Journal of Integrative Neuroscience

J. Integr. Neurosci. **2024**; 23(1): 16
https://doi.org/10.31083/j.jin2301016

Original Research

The Effect of p.G2019S Mutation in the *LRRK2* Gene on the Activity of Lysosomal Hydrolases and the Clinical Features of Parkinson's Disease Associated with p.N370S Mutation in the *GBA1* Gene

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Academic Editor: Gernot Riedel
Submitted: 30 August 2023 Revised: 29 October 2023 Accepted: 31 October 2023 Published: 16 January 2024

Abstract

Background: Mutations in the glucocerebrosidase (*GBA1*) and leucine-rich repeat kinase 2 (*LRRK2*) genes, encoding lysosomal enzyme glucocerebrosidase (GCase) and leucine-rich repeat kinase 2 (LRRK2), respectively, are the most common related to Parkinson's disease (PD). Recent data suggest a possible functional interaction between GCase and LRRK2 and their involvement in sphingolipid metabolism. The aim of the present study was to describe the clinical course and evaluate the lysosomal enzyme activities and sphingolipid concentrations in blood of patients with PD associated with dual mutations p.N370S *GBA1* and p.G2019S *LRRK2* (p.N370S/GBA-p.G2019S/LRRK2-PD) as well as in blood of asymptomatic mutation carriers (p.N370S/GBA1-p.G2019S/LRRK2-carrier). **Methods**: One patient with p.N370S/GBA1-p.G2019S/LRRK2-PD and one p.N370S/GBA1-p.G2019S/LRRK2-carrier were enrolled. GBA1-associated PD (GBA1-PD), LRRK2-associated PD (LRRK2-PD), sporadic PD (sPD) patients were described earlier by our research group. A neuropsychiatric examination of the p.N370S/GBA1-p.G2019S/LRRK2-PD patient was carried out using scales (Montreal Cognitive Assessment scale (MoCA), Mini-mental State Examination scale (MMSE), Frontal Assessment Batter scale (FAB), Hospital Anxiety, and Depression Scale (HADS), etc). Lysosomal enzyme activity (GCase, alpha-galactosidase [GLA], acid sphingomyelinase [ASMase], galactosylcerebrosidase [GALC]) and sphingolipid concentrations (hexasylsphingosine [HexSph], lysoglobotriaosylsphingosine [LysoGb3], lysosphingomyelin [LysoSM]) were assessed with high-performance liquid chromatography–tandem mass spectrometry in blood. The following comparison with the previously described groups of GBA1-PD and sPD patients were conducted. **Results**: Clinical features of p.N370S/GBA1-p.G2019S/LRRK2-PD included an early age of onset of the disease (46 years) and mild cognitive and affective disorders (MMSE = 29, MoCA = 23), despite a long (24 years) course of the disease. Interestingly, no differences were found in hydrolase activity and lysosphingolipid concentrations between the p.N370S/GBA1-p.G2019S/LRRK2-PD patient and GBA1-PD patients. However, GCase activity was lower in these groups than in LRRK2-PD, sPD, and controls. Additionally, the p.N370S/GBA1-p.G2019S/LRRK2-PD patient was characterized by a pronounced decreased in ASMase activity and increased LysoSM concentration compared to the p.N370S/GBA1-p.G2019S/LRRK2-carrier ($p = 0.023$, $p = 0.027$, respectively). **Conclusions**: Based on one patient, our results indicate a protective effect of the p.G2019S mutation in the *LRRK2* gene on clinical course of p.N370S/GBA1-PD. The identified pronounced alteration of ASMase activity and LysoSM concentration in p.N370S/GBA1-p.G2019S/LRRK2-PD provide the basis for the further research.

Keywords: Parkinson's disease; *GBA1*; *LRRK2*; lysosomal enzyme activity; lysosphingolipids

1. Introduction

Parkinson's disease (PD) is a common progressive neurodegenerative disorder caused by the death of dopaminergic neurons in the substantia nigra pars compacta (SNc) accompanied by accumulation and aggregation of alpha-synuclein protein [1,2]. Mutations in the *GBA1* gene are a common genetic risk factor for PD [3–7]. Penetrance of glucocerebrosidase (*GBA1*) mutations is around 10%, depending on the age of onset and the severity of the mutation [8]. The *GBA1* gene encodes the lysosomal enzyme glucocerebrosidase (GCase), which cleaves glucosylceramide (GlcCer) and glucosylsphingosine (GlcSph) to glucose and ceramide or sphingosine, respectively. Homozygous and compound heterozygous mutations in the *GBA1* gene lead to the development of the rare lysosomal storage disease (LSD), Gaucher disease (GD) [9]. p.L444P and p.N370S were reported to be major mutations, accounting for 60–70% of the *GBA1* mutant alleles. Previously, we and other authors have shown that a decrease in GCase activity characterized both patients with PD associated with mutations in the *GBA1* gene (GBA1-PD) and asymptomatic carriers of *GBA1* mutations [10–14]. Simultaneously, lysosphingolipid concentration (HexSph) in the blood, a more sensitive marker of GD [15], was significantly different in GBA1-PD patients compared to asymptomatic carriers [11], indicating a probable functional role of lysosphingolipids in PD pathogenesis. It should also be noted that some studies have shown that patients with sporadic PD (sPD) may also have reduced GCase activity [16]. The clinical course of GBA1-PD is more severe compared to sPD [17–19]. Thus, the clinical course of PD among carriers of mutations in the *GBA1* gene is characterized by an early onset [20] and a higher incidence of early cognitive deficits [21–23]. Recent studies have identified various environmental and genetic modifiers of the penetrance of *GBA1* mutations [24].

