Nigral Pathology and Parkinsonian Signs in Elders without Parkinson Disease

Aron S. Buchman, MD,[1,2] Joshua M. Shulman, MD, PhD,[3,4] Sukriti Nag, MD, PhD,[1,5]
Sue E. Leurgans, PhD,[1,2] Steven E. Arnold, MD,[5] Martha C. Morris, ScD,[6]
Julie A. Schneider, MD, MS,[1,2,7] and David A. Bennett, MD[1,2]

Objective: Motor symptoms such as mild parkinsonian signs are common in older persons, but little is known about their underlying neuropathology. We tested the hypothesis that nigral pathology is related to parkinsonism in older persons without Parkinson disease (PD).

Methods: More than 2,500 persons participating in the Religious Orders Study or the Memory and Aging Project agreed to annual assessment of parkinsonism with a modified version of the Unified Parkinson Disease Rating Scale and brain donation. Brains from 744 deceased participants without PD were assessed for nigral neuronal loss and α-synuclein immunopositive Lewy bodies.

Results: Mean age at death was 88.5 years. Mean global parkinsonism was 18.6 (standard deviation, 11.90). About $\frac{1}{3}$ of cases had mild or more severe nigral neuronal loss, and about 17% had Lewy bodies. In separate regression models that adjusted for age, sex, and education, nigral neuronal loss and Lewy bodies were both related to global parkinsonism (neuronal loss: estimate, 0.231; standard error [SE], 0.068; $p < 0.001$; Lewy bodies: estimate, 0.291; SE, 0.133; $p = 0.029$). Employing a similar regression model that included both measures, neuronal loss remained associated with global parkinsonism (neuronal loss: estimate, 0.206; SE, 0.075; $p = 0.006$). By contrast, the association between Lewy bodies and global parkinsonism was attenuated by >60% and was no longer significant (Lewy bodies: estimate, 0.112; SE, 0.148; $p = 0.447$), suggesting that neuronal loss may mediate the association of Lewy bodies with global parkinsonism.

Interpretation: Nigral pathology is common in persons without PD and may contribute to loss of motor function in old age.

The growing burden of motor impairment in our aging population is an urgent public health challenge. The specific motor abilities impaired in old age vary and can include reduced gait speed and loss of muscle strength and bulk, balance, and dexterity. Currently, there is no single, universally accepted scale for documenting motor impairment in old age. Several community-based studies report that mild parkinsonian signs, including motor slowing (bradykinesia), gait and posture disturbances, rigidity, and rest tremor are common in old age.[1–3] Depending on the definition employed, mild parkinsonian signs may be present in up to 50% of community-dwelling older persons without Parkinson disease

(PD) over age 85 years.[3] Although the literature labels these signs as "mild" they are not benign, but rather are associated with a wide range of adverse health outcomes, including an increased risk of death and development of disability, mild cognitive impairment, Alzheimer disease (AD), and cognitive decline.[1,4–7]

Despite the growing awareness of the scope and significance of mild parkinsonian signs in older persons, treatments are limited, because little is known about their underlying neuropathology. Current information about the neuropathology of parkinsonism comes almost exclusively from studies of the substantia nigra from persons with clinically diagnosed PD.[8] Neuropathologically, PD

is characterized by loss of nigral melanin-pigmented do-paminergic neurons that project to the striatum, and by the formation of Lewy bodies, intracytoplasmic aggregated α-synuclein protein in these same neurons.[9,10] The prevalence of PD is estimated to be from 2 to 5% by age 85 years, suggesting that it would not account for reports of parkinsonism affecting up to 50% of older individuals. Few studies have examined whether nigral neuronal loss[11,12] and Lewy bodies[13] are related to parkinsonism in older persons without PD.

