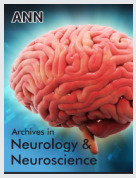

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Research Article

GBA Mutation Analysis in Turkish Parkinson's Disease Patients: Comparison of Clinical and Cognitive Performance

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Abstract

Background: Recent studies have established a substantial relationship between *GBA* gene and Parkinson's Disease (PD). Mutations in the *GBA* play an important role in the pathogenesis of PD.

Methods: 33 young onset and 38 idiopathic PD patients and 76 healthy controls were screened with real time PCR for 11 *GBA* mutations (N370S, L444P, D409H, 84insGG, IVS2+1G>A, V394L, IVS10-1 G>A, IVS10-4 C>T, R120W, V460V and R496H). Leukocyte *GBA* expression levels were also investigated. All patients underwent a comprehensive assessment based on the Unified Parkinson's Disease Rating Scale (UPDRS), H&Y scale, Hamilton's Depression Scale (HDS), Non-Motor Symptom Questionnaire, Mini Mental State Examination (MMSE) and Addenbrooke's Cognitive Examination (ACE-R).

Results: Mutation screening showed that 15.5% of PD patients and 0% of controls had *GBA* mutations (p=0,000). The most common *GBA* mutations were R496H and L444P mutations. Leukocyte *GBA* expression was significantly lower in patients with respect to the control (p=0,04). There was no difference in terms of clinical manifestations between mutation positive and negative PD patients.

Conclusions: This study is the first study to investigate the relationship between Parkinson's disease and *GBA* mutations in Turkey. The results show that R496H mutation is frequent (14%) among Turkish PD patients. This mutation is related with neither early onset nor clinical manifestations. Regardless from mutation presence most of the PD patients (84.5 %) have cognitive impairment when tested with ACE-R.

Keywords: Parkinson's disease; Glucocerebrosidase; *GBA* mutation; ACE-R

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease, characterized by resting tremor, bradykinesia, rigidity and postural instability. These symptoms result predominantly from selective loss of dopaminergic neurons in the substantia nigra pars compacta and subsequent depletion of dopamine in their projections. The pathogenesis of PD remains unclear. An interaction between environmental factors and genetic predisposition is thought to contribute to disease development. Causal mutations in the genes for α-synuclein, parkin, DJ-1, PTEN induced kinase 1 and leucine-rich repeat kinase-2 have been identified [1]. However, mutations of these genes do not account for the occurrence of PD in all patients. Therefore, identifying novel PD genetic risk factors is important to understand its pathogenesis.

Mutations and rearrangements in β-glucocerebrosidase (*GBA*) gene cause Gaucher disease (GD), a autosomal recessively inherited deficiency of the lysosomal enzyme, glucocerebrosidase

[2]. Clinically, GD is characterized by vast phenotypic heterogeneity and is classified into three types based on the severity of associated neurological symptoms. There are multiple independent studies reporting the association between *GBA* mutations and parkinsonism [3-5]. Although the molecular pathogenic mechanism causing PD in *GBA* mutation carriers remains unclear, clinical observations, neuropathologic evidence, and genetic studies have implicated mutations in the *GBA* gene in parkinsonian phenotypes and in Parkinson disease (PD) susceptibility. Together with family studies revealing a significant frequency of parkinsonian symptoms in obligate or confirmed *GBA* mutation carrier relatives of patients with GD [6-8] and Parkinsonian manifestations reported in genotypically heterogeneous patients with GD [6,7,9], suggested an association between GD and PD. Furthermore, brain samples from autopsy-confirmed PD cases revealed significantly higher carrier frequencies than the estimated *GBA* mutation carrier frequency in the general population [10]. These findings strongly suggest that heterozygosity for mutations in the glucocerebrosidase gene may be a risk factor for parkinsonism.

In this case-control study, we aimed to investigate the possible association between the *GBA* gene mutations and PD in Turkey. We examined the frequency of most common 11 mutations in *GBA* gene in PD patients and healthy controls.

Materials and Methods

Here we report the results of mutation analysis of *GBA* in a series of 71 Turkish patients with PD, collected sequentially in Pamukkale University Hospital in Turkey and 76 healthy controls. All patients were Caucasian and of apparent Turkish ancestry. Patients were diagnosed according to the United Kingdom Brain Bank criteria. Inclusion criteria for patients were; (1) having the diagnosis of idiopathic PD, (2) responsive to L-dopa, (3) no history of recurrent stroke, encephalitis or head trauma (4) no history of neuroleptic or toxic exposure. The inclusion criteria for the control group was; (1) no history or finding suggesting parkinsonism, (2) no history of other neurological diseases, (3) no family history of PD.

