Heterozygosity for a mutation in the parkin gene leads to later onset Parkinson disease

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Abstract—Background: The vast majority of the parkin mutations previously identified have been found in individuals with juvenile or early onset PD. Previous screening of later onset PD cohorts has not identified substantial numbers of parkin mutations. Methods: Families with at least two siblings with PD were ascertained to identify genes contributing to PD susceptibility. Screening of the parkin gene, by both quantitative PCR and exon sequencing, was performed in those families with either early onset PD (age onset ≤50 years) or positive lod score with a marker in intron 7 of the parkin gene. Results: A total of 25 different mutations in the parkin gene were identified in 103 individuals from 47 families. Mutations were found in both parkin alleles in 41 of the individuals, whereas a single mutation in only one of the two parkin alleles was observed in 62 individuals. Thirty-five of the subjects (34%) with a parkin mutation had an age at onset of 60 years or above with 30 of these 35 (86%) having a detectable mutation on only one parkin allele. Few significant clinical differences were observed among the individuals with two, one, or no mutated copies of the parkin gene. Conclusions: Mutations in the parkin gene occur among individuals with PD with an older age at onset (≥60 years) who have a positive family history of the disease. In addition, the clinical findings of parkin-positive individuals are remarkably similar to those without mutations.

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Mutations in the parkin gene (PARK2) on chromosome 6 were initially reported to result in autosomal recessive, juvenile onset PD.1 Subsequently, studies performed using samples from a number of PD cohorts have identified many different mutations in the parkin gene. These have included point mutations as well as exon rearrangements, with both deletions and duplications reported.2-9

Studies focusing on early onset PD cases consistent with an autosomal recessive mode of inheritance have found mutations in the parkin gene in nearly 50% of the subjects tested.4 Additional studies have confirmed that a substantial number of subjects with early onset have mutations in the parkin gene. Genotype-phenotype comparisons of the parkin gene, primarily among early onset individuals, have not identified distinct domains within the gene or types of mutations, either missense or truncating, leading to differential clinical findings.3

Thirteen patients with parkin mutations and an age at onset after 40 years were reported, with the oldest parkin-positive subject having an age at onset of 58 years.4 However, in that sample, the mean age at onset of the parkin-positive sample was only 38 years. Most subsequent studies of later onset PD subjects, typically those with an age at onset greater than 45 or 50 years, have not found mutations in the parkin gene.10,11 Importantly, most of these studies have examined individuals with sporadic, later onset PD and not all have rigorously evaluated the parkin gene by gene dosage experiments.

In our study, we ascertained sibling pairs with PD. Thus, our sample provides the opportunity to examine whether individuals, regardless of their age at onset, who have an affected sibling have a higher frequency of parkin mutations. We also examined the hypothesis that individuals with a later onset of PD are more likely to have a single parkin mutation whereas individuals with the more typical early onset PD have mutations in both alleles of the parkin gene.

Methods. Subjects. Families consisting of at least one pair of living siblings diagnosed with PD were recruited through 60 Parkinson Study Group (PSG) sites located throughout North America. Informed consent was obtained from all study participants. A family history questionnaire was completed by at least one individual in each family and identified all other family members diagnosed with or believed to be showing signs of PD. All study

See Appendix 1 for a list of Parkinson Study Group Investigators.

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patients completed a uniform clinical evaluation that con-
sisted of Parts II and III of the Unified PD Rating Scale
(UPDyS),12 the Schwab and England Activities of Daily Living,13
and the Hoehn and Yahr Scale14 as standardized instruments
to estimate the severity of parkinsonian motor signs, symptoms, and
disability.

A diagnostic checklist (Appendix 2) was also completed by a
PSG movement disorder specialist with inclusion criteria consist-
ing of clinical features highly associated with autopsy-confirmed
PD and exclusion criteria highly associated with other non-PD
pathologic diagnoses.15,16 Previously, high inter-rater reliability
(pairwise kappa = 0.83, range 0.47 to 1.0) of the diagnostic instru-
ment was demonstrated, providing reassurance that error in diag-
nosis was kept at a minimum.17 Subsequently, neuropathologic
evaluation was completed in two study participants. One subject
was classified as nonverified PD (NVPD) based on the results of
the diagnostic checklist, although the neuropathologic examina-
tion was completed in two study participants. One subject
screened and found not to have a mutation detected in the
parkin gene (n = 171) or were not tested for mutations in the gene
due to the family’s negative lod score with the marker in intron 7 of
the parkin gene (n = 285). There were no significant differences in
the racial or sex distribution or in the proportion of subjects meeting
VPD when comparing the sample of individuals who were screened
and found not to have a parkin mutation and those who were not tested for mutations in the gene
due to the family’s negative lod score and later age at onset.

