

# Reduced $\alpha 4\beta 2^*$ -Nicotinic Acetylcholine Receptor Binding and Its Relationship to Mild Cognitive and Depressive Symptoms in Parkinson Disease

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**Context:** Cognitive or depressive disorders are frequently noted in patients with Parkinson disease (PD) and may be related to altered signaling through  $\alpha 4\beta 2^*$ -nicotinic acetylcholine receptors ( $\alpha 4\beta 2^*$ -nAChRs).

**Objective:** To assess the availability of  $\alpha 4\beta 2^*$ -nAChRs and their relationship to mild cognitive and mild depressive symptoms in vivo in patients with PD.

**Design:** Crossover comparison between patients with PD and healthy volunteers (control group) using the  $\alpha 4\beta 2^*$ -nAChR-specific radioligand 2-[ $^{18}\text{F}$ ]fluoro-3-(2[S]-2-azetidylmethoxy)-pyridine (2-[ $^{18}\text{F}$ ]FA-85380) and positron emission tomography.

**Setting:** Departments of Neurology and Nuclear Medicine, University of Leipzig, Leipzig, Germany.

**Participants:** Twenty-two nonsmoking patients with PD and 9 nonsmoking healthy volunteers.

**Main Outcome Measures:** Level of 2-[ $^{18}\text{F}$ ]FA-85380 binding potential (2-FA BP), a measure of  $\alpha 4\beta 2^*$ -nAChR availability. The relationship between severity of cognitive symptoms as rated using the Mini-Mental State Examination and DemTect scale and the level of depressive symptoms as indicated using the Beck Depression Inventory, and 2-FA BP were assessed.

**Results:** In patients with PD compared with healthy volunteers, there was widespread reduced 2-FA BP, especially in the midbrain, pons, anterior cingulate cortex, frontoparietal cortex, and cerebellum. In subgroups of patients with PD with possible depression, reduced 2-FA BP was most pronounced in the cingulate cortex and frontoparieto-occipital cortex, whereas in patients with PD with mild cognitive impairment, 2-FA BP was reduced in the midbrain, pons, and cerebellum. In patients with PD, the strongest associations between depressive symptoms and reduced 2-FA BP were noted in the anterior cingulate cortex, putamen, midbrain, and occipital cortex. In contrast, cognitive symptoms correlated only weakly with reduced 2-FA BP in the thalamus, midbrain, temporal cortex, hippocampus, and cerebellum.

**Conclusions:** There is a broad reduction of  $\alpha 4\beta 2^*$ -nAChR availability in patients with PD without clinically manifest dementia or depression compared with healthy volunteers. Reduced  $\alpha 4\beta 2^*$ -nAChR binding in patients with PD within the subcortical and cortical regions is associated with the severity of mild cognitive or depressive symptoms. These results provide novel in vivo evidence for a role of the cholinergic neurotransmission in psychiatric comorbidity of PD.

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**M**ILD COGNITIVE OR depressive symptoms are common in Parkinson disease (PD) and may represent a pre-stage for dementia or depression.<sup>1-3</sup> Patients with PD with cognitive and depressive symptoms are at higher risk of developing clinically manifest dementia or depression.<sup>1-3</sup> Mild cognitive or depressive symptoms in PD that do not fulfill the DSM-IV criteria for dementia or depression can be defined by performing validated neuropsychological screening scales using the Mini-Mental State Examination (MMSE)<sup>4</sup> or DemTect<sup>5</sup> scale and the

Beck Depression Inventory (BDI),<sup>6,7</sup> respectively.

The  $\alpha 4\beta 2^*$  subunits of the neuronal nicotinic acetylcholine receptors ( $\alpha 4\beta 2^*$ -nAChRs) are widely abundant in the human brain, may have a key role in mediating or modulating neurotransmission in various neuronal systems, and have been implicated in a variety of brain functions including mood, attention, learning, and memory.<sup>8</sup> There is evidence from post-mortem studies that normal human brain aging and neurodegenerative diseases such as PD, dementia with Lewy bodies, and Alzheimer disease are associated with a substantial decrease in binding to nAChRs

in cortical and subcortical brain areas.<sup>9-13</sup> Drugs that selectively target  $\alpha 4\beta 2^*$ -nAChRs may be useful in reducing nonmotor symptoms such as cognitive and mood dysfunction in neurodegenerative disease.<sup>14,15</sup> Because they contribute substantially to the severity of disability in PD, there is an unmet need to determine the association between  $\alpha 4\beta 2^*$ -nAChR availability and those symptoms.

Previously, single-photon emission computed tomography (SPECT) demonstrated widespread decreased in vivo availability of  $\alpha 4\beta 2^*$ -nAChRs in small groups of patients with mild to moderate PD without dementia. However, no association between  $\alpha 4\beta 2^*$ -nAChR decrease and mental dysfunction was noted.<sup>16,17</sup> Until recently, in vivo quantification of nAChRs with positron emission tomography (PET) was hampered by the lack of suitable radioligands. The relatively new radioligand 2-[<sup>18</sup>F]fluoro-3-(2-[S]-2-azetidylmethoxy)-pyridine (2-[<sup>18</sup>F]FA-85380 or 2-FA) is characterized by its high affinity and specificity to the  $\alpha 4\beta 2^*$  subunits of the nAChRs and is highly suitable for in vivo quantitative assessment of  $\alpha 4\beta 2^*$ -nAChR availability in the human brain.<sup>18</sup>

In a primarily exploratory approach, using clinical rating scales to assess cognitive and depressive symptoms and the recently developed radioligand 2-FA and PET, we demonstrate for the first time, to our knowledge, in vivo decrease in  $\alpha 4\beta 2^*$ -nAChR binding and its association with mild cognitive and depressive symptoms in PD.

## METHODS

### HEALTHY VOLUNTEERS AND PATIENTS WITH PD

The study included 22 nonsmoking patients with PD with mild to moderate motor symptoms (age, 39-74 years) and 9 nonsmoking healthy volunteers (age, 43-69 years) with no history of neuropsychiatric disorders and medication (**Table 1**).<sup>19,20</sup> Each study participant underwent a clinical interview to exclude DSM-IV criteria for dementia and depression. We used the MMSE,<sup>4</sup> DemTect scale,<sup>5</sup> and BDI<sup>6,7</sup> to screen for cognitive and depressive symptoms. All patients with PD had MMSE scores of 24 or higher and DemTect scale scores of 8 or higher, which is within the normal range or the range of mild cognitive impairment (**Table 2** and supplementary Table 1 [The supplementary tables are available on the authors' Web site at [http://nuklmed.uniklinikum-leipzig.de/download/YOA90008\\_Meyer\\_etal\\_Suppl.pdf](http://nuklmed.uniklinikum-leipzig.de/download/YOA90008_Meyer_etal_Suppl.pdf)]). Because the DemTect scale comprises 5 subtests that measure various aspects of cognitive impairment including memory, attention, and executive function, an accordant subtest analysis was also performed (Table 2). We characterized visuoconstructive ability and executive function using the Clock Drawing Test, CERAD-NP, part V (Consortium to Establish a Registry for Alzheimer Disease, neuropsychological battery), and Trail Making Test part B-A time scores (Table 2 and supplementary Table 1). All patients with PD had BDI scores of 16 or lower, which is below the cutoff score of 16/17 (BDI score >16 is diagnostic of depression with high specificity).<sup>7</sup> For better differentiation within the PD group, a second cutoff score of 8/9 was used (BDI 9-16 is diagnostic of possible depression with high sensitivity but low specificity).<sup>7</sup> Patients with PD with a BDI score lower than 9 were considered nondepressive (Table 2 and supplementary Table 1). Nineteen patients with PD received levodopa, dopamine agonists, or other anti-PD

**Table 1. Epidemiologic and Motor Function Characteristics in Patients With PD and Healthy Volunteers<sup>a</sup>**

Characteristic	Patients With PD (n=22)	Healthy Volunteers (n=9)
Sex		
Male	16	5
Female	6	4
Age, y	63.3 (9.2)	59.1 (9.9)
Educational achievement level, y	13.3 (3.7)	15.8 (2.2)
Disease duration, y	7.3 (4.9)	NA
Age at disease onset, y	56.0 (10.3)	NA
Hoehn and Yahr stage	2.6 (0.8)	NA
UPDRS-III without/with medication	30.3 (14.4)/20.6 (9.9)	NA
Motor type, rigid-akinetic/equivalent or tremor-dominant	10/12	NA
Clinically more affected side, right/left	11/11	NA
Handedness, right/left	21/1	9/0
Anti-PD medications (n=19)		
Levodopa	15	NA
Dopamine agonists	13	NA
Other	12	NA
Levodopa equivalent daily dosage	662.5 (570.1)	NA
Antidepressive medications	3	NA
Cognitive enhancers	0	NA

Abbreviations: NA, not applicable; PD, Parkinson disease; UPDRS-III, Unified Parkinson's Disease Rating Scale, part III.  
<sup>a</sup>Data are given as mean (SD). Unpaired 2-sample *t* test for comparison of patients with PD with healthy volunteers; significant at  $P \leq .05_{\text{uncorrected}}$ .

drugs. Three patients received antidepressant agents to treat sleep disturbances or chronic pain. Depressive episodes were absent in the medical history. Therefore, these patients with PD were included in the study. No cognitive enhancers were given to patients with PD (Table 1). Informed consent was obtained from all participants. The study was approved by the local ethics committee and the competent authorities of the Federal Republic of Germany.

