A Family With Parkinson Disease, Essential Tremor, Bell Palsy, and Parkin Mutations

Hao Deng, PhD, MD; Wei-Dong Le, MD, PhD; Christine B. Hunter, RN; Nicte Mejia, MD; Wen-Jie Xie, MD; Joseph Jankovic, MD

Background: Mutations in the parkin gene cause autosomal recessive early-onset Parkinson disease (EOPD). The A265G variant in the HS1 binding protein 3 gene (HS1BP3) is common in essential tremor (ET).

Objective: To investigate the presence of mutations in the parkin gene and the A265G variant in the HS1BP3 gene in a Mexican family with EOPD, ET, and Bell palsy.

Design: Direct sequencing, semiquantitative polymerase chain reaction, and reverse transcription–polymerase chain reaction were performed in the 14 members of this family.

Setting: Mexican family.

Patients: Two patients with EOPD were analyzed.

Results: Compound heterozygous mutations (EX 3_6 del and EX 5 del) in the parkin gene were identified in 2 patients with EOPD, characterized by beneficial response to levodopa, relatively slow progression, and motor complications. Although heterozygous EX 3_6 del and homozygous EX 5 del mutations in the parkin gene have been previously described, to our knowledge, this is the first report of these mutations in compound heterozygotes. Seven heterozygous A265G variants in the HS1BP3 gene were found in this pedigree, but they did not cosegregate with ET, Parkinson disease, or Bell palsy, supporting the conclusion that this variant is not associated with ET.

Conclusions: Compound heterozygous parkin mutations (EX 3_6 del and EX 5 del) caused EOPD in this family, but the A265G variant in the HS1BP3 gene, previously considered to be responsible for ET, was probably not pathogenically related to the ET in this family.

Arch Neurol. 2007;64:421-424

METHODS

PEDIGREE, PATIENTS, AND HEALTHY CONTROLS

Fourteen members of a 3-generation Mexican family, including 2 members affected with EOPD (II:3, II:6), 3 with ET (II:2, III:4, III:10), and 6 with BP (I:2, II:2, III:3, III:5, III:6, III:8) (Figure 1), were screened for parkin mutations and the A265G variant in the HS1BP3 gene. These patients were compared with 60 healthy controls (male/female: 29/31; mean±SD years of age, 51.9±14.1). The diagnoses of PD, ET, and BP were made according to common diagnostic criteria. The Baylor College of Medicine institutional review board approved the study, and all participating individuals provided informed consent.

GENETIC ANALYSIS

Genomic DNA was isolated from peripheral blood leukocytes using the standard method.

Author Affiliations: Department of Neurology, Baylor College of Medicine, Houston, Tex.
HS1BP3 A265G
Parkinson Mutations

II:1
II:2 A/G
W/D2
II:3
II:4 A/G
W/D2
II:5
II:6 A/G
W/D2
II:7
II:8
II:9
II:10
II:11
II:12
II:13
III:1
III:2
III:3 A/G
D1/D2
III:4 A/A
D1/W
III:5 A/A
W/D2
III:6
III:7 A/G
D1/W
III:8
III:9 A/G
W/D2
III:10 A/G
D1/W
III:11 A/A
W/D2
III:12 A/A
W/D2
III:13 A/A
D1/W

Figure 1. Genetic analysis of a family with early-onset Parkinson disease, essential tremor, and Bell palsy. Squares represent men; circles, women. W indicates wild-type allele; A, 256A allele in the HS1BP3 gene; G, 256G allele in the HS1BP3 gene; D1, EX 3_6 del allele in the parkin gene; and D2, EX 5 del allele in the parkin gene.

