Gait speed in Parkinson disease correlates with cholinergic degeneration

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ABSTRACT

Objective: We investigated dopaminergic and cholinergic correlates of gait speed in Parkinson disease (PD) and non-PD control subjects to test the hypothesis that gait dysfunction in PD may result from multisystem degeneration.

Methods: This was a cross-sectional study. Subjects with PD but without dementia ($n = 125$, age 65.6 ± 7.3 years) and elderly subjects without PD ($n = 32$, age 66.0 ± 10.6 years) underwent [^{11}C]dihydrotetrabenazine dopaminergic and [^{11}C]methyl-4-piperidiny propionate acetylcholinesterase PET imaging, and cognitive and clinical testing, including an 8.5-m walk in the dopaminergic “off” state. The fifth percentile of cortical cholinergic activity in the elderly without PD was used to define normal-range activity in the subjects with PD.

Results: Normal-range cortical cholinergic activity was present in 87 subjects with PD (69.6%). Analysis of covariance using gait speed as the dependent variable demonstrated a significant model ($F = 6.70$, $p < 0.0001$) with a significant group effect ($F = 3.36$, $p = 0.037$) and significant slower gait speed in the low cholinergic PD subgroup (0.97 ± 0.22 m/s) with no significant difference between the normal-range cholinergic PD subgroup (1.12 ± 0.20 m/s) and control subjects (1.17 ± 0.18 m/s). Covariate effects were significant for cognition ($F = 6.58$, $p = 0.011$), but not for striatal dopaminergic innervation, sex, or age.

Conclusion: Comorbid cortical cholinergic denervation is a more robust marker of slowing of gait in PD than nigrostriatal denervation alone. Gait speed is not significantly slower than normal in subjects with PD with relatively isolated nigrostriatal denervation. *Neurology*® 2013;81:1611-1616

GLOSSARY

AChE = acetylcholinesterase; **ANCOVA** = analysis of covariance; **DTBZ** = dihydrotetrabenazine; **PD** = Parkinson disease; **PMP** = methyl-4-piperidiny propionate; **PPN** = pedunclopontine nucleus; **VMAT2** = vesicular monoamine transporter type 2.

The cardinal motor symptoms of Parkinson disease (PD) often develop at different times during disease progression. Upper extremity bradykinesia and rigidity typically precede axial and bilateral lower extremity motor changes, such as limitations in gait.^{1,2} Imaging studies show that nigrostriatal denervation is already severe in early clinical stage PD.³ Increased activity of other brain systems may compensate for nigrostriatal degeneration.^{4,5} If gait speed functions are not prominently affected in early PD despite significant dopaminergic losses, subsequent slowing of gait speed implicates later degeneration of nondopaminergic pathways. A recent study suggested that cholinergic dysfunction is an important contributor to gait dysfunction in PD.⁶ We reported previously that subjects with PD with combined basal forebrain cholinergic and dopaminergic deficits had lower global cognitive performance, more severe nigrostriatal denervation, and a trend toward more severe motor impairments, including slower gait, when compared to subjects with PD with normal-range cortical cholinergic innervation.⁷ In our prior study, there was no comparison with a non-PD control group and findings were not corrected for the degree of nigrostriatal denervation.

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Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

We set out to examine possible differences in gait speed among subjects with PD with relatively isolated nigrostriatal degeneration, healthy control subjects, and subjects with PD with comorbid basal forebrain cholinergic degeneration while considering the degree of nigrostriatal denervation. We hypothesized that gait speed is preserved in subjects with PD with relatively isolated nigrostriatal denervation.