### 2-[<sup>18</sup>F]FA-85380 PET

The radiosynthesis of 2-FA was performed as previously described in detail.<sup>21</sup> The PET scans were obtained with a tomograph (ECAT EXACT HR<sup>+</sup>; CTI/Siemens, Knoxville, Tennessee) in 2-dimensional mode simultaneously collecting 63 sections with an axial resolution of 5 mm full width at half maximum and an in-plane resolution of 4.6 mm. The 2-FA was injected into the cubital vein as a continuous short infusion over 90 seconds. The mean tracer dose was 370 MBq with a total substance amount of less than 0.1 nmol/kg of body weight. The first dynamic series was acquired with blood sampling times of 4 × 15, 4 × 60, 5 × 120, 5 × 300, and 8 × 600 seconds for a total acquisition of 120 minutes. A second series was performed 6 hours postinfusion with four 15-minute images after repositioning the patient in the scanner. An individual thermoplastic mask with a special head-holder system was used to prevent head-movement artifacts.<sup>22-24</sup> All dynamic images were corrected for attenuation using 10-minute transmission scans with 3 rotating germanium 68 rod sources acquired before the first dynamic and the second series, respectively. The PET data sets were reconstructed using Hann-filtered backprojection (4.9 mm full width at half maximum) in a 128 × 128 matrix (voxel 2.57 × 2.57 × 2.43 mm<sup>3</sup>). Blood sampling and analysis of individual binding of 2-FA to plasma proteins and radiolabeled metabolites was performed as previously described in detail.<sup>25-27</sup>

**Table 2. Nonmotor Symptom (Cognitive and Mood) Characteristics in Patients With PD and Healthy Volunteers<sup>a</sup>**

Clinical Scales	Patients With PD (n=22)	Healthy Volunteers (n=9)	ANCOVA <sup>b</sup>	
			F Score	P Value
BDI <sup>c</sup>	7.7 (3.7) <sup>d</sup>	3.7 (2.1)	2.85	.05
MMSE <sup>c</sup>	27.8 (2.0)	29.4 (1.3)	1.33	.29
DemTect <sup>c</sup>	13.4 (2.8) <sup>d</sup>	16.5 (1.9)	4.16	.01
DemTect subtests, total No.				
Word list, immediate recall	10.6 (3.3) <sup>e</sup>	13.9 (2.8)	5.76	.002
Number transcoding	3.2 (1.1)	4.0 (0.0)	1.79	.16
Semantic task fluency record-verbal fluency	21.7 (5.8) <sup>d</sup>	27.1 (4.9)	3.28	.03
Digit span backward condition	4.6 (1.3)	5.7 (0.7)	1.55	.22
Word list, delayed recall	3.5 (1.8) <sup>e</sup>	5.4 (1.7)	4.89	.004
Clock Drawing Test	1.4 (0.7)	1.0 (0.0)	0.91	.48
CERAD, part V, visuospastic praxis, delayed recall	7.9 (2.7) <sup>d</sup>	10.2 (1.4)	3.18	.03
Trail Making Test, part B-A, time scores	81.3 (49.7)	41.6 (17.6)	1.70	.19

Abbreviations: ANCOVA, analysis of covariance; BDI, Beck Depression Inventory; CERAD, Consortium to Establish a Registry for Alzheimer Disease; MMSE, Mini-Mental State Examination; PD, Parkinson disease.

<sup>a</sup>Data are given as mean (SD).

<sup>b</sup>ANCOVA (age, sex, and educational achievement level as covariates) for comparison of patients with PD with healthy volunteers.

<sup>c</sup>BDI score: 9-16, possible depression with high sensitivity but low specificity; <9, no depression. MMSE score: ≤23, probable dementia; 24-26, cognitive impairment; 27-30, normal. DemTect score: <8, probable dementia; 8-13, mild cognitive impairment; 14-18, normal.

<sup>d</sup>Significant at  $P \leq .05_{\text{uncorrected}}$ .

<sup>e</sup>Significant at  $P \leq .05_{\text{corrected}}$  for multiple comparisons ( $P \leq .005_{\text{uncorrected}}$ ).

## ESTIMATION OF PARAMETERS AND ANALYSIS

Kinetic analysis of the tissue response time-activity curves was performed using commercially available software (Pmod; Pmod Technologies, Ltd, Zürich, Switzerland). Because of the slow kinetics of 2-FA, PET studies should take as long as 7 hours to enable accurate quantification of 2-FA-specific binding using graphical analysis.<sup>25</sup> Parametric images of the regional 2-FA distribution volume (2-FA DV) were calculated using the Logan plot (10 points between 1-7 hours postinfusion) with the arterial input function corrected for decay, individual plasma protein binding, and radiolabeled metabolites.<sup>25,28</sup> The parametric 2-FA DV images were coregistered to the individual anatomical magnetic resonance images (MRIs) using the mutual information algorithm implemented using imaging software (MultiModality; Hermes Medical Solutions AB, Stockholm, Sweden). Anatomical information from the individual MRIs was used to manually trace sets of single regions of interest (ROIs) bilaterally on 4 to 8 consecutive planes for each region. The ROIs are shown in **Figure 1** on the ipsilateral side in 3 exemplary MRI sections of the brain in a healthy volunteer and a patient with PD. The ROIs were carefully drawn only in areas containing brain tissue on the MRI, thereby minimizing partial-volume effects. These MRI-defined ROIs were overlaid simultaneously on the PET data set. The following ROIs were chosen because they are implicated in the pathogenesis of PD or in cognitive or affective dysfunction: anterior cingulate cortex (encompassing the subgenual and pregenual portions of the anterior cingulate cortex and the anterior mid-cingulate cortex), posterior cingulate cortex (encompassing the ventral portion of the splenium of the corpus callosum to the retrosplenial portion of the posterior cingulate cortex), frontal lobe (medial prefrontal, orbitofrontal, and dorsolateral cortices), parietal lobe (superior and inferior parietal cortices), temporal lobe (superior, middle, and inferior temporal cortices), occipital lobe (cuneus and visual cortices), corpus callosum (splenium and genu), hippocampus, amygdala, ventral and dorsal striata (caudate and putamen), thalamus, midbrain (substantia nigra and median raphe), pons, and cerebellum (cerebellar hemisphere). The corpus callosum has the lowest density of nAChRs and, consequently, the lowest distribution volume, and the tracer 2-FA

is almost nondisplaceable by nicotine.<sup>29</sup> Therefore, the corpus callosum was used as a reference region to calculate the binding potential (2-FA BP) as follows:  $2\text{-FA BP} = 2\text{-FA DV}_{\text{ROI}} / 2\text{-FA DV}_{\text{corpus callosum}} - 1$ .<sup>29,30</sup>

