RESEARCH

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Investigation of LRRK2 G2019S Mutation in the Patients with Sporadic Parkinson's Disease in Turkey

ABSTRACT
Objective: Recently understanding genetic forms and pathogenic mutations has been providing growing knowledges about etiopathogenesis of Parkinson's disease. *Leucine-rich repeat kinase 2 gene (LRRK2)* G2019S mutation is the most commonly reported mutation amongst autosomal dominant and sporadic Parkinson's disease patients. Aims of our study are to identify the frequency of the *LRRK2* G2019S mutation in sporadic late onset Parkinson's disease patients from the Eskisehir, diagnostic utility of this mutation and to confer genetic counselling to the mutation carier patients.
Methods: We investigated 83 patients with sporadic Parkinson's disease and 50 normal (healty) controls unrelated to patients. *LRRK2* exon 41 was investigated with direct sequencing method.
Results: Any point mutation or polymorphism was not detected in the *LRRK2* exon 41 amongst patients and control subjects.
Conclusion: Our findings suggest that the frequency of *LRRK2* G2019S mutation is very lower in Turkish patients with Parkinson's disease.
Key Words: G2019S, *LRRK2*, Parkinson's disease

Türkiye'de sporadik Parkinson Hastalığı Olan Hastalarda *LRRK2* Geni G2019S Mutasyonunun Araştırılması

ÖZET
Amaç: Son yıllarda genetik formlarının ve patojenik mutasyonların tanımlanması ile Parkinson Hastalığının etiyopatogenezine ilişkin bilgiler artmıştır. LRRK2 geni G2019S mutasyonu otozomal dominant ve sporadik Parkinson Hastalığında en sık bildirilen nokta mutasyonudur. Çalışmamızın amacı; Eskişehir bölgesinde sporadik geç başlangıçlı Parkinson Hastalığı tanısı alan olgulardaki LRRK2 genindeki G2019S mutasyonlarının sıklığının belirlenmesi, tanısal amaçlı kullanılabilirliği ve ayrıca mutasyon saptanan olgulara hastalığı ve mutasyonu ile ilgili genetik danışmanın verilmesi amaçlanmıştır.
Yöntem: Çalışmamızda sporadik Parkinson Hastalığı tanısı almış 83 olgu ve 50 normal sağlıklı kontrol bireyi incelenmiştir. LRRK2 geni 41. ekzonu DNA dizileme yöntemi ile çalışılmıştır.
Bulgular: Ne hastalarda ne de kontrol grubunda herhangi bir nokta mutasyon ya da polimorfizm saptanmamıştır.
Sonuç: Çalışmamız, LRRK2 geni G2019S mutasyonunun bölgemizdeki sporadik Parkinson Hastalığı tanısı alan olgulardaki sıklığının çok düşük olduğunu göstermektedir.
Anahtar Kelimeler: G2019S, LRRK2, Parkinson Hastalığı

INTRODUCTION

Parkinson Disease (PD), the second most common neurodegenerative disease after Alzheimer disease, is a severe, progressive, age dependent disorder and characterized clinically by resting tremor, bradykinesia, muscle rigidity, and postural instability. In addition these findings multiple motor, cognitive, sensory, and autonomic symptoms can be seen. Symptoms are caused mainly by loss of dopaminergic neurons in the substantia nigra (1). The gold standard of PD diagnosis remains the presence of brainstem neuronal Lewy body inclusions (2). Although there are many identified risk factors, the etiology of PD is unknown. Environmental factors such as industrial chemicals, well water and heavy metals, living in a rural area, pesticide, herbicide and head trauma are associated agents with an increased risk to develop PD (3,4). Familial aggregation, genetic association studies and identification of families with clear mendelian inheritance of the disorder are shown that genetic factors play an important role in the etiology of PD. At east 13 chromosomal loci linked to familial PD and nine causative genes have been identified, of which seven genes are responsible for dominantly inherited PD (5).

