Parkin Disease

A Clinicopathologic Entity?

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Importance: Mutations in the gene encoding parkin (PARK2) are the most common cause of autosomal recessive juvenile-onset and young-onset parkinsonism. The few available detailed neuropathologic reports suggest that homozygous and compound heterozygous parkin mutations are characterized by severe substantia nigra pars compacta neuronal loss.

Objective: To investigate whether parkin-linked parkinsonism is a different clinicopathologic entity to Parkinson disease (PD).

Design, Setting, and Participants: We describe the clinical, genetic, and neuropathologic findings of 5 unrelated cases of parkin disease and compare them with 5 pathologically confirmed PD cases and 4 control subjects. The PD control cases and normal control subjects were matched first for age at death then disease duration (PD only) for comparison.

Results: Presenting signs in the parkin disease cases were hand or leg tremor often combined with dystonia. Mean age at onset was 34 years; all cases were compound heterozygous for mutations of parkin. Freezing of gait, postural deformity, and motor fluctuations were common late features. No patients had any evidence of cognitive impairment or dementia. Neuronal counts in the substantia nigra pars compacta revealed that neuronal loss in the parkin cases was as severe as that seen in PD, but relative preservation of the dorsal tier was seen in comparison with PD (P = .04). Mild neuronal loss was identified in the locus coeruleus and dorsal motor nucleus of the vagus, but not in the nucleus basalis of Meynert, raphe nucleus, or other brain regions. Sparse Lewy bodies were identified in 2 cases (brainstem and cortex).

Conclusions and Relevance: These findings support the notion that parkin disease is characterized by a more restricted morphologic abnormality than is found in PD, with predominantly ventral nigral degeneration and absent or rare Lewy bodies.


For editorial comment see page 551

Video available online at www.archdermatol.com

The parkin gene has 12 coding exons and the subsequent protein comprises 3 RING fingers separated by an in-between domain at the carboxyl terminal. Parkin plays an important role in mitochondrial functioning, as well as the ubiquitin proteasome system, where it acts as a ubiquitin E3 ligase. Impaired autophagy and mitophagy, protein accumulation, and mitochondrial dysfunction are the main mechanisms proposed in the development of parkinsonism due to mutations of parkin.
Parkinson disease (PD) is characterized pathologically by severe loss of dopaminergic neurons in the substantia nigra (SN), with numerous cytoplasmic inclusions containing \(H\alpha\)-synuclein, known as Lewy bodies (LBs), in the surviving neurons. Autopsy reports of patients with parkinsonism carrying 2 mutations in the \(par\)kin gene described localized severe nigral degeneration with gliosis, mild neuronal loss, and depigmentation of the locus coeruleus (LC) but an absence of LBs in most cases (Table 1 and Table 2).

We conducted a detailed clinicopathologic study in 5 cases with compound heterozygous mutations of \(par\)kin to define the clinical features, late disease course, and pathologic lesion in parkin disease.

**METHODS**

**MATERIALS**

Cases with 2 confirmed mutations of \(par\)kin with clinical data and pathologic material were included. Three cases were identified from the Queen Square Brain Bank and 2 from the Dublin Brain Bank, where tissue is collected using ethically approved protocols and material stored under a license issued by...
GENETIC, NEUROPATHOLOGIC, AND CLINICAL METHODS

Genomic DNA was extracted using standard methods from frozen brain tissue in all suspected cases. Parkin coding region and splice sites were screened for point mutations by polymerase chain reaction and subsequent bidirectional sequencing using BigDye Terminator version 3.1 (Applied Biosystems) sequencing chemistry. Exon rearrangements were detected by multiplex ligation-dependent probe amplification using the P051 and P052 Salsa MLPA Parkinson probe sets, according to the manufacturer’s instructions (MRC-Holland).

Brain tissue fixed with 10% buffered formalin was sampled, processed for histology, and stained according to standard protocols. Formalin-fixed paraffin-embedded tissue sections from cortical, subcortical, brainstem, and cerebellar regions were stained using routine histologic stains (hematoxylin and eosin and Luxol fast blue/cresyl violet) and supplemented by immunohistochemical staining using the following primary antibodies: glial fibrillary acidic protein, α-synuclein, tau (AT8), amyloid-β, ubiquitin, p62, neurofilaments, IC2, fused in sarcoma (FUS), and TAR DNA-binding protein 43 (full details are in eAppendix 1, http://www.jamaneuro.com). All cases were assessed by an experienced neuropathologist who was blind to the diagnosis and graded regional neuronal loss and gliosis using a 4-point semiquantitative scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe, based on previously published studies). α-Synuclein, tau, ubiquitin, p62, and amyloid-β immunoreactive structures were analyzed in selected brain areas. Lewy body pathology was assessed according to the recommendations of the third report of the Dementia with Lewy Bodies Consortium and also by Braak stage.

