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Postural and gait symptoms in *de novo* Parkinson's disease patients correlate with cholinergic white matter pathology

Muhammad Nazmuddin [a,*], Jan-Willem van Dalen [b,c], Ronald J.H. Borra [d], Gilles N. Stormezand [e], Harm Jan van der Horn [a], Sygrid van der Zee [a], Jeffrey Boertien [a], Teus van Laar [a]

[a] Department of Neurology, Parkinson Expertise Center, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands
[b] Department of Neurology, Donders Institute for Brain, Behaviour, and Cognition, Radboud University Medical Center, Nijmegen, the Netherlands
[c] Department of Neurology, Amsterdam UMC, Location AMC, Amsterdam, the Netherlands
[d] Department of Radiology, University Medical Center Groningen, University of Groningen, the Netherlands
[e] Department of Nuclear Medicine and Molecular Imaging, Medical Imaging Center, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

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ABSTRACT

Introduction: The postural instability gait difficulty motor subtype of patients with Parkinson's disease (PIGD-PD) has been associated with more severe cognitive pathology and a higher risk on dementia compared to the tremor-dominant subtype (TD-PD). Here, we investigated whether the microstructural integrity of the cholinergic projections from the nucleus basalis of Meynert (NBM) was different between these clinical subtypes.

Methods: Diffusion-weighted imaging data of 98 newly-diagnosed unmedicated PD patients (44 TD-PD and 54 PIGD-PD subjects) and 10 healthy controls, were analysed using diffusion tensor imaging, focusing on the white matter tracts associated with cholinergic projections from the NBM (NBM-WM) as the tract-of-interest. Quantitative tract-based and voxel-based analyses were performed using FA and MD as the estimates of white matter integrity.

Results: Voxel-based analyses indicated significantly lower FA in the frontal part of the medial and lateral NBM-WM tract of both hemispheres of PIGD-PD compared to TD-PD. Relative to healthy control, several clusters with significantly lower FA were observed in the frontolateral NBM-WM tract of both disease groups. Furthermore, significant correlations between the severity of the axial and gait impairment and NBM-WM FA and MD were found, which were partially mediated by NBM-WM state on subjects' attentional performance.

Conclusions: The PIGD-PD subtype shows a loss of microstructural integrity of the NBM-WM tract, which suggests that a loss of cholinergic projections in this PD subtype already presents in *de novo* PD patients.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disease with a heterogeneous clinical and neuropathological manifestation [1–3]. To understand the underlying mechanisms, prognosis, and personalized therapeutic strategies, many empirical classifications have been made to classify PD into several subtypes. One of the most common classifications is based on the prominent motor symptoms, which differentiate between the tremor-dominant (TD-PD) and the postural instability and gait disorders (PIGD-PD) subtype [4].

Previous studies have shown that the PIGD-PD is a more malignant subtype, compared to TD-PD, characterized mainly by more disabling axial symptoms, gait impairment and more severe non-motor symptoms, including cognitive dysfunction, finally leading to a greater impact on quality of life and a higher demand on nursing home placement [5,6]. Furthermore, levodopa and deep brain stimulation provide limited effects on the balance and gait symptoms in PIGD-PD. This suggests that the underlying pathophysiology at least also involves non-dopaminergic systems [7–9]. Post-mortem data and *in vivo* neuroimaging suggest that disruptions of the mesencephalic and the basal forebrain cholinergic systems are the main findings associated with cognitive, gait and postural impairments in PD [10–16]. Therefore, structural

* Corresponding author. Department of Neurology, UMCG, Hanzeplein 1 AB-51, 9700RB, Groningen, the Netherlands.
E-mail address: m.nazmuddin@umcg.nl (M. Nazmuddin).

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measurements of these cholinergic projections, using neuroimaging modalities like diffusion tensor imaging MRI (DTI-MRI), may serve as a distinctive marker for the PIGD-PD subtype.

In this study, we compared the microstructural integrity of the white matter tracts that are associated with the cortically-projecting cholinergic structure in the basal forebrain, the nucleus basalis of Meynert (NBM), between TD-PD and PIGD-PD subtypes, using DTI-MRI. This technique allows *in vivo* measurement of water molecule diffusion which is restricted along coherently oriented axonal fibers in normal white matter, thus highly anisotropic. Microstructural damages in white matter cause less constraint of water molecule motion and thus, will be observed in measurable DTI parameters as a decrease in fractional anisotropy (FA) and an increase in mean diffusivity (MD).