Most recently, mutations in the *leucine-rich repeat kinase 2* (*LRRK2*) gene at the *PARK8* locus were identified in late onset autosomal dominant PD families, but also sporadic cases (6,7). The most common mutation in this gene is G2019S mutation accounts 2-5% of familial and 1-2% sporadic PD in Europe**.** The clinical and pathological presentation are similar in the patients with idiopathic PD, including age at onset, good response to L-dopa and Lewy body pathology (8). But prevalence of the G2019S has marked heterogeneity between ethnicities, very rare in South India, South Africa, Asia, some European countries, such as Germany, Greece and Poland. It accounts for 30-40% both of familial and sporadic PD cases from North Africa and 10-30% of Ashkenazi Jews (9-15). To the best of our knowledge there is no systematically studied knowledge about Turkey. Because of this reason, we aimed to investigate mutations in exon 41 *LRRK2* gene among Turkish late-onset PD patients.

MATERIALS AND METHODS

We screened 83 consecutive, unrelated late-onset (>45 years old) patients with PD. All the patients were examined by a neurologist specialized in movement disorders at the Eskisehir Osmangazi University Hospital, Department of Neurology (Author S.O.). Clinical diagnosis had been made according to London Brain Bank criteria and severity of the disease was rated according to the Unified Parkinson's Disease Rating Scale (UPDRS) and Hoehn and Yahr staging (16). The patients who have family history were excluded. All the cases were idiopathic. Control subjects (n=50) had similar age and the same ethnicity as the cases, and no neurodegenerative disease or family history of Parkinson's tremors. Informed consent was taken from all cases, and this study was approved by the Ethics Committee of the Eskisehir Osmangazi University.

Genomic DNA was isolated from peripheral blood using organic extraction protocols. For amplification of LRRK2 exon 41 following primers was used: 5'-TTTTGATGCTTGACATAGTGGAC-3' (forward) and 5'-CACATCTGAGGTCAGTGGTTATC-3' (reverse). Cycle sequencing in forward and reverse directions was performed on purified PCR products using Big Dye Terminator ver.3.1 and run on an ABI3130 Genetic Analyser and analysed with DNA Sequencing Analysis (ver5.1) software (Applied Biosystems).

RESULTS

The mean age of the patients was (n=83; 43 males, 40 females) 63.4±9.2 years and control was (n=50; 25 males, 25 females) 61.4±8.1 years. All the patients were late-onset (>45 years); the mean age at onset was 57.9±9.4 years (range 49-69). The mean UPDRSIII motor score at last examination was 34.5±11.3 and the Hoehn and Yahr score was 2.3±0.7. Demographic characteristics are detailed in table 1. All the patients were living in Eskisehir region and of apparent Turkish ancestry, although a detailed genealogical history outside of the nuclear family was not taken. In exon 41 of the *LRRK2* gene there were many determined pathogenic mutations such as G2019S and I2020T. We used direct sequencing technique for all DNA samples but no mutation was detected in exon 41 of the *LRRK2* gene studied in any of the patients and control subjects. No polymorphisms were found either.

Table 1. Demographic characteristics of patients

Characteristic (mean ± SD)	**Patients (n=83)**
Age at collection	63.4±9.2
Age at onset	57.9±9.4
Range of age at onset	49–69
UPDRSIII score	34.5±11.3
Hoehn Yahr score	2.3±0.7

DISCUSSION

Familial aggregation studies, genetic association studies and identification of families with clear mendelian inheritance of the disorder show that genetic factors play an important role in the etiology of PD. Today, at least 13 loci and 9 genes related to PD have been identified. In all of the PD, 5-10% of patients reported to carry mutations in one of these genes (17).

In two different studies in the Portuguese cohorts showed that G2019S mutation frequency of 6% among the PD patients (18). Squillaro et al. investigated the frequency of the G2019S mutation

in unrelated 98 Italian PD patients (12 familial 12%, 86 sporadic 88%) and they detected the G2019S mutation in only one sporadic female patient (1.2%). Eventually they suggest that the G2019S mutation is a relevant cause of sporadic PD cases in the Italian population and stress the importance of performing this genetic test, which has important implications for genetic counseling (19). The penetrance of the *LRRK2* G2019S mutation is known to be a very important issue for genetic counseling. International *LRRK2* Consortium determined the age specific penetrance of the *LRRK2* G2019S mutation to be 28% at 59, 51% at 69 and 74% at 79 years with no effect of the sex (8).