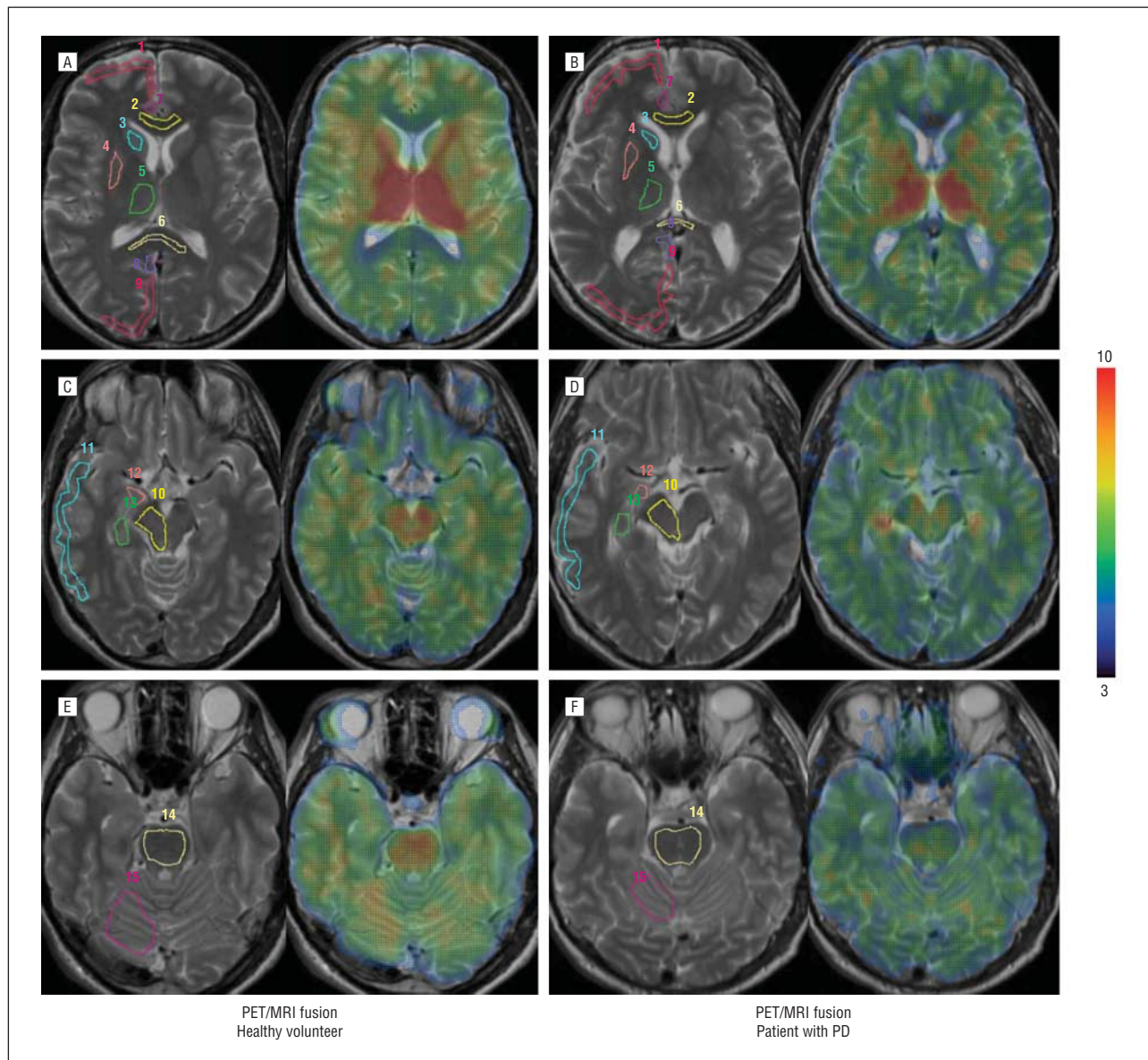
## STATISTICAL PARAMETRIC MAPPING

A voxelwise statistical analysis was performed using statistical parametric mapping (SPM) software (SPM99; Wellcome Trust Centre for Neuroimaging, University College, London, England [http://www.fil.ion.ucl.ac.uk/spm/]). Parametric 2-FA BP images of all patients with PD and healthy volunteers were included in the SPM analysis. The 2-FA BP images were generated from 2-FA DV images using the equation  $2\text{-FA BP} = 2\text{-FA DV}_{\text{ROI}} / 2\text{-FA DV}_{\text{corpus callosum}} - 1$  and the individual reference region, the corpus callosum, as obtained at ROI analysis (Hermes Medical Solutions AB). For SPM analysis, all brain images of patients with PD and healthy volunteers were spatially normalized to the Talairach space on the [<sup>15</sup>O]H<sub>2</sub>O SPM PET template and smoothed (12 mm full width half maximum) to adjust the anatomical variability between the individual brains and to enhance the signal-to-noise ratio for the analysis. Brain areas with substantially decreased or increased 2-FA BP were detected using different contrast vectors (healthy volunteers > patients: [1 -1 0 0 0]; healthy volunteers < patients: [-1 1 0 0 0]) and displayed as glass brain images in 3 views and on a 3-dimensional standard MRI brain image provided by SPM99 (eFigure 1: http://www.archgenpsychiatry.com). The relevant brain areas were displayed with Montreal Neurological Institute atlas coordinates (in millimeters) in the stereotactic space using the automated anatomical labeling toolbox (supplementary Table 2).<sup>31</sup>

## MAGNETIC RESONANCE IMAGING

Each study participant underwent MRI of the brain for anatomical correlation and to rule out any pathologic conditions other than PD. All MRI examinations were carried out using a 1.5-T MRI scanner (Magnetom Symphony; Siemens Medical Solutions, Erlangen, Germany). The imaging protocol consisted of a transverse T2-weighted turbo spin-echo sequence





**Figure 1.** Transaxial sections of positron emission tomography (PET) and magnetic resonance imaging (MRI) 2-[<sup>18</sup>F]fluoro-3-(2[S]-2-azetidylmethoxy)-pyridine (2-[<sup>18</sup>F]FA-85380) distribution volume (2-FA DV) fusion images in a healthy volunteer (A, C, and E) and a patient with Parkinson disease (PD) (B, D, and F) demonstrate widespread cortical and subcortical reduction of 2-FA DV in the patient with PD compared with the healthy volunteer. The 2-FA DV values range from low (blue) to high (red) as indicated by the pseudocolored bar. Manually drawn regions of interest for data analysis are shown in the ipsilateral side of the brain in 3 exemplary transaxial sections of individual MRIs in a healthy volunteer and a patient with PD. Regions of interest are labeled with numbers, and anatomical locales are assigned as follows: 1, frontal lobe; 2, corpus callosum (genu); 3, caudate nucleus; 4, putamen; 5, thalamus; 6, corpus callosum (splenium); 7, anterior cingulate cortex; 8, posterior cingulate cortex; 9, occipital lobe; 10, midbrain; 11, temporal lobe; 12, amygdala; 13, hippocampus; 14, pons; and 15, cerebellum. Regions of interest in the parietal lobe are not shown.

(repetition time, 4000 ms; echo time, 84 ms; 40 sections; section thickness, 3 mm; and field of view, 280 mm) and T1-weighted magnetization prepared rapid gradient echo 3-dimensional sequence (repetition time, 2280 ms; echo time, 3.93 ms; 192 sections; section thickness, 0.8 mm; and field of view, 256 mm), which were used for PET data coregistration. The MRI-based brain atrophy ratings were determined according to published criteria.<sup>32</sup> The degree of global brain atrophy was visually assessed by an experienced rater (D.L.) who was blinded to the individual diagnosis. A 4-point scale was used (0, no atrophy; 1, mild atrophy; 2, moderate atrophy; and 3, severe global atrophy) in comparison with a standard MRI obtained in a healthy volunteer.

