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. 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.

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. 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 LRRK2-GBA-PD and least common for LRRK2-GBA-PD. 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 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, 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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E-mail address: gyahalom@gmail.com (G. Yahalom).
Table 1

<table>
<thead>
<tr>
<th>LRRK2 PD</th>
<th>GBA-PD</th>
<th>LRRK2-GBA-PD</th>
<th>MNPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBAPD</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Number</td>
<td>236</td>
<td>66</td>
<td>50</td>
</tr>
<tr>
<td>Males, % (n)</td>
<td>60.2 (142)</td>
<td>64 (52)</td>
<td>65 (52)</td>
</tr>
<tr>
<td>PD duration, years (mean ± SD)</td>
<td>12.2 ± 7.3</td>
<td>14.4 ± 8.3</td>
<td>11.5 ± 7.0</td>
</tr>
<tr>
<td>Age at symptom onset, years (mean ± SD)</td>
<td>59.1 ± 10.9</td>
<td>57.7 ± 10.8</td>
<td>58.6 ± 10.0</td>
</tr>
<tr>
<td>Motor UPDRS, mean ± SD</td>
<td>31.7 ± 15.9</td>
<td>30.9 ± 19.1</td>
<td>37.3 ± 18.1</td>
</tr>
</tbody>
</table>

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.

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 Genalyx 3.3b software.
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 variant 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 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 3

Parkinson’s disease genotype groups: clinical characteristics.

<table>
<thead>
<tr>
<th>LRRK2-GBA-</th>
<th>LRRK-GBA-</th>
<th>GBA-</th>
<th>LRRK2-GBA</th>
<th>LRRK2-</th>
<th>MNPD</th>
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<tbody>
<tr>
<td>Number</td>
<td></td>
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<tr>
<td>Tremor as</td>
<td>N = 62</td>
<td>N = 12</td>
<td>N = 80</td>
<td>N = 63</td>
<td>N = 78</td>
</tr>
<tr>
<td>1st sign, %</td>
<td>43.5</td>
<td>50.6</td>
<td>25</td>
<td>57.5</td>
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<td></td>
<td>(0.12)</td>
<td>(0.42)</td>
<td>(0.24)</td>
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<td>(0.47)</td>
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<td>FOG, %</td>
<td>N = 76</td>
<td>N = 78</td>
<td>N = 78</td>
<td>N = 66</td>
<td>N = 55</td>
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<tr>
<td>33.3</td>
<td>66.7</td>
<td>66.7</td>
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<td>(0.57)</td>
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<td>Probable RBD, %</td>
<td>N = 60</td>
<td>N = 78</td>
<td>N = 78</td>
<td>N = 66</td>
<td>N = 55</td>
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<tr>
<td>Psychosis, %</td>
<td>N = 63</td>
<td>N = 12</td>
<td>N = 78</td>
<td>N = 66</td>
<td>N = 55</td>
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<td>19.0</td>
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<tr>
<td></td>
<td>(0.003)</td>
<td>(0.04)</td>
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**Abbreviations:** N = number; MNPD = Motor Neuron Disease; 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 of differences between single or double mutation groups, not reaching statistical significance for multiple testing for LRRK2-GBA-PD group (8.3%, p = 0.04), probably due to the small size of this group. Significance following correction for multiple testing 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 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 compared 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 homogeneous (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 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 sub-scores 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 heterogeneous 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.

Drafting the manuscript: Yahalom.

Critical revision of the manuscript for important intellectual content: Gan-Or, Greenbaum, Hassin-Baer.

Statistical analysis: Yahalom, Gan-Or.

Obtained funding: None.

Study supervision: Hassin-Baer, Gan-Or.

Conflict of interest disclosures

Dr. Gilad Yahalom and Dr. Simon Israeli-Korn received consultancy fees from Abbvie biopharmaceuticals Inc., Dr. Gan-Or received consultancy fees from Sanofi/Genzyme and Lysosomal Therapeutics Inc. (LTI). Prof. Sharon Hassin-Baer received consultancy fees from Abbvie biopharmaceuticals Inc., Boston scientific, Medtronic and Actelion LTD. No other disclosures were reported.

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