Parkin mutations are frequent in patients with isolated early-onset parkinsonism

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Summary

Parkin gene mutations are reported to be a major cause of early-onset parkinsonism (age at onset ≤45 years) in families with autosomal recessive inheritance and in isolated juvenile-onset parkinsonism (age at onset <20 years). However, the precise frequency of parkin mutations in isolated cases is not known. In order to evaluate the frequency of parkin mutations in patients with isolated early-onset parkinsonism according to their age at onset, we studied 146 patients of various geographical origin with an age at onset ≤45 years. All were screened for mutations in the parkin gene using semi-quantitative polymerase chain reaction combined with sequencing of the entire coding region. We identified parkin mutations in 20 patients including three new exon rearrangements and two new missense mutations. These results, taken in conjunction with those of our previous study (Lücking et al., 2000) show that parkin mutations account for at least 15% (38 out of 246) of our early-onset cases without family history, but that the proportion decreases significantly with increasing age at onset. There were no clinical group differences between parkin cases and other patients with early-onset parkinsonism. However, a single case presenting with cerebellar ataxia several years before typical parkinsonism extends the spectrum of parkin related-disease.

Keywords: parkin; mutation frequency; isolated early-onset parkinsonism

Abbreviations: PCR = polymerase chain reaction

Introduction

Parkinson’s disease is a common neurodegenerative disorder, with a prevalence of nearly 2% after age 65 years (Elbaz et al., 1999). Clinically, it is characterized by resting tremor, rigidity and bradykinesia—a triad of neurological symptoms caused by the progressive and selective degeneration of dopaminergic neurons in the substantia nigra pars compacta.
(Lang and Lozano, 1998). Lewy bodies—neuronal cytoplasmic inclusions containing aggregated ubiquitinated proteins—are a pathological hallmark of the disease.

The aetiology of idiopathic Parkinson’s disease remains obscure, but new insights into the molecular mechanisms of its pathogenesis have recently been provided by the discovery of several families with clearly established monogenic inheritance (Lansbury and Brice, 2002). Autosomal dominant forms of the disease are extremely rare. Missense mutations in the α-synuclein gene (A30P, A53T) have been described in a subset of Parkinson’s disease families with autosomal dominant transmission (Polymeropoulos et al., 1997; Krüger et al., 1998). Despite the relative rarity of this gene in familial Parkinson’s disease, the observation that normal α-synuclein is a major component of Lewy bodies indicates a more general role of this protein in the pathogenesis of sporadic Parkinson’s disease (Spillantini et al., 1998). A point mutation (I93M) in the ubiquitin carboxyterminal hydrolase L1 (UCHL-1) has been found in a family with autosomal dominant Parkinson’s disease (Leroy et al., 1998), but its role in the pathogenesis of the disease remains uncertain as it has not been found in other familial or sporadic cases with Parkinson’s disease. Autosomal dominant parkinsonism has also been linked to three other loci: PARK3 on chromosome 2p13 (Gasser et al., 1998); PARK 4 on chromosome 4p14–16.3 (Farrer et al., 1999); and PARK8 on chromosome 12p11.2-q13.1 (Funayama et al., 2002). In families with autosomal recessive inheritance, one locus, PARK6, and one gene PARK7 or DJ-1, have recently been identified both on chromosome 1p35–36 (Valente et al., 2001; van Duijn et al., 2001; Bonifati et al., 2002)—but to date, the majority of such cases are caused by mutations in the parkin gene (PARK2), which result in autosomal recessive early-onset parkinsonism (Kitada et al., 1998; Hattori et al., 1998a; Lücking et al., 1998, 2000; Abbas et al., 1999). The phenotype associated with parkin gene mutations is variable, but is usually characterized by early-onset parkinsonism (mean age at onset around 30 years) and slow disease progression (Ishikawa and Tsuji, 1996; Lücking et al., 2000). Several post mortem brain studies have shown that parkin patients do not have Lewy bodies (Mori et al., 1998; van de Warrenburg et al., 2001), except for one case with a particular parkin mutation (Farrer et al., 2001). Lewy bodies are ubiquitinated cytoplasmic inclusions consisting of aggregates containing a number of proteins, including α-synuclein. Parkin is an E3 ubiquitin–protein ligase (Shimura et al., 2000) that ubiquitinates specific substrates that are targeted for their degradation through the ubiquitin–proteasome pathway (Joazeiro and Weissman, 2000). Interestingly, one of its recently identified substrates is a glycosylated form of α-synuclein, establishing a direct link between parkin and idiopathic Parkinson’s disease (Shimura et al., 2001), although the role of this minor form of α-synuclein has not been clarified.