## STATISTICAL ANALYSIS

With the large number of regions studied and the lack of information on this topic before this study, this analysis was primarily exploratory. Group comparisons of clinical and imaging data between patients with PD and healthy volunteers were performed using analysis of covariance (ANCOVA) with age and sex as covariates, (un)paired 2-sample *t* test, and analysis of variance (ANOVA) with Bonferroni correction (**Tables 1, 2, 3, and 4** and supplementary Tables 1 and 2). For group comparisons of clinical data between patients with PD and healthy volunteers, significance was accepted at

**Table 3. A Priori Hypothesis Selected and Exploratory ROI-Based Data Comparisons Show Widespread Reductions in In Vivo Regional  $\alpha$ 4 $\beta$ 2\*-nAChR Availability in Patients With PD Compared With Healthy Volunteers<sup>a</sup>**

Region	Brain Side	Patients With PD (n=22)	Healthy Volunteers (n=9)	Mean Change, % <sup>b</sup>	ANCOVA <sup>c</sup>	
					F Score	P Value
Caudate nucleus <sup>d</sup>	Right	0.44 (0.20)	0.70 (0.18)	-37.1	4.04	<.02 <sup>e</sup>
	Left	0.43 (0.21)	0.59 (0.21)	-27.1	1.12	.36
Putamen <sup>d</sup>	Right	0.50 (0.13)	0.64 (0.14)	-21.9	2.64	.07
	Left	0.51 (0.13)	0.64 (0.15)	-20.3	2.03	.13
Thalamus	Right	1.63 (0.22)	1.74 (0.29)	-6.3	2.60	.07
	Left	1.66 (0.20)	1.73 (0.31)	-4.0	2.22	.11
Midbrain <sup>d</sup>	Right	0.53 (0.15)	0.75 (0.19)	-29.3	7.95	<.001 <sup>e</sup>
	Left	0.54 (0.14)	0.77 (0.17)	-29.9	9.27	<.001 <sup>e</sup>
Pons	NA	0.50 (0.13)	0.69 (0.17)	-27.5	8.92	<.001 <sup>f</sup>
ACC	Right	0.31 (0.09)	0.39 (0.07)	-20.5	2.07	.13
	Left	0.22 (0.09)	0.38 (0.07)	-42.1	6.90	<.001 <sup>f</sup>
PCC	Right	0.28 (0.10)	0.35 (0.06)	-20.0	1.28	.30
	Left	0.23 (0.13)	0.41 (0.09)	-43.9	4.64	<.01 <sup>g</sup>
Frontal lobe <sup>d</sup>	Right	0.25 (0.11)	0.41 (0.10)	-39.0	4.75	<.01 <sup>e</sup>
	Left	0.22 (0.10)	0.38 (0.08)	-42.1	6.01	<.002 <sup>g</sup>
Parietal lobe	Right	0.17 (0.12)	0.33 (0.06)	-48.5	5.73	<.004 <sup>g</sup>
	Left	0.17 (0.09)	0.35 (0.07)	-51.4	11.17	<.001 <sup>f</sup>
Temporal lobe <sup>d</sup>	Right	0.20 (0.10)	0.33 (0.07)	-39.4	4.26	<.01 <sup>e</sup>
	Left	0.25 (0.08)	0.35 (0.07)	-28.6	3.45	<.03 <sup>e</sup>
Occipital lobe	Right	0.25 (0.07)	0.32 (0.06)	-21.9	3.20	<.04 <sup>g</sup>
	Left	0.23 (0.06)	0.34 (0.08)	-32.4	5.55	<.004 <sup>g</sup>
Cerebellum	Right	0.42 (0.12)	0.59 (0.14)	-28.8	6.67	<.002 <sup>f</sup>
	Left	0.41 (0.11)	0.59 (0.15)	-30.5	8.95	<.001 <sup>f</sup>
Hippocampus	Right	0.31 (0.11)	0.44 (0.11)	-29.5	3.15	<.04 <sup>g</sup>
	Left	0.34 (0.08)	0.42 (0.16)	-19.0	1.63	.21
Amygdala	Right	0.17 (0.10)	0.30 (0.08)	-43.3	4.32	<.01 <sup>g</sup>
	Left	0.17 (0.11)	0.26 (0.09)	-34.6	1.86	.16

Abbreviations: ACC, anterior cingulate cortex; ANCOVA, analysis of covariance; NA, not applicable; nAChR, nicotinic acetylcholine receptor; PCC, posterior cingulate cortex; PD, Parkinson disease; ROI, region of interest.

<sup>a</sup>Data for 2-[<sup>18</sup>F]fluoro-3-(2-[S]-2-azetidylmethoxy)-pyridine binding are given as mean (SD).

<sup>b</sup>Mean change in patients with PD (healthy volunteers=100%).

<sup>c</sup>ANCOVA using age and sex as covariates was applied for comparison of patients with PD and healthy volunteers.

<sup>d</sup>ROI selected according to a priori hypothesis.

<sup>e</sup>Significant at  $P \leq .05_{\text{uncorrected}}$  (a priori ROIs).

<sup>f</sup>Significant at  $P \leq .002_{\text{uncorrected}}$  (post hoc ROIs;  $P \leq .05_{\text{corrected}}$  for multiple comparisons).

<sup>g</sup>Significant at  $P \leq .05_{\text{uncorrected}}$  (post hoc ROIs).

$P \leq .05_{\text{uncorrected}}$  (Tables 1 and 2 and supplementary Table 1). For group comparisons of ROI data between patients with PD and healthy volunteers, a priori hypothesis-driven ROIs were selected according to results of previous postmortem studies that demonstrated decreased nAChRs in PD in the striatum, midbrain, and frontal and temporal lobes.<sup>9-13</sup> For these ROIs, significance was accepted at  $P \leq .05_{\text{uncorrected}}$ . In addition, exploratory post hoc analyses were carried out within further subcortical and cortical ROIs that may be of relevance for PD or mental dysfunction.<sup>9-13,16,17,33-39</sup> Significance within these exploratory ROIs was accepted at  $P \leq .002_{\text{uncorrected}}$  or  $P \leq .05_{\text{corrected}}$  (for multiple comparisons) (ANCOVA with age and sex as covariates) (Table 3). For exploratory group comparisons of voxel-based SPM data between patients with PD and healthy volunteers, statistical significance was accepted at  $P \leq .001_{\text{uncorrected}}$  and  $P \leq .001_{\text{set-level corrected}}$  with an extent threshold of  $\kappa=5$  voxels and a voxel size of 8 mm<sup>3</sup> (ANCOVA with age and sex as covariates [supplementary Table 2]). For exploratory group comparisons between the 3 PD subgroups (patients with PD without possible depression and without mild cognitive impairment [ND/nMCI], patients with possible depression [D], and patients with mild cognitive impairment [MCI]) and healthy volunteers (Table 4), 1-way ANOVA with Bonferroni correction between groups and correction for multiple testing within the 27 ROIs investigated earlier was per-

formed. Significance was accepted at  $P \leq .002_{\text{uncorrected}}$  or  $P \leq .05_{\text{corrected}}$  (for multiple comparisons) (Table 4). Exploratory ROI-based regression analyses of 2-FA BP within the 27 ROIs and psychometric parameters (BDI, MMSE, and DemTect) were performed using the partial correlation test and age and sex as covariates. Significance was accepted at  $P \leq .002_{\text{uncorrected}}$  or  $P \leq .05_{\text{corrected}}$  (Table 5). No such regression analyses were performed in the healthy volunteers.

Statistical analysis was performed using commercially available software (SPSS version 14.0.1; SPSS, Inc, Chicago, Illinois). Values are given as mean (SD).

## RESULTS

### CLINICAL DATA FOR PATIENTS WITH PD AND HEALTHY VOLUNTEERS

In the group of patients with PD, the male-to-female ratio was approximately 3:1; in the group of healthy volunteers the ratio was 5:4. Patients with PD and healthy volunteers were comparable in age (63±9 years vs 59±10 years), and age did not differ significantly between the groups. However, because patients

**Table 4. Exploratory ROI-Based Data Comparisons of In Vivo Regional  $\alpha 4\beta 2^*$ -nAChR Binding Between 3 Subgroups of Patients With PD and Healthy Volunteers<sup>a</sup>**

