

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.

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AUTOSOMAL RECESSIVE FAMILIES of juvenile-onset or young-onset parkinsonism were reported from Japan almost 20 years ago.¹ These patients had a relatively benign course, sleep benefit, and a sustained response to low doses of levodopa but with early development of interdose choreoathetosis and dystonia. Linkage studies pinpointed a locus on the long arm of chromosome 6 (6q25.2q27) and were followed by identification of a gene, which was named *parkin*.^{2,3} From the large number of parkin cases subsequently reported worldwide, a distinctive phenotype has been proposed, characterized by prominent leg tremor,⁴ foot dystonia,^{5,6} normosmia,⁷ and marked behavioral disturbances,^{5,8} although it has been hypothesized that the early age at onset rather than the presence of a *parkin* mutation⁹ is the critical determinant of the clinical pic-

ture. In some cases, there may be no detectable family history and rarely has onset in the fifth and sixth decades of life been reported.¹⁰⁻¹²

*For editorial comment
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Video available online at
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The *parkin* gene has 12 coding exons and the subsequent protein comprises 3 RING fingers¹³ 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.¹⁴ Impaired autophagy and mitophagy, protein accumulation, and mitochondrial dysfunction are the main mechanisms proposed in the development of parkinsonism due to mutations of *parkin*.¹⁵

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Table 1. Pathological Reports of Published Parkin Cases

Source	Yamamura et al, ¹⁶ 1998	Mori et al, ¹⁷ 1998	Hayashi et al, ¹⁸ 2000	Van de Warrenburg et al, ¹⁹ 2001	Farrer et al, ²⁰ 2001	Mori et al, ²¹ 2003	Gouider-Khouja et al, ²² 2003
Parkin status	Homozygous	Homozygous	Homozygous	Compound heterozygous	Compound heterozygous	Compound heterozygous	Homozygous
Sex	Female	Male	Male	Male	Male	Male	Male
Ethnicity	Japanese	Japanese	Japanese	Dutch	North American	Japanese	Tunisian
Siblings affected	2 of 6	3 of 5	3 of 8	2 of 3	0 of 1 (+father)	0	2 of 3
Age at disease onset, y	20	24	32	18	41	30	34
Age at death, y	52	62	70	75	52	47	47
Disease duration, y	33	38	38	57	11	17	13
Cause of death	NA	Ileus	Pneumonia	Cardiac failure	Accident	NA	Cerebral abscess
Presenting symptom	Slowness	Foot tremor	Gait difficulty	Leg tremor	Gait difficulty	Gait difficulty	Hand tremor
SNpc depigmented	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Neuronal loss in SN	Yes	Yes	Yes	Yes	Yes	Yes	Yes
SN tier most depleted	VL	VL	VL	NA	NA	VL	NA
SN gliosis	Yes	Yes	Yes	Yes	NA	Yes	Yes
Neuronal loss in LC	Yes	Yes	Yes	No	Yes	Yes	Yes
Lewy bodies present	No	No	No	No	Yes	No	No
Tau deposition	NA	Yes	Yes	Yes	No	No	No

Abbreviations: LC, locus coeruleus; NA, not available; SN, substantia nigra; SNpc, substantia nigra pars compacta; VL, ventrolateral.

Table 2. Additional Pathological Reports of Published Parkin Cases

Source	Sasaki et al, ²³ 2004	Pramstaller et al, ²⁴ 2005	Orimo et al, ²⁵ 2005	Orimo et al, ²⁵ 2005	Torres et al, ²⁶ 2011	Miyakawa et al, ²⁷ 2013
Parkin status	Homozygous	Compound heterozygous	Homozygous	Homozygous	Compound heterozygous	Homozygous
Sex	Female	Male	Male	Male	Female	Female
Ethnicity	Japanese	Italian	Japanese	Japanese	Peruvian	Japanese
Siblings affected	1 of 1	NA	3	NA	2 of 4	0 ^a
Age at disease onset, y	33	49	24	28	About 15	61
Age at death, y	70	73	73	66	NA	72
Disease duration, y	37	23	49	38	>40	11
Cause of death	Pneumonia	Embolus	NA	NA	NA	Pneumonia
Presenting symptom	Gait difficulty	Arm tremor	Hand tremor	Tremor	NA	Gait difficulty
SNpc depigmented	Yes	Yes	Yes	Yes	NA	Yes
Neuronal loss in SN	Yes	Yes	Yes	Yes	Yes	Yes
SN tier most depleted	VL	NA	NA	NA	NA	NA
SN gliosis	Yes	Yes	Yes	Yes	NA	NA
Neuronal loss in LC	Yes	Yes	Yes	Yes	No	Yes
Lewy bodies present	No ^b	Yes	No	No	No	Yes
Tau deposition	No	No	NA	NA	NA	Yes