Mutations in the LRRK2 Gene

Mutations in the leucine-rich repeat kinase 2 (*LRRK2*) gene, which encodes the leucine repeat kinase, lead to the development of an autosomal dominant form of PD (LRRK2-PD). The most common mutation in the *LRRK2* gene is p.G2019S [25,26]. Previously, we identified the frequency of the p.G2019S mutation in Russia, which was 5.7% for familial cases, and 0.7% among sPD cases, which corresponds to data in other populations around the world [7]. p.G2019S has been shown to increase kinase activity of LRRK2, which is associated with an increase in the phosphorylation level of LRRK2 substrates, such as the Rab10 protein. Rab10 is involved in the transport of proteins into lysosomes and the process of degradation of molecules through autophagy [27–29]. Rab10 hyperphosphorylation may affect the transport and, consequently, the activity of lysosomal enzymes, such as GCase [30–32]. LRRK2-PD cases appear to be clinically and symptomatically indistinguishable from those of sPD [26,33,34].

Functional interactions between leucine-rich repeat kinase 2 (LRRK2) and GCase have been suggested [31]. Currently, a possible protective effect of LRRK2 p.G2019S mutation on the clinical course of GBA1-PD has been discussed in studies analyzing the clinical course of GBA1-LRRK2-PD [35–38]. We have previously shown the effect of exonic variants of the *LRRK2* gene on the activity of lysosomal enzymes and the concentration of corresponding sphingolipids in blood of sPD patients [39]. However, it remains unknown whether variations in the clinical course of LRRK2-GBA1-PD may be explained by the influence of *LRRK2* mutations on GCase activity.

Here, we suggest that p.G2019S in the *LRRK2* gene may influence the activity of lysosomal hydrolases and consequently the clinical course of LRRK2-GBA1-PD. In the present study, we described the clinical course of p.N370S/GBA1-p.G2019S/LRRK2-PD, estimated the activity of GCase and other lysosomal enzymes (alpha-galactosidase [GLA], acid sphingomyelinase [ASMase], galactosylcerebrosidase [GALC]), and the concentration of sphingolipids (hexasylsphingosine [HexSph], lysoglobotriaosylsphingosine [LysoGb3], lysosphingomyelin [LysoSM]) in the blood of a patient with p.N370S/GBA1-p.G2019S/LRRK2-PD and a patient that was an asymptomatic carrier of dual mutations p.N370S the *GBA1* gene and p.G2019S in the *LRRK2* gene (p.N370S/GBA1-p.G2019S/LRRK2-carrier). These values were compared to those in patients with GBA1-PD, LRRK2-PD, and sPD that were previously described.

2. Materials and Methods

2.1 Studied Groups

The study included one patient with p.N370S/GBA1-p.G2019S/LRRK2-PD and a patient that was a p.N370S/GBA1-p.G2019S/LRRK2-carrier. The comparison groups included 8 patients with p.N370S/GBA1-PD, 10 patients with LRRK2-PD, and 197 sPD patients that were described by us earlier [7,39]. The p.N370S/GBA1-p.G2019S/LRRK2-PD patient was identified by a screening for major mutations in the *GBA1* and *LRRK2* genes using allele-specific real-time polymerase chain reaction (PCR) or PCR and restriction analysis in PD patients as described previously [7,10,11]. These analyses were confirmed by Sanger sequencing (**Supplementary Fig. 1**).

The control group consisted of patients who were observed in the consultative and diagnostic center of First Pavlov State Medical University of St. Petersburg and were screened for two major mutations in the *GBA1* gene (p.L444P and p.N370S) and p.G2019S in the *LRRK2* gene. To exclude patients with a diagnosis of PD and other neurodegenerative diseases, all patients in the control group were examined by a neurologist (Table 1).

Table 1. Clinical characteristics of the studied groups.

Groups	Mutations	Sex (Male:Female)	Age at exam, y.o.	Age at onset, y.o.	Duration
p.N370S/GBA1-p.G2019S/LRRK2-PD (N = 1)	p.N370S/N + p.G2019S/N (N = 1)	0:1	65	46	24
p.N370S/GBA1-p.G2019S/LRRK2-carrier (N = 1)	p.N370S/N + p.G2019S/N (N = 1)	1:0	48	-	-
p.N370S/GBA1-PD (N = 8)	p.N370S/N (N = 8)	3:5	68.2 ± 9.26	61 ± 9.3	6.80 ± 5.17
LRRK2-PD (N = 10)	p.G2019S/N (N = 10)	3:7	71.0 ± 4.36	68.33 ± 3.06	2.67 ± 2.08
sPD (N = 197)	-	78:119	64.87 ± 10.14	59.83 ± 10.60	6.0 ± 5.07
Controls (N = 179)	-	74:105	64.88 ± 10.15	-	-

GBA1-PD, GBA1-associated PD; LRRK2-PD, leucine-rich kinase kinase 2-associated PD; sPD, sporadic PD.

