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Altered cholinergic innervation in de novo Parkinson's disease with and without cognitive impairment

Running title: Altered cholinergic innervation in de novo Parkinson

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Abstract

Background: Altered cholinergic innervation plays a putative role in cognitive impairment in Parkinson's disease (PD), at least in advanced stages. Identification of the relationship between cognitive impairment and cholinergic innervation early in the disease will provide better insight in disease prognosis and possible early intervention.

Objective: To assess regional cholinergic innervation status in de novo patients with PD, with and without cognitive impairment.

Methods: 57 newly diagnosed, treatment-naïve, PD patients (32 males, mean age 64.6 ± 8.2 years) and 10 healthy control subjects (5 males, mean age of 54.6 ± 6.0 years) were included. All participants underwent cholinergic [^{18}F]Fluoroethoxybenzovesamicol PET, and detailed neuropsychological assessment. PD patients were classified as either cognitively normal (PD-NC) or mild cognitive impairment (PD-MCI). Whole brain voxel-based group comparisons were performed.

Results: Results show bidirectional cholinergic innervation changes in PD. Both PD-NC and PD-MCI groups showed significant cortical cholinergic denervation compared to controls ($p<0.05$, FDR corrected), primarily in the posterior cortical regions. Higher than normal binding was most prominently present in PD-NC in both cortical and sub-cortical regions, including the cerebellum, cingulate cortex, putamen, gyrus rectus, hippocampus and amygdala.

Conclusion: Altered cholinergic innervation is already present in de novo patients with PD. Posterior cortical cholinergic losses were present in all patients independent of cognitive status. Higher than normal binding in cerebellar, frontal and subcortical regions in cognitively intact patients may reflect compensatory cholinergic upregulation in early-stage PD. Limited or failing cholinergic upregulation may play an important role in early, clinically evident cognitive impairment in PD.

Abbreviations:

[^{18}F]FEOBV: [^{18}F]Fluoroethoxybenzovesamicol

FDR: False Discovery Rate

HC: healthy controls

LED: Levodopa Equivalent Dose

MDS-UPDRS-III: Movement Disorder Society - Revised Unified Parkinson's Disease Rating Scale part III

PD: Parkinson's disease

PD-MCI: Parkinson's disease Mild Cognitive Impairment

PC-NC: Parkinson's disease Normal Cognition

PET: Positron Emission Tomography

VAChT: Vesicular Acetylcholine Transporter

Introduction

Cognitive impairment is common in Parkinson's disease (PD) and a major source of disability and lower quality of life (1,2). Mild cognitive impairment (PD-MCI) is already present in 25-30% of newly diagnosed patients and is a major risk factor for the development of PD dementia (PDD) (3–5). Cognitive impairment in PD is heterogeneous with multiple domains affected and great variability in onset and progression (6).

It has been increasingly recognized that comorbid cholinergic dysfunction is a major contributor to the pathophysiology of cognitive impairment in PD (7,8). The four major human brain cholinergic systems are the basal forebrain corticopetal (BF) projection system, cholinergic efferents of the peduncupontine-laterodorsal tegmental complex (PPN/LDTC), medial vestibular nucleus (MVN) cholinergic neurons projecting to the cerebellum, and striatal cholinergic interneurons (9–12).

Previous in vivo neuroimaging assessment of cholinergic innervation, using acetylcholinesterase PET, has demonstrated predominantly posterior cholinergic denervation in PD, with more severe cholinergic degeneration in PDD (13–16). There is additional evidence that the cholinergic system is a major driver of cognitive impairment in PD even in the absence of dementia (17). We previously showed that deficits in attention, executive functioning and memory correlated with loss of cholinergic activity on both a global cortical level (16,18) as well as on a regional (sub)cortical level (19). Although cholinergic denervation has been shown in cognitively impaired patients with PD, less is known about cholinergic innervation changes in early-stage disease and before the onset of cognitive changes. Elucidation of the relationship between cognitive impairment and cholinergic integrity in a very early stage of the disease may provide new clues that may inform novel therapeutic strategies.

The goal of this study was to characterize cholinergic innervation status in newly diagnosed, treatment-naïve PD patients with and without cognitive deficits. We compared vesicular acetylcholine transporter (VAChT) [^{18}F]Fluoroethoxybenzovesamicol ([^{18}F]FEOBV) PET imaging between de novo patients (with and without mild cognitive impairment) and healthy control (HC) subjects. Unlike previously used acetylcholinesterase PET ligands, [^{18}F]FEOBV PET allows for detailed assessment of not only low level cortical but also high binding subcortical structures, such as the basal ganglia and the cerebellum (20–22).

Methods

Participants

57 Newly diagnosed patients with PD and 10 healthy control (HC) subjects were included in this cross-sectional study. Patients were enrolled in the Dutch Parkinson Cohort (DUPARC) study between 2017 and 2019 (For details: Boertien et al., 2020) (23). Inclusion criteria for patients consisted of PD diagnosis by a movement disorders specialist according to Movement Disorder Society (MDS) Clinical Diagnosis Criteria for PD (24) and with a confirmed dopaminergic striatal deficit on 3,4-dihydroxy-6-18F-fluoro-1-phenylalaninie (^{18}F-FDOPA) PET. HC had a normal neurological examination and did not have a history of neurological or psychiatric disorders. Exclusion criteria for both PD and HC subjects included the inability to provide written informed consent, the use of dopaminergic and (anti-)cholinergic medication and an estimated low premorbid intelligence level (estimated IQ <70, on the Dutch Adult Reading test (25)). All subjects gave written informed consent and the study was approved by the local ethics committee.

Clinical examination

All patients underwent comprehensive neuropsychological assessment covering all cognitive domains (23). A selection of outcome measures of tests and subtests of the cognitive test battery was made a priori, meeting level II criteria for PD-MCI (26,27), listed in table 1. Subject scores for each of the cognitive tests were compared to established test-specific normative data generated by age, gender and education. A performance of >1.5 standard deviation (SD) below normative values was considered abnormal. Patients were categorized as either PD with normal cognition (PD-NC) or PD-MCI. PD-MCI was based on level II criteria for PD-MCI and required below-threshold performance on at least two neuropsychological tests (26). Any patient or clinical characteristics possibly influencing performance on the neuropsychological assessments and MCI grouping, including visual difficulties, color blindness, speech problems and significant mood disorders were taken into account at the time of assessment and prior to data analysis, and if necessary excluded. HC subjects underwent cognitive testing using the Montreal Cognitive Assessment (MoCA) test.

Additional clinical assessment included the subjective duration of motor complaints before PD diagnosis and the Hospital Anxiety and Depression scale (HADS) (28). All PD subjects underwent motor examination using the MDS-revised Unified Parkinson's Disease Rating Scale part III (MDS-UPDRS-III). In addition, specific items from the MDS-UPDRS parts II and III were used for the classification of motor phenotypetype, using criteria previously formulated by Stebbins et al. (29). Patients were classified into tremor dominant (TD), postural instability and gait difficulty (PIGD), and indeterminate motor phenotypes

Image acquisition

All subjects underwent brain MRI and VAChT PET imaging, using ([^{18}F]FEOBV). MR imaging of PD subjects was acquired using Siemens Magnetom Prisma 3-Tesla magnetic resonance imaging scanners (Best, Netherlands) equipped with SENSE-8 channel head coils. For each subject, anatomical T1-weighted images were obtained using a sagittal 3-dimensional gradient-echo T1-weighted sequence with 0.9 x 0.9 x 0.9 mm acquisition. HC subjects underwent a T1-weighted MRI scan (3T Intera, Philips, The Netherlands) with 1.0 x 1.0 x 1.0 mm acquisition. [^{18}F]FEOBV imaging was performed on the same day as MR imaging. After a low-dose CT for attenuation and scatter correction, participants were scanned using either a Biograph 40-mCT or 64-mCT (Siemens Healthcare, USA). Both these systems are EARL certified and identical in software version, acquisition- and reconstruction-protocols and PET detectors, and only differ in the number of CT slices. Patient and control subjects were randomly divided over the two PET scanners. [^{18}F]FEOBV was injected using an intravenous bolus and delayed imaging was performed over 30 minutes (in six 5-minute frames) starting 210 minutes after injection.