Concomitant pathologies were assessed (eAppendix 1).

Detailed study of the severity of nigral neuronal loss was carried out in the PD and parkin cases only. A single, transverse 7-μm thick section of midbrain was taken at the level where the fascicles of the third cranial nerve emerge from the midbrain, allowing evaluation of pertinent nuclear groups at a defined level. These sections were stained with the Luxol fast blue/cresyl violet and supplemented by immunohistochemical staining using the following primary antibodies: glial fibrillary acidic protein, α-synuclein, tau (AT8), amyloid-β, ubiquitin, p62, and neurofilaments, IC2, fused in sarcoma (FUS), and TAR DNA-binding protein 43 (full details are in eAppendix 1, http://www.jamaneuro.com). All cases were assessed by an experienced neuropathologist who was blind to the diagnosis and graded regional neuronal loss and gliosis using a 4-point semiquantitative scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe, based on previously published studies). α-Synuclein, tau, ubiquitin, p62, and amyloid-β immunoreactive structures were analyzed in selected brain areas. Lewy body pathology was assessed according to the recommendations of the third report of the Dementia with Lewy Bodies Consortium and also by Braak stage.

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Clinical record review was undertaken in the parkin, PD, and control cases for details of disease presentation, response to medication, progression, and late features. Full case descriptions were summarized for the parkin cases.

STATISTICAL METHODS

Using a semiquantitative approach, we first compared the severity (ie, none, mild, moderate, or severe) of neuronal loss and gliosis among the 3 groups: parkin disease (5 cases), PD (5 cases), and control (4 cases). In each case, 9 brain areas were selected for examination. The grades of neuronal loss (or gliosis) in each brain area were pooled and an ordinal logistic regression model with a robust variance estimator was used to test the increased odds of having more severe neuronal loss (or gliosis) and take into account clustering among individuals.

RESULTS

The clinical features and genetic mutations in the 5 parkin cases are summarized in Table 3. The clinical description of the first parkin case is detailed here (descriptions of cases 2-5 are available in eAppendix 2 and Video 1 and Video 2 of case 5 in the online-only material).

CASE 1: CLINICAL SUMMARY

At age 36 years, this woman noticed the toes on her left foot turning up. Initial examination revealed involuntary dorsiflexion of her left great toe associated with a coarse tremor of her left foot and leg. Focal dystonia was diagnosed, and she was treated with anticholinergic medication to good effect. Ten years later, a diagnosis of PD was considered when reduced arm swing and arm tremor were noted; however, levodopa treatment was not started until the age of 56 years, when she had an excellent response. While in her 70s, she began to experience falls and freezing of gait, but she still walked unaided when taking medication. Examination in the year she died revealed occasional tremor of both hands with bilateral rigidity and bradykinesia, and she reported that her symptoms. In her last year of life, she had an episode of confusion and disorientation and experienced some visual hallucinations, but these were short lived. She was not demented and denied any memory problems. She died of ischemic heart disease 4 days following repair of a fractured neck of femur at age 86 years.
Four different parkin mutations were detected: 2 missense changes (c.823C>T p.R275W; c.1289G>A, p.G430D), 1 frameshift deletion causing the premature introduction of a stop codon (c.337_376del; p.Pro113fs), and 1 whole-exon rearrangement (exon 6 deletion). All the identified mutations have been previously described and shown (when homozygous or compound heterozygous) to be associated with early-onset parkinsonism.

