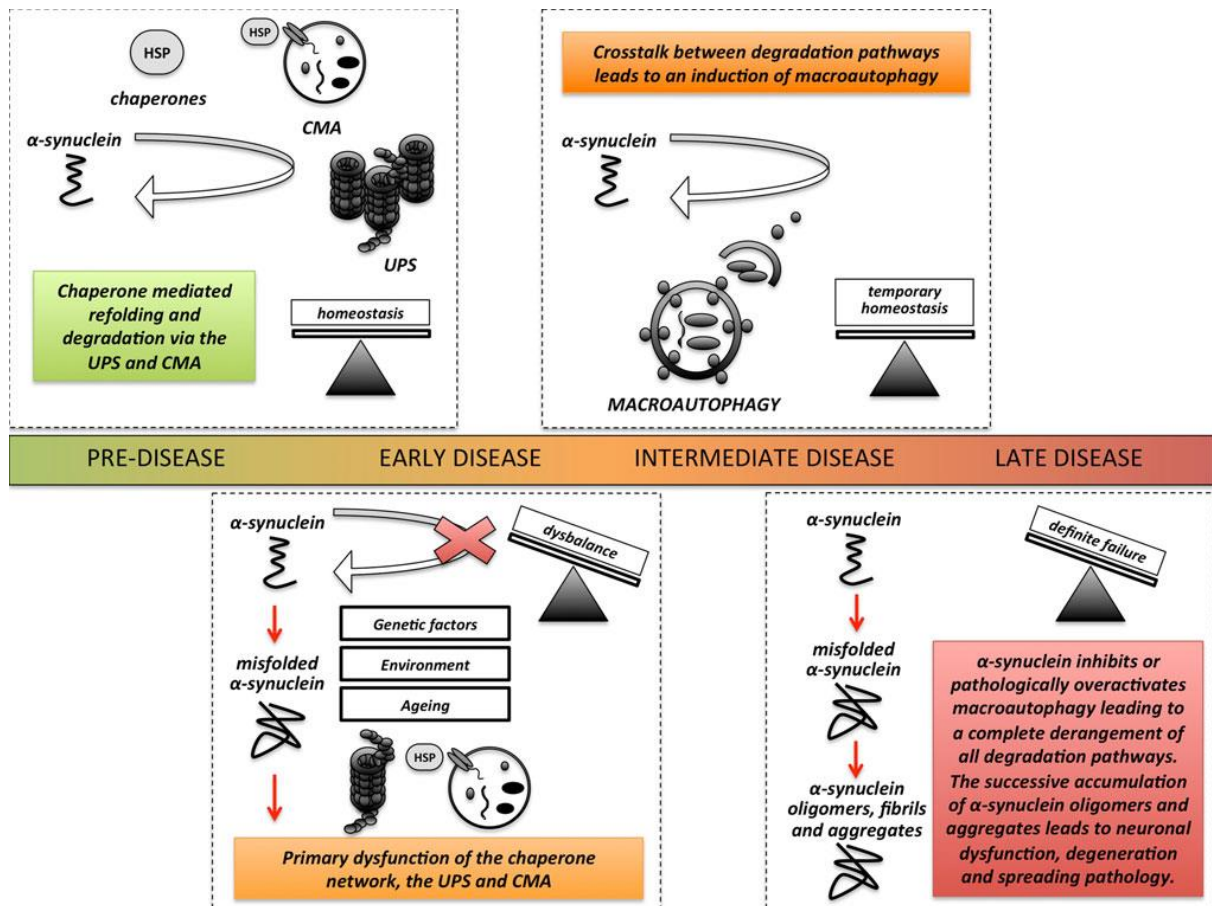


Figure 3. The imbalance of cellular homeostasis in PD mediated by the failure of protein degradation pathways seems to be a key factor in the pathological cascade of the disease. This process leads to the accumulation of toxic α -Syn and finally neuronal dysfunction. This whole process seems to interact reciprocally, feeding back and disseminating neurodegeneration as the disease progresses. UPS: ubiquitin–proteasome system; CMA: chaperone-mediated autophagy; HSP: mediatory heat shock proteins. Extracted from Ebrahimi-Fakhari et al.2012.

3.2. Implications of lysosomal dysfunction as a pathological mechanism of sporadic PD.

As indicated, one of the most significant gene associated with PD is *GBA1*, which encodes for the lysosomal enzyme β -GCase (Sidransky et al., 2009). The discovery of this association took place in the clinic, with the identification of parkinsonian symptoms in patients with Gaucher disease (GD). GD is a lysosomal storage disorder caused by biallelic

mutations in the same *GBA1* gene. Further studies also determined that in relatives of these patients (many of them being carriers of *GBA1* mutations), there was a higher incidence of PD (Goker-Alpan et al., 2004). Cellular and animal models with deficiency in β -GCase activity tend to accumulate α -Syn in the cytoplasm. It has been suggested that this enzyme binds directly to α -Syn in the lysosome, and contributes to the stabilization of oligomeric soluble forms, considered highly neurotoxic (Yap et al., 2011). On the other hand, other studies have shown that α -Syn forms a bond to the lysosomal membrane and interacts with β -GCase, resulting in its inhibition (Yap et al., 2013). Gegg et al. (2012) reported a reduction in the enzymatic activity of β -GCase in the brain of PD patients with mutations in *GBA1*, as well as in sporadic cases. Subsequently, Murphy et al. (2014) corroborated these results, demonstrating that there was a deficit of β -GCase activity in the brain of patients with sporadic PD, being responsible for the accumulation of α -Syn and alterations in lipid metabolism. In a recent work, Bae et al. (2014) demonstrated that β -GCase depletion promotes the propagation of α -Syn aggregates between cells, suggesting that this toxic role could be liable for the disease progression. All these results clearly indicate a strong relationship between β -GCase activity and α -Syn, although it remains to be determined whether the formation of protein aggregates is consequence of a failure in the lysosomal activity or, conversely, that the increase in α -Syn concentration is promoting the β -GCase impairment.

Recent studies describe evidence linking other lysosomal enzymes with different types of Parkinsonism or associated pathologies. For example, they have associated deficiencies in the enzymes **ATP13A2**, **β -hexosaminidase**, **β -galactosidase**, and **cathepsin D** with α -Syn deposits. Kufor-Rakeb syndrome is a type of autosomal recessive inherited Parkinsonism caused by mutations in the gene *ATP13A2* (PARK9), which codes for a lysosomal transmembrane protein. Different studies have shown that a loss of function of this protein

leads to a decrease in the degradation capacity of the lysosome, and therefore resulting in an accumulation of α -Syn (Usenovic et al., 2012; Dehay et al., 2012). Sandhoff's disease, a type of hereditary GM2 gangliosidosis, is another lysosomal disease caused by deficiency of the enzyme β -hexosaminidase, leading to the accumulation of gangliosides in the peripheral and CNS. In knockout mice for this gene, it has been shown the presence of aggregates of α -Syn in a wide distribution of brain areas, including caudate, putamen, and substantia nigra (Suzuki et al., 2003). GM1 gangliosidosis, caused by deficiency of the enzyme β -galactosidase, is characterised by a generalised dystonia and Parkinsonism among other symptoms (Roze et al., 2005). Choroidal neuronal lipofuscinosis includes another heterogeneous group of inherited lysosomal diseases characterised clinically due to a decrease in cognitive abilities, epilepsy and loss of vision due to retinal degeneration. One of the genes identified by the cause of this disorder is *CTSD*, which codes for cathepsin D (Siintola et al., 2006). In vitro experiments have shown that cathepsin D plays an important role in the degradation of α -Syn, in such a way that its deficiency could contribute to the toxic effect of these protein aggregates within dopaminergic cells (Cullen et al., 2009). Furthermore, Chu et al. (2009) found a greater reduction of the expression of cathepsin D in nigral neurons, positive for inclusions of α -Syn in PD patients compared to healthy subjects.

However, the extent of these genes in the development of the sporadic variety of PD is less known. In general, in all these metabolic diseases described, α -Syn deposits and neurodegeneration have been observed that suppose that any defect in any of these lysosomal enzymes could also contribute to the development of the PD.

In addition to the above described, protein aggregation is not the only consequence of lysosomal dysfunction. Most of these enzymes play a very important role in the catabolism of lipids and glycosphingolipids. For example, lysosomal β -GCase catalyses the conversion

of glucosylceramide to ceramide and glucose. The ceramide, together with the cholesterol is responsible for most of the biophysical properties of cell membranes conditioning many of the lipid-protein interactions and cell signalling processes (Castro et al., 2014). Therefore, the reduction of the enzyme can contribute to the loss of these properties and failing that, to cell death. The impaired ceramide metabolism has been linked to impaired cognitive characteristics of AD (Yadav & Tiwari, 2014). Mielke et al. (2013) in a pilot study, also demonstrates its link with cognitive damage in a sporadic variety of PD. In a recent work in an animal model of GD, it has been observed how the accumulation of glucosylceramide leads to a neurodegenerative process (Farfel-Becker et al., 2014). In the same way, other lysosomal enzyme deficiencies leading to lipid accumulation and cholesterol, also present with neurodegeneration and dementia (Sun et al., 2001; Kollmann et al., 2012). It is possible that PD-associated lysosomal dysfunction also leads to increased metabolism lipid defects that is responsible for the cognitive dysfunction found in some patients with the disease.

3.3. Cofactors or activating proteins of sphingolipids necessary for enzymatic hydrolysis: Saposins

In order for these lysosomal enzymes to carry out the hydrolysis of the different substrates, in this case sphingolipids, they require cofactors or Sphingolipid Activator Proteins called saposins (SAPs).

These small thermostable glycoproteins (12-14 kDa) called saposins A, B, C and D, are generated in the lysosome by proteolytic cleavage of the precursor protein Prosaposin (PSAP) (Sandhoff, 2012). These act by facilitating the interaction between the sphingolipids and the enzyme for their degradation (Morimoto et al., 1990; Sandhoff & Kolter, 2003). In particular, Saposin C plays an activating role in the hydrolysis of glucosylceramide (GlcCer)

by the enzyme GCase and of galactocerebrosides by β -galactosidase (Kishimoto et al., 1992). Alterations in these activating proteins have been associated with different lysosomal storage disorders, as occurred in GD (Carvelli et al., 2015). Some of the genetic variants of *GBA1* related to PD (such as p. T369M, p. N370S and p. L444P) have revealed a close relationship with Saposin C, since they are located at or very close to its binding site (Lieberman, 2011; Ouled Amar Bencheikh et al., 2018). This fact suggests that the alteration in the union of the Saposin C and GCase complex could cause a decrease in the activity of the enzyme in carriers of these variants and lead to a higher risk of developing PD

Saposins from the beginning: PSAP protein

The saposin precursor protein PSAP is encoded from the *PSAP* gene, located on chromosome 10q21. In this gene, we find four homologous domains that correspond to each of the saposins for which it codes (Füerst & Sandhoff, 1992). PSAP protein consists of 524 amino acids and has 5 glycosylation sites, two in the sap A domain and one in the others (B,C,D) (Figure 4). Once synthesised, the protein is transported through the ER to the Golgi apparatus, where the glycosylation process takes place. There are studies that support that depending on how this process is carried out, the transport of this protein to the lysosome through the sortilin membrane receptor will be better or worse (Igdoura et al., 1996; Lefrancois et al., 2003). Once there, it is the enzyme Cathepsin D that is responsible for the cleavage of PSAP in its multiple saposin domains (Tamargo et al., 2012).

Prosaposin is also known for other functions that it performs in the organism, such as its relevant role in spermatogenesis (Morales et al., 2000). There are even studies that support its action in early brain development and in hippocampal regeneration processes, considering it a potential neurotrophic factor (O'Brien et al., 1994; Kotani et al., 1996; Xue et al., 2011).

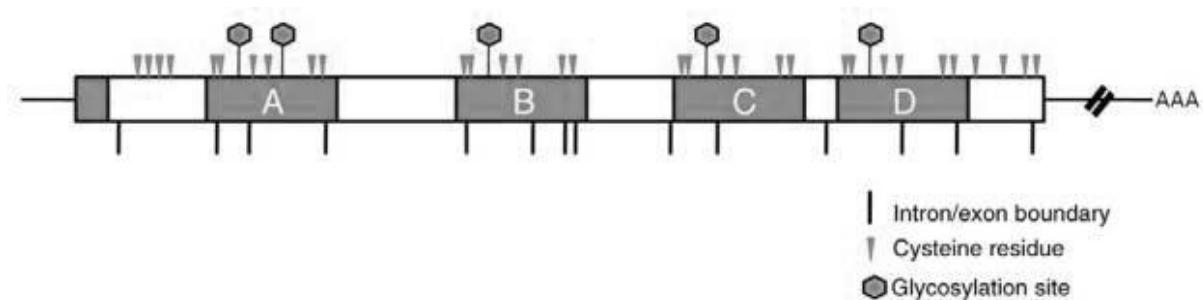


Figure 4. **Structure of the PSAP gene.** The different domains A, B, C and D, cysteine residues and glycosylation sites indicated in the image. Extracted from Tamargo RJ. et al., 2012.

Saposin C: Structure and functions

First described in 1971, as a soluble factor necessary for GCase activation, it is very similar to its other homologues (A, B and D). They are mainly small molecules (8-12 kDa) composed of amino acids, glycosylation sites with highly conserved stable prolines in each of them, in addition to six identically located cysteines. In addition, it is assumed that they are highly compact and rigid proteins, thanks to the disulfide bridges that make up their structure and that give them high thermostability (O'Brien & Kishimoto, 1991; Vaccaro et al., 1995). However, despite the structural similarity, the molecular mechanisms and the interaction with their respective substrates or lysosomal enzymes are different.

Among their functions, saposins share properties related to the union and interaction with certain lipids and/or cell membranes. Specifically, these activating proteins act on intralysosomal molecules, facilitating the selective degradation of certain lipids such as ceramides and sphingolipids, without interfering with the integrity of the lysosome. Specifically, saposin C is known to carry out the metabolism of glucosylceramide in the lysosome, acting directly on the GCase enzyme as an allosteric modulator.

Some models address how this enzyme activation occurs, based mainly on the location of the saposin-mediated lipid-hydrolase interaction, being able to distinguish between two possible hypotheses. In a first approximation, the target molecules would be extracted from the membranes by the saposins. These would act in a similar way to the action of a detergent, solubilizing and/or permeabilizing the membranes, presenting the different residues to be degraded by the digestive enzymes, such as protein complexes or soluble lipids. In a second model, the binding of the enzyme to the membrane would be necessary, where the saposin would facilitate the access to lipid substrates. However, although saposin C has shown high membrane-binding capacity (Wilkening et al., 1998; de Alba et al., 2003; You et al., 2004) and appears to require enzyme binding to facilitate lipid catabolism, it remains unclear whether activation of the enzyme is produced according to one of the previous models.

In addition to lipid interaction and sphingolipid catabolism, saposin C is also considered a protein with a neurotrophic function, since the responsible sequence is found at the amino-terminal end of said domain in the precursor protein. Misasi et al. (2004) observed that PSAP prevents cell death induced by the tumour necrosis factor TNF α via stimulation of signal cascades involving protein kinases. Similarly, saposin C was shown to prevent cell death in cellular models of prostate cancer (Lee et al., 2004).

Saposin C and PD

The relationship between Saposin C and PD has its origin in clinical practice, with GD, as well as with the discovery of the *GBA1* gene in heterozygosis as the main genetic risk factor for PD, as we discussed previously in this chapter.

At the molecular level, GD is characterized by a marked reduction in the lysosomal enzymatic activity of GCase, derived from mutations in the *GBA1* gene. As a consequence of the different mutations that compromise this disease, biochemical and/or structural changes

are even produced, capable of affecting the activation mechanisms of the enzyme and the interaction with saposin C (Salvioli et al., 2005; Liou et al., 2006). Additionally, saposin C deficiency alone is capable of promoting symptoms compatible with GD, highlighting the importance of this protein in the catabolism of glucosylceramide, according to a study carried out by Tylki-Szymańska et al. (2007).

Given the evidences supporting the relationship between GCase and α -Syn protein, saposin C acquires a key role in the possible lysosomal dysfunction and pathogenic process of PD. In addition, due to the neurotrophic character and multiple beneficial effects of PSAP in the nervous system (reviewed in Meyer et al., 2014), as well as the close relationship with GCase, the *PSAP* gene, protein and the sap C domain are great logical candidates for a therapeutic approach in PD for its potential role in lysosomal function.

II. HYPOTHESIS AND OBJECTIVES

Over the past years, many studies have shown the involvement of ALP in the pathology of Parkinson's disease. Recently, lysosomal dysfunction has been proposed as one of the main pathogenic mechanisms of the disease, since the mutation in the GBA1 gene has been considered the main genetic risk factor for its development. This gene codes for one of the main lysosomal enzymes responsible for the correct elimination of substrates, including α -Syn, within the cell. Despite the genetic and molecular evidence described so far, little is known about the specific mechanisms involved in lysosomal dysfunction and how it contributes to the aetiology of Parkinson's disease.

In this doctoral thesis, we decided to study the implication of the lysosomal enzymes to the pathogenesis of PD. For this, a cell model of patients with PD in early stages of the disease was used. Furthermore, a cell model of H4 human neuroglioma was used, subjected to a process of autophagy induction through inhibition of LRRK2 kinase activity, to elucidate how lysosomal dysfunction may contribute to PD. We hypothesised that the activity of lysosomal enzymes may be impaired in PD, favouring the aggregation of α -Syn.

To carry out this study, the following objectives were proposed:

1. To determine the activities of β -glucocerebrosidase (GCase; EC 3.2.1.45), β -galactosidase (β -gal; EC 3.2.1.23), β -hexosaminidase (Hex B; EC 3.2.1.52), and Cathepsin D (CatD; EC 3.4.23.5) in leukocytes obtained from whole blood of patients in early stages of sporadic PD (HY I-II) and compare it with healthy subjects.
2. To analyse if the changes in the previous enzymatic activities are accompanied by changes in their protein concentration.
3. To determine the protein concentration of α -Syn in leukocytes of patients and compare it with that of healthy subjects.

4. To determine the protein concentration of PSAP and Saposin C in leukocytes of patients and compare it with that of healthy subjects.
5. To determine the possible changes in the mentioned indicators between PD states under study (HY I-II)
6. To explore possible associations between the assessed proteins in order to estimate the pathogenicity of PD attributable to the lysosomal dysfunction assessed in these patients
7. To analyse the impact of LRRK2 kinase activity to GCase by using specific inhibitors (LRRK2in1, MLi-2 and CBE) in human neuroglioma cell lines
8. To evaluate the enzymatic activity and protein concentration levels of GCase after treatment with the specific inhibitors of LRRK2 kinase activity in the cell model
9. To assess the implications of the inhibition treatment in the lysosomal expression/distribution and in the macroautophagy process

III. ANALYSIS OF LYSOSOMAL ENZYME ACTIVITY IN PATIENTS WITH PD

3.1. Material and methods

3.1.1. Study Population and Blood Sample Collection

Human whole blood samples were obtained from 42 patients affected by PD, recruited at the Movement Disorders Unit of the Service of Neurology of the University Hospital San Cecilio (Granada, Spain). Inclusion criteria comprised: the diagnosis of PD, according to the UK PD Society Brain Bank (Gelb et al., 1999) ; no reported family history of PD; age between 50 and 80 years old; in Hoehn and Yahr (HY) stages I and II (early stages) (Hoehn & Yahr, 2001); and under treatment with dopamine agonists and levodopa. The control group comprised 37 healthy individuals, partners of PD patients, and/or volunteers with no clinical or neurological signs of Parkinsonism. They were age- and sex-matched with PD patients. All the individuals under study were negative for GBA mutations. The study was approved by the Human Ethics Committee of the hospital and written informed consent was obtained from all participants.

3.1.2. White Blood Cells Extraction

Blood samples were drawn from the cubital vein into EDTA tubes and refrigerated at 4 °C until processed. For white blood cell (WBC) extraction, up to 4 mL of each blood sample were processed in Falcon tubes containing 6 mL of lysis solution for red blood cells (RBC) (RBC lysis solution: 1 mM EDTA solution obtained by a 0.5 M EDTA solution at pH 8.0, prepared with EDTA disodium salt dihydrate from Sigma E-5134, and stored at room temperature). Tubes were mixed by inversion 2 times, incubated for 2 min at room temperature, mixed once more time by inversion and centrifuged at 2000 g (3161 rpm) for 20 min at room temperature. After centrifugation, supernatants were removed, leaving the WBC

pellets at the bottom of the tubes. This procedure was repeated at least 3 or 4 times. Cells were suspended in 1 mL of 1 M NaCl Solution, aliquoted and frozen at -20 °C until use.

3.1.3. Enzymatic Assays

After extraction, NaCl Solution was removed from WBC pellets, and then they were resuspended in RIPA lysis buffer (150 Mm NaCl, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 Mm Tris pH 7.5) together with phosphatase inhibitors (Halt phosphatase inhibitor cocktail, Pierce). Samples were incubated on ice for 30 min and subsequently centrifuged at maximum speed for 20 min at 4 °C. Supernatants were collected and total protein amount was measured using a Bicinchoninic acid (BCA) protein assay kit (Pierce BCA Protein Assay Kit, Thermo Scientific ref. 23227) according to the manufacturer's instructions. All samples were tested for the following lysosomal enzymes: β -GCase and β -gal enzymatic activities were measured as previously described (van Dijk et al., 2013), whereas CatD and Hex B enzymatic activities were measured by commercial enzymatic assay kits (Cathepsin D Activity Assay Kit ab65302 from Abcam and β -N-Acetylglucosaminidase Assay Kit CS0780 from Sigma-Aldrich). Briefly, 20 μ L per sample were incubated in presence of 40 μ L of substrate (4-methylumbelliferyl- β -D-glucopyranoside in citrate/phosphate buffer pH 4.5 plus 0.2% taurodeoxicolate for GCase enzymatic activity, and 4-methylumbelliferyl- β -D-galactoside in citrate/phosphate buffer pH 4.5 for β -gal enzymatic activity) in a black 96-well plate for 90 min at 37 °C. Thereafter, reaction was stopped, 240 μ L of a stop buffer solution (0.2 M Glycine-NaOH, pH 10.4) was added, and fluorescence was measured on a TECAN infiniteM200PRO plate reader (360 nm excitation; 446 nm emission). Activity was expressed as nanomoles of substrate hydrolyzed per minute per milligram of protein (nmol/min/mg). Regarding the commercial enzymatic assay kits, Hex

B activity was measured after the hydrolysis of the substrate N-acetyl- β -D-glucosaminidase, releasing p-nitrophenol, which was detected colorimetrically at 405 nm emission. Activity was expressed in units per milliliter (one unit refers to 1.0 μ mole of substrate hydrolysed to p-nitrophenol per minute at pH 4.7 and 37 °C). CatD activity was quantified after the cleavage of its synthetic substrate containing the sequence GKPILFFRLK (Dnp)-D-R-NH₂, labelled with the fluorescent compound 7-Methoxycoumarin-4-acetic acid (MCA) and using a fluorescence plate reader at 328 nm excitation/460 nm emission. Activity was expressed as relative fluorescence units (RFU) per milligram of protein per millilitre (RFU/mg/mL). All measurements were performed in triplicates. The specific details of every enzymatic assay are summarised in Table 2.

Lysosomal Enzyme	Substrate	Reaction Buffer	Stop Solution	Sample (μ L)	Substrate (μ L)	Stop Solution (μ L)	Time (min)	Temperature (°C)
β -GCase	3 mM 4-methylumbelliferyl- β -D-glucopyranoside	Citrate/phosphate buffer pH 4.5 plus 0.2% taurodeoxicolate	0.2 M Glycine-NaOH pH 10.4	20	40	240	90	37
β -gal	3 mM 4-methylumbelliferyl- β -D-galactoside	Citrate/phosphate buffer pH 4.5	0.2 M Glycine-NaOH pH 10.4	20	40	240	90	37
Hex B	1 mg/mL N-acetyl- β -D-glucosaminidase	Citrate buffer solution (C2488)	Sodium carbonate solution (S2127)	5	95	200	10	37
CatD	1 mM GKPILFFRLK (Dnp)-D-R-NH ₂ with MCA	CD Reaction Buffer (ab65302)	-	50	52	-	90	37

Table 2. *Specific details of every enzymatic assay.*

3.1.4 Antibodies

The antibodies used were as follows: rabbit anti-human GBA antibody (sc-32883; Santa Cruz Biotechnology), mouse anti-human BGAL antibody (sc- 377257; Santa Cruz Biotechnology), mouse anti-human CTSD antibody (sc-377299; Santa Cruz Biotechnology), rabbit anti-human HEXB antibody (PA-36146; ThermoFisher), rabbit anti-human Saposin C antibody (sc-32875; Santa Cruz Biotechnology), mouse anti-human β -Actin antibody (A1978-200UL; Sigma-Aldrich), anti-rabbit antibody (sc-2004; Santa Cruz Biotechnology), and anti-mouse antibody (A2304; Sigma-Aldrich). All antibodies were used at 1:500 in 5% protein blocker in Tris-buffered saline with 0.1 % Tween 20, except for mouse anti-human β -Actin antibody, which was used at 1:50.000 in 5% protein blocker in Phosphate buffered saline with 0.1% Tween 20.