Image processing was performed using Statistical Parametric Mapping (SPM) software (30). [^{18}F]FEOBV PET imaging frames were spatially coregistered within subjects with a rigid-body transformation. The cropped T1-weighted MR scan was coregistered with the subject PET image. Freesurfer software package (Laboratory for Computational Neuroimaging, Athinoula A. Martinos Center for Biomedical Imaging, Boston, MA, USA) was used to segment MRI into cortical and subcortical brain regions. We calculated the distributed volume ratio (DVR) of each grey matter target region using the summed six delayed imaging frames and the white matter reference region as previously described (21,31). A parametric image for individual subjects was created by using the average of six delayed imaging frames divided by the mean of the white matter reference region. Partial volume correction on our parametric images was done using the Muller-Gartner method (32).

Imaging analysis

Voxel-based analysis was performed as previously described (33). Parametric PET images were spatially normalized to the Montreal Neurological Institute stereotactic template and smoothed with a Gaussian kernel of 8 mm full width half maximum. The relevant brain areas were displayed in Montreal Neurological Institute atlas coordinates (in millimeters) in the stereotactic space using the automated anatomical labeling toolbox.

Statistical analyses

To evaluate brain cholinergic innervation in *de novo* patients with PD, we first compared HC, PD-NC and PD-MCI groups on baseline demographic and clinical characteristics. Comparisons between all

three groups were performed using ANOVA for parametric variables and χ^2 testing for dichotomous variables. Comparisons between the two PD groups were performed using an independent samples t-test or a Mann-Whitney U test, for either normal and skewed distributed data, respectively. Statistical analysis was considered significant for $\alpha<0.05$. Statistical analyses were performed using SPSS Statistics for Windows, Version 24.0 (IBM Statistics, USA) and SPM software.

Whole brain voxel-based analyses were performed to assess difference in cholinergic innervation between groups. Parametric [^{18}F]FEOBV DVR images were used for a two-sample voxel-wise statistical comparison between groups, in both positive and negative directions, controlling for age. The minimum cluster size was set to 50. The false discovery rate (FDR) approach was used for correction for multiple testing effects in the voxel-based analyses. Additional post-hoc Freesurfer-based volume-of-interest (VOI) analysis was performed on regions selected based on the voxel-based analysis. The percentage change of these regions was calculated by dividing the difference between the mean PD and HC group by the mean HC group.

Results

Demographic and clinical characteristics

The 57 *de novo* PD patients (32 males) had a mean (SD) age of 64.6 (8.2) years and a mean (SD) MDS-UPDRS-III score of 30.8 (11.4). 17 (29.8%) patients were classified as PD-MCI. Demographic and clinical characteristics of the different groups are listed in table 2. HC subjects had a significantly lower age and higher MoCA score compared to both PD groups ($p<0.001$). The PD-MCI group had higher MDS-UPDRS-III scores ($p<0.01$) and Hoehn and Yahr stage ($p=0.05$) compared to the PD-NC group, reflecting more severe motor impairment in the PD-MCI group. No significant difference was found between the PD groups on the motor phenotype (TD, PIGD or indeterminate). Information on duration of motor complaints was missing for 11 patients. Available data showed no significant difference in the duration of motor symptom complaints prior to PD diagnosis.

Cognitive functioning

Overall, 15 out of 17 patients classified as PD-MCI showed multi-domain cognitive impairment, reflecting impaired scores in two or more cognitive domains. The most frequently affected domains were memory and executive functions, with 12 and 10 patients presenting impairments in these domains, respectively. The attention and visuospatial domains were affected in 7 out of 17 PD-MCI patients. Language was the least affected domain with 4 PD-MCI patients showing impaired scores. Detailed cognitive performance on the neuropsychological tests for both PD groups is listed in the supplementary materials.

Cholinergic innervation compared to controls.

Whole brain voxel-based analyses were performed to compare regional brain VAChT binding between the PD groups (PD-MCI and PD-NC) and the HC subjects in positive and negative directions, while controlling for age. The PD groups showed evidence of bidirectional changes in different topographic patterns compared to the control subjects.

Significantly lower VAChT binding was found in both PD groups, primarily in the temporal and posterior cortical regions, compared to HC (Figure 1; $p<0.05$, FDR corrected). The topographic profiles for PD-NC and PD-MCI were comparable, with main affected regions including the parietal, parieto-occipital junction and lateral temporal cortices. PD-NC presented with additional lower binding in the frontal cortex, insula and caudate (tail), and PD-MCI with additional lower binding in the pons-medulla regions. A detailed overview of significant clusters is provided in the supplemental data.

Reverse direction voxel-based comparison between HC and both PD groups showed significantly higher VAChT binding in PD subjects compared to HC ($p<0.05$, FDR corrected). This higher cholinergic innervation was most prominently apparent in the PD-NC group (Figure 2A), including the cerebellum, the cingulate cortex, the gyrus rectus, the anteroventral striatum, putamen, the dorsal tegmentum, thalamus, metathalamus (lateral geniculate nucleus (LGN) and medical geniculate nucleus (MGN)), pulvinar, the amygdala, the hippocampus and parahippocampal regions. The PD-MCI groups showed a more limited topography of higher VAChT binding, including the cerebellar vermis, the right gyrus rectus, amygdala, hippocampus and metathalamus, dorsal tegmentum, right inferior thalamus/pulvinarthalamus, and the left precentral gyrus (figure 2B). A detailed overview of significant clusters is provided in the supplemental data.

Most prominent representative examples of the magnitude of absolute binding changes with HC based on VOI comparison include reduced binding in the lateral occipital gyri (-13.3%), the pericalcarine (-19.0%), the transverse temporal gyri (-23.1%), and increases in the cerebellar cortex (+14.2%), the medial and lateral orbitofrontal region (resp +8.8% and +6.5%), frontal pole (+6.1%), the bilateral amygdala (+4.8%), and anterior cingulate (+3.8%) in the PD-NC group. For the PD-MCI group, representative examples include the lateral occipital gyri (-15.7%), the cuneus (-19.6%), pericalcarine (-23.9%), the middle temporal gyri (-14.2%) and the inferior parietal gyri (-15.4%) with lower VAChT binding in PD-MCI, and increases in the cerebellar cortex (+13.2%), the right medial and lateral orbitofrontal region (resp +2.6% and +3.9%), and the right amygdala (+2.9%).

PD-MCI vs PD-NC group comparison

Lower VAChT binding was found in the PD-MCI group in the fusiform and hippocampal region, the anterior insula and the left prefrontal cortex (uncorrected, $p<0.001$). No significant differences were found after correction for multiple comparisons.

Post hoc: controlling for motor impairment

Clinical characteristics comparing both PD groups demonstrated a significant higher MDS-UPDRS-III score in patients with PD-MCI compared to PD-NC (table 2), indicating more severe motor impairment in the PD-MCI group. Bidirectional whole brain voxel-based analysis comparing both PD groups with controls was therefore repeated, controlling for both age and MDS-UPDRS-III.

Limited lower VAChT binding was found in PD-NC and PD-MCI compared to HC ($p<0.001$, uncorrected), including small regional differences in occipital and temporal regions.

Reverse direction analysis showed higher VAChT binding in both PD-NC and PD-MCI compared to controls (figure 3; $p<0.001$, uncorrected). PD-NC showed most extensive higher binding when compared to controls, with a cholinergic topography including the brainstem, gyrus rectus, posterior cingulate cortex, amygdala, and hippocampus and right parahippocampal region. The PD-MCI group demonstrated less widespread higher VAChT binding, limited to the right superior temporal region, the left pulvinar, left optic radiation/occipital region and precentral gyrus.

Discussion

We investigated the topography of regional cholinergic innervation status in newly diagnosed, treatment-naïve patients with PD, with and without cognitive impairment, compared to healthy control subjects. We found altered cholinergic innervation, demonstrated by both higher and lower binding cholinergic brain regions. First, we demonstrate that cortical cholinergic denervation is already present at the start of the disease in both cognitively impaired and cognitively unaffected patients with PD. Second, we also found evidence of increased cholinergic binding in the PD groups. Cognitively unimpaired PD patients showed most robust evidence of higher-than-normal VAChT binding compared to the PD-MCI group.