The G2019S mutation is observed in very rare in Far Eastern countries like Japan, China and India (8,20,18). Vijayan et al. investigated the G2019S mutation in 100 sporadic and 86 familial PD patients in South India. They found no G2019S mutation among the patients. Therefore they suggest that G2019S-associated PD may be population specific (20). In these regions the G2385R mutation is more frequently seen than G2019S mutation. For instance in Taiwan G2019S mutation was not detected in a study including 305 patients with sporadic PD while G2385R mutation was detected in 27 patients (9%) (21). Our study showed that Turkish PD patients have similar features with Far Eastern populations.

Three different haplotypes are found in G2019S mutation carriers. Commonly observed haplotype 1 is shared by 95% of G2019S mutation carriers of European, North and South African and Ashkenazi Jewish origin. The second rare haplotype, so-called haplotype 2, is found in a total of five families of West European ancestry. The third haplotype, which differs from the European and North African haplotypes, was primarily identified in Japanese carriers. Up to now, G2019S mutation has only been detected in a Turkish patient with familial PD. In this study only familial cases was evaluated (32 autosomal dominant, 20 autosomal recessive) and it is found that the carrier with G2019S mutation has haplotype 3 (22). In terms of G2019S mutation status, these findings suggest that Turkish PD patients have similar genotypic features with Far Eastern countries.

Consequently G2019S mutation is not frequent in Turkish PD patients. We probably suggest that G2019S-related PD may be population specific manner. So, LRRK2-related PD seems to be very rare in the Turkish population. Turkish patients have similar haplotype with Far East countries and different mutations, like G2385R, are seen more frequently in these countries. The G2385R mutation is not in the exon 41 of the LRRK2 gene so we could not investigate it. Eventually we can say that, Turkish PD patients may share similar mutations, like G2385R, with Far East and India population. The limitation of this study included restricted number of patients.

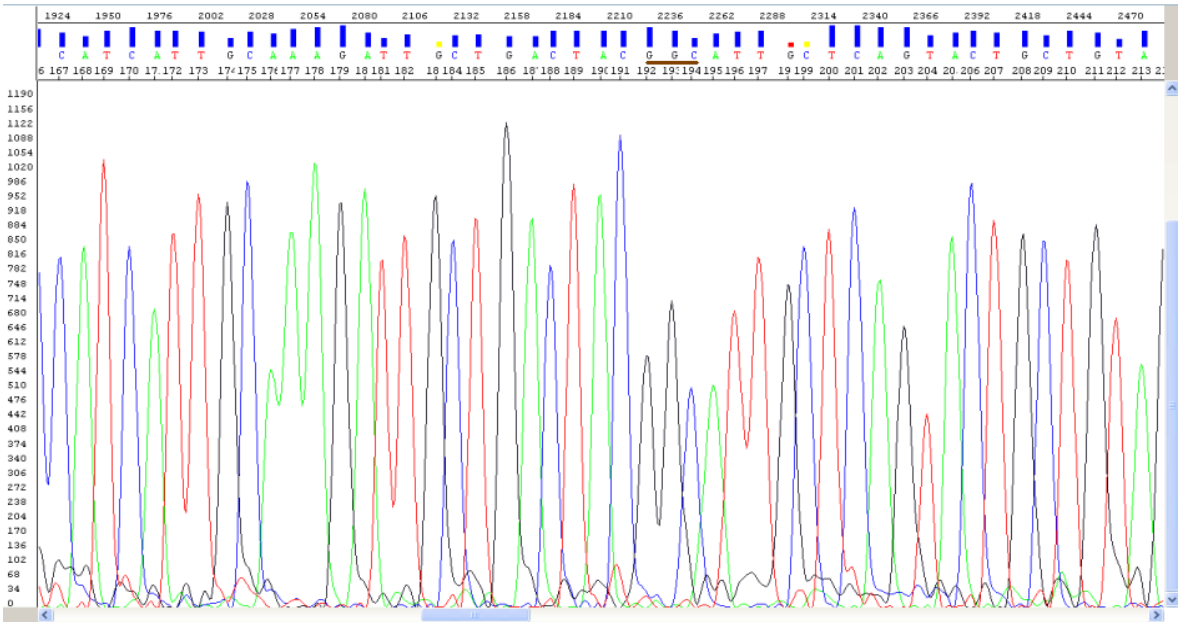

Figure 1. Sequence analysis of the exon 41 of the LRRK2 gene. There was no mutation in the G2019S mutation zone to the patient (Brownish line shows G2019S mutation zone).

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