

**An assessment of the frequency of mutations in the GBA and VPS35 genes
in Hungarian patients with sporadic Parkinson's disease**

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Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disorder, with cases of either familial or sporadic origin. Several polymorphisms in a number of genes have been proved to have an important role in the development of PD. Particular attention has recently been paid to genes of the glucocerebrosidase (GBA) and the vacuolar protein sorting-associated protein 35 (VPS35).

In this study, the three most common mutations (L444P, N370S and R120W) of the GBA gene and the D620N mutation of the VPS35 gene were examined in 124 Hungarian patients diagnosed with sporadic PD (SPD) and 122 control subjects.

The frequency of the L444P mutation of the GBA gene proved to be higher in the PD patients (2.4%) than in the controls (0%), although the difference was not statistically significant. All the patients who carried the mutant allele were in the early-onset PD (EOPD) group. However, neither the R120W nor the N370S variant of the GBA gene nor D620N mutation of the VPS35 gene were detected among the PD cases or the controls.

Even though these results suggest that the studied mutations are quite rare in SPD patients, the most frequent L444P mutation of the GBA gene may be associated with the development of EOPD in the Hungarian population.

Keywords

Parkinson's disease, GBA mutations, L444P, VPS35 mutation

Introduction

The most prominent neuropathological features of Parkinson's disease (PD), the second most common neurodegenerative disorder, are the loss of dopaminergic neurons in the substantia nigra pars compacta and the presence of Lewy bodies, although the precise pathomechanism is still not fully understood. Gene–environment interactions are certainly implicated in the multifactorial pathogenesis of PD [6].

The majority of PD cases are sporadic; only 15-20% of the cases are identified as familial. In the background of these familial cases, numerous mutations have been identified in several genes that cause autosomal dominant (PARK1, PARK3, PARK4, PARK5, PARK8 and PARK11) or autosomal recessive (PARK2, PARK6, PARK7, PARK9, PARK14 and PARK15) forms of PD [9]. The D620N mutation in the vacuolar protein sorting-associated protein 35 (VPS35) was recently discovered as a new cause of PD [12]. Furthermore, genetic risk factors have been identified in sporadic forms of PD (SPD) [9]. The gene coding for glucocerebrosidase (GBA) has been reported to have a potential role in the development of PD [5].

Gaucher's disease (GD) is an autosomal recessively inherited glycolipid storage disorder caused by a deficiency of the lysosomal enzyme GBA. Several studies have reported a clinical, neuropathological or genetic association between GD and PD [5, 8, 20].

The GBA gene is localized at chromosome 1q21 and has more than 300 mutations [16]. Numerous genotyping studies have demonstrated associations between several GBA mutations and PD in different ethnic groups. As an example, the frequencies of GBA mutations in PD patients were highest amongst Ashkenazi Jews (ranging from 13.7% to 31.3%) [1, 7, 13]. Furthermore, several studies have indicated that a high frequency of GBA

mutations is related to an increased risk of PD amongst Japanese, Canadian, Portuguese, Greek, Norwegian, Italian, Serbian and Chinese populations [4, 11, 18, 25, 26, 28, 35, 38, 41].

N370S has been demonstrated to be the most common mutation among Ashkenazi Jewish PD patients (14.1%), and it is also the most frequent mutation in several European (e.g. Serbian (1.9%), French (2.9%) and Portuguese (2.2%)) populations [4, 18, 20, 32]. A Japanese study detected a significant association between the R120W mutation and PD [25]. L444P is the most common variant among non-Ashkenazi Jewish patients. The possible association between this mutation and PD has been investigated in a number of studies [23, 38, 39, 41].

The VPS35 gene, which is involved in the development of many neurodegenerative diseases, including Alzheimer's disease and PD [12, 33], is localized to 16q11.2, and various mutations have been reported in it [40]. The VPS35 protein is a key component of the retromer complex which mediates the retrograde transport of proteins from endosomes to the trans-Golgi network [3]. Amongst the mutations of the VPS35 gene, the D620N missense mutation has been reported to be pathogenic for PD [37, 42], mainly in the autosomal dominantly inherited cases, but it has additionally been detected in some sporadic PD cases [2, 19, 21, 31, 37, 42]. A recent multicentre study determined the frequencies of VPS35 mutations in PD in various populations. The D620N mutation was found in 5 familial and 2 sporadic cases [30]. In contrast, other studies suggest that there is no such mutation in SPD in the Caucasian population [19, 31].