**NEUROPATHOLOGY**

**Parkin-Linked Cases**

Macroscopically, the changes in the parkin cases appeared identical to that of PD, with marked nigral (all cases) and LC (2 of the 5 cases) pallor. The histologic findings in the parkin cases, the most striking feature being the loss of pigmented neurons in the SN (moderate to severe); in all cases, the pattern was one of the ventral tier being most severely affected (Figure 1A and B). This was accompanied by mild to moderate cell loss in the LC with pigment incontinence. Other structures affected by neuronal loss included the dorsal motor nucleus of the vagus (DMV) (mild in 2 of 3 cases examined) and the cerebellar cortex (examined at the level of the dentate nucleus and the superior cerebellar peduncle), which showed mild Purkinje cell loss with empty baskets. In no other region examined was any appreciable neuronal loss evident including the nucleus basalis of Meynert (NBM) (4 cases examined; Figure 2A). Mild to moderate gliosis accompanied the neuronal loss previously described, but there was also evidence of gliosis (in the absence of detectable neuronal loss) in the raphe nucleus, NBM, striatum, globus pallidus, dentate nucleus, amygdala, hippocampus, and cerebral cortices (Figure 2B). None of the parkin disease cases looked similar to PD when immunohistochemistry for α-synuclein was performed. The results from 2 cases were negative for α-synuclein (cases 1 and 2). 1 had sparse Lewy neurites in the SN but no LBs (case 4) and 1 had sparse Lewy neurites and a total of 2 LBs (midbrain periaqueductal gray matter and transentorhinal cortex) (case 3). Case 5 had brainstem-predominant LB disease according to McKeith et al criteria.

### Table 3. Clinical and Genetic Details of Parkin Disease Cases

<table>
<thead>
<tr>
<th>Case</th>
<th>Nationality</th>
<th>Sex</th>
<th>Age at onset, y</th>
<th>Age at death, y</th>
<th>Disease duration, y</th>
<th>Presenting symptoms</th>
<th>Presenting signs</th>
<th>Posture</th>
<th>Visual hallucinations</th>
<th>Dementia</th>
<th>Late disease features</th>
<th>Initial diagnosis</th>
<th>Final clinical diagnosis</th>
<th>Siblings affected (No.)</th>
<th>Mutation 1</th>
<th>Mutation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>British</td>
<td>Female</td>
<td>36</td>
<td>86</td>
<td>50</td>
<td>Toes turning up, leg pain</td>
<td>Foot dystonia, leg tremor</td>
<td>Kyphoscoliotic</td>
<td>Yes, brief</td>
<td>No</td>
<td>No</td>
<td>Falls, freezing of gait</td>
<td>Dystonia</td>
<td>PD</td>
<td>No (1 of 5)</td>
<td>R275W</td>
</tr>
<tr>
<td>2</td>
<td>British</td>
<td>Female</td>
<td>25</td>
<td>62</td>
<td>37</td>
<td>Hand spasm and tremor</td>
<td>Hand tremor and dystonia</td>
<td>Kyphoscoliotic</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Painful dystonia</td>
<td>YOPD</td>
<td>YOPD</td>
<td>Yes (1)</td>
<td>R275W</td>
</tr>
<tr>
<td>3</td>
<td>Irish</td>
<td>Female</td>
<td>33</td>
<td>60</td>
<td>27</td>
<td>Hand and leg tremor</td>
<td>Hand tremor and dystonia</td>
<td>Kyphoscoliotic</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Painful dystonia</td>
<td>ET</td>
<td>YOPD</td>
<td>Yes (2 of 7)</td>
<td>G430D</td>
</tr>
<tr>
<td>4</td>
<td>Irish</td>
<td>Male</td>
<td>32</td>
<td>68</td>
<td>36</td>
<td>Tremor, gait difficulties</td>
<td>Tremor, gait difficulties</td>
<td>Kyphoscoliotic</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Rapid on/off, dystonia</td>
<td>YOPD</td>
<td>YOPD</td>
<td>No (1)</td>
<td>R275W</td>
</tr>
<tr>
<td>5</td>
<td>British</td>
<td>Male</td>
<td>46</td>
<td>82</td>
<td>36</td>
<td>Leg tremor</td>
<td>Tremor</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Falls, freezing of gait, pain</td>
<td>PD</td>
<td>PD</td>
<td>No (2 of 7)</td>
<td>R275W</td>
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</table>

