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SHORT COMMUNICATION

LRRK2 and *GBA* mutations differentially affect the initial presentation of Parkinson disease

Z. Gan-Or · A. Bar-Shira · A. Mirelman · T. Gurevich · M. Kedmi · N. Giladi · A. Orr-Urtreger

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Abstract *GBA* and *LRRK2* mutations increase susceptibility to Parkinson disease (PD), which is characterized by various disabling symptoms. An extended cohort of 600 Ashkenazi PD patients was screened for the *LRRK2* G2019S and for eight *GBA* mutations. Reported initial symptoms were compared between three genotypic groups of patients: carriers of *GBA* mutations, carriers of *LRRK2* G2019S mutation, and non-carriers. More *LRRK2* G2019S carriers reported muscle stiffness (rigidity, $p=0.007$) and balance disturbances ($p=0.008$), while more *GBA* mutation carriers reported slowness (bradykinesia, $p=0.021$). These results suggest distinct effects of *LRRK2* or *GBA* mutations on the initial symptoms of PD.

Keywords Parkinson's disease · Initial symptoms · GBA · Glucocerebrosidase · LRRK2

Introduction

GBA mutations and the *LRRK2* G2019S mutation are among the most common genetic alterations associated with increased susceptibility to Parkinson's disease (PD) [1, 2]. Together, these mutations are most common among the Ashkenazi-Jewish population [3–6]. *GBA* encodes the lysosomal enzyme *β-glucocerebrosidase*, and carriage of homozygous or compound heterozygous *GBA* mutations cause type I, II or III Gaucher's disease [7]. *LRRK2* encodes *dardarin*, a multi-domain protein kinase with GTPase activity, and mutations in the kinase domain that lead to gain-of-function of the kinase activity, as well as other mutations in this gene, have been associated with PD [8]. Motor symptoms are among the most disabling clinical features in PD, and it was hypothesized that mutations in *LRRK2* and *GBA* might differentially affect the clinical course of PD. However, only few reports have suggested distinct clinical signs specific to PD patients with either *LRRK2* or *GBA* mutations [1, 5, 9]. Furthermore, a comparison of these two groups of patients within the same cohort was not reported to date. We screened an extended cohort of 600 Ashkenazi-Jewish PD patients for eight founder *GBA* mutations and for the *LRRK2* G2019S mutation, and analyzed the initial symptoms in three different genotypic groups of patients: *GBA* mutation carriers, *LRRK2* G2019S carriers and non-carriers of these mutations.

Materials and methods

The study population included 600 consecutive PD patients, all of full Ashkenazi-Jewish ancestry. Part of this cohort (420 patients) was previously reported and details regarding their recruitment, diagnostic criteria and interview procedure were previously described [5]. The epidemiologic variables of the patients are presented in Table 1. Initial symptoms that could be retrospectively associated

Z. Gan-Or · A. Bar-Shira · M. Kedmi · A. Orr-Urtreger (✉)
Genetic Institute, Tel Aviv Sourasky Medical Center,
6 Weizmann Street,
Tel Aviv 64239, Israel
e-mail: aviorr@tasmc.health.gov.il

A. Mirelman · T. Gurevich · N. Giladi
Movement Disorders Unit Parkinson Center Department of Neurology, Tel Aviv Sourasky Medical Center,
Tel Aviv, Israel

Z. Gan-Or · T. Gurevich · N. Giladi · A. Orr-Urtreger
The Sackler Faculty of Medicine, Tel Aviv University,
Tel Aviv, Israel

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Table 1 Epidemiologic variables of 600 Ashkenazi-Jewish Parkinson's disease patients