2.2 Clinical Features of p.N370S/GBA1-p.G2019S/LRRK2-PD Patient

The p.N370S/GBA1-p.G2019S/LRRK2-PD patient was examined during the patient's best imaginable health state. The PD diagnosis was established according to the UK Brain Bank Criteria [40,41]. Disease severity was measured according to the unified PD rating scale (UPDRS), parts I—IV [42]. The stage of the disease was assessed according to the Hoehn and Yahr scale with Lindval modifications [43]. Cognitive function was assessed using the Montreal Cognitive Assessment scale (MoCA) and the Mini-mental State Examination scale (MMSE). The Frontal Assessment Batter scale (FAB) was used to comparatively assess cognitive impairment with a primary lesion in the frontal lobe or subcortical structures. The severity of anxiety-depressive disorders was assessed using the Mini Geriatric Depression Scale (GDS-SF), the Sheehan Clinical Anxiety Scale (ShARS), the Beck Depression Inventory (BDI), and the Hospital Anxiety and Depression Scale (HADS). Other non-motor manifestations were assessed using the PD Non-Motor Symptom Assessment Questionnaire (PD-NMS). Rapid eye movement (REM) sleep disorders were assessed using a questionnaire (RBDSQ, RBD-Screening Questionnaire).

2.3 Assessment of Enzyme Activities and Lysosphingolipid Concentration in Blood

Venous blood samples were collected from each participant in ethylenediaminetetraacetic acid (EDTA) tubes. Dry blood spot (DBS) cards were prepared by pipetting 40 µL of the whole blood on each spot. DBS were dried in open air at room temperature for 2 h and then stored at –20 °C before extraction. The enzymatic activities of glucocerebrosidase (GCase, EC 3.2.1.45, deficient in Gaucher disease), alpha-galactosidase A (GLA, EC 3.2.1.22 deficient in Fabry disease), acid sphingomyelinase (ASMase, EC 3.1.4.12, deficient in Neimann-Pick disease types A and B), and galactosylceramidase (GALC, EC 3.2.1.46, deficient in Krabbe disease) as well as the concentration of sphingolipids (hexasylsphingosine [HexSph]), a mixture of glycosylsphingosine (GlcSph) and galactosylsphingosine (GalSph), lysosphingomyelin (LysoSM), and lysoglobotriaosylsphingosine (LysoGb3) were estimated by liquid chromatography tandem-mass spectrometry (LC-MS/MS) in blood as previously described [44,45]. Enzymatic activities and substrate concentrations for both the patient with p.N370S/GBA1-p.G2019S/LRRK2-PD and the patient that was a p.N370S/GBA1-p.G2019S/LRRK2-carrier were evaluated in triplicate in different time. The activity of enzymes in the sPD, p.N370S/GBA1-PD, and LRRK2-PD groups were assessed in our previous studies [10,11,39,46].

2.4 Statistical Analysis

All statistical analyses were performed using the bioinformatics environment R (vs. 4.1.2, R Core Team, Vienna, Austria). The difference between the groups were assessed using a Mann-Whitney U-test. The differences were considered statistically significant at $p < 0.05$. Clinical and demographic characteristics are presented as mean ± standard deviation. Experimental data are presented as median (min-max).

3. Results

3.1 The Clinical Features of p.N370S/GBA1-p.G2019S/LRRK2-PD

A 70-year-old ethnically Russian woman, with no family history of PD or other neurodegenerative diseases, noticed awkwardness in her right hand at the age of 46. This was followed by stiffness in her right hand. Over the course of six months, the stiffness spread to the muscles of the trunk and the left arm. Later, slowness in the left arm and general slowness gradually increased.

The patient was diagnosed with PD in accordance with the United Kindom Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria (UKPDS) [41]. The patient had been receiving oral levodopa therapy from the age of 47 and noted almost complete regression of symptoms. However, after 1.5 years, stiffness in the muscles of the limbs and trunk began to increase again. Starting at the age of 51, as the dosage of levodopa increased due to progression of motor symptoms, motor fluctuation appeared and increased in intensity and duration. At the same time, the patient noticed pain in the limbs and increased anxiety during "off" periods. At the age of 55, choreiform dyskinesias gradually appeared and began to increase, first in the muscles of the

Table 2. UPDRS score and Hoehn and Yar scale modified by Lindval, (points, median (min-max)).

Scale	p.N370S/GBA1-p.G2019S/LRRK2-PD (N = 1)	GBA1-PD (N = 14)	sPD (N = 33)
Duration			
	24	12 (3–18)	4 (1–20)
Score according to the UPDRS scale and the Hoehn and Yahr scale in the Lindval modification, (points, median (min-max))			
UPDRS I	8	16.6 (4–25)	10 (3–25)
UPDRS II	18	12 (5–27)	10.5 (0–33)
UPDRS III	40	35.5 (6–56)	28.0 (3–62)
UPDRS IV	8	2 (0–12)	0 (0–12)
UPDRS, total score	74	69.5 (32–106)	48.5 (22–115)
Hoehn-Yar	3–4	2.75 (1–4)	2 (1–4)
The results of the assessment of cognitive functions, (points, median (min-max))			
MoCA	23	22.5 (19–28)	25 (13–30)
MMSE	29	24.5 (21–30)	27.5 (18–30)
FAB	17	16.5 (9–17)	16.5 (10–18)
Neuropsychological examination, (scores, median (min-max))			
ShARS	30	32.0 (11–66)	18.5 (1–38)
HADS «A»	7	9.5 (3–15)	5 (0–10)
HADS «D»	7	7.5 (3–15)	6.5 (2–14)
BDI	12	13.5 (5–25)	12.5 (2–26)
GDS-SF	4	7.5 (4–13)	6.5 (0–13)
Assessment of non-motor symptoms, (scores, median (min-max))			
RBDSQ	7	5.5 (2–10)	4.5 (1–11)
PD NMS	12	16.5 (2–25)	11 (1–23)