No study has been conducted previously to assess the frequency of GBA or VPS35 gene mutations in Hungarian PD patients. The aim of the current preliminary study was therefore to investigate whether any of the L444P, N370S and R120W mutations of the GBA gene or the D620N mutation of the VPS35 gene is present in SPD in the Hungarian population, which belongs in the Caucasian race.

Material and methods

Subjects

124 SPD patients (mean age: 66.5 ± 9.5 years) were enrolled in the study. The mean age at onset of PD was 59.1 ± 10.9 years. Early-onset PD (EOPD) was defined as an age at onset ≤ 60 years (67 subjects) and late-onset PD (LOPD) as an age at onset > 60 years (57 subjects). All of the patients were examined by movement disorder specialists, who confirmed the diagnosis of SPD. Secondary forms of parkinsonism were excluded. The PARK2 and PARK8 mutations were not present in the assessed patient population.

The control group comprised 122 volunteer individuals (mean age: 64.3 ± 8.2 years) who had no history of neurological or psychiatric disorders (Table 1). All the patients with SPD and all the controls were of Hungarian origin and were enrolled in the Department of Neurology at the University of Szeged.

The study protocol was approved by the Medical Research Council Scientific and Research Ethics Committee (47066-3/2013/EKU (556/2013)) and all study participants gave their written informed consent in accordance with the Helsinki Declaration.

Table 1.
Characteristics of groups of PD patients and controls

	PD group	Control group
No.	124	122
Age (years, mean \pm SD)	66.5 ± 9.5	64.3 ± 8.2
Age at onset ≤ 60 years	67 (54%)	
Age at onset > 60 years	57 (46%)	

DNA isolation

Genomic DNA was extracted from peripheral blood by a standard desalting method [24], and stored at –20 °C until further use.

Analysis of GBA mutations

The L444P, R120W and N370S polymorphisms of the GBA gene were determined in all the patients and the controls by using the polymerase chain reaction and restriction fragment-length polymorphism (PCR-RFLP) techniques. Three previously described primer pairs were used separately to amplify the DNA region of each mutation [1, 39]. The PCR primers, annealing temperatures, restriction enzymes and fragment lengths are listed in Table 2. The digested products were separated by agarose gel electrophoresis.

Place of Table 2

Detection of VPS35 mutation

The D620N mutation in the VPS35 gene was determined by PCR-RFLP. The PCR conditions and primers were as described previously [34]. The PCR products were digested with the HinFI restriction enzyme (Thermo Scientific, Waltham, MA, USA) at 37 °C for 30 min. The digested products were separated by agarose gel electrophoresis. The genotypes were defined as GG (257 bp, 231 bp, 139 bp, 74 bp, 18 bp), GA (257 bp, 231 bp, 213 bp, 139 bp, 74 bp, 18 bp) or AA (257 bp, 231 bp, 213 bp, 18 bp).

Statistical analysis

SPSS software version 22.0 was applied for the evaluation of the data populations. The genotype frequencies in the patients and the controls were analysed by using the Fisher exact test. The normality of the data was checked with the Kolmogorov-Smirnov test. Since the data

exhibited Gaussian distribution, and the Levene test did not reveal significant differences in the homogeneity of variances, we applied the independent *t* test for the comparison of the difference in age between the PD groups and the controls. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated to test for an association between the GBA mutation and PD. A *p* value of less than 0.05 was considered statistically significant.

Results

GBA gene mutations

Among the PD patients, 3 individuals (2.4%) carried a heterozygous mutant GBA allele: in all 3 cases the L444P substitution. In contrast, no mutations were detected in the control group. The difference in mutation frequencies between the patients (2.4%) and controls (0%) was not statistically significant (*p* = 0.247). However, the carriers of the GBA mutation were at an increased risk of developing PD (OR = 6.05, 95% CI 0.300 to 122.06). Moreover, all the patients who carried the mutant allele were in the EOPD group. The frequency was significantly higher in the EOPD group than in the controls (*p*=0.042). However, a comparison of the frequency between the EOPD group and the LOPD group failed to reveal any difference (*p* = 0.247).

Neither the R120W nor the N370S variant of the GBA gene was identified among the assessed PD cases and controls.