Previous DTI studies have highlighted the distinctive tissue integrity and connectivity within the striato-nigro-thalamo-cortical circuit between these two subtypes [17]. However, the alteration within the NBM-associated white matter tract (NBM-WM) in PD has not been investigated yet. We hypothesize that the microstructural integrity of the NBM-WM is decreased in PIGD-PD, compared to TD-PD. Secondly, we predict that the decrease of the NBM-WM tract integrity is significantly correlated with the severity of PIGD-associated symptoms. Finally, as on one hand, the NBM cholinergic neurons are responsible for maintaining the attentional function, and on the other hand, the attentional state contributes to gait impairment in PD [18,19], we predict that the significant association between the NBM-WM tract integrity on PIGD severity is, at least, partially mediated by subjects' attentional state.

2. Methods

2.1. Subjects and clinical assessment

One-hundred fourteen *de novo* dopa-naïve PD patients from the DUtch PARkinson Cohort (DUPARC) with complete clinical and neuroimaging assessments by the time of study analysis were included in this study [20]. A focused interview was performed to obtain demographic information and symptom as well as medication history. The Movement Disorder Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) was used to examine the severity of motor symptoms [21]. PD diagnosis was established according to the Movement Disorder Society Clinical Diagnostic Criteria for PD. The clinical diagnosis was confirmed by a 3,4-dihydroxy-6-18F-fluoro-L-phenylalanine (^{18}F-FDOPA) PET scan, providing an uptake ratio between the putamen and occipital cortex of each brain hemisphere.

Cognitive functioning of every subject was assessed by a trained neuropsychologist using a series of standardized neuropsychological tests including the Montreal Cognitive Assessment (MOCA) as a global cognitive measure, and the color-word part of the Stroop Test, the determination test (S1 and S3) and the DS of the Vienna Test System (VTS) to measure attention [22–24]. The attentional percentile score was derived from the average percentile score of each test normalized with age and education level.

We classified the participants clinically according to their prominent motor phenotype (TD-PD/PIGD-PD) [4]. Based on this classification approach, we excluded subjects with an indeterminate motor phenotype (n = 16), which resulted in the inclusion of 54 PIGD-PD and 44 TD-PD subjects. Subjects with (a)symmetrical motor symptoms were additionally identified [25].

As a complementary analysis for comparisons between two PD subtypes, we included diffusion-weighted imaging (DWI) data of 10 healthy control (HC) subjects, aged 28.5 (SD ± 7.1) years, obtained locally from the same scanner and image acquisition parameters. The study was approved by the medical ethics committee of the University Medical Center Groningen, the Netherlands. All participants provided written informed consent.

2.2. MRI image acquisition

MRI scans were performed on 3T Siemens Prisma. DWI were acquired by using a dual spin-echo, single-shot, echo-planar sequence with 30 directions, b-value 1000 s/m2, twelve non-diffusion weighted image (b = 0 s/mm2), repetition time 3300 ms, echo time 69 ms, voxel size 1.7 x 1.7 × 5.2 mm3 T1-weighted images were acquired using magnetization-prepared rapid gradient echo sequence (MPRAGE) with repetition time 2300 ms, echo time 2.3 ms, flip angle 8°, slice thickness 0.9 mm, and voxel size 0.9 x 0.9 × 0.9 mm^3. FLAIR images were acquired with the following parameters: repetition time 5000 ms, echo time 398 ms, inversion time 1800 ms, flip angle 120°, and voxel size 0.9 x 0.9 × 0.9 mm^3.

2.3. Neuroimaging analysis

DWI, T1, and FLAIR images were anonymized and converted from DICOM to NIFTI format with MRIcron (https://people.cas.sc.edu/rorden/mricron/install.html). Deformations of the DWIs induced by signal drift, Gibbs ringing artifact, head motion and eddy current were corrected with exploreDTI (http://www.ExploreDTI.com). Afterwards, MRI data were processed with the FMRIB Software Library, version 6.0 (FSL; www.fmrib.ox.ac.uk/fsl). A diffusion tensor model throughout the brain was fitted using FDTs DTIFIT, to generate FA and MD maps. Subsequently, all FA and MD maps were non-linearly registered into the FMRIB58_FA standard-space image.