Notes: Patients with GBA1-PD and sPD were described by us earlier and presented in the table for a visual comparison of the clinical course of p.N370S/GBA1-p.G2019S/LRRK2-PD with GBA1-PD and sPD [23]. UPDRS, unified PD rating scale; MoCA, Montreal Cognitive Assessment scale; MMSE, Mini-mental State Examination scale; FAB, Frontal Assessment Batter scale; ShARS, Sheehan Clinical Anxiety Scale; HADS, Hospital Anxiety and Depression Scale; BDI, Beck Depression Inventory; GDS-SF, Geriatric Depression Scale; RBDSQ, RBD-Screening Questionnaire; PD NMS, Parkinson's disease Non-Motor Symptom Assessment Questionnaire.

limbs, then in the muscles of the trunk. At the same age, 300 mg of amantadine was added to levodopa-based therapy. At the age of 57, the dose of levodopa was increased to 1200 mg. After 5 years the dose was increased a final time to 1600 mg. From the age of 63, 3 mg of Mirapex was added to the therapy. However, the dose was reduced due to severe dyskinesias. Thus, dominating in the clinical picture was akinetic-rigid syndrome in the absence of trembling, moderate postural disorders, and pronounced motor fluctuations with disabling dyskinesias during the "on" periods. The appearance of the non-motor fluctuations during the "off" periods, namely increased pain and anxiety, was also noteworthy

According to the new diagnostic criteria of the International Society for the Study of Movement Disorders, the clinical picture corresponds to stage 3 during "on" periods and stage 4 during "off" periods according to the Hoehn and Yahr scale. Assessment of cognitive function, affective component, and motor and non-motor manifestations of PD were conducted using the scales presented in Table 2.

Comparison of the clinical picture of p.N370S/GBA1-p.G2019S/LRRK2-PD with clinical pictures of GBA1-PD and sPD, that were described earlier [21,23], revealed differences in several parameters (Table 2, Ref. [23]). When assessing cognitive function using the MoCA and FAB scales, the average score when comparing the patient with p.N370S/GBA1-p.G2019S/LRRK2-PD and patients with GBA1-PD and sPD was comparable. In the MMSE survey, the patient with p.N370S/GBA1-p.G2019S/LRRK2-PD performed better compared to those with GBA1-PD and sPD. Analysis of the neuropsychological testing by Hospital Anxiety and Depression Scale - Anxiety (HADS-A) and Sheehan Disability Scale (SDS) scales revealed the development of anxiety disorders among patients with GBA1-PD, but not among patients with sPD, and a patient with p.N370S/GBA1-p.G2019S/LRRK2-PD obtained a borderline value. When assessing the manifestations of depression according to Hospital Anxiety and Depression Scale - Depression (HADS-D), Beck Depression Inventory (BDI), and Geriatric Depression Scale Short Form (GDS-SF) no significant differences were found among all patient groups. According to the results of the Rapid eye movement (REM) Sleep Behavior Disorder Screening Questionnaire (RBDSQ) questionnaire for assessing REM phase disturbances, no statistically significant difference was found. The severity of non-motor symptoms according to the results of the Parkinson's disease nonmotor symptoms questionnaire (PD-NMS) was significant among the GBA1-PD patients.

Table 3. Enzyme activities and lysosphingolipid concentrations in blood of the study groups.

