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The contribution of Niemann-Pick *SMPD1* mutations to Parkinson disease in Ashkenazi Jews

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ABSTRACT

Introduction: Parkinson disease is noted for its association with mutations in GBA and the p.G2019S mutation in *LRRK2*. This study aimed to evaluate the frequency of Ashkenazi founder mutations in sphingomyelin phosphodiesterase 1 (*SMPD1*) in Ashkenazi patients diagnosed with Parkinson's disease (PD); and their impact on PD phenotypic expression. *SMPD1* underlies the lysosomal storage disease - Niemann-Pick.

Methods: A case (n = 287) control (n = 400) study was undertaken. All patients underwent a physical, neurobehavioral and neurologic examination that incorporated the Unified Parkinson's Disease Rating Scale. Three founder *SMPD1* Ashkenazi mutations (c.996delC (fsP330), p.L302P and p.R496L) were investigated in patients and controls, previously evaluated for carriage of founder mutations in *GBA* and the p.G2019S mutation in *LRRK2*.

Results: Nine (3.1%) PD patients compared to two (0.5%) individuals from the control group were found to carry one of the three Ashkenazi *SMPD1* founder mutations (p = 0.007). The overall clinical characteristics of PD patients carrying *SMPD1* mutations were similar to those of PD patients with no mutations in *SMPD1*, *GBA* and *LRRK2* (n = 189).

Conclusion: We maintain that disruptive mutations in *SMPD1* constitute a risk factor for PD.

1. Introduction

Parkinson is a multifactorial disease that affects 1% of the population over 65 years of age [1]. Symptoms include tremor at rest, muscle stiffness, bradykinesia and impaired balance upon walking [2,3]. Mutations in genes designated PARK1 to 14, were shown to be linked to Parkinson's disease (PD), of which α-synuclein was first to be identified (*SNCA*/PARK1/4). Variants in these genes are basically rare [4–6]. In patients of Ashkenazi descent, a strong association between PD, the p.G2019S mutation in leucine-rich repeat kinase 2 (*LRRK2*) [7–9] and/or founder mutations in glucocerbrosidase (*GBA*) [10–14] is well documented. About 15% and 20% of Ashkenazi PD patients carry the p.G2019S mutation in *LRRK2* and/or a founder mutation in *GBA*, respectively [9,12–14].

Two missense mutations, p.R496L, p.L302P, and one base deletion c.996delC (fsP330) account for over 95% of mutant *SMPD1* alleles in Ashkenazi type A Niemann-Pick patients, with a carrier frequency of 1:90 [15–17]. Recently, the p.L302P mutation [18] and the rare variant p.R591C, in *SMPD1*, were independently reported to occur in excess in PD patients [18,19]. *SMPD1* encodes the lysosomal enzyme sphingomyelin phosphodiesterase 1 (acid-sphingomyelinase-ASMase), that catalyzes the cleavage of sphingomyelin to generate ceramide [16,17,20–22]. Niemann-Pick and Gaucher disease (GD) belong to a group of lysosomal storage diseases (LSDs), typically inherited as autosomal recessive traits. Mutations in these genes result in cellular accumulation of sphingolipids, thereby causing neuro- and/or dysmyelinative degeneration [21,22].

Here we examined the prevalence of *SMPD1* Ashkenazi founder mutations, in a subset of Ashkenazi PD patients compared to that observed in healthy Ashkenazi individuals. To isolate the impact of *SMPD1* mutations on the phenotypic expression of PD, we

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compared the clinical characteristics of Ashkenazi PD patients, carriers of a founder mutation in *SMPD1* to non-carriers of any of the mutations at interest (*SMPD1*, *GBA* and *LRRK2*) [9,12–14,18].

2. Methods

2.1. Participants

The study was conducted at the Genetics Institute, the Department of Neurology, and the Cognitive Neurology Institute, at Rambam Health Care Campus, Haifa, Israel, following approval by the hospital's ethics committee. The *study group* consisted of 287 Ashkenazi PD patients (169 men and 118 women; 62.54 ± 10.78 mean age at diagnosis); 99 of whom previously participated in our study linking *GBA* Ashkenazi founder mutations to PD [12] and 188 PD patients recruited thereafter, until 2013. Parkinson's disease was defined by the UK PD Society Brain Bank Clinical Diagnostic Criteria [23]. The *control group* comprised of 400 healthy individuals of Ashkenazi descent (78 men and 322 women; 30.08 ± 3.96 mean age at molecular testing), without a known personal or family history of a neurological illness, who were screened at the genetic institute, Rambam Health Care Campus, for common Ashkenazi autosomal recessive disorders.