The clinical presentation of parkin patients is highly variable with an age at onset that ranges from 7 to 72 years (Klein et al., 2000; Lücking et al., 2000; Nichols et al., 2002). The mutations in the parkin gene are also extremely varied and include many different point mutations and exon rearrangements affecting all 12 of the coding exons (Kitada et al., 1998; Abbas et al., 1999; Lücking et al., 2000; Maruyama et al., 2000; Klein et al., 2000; Periquet et al., 2001; Hedrich et al., 2002; West et al., 2002). The frequency of these mutations in Europe was estimated at 50% in families with early onset parkinsonism with potentially recessive inheritance and 18% in patients with isolated early-onset parkinsonism with onset prior to 45 years (Lücking et al., 2000).

In order to evaluate more accurately the frequency of parkin mutations in patients with isolated early onset parkinsonism, we studied 146 patients selected with an age at onset ≤45 years. All subjects were screened for mutations in the parkin gene with the use of semi-quantitative polymerase chain reaction (PCR) combined with the sequencing of the entire coding region.

### Subjects and methods

#### Patients and families

One hundred and forty-six isolated patients with early-onset parkinsonism were selected according to the following criteria: (i) parkinsonism, defined by at least two of the following signs: akinesia, rigidity or rest tremor, and a good response to levodopa treatment (>30% of improvement); (ii) no first degree family history of Parkinson’s disease; and (iii) age at onset ≤45 years. There were eight cases with known consanguinity. The majority of patients were from France (n = 92). The remainder were from Italy (n = 13), Brazil (n = 15), Algeria (n = 13), United Kingdom (n = 6), Spain (n = 3), Turkey (n = 2), Portugal (n = 1) and Israel (n = 1).

#### Molecular analysis

Blood samples were taken with written informed consent from all the 146 patients and genomic DNA was extracted from peripheral blood leukocytes using standard procedures. The patients were screened for parkin mutations with the use of the semi-quantitative PCR assay established in our laboratory (Lücking et al., 2000). Briefly, exons 2 to 12 are amplified by PCR simultaneously, associated in groups defined by the amplification conditions: group 1 includes exons 4, 7, 8 and 11; group 2 includes exons 5, 6, 8 and 10; and group 3 includes exons 2, 3, 9 and 12, and an external control, the transthyretin gene. PCR conditions were set up so that amplification was exponential for all of the exons that are co-amplified. The DNA from a patient known to have a heterozygous deletion of exons 8 and 9 was always processed in parallel as an internal control. To identify the mutations, the PCR products were analysed on denaturing polyacrylamide gels on a ABI 377 automated sequencer with GENESCAN 3.1 and GENOTYPER 1.1.1 softwares (all from Applied Biosystems, Foster City, CA, USA). The ratios
between the heights of the peaks corresponding to each of the exons amplified in a given reaction were calculated and then compared with the ratios measured with the non-rearranged exons in the control sample from a normal subject. For a detailed description of this method, see LuÈcking and Brice (2002).

In the patients in whom the dosage technique detected only one or no mutations, the entire coding sequence was analysed by sequencing as described previously (Abbas et al., 1999).

Brie¿ly, the 12 exons and the intron±exon boundaries of the parkin gene were ampli¿ed and sequenced on both strands using a Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems), according to the manufacturer’s recommendations, on an ABI 377 automated sequencer with Sequence Analysis 3.0 software (Applied Biosystems).

In addition, 162 European and 68 North African control chromosomes were analysed by restriction assay for the new point mutation Ala398Thr. Primers were chosen to create a restriction site for BalI enzyme on the mutant allele giving a product of 207 bp instead of 228 bp in the wild-type control. The restriction products were analysed by electrophoresis on 2.5% agarose gel. For the new Thr240Met mutation, 106 European control chromosomes were analysed by direct sequencing of exon 6.