Previous studies have established the importance of the cholinergic system in cognitive impairment in PD, even in the absence of dementia (13,34). However, the majority of previous in vivo imaging studies are based on PET imaging using acetylcholinesterase tracers, which limit accurate estimates of high binding brain areas. The use of a VAChT PET tracer, like [^{18}F]FEOBV, allows for more reliable and regionally specific cholinergic assessment, especially in high binding areas such as the striatum and cerebellum (20–22). To our knowledge, this is the first assessment of regional cholinergic innervation in de novo patients with PD, with and without cognitive impairment, on a detailed cortical and subcortical level.

29.8% of patients in our study met PD-MCI criteria, which is in line with previous research on the cognitive impairment in early PD (3). In addition, the PD-MCI group presented with more severe motor impairment. However, there was no difference between PD-NC and PD-MCI in terms of motor phenotype (TD, PIGD or indeterminate) or duration of subjective motor complaints prior to PD diagnosis.

A striking finding in our study was the higher-than-normal cholinergic binding in de novo patients with PD compared to controls, most prominent in the PD-NC group. The brain topography represented by higher VAChT binding included the cerebellum, parts of the thalamic complex, putamen, hippocampus and parahippocampal region, amygdala, gyrus rectus and cingulate cortex. Higher VAChT binding in the cerebellum and thalamus indicate a substantial role of the cholinergic projections from the medial vestibular nucleus and PPN/LDTC, respectively (10,11). In addition, the involvement of the hippocampus, parahippocampal region, amygdala, gyrus rectus and cingulate cortex suggests involvement of projections originating from the basal forebrain, including Ch1-2 and Ch4 (Nucleus basalis of Meynert) groups (12). From the Ch4 cell group two major pathways can be identified; the medial pathway joining the white matter of the gyrus rectus and projecting to the

cingulum, and the lateral pathway of which the capsular division projects to the amygdala and temporal lobe (11). Finally, the altered [^{18}F]FEOBV uptake in the putamen and caudate nucleus indicate a role of intrinsic cholinergic striatal interneurons or PPN/LDTC projections (35). A possible role of the dopaminergic system should also be considered, as previous studies have shown an intricate interaction between the dopaminergic and cholinergic system; it is the multiplicative and interacting effects of the two systems that lead to cognitive deficits (17,36).

The higher cholinergic innervation in de novo PD suggests a compensatory cholinergic upregulation in the early phase of the disease. A compensatory role of the cholinergic system has previously been suggested in Alzheimer's disease (AD) where cholinergic upregulation was found in prodromal patients with MCI (37,38). Bohnen et al. (2015) and Kim et al. (2019) expanded on these findings by showing both independent and interactive roles of the cholinergic and dopaminergic system in cognitive functioning in PD (17,39). In the context of dopaminergic losses, preservation or even upregulation of cholinergic innervation may help preserve cognitive functioning (40). In addition, Liu et al (2018) reported increased cortical acetylcholinesterase activity related to LRRK2 gene mutation in (premanifest) PD (41). Bedard et al. (2019) recently showed significantly higher [^{18}F]FEOBV uptake in patients with idiopathic REM sleep behavior disorder (RBD) (42). RBD is considered an important marker of prodromal PD (43) and associated with cognitive impairment conversion (44). Our study is the first to demonstrate evidence of higher-than-normal cholinergic innervation in PD patients, substantiating the hypothesis of a compensatory cholinergic upregulation in very early PD.

Interestingly, the higher cholinergic VAChT binding was less profound in the PD-MCI subgroup than in the cognitively unimpaired PD group, even though both groups consisted of newly diagnosed, treatment-naïve patients with PD with a similar duration of motor complaints prior to PD diagnosis. These findings suggest that a possible lack of compensatory cholinergic upregulation may be related to clinically evident cognitive impairment in early PD patients. In addition, the demonstrated denervation pattern in both PD-NC and PD-MCI groups provides further support for this hypothesis, as the relative extent of the denervation changes in the two PD subgroups had remarkable overlap and PD-MCI did not show more profound cholinergic denervation than PD-NC when compared to controls. In contrast, the PD-NC group showed slightly more extent denervation changes, possibly related to the higher number of subjects in this group providing more statistical power.

However, it should be noted that an alternative explanation for the difference in higher-than-normal VAChT binding between PD-NC and PD-MCI may be related to the severity of motor impairment. The PD-MCI group presented with a higher MDS-UPDRS-III score than PD-NC, indicating more severe motor impairment. Therefore, a post-hoc analysis was performed, controlling for motor

impairment. Our data showed that the higher cholinergic binding was still present in PD-NC, but less widespread and robust. The upregulation in the cerebellum and thalamic complex was less profound, as well as the striatal regions, suggesting an important role of increased PPN cholinergic innervation and cholinergic striatal interneurons in motor performance in early PD. This is in line with previous research showing cholinergic PPN projections and striatal interneurons strongly contributing to motor symptoms in PD, especially in the motor subtype presenting with postural instability and gait disorder (31,45). In contrast, regions with basal forebrain cholinergic projections, including the posterior cingulate cortex, gyrus rectus, amygdala, hippocampus and parahippocampal region remained present after correction for this confounder variable. Interestingly, these regions show partial overlap with the regional cholinergic topography we previously found to be related to cognitive functioning in PD on the level of multiple cognitive domains (19), substantiating the cholinergic role of these regions in cognitive functioning in PD. On the other hand, controlling for MDS-UPDRS-III scores might cause overcorrection, as more severe motor and cognitive impairment often coincide (46,47) and MDS-UPDRS-III scores are significantly correlated with the majority of included cognitive tests (supplementary). The post-hoc analysis controlling for motor performance may therefore give an underestimation of the regional topography of higher cholinergic binding related to cognitive status.

In contrast to earlier findings (14,15,48), we found only limited cortical cholinergic denervation in patients with PD-MCI, without a substantial VAChT binding differences versus PD-NC. A possible explanation for this lack of difference may be the heterogeneity of cognitive performance in patients with PD. In our study, the majority of patients with PD-MCI showed multidomain cognitive impairment, with a variety of domains affected. In addition, not only heterogeneity in cognitive functioning, but also in other PD symptoms, including motor profile and non-motor symptoms, may result in cholinergic heterogeneity (49). The finding of a limited cholinergic denervation pattern may also contribute to the understanding why previous studies have found limited effectiveness of cholinesterase inhibitors in PD-MCI (50,51). We suggest that early cognitive decline is the result of failing cholinergic compensation, rather than cholinergic denervation per se. A better stratification, including the specific profile of dopaminergic and cholinergic innervation, in addition to clinical cognitive performance, might therefore improve the effectiveness of cholinergic treatment in PD.

Limitations

One limitation of this study is the relatively small sample size of the PD subgroups. In addition, the PD-MCI group presented with a heterogeneous cognitive profile, affecting multiple domains. The clinical heterogeneity of this group and the relatively small sample size of the subgroups may have

contributed to the limited difference found between PD-MCI and PD-NC. Future studies with a larger sample size can allow for more detailed stratification and improve our understanding on possible compensatory mechanisms specific to cognitive functioning across different domains. Second, even though MDS guidelines were followed, the grouping of patients into PD-NC and PD-MCI is an arbitrary process, as cognitive changes occur along a continuous spectrum. This might add to the heterogeneity. Furthermore, the cross-sectional design of our study does not allow assessment of temporal changes. More detailed data on cognitive performance over time and the progression to PD-MCI and PDD will enhance our understanding of the cholinergic role in the progression of the disease and the suggested compensatory mechanism. Finally, the HC group had a significantly lower age than both PD groups. We previously demonstrated an important role of age in the relationship between cognition and cholinergic innervation (19). Although the analyses we corrected for age, a possible role of the age difference can not be ruled out.