3.1.5. Western Blot Analysis

The protein concentration of WBC lysates was measured by BCA protein assay (Pierce BCA Protein Assay Kit, 23225 Thermofisher Scientific). An amount of 15 μ g of proteins was loaded in every lane of 10% SDS-PAGE gels. After electrophoresis, proteins were transferred to PVDF membranes (0.2 μ m Immuno-Blot PVDF Membrane 162-0177 Bio-Rad) for 30 min. Membranes were blocked in 5% non-fat dried milk for 1 h, and then incubated in the appropriate primary antibodies overnight. Immunoblots were performed for proteins GBA, BGAL, HEXB, CTSD, α -Syn, and β -Actin as protein loading controls. Furthermore, PSAP and Saposin C were also assayed due their activator role in the hydrolysis of glucosylceramide and galactocerebroside by β -GCase and β -gal enzymes, respectively. Membranes were developed after incubation with the respective horseradish peroxidase-linked secondary antibodies using enhanced chemiluminescence (ECL). Bands were

visualized using the digital imaging system Kodak Image Station 4000 MM PRO and then quantified using the ImageJ software.

3.1.6. Statistical Analysis

Statistical analysis was performed using GraphPad Prism, version 5 (GraphPad Software, San Diego, CA, USA). All quantitative data were averaged across multiple experiments, specifying the number of experiments in the corresponding figure legends. To determine statistically significant differences between groups, p values were calculated using the non-parametric Mann–Whitney U test and the Kruskal–Wallis post hoc test. Differences were considered significant at $p < 0.05$. The Spearman correlation coefficient was used when appropriate to test the association between demographic and experimental variables.

3.2. Results

Demographic and clinical data are summarized in Table 3. The mean age at PD onset was 60.06 ± 10.77 years (mean $\pm$ SD). All PD patients recruited were categorized as early I and II disease stages according to the Hoehn and Yahr scale. Total WBC protein concentration was significantly higher in PD patients compared to controls. There were no statistical differences between groups for gender and age.

	Controls	PD Patients	p-Value
Number of individuals	37	42	NA
Men	11	22	0.06
Women	26	20	
* Age (y)	68.3 ± 7.44	66.4 ± 10.28	0.35
* Age at onset (y)	-	60.06 ± 10.77	NA
Hoehn and Yahr stage			NA
I	-	21	
II	-	21	
* WBC total protein $\mu\text{g}/\mu\text{L}$	0.96 ± 0.33	1.16 ± 0.39	0.01

Table 3. **Demographic and clinical data of the study cohort.** * Data were reported as mean $\pm$ SD; NA: not applicable.

3.2.1. Evaluation of the Lysosomal Enzymatic Activities

The β -GCase, β -gal, Hex B and CatD enzymatic activities in WBC from controls and PD patients are reported in Figure 5.

β -GCase and β -gal enzymatic activities were significantly lower in PD patients compared to controls (β -GCase: controls = 13.02 ± 0.89 nmol/min/mg; PD = 10.44 ± 0.63 nmol/min/mg; $p < 0.05$; β -gal: controls = 20.37 ± 1.06 nmol/min/mg; PD = 18.02 ± 0.10 nmol/min/mg; $p < 0.05$), both showing a reduction of 20%. (Figure 5 A, C). No significant differences were found for Hex B activity between PD patients and controls (controls = 0.98 ± 0.09 U/mL; PD = 0.90 ± 0.12 U/mL; $p = 0.122$) (Figure 5B). Surprisingly, CatD activity

was significantly higher in PD patients than in controls (controls = 51646 ± 11320 UFR/mg/mL; PD = 102024 ± 11985 UFR/mg/mL, $p < 0.001$), reaching an increase of 100% (Figure 1D).

We also examined whether age and gender were associated with lysosomal enzymatic activities among PD patients and controls, but we did not find any significant association.

3.2.2. Determination of GBA, BGAL, HEXB, CTSD, and α -Syn Protein Levels

We determined the protein levels of the above referred enzymes through Western blot analyses (Figure 6). We also evaluated PSAP and Saposin C protein levels, since they are the natural cofactors of both β -GCase and β -gal enzymes, and α -Syn protein level, whose metabolism is linked to lysosome activity (Figure 7).

Blots revealed no significant differences in GBA and HEXB protein levels between PD patients and controls (Figure 6 B, C). Conversely, BGAL and CTSD protein levels were significantly higher in PD patients, with an increase of 50% compared to controls (Figure 6 C, D). We also explored BGAL and CTSD protein levels within PD patients, distinguishing between HY stages I and II, and observed that only PD patients at HY stage I showed a significant increase with respect to controls.

In relation to PSAP and Saposin C protein levels, both experienced a significant decrease of approximately 40% in PD patients compared to controls (PSAP: controls = $1,139 \pm 0,2170$; PD = $0,6878 \pm 0,05014$, $p < 0.05$; Saposin C: controls = $0,2255 \pm 0,04037$; PD = $0,1203 \pm 0,01337$, $p < 0.05$). No differences were found between disease stages (Figure 7 C, D).

Finally, as expected, a higher level of α -Syn protein was found in PD patients in comparison to controls (controls = $0,7898 \pm 0,1195$; PD = $1,477 \pm 0,1738$, $p < 0.01$), which was consistent with the disease progression (Figure 7 E).

In order to explore possible associations between the assessed proteins, we performed Spearman's Rank Correlation Coefficients. The results showed a strong negative correlation between PSAP and α -Syn protein levels ($r = -0.6357$, $p = 0.01$), in addition to a significant positive correlation between CTSD and α -Syn protein levels ($r = 0.6485$, $p = 0.04$) (Figure 8 A, B).

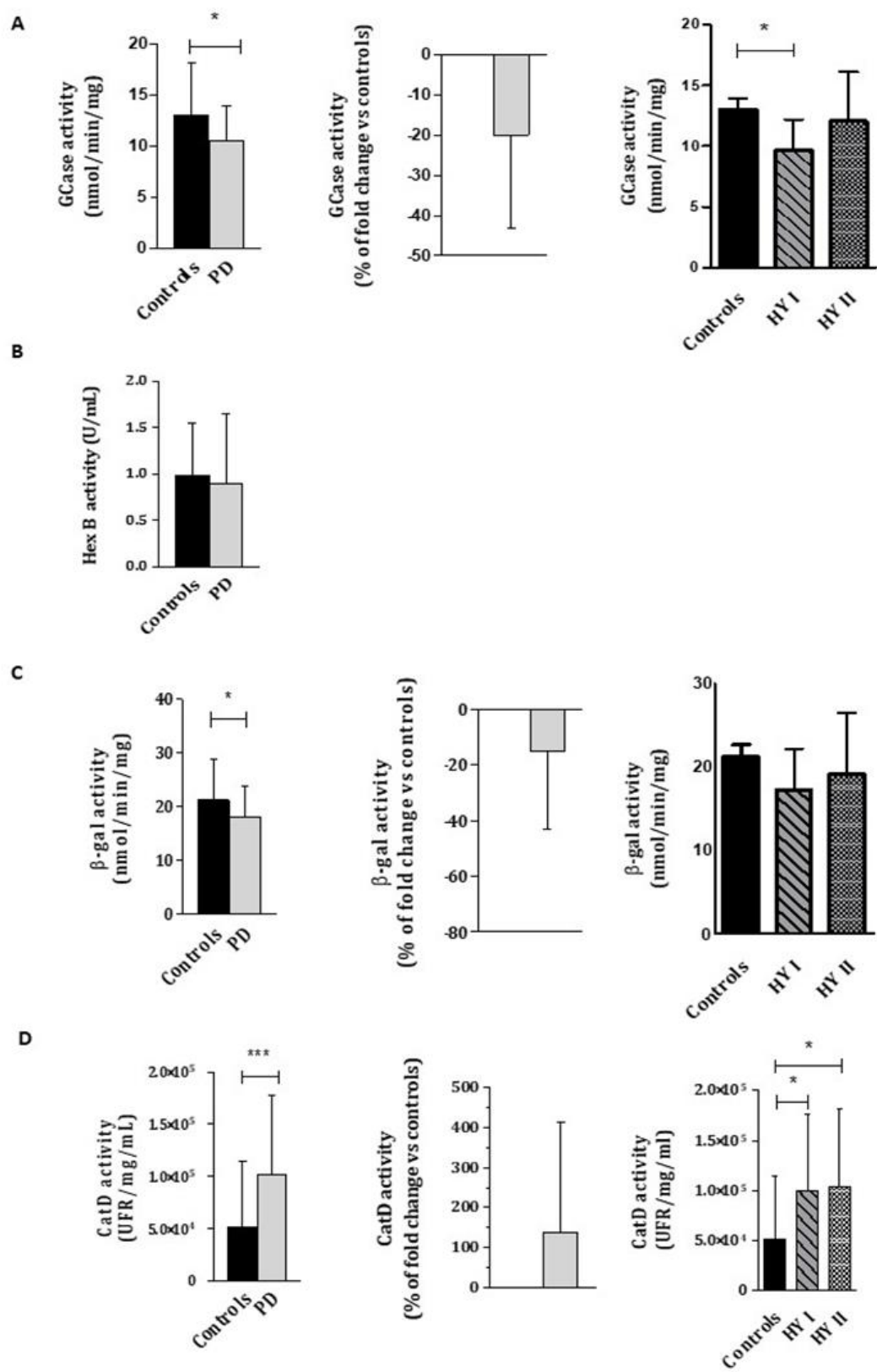


Figure 5. *Lysosomal enzymatic activity of β -GCase (A), Hex B (B), β -gal (C), and CatD (D) in WBC from PD patients compared to healthy controls. The percentage of fold change over controls was calculated as follows: (PD value—control activity average)/control activity average x 100. Data show mean $\pm$ SEM. * $p < 0.05$; *** $p < 0.001$.*

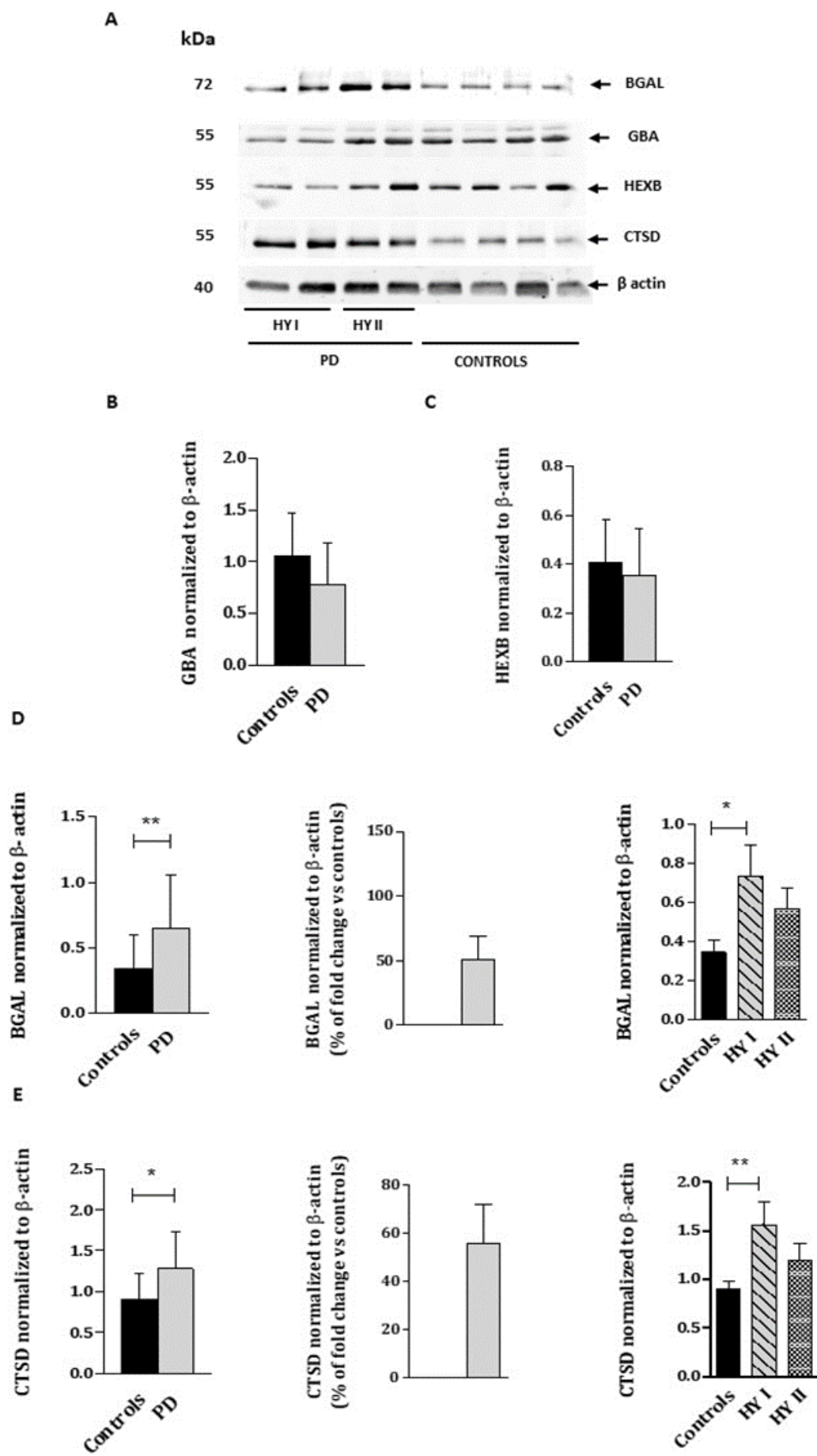


Figure 6. Immunoblot analysis of GBA, BGAL, HEXB, and CTSD proteins from the WBC lysates of PD patients and healthy controls (A). Molecular weight markers are indicated in kilodaltons. Quantification of GBA (B), BGAL (C), HEXB (D), and CTSD (E) protein levels normalized to loading control β -actin. HYI and HYII corresponded PD patients at Hoehn and Yahr stages I and II. Quantifications were based on four independent experiments (N = 16 per group). The percentage of fold change over controls was calculated as follows: (PD value– control protein average)/control protein average $\times$ 100. Values represent mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$.

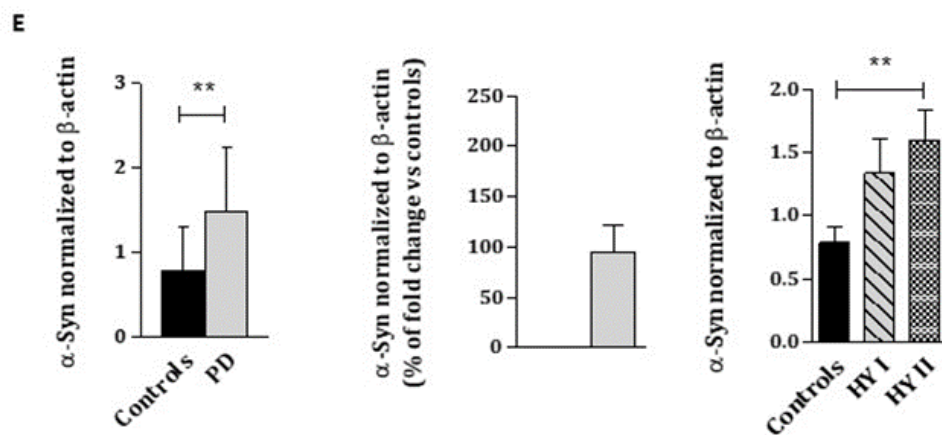
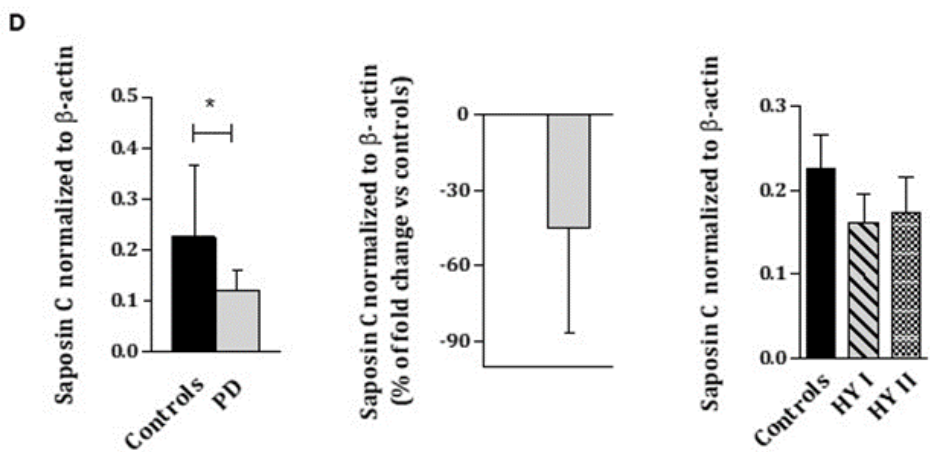
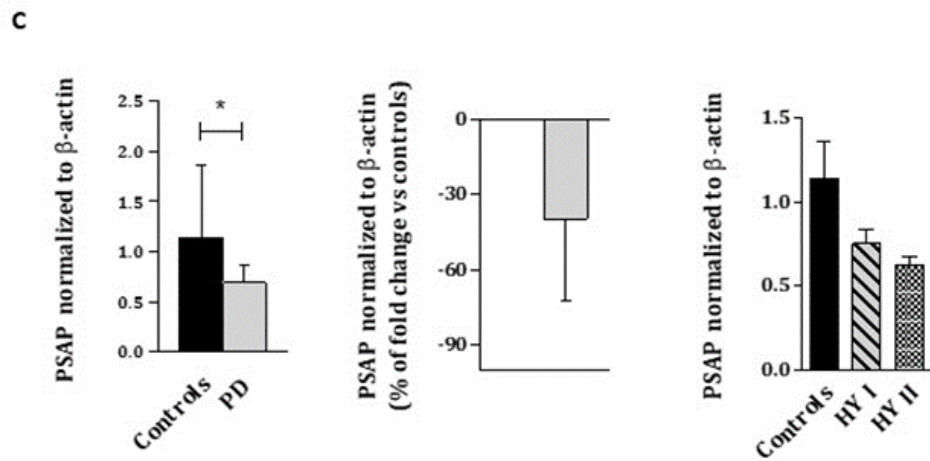
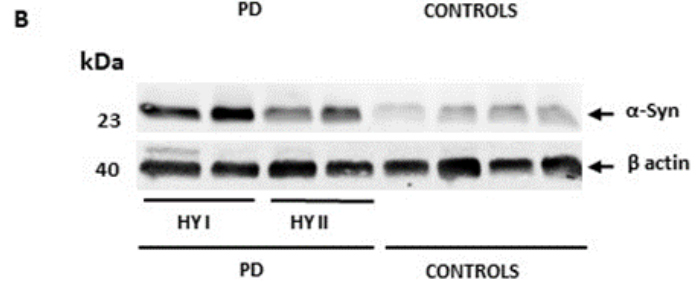
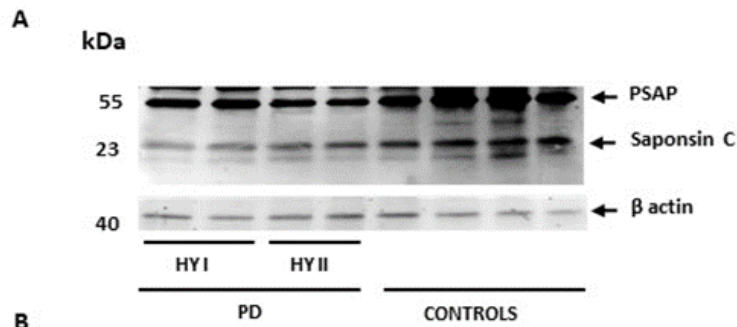


Figure 7. *Immunoblot analysis of PSAP, Saposin C (A), and α -Syn (B) protein levels from the WBC lysates of PD patients and healthy controls. Molecular weight markers are indicated in kilodaltons. The quantification of PSAP (C), Saposin C (D), and α -Syn (E) protein levels normalized to loading control β -actin. HYI and HYII correspond to PD patients at Hoehn and Yahr stages I and II. Quantifications were based on four independent experiments (N = 16 per group). The percentage of fold change over controls was calculated as follows: (PD value—control protein average)/control protein average $\times$ 100. Values represent mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$.*

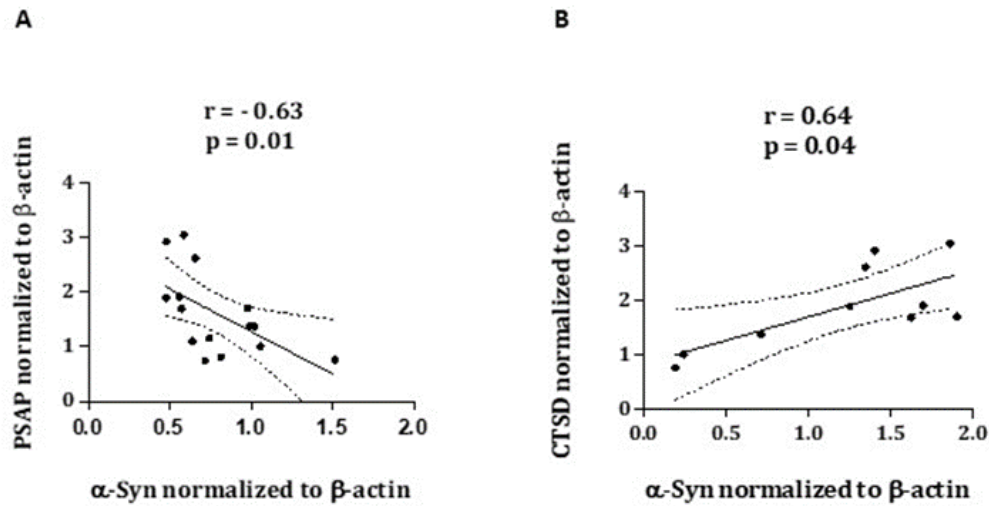


Figure 8. *Correlation analysis of PSAP and α -Syn (A) and CTSD and α -Syn (B), obtained from Western blots from 15 and 10 PD patients, respectively.*

3.3. Discussion

A number of recent investigations have reported a close relationship between lysosomal dysfunction, α -Syn aggregation, and PD (Bellomo et al., 2020; Moors et al., 2016). In this sense, lysosome play an essential role in α -Syn degradation, and furthermore, within the lysosomal enzymes, β -GCase and CatD have been directly involved in the α -Syn metabolism. Reduced β -GCase activity has been observed in the brain and cerebrospinal fluid of PD patients with GBA mutations, as well as in sporadic PD patients (Gegg et al., 2012; Murphy et al., 2014; van Dijk et al., 2013). However, when this activity is explored in peripheral tissues, results are incongruous (Niimi et al., 2019; Wu et al., 2008). In this study, we provide evidence supporting that PD is associated with impaired lysosomal enzymatic activity, along with increased α -Syn and reduced PSAP and saposin C protein levels. We observed a significant decrease in both β -GCase and β -gal enzymatic activities in the WBC lysates of PD patients, whereas CatD enzymatic activity was significantly increased in comparison to controls. No significant difference was found for Hex B enzymatic activity. Moreover, when we explored their corresponding protein levels, significant changes were only observed for BGAL and CTSD proteins, as they were increased in the WBC lysates of PD patients in comparison to controls. Supporting our results, as is mentioned above, reduced β -GCase activity has been reported in PD patients compared to controls (van Dijk et al., 2013; Parnetti et al., 2014:2017; Balducci et al., 2007). This decreased activity seems to be promoting the accumulation of α -Syn, compromising the protein degradation capacity of lysosome and consequently favoring the formation of α -Syn aggregates. The cause underlying the decrease in β -GCase activity might be due to an impaired trafficking of GBA through the endoplasmic reticulum (ER)/Golgi apparatus (GA) that hinders its correct localization in the

lysosome (Gegg et al., 2012; Mazzulli et al., 2011). Although the aim of this study was not to investigate how GBA and α -Syn interact, we reported a significant increase of α -Syn levels in PD patients in comparison to controls. Likewise, β -gal activity was significantly reduced in the WBC lysates of PD patients compared to controls. Previous studies have reported similar results in the substantia nigra (Huebecker et al., 2019) as well as in CSF of PD patients (Parnetti et al., 2014; Balducci et al., 2007). Furthermore, the decrease observed in both β -GCase and β -gal activities was linked with a significant reduction in the PSAP and Saposin C protein levels. Surprisingly, we also found a negative correlation between PSAP and α -Syn protein levels. Taken together, the depletion observed in these enzymes might result from a reduction in PSAP and Saposin C protein levels. Since Saposin C deficiency has been associated with GD and GD-like diseases (Tamargo et al., 2012; Motta et al., 2014; Vaccaro et al., 2010), several studies have investigated the contribution of this natural cofactor to PD. Although there is no strong evidence that supports a genetic association between rare or common variants in PSAP gene and PD (Sosero et al., 2020; Chen et al., 2021), several cell models have showed how Saposin C protects β -GCase from protease degradation in the lysosome and prevents its inhibition mediated by α -Syn (Sun et al., 2003; Yap et al., 2013). In a recent work on peripheral blood mononuclear cells of PD patients, Avenali et al. (2021) found a significant reduction in Saposin C levels in PD patients carrying GBA mutations in comparison to non-mutated PD patients but not versus healthy controls. However, exosomal α -Syn protein levels were significantly elevated in both GBA-PD and non-mutated PD patients compared to healthy controls. The deficiency observed in Saposin C could be a consequence of a negative feedback loop from increased α -Syn protein levels. It has been shown that α -Syn perturbs protein trafficking machinery downstream at the GA, resulting in the accumulation of immature proteins (Mazzulli et al., 2011; Stojkovska et al., 2021). Thus,

it is possible that PSAP does not correctly reach the lysosome where it is processed into active saposins, contributing in part to the reduction in β -GCase activity observed in PD patients.