Number of individuals in the studied groups, N	Enzyme activity, mM/L/h, median (min-max)				Lysosphingolipid concentrations, ng/mL, median (min-max)		
	GCase	GALC	GLA	ASMase	LysoSM	LysoGb3	HexSph
p.N370S/GBA1-p.G2019S/LRRK2-PD patient (All parameters were measured in triplicate in differenttime) (N = 1)	3.68 (3.10–4.10) $p = 0.039^a$ $p = 0.012^b$ $p = 0.031^c$	3.90 (3.42–4.47) $p = 0.015^a$ $p = 0.0095^b$	8.21 (7.69–8.71) $p = 0.012^a$ $p = 0.026^b$	3.22 (2.80–3.79) $p = 0.023^e$	10.86 (10.41–11.30) $p = 0.003^a$ $p = 0.005^b$ $p = 0.027^e$	0.98 (0.88–1.1)	3.96 (3.40–4.24) $p = 0.023^e$
p.N370S/GBA1-p.G2019S/LRRK2-carrier (All parameters were measuredin triplicate in different time) (N = 1)	5.89 (5.48–8.31)	1.93 (1.87–3.08)	10.17(8.32–11.68) $p = 0.0057^a$ $p = 0.0092^b$ $p = 0.03^d$	4.82 (3.69–5.14) $p = 0.032^d$	4.96 (3.69–9.05) $p = 0.036^b$	0.93 (0.67–2.53)	8.40 (5.86–10.42) $p = 0.0003^a$ $p = 0.0002^b$ $p = 0.0007^c$
p.N370S/ GBA1-PD (N = 8)	4.54 (1.29–7.41) $p = 0.0023^a$ $p = 0.00048^b$ $p = 0.0015^c$	3.94 (1.05–6.87) $p = 0.002^a$ $p = 0.012^b$ $p = 0.029^c$	7.37 (1.29–10.07) $p = 0.0004^a$ $p = 0.0072^b$	3.09 (1.83–4.59) $p = 0.015^a$ $p = 0.0014^b$	4.46 (3.81–18.17)	1.07 (0.75–43.95) $p = 0.0028^a$ $p = 0.0022^b$	4.15 (0.08–13.64) $p = 0.02^a$ $p = 0.0075^b$
LRRK2-PD (N = 10)	7.17 (3.15–12.69)	2.00 (0.8–4.89)	5.55 (2.15–11.65)	3.77 (1.83–4.59) $p = 0.024^b$	4.24 (2.35–11.64)	1.29 (0.54–40.77) $p = 0.00031^a$ $p = 0.00046^b$	3.08 (0.49–9.23)
sPD (N = 197)	6.82 (2.07–23.08)	2.18 (0.21–12.68) $p = 0.015^a$	4.8 (1.33–36.39)	4.75 (1.53–13.25)	3.61 (0.72–16.08) $p = 0.0088^a$	0.76 (0.04–3.73)	2.64 (0.87–13.23)
Controls (N = 179)	6.35 (1.55–32.13)	1.90 (0.24–9.35)	4.08 (1.03–14.81)	4.20 (1.4–12.39)	3.98 (0.59–11.6)	0.79 (0.03–2.31)	2.97 (0.57–15.36)

^{a}-compared to controls, ^{b}-compared to sPD, ^{c}-compared to LRRK2-PD, ^{d}-compared to GBA1-PD (p.N370S/N), ^{e}-p.N370S/GBA1-p.G2019S/LRRK2-carrier.

ASMase, acid sphingomyelinase; GCase, glucocerebrosidase; GLA, acid alpha-galactosidase; GALC, galactocerebrosidase; HexSph, hexasylsphingosine; LysoSM, lysosphingomyelin; LysoGb3, lysoglobo-triaosylsphingosine.

The p.N370S/GBA1-p.G2019S/LRRK2-PD patient was also characterized by more pronounced motor symptoms of parkinsonism (parts II and III of the UPDRS) and a greater severity of motor fluctuations and dyskinesias (part IV of the UPDRS) (Table 2). The p.N370S/GBA1-p.G2019S/LRRK2-PD patient was also characterized by the development of cognitive deficits that were similar in severity to that observed among GBA1-PD patients. However, the duration of the disease was twice as long as the matched group with GBA1-PD (24 years vs 12 years).

3.2 Lysosomal Enzyme Activities and Lysphingolipid Concentrations

In the current study we evaluated the activity of lysosomal enzymes (GCase, GALC, GLA, and ASMase) and lysosphingolipid concentrations (LysoSM, LysoGb3, HexSph) in blood of a patient with p.N370S/GBA1-p.G2019S/LRRK2-PD and a patient identified as a p.N370S/GBA1-p.G2019S/LRRK2 carrier. These values were compared with those in the blood of patients with sPD, p.N370S/GBA1-PD, LRRK2-PD and controls that were previously described [39] (Table 3). GALC, GLA, ASMase, and GCase are involved in the metabolism of sphingolipids (Fig. 1).

As expected, we showed a decreased GCase activity in blood of the p.N370S/GBA1-p.G2019S/LRRK2-PD patient compared to patients with sPD, LRRK2-PD, and controls (p = 0.012, p = 0.039, p = 0.031, respectively). The p.N370S/GBA1-p.G2019S/LRRK2-PD patient was characterized by increased GALC and GLA activities compared to sPD patients and controls ($p < 0.05$). The p.N370S/GBA1-p.G2019S/LRRK2-PD patient was also characterized by pronounced increase of LysoSM concentration, the substrate of ASMase enzyme, compared to sPD, LRRK2-PD, and control patients (p = 0.005, p = 0.027, p = 0.003, respectively). There were no differences in LysoGb3 concentration in the p.N370S/GBA1-p.G2019S/LRRK2-PD patient compared to other studied groups ($p > 0.05$). Blood HexSph concentration was elevated in the p.N370S/GBA1-p.G2019S/LRRK2-PD patient compared to the LRRK2-PD patients (p = 0.023).

Interesting, the p.N370S/GBA1-p.G2019S/LRRK2-carrier was characterized by decreased GCase activity compared to sPD, LRRK2-PD patients and controls but without significant differences ($p > 0.05$) and increased GLA activity compared to sPD, p.N370S/GBA1-PD, and control patients (p = 0.0092, p = 0.032, p = 0.0057, respectively). There was also a pronounced increase of HexSph concentration compared to sPD, LRRK2-PD, and control patients (p = 0.0002, p = 0.0007, p = 0.0003, respectively). Additionally, the p.N370S/GBA1-p.G2019S/LRRK2-PD patient was characterized by decreased ASMase activity and elevated LysoSM concentration compared to the p.N370S/GBA1-p.G2019S/LRRK2-carrier (p = 0.023, p = 0.027, respectively) (Table 3).

