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Carriers of both *GBA* and *LRRK2* mutations, compared to carriers of either, in Parkinson's disease: Risk estimates and genotype-phenotype correlations

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ABSTRACT

Introduction: The clinical characteristics of Parkinson's disease (PD) associated with both the *LRRK2* p.G2019S mutation and a *GBA* variant (*LRRK2-GBA*-PD) have not yet been determined.

Methods: In this retrospective observational study of Ashkenazi-Jewish (AJ) PD patients, we describe the clinical course and characteristics of *LRRK2-GBA*-PD and estimate the risk to develop PD for the double mutation carriers. Odds ratios (OR) were estimated using published data on frequencies of *GBA* and *LRRK2* mutations. Demographic and clinical data was retrieved from medical records and from rating at last visit.

Results: Our analysis included 236 PD patients, divided into four groups: *LRRK2*-PD (n = 66), *GBA*-PD (n = 78), *GBA-LRRK2*-PD (n = 12) and mutation-negative PD (MNPD, n = 80 randomly selected). The estimated ORs in different models for *GBA-LRRK2* PD were 15–28 (95% CI 6.7–72.0, $p < 0.0001$), compared to AJ controls. Using logistic regression (while controlling for sex, age at onset and PD duration), we found that probable REM-sleep behavior disorder (RBD) was significantly more common for *GBA*-PD than for *LRRK2*-PD, while none of the *GBA-LRRK2*-PD patients reported RBD. Dementia was significantly more common for *GBA*-PD than for the *LRRK2*-PD and MNPD. Psychosis was the most common for *GBA*-PD and least common for *LRRK2-GBA*-PD.

Conclusions: While *GBA*-PD is characterized by higher rates of dementia, probable RBD and psychosis, it seems that compared to the other groups, these features are less common for *LRRK2-GBA*-PD. This may imply to a possible protective effect of *LRRK2* p.G2019S mutation among *GBA* variant carriers.

1. Introduction

Parkinson's disease (PD) is a common neurodegenerative disorder associated with multifactorial etiology. In most populations, only a small proportion of PD cases can be attributed to a single genetic mutation [1]. About one-third of Ashkenazi Jewish (AJ) patients with sporadic PD and about 40% of those with familial PD have been found to carry a relevant mutation [2]. The p.G2019S mutation in the leucine-rich repeat kinase 2 (*LRRK2*) gene and several variations in the glucocerebrosidase (*GBA*) gene are frequent among AJ patients [3,4]. The frequency of *GBA* variants in AJ PD patients is 17–20% [5,6] and the *LRRK2* p.G2019S mutation is detected in 10–14% of sporadic and in 26–30% in familial PD patients of AJ origin [7,8].

A limited number of studies compared the clinical manifestations of *LRRK2* and *GBA* associated PD [9–13]. To the best of our knowledge, the clinical course and features of PD in patients carrying both the *LRRK2* p.G2019S mutation and a *GBA* variant has not been reported.

In this retrospective study, we aimed to identify the characteristic features of PD patients carrying both a *GBA* variant and the *LRRK2* p.G2019S mutation, and to compare them to the features of PD associated with either gene mutation or to none of the two (mutation negative PD, MNPD). Since the patient sample analyzed has been in long-term follow up, we focused mainly on late-disease motor, cognitive and psychiatric complications.

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Table 1
Parkinson's disease genotype groups: clinical demographics.

	Total	*LRRK2*-PD	*GBA*-PD	*LRRK-GBA*-PD	MNPD	P-value (four groups comparison)*	p-value1	p-value2	p-value3	p-value4	p-value5	p-value6
Number	236	66	78	12	80							
Males, % (n)	60.2 (142)	57.6 (38)	60.3 (47)	41.7 (5)	65 (52)	0.44	0.87	0.36	0.40	0.35	0.62	0.20
PD duration, years (mean ± SD)	12.2 ± 7.3	14.4 ± 8.3	11.5 ± 7.0	11.8 ± 6.9	11.3 ± 6.5	0.13	0.04	0.34	0.04	0.84	0.94	0.79
Age at symptom onset, years (mean ± SD)	59.1 ± 10.9	57.7 ± 10.8	58.6 ± 10.0	54.4 ± 10.5	61.4 ± 11.7	0.04	0.34	0.58	0.02	0.26	0.06	0.06
Motor UPDRS, mean ± SD	31.7 ± 15.9	30.9 ± 19.1 (N = 43)	37.3 ± 18.1 (N = 32)	28.4 ± 11.4 (N = 8)	29.6 ± 11.2 (N = 53)	0.23	0.07	0.96	0.69	0.28	0.08	0.66