Region	Brain Side	Patients With PD <sup>b</sup> (n=22)			Healthy Volunteers (n=9)	ANOVA <sup>c</sup>	
		ND/nMCI (n=7)	D (n=7)	MCI (n=8)		F Score	P Value
Caudate nucleus	Right	0.47 (0.14)	0.42 (0.26)	0.43 (0.21)	0.70 (0.18)	3.69	.02
	Left	0.55 (0.15)	0.37 (0.23)	0.38 (0.22)	0.59 (0.21)	2.41	.09
Putamen	Right	0.48 (0.07)	0.48 (0.09)	0.53 (0.19)	0.64 (0.14)	2.62	.07
	Left	0.56 (0.12)	0.43 (0.14) <sup>d</sup>	0.55 (0.09)	0.64 (0.15)	3.58	.03
Thalamus	Right	1.63 (0.25)	1.62 (0.21)	1.63 (0.22)	1.74 (0.29)	.40	.80
	Left	1.72 (0.19)	1.69 (0.21)	1.59 (0.19)	1.73 (0.31)	.64	.60
Midbrain	Right	0.64 (0.09)	0.56 (0.12)	0.40 (0.12) <sup>e,f</sup>	0.75 (0.19)	8.87	<.001
	Left	0.65 (0.15)	0.53 (0.07) <sup>d</sup>	0.46 (0.11) <sup>e</sup>	0.77 (0.17)	8.66	.001
Pons	NA	0.58 (0.12)	0.52 (0.13)	0.42 (0.11) <sup>e</sup>	0.69 (0.17)	5.65	.004
ACC	Right	0.36 (0.10)	0.24 (0.10) <sup>d</sup>	0.31 (0.06)	0.39 (0.07)	4.87	.008
	Left	0.29 (0.10)	0.15 (0.07) <sup>e,f</sup>	0.24 (0.06) <sup>d</sup>	0.38 (0.07)	12.70	<.001
PCC	Right	0.35 (0.08)	0.21 (0.10) <sup>d,f</sup>	0.27 (0.07)	0.35 (0.06)	6.16	.002
	Left	0.32 (0.15)	0.17 (0.09) <sup>e</sup>	0.21 (0.10) <sup>d</sup>	0.41 (0.09)	8.60	<.001
Frontal lobe	Right	0.27 (0.07)	0.21 (0.17) <sup>d</sup>	0.26 (0.10) <sup>d</sup>	0.41 (0.10)	4.93	.007
	Left	0.23 (0.10) <sup>d</sup>	0.16 (0.10) <sup>e</sup>	0.25 (0.08) <sup>d</sup>	0.38 (0.08)	8.29	<.001
Parietal lobe	Right	0.22 (0.12)	0.10 (0.11) <sup>e</sup>	0.18 (0.09) <sup>d</sup>	0.33 (0.06)	7.93	<.001
	Left	0.22 (0.06) <sup>d</sup>	0.11 (0.10) <sup>e</sup>	0.17 (0.07) <sup>e</sup>	0.35 (0.07)	13.89	<.001
Temporal lobe	Right	0.23 (0.08)	0.17 (0.14) <sup>d</sup>	0.20 (0.07) <sup>d</sup>	0.33 (0.07)	5.03	.007
	Left	0.30 (0.08)	0.22 (0.09) <sup>d</sup>	0.23 (0.07) <sup>d</sup>	0.35 (0.07)	5.26	.005
Occipital lobe	Right	0.26 (0.07)	0.19 (0.07) <sup>e,g</sup>	0.29 (0.06)	0.32 (0.06)	5.86	.003
	Left	0.26 (0.07)	0.19 (0.05) <sup>e</sup>	0.23 (0.05) <sup>d</sup>	0.34 (0.08)	7.67	<.001
Cerebellum	Right	0.50 (0.13)	0.41 (0.07) <sup>d</sup>	0.35 (0.12) <sup>e</sup>	0.59 (0.14)	5.94	.003
	Left	0.48 (0.14)	0.40 (0.07) <sup>d</sup>	0.38 (0.09) <sup>e</sup>	0.59 (0.15)	5.72	.004
Hippocampus	Right	0.35 (0.11)	0.31 (0.13)	0.27 (0.07) <sup>d</sup>	0.44 (0.11)	4.15	.02
	Left	0.36 (0.08)	0.34 (0.10)	0.33 (0.08)	0.42 (0.16)	1.15	.35
Amygdala	Right	0.12 (0.09) <sup>d</sup>	0.19 (0.10)	0.19 (0.10)	0.30 (0.08)	5.28	.005
	Left	0.20 (0.12)	0.15 (0.14)	0.17 (0.09)	0.26 (0.09)	1.75	.18

Abbreviations: ACC, anterior cingulate cortex; ANOVA, analysis of variance; BDI, Beck Depression Inventory; D, PD with possible depression; MCI, PD with mild cognitive impairment; NA, not applicable; nAChR, nicotinic acetylcholine receptor; ND/nMCI, PD without possible depression and without mild cognitive impairment; PCC, posterior cingulate cortex; PD, Parkinson disease; ROI, region of interest.

<sup>a</sup>Data for 2-[<sup>18</sup>F]fluoro-3-(2[<sup>1</sup>S]-2-azetidylmethoxy)-pyridine binding are given as mean (SD).

<sup>b</sup>Subgroups of PD are defined clinically by BDI and DemTect cutoff scores. ND/nMCI: BDI <9; DemTect >13; and MMSE >26. D: BDI ≥9. MCI: DemTect ≤13 or MMSE 24-26.

<sup>c</sup>ANOVA with Bonferroni correction between subgroups with significance accepted at  $P \leq .05_{\text{corrected}}$  and within various regions.

<sup>d</sup>Significantly lower than healthy volunteer group;  $P \leq .05_{\text{uncorrected}}$ .

<sup>e</sup> $P \leq .05_{\text{corrected}}$  ( $P \leq .002$ ) (see also footnote d).

<sup>f</sup>Significantly lower than ND/nMCI group;  $P \leq .05_{\text{uncorrected}}$ .

<sup>g</sup>Significantly lower than MCI group;  $P \leq .05_{\text{uncorrected}}$ .

with PD tended to be older than healthy volunteers (Table 1), age and sex were included as covariates for data analyses. Compared with healthy volunteers, in patients with PD, the scores on the cognitive scales were altered, indicating mild cognitive symptoms. Significance was reached for the DemTect scale, DemTect scale subtests (Word list, immediate and delayed recall; and Semantic task fluency record, verbal fluency), and CERAD part V ( $P \leq .05_{\text{corrected}}$  and  $uncorrected$ ) (Table 2). Nine patients with PD (40.9%) had DemTect scale scores of 8 to 13, indicating mild cognitive impairment. The BDI scores were significantly higher in patients with PD compared with healthy volunteers, indicating depressive symptoms ( $P \leq .05_{\text{uncorrected}}$ ) (Table 2). Eight patients with PD (36.4%) had scores above the cutoff score of 8/9, indicating possible depression. Two patients with PD (9.1%) fulfilled criteria for mild cognitive impairment (DemTect scale score, 8-13) and possible depression (BDI score, 9-16).

Cognitive function (MMSE) correlated negatively with duration of disease ( $r = -0.50$ ;  $P = .01_{\text{uncorrected}}$ ). There were no other correlations between the nonmotor symptom (cognition and mood) characteristics and epidemiologic measures.

## MRI FINDINGS

Negligible global atrophy was present in patients with PD (0.50 [0.74]) and healthy volunteers (0.11 [0.33]). This did not differ significantly between groups.

## 2-FA BP IN PATIENTS WITH PD AND HEALTHY VOLUNTEERS

Compared with healthy volunteers (Figure 1A, C, and E), in a priori-selected ROIs, patients with PD exhibited reduced 2-FA BP ranging from -20.3% in the left putamen (not significant) to -42.1% in the left frontal lobe (Figure 1B, D, and F), reaching highest significance in

**Table 5. Exploratory Correlations Between In Vivo Regional  $\alpha 4\beta 2$ -nAChR Availability and Psychometric Parameters in 22 Patients With PD<sup>a</sup>**