For two patients (SPD-169±03 and SPD-166±10), total RNA was isolated from lymphoblastoid cell lines and cDNA was prepared using the Super script II reverse transcriptase±polymerase chain reaction (RT±PCR) system (Invitrogen, Glasgow, UK) in order to determine the phase of transmission. A first-round PCR was performed with the forward and reverse primers Ex1iFor (5¢-CGCGCATGGGCGCTGTCCT-3¢) and Ex9iRev (5¢-CCATACTGCCTGTTACCGGTTG-3¢). A second-round PCR was performed with primers Ex1iForNes (5¢-CAGCCGCCACCTACCCAGT-3¢) and Ex5iRev (5¢-GATTGGCATTCACCACTCATCC-3¢). The PCR products were sequenced directly using the second-round PCR primers and a Big Dye Terminator Cycle Sequencing Ready Reaction DNA Sequencing Kit (Applied Biosystems).

The five patients with the Arg275Trp mutation were genotyped for marker D6S305. The genotyping was performed by PCR using the primers speci¿ed in the Genome Database (GDB). The primers were labelled ¯uorescently and PCR products were analysed on an ABI377 automated sequencer with GENESCAN 3.1 and GENOTYPER 1.1.1 software.

Statistical analysis
Raw data for means and for proportions were compared with Student’s t-test and the \( \chi^2 \) test or the Fisher exact test, respectively.

Results
Frequency of mutations in the parkin gene
Among the 146 patients with early-onset parkinsonism but without family history, 20 (14%) had mutations in the parkin gene (Table 1). None of the eight patients with consanguinity carried a parkin mutation. Two mutations of the parkin gene

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at onset (years)</th>
<th>Country of origin</th>
<th>Dosage exon 2±12</th>
<th>Sequence exon 1±12</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPD-145-012</td>
<td>12</td>
<td>France</td>
<td>Ex 3 het del</td>
<td>Arg275Trp het</td>
</tr>
<tr>
<td>SPD-134-10a</td>
<td>30</td>
<td>France</td>
<td>Ex 2-4 het dupl/Ex3 het del</td>
<td>ND</td>
</tr>
<tr>
<td>SPD-146-005</td>
<td>35</td>
<td>France</td>
<td>Ex 3-4 het del</td>
<td>Arg275Trp het</td>
</tr>
<tr>
<td>SPD-164-10</td>
<td>27</td>
<td>France</td>
<td>Ex 6 het del</td>
<td>Normal</td>
</tr>
<tr>
<td>SPD-169-003b</td>
<td>29</td>
<td>France</td>
<td>Ex 2 het del/Ex3 het del</td>
<td>Normal</td>
</tr>
<tr>
<td>SPD-188-008</td>
<td>39</td>
<td>France</td>
<td>Normal</td>
<td>Arg256Cys het</td>
</tr>
<tr>
<td>JMP28</td>
<td>28</td>
<td>Italy</td>
<td>Ex 3 hom del</td>
<td>ND</td>
</tr>
<tr>
<td>BHAM 18c</td>
<td>18</td>
<td>UK</td>
<td>Ex 3,4,5 het del</td>
<td>Normal</td>
</tr>
<tr>
<td>SPD-099-003</td>
<td>28</td>
<td>Italy</td>
<td>Ex 6 het dupl</td>
<td>Normal</td>
</tr>
<tr>
<td>SPD-100-003</td>
<td>40</td>
<td>France</td>
<td>Normal</td>
<td>Arg275Trp het</td>
</tr>
<tr>
<td>SPD-110-001a</td>
<td>34</td>
<td>Algeria</td>
<td>Ex 3 het del</td>
<td>Normal</td>
</tr>
<tr>
<td>SPD-112-001</td>
<td>36</td>
<td>France</td>
<td>Ex 10 het del</td>
<td>Normal</td>
</tr>
<tr>
<td>SPD-123-007</td>
<td>30</td>
<td>France</td>
<td>Ex 3 het dupl</td>
<td>Normal</td>
</tr>
<tr>
<td>SPD-166-010b</td>
<td>45</td>
<td>France</td>
<td>Ex 2 het del/Ex 3-4 het del</td>
<td>Normal</td>
</tr>
<tr>
<td>SPD-181-009</td>
<td>27</td>
<td>France</td>
<td>Ex 4 het del</td>
<td>Arg275Trp het</td>
</tr>
<tr>
<td>SPD-187-003c</td>
<td>29</td>
<td>France</td>
<td>Ex 3,4 het dupl</td>
<td>Normal</td>
</tr>
<tr>
<td>SPD-225-008</td>
<td>38</td>
<td>France</td>
<td>Normal</td>
<td>Arg275Trp het</td>
</tr>
<tr>
<td>SPD-229-003</td>
<td>45</td>
<td>Algeria</td>
<td>Normal</td>
<td>Ala398Thr het</td>
</tr>
<tr>
<td>SPD-245-001c</td>
<td>43</td>
<td>Brazil</td>
<td>Ex 2,3 het dupl</td>
<td>Normal</td>
</tr>
<tr>
<td>JMP 37</td>
<td>7</td>
<td>Italy</td>
<td>Ex 2-4 het del</td>
<td>Thr240Met het</td>
</tr>
</tbody>
</table>