Abbreviations: PD = Parkinson's disease; MNPD = mutation negative Parkinson's disease; SD = standard deviation; UPDRS = unified Parkinson's disease rating scale; (N =) the number of patients per group for which motor UPDRS score was available.
*Kruskal-Wallis and Man-Whitney *U* test for continuous demographic variables, chi-square or Fisher's exact analysis for differences in sex. Linear regression model was used to compute differences in UPDRS and were adjusted to age at symptom onset, disease duration and sex.
P-value1 represents comparison between *LRRK2* –PD and *GBA*-PD.
P-value2 represents comparison between *LRRK2*-PD and *LRRK2-GBA*-PD.
P-value3 represents comparison between *LRRK2*-PD and MNPD.
P-value4 represents comparison between *GBA*-PD and *LRRK2-GBA*-PD.
P-value5 represents comparison between *GBA*-PD and MNPD.
P-value6 represents comparison between *LRRK2-GBA*-PD and MNPD.

2. Methods

2.1. Population

Consecutive patients diagnosed with idiopathic PD, based on the United Kingdom Parkinson's Disease Society Brain Bank criteria [14], attending the Sheba Medical Center Movement Disorders Institute, were consecutively recruited between 2008 and 2017. Informed consent was obtained from all patients and the study was approved by the local ethics committee. Blood samples were obtained for DNA extraction and genetic analysis.

For this study, only AJ patients were included. The patients in our institute visit the clinic at least twice a year and visit intervals do not exceed 6 months. Clinical characteristics were compared between four study populations identified, according to their genotype (Table 1): patients with a single *GBA* variant, negative for *LRRK2* p.G2019S mutation (*GBA*-PD); patients positive for the *LRRK2* p.G2019S mutation but negative for *GBA* variants (*LRRK2*-PD), patients positive for both gene mutations (*LRRK2*- *GBA*-PD) and patients negative for both gene mutations (MNPD), who were randomly selected from the available cohort through a random list generator.

The medical data was accessed and recorded from the patient files by a single movement disorders specialist (GY), blinded for genotype. Patients' demographic and clinical data was retrieved from medical records, including the age at symptom onset (AAO), the age and disease duration at the last visit, and the score of part III (motor examination) of the Unified PD rating scale (UPDRS) when patients were at "on" state.

An additional set of categorical variables was examined, consisting of whether patients had tremor as the first motor symptom, whether they had reached Hoehn & Yahr stage 3 (HY3), whether they had ever developed the following PD characteristics, during the course of PD: dementia, probable REM-sleep behavior disorder (RBD, based on a single-question screen which showed a sensitivity of 93.8% and a specificity of 87.2%) [15], psychosis, freezing of gait (FOG), motor fluctuations (MF) and levodopa-induced dyskinesia (LID). A dichotomized (Yes/No) definition of these variables was used, since we did not have documentation of time to symptom in the medical record of many patients. The diagnosis of dementia was based on score ≥ 2 of question 1 of the UPDRS - Part I, answered by the patient/caregiver, along with a description of additional functional deficits, evolving from dementia or an abnormal Montreal Cognitive Assessment – MoCA (≤ 26) or Mini-Mental State Examination – MMSE (≤ 24) score, if available.

