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Mutation analysis of the *PARKIN*, *PINK1*, *DJ1*, and *SNCA* genes in Turkish early-onset Parkinson's patients and genotype-phenotype correlations

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ABSTRACT

Objective: Variations in PARK genes (*PRKN*, *PINK1*, *DJ-1*, and *SNCA*) cause early-onset Parkinson's disease (EOPD) in different populations. In the current study, we aimed to evaluate the frequencies of variations in PARK genes and the effects of these variations on the phenotypes of Turkish EOPD patients.
Methods: All coding regions and exon-intron boundaries of the *PRKN*, *PINK1*, *DJ-1*, and *SNCA* genes were screened by heteroduplex analysis followed by direct sequencing of the detected variants in 50 Turkish EOPD patients. These variants were evaluated using SIFT, PolyPhen, HSF, and LOVD web-based programs.
Results: The frequency of EOPD-associated variations in the *PRKN* gene was 34%. Among these variations, p.A82E in exon 3 and p.Q409X in exon 11 was determined to be pathogenic. We also defined previously unknown cryptic variations, including c.872-35 G > A and c.872-28T > G in exon 8 of *PRKN* and c.252 + 30 T > G and c.322 + 4 A > G in exons 4 and 5 of *DJ1*, respectively, that were associated with EOPD. Although no significant association was observed between the PARK gene mutations and clinical features (P > 0.05), the alterations were related to the clinical symptoms in each patient.
Conclusion: An increasing number of studies report that *PRKN*, *PINK1*, *DJ1* and *SNCA* mutations are associated with early-onset Parkinson's disease; however, a limited number of studies have been conducted in Turkey. Additionally, our study is the first to evaluate the frequency of SNCA mutations in a Turkish population. The aim of this study was determine the frequency distributions of the *PRKN*, *PINK1*, *DJ1*, and *SNCA* gene mutations and to analyze the relationships between these genetic variations and the clinical phenotype of EOPD in Turkish patients.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder. Its clinical signs include bradykinesia, resting tremor, rigidity and impairment of postural reflexes in association with a good response to levodopa treatment [1]. Although much of the etiology of PD remains unknown, early-onset PD (EOPD) (onset before 50 years of age), which accounts for approximately 5–10% of all PD cases, can be explained by monogenic causes [2,3]. Recent epidemiologic studies have highlighted the effects of interactions between genetic and environmental factors on the clinical signs of EOPD [4]. To date, several mutations associated with EOPD have been described in genes including *PRKN* (PARK2), *PINK1* (PARK6), *DJ1* (PARK7) and *SNCA* (PARK¼) in different populations [5–7].

Mutations in the *PRKN* gene are the most frequent genetic causes of EOPD, particularly in patients with a positive family history. These mutations account for 18% of cases with onset before the age of 45 and 77% of those with onset before the age of 20 among sporadic PD cases [6,8]. *PINK1* mutations are the second most frequent mutations causing EOPD after *PRKN* mutations, accounting

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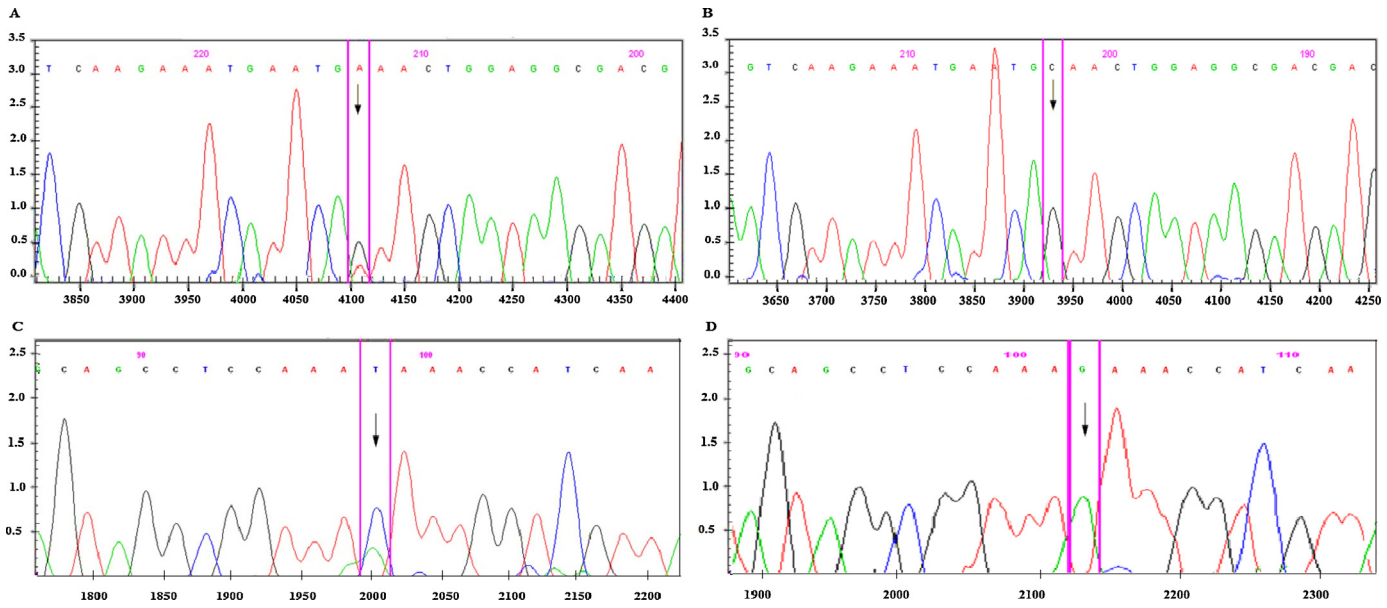