Because age at onset was one of the criteria used to select subjects
for mutation screening, it is not surprising that those individuals
screened and found to be negative for a parkin mutation had a
younger age at onset of PD as compared with subjects who were
not screened (60.7 vs 65.0, p = 0.0001). Similarly, the individuals
screened and found not to have a parkin mutation also had an
earlier age at diagnosis than the subjects who were not screened
(62.4 vs 67.1, p = 0.0001).

The most common mutation detected in our PD cohort involved
exon 8. Nineteen of the 47 families with parkin mutations had
either a deletion or duplication of exon 8 (see table 1). Other
common deletions and duplications involved exons 3 (11 families),
4 (7 families), 5 (5 families), and 10 (5 families). Nine different
point mutations were also observed in seven different exons. Ten
of the 25 mutations identified in this sample were novel
parkin mutations. There was no difference in the distribution of missense
data nonsense mutations in the individuals having one as com-
pared with two mutant parkin alleles.

Families with the most common parkin mutation, a deletion of
exon 8, were of varied Caucasian ancestry, including Italian,
French Canadian, and Hispanic. Although the sample size is mod-
est (n = 12 families), there did not appear to be preferential
transmission of a particular allele at the D6S305 marker in the
parkin gene in the families inheriting an exon 8 deletion. Thus,
we did not have evidence of a founder effect for the most common
mutation in our sample.

Peripheral blood was obtained from all individuals after the
completion of appropriate written informed consent approved by
each individual institution’s Institutional Review Board. DNA was
prepared using standard methods.18

Parkin screening. A short tandem repeat marker in intron 7
of the parkin gene (D6S305) was genotyped in all study subjects.

Families with positive lod scores at this marker under an autosomal
recessive model of disease inheritance (n = 94) and families with
at least one evaluated and affected family member with an
age at onset of 50 years or less (n = 66) were screened for parkin
mutations using both direct sequencing and fluorescent dosage
analysis, as previously described.19 For direct sequence analysis,
all 12 exons of the parkin gene were PCR amplified. The resulting
PCR products were purified using the QIAquick 96 PCR purifica-
tion kit (QIAGEN, Santa Clara, CA) and sequenced on an ABI
3700A DNA analyzer using the Applied Biosystems BigDye Termi-
nator version 2.0 kit (Applied Biosystems, Foster City, CA). We
confirmed segregation of the mutations within families, and ex-
cluded the presence of the mutations in a panel of 182 normal
chromosomes, by PCR amplification of the relevant exon followed
by either mutation specific restriction fragment length polymor-
phism (RFLP) analysis or direct sequencing. Fluorescent dosage
PCR was performed as previously described.20 Briefly, 125 ng of
genomic DNA was PCR amplified using a fluorescently labeled
forward PCR primer. Multiplex PCR reactions were performed for
exons 2 through 12 of the parkin gene in two different groups with
group 1 containing exons 4, 6, 7, 8, 9, and 12 and group 2 contain-
ing exons 2, 3, 5, 10, and 11. PCR products were electrophoresed on
an ABI 377 DNA Analyzer. Data were analyzed using the Scan and Genotyper software (Applied Biosystems)
to produce electropherograms showing the size in base pairs of the peaks and
areas under the peaks representing the amount of PCR product
present. To determine gene dosage for each exon, samples were
compared to each other to obtain the dosage quotients. Each
sample was repeated three times. Any individual exon sample giving
an ambiguous or uninterpretable result was repeated an addi-
tional three times. Thirty-three families had both a positive lod
score as well as at least one family member with an age at onset of
50 or less.

Statistical analyses. Statistical analyses were designed to
address specific hypotheses. Initial analyses through to identify signifi-
cant difference in number of variables between those subjects
with and without a parkin mutation. Individuals with one or two
parkin mutations were grouped together and compared with sub-
jects screened and found not to have a parkin mutation. For con-
tinuous variables such as age at onset, Student t-test was used to
test for significant differences between the two groups. For cate-
gorical variables, the individual disease diagnosis (vs normal
= 0.0001) and duration of disease (vs 10 or more
= 0.0001) as compared with individuals having only one mutated
parkin allele. A greater proportion of the individuals with two
parkin mutations had a clinical course of 10 or more years (46.6% vs
78.1%, p = 0.002) as compared to those with only one mutation.
Similar to the analyses comparing parkin mutation–positive and
negative groups, no difference was found between the propor-
tion of subjects meeting criteria for VPD between the two
groups. As a result, no further statistical analyses were performed.
different parkin mutation–positive groups (one mutant allele vs two mutant alleles, \( p = 0.68 \)).