**Table 4. Neuropathology of Parkin Disease Cases**

<table>
<thead>
<tr>
<th>Case</th>
<th>Neuronal loss in SN</th>
<th>Neuronal loss in LC</th>
<th>Lewy bodies present</th>
<th>VNN density, neurons/mm²</th>
<th>DNN density, neurons/mm²</th>
<th>Total nigral neuronal density, neurons/mm²</th>
<th>Ratio of VNN/DNN density</th>
<th>Aβ diffuse deposits</th>
<th>Aβ mature deposits</th>
<th>CAA</th>
<th>Braak &amp; Braak AD stage</th>
<th>Small-vessel disease</th>
<th>TDP-43-positive inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moderate</td>
<td>Mild</td>
<td>No</td>
<td>5.55</td>
<td>16.72</td>
<td>12.21</td>
<td>0.33</td>
<td>Severe</td>
<td>Mild</td>
<td>None</td>
<td>II</td>
<td>Mild</td>
<td>None</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>Mild</td>
<td>No</td>
<td>12.83</td>
<td>21.55</td>
<td>18.25</td>
<td>0.60</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>Severe</td>
<td>Moderate</td>
<td>Yes</td>
<td>2.60</td>
<td>20.88</td>
<td>12.53</td>
<td>0.12</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>I</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>Severe</td>
<td>Moderate</td>
<td>No</td>
<td>7.67</td>
<td>15.45</td>
<td>12.63</td>
<td>0.50</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>Severe</td>
<td>Mild</td>
<td>Yes (2 of 7)</td>
<td>Severe</td>
<td>Mature</td>
<td>16.13</td>
<td>0.52</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

**Table 4.** Table 4, Figure 1, and Figure 2 provide a summary of the histologic findings in the parkin cases, the most striking feature being the loss of pigmented neurons in the SN (moderate to severe); in all cases, the pattern was one of the ventral tier being most severely affected (Figure 1A and B). This was accompanied by mild to moderate cell loss in the LC with pigment incontinence. Other structures affected by neuronal loss included the dorsal motor nucleus of the vagus (DMV) (mild in 2 of 3 cases examined) and the cerebellar cortex (examined at the level of the dentate nucleus and the superior cerebellar peduncle), which showed mild Purkinje cell loss with empty baskets. In no other region examined was any appreciable neuronal loss evident including the nucleus basalis of Meynert (NBM) (4 cases examined; Figure 2A). Mild to moderate gliosis accompanied the neuronal loss previously described, but there was also evidence of gliosis (in the absence of detectable neuronal loss) in the raphe nucleus, NBM, striatum, globus pallidus, dentate nucleus, amygdala, hippocampus, and cerebral cortices (Figure 2B). None of the parkin disease cases looked similar to PD when immunohistochemistry for α-synuclein was performed. The results from 2 cases were negative for α-synuclein (cases 1 and 2). 1 had sparse Lewy neurites in the SN but no LBs (case 4) and 1 had sparse Lewy neurites and a total of 2 LBs (midbrain periaqueductal gray matter and transentorhinal cortex) (case 3). Case 5 had brainstem-predominant LB disease according to McKeith et al criteria.

**GENETIC ANALYSIS**

Four different parkin mutations were detected: 2 missense changes (c.823C>T p.R275W; c.1289G>A, p.G430D), 1 frameshift deletion causing the premature introduction of a stop codon (c.337_376del; p.Pro113fs), and 1 whole-exon rearrangement (exon 6 deletion). All the identified mutations have been previously described and shown (when homozygous or compound heterozygous) to be associated with early-onset parkinsonism.
but was unusual for PD because the severe loss of pigmented nigral neurons was accompanied by only very sparse LBs (Figure 1C and D). Mild neuronal loss in the LC was associated with moderate numbers of LBs (arrows) and Lewy neurites (F). The pattern of pathology did not conform well to the Braak PD staging scheme as the density of brainstem LBs did not show the expected increase when LB pathology extended beyond the brainstem.30

There was either no (2 cases) or only mild (3 cases) deposition of hyperphosphorylated tau, which did not

Figure 1. Histologic findings in the parkin cases. Case 2 substantia nigra illustrates the more severe neuronal loss in the ventral tier (white arrow) compared with the dorsal tier (black arrow) (A), accompanied by severe gliosis (white arrow) in the ventral tier compared with the dorsal tier (black arrow) (B). Case 5 showed severe neuronal loss in the ventrolateral substantia nigra (C) and only sparse α-synuclein pathology (arrows) (D), with mild cell dropout in the locus coeruleus (E) accompanied by moderate numbers of Lewy bodies (arrows) and Lewy neurites (F). The scale bar represents 260 µm in parts A and B, 50 µm in parts C and D, 100 µm in parts E and F, and 25 µm in the inset in part F. Hematoxylin-eosin staining in parts A, C, and E; glial fibrillary acidic protein in part B; and α-synuclein in parts D and F.
Ubiquitin and p62 stains highlighted small irregular neuronal intracytoplasmic inclusions in the cytoplasm of pigmented nigral neurons in 3 cases. These inclusions were not recognized in immunohistochemical preparations for α-synuclein, tau, IC2 (recognizing polyglutamine-repeat–containing proteins), neurofilaments, TAR DNA-binding protein 43, or FUS, and they were also observed in 3 of 4 control cases, indicating that they are not disease specific.