Abbreviations: LC, locus coeruleus; NA, not available; SN, substantia nigra; SNpc, substantia nigra pars compacta; VL, ventrolateral.

^aParents were first cousins; mother possibly had Parkinson disease.

^bBasophilic inclusions in the pedunculopontine nucleus (α -synuclein and ubiquitin positive).

Parkinson disease (PD) is characterized pathologically by severe loss of dopaminergic neurons in the substantia nigra (SN), with numerous cytoplasmic inclusions containing α -synuclein, known as Lewy bodies (LBs), in the surviving neurons. Autopsy reports of patients with parkinsonism carrying 2 mutations in the *parkin* 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**).¹⁶⁻²⁷

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 *parkin* 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

the Human Tissue Authority. None had received a diagnosis of parkin disease in life, but they were genetically tested following suspicion raised on review of available clinical and family history data. Five PD control cases and 4 normal control cases matched first for age at death then disease duration (PD only) were also selected for comparison.

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).²⁸ 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 Consortium²⁹ and also by Braak stage.³⁰ 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- μ m 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 method; single-section counts of all neuromelanin-containing neurons, with or without a visible nucleus, in the SN pars compacta were obtained by using Image-Pro Plus software package (Media Cybernetics). All the counts were performed twice by the same investigator blinded to clinical data. Each SN pars compacta was outlined and further divided into ventral and dorsal tiers as described in detail elsewhere.³¹ Pars lateralis was excluded from the analysis because, in this region, there is a considerable mixing of neuronal types. The software automatically divided each examined area into a number of nonoverlapping counting squares of equal size of 300 μ m $\times$ 300 μ m, where all pigmented neurons were counted at $\times$ 200 magnification. The number of these squares was used to determine the surface area and, finally, the neuronal density was expressed as neurons per square millimeter. Single-section counting has been shown to be as reliable as the disector method in evaluating the neuronal loss from SN.³²

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 assess the increased odds of having more severe neuronal loss (or gliosis) and take into account clustering among individuals.

In the second analysis, only parkin and PD groups were compared with regard to the neuronal densities in the ventral, dorsal, and total (ventral and dorsal combined) nigral tiers. Neuronal counts in the SN were performed twice on each case (5 parkin and 5 PD). Intrarater reliability for the nigral neuronal counts was assessed with intraclass correlation. Intraclass correlation coefficients were greater than 0.80 for all the ratings performed, reflecting high reliability. The means of the 2 counts for each given nigral area were then plotted and assessed for normality. Independent-sample *t* tests or Mann-Whitney *U* tests were used depending on the distribution. *P* values of less than .05 were considered to indicate a significant trend. Linear regression was used to assess what proportion of the variation of each outcome variable was explained by each predictor variable (*R*²) and to quantify differences between individuals with parkin disease and those with PD. SPSS version 19 (SPSS Inc) and STATA version 11.2 (StataCorp) statistical programs were used to analyze the data.

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 medication (combined levodopa/carbidopa/entacapone, 150/37.5/200 mg, 4 times per day, and selegiline hydrochloride, 5 mg, twice daily) still improved 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.