**Discussion.** Since the initial identification of parkin mutations in patients with PD, there has been keen interest in delineating the spectrum of clinical findings among those with and without mutations. Although studies have consistently reported the important role of parkin mutations in the etiology of early onset PD, only a limited number of studies have examined the contribution of parkin mutations to later onset PD. Recently, results were reported in a sample with 95 sporadic PD patients and 23 subjects with at least one affected sibling. All tested subjects had onset of PD after age 45, and no parkin mutations were identified in any of the subjects. Importantly, molecular studies were limited and did not rigorously assess parkin mutations through gene dosage experiments. Therefore, this study would have failed to detect many potential heterozygous mutations in their sample.

In contrast, we identified parkin mutations in 18.4% (103/559) of our familial PD subjects. Eighty-four individuals reported an age at onset less than 50 years. Among these, mutations were identified in 52 individuals (62%). In the larger cohort of subjects with an onset of 50 years or later, mutations were identified in 50 individuals (50/448/11.2%). Of the 47 families in which parkin mutations were detected, 16 families reported all affected individuals to have an age at onset of 50 years or older. This estimate is substantially higher than that reported in previous studies of late onset PD subjects. We included both gene sequencing and dosage studies to ensure that we would detect all parkin mutations. Given the high frequency of exon rearrangements in our sample as well as that of others, it is apparent that thorough screening of the parkin gene must include dosage studies.

Our estimate of the proportion of subjects with a parkin mutation is very likely an underestimate because all subjects were not screened for mutations. Rather, through the use of age at onset information and evidence of linkage to a marker in the parkin gene, a subset of individuals and families were identified who were more likely to have a parkin muta-

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**Table 1** Identified parkin mutations in the study sample

<table>
<thead>
<tr>
<th>Exon</th>
<th>Mutation type</th>
<th>Nucleotide change</th>
<th>Protein change</th>
<th>Number of families</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Frameshift</td>
<td>c.154del A</td>
<td>Truncation at aa 52</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Frameshift</td>
<td>c.101–102del AG</td>
<td>Truncation at aa 34</td>
<td>1</td>
</tr>
<tr>
<td>2, 3</td>
<td>Deletion</td>
<td></td>
<td>Truncation at aa 3</td>
<td>2</td>
</tr>
<tr>
<td>2, 3, 4</td>
<td>Duplication↑</td>
<td>Duplication of aa 3–176, truncation at aa 177</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Duplication</td>
<td></td>
<td>Duplication of aa 58–136, truncation at aa 137</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Nonsense</td>
<td>G235T</td>
<td>Glu79Stop</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Deletion</td>
<td>c.236–276del</td>
<td>Truncation at aa 79</td>
<td>3</td>
</tr>
<tr>
<td>3, 4</td>
<td>Deletion</td>
<td></td>
<td>In frame deletion of aa 58–176</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Missense</td>
<td>G500A</td>
<td>Ser167Asn</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Deletion</td>
<td></td>
<td>Truncation at aa 136</td>
<td>1</td>
</tr>
<tr>
<td>4, 5, 6</td>
<td>Deletion</td>
<td></td>
<td>Truncation at aa 136</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Duplication</td>
<td></td>
<td>In frame duplication of aa 177–204</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Missense</td>
<td>A574G</td>
<td>Met192Val</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Missense</td>
<td>A633T</td>
<td>Lys211Asn</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Deletion</td>
<td></td>
<td>Truncation at aa 205</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Missense</td>
<td>C718T</td>
<td>Thr240Met</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Duplication</td>
<td></td>
<td>Duplication of aa 243–289, truncation at aa 290</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Missense</td>
<td>C823T</td>
<td>Arg275Trp</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>Deletion</td>
<td></td>
<td>Truncation at aa 243</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Deletion</td>
<td></td>
<td>Truncation at aa 298</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>Duplication</td>
<td></td>
<td>Duplication of aa 289–309, truncation at aa 310</td>
<td>6</td>
</tr>
<tr>
<td>8, 9</td>
<td>Deletion</td>
<td></td>
<td>Truncation at aa 289</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Duplication</td>
<td></td>
<td>In frame duplication of aa 360–389</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>Deletion</td>
<td></td>
<td>In frame deletion of aa 360–389</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>Missense</td>
<td>C1310T</td>
<td>Pro437Leu</td>
<td>1</td>
</tr>
</tbody>
</table>

Mutations shown in bold represent novel mutations not previously reported.