Bold characters indicate new mutations. *The phase of transmission was deduced by co-segregation analysis. †The phase of transmission was deduced by RT±PCR analysis. ‡The phase of transmission is not known for these patients who may be compound heterozygotes. het = heterozygous; hom = homozygous; del = deletion; dupl = duplication; ND = not determined.
were identified in eight cases, but in nine, a single mutation was detected. However, in three patients, the mutation was a rearrangement involving consecutive exons and it was not possible to determine if there were rearrangements of different consecutive exons on the two alleles or a single rearrangement on the same allele. Unfortunately, the unaffected relatives or the cell lines for RT-PCR experiments were unavailable for these ambiguous cases. In this study, all patients with one mutation were considered to have parkin-related disease, based on the assumption that the second mutation could not be detected by the methods.

The frequency of mutations in the patients of our series decreased with increasing age at onset. Three out of seven patients with onset before 20 years of age had parkin mutation compared with only 7 out of 75 with onset after 29 years. When our results are combined with those of our previous study (Lücking et al., 2000), which used the same inclusion criteria, the frequency of parkin gene mutations in isolated cases with onset \( \leq 45 \) years of age is 15% (38 out of 246) (Table 2). Taken together, these two studies demonstrate a significant negative correlation between age at onset and the presence of mutations in the parkin gene: mutations were detected in 67% with onset before 20 years of age, but only in 7% with onset after 29 years \( (P < 0.001, \text{Table 2}) \).

### Table 2: Frequency of parkin gene mutations in patients with isolated early-onset parkinsonism, by age at onset

<table>
<thead>
<tr>
<th>Age at onset (years)</th>
<th>Patients with parkin mutations</th>
<th>Patients without parkin mutation</th>
<th>Total*</th>
<th>Frequency (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>(&lt;20)</td>
<td>10</td>
<td>5</td>
<td>15</td>
<td>67</td>
<td>38–88</td>
</tr>
<tr>
<td>20–24</td>
<td>4</td>
<td>11</td>
<td>15</td>
<td>27</td>
<td>8–55</td>
</tr>
<tr>
<td>25–29</td>
<td>9</td>
<td>29</td>
<td>38</td>
<td>24</td>
<td>11–40</td>
</tr>
<tr>
<td>30–34</td>
<td>4</td>
<td>49</td>
<td>53</td>
<td>8</td>
<td>2–18</td>
</tr>
<tr>
<td>35–39</td>
<td>4</td>
<td>67</td>
<td>71</td>
<td>6</td>
<td>2–14</td>
</tr>
<tr>
<td>40–45</td>
<td>5</td>
<td>46</td>
<td>51</td>
<td>9</td>
<td>3–21</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>38</strong></td>
<td><strong>208</strong></td>
<td><strong>246</strong></td>
<td><strong>15</strong></td>
<td><strong>11–20</strong></td>
</tr>
</tbody>
</table>

*Age at onset was not known for 2, 1 and 3 patients, respectively, who were younger than 45 years when examined. *Including 100 cases from Lücking et al. (2000). CI = confidence interval.

**Clinical studies**

Patients were identified not only in Europe, but also in Algeria and Brazil, indicating the widespread distribution of parkin mutations. The relative frequency of parkin cases was similar in French (18 out of 114, 16%), Italian (4 out of 31, 13%), North African (3 out of 14, 21%), and Brazilian (1 out of 13, 8%) cases. There were 88 males and 58 females (Table 3). Age at onset was not significantly different between the 20 parkin patients and those without mutation (31 \( \pm 10 \) versus 34 \( \pm 7 \) years). There were no significant group differences in clinical features between parkin cases and other patients (Table 3). In addition, at the individual level, there was no clinical feature that distinguished the two groups.

