T313M PINK1 Mutation in an Extended Highly Consanguineous Saudi Family With Early-Onset Parkinson Disease

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Background: To date, 5 well-confirmed genes for Parkinson disease (PD) have been identified, including 3 autosomal recessive genes: PTEN-induced putative kinase 1 (PINK1), parkin, and DJ-1. Almost nothing is known about the genetics of PD in Saudi Arabia; however, consanguineous families, not infrequent in this population, could be important in the evaluation of known PD genes and the search for new PD factors in the future.

Objective: To investigate known recessive PD genes in 5 consanguineous Saudi families with PD.

Design: The entire open frame as well as the untranslated region and all 5’ and 3’ intron-exon boundaries of the PINK1, parkin, and DJ-1 genes were sequenced in 5 probands in Saudi families.

Results: Four of 5 probands tested negative for PINK1, parkin, and DJ-1 mutations. However, in a large Saudi family with PD with at least 3 consanguineous marriages between first cousins, we detected a threonine to methionine substitution at codon 313 (T313M) PINK1 mutation that affected the kinase domain. Manifestations of the disease in this family included early onset (age, 28-38 years), tremulous movement, slow progression, diurnal fluctuations, bradykinesia, good response to levodopa therapy, and only mild dyskinesias. A neurologist blinded to genetic status clinically evaluated 15 family members, all older than 20 years, and diagnosed PD only in individuals who were later found to be homozygous for the T313M mutation. None of the 13 heterozygotes demonstrated any sign of PD.

Conclusion: A homozygous T313M mutation is responsible for PD in this large Saudi family. However, the heterozygous T313M mutation does not act as a PD susceptibility factor, which is in contrast to several reports of mutations affecting only 1 PINK1 allele discovered in sporadic PD.

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Parkinson disease (PD) is the most common progressive movement disorder, characterized by the predominant loss of midbrain dopaminergic neurons and presence of intraneuronal Lewy body inclusions. The disease is mostly sporadic; fewer than 10% of PD cases are familial.1 Five well-confirmed PD genes have been identified. Mutations in α-synuclein2 and LRRK23 are responsible for autosomal dominant PD, and mutations in PTEN-induced putative kinase 1 (PINK1),4 parkin,5 and DJ-16 are associated with autosomal recessive early-onset PD.

PINK1 is located within the PARK6 locus on chromosome 1p36 and encodes a protein with a conserved protein kinase domain (G193-L507). PINK1 mutations were first described in consanguineous families of Italian and Spanish origin and are responsible for typical slowly progressive PD with onset before age 50 years.7,8 Subsequently, PINK1 mutations have been reported in Philippine, Taiwanese, Israeli, Japanese, Irish, and North American populations.9 Most PINK1 mutations have been detected in families with a recessive form of PD; however, there are several reports of mutations affecting only 1 PINK1 allele discovered in sporadic PD.10 PINK1 protein is localized to the mitochondria and may have a protective effect against cell death; it reduces the basal neuronal proapoptotic activity and protects neurons from staurosprone-induced apoptosis.11 Loss of this protective function may lead to the neurodegeneration noted in patients with PINK1 mutations.

To our knowledge, there is only 1 report of PD genetics in Saudi families in which PD was linked to the parkin locus.12 We investigated 3 recessive PD genes in 5 consanguineous Saudi families with PD and report the clinical and genetic findings in a family with a T313M PINK1 mutation.
METHODS

SUBJECTS

Informed consent for research purposes was obtained from all individuals involved in the study, which was approved by ethics and basic research committees. The subjects are members of 5 extended consanguineous families native to Saudi Arabia with PD with onset before age 50 years. All participants underwent standard neurologic examinations at King Faisal Specialist Hospital and Research Center in Riyadh, Saudi Arabia. Blood samples and clinical information were obtained by a neurologist (S.B.) blinded to genetic status. Diagnosis of PD was based on published criteria.13

GENETIC ANALYSIS

Genomic DNA was extracted from blood samples using a QIAGEN kit (Venlo, the Netherlands). The entire open frames, as well as the untranslated region and all 5’ and 3’ intron-exon boundaries of PINK1, parkin, and DJ-1, were sequenced in 5 probands of Saudi families as previously described.14 Mutations were detected by direct inspection of the fluorescent chromatographs using Lasergene software (DNASTAR Inc, Madison, Wis). The frequency of the T313M mutation was evaluated in 110 healthy control subjects by using a restriction digest assay. PINK1 exon 4 was amplified13 and then digested with Fok1, and the restriction fragments were resolved on a 2% agarose gel (373 base pair [bp] for the wild type, and 118 bp and 255 bp for the mutant allele).