The study is approved by the local ethics committee and all of the patients and controls gave their informed consents.

Clinical evaluation

All of the patients were evaluated by the study neurologist (G.D.) A full history, including clinical data, family history, and neurologic examination, was recorded for each patient. All patients underwent a comprehensive assessment comprising motor rating based on the Unified Parkinson's Disease Rating Scale (UPDRS), clinical stage based on Hoehn and Yahr scale. Hamilton's Depression Scale (HDS), Non-Motor Symptom Questionnaire (NMS), Mini Mental State Examination (MMSE) and Addenbrooke's Cognitive Examination (ACE-R) were also applied. The cut off values for MMSE were 18/19 and 22/23 for patients having less and more than 5 year education, respectively [11]. The cut off values for ACE-R were 72 for patients who had less than 5 years of education; 82 for 5-12 years of education and 88 for more than 12 years of education [12-14]. The physical examination and tests were applied during "on" periods.

Methods

Genomic DNA was extracted from peripheral blood by using Roche High Pure PCR Template Preparation Kit as specified in the manufacturer's protocol. All subjects were investigated for 11 common *GBA* mutations [N370S (rs76763715), L444P (rs421016), D409H (rs1064651), 84insGG (rs387906315), IVS2+1 G>A (rs104886460), V394L (rs80356769), IVS10-1 G>A (rs189380051), IVS10-4 C>T (rs755265316), R120W (397515515), V460V (1135675) and R496H (rs7582236)] with real-time PCR using LightCycler 480 Real Time PCR system, Roche Diagnostics. Melting curve analysis was used to detect genotypes as wild type, heterozygous mutant or homozygous mutant. Genomic RNA was extracted from peripheral blood by using QIAamp RNA Blood Mini Kit, QIAGEN as specified in the manufacturer's protocol. *GBA* expression analysis was performed on leukocyte RNA from patients who were positive for any of these *GBA* mutations and from healthy controls with real-time PCR using LightCycler 480 Real Time PCR system, Roche Diagnostics.

The raw data are evaluated in 3 steps. First, we identified the rate of 11 *GBA* gene mutations in Parkinson's patients and compared with the healthy controls. In the second step, the mutation rates are compared between patients with young onset PD (≤ 50 years of age) (EOPD) and late onset PD (>50years of age) (LOPD). In the third step, independent from age at disease onset, we compared the clinical parameters such as onset symptom, disease subtype, disease progression (H & Y score), family history, DBS requirement and cognitive performance between mutation positive and negative patients.

Statistical analysis

The data were analyzed by SPSS software program. Continuous variables were expressed as mean ± standard deviation, median (minimum and maximum values), and categorical variables as number and percentage. Student-t test was used for comparing parametric test assumptions and independent group differences; Mann-Whitney U test was used to compare independent group differences when parametric test assumptions were not provided. Differences between categorical variables were analyzed by Chi square analysis. In all analyzes $p < 0.05$ was considered statistically significant.

Results

GBA Mutation Rate in PD patients

The demographic characteristics of the patient and control groups are summarized in Table 1. All subjects were investigated for 11 most common *GBA* mutations. A total of 11/71 (%15,5) PD patients were heterozygous for two common *GBA* mutations as shown in Table 1. As shown in Table 1 compared with the PD patients there were no *GBA* mutations in healthy control subjects (p=0,000). The most remarkable finding in *GBA* mutation positive patients was the high rate of R496H mutation detected in 10 of 11 mutation positive PD patients. Ten out of 11 *GBA* mutation positive PD patients only 1 (%9) had L444P mutation. Leukocyte *GBA* expression was significantly lower in patients with respect to the control individuals (p=0,04) (Table 2).

Table 1: The demographic characteristics and *GBA* mutation rates of the patient and control groups.

Characteristics	Patients (n=71)	Control (n=116)	p value
Age, mean (SD)	61,8 ± 10,7	62,47 ± 8,7	0,587
Age, median (min-max)	64 (36-90)	61 (50 - 87)	
Sex, no (%)			0,968
Male	46 (%64,8)	49 (%64,5)	
Female	25 (%35,2)	27 (%35,5)	
Mutation positive, no (%)	11 (%15,5)	0 (%0)	0,000*
Type of mutation, no (%)			
R496H	10 (%14)	-	
L444P	1 (%1,4)	-	

Table 2: *GBA* ΔCT values of the patients and healthy control subjects.