More than 2,500 persons have been recruited to participate in the Religious Orders Study (ROS) or the Rush Memory and Aging Project (MAP), 2 clinicopathologic cohort studies of chronic conditions of aging. Participants in both studies agreed to annual evaluation and brain donation. For the current study, we used clinical and postmortem data from the first 744 cases without PD to test the hypothesis that nigral neuronal loss and Lewy bodies are related to parkinsonism in older persons without PD. In further analyses, we examined whether nigral neuronal loss and Lewy bodies had separate effects when considered together or whether neuronal loss might mediate the association of Lewy bodies with parkinsonism.

Subjects and Methods

Subjects

Participants are from 2 ongoing studies of aging approved by the institutional review board of Rush University Medical Center. Each subject signed an informed consent for annual exam and an anatomic gift act for donation of brain at the time of death. Both studies employ common antemortem and postmortem data collection, allowing analyses of data from the combined cohorts.

At the time of this study, completed postmortem data were available for 756 persons (ROS, n = 467; MAP, n = 289). Because our focus was on individuals without PD, we excluded 12 persons (ROS, n = 7 [1.5%]; MAP, n = 5 [1.7%]) with both clinical PD (self-report history of PD including L-dopa treatment at any time prior to death) and postmortem evidence for PD (nigral Lewy bodies with moderate or severe neuronal loss). Our primary analyses included 15 participants with clinically diagnosed PD but without postmortem evidence of PD. None had nigral Lewy bodies, but 9 had mild (n = 5) or moderate (n = 4) neuronal loss. Compared to MAP participants, ROS participants were younger at death and had higher levels of education, but they did not differ with respect to sex, level of parkinsonian signs, or Mini-Mental State Examination proximate to death (results not shown).

Clinical Evaluation and Assessment of Parkinsonian Signs

Participants in both studies undergo a uniform structured clinical evaluation each year that includes medical history, neuro-

logic examination, and neuropsychological performance tests.[14,15] Trained nurses assessed 26-items from the motor section of the Unified Parkinson Disease Rating Scale (UPDRS) on a 0 to 5 scale (Supplementary Table 1).[16] Four previously established parkinsonian sign scores (gait disturbance, bradykinesia, rigidity, and tremor) were derived from these 26 items. A summary global parkinsonian sign score was constructed by averaging these 4 scores (Supplementary Methods). The average interval between last clinical examination and death was on average 10.8 months (standard deviation [SD], 11.29 months).

Postmortem Evaluation

The average postmortem interval was 8.3 hours (SD, 8.24 hours). A complete neuropathologic evaluation was performed.[17] Dissection of diagnostic blocks included a hemisection of midbrain, which included substantia nigra. Nigral neuronal loss was assessed in the substantia nigra in the mid to rostral midbrain near or at the exit of the 3rd nerve using hematoxylin & eosin stain and 6μm sections using a semiquantitative scale (0–3) shown in the Figure.[17] Nigral neuron density of tyrosine hydroxylase immunoreactive neurons was determined in 4 quadrants in a subset of cases (Supplementary Methods). Lewy bodies were identified with antibodies to α-synuclein using alkaline phosphatase as the chromogen.[17] A tissue diagnosis of PD was based on the presence of nigral Lewy bodies and moderate or severe nigral neuronal loss.[9]

Postmortem indices of AD pathology and cerebrovascular disease were collected as previously described.[17] Neuron density measures were also obtained in several cortical and spinal cord regions (Supplementary Methods).

Statistical Analysis

We used regression analyses of global parkinsonism and each of the 4 parkinsonian signs with terms for demographic variables. We used a generalized logit model for the degree of nigral neuronal loss with terms for Lewy bodies and for demographic variables to assess the association of nigral neuronal loss with Lewy bodies.

