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LRRK2 G2019S, I2020T, R1441C GENE mutation analysis in patients with idiopathic Parkinson's in Turkey

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Abstract

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease. The number of genetic studies on the etiopathogenesis of the disease has increased in the recent years. Leucine-rich repeat kinase 2 (LRRK2) gene mutation is the most common in autosomal dominant and sporadic PD. In this study, we aim to investigate the LRRK2 mutation frequency in patients diagnosed with idiopathic PD in and around Malatya province in eastern Turkey and to determine certain parameters in positive cases such as phenotype characteristics and treatment efficacy. A total of 170 patients (106 male and 64 female) were included. The most common loci of the LRRK2 gene (G2019S, I2020T and R1441C) were examined. To identify mutations, genotyping studies were performed by targeting related gene regions in isolated DNA using real-time polymerase chain reaction. No mutation was detected in any patient. Therefore, the probability of G2019S, I2020T and R1441C point mutations in the LRRK2 gene was very low in PD patients in Malatya region, which is a region of Turkey closer to the Middle East. Future studies investigating mutations involved in other loci of the LRRK2 gene with larger sample size in a wider geography in Turkey will provide more information about the genotype–phenotype relationship, incidence and carrier characteristics.

Keywords: Gene, LRRK2, Parkinson's Disease

Introduction

Idiopathic Parkinson's disease (PD) is a chronic progressive movement disorder clinically characterized by resting tremor, rigidity, bradykinesia and postural instability, with an incidence of 3% in individuals aged >65 years [1]. Although most cases are sporadic, genetic factors contribute to the pathogenesis of the disease. In genome-wide association studies, 26 gene loci have been identified that increase the risk of PD [2]. Gene mutations in eight of these loci (SNCA, LRRK2, EIF4G1, VPS35, PRKN, DJ1, PINK1 and ATP13A2) cause familial PD. In addition, variations in the α-Synuclein (SNCA), microtubule associated protein Tau (MAPT) and LRRK2 genes are risk factors for PD [3].

LRRK2 mutation was discovered in 2002. Funayama et al. identified a new locus on chromosome 12p11.2 – q13.1 in individuals with autosomal dominant Parkinsonism in a family living in Sagamihara, Japan [4]. Generally, patients with LRRK2 mutation and sporadic Parkinson show similar phenotypic features. However, carriers of this mutation have a benign prognosis in which the disease manifests at a later age, usually after the age of 65 years and then progresses slowly [5]. The most common mutation in this gene is the mutation at the G2019S locus [6]. In this mutation, Guanine (G) in nucleotide 6055 is replaced by Adenine (A). This replacement results in a missense mutation that causes glycine at position 2019 in the polypeptide sequence to be replaced by serine (G2019S). R1441C mutation is the second most common mutation in the LRRK2 gene after G2019S. This mutation (R1441C) causes cytosine (C) in nucleotide 4321 of the LRRK2 gene to be replaced by thymine (T) (4321C>T). This replacement results in a missense mutation that causes arginine at position 1441 in the polypeptide sequence to be replaced by cysteine (R1441C). Another missense mutation identified in the LRRK2 gene is I2020T mutation. In this mutation, T in nucleotide 6059 of the LRRK2 gene is replaced by C (6059T>C). This replacement results in a missense mutation that causes isoleucine at position 2020 in the polypeptide sequence to be replaced by threonine (I2020T).

There are very few studies on this subject in Turkey. In the present study, the presence of LRRK2 mutations in PD patients living in the Eastern Anatolian Region of Turkey close to the Middle East was investigated.

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Materials and Methods

Idiopathic PD in patients aged >20 years, with or without a family history, who were followed in our Movement Disorders outpatient clinic were included. A total of 170 patients were included, and the mutations 6055G>A (G2019S) rs34637584, 6059T>C (I2020T), rs35870237 and 4321C>T (R1441C) previously reported in the LRRK2 gene in different populations were screened.

For further analysis, 2 ml peripheral blood samples were collected from all patients and collected into EDTA tubes. High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Germany), was used for DNA isolation according to the manufacturer's protocol and isolated DNA was stored at −15°C/−25°C for further analysis. To identify the mutations, genotyping studies were performed on isolated DNA by targeting the related gene regions using polymerase chain reaction. Genotyping of the cases for each mutation was determined according to the melting temperature (Tm) after the study. (Figure-1)

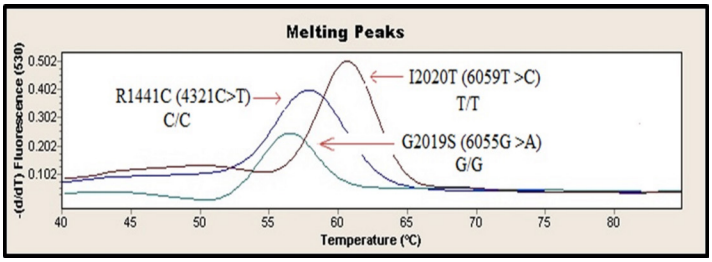

Figure 1. LRRK2 R1441C (4321C>T), I2020T (6059T >C) and G2019S (6055G >A) genotyping by melting curve analysis

Results

A total of 105 male and 65 female patients were included. Most of the participants were born in the Eastern Anatolia region (73% Malatya, 27% surrounding provinces). The mean (SD) age of disease onset was 56.6 (11.15) (min–max age: 25–88) and the mean (SD) age was 62.8 (10.4) years. (Table-1) A total of 16 patients had a family history of PD.