4. Discussion

We studied the features of the clinical course of GBA1-LRRK2-PD, and estimated the activities of lysosomal hydrolases and lysosphingolipid concentrations in blood of a p.N370S/GBA1-p.G2019S/LRRK2-PD patient and a p.N370S/GBA1-p.G2019S/LRRK2-carrier compared to GBA1-PD, LRRK2-PD, sPD, and control patients groups. An important aspect of the course of p.N370S/GBA1-p.G2019S/LRRK2-PD was the mildness of cognitive (MoCA score = 23) and affective (HADS 7/7) features, despite the early onset and long course of the disease (24 years). It should be noted that the neurological symptoms were stable over the past 7 years. Three independent studies also demonstrated that PD patients carrying dual *GBA1* and *LRRK2* mutations manifest a milder phenotype compared to PD patients carrying mutations in the *GBA1* gene only [35,37,38]. The authors of these studies concluded that mutations in the *LRRK2* gene might have a modifying role in GBA1-PD, attenuating the clinical course. However, the mechanism of the interaction between LRRK2 and GCase remains unknown.

Our data of the psychomotor examination of a p.N370S/GBA1-p.G2019S/LRRK2-PD patient are consistent with previously obtained data on the protective effect of p.G2019S *LRRK2* mutation on the clinical course of PD among carriers of *GBA1* mutations [35,37,38]. In the MMSE survey, the p.N370S/GBA1-p.G2019S/LRRK2-PD patient performed better compared to the GBA1-PD and sPD patients, however this could be explained by the low sensitivity of this scale while testing patients with mild cognitive impairment.

The penetrance of *GBA1* and *LRRK2* mutations is incomplete. Therefore, genetic and environmental modifiers may play a role in PD risk and severity. Identifying modifiers for the disease is crucial for better understanding the mechanism linking the *GBA1* and *LRRK2* genes to PD. There were no differences between behavioral characteristics of the p.N370S/GBA1-p.G2019S/LRRK2-PD patient, and GBA1-PD and LRRK2-PD patients [47].

We showed that lysosomal enzyme activity and substrate concentrations in the p.N370S/GBA1-p.G2019S/LRRK2-PD patient were comparable with the group of patients with p.N370S/GBA1-PD. We found that patients with p.N370S/GBA1-p.G2019S/LRRK2-PD and p.N370S/GBA1-PD were characterized by a decrease in GCase activity, and increased activity of GLA and GALC enzymes compared with sPD patients and controls. In contrast, p.N370S/GBA1-PD patients and patients with p.N370S/GBA1-p.G2019S/LRRK2-PD were characterized by an increase in LysoSM concentration compared to sPD and control patients. These results suggested that the presence of mutations in the *LRRK2* gene does not lead to pronounced change in the activity of enzymes and the concentration of sphingolipids in p.N370S/GBA1-p.G2019S/LRRK2-PD. In turn, the p.N370S/GBA1-p.G2019S/LRRK2-carrier was char-