Table 3. Clinical and Genetic Details of Parkin Disease Cases

Case	1	2	3	4	5
Nationality	British	British	Irish	Irish	British
Sex	Female	Female	Female	Male	Male
Age at onset, y	36	25	33	32	46
Age at death, y	86	62	60	68	82
Disease duration, y	50	37	27	36	36
Presenting symptoms	Toes turning up, leg pain	Hand spasm and tremor	Hand and leg tremor	Tremor, gait difficulties	Leg tremor
Presenting signs	Foot dystonia, leg tremor	Hand tremor and dystonia	Hand and arm tremor	Tremor	Bilateral leg tremor
Posture	Kyphoscoliotic	Kyphoscoliotic	Stooped	Normal	Normal
Visual hallucinations	Yes, brief	No	No	No	No
Dementia	No	No	No	No	No
Late disease features	Falls, freezing of gait	Painful dystonia	Painful dystonia	Rapid on/off, dystonia	Falls, freezing of gait, pain
Initial diagnosis	Dystonia	YOPD	ET	YOPD	BTP
Final clinical diagnosis	PD	YOPD	PD	YOPD	PD
Siblings affected (No.)	No	Yes (1 of 5)	Yes (1)	Yes (2 of 7)	No
Mutation 1	R275W	R275W	R275W	G430D	R275W
Mutation 2	Del exon 6	Pro113fs	G430W	Pro113fs	Del exon 6

Abbreviations: BTP, benign tremulous parkinsonism; ET, essential tremor; PD, Parkinson disease; YOPD, young-onset PD.

Table 4. Neuropathology of Parkin Disease Cases

Case	1	2	3	4	5
Neuronal loss in SN	Moderate	Moderate	Severe	Severe	Severe
Neuronal loss in LC	Mild	Mild	Moderate	Moderate	Mild
Lewy bodies present	No	No	Yes	No	Yes
VNN density, neurons/mm ²	5.55	12.83	2.60	7.67	11.11
DNN density, neurons/mm ²	16.72	21.55	20.88	15.45	21.23
Total nigral neuronal density, neurons/mm ²	12.21	18.25	12.53	12.63	16.13
Ratio of VNN/DNN density	0.33	0.60	0.12	0.50	0.52
A β diffuse deposits	Severe	None	None	Severe	Mild
A β mature deposits	Mild	None	None	Severe	None
CAA	None	None	Mild	None	None
Braak & Braak AD stage	II	None	None	I	I
Small-vessel disease	Mild	Mild	None	None	Mild
TDP-43-positive inclusions	None	None	None	None	None

Abbreviations: A β , amyloid- β ; AD, Alzheimer disease; CAA, cerebral amyloid angiopathy; DNN, dorsal nigral neuronal; LC, locus coeruleus; SN, substantia nigra; TDP-43, TAR DNA-binding protein 43; VNN, ventral nigral neuronal.

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.

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. **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 immunochemistry 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 criteria²⁹