2.2. Genetic analysis

DNA was extracted using a standard protocol. The full coding sequences of *GBA* and *LRRK2* were sequenced using targeted next generation sequencing (NGS), and exons 10 and 11 of *GBA* were also sequenced using Sanger sequencing due to reduced coverage of the NGS data [5]. Molecular inversion probes (MIPs) were designed as previously described [16], and the sequences and targets of the probes are available upon request. To capture the target sequences, MIPs were hybridized to 100 ng of genomic DNA, and generated circular DNA including the target regions. Linear DNA which included the non-targeted regions was degraded using exonucleases, and the targeted captured DNA was amplified by PCR. Sequencing was performed with the use of the Illumina HiSeq 2500 platform at the McGill University and Genome Quebec Innovation Center. The full protocol is available upon request. To process the raw sequence reads, Burrows-Wheeler aligner, the Genome Analysis Toolkit (GATK, v.2.6.4), and ANNOVAR (annotation variant software) were used. Variants from the targeted MIP sequencing were visualized using the Integrative Genomics Viewer (http://software.broadinstitute.org/software/igv/). The Sanger sequencing chromatograms were analyzed using the Genalys 3.3b software.

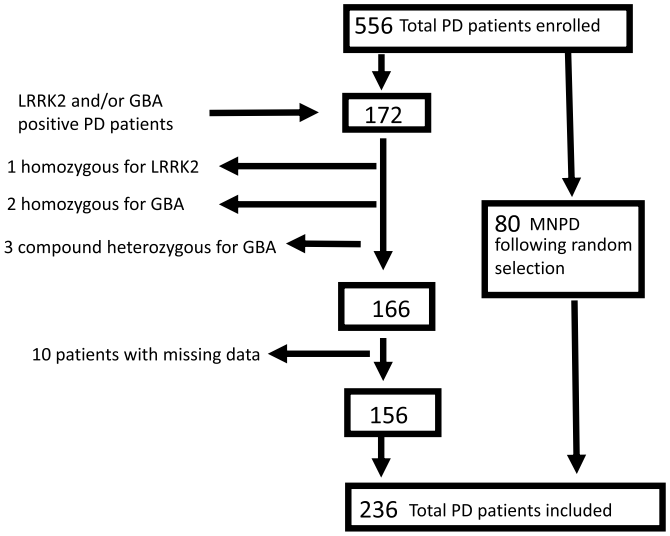

Fig. 1. This flow diagram represents the pathway through which patients were selected and included in the study. The group of mutation negative Parkinson's disease was selected using random selection generator.

2.3. Statistical analysis

Demographic and clinical features of the 4 subgroups were computed using summary statistics and frequency tables. Differences in AAO and disease duration were compared by non-parametric Mann-Whitney U and Kruskal-Wallis tests. Differences in sex and in other dichotomous variables were compared by crosstab chi-square or Fisher's exact tests.

Linear regression model was implemented for continuous variables and logistic regression model for the dichotomous variables, to compare groups for significant differences in dependent variables, with adjustment to AAO, disease duration and sex. Simulation of odds ratio (OR) for carriers of both a *GBA* variant and the *LRRK2* p.G2019S mutation was performed using published frequencies of *GBA* variants and the *LRRK2* p.G2019S mutation from the literature [3,5–7], as well as public databases (https://ibd.broadinstitute.org/). P-value ≤ 0.05 was defined as statistically significant. For the clinical variables (tremor as first motor symptom, reaching HY3, dementia, probable RBD, psychosis, FOG, MF LID and UPDRS), post-hoc correction for multiple testing was implemented and p-value ≤ 0.008 (0.05/6 due to 6 pairwise comparisons within each variable) was defined as statistically significant. All p-values were 2-sided. All statistical analyses were performed using SPSS software (v. 25, IBM® SPSS® Inc., Chicago, IL).

3. Results

The cohort consisted of 556 PD patients of AJ descent, and 156 (28.1%) were found to be carriers of one or more of the *LRRK2* p.G2019S mutation or a *GBA* variant.