**PD and Control Cases**

The pathologically confirmed PD cases were retrospectively reviewed, and the demographics and mean SN neuronal density measurements are given in **Table 5**. The clinical details were consistent with PD and none of the cases had a positive family history of parkinsonism. The pathologic findings were also classic for PD in all cases without the finding of other significant pathology.

The causes of death in the control cases were metastatic bowel cancer in 2 and myocardial infarction in 2. None had experienced any symptoms suggestive of parkinsonism or other neurodegenerative diseases. One control case was found to have sparse cortical and brainstem LBs without significant SN or LC neuronal loss. There was no clinical correlate to these findings; the patient was 81 years old at death and the pathologic diagnosis of incidental LB disease was made.

**STATISTICAL ANALYSES**

Figure 2 shows the distribution and severity of neuronal loss and gliosis in the 3 groups. The PD group had the greatest severity of neuronal loss and the control group had the least severe: after adjusting for age at death, the odds ratios of having an increased severity of neuronal loss were 1.2 (95% CI, 0.8-1.8) and 0.5 (95% CI, 0.4-0.7) for PD and control cases, respectively, relative to parkin disease (global \( P < .001 \), averaged over all brain areas examined). Gliosis also differed significantly between the 3 diagnoses (\( P = .02 \)), with a 1.4 increased odds ratio (95% CI, 0.6-3.1) of having more severe gliosis in PD compared with parkin disease cases and a 0.4 decreased odds ratio (95% CI, 0.1-0.9) for control compared with parkin disease cases. Detailed statistical analysis in each region was limited by small case number, but the largest differences in severity were observed in the SN, LC, and NBM for neuronal loss (Figure 2A), as well as in the SN, LC, and NBM for gliosis (Figure 2B).

Table 5 and **Figure 3** illustrate the neuronal densities in the different tiers of the SN in the PD and parkin disease cases. Analysis revealed a significantly greater mean cell density in the dorsal tier of the parkin disease cases (19.16 neurons/mm\(^2\)) than in the PD cases (14.28 neurons/mm\(^2\)) (\( P = .04 \); Table 5). The neuronal density of the ventral nigra did not differ between parkin and PD cases (\( P = .89 \)). An unadjusted standard linear regression model found that diagnosis (parkin or PD) predicted a mean 4.88 neurons/mm\(^2\) (95% CI, 0.36-9.41; \( P = .04 \)) higher dorsal SN density in parkin compared with PD cases.

![Image](image-url)
Parkin disease has recently been described as a nigropathy owing to its restricted pathology.33 Previous neuropathologic reports described marked SN neuronal loss (5 noted that the ventrolateral tier was most severely affected16,18,21,23), mild to moderate neuronal loss of the LC (11 of 13 cases), limited tau pathology27 (few neurofibrillary tangles17,18 and occasional tau-positive astrocytes19), and LBs staining positively with α-synuclein in only 3 cases.20,24,27 To our knowledge, this is the largest and most detailed neuropathologic study of parkin disease to date, and our comparison with PD and control cases led us to consider parkin disease as a ventral-predominant nigropathy with additional involvement of the LC. In contrast to PD, marked neuronal loss of the DMV, NBM, and the midbrain raphe was not found; LBs were rare and, when observed, were sparse.

Lewy bodies have been observed in other genetic forms of parkinsonism: neurodegeneration with brain iron accumulation associated with the PLA2G6 mutation (NBIA type 2 or PARK14)34 and recently in a young patient (age, 39 years) with compound heterozygous PINK1 mutations.35 In PARK8 (leucine-rich repeat kinase 2), LBs appear to be more commonly found than in parkin disease36 (especially in those with the G2019S mutation); however, limited nigral and LC neuronal loss in the absence of other pathology is also described. Tau pathology has been reported in a large proportion of leucine-rich repeat kinase 2 cases,37 whereas our series combined with existing reported cases would suggest there is much less tau burden in parkin disease.