		Mean ± Standard deviation	Med (min - max)	p value
***GBA* ΔCT values**	Patients (n=8)	0,11 ± 0,03	0,1 (0,08 - 0,15)	0,04*
	Controls (n=7)	0,17 ± 0,07	0,14 (0,12 - 0,3)	

Table 3: Comparison of parameters of *GBA* mutation-detected and non-mutated patients.

	GBA mutation positive (n=11)	*GBA* mutation negative (n=60)	p value
Age, year, mean (SD)	58,91 ± 11,34	62,33 ± 10,71	0,337
Age at onset, mean (SD)	53,09 ± 11,55	52,72 ± 12,19	0,925
Motor UPDRS, mean (SD)	26,36 ± 13,79	33,13 ± 21,61	0,238
Total UPDRS, mean (SD)	43,36 ± 19,24	61,27 ± 29,47	0,057
Non-Motor Survey, mean (SD)	23,2 ± 12,25	36,51 ± 28,5	0,138

GBA Mutation and Clinical Manifestations in Early and Late Onset PD

As shown in Figure 1, there was no difference in *GBA* mutation rate between EOPD and LOPD patients. Our comparisons revealed that clinical manifestations were also similar in both groups. Asymmetric rest tremor was the first symptom in both EOPD and LOPD patients, and it was more frequent than bradykinesia as the initial symptom in LOPD patients. (p=0,011). There was no difference in positive family history and cognitive impairment or depression symptoms between EOPD and LOPD patients.

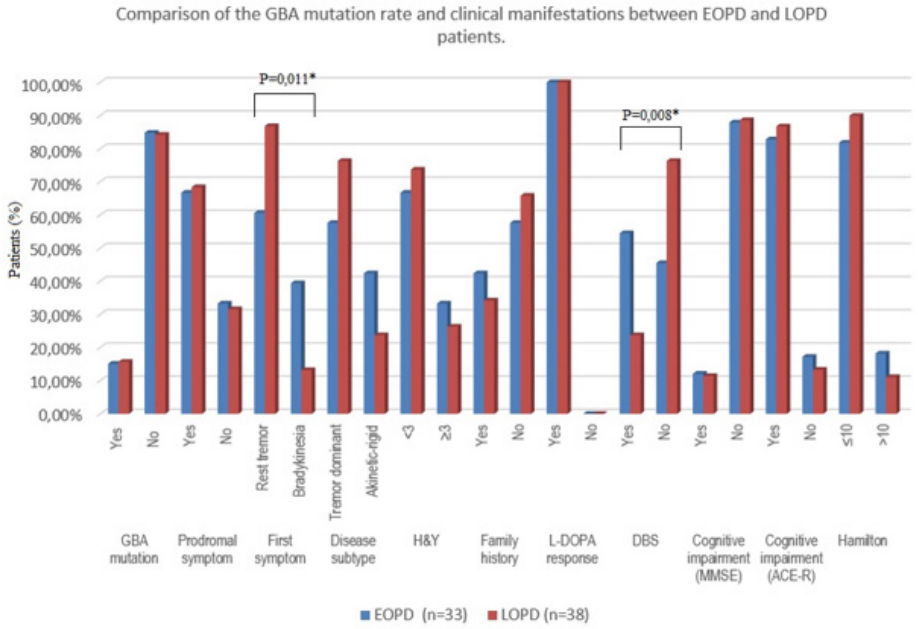

Figure 1: Comparison of the GBA mutation rate and clinical manifestations between EOPD and LOPD patients.

Clinical Manifestations in *GBA* Mutation Positive and Negative PD Patients

In the third step of the analysis, independent from age at disease onset, we compared the prodromal symptoms, symptom at disease onset, disease subtype, disease severity (H & Y score), positive family history, depression, non-motor symptoms, DBS requirement and cognitive performance between mutation positive and negative patients.

Prodromal symptoms such as mask face, constipation and anosmia, were present in 45.5 % of mutation positive and 71,7% of mutation negative patients. In all *GBA* mutation positive PD patients, asymmetric rest tremor was the initial symptom of the disease. However, the first symptom was rest tremor in 70% of the PD patients who were negative for *GBA* mutations (p=0,05). The family history for PD was similar in both mutation positive and negative patients (Figure 2). Despite the high and consistent mutation rate, there was a remarkable similarity in age at disease onset, "on" UPDRS scores, non-motor symptoms scores, depression scores and family history between mutation positive and negative patients (Table 3 and Figure 2).