Table. Characteristics of Parkinson Disease Patients With Compound Heterozygous Parkin Mutations

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patient III:3</th>
<th>Patient III:6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>EX 5 del and EX 3_6 del</td>
<td>EX 5 del and EX 3_6 del</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Female</td>
</tr>
<tr>
<td>Age at last examination, y</td>
<td>47</td>
<td>40</td>
</tr>
<tr>
<td>Age at onset, y</td>
<td>36</td>
<td>27</td>
</tr>
<tr>
<td>Presenting symptom</td>
<td>Left tremor</td>
<td>Right leg tremor</td>
</tr>
<tr>
<td>UPDRS part I mentation score</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Levodopa response</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Drug-induced dyskinesia†</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>UPDRS part II ADL score</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>UPDRS part III motor score</td>
<td>39</td>
<td>22</td>
</tr>
<tr>
<td>Hoehn and Yahr stage</td>
<td>1.5</td>
<td>2.5</td>
</tr>
<tr>
<td>UPDRS part IV complications score</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations: ADL, activities of daily living; UPDRS, Unified Parkinson Disease Rating Scale.

*All characteristics are noted at last examination, with the exception of presenting tremor, which is the location of tremor at initial presentation.

†The drug-induced dyskinesia rating was taken from question 32 of the UPDRS, which describes the proportion of the waking day with dyskinesias: 0 indicates none; 1, 1%-25%; 2, 26%-50%; 3, 51%-75%; and 4, 76%-100%. The UPDRS motor score was measured with the patient in the “on” state.

All 12 exons of the parkin gene and the A265G allele of the HS1BP3 gene were sequenced using primers.6 7 Semi-quantitative polymerase chain reaction (PCR) was used for identification of the parkin genomic DNA and the dose of the parkin exons relative to glyceraldehyde 3-phosphate dehydrogenase normalized to control DNA. For evaluation of our assay, we used samples with LRRK2 R1441G and G2019S mutations as diploid controls.8 The DNA levels were quantified by densitometric analysis using a GS-700 densitometer (Bio-Rad, Richmond, Calif).7,9

To determine the deletion of the parkin gene changes in messenger RNA (mRNA) splice, we extracted RNA and synthesized complementary DNA (cDNA) by reverse transcription (RT)–PCR. The transcribed parkin fragments from exon 2 to exon 7 and from exon 4 to exon 6 were amplified using paired primers (5’T-GCATTTCCAGCTCAAGGAG - 3’ and 5’T-GAAACAGTCTAAGCAATCAG - 3’ and 5’T-AAAGGC-CCTCTGCAAAGAGT - 3’ and 5’T-GTTTCTTGTCAGAGG-G 3’, respectively). The PCR products were analyzed by gel purification and sequencing. In addition, 60 healthy controls underwent analysis and sequencing to evaluate the existence of exon 5–containing splicing at the mRNA level.

RESULTS

The 2 parkinsonian patients from this family had typical EOPD with a young age at onset, a beneficial response to levodopa, marked motor and nonmotor fluctuations, and relatively slow progression (Figure 1 and Table). Both patients harbored the compound heterozygous mutations (EX 3_6 del and EX 5 del). The RTPCR showed the EX 3_6 del and EX 5 del cDNA (Figure 2). The heterozygous EX 3_6 del mutation was also present in the other 4 family members not affected with PD, including 1 with ET, 1 with BP, and 2 unaffected individuals; the heterozygous EX 5 del mutation was present in 6 family members without PD, including 1 with ET and BP, 2 with BP, and 3 unaffected (Figure 1).

To determine the importance of exon 5–containing parkin for maintaining normal function, we analyzed 60 healthy controls and found that all had the related mRNA splicing and that exon 5–containing mRNA (by cDNA analysis) doses were more than 10-fold higher than those with exon 5 deletion.

The heterozygous A265G variant in the HS1BP3 gene was identified in 7 members of this family, but the 265G allele did not cosegregate with ET, EOPD, or BP; only 1 of 3 patients with ET harbored this variant (Figure 1).

COMMENT

In this study of a 3-generation Mexican family with a unique complex neurological phenotype manifested by EOPD, ET, and BP, we identified compound heterozygous mutations (EX 3_6 del and EX 5 del) in the parkin gene in 2 members with EOPD. These patients share simi-
lar clinical features with other patients with the parkin gene mutations, including onset in the third to fourth decade of life, a beneficial response to levodopa, occurrence of levodopa-related motor complications, diurnal fluctuations, and slow progression of disease.