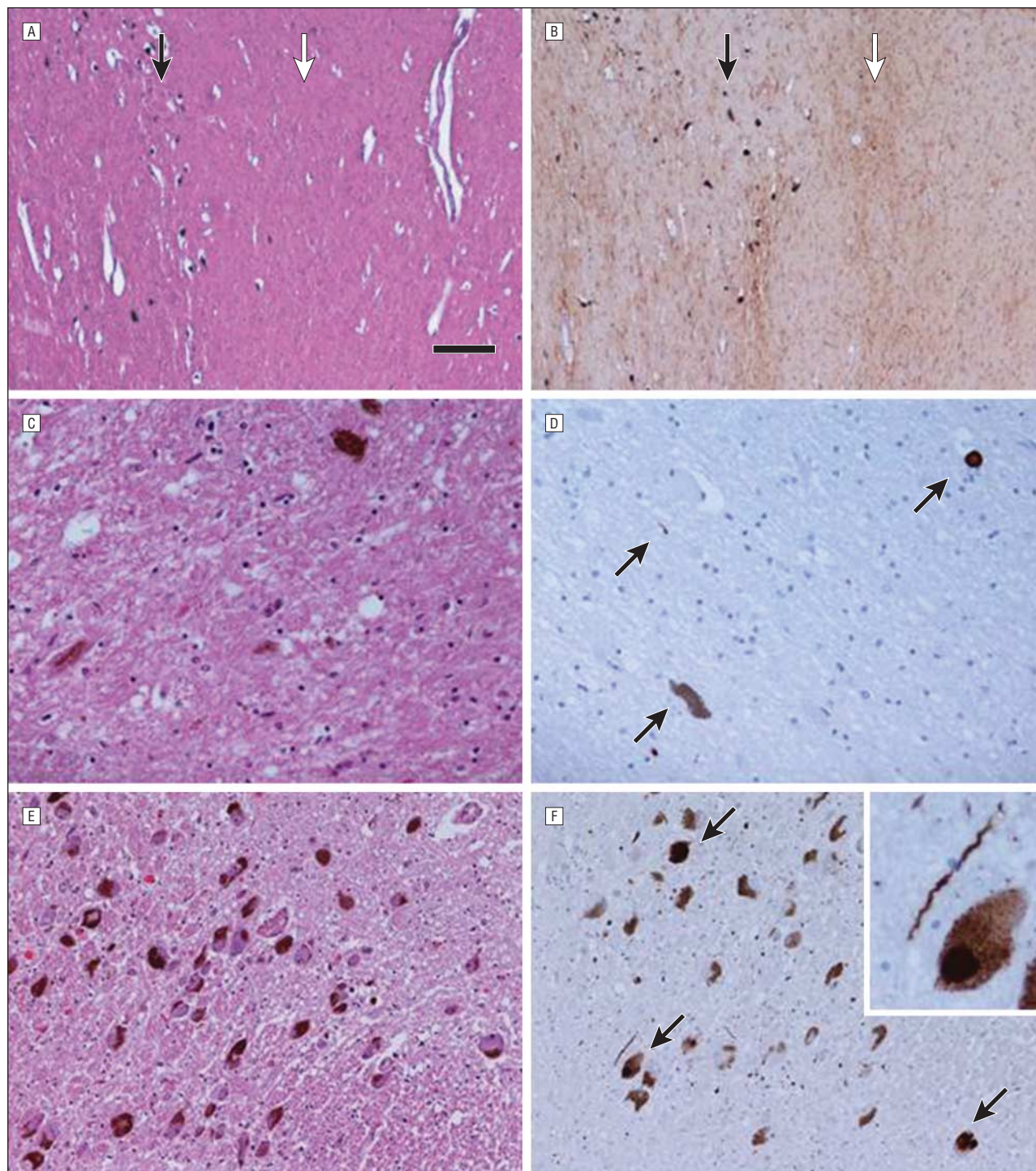


Figure 1. Histologic findings in the parkin cases. Case 2 substantia nigra illustrates the more severe neuronal loss in the ventral tier (white arrow) compared with the dorsal tier (black arrow) (A), accompanied by severe gliosis (white arrow) in the ventral tier compared with the dorsal tier (black 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 μ m in parts A and B, 50 μ m in parts C and D, 100 μ m in parts E and F, and 25 μ m 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.

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 (Figure 1E and F). Only 1 or 2 LBs were identified in the NBM, transentorhinal cortex, amygdala, and cingulate cortex sections, and the pat-

