Heterogeneous Phenotype in a Family With Compound Heterozygous Parkin Gene Mutations

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Background: Mutations in the parkin gene (PRKN) cause autosomal recessive early-onset Parkinson disease (EOPD).

Objective: To investigate the presence of mutations in the PRKN gene in a white family with EOPD and the genotype-phenotype correlations.

Design: Twenty members belonging to 3 generations of the EOPD family with 4 affected subjects underwent genetic analysis. Direct genomic DNA sequencing, semi-quantitative polymerase chain reaction, real-time quantitative polymerase chain reaction, and reverse-transcriptase polymerase chain reaction analyses were performed to identify the PRKN mutation.

Results: Compound heterozygous mutations (T240M and EX 5_6 del) in the PRKN gene were identified in 4 patients with early onset (at ages 30-38 years). Although heterozygous T240M and homozygous EX 5_6 del mutations in the PRKN gene have been previously described, this is, to our knowledge, the first report of these mutations in compound heterozygotes. The phenotype of patients was that of classic autosomal recessive EOPD characterized by beneficial response to levodopa, relatively slow progression, and motor complications. All heterozygous mutation carriers (T240M or EX 5_6 del) and a 56-year-old woman who was a compound heterozygous mutation carrier (T240M and EX 5_6 del) were free of any neurological symptoms.

Conclusions: Compound heterozygous mutations (T240M and EX 5_6 del) in the PRKN gene were found to cause autosomal recessive EOPD in 4 members of a large white family. One additional member with the same mutation, who is more than 10 years older than the mean age at onset of the 4 affected individuals, had no clinical manifestation of the disease. This incomplete penetrance has implications for genetic counseling, and it suggests that complex gene-environment interactions may play a role in the pathogenesis of PRKN EOPD.

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EARLY-ONSET PARKINSON disease (EOPD), beginning before 50 years of age, is clinically and genetically heterogeneous. At least 5 genes have been identified as causal genes for EOPD, including α-synuclein (PARK1), parkin (PRKN, PARK2), DJ-1 (PARK7), PTEN-induced kinase 1 (PINK1, PARK6), and leucine-rich repeat kinase 2 (LRRK2, PARK8). Homozygous and compound heterozygous mutations in the PRKN gene are responsible for 49% of familial EOPD and 18% of sporadic EOPD, and at least 109 different mutations have been identified in the Human Gene Mutation Database, including 38 nonsense/missense mutations and 38 gross deletions (available at: http://archive.uwcm.ac.uk/uwcm /mg/hgmd0.html), whereas mutations in late-onset cases are rare. We describe herein a large family with EOPD caused by compound heterozygous mutations in the PRKN gene: a missense mutation (T240M) and a gross deletion (EX 5_6 del; deletion of exons 5 and 6). This family draws attention to the broad spectrum of phenotypes in the PRKN group of EOPD.

METHODS

PEDIGREE, PATIENTS, AND HEALTHY CONTROL SUBJECTS

A 3-generation, 20-member family in which 4 members had EOPD (the mother is Irish American and the father is Dutch and American Indian) underwent screening for PRKN mutations. They were compared with 208 patients with PD, including 106 with EOPD (male-female ratio, 55:51; mean ± SD onset age, 40.2 ± 7.2 years) and 102 with late-onset PD (male-female ratio, 52:50; mean ± SD onset age, 63.2 ± 8.7 years), and 134 healthy control subjects (male-female ratio, 69:65; mean ± SD age, 57.2 ± 11.2 years). Patients were diagnosed as having PD according to common diagnostic cri-
Genomic DNA was isolated from lymphocytes using standard methods. Polymerase chain reaction (PCR) amplification of the PRKN gene was performed with the use of a thermocycler system (MyCycle; Bio-Rad Laboratories, Hercules, Calif) for 32 cycles at 95°C for 45 seconds, 58°C for 45 seconds, and 72°C for 45 seconds, for 100 ng of genomic DNA, and 10-pmol primers. Gene dosage alternations were confirmed after triple analysis. The sequences of the primers are 5′-ACATCGATGCTTTGACCTG-3′ for PRKN6F; 5′-GGGGAGATGATGTATTTTT-3′ for PRKN6R; 5′-CAATAGTGGACATGACCTTG-3′ for PRKN probe; 5′-ATTCCACCCATGCGAAAATT-3′ for GAPDH-F; 5′-AGGCCACCATCTGATT-3′ for GAPDH-R; and 5′-CAAGCTTCCCCTTACGAC-3′ for GAPDH probe.

To determine the deletion of the PRKN gene changes per messenger RNA (mRNA) splice, lymphocytes from peripheral blood were isolated from the patients and total RNA was extracted with TRIZOL reagent (Invitrogen, Carlsbad, Calif). The complementary DNA (cDNA) synthesis was completed as per the instructions of the cDNA synthesis kit (Iscript; Bio-Rad Laboratories) using 1 µg of total RNA. Polymerase chain reaction amplification from exon 1 to exon 11 was conducted using paired primers 5′-ACATCGATGCTTTGACCTG-3′ for forward primer PRKN1-11F and 5′-ACAGGTGGCTGGTGTTTC-3′ for reverse primer cPRKN1-11R. The thermocycling profile was a 3-step PCR (94°C for 1.5 minutes, 58°C for 1.5 minutes, and 72°C for 2.5 minutes) for 32 cycles. We used 1 µL of the first reverse-transcriptase PCR products as a template for second amplification with paired primers (5′-TGGACATGGGTGATTGTTT-3′ for cPRKN2-11F and 5′-GGTGTTCGTTGAGCGG-3′ for cPRKN2-11R) and denatured the PCR products initially at 95°C followed by 28 thermocycles of 1.5 minutes at 94°C, 1.5 minutes at 60°C, 2 minutes at 72°C, and a final elongation of 5 minutes at 72°C. The PCR products were analyzed by gel purification and sequencing.