RESULTS

MUTATION ANALYSIS

Four of 5 probands tested negative for mutations in PINK1, parkin, and DJ-1; however, in proband PD3, we identified a homozygous ACG-to-ATG PINK1 mutation in exon 4 at nucleotide position 1032 (messenger RNA Accession No. NM_032409), resulting in substitution of threonine to methionine at codon 313 (T313M) (Figure 1). This proband is a member of a highly consanguineous family with 21 individuals available for study (Figure 2). The proband is married to a first cousin heterozygous for the T313M mutation and has 6 children (3 heterozygous and 3 homozygous for the mutation who are too young to have developed PD). The proband’s affected sibling (PD2) has a spouse who is not a mutation carrier, and all 4 children are heterozygous for the T313M mutation. This mutation was absent in 110 unrelated healthy control subjects of Saudi origin.

Figure 1. DNA sequence fluorescent chromatogram of a PTEN-induced putative kinase 1 (PINK1) homozygous threonine to methionine substitution at codon 313 (T313M) mutation observed in proband PD3 shown with a heterozygous and a wild-type sequence. Sequence around the mutation site is shown, with the arrow pointing to substitution.

Figure 2. Pedigree structure of a PTEN-induced putative kinase 1 (PINK1)-linked consanguineous Saudi family with early-onset Parkinson disease. Solid symbols indicate affected individuals and double lines indicate consanguineous marriages. Genotypes for the PINK1 threonine to methionine substitution at codon 313 (T313M) mutation are shown for all assessed individuals. The gender of individuals has been masked to protect family confidentiality. Numbers indicate the participant’s age at onset.
CLINICAL FINDINGS

The PINK1-linked Saudi kindred originate from the western part of the Arabian Peninsula (Mecca). Parkinson disease was diagnosed in 4 family members (age at onset, 28-34 years; mean age, 32 ± 7 years). The family includes at least 3 consanguineous marriages between first cousins.

The proband (PD3) is 46 years old. At age 34 years, the proband began to notice intermittent tremulous movement of the right great toe with involuntary dorsiflexion of that toe. A year later, he noticed that the right side of his body was heavy and tremulous. The proband responded well to levodopa therapy and was able to resume normal daily activities. No depression, cognitive impairment, or psychiatric illness were noted. Motor fluctuation in the form of end-of-dose deterioration was noted 4 years after initiation of levodopa therapy. Mild peak-dose dyskinesia, off-period tremor, and dystonia were noted about 5 years later (9 years after disease onset). The proband is employed full time with no physical limitations. The proband is married to a first cousin and has 6 children aged 5 to 14 years. The proband's parents are also first cousins. Both are alive, at ages 67 and 70 years, and are unaffected carriers of the mutation.

Two cousins of the proband (unavailable for genetic study), who are also the offspring of a consanguineous marriage, have a similar disease, with tremor and bradykinesia beginning at about age 28 years. After more than 15 years of levodopa therapy, they are ambulatory. A neurologist (S.B.), blinded to genetic status, clinically assessed 13 family members, all older than 20 years, who are heterozygous for the mutation. None of these individuals showed any sign of PD. The oldest carrier of the mutation is 70 years.

The mutation survey of known recessive PD genes in 5 consanguineous Saudi families uncovered a non-synonymous T313M PINK1 substitution in a family with early-onset PD manifested by asymmetric tremor, dystonia, and rigidity. No cognitive impairment or major axial symptoms such as falling, impairment of postural reflexes, or dysarthria were observed.

A heterozygous T313M mutation in the Saudi family (13 carriers of the mutation) does not act as a susceptibility factor for development of PD, in contrast to reports of heterozygous mutations affecting only 1 PINK1 allele discovered in patients with sporadic PD. Current data suggest that, in patients with a single PINK1 mutation, additional genetic or environmental factors may exist.

The T313M mutation was also recently reported in a Chinese family with recessive early-onset PD and clinical findings similar to those observed in the family members, which further confirms the pathologic role of the T313M mutation in PD and indicates that codon 313 in PINK1 may be a mutational hot spot because it is affected in families of different ethnic origin.

The value of discovering large families such as the Saudi kindred is the possibility of a future longitudinal study of PINK1-linked PD. For example, the investigation of pre-symptomatic homozygous carriers could include testing the predictive value of neuroimaging techniques such as transcranial ultrasound examination of the substantia nigra and functional neuroimaging using positron emission tomography.

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