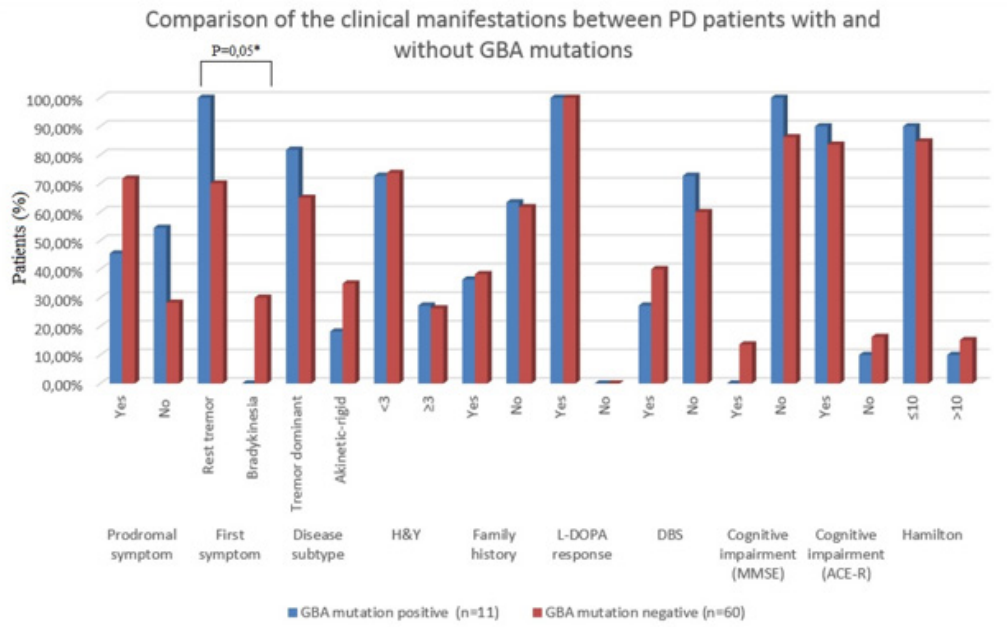

Figure 2: Comparison of the clinical manifestations between PD patients with and without *GBA* mutation.

In cognitive evaluation we used MMSE and ACE-R tests. According to MMSE results only 6.7% of PD patients had cognitive impairment, however with ACE-R test we identified that 84.5% of the PD patients had cognitive impairment. In other words, MMSE missed 63% (45 of 71 patients) of patients who had cognitive impairment (Table 4). Mutation positivity was not a significant factor on cognitive performance.

Table 4: Comparison of MMSE and ACE-R tests.

Cognitive impairment, no (%)		MMSE		Total	p value
		Normal, no (%)			
ACE-R	Cognitive impairment, no (%)	4 (6,9)	45 (77,6)	49 (84,5)	0,00*
	Normal, no (%)	0 (0,0)	9 (15,5)	9 (15,5)	
Total		4 (6,9)	54 (93,1)	58 (100)	

Discussion

In recent years researchers have found a strong association between PD and the *GBA* gene, responsible for Gaucher's disease which is a lysosomal storage disease. Sidransky and colleagues found that parents of Gaucher patients and their second-degree relatives were PD patients. When they performed *GBA* mutation analysis on these family members with PD, they found that they carried heterozygous *GBA* mutation [7]. After this study, following studies have revealed a strong relationship between PD and *GBA* that is not exclusive to a specific ethnic group or a specific *GBA* mutation [5,15,16]. Although there are differences between populations, *GBA* mutations are seen in approximately 5-10% of PD cases [17,18]. The high frequency of mutations among ethnically diverse, heterogeneous series of PD patients makes this gene the most common genetic risk factor for PD. Mutation detection rates in the healthy control group also differ between populations [15]. *GBA* mutation rate in PD is highest in Ashkenazi Jews with 10.7-31.3% [19]. In this population, about 3% of *GBA* mutation carriers are also found in healthy individuals. *GBA* mutation carriers have 5 times higher risk for PD than in the normal population [20]. *GBA*

mutation rates in early-onset patients were two-fold higher than late-onset patients [21].