Conclusion

This study demonstrates evidence of bidirectional changes in cholinergic innervation in de novo patients with PD, with and without cognitive impairment, compared to healthy controls. Increased cholinergic binding in early PD, especially in the cognitively intact patients, suggests a compensatory cholinergic upregulation in this group. Taken together, we postulate that in early, treatment-naïve patients with PD, the clinical syndrome of PD-MCI may be related to limited or failing cholinergic upregulation, instead of a more progressed (posterior cortical) cholinergic denervation.

Author contributions

Study concept and design: van der Zee, Boertien, Gerritsen, Spikman, van Laar

Acquisition, analysis or interpretation of the data: van der Zee, Kanel, Slomp, Boertien, van Laar

Drafting of the manuscript: van der Zee

Critical revision of the manuscript: all authors

Statistical analysis: van der Zee, Kanel

Obtained funding: van der Zee, van Laar

Conflict of Interest Disclosures

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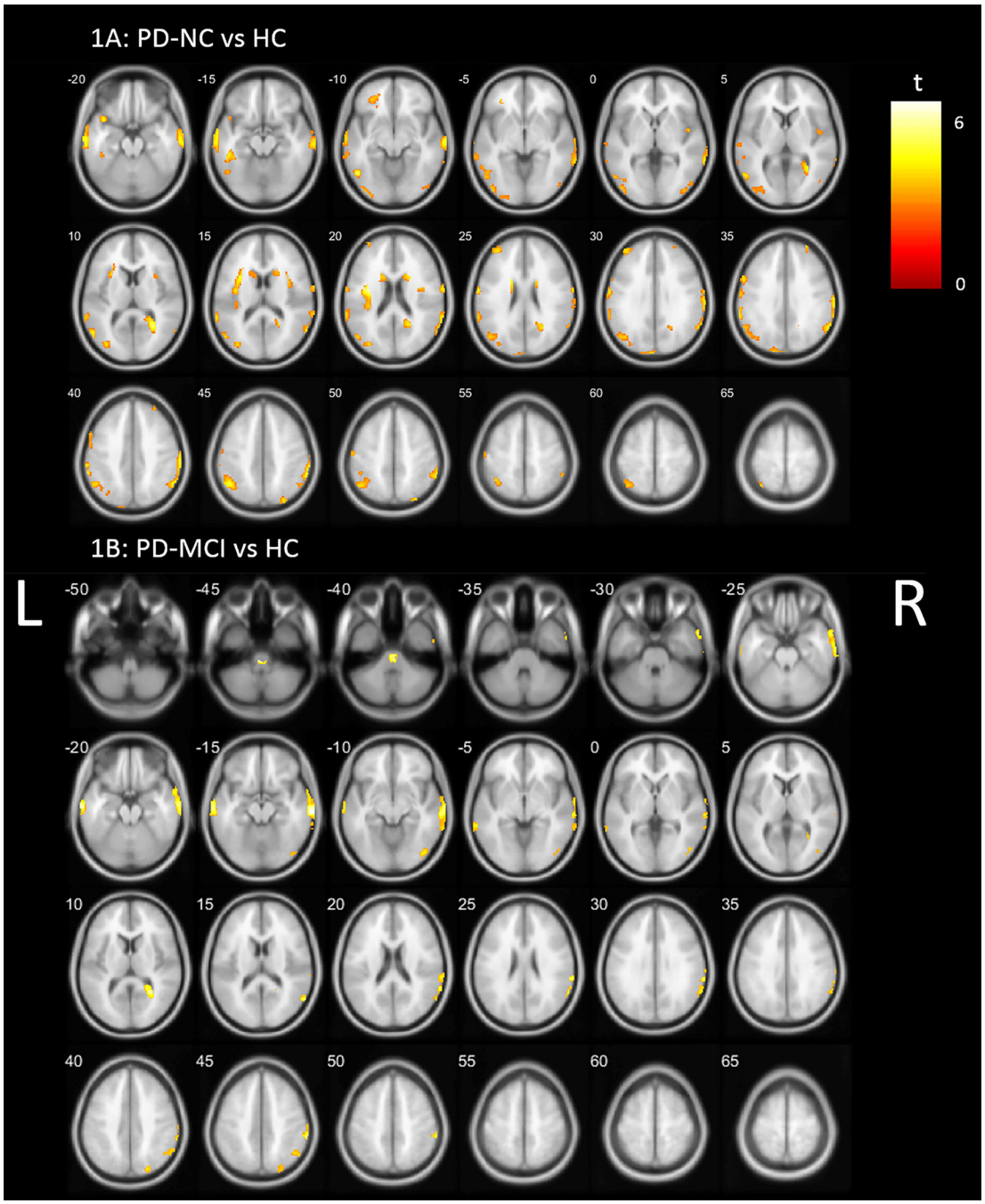

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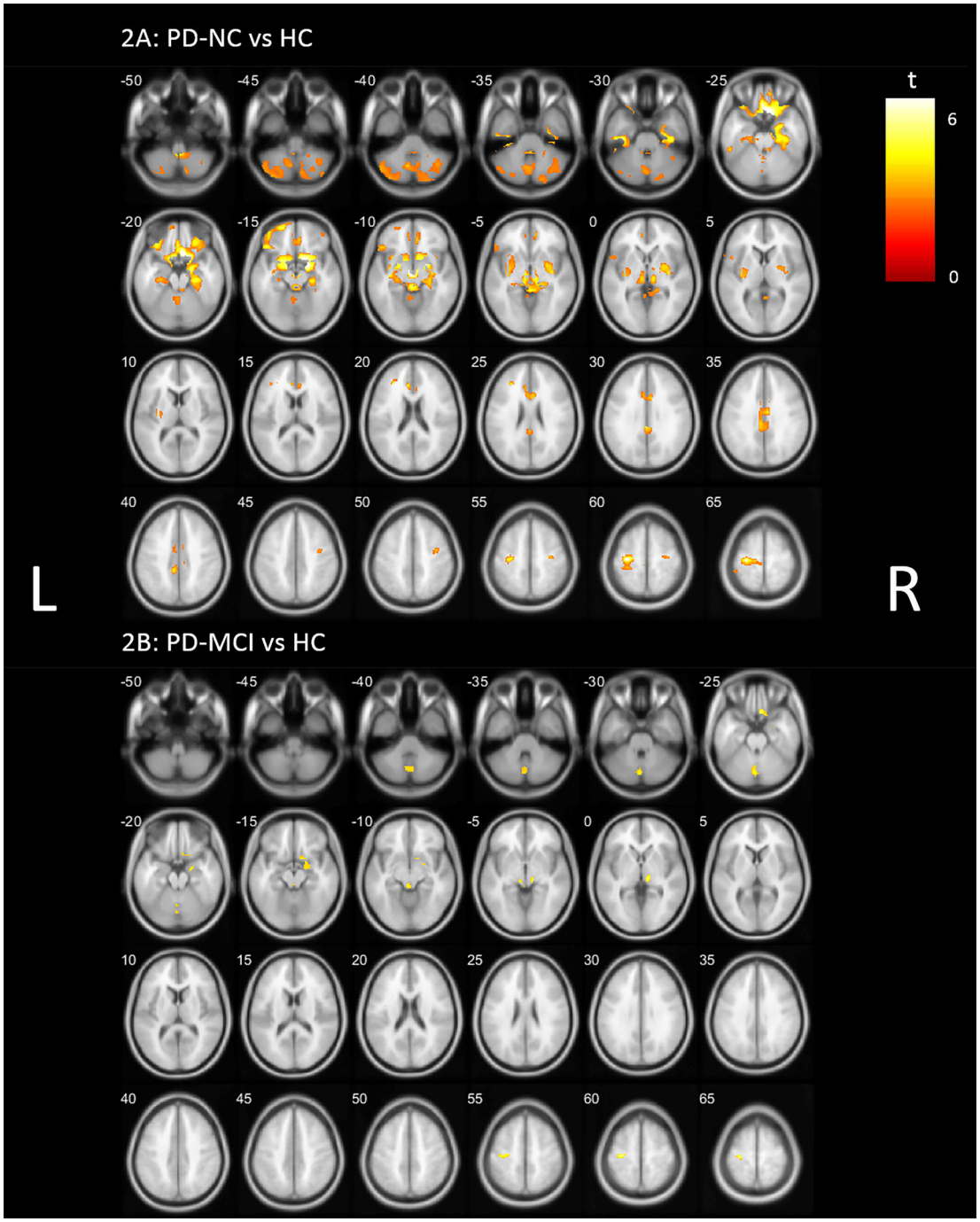