To determine the T240M change at the mRNA level, the transcribed PRKN fragment from exon 6 to exon 11 was amplified using paired primers (for cPRKN6F, 5′-CCACCTCTGACAGGAAAC-3′, and cPRKN1-11R) and a second amplification with paired primers (for PRKN6F and cPRKN2-11R) and sequencing.
The patients had a beneficial response to levodopa, relatively slow progression of the disease, and marked motor and nonmotor fluctuations, all of which are typically present in patients with the PRKN mutations. Direct sequencing of the PRKN gene identified a C>T (NT_007422, nt 754455) substitution in exon 6 (Figure 1A), changing a threonine codon (ACG) to a methionine codon (ATG) at amino acid position 240 (T240M), rather than changing the mRNA splice site (predicted by http://hgsc.bcm.tmc.edu). The mRNA sequence was also changed by the reverse-transcriptase PCR and sequencing assay. This mutation was absent in 208 unrelated patients with PD and 134 healthy controls. Five healthy family members were heterozygous for the T240M mutation (age, 22-77 years; mean age, 38.8 years).

Semiquantitative and quantitative PCR showed the deletion of exons 5 and 6 in the PRKN gene in this pedigree (Figure 2). To evaluate the change of the mRNA splice, we conducted nest reverse-transcriptase PCR to obtain the abnormal splice and identified the EX 5_6 del mutation in complementary RNA (Figure 1B). All 4 patients harbored the compound heterozygous mutations (T240M and EX 5_6 del) (Table 2), but a 56-year-old woman (II:1 in Figure 2) with the compound heterozygous mutations had no parkinsonian features. The heterozygous EX 5_6 del mutation was present in 10 healthy family members (age range, 13-75 years; mean age, 34.1 years).

The PRKN gene (PARK2) was mapped to chromosome 6q34 and encodes an E3 ubiquitin-proteasome system. It contains 12 exons spanning about 1.4 centimorgans and encodes a 466–amino acid protein. Parkin protein appears to have 6 phosphorylation sites (3 sites in exon 3, 1 in exon 5, and 2 in exon 6) for casein kinase II, a serine or threonine kinase that is found in the nucleus and cytoplasm of eukaryotic cells and has been implicated to play roles in regulating various cellular functions. Mutations in this gene account for half of familial cases of EOPD.

We identified compound heterozygous mutations (T240M and EX 5_6 del) in the PRKN gene in a large white EOPD pedigree. Patients in this family began to have symptoms in their fourth decade of life, typical of PRKN EOPD. Our cases also share similar clinical features to other PRKN cases, including symptoms at disease onset, beneficial response to levodopa, and the occurrence of levodopa-related motor complications.

The T240M mutation, which presumably eliminates a phosphorylation site for casein kinase II, was found in a patient with late-onset onset PD from North America, and a homozygous T240R mutation in PRKN was previously reported in a Turkish family with EOPD, indicating that this gene site contains an important functional domain of
the parkin protein. Expression of parkin, but not its T240R mutant, significantly alleviated detrimental effects of the misfolded dopamine transporter, indicating that the 240–amino acid position is very important for the maintenance of normal parkin protein function. Among 38 known nonsense/missense mutations in the PRKN gene, 3 nucleotides may be substituted by at least 2 different nucleotides, resulting in 2 mutated amino acids, including T240, which indicates that this position is highly liable to produce mutations. The T240M and T240R mutations were not present in 208 unrelated patients with PD or 134 healthy controls, suggesting that they are indeed mutations rather than polymorphisms, as they seem to be rare in general population. Three alternative splicing variants (NM_004562, NM_013987, and NM_013988) of the PRKN gene were described in the literature, and each of them contains exon 6.

To explore the possibility of deletions or multiplications of 1 or more exons, we conducted gene dose studies and found the deletion of exons 5 and 6 of the PRKN gene in this pedigree. Homozygous deletion of exons 5 and 6 was previously found in 2 non–North American patients with EOPD by genome DNA analysis, but the status of the transcripts was unknown. We found that EX 5_6 del carriers in our pedigree can change the mRNA splice by reverse-transcriptase PCR and sequencing.

The PRKN mutations vary from point mutations to complex rearrangements, including deletions and/or multiplications of complete exons. Previous studies suggested that a single mutation may cause EOPD or represent a risk factor for late-onset PD. In a few patients, only heterozygous mutations have been detected, suggesting that a second mutation has escaped detection by the methods used or that some mutations in heterozygous forms are sufficient to cause this disorder. Our study suggests that the heterozygous T240M or EX 5_6 del mutation is of minor importance in EOPD because 5 heterozygous T240M and 10 heterozygous EX 5_6 del carriers were all exempted from this disorder (the oldest ages of neurologically healthy family members with heterozygous T240M and heterozygous EX 5_6 del were 77 and 75 years, respectively), consistent with loss of function of the PRKN gene. The observation that a 36-year-old compound heterozygous female carrier does not at present manifest any clinical features of PD suggests some inter-action of PRKN with other genes (epistasis), that certain environmental effects somehow protect this woman from developing PD, or both.

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Table 2. Clinical Characteristics of Patients With PRKN Mutations

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Abbreviations: MMSE, Mini-Mental State Examination; NE, not examined; PRKN, parkin gene.

REFERENCES


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