The NBM-WM tract map as the tract-of-interest (TOI) was based on previous study [26]. The NBM-WM tract map was derived from the probabilistic tractography of DWI's of a patient cohort from the memory clinic of the Amsterdam Academic Medical Center (age 70–83). The resulted reconstructions of the medial and lateral NBM-WM pathways of each individual were combined and were non-linearly registered into the Montreal Neurological Institute (MNI) standard [27,28]. The population image was normalized at a threshold of 5% of the maximum intensity, to generate the final NBM-WM tract map. This map fitted to the histological description of the anatomical projection of the NBM-WM [29]. Furthermore, the NBM-WM map was divided into a proximal and distal region, to evaluate the possible regional degradation susceptibility to its clinical impact, as reported previously (Suppl. Fig. 1). The proximal NBM-WM region consists of white matter projections to the frontal and limbic area, while the distal region mainly projects to the parietal and occipital regions. For TOI-based analyses, the average of MD and FA values of voxels within the proximal, and distal NBM-WM tract were extracted as measures of the NBM-WM tract integrity. Detailed analysis methods on T1 and FLAIR images to measure the total volume of brain white matter, NBM-WM tract and white matter hyperintensity (WMH) volume are available in the supplementary file.

Furthermore, voxel-wise statistics on normalized FA and MD maps were applied to analyze the spatial microstructural integrity of the NBM-WM tract between both groups. Permutation-based testing (number of permutations = 5000) was performed using threshold-free cluster enhancement, corrected for multiple comparisons using the family-wise error correction ($P_{FWE} < 0.05$) [30]. Age, sex, and motor symptom duration were included as covariates when comparing between TD-PD and PIGD-PD groups.

2.4. Statistical analysis

Statistical analyses were conducted using R (version 3.0.2; R Foundation for Statistical Computing, Vienna, Austria). The normality of the continuous variables was assessed using the Shapiro-Wilk test. Data are presented as mean ± standard deviation (SD) if normally distributed or median ± interquartile range (IQR) if non-normally distributed. Comparisons between TD-PD and PIGD-PD on demographics, clinical characteristics, and neuroimaging volumetric indices were assessed using independent sample *t*-test, or Mann-Whitney *U* test for continuous

variables, where appropriate. Pearson's Chi-squared test was applied for categorical variables. One-way analysis of variance (ANOVA) were applied when comparing NBM-WM FA and MD between HC and the two disease groups. Tukey's honestly significant differences (HSD) multiple-comparison procedure was applied if the result of one-way ANOVA was significant.

We additionally tested if the NBM-WM microstructural alteration was lateralized in PD subjects with asymmetric symptoms. For each subtype (TD-PD and PIGD-PD), the NBM-WM FA and MD of the ipsilateral and contralateral hemispheres relative to the predominant side of motor symptoms were compared using paired sample *t*-test. The cross-hemispheric comparison of the striatal-to-occipital ratio of the ^{18}F-FDOPA PET was additionally made to validate the approach.

We hypothesized that the NBM-WM tract degeneration would uniquely associate with postural-and-gait dysfunction while correcting for the effect of age, sex, and duration of motor complaints. Thus, binary logistic regression analyses were applied to assess the relationship of NBM-WM FA and MD with the likelihood of PIGD-PD occurrence. Furthermore, the associations of the NBM-WM FA and MD to the severity of PIGD as well as tremor symptoms were assessed using multiple linear regression. All outcomes and predictors were converted to Z-scores to obtain standardized regression coefficients. Explorative analyses on the functional implication of the degeneration of the NBM-WM subregions (proximal and distal) were additionally performed. An additional regression model was assessed when a significant difference on either NBM-WM tract volume, total white matter volume, or total WMH volume between two PD subtypes was found. This model was applied to investigate the effect of these indices in affecting the estimated effect of DTI indices measured in the first model.