The lysosomal enzyme CatD has recently emerged as a key mediator in α -Syn proteolysis (Sevlever et al., 2008; Cullen et al., 2009). Huarcaya et al., (2022) showed that the amount of mature CTSD protein was proportional to the reduction of α -Syn protein level in H4 neuroglioma cells overexpressing α -Syn protein. Moreover, Chu et al. (2009) found a higher reduction in CTSD expression levels of nigral neurons positive for α -Syn inclusions in PD patients compared to healthy subjects.

Here, we found that: (1) CatD enzymatic activity was significantly increased in PD patients compared to controls, being also parallel to its protein levels, and (2) there was a significant positive correlation between both CTSD and α -Syn proteins. These results were surprisingly opposed to the most scientific evidence focused on lysosomal enzymes, in which CatD activity and CTSD protein levels were reduced in PD (Parnetti et al., 2017; Balducci et al., 2007; Chu et al., 2009). Nevertheless, when we explored CTSD expression taking PD progression into consideration, it was notably higher in PD patients at HY stage I, being similar to controls in PD patients at HY stage II. It is possible that this observed increase responds to a positive regulation mechanism in an early attempt of the lysosome to restore the α -Syn clearance, and subsequently, in advanced stages, CTSD shows a reduction (Moors et al., 2018). Several studies have documented that endogenous ceramide levels in endolysosomal compartments regulate CatD enzymatic activity. Ceramides seem to interact with pre-pro CTSD isoforms, promoting their proteolytic processing to mature and active isoforms (Heinrich et al., 1999:2000). Thus, the expected increase in monohexyl-ceramides derived from the deficiency of both β -GCase and β -gal activities observed in PD patients,

might induce a rapid activation of CTSD, eventually resulting in the higher activity of the enzyme.

VI. ANALYSIS OF THE INHIBITION OF LRRK2
KINASE ACTIVITY ON GCASE ACTIVITY IN
HUMAN NEUROGLIOMA CELL LINES

4.1. Material and methods

4.1.1. Inhibitors

LRRK2in1 and MLi-2 were purchased from the Eurodiagnostico (HY-10875 100mg and HY-100411 5mg respectively). Conduitirol-beta-epoxide (CBE, sc-201356) was purchased from Santa Cruz. All compounds used were dissolved in dimethyl sulfoxide (DMSO).

4.1.2. Antibodies

Antibodies used were as follows: rabbit anti-human LC3 antibody (NB100-2220, Novus Biologicals), mouse anti-human LAMP-1 antibody [H4A3] (ab 25630, Abcam), mouse anti-human β -Glucocerebrosidase antibody (GBA) (B-6) (sc-166407, Santa Cruz), rabbit recombinant anti-rab10 (phospho T73) antibody (ab230261, Abcam), rabbit monoclonal anti-Rab10 (8127S, Cell Signaling), mouse anti-human β - Actin antibody (A1978-200UL, Sigma), anti-rabbit antibody (A0545, sigma) and anti-mouse antibody (A3682, Sigma).

4.1.3. Cell culture and pharmacological treatments

H4 human neuroglioma cells were grown in DMEM containing 10% of Fetal Bovine Serum (FBS). H4 cells (ATCC number HTB-148) were seeded into six-well plates (2 ml for each well) once they reached the 90% of confluence (2×10^5 cells/ml approximately). After 24 hours from plating, cells were treated with LRRK2in1, MLi-2 (both selective LRRK2 inhibitors with unrelated structures), CBE (an irreversible β -GCase enzyme inhibitor) or vehicle (DMSO as control) under 24 or 72 h treatments. For 72 h treatment, medium was replaced with LRRK2in1, MLi-2 and CBE, and cells were incubated for 72 hours. For short-term treatment, medium was replaced as above and cells were incubated for 3 days.

Information about concentrations of every inhibitor is reported in Table 4. LRRK2in1 and CBE concentrations used for both experimental conditions were previously described and tested in several studies finding no signs of toxicity for cells (Manzoni et al., 2013, 2016; Smith et al., 2016). MLi-2 was used at a concentration of 600 nM based on previous works (Volpicelli-Daley et al., 2016; Kluss et al., 2018; Bright et al., 2021). Cell viability was analyzed by Trypan blue assay.

72 h treatment		
Inhibitor	Concentration	Pharmacological effect
LRRK2in1	5 μ M	LRRK2 kinase domain inhibitor
MLi-2	600 nM	LRRK2 kinase domain inhibitor
CBE	75 nM	β -GCase enzyme inhibitor
DMSO	0.15% v/v	Organic solvent
24 h treatment		
Inhibitor	Concentration	Pharmacological effect
LRRK2in1	5 μ M	LRRK2 kinase domain inhibitor
MLi-2	600 nM	LRRK2 kinase domain inhibitor
CBE	75 nM	β -GCase enzyme inhibitor
DMSO	0.15% v/v	Organic solvent

Table 4. Inhibitor Concentrations for the 24 h and 72 h treatments.

After treatment, cells were washed in Dulbecco's phosphate buffered saline (DPBS), collected in RIPA lysis buffer: 150 Mm NaCl, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 50 Mm Tris pH 7.5, protease inhibitors (cOmplete, protease inhibitor cock-tail, Roche) and phosphatase inhibitors (Halt phosphatase inhibitor cocktail, Pierce), and incubated on ice for 30 minutes. After centrifugation at 17,000 g, 4°C for 20 minutes, the Triton-soluble supernatant was stored at -20°C until analysis. Both experimental conditions (24 and 72 h treatments) were repeated to obtain independent replicates.

4.1.4. β -Glucocerebrosidase enzymatic assay

GCase activity was determined using the artificial substrate 4-Methylumbelliferyl (4-MU) β -D-glucopyranoside (M3633, Sigma Aldrich) as previously described by Dijk et al. (2013). Briefly, the collected samples were incubated with the substrate in citrate phosphate buffer pH: 4.5 (0.1 M citric acid / 0.2 M solution dibasic sodium phosphate) with 0.2% of Sodium taurodeoxycholate hydrate (W3026000, Sigma Aldrich) in a 96 well plate at 37°C° for 90 minutes. After incubation, the reaction was stopped with glycine-NaOH buffer 0.2 M pH: 10.4 (0.2 M solution Glycine/ 0.2 M NaOH). Fluorescence was measured using an Infinite M200Pro TECAN plate reader with 360 nm excitation wavelength 446 nm emission wavelength. A standard curve of free 4-MU was used as reference to calculate the enzyme activity. Results were normalized to protein content.

4.1.5. Endo-H treatment

10 μ g of proteins from cell lysates were subjected to one hour incubation at 37°C with Endoglycosidase-H (Endo-H) according to the manufacturer's instructions (Endo H, P0702S, New England BioLabs).

4.1.6. SDS-PAGE and western blot analysis

Protein concentration of cell lysates was measured by BCA protein assay (Pierce BCA Protein Assay Kit, 23225 Thermofisher Scientific). 10 μ g of proteins were loaded in each lane of 4-12% Bis-Tris NuPAGE gels (Invitrogen) and separated according to the manufacturer's instructions. After electrophoresis, proteins were transferred to PVDF membranes (IPVH00010, Immobilon Millipore) for 2 h. Membranes were blocked in 5% milk for 1 h, and then incubated in the appropriate primary antibodies (diluted in Superblock solution,

ThermoFisher Scientifics) overnight. Bands were visualized after incubation with the respective horseradish peroxidase-linked secondary antibodies using Enhanced Chemiluminescence (ECL) in the digital imaging system Kodak Image Station 4000MM PRO. Then, bands were quantified using the ImageJ software (NIH).

4.1.7. Statistical analyses

Data from each experimental condition were pooled and normalized to perform analysis of variance (ANOVA) followed by Tukey and Dunnett post hoc tests. Values are represented as the mean and standard deviation (SD), where $p < 0.05$ was considered statistically significant. All statistical analyses were performed with GraphPad Prism (GraphPad Software, San Diego, CA, USA).

4.2. Results

To examine the impact of LRRK2 inhibition upon GCase activity, H4 neuroglioma cells were exposed to 24 hours (h) and 72 h treatments with LRRK2in1 and MLi-2, two inhibitors of LRRK2 kinase activity (Deng et al., 2011; Fell et al., 2015). 24 h inhibition of LRRK2 kinase activity did not result in any significant alteration in GCase activity (Figure 9A). Following extended treatments with LRRK2in1 and MLi2 over 72 h, GCase activity was also comparable to DMSO treated cells (Figure 9B). As expected, CBE treated cells showed reduced GCase activity in both experimental conditions. To assess possible changes in protein expression levels after treatments with the inhibitors, immunoblot analysis of GBA was performed alongside the lysosomal marker LAMP1 and the marker for macroautophagy LC3 (Figure 9C-D). When 24 h treatment with LRRK2in1 was applied, only LC3-II displayed significantly increased protein levels compared to DMSO treated cells (Figure 9I), while 72 h treatment resulted in significantly increased protein levels for both GBA and LC3-II when compared to DMSO treated cells (Figure 1F, J), and LAMP1 when compared to CBE treated cells (Fig 9H). However, the treatment with MLi-2 did not show significant changes in any of the three proteins for both experimental conditions (Figure 9E-J).

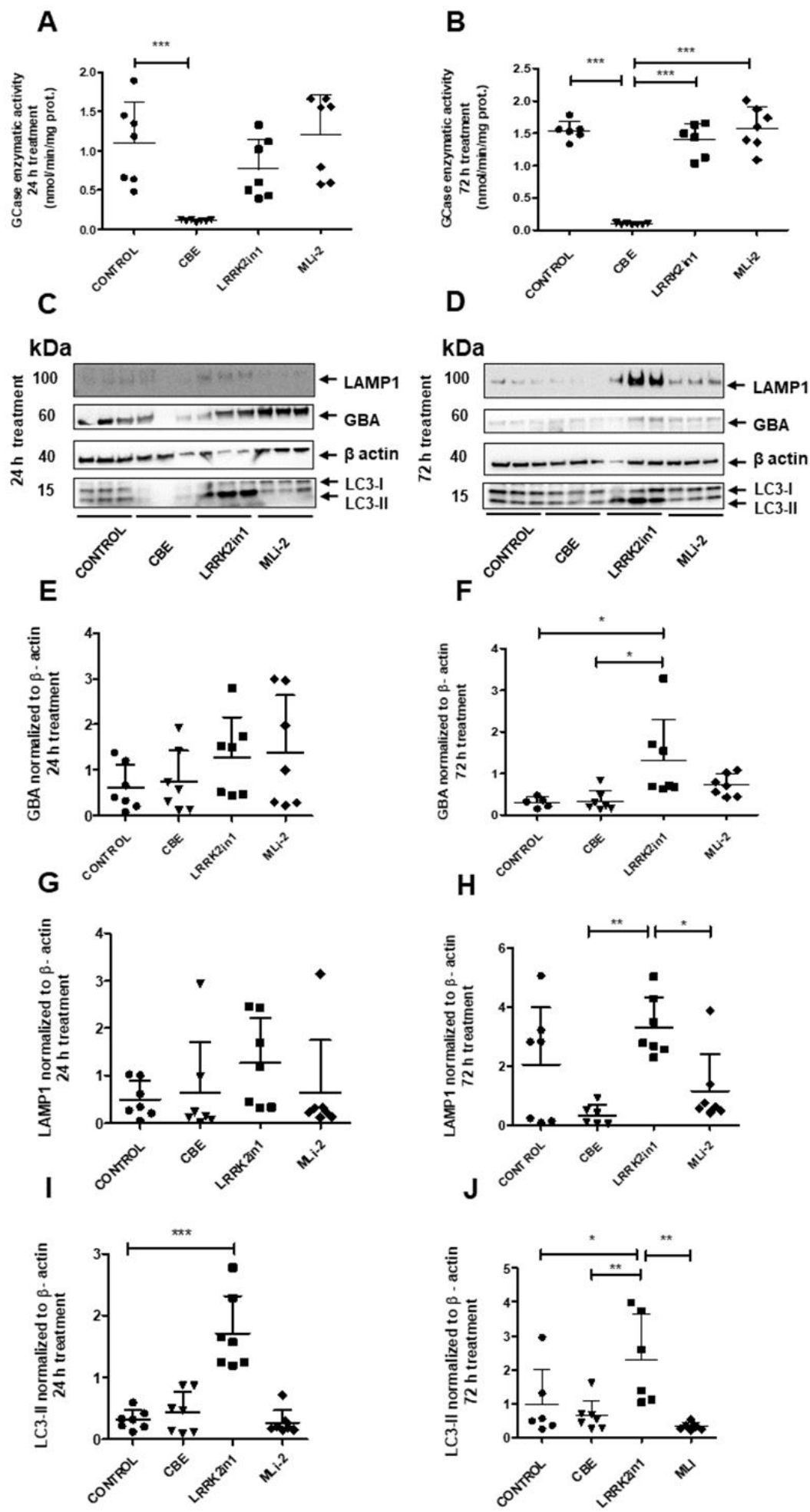


Figure 9. Data obtained from enzymatic activities and protein expression levels assessed 24 and 72 hours post treatments in H4 cells. (A) 24 h inhibition with both LRRK2-in1 and MLI2 produced no significant increase of GCase enzymatic activity compared to control cells. Quantifications were based on seven independent experiments. (B) Inhibition of LRRK2 kinase activity did not show significant changes of GCase activity in H4 neuroglioma cells after exposure to 72 treatment when compared with control cells. Quantifications were based on seven independent experiments. (C, D) Immunoblot analysis of GBA, LAMP1 and LC3-II proteins from cell lysates 24 and 72 h treatments. Molecular weight markers are indicated in kilodaltons. (E-J) Quantification of GBA, LAMP1 and LC3-II protein levels in cell lysates after treatment normalized to loading control β -actin. 24 h inhibition of the kinase activity of LRRK2 increased significantly the LC3-II protein levels when LRRK2in1 was applied (I). However, after 72h inhibition, both LC3-II and GBA protein levels were significantly increased (F, J). Data of each experimental condition were grouped and normalized to perform analysis of variance followed by Tukey and Dunnett post hoc tests (values represent mean and SD, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

We also examined the phosphorylation status of Rab10 as a direct readout for LRRK2 kinase activity. As expected, treatments with LRRK2in1 and MLI2 decreased the phosphorylation of Rab10 (pRab10), confirming the efficacy of both inhibitors (Figure 10).

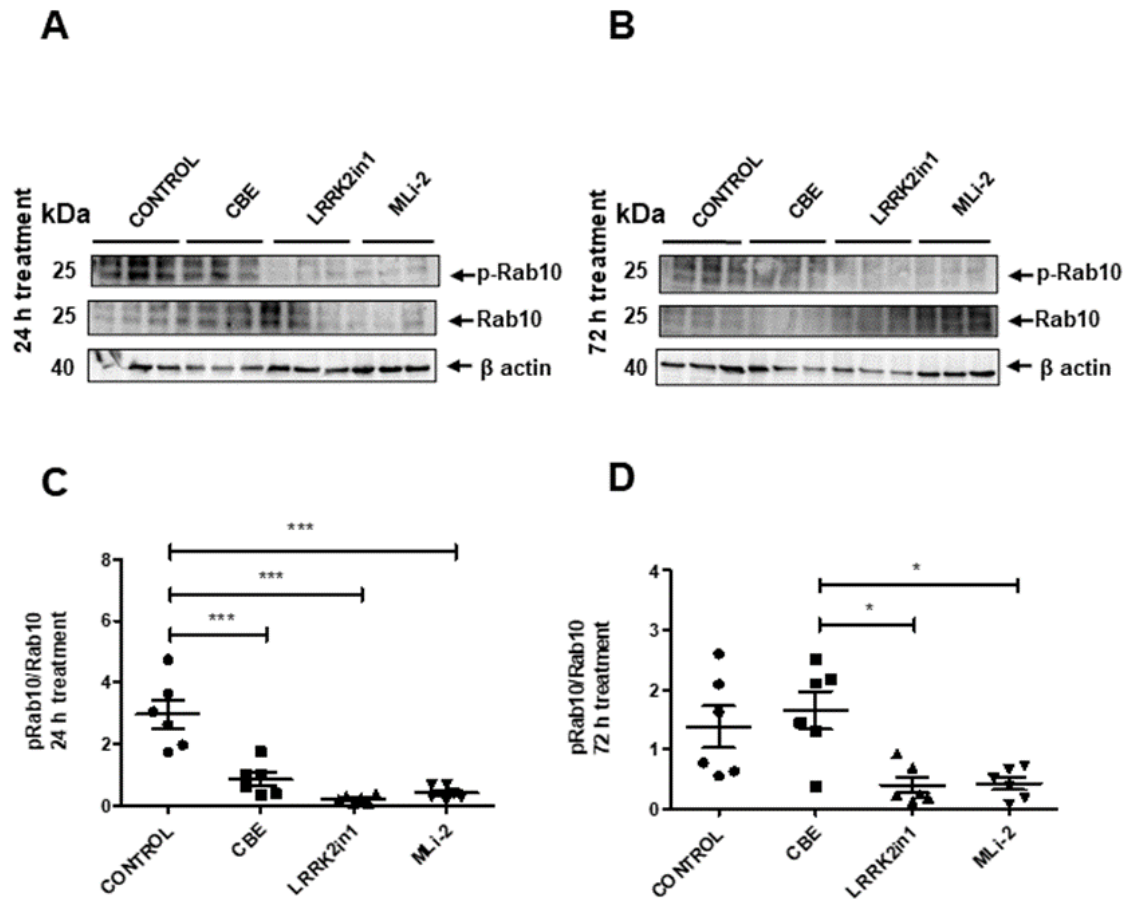


Figure 10. *Phosphorylation status of Rab10 as a direct readout for LRRK2 kinase activity.* (A, B) Representative western blot of pRab10 and total Rab10 in cell lysates treated with LRRK2in1, MLI2 and CBE. (C, D) Images were quantified by normalization to total Rab10 protein levels from six independent experiments. Data demonstrated a significant reduction of the pRab10/Rab10 ratio in cell lysates treated with both LRRK2 inhibitors.

To recapitulate both results in enzymatic activity and in protein levels we examined the magnitude of change in specific GCase activity dividing the average GCase activity by the estimate of GBA protein quantification. Following 24 h treatment, control cells showed a

GCase activity/GBA estimate ratio of 4.694 while cells treated with LRRK2in1 and MLI-2 inhibitors showed a GCase activity/GBA estimate ratio of 1.200 and 2.902, respectively. After 72 h treatment, control cells showed a GCase activity/GBA estimate ratio of 4.863, while cells treated with LRRK2in1 and MLI-2 inhibitors showed a GCase activity/GBA estimate ratio of 1.375 and 2.423, respectively (Figure 11).

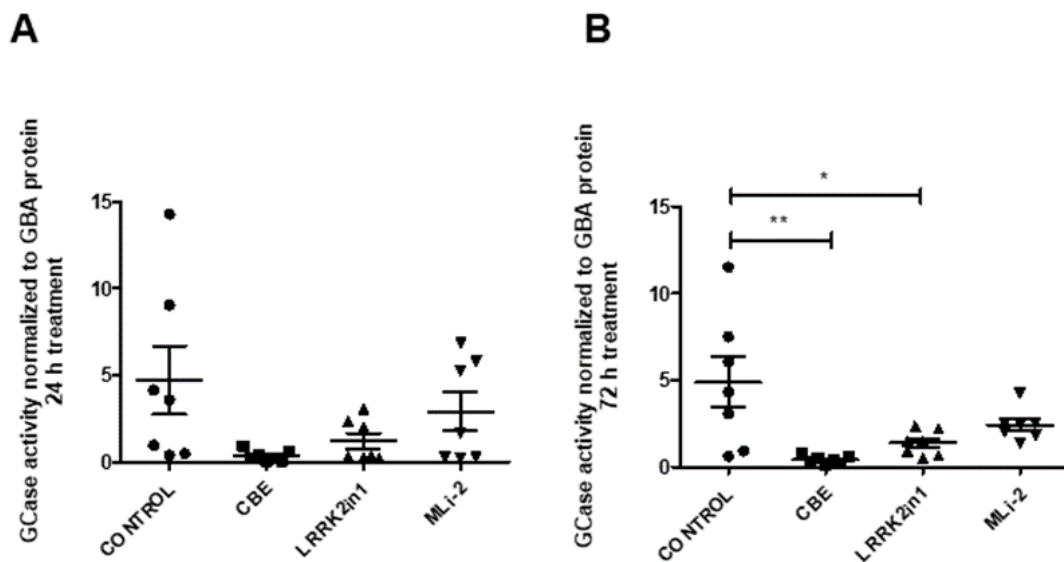


Figure 11. GCase activity/GBA estimate ratio of cell lysates treated with LRRK2in1, MLI2 and CBE (A, 24 h treatment; B, 72 h treatment). Specific GCase activity is significantly reduced in cell lysates after 72 h treatment with LRRK2in1 compared to DMSO treated cells.

To explore if the observed decrease in specific GCase activity was due to an accumulation of immature GBA caused by defective protein trafficking/sorting through Golgi apparatus, cell lysates were subjected to Endo-H digestion. The results presented in Figure 12, showed no relevant changes in the Endo-H cleavage pattern, suggesting that most of the GBA protein is being correctly sorted to the mid Golgi and matured to lysosomal GBA.