Variable	Heterozygous carriers		Homozygous or compound heterozygous carriers		Carriers of both *GBA* and *LRRK2*	Non-carriers	Total
	GBA	*LRRK2*	*GBA*	*LRRK2*			
Number (%)	109 (18.2)	74 (12.3)	6 (1.0)	1 (0.2)	8 (1.3)	402 (67.0)	600
Age at onset, years (±SD) range in years	57.7 (±10.7) 30–79	57.5[a] (±11.7) 32–91	51.2 (±9.7) 41–68	58.0 (–) –	57.4 (±6.4) 51–69	61.0[b](±11.0) 21–94	59.8[c] (±11.1) 21–94
Age at enrollment, years (±SD) range in years	65.9 (±10.2) 47–89	67.2 (±10.4) 43–93	58.3 (±8.7) 47–69	63.0 (–) –	65.1 (±7.7) 53–77	69.2 (±9.8) 41–96	68.2 (±10.1) 41–96
Early onset PD, % (*n*)	23.9 (26)	23.3 (17)	50.0 (3)	–	–	14.6 (58)	17.5 (104)
Gender							
Men, % (*n*)	62.4 (68)	50.0 (37)	66.7 (4)	–	50.0 (4)	65.2 (262)	62.5 (375)
Men/Women ratio	1.66	1.00	–		–	1.87	1.67
Family history of PD							
1st degree relative with PD, % (*n*)	15.0[d] (16)	33.8 (25)	33.3 (2)	–	25.0 (2)	14.3[e] (57)	17.1[c] (102)
2nd degree relative with PD, % (*n*)	11.2[d] (12)	16.2 (12)	16.7 (1)	–	37.5 (3)	9.3[b] (37)	10.9[f] (65)
Total with family history of PD, % (*n*)	25.2[d] (27)	45.9 (34)	33.3 (2)	–	50.0 (4)	21.4[b] (85)	25.6[f] (152)
Smoking, past or present, % (*n*)	36.4[d] (39)	40.5 (30)	50.0 (3)	–	50.0 (4)	42.4[e] (169)	41.2[c] (245)

n number of patients, *SD* standard deviation

[a] Data were not available for one patient

[b] Data were not available for four patients

[c] Data were not available for five patients

[d] Data were not available for two patients

[e] Data were not available for three patients

[f] Data were not available for six patients

with the onset of PD were collected by a structured interview of the patient and the spouse at the time of enrollment. All patients signed an informed consent before entering the study, and The Institutional and National Supreme Helsinki Committees for Genetic Studies approved the study protocols and the informed consents.

Detection of *GBA* mutations (84GG, IVS2+1, N370S, V394L, D409H, L444P, R496H and RecTL) and *LRRK2* G2019S mutation was done as previously described [5, 6]. Statistical analysis of continuous and categorical variables was done as previously published [5]. Confirmations of significant variations detected in previous publications [5, 6] are not presented here. FDR correction for multiple comparisons was applied for the analysis of the initial symptoms. In order to avoid a possible effect of age at motor symptoms onset (AAO) on the presenting symptoms, a group of 236 PD patients, non-carriers of either *LRRK2* G2019S or *GBA* mutations and matched for AAO, was randomly selected for comparison out of the 402 non-carrier patients.

Results

One- third of Ashkenazi PD patients carry *GBA* and/or *LRRK2* G2019S mutations

Of the 600 PD patients, 117 (19.5%) were heterozygous for *GBA* mutations and 82 (13.7%) were heterozygous for the *LRRK2* G2019S mutation, including eight patients carrying both *GBA* and *LRRK2* mutations (Table 1). There were six (1.0%) homozygotes or compound heterozygotes *GBA* mutations carriers, and 1 (0.2%) patient homozygote for the *LRRK2* G2019S mutation. As previously reported [4, 5], carriers of *LRRK2* G2019S and carriers of *GBA* mutations had a significantly earlier average AAO (57.5 and 57.7 years) than non-carriers of these mutations (61.0 years). The eight patients that carried both *GBA* N370S and *LRRK2* G2019S mutations had a similar average AAO (57.4±6.4 years) and none had an Early Onset PD (EOPD, defined as an AAO<50 years). EOPD was present in 18.8% and 23.3% of those carrying *GBA* N370S or *LRRK2* G2019S mutation, compared to 14.6% among the non-carrier PD patients. Interestingly, the *LRRK2* G2019S carriers were significantly under-represented among the *GBA* mutation carriers. The percentage of *LRRK2* G2019S carriers among patients without *GBA* mutations (75/477, 15.7%) was significantly higher compared to their frequency among carriers of one or more *GBA* mutant allele (8/123, 6.5%, p=0.008, Fisher's Exact Test), suggesting the absence of an epistatic effect between *GBA* and *LRRK2* in Ashkenazi-Jewish PD patients. Our previous findings of significant gender (female) and family history effects on the increased frequency of *LRRK2* G2019S mutation and the differential effects of severe versus mild *GBA* mutations [5, 6] were confirmed in this larger cohort.