METHODS Subjects and clinical test battery. This cross-sectional study involved 125 subjects with PD (95 males and 30 females), mean age 65.6 ± 7.3 (range 50–84) years and 32 control non-PD subjects (18 males and 14 females), mean age 66.0 ± 10.6 (range 50–84) years. Subjects with PD met the UK Parkinson's Disease Society Brain Bank clinical diagnostic criteria.⁸ Specifically, none of the patients had signs or symptoms to indicate an atypical parkinsonism disorder, such as multiple system atrophy or corticobasal degeneration. Use of (+)-[¹¹C]dihydrotetrabenazine (DTBZ) vesicular monoamine transporter type 2 (VMAT2) PET confirmed the presence of nigrostriatal dopaminergic denervation in the subjects with PD. Forty-two subjects with PD were taking a combination of dopamine agonist and carbidopa-levodopa medications, 63 were using carbidopa-levodopa alone, 12 were taking dopamine agonists alone, and 8 were not receiving dopaminergic drugs. No subjects were being treated with anticholinergic or cholinesterase inhibitor drugs. Most subjects had moderate severity of disease: 4 patients in stage 1, 7 in stage 1.5, 30 in stage 2, 56 in stage 2.5, 27 in stage 3, and 1 in stage 4 of the modified Hoehn and Yahr classification.⁹ The mean duration of disease was 6.0 ± 4.2 (range 0.5–20) years. Subjects receiving dopaminergic medications were examined and imaged first with the (+)-[¹¹C]DTBZ VMAT2 PET ligand in the morning after dopaminergic medications had been withheld overnight. Each patient also underwent [¹¹C]methyl-4-piperidinyl propionate (PMP) acetylcholinesterase (AChE) PET and brain MRI.

Each patient underwent a neuropsychological examination similar to one described previously.⁷ A global cognitive composite *z* score was calculated based on normative data. Patients with evidence of dementia as defined by a global composite *z* score of less than -2 and significant impairments of instrumental activities of daily living were not eligible for this study.

Standard protocol approvals, registrations, and patient consents. The subjects were pooled from different clinical studies, including ClinicalTrials Identifier NCT01565473 and NCT01565473, which used common imaging procedures and cognitive testing, but subjects did not undergo identical PD motor rating scale assessments. A common assessment, however, was a timed 8.5-m walk in the dopaminergic “off” state at usual speed. This study was approved by the Institutional Review Boards of the University of Michigan and Ann Arbor VA Medical Center for studies involving human subjects. Written informed consent was obtained from all subjects.

Imaging techniques. All subjects underwent brain MRI and [¹¹C]PMP (AChE) and [¹¹C]DTBZ (VMAT2) PET. MRI was performed on a 3T Philips Achieva system (Philips, Best, the Netherlands). A standard T1-weighted series of a 3-dimensional inversion recovery–prepared turbo field-echo was performed in the sagittal plane using repetition time/echo time/inversion time = 9.8/4.6/1,041 milliseconds; turbo factor = 200; single average; field of view = $240 \times 200 \times 160$ mm; acquired matrix = 240×200 .

One hundred sixty slices were reconstructed to 1-mm isotropic resolution. This sequence maximizes contrast among gray matter, white matter, and CSF and provides high-resolution delineation of cortical and subcortical structures. None of the subjects with PD had MRI evidence of atypical parkinsonian disorders, such as corticobasal degeneration or multiple system atrophy.

[¹¹C]PMP and [¹¹C]DTBZ PET imaging were performed in 3-dimensional imaging mode using an ECAT Exact HR + tomograph (Siemens Molecular Imaging, Inc., Knoxville, TN), which acquires 63 transaxial slices (slice thickness: 2.4 mm; intrinsic in-plane resolution: 4.1 mm) full width at half maximum over a 15.2-cm axial field of view. A NeuroShield (Scanwell Systems, Montreal, Canada) head-holder/shielding unit was attached to the patient's bed to reduce the contribution of detected photon events originating from the body outside the scanner field of view.¹⁰ Before the DTBZ and PMP injections, a 5-minute transmission scan using rotating ⁶⁸Ge rods was acquired for attenuation correction of emission data using the standard vendor-supplied segmentation and re-projection routines.