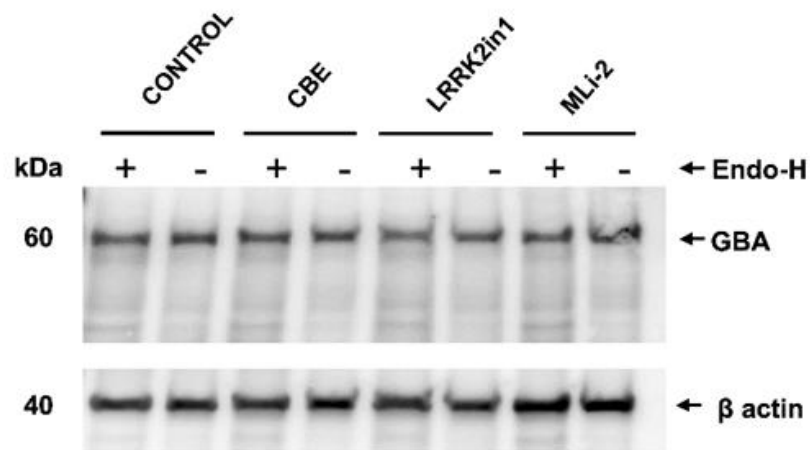
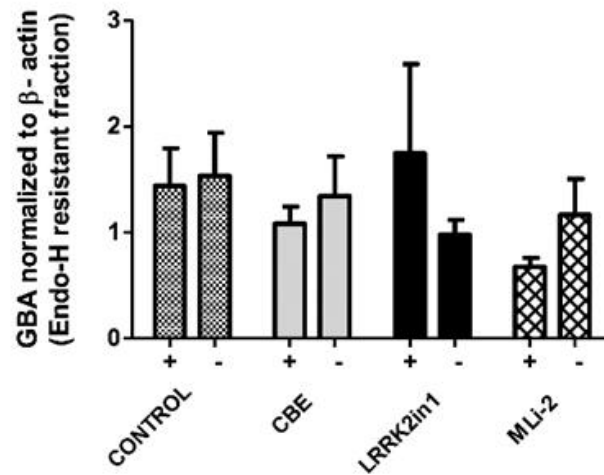
A**B**

Figure 12. *Endo-H resistance of GBA protein in cell lysates.* (A) Cell lysates containing the same amount of protein were subjected to endo-H digestion and western blot analysis with anti-GBA antibody. (B) Blots were scanned and the intensity of each band was measured. Bars (+) represent Endo-H resistant fraction of GBA protein normalized to loading control β -actin from three independent experiments. Data show no significant differences in GBA protein levels between any of the treatments applied.

4.3. Discussion

In this study, the impact of inhibiting LRRK2 kinase activity upon GCase levels in a cell model for human neuroglioma was investigated. 24 and 72 h inhibition of LRRK2 kinase activity resulted in no significant increase in overall GCase activity when compared to DMSO treated cells. However, it was observed a significant increase in GBA protein levels following 72 h treatment with LRRK2in1. These data were combined normalizing GCase activity levels to GBA protein levels, showing a striking reduced specific GCase activity after LRRK2 kinase inhibition. Concomitant with these changes, an increase in protein markers for macroautophagy activation (LC3-II) was observed. These data complement, and expand upon, a growing literature examining the link between LRRK2 and GBA1. At a genetic level, the population frequency of mutations in both genes, especially in specific populations such as the Ashkenazim and Imazighen, has resulted in a number of individuals harboring PD associated mutations in LRRK2 and GBA1, and there is evidence from the literature that supports an interaction at a clinical level between the impact of mutations in the two genes (Omer et al., 2020; Yahalom et al., 2019). At a biochemical level, this has been examined in both human tissue and in a cellular context. Two studies reported analyses of GBA and GCase activity in human samples from carriers of mutations in LRRK2. Alcalay and co-workers examined GCase activity in dried blood spots from 36 carriers of the G2019S mutation, reporting that this was elevated compared to non-mutation carriers (13.69 $\mu\text{mol/l/h}$ versus 11.93 $\mu\text{mol/l/h}$, P value 0.002) (Alcalay et al., 2015). Zhao and colleagues (2018) investigated GBA protein levels in the brains of 17 individuals with the G2019S and I2020T mutations (n = 12 and n = 5 respectively), revealing a significantly reduced level of Tris buffered saline soluble GBA protein in frontal cortex samples from LRRK2 mutation carriers versus control.

A study of the relationship between LRRK2 and GCase activity in human neuronal cultures by Ysselstein and co-workers reported that cells carrying the G2019S mutation in LRRK2 had reduced GCase activity, and that inhibition of LRRK2 kinase activity increased GCase activity (Ysselstein et al., 2019). A further study by Sanyal and colleagues reported that inhibition of LRRK2 kinase activity in cells that harbor a mutation in GBA1 boosts GCase activity (Sanyal et al., 2020).

The results from this current study suggest that there is a complex, and multifaceted, relationship between LRRK2 and GBA. First, there is a delay between inhibition of LRRK2 kinase activity and alterations in GBA, with changes only observed after 72 hours of inhibition. This suggests that any links between LRRK2 and GBA are not immediately proximal to LRRK2 kinase activity, instead being a downstream event. Secondly, in the human neuroglioma cell model under examination there were two outcomes of LRRK2 kinase inhibition upon GBA: an increase in GBA protein levels, and a decrease in the specific GCase activity of GBA. Intriguingly, the decrease in specific activity may mirror data from post mortem studies of LRRK2 mutation carriers, where the G2019S mutation is associated with decreased GBA protein levels (Zhao et al., 2018), and increased GCase activity (Alcalay et al., 2015). As the G2019S mutation causes an increase in LRRK2 kinase activity, the prediction from these studies would be that inhibition of LRRK2 kinase activity would result in an increase in GBA protein levels and a decrease in specific activity. In a recent work, Kedariti et al. showed that pharmacological inhibition of G2019S LRRK2 in HEK293T cells caused a significant reduction of GCase activity when it was normalized by GBA levels, being consistent with the results in our study (Yahalom et al., 2019).

The precise nature of the relationship between LRRK2 kinase activity and GBA GCase activity is not known. The requirement for 72 h inhibition of LRRK2 kinase activity to elicit a change in GBA biology suggests that there may be a number of steps separating LRRK2 and GBA within the cell. Moreover, it is interesting to clear up whether the selective inhibition of LRRK2-dependent phosphorylation on endolysosomal function can be influenced by the chemical structure of the inhibitors used. This could explain why treatment with MLI-2 does not exert the same effect than LRRK2in-1 on autophagy markers. Yao et al. observed differences in efficacy and pharmacodynamics of two LRRK2 inhibitors, TTT-3002 and LRRK2in-1, upon evaluation of dopaminergic neurodegeneration caused by different LRRK2 mutations using transgenic *C. elegans* models. Although both inhibitors were able to rescue the pathological phenotype manifested by the expression of mutants LRRK2 G2019S and R1441C, results suggested that TTT-3002 showed higher affinity for LRRK2 proteins and higher potency of inhibition over kinase activity of LRRK2 (Yao et al., 2013). Otherwise, Mercatelli et al. found that LRRK2in-1 and GSK2578215A, two structurally unrelated LRRK2 kinase activity inhibitors, differentially affected the neurotransmitter release as well as pSer935 levels in synaptosomes from mouse striatum and cerebral cortex (Mercatelli et al., 2019). These results would indicate that every inhibitor might have a different mode to interact with LRRK2 kinase pocket. Furthermore, it remains unresolved whether the observed changes following LRRK2 kinase inhibitor treatment are entirely due to kinase activity inhibition or are also related to off-target effects. Due to the contribution of kinase proteins in the regulation of many subcellular functions, the design of highly selective inhibitors is essential to specifically block LRRK2 kinase activity. Recent works have identified hundreds of proteins susceptible to being phosphorylated by LRRK2 kinase in order to detect changes in their phosphorylation levels upon LRRK2 kinase activity inhibition (Fell et al., 2015;

Luerman et al., 2014; Tasegian et al., 2021). Despite the widespread use of LRRK2in-1 and MLi-2 in exploring LRRK2 biology, their pharmacological impact on other molecular pathways should be further studied. Finally, given the reported role for LRRK2 in responding to lysosomal damage (Herbst et al., 2020; Eguchi et al., 2018), one potential mechanism whereby inhibition of LRRK2 would result in alterations in GBA could be impairments in the basal turnover of damaged lysosomes leading to accumulation of GBA protein and a paradoxical decrease in activity. Further investigations are required to clarify the mechanisms connecting LRRK2 and GBA. Being known the status of both LRRK2 and GBA as drug targets for PD, such investigations should be prioritized in the coming years.

V. GENERAL DISCUSSION

In this thesis, two models have been used to delve into the possible potential mechanisms in the pathology of sporadic Parkinson's disease as a result of the affected lysosomal function in its early stages.

First, a cell model of leukocytes or WBC from patients with sporadic PD, obtained from a blood draw from the cubital vein, was used to achieve one of the main objectives of this work: to study the contribution of the lysosome to the pathogenesis of PD through the analysis of a group of lysosomal enzymes. Recently in the literature, different models are proposed to characterize lysosomal dysfunction in the neurodegenerative process (Reviewed in Udayar et al., 2022). Even though there are different approaches, the functional study of the lysosome via analysis of enzymatic activity has been carried out in post-mortem brain tissue, CFS (cerebrospinal fluid), in neuronal and non-neuronal cell models (such as iPSCs, microglial and astrocytic models), as well as in patient blood. Although detection in post-mortem tissue would provide greater validity to this study, the need to establish biomarkers at early levels of the disease would support prevention, detection and future therapeutic improvement in these patients. The use of WCB reduces some of the variables that could be involved in the processing and collection of the sample, as well as the subsequent analysis: (1) with this collection method, we reduce the patient's aversion to taking the sample (since obtaining CFS entails a rather more invasive and complex technique such as lumbar puncture), in addition to being an economical, accessible and easy method to perform. (2) Given the speed, simplicity and standardization when processing this type of sample, defects in the analysis derived from its degradation can be avoided. Some previous works ensure that variables such as storage delay, frozen aspects, or the time that passes from processing to analysis, alter the data collected later. Taking into account that this phenomenon especially affects these studies linked to the analysis of autophagy processes and degradation

mechanisms in the cell (Zhao et al., 2018), the determination using this model can better reproduce the real molecular situation. (3) In addition to that, given the nature of sporadic PD, this model is very useful, since WBCs not only contain the entire genetic scenario of the individual, but also have possible changes or molecular events derived from aging or the environment, that are useful for their understanding. In addition to the reasons mentioned, this type of cell has been suggested as a good model for the study of markers involved in ALP and lysosomal dysfunction by other authors (Papagiannakis et al., 2015; Miki et al., 2018; Avenali et al., 2021; Deen et al., 2022; Vavilova et al., 2022; Usenko et al., 2022).

In the second study, we used a model of human neuroglioma to delve, once proven lysosomal enzyme deficiency in PD patients, into potential mechanisms in the pathogenesis of PD through the use of specific inhibitors. We selected glial cells to carry out our study for different reasons, according to the literature (Doll & Smith, 1993; Booth et al., 2017; Kam et al., 2020): (1) there is great evidence of the relationship between glial cells and the neurodegenerative process of PD, since they also share genes and expressed proteins involved with PD (*SNCA*, *LRRK2*, *DJ-1*, *Parkin*, *ATP13A2*, etc.); (2) astrocytes play a very important role in the maintenance of neurons, especially DA. They are involved in structural and biochemical support processes, vesicular trafficking, membrane transport, and in the blood–brain barrier function; (3) the *LRRK2* protein is abundantly expressed in neurons and astrocytes, making it a useful model to explore its function; (4) additionally, astroglial activation is a hallmark in certain lysosomal storage disorders, which have been observed to trigger neuronal degeneration. In addition, like the *LRRK2* protein, *GCase* is also physiologically present in them, which allows studying the possible relationship between both. These findings further support the involvement of these glial cells in the autophagy process. Finally, other aspects were assessed, such as the lack of viability of establishing a

primary culture derived from post-mortem brain tissue, the variability of the expression of genes related to PD in glial cells and neurons between human and animal models (reviewed in Booth et al., 2017), as well as the accessibility of this type of cells, led us to choose this model as the most appropriate for our study.

Collectively, the results derived from this thesis have allowed us to first carry out one of its main objectives, to establish the contribution of the lysosome to PD through the study of certain lysosomal enzymes.

The data from the first study showed a deficiency in the enzymatic activity of GCase and β -gal in WBC from patients, however, their protein levels were not decreased. Interestingly in the second study, inhibition of LRRK2 kinase activity produced a significant increase in GBA protein levels 72 hours later. However, despite the increase in protein levels, not only were no changes in enzyme activity found, but also the ratio between the two showed a decreased specific enzyme activity. These results led us to consider whether the defective activity of the enzyme was due to a possible impaired trafficking of GBA protein from the ER/Golgi to the lysosome. However, in the second study there were no changes in GBA protein expression upon treatment with Endoglycosidase H, which indicates that the expressed protein corresponds to the lysosomal fraction. Another potential mechanism for the involvement of LRRK2 with the GBA protein derives from its evidence in the response to the maintenance and elimination of damaged lysosomes (Herbst et al., 2020; Eguchi et al., 2018). It is possible that the inhibition of this protein affects the correct basal renewal of the damaged lysosomes, causing an increase in the dysfunctional GBA protein, reflected in a decrease in the activity of the enzyme.

Interestingly, in the first study we found decreased PSAP protein and saposin C expression levels in PD patients. In addition to that, a negative correlation was established

between the levels of PSAP and α -Syn, increased in these patients. According to this data, some studies suggest that Saposin C seems to exert a protective effect on GCase from degradation by proteases, also preventing its inhibition by α -Syn (Sun et al., 2003; Yap et al., 2013). Although there is still no genetic evidence to support the contribution of PSAP and PD disease, saposin C seems to have a potential key role in the pathogenesis of PD through α -Syn aggregation.

Additionally, through the findings obtained in this study, a possible regulation of GBA and LRRK2 is shown, beyond the kinase activity of the LRRK2 protein itself, where different pathways through the ALP process could be potential mechanisms under study for the pathogenesis of the PD. There is evidence of the involvement of LRRK2 in sporadic PD and lysosomal dysfunction (Nalls et al., 2019). However, the relationship between the LRRK2 protein and GBA is complex and multifactorial. To date, there are no studies linking saposin C with the LRRK2 protein. Regarding the results obtained in this work, it is proposed as a very interesting challenge to establish a relationship between the two, due to the implication of the LRRK2 protein in different cellular processes, such as the autophagy process and vesicular trafficking, being a potential mechanism to elucidate the pathology of sporadic PD in the future.

This study brings to light the contribution of the lysosomal dysfunction to the pathogenesis of sporadic PD, showing a pattern of enzymatic activity that could be specific to early stages of the disease. It is remarkable that the significant decrease in Saposin C protein levels is directly affecting GBA function and α -Syn clearance. Saposin C would be capable of restoring GCase function, competing for it with α -Syn. The manipulation of Saposin C levels in patients could enhance the activity of the mutated GCase and favour the clinical status of patients. Although we are aware that these results might not be completely

reproducible in the brain and they also need to be replicated in larger independent cohorts, including early and advanced PD patients, the identification through the WBCs of a PD-linked biochemical profile it is highly encouraging because of the possible clinical implications.

Great advances have been made in recent years regarding sporadic PD, including genetic approaches. However, there is still a long and arduous path to unravel. New non-invasive methods for detecting early biomarkers are necessary for a better understanding of the molecular mechanisms involved in PD, which will also allow an improvement in the development of new therapeutic targets and strategies.

VI. CONCLUSIONS

In this Thesis, some of the alterations in the lysosomal function that may be related to the aetiology of PD have been evaluated in different cell models. We suggest that, taken together, these findings may have practical implications for a better understanding of the pathogenesis of sporadic PD in patients.

Our results allowed us to reach the following conclusions:

1. Neurodegenerative diseases are a major burden for our society, affecting millions of people worldwide. A main goal of past and current research remains to enhance our understanding of the mechanisms underlying proteotoxicity, a common theme among these incurable and debilitating conditions.
2. Sporadic PD was associated with specific impaired lysosomal enzyme activity, showed as decreased GCase and β -gal activities, but no differences in protein expression levels in WBCs of patients versus controls.
3. We found elevated expression levels of α -Syn protein while PSAP and saposin C were decreased in patients. Furthermore, a negative correlation was established between them, suggesting a possible negative feedback loop due to increased toxicity of α -Syn that would alter GBA function.
4. In addition to increased CatD activity, its protein expression levels were notably overexpressed in patients at HY stage I, showing a possible positive regulation mechanism carried out by the lysosome to restore the α -Syn clearance, whereas with the disease progression (HY II) it showed a reduction.
5. The chronic inhibition of LRRK2 kinase activity accompanied by an activation of ALP, causes a significant increase in GBA, however it reduces its specific enzymatic activity.

6. The reduction in the specific activity of GCase does not seem to be determined by a failure in the maturation process of the protein or its transport to the lysosome.
7. Our findings support the contribution of an altered lysosomal activity pattern in early stages of PD, highlighting the importance of the cofactors PSAP and saposin C in the correct GBA function and appropriate α -Syn degradation.
8. To restore the normality of pathways that have disrupted protein quality control, such as increased proteasome activity, improved cellular mechanisms through upregulation of chaperones, and cascade elimination pathways, as well as controlling the ability of misfolded proteins to spread to nearby cells, are key to avoid progressive neuronal degeneration.
9. Although more studies are needed to elucidate how lysosomal alterations contribute to the disease, the identification of molecular patterns linked to PD in white blood cells could help to establishing new targets for a better understanding of the disease and efficient strategies for its treatment.

VII. ACKNOWLEDGMENTS/AGRADECIMIENTOS

En primer lugar y como corresponde, quiero agradecer a mis directores de tesis Paco y Raquel, por su apoyo incondicional a nivel profesional, personal y emocional durante todo este tiempo. Me acogisteis sin conocerme desde otra tesis, y me ayudasteis a salir de aquel pozo donde ni yo creí poder salir, y me habéis apoyado inconmensurablemente durante todas las adversidades que han surgido durante todo este proceso. Increíbles profesionales, mejores personas. Gracias, gracias y gracias.

Durante el desempeño de esta tesis, han sido muchas las experiencias vitales a las que me he tenido que enfrentar, y aunque no todas académicas, me encuentro en la necesidad de plasmarlas en esta tesis: un acompañamiento hospitalario de varios años con mi madre sobrevenida por un cáncer de mama; una primera toma de contacto con el doctorado traumática; y finalmente, un embarazo que a su término, no solo trajo lo mejor que he conocido nunca, sino también una de las peores noticias que pude recibir durante el transcurso de esta tesis. Mi marido y compañero de vida durante 11 años, padre de mi hija y el principal pilar en esta tesis, fue diagnosticado de un cáncer atípico (Sarcoma de Ewing), y apenas dos años después, tras un largo y duro proceso de acompañamiento que me ausentó de los laboratorios y del resto del mundo, dejó este mundo, no sin antes hacerme prometer que terminaría esta tesis. Estuviste físicamente durante casi todo el proceso, y emocionalmente en todo momento.

Gracias Jonathan, por demostrarme aun sin estar presente, que podía (podíamos) llegar, aunque aún no me lo crea. Gracias por mostrar siempre al mundo un orgullo inmenso por mí, por el mero hecho de estar haciendo una tesis doctoral. Gracias por escucharme, darme aliento y aconsejarme, aun cuando ni siquiera entendías en detalle lo que realizaba día a día en mi trabajo. Quiero agradecer también a tu familia, que ha sido un gran apoyo, y lo sigue siendo, pues aunque cada uno tiene una forma de gestionar el dolor, ¿quién está preparado

para hacerlo, después de todo lo que hemos sufrido? Doy las gracias por aprender junto a ti, que la felicidad en la vida no son estados, sino momentos en los que hay que parar y disfrutar al máximo. Y gracias por darme la mayor lección de vida que he aprendido y que no quiero abandonar nunca *“mientras hay vida hay que vivir, luchar y amar sin medida”*, que es, lo que has hecho tú, de forma incondicional hasta que te fuiste. Al principio me sentí perdida, desconsolada y enfadada, pues yo te prometí acabar esta tesis y tú me prometiste que jamás me ibas a dejar sola. Con el tiempo entendí que estaba equivocada, pues ambos hemos cumplido nuestras promesas: yo con este trabajo, y tú que sigues vivo y me acompañas en todo momento a través de nuestra pequeña Martina. Gracias papá, te amamos 3000.

A mis padres. Mamá, papá, he vivido pocos años pero si muchas experiencias y creo que no conozco a nadie que haya sufrido tanto como vosotros. Si algo me ha enseñado esta tesis, es que la genética es difícil de esquivar, y desgraciadamente en eso he heredado algo de vosotros. Pero siempre recuerdo, que aunque hayan sido muchas y graves las adversidades, habéis sido resilientes y habéis afrontado la vida con decisión y de frente, y en eso también tengo que darle las gracias a la genética. Me va a faltar vida para poder agradecer todos los esfuerzos que habéis hecho por mí, por dejarme esta herencia que siempre habéis dicho que era mi formación, por ser escudo, ocuparos de todo cuando yo no estaba ni para mí misma y por ser también padres de mi hija. Sé que no soy ni la mitad de cercana o cariñosa que era antes, que guarde mis sentimientos o esté insoportable los días enteros, la vida me ha golpeado duro estos años, pero quiero que sepáis que sigo aquí, queriéndoo como siempre y como nunca. Espero que algún día, estéis la mitad de orgullosos de mí, que lo que yo lo estoy de vosotros. Gracias infinitas.

A todo el departamento de fisiología de la facultad, y en especial a la más bella flor, mi Rosa favorita. Desde el minuto uno has tenido palabras de amor y aliento para mí. Aunque

no he aceptado jamás tu ayuda, porque soy dura para hacerlo, le conste a todo el mundo que te ha dolido la boca de ofrecérmela. Quiero que sepas que te la agradezco, aunque haya sido terca para aceptarla. Eres una persona con mucha luz y te deseo lo mejor en la vida.

Gracias a Paco Barrero, Javier Pelegrina, Paco Escamilla y todos los neurólogos compañeros del IBS, por su paciencia y dedicación.

A mis queridos hermanos de la salita 39: Tomás, Emma, Inma, Antonio, Alejandro, Sergio, Ester, Nuria, Miriam, Andrea, y muy especialmente a Marisol, con la que he compartido horas y horas en el laboratorio, experimentos clandestinos en época de pandemia y desventuras varias. Os amo, y aunque sea la imagen principal del grupo de WhatsApp disfrazada de pitonisa, habéis hecho que entre todos los días con una sonrisa en el CIBM, solo por ver esas caras a la hora de la comida. Nadie haciendo una tesis ha tenido tanta suerte de tener tan buenos compañeros y amigos.

Gracias también a Leto, mi Alejandro, por todo. Porque fue un trampolín para que yo llegara al CIBM, y la vida caprichosamente quiere tenernos cerca el uno del otro, incluso laboratorio con laboratorio. No dejes nunca de estar en mi vida.

Mil gracias a Jose Luis (Tuflo), gran parte de esta tesis es gracias a ti. Por las horas ininterrumpidas en el laboratorio, por aguantarme, por sufrirme y por darle nueva música a mi vida. También a Antonio, el mejor técnico del universo. Y a todos los que han pasado por el laboratorio 152, pues siempre he aprendido algo de ellos.

Este trabajo también merece unas palabras para Sara, gracias amiga. Has sido un apoyo incondicional durante toda mi estancia en el CIBM, he tenido la suerte de conocerte como profesional y como persona, y no sabría decir en cuál de las dos categorías destacas más. El primer trabajo que da apoyo a esta tesis, es gracias a ti, quiero que sepas que te deseo lo mejor, eres una persona maravillosa en todos los aspectos.

A Dani, Ana, Laura, Diego y muy especialmente a Cecilia, compañeros del CIBM y de laboratorio.

También quiero agradecer a mi primer director de tesis, el que dijo que no llegaría, que jamás publicaría un trabajo en una revista ni me dedicaría a esto. Gracias a él me he dado cuenta de que soy una persona resiliente, que adora la investigación y la docencia, y pude conocer a unas personas maravillosas como Paco y Raquel.

Muchísimas gracias a Paco Arrebola, que me confió totalmente a ciegas su laboratorio de cultivos y estuvo dispuesto a enseñarme cualquier cosa que yo le demandaba.

Necesito dedicar también unas palabras de gratitud a todos los compañeros/as que conocí durante mi estancia en el University College of London: David, Chou, Caro, Noemí, Phil, Lesley, Lauren Mary, Sebastian, Alan, Luci, Demis, y que me perdonen los que haya podido dejar en el tintero. Pero muy en especial a Claudia Manzoni, Patrick Lewis, John Hardy, Susanna Cogo y James Tomkins: Beautiful people, better scientists. Thank you.

Quiero dar las gracias a Gregorio, Ricardo y a Paco Mora, ya que fue gracias a ellos que descubrí este apasionante mundo de la investigación biomédica durante mis prácticas en el máster de Neurociencia en la Universidad Complutense de Madrid. Me hicieron sentir muy especial y me enseñaron todo el trabajo de laboratorio necesario para desenvolverse durante mi tesis con unos buenos cimientos y no morir en el intento. He podido con el apoyo especial de Ricardo incluso durante mi tesis, para lidiar con el western blot. Mil gracias.