Finally, mediation analyses were applied to test if the significant association between the PIGD severity and diffusion metrics was mediated by subjects' attentional function [31]. The path coefficients between the independent (NBM-WM FA/MD), dependent (PIGD score), and mediator (attentional percentile score) variables were calculated through three regression equations. The significance of the effect of NBM-WM FA/MD on attention, the effect of NBM-WM FA/MD on PIGD score, and the effect of attention on PIGD score while controlling for NBM-WM FA/MD were determined. In all regression equations, age, sex, and duration of motor symptoms before diagnosis were included as covariates. If all effects were significant, the significance of the mediation effect was assessed using a bootstrap estimation approach with 1000 samples, yielding an indirect effect estimate of NBM-WM FA and MD on the PIGD severity which was mediated by attentional performance. Because of the high inter-correlation of FA and MD and the explorative nature of the subregion analyses, the significance threshold was set at $P < 0.05$.

3. Results

3.1. Demographic and clinical characteristics

The demographic and clinical characteristics of the participants are summarized in Table 1. The age of PIGD-PD group was significantly older compared to TD-PD group ($P = 0.020$). PIGD-PD subjects had a significantly lower MOCA score ($P = 0.004$) and higher Hoehn and Yahr stage relative to TD-PD subjects ($P < 0.001$). No significant differences were found on the MDS-UPDRS III scores, education level, duration of motor complaints before diagnosis, and motor symptom laterality between both *de novo* PD groups. No cholinergic medication was used by any of the participants. As expected, both the tremor subscore and the PIGD subscore were significantly different between the two subtypes. The profiles of tremor and PIGD assessments based on MDS-UPDRS II and III are presented in Suppl. Figure. 2.

Table 1
Demographics and clinical characteristics.

Characteristic	PIGD-PD (n = 54)	TD-PD (n = 44)	*P*
Male, %	72%	66%	0.831
Age, years (SD)	66.9 (9.26)	62.7 (8.28)	0.020
Level of education (IQR)[a]	5 (2)	5 (2)	0.135
MDS-UPDRS III (SD)	32.09 (11.17)	31.32 (12.52)	0.75
MDS-UPDRS tremor sub-score (IQR)[b]	1 (2)	9 (7)	<0.001
MDS-UPDRS PIGD sub-score (IQR)[b]	3 (2.75)	2 (1.25)	<0.001
Motor complaint duration, months	24 (12)	24 (17)	0.284
Hoehn & Yahr (%)			
1/2/3/4/5	15/60/19/6/0	39/59/0/2/0	<0.001
Motor-symptom laterality (%)			
LD/ID/RD	4824/28	43/16/41	0.339
MOCA (SD)	24.4 (3.38)	26.0 (2.04)	0.004
Attentional percentile score (SD)	37.8 (18.48)	47.6 (14.13)	0.003

Abbreviations: HC, healthy control; PD, Parkinson's disease; TD, tremor dominant; PIGD, postural instability and gait difficulty; LD, left-dominant; ID, indeterminate; RD, right-dominant; MDS-UPDRS-III, Movement Disorder Society-Unified Parkinson's Disease Rating Scale–Motor Subscale; MOCA, Montreal Cognitive Assessment; MCI, mild cognitive impairment.

[a] Educational level according to the Dutch Verhage Scale (Verhage, 1964).

[b] Total scores of MDS-UPDRS items assessing tremor (2.10, 3.15, 3.16, 3.17, and 3.18) or PIGD (2.12, 2.13, 3.10, 3.11, 3.12).

3.2. Differences in the NBM-WM structural integrity, volume, and WMH load at group level

The one-way ANOVA test showed that there were significant differences in FA [$F_{(2, 105)} = 8.74$, $P < 0.001$) and MD [$F_{(2, 105)} = 5.83$, $P = 0.004$] between HC and both PD groups. Post-hoc analyses revealed that PIGD-PD group had significantly lower NBM-WM FA ($P_{adj.} < 0.001$) and higher NBM-WM MD ($P_{adj.} = 0.004$) compared to TD-PD (Suppl. Fig. 3A and 3B). The TD-PD group however did not differ significantly from the HC group in FA and MD. No significant difference was found between TD-PD and PIGD-PD on the volume of the NBM-WM tract ($P = 0.619$) and the total white matter ($P = 0.781$). However, PIGD-PD had significantly higher WMH load compared to TD-PD (4.10 ± 7.20 vs. 1.56 ± 3.07, $P = 0.036$) (Suppl. Fig. 3C, 3D, & 3E).