Region	Brain Side	BDI <sup>b</sup>	MMSE <sup>b</sup>	DemTect <sup>b</sup>
ACC	Right	-0.64 (<.001) <sup>c</sup>	0.10 (.34)	0.03 (.45)
	Left	-0.64 (<.001) <sup>c</sup>	0.07 (.39)	0.05 (.41)
PCC	Right	-0.53 (<.01) <sup>d</sup>	0.14 (.28)	0.14 (.28)
	Left	-0.39 (<.05) <sup>d</sup>	0.09 (.36)	0.15 (.27)
Caudate nucleus	Right	-0.35 (.07)	0.22 (.17)	0.06 (.40)
	Left	-0.46 (<.03) <sup>d</sup>	0.02 (.46)	0.29 (.10)
Putamen	Right	-0.25 (.14)	0.07 (.30)	-0.34 (.07)
	Left	-0.66 (<.001) <sup>c</sup>	0.00 (.50)	-0.09 (.35)
Thalamus	Right	-0.46 (<.02) <sup>d</sup>	0.21 (.19)	0.18 (.22)
	Left	-0.31 (.09)	0.12 (.30)	0.47 (<.02) <sup>d</sup>
Midbrain	Right	-0.21 (.11)	0.40 (<.04) <sup>d</sup>	0.46 (<.02) <sup>d</sup>
	Left	-0.61 (<.002) <sup>c</sup>	0.01 (.48)	0.31 (.09)
Pons	NA	-0.38 (<.05) <sup>d</sup>	0.33 (.08)	0.34 (.07)
Frontal lobe	Right	-0.49 (<.02) <sup>d</sup>	-0.02 (.47)	0.11 (.32)
	Left	-0.47 (<.02) <sup>d</sup>	-0.23 (.16)	-0.21 (.18)
Parietal lobe	Right	-0.39 (<.05) <sup>d</sup>	-0.31 (.10)	0.13 (.29)
	Left	-0.42 (<.03) <sup>d</sup>	-0.03 (.45)	0.18 (.22)
Temporal lobe	Right	-0.29 (.11)	0.03 (.44)	0.40 (<.04) <sup>d</sup>
	Left	-0.32 (.09)	0.09 (.35)	0.36 (.06)
Occipital lobe	Right	-0.63 (<.002) <sup>c</sup>	-0.12 (.32)	-0.06 (.41)
	Left	-0.46 (<.02) <sup>d</sup>	0.03 (.44)	0.21 (.19)
Cerebellum	Right	-0.54 (.007) <sup>d</sup>	-0.11 (.33)	0.37 (<.05) <sup>d</sup>
	Left	-0.58 (.004) <sup>d</sup>	-0.07 (.39)	0.19 (.21)
Hippocampus	Right	-0.36 (.06)	0.45 (<.02) <sup>d</sup>	0.31 (.09)
	Left	-0.10 (.33)	0.06 (.41)	-0.02 (.47)
Amygdala	Right	-0.05 (.42)	0.17 (.23)	-0.21 (.19)
	Left	-0.36 (.05) <sup>d</sup>	-0.03 (.45)	0.21 (.19)

Abbreviations: ACC, anterior cingulate cortex; BDI, Beck Depression Inventory; MMSE, Mini-Mental State Examination; NA, not applicable; nAChR, nicotinic acetylcholine receptor; PCC, posterior cingulate cortex; PD, Parkinson disease.

<sup>a</sup>Data are given as the correlation coefficient *r* (*P* value).

<sup>b</sup>Partial correlation test after correction for age and sex.

<sup>c</sup>*P* ≤ .05<sub>corrected</sub> for multiple comparisons (*P* ≤ .002).

<sup>d</sup>*P* ≤ .05<sub>uncorrected</sub>.

the left side of the midbrain (*P* < .001). The 2-FA BP was significantly lower in patients with PD compared with healthy volunteers in the right caudate nucleus, midbrain, and frontal and temporal lobes (a priori ROIs, *P* ≤ .05<sub>uncorrected</sub> and *corrected*) (Table 3). Exploratory analyses revealed additional pronounced and widespread reduced 2-FA BP in patients with PD compared with healthy volunteers (eg, in the left parietal cortex, -51.4%), reaching significance in the pons, left anterior cingulate cortex, left parietal cortex, and cerebellum (ANCOVA with age and sex as covariates, *P* ≤ .002 or *P* ≤ .05<sub>corrected</sub>) (Table 3). Without controlling for age and sex in patients with PD compared with healthy volunteers, 2-FA BP was lower, with high significance in the right caudate, midbrain, cingulate cortex, cerebral lobes, cerebellum, and right amygdala (*P* ≤ .05<sub>corrected</sub> [data not shown]). The 2-FA BP in 3 patients with PD receiving antidepressive medication did not differ significantly from that in those not receiving antidepressive medication. Neither retaining nor excluding these 3 patients had a significant effect on the findings for nAChR availability compared with that in healthy volunteers. In patients with PD, asymmetries of 2-FA BP were found in the anterior (*P* ≤ .05<sub>corrected</sub>; left < right) and posterior (*P* ≤ .05<sub>uncorrected</sub>; left < right) cingulate cortices and in the temporal lobe (*P* ≤ .05<sub>corrected</sub>; right < left; paired dependent *t* test).

## EXPLORATORY SPM ANALYSIS

The a priori and exploratory ROI-based 2-FA BP reductions in patients with PD were all confirmed at exploratory voxel-based SPM analysis. In addition, there were significant clusters of 2-FA BP reduction in patients with PD compared with healthy volunteers in the putamen, insular cortex, medial thalamus, posterior cingulate cortex, occipital cortex, hippocampus, and amygdala that were not discovered at ROI analyses (*P* ≤ .001<sub>uncorrected</sub>) (eFigure 1 and supplementary Table 2).

## CLINICAL DATA IN PD SUBGROUPS

Subgroups of patients with PD were identified on the basis of BDI, MMSE, and DemTect scale scores (supplementary Table 1). These PD subgroups (ND/nMCI, D, and MCI) differed significantly from each other insofar as BDI or DemTect scale scores according to the definition of the subgroups. Only the MCI group had significantly altered values on the Clock Drawing Test, CERAD part V, and the Trail Making Test part B-A time scores (*P* ≤ .05<sub>corrected</sub> and *uncorrected*). Of note, PD subgroups did not differ significantly between each other in all other clinical parameters.

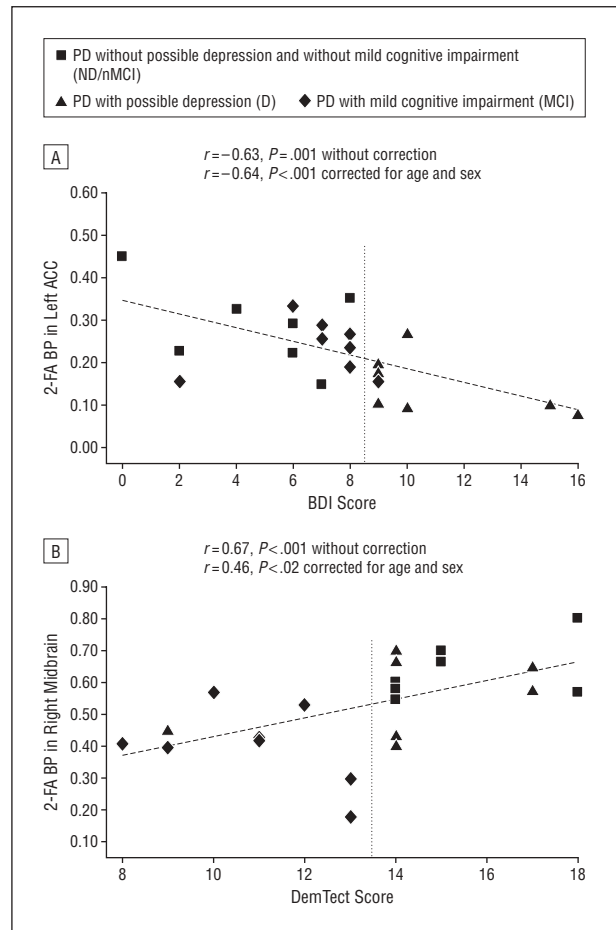


## EXPLORATORY ROI ANALYSES OF 2-FA BP IN PD SUBGROUPS

Data are given in Table 4 and eFigure 2. The objective of the PD subgroup ROI analysis was to identify brain regions that differed specifically between groups. Compared with healthy volunteers, all 3 PD subgroups demonstrated reduced 2-FA BP except within the right posterior cingulate cortex in the ND/nMCI group. Overall, 2-FA BP was lower in the D and MCI groups vs the ND/nMCI group. In addition, in the D group, there was lower binding than in the MCI group in most cortical areas, whereas the MCI group exhibited lower 2-FA BP in most subcortical areas and the cerebellum (eFigure 2). Analysis of variance (Table 4) revealed significant reductions in 2-FA BP in the D group vs the healthy volunteers in the left anterior and posterior cingulate cortices, left frontal lobe, and bilateral parietal and occipital lobes ( $P \leq .05_{\text{corrected}}$ ). Significant reductions in 2-FA BP in the MCI group compared with the healthy volunteers were observed in the midbrain, pons, left parietal cortex, and cerebellum ( $P \leq .05_{\text{corrected}}$ ). Similar significant differences were not observed when comparing the ND/nMCI group with the healthy volunteers. Analysis of variance demonstrated significantly reduced 2-FA BP in the D group vs the MCI group in the right occipital lobe ( $P \leq .05_{\text{uncorrected}}$ ). Significantly reduced 2-FA BP was present in the D group vs the ND/nMCI group in the left anterior and right posterior cingulate cortices ( $P \leq .05_{\text{uncorrected}}$ ). In the MCI group vs the ND/nMCI group, 2-FA BP was significantly more reduced only in the right midbrain ( $P \leq .05_{\text{uncorrected}}$ ). Especially in the D or MCI groups compared with the healthy volunteers, 2-FA BP was reduced in further regions ( $P \leq .05_{\text{uncorrected}}$ ) (Table 4).