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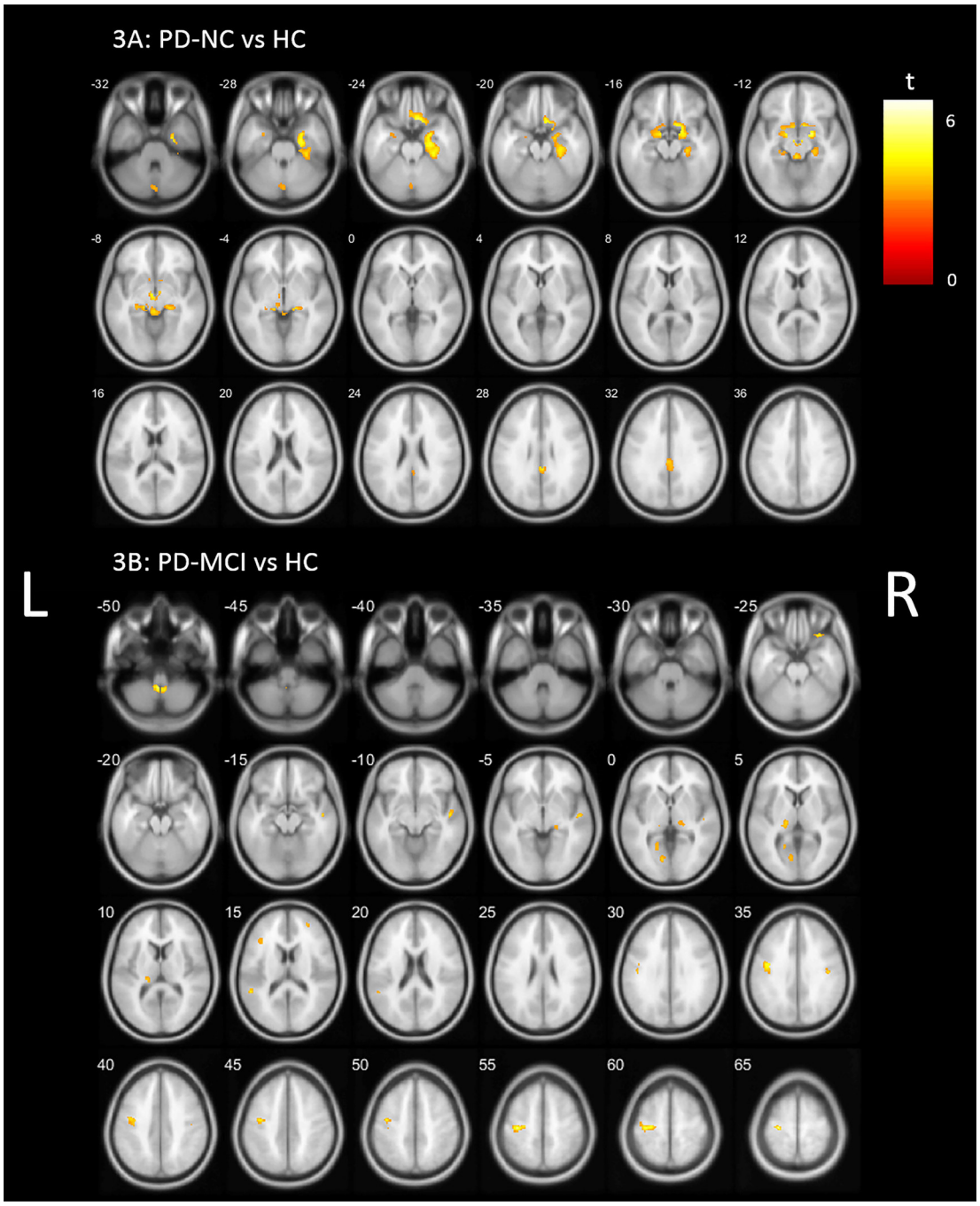

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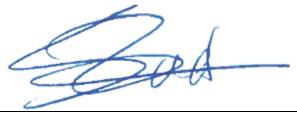

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Abstract

Background: Altered cholinergic innervation plays a putative role in cognitive impairment in Parkinson's disease (PD), at least in advanced stages. Identification of the relationship between cognitive impairment and cholinergic innervation early in the disease will provide better insight in disease prognosis and possible early intervention.

Objective: To assess regional cholinergic innervation status in de novo patients with PD, with and without cognitive impairment.

Methods: 57 newly diagnosed, treatment-naïve, PD patients (32 males, mean age 64.6 ± 8.2 years) and 10 healthy control subjects (5 males, mean age of 54.6 ± 6.0 years) were included. All participants underwent cholinergic [^{18}F]Fluoroethoxybenzovesamicol PET, and detailed neuropsychological assessment. PD patients were classified as either cognitively normal (PD-NC) or mild cognitive impairment (PD-MCI). Whole brain voxel-based group comparisons were performed.

Results: Results show bidirectional cholinergic innervation changes in PD. Both PD-NC and PD-MCI groups showed significant cortical cholinergic denervation compared to controls ($p<0.05$, FDR corrected), primarily in the posterior cortical regions. Higher than normal binding was most prominently present in PD-NC in both cortical and sub-cortical regions, including the cerebellum, cingulate cortex, putamen, gyrus rectus, hippocampus and amygdala.

Conclusion: Altered cholinergic innervation is already present in de novo patients with PD. Posterior cortical cholinergic losses were present in all patients independent of cognitive status. Higher than normal binding in cerebellar, frontal and subcortical regions in cognitively intact patients may reflect compensatory cholinergic upregulation in early-stage PD. Limited or failing cholinergic upregulation may play an important role in early, clinically evident cognitive impairment in PD.

Abbreviations:

[^{18}F]FEOBV: [^{18}F]Fluoroethoxybenzovesamicol

FDR: False Discovery Rate

HC: healthy controls

LED: Levodopa Equivalent Dose

MDS-UPDRS-III: Movement Disorder Society - Revised Unified Parkinson's Disease Rating Scale part III

PD: Parkinson's disease

PD-MCI: Parkinson's disease Mild Cognitive Impairment

PC-NC: Parkinson's disease Normal Cognition

PET: Positron Emission Tomography

VAChT: Vesicular Acetylcholine Transporter

Introduction

Cognitive impairment is common in Parkinson's disease (PD) and a major source of disability and lower quality of life (1,2). Mild cognitive impairment (PD-MCI) is already present in 25-30% of newly diagnosed patients and is a major risk factor for the development of PD dementia (PDD) (3–5). Cognitive impairment in PD is heterogeneous with multiple domains affected and great variability in onset and progression (6).

It has been increasingly recognized that comorbid cholinergic dysfunction is a major contributor to the pathophysiology of cognitive impairment in PD (7,8). The four major human brain cholinergic systems are the basal forebrain corticopetal (BF) projection system, cholinergic efferents of the peduncupontine-laterodorsal tegmental complex (PPN/LDTC), medial vestibular nucleus (MVN) cholinergic neurons projecting to the cerebellum, and striatal cholinergic interneurons (9–12).

Previous in vivo neuroimaging assessment of cholinergic innervation, using acetylcholinesterase PET, has demonstrated predominantly posterior cholinergic denervation in PD, with more severe cholinergic degeneration in PDD (13–16). There is additional evidence that the cholinergic system is a major driver of cognitive impairment in PD even in the absence of dementia (17). We previously showed that deficits in attention, executive functioning and memory correlated with loss of cholinergic activity on both a global cortical level (16,18) as well as on a regional (sub)cortical level (19). Although cholinergic denervation has been shown in cognitively impaired patients with PD, less is known about cholinergic innervation changes in early-stage disease and before the onset of cognitive changes. Elucidation of the relationship between cognitive impairment and cholinergic integrity in a very early stage of the disease may provide new clues that may inform novel therapeutic strategies.