3.1. Estimated risk for PD in carriers of both LRRK2 and GBA mutations

Twelve patients (2%) were carriers of both *GBA* variant and the *LRRK2* p.G2019S mutation. In an AJ control population (n = 662) no carriers of both *GBA* and *LRRK2* variants were identified [5]. To estimate the effect of carriage of both *GBA* and *LRRK2* variants, and due to the lack of data from the literature on carriage of both in the general AJ population [5–7,17], we simulated this frequency based on the known frequencies of *GBA* and *LRRK2* mutations in the general AJ population. *GBA* variants are found in 6.4% of 3805 AJ controls [17], and since the p.E326K was not screened in this population, we added its frequency in the AJ control population based on our recent study (0.3%) [5], to a total of 6.7%. The frequency of the *LRRK2* p.G2019S mutation in AJ controls was reported as 2% [7], 1.3% [3] and 1.08% in the IBD exomes browser (https://ibd.broadinstitute.org/) which includes data on 3044 AJ controls. Based on the higher estimation of 2%, the expected frequency of carriers of both *GBA* and *LRRK2* mutations in the AJ control population is 0.134%. Comparing the frequency of carriers of both mutations observed in our PD population (2%) with a simulated population of 10,000 AJ controls with frequency of 0.134% yielded an OR of 15.0 (95% CI 6.7–33.5, $p < 0.0001$). Simulating with the lower boundary of *LRRK2* p.G2019S frequency of 1.08% in the AJ population, the expected frequency of carriers of both *GBA* and *LRRK2* mutations in the AJ control population is 0.072%. Comparing the frequency of carriers of both mutations observed in our PD population (2%) with a simulated population of 10,000 AJ controls with frequency of 0.072% yielded an OR of 28.0 (95% CI 10.9–72.0, $p < 0.0001$). Considering the published ORs in AJ for *GBA* mutations alone of 2.7–3.5 [5,6] and the OR for the *LRRK2* p.G2019S mutation alone, 8.6 [7], our simulation suggests at least an additive effect on risk for carriers of both *GBA* and *LRRK2* mutations.

3.2. Genotype-phenotype correlations

Of the 556 PD patients, one patient homozygous for the *LRRK2* p.G2019S mutation, 2 patients homozygous and 3 compound heterozygous for a *GBA* variants and an additional 10 patients (2 *LRRK2*-PD, 7 GBA-PD, 1 homozygous GBA-PD) with incomplete clinical data were excluded (Fig. 1).

Of note, several other private *LRRK2* variants were identified, none of which is associated with PD.

A total of 236 patients (142 males, mean age 72.2 ± 11.2 years) were included in the final analysis. Table 1 summarizes the clinical demographics of the four groups. The *LRRK2*-PD group consisted of 66 patients, the *GBA*-PD group consisted of 78 patients, the *LRRK2-GBA*-PD consisted of 12 patients and the MNPD group consisted of 80 patients. Table 2 summarizes the various *GBA* variants identified in the *GBA*-PD and the *LRRK2-GBA*-PD groups. The N370S *GBA* variant was the most common with a frequency of 66.7% in the *GBA*-PD group and 75% in the *LRRK2-GBA*-PD group.

We observed that AAO was older for the MNPD compared to *LRRK2*-PD group (p = 0.02) and less significantly so to the *GBA*-PD group (p = 0.06) and to the *LRRK2-GBA*-PD group (p = 0.06) (Table 1). PD duration was longer for the *LRRK2* than for the *GBA*-PD and MNPD (p = 0.04 for each). No significant differences were observed in motor UPDRS at last visit among the groups.

Table 3 summarizes the clinical characteristics of all four groups. Sex did not affect any of the variables tested.

Using logistic regression (controlling for sex, AAO and disease duration), we found no significant differences among the groups in the prevalence of tremor as first sign, MF, FOG and patients reaching HY3.

LID was less common in the MNPD group (32.1%) than in the *GBA*-PD group- 57.9% (p < 0.001) and in the *LRRK2*-PD group- 61.9%

Table 2
Frequency of *GBA* variants according to groups.

GBA variants	*GBA*-PD n (%)	*LRRK2-GBA*-PD n (%)
All variants	78 (100)	12 (100)
p.N370S	52 (66.7)	9 (75)
p.E326K	6 (7.7)	2 (16.7)
p.V394L	1 (1.3)	
p.E388K	1 (1.6)	
p.L444P	2 (3.2)	
p.A384D	1 (1.3)	
p.N188S	1 (1.3)	
p.R496H	3 (3.8)	1 (8.3)
p.R44C	1 (1.3)	
c.84dupG	10 (12.8)	

Table 3
Parkinson's disease genotype groups: clinical characteristics.