In our primary analyses, we employed separate regression analyses controlling for age, sex, and education to document the association of nigral neuronal loss and of Lewy bodies with global parkinsonism. Next we tested the hypothesis that neuronal loss mediates the association of Lewy bodies with parkinsonian signs. We included terms for both neuronal loss and Lewy bodies in a single model and examined the effect of neuronal loss on the association of Lewy bodies and global parkinsonism. If neuronal loss mediates the association (ie, is a critical step in the causal chain linking Lewy bodies to parkinsonism), then the effect of Lewy bodies on parkinsonism should be markedly reduced. Although mediation and confounding are identical statistically, they can be distinguished on conceptual grounds.[18] Recent reports support the plausibility of a causal sequence in which the presence of Lewy bodies leads to neuronal loss, which leads to clinical parkinsonism.[8,19] We then repeated the previous models in a series of sensitivity analyses to determine whether subsets of nigral pathology affected our findings. We
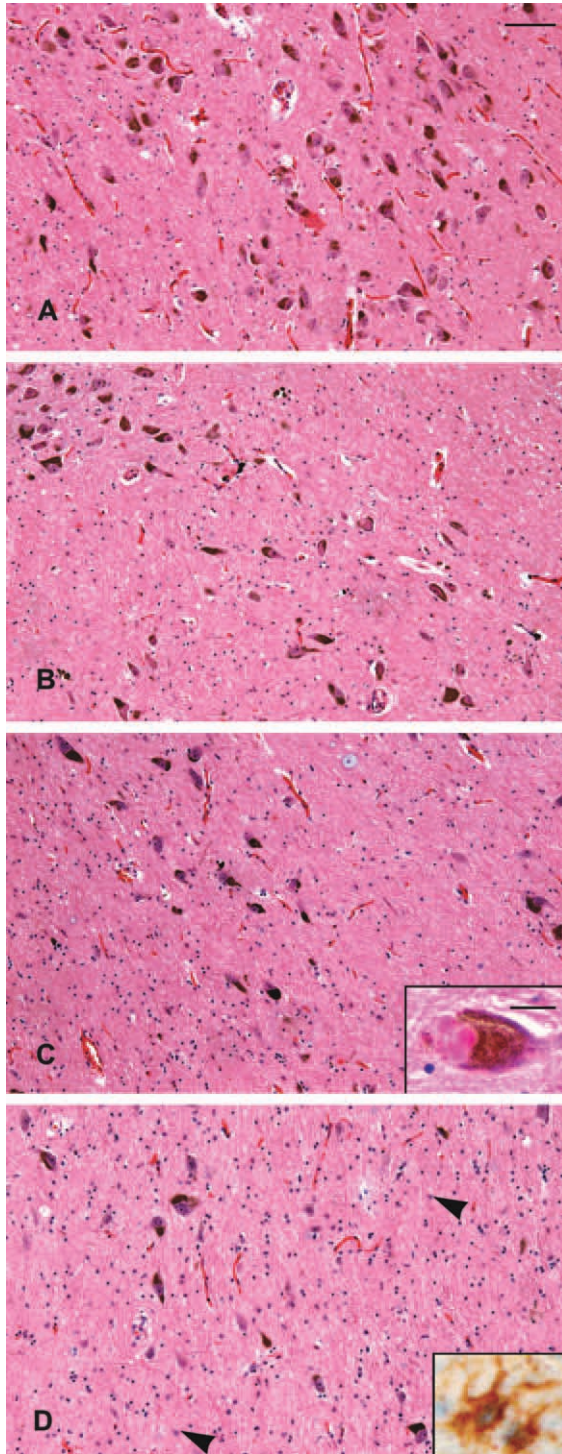
FIGURE: Spectrum of nigral neuronal degeneration. Substantia nigra showing (A) no neuronal loss, (B) mild neuronal loss, (C) moderate neuronal loss, and (D) severe neuronal loss. The inset in C shows a neuron containing Lewy bodies. Severe neuronal loss in D is associated with atrophy of remaining neurons and astrogliosis *(arrowheads)*. The inset in D shows fibrillary astrocytes with immunoreactivity with glial fibrillary acid protein (Novacastra Laboratories, Newcastle upon Tyne, UK; 1:1,000 antibody dilution). Scale bar for panels A–D = 200μm; scale bar for both insets = 12.5μm.

