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SHORT COMMUNICATION

Glucocerebrosidase mutations in a Serbian Parkinson's disease population

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Background and purpose: To screen for *glucocerebrosidase* (*GBA*) mutations in a Serbian Parkinson's disease (PD) population.

Methods: *Glucocerebrosidase* exons 8–11 harbouring the most common mutations were sequenced in 360 patients with PD and 348 controls from Serbia. Haplotype analysis was performed for the N370S mutation and compared with German and Ashkenazi Jewish carriers.

Results: *Glucocerebrosidase* mutations were significantly more frequent in patients with PD (21/360; 5.8%) vs. controls (5/348; 1.4%; OR = 4.25; CI, 1.58–11.40; $P = 0.0041$). Two patients with PD carried homozygous or compound heterozygous mutations in *GBA*. The N370S mutation accounted for about half of the mutated alleles in patients (10/23) but was absent amongst controls. Three novel variants were detected including two non-synonymous variants (D380V, N392S) in the patient group and one synonymous change (V459V) in a control. Carriers of the D409H mutation were also sequenced for H255Q, and all were found to carry the [D409H; H255Q] double-mutant allele. Genotyping suggested a common haplotype for all N370S carriers.

Conclusion: *Glucocerebrosidase* mutations represent a PD risk factor in the Serbian population.

Introduction

Gaucher disease (GD) is caused by homozygous or compound heterozygous mutations in the *β-glucocerebrosidase* (*GBA*) gene. *GBA* mutations can be classified according to phenotypic effects as mild (associated with 'non-neuronopathic' Type 1 GD) and severe or null (neuronopathic Type 2 or 3 disease) [1]. In addition to causing GD, *GBA* mutations are also susceptibility factors for Parkinson's disease (PD) [2,3]. Most studies show an association between *GBA* mutations and PD, although this association was not found in a Norwegian population [4]. The frequency of the different *GBA* mutations varies according to ethnicity. In Ashkenazi Jewish (AJ) and French populations, N370S is the most frequent mutation [2,3], whereas in Asian populations, the L444P mutation is more common [3]. PD patients with *GBA* mutations present earlier and are more likely to have a positive family history and cognitive changes [3]. A common founder has been observed for the N370S mutation in AJs and in several European populations [5–7].

In the present study, we elucidated, for the first time, the role of *GBA* mutations in PD in the Serbian population, including mutational analysis of exons 8–11, genotype–phenotype comparisons, and haplotyping for the N370S mutation.

Methods

Unrelated PD subjects were recruited from a tertiary referral centre in Belgrade, Serbia. Unrelated, ethnically matched controls (Table S1) underwent a medical examination, and those with signs of a

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neurological disorder were excluded. Ethnicity was self-reported, and none of the subjects were of AJ ancestry. PD was defined according to UK Brain Bank Criteria [8], with the exception that a positive family history was not part of the exclusion criteria. DNA samples from known non-AJ German N370S heterozygotes and an AJ N370S homozygote were procured from Lübeck (Germany) and New York (USA), respectively. The study was approved by the institutional ethics committees, and written informed consent was obtained from each subject.

Clinical examination of patients with PD included the Unified Parkinson's Disease Rating Scale (UPDRS), Hoehn and Yahr Scoring (H&Y), Mini-Mental State Examination (MMSE), Hamilton depression rating scale (HAMD) and Hamilton anxiety rating scale (HAMA). The clinical impression of the treating neurologist was used to determine whether patients were categorized as levodopa-responsive or levodopa-unresponsive. Sequence analysis was performed for exons 8–11. Primers used for amplification of exons 8 and 9 (Table S2) were specific to the functional gene (*GBA*) rather than the pseudogene (*GBAP*). Exons 10 and 11 were amplified using nested PCR with partially mismatched primers to avoid co-amplification of the *GBAP*. Subjects identified with the D409H mutation were also sequenced for H255Q (Table S2) given the high frequency of the [D409H; H255Q] allele in Balkan patients [9].