3.3. Voxel-based analyses of TD-PD, PIGD-PD, and HC

Voxel-based analyses of the FA maps within the NBM-WM pathways as the TOI showed clusters with significantly lower FA bilaterally at the proximal portion of the medial NBM tract and the frontotemporal portion of the lateral NBM-WM pathways of PIGD-PD compared to TD ($P_{FWE} < 0.05$, Fig. 1). No significant difference of MD within the NBM-WM tract was observed.

Compared to HC, PIGD-PD showed voxel clusters with significantly higher MD, primarily in the fronto-lateral NBM-WM tract of the left hemisphere. (Suppl. Fig. 4). No significant cluster was found when TD-PD was compared to HC. Similar comparisons with the FA maps indicated several voxel clusters with significantly lower FA along the fronto-lateral NBM-WM tract of TD-PD and PIGD-PD compared to HC. These clusters were more widespread when comparing HC to PIGD-PD instead of HC to the TD-PD group (Suppl. Fig. 5).

3.4. The NBM-WM integrity and its relationship with asymmetry in motor symptoms

Complementary analyses on the DTI data of PD patients with lateralized motor symptoms were conducted (TD-PD = 37; PIGD-PD = 41) to check if the observed decrease of structural integrity was associated with the (a)symmetry in clinical symptoms. The validation with ^{18}F-DOPA

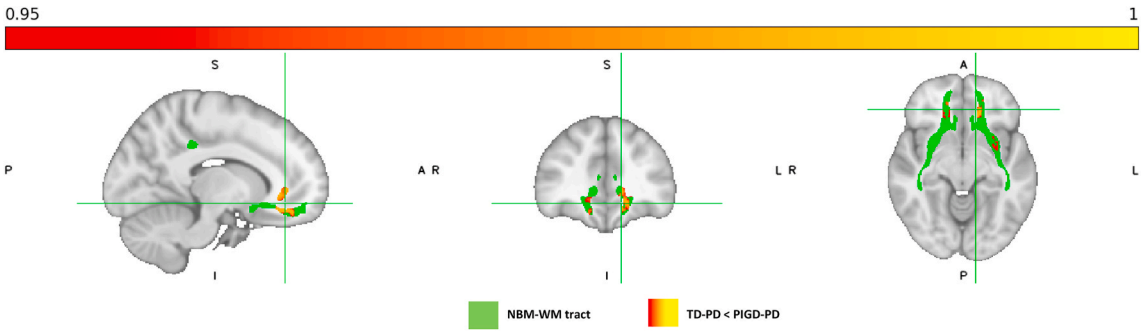

Fig. 1. Voxel-based analyses of fractional anisotropy (FA) maps of the NBM-WM tract comparing TD-PD and PIGD-PD. The PIGD-PD group showed significantly lower FA in the proximal-medial and -lateral NBM-WM tract of both hemispheres (showed in red), compared to the TD-PD group ($P_{FWE} < 0.05$). No significant MD difference was found between the two PD subtypes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

PET data is shown in Suppl. Figure 6A. Both the lateralized PIGD-PD and the TD-PD groups showed a clear decrease in the striatal-occipital ratio (SOR) on the contralateral side, as expected. The averaged FA and MD of the NBM-WM tract between the ipsilateral and contralateral hemispheres did not significantly differ between both PIGD-PD and TD-PD groups (Suppl. Fig. 6B and 6C).

3.5. *The NBM-WM tract integrity and its relationship with postural and gait impairment*

Results of binary logistic regression analyses showed that lower FA and higher MD values of the NBM-WM projections were associated with the PIGD-PD subtype (Table 2, model 1). Subregional analyses indicated that FA and MD of the proximal NBM-WM tract and FA of the distal NBM-WM tract significantly predicted the occurrence of PIGD-PD. Adding total WMH volume in the second model, odds ratio for having PIGD-PD subtype remained significant only for FA of overall and NBM-WM subregions.

Our first linear regression model showed that a higher NBM-WM FA and lower NBM-WM MD, regardless of the localization within the NBM-WM tract, were significantly associated with more severe postural and gait impairment (Table 2). A similar analysis did not reveal any association between the severity of tremor and the integrity of the NBM white matter tract expressed by the FA and MD values (Suppl. Table 1).