* Nucleotides are numbered according to GenBank Accession AB009973 with the A of the initiator ATG numbered as +1.
† Indicates mutation for which phase could not be determined.
tion. After analyzing our results and identifying substantial numbers of heterozygous mutations, we performed linkage analyses using an autosomal dominant model of disease inheritance. We identified 12 families with positive lod scores under the autosomal dominant model who did not have a positive lod score under the autosomal recessive model that had been used to identify families for parkin screening. These results suggest that further parkin screening might identify additional families with heterozygous mutations.

Statistical analyses were also performed to more broadly compare individuals with a parkin mutation to the sample of individuals unlikely to have a parkin mutation. The later group included the 171 individuals who were screened and found not to have a mutation as well as the 285 individuals who had an age at onset over 50 years and had a negative lod score with D6S305 using an autosomal recessive model. Results were very similar to those obtained when the mutation-negative group was limited to only the 171 screened individuals. The subjects with at least one mutant parkin allele were younger (50.3 vs 60.7, \( p < 0.0001 \)) and had an earlier age at diagnosis (52.3 vs 62.9, \( p = 0.0001 \)) than the more broadly defined group unlikely to have a parkin mutation. The mutation-positive individuals were also more likely to have a disease duration of 10 or more years (37.3% vs 59.6%, \( p = 0.001 \)).

Those subjects in whom parkin mutations were identified had an age at onset as late as 78. In fact, mutations in the parkin gene were found in 50 individuals with age at onset 50 years or above. Notably, the clinical features of those with one or two parkin mutations differed significantly on only a few questions from the diagnostic checklist as compared with those lacking parkin mutations. Because the subjects with a parkin mutation had an earlier age at onset of PD, it is not surprising that a higher proportion of them had a disease course of 10 or more years (\( p = 0.001 \)). Similarly, when comparing subjects with one and two parkin mutations, the proportion of subjects with a 10-year disease course was significantly greater among those with two as compared with one parkin mutation (\( p = 0.007 \)). Importantly, the proportion of subjects meeting criteria for VPD and conversely NVPD were not significantly different between the mutation-positive and mutation-negative groups as well as those with one as compared with two mutations. This suggests that individuals with a parkin mutation or mutations presented with what appeared clinically to be idiopathic PD.

### Table 2 Comparison of clinical features among study subjects with 0, 1, or 2 parkin mutations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parkin Number of parkin mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Number of individuals</td>
<td>103</td>
</tr>
<tr>
<td>Number of additional affected family members beyond a sibling pair</td>
<td>1.26</td>
</tr>
<tr>
<td>Age at onset, y</td>
<td>50.3</td>
</tr>
<tr>
<td>Age at diagnosis, y</td>
<td>52.3</td>
</tr>
<tr>
<td>Current age, y</td>
<td>65.5</td>
</tr>
<tr>
<td>Schwab and England‡</td>
<td>78.1</td>
</tr>
<tr>
<td>Hoehn and Yahr‡</td>
<td>2.4</td>
</tr>
<tr>
<td>Percent with Onset w/tremor</td>
<td>63.4</td>
</tr>
<tr>
<td>Bradykinesia</td>
<td>92.0</td>
</tr>
<tr>
<td>Muscular rigidity</td>
<td>96.0</td>
</tr>
<tr>
<td>Postural instability</td>
<td>62.6</td>
</tr>
<tr>
<td>Persistent asymmetry of signs</td>
<td>59.6</td>
</tr>
<tr>
<td>Rest tremor</td>
<td>83.0</td>
</tr>
<tr>
<td>Strictly unilateral features after 3 y</td>
<td>6.3</td>
</tr>
<tr>
<td>Levodopa response ( \geq 5 ) y</td>
<td>64.2</td>
</tr>
<tr>
<td>Clinical course ( \geq 10 ) y</td>
<td>59.6</td>
</tr>
<tr>
<td>Likelihood of PD &gt; 90%</td>
<td>75.5</td>
</tr>
<tr>
<td>Verified PD</td>
<td>70.9</td>
</tr>
</tbody>
</table>

* Negative includes individuals who were screened molecularly and no parkin mutation was identified.
† Bold indicates statistical comparisons that are still significant after adjusting for multiple testing (i.e., \( p < 0.003 \)).
‡ Statistical analysis includes disease duration as a covariate.
tations. We neither clinically examined nor obtained a DNA sample from the apparently unaffected siblings, parents, or offspring of affected individuals. Therefore, we cannot determine what proportion of these individuals were in fact clinically unaffected despite having inherited one or even two mutant *parkin* alleles. This is a critical question that will be addressed in our future studies.

