New parkin mutations and atypical phenotypes in families with autosomal recessive parkinsonism

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Abstract—The frequency of parkin mutations was evaluated in 30 families of highly diverse geographic origin with early-onset autosomal recessive parkinsonism. Twelve different mutations, six of which were new, were found in 10 families from Europe and Brazil. Patients with parkin mutations had significantly longer disease duration than patients without the mutation but with similar severity of disease, suggesting a slower disease course. Two patients with parkin mutations had atypical clinical presentation at onset, with predominant tremor when standing.

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Parkin, a gene initially identified in Japanese families with autosomal recessive juvenile parkinsonism, accounts for 50% of familial and 15% of European isolated cases with onset before the age of 45 years.1,2 This disease, often indistinguishable from idiopathic PD, is characterized by selective neuronal death in the substantia nigra pars compacta. Except for one case with a specific mutation,3 patients with the parkin gene do not have Lewy bodies, the pathologic hallmark of idiopathic PD.4 Parkin is an E3 ubiquitin-protein ligase that targets specific substrates for degradation through the ubiquitin-proteasome pathway.5 One of its substrates is a glycosylated form of α-synuclein, establishing a link between parkin and idiopathic PD,6 although the role of this minor form of α-synuclein has not been clarified. The clinical presentation of patients with the parkin gene is highly variable, with ages at onset ranging from 7 to 72 years.7–7 Parkin mutations include many different point mutations and exon rearrangements affecting all 12 of the coding exons.1,2,5 In this study, we screened 30 new families with early-onset autosomal recessive (EO-AR) parkinsonism for mutations in the parkin gene using semiquantitative PCR combined with sequencing of the entire coding region and the corresponding exon-intron boundaries.

Methods. The families were selected according to the following criteria: parkinsonian symptoms that were reduced by at least 30% with levodopa treatment; a mode of inheritance compatible with AR transmission; and onset before the age of 45 years in at least one affected family member. The families came from France (n = 11), Brazil (n = 4), Portugal (n = 4), Italy (n = 4), the Netherlands (n = 2), North Africa (n = 2), Spain (n = 2), and Turkey (n = 1); four families were known to be consanguineous. In addition, an index patient (FPD 029 004) with a single exon rearrangement detected in a previous screen of families with EO-AR parkinsonism2 was included in the sequence analysis. Clinical information and peripheral blood were collected with a standard protocol for each patient and DNA was extracted from leukocytes according to standard procedures.

All index cases were screened for exon rearrangements in the parkin gene with a semiquantitative multiplex PCR7 in which exon 1 can now be analyzed by coamplification with exon 3.8 In the patients in whom the dosage technique detected only one or no mutations, the entire coding sequence, including the intron-exon boundaries, was analyzed by sequencing as previously reported.9

See the Appendix for a list of The French Parkinson’s Disease Genetics Study Group and the European Consortium on Genetic Susceptibility in Parkinson’s Disease group members.

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**Table 1 Analysis of four variants in the parkin gene**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Location</th>
<th>Primers*</th>
<th>Restriction enzyme</th>
<th>Wild type, bp</th>
<th>Mutated, bp</th>
<th>Control chromosomes examined, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.102A&gt;T</td>
<td>Ex 1</td>
<td>Ex1ForMut/Ex1Rev</td>
<td>Bal 1</td>
<td>53 and 41</td>
<td>94</td>
<td>102</td>
</tr>
<tr>
<td>IVS7-1G→C†</td>
<td>Intr 7</td>
<td>Ex7For/Ex7Rev</td>
<td>Pvu II</td>
<td>124 and 82</td>
<td>206</td>
<td>188</td>
</tr>
<tr>
<td>Pro437Leu</td>
<td>Ex 12</td>
<td>Ex12For/Ex12Rev</td>
<td>Pst I</td>
<td>255</td>
<td>158 and 97</td>
<td>144</td>
</tr>
<tr>
<td>Trp445Stop‡</td>
<td>Ex 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>154</td>
</tr>
</tbody>
</table>

* Primer sequences in reference 2, except for Ex1ForMut (5′-CCGCCACCTACCCAGTGCCC-3′).
† Reported in reference 9. Verified by restriction digestion.
‡ Verified by sequence analysis.