A Puri Checa. Gracias Puri, cuando estuve al borde de renunciar a mi FPU en mi primera toma de contacto con una tesis, me llamaste sin conocerme personalmente, y me animaste a seguir luchando. Tu eres una de las personas que finalmente me animó a decidirme y te estaré eternamente agradecida.

Desi, te conocí en mi estancia en Londres más en profundidad, fuimos apoyo la una de la otra en un país que no era el nuestro y desgraciadamente la vida nos puso la misma piedra en el camino y perdimos a grandes hombres de nuestra vida afectados por el cáncer. Le agradezco enormemente a la vida por habernos encontrado.

Anaí y Jose (y ahora también la pequeña Sofía), sois personas increíbles, muchas gracias por hacerme sentir como un miembro más de vuestra familia.

Y porque lo mejor hay que dejarlo para el final, quiero agradecer enormemente a mi gente más querida, en especial a Loli, Estela, Jose, Loren y Bol. Aunque como hermanos a veces tenemos nuestras riñas, también somos un grupo muy unido. Jamás voy a olvidar las veces que me habéis metido en la ducha, hecho los quehaceres de mi casa y sacado de la misma en ocasiones para respirar aire puro, cuando el de mi casa estaba más viciado que nunca. También a Sheila, Belén, Andrea, Alba, Juan, Desi, Mari, Patri, y a todos los amigos y gente de mi pueblo que no podría nombrar, porque daría para otra tesis. Gracias infinitas.

Papaya, a ti te dedico un espacio especial, eres la hermana que siempre he necesitado, que mi madre no ha traído al mundo, pero que he tenido a mi lado de forma incondicional. Gracias por estar en este mundo y cerca de mí. Te amo.

Juandy, también quiero agradecerte a ti, que tu paso en esta tesis ha sido la más corta pero de las más importantes sin duda alguna. No sé si en esta vida podré darte las gracias por tanto, pues has sido uno de mis principales puntos de apoyo en este tiempo, sin los cuales tal vez hubiera cometido alguna locura contra mi misma y no hubiera encontrado fuerzas para nada, mucho menos para acabar esta tesis. Gracias por devolverme la ilusión, por regalarme vida. Estemos donde nos depare la vida, siempre vas a tener mi amor incondicional.

Finalmente, agradecer a todo aquel que haya tenido paciencia para llegar a estas últimas líneas, a aquellos que no haya nombrado y que siempre me han apoyado y creído en

mí en esta gesta que ha sido esta tesis. Durante todo este proceso, he podido extraer una lista de las numerosas cualidades que debe poseer una persona que quiera dedicarse a esta profesión que es la investigación: alta tolerancia a la frustración y al estrés, optimismo, resiliencia, compañerismo, dedicación, pasión, paciencia, honestidad, curiosidad, compromiso, implicación y sobretodo constancia. Como cualquier otra persona, no soy perfecta, pero quiero dar gracias por lo que soy y lo que no, por lo que tengo, lo que he vivido y aprendido. La vida, como los viajes, pueden ser extremadamente largos, pero todos comienzan con un sueño y un primer paso, y el mío no iba tan desencaminado.



Figura 13. La doctoranda a los 5 años.

VIII. REFERENCES

- Aarsland D, Larsen JP, Tandberg E, Laake K. Predictors of nursing home placement in Parkinson's disease: a population-based, prospective study. *J Am Geriatr Soc*. 2000 Aug;48(8):938-42. doi: 10.1111/j.1532-5415.2000.tb06891.x. PMID: 10968298.
- Alcalay RN, Levy OA, Waters CC, Fahn S, Ford B, Kuo SH, Mazzoni P, Pauciulo MW, Nichols WC, Gan-Or Z, Rouleau GA, Chung WK, Wolf P, Oliva P, Keutzer J, Marder K, Zhang X. Glucocerebrosidase activity in Parkinson's disease with and without GBA mutations. *Brain*. 2015 Sep;138(Pt 9):2648-58. doi: 10.1093/brain/awv179. Epub 2015 Jun 27. PMID: 26117366; PMCID: PMC4564023.
- Alegre-Abarrategui J, Christian H, Lufino MM, Mutihac R, Venda LL, Ansorge O, Wade-Martins R. LRRK2 regulates autophagic activity and localizes to specific membrane microdomains in a novel human genomic reporter cellular model. *Hum Mol Genet*. 2009 Nov 1;18(21):4022-34. doi: 10.1093/hmg/ddp346. Epub 2009 Jul 29. PMID: 19640926; PMCID: PMC2758136.
- Angot E, Brundin P. Dissecting the potential molecular mechanisms underlying alpha-synuclein cell-to-cell transfer in Parkinson's disease. *Parkinsonism Relat Disord*. 2009 Dec;15 Suppl 3:S143-7. doi: 10.1016/S1353-8020(09)70802-8. PMID: 20082977.
- Avenali M, Cerri S, Ongari G, Ghezzi C, Pacchetti C, Tassorelli C, Valente EM, Blandini F. Profiling the Biochemical Signature of GBA-Related Parkinson's Disease in Peripheral Blood Mononuclear Cells. *Mov Disord*. 2021 May;36(5):1267-1272. doi: 10.1002/mds.28496. Epub 2021 Feb 22. PMID: 33617695; PMCID: PMC8247888.

- Bae EJ, Yang NY, Song M, Lee CS, Lee JS, Jung BC, Lee HJ, Kim S, Masliah E, Sardi SP, Lee SJ. Glucocerebrosidase depletion enhances cell-to-cell transmission of α -synuclein. *Nat Commun*. 2014 Aug 26;5:4755. doi: 10.1038/ncomms5755. PMID: 25156829; PMCID: PMC4452288.
- Bagheri H, Damase-Michel C, Lapeyre-Mestre M, Cismondo S, O'Connell D, Senard JM, Rascol O, Montastruc JL. A study of salivary secretion in Parkinson's disease. *Clin Neuropharmacol*. 1999 Jul-Aug;22(4):213-5. PMID: 10442250.
- Balducci C, Pierguidi L, Persichetti E, Parnetti L, Sbaragli M, Tassi C, Orlacchio A, Calabresi P, Beccari T, Rossi A. Lysosomal hydrolases in cerebrospinal fluid from subjects with Parkinson's disease. *Mov Disord*. 2007 Jul 30;22(10):1481-1484. doi: 10.1002/mds.21399. PMID: 17546678.
- Bandres-Ciga S, Diez-Fairen M, Kim JJ, Singleton AB. Genetics of Parkinson's disease: An introspection of its journey towards precision medicine. *Neurobiol Dis*. 2020 Apr;137:104782. doi: 10.1016/j.nbd.2020.104782. Epub 2020 Jan 25. PMID: 31991247; PMCID: PMC7064061.
- Barone P, Antonini A, Colosimo C, Marconi R, Morgante L, Avarello TP, Bottacchi E, Cannas A, Ceravolo G, Ceravolo R, Cicarelli G, Gaglio RM, Giglia RM, Iemolo F, Manfredi M, Meco G, Nicoletti A, Pederzoli M, Petrone A, Pisani A, Pontieri FE, Quatralo R, Ramat S, Scala R, Volpe G, Zappulla S, Bentivoglio AR, Stocchi F, Trianni G, Dotto PD; PRIAMO study group. The PRIAMO study: A multicenter assessment of nonmotor symptoms and their impact on quality of life in Parkinson's

disease. *Mov Disord.* 2009 Aug 15;24(11):1641-9. doi: 10.1002/mds.22643. PMID: 19514014.

Bartels T, Choi JG, Selkoe DJ. α -Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. *Nature.* 2011 Aug 14;477(7362):107-10. doi: 10.1038/nature10324. PMID: 21841800; PMCID: PMC3166366.

Beilina A, Rudenko IN, Kaganovich A, Civiero L, Chau H, Kalia SK, Kalia LV, Lobbestael E, Chia R, Ndukwe K, Ding J, Nalls MA; International Parkinson's Disease Genomics Consortium; North American Brain Expression Consortium, Olszewski M, Hauser DN, Kumaran R, Lozano AM, Baekelandt V, Greene LE, Taymans JM, Greggio E, Cookson MR. Unbiased screen for interactors of leucine-rich repeat kinase 2 supports a common pathway for sporadic and familial Parkinson disease. *Proc Natl Acad Sci U S A.* 2014 Feb 18;111(7):2626-31. doi: 10.1073/pnas.1318306111. Epub 2014 Feb 7. PMID: 24510904; PMCID: PMC3932908.

Belarbi K, Cuvelier E, Bonte MA, Desplanque M, Gressier B, Devos D, Chartier-Harlin MC. Glycosphingolipids and neuroinflammation in Parkinson's disease. *Mol Neurodegener.* 2020 Oct 17;15(1):59. doi: 10.1186/s13024-020-00408-1. PMID: 33069254; PMCID: PMC7568394.

Bellomo G, Paciotti S, Gatticchi L, Parnetti L. The vicious cycle between α -synuclein aggregation and autophagic-lysosomal dysfunction. *Mov Disord.* 2020 Jan;35(1):34-44. doi: 10.1002/mds.27895. Epub 2019 Nov 15. PMID: 31729779.

Benito-León J, Bermejo-Pareja F, Morales-González JM, Porta-Etessam J, Trincado R, Vega S, Louis ED; Neurological Disorders in Central Spain (NEDICES) Study Group. Incidence of Parkinson disease and parkinsonism in three elderly populations of central Spain. *Neurology*. 2004 Mar 9;62(5):734-41. doi: 10.1212/01.wnl.0000113727.73153.68. PMID: 15007123.

Benito-Leon J. Epidemiologia de la enfermedad de Parkinson en España y su contextualizacion mundial [Epidemiology of Parkinson's disease in Spain and its contextualisation in the world]. *Rev Neurol*. 2018 Feb 16;66(4):125-134. Spanish. PMID: 29435969.

Benskey MJ, Perez RG, Manfredsson FP. The contribution of alpha synuclein to neuronal survival and function - Implications for Parkinson's disease. *J Neurochem*. 2016 May;137(3):331-59. doi: 10.1111/jnc.13570. Epub 2016 Mar 23. PMID: 26852372; PMCID: PMC5021132.

Berardelli A, Rothwell JC, Thompson PD, Hallett M. Pathophysiology of bradykinesia in Parkinson's disease. *Brain*. 2001 Nov;124(Pt 11):2131-46. doi: 10.1093/brain/124.11.2131. PMID: 11673316.

Bindoff LA, Birch-Machin MA, Cartlidge NE, Parker WD Jr, Turnbull DM. Respiratory chain abnormalities in skeletal muscle from patients with Parkinson's disease. *J Neurol Sci*. 1991 Aug;104(2):203-8. doi: 10.1016/0022-510x(91)90311-t. PMID: 1658241.

- Binolfi A, Theillet FX, Selenko P. Bacterial in-cell NMR of human α -synuclein: a disordered monomer by nature? *Biochem Soc Trans.* 2012 Oct;40(5):950-4. doi: 10.1042/BST20120096. PMID: 22988846.
- Bjorklund G, Stejskal V, Urbina MA, Dadar M, Chirumbolo S, Mutter J. Metals and Parkinson's Disease: Mechanisms and Biochemical Processes. *Curr Med Chem.* 2018;25(19):2198-2214. doi: 10.2174/0929867325666171129124616. PMID: 29189118.
- Blackinton J, Lakshminarasimhan M, Thomas KJ, Ahmad R, Greggio E, Raza AS, Cookson MR, Wilson MA. Formation of a stabilized cysteine sulfinic acid is critical for the mitochondrial function of the parkinsonism protein DJ-1. *J Biol Chem.* 2009 Mar 6;284(10):6476-85. doi: 10.1074/jbc.M806599200. Epub 2009 Jan 5. PMID: 19124468; PMCID: PMC2649108.
- Bolam JP, Pissadaki EK. Living on the edge with too many mouths to feed: why dopamine neurons die. *Mov Disord.* 2012 Oct;27(12):1478-83. doi: 10.1002/mds.25135. Epub 2012 Sep 24. PMID: 23008164; PMCID: PMC3504389.
- Booth HDE, Hirst WD, Wade-Martins R. The Role of Astrocyte Dysfunction in Parkinson's Disease Pathogenesis. *Trends Neurosci.* 2017 Jun;40(6):358-370. doi: 10.1016/j.tins.2017.04.001. Epub 2017 May 17. PMID: 28527591; PMCID: PMC5462417.
- Bostantjopoulou S, Katsarou Z, Karakasis C, Peitsidou E, Milioni D, Rossopoulos N. Evaluation of non-motor symptoms in Parkinson's Disease: An underestimated

necessity. Hippokratia. 2013 Jul;17(3):214-9. PMID: 24470730; PMCID: PMC3872456.

Bourdenx M, Koulakiotis NS, Sanoudou D, Bezard E, Dehay B, Tsarbopoulos A. Protein aggregation and neurodegeneration in prototypical neurodegenerative diseases: Examples of amyloidopathies, tauopathies and synucleinopathies. Prog Neurobiol. 2017 Aug;155:171-193. doi: 10.1016/j.pneurobio.2015.07.003. Epub 2015 Jul 21. PMID: 26209472.

Braak H, Sastre M, Del Tredici K. Development of alpha-synuclein immunoreactive astrocytes in the forebrain parallels stages of intraneuronal pathology in sporadic Parkinson's disease. Acta Neuropathol. 2007 Sep;114(3):231-41. doi: 10.1007/s00401-007-0244-3. Epub 2007 Jun 19. PMID: 17576580.

Breydo L, Wu JW, Uversky VN. A-synuclein misfolding and Parkinson's disease. Biochim Biophys Acta. 2012 Feb;1822(2):261-85. doi: 10.1016/j.bbadis.2011.10.002. Epub 2011 Oct 12. PMID: 22024360.

Bright JM, Carlisle HJ, Toda AMA, Murphy M, Molitor TP, Wren P, Andruska KM, Liu E, Barlow C. Differential Inhibition of LRRK2 in Parkinson's Disease Patient Blood by a G2019S Selective LRRK2 Inhibitor. Mov Disord. 2021 Jun;36(6):1362-1371. doi: 10.1002/mds.28490. Epub 2021 Feb 11. PMID: 33836114; PMCID: PMC8248170.

Burré J, Sharma M, Südhof TC. α -Synuclein assembles into higher-order multimers upon membrane binding to promote SNARE complex formation. Proc Natl Acad Sci U S

- A. 2014 Oct 7;111(40):E4274-83. doi: 10.1073/pnas.1416598111. Epub 2014 Sep 22. PMID: 25246573; PMCID: PMC4210039.
- Burré J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Südhof TC. Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. *Science*. 2010 Sep 24;329(5999):1663-7. doi: 10.1126/science.1195227. Epub 2010 Aug 26. PMID: 20798282; PMCID: PMC3235365.
- Burté F, De Girolamo LA, Hargreaves AJ, Billett EE. Alterations in the mitochondrial proteome of neuroblastoma cells in response to complex 1 inhibition. *J Proteome Res*. 2011 Apr 1;10(4):1974-86. doi: 10.1021/pr101211k. Epub 2011 Mar 10. PMID: 21322648.
- Cacabelos R. Parkinson's Disease: From Pathogenesis to Pharmacogenomics. *Int J Mol Sci*. 2017 Mar 4;18(3):551. doi: 10.3390/ijms18030551. PMID: 28273839; PMCID: PMC5372567.
- Cartier AE, Ubhi K, Spencer B, Vazquez-Roque RA, Kosberg KA, Fourgeaud L, Kanayson P, Patrick C, Rockenstein E, Patrick GN, Masliah E. Differential effects of UCHL1 modulation on alpha-synuclein in PD-like models of alpha-synucleinopathy. *PLoS One*. 2012;7(4):e34713. doi: 10.1371/journal.pone.0034713. Epub 2012 Apr 13. PMID: 22514658; PMCID: PMC3326048.
- Carvelli L, Libin Y, Morales CR. Prosaposin: a protein with differential sorting and multiple functions. *Histol Histopathol*. 2015 Jun;30(6):647-60. doi: 10.14670/HH-30.647. Epub 2014 Dec 18. PMID: 25519158.

- Castro BM, Prieto M, Silva LC. Ceramide: a simple sphingolipid with unique biophysical properties. *Prog Lipid Res.* 2014 Apr;54:53-67. doi: 10.1016/j.plipres.2014.01.004. Epub 2014 Feb 7. PMID: 24513486.
- Chai C, Lim KL. Genetic insights into sporadic Parkinson's disease pathogenesis. *Curr Genomics.* 2013 Dec;14(8):486-501. doi: 10.2174/1389202914666131210195808. PMID: 24532982; PMCID: PMC3924245.
- Chandra S, Gallardo G, Fernández-Chacón R, Schlüter OM, Südhof TC. Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. *Cell.* 2005 Nov 4;123(3):383-96. doi: 10.1016/j.cell.2005.09.028. PMID: 16269331.
- Chen YP, Gu XJ, Ou RW, Zhang LY, Hou YB, Liu KC, Cao B, Wei QQ, Song W, Zhao B, Wu Y, Cheng JQ, Shang HF. Genetic Analysis of Prosaposin, the Lysosomal Storage Disorder Gene in Parkinson's Disease. *Mol Neurobiol.* 2021 Apr;58(4):1583-1592. doi: 10.1007/s12035-020-02218-4. Epub 2020 Nov 20. PMID: 33219486.
- Cheng F, Vivacqua G, Yu S. The role of α -synuclein in neurotransmission and synaptic plasticity. *J Chem Neuroanat.* 2011 Dec;42(4):242-8. doi: 10.1016/j.jchemneu.2010.12.001. Epub 2010 Dec 16. PMID: 21167933.
- Cheng HC, Ulane CM, Burke RE. Clinical progression in Parkinson disease and the neurobiology of axons. *Ann Neurol.* 2010 Jun;67(6):715-25. doi: 10.1002/ana.21995. PMID: 20517933; PMCID: PMC2918373.
- Chu Y, Dodiya H, Aebischer P, Olanow CW, Kordower JH. Alterations in lysosomal and proteasomal markers in Parkinson's disease: relationship to alpha-synuclein

inclusions. *Neurobiol Dis.* 2009 Sep;35(3):385-98. doi: 10.1016/j.nbd.2009.05.023. Epub 2009 Jun 6. PMID: 19505575.

Chu Y, Morfini GA, Langhamer LB, He Y, Brady ST, Kordower JH. Alterations in axonal transport motor proteins in sporadic and experimental Parkinson's disease. *Brain.* 2012 Jul;135(Pt 7):2058-73. doi: 10.1093/brain/aws133. Epub 2012 Jun 19. PMID: 22719003; PMCID: PMC4571141.

Ciechanover A, Kwon YT. Degradation of misfolded proteins in neurodegenerative diseases: therapeutic targets and strategies. *Exp Mol Med.* 2015 Mar 13;47(3):e147. doi: 10.1038/emm.2014.117. PMID: 25766616; PMCID: PMC4351408.

Colla E, Coune P, Liu Y, Pletnikova O, Troncoso JC, Iwatsubo T, Schneider BL, Lee MK. Endoplasmic reticulum stress is important for the manifestations of α -synucleinopathy in vivo. *J Neurosci.* 2012 Mar 7;32(10):3306-20. doi: 10.1523/JNEUROSCI.5367-11.2012. PMID: 22399753; PMCID: PMC3461828.

Cook C, Stetler C, Petrucelli L. Disruption of protein quality control in Parkinson's disease. *Cold Spring Harb Perspect Med.* 2012 May;2(5):a009423. doi: 10.1101/cshperspect.a009423. PMID: 22553500; PMCID: PMC3331692.

Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, Bhullar B, Liu K, Xu K, Strathern KE, Liu F, Cao S, Caldwell KA, Caldwell GA, Marsischky G, Kolodner RD, Labaer J, Rochet JC, Bonini NM, Lindquist S. Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science.* 2006 Jul 21;313(5785):324-

8. doi: 10.1126/science.1129462. Epub 2006 Jun 22. PMID: 16794039; PMCID: PMC1983366.

Cooper JA, Sagar HJ, Tidswell P, Jordan N. Slowed central processing in simple and go/no-go reaction time tasks in Parkinson's disease. *Brain*. 1994 Jun;117 (Pt 3):517-29. doi: 10.1093/brain/117.3.517. PMID: 8032862.

Cullen V, Lindfors M, Ng J, Paetau A, Swinton E, Kolodziej P, Boston H, Saftig P, Woulfe J, Feany MB, Myllykangas L, Schlossmacher MG, Tyynelä J. Cathepsin D expression level affects alpha-synuclein processing, aggregation, and toxicity in vivo. *Mol Brain*. 2009 Feb 9;2:5. doi: 10.1186/1756-6606-2-5. PMID: 19203374; PMCID: PMC2644690.

Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron*. 2003 Sep 11;39(6):889-909. doi: 10.1016/s0896-6273(03)00568-3. PMID: 12971891.

de Lau LM, Breteler MM. Epidemiology of Parkinson's disease. *Lancet Neurol*. 2006 Jun;5(6):525-35. doi: 10.1016/S1474-4422(06)70471-9. PMID: 16713924.

de Lau LM, Giesbergen PC, de Rijk MC, Hofman A, Koudstaal PJ, Breteler MM. Incidence of parkinsonism and Parkinson disease in a general population: the Rotterdam Study. *Neurology*. 2004 Oct 12;63(7):1240-4. doi: 10.1212/01.wnl.0000140706.52798.be. PMID: 15477545.

Deen MC, Zhu Y, Gros C, Na N, Gilormini PA, Shen DL, Bhosale S, Anastasi N, Wang R, Shan X, Harde E, Jagasia R, Lynn FC, Vocadlo DJ. A versatile fluorescence-quenched substrate for quantitative measurement of glucocerebrosidase activity within live cells.

Proc Natl Acad Sci U S A. 2022 Jul 19;119(29):e2200553119. doi: 10.1073/pnas.2200553119. Epub 2022 Jul 12. PMID: 35858317; PMCID: PMC9304032.

Dehay B, Ramirez A, Martinez-Vicente M, Perier C, Canon MH, Doudnikoff E, Vital A, Vila M, Klein C, Bezdard E. Loss of P-type ATPase ATP13A2/PARK9 function induces general lysosomal deficiency and leads to Parkinson disease neurodegeneration. Proc Natl Acad Sci U S A. 2012 Jun 12;109(24):9611-6. doi: 10.1073/pnas.1112368109. Epub 2012 May 30. PMID: 22647602; PMCID: PMC3386132.

DeKosky ST, Marek K. Looking backward to move forward: early detection of neurodegenerative disorders. Science. 2003 Oct 31;302(5646):830-4. doi: 10.1126/science.1090349. PMID: 14593169.

Deng H, Wang P, Jankovic J. The genetics of Parkinson disease. Ageing Res Rev. 2018 Mar;42:72-85. doi: 10.1016/j.arr.2017.12.007. Epub 2017 Dec 26. PMID: 29288112.

Deng X, Dzamko N, Prescott A, Davies P, Liu Q, Yang Q, Lee JD, Patricelli MP, Nomanbhoy TK, Alessi DR, Gray NS. Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2. Nat Chem Biol. 2011 Apr;7(4):203-5. doi: 10.1038/nchembio.538. Epub 2011 Mar 6. PMID: 21378983; PMCID: PMC3287420.

Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, Spencer B, Masliah E, Lee SJ. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. Proc Natl Acad Sci U S A. 2009 Aug

4;106(31):13010-5. doi: 10.1073/pnas.0903691106. Epub 2009 Jul 27. Erratum in: Proc Natl Acad Sci U S A. 2009 Oct 13;106(41):17606. PMID: 19651612; PMCID: PMC2722313.

Dickson DW. Neuropathology of Parkinson disease. *Parkinsonism Relat Disord*. 2018 Jan;46 Suppl 1(Suppl 1):S30-S33. doi: 10.1016/j.parkreldis.2017.07.033. Epub 2017 Aug 1. PMID: 28780180; PMCID: PMC5718208.

Dodson MW, Leung LK, Lone M, Lizzio MA, Guo M. Novel ethyl methanesulfonate (EMS)-induced null alleles of the *Drosophila* homolog of LRRK2 reveal a crucial role in endolysosomal functions and autophagy in vivo. *Dis Model Mech*. 2014 Dec;7(12):1351-63. doi: 10.1242/dmm.017020. Epub 2014 Oct 2. PMID: 25288684; PMCID: PMC4257004.