The goal of this study was to characterize cholinergic innervation status in newly diagnosed, treatment-naïve PD patients with and without cognitive deficits. We compared vesicular acetylcholine transporter (VAChT) [^{18}F]Fluoroethoxybenzovesamicol ([^{18}F]FEOBV) PET imaging between de novo patients (with and without mild cognitive impairment) and healthy control (HC) subjects. Unlike previously used acetylcholinesterase PET ligands, [^{18}F]FEOBV PET allows for detailed assessment of not only low level cortical but also high binding subcortical structures, such as the basal ganglia and the cerebellum (20–22).

Methods

Participants

57 Newly diagnosed patients with PD and 10 healthy control (HC) subjects were included in this cross-sectional study. Patients were enrolled in the Dutch Parkinson Cohort (DUPARC) study between 2017 and 2019 (For details: Boertien et al., 2020) (23). Inclusion criteria for patients consisted of PD diagnosis by a movement disorders specialist according to Movement Disorder Society (MDS) Clinical Diagnosis Criteria for PD (24) and with a confirmed dopaminergic striatal deficit on 3,4-dihydroxy-6-18F-fluoro-1-phenylalaninie (^{18}F-FDOPA) PET. HC had a normal neurological examination and did not have a history of neurological or psychiatric disorders. Exclusion criteria for both PD and HC subjects included the inability to provide written informed consent, the use of dopaminergic and (anti-)cholinergic medication and an estimated low premorbid intelligence level (estimated IQ <70, on the Dutch Adult Reading test (25)). All subjects gave written informed consent and the study was approved by the local ethics committee.

Clinical examination

All patients underwent comprehensive neuropsychological assessment covering all cognitive domains (23). A selection of outcome measures of tests and subtests of the cognitive test battery was made a priori, meeting level II criteria for PD-MCI (26,27), listed in table 1. Subject scores for each of the cognitive tests were compared to established test-specific normative data generated by age, gender and education. A performance of >1.5 standard deviation (SD) below normative values was considered abnormal. Patients were categorized as either PD with normal cognition (PD-NC) or PD-MCI. PD-MCI was based on level II criteria for PD-MCI and required below-threshold performance on at least two neuropsychological tests (26). Any patient or clinical characteristics possibly influencing performance on the neuropsychological assessments and MCI grouping, including visual difficulties, color blindness, speech problems and significant mood disorders were taken into account at the time of assessment and prior to data analysis, and if necessary excluded. HC subjects underwent cognitive testing using the Montreal Cognitive Assessment (MoCA) test.

Additional clinical assessment included the subjective duration of motor complaints before PD diagnosis and the Hospital Anxiety and Depression scale (HADS) (28). All PD subjects underwent motor examination using the MDS-revised Unified Parkinson's Disease Rating Scale part III (MDS-UPDRS-III). In addition, specific items from the MDS-UPDRS parts II and III were used for the classification of motor phenotypetype, using criteria previously formulated by Stebbins et al. (29). Patients were classified into tremor dominant (TD), postural instability and gait difficulty (PIGD), and indeterminate motor phenotypes

Image acquisition

All subjects underwent brain MRI and VAChT PET imaging, using ([^{18}F]FEOBV). MR imaging of PD subjects was acquired using Siemens Magnetom Prisma 3-Tesla magnetic resonance imaging scanners (Best, Netherlands) equipped with SENSE-8 channel head coils. For each subject, anatomical T1-weighted images were obtained using a sagittal 3-dimensional gradient-echo T1-weighted sequence with 0.9 x 0.9 x 0.9 mm acquisition. HC subjects underwent a T1-weighted MRI scan (3T Intera, Philips, The Netherlands) with 1.0 x 1.0 x 1.0 mm acquisition. [^{18}F]FEOBV imaging was performed on the same day as MR imaging. After a low-dose CT for attenuation and scatter correction, participants were scanned using either a Biograph 40-mCT or 64-mCT (Siemens Healthcare, USA). Both these systems are EARL certified and identical in software version, acquisition- and reconstruction-protocols and PET detectors, and only differ in the number of CT slices. Patient and control subjects were randomly divided over the two PET scanners. [^{18}F]FEOBV was injected using an intravenous bolus and delayed imaging was performed over 30 minutes (in six 5-minute frames) starting 210 minutes after injection.

Image processing was performed using Statistical Parametric Mapping (SPM) software (30). [^{18}F]FEOBV PET imaging frames were spatially coregistered within subjects with a rigid-body transformation. The cropped T1-weighted MR scan was coregistered with the subject PET image. Freesurfer software package (Laboratory for Computational Neuroimaging, Athinoula A. Martinos Center for Biomedical Imaging, Boston, MA, USA) was used to segment MRI into cortical and subcortical brain regions. We calculated the distributed volume ratio (DVR) of each grey matter target region using the summed six delayed imaging frames and the white matter reference region as previously described (21,31). A parametric image for individual subjects was created by using the average of six delayed imaging frames divided by the mean of the white matter reference region. Partial volume correction on our parametric images was done using the Muller-Gartner method (32).

Imaging analysis

Voxel-based analysis was performed as previously described (33). Parametric PET images were spatially normalized to the Montreal Neurological Institute stereotactic template and smoothed with a Gaussian kernel of 8 mm full width half maximum. The relevant brain areas were displayed in Montreal Neurological Institute atlas coordinates (in millimeters) in the stereotactic space using the automated anatomical labeling toolbox.

Statistical analyses

To evaluate brain cholinergic innervation in *de novo* patients with PD, we first compared HC, PD-NC and PD-MCI groups on baseline demographic and clinical characteristics. Comparisons between all

three groups were performed using ANOVA for parametric variables and χ^2 testing for dichotomous variables. Comparisons between the two PD groups were performed using an independent samples t-test or a Mann-Whitney U test, for either normal and skewed distributed data, respectively. Statistical analysis was considered significant for $\alpha<0.05$. Statistical analyses were performed using SPSS Statistics for Windows, Version 24.0 (IBM Statistics, USA) and SPM software.

Whole brain voxel-based analyses were performed to assess difference in cholinergic innervation between groups. Parametric [^{18}F]FEOBV DVR images were used for a two-sample voxel-wise statistical comparison between groups, in both positive and negative directions, controlling for age. The minimum cluster size was set to 50. The false discovery rate (FDR) approach was used for correction for multiple testing effects in the voxel-based analyses. Additional post-hoc Freesurfer-based volume-of-interest (VOI) analysis was performed on regions selected based on the voxel-based analysis. The percentage change of these regions was calculated by dividing the difference between the mean PD and HC group by the mean HC group.

Results

Demographic and clinical characteristics

The 57 *de novo* PD patients (32 males) had a mean (SD) age of 64.6 (8.2) years and a mean (SD) MDS-UPDRS-III score of 30.8 (11.4). 17 (29.8%) patients were classified as PD-MCI. Demographic and clinical characteristics of the different groups are listed in table 2. HC subjects had a significantly lower age and higher MoCA score compared to both PD groups ($p<0.001$). The PD-MCI group had higher MDS-UPDRS-III scores ($p<0.01$) and Hoehn and Yahr stage ($p=0.05$) compared to the PD-NC group, reflecting more severe motor impairment in the PD-MCI group. No significant difference was found between the PD groups on the motor phenotype (TD, PIGD or indeterminate). Information on duration of motor complaints was missing for 11 patients. Available data showed no significant difference in the duration of motor symptom complaints prior to PD diagnosis.

Cognitive functioning

Overall, 15 out of 17 patients classified as PD-MCI showed multi-domain cognitive impairment, reflecting impaired scores in two or more cognitive domains. The most frequently affected domains were memory and executive functions, with 12 and 10 patients presenting impairments in these domains, respectively. The attention and visuospatial domains were affected in 7 out of 17 PD-MCI patients. Language was the least affected domain with 4 PD-MCI patients showing impaired scores. Detailed cognitive performance on the neuropsychological tests for both PD groups is listed in the supplementary materials.

Cholinergic innervation compared to controls.

Whole brain voxel-based analyses were performed to compare regional brain VAChT binding between the PD groups (PD-MCI and PD-NC) and the HC subjects in positive and negative directions, while controlling for age. The PD groups showed evidence of bidirectional changes in different topographic patterns compared to the control subjects.