**Table 2 Parkin mutations detected in families with EO-AR parkinsonism**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at onset, y</th>
<th>Origin</th>
<th>Dosage exons 1–12</th>
<th>Sequence exons 1–12</th>
<th>Excluding</th>
<th>Excluding</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPD 214 003</td>
<td>25</td>
<td>France</td>
<td>ex6 het del</td>
<td>c.1385insA het</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPD 227 007*</td>
<td>43</td>
<td></td>
<td>ex4-7 het del</td>
<td>IVS7-1G→C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPD 235 013</td>
<td>16</td>
<td>Portugal</td>
<td>ex3-6 het del</td>
<td>c.255delA het</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPD 256 001</td>
<td>37</td>
<td>The Netherlands</td>
<td>Normal</td>
<td>Arg275Trp hom/Pro437Leu het</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPD 264 005</td>
<td>45</td>
<td>Brazil</td>
<td>ex3-4 het del</td>
<td>c.255delA het</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPD 267 016</td>
<td>11</td>
<td>Brazil</td>
<td>ex4 hom del</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPD 271 004</td>
<td>16</td>
<td>Brazil</td>
<td>ex6 het del</td>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPD 276 001†</td>
<td>31</td>
<td>France</td>
<td>ex3-5 hom del</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IT 048 099‡</td>
<td>47</td>
<td>Italy</td>
<td>ex3-4 hom del</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPD 306 005</td>
<td>34</td>
<td>France</td>
<td>Normal</td>
<td>Trp445Stop hom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FPD 029 004¶</td>
<td>29</td>
<td>France</td>
<td>ex4 het del</td>
<td>c.102A&gt;T</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Sequenced in reference 9.
† Consanguineous family.
‡ Age at onset of his affected brother <45.
§ Patient with a previously described heterozygous exon rearrangement.
het = heterozygous; hom = homozygous; del = deletion; ND = not determined.

Results. Mutations in the parkin gene were detected in 10 of the 30 families with EO-AR parkinsonism and in one previously examined patient with a heterozygous mutation (table 2). Four of the point mutations, including one already reported, and two of the exon rearrangements were observed for the first time. None of the new point mutations were found on more than 14 different European control chromosomes (see table 1). A heterozygous Pro437Leu variant was also found in a nonconsanguineous family (FPD 256 001) carrying a homozygous Arg275Trp mutation known to be causative. The biological significance of the Pro437Leu variant remains therefore to be determined, although it was already reported in a patient with the parkin gene (table 1). The c.102A>T mutation is predicted to produce no full-length parkin. The use of the next in-frame ATG codon at position 80 would result in a parkin protein lacking the ubiquitin-like domain. The putative IVS7–1G→C splicing mutation was further analyzed by RT-PCR in patient FPD 227 007 (figure). A 483-bp major PCR product was detected that lacked exon 8, resulting in a frameshift producing a protein truncated at codon 325. An alternative 400-bp transcript that lacked exons 5 and 8 was also detected. Because the exons 4 through 7 were deleted on the other allele, the corresponding transcript could not be amplified with primers used.

Age at onset tended to be earlier in patients with the parkin gene (29 ± 12 years) than in those without (38 ± 14 years) although the difference was not significant. The overall clinical features were similar in both groups but some variations were observed: patients with the parkin gene had more micrography (9/24 vs 0/11) and tremor (10/11 vs 13/22) at onset (both p < 0.05). Although patients with the parkin gene were examined significantly later in the course of their disease (17 ± 11 vs 9 ± 8 years after onset), the mean daily dose of levodopa was similar (495 ± 390 vs 520 ± 330 mg). Two patients had atypical presentations. Patient FPD 235 007, examined at age 53 years, had resting tremor in both legs at age 27 years that increased markedly when standing. Tremor was not present while the patient was walking and did not respond to levodopa. Twelve years later he developed dopa-responsive parkinsonism. In the second patient, IT 048 099, disease began at age 47 years with tremor in the right leg, then in the left leg 1 year later. Tremor was sometimes present at rest but was mostly observed when standing. The diagnosis of orthostatic tremor was considered, but the frequency of the tremor (5–6 c/s) was lower than usual for this condition (12–16 c/s). At age 52 years, the patient developed dopa-responsive parkinsonism.
Several new exon rearrangements and point mutations were detected not only in European patients, but also, and for the first time, in three Brazilian index cases. Although the sample was small, there was a tendency for earlier onset and slower disease progression in patients with the \textit{parkin} gene compared with those without. In addition, the mean daily doses of levodopa were similar in both groups of patients despite the fact that the \textit{parkin}-positive patients had been treated almost twice as long. This might be explained by slower degeneration of the dopaminergic nigrostriatal pathway, a better response to levodopa due to increased presynaptic availability of levodopa, or to hypersensitivity of postsynaptic dopaminergic receptors in patients with the \textit{parkin} gene compared with others. Although all patients had parkinsonism, two had atypical tremor in the lower limbs at onset that was particularly prominent when standing. However, they did not complain of unsteadiness, and tremor frequency, recorded in one patient, was lower than in orthostatic tremor.

