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Glucocerebrosidase mutations are not a common risk factor for Parkinson disease in North Africa

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Abstract

Mutations in the Glucocerebrosidase gene (*GBA*) have recently been associated with an increased risk of Parkinson disease (PD). *GBA* mutations have been observed to be particularly prevalent in the Ashkenazi Jewish population. Interestingly, this population also has a high incidence of the *Lrrk2* p.G2019S mutation which is similar in North African Arab-Berber populations. Herein, our sequencing of the *GBA* gene, in 33 North African Arab-Berber familial parkinsonism probands, identified two novel mutations in three individuals (p.K-26R and p.K186R). Segregation analysis of these two variants did not support a pathogenic role. Genotyping of p.K-26R, p.K186R and the common p.N370S in an ethnically matched series consisting of 395 patients with PD and 372 control subjects did not show a statistically significant association ($P > 0.05$). The p.N370S mutation was only identified in 1 sporadic patient with PD and 3 control subjects indicating that the frequency of this mutation in the North African Arab-Berber population is much lower than that observed in Ashkenazi Jews, and therefore arose in the latter after expansion of the *Lrrk2* p.G2019S variant in North Africa.

Keywords

Parkinson disease; Gaucher disease; genetics

Introduction

Parkinson disease (PD) is the most common form of age-related neurodegenerative parkinsonism [10]. Mutations in several genes have been reported to result in familial forms of parkinsonism; leucine-rich repeat kinase 2 (*LRRK2*), alpha-synuclein (*SNCA*), parkin (*PRKN*), PTEN-induced kinase 1 (*PINK1*), and *DJ-1* [8]. Gaucher disease (GD) is a recessively inherited disorder of glycolipid storage caused by mutations and rearrangements in the glucocerebrosidase gene (*GBA*) [23]. Interestingly, some GD patients have parkinsonism as a

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symptomatic feature of their disease and both disorders share similar pathological features [23]. To date, several reports have concluded that heterozygous *GBA* mutations are a risk factor for PD [1,12,21].

The frequency of *GBA* mutations varies depending on population ethnicity with a remarkably high frequency in individuals of Ashkenazi Jewish descent. Indeed it was this population that first noted a high number of *GBA* mutation carriers (31.3%) among Ashkenazi Jewish PD patients [1]. A number of studies have now reported the frequency of *GBA* mutations in PD patients across populations [2,5-7,9,17-22,24,26,27,30]. Given the presence of a neighboring *GBA* pseudo-gene, whole gene sequencing is not straightforward so many studies limit their analysis to known mutations, which in most populations may be representative of the complete mutation burden. Therefore given differences in analytical methods, the allele frequencies of all mutations, independently or combined, are not always reported for the *GBA* locus. Rather point variations are typically studied, the most common of these mutations (p.N370S) has been analyzed in most studies and the global frequency is presented in Figure 1. Although the p.N370S mutation may not be representative of the total burden of *GBA* mutations within the locus, particularly in Asian populations, and other mutations may be highly prevalent in specific populations; these results do give us insight into the general frequency of mutations in different ethnicities.

Interestingly, Ashkenazi Jewish patients with PD also have a remarkably high frequency of Lrrk2 p.G2019S (hereditary 28%, sporadic 10%) [11]. This corresponds to a similarly high frequency in Tunisian Arab-Berbers of which 30% of patients with PD harbor Lrrk2 p.G2019S [14]. Moreover, the study of *GBA* mutations has identified p.N370S as the most frequent mutation in Gaucher's patients from Tunisia [3,4]. Considering this observed genetic distribution and a common ancestral founder haplotype for *LRRK2* p.G2019S [28], we hypothesized that patients with PD from Tunisia also may have the higher frequency of *GBA* mutations, performing complete gene sequencing on a series of familial affected probands previously screened for mutations in all known genetic forms of PD.

Material and method

Subjects

A total of 89 families, 240 sporadic patients with PD from the Institut National de Neurologie, Tunis, and 372 control participants from the same geographic region were included in the study. The Institut National de Neurologie, Tunis provides a specialized neurological service to the entire country of Tunisia [16]. The site obtained local ethics committee approval before beginning recruitment. Informed written or proxy consent for the study was given by all subjects. Individuals were diagnosed as “affected” if they satisfied the United Kingdom PD Society Brain Bank (UKPDS) criteria [13] or “controls” if all signs of parkinsonism were absent and there was no family history of parkinsonism.

Sequencing and genotyping

We selected 33 familial probands with PD (age at onset; 53.0 ± 15.2 (average $\pm$ S.D). M:F=12:21). Genomic DNA was extracted from peripheral blood lymphocytes using standard protocols. All 33 probands were previously screened negative for mutations in *LRRK2*, *SNCA*, *PINK1*, *DJ-1*, and *PRKN* [14,15] (and unpublished data). We sequenced the entire *GBA* coding sequence (exons 1-11) as previously reported [25]. PCR products were purified from unincorporated nucleotides using Agencourt bead technology (Beverly, MA) with Biomek FX automation (Beckman Coulter, Fullerton, CA). Electropherograms were analyzed with SeqScape v2.1.1 using 3730 DNA Analyzer (ABI, Applied Biosystems, Foster City, CA, USA).