Clinical and demographic details of mutation carriers were compared with those of non-mutation carriers. Odds ratios (OR) were calculated for an association between *GBA* mutations and PD, Fisher's exact test was utilized for categorical variables and the Mann–Whitney *U*-test for continuous variables. Medcalc® software (version 11.6, MedCalc Software, Mariakerke, Belgium) was used for statistical analyses. Genotyping was performed using established microsatellite markers [5–7].

Results

The frequency of *GBA* mutations was significantly higher (OR = 4.25; CI 1.58–11.40; $P \leq 0.0041$) amongst patients with PD (5.8%; 21/360) compared with controls (1.4%; 5/348), and this result remained significant after exclusion of novel variants or variants of uncertain pathogenicity (OR 4.79, CI 1.61–14.23). One patient was found to be an N370S homozygote and one was identified as an N370S/[D409H; H255Q] compound heterozygote. The following known mutations were identified in the heterozygous state in patients versus controls: N370S (7/360 vs. 0/348), [D409H; H255Q] (6/360 vs. 2/348), L444P (2/360 vs. 1/348), A456P (0/360 vs. 1/348), R463C (1/360 vs. 0/348) and Rec*Nci*I (L444P + A456P + V460V, 1/360 vs. 0/348). When analysed independently, the L444P and D409H mutations were not significantly associated with PD. The allelic frequency of the T369M substitution was similar for patients (2.08%) and controls (1.72%, OR 1.21, CI 0.56–2.61). A non-synonymous change of uncertain pathogenicity (E388K) was identified in the heterozygous state in a control. Additionally, we detected three novel variants in the heterozygous state including two missense changes (c.1256A>T, D380V and c.1292A>G, N392S) in one patient each and one silent variant (c.1497G>A, V459V) in a control.

Comparison of demographic and clinical features between mutation carriers and non-carriers demonstrated a higher frequency of rigidity at disease onset and postural instability on examination (Table 1). Patients carrying a severe heterozygous *GBA* mutation (L444P, [D409H; H255Q], R463C, Rec*Nci*l) had a significantly ($P < 0.001$) earlier age at onset (AAO) than those with a mild heterozygous (N370S) change (45.1 ± 8.32 vs. 56.17 ± 7.63). Additionally, the N370S/[D409H; H255Q] compound heterozygote with AAO of PD 39 years was found to have features of GD (thrombocytopenia, leukopenia, and splenomegaly) at age 41 years, whereas the N370S homozygote (AAO of PD 54 years, examined at age 58 years) had no definite clinical manifestations of GD.

Haplotype analysis showed a putative shared haplotype for three markers flanking *GBA* for non-AJ Serbian, German and AJ N370S alleles.

Discussion

This study provides further evidence that mutations in the *GBA* gene are an independent risk factor for PD by establishing an association between *GBA* mutations and PD in a Serbian sample. The total number of mutations may have been underestimated by selective exon screening [3]. However, the most common mutations [2] are included by screening of exons 8–11, and an association with additional rare variants detected by screening of the remaining exons would be difficult to validate in a cohort of this size [4]. Controls were younger than patients, but given that the lifetime prevalence of PD is ~2% it is probable that only a small minority of controls will develop PD in the future, and consequently the effect upon the results is likely to be negligible.

Of the *GBA* mutations, N370S was the most common change amongst patients. In contrast, N370S was absent in the control sample; the reason for this finding is unclear. The L444P mutation was

Table 1 Continuous variables are given as mean ± standard deviation with the range in parentheses.