However, clinical presentation was highly atypical in one patient (JMP28) (Table 1). This 35-year-old Italian woman presented the initial signs of the disease at 28 years (immediately after a second pregnancy), with unsteadiness, inconstant hand tremor, motor slowing and difficulty with fine movements at the left side, distal numbness in all limbs. Symptoms were absent at awakening, worsened during the day and culminated in severity in the evening. At age 30 years, examination showed unsteadiness and retropulsion, nystagmus and slight left limb dyssymetria. Other investigations were normal except cerebral MRI, which showed bilateral sickle-shaped areas of abnormal signal (decreased in \( T_1 \)-weighted images, increased in \( T_2 \)-weighted images) in the cerebellum. She was diagnosed as having idiopathic cerebellar ataxia. At age 33 years, neurological examination revealed left limb increased tone and left foot dystonia. Levodopa treatment (150 mg daily) improved the symptoms and she was...
diagnosed as having dopa-responsive dystonia. At age 34 years, she had mild nystagmus on lateral gaze, slight bradykinesia and rigidity on the left side and left foot dystonia. Tendon reflexes were moderately increased; eye movements, speech, motor coordination, sensation and plantar responses were normal. The Unified Parkinson’s Disease Rating Scale Motor Section score was 16 and the Hoehn and Yahr stage was 1, while she was taking levodopa. Extrapyramidal symptoms fluctuated during the day and mild limb dyskinesias were present. She was diagnosed as having juvenile onset parkinsonism, confirmed by the presence of a homozygous deletion of exon 3 in the parkin gene.

The frequency of mutations in the patients decreased with increasing age at onset. Mutations were detected in 67% with onset before 20 years, but only in 7% with onset after 29 years (P < 0.001, Table 2). This raises the question of the frequency of parkin mutations in late-onset cases, which is considered to be rare (Oliveri et al., 2001). However, ages at onset <72 years have been described in parkin cases (Klein et al., 2001; Nichols et al., 2002) and no systematic study of a large group of late-onset cases has been performed with the appropriate molecular tools (e.g. combining sequence analysis and exon dosage). Nevertheless, although the frequency of parkin mutations might be underestimated because of undetected mutations, our results demonstrate that parkin is the most important known aetiological factor for early-onset parkinsonism, which represents at least 10% of cases with Parkinson’s disease (Lang and Lozano, 1998).

Nine out of the 20 patients with parkin mutations unambiguously carry a single mutation. This raises two questions: (i) do other mutations remain to be discovered in other regions of the parkin gene or (ii) are single mutations in parkin sufficient to cause the phenotype? The observation of patients with both normal and mutant alleles may reflect that haploinsufficiency is a risk factor for disease or that certain mutations are dominant, conferring dominant-negative or toxic gain of function. A kindred has been reported recently with a novel mutation in the parkin gene and autosomal dominant inheritance of Parkinson’s disease with Lewy bodies (Farrer et al., 2001). Since none of our patients had

<table>
<thead>
<tr>
<th>Clinical signs at examination</th>
<th>With (n = 20)</th>
<th>Without (n = 126)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinesia</td>
<td>13 out of 20 (65%)</td>
<td>71 out of 106 (67%)</td>
</tr>
<tr>
<td>Tremor</td>
<td>10 out of 20 (50%)</td>
<td>67 out of 105 (64%)</td>
</tr>
<tr>
<td>Dystonia</td>
<td>3 out of 19 (16%)</td>
<td>13 out of 104 (13%)</td>
</tr>
<tr>
<td>Asymmetry of clinical signs</td>
<td>19 out of 20 (95%)</td>
<td>103 out of 106 (97%)</td>
</tr>
</tbody>
</table>

Discussion

Combining our results with those obtained by Lücking et al. (2000) results in a frequency of parkin gene mutations of 15% (38 out of 246) in patients with early-onset parkinsonism with an age at onset ≤45 years. Since our population was selected for patients with juvenile and early onset parkinsonism, parkin cases might be over-represented compared with the general population of patients with early-onset parkinsonism. However, our calculation of the relative frequency of parkin cases in 5-year classes excludes this potential bias. In addition, the frequency might also be underestimated since current techniques might not detect all mutations. To our knowledge, this is the first study in which the frequency of parkin gene mutation is evaluated in such large series of patients.
family histories of Parkinson’s disease, the hypothesis of autosomal dominant transmission is unlikely, except in the case of reduced penetrance or \textit{de novo} mutations. Furthermore, the nature and putative consequences of these mutations does not appear to differ from those detected in cases with mutations on both alleles. However, mutations in unexplored regions of the \textit{parkin} gene, including the promotor, cannot be excluded, although a recent study exploring a large portion of the promotor region found no disease causing mutations (West \textit{et al.}, 2002).