## EXPLORATORY REGRESSION ANALYSIS BETWEEN 2-FA BP AND NEUROPSYCHOLOGICAL SCALES IN PD

Data are given in Table 5, and 2 correlational graphs are shown in **Figure 2**. In patients with PD, strong and highly significant correlations between the severity of depressive symptoms (BDI scores) and 2-FA BP reductions in the anterior cingulate cortex, left putamen, left midbrain, and right occipital lobe were measured ( $P \leq .05_{\text{corrected}}$ ). There were several additional correlations between 2-FA BP and BDI scores in cortical and subcortical regions in patients with PD that did not remain significant when correcting for multiple comparisons ( $P \leq .05_{\text{uncorrected}}$ ) (Table 5). Correlations between the severity of cognitive symptoms (MMSE or DemTect scale scores) and reduction of 2-FA BP in the left thalamus, right midbrain, right temporal lobe, right hippocampus, and right cerebellum did not remain significant when correcting for multiple comparisons (Table 5). Correlations between reduction of 2-FA BP and BDI or MMSE scores were similar independent of age and sex. Without controlling for age and sex, there were correlations between reduction of 2-FA BP and DemTect scale scores in the thalamus and midbrain, with higher significance ( $P \leq .05_{\text{corrected}}$ ), and also in the pons ( $P \leq .05_{\text{uncorrected}}$  [data not shown]).



**Figure 2.** Two exemplary correlation graphs in Parkinson disease (PD) demonstrate the reduced 2-[<sup>18</sup>F]fluoro-3-(2[*S*]-2-azetidinylmethoxy)-pyridine (2-[<sup>18</sup>F]FA-85380) binding potential (2-FA BP) and its relationship to depressive and cognitive symptoms in PD. The graphs show the association between the reduced 2-FA BP in the left anterior cingulate cortex (ACC) and the degree of depressive symptoms as indicated by higher Beck Depression Inventory (BDI) scores (A) and the relationship between the decreased 2-FA BP in the right midbrain and the degree of cognitive symptoms as indicated by lower DemTect scores (B). Data assigned to the various PD subgroups are indicated by different symbols. The dotted lines in A and B indicate, respectively, the BDI cutoff score of 8/9 (BDI <9, PD without depression; BDI ≥9, PD with possible depression) and the DemTect cutoff score of 13/14 (DemTect >13, PD without cognitive impairment; DemTect ≤13, PD with mild cognitive impairment). D indicates PD with possible depression (BDI score 9-16); MCI, PD with mild cognitive impairment (DemTect score 8-13); ND/nMCI, PD without possible depression and without mild cognitive impairment (BDI score <9; DemTect score >13). Correlation coefficients and *P* values with and without correction for age and sex are shown to demonstrate the influence of these covariates on the correlation analyses.

In healthy volunteers, reduction of 2-FA BP with aging (5.0%-13.4% per decade) was found in all regions except the occipital lobe, reaching low significance in the caudate nucleus, thalamus, cerebellum, midbrain, and pons ( $r > -0.57$ ;  $P \leq .05_{\text{uncorrected}}$ ; right/left data pooled). In patients with PD, no significant correlations between aging and 2-FA BP were found except in the pons ( $r = -0.44$ ;  $P = .02_{\text{uncorrected}}$ ; reduction of 8.6% per decade). In healthy volunteers but not in patients with PD, there was a trend for lower 2-FA BP in men compared with women in the cingulate cortex, neocortex, putamen, hippocampus, and amygdala ( $P = .06-.10$ ). No significant correlations between 2-FA BP level and the levodopa equivalent daily dosage were found in patients with PD.



The present mainly exploratory 2-FA PET study demonstrates broad differences in  $\alpha 4\beta 2^*$ -nAChR binding, especially in the midbrain, pons, anterior cingulate cortex, frontoparietal lobe, and cerebellum in patients with PD without clinically manifest dementia or depression compared with healthy volunteers. Furthermore, these  $\alpha 4\beta 2^*$ -nAChR diminutions are most pronounced in subgroups of patients with PD with possible depression, especially in the cingulate cortex and frontoparieto-occipital cortex, and in those with mild cognitive impairment, especially in the midbrain, pons, and cerebellum, and they are related to the degree of mild depressive and mild cognitive symptoms as indicated at correlation analyses.

Our study findings agree with those of 2 previous SPECT investigations in patients with PD without dementia.<sup>16,17</sup> The latter studies documented reductions in nAChR binding primarily in the brainstem and the frontal, cingulate, and temporal cortices<sup>17</sup> or in virtually all brain regions investigated.<sup>16</sup> However, owing to the limited spatial resolution of SPECT and few study participants, these authors did not find associations with non-motor symptoms such as depression or cognitive impairment. In addition, there is postmortem evidence for less  $\alpha 4\beta 2^*$ -nAChR binding in the striatum, midbrain, thalamus, cerebral cortex, and hippocampus in patients with PD with and without dementia using autoradiography and immunohistochemistry.<sup>9-13,39</sup>

In PD, significantly reduced  $\alpha 4\beta 2^*$ -nAChRs within the a priori-selected ROIs were expected and proved in the midbrain, right caudate nucleus, and putamen, most likely attributed to the loss of  $\alpha 4\beta 2^*$ -nAChRs on degenerated nigrostriatal dopaminergic neurons.<sup>9-12</sup> Reductions in the cingulate cortex, amygdala, hippocampus, and cerebral cortex may also be related to degenerated mesocorticolimbic dopaminergic neurons.<sup>16,17,40,41</sup>

Alternatively, these alterations in PD may represent degeneration of major cholinergic projection systems. One arises from the basal nucleus of Meynert making broad projections throughout the cerebral cortex and hippocampus. The second system arises from neurons in the pedunclopontine tegmentum and dorsolateral pontine tegmentum giving widespread innervation to the thalamus, midbrain, caudal pons, and brainstem. The third system arises from the cholinergic interneurons in the striatum.<sup>8,11</sup> This is supported by findings of in vivo PET and SPECT studies with various radiotracers demonstrating subcortical and cortical cholinergic deficits in patients with PD with and without dementia.<sup>42-46</sup>

Because  $\alpha 4\beta 2^*$ -nAChRs are widely abundant in the human brain in presynaptic, axonal, and somatodendritic locations and are not restricted to cholinergic or dopaminergic neurons,<sup>8</sup> multiregional reductions in  $\alpha 4\beta 2^*$ -nAChRs in patients with PD without full-blown dementia or depression may represent degeneration of multiple neurotransmitter systems (eg, noradrenergic, serotonergic, glutamatergic, or transmitting  $\gamma$ -aminobutyric acid [GABAergic]) to which the nAChRs are coupled.<sup>8,47,48</sup> Therefore, multiple tracer imaging studies combining cholinergic and noncholinergic PET in parallel with 2-FA PET may

enable better understanding of the  $\alpha 4\beta 2^*$ -nAChR alteration patterns in PD determined in this study.<sup>49</sup>

Our findings are of particular interest because (1) some of these brain regions are implicated in cognitive and affective disorders, as revealed by preclinical, postmortem, and PET studies,<sup>12,33-39,42-46</sup> and (2) only patients with PD with mild cognitive and depressive symptoms not meeting clinical criteria for dementia or depression were studied. As revealed in the PD subgroup analysis and the correlations, the widespread  $\alpha 4\beta 2^*$ -nAChR reductions may be related to mental dysfunction in PD. However, despite focusing on nonmotor symptoms, patients with PD without possible depression and without possible mild cognitive impairment (ND/nMCI) still had altered 2-FA BP in several brain areas to a milder degree.