Different initial symptoms among *LRRK2* or *GBA* mutation carriers

The following initial symptoms were reported in the entire cohort of 600 patients: tremor (56.5%), muscle stiffness (rigidity, 26.5%), slowness (bradykinesia, 16.5%), gait disturbances (16.2%), depressed mood (7.3%), balance disturbances (6.7%), subjective sense of weakness in a limb (6.3%), micrographia (6.3%), pain (5.7%), urination disturbances (3.5%), confusion (2.7%), fatigue (2.2%), loss of smell sense (2.0%), and constipation (1.5%). Three genotypic groups of patients were compared: *GBA* mutation carriers (n=109), *LRRK2* G2019S carriers (n=74) and non-carriers of either (n=402, Table 2). Due to small numbers, and in order to analyze the effect of mutations in either *GBA* or *LRRK2* separately, patients homozygous or compound-heterozygous for *GBA* (n=6) or *LRRK2* G2019S (n=1) mutations, and carriers of both *GBA* and *LRRK2* mutations (n=8) were excluded from the analysis. Symptoms that occurred in at least 10% of patients in any one of the groups were included in the statistical analysis to avoid analyzing small numbers that might result in unstable estimates. A cutoff p value of 0.025 was set after applying FDR correction for multiple comparisons.

LRRK2 G2019S carriers reported higher frequencies of muscle stiffness (2.1- and 1.5-fold, χ^2=9.90, df=2, p=0.007) and balance disturbances (7.5- and 2.0-fold, χ^2=9.67, df=2, p=0.008) compared to patients with *GBA* mutations and non-carrier patients (Table 2). *GBA* mutation carriers had a higher frequency of slowness (1.6- and 2.3-fold) than the two other groups (χ^2=7.69, df=2, p=0.021). The frequency of patients reporting a subjective sense of weakness in a limb was more than twofold higher among *GBA* mutation carriers, but this did not reach a level of significance (p=0.064). More loss of smell sense (4.6% compared to 1.4% and 1.2%, not statistically analyzed) was also reported by this group.

Finally, we examined the possibility that AAO affects the type of initial symptoms. For this analysis, a group of PD patients non-carriers of either *GBA* or *LRRK2* G2019S mutations was randomly selected (n=236, average AAO 57.8±11.2 years) to match the AAO of the two groups of carrier patients (one-way ANOVA, $F_{(2,415)}$=0.019, p=0.98). Again, carriers of the *LRRK2* G2019S mutation had higher frequencies of muscle stiffness (p=0.008) and balance disturbances (p=0.004), and *GBA* mutation carriers had a higher frequency of slowness (p=0.032), thus, excluding the possibility that the AAO affects the differential presentation of PD in *LRRK2* G2019S and *GBA* mutation carriers.

Table 2 Comparison between Ashkenazi Parkinson's disease patients, carriers of either *LRRK2* G2019S or *GBA* mutations, and non-carriers

	GBA mutations carriers	*LRRK2* G2019S carriers	Non-carriers of *GBA* or *LRRK2* G2019S mutations	Total	*p* value
Number	109	74	402	585	
Initial symptoms, % (*n*)					
Tremor	52.3% (57)	50.0% (37)	58.7% (236)	56.4% (330)	0.241
Gait disturbances	19.3% (21)	18.9% (14)	15.4% (62)	16.6% (97)	0.535
Muscle stiffness (rigidity)	18.3% (20)	39.2%[a] (29)	26.1% (105)	26.3% (154)	0.007[a,b]
Slowness of movement (bradykinesia)	24.8%[c] (27)	10.8% (8)	15.2% (61)	16.4% (96)	0.021[b,c]
Balance disturbances	1.8% (2)	13.5%[a] (10)	6.7% (27)	6.7% (39)	0.008[a,b]
Subjective sense of weakness in a limb	11.0% (12)	5.4% (4)	5.0% (20)	6.2% (36)	0.064