	LRRK2-PD	*GBA*-PD	*LRRK-GBA*-PD	MNPD	P-value (four groups compari-son)[a]	p-value1	p-value2	p-value3	p-value4	p-value5	p-value6
Number	66	78	12	80							
Tremor as first sign, %	**43.5** (N = 62)	**50.6** (N = 77)	**25** (N = 12)	**57.5** (N = 80)	0.12	0.42	0.24	0.06	0.21	0.47	**0.03**
MF, %	**68.3** (N = 63)	**64.5** (N = 76)	**66.7** (N = 12)	**51.3** (N = 78)	0.17	0.53	0.92	0.23	0.89	0.08	0.20
LID, %	**61.9** (N = 63)	**57.9** (N = 76)	**66.7** (N = 12)	**32.1** (N = 78)	**0.001**[c]	0.35	0.50	**0.004**[c]	0.78	< **0.001**[c]	0.06
Reaching HY3, %	**53.0** (N = 66)	**56.8** (N = 70)	**66.7** (N = 12)	**46.3** (N = 80)	0.43	0.06	0.14	0.94	0.37	**0.05**	0.07
FOG, %	**45.0** (N = 60)	**33.8** (N = 74)	**33.3** (N = 12)	**35.9** (N = 78)	0.56	0.51	0.66	0.36	0.52	0.91	0.99
Probable RBD, %	**16.4** (N = 55)	**50.0** (N = 60)	**0** (N = 11)	**37.5** (N = 72)	< **0.001**[c]	< **0.001**[c]	NA[b]	**0.01**	NA[b]	0.21	NA[b]
Psychosis, %	**19.0** (N = 63)	**45.9** (N = 74)	**8.3** (N = 12)	**17.7** (N = 79)	< **0.001**[c]	< **0.001**[c]	0.59	0.99	**0.003**[a]	< **0.001**[a]	0.39
Dementia, %	**6.5** (N = 62)	**31.6** (N = 76)	**8.3** (N = 12)	**11.5** (N = 78)	< **0.001**[c]	< **0.001**[c]	0.58	0.18	**0.04**	**0.001**[a]	0.83

Abbreviations: N = number; MNPD = mutation negative Parkinson's disease; MF = motor fluctuations; LID = levodopa-induced dyskinesia; HY3=Hoehn & Yahr stage 3; FOG = freezing of gait; RBD = REM-sleep behavior disorder; NA = not applicable; (N =): Number of patients per group for which data was available for analysis.
Regression models were performed to calculate significance level of all dependent variables with adjustment to age at symptom onset, disease duration and sex. All p-values were 2-sided.
P-value1 represents comparison between *LRRK2*–PD and *GBA*-PD.
P-value2 represents comparison between *LRRK2*-PD and *LRRK2-GBA*-PD.
P-value3 represents comparison between *LRRK2*-PD and MNPD.
P-value4 represents comparison between *GBA*-PD and *LRRK2-GBA*-PD.
P-value5 represents comparison between *GBA*-PD and MNPD.
P-value6 represents comparison between *LRRK2-GBA*-PD and MNPD.
[a] Chi square analysis, not adjusted to covariates.
[b] Analysis was not applicable.
[c] Significant also following multiple testing correction.

(p = 0.004). The *LRRK2-GBA*-PD group had highest LID prevalence (66.7%), reaching nearly nominal significant level (p = 0.06).

Probable RBD was significantly less common in the *LRRK2*-PD (16.4%) than in the *GBA*-PD (50.0%, p < 0.001), not reaching statistically significance level for the MNPD group, following correction for multiple testing (37.5%, p = 0.01). None of the *LRRK2-GBA*-PD patients (n = 11) answered affirmatively to the RBD question and thus none of the double mutation carriers had probable RBD, but statistical comparison for this variable was unattainable.