We found a frequency of 15.5 % (11 of 71) pathogenic *GBA* mutationin Turkish PD series and no mutation in healthy controls (P=0,000). The most common mutation identified was R496H. Ten out of 11 *GBA* mutation positive PD patients (91 %) had R496H mutation and only 1 (%9) had L444P mutation. These results represent a significantly higher frequency of R496H mutation in *GBA* in Turkish PD patients. Additionally leukocyte *GBA* expression was significantly lower in patients with respect to the control individuals (p=0,04). Chiasserini et al. have found that GCase enzyme levels were normal despite low expression levels [22]. They have found low *GBA* expression levels at frontal cortex, caudate nucleus, hippocampus, substantia nigra and cerebellum in *GBA* mutation positive PD patients. However GCase enzyme levels at these brain regions were normal, except substantia nigra and cerebrospinal fluid. Another study showed a negative association between with GCase activity in leucocytes and measurement of oligomeric alpha-synuclein in plasma of GD patients, postulating that the reduced GCase activity might be linked to the peripheral accumulation of toxic alpha-synuclein species [23].

Previously, R496H mutation was reported as a frequent mutation in Ashkenazi population, but a large multi-center analysis with 16 participating centers revealed that L444P and N370S are the most common *GBA* mutation among Ashkenazi Jewish subjects and either mutation was found in 15% of patients and 3% of controls [15]. Hence there is no previous data about *GBA* mutation in PD among Turkish patients, the frequency of most common *GBA* mutations is unknown. Many studies in the literature report results about genotype-phenotype relationship among the L444P and N370S mutation carriers with PD. Some studies state that R496H mutation is one of the frequent "mild" mutations but this mutation is not separately evaluated in terms of clinical parameters.

To understand the genotype-phenotype relationship, we compared prodromal symptoms, age at disease onset, initial symptom at onset, disease subtype, motor impairment, presence of family history, non-motor symptoms and cognitive performance in mutation positive and negative PD patients. Our results revealed that PD patients with R496H mutation did not differ from patients without mutation in terms of these clinical findings and scores, except initial symptom at onset.

All of the patients with R496H mutation had asymmetric hand tremor at onset. Despite the fact that the onset symptom was rest tremor in 100% of the *GBA* mutation positive PD patients, 81.8% was with tremor predominant subtype. In other words, the subtype of the disease might have evolved in the later stages of the disease, regardless of the onset symptom.

Previous studies report that *GBA* mutations predispose to a younger age at motor onset which occurs about 1.7-6.0 years earlier than in PD patients without *GBA* mutation [3,24,25]. Furthermore, studies with early age at onset PD strengthened this finding [26,27]. However our results are different from previous reports which have found the above mentioned results in mostly L444P and N370S mutation carriers. In our series, R496H mutation which is a mild mutation according to definitions of Beutler et al, was the only predominant mutation. Clinical correlation analysis revealed that it is not possible to differentiate R496H mutation carriers clinically from non-carriers. Several studies have demonstrated the differential effects of severe versus mild *GBA* mutations on the risk and age at onset of Parkinson's disease [28,29]. Carriers of severe *GBA* mutations tend to have an earlier age at onset, however phenotypic presentation of mild mutations may not be differentiated from sporadic PD.

Similar to our findings, Sidransky et al reported that severity of motor impairment or Hoehn and Yahr staging revealed no significant differences between *GBA* mutation carries and non-carriers, although one study reported *GBA*- associated PD patients to show more bradykinesia [15]. PD patients carrying a *GBA* mutation have a higher prevalence of cognitive impairment compared to non-mutation carriers [25,28-30]. Genotype-phenotype correlations indicated severe mutations (p-L444P) to predispose to more frequent cognitive decline [27]. There is no sufficient data about R496H mutation and cognitive impairment, but our results revealed that there was a high frequency (84.5 %) of cognitive impairment in PD patients regardless from presence of mutation.

Conclusion

In conclusion, this study is the first study to investigate the relationship between Parkinson's disease and *GBA* mutations in Turkey. The results show that R496H mutation is frequent (14%) among Turkish PD patients. This mutation is related with neither early onset nor clinical manifestations. Regardless from mutation presence, most of the PD patients (84.5 %) have cognitive impairment. MMSE test may not be adequate, therefore more detailed neuropsychiatric test are required to determine the cognitive deficits in PD patients. The study have some limitations. First of all is that, the sample size is not large enough for represent the Turkish population. The other limitation is that, because of cost effectiveness we didn't able to evaluate *GBA* expression levels from all subjects. Limitations of our study should be overcome in future with larger cohorts.

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Conflict of Interest

No Conflict of interest.

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