In linear regression model 2, the standardized β was only significant for FA and MD of the overall NBM-WM and the MD of the proximal NBM-WM. Furthermore, the explanatory power of the second model which added the total WMH volume as an additional independent variable, increased the explanatory power significantly in all analyses as indicated by an increase of R^2 by 0.04–0.07 (Suppl. Table 2).

Finally, the mediation analysis revealed that the significant effect of the NBM-WM FA and MD on the severity of PIGD was partially mediated through attentional performance (Fig. 2). The regression coefficient between NBM-WM FA and PIGD score and the regression coefficient between NBM-WM and attentional performance were all significant. Additionally, the effect of NBM-WM FA and MD was still significant on PIGD score after controlling for attentional performance, suggesting partial mediation effect of attentional function. The bootstrapped standardized indirect effect (β) was −0.063 [95% CI: −0.15 to −0.01, $P = 0.026$] for NBM-WM FA and 0.063 [95% CI: 0.006–0.15, $P = 0.024$] for NBM-WM MD.

4. Discussion

The present study investigated the microstructural integrity of the NBM-WM pathway using DTI in *de novo* unmedicated PD patients with different motor subtypes. Both voxel- and tract-based analyses showed that the NBM-WM microstructural degeneration was present bilaterally

Table 2
Results of binary logistic and multiple linear regression models on the association between NBM-WM DTI indices and PIGD-PD subtype and its symptom-associated severity.

DTI Par.	Structure	Logistic regression of the event of PIGD-PD among *de novo* PD							
		model 1 (unadjusted for WMH)				model 2 (adjusted for WMH)			
		OR	95% CI		P	OR	95% CI		P
FA	NBM	0.409	0.209	0.735	**0.005**	0.439	0.217	0.823	**0.015**
	NBM-prox	0.463	0.249	0.808	**0.01**	0.502	0.263	0.9	**0.027**
	NBM-dist	0.377	0.19	0.683	**0.003**	0.384	0.18	0.753	**0.008**
MD	NBM	2.289	1.19	4.846	**0.019**	2.047	0.995	4.604	0.064
	NBM-prox	1.958	1.096	3.724	**0.03**	1.746	0.933	3.465	0.093
	NBM-dist	1.541	0.918	2.68	0.11	1.316	0.719	2.451	0.375
		Multiple linear regression of PIGD severity							
		β	**95% CI**		**P**	**β**	**95% CI**		**P**
FA	NBM	−0.386	−0.593	−0.181	**< 0.001**	−0.255	−0.474	−0.037	**0.023**
	NBM-prox	−0.32	−0.527	−0.112	**0.003**	−0.198	−0.409	0.012	0.065
	NBM-dist	−0.383	−0.59	−0.177	**< 0.001**	−0.222	−0.459	0.014	0.065
MD	NBM	0.473	0.247	0.7	**< 0.001**	0.323	0.074	0.573	**0.012**
	NBM-prox	0.383	0.169	0.597	**< 0.001**	0.249	0.025	0.473	**0.03**
	NBM-dist	0.352	0.146	0.557	**< 0.001**	0.195	−0.032	0.422	0.092

Age, sex, and motor symptom duration were included as covariates in both models. NBM: the bilateral NBM-WM tract; NBM-prox: the bilateral proximal NBM-WM tract; NBM-dist: the bilateral distal NBM-WM tract. FA: fractional anisotropy; MD: mean diffusivity; OR: odds ratio; CI: confidence interval. PIGD: postural instability and gait disorder; WMH: white matter hyperintensity.