Dementia was more common in the *GBA*-PD group (31.6%) than in the *LRRK2*-PD (6.5%, p < 0.001), and MNPD (11.5%, p = 0.001) groups, not reaching statistical significance following correction for multiple testing for the *LRRK2-GBA*-PD group (8.3%, p = 0.04), probably due to the small size of this group. Prevalence of psychosis was highest in the *GBA*-PD (45.9%) group comparing to the *LRRK2*-PD (19.0%, p < 0.001), *LRRK2-GBA*-PD (8.3%, p = 0.003), and MNPD groups (17.7%, p < 0.001).

Importantly, in sequencing of the following PD gene, no pathogenic *SNCA* mutations and no biallelic *PARK2, PINK2* and *PARK7* mutations (either homozygous or compound heterozygous) were identified in any of the patients included in the study.

4. Discussion

We present the results of clinical genetic analysis of *GBA* and *LRRK2* mutations in a large AJ cohort of PD patients followed up in a single movement disorders center in Israel. Obtaining data from many mutation carriers, we were able to estimate the susceptibility for carriers of both *GBA* and *LRRK2* mutations to PD, and studied the clinical features of four different genotype groups according to presence of *GBA* variants and the *LRRK2* p.G2019S mutation.

The OR simulations suggest that there is at least an additive risk for carriers of both a *GBA* variant and the *LRRK2* p.G2019S mutation to develop PD. A limitation of this analysis is that carriers of both *GBA* and *LRRK2* variants were not identified in AJ control populations, and the analysis was performed using publicly available data. To accurately estimate the risk, a genetic analysis of at least 10,000 healthy, elderly AJ controls should have been performed.

The mutation –positive PD groups displayed a younger AAO than MNPD, a difference which was significant for *LRRK2*-PD and nearly significant for *GBA*-PD and *LRRK2-GBA*-PD; moreover, the mean AAO was 4–5 years younger for the double mutation group than for the single mutation groups (but it did not reach statistical significance). It is well established from previous studies that both *LRRK2*- and *GBA*-associated PD display a younger AAO than MNPD [6,10,17]. It is not clear if the burden of two different gene mutations may or may not have a further effect on AAO, as the *LRRK2-GBA*-PD sample size is limited. AAO for PD patients homozygous or compound heterozygous for mutations in *GBA*, exhibiting either clinical or subclinical Gaucher disease, has been found to be significantly younger [18].

Our findings demonstrate that single variant *GBA*-associated PD is characterized by higher rates of some non-motor features, such as dementia, probable RBD and psychosis, compared to *LRRK2*-PD or MNPD. Probable RBD and psychosis are known to be risk factors of dementia [19–22]. Our findings are in agreement with previous studies on *GBA*-associated PD [23–25] and Lewy body dementia [26].

Tremor as an initial motor manifestation was not different among the different groups. This finding is in agreement with other studies [9,10]. Disease progression as reflected by the percentage of patients reaching HY3 showed that the *GBA*-PD and *LRRK2-GBA*-PD groups tended to deteriorate faster than MNPD. However, the correction for multiple testing discarded statistical significance. We could not perform cox analysis to study the rate of deterioration over time due to limitation of our dataset (lack of data regarding the time from onset to HY3). A previous study [27] showed a faster deterioration to HY3 in *GBA* associated PD (n = 14) as compared to non-carriers of the *GBA*

mutation. Our group studied disease progression of *LRRK2* vs. *LRRK2*-negative PD and found no significant difference in the time from onset to HY3 [28].

We found that LID was less common for the MNPD group than for the other groups while controlling for PD duration. In a large consortium including 530 *LRRK2* carriers, the *LRRK2* group had more commonly severe LID than the MNPD group [29]. LID was found to be more prevalent in *GBA*-associated PD than *GBA*-negative PD patients [30]. On the other hand, studies performed on Chinese and Brazilian PD samples did not detect differences in prevalence of LID among *LRRK2*-PD, *GBA*-PD or MNPD patients [9,10].