also examined several potential confounding variables obtained at the last visit prior to death (Supplementary Methods). A similar analytic approach was employed to examine the association of nigral pathology with each of the 4 individual parkinsonian signs. We employed linear regression models to examine parkinsonian gait, tobit regression models for bradykinesia, and logistic regressions for presence or absence of tremor and rigidity. We compared nigral neuronal loss to neuron density in several other cortical and spinal cord regions. Model assumptions of linearity, normality, independence of errors, and homoscedasticity of errors were examined graphically and analytically and were adequately met. All analyses were carried out using SAS/STAT software version 9 (SAS Institute, Cary, NC) on a Hewlett Packard ProLiant ML350 server running LINUX.[20]

Results

Summary of Parkinsonian Signs and Nigral Pathology Measures

There were 744 participants (62.5% female) without PD included in the primary analyses, and their clinical characteristics proximate to death and measures of nigral pathology are included in Table 1. Global parkinsonism was associated with age (estimate, 0.07; standard error [SE], 0.008; $p < 0.001$) but not with sex (estimate, −0.03; SE, 0.105; $p = 0.769$) or education (estimate, −0.003; SE, 0.014; $p = 0.834$).

Nigral pathology was common, with 39% of cases showing at least some degree (Table 2). However, the combination of both neuronal loss and Lewy bodies was observed in only 10% of cases (group 4). About 30% had either Lewy bodies alone with preserved neuronal numbers (group 2) or neuronal loss alone without Lewy bodies (group 3). The distributions of the individual modified UPDRS item scores for each of these 4 groups are shown in Supplementary Table 2.

The semiquantitative measure used to assess nigral neuron loss was associated with nigral neuron density based on computer-assisted counts (ρ, 0.43, $p < 0.001$). In particular, using a method adapted from Ross et al[12] (Supplementary Methods), the semiquantitative measure was related to neuron density in the ventral medial and lateral quadrants and dorsal medial quadrant (all $p < 0.01$) but not in the dorsal lateral quadrant ($p = 0.251$). Comparing cases with and without nigral neuronal loss, neuron density was reduced in the ventral tier in both the lateral and medial quadrants as well as in the dorsal medial quadrant (Supplementary Table 3A). Neuron density in cases with both Lewy bodies and neuronal loss (group 4) was reduced by 30 to 60% in all 4 quadrants as compared to cases without nigral pathology (see Supplementary Table 3B); however, these latter differences

TABLE 1: Clinical Characteristics of the Cohort Proximate to Death (N = 744)

Variable	Value
Age at death, mean yr (SD)	88.5 (5.38)
White, non-Hispanic, No. [%]	79 [96.3]
Education, mean yr (SD)	13.9 (3.14)
Last Mini-Mental State Exam, max 30 (SD)	22.7 (8.86)
Global parkinsonism, max 100 (SD)	18.3 (11.90)
Parkinsonian gait, max 100 (SD)	41.2 (23.58)
Rigidity, max 100 (SD)	12.2 (17.74)
Tremor, max 100 (SD)	4.4 (7.51)
Bradykinesia, max 100 (SD)	20.0 (16.49)
Medical conditions, No. [%]	
Cancer	308 [41.5]
Hypertension	342 [46.2]
Stroke	178 [24.0]
Myocardial infarction	174 [23.4]
Thyroid	157 [21.1]
Diabetes	141 [19.0]
Head injury	55 [7.4]
Musculoskeletal pain	237 [32.0]
Assistive devices for walking at last visit	281 [38.0]

SD = standard deviation.

reached statistical significance for only the ventral lateral and medial quadrants (see Supplementary Table 3C).