Fig. 1. Sequence images of pathogenic PRKN mutations A. c.245 C > A; p.A82E in exon 3, B. wild type exon 3, C. c. 1225 G > T; p.Q409X in exon 11, D. wild type exon 11.

for 14% of EOPD cases [7]. Further, *DJ1, LRRK2* and *SNCA* mutations are each less frequent than *PINK1* mutations. However, there is clear evidence that the etiologic factors may vary depending on the geographic and ethnic backgrounds of the studied population; in particular, the prevalence rates of disease-causing mutations may vary considerably between populations [9]. For example, a study conducted in Japan reported a 10% prevalence of *PINK1* mutations among parkin-negative EOPD cases in their population [10,11]. In addition, *LRRK2* mutations are uncommon in Turkey [12].

The aim of this study was to determine the frequency distributions of *PRKN*, *PINK1*, *DJ1,* and *SNCA* gene mutations and to analyze the relationships between these genetic variations and clinical phenotypes in EOPD patients from a Turkish population.

2. Materials and methods

2.1. Patient selection

We analyzed 50 Turkish patients (26 males and 24 females) with EOPD (parkinsonism manifesting before 50 years of age) from Parkinson's disease and movement disorders outpatient Neurology clinics from January 2013 to December 2014. EOPD was diagnosed according to the Queen Square Brain Bank criteria. Clinical evaluations were performed using the Unified Parkinson's Disease Rating Scale (UPDRS), with the participants in the "on" stage. All patients originated from Turkey, and two sets of cases were siblings (cases 13–14 and cases 37–38). Family history was considered positive if parkinsonism was reported in a first- or second- degree relative, depending on the pedigree. In addition, genomic DNA isolated from blood samples collected from 47 unaffected age-matched healthy volunteers with no family history of PD was also analyzed for *PRKN*, *PINK1*, *DJ1*, and *SNCA* gene variations. This study was approved by the Medical Ethics Committee of Uludag University (2013-3/19) and conformed to the ethical standards of the Helsinki Declaration. All patients and healthy volunteers provided written informed consent.

2.1.1. DNA extraction and PCR

A blood sample was collected from a peripheral vein of each patient using venipuncture, and genomic DNA was extracted according to the kit protocol (Roche, Cat No. 11796828001, Germany). Polymerase chain reaction (PCR) was performed to amplify all of the coding exons and intronic splice sites of the *PRKN* (OMIM: 602544; GenBank: NM_004562.2), *PINK1* (OMIM: 608309; GenBank: NM_032409.2), *DJ1* (OMIM: 606324; GenBank: NM_001123377.1), and *SNCA* (OMIM: 163890; GenBank: NM_001146055.1) genes. The 15-μL PCR mixture contained deoxyribonucleoside triphosphates (0.15 mM each) (DNTP-Promega U1515, USA), primers (10 pmol each) (Supplementary Table 1), 500 U/5 μL of Taq DNA polymerase (Promega-M8305, USA), and 150 ng of genomic DNA. The quality of the PCR products was analyzed by 2% agarose gel electrophoresis.

2.1.2. Heteroduplex analyses

The PCR products were assessed by heteroduplex analysis (HDA). For heteroduplex formation, 11 μL of each PCR product was heated to 96 °C for 6 min and then cooled to 50 °C for 10 min, 37 °C for 15 min, and 20 °C for 30 min. The reaction was terminated with 2 μL of stop solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol) and kept on ice until the sample was loaded into the gel. A 3.5-μL aliquot of each experimental sample and a 3.5-μL aliquot of the unmutated control sample were electrophoresed in 1X MDE gels (BMA, Rockland, Maine, USA) at 900 V for 9–16 h at room temperature. The resulting bands were visualized using a non-radioactive silver staining technique according to a standard protocol. Samples with bands that differed from the wild-type bands were identified as HDA-positive. HDA analyses were performed in duplicate for all positive samples to avoid contamination risk.

2.1.3. Sequence analyses

Each different haplotype identified in the population was sequenced as described previously [13]. Brifely, 20-μL sequencing reaction mixture consisted of 8 μL of Quick Start Mix (DTCS, Quick Start Mix- M010812, USA), 2 μL each of 10 pmol forward and reverse primers, and 2.5 μL of 10 ng/μL template DNA. The reaction cycle parameters were as follows: 30 cycles at 96 °C for 20 s, 50 °C for 20 s, and 60 °C for 4 min. DNA sequencing was performed using a CEQ8000 DNA Analysis System (Beckman Coulter, Inc., Fullerton, CA, USA). The associations between the identified variations and the risk of PD were confirmed using Ensemble mutation database (http://www.ensembl.org/).