

# Monitoring of 30 marker candidates in early Parkinson disease as progression markers

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## ABSTRACT

**Objective:** This was a longitudinal single-center cohort study to comprehensively explore multi-modal progression markers for Parkinson disease (PD) in patients with recently diagnosed PD (n = 123) and age-matched, neurologically healthy controls (HC; n = 106).

**Methods:** Thirty tests at baseline and after 24 months covered nonmotor symptoms (NMS), cognitive function, and REM sleep behavior disorder (RBD) by polysomnography (PSG), voxel-based morphometry (VBM) of the brain by MRI, and CSF markers. Linear mixed-effect models were used to estimate differences of rates of change and to provide standardized effect sizes (d) with 95% confidence intervals (CI).

**Results:** A composite panel of 10 informative markers was identified. Significant relative worsening (PD vs HC) was seen with the following markers: the Unified Parkinson's Disease Rating Scale I (d 0.39; CI 0.09–0.70), the Autonomic Scale for Outcomes in Parkinson's Disease (d 0.25; CI 0.06–0.46), the Epworth Sleepiness Scale (d 0.47; CI 0.24–0.71), the RBD Screening Questionnaire (d 0.44; CI 0.25–0.64), and RBD by PSG (d 0.37; CI 0.19–0.55) as well as VBM units of cortical gray matter (d –0.2; CI –0.3 to –0.09) and hippocampus (d –0.15; CI –0.27 to –0.03). Markers with a relative improvement included the Nonmotor Symptom (Severity) Scale (d –0.19; CI –0.36 to –0.02) and 2 depression scales (Beck Depression Inventory d –0.18; CI –0.36 to 0; Montgomery-Åsberg Depression Rating Scale d –0.26; CI –0.47 to –0.04). Unexpectedly, cognitive measures and select laboratory markers were not significantly changed in PD vs HC participants.

**Conclusions:** Current CSF biomarkers and cognitive scales do not represent useful progression markers. However, sleep and imaging measures, and to some extent NMS, assessed using adequate scales, may be more informative markers to quantify progression. *Neurology*<sup>®</sup> 2016;87:168–177

## GLOSSARY

**24FU** = 24 months follow-up; **α-syn** = α-synuclein; **AD** = Alzheimer disease; **BDI** = Beck Depression Inventory; **BL** = baseline; **DeNoPa** = de novo Parkinson; **ESS** = Epworth Sleepiness Scale; **HC** = healthy control; **LED** = levodopa equivalent dosage; **MADRS** = Montgomery-Åsberg Depression Rating Scale; **MDS** = Movement Disorder Society; **MMSE** = Mini-Mental State Examination; **MoCA** = Montreal Cognitive Assessment Test; **NMS** = nonmotor signs; **NMSS** = Nonmotor Symptom Scale; **p-tau** = tau protein phosphorylated at threonine 181; **PD** = Parkinson disease; **PSG** = polysomnography; **RBD** = REM sleep behavior disorder; **RBD-SQ** = REM Sleep Behavior Disorder Screening Questionnaire; **Scopa-AUT** = Autonomic Scale for Outcomes in Parkinson's Disease; **t-tau** = total tau protein; **UPDRS** = Unified Parkinson's Disease Rating Scale; **VBM** = voxel-based morphometry.

One of the great challenges in Parkinson disease (PD) therapeutics is the definition of clinically meaningful endpoints that measure progression, or lack thereof.<sup>1</sup> To date, numerous clinical trials with putative neuroprotective agents have yielded negative results.<sup>2–4</sup> It remains unclear whether trial design or the agent tested, or both, are the reasons for past failures.

Currently, several parameters are typically selected as endpoints for use in symptomatic and neuroprotective treatment trials, e.g., the Unified Parkinson's Disease Rating Scale (UPDRS), quality of life measurements,<sup>5</sup> or the “need for levodopa therapy.”<sup>2</sup>

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Supplemental data  
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Reports of selective, longitudinal investigations of nonmotor signs (NMS),<sup>6–8</sup> neuroimaging, and laboratory analyses<sup>9</sup> have been previously published as potential markers of progression.<sup>10</sup> These investigations were largely conducted in smaller cohorts and focused on single symptoms/measures and frequently relied on more advanced PD cases, and lacked age-matched healthy controls.

The de novo Parkinson (DeNoPa) cohort represents a prospective, longitudinal, single-center, observational study of participants with de novo PD and matched neurologically healthy controls (HC). The study's aim is to improve early diagnosis and to assess proper progression markers, scored at baseline with biannual follow-up.<sup>11</sup>

We hypothesized that a composite panel of multimodal progression markers covering overall NMS, cognitive measures, sleep, imaging, and CSF indices would be instructive in understanding the natural history of early PD, and to thereby delineate specific and sensitive outcome measures for future intervention trials. To this end, we applied the same panel of tests to a group of HC to separate PD changes from the process of normal aging.