[a] Chi-square, significant after FDR correction that sets the cutoff *p* value on 0.025. The source of significance was the group of the *LRRK2* mutation carriers

[b] Statistically significant *p* values

[c] Chi-square, significant after FDR correction that sets the cutoff *p* value on 0.025. The source of significance was the group of the *GBA* mutations carriers

Discussion

The initial symptoms in PD vary, and the three cardinal motor initial symptoms were reported at different frequencies [10–12]: tremor (49–58%), rigidity (5–37%), and bradykinesia (13–21%). These initial symptoms were reported in our entire cohort of Ashkenazi PD patients in similar frequencies (56.5%, 26.5%, and 16.5%). Until now, only few studies detected an effect of a specific genetic alteration on the initial presentation of the disease. PD patients with the *SNCA* A53T mutation were found to have significantly higher frequencies of rigidity and bradykinesia and a lower frequency of tremor as presenting symptoms compared to sporadic PD patients [13]. Recently, 33 patients of North-African origin who carried the *LRRK2* G2019S mutation were compared to 72 non-carriers. Four presenting symptoms were included in that analysis (tremor, bradykinesia, micrographia, and dystonia), and no significant differences were detected [9]. Interestingly, a significantly increased frequency of dyskinesia was found among the carriers [9], further suggesting a possible genotype–phenotype correlation between the *LRRK2* G2019S mutation and the response to anti-parkinsonian treatment. An additional study that compared familial and sporadic PD cases also failed to detect different frequencies of initial symptoms between these two groups of patients [14]. These studies, however, were done on a relatively small number of patients that were not always genotyped for the common mutations.

To test the hypothesis that mutations in PD-associated genes might differentially affect the initial presentation of the disease, we studied the largest cohort of Ashkenazi PD patients analyzed to date. Furthermore, knowing the *LRRK2* and *GBA* mutations status of our patients, we were able to compare the effects of these two different mutated genes in the same cohort. Dividing and analyzing our patient population into three different genotypic groups also prevented a possible masking effect that occurred when some of the control patient population carried a frequent but undetected mutation [5, 6]. Finally, by applying stringent statistical criteria, we demonstrated that higher frequencies of muscle stiffness (rigidity) and balance disturbances and higher frequency of slowness (bradykinesia) were detected as initial symptoms in *LRRK2* G2019S and *GBA* mutation carriers.

Functional studies of *LRRK2* suggested its involvement in several PD-related pathways and mechanisms, such as neuronal toxicity [15], apoptosis [16], and oxidative stress [17]. *GBA* mutations might result in endoplasmic reticulum retention [18], a condition that can potentially lead to cell death. In addition, lysosomal and autophagy dysfunction and the ceramide metabolism pathway might be related to PD pathogenesis [19, 20]. The suggested different models for *LRRK2*- and *GBA*-associated PD support our hypothesis that distinct cellular pathways might underlie the differences in the initial symptoms. However, the occurrence of Lewy-bodies in brains of both *LRRK2* and *GBA* mutation carriers suggests that the end result of these pathways might be α-synuclein accumulation [15, 20].

To conclude, our data suggest that *LRRK2* and *GBA* mutations constitute not only important risk factors, but also affect the phenotype of the disease, specifically, the initial symptoms. Since these symptoms are markers of the long-term clinical course of PD, the different presentation among the three genotypic groups further stress the need to elucidate the specific roles of *LRRK2* and *GBA* in the pathogenesis of PD.

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Declaration The experiments presented herein comply with the current laws of Israel, the country in which they were performed.

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