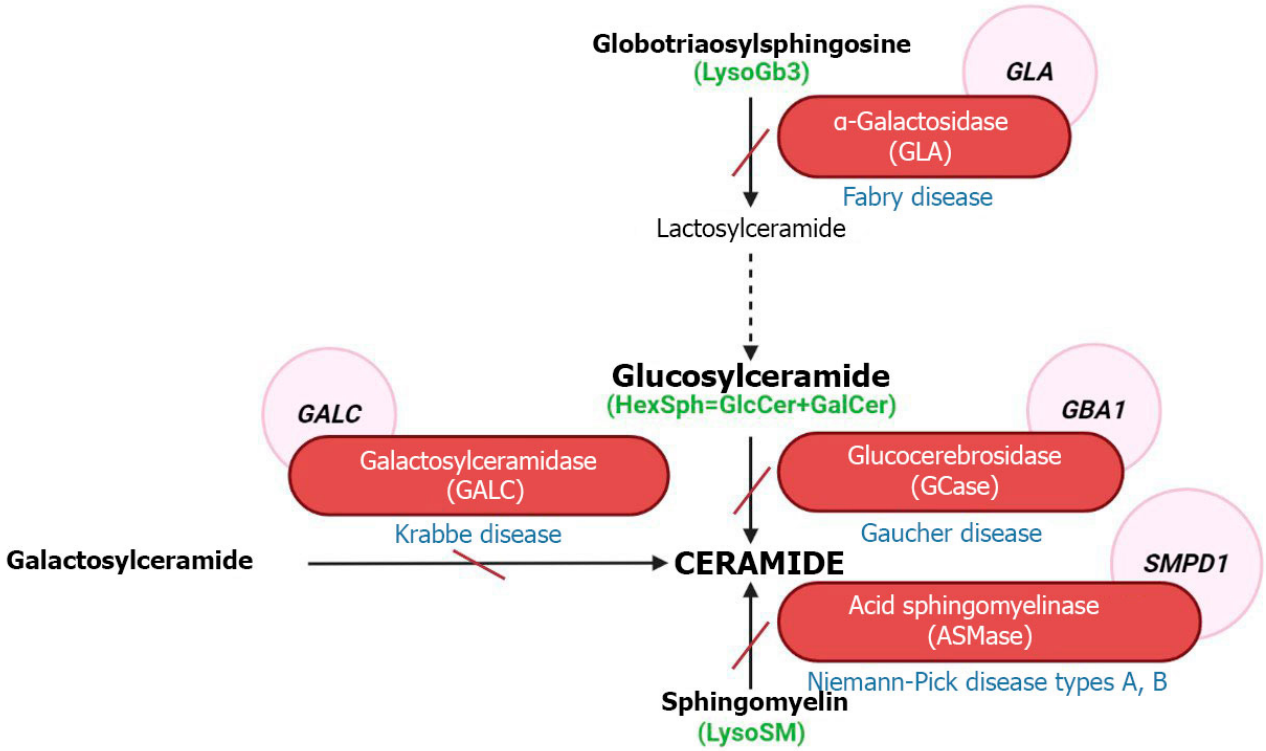

Fig. 1. Role of lysosomal hydrolases in ceramide metabolism. The lysosomal hydrolases included in this study (acid sphingomyelinase [ASMase], glucocerebrosidase [GCase], acid alpha-galactosidase [GLA], and galactocerebrosidase [GALC]) are involved in the metabolism of sphingolipids in lysosomes (indicated in red). The genes that code for these lysosomal hydrolases are shown in purple. Substrates of lysosomal hydrolases are shown in green. It is known that homozygous or compound mutations in the genes, encoding these enzymes, lead to the development of lysosomal storage diseases (LSD) due to a deficiency in the enzymatic activities of the corresponding enzymes. The multiplex assay used for activity screening included four enzymes involved in the sphingolipid pathway as above: GCase, ASMase, GLA, GALC, and lysosphingolipids: hexasylsphingosine (HexSph) (a mixture of glycosylsphingosine [GlcSph] and galactosylsphingosine [GalSph]), lysosphingomyelin (LysoSM), and lysoglobotriaosylsphingosine (LysoGb3). SMPD1, sphingomyelin phosphodiesterase 1; GBA1, glucocerebrosidase.

acterized by an increase in GLA activity and HexSph concentration compared to sPD, LRRK2-PD, and control patients.

Recent data indicated a possible functional interaction between the GCase and LRRK2 enzymes. However, these data are contradictory. Both of them are involved in sphingolipid metabolism [48,49]. For example, Alcalay and colleagues found an increase in GCase activity in the blood of patients with LRRK2-PD [50]. However, Yysselstein and colleagues [31] showed a decrease in GCase activity in a culture of dopaminergic neurons differentiated from pluripotent stem cells from patients with LRRK2-PD. In the present study, we did not observe a change in the activity of the GCase enzyme in the blood of patients with LRRK2-PD [39]. The functions of LRRK2 have remained unknown. However, recently LRRK2 has been shown to be involved in the intracellular transport of cytoplasmic molecules to the lysosome for their degradation and may be related to sphingolipids metabolism [41,48,51,52]. Rab10 protein, which is considered the main substrate of LRRK2, performs many functions in the cell. In particular, Rab10 is involved in endolysosomal traffic [53,54]. An abnormal level of phosphorylated Rab10 protein due to alterations in the kinase activity of LRRK2 might lead to an impairment of the endolysosomal pathway, including transport of newly synthesized lysosomal enzymes from endoplasmic reticulum to lysosomes, which can lead to disruption of sphingolipid metabolism, lysosome dysfunction, and the formation of stable forms of oligomeric alpha-synuclein—around 50% of which can be degraded by the autophagy [55–57]. In a primary culture of peripheral blood macrophages of patients, in the culture of dopaminergic neurons differentiated from pluripotent stem cells of patients with GBA1-PD, as well as in a culture of astrocytes obtained from homozygous and heterozygous mice with a knock-in mutation in the *GBA1* p.D409V gene, inhibition of LRRK2 kinase activity led to a decrease in the phosphorylation level of the Rab10 protein and an increase in GCase activity, suggesting a negative convergence between LRRK2 and GCase [31,46,58].

GALC and GLA are encoded by the *GALC* and *GLA* genes, respectively, and whose mutations are also associated with the risk of PD [59]. However, data on the activity

of these lysosomal hydrolases in PD remain controversial. Mutations in the *GLA* gene lead to the development of a rare disease related to LSD, Fabry disease, which is characterized by the accumulation of glycosphingolipids, including globotriaosylceramide (Gb3) and LysoGb3 [60]. Interestingly, Alcalay *et al*. [50] found an increase in GLA activity in blood of patients with sPD and LRRK2-PD. In the present study, we showed accumulation of LysoGb3 substrate without concomitant changes in the activity of the GLA enzyme in blood of LRRK2-PD patients [39]. GALC catalyzes the hydrolysis of GalCer and galactosylsphingosine. Deficiency in lysosomal GALC activity due to mutations in the *GALC* gene in patients homozygous for Krabbe disease results in the rapid accumulation of galactosylsphingosine, a neurotoxic sphingolipid, in neurons and myelinating cells [61]. The reason for the increase in GALC activity in PD patients with mutations in the *GBA1* gene remains unknown but a compensatory mechanism in response to a decrease in GCase activity could be assumed.