**METHODS Study participants.** Longitudinal data were collected at baseline (BL) and at 24 months follow-up (24FU). Detailed inclusion criteria have been described previously<sup>11</sup> and are summarized in appendix e-1 on the *Neurology*<sup>®</sup> Web site at [Neurology.org](http://Neurology.org).

After BL assessments, all patients started dopaminergic therapy according to accepted guidelines<sup>12</sup> with stable medication until the 24FU visit. The levodopa equivalent dosage (LED) at 24FU was determined as described.<sup>13</sup> The clinical diagnosis was reassessed in the on state for all patients at 24FU by consensus of 2 teams of independent neurologists (C.T./F.S.-D. and B.M./J.E.; for details, see appendix e-1).

**Standard protocol approvals, registrations, and patient consents.** We received approval from the ethical standards committee on human experimentation (Landesärztekammer Frankfurt) for all experiments using human participants. Written informed consent was obtained from all study participants (consent for research).

**Procedures.** Protocols, data collection, and data/sample storage have been described previously.<sup>11</sup> Briefly, our approach is of an exploratory nature. We examined longitudinal assessments using validated and published tools: NMS were the most relevant diagnostic tests in DeNoPa<sup>11</sup> and were also investigated longitudinally by means of the Movement Disorder Society (MDS) UPDRS part I,<sup>14</sup> the NMS Questionnaire, the Nonmotor Symptom Scale (NMSS), and the Autonomic Scale for Outcomes in Parkinson's Disease (Scopa-AUT). The PD Questionnaire, which has been the primary endpoint in randomized studies,<sup>5</sup> was used together

with assessment of depression by the self-reported Beck Depression Inventory (BDI) and the Montgomery-Åsberg Depression Rating Scale (MADRS). Cognition was tested with the Mini-Mental State Examination (MMSE), the Montreal Cognitive Assessment Test (MoCA), and the Clock Drawing Test. Further tests for different cognitive domains are given in appendix e-1. Sleep was assessed using the Parkinson's Disease Sleep Scale, the Epworth Sleepiness Scale (ESS), and the REM Sleep Behavior Disorder (RBD) Screening Questionnaire (RBD-SQ). In addition, we performed video-supported polysomnography (PSG) to classify RBD. Voxel-based morphometry (VBM) of gray matter and hippocampus by 1.5T volumetric MRI was included because significant atrophy has been described in correlation with PD progression and cognitive decline.<sup>15</sup> We also investigated serum urate.<sup>16</sup> We tested known CSF markers that have been previously discussed as potential diagnostic markers of PD state or its progression, including total  $\alpha$ -synuclein ( $\alpha$ -syn),  $\beta$ -amyloid 1-42, total and phosphorylated tau protein (t-tau and p-tau), as well as neurofilament light chain proteins.<sup>17</sup> See appendix e-1 for experimental details.

**Statistical analysis.** We used linear mixed-effect models to identify differences in the rates of change in selected markers between PD and controls. For each marker, we estimated a random intercept model including group (PD vs HC), time (BL vs 24FU), and their interaction as predictors. Models were estimated using restricted maximum likelihood estimation, taking into account all available data points at BL and 24FU. Differential change in PD is apparent in a significant group multiplied by the time interaction. We present model-based effect sizes for differential change, standardized by the observed SD in the PD group at baseline.<sup>18</sup> Significance testing was based on 95% confidence intervals using parametric bootstrap with 2,000 resamples.

In addition, we explored whether changes in individual markers were influenced by selected BL characteristics. To this end, we conducted robust multiple regression analyses in the PD sample predicting 24FU scores of individual markers (while controlling for respective BL scores) from LED and dopamine agonists at 24FU and selected sociodemographic and clinical factors at BL. Robust regression was used to deal with non-normality (e.g., outliers) in the distribution of markers.<sup>19</sup> All analyses were carried out with the statistical platform R,<sup>20</sup> using the package lme4 for linear mixed models and robustbase for robust regressions.

We did not correct for multiple testing since our approach was of an exploratory nature (see also the Discussion and appendix e-1).<sup>21</sup> However, marker candidates were chosen according to their clinical relevance and following a review of the literature.

**RESULTS** Of the 269 recruited participants, 254 (94%) underwent follow-up assessments (147 PD and 107 HC). Four patients and 2 controls died before 24FU. Reassessment of the clinical working diagnosis led to the identification of a distinct neurologic disorder in 24 (16%) patients shown in figure e-1. The reassessment of our previously described panel of diagnostic markers<sup>11</sup> in the remaining 123 patients with PD and 106 HC at BL remained significant.