The spectrum of causative mutations in the \textit{parkin} gene is very wide but no single signs distinguish \textit{parkin} from other types of EO-AR parkinsonism, and molecular analysis is necessary to identify atypical \textit{parkin} cases.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Functional study of the IVS7–1G→C splicing mutation. Parkin mRNA extracted from lymphoblasts of a control subject (C) and patient FPD 227 007 with the IVS7–1G→C mutation and an exon 4 through 7 deletion was amplified with primers in exons 4 and 9. Reverse transcriptase PCR products: 545 bp, normal; 483 bp, exon 8 deleted; 400 bp, exon 5 and exon 8 deleted. Deletions were confirmed by sequencing.}
\end{figure}

\section*{Discussion}
\textit{Parkin} mutations were found in 10 of 30 families with AR parkinsonism with onset before age 45 years, confirming their importance in this form of parkinsonism. \textit{Parkin} mutations were detected not only in European patients, but also, and for the first time, in three Brazilian index cases. Several new exon rearrangements and point mutations were detected, increasing the list of known mutations in the \textit{parkin} gene. Two mutations are of particular interest. The first, a c.102A>T mutation, should result in the complete absence of parkin. It was found in combination with a heterozygous deletion of exon 4 that should produce a highly truncated protein. Surprisingly, these mutations were not associated with a particularly severe phenotype or juvenile onset in index patient FPD 029 004. The second is an IVS7–1G→C splicing mutation in patient FPD 227 007\textsuperscript{a} that causes deletion of exon 8. This was confirmed by RT-PCR on lymphoblast mRNA, which also evidenced a splice variant in which exon 5 was lost as well. Transcripts lacking exon 5 were previously reported in brain.\textsuperscript{19}

Although the sample was small, there was a tendency for earlier onset and slower disease progression in patients with the \textit{parkin} gene compared with those without. In addition, the mean daily doses of levodopa were similar in both groups of patients despite the fact that the \textit{parkin}-positive patients had been treated almost twice as long. This might be explained by slower degeneration of the dopaminergic nigrostriatal pathway, a better response to levodopa due to increased presynaptic availability of levodopa, or to hypersensitivity of postsynaptic dopaminergic receptors in patients with the \textit{parkin} gene compared with others. Although all patients had parkinsonism, two had atypical tremor in the lower limbs at onset that was particularly prominent when standing. However, they did not complain of unsteadiness, and tremor frequency, recorded in one patient, was lower than in orthostatic tremor.

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\section*{Appendix}

Members of the European Consortium on Genetic Susceptibility in Parkinson’s Disease are as follows: N.W. Wood and J.R. Vaughan (United Kingdom); A. Brice, A. Durr, M. Martinez, and Y. Agid (France); T. Gasser and B. Müller-Myhsok (Germany); M. Breteler, S. Harhangi, and B. Oostra (The Netherlands); V. Bonifati, N. Vanacore, G. De Michele, E. Fabrizio, A. Filli, and G. Meco (Italy).