The disparity between the severity of nigral loss with sparse or no LBs that we observed supports the notion that abnormal α-synuclein deposition is not an integral component of the pathology of parkin disease; therefore, it is innately different from PD. It can be argued that the LBs we identified were incidental owing to their paucity in comparison with PD. Case 5 died at age 82 years, an age at which the finding of incidental LB disease post mortem is known to occur in at least 15% of normal control subjects,38–41 and case 3 died at age 61 years and only 2 LBs were identified after thorough study. Incidental LBs were also identified in 1 of our control cases with age at death of 81 years. Incidental LB disease may also explain 2 of the LB-positive parkin cases described in the literature.24,27 Age at disease onset is another possible factor that has been linked to the finding of LBs post mortem in parkin disease (Christine Klein, MD, oral communication, June 2012). Combining our 5 cases with those previously reported and comparing those that had LBs at post mortem with those that did not, there was a significant difference in age at disease onset between the groups (mean age at onset: LB-positive cases, 46 years; LB-negative cases, 27 years; P < .001; t test). Parkin cases with LBs discovered at pathologic examination were on average 19 years older at presentation.

An incomplete loss of ubiquitin ligase activity, recognized to occur with certain missense mutations of parkin,42 might permit the formation of LBs. In vitro studies have shown that different mutant parkin isoforms have different levels of enzymatic activity, with certain point mutations exhibiting only partial loss of function or possibly toxic gain of function.42 This is exemplified by the p.R275W mutation, which retains a functional RING domain (critical for E3 ligase activity) and has preserved

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**Table 5. Comparison of Parkin and Parkinson Disease Cases**

<table>
<thead>
<tr>
<th>Case</th>
<th>Mean (Range)</th>
<th>Parkinson Disease</th>
<th>P Value</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2</td>
<td>5</td>
<td>.90</td>
<td>t test</td>
</tr>
<tr>
<td>Female</td>
<td>3</td>
<td>0</td>
<td>.84</td>
<td>MWU</td>
</tr>
<tr>
<td>Age at onset, y</td>
<td>33.6 (25-46)</td>
<td>45.2 (36-64)</td>
<td>.09</td>
<td>t test</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>38.3 (27-50)</td>
<td>26.1 (21-31)</td>
<td>.03</td>
<td>MWU</td>
</tr>
<tr>
<td>Age at death, y</td>
<td>71.9 (60-86)</td>
<td>71.3 (61-81)</td>
<td>.04</td>
<td>MWU</td>
</tr>
<tr>
<td>VNN density, neurons/mm²</td>
<td>8 (3-13)</td>
<td>7.7 (6-11)</td>
<td>.89</td>
<td>t test</td>
</tr>
<tr>
<td>DNN density, neurons/mm²</td>
<td>19.2 (15-22)</td>
<td>14.3 (10-20)</td>
<td>.04</td>
<td>t test</td>
</tr>
<tr>
<td>Total nigra neuronal density, neurons/mm²</td>
<td>14.3 (12-18)</td>
<td>11.7 (9-14)</td>
<td>.12</td>
<td>t test</td>
</tr>
<tr>
<td>Ratio of VNN/DNN</td>
<td>0.41 (0.12-0.60)</td>
<td>0.55 (0.39-0.77)</td>
<td>.26</td>
<td>t test</td>
</tr>
</tbody>
</table>

**Abbreviations:** DNN, dorsal nigral neuronal; MWU, Mann-Whitney U test; VNN, ventral nigral neuronal.

*Significant at the P < .05 level.

**Figure 3. Clustered box plot illustrating neuronal density in ventral, dorsal, and combined (total) nigral tiers in the parkin and Parkinson disease (PD) cases.** The asterisk indicates an extreme score (ie, the value is more than 3 box lengths from the upper quartile) and the black dot indicates an outlier (ie, the value is more than 1.5 box lengths from the lower quartile).
ubiquitylation activity and the capacity to produce aggresomes. Alternatively, it has been proposed that parkin mutations resulting in loss of RING domain function are unable to form LBs as α-synuclein cannot be ubiquitinated. The p.R275W missense mutation was present in 4 of our cases (2 of which had LBs) and also in the case with LBs reported by Farrer and colleagues. We postulate that if the p.R275W-mutated protein is able to ubiquitinate some of its substrates to form an aggresome, then LBs could occur.

Another consideration is that 2 of the LB-positive cases descended from pedigrees not reflective of simple autosomal recessive inheritance—parkinsonism was seen in consecutive generations.