[¹¹C]PMP was prepared in high radiochemical purity (>95%) by *N*-[¹¹C]methylation of piperidin-4-yl propionate using a previously described method.¹¹ Dynamic PET scanning was performed for 70 minutes as previously reported.¹² No-carrier-added (+)-[¹¹C]DTBZ (250–1,000 Ci/mmol at the time of injection) was prepared as reported previously.¹¹ Dynamic PET scanning was performed for 60 minutes as reported previously.¹³

Analysis. All image frames were spatially coregistered within subjects with a rigid-body transformation to reduce the effects of subject motion during the imaging session.¹⁴ Interactive Data Language image analysis software (Research Systems, Inc., Boulder, CO) was used to manually trace volumes of interest on MRIs to include the thalamus, caudate nucleus, and putamen of each hemisphere. Total neocortical volumes of interest were defined using semiautomated threshold delineation of the cortical gray matter signal on the MRI scan. AChE [¹¹C]PMP hydrolysis rates (*k*₃) were estimated using the striatal volume of interest (defined by manual tracing on the MRI scan of the putamen and caudate nucleus) as the tissue reference for the integral of the precursor delivery.¹⁵ There are 2 major brain cholinergic projection systems. The first arises in the basal forebrain complex, providing the principal cholinergic input of the cortical mantle and is known to degenerate in PD. The second arises in the pedunculopontine nucleus, a brainstem locomotor center, and provides cholinergic inputs to the thalamus, cerebellum, basal ganglia, other brainstem nuclei, and the spinal cord.¹⁶ AChE PET imaging assesses cholinergic terminal integrity with cortical uptake reflecting largely basal forebrain neuron integrity and thalamic uptake principally reflecting pedunculopontine nucleus integrity.

[¹¹C]DTBZ distribution volume ratios were estimated using the Logan plot graphical analysis method with the striatal time-activity curves as the input function and the total neocortex as reference tissue, a reference region overall low in VMAT2 binding sites, with the assumption that the nondisplaceable distribution is uniform across the brain at equilibrium.¹⁷

Cognitive characteristics, motor features, and normative [¹¹C]PMP values in PD have been reported previously by our group in a smaller subset of this cohort and form the basis for the cholinergic system classification used in the present study based on a fifth percentile cutoff in the non-PD control group.⁷ The use of dual radiotracer dopaminergic and cholinergic imaging in this study and an imaging-based definition of normal-range cholinergic activity levels allows for the classification of subjects with PD within a predominant hypodopaminergic but preserved cortical cholinergic innervation group compared to those with combined deficits of both of these projections.

Analysis of covariance (ANCOVA) was performed with gait speed as a dependent variable, cholinergic subgroup (normal control, normocholinergic and hypocholinergic PD groups) as an independent variable, and striatal VMAT2 distribution volume ratio, age, sex, and cognitive composite z score as covariates. Duncan multiple range post hoc testing was performed to assess differences between subgroups.

RESULTS Cortical cholinergic denervation was heterogeneous, with 38 subjects with PD (30.4%) exhibiting cortical AChE activity below normal range. Figures 1 and 2 show distribution plots for the non-PD control, relatively isolated dopaminergic, and combined dopaminergic and cholinergic degeneration PD groups.

Table 1 lists mean age, Hoehn and Yahr stage, global cognitive z score, and sex distribution in the non-PD control, relatively isolated dopaminergic, and combined dopaminergic and cholinergic degeneration PD groups.

Age-, sex-, and cognition-adjusted ANCOVA comparing gait speed among the 3 groups showed a significant overall model ($F = 6.70, p < 0.0001$) with a significant cortical AChE group effect ($F = 3.36, p = 0.037$) and significant slower gait speed in the low cortical cholinergic innervation PD subgroup compared with the normal-range cortical cholinergic innervation PD subgroup and non-PD control subjects (table 2). There was a significant cognition effect

($F = 6.58, p = 0.011$) on gait speed but no significant striatal dopaminergic ($F = 1.30, p = 0.26$), sex ($F = 3.44, p = 0.07$), or age ($F = 3.62, p = 0.06$) covariate effects.