Doll RF, Smith FI. Regulation of expression of the gene encoding human acid beta-glucosidase in different cell types. *Gene*. 1993 May 30;127(2):255-60. doi: 10.1016/0378-1119(93)90729-m. PMID: 8500768.

Ebrahimi-Fakhari D, McLean PJ, Unni VK. Alpha-synuclein's degradation in vivo: opening a new (cranial) window on the roles of degradation pathways in Parkinson disease. *Autophagy*. 2012 Feb 1;8(2):281-3. doi: 10.4161/auto.8.2.18938. Epub 2012 Feb 1. PMID: 22301995; PMCID: PMC3336083.

Edison P, Ahmed I, Fan Z, Hinz R, Gelosa G, Ray Chaudhuri K, Walker Z, Turkheimer FE, Brooks DJ. Microglia, amyloid, and glucose metabolism in Parkinson's disease with

- and without dementia. *Neuropsychopharmacology*. 2013 May;38(6):938-49. doi: 10.1038/npp.2012.255. Epub 2012 Dec 6. PMID: 23303049; PMCID: PMC3629382.
- Eftekharzadeh B, Hyman BT, Wegmann S. Structural studies on the mechanism of protein aggregation in age related neurodegenerative diseases. *Mech Ageing Dev*. 2016 Jun;156:1-13. doi: 10.1016/j.mad.2016.03.001. Epub 2016 Mar 19. PMID: 27005270.
- Eguchi T, Kuwahara T, Sakurai M, Komori T, Fujimoto T, Ito G, Yoshimura SI, Harada A, Fukuda M, Koike M, Iwatsubo T. LRRK2 and its substrate Rab GTPases are sequentially targeted onto stressed lysosomes and maintain their homeostasis. *Proc Natl Acad Sci U S A*. 2018 Sep 25;115(39):E9115-E9124. doi: 10.1073/pnas.1812196115. Epub 2018 Sep 12. PMID: 30209220; PMCID: PMC6166828.
- Eisele YS, Monteiro C, Fearn C, Encalada SE, Wiseman RL, Powers ET, Kelly JW. Targeting protein aggregation for the treatment of degenerative diseases. *Nat Rev Drug Discov*. 2015 Nov;14(11):759-80. doi: 10.1038/nrd4593. Epub 2015 Sep 4. PMID: 26338154; PMCID: PMC4628595.
- Elkouzi A, Vedam-Mai V, Eisinger RS, Okun MS. Emerging therapies in Parkinson disease - repurposed drugs and new approaches. *Nat Rev Neurol*. 2019 Apr;15(4):204-223. doi: 10.1038/s41582-019-0155-7. PMID: 30867588; PMCID: PMC7758837.
- Elmore SP, Qian T, Grissom SF, Lemasters JJ. The mitochondrial permeability transition initiates autophagy in rat hepatocytes. *FASEB J*. 2001 Oct;15(12):2286-7. doi: 10.1096/fj.01-0206fje. Epub 2001 Aug 17. PMID: 11511528.

Farfel-Becker T, Vitner EB, Kelly SL, Bame JR, Duan J, Shinder V, Merrill AH Jr, Dobrenis K, Futerman AH. Neuronal accumulation of glucosylceramide in a mouse model of neuronopathic Gaucher disease leads to neurodegeneration. *Hum Mol Genet*. 2014 Feb 15;23(4):843-54. doi: 10.1093/hmg/ddt468. Epub 2013 Sep 24. PMID: 24064337; PMCID: PMC3900102.

Fauvet B, Mbefo MK, Fares MB, Desobry C, Michael S, Ardah MT, Tsika E, Coune P, Prudent M, Lion N, Eliezer D, Moore DJ, Schneider B, Aebischer P, El-Agnaf OM, Masliah E, Lashuel HA. α -Synuclein in central nervous system and from erythrocytes, mammalian cells, and *Escherichia coli* exists predominantly as disordered monomer. *J Biol Chem*. 2012 May 4;287(19):15345-64. doi: 10.1074/jbc.M111.318949. Epub 2012 Feb 7. PMID: 22315227; PMCID: PMC3346117.

Fell MJ, Mirescu C, Basu K, Cheewatrakoolpong B, DeMong DE, Ellis JM, Hyde LA, Lin Y, Markgraf CG, Mei H, Miller M, Poulet FM, Scott JD, Smith MD, Yin Z, Zhou X, Parker EM, Kennedy ME, Morrow JA. MLI-2, a Potent, Selective, and Centrally Active Compound for Exploring the Therapeutic Potential and Safety of LRRK2 Kinase Inhibition. *J Pharmacol Exp Ther*. 2015 Dec;355(3):397-409. doi: 10.1124/jpet.115.227587. Epub 2015 Sep 25. PMID: 26407721.

Gan-Or Z, Dion PA, Rouleau GA. Genetic perspective on the role of the autophagy-lysosome pathway in Parkinson disease. *Autophagy*. 2015;11(9):1443-57. doi: 10.1080/15548627.2015.1067364. PMID: 26207393; PMCID: PMC4590678.

Gao X, Chen H, Schwarzschild MA, Ascherio A. Use of ibuprofen and risk of Parkinson disease. *Neurology*. 2011 Mar 8;76(10):863-9. doi:

10.1212/WNL.0b013e31820f2d79. Epub 2011 Mar 2. PMID: 21368281; PMCID: PMC3059148.

Garcia-Reitböck P, Anichtchik O, Bellucci A, Iovino M, Ballini C, Fineberg E, Ghetti B, Della Corte L, Spano P, Tofaris GK, Goedert M, Spillantini MG. SNARE protein redistribution and synaptic failure in a transgenic mouse model of Parkinson's disease. *Brain*. 2010 Jul;133(Pt 7):2032-44. doi: 10.1093/brain/awq132. Epub 2010 Jun 9. PMID: 20534649; PMCID: PMC2892942.

Gegg ME, Burke D, Heales SJ, Cooper JM, Hardy J, Wood NW, Schapira AH. Glucocerebrosidase deficiency in substantia nigra of parkinson disease brains. *Ann Neurol*. 2012 Sep;72(3):455-63. doi: 10.1002/ana.23614. PMID: 23034917; PMCID: PMC3638323.

Gegg ME, Schapira AHV. The role of glucocerebrosidase in Parkinson disease pathogenesis. *FEBS J*. 2018 Oct;285(19):3591-3603. doi: 10.1111/febs.14393. Epub 2018 Feb 19. PMID: 29385658.

Gelb DJ, Oliver E, Gilman S. Diagnostic criteria for Parkinson disease. *Arch Neurol*. 1999 Jan;56(1):33-9. doi: 10.1001/archneur.56.1.33. PMID: 9923759.

Gerhard A, Pavese N, Hotton G, Turkheimer F, Es M, Hammers A, Eggert K, Oertel W, Banati RB, Brooks DJ. In vivo imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol Dis*. 2006 Feb;21(2):404-12. doi: 10.1016/j.nbd.2005.08.002. Epub 2005 Sep 21. PMID: 16182554.

- Giladi N, Kao R, Fahn S. Freezing phenomenon in patients with parkinsonian syndromes. *Mov Disord*. 1997 May;12(3):302-5. doi: 10.1002/mds.870120307. PMID: 9159723.
- Giladi N, McDermott MP, Fahn S, Przedborski S, Jankovic J, Stern M, Tanner C; Parkinson Study Group. Freezing of gait in PD: prospective assessment in the DATATOP cohort. *Neurology*. 2001 Jun 26;56(12):1712-21. doi: 10.1212/wnl.56.12.1712. PMID: 11425939.
- Gilks WP, Abou-Sleiman PM, Gandhi S, Jain S, Singleton A, Lees AJ, Shaw K, Bhatia KP, Bonifati V, Quinn NP, Lynch J, Healy DG, Holton JL, Revesz T, Wood NW. A common LRRK2 mutation in idiopathic Parkinson's disease. *Lancet*. 2005 Jan 29-Feb 4;365(9457):415-6. doi: 10.1016/S0140-6736(05)17830-1. PMID: 15680457.
- Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev*. 2002 Apr;82(2):373-428. doi: 10.1152/physrev.00027.2001. PMID: 11917093.
- Goers J, Manning-Bog AB, McCormack AL, Millett IS, Doniach S, Di Monte DA, Uversky VN, Fink AL. Nuclear localization of alpha-synuclein and its interaction with histones. *Biochemistry*. 2003 Jul 22;42(28):8465-71. doi: 10.1021/bi0341152. PMID: 12859192.
- Goker-Alpan O, Schiffmann R, LaMarca ME, Nussbaum RL, McInerney-Leo A, Sidransky E. Parkinsonism among Gaucher disease carriers. *J Med Genet*. 2004 Dec;41(12):937-40. doi: 10.1136/jmg.2004.024455. PMID: 15591280; PMCID: PMC1735652.

- Gómez-Sánchez R, Yakhine-Diop SM, Bravo-San Pedro JM, Pizarro-Estrella E, Rodríguez-Arribas M, Climent V, Martín-Cano FE, González-Soltero ME, Tandon A, Fuentes JM, González-Polo RA. PINK1 deficiency enhances autophagy and mitophagy induction. *Mol Cell Oncol*. 2015 Aug 20;3(2):e1046579. doi: 10.1080/23723556.2015.1046579. PMID: 27308585; PMCID: PMC4905358.
- Gosavi N, Lee HJ, Lee JS, Patel S, Lee SJ. Golgi fragmentation occurs in the cells with prefibrillar alpha-synuclein aggregates and precedes the formation of fibrillar inclusion. *J Biol Chem*. 2002 Dec 13;277(50):48984-92. doi: 10.1074/jbc.M208194200. Epub 2002 Sep 25. PMID: 12351643.
- Greenbaum J, Sidney J, Chung J, Brander C, Peters B, Sette A. Functional classification of class II human leukocyte antigen (HLA) molecules reveals seven different supertypes and a surprising degree of repertoire sharing across supertypes. *Immunogenetics*. 2011 Jun;63(6):325-35. doi: 10.1007/s00251-011-0513-0. Epub 2011 Feb 9. PMID: 21305276; PMCID: PMC3626422.
- Gu XL, Long CX, Sun L, Xie C, Lin X, Cai H. Astrocytic expression of Parkinson's disease-related A53T alpha-synuclein causes neurodegeneration in mice. *Mol Brain*. 2010 Apr 21;3:12. doi: 10.1186/1756-6606-3-12. PMID: 20409326; PMCID: PMC2873589.
- Haaxma CA, Bloem BR, Borm GF, Oyen WJ, Leenders KL, Eshuis S, Booij J, Dluzen DE, Horstink MW. Gender differences in Parkinson's disease. *J Neurol Neurosurg Psychiatry*. 2007 Aug;78(8):819-24. doi: 10.1136/jnnp.2006.103788. Epub 2006 Nov 10. PMID: 17098842; PMCID: PMC2117736.

- Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, Okano H, Mizushima N. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature*. 2006 Jun 15;441(7095):885-9. doi: 10.1038/nature04724. Epub 2006 Apr 19. PMID: 16625204.
- He Y, Appel S, Le W. Minocycline inhibits microglial activation and protects nigral cells after 6-hydroxydopamine injection into mouse striatum. *Brain Res*. 2001 Aug 3;909(1-2):187-93. doi: 10.1016/s0006-8993(01)02681-6. PMID: 11478935.
- Heinrich M, Wickel M, Schneider-Brachert W, Sandberg C, Gahr J, Schwandner R, Weber T, Saftig P, Peters C, Brunner J, Krönke M, Schütze S. Cathepsin D targeted by acid sphingomyelinase-derived ceramide. *EMBO J*. 1999 Oct 1;18(19):5252-63. doi: 10.1093/emboj/18.19.5252. Erratum in: *EMBO J* 2000 Jan 17;19(2):315. PMID: 10508159; PMCID: PMC1171596.
- Heinrich M, Wickel M, Winoto-Morbach S, Schneider-Brachert W, Weber T, Brunner J, Saftig P, Peters C, Krönke M, Schütze S. Ceramide as an activator lipid of cathepsin D. *Adv Exp Med Biol*. 2000;477:305-15. doi: 10.1007/0-306-46826-3_33. PMID: 10849758.
- Henry AG, Aghamohammadzadeh S, Samaroo H, Chen Y, Mou K, Needle E, Hirst WD. Pathogenic LRRK2 mutations, through increased kinase activity, produce enlarged lysosomes with reduced degradative capacity and increase ATP13A2 expression. *Hum Mol Genet*. 2015 Nov 1;24(21):6013-28. doi: 10.1093/hmg/ddv314. Epub 2015 Aug 6. PMID: 26251043.

- Herbst S, Campbell P, Harvey J, Bernard EM, Papayannopoulos V, Wood NW, Morris HR, Gutierrez MG. LRRK2 activation controls the repair of damaged endomembranes in macrophages. *EMBO J*. 2020 Sep 15;39(18):e104494. doi: 10.15252/embj.2020104494. Epub 2020 Jul 9. PMID: 32643832; PMCID: PMC7507578.
- Hoehn MM, Yahr MD. Parkinsonism: onset, progression, and mortality. 1967. *Neurology*. 2001 Nov;57(10 Suppl 3):S11-26. PMID: 11775596.
- Hsu LJ, Sagara Y, Arroyo A, Rockenstein E, Sisk A, Mallory M, Wong J, Takenouchi T, Hashimoto M, Masliah E. alpha-synuclein promotes mitochondrial deficit and oxidative stress. *Am J Pathol*. 2000 Aug;157(2):401-10. doi: 10.1016/s0002-9440(10)64553-1. PMID: 10934145; PMCID: PMC1850140.
- Huebecker M, Moloney EB, van der Spoel AC, Priestman DA, Isacson O, Hallett PJ, Platt FM. Reduced sphingolipid hydrolase activities, substrate accumulation and ganglioside decline in Parkinson's disease. *Mol Neurodegener*. 2019 Nov 8;14(1):40. doi: 10.1186/s13024-019-0339-z. Erratum in: *Mol Neurodegener*. 2020 Jan 15;15(1):6. PMID: 31703585; PMCID: PMC6842240.
- Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry*. 1992 Mar;55(3):181-4. doi: 10.1136/jnnp.55.3.181. PMID: 1564476; PMCID: PMC1014720.

Hunn BH, Cragg SJ, Bolam JP, Spillantini MG, Wade-Martins R. Impaired intracellular trafficking defines early Parkinson's disease. *Trends Neurosci.* 2015 Mar;38(3):178-88. doi: 10.1016/j.tins.2014.12.009. Epub 2015 Jan 29. PMID: 25639775; PMCID: PMC4740565.

Inestrosa NC, Marzolo MP, Bonnefont AB. Cellular and molecular basis of estrogen's neuroprotection. Potential relevance for Alzheimer's disease. *Mol Neurobiol.* 1998 Winter;17(1-3):73-86. doi: 10.1007/BF02802025. PMID: 9887447.

Janezic S, Threlfell S, Dodson PD, Dowie MJ, Taylor TN, Potgieter D, Parkkinen L, Senior SL, Anwar S, Ryan B, Deltheil T, Kosillo P, Cioroch M, Wagner K, Ansorge O, Bannerman DM, Bolam JP, Magill PJ, Cragg SJ, Wade-Martins R. Deficits in dopaminergic transmission precede neuron loss and dysfunction in a new Parkinson model. *Proc Natl Acad Sci U S A.* 2013 Oct 15;110(42):E4016-25. doi: 10.1073/pnas.1309143110. Epub 2013 Sep 30. PMID: 24082145; PMCID: PMC3801069.

Jankovic J. Parkinson's disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry.* 2008 Apr;79(4):368-76. doi: 10.1136/jnnp.2007.131045. PMID: 18344392.

Johnson IP. Age-related neurodegenerative disease research needs aging models. *Front Aging Neurosci.* 2015 Sep 2;7:168. doi: 10.3389/fnagi.2015.00168. PMID: 26388766; PMCID: PMC4557095.

- Kalia LV, Lang AE. Parkinson's disease. *Lancet*. 2015 Aug 29;386(9996):896-912. doi: 10.1016/S0140-6736(14)61393-3. Epub 2015 Apr 19. PMID: 25904081.
- Kam TI, Hinkle JT, Dawson TM, Dawson VL. Microglia and astrocyte dysfunction in parkinson's disease. *Neurobiol Dis*. 2020 Oct;144:105028. doi: 10.1016/j.nbd.2020.105028. Epub 2020 Jul 28. PMID: 32736085; PMCID: PMC7484088.
- Katzenschlager R, Lees AJ. Treatment of Parkinson's disease: levodopa as the first choice. *J Neurol*. 2002 Sep;249 Suppl 2:II19-24. doi: 10.1007/s00415-002-1204-4. PMID: 12375059.
- Kishimoto Y, Hiraiwa M, O'Brien JS. Saposins: structure, function, distribution, and molecular genetics. *J Lipid Res*. 1992 Sep;33(9):1255-67. PMID: 1402395.
- Klingelhoefer L, Reichmann H. Parkinson's disease as a multisystem disorder. *J Neural Transm (Vienna)*. 2017 Jun;124(6):709-713. doi: 10.1007/s00702-017-1692-0. Epub 2017 Feb 2. PMID: 28155133.
- Kluss JH, Conti MM, Kaganovich A, Beilina A, Melrose HL, Cookson MR, Mamais A. Detection of endogenous S1292 LRRK2 autophosphorylation in mouse tissue as a readout for kinase activity. *NPJ Parkinsons Dis*. 2018 Apr 19;4:13. doi: 10.1038/s41531-018-0049-1. PMID: 29707617; PMCID: PMC5908918.
- Kollmann K, Damme M, Markmann S, Morelle W, Schweizer M, Hermans-Borgmeyer I, Röcher AK, Pohl S, Lübke T, Michalski JC, Käkälä R, Walkley SU, Bräulke T. Lysosomal dysfunction causes neurodegeneration in mucopolipidosis II 'knock-in' mice.

Brain. 2012 Sep;135(Pt 9):2661-75. doi: 10.1093/brain/aws209. PMID: 22961545; PMCID: PMC3437033.

Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, Tanaka K. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature. 2006 Jun 15;441(7095):880-4. doi: 10.1038/nature04723. Epub 2006 Apr 19. PMID: 16625205.

Komatsu M, Wang QJ, Holstein GR, Friedrich VL Jr, Iwata J, Kominami E, Chait BT, Tanaka K, Yue Z. Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. Proc Natl Acad Sci U S A. 2007 Sep 4;104(36):14489-94. doi: 10.1073/pnas.0701311104. Epub 2007 Aug 28. PMID: 17726112; PMCID: PMC1964831.

Kontopoulos E, Parvin JD, Feany MB. Alpha-synuclein acts in the nucleus to inhibit histone acetylation and promote neurotoxicity. Hum Mol Genet. 2006 Oct 15;15(20):3012-23. doi: 10.1093/hmg/ddl243. Epub 2006 Sep 7. PMID: 16959795.

Krebiehl G, Ruckerbauer S, Burbulla LF, Kieper N, Maurer B, Waak J, Wolburg H, Gizatullina Z, Gellerich FN, Voitalla D, Riess O, Kahle PJ, Proikas-Cezanne T, Krüger R. Reduced basal autophagy and impaired mitochondrial dynamics due to loss of Parkinson's disease-associated protein DJ-1. PLoS One. 2010 Feb 23;5(2):e9367. doi: 10.1371/journal.pone.0009367. PMID: 20186336; PMCID: PMC2826413.

Krige D, Carroll MT, Cooper JM, Marsden CD, Schapira AH. Platelet mitochondrial function in Parkinson's disease. The Royal Kings and Queens Parkinson Disease Research

Group. *Ann Neurol.* 1992 Dec;32(6):782-8. doi: 10.1002/ana.410320612. PMID: 1471869.

Kritsilis M, V Rizou S, Koutsoudaki PN, Evangelou K, Gorgoulis VG, Papadopoulos D. Ageing, Cellular Senescence and Neurodegenerative Disease. *Int J Mol Sci.* 2018 Sep 27;19(10):2937. doi: 10.3390/ijms19102937. PMID: 30261683; PMCID: PMC6213570.

Kumar V, Sami N, Kashav T, Islam A, Ahmad F, Hassan MI. Protein aggregation and neurodegenerative diseases: From theory to therapy. *Eur J Med Chem.* 2016 Nov 29;124:1105-1120. doi: 10.1016/j.ejmech.2016.07.054. Epub 2016 Jul 26. PMID: 27486076.

Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science.* 1983 Feb 25;219(4587):979-80. doi: 10.1126/science.6823561. PMID: 6823561.

Lashuel HA, Overk CR, Oueslati A, Masliah E. The many faces of α -synuclein: from structure and toxicity to therapeutic target. *Nat Rev Neurosci.* 2013 Jan;14(1):38-48. doi: 10.1038/nrn3406. PMID: 23254192; PMCID: PMC4295774.

Lee A, Gilbert RM. Epidemiology of Parkinson Disease. *Neurol Clin.* 2016 Nov;34(4):955-965. doi: 10.1016/j.ncl.2016.06.012. Epub 2016 Aug 18. PMID: 27720003.

Lee CC, Nayak A, Sethuraman A, Belfort G, McRae GJ. A three-stage kinetic model of amyloid fibrillation. *Biophys J.* 2007 May 15;92(10):3448-58. doi:

10.1529/biophysj.106.098608. Epub 2007 Feb 26. PMID: 17325005; PMCID: PMC1853138.

Leroy E, Boyer R, Auburger G, Leube B, Ulm G, Mezey E, Harta G, Brownstein MJ, Jonnalagada S, Chernova T, Dehejia A, Lavedan C, Gasser T, Steinbach PJ, Wilkinson KD, Polymeropoulos MH. The ubiquitin pathway in Parkinson's disease. *Nature*. 1998 Oct 1;395(6701):451-2. doi: 10.1038/26652. PMID: 9774100.

Lewis PA. Leucine rich repeat kinase 2: a paradigm for pleiotropy. *J Physiol*. 2019 Jul;597(14):3511-3521. doi: 10.1113/JP276163. Epub 2019 Jun 11. PMID: 31124140.

Li SC, Schoenberg BS, Wang CC, Cheng XM, Rui DY, Bolis CL, Schoenberg DG. A prevalence survey of Parkinson's disease and other movement disorders in the People's Republic of China. *Arch Neurol*. 1985 Jul;42(7):655-7. doi: 10.1001/archneur.1985.04060070045013. PMID: 4015461.

Lindersson E, Beedholm R, Højrup P, Moos T, Gai W, Hendil KB, Jensen PH. Proteasomal inhibition by alpha-synuclein filaments and oligomers. *J Biol Chem*. 2004 Mar 26;279(13):12924-34. doi: 10.1074/jbc.M306390200. Epub 2004 Jan 7. PMID: 14711827.

Lippai M, Szatmári Z. Autophagy-from molecular mechanisms to clinical relevance. *Cell Biol Toxicol*. 2017 Apr;33(2):145-168. doi: 10.1007/s10565-016-9374-5. Epub 2016 Dec 12. PMID: 27957648.

Liu G, Zhang C, Yin J, Li X, Cheng F, Li Y, Yang H, Ueda K, Chan P, Yu S. alpha-Synuclein is differentially expressed in mitochondria from different rat brain regions and dose-

dependently down-regulates complex I activity. *Neurosci Lett*. 2009 May 1;454(3):187-92. doi: 10.1016/j.neulet.2009.02.056. Epub 2009 Feb 28. PMID: 19429081.

Luerman GC, Nguyen C, Samaroo H, Loos P, Xi H, Hurtado-Lorenzo A, Needle E, Stephen Noell G, Galatsis P, Dunlop J, Geoghegan KF, Hirst WD. Phosphoproteomic evaluation of pharmacological inhibition of leucine-rich repeat kinase 2 reveals significant off-target effects of LRRK-2-IN-1. *J Neurochem*. 2014 Feb;128(4):561-76. doi: 10.1111/jnc.12483. Epub 2013 Nov 11. PMID: 24117733.

MacLeod DA, Rhinn H, Kuwahara T, Zolin A, Di Paolo G, McCabe BD, Marder KS, Honig LS, Clark LN, Small SA, Abeliovich A. RAB7L1 interacts with LRRK2 to modify intraneuronal protein sorting and Parkinson's disease risk. *Neuron*. 2013 Feb 6;77(3):425-39. doi: 10.1016/j.neuron.2012.11.033. Erratum in: *Neuron*. 2013 Mar 6;77(5):994. MacCabe, Brian D [corrected to McCabe, Brian D]. PMID: 23395371; PMCID: PMC3646583.