Here, we first compared hydrolase activity and lysosphingolipid concentrations in a p.N370S/GBA1-p.G2019S/LRRK2-PD patient and a p.N370S/GBA1-p.G2019S/LRRK2-carrier, and revealed increased LysoSM concentration, which is a substrate of the ASMase enzyme, and decreased ASMase activity in blood of patients with p.N370S/GBA1-p.G2019S/LRRK2-PD. Mutations in the sphingomyelin phosphodiesterase 1 (*SMPD1*) gene encoding ASMase activity cause a rare, autosomal recessive LSD Niemann–Pick disease that is characterized by ASMase deficiency and the accumulation of sphingomyelin and LysoSM [62]. Mutations in the *SMPD1* gene are associated with PD risk [63–65]. In our previous study, we demonstrated a decrease in ASMse activity in LRRK2-PD patients [39]. Earlier, we found decreased ASMase activity in patients with multiple system atrophy and dementia with Lewy bodies that belong to synucleinophaties as PD [46]. At the same time, ASMase activity was not different between *GBA1* mutation carriers and non-carriers [66]. As previously discussed, mutations in the *GBA1* and *LRRK2* genes are characterized by incomplete penetrance, and we can speculate that a pronounced change in the lysosphingolipid profile may be a potential PD modifier in p.N370S/GBA1-p.G2019S/LRRK2-carriers.

The main limitation of the current study is the number of *GBA1* and *LRRK2* double mutations carriers. It should be noted that the co-occurrence of a p.G2019S mutation in the *LRRK2* gene and a p.N370S mutation in the *GBA1* gene is a rare event that occurs in approximately 2% of GBA1-PD [35,37,38].

5. Conclusions

Our results suggest a protective effect of a p.G2029S mutation in the *LRRK2* gene in the clinical features of PD associated with carrying both *GBA1* and *LRRK2* mutations. The *GBA1* and *LRRK2* genes are the most intensely studied genes among all PD genes, and they point to an involvement of endolysosomal pathway disruption in PD pathogenesis. The activity of lysosomal enzymes (GCase, ASMase, GALC, and GLA) and lysosphingolipids concentrations (HexSph, LysoGb3, LysoSM) in the blood of a p.N370S/GBA1-p.G2019S/LRRK2-PD patient and a p.N370S/GBA1-p.G2019S/LRRK2-carrier were assessed and compared with those in the blood of sPD, GBA1-PD, LRRK2-PD, and control patients. GCase activity and HexSph concentration in the p.N370S/GBA1-p.G2019S/LRRK2-PD carrier was comparable with the group of patients with p.N370S/GBA1-PD. Interestingly, increased LysoSM concentration and decreased ASMase activity were found in the p.N370S/GBA1-p.G2019S/LRRK2-PD patient compared to p.N370S/GBA1-p.G2019S/LRRK2-carrier. The pronounced alteration in LysoSM concentration observed in p.N370S/GBA1-p.G2019S/LRRK2-PD was not related with clinical course of PD. The critical limitation single cases of dual *GBA1* and *LRRK2* mutation carriers that have been examined must be kept in mind when making conclusions. Therefore, an examination of lysosomal hydrolases activitiy in p.N370S/GBA1-p.G2019S/LRRK2-PD patients and p.N370S/GBA1-p.G2019S/LRRK2-carriers on extended groups are required.

Abbreviations

GCase, glucocerebrosidase; LRRK2, leucine-rich kinase kinase 2; PD, Parkinson's disease; p.N370S/GBA1-p.G2019S/LRRK2-PD, PD associated with dual mutations p.N370S GBA1 and p.G2019S LRRK2; asymptomatic p.N370S GBA1 and p.G2019S LRRK2 mutations carrier, p.N370S/GBA1-p.G2019S/LRRK2-carrier; GLA, alpha-galactosidase; ASMase, acid sphingomyelinase; GALC, galactosylcerebrosidase; HexSph, hexasylsphingosine; LysoGb3, lysoglobotriaosylsphingosine; LysoSM, lysosphingomyelin; SNc, substantia nigra pars compacta; GlcCer, glucosylceramide; GlcSph, glucosylsphingosine; LSD, lysosomal storage disease; GD, Gaucher disease; GBA1-PD, PD associated with mutations in the GBA1 gene; sPD, sporadic PD; LRRK2-PD, PD associated with mutations in the LRRK2 gene; UPDRS, the unified PD rating scale; MoCA, Montreal Cognitive Assessment scale; MMSE, Mini-mental State Examination scale; FAB, Frontal Assessment Batter scale; GDS-SF, Mini Geriatric Depression Scale; ShARS, Sheehan Clinical Anxiety Scale; BDI, Beck Depression Inventory; HADS, Hospital Anxiety and Depression Scale; PD-NMS, PD Non-Motor Symptom Assessment Questionnaire; REM, rapid eye movements; DBS, Dry blood spots; GalSph, galactosylsphingosine; LC-MS/MS, liquid chromatography tandem-mass spectrometry.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

TSU: investigation, formal analysis, visualization, writing-original draft, writing-review and editing; AT: investigation, writing-review and editing; MB, GB, AB, MG, AE, EZ, IM: investigation; KB: investigation, statistical analysis visualization, SP: supervision, investigation, writing-review and editing. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by Pavlov First Saint-Petersburg State Medical University (approval number: 261, data: 25.04.2022). A formal written consent form was provided to all included subjects to read and sign prior to the study.

Acknowledgment

The authors are grateful to all patients whose participation in the study made this analysis possible.

Funding

The study was supported by the Russian Science Foundation Grant No. 22-25-00501.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.jin2301016.

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