This screening yielded three new exon rearrangements (duplications of exons 2, duplication of exons 2–4 and deletion of exon 10) and two point mutations (Ala398Thr and Thr240Met) (Fig. 1) to be added to the growing list of known mutations in the \textit{parkin} gene which now includes 45 different point mutations and 34 exon rearrangements (Kitada \textit{et al.}, 1998; Abbas \textit{et al.}, 1999; Lücking \textit{et al.}, 2000; Klein \textit{et al.}, 2000; Maruyama \textit{et al.}, 2000; Periquet \textit{et al.}, 2000; Hedrich \textit{et al.}, 2001; West \textit{et al.}, 2002). The Ala398Thr mutation concerns an amino acid located in exon 11, which is conserved among species (human, rat and mouse). The Thr240Met mutation, located in exon 6, is a variant of the previously reported Thr240Arg mutation (Hattori \textit{et al.}, 1998b). Four out of five patients with the Arg275Trp mutation carried the very rare 220 bp allele at marker D6S305 located in intron 7 close to the mutation. This mutation is the most common point mutation of the \textit{parkin} gene in Europe, where it probably results from a founder effect (Periquet \textit{et al.}, 2001), and was already detected in 8 out of 8 patients previously analysed (Periquet \textit{et al.}, 2001). The present results confirm the existence of this founder effect.

There were no significant group differences in clinical features of \textit{parkin} cases and other patients (Table 3). In addition, at the individual level, no clinical features distinguished the two groups. These results demonstrate that \textit{parkin} cases do not represent a phenotypically distinct group, as was reported in Japanese families (Ishikawa and Tsuji, 1996). This could have an impact on molecular analyses for \textit{parkin} mutations which are useful for genetic counselling. Since there is no obvious genotype–phenotype correlation, the decision to perform \textit{parkin} analysis can only be based on the frequency of \textit{parkin} cases as a function of age at onset that sharply decreases after 30 years of age.

\begin{center}
\textbf{Fig. 1} Point mutations and exon rearrangements in the \textit{parkin} gene found in this study. (A) The two new point mutations are indicated in bold characters. (B) The deletions and duplications are represented by lines indicating their sizes and positions. Dashed lines represent new deletions or duplications. The functional domains of \textit{parkin} are indicated below the schematic representation: UBL = ubiquitin-like domain; RING-IBR-RING = RING-in between RING-RING; UTR = untranslated region.
\end{center}
Clinical analysis of patient JMP28 indicates that a parkin analysis should also be considered in patients with a highly atypical presentation. This patient was diagnosed on clinical grounds as having cerebellar ataxia, levodopa-responsive dystonia and, finally, levodopa-responsive parkinsonism. The difficulty in distinguishing patients with levodopa-responsive dystonia from those with parkin mutations has been reported previously (Tassin et al., 2000) and is confirmed by this case.

This study demonstrates that a monogenic form of parkinsonism caused by parkin mutations represents an important cause of early-onset parkinsonism without family history, especially before the age of 30 years. However, even when onset occurs between age 30 and 45 years, parkin cases still account for 8% of isolated parkinsonism. The parkin phenotype is variable, but cannot be distinguished from non-parkin cases. Therefore, molecular analysis is necessary to identify parkin cases, whose presentations are sometimes highly atypical. However, even with the combination of gene dosage and sequencing, a significant proportion of mutations might remain undetected due to the size and the complexity of the parkin gene and negative results should be interpreted with caution.

Electronic database information
Accession numbers and URLs for data in this article are as follows: The Genome Database (GDB) (for the primer sequences of marker D6S305), http://www.gdb.org; The DNA Databank of Japan (DDBJ) (for the cDNA sequence of the parkin gene, accession no. AB009973), http://www.ddbj.nig.ac.jp.

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References


Lansbury PT Jr, Brice A. Genetics of Parkinson’s disease and