We performed a post hoc analysis to assess for possible covariate effects of duration of disease in ANCOVA limited to the 2 PD subgroups. Similar findings of significant slowing of gait speed in the cortical hypocholinergic group were seen compared with the normal-range PD group with total model effect $F = 5.99 (p < 0.0001)$, significant cortical AChE effect ($F = 5.66, p = 0.019$), significant duration of disease ($F = 4.88, p = 0.029$), and global cognition ($F = 8.41, p = 0.0045$), but no significant striatal dopaminergic ($F = 0.03, p = 0.87$), sex ($F = 3.06, p = 0.082$), or age ($F = 0.71, p = 0.40$) covariate effects.

Finally, we performed a cholinergic subgroup analysis of low- vs normal-range pedunculopontine nucleus (PPN)-thalamic cholinergic subgroup effects to assess for possible specific PPN-thalamic cholinergic effects. There were a total of 21 subjects with PD with PPN-thalamic AChE activity below normal range. ANCOVA comparing gait speed among the 3 groups showed a significant overall model ($F = 5.12, p < 0.0001$) with no significant PPN-thalamic AChE group effect ($F = 0.29, p = 0.75$) and significant global cognitive composite z score ($F = 9.53, p = 0.0024$) effect. There were no significant striatal dopaminergic ($F = 2.08, p = 0.16$), sex ($F = 2.62, p = 0.11$), or age ($F = 3.41, p = 0.07$) covariate effects.

Figure 1 Distribution of cortical cholinergic innervation in the different groups

Group scatter plot of distribution of cortical acetylcholinesterase activity (k_3 hydrolysis rate, min^{-1}) in non-Parkinson disease control (Non-PD), relatively isolated dopamine (Low DA PD), and combined DA and acetylcholine (Low DA & Low ACH PD) degeneration PD groups.

DISCUSSION Our findings indicate that the PD subgroup with preserved cortical cholinergic innervation does not have significant slowing of gait speed compared with non-PD control subjects. In contrast, significant slowing of gait speed compared with non-PD control subjects was seen in the subgroup with combined nigrostriatal and basal forebrain cortical cholinergic denervation. These findings are consistent with the general hypothesis that the clinical heterogeneity of PD results from variable involvement of different brain systems. These results provide support for the notion that emergence of dopamine nonresponsive gait problems in PD reflects transitions from a predominantly hypodopaminergic disorder to a multisystem neurodegenerative disorder.

We found that the multisystem hypocholinergic effect on gait speed was driven by basal forebrain but not by PPN-thalamic denervation effects. Degeneration of the PPN and its thalamic projections is associated with impaired postural control,^{18,19} while cortical cholinergic denervation associates with cognitive impairments.²⁰ Alterations in cognitive function are linked to gait disturbances²¹; gait speed is sensitive to cognitive dysfunction and gait speed slowing predicts cognitive decline in initially unimpaired older

Figure 2 Distribution of nigrostriatal dopaminergic innervation in the different groups

Group scatter plot of distribution of striatal vesicular monoamine type 2 distribution volume ratio in non-Parkinson disease control (Non-PD), relatively isolated dopamine (Low DA PD), and combined DA and acetylcholine (Low DA & Low ACH PD) degeneration PD groups. Although 7 subjects with PD had average striatal binding values in the low normal range, these subjects had evidence of more posterior putaminal dopaminergic denervation patterns consistent with the diagnosis of PD.

adults.^{22,23} Because cognitive performance is also a significant predictor of gait speed in PD,²⁴ our findings that comorbid cortical cholinergic denervation is a more robust correlate of slower gait speed in PD than nigrostriatal denervation alone may reflect the impact of impaired cognitive processing during ambulation.