A generalized logit model that controlled for age, sex, and education showed that the presence of Lewy bodies increased the ratio of the probability of each level of neuronal loss relative to the probability of no neuronal loss ($p = 0.002$). The ratio for mild neuronal loss is doubled in the presence of Lewy bodies, is increased 16-fold for moderate neuronal loss, and is increased 45-fold for severe neuronal loss.

Association of Nigral Pathology and Global Parkinsonism

In separate regression analyses adjusting for age, sex, and education, neuronal loss and Lewy bodies were each associated with global parkinsonism (Table 3, models A and B). In current models of the pathogenesis of PD, the development of Lewy bodies is proposed as part of a causal sequence leading to neuronal loss and clinical manifestations of PD.[8,19] To test whether a similar causal sequence might occur in individuals without PD, we conducted a form of mediation analysis by including terms for both neuronal loss and Lewy bodies in a single model. In the joint model, the association of Lewy bodies with global parkinsonism was reduced by >60% and was no longer statistically significant, whereas the association of neuronal loss with global parkinsonism was essentially unchanged (see Table 3, model C). These data suggest that neuronal loss may be a step in the causal chain linking or mediating the association of Lewy bodies with global parkinsonism. In further analyses, we added an interaction term to the previous model, which showed that the association of neuronal loss and global parkinsonism score did not vary with the presence of Lewy bodies (estimate, 0.091; SE, 0.152; $p = 0.549$).

To ensure that the inadvertent inclusion of cases with PD did not account for our results, we undertook a series of secondary analyses in which we also excluded cases that had 1 or more of the 3 elements used to diagnose PD. Because limitations in our postmortem exam could underestimate tissue evidence of PD, we excluded all participants who had a clinical history of PD regardless of whether there was postmortem evidence of PD. Our findings were unchanged (Supplementary Table 4, model 2). Next, because our clinical ascertainment of PD might underestimate its prevalence, we also excluded cases without a clinical history of PD but that showed 1 or both elements of nigral pathology. Excluding cases with moderate or severe neuronal loss with or without Lewy bodies (groups 3B or 4B) attenuated but did not eliminate the association of neuronal loss with global parkinsonism (see Supplementary Table 4, models 3–5).

TABLE 2: Distribution of Nigral Neuronal Loss and Lewy Bodies

Lewy Bodies	Nigral Neuronal Loss			Total
	None	Mild	Moderate or Severe	
No	Group 1, 454 (61.0%)	Group 3A, 144 (19.3%)	Group 3B, 23 (3.1%)	621 (83.5%)
Yes	Group 2, 41 (5.5%)	Group 4A, 31 (4.2%)	Group 4B, 51 (6.9%)	123 (16.5%)
Total	495 (66.5%)	175 (23.5%)	74 (10.0%)	

TABLE 3: Relation of Nigral Pathology and Global Parkinsonism Score

Term	Model A	Model B	Model C
Age	0.067 (0.008, <0.001)	0.069 (0.008, <0.001)	0.067 (0.008, <0.001)
Sex	−0.042 (0.104, 0.689)	−0.038 (0.105, 0.720)	−0.043 (0.104, 0.680)
Education	−0.004 (0.014, 0.778)	−0.002 (0.014, 0.874)	−0.003 (0.014, 0.780)
Neuronal loss	0.231 (0.068, <0.001)		0.206 (0.075, 0.006)
Lewy bodies		0.291 (0.133, 0.029)	0.112 (0.148, 0.447)

Estimated from regression models adjusted for age, sex, and education. Estimate (standard error, *p* value).

Lastly, we excluded 3 cases of Lewy body dementia from our primary analyses, and our results were again unchanged (see Supplementary Table 4, model 6).

Association of Nigral Pathology, Other Covariates, and Global Parkinsonism

We repeated the core models (see Table 3, models A and B) while adding terms for potential confounding variables to examine whether these conditions affected the associations of nigral pathology and global parkinsonism. These associations were unchanged when we included terms for body mass index and each of the 8 chronic conditions listed in Table 1 in a single model (neuronal loss: estimate, 0.205; SE, 0.067; $p = 0.002$; Lewy bodies: estimate, 0.329; SE, 0.133; $p = 0.014$).