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\section*{References}
Benign adult familial myoclonic epilepsy

Genetic heterogeneity and allelism with ADCME

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Abstract—Benign adult familial myoclonic epilepsy (BAFME) has been mapped to chromosome 8q24; however, genetic heterogeneity has been recently suggested. The authors report a clinical and electrophysiologic study of two Italian BAFME families showing linkage to chromosome 2p11.1–q12.2. Their report supports the evidence of non-Japanese families with BAFME and suggests a possible allelism with the recently described autosomal dominant cortical myoclonus and epilepsy syndrome.

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Benign adult familial myoclonic epilepsy (BAFME) is an autosomal dominant syndrome characterized by adult-onset cortical tremor and generalized seizures, and is mapped on chromosome 8q24.1,2 Recently, the same phenotype has been reported in families outside Japan without evidence of linkage on chromosome 8.3 A similar condition, autosomal dominant cortical myoclonus and epilepsy (ADCMC), showed linkage to chromosome 2p11.1–q12.2.5 We report two Italian BAFME families showing linkage to chromosome 2 and suggest allelism with ADCME.

Patients and methods. We investigated two unrelated families (Family A and B) originating from the Naples province in Italy.

Electrophysiologic study. Nine patients from Family A and five from Family B underwent EEG or videopolysomnographic study, somatosensory evoked potentials (SEPs), and C-reflex. Off-line jerk-locked averaging analysis (JLA) was performed in six patients from Family A and in three from Family B. From Patient A-II-2, only one EEG recording was available. SEPs were judged as “giant” when the components N20-P25 and P25-N33 were >8.6 μV and 8.4 μV, respectively.

Genotyping and linkage analysis. Fourteen microsatellite markers were used to investigate BAFME and ADCME loci on chromosomes 8q24 (D8S556, D8S1850, D8S555, D8S281, D8S1694, D8S7514, D8S1804) and 2p11.1–q12.2 (D2S2161, D2S388, D2S2216, D2S2175, D2S113, D2S2264, D2S1897) as described.3,5 According to the juvenile–adult onset of the disease, asymptomatic subjects aged <40 years were not included in the linkage study. Multipoint linkage analysis was performed using Allegro 1.0 software assuming an AD allele with high penetrance and frequency of 0.001 and equifrequent marker alleles.

Results. Pedigrees of the families (figure 1) did not have consanguineous marriages. Eleven members (10 living, 10 investigated) of Family A and 10 (7 living, 5 investigated) of Family B had hand tremors, and seizures occurred in 5 patients from each family. All patients referred to our centers (n = 15) had normal psychomotor development, no signs of cognitive impairment, and negative neuropsychologic examination. Clinical and neurophysiologic data are summarized in table 1.

Cortical tremor. Cortical tremor was the onset symptom in all affected patients, appearing at age 15 to 40 years (mean, 25.1 years) in Family A and at age 11 to 25 years (mean, 18 years) in Family B. A decrease in the age at onset was observed through generations, probably the result of early recognition of symptoms in younger family members. Tremor was neither significantly progressive nor reduced by β-blockers but was responsive to valproate (VPA) and clonazepam (CZP). All patients also had distal arrhythmic myoclonic jerks at upper limbs, which were enhanced by posture.

Seizures. Of the patients referred to our centers, four (40%) from Family A and three (60%) from Family B experienced seizures. These were rare (one to five episodes) but present in >85% of members aged ≥25 years (seven of eight), with onset ranging from 30 to 50 years (mean, 42.5 years). In Family A, seizures were sleep related, whereas they occurred randomly in Family B. Provoking factors, such as sleep deprivation, emotional stress, and photic stimulation, were often reported.

Neuroradiologic examination. Brain MRIs were normal for the five members from Family A and for the four from Family B who were investigated.

Antiepileptic drug history. Nine patients were prescribed antiepileptic treatment. Three were treated with VPA and two with phenobarbital (PB); four were taking a bitherapy (VPA + CNZ, VPA + PB, or carbamazepine + PB). No further seizures occurred during therapy.

Electrophysiologic findings. EEG background activity was normal for 6 patients and slightly slowed, in the slower alpha band, for 10 patients. Interictal generalized paroxysmal activity (spike and wave complexes, in short sequences) were seen in these 10 patients. A photoparoxysmal response, diffuse or mostly occipital (type 3 or 1–2 of Waltz6), was seen in eight of the aferen-
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