An association study for the two novel variants detected by sequencing and the common *GBA* p.N370S mutant was performed in 240 sporadic PD (Age at onset; 57.1 ± 13.0 , M:F=116:124) and 372 normal control subjects (Age at exam; 58.3 ± 11.0 , M:F=190:182). Screening and segregation of these variants was also evaluated 50 additional PD probands and 122 additional family members diagnosed with PD (Age at onset; 53.7 ± 17.9 , M:F=64:58) from 76 pedigrees. Of those additional kindreds, 33 are *Lrrk2* p.G2019S positive, 13 are *PINK1* positive, and 4 are *PRKN* positive [14,15].

Genotyping was performed by Taqman Assay-by-Design SNP method using SDS 2.2.2 software on ABI 7900 HT Fast Real-Time PCR system (Applied Biosystems, CA). Probes are purchased from Applied Biosystems. Sequence information for the probes is given in supplementary table-1. Following genotyping, direct sequencing was performed to confirm the presence of the mutation in the functional gene.

Results

Complete *GBA* gene sequencing of 33 familial PD probands detected two novel non-synonymous variants; (p.K-26R, traditional nomenclature; p.K13R, protein nomenclature including the 39-residue signal peptide) in two patients and (p.K186R; p.K225R) in one patient (Figure 2). The p.N370S substitution commonly identified in the Ashkenazi Jewish population, was not observed in our sequencing analysis. We then assessed the frequency of p.K-26R, p.K186R, and p.N370S among 155 familial (including the 33 sequenced probands) and 240 sporadic patients with PD and 372 normal control subjects by SNP genotyping. Genotyping of these variants identified the p.K-26R mutation in a total of 14 patients with PD (3.54%) of which 5 are familial and 9 controls (4.30%), p.K186R was identified only in two familial samples (0.51%), and the p.N370S mutation was observed in one sporadic patient with PD (0.25%) and three control subjects (0.81%). Direct sequencing confirmed all mutations were in the functional *GBA* gene and segregation with disease in families was equivocal. The three unaffected individuals carrying the p.N370S were 58, 64 and 76 years of age at examination. Statistical comparisons between patients and control subjects did not identify significant associations between *GBA* variants and risk of PD ($P > 0.05$). As none of the 50 kindreds harboring known pathogenic mutations for PD presented with the *GBA* mutations identified in this study we were unable to assess their potential role as disease modifiers.

Discussion

Our study suggests the frequency of *GBA* gene mutations in the Tunisian Arab-Berber population is much lower than that of the Ashkenazi Jewish population. Our sequence analysis identified two novel variants (p.K-26R and p.K186R); whereas the p.K-26R variant appears to be present at similar frequencies in cases and controls (~3-4%), p.K186R is rare and was only observed in two patients. In those families with additional family members available to study, segregation analysis did not support a pathogenic role for p.K-26R. No additional family members were available for the assessment of p.K186R segregation. Despite not identifying any control subjects harboring this mutation, pathogenicity for p.K186R still remains to be proven.

The *GBA* p.N370S mutant is common in Ashkenazi Jews and is one of the most frequently assayed variants. Therefore focusing on its ethnic distribution may provide a greater perspective for our findings with respect to other global frequencies of *GBA* mutations in PD patients (Figure 1). These data show that p.N370S is less frequent in Caucasian populations, and absent in Asian populations. We now need to determine the ethnic distribution of mutations and influence of the *GBA* gene on PD risk globally to advise on genetic testing and diagnosis.

Although our study does not support the growing body of evidence showing that *GBA* variants increase susceptibility to PD, this could be due to the high level of consanguinity, sample size or phenotypic differences in our population. Therefore it is crucial to examine if specific phenotypic features exist in *GBA* mutation carriers; this may help resolve the pathomechanism involved in PD risk. Further studies evaluating the risk of PD in relatives of Gaucher's disease patients, and the potential use of therapeutics which target *GBA* to lower their risk are warranted. The collection of comprehensive clinical data from large populations of carriers to assess correlates of clinical presentation and the *GBA* genotype are required. Clinically, the age of onset of p.N370S seems to slightly earlier than typical PD with previous reports of 54.4 ± 13.7 (mean $\pm$ S.D.) of p.N370S [7,19,22,24,26,27]. Of note, p.N370S transgenic mice models display a severe phenotype with mutant mice dying soon after birth [29].

Herein we show that *GBA* mutations are rare in North African Berber-Arabs from Tunisia. Given the shared ancestry and high frequency of *Lrrk2* p.G2019S in both Arab-Berber and Ashkenazi Jews, these results suggest that the *Lrrk2* p.G2019S founder existed prior to the expansion of *GBA* mutations in the Ashkenazi Jewish community. We conclude that *GBA* mutations are not a major risk factor for PD in North African Berber-Arabs from Tunisia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Global distribution of GBA p.N370S mutant. The frequency of p.N370S in patients with PD followed by the frequency in control subjects is given for each population [1,2,5-7,9,17-22, 24,26,27,30]. *Ashkenazi Jewish population.

Figure 2. Schematic of *GBA* (NM_000157). Position of the novel mutations identified and p.N370S are given. Double nomenclature with and without the absence of the signal peptide is given for all mutations. Exons encoding the signal peptide are shaded.