Significantly lower VAChT binding was found in both PD groups, primarily in the temporal and posterior cortical regions, compared to HC (Figure 1; $p<0.05$, FDR corrected). The topographic profiles for PD-NC and PD-MCI were comparable, with main affected regions including the parietal, parieto-occipital junction and lateral temporal cortices. PD-NC presented with additional lower binding in the frontal cortex, insula and caudate (tail), and PD-MCI with additional lower binding in the pons-medulla regions. A detailed overview of significant clusters is provided in the supplemental data.

Reverse direction voxel-based comparison between HC and both PD groups showed significantly higher VAChT binding in PD subjects compared to HC ($p<0.05$, FDR corrected). This higher cholinergic innervation was most prominently apparent in the PD-NC group (Figure 2A), including the cerebellum, the cingulate cortex, the gyrus rectus, the anteroventral striatum, putamen, the dorsal tegmentum, thalamus, metathalamus (lateral geniculate nucleus (LGN) and medical geniculate nucleus (MGN)), pulvinar, the amygdala, the hippocampus and parahippocampal regions. The PD-MCI groups showed a more limited topography of higher VAChT binding, including the cerebellar vermis, the right gyrus rectus, amygdala, hippocampus and metathalamus, dorsal tegmentum, right inferior thalamus/pulvinarthalamus, and the left precentral gyrus (figure 2B). A detailed overview of significant clusters is provided in the supplemental data.

Most prominent representative examples of the magnitude of absolute binding changes with HC based on VOI comparison include reduced binding in the lateral occipital gyri (-13.3%), the pericalcarine (-19.0%), the transverse temporal gyri (-23.1%), and increases in the cerebellar cortex (+14.2%), the medial and lateral orbitofrontal region (resp +8.8% and +6.5%), frontal pole (+6.1%), the bilateral amygdala (+4.8%), and anterior cingulate (+3.8%) in the PD-NC group. For the PD-MCI group, representative examples include the lateral occipital gyri (-15.7%), the cuneus (-19.6%), pericalcarine (-23.9%), the middle temporal gyri (-14.2%) and the inferior parietal gyri (-15.4%) with lower VAChT binding in PD-MCI, and increases in the cerebellar cortex (+13.2%), the right medial and lateral orbitofrontal region (resp +2.6% and +3.9%), and the right amygdala (+2.9%).

PD-MCI vs PD-NC group comparison

Lower VAChT binding was found in the PD-MCI group in the fusiform and hippocampal region, the anterior insula and the left prefrontal cortex (uncorrected, $p<0.001$). No significant differences were found after correction for multiple comparisons.

Post hoc: controlling for motor impairment

Clinical characteristics comparing both PD groups demonstrated a significant higher MDS-UPDRS-III score in patients with PD-MCI compared to PD-NC (table 2), indicating more severe motor impairment in the PD-MCI group. Bidirectional whole brain voxel-based analysis comparing both PD groups with controls was therefore repeated, controlling for both age and MDS-UPDRS-III.

Limited lower VAChT binding was found in PD-NC and PD-MCI compared to HC ($p<0.001$, uncorrected), including small regional differences in occipital and temporal regions.

Reverse direction analysis showed higher VAChT binding in both PD-NC and PD-MCI compared to controls (figure 3; $p<0.001$, uncorrected). PD-NC showed most extensive higher binding when compared to controls, with a cholinergic topography including the brainstem, gyrus rectus, posterior cingulate cortex, amygdala, and hippocampus and right parahippocampal region. The PD-MCI group demonstrated less widespread higher VAChT binding, limited to the right superior temporal region, the left pulvinar, left optic radiation/occipital region and precentral gyrus.

Discussion

We investigated the topography of regional cholinergic innervation status in newly diagnosed, treatment-naïve patients with PD, with and without cognitive impairment, compared to healthy control subjects. We found altered cholinergic innervation, demonstrated by both higher and lower binding cholinergic brain regions. First, we demonstrate that cortical cholinergic denervation is already present at the start of the disease in both cognitively impaired and cognitively unaffected patients with PD. Second, we also found evidence of increased cholinergic binding in the PD groups. Cognitively unimpaired PD patients showed most robust evidence of higher-than-normal VAChT binding compared to the PD-MCI group.

Previous studies have established the importance of the cholinergic system in cognitive impairment in PD, even in the absence of dementia (13,34). However, the majority of previous in vivo imaging studies are based on PET imaging using acetylcholinesterase tracers, which limit accurate estimates of high binding brain areas. The use of a VAChT PET tracer, like [^{18}F]FEOBV, allows for more reliable and regionally specific cholinergic assessment, especially in high binding areas such as the striatum and cerebellum (20–22). To our knowledge, this is the first assessment of regional cholinergic innervation in de novo patients with PD, with and without cognitive impairment, on a detailed cortical and subcortical level.

29.8% of patients in our study met PD-MCI criteria, which is in line with previous research on the cognitive impairment in early PD (3). In addition, the PD-MCI group presented with more severe motor impairment. However, there was no difference between PD-NC and PD-MCI in terms of motor phenotype (TD, PIGD or indeterminate) or duration of subjective motor complaints prior to PD diagnosis.

A striking finding in our study was the higher-than-normal cholinergic binding in de novo patients with PD compared to controls, most prominent in the PD-NC group. The brain topography represented by higher VAChT binding included the cerebellum, parts of the thalamic complex, putamen, hippocampus and parahippocampal region, amygdala, gyrus rectus and cingulate cortex. Higher VAChT binding in the cerebellum and thalamus indicate a substantial role of the cholinergic projections from the medial vestibular nucleus and PPN/LDTC, respectively (10,11). In addition, the involvement of the hippocampus, parahippocampal region, amygdala, gyrus rectus and cingulate cortex suggests involvement of projections originating from the basal forebrain, including Ch1-2 and Ch4 (Nucleus basalis of Meynert) groups (12). From the Ch4 cell group two major pathways can be identified; the medial pathway joining the white matter of the gyrus rectus and projecting to the

cingulum, and the lateral pathway of which the capsular division projects to the amygdala and temporal lobe (11). Finally, the altered [^{18}F]FEOBV uptake in the putamen and caudate nucleus indicate a role of intrinsic cholinergic striatal interneurons or PPN/LDTC projections (35). A possible role of the dopaminergic system should also be considered, as previous studies have shown an intricate interaction between the dopaminergic and cholinergic system; it is the multiplicative and interacting effects of the two systems that lead to cognitive deficits (17,36).

The higher cholinergic innervation in de novo PD suggests a compensatory cholinergic upregulation in the early phase of the disease. A compensatory role of the cholinergic system has previously been suggested in Alzheimer's disease (AD) where cholinergic upregulation was found in prodromal patients with MCI (37,38). Bohnen et al. (2015) and Kim et al. (2019) expanded on these findings by showing both independent and interactive roles of the cholinergic and dopaminergic system in cognitive functioning in PD (17,39). In the context of dopaminergic losses, preservation or even upregulation of cholinergic innervation may help preserve cognitive functioning (40). In addition, Liu et al (2018) reported increased cortical acetylcholinesterase activity related to LRRK2 gene mutation in (premanifest) PD (41). Bedard et al. (2019) recently showed significantly higher [^{18}F]FEOBV uptake in patients with idiopathic REM sleep behavior disorder (RBD) (42). RBD is considered an important marker of prodromal PD (43) and associated with cognitive impairment conversion (44). Our study is the first to demonstrate evidence of higher-than-normal cholinergic innervation in PD patients, substantiating the hypothesis of a compensatory cholinergic upregulation in very early PD.

Interestingly, the higher cholinergic VAChT binding was less profound in the PD-MCI subgroup than in the cognitively unimpaired PD group, even though both groups consisted of newly diagnosed, treatment-naïve patients with PD with a similar duration of motor complaints prior to PD diagnosis. These findings suggest that a possible lack of compensatory cholinergic upregulation may be related to clinically evident cognitive impairment in early PD patients. In addition, the demonstrated denervation pattern in both PD-NC and PD-MCI groups provides further support for this hypothesis, as the relative extent of the denervation changes in the two PD subgroups had remarkable overlap and PD-MCI did not show more profound cholinergic denervation than PD-NC when compared to controls. In contrast, the PD-NC group showed slightly more extent denervation changes, possibly related to the higher number of subjects in this group providing more statistical power.