Table 1. Clinical Features

Number of patients (M/F)	170 (105/65)
Age of disease onset	56.65 ± 11.15
Age	62.83 ± 10.47
Initial symptom n (%)	
• **tremor**	103 (60.6%)
• **bradykinesia**	63 (37.1%)
• **other**	4 (2.3%)
Hoehn & Yahr stage	2.29 ± 1.043

No mutations were detected in the I2020T, G2019S and R1441C loci of the LRRK2 gene in any patient. Normal homozygous results were obtained in the examined loci. (Table-2)

Table 2. Genotype and allele frequencies of the LRRK2 gene for I2020T, G2019S and R1441C locus mutations

Number of patients (M/F)	Genotypes	N (%)	Allele	N
6059T>C (I2020T)	T/T	170 (100)	T	340
	T/C	0	C	0
	C/C	0		
6055G>A (G2019S)	G/G	170 (100)	G	340
	G/A	0	A	0
	A/A	0		
4321C>T (R1441C)	C/C	170 (100)	C	340
	C/T	0	T	0
	T/T	0		

Discussion

Although most idiopathic PDs are sporadic, chain analyses have shown pathogenic mutations in various Mendelian genes. Mutations in the LRRK2 gene are the most common cause of autosomal dominant PD and account for an average of 10% of all familial forms with dominant inheritance [7]. This gene contains 51 exons and encodes a large protein called LRRK2 (Dardarin). The LRRK2 protein contains a kinase, a GTPase and several protein–protein interaction sites. Mutations in this gene are thought to cause PD by increasing kinase activity.

In this study, no mutations in LRRK2 gene were detected in these three loci (G20119S, R1441C and I2020T). This result is consistent with similar studies previously conducted in Turkey. In a 2009 study conducted for the first time in Turkey, G2019S mutation was investigated in 52 patients with familial PD. In this study, G2019S mutation in the LRRK2 gene was detected in only one patient [8]. Both parents of this index patient had a diagnosis of late-onset PD. In addition, 91 patients were evaluated in another study published in Turkey in 2017. In this study, LRRK2 was examined using whole DNA (exon 1–51) sequencing method and G2019S mutation was detected in only one patient. In the same study, less common T2494 and R1067Q mutations were also detected in Turkey for the first time [9]. In another study conducted in Eskişehir in 2014, 83 patients and 50 control subjects were examined and G2019S mutation was found in exon 41 of the LRRK2 gene, but no mutation was detected in any patient [10]. Individuals with a positive family history were screened in these three studies in Turkey.

Two different cohort studies in Portugal showed that the frequency of G2019S mutation in individuals with familial PD was approximately 6% [11]. In a large multinational study involving French-based Mediterranean countries, 1230 patients and 391 control subjects were examined, and mutations were detected in 125 (120 heterozygous and 5 homozygous) patients. In the control group, one patient was found to be a carrier. In this study, G2019S mutation was detected in people from North African, European and Middle Eastern origin [12].

R1441C mutation is the second most common mutation in the LRRK2 gene after G2019S. Although this mutation is most

common in Spanish individuals, it is also detected in individuals of different ethnicities [13]. A study in 2009 reported that the prevalence of R1441C mutation in Spain was approximately 20% among those with familial PD. The clinical symptoms appeared at a slightly later age in individuals with this mutation than in G2019S carriers [14].

Another mutation associated with the LRRK2 gene is the I2020T mutation. Unlike the G2019S haplotype, this mutation is more common in Asians, particularly in Japanese individuals [6]. In a multicenter study in the Asian continent with 763 patients, I2020T mutation was detected in two patients. These two patients were of Japanese origin and from the same family [15]. Bialecka et al. examined G2019S and I2020T loci in 174 Polish patients and 190 healthy control groups, but no mutation was detected [16]. Although certain mutations are more common in some geographies, LRRK2 carriers are generally detected worldwide. Most studies have shown that individuals with a family history have a significantly higher risk of being carriers. When we look at carriers of all variants of LRRK2 mutations, this mutation is observed in 5%–13% of those with familial PD and in 1%–5% of individuals with sporadic PD [17]. It may be considered that most asymptomatic individuals and especially individuals with autosomal dominant inheritance carry a haplotype we do not still know. Because LRRK2 gene comprises 51 exons, it is important to decide which exon should be screened for the first mutation analysis.

Based on the data of previous studies that G2019S mutation can be found in individuals of Middle Eastern origin, we thought that this mutation could be detected in Turkey, especially in and around Malatya province, which is closer to the Middle East. However, this mutation was not detected in our study. The most important limitation of our study was the limited number of PD patients with a family history. Identifying the geographical distribution of genetic mutations is extremely important for determining the etiopathogenesis of our patients. Further comprehensive studies in our region will help us perform genetic screening by considering ethnicity and geographic features.

Conflict of interests
The authors declare that they have no conflict of interest

Financial Disclosure
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Ethical approval
Inonu Universıty Faculty of Medicine Clinical Research Ethics N:2017/12

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