Maday S, Wallace KE, Holzbaur EL. Autophagosomes initiate distally and mature during transport toward the cell soma in primary neurons. *J Cell Biol*. 2012 Feb 20;196(4):407-17. doi: 10.1083/jcb.201106120. Epub 2012 Feb 13. PMID: 22331844; PMCID: PMC3283992.

Malik BR, Maddison DC, Smith GA, Peters OM. Autophagic and endo-lysosomal dysfunction in neurodegenerative disease. *Mol Brain*. 2019 Nov 29;12(1):100. doi: 10.1186/s13041-019-0504-x. PMID: 31783880; PMCID: PMC6884906.

Manzoni C, Mamais A, Dihanich S, Abeti R, Soutar MPM, Plun-Favreau H, Giunti P, Tooze SA, Bandopadhyay R, Lewis PA. Inhibition of LRRK2 kinase activity stimulates macroautophagy. *Biochim Biophys Acta*. 2013 Dec;1833(12):2900-2910. doi: 10.1016/j.bbamcr.2013.07.020. Epub 2013 Aug 1. PMID: 23916833; PMCID: PMC3898616.

Manzoni C, Mamais A, Roosen DA, Dihanich S, Soutar MP, Plun-Favreau H, Bandopadhyay R, Hardy J, Tooze SA, Cookson MR, Lewis PA. mTOR independent regulation of macroautophagy by Leucine Rich Repeat Kinase 2 via Beclin-1. *Sci Rep*. 2016 Oct 12;6:35106. doi: 10.1038/srep35106. PMID: 27731364; PMCID: PMC5059726.

Martin WE, Loewenson RB, Resch JA, Baker AB. Parkinson's disease. Clinical analysis of 100 patients. *Neurology*. 1973 Aug;23(8):783-90. doi: 10.1212/wnl.23.8.783. PMID: 4578348.

Matta S, Van Kolen K, da Cunha R, van den Bogaart G, Mandemakers W, Miskiewicz K, De Bock PJ, Morais VA, Vilain S, Haddad D, Delbroek L, Swerts J, Chávez-Gutiérrez L, Esposito G, Daneels G, Karran E, Holt M, Gevaert K, Moechars DW, De Strooper B, Verstreken P. LRRK2 controls an EndoA phosphorylation cycle in synaptic endocytosis. *Neuron*. 2012 Sep 20;75(6):1008-21. doi: 10.1016/j.neuron.2012.08.022. PMID: 22998870.

Mazzulli JR, Xu YH, Sun Y, Knight AL, McLean PJ, Caldwell GA, Sidransky E, Grabowski GA, Krainc D. Gaucher disease glucocerebrosidase and α -synuclein form a bidirectional pathogenic loop in synucleinopathies. *Cell*. 2011 Jul 8;146(1):37-52. doi:

10.1016/j.cell.2011.06.001. Epub 2011 Jun 23. PMID: 21700325; PMCID: PMC3132082.

McGeer PL, Itagaki S, Boyes BE, McGeer EG. Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology*. 1988 Aug;38(8):1285-91. doi: 10.1212/wnl.38.8.1285. PMID: 3399080.

Melrose HL, Kent CB, Taylor JP, Dachsel JC, Hinkle KM, Lincoln SJ, Mok SS, Culvenor JG, Masters CL, Tyndall GM, Bass DI, Ahmed Z, Andorfer CA, Ross OA, Wszolek ZK, Delldonne A, Dickson DW, Farrer MJ. A comparative analysis of leucine-rich repeat kinase 2 (Lrrk2) expression in mouse brain and Lewy body disease. *Neuroscience*. 2007 Jul 29;147(4):1047-58. doi: 10.1016/j.neuroscience.2007.05.027. Epub 2007 Jul 3. PMID: 17611037.

Menzies FM, Fleming A, Caricasole A, Bento CF, Andrews SP, Ashkenazi A, Füllgrabe J, Jackson A, Jimenez Sanchez M, Karabiyik C, Licitra F, Lopez Ramirez A, Pavel M, Puri C, Renna M, Ricketts T, Schlotawa L, Vicinanza M, Won H, Zhu Y, Skidmore J, Rubinsztein DC. Autophagy and Neurodegeneration: Pathogenic Mechanisms and Therapeutic Opportunities. *Neuron*. 2017 Mar 8;93(5):1015-1034. doi: 10.1016/j.neuron.2017.01.022. PMID: 28279350.

Mercatelli D, Bolognesi P, Frassinetti M, Pisanò CA, Longo F, Shimshek DR, Morari M. Leucine-rich repeat kinase 2 (LRRK2) inhibitors differentially modulate glutamate release and Serine935 LRRK2 phosphorylation in striatal and cerebrocortical synaptosomes. *Pharmacol Res Perspect*. 2019 May 27;7(3):e00484. doi: 10.1002/prp2.484. PMID: 31149340; PMCID: PMC6536420.

- Mielke MM, Maetzler W, Haughey NJ, Bandaru VV, Savica R, Deuschle C, Gasser T, Hauser AK, Gräber-Sultan S, Schleicher E, Berg D, Liepelt-Scarfone I. Plasma ceramide and glucosylceramide metabolism is altered in sporadic Parkinson's disease and associated with cognitive impairment: a pilot study. *PLoS One*. 2013 Sep 18;8(9):e73094. doi: 10.1371/journal.pone.0073094. PMID: 24058461; PMCID: PMC3776817.
- Miki Y, Shimoyama S, Kon T, Ueno T, Hayakari R, Tanji K, Matsumiya T, Tsushima E, Mori F, Wakabayashi K, Tomiyama M. Alteration of autophagy-related proteins in peripheral blood mononuclear cells of patients with Parkinson's disease. *Neurobiol Aging*. 2018 Mar;63:33-43. doi: 10.1016/j.neurobiolaging.2017.11.006. Epub 2017 Nov 21. PMID: 29223072.
- Miklossy J, Arai T, Guo JP, Klegeris A, Yu S, McGeer EG, McGeer PL. LRRK2 expression in normal and pathologic human brain and in human cell lines. *J Neuropathol Exp Neurol*. 2006 Oct;65(10):953-63. doi: 10.1097/01.jnen.0000235121.98052.54. PMID: 17021400; PMCID: PMC7185781.
- Miller DB, O'Callaghan JP. Biomarkers of Parkinson's disease: present and future. *Metabolism*. 2015 Mar;64(3 Suppl 1):S40-6. doi: 10.1016/j.metabol.2014.10.030. Epub 2014 Oct 31. PMID: 25510818; PMCID: PMC4721253.
- Moiso N, Fedele V, Edwards J, Martins LM. Loss of PINK1 enhances neurodegeneration in a mouse model of Parkinson's disease triggered by mitochondrial stress. *Neuropharmacology*. 2014 Feb;77:350-7. doi: 10.1016/j.neuropharm.2013.10.009. Epub 2013 Oct 23. PMID: 24161480; PMCID: PMC3878764.

- Moon HE, Paek SH. Mitochondrial Dysfunction in Parkinson's Disease. *Exp Neurobiol*. 2015 Jun;24(2):103-16. doi: 10.5607/en.2015.24.2.103. Epub 2015 Jun 8. PMID: 26113789; PMCID: PMC4479806.
- Moors T, Paciotti S, Chiasserini D, Calabresi P, Parnetti L, Beccari T, van de Berg WD. Lysosomal Dysfunction and α -Synuclein Aggregation in Parkinson's Disease: Diagnostic Links. *Mov Disord*. 2016 Jun;31(6):791-801. doi: 10.1002/mds.26562. Epub 2016 Feb 29. PMID: 26923732.
- Moors TE, Paciotti S, Ingrassia A, Quadri M, Breedveld G, Tasegian A, Chiasserini D, Eusebi P, Duran-Pacheco G, Kremer T, Calabresi P, Bonifati V, Parnetti L, Beccari T, van de Berg WDJ. Characterization of Brain Lysosomal Activities in GBA-Related and Sporadic Parkinson's Disease and Dementia with Lewy Bodies. *Mol Neurobiol*. 2019 Feb;56(2):1344-1355. doi: 10.1007/s12035-018-1090-0. Epub 2018 Jun 8. PMID: 29948939; PMCID: PMC6400877.
- Morimoto S, Kishimoto Y, Tomich J, Weiler S, Ohashi T, Barranger JA, Kretz KA, O'Brien JS. Interaction of saposins, acidic lipids, and glucosylceramidase. *J Biol Chem*. 1990 Feb 5;265(4):1933-7. PMID: 2298731.
- Moscovitz O, Ben-Nissan G, Fainer I, Pollack D, Mizrahi L, Sharon M. The Parkinson's-associated protein DJ-1 regulates the 20S proteasome. *Nat Commun*. 2015 Apr 2;6:6609. doi: 10.1038/ncomms7609. PMID: 25833141.
- Motta M, Camerini S, Tatti M, Casella M, Torrerri P, Crescenzi M, Tartaglia M, Salvioli R. Gaucher disease due to saposin C deficiency is an inherited lysosomal disease caused

by rapidly degraded mutant proteins. *Hum Mol Genet.* 2014 Nov 1;23(21):5814-26. doi: 10.1093/hmg/ddu299. Epub 2014 Jun 12. PMID: 24925315.

Murphy KE, Gysbers AM, Abbott SK, Tayebi N, Kim WS, Sidransky E, Cooper A, Garner B, Halliday GM. Reduced glucocerebrosidase is associated with increased α -synuclein in sporadic Parkinson's disease. *Brain.* 2014 Mar;137(Pt 3):834-48. doi: 10.1093/brain/awt367. Epub 2014 Jan 28. PMID: 24477431; PMCID: PMC3927701.

Nalls MA, Blauwendraat C, Vallerga CL, Heilbron K, Bandres-Ciga S, Chang D, Tan M, Kia DA, Noyce AJ, Xue A, Bras J, Young E, von Coelln R, Simón-Sánchez J, Schulte C, Sharma M, Krohn L, Pihlstrøm L, Siitonen A, Iwaki H, Leonard H, Faghri F, Gibbs JR, Hernandez DG, Scholz SW, Botia JA, Martinez M, Corvol JC, Lesage S, Jankovic J, Shulman LM, Sutherland M, Tienari P, Majamaa K, Toft M, Andreassen OA, Bangale T, Brice A, Yang J, Gan-Or Z, Gasser T, Heutink P, Shulman JM, Wood NW, Hinds DA, Hardy JA, Morris HR, Gratten J, Visscher PM, Graham RR, Singleton AB; 23andMe Research Team; System Genomics of Parkinson's Disease Consortium; International Parkinson's Disease Genomics Consortium. 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 Dec;18(12):1091-1102. doi: 10.1016/S1474-4422(19)30320-5. PMID: 31701892; PMCID: PMC8422160.

Nalls MA, Pankratz N, Lill CM, Do CB, Hernandez DG, Saad M, DeStefano AL, Kara E, Bras J, Sharma M, Schulte C, Keller MF, Arepalli S, Letson C, Edsall C, Stefansson H, Liu X, Pliner H, Lee JH, Cheng R; International Parkinson's Disease Genomics Consortium (IPDGC); Parkinson's Study Group (PSG) Parkinson's Research: The

Organized GENetics Initiative (PROGENI); 23andMe; GenePD; NeuroGenetics Research Consortium (NGRC); Hussman Institute of Human Genomics (HIHG); Ashkenazi Jewish Dataset Investigator; Cohorts for Health and Aging Research in Genetic Epidemiology (CHARGE); North American Brain Expression Consortium (NABEC); United Kingdom Brain Expression Consortium (UKBEC); Greek Parkinson's Disease Consortium; Alzheimer Genetic Analysis Group, Ikram MA, Ioannidis JP, Hadjigeorgiou GM, Bis JC, Martinez M, Perlmutter JS, Goate A, Marder K, Fiske B, Sutherland M, Xiromerisiou G, Myers RH, Clark LN, Stefansson K, Hardy JA, Heutink P, Chen H, Wood NW, Houlden H, Payami H, Brice A, Scott WK, Gasser T, Bertram L, Eriksson N, Foroud T, Singleton AB. Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet.* 2014 Sep;46(9):989-93. doi: 10.1038/ng.3043. Epub 2014 Jul 27. PMID: 25064009; PMCID: PMC4146673.

Narhi L, Wood SJ, Steavenson S, Jiang Y, Wu GM, Anafi D, Kaufman SA, Martin F, Sitney K, Denis P, Louis JC, Wypych J, Biere AL, Citron M. Both familial Parkinson's disease mutations accelerate alpha-synuclein aggregation. *J Biol Chem.* 1999 Apr 2;274(14):9843-6. doi: 10.1074/jbc.274.14.9843. Erratum in: *J Biol Chem* 1999 May 7;274(19):13728. PMID: 10092675.

Nemani VM, Lu W, Berge V, Nakamura K, Onoa B, Lee MK, Chaudhry FA, Nicoll RA, Edwards RH. Increased expression of alpha-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle recluster after endocytosis. *Neuron.* 2010 Jan 14;65(1):66-79. doi: 10.1016/j.neuron.2009.12.023. PMID: 20152114; PMCID: PMC3119527.

Nichols WC, Pankratz N, Marek DK, Pauciulo MW, Elsaesser VE, Halter CA, Rudolph A, Wojcieszek J, Pfeiffer RF, Foroud T; Parkinson Study Group-PROGENI Investigators. Mutations in GBA are associated with familial Parkinson disease susceptibility and age at onset. *Neurology*. 2009 Jan 27;72(4):310-6. doi: 10.1212/01.wnl.0000327823.81237.d1. Epub 2008 Nov 5. PMID: 18987351; PMCID: PMC2677501.

Niimi Y, Ito S, Mizutani Y, Murate K, Shima S, Ueda A, Satake W, Hattori N, Toda T, Mutoh T. Altered regulation of serum lysosomal acid hydrolase activities in Parkinson's disease: A potential peripheral biomarker? *Parkinsonism Relat Disord*. 2019 Apr;61:132-137. doi: 10.1016/j.parkreldis.2018.10.032. Epub 2018 Nov 2. PMID: 30415794.

Obeso JA, Stamelou M, Goetz CG, Poewe W, Lang AE, Weintraub D, Burn D, Halliday GM, Bezard E, Przedborski S, Lehericy S, Brooks DJ, Rothwell JC, Hallett M, DeLong MR, Marras C, Tanner CM, Ross GW, Langston JW, Klein C, Bonifati V, Jankovic J, Lozano AM, Deuschl G, Bergman H, Tolosa E, Rodriguez-Violante M, Fahn S, Postuma RB, Berg D, Marek K, Standaert DG, Surmeier DJ, Olanow CW, Kordower JH, Calabresi P, Schapira AHV, Stoessl AJ. Past, present, and future of Parkinson's disease: A special essay on the 200th Anniversary of the Shaking Palsy. *Mov Disord*. 2017 Sep;32(9):1264-1310. doi: 10.1002/mds.27115. PMID: 28887905; PMCID: PMC5685546.

Omer N, Giladi N, Gurevich T, Bar-Shira A, Gana-Weisz M, Goldstein O, Kestenbaum M, Cedarbaum JM, Orr-Urtreger A, Mirelman A, Thaler A. A Possible Modifying Effect

of the G2019S Mutation in the LRRK2 Gene on GBA Parkinson's Disease. *Mov Disord.* 2020 Jul;35(7):1249-1253. doi: 10.1002/mds.28066. Epub 2020 Apr 30. PMID: 32353202.

Orenstein SJ, Kuo SH, Tasset I, Arias E, Koga H, Fernandez-Carasa I, Cortes E, Honig LS, Dauer W, Consiglio A, Raya A, Sulzer D, Cuervo AM. Interplay of LRRK2 with chaperone-mediated autophagy. *Nat Neurosci.* 2013 Apr;16(4):394-406. doi: 10.1038/nn.3350. Epub 2013 Mar 3. PMID: 23455607; PMCID: PMC3609872.

Papagiannakis N, Xilouri M, Koros C, Stamelou M, Antonelou R, Maniati M, Papadimitriou D, Moraitou M, Michelakakis H, Stefanis L. Lysosomal alterations in peripheral blood mononuclear cells of Parkinson's disease patients. *Mov Disord.* 2015 Nov;30(13):1830-4. doi: 10.1002/mds.26433. PMID: 26769460.

Park SM, Ahn KJ, Jung HY, Park JH, Kim J. Effects of novel peptides derived from the acidic tail of synuclein (ATS) on the aggregation and stability of fusion proteins. *Protein Eng Des Sel.* 2004 Mar;17(3):251-60. doi: 10.1093/protein/gzh029. Epub 2004 Apr 5. PMID: 15067107.

Park SM, Jung HY, Chung KC, Rhim H, Park JH, Kim J. Stress-induced aggregation profiles of GST-alpha-synuclein fusion proteins: role of the C-terminal acidic tail of alpha-synuclein in protein thermosolubility and stability. *Biochemistry.* 2002 Mar 26;41(12):4137-46. doi: 10.1021/bi015961k. PMID: 11900557.

Parkinson J. An essay on the shaking palsy. 1817. *J Neuropsychiatry Clin Neurosci.* 2002 Spring;14(2):223-36; discussion 222. doi: 10.1176/jnp.14.2.223. PMID: 11983801.

Parnetti L, Chiasserini D, Persichetti E, Eusebi P, Varghese S, Qureshi MM, Dardis A, Deganuto M, De Carlo C, Castrioto A, Balducci C, Paciotti S, Tambasco N, Bembi B, Bonanni L, Onofri M, Rossi A, Beccari T, El-Agnaf O, Calabresi P. Cerebrospinal fluid lysosomal enzymes and alpha-synuclein in Parkinson's disease. *Mov Disord*. 2014 Jul;29(8):1019-27. doi: 10.1002/mds.25772. Epub 2014 Jan 16. PMID: 24436092; PMCID: PMC4282452.

Parnetti L, Paciotti S, Eusebi P, Dardis A, Zampieri S, Chiasserini D, Tasegian A, Tambasco N, Bembi B, Calabresi P, Beccari T. Cerebrospinal fluid β -glucocerebrosidase activity is reduced in parkinson's disease patients. *Mov Disord*. 2017 Oct;32(10):1423-1431. doi: 10.1002/mds.27136. Epub 2017 Aug 26. PMID: 28843015.

Piccoli G, Condliffe SB, Bauer M, Giesert F, Boldt K, De Astis S, Meixner A, Sarioglu H, Vogt-Weisenhorn DM, Wurst W, Gloeckner CJ, Matteoli M, Sala C, Ueffing M. LRRK2 controls synaptic vesicle storage and mobilization within the recycling pool. *J Neurosci*. 2011 Feb 9;31(6):2225-37. doi: 10.1523/JNEUROSCI.3730-10.2011. PMID: 21307259; PMCID: PMC6633036.

Pieri L, Madiona K, Bousset L, Melki R. Fibrillar α -synuclein and huntingtin exon 1 assemblies are toxic to the cells. *Biophys J*. 2012 Jun 20;102(12):2894-905. doi: 10.1016/j.bpj.2012.04.050. Epub 2012 Jun 19. PMID: 22735540; PMCID: PMC3379023.

Plowey ED, Cherra SJ 3rd, Liu YJ, Chu CT. Role of autophagy in G2019S-LRRK2-associated neurite shortening in differentiated SH-SY5Y cells. *J Neurochem*. 2008

May;105(3):1048-56. doi: 10.1111/j.1471-4159.2008.05217.x. Epub 2008 Jan 7.
PMID: 18182054; PMCID: PMC2361385.

Poewe W. Treatments for Parkinson disease--past achievements and current clinical needs.
Neurology. 2009 Feb 17;72(7 Suppl):S65-73. doi: 10.1212/WNL.0b013e31819908ce.
PMID: 19221317.

Postuma RB, Berg D, Stern M, Poewe W, Olanow CW, Oertel W, Obeso J, Marek K, Litvan
I, Lang AE, Halliday G, Goetz CG, Gasser T, Dubois B, Chan P, Bloem BR, Adler
CH, Deuschl G. MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord*.
2015 Oct;30(12):1591-601. doi: 10.1002/mds.26424. PMID: 26474316.

Prieto Huarcaya S, Drobny A, Marques ARA, Di Spiezio A, Dobert JP, Balta D, Werner C,
Rizo T, Gallwitz L, Bub S, Stojkovska I, Belur NR, Fogh J, Mazzulli JR, Xiang W,
Fulzele A, Dejung M, Sauer M, Winner B, Rose-John S, Arnold P, Saftig P, Zunke F.
Recombinant pro-CTSD (cathepsin D) enhances SNCA/ α -Synuclein degradation in α -
Synucleinopathy models. *Autophagy*. 2022 May;18(5):1127-1151. doi:
10.1080/15548627.2022.2045534. Epub 2022 Apr 28. PMID: 35287553; PMCID:
PMC9196656.

Pukaß K, Richter-Landsberg C. Inhibition of UCH-L1 in oligodendroglial cells results in
microtubule stabilization and prevents α -synuclein aggregate formation by activating
the autophagic pathway: implications for multiple system atrophy. *Front Cell
Neurosci*. 2015 May 5;9:163. doi: 10.3389/fncel.2015.00163. PMID: 25999815;
PMCID: PMC4419839.

- Qi Z, Yang W, Liu Y, Cui T, Gao H, Duan C, Lu L, Zhao C, Zhao H, Yang H. Loss of PINK1 function decreases PP2A activity and promotes autophagy in dopaminergic cells and a murine model. *Neurochem Int.* 2011 Oct;59(5):572-81. doi: 10.1016/j.neuint.2011.03.023. Epub 2011 Jun 13. PMID: 21672589.
- Rajput AH, Rozdilsky B, Ang L. Occurrence of resting tremor in Parkinson's disease. *Neurology.* 1991 Aug;41(8):1298-9. doi: 10.1212/wnl.41.8.1298. PMID: 1866022.
- Ramonet D, Daher JP, Lin BM, Stafa K, Kim J, Banerjee R, Westerlund M, Pletnikova O, Glauser L, Yang L, Liu Y, Swing DA, Beal MF, Troncoso JC, McCaffery JM, Jenkins NA, Copeland NG, Galter D, Thomas B, Lee MK, Dawson TM, Dawson VL, Moore DJ. Dopaminergic neuronal loss, reduced neurite complexity and autophagic abnormalities in transgenic mice expressing G2019S mutant LRRK2. *PLoS One.* 2011 Apr 6;6(4):e18568. doi: 10.1371/journal.pone.0018568. PMID: 21494637; PMCID: PMC3071839.
- Roy B, Jackson GR. Interactions between Tau and α -synuclein augment neurotoxicity in a *Drosophila* model of Parkinson's disease. *Hum Mol Genet.* 2014 Jun 1;23(11):3008-23. doi: 10.1093/hmg/ddu011. Epub 2014 Jan 14. PMID: 24430504; PMCID: PMC4014195.
- Roze E, Paschke E, Lopez N, Eck T, Yoshida K, Maurel-Ollivier A, Doummar D, Caillaud C, Galanaud D, Billette de Villemeur T, Vidailhet M, Roubergue A. Dystonia and parkinsonism in GM1 type 3 gangliosidosis. *Mov Disord.* 2005 Oct;20(10):1366-9. doi: 10.1002/mds.20593. PMID: 15986423.

Ryan E, Seehra G, Sharma P, Sidransky E. GBA1-associated parkinsonism: new insights and therapeutic opportunities. *Curr Opin Neurol.* 2019 Aug;32(4):589-596. doi: 10.1097/WCO.0000000000000715. PMID: 31188151.

Sandhoff K, Kolter T. Biosynthesis and degradation of mammalian glycosphingolipids. *Philos Trans R Soc Lond B Biol Sci.* 2003 May 29;358(1433):847-61. doi: 10.1098/rstb.2003.1265. PMID: 12803917; PMCID: PMC1693173.

Sanyal A, DeAndrade MP, Novis HS, Lin S, Chang J, Lengacher N, Tomlinson JJ, Tansey MG, LaVoie MJ. Lysosome and Inflammatory Defects in GBA1-Mutant Astrocytes Are Normalized by LRRK2 Inhibition. *Mov Disord.* 2020 May;35(5):760-773. doi: 10.1002/mds.27994. Epub 2020 Feb 8. PMID: 32034799; PMCID: PMC8167931.