tern 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

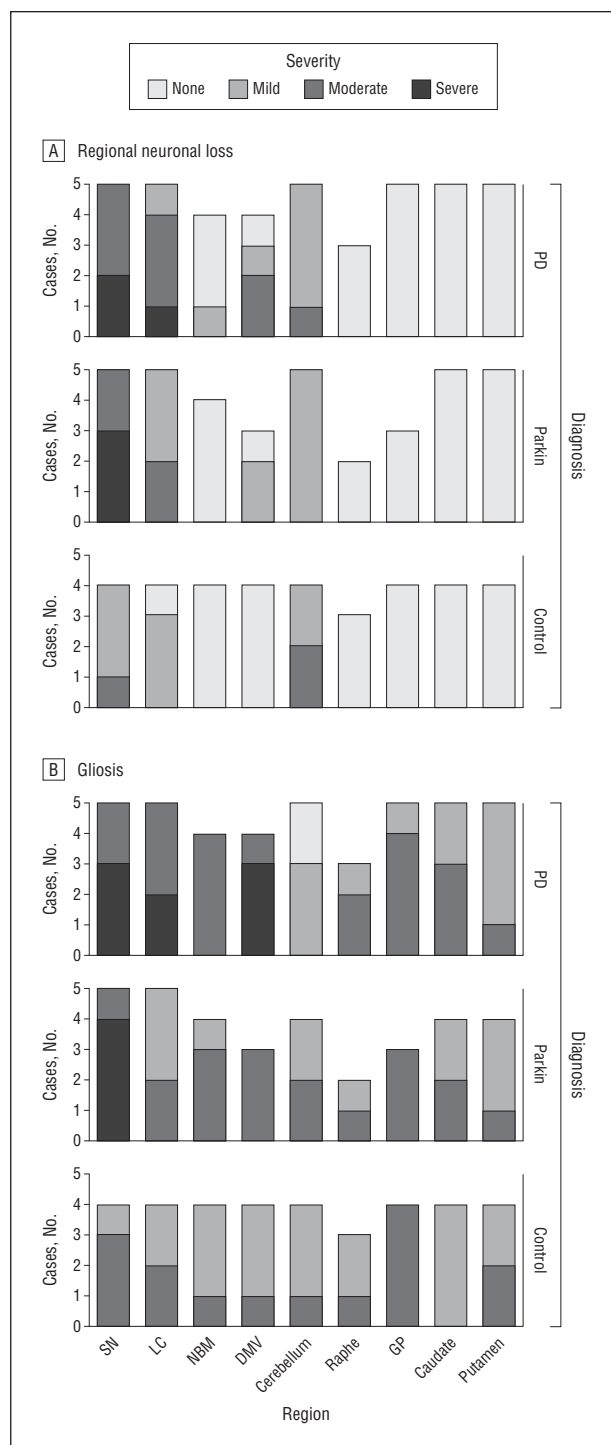


Figure 2. Regional neuronal loss (A) and gliosis (B) in parkin, Parkinson disease (PD), and control cases. A, The severity of neuronal loss in various brain regions in the 5 parkin, 5 PD, and 4 control cases (not all structures were available for examination in all cases [eg, the raphe was only available for examination in 3 parkin, 2 PD, and 3 control cases]). B, The severity of gliosis in various brain regions in the parkin, PD, and control cases. GP indicates globus pallidus; DMV, dorsal motor nucleus of the vagus; LC, locus coeruleus; NBM, nucleus basalis of Meynert; SN, substantia nigra.

exceed Braak and Braak stage II pathology. Sparse tau immunoreactive neuropil threads were identified in the SN in 2 cases (cases 1 and 4) and in the LC in case 5. Tau pathology characteristic of other tauopathies, such as tufted astrocytes or astrocytic plaques, was not observed.

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 DMV 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/mm²) than in the PD cases (14.28 neurons/mm²) ($P = .04$; 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/mm² (95% CI, 0.36-9.41; $P = .04$) higher dorsal SN density in parkin compared with PD cases.

Table 5. Comparison of Parkin and Parkinson Disease Cases

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

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

^aSignificant at the *P* < .05 level.

COMMENT

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 affected^{16-18,21,23}), mild to moderate neuronal loss of the LC (11 of 13 cases), limited tau pathology²⁷ (few neurofibrillary tangles^{17,18} 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

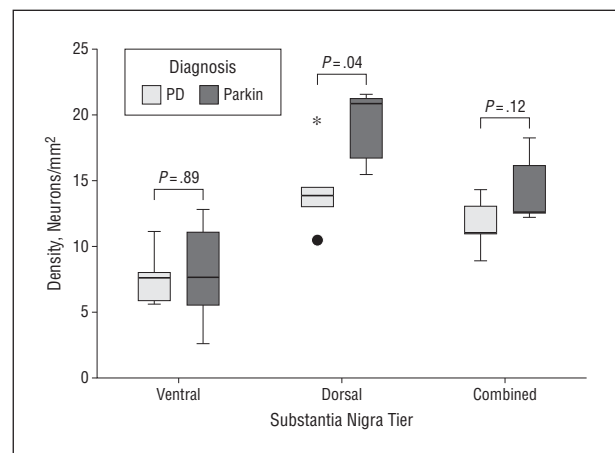


Figure 3. Clustered box plot illustrating neuronal density in ventral, dorsal, and combined (total) nigral tiers in the parkin and Parkinson disease (PD) cases. The asterisk indicates an extreme score (ie, the value is more than 3 box lengths from the upper quartile) and the black dot indicates an outlier (ie, the value is more than 1.5 box lengths from the lower quartile).

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

ubiquitylation activity⁴² and the capacity to produce aggresomes.⁴³ 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.⁴⁴ 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.²⁰ 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^{20,24} 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 allay some patient fears in considering the long-term aspects of their parkin disease and its management.

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.

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