However, it should be noted that an alternative explanation for the difference in higher-than-normal VAChT binding between PD-NC and PD-MCI may be related to the severity of motor impairment. The PD-MCI group presented with a higher MDS-UPDRS-III score than PD-NC, indicating more severe motor impairment. Therefore, a post-hoc analysis was performed, controlling for motor

impairment. Our data showed that the higher cholinergic binding was still present in PD-NC, but less widespread and robust. The upregulation in the cerebellum and thalamic complex was less profound, as well as the striatal regions, suggesting an important role of increased PPN cholinergic innervation and cholinergic striatal interneurons in motor performance in early PD. This is in line with previous research showing cholinergic PPN projections and striatal interneurons strongly contributing to motor symptoms in PD, especially in the motor subtype presenting with postural instability and gait disorder (31,45). In contrast, regions with basal forebrain cholinergic projections, including the posterior cingulate cortex, gyrus rectus, amygdala, hippocampus and parahippocampal region remained present after correction for this confounder variable. Interestingly, these regions show partial overlap with the regional cholinergic topography we previously found to be related to cognitive functioning in PD on the level of multiple cognitive domains (19), substantiating the cholinergic role of these regions in cognitive functioning in PD. On the other hand, controlling for MDS-UPDRS-III scores might cause overcorrection, as more severe motor and cognitive impairment often coincide (46,47) and MDS-UPDRS-III scores are significantly correlated with the majority of included cognitive tests (supplementary). The post-hoc analysis controlling for motor performance may therefore give an underestimation of the regional topography of higher cholinergic binding related to cognitive status.

In contrast to earlier findings (14,15,48), we found only limited cortical cholinergic denervation in patients with PD-MCI, without a substantial VAChT binding differences versus PD-NC. A possible explanation for this lack of difference may be the heterogeneity of cognitive performance in patients with PD. In our study, the majority of patients with PD-MCI showed multidomain cognitive impairment, with a variety of domains affected. In addition, not only heterogeneity in cognitive functioning, but also in other PD symptoms, including motor profile and non-motor symptoms, may result in cholinergic heterogeneity (49). The finding of a limited cholinergic denervation pattern may also contribute to the understanding why previous studies have found limited effectiveness of cholinesterase inhibitors in PD-MCI (50,51). We suggest that early cognitive decline is the result of failing cholinergic compensation, rather than cholinergic denervation per se. A better stratification, including the specific profile of dopaminergic and cholinergic innervation, in addition to clinical cognitive performance, might therefore improve the effectiveness of cholinergic treatment in PD.

Limitations

One limitation of this study is the relatively small sample size of the PD subgroups. In addition, the PD-MCI group presented with a heterogeneous cognitive profile, affecting multiple domains. The clinical heterogeneity of this group and the relatively small sample size of the subgroups may have

contributed to the limited difference found between PD-MCI and PD-NC. Future studies with a larger sample size can allow for more detailed stratification and improve our understanding on possible compensatory mechanisms specific to cognitive functioning across different domains. Second, even though MDS guidelines were followed, the grouping of patients into PD-NC and PD-MCI is an arbitrary process, as cognitive changes occur along a continuous spectrum. This might add to the heterogeneity. Furthermore, the cross-sectional design of our study does not allow assessment of temporal changes. More detailed data on cognitive performance over time and the progression to PD-MCI and PDD will enhance our understanding of the cholinergic role in the progression of the disease and the suggested compensatory mechanism. Finally, the HC group had a significantly lower age than both PD groups. We previously demonstrated an important role of age in the relationship between cognition and cholinergic innervation (19). Although the analyses we corrected for age, a possible role of the age difference can not be ruled out.

Conclusion

This study demonstrates evidence of bidirectional changes in cholinergic innervation in de novo patients with PD, with and without cognitive impairment, compared to healthy controls. Increased cholinergic binding in early PD, especially in the cognitively intact patients, suggests a compensatory cholinergic upregulation in this group. Taken together, we postulate that in early, treatment-naïve patients with PD, the clinical syndrome of PD-MCI may be related to limited or failing cholinergic upregulation, instead of a more progressed (posterior cortical) cholinergic denervation.

Author contributions

Study concept and design: van der Zee, Boertien, Gerritsen, Spikman, van Laar

Acquisition, analysis or interpretation of the data: van der Zee, Kanel, Slomp, Boertien, van Laar

Drafting of the manuscript: van der Zee

Critical revision of the manuscript: all authors

Statistical analysis: van der Zee, Kanel

Obtained funding: van der Zee, van Laar

Conflict of Interest Disclosures

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Table 1: Selection of cognitive tasks used for the assessment of PD-MCI

Domain	Function	(Sub)test
Learning and memory	Verbal learning	RAVLT immediate recall [a]
	Verbal recall	RAVLT delayed recall [a]
	Visual learning	LLT(52) immediate recall [b]
	Visual recall	LLT delayed recall [b]
Attention and processing speed	Basic processing speed	Vienna test system Reaction time test(53): S1 decision time [c]
	Complex information processing speed	Vienna test system Reaction time test: S3 decision time [c]
	Selective attention	Stroop III: color-word
	Attention and memory span	Digit Span
Executive function	Set Shifting	WCST perseverative errors [d]
	Problemsolving	WCST trials to complete first category [d]
	Flexibility	Trail Making Test part B
Visual perception	Visuospatial perception	Judgment of Line orientation
	Visual search and attention	TEA Map search
Language	Verbal fluency	Category fluency: animal naming
	Naming ability	Boston Naming test

[a, b, c, d] – concerns two subtasks within one cognitive test. If both scores are considered impaired, it will be counted as one impaired test.
PD-MCI: Parkinson's disease Mild Cognitive Impairment; RAVLT: Rey Auditory Verbal Learning Test; LLT: Location Learning Test; WCST: Wisconsin Card Sorting Test; TEA: Test of everyday attention.

Table 2. Demographic and Clinical Characteristics of the study participants[a]

	HC (n=10)	PD-NC (n=40)	PD-MCI (n=17)	P Value
Age, y	54.6 (6.0)	63.4 (7.4)	67.5 (9.6)	**<0.001[d]**
Gender, male n (% male)	5 (50%)	19 (47.5%)	13 (76.5%)	0.124
Educational level[b]	5.0 (1.0)	5.0 (2.0)	6.0 (1.0)	0.704
Motor symptom duration, months		20.2 (11.8)	25.1 (17.2)	0.273
MDS-UPDRS-III, total score		28.2 (9.1)	36.8 (14.1)	**0.008**
Motor phenotype, n TD/PIGD/Indeterminate		15/18/7	4/12/1	0.189
Hoehn and Yahr stage[c]		2.0 (1.0); 26.7	2.0 (1.0); 34.5	**0.050**
MoCA, total score	28.4 (0.8)	26.1 (2.6)	23.7 (3.2)	**< 0.001[e]**
HADS anxiety, total score		4.5 (2.6)	4.7 (2.4)	0.778
HADS depression, total score		3.4 (2.4)	4.6 (2.6)	0.085

Abbreviations: HC, healthy controls; PD-NC, Parkinson's Disease Normal Cognition; PD-MCI, Parkinson's Disease Mild Cognitive Impairment; MDS-UPDRS-III, Movement Disorder Society Unified Parkinson's Disease Rating Scale part III; TD, Tremor Dominant; PIGD, Postural Instability and Gait Difficulty; MoCA, Montreal Cognitive Assessment; HADS, Hospital Anxiety and Depression Scale;

[a] Data are presented as mean (standard deviation) unless otherwise indicated.

[b] Educational level according to the Dutch Verhage scale (Verhage, 1964), listed as median (interquartile range)

[c] Hoehn and Yahr stage is listed as median (interquartile range); mean rank

[d] Post-hoc analysis: HC<PD-NC; HC<PD-MCI

[e] Post-hoc analysis: HC>PD-NC>PD-MCI

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