A recent study of a community-based sample of 111 early onset PD subjects (age at onset <50 years) found *parkin* mutations in 9% of their subjects, with individuals identified with compound mutations as well as a single mutated allele. Interestingly, the authors also found a higher proportion of *parkin* mutations among subjects with a positive family history of PD. Another study also examined the role of *parkin* mutations in early onset PD cases. In a sample of 50 PD subjects with age at onset under 50 years, individuals with either one or two mutated copies of the *parkin* gene were identified. Similar to our findings, the authors reported earlier age at onset among those with two *parkin* mutations as compared with those with only one mutation. In contrast to these studies, we have now examined a much larger cohort of patients with PD and have not limited the age at onset to less than 50 years. In this way, we have identified 50 individuals who had onset at age 50 or later. Among these, 42 had a single detected mutation and 8 had a mutation in both *parkin* alleles.

Review of our family data identified two families in which the affected family members differed in their inherited *parkin* mutations. In one kindred, two siblings with PD were heterozygous for a deletion in exon 8 whereas the third affected sibling did not inherit a *parkin* mutation. Linkage analysis performed in this family under both an autosomal recessive and autosomal dominant model of inheritance with the marker D6S305 in the *parkin* gene yielded negative lod scores, consistent with at least one family member lacking linkage to this chromosomal region. The two family members with *parkin* mutations had older ages at onset (>40 years) as compared with their sibling who lacked a mutation (<40 years). In the second family with discordant *parkin* mutation results, two siblings had a heterozygous deletion of exon 10 whereas their sibling lacked a *parkin* mutation. In this family, all affected members had onset of PD between the ages of 30 and 46. The lod score in this family is slightly positive under both an autosomal recessive and autosomal dominant model of inheritance; however, marker information is incomplete in this region. Given the relative frequency of PD in the general population, it is to be expected that some individuals, even within a family, might have inherited PD owing to nongenetic factors. These individuals would be termed phenocopies. There was evidence of a potential phenocopy in the initially reported Italian kindred that segregated an autosomal dominant form of PD due to a mutation in the alpha synuclein gene. In that kindred, there was one individual, with a substantially later age at onset than the other family members who had not inherited a mutation in the alpha synuclein gene and who was therefore presumed to be a phenocopy.

Twenty-five families in this study had three or more affected members who completed a study visit. In six families with three or more examined members with PD, all family members in a particular kindred had the same mutation on both their *parkin* alleles. In one family, all three examined affected individuals had only one mutation, and it was the same in all affected members. In two families, some but not all family members had a *parkin* mutation. In the remaining 16 families, either no *parkin* mutation was identified through molecular screening or the family was not screened due to the later age at onset of all affected individuals (>50 years) and negative lod scores with a marker in the *parkin* gene.

To date, subjects with late onset PD who have been examined for *parkin* mutations primarily had sporadic PD. Our study is unique in its use of siblings, both of whom had PD and who therefore likely had a greater genetic contribution to disease as compared with patients with sporadic PD. In addition, subjects with a wide range in age at onset were examined for *parkin* mutations. We did not limit our study of the *parkin* gene to individuals with early PD onset. We tested for mutations in all families having at least one member with an age at onset of 50 years or less. In addition, we broadened our molecular screening to include all those families who demonstrated evidence of linkage to a marker in an intron of the *parkin* gene as well. For this reason, we were able to demonstrate that *parkin* mutations contribute to PD etiology even among individuals with later onset PD. Thus, it is important to consider *parkin* mutations when evaluating patients with later onset PD, particularly those reporting a close family history of PD.

Appendix 1: Parkinson Study Group investigators

**Steering committee:** Lawrence Golbe, MD, UMDNJ Robert Wood Johnson Medical Center, New Brunswick, NJ; William Koller, MD, Kelly Lyons, PhD, University of Miami, FL; Karen Marder, MD, Columbia-Presbyterian Medical Center, New York, NY; Frederick Marshall, MD, David Oakes, PhD, Alice Rudolph, PhD, University of Rochester, NY; Cliff Shults, MD, University of California, San Diego; Aileen Shinaman, JD, University of Rochester, NY; Eric Siemers, Eli Lilly & Company, Indianapolis, IN.

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Exclusion criteria

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References

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