	GBA carriers $n = 21$	n_a	*GBA* non-carriers $n = 339$	n_a	*P*-value (significant values in bold)
Sex ratio (male/female)	15:6	21	214:122	336	0.64
Family history (%)	16.7	18	18.18	297	1.00
Age at onset (years)	49.68 ± 9.68 (34–66)	19	52.40 ± 11.10 (10–76)	302	0.27
Age at examination (years)	59.89 ± 9.13 (41–75)	19	60.41 ± 10.68 (28–80)	304	0.78
Disease duration (years)	10.11 ± 5.63 (2–22)	19	8.00 ± 5.86 (0.25–45)	302	0.073
Signs at onset (%)					
Rigidity	46.67	15	16.07	280	**0.0074**
Bradykinesia	26.67	15	27.86	280	1.00
Tremor	40.00	15	53.93	280	0.30
Signs at examination (%)					
Asymmetric parkinsonism	78.95	19	74.56	287	0.79
Dyskinesia	5.26	19	11.85	287	0.71
Dystonia	0	19	0.70	287	1.00
Gait freezing	0	19	2.79	287	1.00
Postural instability	42.11	19	20.91	287	**0.044**
UPDRS total 'on'	73.90 ± 39.11 (31–159)	10	57.54 ± 26.58 (3–152)	171	0.22
H&Y 'on'	2.74 ± 0.77 (1.5–4)	19	2.40 ± 0.79 (1–4)	303	0.076
H&Y 'off'	3.21 ± 1.14 (1.5–5)	12	3.11 ± 1.10 (1–5)	295	0.75
MMSE	26.27 ± 4.38 (16–30)	11	27.69 ± 3.20 (11–30)	222	0.16
HAMD	11.22 ± 9.51 (1–28)	9	10.96 ± 9.00 (0–46)	182	0.91
HAMA	9.29 ± 8.12 (1–24)	7	7.58 ± 5.94 (0–23)	106	0.59
Treatment response (%)	94.73	19	97.55	286	0.08

n_a, number of subjects available for assessment.

not a significant independent risk factor in this population, in contrast to other studies which have shown that the OR is highest for this mutation [3]. PD subjects with a severe heterozygous *GBA* mutation had an earlier AAO compared with those with a mild heterozygous mutation, consistent with a differential phenotype for severe versus mild mutations [10]. All D409H carriers also carried H255Q, which coexists in cis on the mutant allele [9]. Homozygosity for the [D409H; H255Q] allele correlates with a more severe GD phenotype than homozygosity for D409H alone, and it has been shown that these two mutations have a detrimental cumulative effect upon enzymatic activity [9]. The N392S is a novel mutation, although N392I has been described in Spanish GD patients [7]. The D380V variant is also a novel change, and several substitutions at this site (D380N, D380H, D380Y and D380A) have been associated with GD. The pathogenicity of the E388K mutation is uncertain given that it has been identified in the control population in this cohort and in previous studies [2,11]. The T369M allele is likely to be a benign polymorphism [3].

Glucocerebrosidase mutation carriers had a higher frequency of rigidity at disease onset and an increased frequency of postural instability at the time of examination. To our knowledge, this is the first time that these aspects of the PD phenotype have been associated with *GBA* carrier status. In contrast to previous studies [2,3], there was no difference in the frequency of a positive family history (mutation carriers versus non-mutation carriers), underlining that *GBA* mutations can be identified in a proportion of sporadic cases. Additionally, it appears that carriers of two mutant *GBA* alleles can present with PD and have minimal clinical evidence of GD, as occurred in the case of the N370S homozygote, consistent with reports that manifestations of PD can precede those of GD by many years [12]. Also, genotyping was consistent with a common haplotype for N370S, supporting a panethnic origin of this mutation. Given the relatively high penetrance [13] and frequency of *GBA* mutation carriers, *GBA*-associated PD should be targeted for future study.

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Disclosure of conflict of interest

The authors declare no financial or other conflict of interests.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Demographic information for PD patients and control subjects.

Table S2. Primer sequences for *Glucocerebrosidase* sequencing. For exons 10 and 11, nested PCR and partially mismatched primers were utilized in order to avoid co-amplification of the pseudogene. The mismatched primers (mis) are listed, with the original sequence below and the mismatched sites underlined. Sequencing was performed in a forward direction and mutations were confirmed in a reverse direction. Positive and negative controls were used to validate *GBA* sequencing.

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