Scherzer C. Interview with Clemens Scherzer--searching for biomarkers in Parkinson's disease. *Biomark Med.* 2009 Apr;3(2):113-4. doi: 10.2217/bmm.09.10. PMID: 20477503.

Schulte C, Gasser T. Genetic basis of Parkinson's disease: inheritance, penetrance, and expression. *Appl Clin Genet.* 2011 Jun 1;4:67-80. doi: 10.2147/TACG.S11639. PMID: 23776368; PMCID: PMC3681179.

Scott D, Roy S. α -Synuclein inhibits intersynaptic vesicle mobility and maintains recycling-pool homeostasis. *J Neurosci.* 2012 Jul 25;32(30):10129-35. doi: 10.1523/JNEUROSCI.0535-12.2012. PMID: 22836248; PMCID: PMC3426499.

Scott DA, Tabarean I, Tang Y, Cartier A, Masliah E, Roy S. A pathologic cascade leading to synaptic dysfunction in alpha-synuclein-induced neurodegeneration. *J Neurosci.* 2010

Jun 16;30(24):8083-95. doi: 10.1523/JNEUROSCI.1091-10.2010. PMID: 20554859; PMCID: PMC2901533.

Sevlever D, Jiang P, Yen SH. Cathepsin D is the main lysosomal enzyme involved in the degradation of alpha-synuclein and generation of its carboxy-terminally truncated species. *Biochemistry*. 2008 Sep 9;47(36):9678-87. doi: 10.1021/bi800699v. Epub 2008 Aug 15. PMID: 18702517; PMCID: PMC2630205.

Sidransky E, Lopez G. The link between the GBA gene and parkinsonism. *Lancet Neurol*. 2012 Nov;11(11):986-98. doi: 10.1016/S1474-4422(12)70190-4. PMID: 23079555; PMCID: PMC4141416.

Sidransky E, Nalls MA, Aasly JO, Aharon-Peretz J, Annesi G, Barbosa ER, Bar-Shira A, Berg D, Bras J, Brice A, Chen CM, Clark LN, Condroyer C, De Marco EV, Dürr A, Eblan MJ, Fahn S, Farrer MJ, Fung HC, Gan-Or Z, Gasser T, Gershoni-Baruch R, Giladi N, Griffith A, Gurevich T, Januario C, Kropp P, Lang AE, Lee-Chen GJ, Lesage S, Marder K, Mata IF, Mirelman A, Mitsui J, Mizuta I, Nicoletti G, Oliveira C, Ottman R, Orr-Urtreger A, Pereira LV, Quattrone A, Rogaeva E, Rolfs A, Rosenbaum H, Rozenberg R, Samii A, Samaddar T, Schulte C, Sharma M, Singleton A, Spitz M, Tan EK, Tayebi N, Toda T, Troiano AR, Tsuji S, Wittstock M, Wolfsberg TG, Wu YR, Zabetian CP, Zhao Y, Ziegler SG. Multicenter analysis of glucocerebrosidase mutations in Parkinson's disease. *N Engl J Med*. 2009 Oct 22;361(17):1651-61. doi: 10.1056/NEJMoA0901281. PMID: 19846850; PMCID: PMC2856322.

Siintola E, Partanen S, Strömme P, Haapanen A, Haltia M, Maehlen J, Lehesjoki AE, Tyynelä J. Cathepsin D deficiency underlies congenital human neuronal ceroid-lipofuscinosis.

Brain. 2006 Jun;129(Pt 6):1438-45. doi: 10.1093/brain/awl107. Epub 2006 May 2. PMID: 16670177.

Smith GA, Jansson J, Rocha EM, Osborn T, Hallett PJ, Isacson O. Fibroblast Biomarkers of Sporadic Parkinson's Disease and LRRK2 Kinase Inhibition. Mol Neurobiol. 2016 Oct;53(8):5161-77. doi: 10.1007/s12035-015-9435-4. Epub 2015 Sep 23. PMID: 26399642; PMCID: PMC5012155.

Smith WW, Jiang H, Pei Z, Tanaka Y, Morita H, Sawa A, Dawson VL, Dawson TM, Ross CA. Endoplasmic reticulum stress and mitochondrial cell death pathways mediate A53T mutant alpha-synuclein-induced toxicity. Hum Mol Genet. 2005 Dec 15;14(24):3801-11. doi: 10.1093/hmg/ddi396. Epub 2005 Oct 20. PMID: 16239241.

Sosero YL, Bandres-Ciga S, Hassin-Baer S, Alcalay RN, Gan-Or Z; International Parkinson's Disease Genomics Consortium (IPDGC). Lack of evidence for genetic association of saposins A, B, C and D with Parkinson's disease. Brain. 2020 Sep 1;143(9):e72. doi: 10.1093/brain/awaa214. PMID: 32793944; PMCID: PMC7523699.

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature. 1997 Aug 28;388(6645):839-40. doi: 10.1038/42166. PMID: 9278044.

Stefanis L. α -Synuclein in Parkinson's disease. Cold Spring Harb Perspect Med. 2012 Feb;2(2):a009399. doi: 10.1101/cshperspect.a009399. PMID: 22355802; PMCID: PMC3281589.

- Stern MB, Doty RL, Dotti M, Corcoran P, Crawford D, McKeown DA, Adler C, Gollomp S, Hurtig H. Olfactory function in Parkinson's disease subtypes. *Neurology*. 1994 Feb;44(2):266-8. doi: 10.1212/wnl.44.2.266. PMID: 8309571.
- Stojkowska I, Wani WY, Zunke F, Belur NR, Pavlenko EA, Mwenda N, Sharma K, Francelle L, Mazzulli JR. Rescue of α -synuclein aggregation in Parkinson's patient neurons by synergistic enhancement of ER proteostasis and protein trafficking. *Neuron*. 2022 Feb 2;110(3):436-451.e11. doi: 10.1016/j.neuron.2021.10.032. Epub 2021 Nov 17. PMID: 34793693; PMCID: PMC8815333.
- Su X, Maguire-Zeiss KA, Giuliano R, Prifti L, Venkatesh K, Federoff HJ. Synuclein activates microglia in a model of Parkinson's disease. *Neurobiol Aging*. 2008 Nov;29(11):1690-701. doi: 10.1016/j.neurobiolaging.2007.04.006. Epub 2007 May 29. PMID: 17537546; PMCID: PMC2621109.
- Sun X, Marks DL, Park WD, Wheatley CL, Puri V, O'Brien JF, Kraft DL, Lundquist PA, Patterson MC, Pagano RE, Snow K. Niemann-Pick C variant detection by altered sphingolipid trafficking and correlation with mutations within a specific domain of NPC1. *Am J Hum Genet*. 2001 Jun;68(6):1361-72. doi: 10.1086/320599. Epub 2001 May 9. PMID: 11349231; PMCID: PMC1226123.
- Sun Y, Qi X, Grabowski GA. Saposin C is required for normal resistance of acid beta-glucosidase to proteolytic degradation. *J Biol Chem*. 2003 Aug 22;278(34):31918-23. doi: 10.1074/jbc.M302752200. Epub 2003 Jun 17. PMID: 12813057.

- Surguchov A. Molecular and cellular biology of synucleins. *Int Rev Cell Mol Biol.* 2008;270:225-317. doi: 10.1016/S1937-6448(08)01406-8. PMID: 19081538.
- Suzuki K, Iseki E, Katsuse O, Yamaguchi A, Katsuyama K, Aoki I, Yamanaka S, Kosaka K. Neuronal accumulation of alpha- and beta-synucleins in the brain of a GM2 gangliosidosis mouse model. *Neuroreport.* 2003 Mar 24;14(4):551-4. doi: 10.1097/00001756-200303240-00004. PMID: 12657883.
- Sweeney P, Park H, Baumann M, Dunlop J, Frydman J, Kopito R, McCampbell A, Leblanc G, Venkateswaran A, Nurmi A, Hodgson R. Protein misfolding in neurodegenerative diseases: implications and strategies. *Transl Neurodegener.* 2017 Mar 13;6:6. doi: 10.1186/s40035-017-0077-5. PMID: 28293421; PMCID: PMC5348787.
- Swerdlow RH, Parks JK, Miller SW, Tuttle JB, Trimmer PA, Sheehan JP, Bennett JP Jr, Davis RE, Parker WD Jr. Origin and functional consequences of the complex I defect in Parkinson's disease. *Ann Neurol.* 1996 Oct;40(4):663-71. doi: 10.1002/ana.410400417. PMID: 8871587.
- Tamargo RJ, Velayati A, Goldin E, Sidransky E. The role of saposin C in Gaucher disease. *Mol Genet Metab.* 2012 Jul;106(3):257-63. doi: 10.1016/j.ymgme.2012.04.024. Epub 2012 May 5. PMID: 22652185; PMCID: PMC3534739.
- Tan JM, Wong ES, Lim KL. Protein misfolding and aggregation in Parkinson's disease. *Antioxid Redox Signal.* 2009 Sep;11(9):2119-34. doi: 10.1089/ars.2009.2490. PMID: 19243238.

- Tanaka Y, Engelender S, Igarashi S, Rao RK, Wanner T, Tanzi RE, Sawa A, Dawson V, Dawson TM, Ross CA. Inducible expression of mutant alpha-synuclein decreases proteasome activity and increases sensitivity to mitochondria-dependent apoptosis. *Hum Mol Genet.* 2001 Apr 15;10(9):919-26. doi: 10.1093/hmg/10.9.919. PMID: 11309365.
- Tanner CM, Goldman SM. Epidemiology of Parkinson's disease. *Neurol Clin.* 1996 May;14(2):317-35. doi: 10.1016/S0733-8619(05)70259-0. PMID: 8827174; PMCID: PMC7173037.
- Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, Marras C, Bhudhikanok GS, Kasten M, Chade AR, Comyns K, Richards MB, Meng C, Priestley B, Fernandez HH, Cambi F, Umbach DM, Blair A, Sandler DP, Langston JW. Rotenone, paraquat, and Parkinson's disease. *Environ Health Perspect.* 2011 Jun;119(6):866-72. doi: 10.1289/ehp.1002839. Epub 2011 Jan 26. PMID: 21269927; PMCID: PMC3114824.
- Tasegian A, Singh F, Ganley IG, Reith AD, Alessi DR. Impact of Type II LRRK2 inhibitors on signaling and mitophagy. *Biochem J.* 2021 Oct 15;478(19):3555-3573. doi: 10.1042/BCJ20210375. PMID: 34515301; PMCID: PMC8589421.
- Taylor M, Alessi DR. Advances in elucidating the function of leucine-rich repeat protein kinase-2 in normal cells and Parkinson's disease. *Curr Opin Cell Biol.* 2020 Apr;63:102-113. doi: 10.1016/j.ceb.2020.01.001. Epub 2020 Feb 7. PMID: 32036294; PMCID: PMC7262585.

Thayanidhi N, Helm JR, Nycz DC, Bentley M, Liang Y, Hay JC. Alpha-synuclein delays endoplasmic reticulum (ER)-to-Golgi transport in mammalian cells by antagonizing ER/Golgi SNAREs. *Mol Biol Cell*. 2010 Jun 1;21(11):1850-63. doi: 10.1091/mbc.e09-09-0801. Epub 2010 Apr 14. PMID: 20392839; PMCID: PMC2877643.

Tosatto L, Andrighetti AO, Plotegher N, Antonini V, Tessari I, Ricci L, Bubacco L, Dalla Serra M. Alpha-synuclein pore forming activity upon membrane association. *Biochim Biophys Acta*. 2012 Nov;1818(11):2876-83. doi: 10.1016/j.bbamem.2012.07.007. Epub 2012 Jul 20. PMID: 22820150.

Tsigelny IF, Sharikov Y, Wrasidlo W, Gonzalez T, Desplats PA, Crews L, Spencer B, Masliah E. Role of α -synuclein penetration into the membrane in the mechanisms of oligomer pore formation. *FEBS J*. 2012 Mar;279(6):1000-13. doi: 10.1111/j.1742-4658.2012.08489.x. Epub 2012 Feb 27. PMID: 22251432; PMCID: PMC3925782.

Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J*. 2008 Jan 23;27(2):433-46. doi: 10.1038/sj.emboj.7601963. Epub 2008 Jan 17. PMID: 18200046; PMCID: PMC2234339.

Ueda K, Fukushima H, Masliah E, Xia Y, Iwai A, Yoshimoto M, Otero DA, Kondo J, Ihara Y, Saitoh T. Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease. *Proc Natl Acad Sci U S A*. 1993 Dec 1;90(23):11282-6. doi: 10.1073/pnas.90.23.11282. PMID: 8248242; PMCID: PMC47966.

UN. United Nations. World population ageing 2013. United Nations. Department of Economic and Social Affairs Population Division; Available online at: <http://www.un.org/en/development/desa/population/publications/pdf/ageing/WorldPopulationAgeingReport2013.pdf>

Usenko TS, Senkevich KA, Bezrukova AI, Baydakova GV, Basharova KS, Zhuravlev AS, Gracheva EV, Kudrevatykh AV, Miliukhina IV, Krasakov IV, Khublarova LA, Fursova IV, Zakharov DV, Timofeeva AA, Irishina YA, Palchikova EI, Zalutskaya NM, Emelyanov AK, Zakharova EY, Pchelina SN. Impaired Sphingolipid Hydrolase Activities in Dementia with Lewy Bodies and Multiple System Atrophy. *Mol Neurobiol*. 2022 Apr;59(4):2277-2287. doi: 10.1007/s12035-021-02688-0. Epub 2022 Jan 23. PMID: 35066761.

Usenovic M, Tresse E, Mazzulli JR, Taylor JP, Krainc D. Deficiency of ATP13A2 leads to lysosomal dysfunction, α -synuclein accumulation, and neurotoxicity. *J Neurosci*. 2012 Mar 21;32(12):4240-6. doi: 10.1523/JNEUROSCI.5575-11.2012. PMID: 22442086; PMCID: PMC3462811.

Vaccaro AM, Motta M, Tatti M, Scarpa S, Masuelli L, Bhat M, Vanier MT, Tylki-Szymanska A, Salvioli R. Saposin C mutations in Gaucher disease patients resulting in lysosomal lipid accumulation, saposin C deficiency, but normal prosaposin processing and sorting. *Hum Mol Genet*. 2010 Aug 1;19(15):2987-97. doi: 10.1093/hmg/ddq204. Epub 2010 May 19. PMID: 20484222.

van Dijk KD, Persichetti E, Chiasserini D, Eusebi P, Beccari T, Calabresi P, Berendse HW, Parnetti L, van de Berg WD. Changes in endolysosomal enzyme activities in

- cerebrospinal fluid of patients with Parkinson's disease. *Mov Disord.* 2013 Jun;28(6):747-54. doi: 10.1002/mds.25495. Epub 2013 May 27. PMID: 23712522.
- van Rooijen BD, Claessens MM, Subramaniam V. Membrane Permeabilization by Oligomeric α -Synuclein: In Search of the Mechanism. *PLoS One.* 2010 Dec 13;5(12):e14292. doi: 10.1371/journal.pone.0014292. PMID: 21179192; PMCID: PMC3001441.
- Vavilova JD, Boyko AA, Troyanova NI, Ponomareva NV, Fokin VF, Fedotova EY, Streltsova MA, Kust SA, Grechikhina MV, Shustova OA, Azhikina TL, Kovalenko EI, Sapozhnikov AM. Alterations in Proteostasis System Components in Peripheral Blood Mononuclear Cells in Parkinson Disease: Focusing on the HSP70 and p62 Levels. *Biomolecules.* 2022 Mar 24;12(4):493. doi: 10.3390/biom12040493. PMID: 35454081; PMCID: PMC9030208.
- Vijiaratnam N, Simuni T, Bandmann O, Morris HR, Foltynie T. Progress towards therapies for disease modification in Parkinson's disease. *Lancet Neurol.* 2021 Jul;20(7):559-572. doi: 10.1016/S1474-4422(21)00061-2. PMID: 34146514.
- Volpicelli-Daley LA, Abdelmotilib H, Liu Z, Stoyka L, Daher JP, Milnerwood AJ, Unni VK, Hirst WD, Yue Z, Zhao HT, Fraser K, Kennedy RE, West AB. G2019S-LRRK2 Expression Augments α -Synuclein Sequestration into Inclusions in Neurons. *J Neurosci.* 2016 Jul 13;36(28):7415-27. doi: 10.1523/JNEUROSCI.3642-15.2016. Erratum in: *J Neurosci.* 2022 Jan 26;42(4):718. PMID: 27413152; PMCID: PMC4945663.

Wade-Martins R. Genetics: The MAPT locus-a genetic paradigm in disease susceptibility. Nat Rev Neurol. 2012 Sep;8(9):477-8. doi: 10.1038/nrneurol.2012.169. PMID: 22940644.

Williams-Gray CH, Wijeyekoon R, Yarnall AJ, Lawson RA, Breen DP, Evans JR, Cummins GA, Duncan GW, Khoo TK, Burn DJ, Barker RA; ICICLE-PD study group. Serum immune markers and disease progression in an incident Parkinson's disease cohort (ICICLE-PD). Mov Disord. 2016 Jul;31(7):995-1003. doi: 10.1002/mds.26563. Epub 2016 Mar 21. PMID: 26999434; PMCID: PMC4957620.

Wood SJ, Wypych J, Steavenson S, Louis JC, Citron M, Biere AL. alpha-synuclein fibrillogenesis is nucleation-dependent. Implications for the pathogenesis of Parkinson's disease. J Biol Chem. 1999 Jul 9;274(28):19509-12. doi: 10.1074/jbc.274.28.19509. PMID: 10391881.

Wood-Kaczmar A, Gandhi S, Yao Z, Abramov AY, Miljan EA, Keen G, Stanyer L, Hargreaves I, Klupsch K, Deas E, Downward J, Mansfield L, Jat P, Taylor J, Heales S, Duchon MR, Latchman D, Tabrizi SJ, Wood NW. PINK1 is necessary for long term survival and mitochondrial function in human dopaminergic neurons. PLoS One. 2008 Jun 18;3(6):e2455. doi: 10.1371/journal.pone.0002455. Erratum in: PLoS ONE. 2008;3(7). doi: 10.1371/annotation/17d5aaa1-c6d8-4aad-a9a4-56b2c1220c83. Abramov, Andrey S Y [corrected to Abramov, Andrey Y]. Erratum in: PLoS ONE. 2008;3(7). doi: 10.1371/annotation/ba489c2a-5cf2-481c-aff7-d2c8c4ecdca. PMID: 18560593; PMCID: PMC2413012.

- Wooten GF, Currie LJ, Bovbjerg VE, Lee JK, Patrie J. Are men at greater risk for Parkinson's disease than women? *J Neurol Neurosurg Psychiatry*. 2004 Apr;75(4):637-9. doi: 10.1136/jnnp.2003.020982. PMID: 15026515; PMCID: PMC1739032.
- Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, Choi DK, Ischiropoulos H, Przedborski S. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J Neurosci*. 2002 Mar 1;22(5):1763-71. doi: 10.1523/JNEUROSCI.22-05-01763.2002. PMID: 11880505; PMCID: PMC6758858.
- Wu G, Yan B, Wang X, Feng X, Zhang A, Xu X, Dong H. Decreased activities of lysosomal acid alpha-D-galactosidase A in the leukocytes of sporadic Parkinson's disease. *J Neurol Sci*. 2008 Aug 15;271(1-2):168-73. doi: 10.1016/j.jns.2008.04.011. Epub 2008 May 20. PMID: 18495164.
- Xu K, Xu Y, Brown-Jermyn D, Chen JF, Ascherio A, Dluzen DE, Schwarzschild MA. Estrogen prevents neuroprotection by caffeine in the mouse 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's disease. *J Neurosci*. 2006 Jan 11;26(2):535-41. doi: 10.1523/JNEUROSCI.3008-05.2006. PMID: 16407551; PMCID: PMC6674425.
- Yadav RS, Tiwari NK. Lipid integration in neurodegeneration: an overview of Alzheimer's disease. *Mol Neurobiol*. 2014 Aug;50(1):168-76. doi: 10.1007/s12035-014-8661-5. Epub 2014 Mar 5. PMID: 24590317.

- Yahalom G, Greenbaum L, Israeli-Korn S, Fay-Karmon T, Livneh V, Ruskey JA, Roncière L, Alam A, Gan-Or Z, Hassin-Baer S. Carriers of both GBA and LRRK2 mutations, compared to carriers of either, in Parkinson's disease: Risk estimates and genotype-phenotype correlations. *Parkinsonism Relat Disord*. 2019 May;62:179-184. doi: 10.1016/j.parkreldis.2018.12.014. Epub 2018 Dec 13. PMID: 30573413.
- Yan C, Huo H, Yang C, Zhang T, Chu Y, Liu Y. Ubiquitin C-Terminal Hydrolase L1 regulates autophagy by inhibiting autophagosome formation through its deubiquitinating enzyme activity. *Biochem Biophys Res Commun*. 2018 Mar 4;497(2):726-733. doi: 10.1016/j.bbrc.2018.02.140. Epub 2018 Feb 17. PMID: 29462615.
- Yao C, Johnson WM, Gao Y, Wang W, Zhang J, Deak M, Alessi DR, Zhu X, Mיעאל JJ, Roder H, Wilson-Delfosse AL, Chen SG. Kinase inhibitors arrest neurodegeneration in cell and *C. elegans* models of LRRK2 toxicity. *Hum Mol Genet*. 2013 Jan 15;22(2):328-44. doi: 10.1093/hmg/ddr431. Epub 2012 Oct 12. PMID: 23065705; PMCID: PMC3526163.
- Yap TL, Gruschus JM, Velayati A, Sidransky E, Lee JC. Saposin C protects glucocerebrosidase against α -synuclein inhibition. *Biochemistry*. 2013 Oct 15;52(41):7161-3. doi: 10.1021/bi401191v. Epub 2013 Oct 1. PMID: 24070323; PMCID: PMC3833811.
- Yap TL, Gruschus JM, Velayati A, Westbroek W, Goldin E, Moaven N, Sidransky E, Lee JC. Alpha-synuclein interacts with Glucocerebrosidase providing a molecular link between Parkinson and Gaucher diseases. *J Biol Chem*. 2011 Aug 12;286(32):28080-

8. doi: 10.1074/jbc.M111.237859. Epub 2011 Jun 8. PMID: 21653695; PMCID: PMC3151053.

Yap TL, Velayati A, Sidransky E, Lee JC. Membrane-bound α -synuclein interacts with glucocerebrosidase and inhibits enzyme activity. *Mol Genet Metab*. 2013 Jan;108(1):56-64. doi: 10.1016/j.ymgme.2012.11.010. Epub 2012 Nov 28. PMID: 23266198; PMCID: PMC3552326.

Ysselstein D, Nguyen M, Young TJ, Severino A, Schwake M, Merchant K, Krainc D. LRRK2 kinase activity regulates lysosomal glucocerebrosidase in neurons derived from Parkinson's disease patients. *Nat Commun*. 2019 Dec 5;10(1):5570. doi: 10.1038/s41467-019-13413-w. PMID: 31804465; PMCID: PMC6895201.

Zhang M, Mu H, Shang Z, Kang K, Lv H, Duan L, Li J, Chen X, Teng Y, Jiang Y, Zhang R. Genome-wide pathway-based association analysis identifies risk pathways associated with Parkinson's disease. *Neuroscience*. 2017 Jan 6;340:398-410. doi: 10.1016/j.neuroscience.2016.11.004. Epub 2016 Nov 10. PMID: 27840232.

Zhang X, Zhou JY, Chin MH, Schepmoes AA, Petyuk VA, Weitz KK, Petritis BO, Monroe ME, Camp DG, Wood SA, Melega WP, Bigelow DJ, Smith DJ, Qian WJ, Smith RD. Region-specific protein abundance changes in the brain of MPTP-induced Parkinson's disease mouse model. *J Proteome Res*. 2010 Mar 5;9(3):1496-509. doi: 10.1021/pr901024z. PMID: 20155936; PMCID: PMC2859700.

Zhao Y, Perera G, Takahashi-Fujigasaki J, Mash DC, Vonsattel JPG, Uchino A, Hasegawa K, Jeremy Nichols R, Holton JL, Murayama S, Dzamko N, Halliday GM. Reduced

LRRK2 in association with retromer dysfunction in post-mortem brain tissue from LRRK2 mutation carriers. *Brain*. 2018 Feb 1;141(2):486-495. doi: 10.1093/brain/awx344. PMID: 29253086; PMCID: PMC5837795.