Association of Nigral Pathology, Other Pathology, and Global Parkinsonism

We repeated the core models (see Table 3, models A and B) while adding terms for other common neuropathologies to examine whether these pathologies affected the associations of nigral pathology and global parkinsonism. These associations were unchanged when we included terms alone (results not shown) or together in a single model. These analyses also showed that vascular patholo-

gies and AD pathology have independent effects on the severity of parkinsonism prior to death (Table 4).

Association of Nigral Pathology and Individual Parkinsonian Signs

Next we examined the association of nigral pathology and the individual parkinsonian signs. Nigral degeneration was related to increasing signs of parkinsonian gait impairment, rigidity, and bradykinesia (Table 5, model A). The presence of Lewy bodies was associated with a higher level of rigidity (see Table 5, model B). The association of Lewy bodies and rigidity was attenuated when a term for nigral neuronal loss was added (see Table 5, model C). Tremor was not related to neuronal loss or Lewy bodies.

Neuronal Loss in Other Cortical and Spinal Cord Regions and Global Parkinsonism

To examine the specificity of the association of nigral neuronal loss with global parkinsonism, we examined the association of neuron density from 9 other CNS regions with nigral neuronal loss and global parkinsonism. Nigral neuronal loss was related to neuron density in the inferior temporal cortex and lumbar spinal cord. Of the 10 regions examined, only nigral neuronal loss was related to global parkinsonism (Supplementary Table 5).

TABLE 4: Relation of Nigral Pathology, Other Common Pathology, and Global Parkinsonism Score

Pathology Measure	Model A[a]	Model B[a]
Nigral neuronal loss	0.185 (0.067, 0.006)	
Lewy bodies		0.294 (0.132, 0.026)
Macroscopic infarcts	0.317 (0.109, 0.004)	0.324 (0.109, 0.003)
Microscopic infarcts	0.114 (0.112, 0.307)	0.126 (0.112, 0.261)
Arteriolosclerosis	0.179 (0.051, <0.001)	0.191 (0.051, <0.001)
Alzheimer disease pathology	0.312 (0.079, <0.001)	0.317 (0.079, <0.001)

[a]Estimated from a single regression model that also included terms for age, sex, and education (not shown) with the estimate (standard error, *p* value) for each term.

TABLE 5: Relation of Nigral Pathology and Individual Parkinsonian Signs

Parkinsonian Sign	Term	Model A[a]	Model B[a]	Model C[a]
Parkinsonian gait[b]	Neuronal loss	0.249 (0.098, 0.012)		0.278 (0.109, 0.011)
	Lewy bodies		0.106 (0.193, 0.582)	−0.134 (0.214, 0.533)
[c]Rigidity	Neuronal loss	0.326 (0.112, 0.004)		0.257 (0.123, 0.037)
	Lewy bodies		0.541 (0.217, 0.013)	0.327 (0.239, 0.171)
[d]Bradykinesia	Neuronal loss	0.214 (084, 0.010)		0.229 (0.093, 0.015)
	Lewy bodies		0.136 (0.162, 0.403)	−0.063 (0.181, 0.728)
[c]Tremor	Neuronal loss	−0.167 (0.104, 0.109)		0.073 (0.226, 0.747)
	Lewy bodies		−0.082 (0.202, 0.687)	−0.184 (0.116, 0.113)

Estimate (standard error, p value).
[a]All 3 models (A, B, C) included terms to adjust for age, sex, and education. In addition, model A included a term for nigral neuronal loss, model B included a term for nigral Lewy bodies, and model C included terms for both nigral neuronal loss and Lewy bodies.
[b]Based on a regression model.
[c]Based on a logistic regression model with outcome the presence or absence of rigidity or the presence or absence of tremor.
[d]Based on a tobit model with the outcome bradykinesia.