Prevalence of FOG did not differ among the 4 groups in our study. Da Silva et al. [10] found that FOG was less frequent in *LRRK2*-PD than in *GBA*-PD and MNPD groups. Gan-Or et al., not focusing on FOG, did not detect differences in gait disturbances between *LRRK2*–positive and *GBA*-positive patients [12] and Jesus et al. [30] reported a similar prevalence of FOG in *GBA* variant carriers and non-carriers.

A major goal of this study was to characterize the clinical features and course of *LRRK2-GBA*-PD. We hypothesized that due to the "double hit" of genetic factors in this group, these PD patients will be characterized by an earlier AAO and a more accelerated clinical course than the other groups. This was partially supported by an absolute younger AAO than the other groups (not reaching significance) and by high percentage of patients reaching HY3.

Regarding non-motor features, it was surprising that none of the *LRRK2-GBA*-PD patients reported RBD. Moreover, the *LRRK2-GBA*-PD had the lowest rates of psychosis (reaching statistical significance as compared to *GBA*-PD) and low rates of dementia (not reaching statistical significance as compared to *GBA*-PD, following correction for multiple testing). This may suggest a possible protective effect of the additional *LRRK2* p.G2019S mutation or may be a finding which is the result of chance, given the small number of patients in the double mutation group.

Our study has several important advantages. First, the availability of relatively large numbers of *LRRK2*-PD and *GBA*-PD patients in a single cohort, explained by the high prevalence of these variants in the AJ population. Our sample is homogenous (all AJ individuals) treated and routinely followed-up in a single tertiary movement disorders clinic. All patients live in Israel (although many were not born in Israel, but immigrated during life), reducing heterogeneity in terms of exposure to environmental factors. All the above present a valuable platform for a direct comparison of the different groups of patients included in this study, for a large set of continuous and categorical variables. Another advantage is the long follow-up time period, required to the emergence of long term symptoms such as motor complications (dyskinesias, fluctuations and FOG), dementia and psychosis.

Nevertheless, this study has several limitations. Due to the scarcity of *LRRK2-GBA* double mutation carriers, the group sample size is small and thus significant results cannot lead to firm statements on any feature. Furthermore, due to its small size, we did not include the UPDRS subscores in the analysis as they would have introduced too many variables which will decrease the power of the study. Second, due to its retrospective design, some clinical data was missing and some data was based on patients' recall. Hence, we lacked accurate time-related data and could not perform survival analysis. Instead, we used categorical variables and controlled for disease duration. Third, we labeled different mutations in the *GBA* gene as "*GBA*". There are known clinical differences among the various *GBA* mutations [6,17,19,20], although 2/3 of our *GBA* carriers had the N370S mutation, so our *GBA* cohort was rather homogeneous. Fourth, although *LRRK2* and *GBA* mutations are the most common in PD, we did not screen for other less frequent gene mutations associated with PD such as *Parkin, SNCA, DJ-1* etc. However, these mutations are rare, and therefore not likely to have a significant effect on our results. Fifth, due to its retrospective design, HY and UPDRS were assessed only at "ON" state. We might have found different outcome if we had assessed patients also at "OFF" state.

Further limitation of this and similar studies is that PD is a complex disorder. Multiple genes contribute to PD susceptibility and clinical course, in addition to environmental factors. This was not taken into account. Therefore, the actual effects of *GBA* and *LRRK2* mutations may be either larger or smaller, and future studies are needed to determine the effects of additional factors as modifiers of the phenotype.

5. Conclusion

We identified some clinical characteristics of PD in patients carrying mutations in both the *LRRK2* and the *GBA* genes. Due to a small sample size we were limited in terms of conclusive statements attributed to this rare group of patients. A larger, preferably multi-center study of heterogenous PD cohorts, carrying the *LRRK2-GBA* genotype, encompassing standardized prospective collection of clinical data is needed to confirm our initial findings regarding the natural course of this genetic subtype.

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Author's contribution

Study concept and design: Yahalom, Greenbaum, Gan-Or, Hassin-Baer.

Acquisition, analysis, interpretation of data: Yahalom, Israeli-Korn, Fay-Karmon, Livneh, Ruskey, Roncière, Alam, Gan-Or, Greenbaum, Hassin-Baer.

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