All of our cases had the characteristic clinical features felt to be particular for parkin disease—early-onset tremor often combined with dystonia and sustained response to dopaminergic medication. All experienced troublesome severe l-dopa–induced motor fluctuations. Freezing of gait and painful off-period dystonia were other common features. Three patients were described as having abnormal postures, either stooped forward or laterally flexed (scoliotic), which may reflect the chronicity of truncal dystonia throughout their disease course. The lack of cognitive and psychiatric features experienced in our patients might reflect sparing of the NBM and cerebral cortex. Only 1 patient experienced brief visual hallucinations at the end of her life (while taking levodopa and selegiline therapy), and no patients were reported as having delirium, amnesia, or dementia, despite their long disease duration (range, 27-50 years). This is important for clinical practice and may also reflect the importance of the parkin gene in the pathogenesis of parkinsonism.

In conclusion, parkin disease appears clinically and pathologically distinct from PD. It can be conceptualized as an early-onset, slowly progressive, levodopa-responsive parkinsonism without hallucinations or dementia due to neuronal loss in the ventral SN.

Accepted for Publication: January 21, 2013.
Published Online: March 4, 2013. doi:10.1001 /jamaneurol.2013.172

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Author Contributions: Study concept and design: Doherty, Silveira-Moriyama, Healy, Ahmed, Hardy, Lynch, Revesz, Lees, and Holton. Acquisition of data: Doherty, Parkkinen, Revesz, Mencacci, Brett, Quinn, Counihan, Lynch, Revesz, and Holton. Analysis and interpretation of data: Doherty, Silveira-Moriyama, Parkkinen, Healy, Mencacci, Quinn, Lynch, Fox, Lees, and Holton. Drafting of the manuscript: Doherty, Brett, Hardy, Lynch, and Fox. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: Doherty, Parkkinen, Lynch, and Fox. Obtained funding: Hardy. Administrative, technical, and material support: Parkkinen, Farrell, Ahmed, and Lees. Study supervision: Silveira-Moriyama, Healy, Counihan, Revesz, Lees, and Holton.

Conflict of Interest Disclosures: Dr Doherty’s work is funded by the Reta Lila Weston Trust for Medical Research. She is the beneficiary of an innovation grant from Parkinson’s UK, and in the past 3 years, she has received travel compensation to attend scientific meetings from Teva Pharmaceuticals, Ipsen, Novartis, GlaxoSmithKline, and Orion. Dr Silveira-Moriyama has received honoraria from Britannia Pharmaceuticals; grants from Parkinson’s UK, the Parkinson Disease Foundation, the Reta Lila Weston Trust, FAPESP, and the University of Campinas; and travel support from UCB Pharmaceuticals, Genus Pharmaceuticals, and Abbott Laboratories. She is employed by the Reta Lila Weston Institute of Neurological Studies and the University of Campinas. Dr Parkkinen is a career development fellow funded by the Monumental Trust Award from Parkinson’s UK. Dr Lynch has received honoraria from Abbott Laboratories, Boehringer Ingelheim, Lundbeck, and Orion; educational grants from Bayer-Schering, Biogen Idec, Lundbeck, and Medtronic; and grants from the Irish Institute of Clinical Neuroscience, and Mater College, as well as PRTLI1 funding. She serves on advisory boards for Abbott Laboratories, Novartis, UCB Pharmaceuticals, Teva Pharmaceuticals, Merck Serono, and Biogen Idec. Dr Revesz’s work is supported by grants from the Multiple System Atrophy Trust, Parkinson’s UK, and Alzheimer’s Research UK. Dr Lees serves as a consultant for Genus Pharmaceuticals and advisory board member for Novartis, Teva Pharmaceuticals, Boehringer Ingelheim, GlaxoSmithKline, Ipsen, Lundbeck, Allergan, Orion, BIAL Pharmaceuticals, Noscira, and Roche. Dr Lees has received honoraria from Novartis, Teva Pharmaceuticals, Meda, Boehringer Ingelheim, GlaxoSmithKline, Ipsen, Lundbeck, Allergan, Orion, BIAL Pharmaceuticals, Noscira, and Roche, as well as grants from the PSP Association and the Reta Lila Weston Trust for Medical Research. Dr Holton’s work is supported by the Reta Lila Weston Trust for Medical Research, Parkinson’s UK, the