Discussion

We measured parkinsonian signs in >2,500 older persons participating in 2 longitudinal clinicopathologic studies, of whom 744 persons without PD died and underwent brain autopsy and a complete neuropathologic examination. Almost 40% had either mild or more severe nigral neuronal loss or Lewy bodies or both. When considered separately, both neuronal loss and Lewy bodies were related to parkinsonian signs proximate to death. Further analyses suggested that the association of Lewy bodies with global parkinsonism and rigidity was mediated through nigral neuronal loss. Thus, nigral pathology is common in older persons without PD and may contribute to motor impairment in old age.

The results of the current study have important public health consequences. There are currently about 40 million persons aged >65 years in the United States, and by 2030 there will be >70 million persons aged >65 years.[21] Prior work in these and other cohorts has shown that parkinsonian signs may occur in up to half of older persons without PD aged >85 years. Furthermore, these signs are not benign, but are associated with a wide range of adverse health outcomes.[3,5–7] With a public health problem of this magnitude, it is essential to understand the neuropathology that underlies parkinsonism so as to facilitate the development of treatments to decrease the growing burden of motor impairment in old age.

In the current study, nearly 20% of cases showed evidence of Lewy bodies, which is similar to other postmortem studies.[22] However, few of these studies have investigated the association of nigral Lewy bodies and neuronal loss with parkinsonian signs in older persons without PD.[11,12] An earlier study reported that neuronal loss occurs in older persons with incidental Lewy bodies and controls without PD, but did not assess signs of parkinsonism. A second study reported that nigral neuronal loss was associated with parkinsonian signs in 50 cases without a history of PD or postmortem evidence of Lewy bodies.[11,12] Nigral pathology was common in the current study, being observed in almost 40% of cases without PD. Furthermore, the presence of Lewy bodies and the degree of neuronal loss were related to severity of global parkinsonism and individual parkinsonian signs including parkinsonian gait, bradykinesia, and rigidity. These associations were robust and persisted even after controlling for demographic variables, body composition, and several chronic conditions. Together these findings suggest that nigral pathology is common and related to what is often considered normal age-related motor signs. Thus, subclinical nigral pathology may not be incidental, but may be an unrecognized contributor to the development of motor impairment in older persons without overt PD.[13,23,24]

The basis for the association between nigral pathology and parkinsonian signs in the current study is uncertain. Lewy bodies and neuronal loss are the histopathologic hallmarks of PD, which is reported to occur in up to 5% of older persons before age 85 years.[25] In the current study, we excluded a dozen cases in which there was both clinical and postmortem evidence of PD. Nonetheless, there were still >50 additional cases without a clinical history of PD but with the classic postmortem characteristics of PD (Table 2, Group 4B).[25] Furthermore, we found that cases with nigral neuronal loss showed a reduction in neuron density that extended beyond the

ventral tier (see Supplementary Table 3). However, the most significant reductions in neuron density were observed in the ventral lateral and medial quadrants, which is similar to the pattern previously reported for PD.[11,12,26] Together, these data support the notion of preclinical PD during which nigral pathology accumulates but clinical symptoms may not warrant a clinical diagnosis of PD,[13] analogous to the pathology of AD in persons without dementia.[27] Recent brain imaging studies have reported that older persons with preclinical PD not only have nigral pathology, but have reductions in striatal dopaminergic nerve terminals at levels intermediate between those of brains from subjects with known PD and controls without neuropathology.[13] Because nigral pathology in the current study was related to the severity of parkinsonian signs, this suggests that individuals with a clinical diagnosis of PD may represent the tip of the iceberg, and that nigral degeneration and Lewy bodies may also account for a substantial proportion of motor dysfunction currently considered normal aging. This has important public health implications, because it suggests that there may be a much larger number of older persons who may benefit from treatments developed for PD. This approach has not been adequately studied, because prior studies that have employed L-dopa to ameliorate motor symptoms in older persons without PD have been very small.[28] It is likely that more sensitive clinical measures may help to identify at-risk individuals prior to the development of clinical PD.[29]