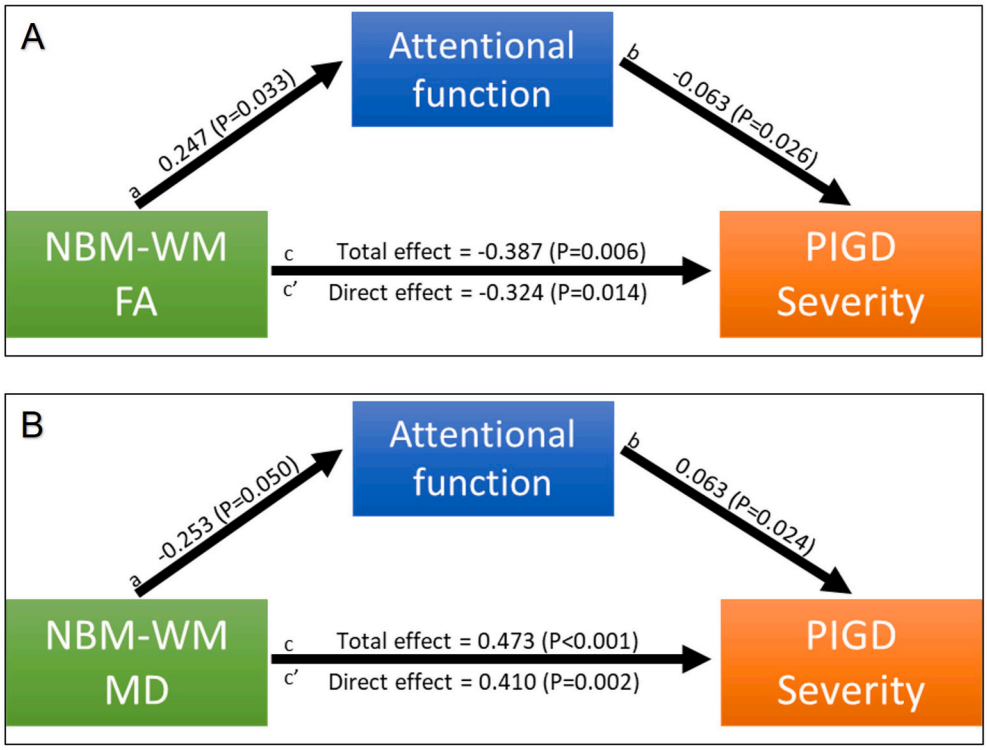

Fig. 2. The mediation effect of attentional function in the effect of NBM-WM FA (A) and MD (B) on PIGD severity. Path a shows coefficient for the effect of NBM-WM FA (β = 0.247 [95%CI = 0.021–0.473], P = 0.033)/MD (β = −0.253 [95%CI = −0.506 – 0.000], P = 0.050) on attentional function. Path b shows the coefficient for the indirect effect of NBM-WM FA (β = −0.063 [95%CI = −0.15 to −0.01], P = 0.026)/MD (β = 0.063 [95%CI = 0.006–0.15], P = 0.024) on PIGD severity that is mediated by attentional function. Paths c and c′ show coefficients for the total (β_{FA} = −0.387 [95%CI_{FA} = −0.599 to −0.14], P_{FA} = 0.006; β_{MD} = 0.473 [95% CI_{MD} = 0.256–0.700], P_{MD}<0.001) and direct (β_{FA} = −0.324 [95%CI_{FA} = −0.546 to −0.07], P_{FA} = 0.014; β_{MD} = 0.410 [95% CI_{MD} = 0.166–0.630], P_{MD} = 0.002) effects of NBM-WM FA/MD on PIGD severity.

in the PIGD-PD group, independent of lateralization of motor symptoms and in the absence of the NBM-WM volumetric difference. Additionally, we were able to show that the severity of the postural and gait symptoms was significantly correlated with lower FA and higher MD in the NBM-WM pathway. Furthermore, the white matter alterations observed were plausibly caused by the presence of vascular lesions. Finally, we confirmed the significant interrelationship between NBM-WM integrity, attentional function, and gait impairment in PD. These findings provide additional evidence on the role of NBM-associated pathology in postural and gait symptomatology in *de novo* PD patients, besides the well-known contribution to cognitive functioning.

Previous studies have consistently indicated the role of the cholinergic system in axial and gait impairment in PD patients [32]. However, little data have been published so far about these cholinergic disturbances in *de novo* PD patients [20]. Our data show that the cholinergic system is already degenerating in *de novo* PD patients, especially in the PIGD subtype, who predominantly suffered from slight-to-moderate postural and gait impairment. This is new information, which adds to the existing data, including the volume of the NBM as a predictor for gait impairment in PD [11] and FEOBV-PET data showing that PD subjects with freezing-of-gait and falls had depletions of the intrastriatal cholinergic interneurons and the pedunculopontine projections to the thalamus nucleus, in addition to a reduced uptake in the bilateral prefrontal cortex [33].