Three alternative splicing variants (NM_004562, NM_013987, and NM_013988) of the parkin gene were described in the literature, and 1 of them (NM_013987) is an EX 5 del splicing variant, which results in a lack of 24 amino acids. To evaluate the role of exon 5–containing mRNA, we analyzed mRNA expression in 60 healthy controls by RT-PCR and found that doses of exon 5–containing cDNA in peripheral lymphocytes were more than 10-fold higher than those with exon 5 deletion, suggesting that the exon 5 position is critically important for the maintenance of normal parkin protein function.

A heterozygous EX 3_6 del mutation, which presumably eliminates parts of the ubiquitin-like domain and the RING–IBR–RING finger motif, was previously found in a European patient with EOPD,1 and a homozygous EX 5 del mutation was reported in a Japanese family with EOPD by genome DNA analysis.10 However, this is, to our knowledge, the first report of these mutations in compound heterozygotes. Because the status of the transcripts was unknown, we analyzed the mRNA transcript in our pedigree and found that both EX 3_6 del and EX 5 del changed the mRNA splice, supporting pathogenic relevance of this mutation. Parkin mutations include missense mutations that involve 1 or a few nucleotides, exonic deletions, duplications, and partial genome triplication.11 Previous studies have suggested that a single mutation might 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 applied methods or that some mutations in heterozygous forms are sufficient to cause this disorder.12 Our study suggests that the heterozygous EX 3_6 del and EX 5 del mutations are of minor importance in EOPD because 4 heterozygous EX 3_6 del and 6 heterozygous EX 5 del carriers were all exempted from this disorder (the oldest ages of neurologically healthy family members with heterozygous EX 3_6 del and heterozygous EX 5 del mutations were 43 and 88 years, respectively), which is consistent with a loss-of-function mechanism of the parkin gene. Our results are supported by the evidence that heterozygous variants occur in patients with late-onset PD and healthy elderly controls with similar frequencies.13 Additionally, the parkin mutations (homozygous or heterozygous) cosegregate with neither ET nor BP.

Seven heterozygous A265G variants in the HS1BP3 gene were found in this family, but they did not cosegregate with ET, PD, or BP. This finding strongly argues against any relevance of this variant in the pathogenesis of ET.3,14 Bell palsy, which is often associated with vascular congestion and secondary ischemia of the facial nerve or with a viral origin, has been found to be familial, with an autosomal dominant pattern of penetrance in 4% to 14% of cases, but no genetic linkage for familial BP has been reported.4 Neither parkin mutations nor the A265G variant cosegregated with BP in our pedigree.

In conclusion, our study of a complex family with EOPD, ET, and BP found unique compound heterozygote mutations (EX 3_6 del and EX 5 del) that caused EOPD. Furthermore, the A265G variant in the HS1BP3 gene does not appear to be pathogenically relevant to ET in this family.

Accepted for Publication: May 31, 2006.

Correspondence: Joseph Jankovic, MD, Parkinson’s Disease Center and Movement Disorders Clinic, Department of Neurology, Baylor College of Medicine, 6550 Fannin, Suite 1801, Houston, TX 77030 (josephj@bcm.tmc.edu).

Author Contributions: Drs Deng and Le contributed equally to this work. Study concept and design: Deng, Le, Hunter, Mejia, and Jankovic. Acquisition of data: Deng, Le, Hunter, Mejia, Xie, and Jankovic. Analysis and interpretation of data: Deng, Le, Hunter, Mejia, Xie, and Jankovic. Drafting of the manuscript: Deng, Hunter, Mejia, Xie, and Jankovic. Critical revision of the manuscript for important intellectual content: Deng, Le, Hunter, Mejia, and Jankovic. Statistical analysis: Deng, Le, and Jankovic. Obtained funding: Le and Jankovic. Administrative, technical, and material support: Deng, Le, Hunter, Mejia, Xie, and Jankovic. Study supervision: Le and Jankovic.

Financial Disclosure: None reported.

Funding/Support: This study was supported by National Institute of Neurological Disorders and Stroke grants NS 043567 and NS 40370, as well as the National Parkinson Foundation, to the Baylor College of Medicine Center of Excellence.
Acknowledgment: We are indebted to the participating individuals who made this work feasible.

REFERENCES