The precise role of Lewy bodies and nigral neuronal loss in producing clinical signs in PD has not been resolved.[22,30] Some have suggested that there is a causal sequence such that Lewy bodies contribute to neuronal loss, which determines the level of parkinsonism, whereas others have suggested a protective role.[8,19,31] In the current study, mediation analyses were to used to examine the extent to which nigral neuronal loss represents a key step in the causal chain linking Lewy bodies with parkinsonism.[32] The results from the current analysis (Table 3, Model C) are consistent with a causal sequence in which Lewy bodies may lead to neuronal loss, which in turn may lead to more severe parkinsonism.[8,19] The sensitivity analyses that were done complement the mediation analyses, because when we excluded the group with more severe neuronal loss (group 4B), our findings were attenuated and no longer significant. Our results might also be consistent with models in which smaller α-synuclein aggregates or soluble synuclein are toxic to nigral neurons, in which case Lewy bodies are a proxy for these toxic species.[33] These analyses do not preclude the possibility that Lewy bodies and neuronal loss may be linked

to parkinsonism through other mechanisms. In this study, many cases showed evidence of either Lewy bodies alone or neuronal loss alone (Table 2, groups 2 and 3). It is unclear whether cases with only Lewy bodies represent an early stage prior to nigral neuronal loss or might be linked with parkinsonian signs through other pathways. Similarly, our mediation analyses do not preclude the possibility that neuronal loss may occur alone due to other mechanisms.

Although there were only a small number of cases, our sensitivity analyses also lend support for the speculation that more severe neuronal loss may contribute to parkinsonism even without the presence of Lewy bodies (group 3B), as has been previously suggested.[12] Our results also suggest that nigral pathology is not the only cause of parkinsonism in old age, because AD and cerebrovascular pathologies measured in other brain regions were also associated with the severity of parkinsonism (Table 4) and may contribute to neuronal loss without Lewy bodies.[34,35] Although these findings were statistically significant, together nigral, AD, and cerebrovascular pathologies explain only a modest amount of the variance of parkinsonism. There are several reasons why our study may have underestimated the contributions of these pathologies to mild parkinsonian signs in old age. The postmortem indices for AD pathology were preferentially collected from traditional cognitive-related brain regions, whereas other motor-related regions rostral and caudal to the substantia nigra were not examined and are likely to make separate contributions to the severity of parkinsonism. Furthermore, other known traditional pathologies such as white matter loss were not measured. Further studies are needed to replicate these findings and to determine the other neuropathologies and mechanisms that contribute to parkinsonian signs in old age.

There are several strengths to the study, including the community-based cohort with large numbers of women and men coming to autopsy following high rates of clinical follow-up and high autopsy rates. Uniform structured clinical procedures were used that included a detailed assessment of parkinsonian signs that has been widely used in other studies. Uniform postmortem procedures assessed several postmortem indices of PD. There are a number of limitations too. The current study only evaluated nigral pathology in 1 region in a hemisection of the substantia nigra, so it may underestimate the presence of both neuronal loss and Lewy bodies in this structure. The study did not assess other brainstem and extranigral regions to allow comparison with other studies with more complete Braak staging of PD pathology.[9,10] It will be important for future studies to fully assess nondopaminergic neurons, that is, noradrenergic neurons

known to be sensitive to α-synuclein pathology. The cohort is selected and replication of these findings in a more general population is needed. This study was large, because on an individual level the effect sizes are small. Nonetheless, from a public policy perspective, given the extent of motor impairments in old age, even the modest effect sizes observed in the current study are likely to be important. Further studies are needed to more fully explicate the types and locations of pathologies that underlie mild parkinsonian signs in old age.

Acknowledgment

Potential Conflicts of Interest

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