#### Relationship Between $\alpha 4\beta 2^*$ -nAChR Availability and Cognitive Symptoms (DemTect Scale and MMSE)

Although the present study did not include patients who fulfilled clinical criteria for dementia, we were able to identify subgroups with mild cognitive impairment who exhibited specific differences in  $\alpha 4\beta 2^*$ -nAChR binding in the midbrain, pons, and cerebellum. Neuronal loss in the midbrain correlates with dementia in PD.<sup>34</sup> In rats, infusions of nicotinic antagonists into the midbrain also impair working memory.<sup>33</sup> The effect of  $\alpha 4\beta 2^*$ -nAChR binding in the midbrain for cognitive function was further supported in that MCI directly compared with ND/nMCI differed only in the midbrain. Accordingly, there was a negative correlation, although not significant when correcting for multiple comparisons, between  $\alpha 4\beta 2^*$ -nAChR binding in the midbrain and the severity of cognitive impairment. Similar weak correlations were detected in the thalamus, temporal lobe, cerebellum, and hippocampus, areas that also may relate to nicotinic effects on cognition.<sup>33</sup> The lack of stronger correlations most likely occurred because (1) cognitive screening tests used in this first study may not be sensitive and selective enough to identify deficits in specific cognitive domains that relate to regional  $\alpha 4\beta 2^*$ -nAChR dysfunction; (2) decreases, especially in subcortical regions (eg, the midbrain), that relate to cognitive impairment might be confounded by the underlying degeneration of dopaminergic neurons; and (3) compensatory effects on  $\alpha 4\beta 2^*$ -nAChR availability may occur as a result of subclinical cholinergic deficits.<sup>50</sup> Further studies will indicate whether 2-FA binding in the midbrain can enable identification of patients at risk for dementia in PD. Overlapping association patterns of altered  $\alpha 4\beta 2^*$ -nAChRs with cognitive or depressive symptoms in the midbrain, thalamus, and cerebellum may reflect a common pathophysiologic mechanism contributing to the clinically often-reported relationship between dementia and depression in PD.<sup>51</sup>

#### Relationship Between $\alpha 4\beta 2^*$ -nAChR Availability and Depressive Symptoms (BDI)

In our subgroup analysis, patients in the D group exhibited the most pronounced differences in  $\alpha 4\beta 2^*$ -nAChR

binding compared with healthy volunteers in the cingulate cortex and frontoparieto-occipital cortex. When patients in the D group were directly compared with those in the ND/nMCI group, differences in  $\alpha 4\beta 2^*$ -nAChR availability were present in the cingulate cortex. Comparing the D group directly with the MCI group revealed respective differences in the occipital lobe. Thus, 2-FA PET assessment of these regions may help to identify patients with PD at risk to develop full-blown depression. The strikingly strong associations between the  $\alpha 4\beta 2^*$ -nAChR reductions and the degree of mild depressive symptoms in PD in the anterior cingulate cortex, putamen, midbrain, and occipital cortex correspond to a frontal corticomesostriatal circuitry. Additional weak associations in the posterior cingulate cortex, caudate nucleus, thalamus, pons, frontoparietal cortex, cerebellum, and amygdala suggest that, in patients with PD with mild depression, this circuitry should be extended to a frontal corticobasal ganglia-limbic-cerebellar circuitry as described for major depressive disorder.<sup>36,37,52-56</sup> Association patterns such as between depressive symptoms and  $\alpha 4\beta 2^*$ -nAChR alterations seem to highlight the relevance of cholinergic modulation of these networks, making  $\alpha 4\beta 2^*$ -nAChRs a possible target for therapeutic interventions.<sup>14,57</sup> In the midbrain, for example, the  $\alpha 4\beta 2^*$ -nAChRs are located on dopaminergic, noradrenergic, GABAergic, and serotonergic neurons.<sup>8</sup> Therefore, by their modulation,  $\alpha 4\beta 2^*$ -nAChRs may be involved in the dysfunction of these various neurotransmitter systems found in PET and SPECT studies in patients with PD with depression.<sup>58-60</sup>

The  $\alpha 4\beta 2^*$ -nAChR reductions in the cerebral cortex, anterior cingulate cortex, striatum, and midbrain and their relationship to depressive symptoms in PD could also result from a genuine disturbance of cholinergic neurons leading to a loss or downregulation of the  $\alpha 4\beta 2^*$ -nAChRs.<sup>61</sup> This interpretation is supported by a previous PET study in patients with PD with and without dementia that suggested that cortical cholinergic deficits are related to depressive symptoms.<sup>42</sup>

In PD, a stronger relationship between depressive symptoms and decrease of  $\alpha 4\beta 2^*$ -nAChRs in the left hemisphere (striatum, midbrain, and amygdala) and reduced  $\alpha 4\beta 2^*$ -nAChR binding in the left anterior cingulate cortex compared with the contralateral side is present, which suggests that the left hemisphere is more associated with depressive symptoms. This is supported by PET and SPECT studies that demonstrated asymmetric alterations of glucose metabolism in the left prefrontal and anterior cingulate cortex in major depression and of dopamine transporters in the left dorsal striatum in patients with PD with depression.<sup>60,62</sup>

In the small group of healthy volunteers studied, there was a weak association between aging and  $\alpha 4\beta 2^*$ -nAChR reduction, primarily in subcortical regions as shown previously with SPECT.<sup>63</sup> In addition, there was a trend for sex-related reduced  $\alpha 4\beta 2^*$ -nAChR binding in men. Comparing patients with PD with healthy volunteers, without controlling for age- or sex-related effects, the number of significantly different regions increased, indicating a potential role for age and sex as confounders. Therefore, age- and sex-related effects on

$\alpha 4\beta 2^*$ -nAChR binding should be considered using PET or SPECT to study nAChRs in aging or neurodegeneration.<sup>63,64</sup> Modulatory effects of dopaminergic therapy might affect  $\alpha 4\beta 2^*$ -nAChR availability.<sup>17,65</sup> Although for optimal investigation of drug-related effects 2-FA PET studies performed before and after drug treatment are needed, major effects of dopaminergic drugs on  $\alpha 4\beta 2^*$ -nAChRs in our patients with PD seem to be unlikely inasmuch as no associations between  $\alpha 4\beta 2^*$ -nAChRs and levodopa equivalent daily dosage were found. Atrophy may contribute to diminish the PET signal as a result of a volume effect. Although major gray matter atrophy was observed in patients with PD with dementia, it was absent in patients with PD without dementia.<sup>66</sup> An effect of gray matter atrophy on  $\alpha 4\beta 2^*$ -nAChR binding in the study patients with PD is unlikely because these patients exhibited negligible global atrophy that did not differ significantly from that in healthy volunteers. More important, ROIs were carefully drawn only within areas containing brain tissue on individual MRIs to which the PET images were coregistered, thereby minimizing gray matter atrophy or partial-volume effects.

## CONCLUSIONS

By quantitative assessment of the  $\alpha 4\beta 2^*$ -nAChR availability using 2-FA and PET in patients with PD, widespread patterns of decreased  $\alpha 4\beta 2^*$ -nAChRs were identified. These patterns are associated with cognitive and depressive symptoms in PD. Most pronounced  $\alpha 4\beta 2^*$ -nAChR decreases are present in subgroups of patients with PD with mild cognitive impairment or possible depression who might be at higher risk to develop full-blown dementia or depression. Given the high frequency of cognitive and depressive symptoms in patients with PD, leading to increased disability and decreased quality of life, early diagnosis of nicotinic alterations enables early specific therapy in patients with PD who are at risk. Our findings may encourage the search for new drugs that target the  $\alpha 4\beta 2^*$ -nAChR system for the treatment of mental dysfunction in PD.

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**Additional Information:** The asterisk used in the receptor nomenclature indicates that the receptor complex may contain additional subunits.

The eFigures are available at <http://www.archgenpsychiatry.com>. The supplementary tables are available on the authors' Web site at [http://nuklmed.uniklinikum-leipzig.de/download/YOA90008\\_Meyer\\_et\\_al\\_Suppl.pdf](http://nuklmed.uniklinikum-leipzig.de/download/YOA90008_Meyer_et_al_Suppl.pdf).

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