2.2. Procedure

Information regarding disease onset, clinical manifestations and family history was obtained from patients, guardians and the subject's medical records in a uniform manner using a structured questionnaire. Patients underwent a physical, neurobehavioral, and neurologic examination that incorporated the Unified Parkinson's Disease Rating Scale [24]. Cognitive status was evaluated using the Mini Mental State Examination (MMSE) [25]. MMSE <26 was considered as cognitive impairment. An explanation regarding the study's significance and procedure was provided to patients or their guardians who then signed a written informed consent, and patients were asked to provide a blood sample.

All participants in the control group previously signed an informed consent allowing for the use of their DNA for future research, subject to the hospital's ethics committee approval. Patients and controls were all tested for the Ashkenazi founder mutations in *SMPD1*, *GBA* and *LRRK2*.

2.3. Molecular analyses

DNA was extracted from whole blood using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), according to the manufacturer's instructions.

DNA samples were subjected to polymerase-chain-reaction (PCR) analyses in order to identify three Ashkenazi founder mutations in *SMPD1* (p.L302P. c.996delC (fsP330) and p.R496L); six Ashkenazi founder *GBA* mutations (p.N370S, p.L444P, c.84 GG, c.115 + 1G > A (IVS2 + 1G > A), p.V394L, and p.R496H); and the p.G2019S mutation in *LRRK2* (Table 1).

Ten primer pairs were designed and used separately to amplify the genomic segments flanking each of the 10 mutations examined. PCR amplification products were separately analyzed. In the majority of cases RLFP was employed (Table 1) to identify the mutant and wild type alleles. PCR primers, restriction enzymes and fragments' sizes before and after cleavage are listed in Table 1.

2.4. Statistical analysis

Descriptive statistics was used to compare PD patients and controls as regards carrier status, and to associate clinical characteristics to *SMPD1* carrier status in PD patients.

Differences among groups as regards continuous or categorical variables were tested using t-test or chi-square analyses when appropriate. For comparison of groups with small numbers of participants, nonparametric Mann–Whitney and Fischer exact test were used. Logistic regression model was used to calculate odds ratio (OR) and confidence interval (CI). Nagelkerke's R^2 was used to estimate the fraction of explained variance of the tested mutations to the phenotypic expression of PD. The Goodness offit test with 1 degree of freedom was applied to look for any deviation from the Hardy–Weinberg equilibrium among the young controls and among patients with PD who were screened for *SMPD1*, *GBA*, and *LRRK2* mutations. SPSS software v.19 (SPSS Inc., Chicago, IL) was used for all data analysis.

3. Results

Of the 287 PD participants, 98 carried at least one mutation in either of *SMPD1*, *GBA* and/or *LRRK2*. The mutations in *SMPD1* were 6-fold more prevalent (n = 9, 3.1%) in PD patients than in controls

Table 1
Primers and measures used for the detection of each mutation in the *SMPD1 (NM_000543.4)*, *GBA (NM_000157.3)* and *LRRK2 (NM_198578.3)* genes.

Gene	Mutation	Primer sequence	Restriction enzymes	Size of fragments	
		Primers		Wild type	Mutant
SMPD1	p.L302P	Detected by custom designed assay using Locked Nucleic Acid (LNA) Fluorescent probes (IDT Integrated DNA Technologies)			
	p.R496L	F- GCATGGGCAGGATGTGTGG R- GTATGGCTCCCGGTATGTTTG	HpyCH4III	105, 83	188
	c.996delC (fsP330)	F- FAM- CCAGACTCGTCAGGACCAAC R- ATAGAGCCAGCGGGAGGAGT	Detected by ABI Prism® 310 Genetic Analyzer (Applied Biosystems).		
GBA	p.V394L	F- GCCTTTGTCCTTACCCTCGa R- GACTGTCGACAAAGTTAGGC	Ban1	91, 22	113
	c.115 + 1G > A (IVS2 + 1G > A)	F- GCTGGCAGCCTCACAGGAaT R- CCAGGCAACAGAGTAAGACTCT	Hph1	141, 64, 39	205, 39
	p.R496H	F- CTGCTGTTGTGGTCGTGCTA R- AAGCTCACACTGGCCCTGCT	Detected by sequencing		
	p.N370S	F- GCCTTTGTCCTTACCCTCGa R- GACTGTCGACAAAGTTAGGC	Xho1	113	97, 16
	p.L444P	F- GGAGGACCCAATTGGGTGCG R- ACGCTGTCTTCAGCCCACTTC	Msp1	622, 45	565, 56, 45
	c.84dupG	F- GCTGGCAGCCTCACAGGAaT R- CCAGGCAACAGAGTAAGACTCT	Bsm1	221, 23	244
LRRK2	p.G2019S	F- CATTGCAAAGATTGCTGACTG R- AGACAGACCTGATCACCTACC	Pst1	97	74, 23