NBM cholinergic denervation has long been associated with cognitive functioning in PD [34]. Voxel-based morphometry analyses showed that the volume of the NBM was associated with cognitive performance, including visuospatial function and attention, and eventually accelerates the progression to dementia [12,35,36]. The link between the attentional capacity and gait functioning in PD was suggested in an animal model with dual depletions of the basal forebrain cholinergic and striatal dopaminergic neurons and was confirmed further in several clinical studies [37–39].

In our study, significant associations between the NBM-WM integrity and the PIGD score as well as attentional performance were found. Furthermore, the results of the mediation analyses indicated that the effect of NBM-WM microstructural state on PIGD severity can only be partially explained by the state of attentional function. Other components, including the direct neuromodulatory effects of NBM cholinergic neurons in sensorimotor cortices and the cortically-projecting non-cholinergic neurons which intermingle into the delineated NBM-WM tract, may explain the remaining significant direct effect observed in our mediation [40,41]. Nevertheless, the results of current analyses confirm that motor and attentional dysfunctions are interconnected phenomena that both rely, at least partially, on the integrity of the cholinergic basal forebrain complex.

The fact that the total WMH volume was significantly higher in PIGD-PD and was associated with PIGD severity does not undermine the interpretation of the plausible cholinergic white matter pathology in this subtype. This may instead explain the extra-somatic cause of cholinergic denervation in addition to the accumulation of pathological proteinopathy in the NBM [42,43]. Future studies should address the significance of WMH as well the impact of cerebrovascular risk factors in *de novo* PD symptomatology.

This study has several limitations. First, concerning the demographics of the study subjects, our PIGD-PD subjects were significantly older than the TD-PD and potentially bias our result, although the analysis was corrected for age. Furthermore, our HC group was small and significantly younger compared to both PD groups, which might have overrated the difference between HC and PD subtypes. However, because all groups had completely similar acquisition parameters of the MRI scanner, we were still able to show that the NBM-WM degeneration severity of PIGD-PD subjects was spatially more extensive than TD-PD relative to HC.

Other limitations are associated with the analysis approach. the mask used to define the NBM-WM tract was derived from a previous study that reconstructed the NBM-associated white matter pathways in patients with Alzheimer disease, rather than from our own subjects [26]. However, the reconstruction methodology applied in this study was consistent with previous attempts to morphologically delineate the NBM tracts in a lateral and medial pathway [29,44]. Therefore, we consider the reconstructed NBM-WM mask as a solid basis for our analysis.

Additionally, the NBM-WM microstructural integrity was estimated in a common space. Although unlikely to be a problem for group comparisons, for individual evaluation and to be used as a valid diagnostic and predictive biomarker, an individual tractography analysis should be pursued.

Of note, the statistical significance level of our multiple regression analyses was not adjusted for multiple comparisons. This approach was chosen because of the cross-correlation between FA and MD as scalar DTI indices, but also considering the exploratory nature of the subregion analyses (proximal and distal NBM-WM). However, even if Bonferroni correction is applied for the six correlation analyses in our logistic and linear regression analysis, the significant associations between the PIGD severity with NBM-WM FA-MD as well as the finding that PIGD-PD subtype is associated with lower NBM-WM FA will still survive.

Finally, our analysis suggests degeneration of the NBM-WM in PIGD-PD patients, which is used as a proxy of cholinergic denervation. Therefore, our conclusions with respect to cholinergic deficiencies in *de novo* PIGD-PD should be made with caution. However, the fact that the microstructural alterations were found in the proximal area of the NBM-WM pathway, increases the likelihood of a real contribution to cholinergic pathology in PIGD-PD patients.

In conclusion, our data show that the NBM-WM tracts seem to degenerate already in *de novo* PD patients, which correlates with gait and postural symptoms in this group. Future studies should include basal forebrain volumetry and PET scans with cholinergic tracers to confirm the gray matter component of these findings, as well as the neurochemical implications of our DTI-MRI findings.

Declaration of competing interest

No funding agency was involved in the design of the study; the collection, analysis, and interpretation of data; or in writing the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2021.11.010.

Author contributions

A. Study design and data collection, B. Data and statistical analysis, C. Writing of the First Draft, D. Review and Critique, E. Editing, F. Final draft, MN: A, B, C, E, F, JWvD: B, D, E, RJHB: A, D, E, GNS: A, D, E, HJvdH: C, D, E, SvdZ: A, D, E, JMB: A, D, E, TvL: A, D, E, F.

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