VPS35 gene mutation

The common VPS35 D620N mutation was not detected either in the PD patients or in the controls in the assessed population.

Discussion

PD is a heterogeneous disorder. The majority of PD cases (75-80%) are sporadic; the remaining 15-20% of the patients have a familial history. Sporadic PD may result from complex interactions between genomic and environmental factors. Genome-wide association studies (GWAS) are carried out to identify rare genetic variants that increase the risk of PD. Multiple GWAS on PD patients and controls have revealed numerous loci, including GBA and MAPT, as risk factors for sporadic PD [36].

Moreover, several genes (PARK1-18) have been identified that can cause autosomal dominant or autosomal recessive forms of PD [17]. They include some genes which play an important role in the pathogenesis of SPD [10, 22]. The D620N mutation in VPS35 (PARK17) was recently discovered as a new cause of PD, mainly in the autosomal dominantly inherited cases, although it may additionally have a role in SPD, but the results are inconsistent [37, 42].

In the present study, we examined the three common mutations (L444P, N370S and R120W) of the GBA gene and the D620N mutation of the VPS35 gene in the Hungarian population (124 patients with SPD and 122 controls).

Several studies have reported that the frequencies of GBA mutations are higher in PD patients (5-10%) than in controls, but the range varies in different ethnic groups [29]. Our results indicated that the PD patients demonstrated a higher frequency (2.4%) of the L444P mutation of the GBA gene as compared with the controls (0%), although the difference was not significant. This finding is similar to those of previous studies that have reported associations between the L444P mutation and PD [23, 38, 39]. The L444P mutation was shown to occur with incidences of 2%, 1.39% and 1.14% among American non-Jewish, Canadian and British

PD patients, respectively [8, 27, 28]. Moreover, an Eastern Canadian study concluded that the frequency of L444P was higher among PD patients (3.1%) than among controls [15].

A number of studies have found that GBA mutations may be considered a risk factor, mainly in EOPD cases [8, 14, 39]. Our study revealed that all the patients who carried the mutant allele were in the EOPD group. These data emphasized the significance of the GBA mutation, particularly in EOPD cases.

Besides the L444P mutation, the other two most frequent mutations in non-Ashkenazi Jewish patients are N370S and R120W [32], and we therefore investigated these two GBA mutations too. We did not detect either the R120W or the N370S variant of the GBA gene among the PD cases or the controls, although the N370S mutation was earlier demonstrated in some European PD patients [18, 26, 32]. These diverse data suggest that the Caucasian population is not homogeneous in this respect.

Mutations in the VPS35 gene have been identified as a causative factor of the development of PD. The mutation carriers have been estimated to account for less than 1% of the PD population [19]. These mutations have been examined in a number of Caucasian populations, with different results [19, 30, 31, 37]. We therefore investigated the presence of the D620N mutation of the VPS35 gene in SPD patients, but we were unable to identify this mutation in any of the patients or controls. This suggests that the D620N mutation of the VPS35 gene is at best a rare cause of SPD.

The differences between the results of the various studies might stem from the different sample size and the different study populations with the possibility of certain ethnic variations.

The genetic causes and risk factors of PD may serve as important tools through which to attain a better understanding of the pathomechanism. Since a genetic background is presumed

in the development of SPD in some cases, it is important to investigate the different genetic factors in the various SPD populations, which may permit the development of new therapeutic targets. Furthermore, the identification of novel genetic risk factors may facilitate a better selection of homogeneous subpopulations for therapeutic studies.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Table 2.

Primers, PCR conditions and restriction enzymes used for the detection of GBA mutations

Mutation	Primer sequence	Annealing temperature (°C)	Restriction enzyme	PCR (bp)	Wild type (bp)	Mutant (bp)
L444P	F: 5'-GGAGGACCCAATTGGGTGCGT-3' R: 5'-ACGCTGTCTTCAGCCCCTTC-3'	59	NciI	637	637	535, 102
R120W	F: 5'-GCAGAGTCCCATACTCTCCT-3' R: 5'-TGGGTGACAGAGAGAGACT-3'	56	NciI	836	454, 300, 82	536, 300
N370S	F: 5'-GCCTTTGTCCTTACCCTC†G-3' R: 5'-GACAAAGTTACGCACCCAA-3'	53	XhoI	105	105	89, 16

† A mismatch was introduced in the primer at one nucleotide to create a restriction site.