Multisystem degeneration in PD may resemble multisystem deteriorations underlying mobility problems and frailty in the elderly.^{25,26} Relatively isolated impairment of a single system may not manifest clinical impairments because of adaptive plasticity in remaining intact systems. Once multisystem impairments occur,

the surviving components of these systems cannot adapt further and clinical morbidity becomes manifest, often in nonlinear fashion as critical thresholds are exceeded.²⁵ Degraded striatal function in PD likely places additional burdens on other brain systems, including those mediating cognitive functions.⁵

Basal forebrain cholinergic neurons are key actors in attentional function and may be increasingly recruited to participate in previously automatic motor tasks, such as simple walking. This apparent automaticity of walking may decompensate in the setting of failing cognitive resources, forcing a slower and more cautious gait. The mechanism of slowing of gait in PD can be conceptualized as a clinical state where degradation of preattentive striatal mechanisms of motor control are initially supplemented by increasing cognitive control mediated by cortical cholinergic mechanisms. With degeneration of compensatory cholinergic systems, gait speed slows. Decreased gait speed may be an early marker of basal forebrain cholinergic deficits in PD and may be informative in identifying those subjects with PD at greater risk for more rapid cognitive decline.^{6,27,28} Further research is needed to also investigate an alternative hypothesis that reduced speed could be related to direct cholinergic speed control in the frontal lobe or other brain regions.

We do not suggest that basal forebrain cholinergic degeneration is the sole mechanism of decline of gait function in PD. Postmortem studies of end-stage PD have shown evidence of multiple and heterogeneous extranigral pathologies, including the presence of cortical Lewy bodies and Lewy neurites, noradrenergic deficits, cholinergic deficits, and Alzheimer pathology.^{29–35} We recently reported an association between the presence of amyloidopathy and postural and gait difficulties in PD.³⁶ Within the framework of multi-system degeneration and declining adaptive capacities, additional degenerations are expected to lower the symptomatic threshold of clinical manifestations,²⁵ and probably explain the increasing dopamine nonresponsiveness of gait difficulties in PD.

Clinical observations that dopaminergic therapy can significantly improve bradykinetic gait in PD

Table 1 Mean age, Hoehn and Yahr stage, global cognitive z score ($\pm$ SD), and sex distribution in the non-PD control, relatively isolated DA, and combined DA and ACh degeneration PD groups

Clinical variable	Non-PD control group (n = 32)	Relatively isolated DA degeneration but normal-range ACh activity PD group (n = 87)	Combined DA and ACh degeneration PD group (n = 38)
Age, y	66.0 $\pm$ 10.6	64.2 $\pm$ 7.1	68.7 $\pm$ 7.0
Sex, females/males	14/18	26/61	4/34
Hoehn and Yahr stage	0	2.35 $\pm$ 0.52	2.51 $\pm$ 0.44
Global cognitive z score	0.06 $\pm$ 0.50	–0.21 $\pm$ 0.70	–0.81 $\pm$ 1.06

Abbreviations: ACh = acetylcholine; DA = dopamine; PD = Parkinson disease.

Table 2 Mean absolute gait speed ($\pm$ SD) in the non-PD control, relatively isolated DA, and combined DA and ACh degeneration PD groups

Outcome variable	Non-PD control group (n = 32)	Relatively isolated DA degeneration but normal-range ACh activity PD group (n = 87)	Combined DA and ACh degeneration PD group (n = 38)
Gait speed, m/s	1.17 $\pm$ 0.18 (1.09) ^a	1.12 $\pm$ 0.20 (1.11) ^a	0.97 $\pm$ 0.2 (1.00)

Abbreviations: ACh = acetylcholine; DA = dopamine; PD = Parkinson disease.

Mean gait speed values adjusted for covariate effects of nigrostriatal denervation, sex, age, and global cognition are shown in parentheses.

^aThese subgroup means are not significantly different. Duncan post hoc testing was performed to assess for differences among subgroups.

may appear to contradict our current findings. According to our multisystem degeneration model of gait impairment, when cholinergic (or other extranigral) degeneration is present, a temporary increase in striatal dopaminergic levels through levodopa replacement therapy may increase gait speed. This is because levodopa pharmacotherapy may restore one system (i.e., dopaminergic neurotransmission) temporarily to normal function levels. However, the degree of dopamine responsiveness of gait functions will depend on the integrity of the remainder of the neural networks supporting mobility functions. Once other systems become severely affected in PD, the beneficial effects of dopamine replacement may no longer offset the loss of adaptive plasticity in other systems.

A limitation of our study is that the main gait parameter was gait speed. Although gait speed = distance/time, this measure does not distinguish those subjects who had hesitations and freezing from those who walked slowly without hesitations and freezing. Therefore, it should be noted that gait abnormalities, such as variability in spatiotemporal gait measures, may exist in PD in the absence of significant overall slowing.

Our findings show that gait speed is not significantly slower than normal in subjects with PD with relatively isolated nigrostriatal dopaminergic denervation. Comorbid cortical cholinergic denervation is a more robust marker of slowing of gait in PD than nigrostriatal denervation alone and may reflect declining capacity for cognitive compensation of motor performance during ambulation. These results also suggest that treatments aimed at enhancing cortical cholinergic function may be useful for treatment of gait disorders in PD.

AUTHOR CONTRIBUTIONS

Dr. Bohnen: obtained funding, study concept and design, acquisition of data, analysis and interpretation, manuscript preparation. Dr. Frey: obtained funding, acquisition of data, study supervision, critical revision of the manuscript for important intellectual content. Dr. Studenski: analysis and interpretation, critical revision of the manuscript for important intellectual content. Dr. Kotagal: critical revision of the manuscript for important intellectual content. Dr. Koeppe and Dr. Scott: acquisition of data, critical revision of the manuscript for important intellectual content, study supervision. Dr. Albin: analysis and interpretation, critical revision of the manuscript for important intellectual content. Dr. Müller:

acquisition of data, analysis and interpretation, critical revision of the manuscript for important intellectual content.

ACKNOWLEDGMENT

The authors thank Christine Minderovic, Virginia Rogers, the PET technologists, cyclotron operators, and chemists for their assistance.

STUDY FUNDING

Supported by the Department of Veterans Affairs, the Michael J. Fox Foundation, and the NIH (grants P01 NS015655 and RO1 NS070856).

DISCLOSURE

N. Bohnen has research support from the NIH, Department of Veteran Affairs, and the Michael J. Fox Foundation. K. Frey has research support from the NIH, GE Healthcare, and Avid Radiopharmaceuticals (Eli Lilly subsidiary). Dr. Frey also serves as a consultant to Avid Radiopharmaceuticals, MIMVista, Inc., Bayer-Schering and GE Healthcare. He also holds equity (common stock) in GE, Bristol-Myers, Merck, and Novo-Nordisk. S. Studenski serves as a consultant to Abbott Laboratories, Eli Lilly, and Biogen. V. Kotagal has research support from the American Academy of Neurology Clinical Research Training Fellowship. R. Koeppe serves on the Board of the International Society of Cerebral Blood Flow and Metabolism; receives research support from NIH (National Institute of Neurological Disorders and Stroke, National Institute on Aging); and is a consultant for Johnson & Johnson and Merck. P. Scott receives editorial royalties from Wiley, is an owner of SynFast Consulting, LLC, and has received research funding from the University of Michigan, GE Healthcare, Bristol-Myers Squibb, Bayer Pharma AG, Eli Lilly, and Molecular Imaging Research. R. Albin received compensation for expert witness testimony in litigation regarding dopamine agonist-induced impulse control disorders. Dr. Albin serves on the editorial boards of *Neurology*[®], *Experimental Neurology*, and *Neurobiology of Disease*. He receives grant support from the NIH and the Department of Veterans Affairs. Dr. Albin served on the Data Safety and Monitoring Boards for the QE3 and HORIZON trials. M. Müller has research support from the NIH and the Department of Veterans Affairs. Go to Neurology.org for full disclosures.

Received May 7, 2013. Accepted in final form July 29, 2013.

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