The demographics, motor characteristics, and details of dopaminergic treatment at 24FU are shown in table 1.

**Panel of progression markers.** Table 2 list the results of 30 tests performed in the entire DeNoPa cohort,

**Table 1** Demographic characteristics, motor symptoms, and information on dopaminergic medication of patients with de novo Parkinson disease (PD, n = 123) and healthy controls (HC, n = 106) at baseline (BL) and 24 months follow-up (24FU)

	PD (n = 123)		HC (n = 106)		p Value, BL PD vs HC
	BL	24FU	BL	24FU	
<b>Demographics</b>					
Age, y	63.7 (9.5, 40-84)	65.8 (9.4, 42-86)	64.6 (6.8, 44-84)	66.7 (6.8, 44-86)	>0.05
Male/female, %	79/44 (64/36)		63/43 (59/41)		>0.05
Follow-up time, mo	25.3 (2.6, 18-34)		25.5 (1.6, 22-31)		>0.05
<b>Motor symptoms</b>					
Duration of first motor symptom until diagnosis, mo	25.2 (36.6, 2-240)		—	—	—
MDS-UPDRS II	8.1 (4.84, 0-29)	9.7 (5.6, 0-28)	0.2 (0.5, 0-2)	1.0 (1.5, 0-9)	<0.001
MDS-UPDRS III	21.5 (11.6, 3-66)	23.6 (13.2, 1-64)	0.6 (1.4, 0-6)	1.5 (2.6, 0-12)	<0.001
MDS-UPDRS total score <sup>a</sup>	35.7 (17.7, 7-88)	43.0 (22.8, 3-109)	3.3 (3.1, 0-15)	6.6 (6.9, 0-37)	<0.001
Hoehn & Yahr stage	1.8 (0.6, 1-3)	1.9 (0.7, 1-4)	—	—	—
<b>Dopaminergic medication</b>					
Levodopa	0	67 (54)	—	—	—
Dopamine agonists	0	86 (70)	—	—	—
Pramipexole	0	56 (46)	—	—	—
Ropinirole	0	9 (7)	—	—	—
Piribedil	0	13 (11)	—	—	—
Rotigotine	0	6 (5)	—	—	—
MAOB inhibitor	0	86 (70)	—	—	—
Total levodopa equivalent dosage	0	372 (243, 0-1,565)	—	—	—

Abbreviations: MAOB = monoamine oxidase B; MDS-UPDRS = Movement Disorder Society Unified Parkinson's Disease Rating Scale. Data are mean (SD, range) or n (%). Age and sex were part of the frequency matching.

<sup>a</sup>We identified 68 (55%) tremor-dominant, 14 (11%) intermediate, and 41 (33%) postural instability and gait disorder subtypes in our patients.

which we used as surrogate markers for PD and its progression. Ten of the 30 markers showed statistically significant rates of change relative to HC over the 2-year observational period, mainly in the categories of NMS, depression, sleep, and imaging (table 2 and figures 1–3).

**Clinical measures.** Nonmotor symptoms significantly worsened in PD vs HC as assessed by the MDS-UPDRS I score (table 2 and figure 1A). The worsening of autonomic features relative to controls was reflected by a statistically significant increase in the Scopa-AUT scores (table 2 and figure 1D). This increase was mainly driven by an increase in cardiovascular items of the Scopa-AUT (data not shown). In contrast, the severity score for NMS (by NMSS) showed improvement in relation to controls (table 2 and figure 1C). Assessment of depression showed a significant improvement in the self-reported BDI and investigator-rated MADRS (table 2 and figure 1, F and G).

Most of our preselected cognitive measures were worse in PD than in controls during follow-up. Interestingly, slopes were parallel for PD and HC,

revealing no significant incremental changes in PD over controls in single tests (table 2). Also, the tests for executive function showed a parallel (and nonsignificant) decrease for both groups.

Excessive daytime sleepiness, as assessed by the ESS, significantly worsened with PD progression compared to controls. RBD significantly increased for the PD group by questionnaire at follow-up, while it decreased in HC (table 2 and figure 2, A–C).

**Technical investigations.** PSG also showed a significant increase of the prevalence of RBD in patients with PD while in HC the prevalence increased only slightly, as expected, with increasing age (table 2 and figure 2D). The severity of RBD by RBD severity score<sup>22</sup> in PD did not differ between baseline and 24FU (data not shown).

Volumetric imaging measures of cortical and hippocampal structures by MRI decreased significantly at 24FU in patients with PD (figure 3, A and B), which was not associated with age, PD phenotype (table e-1), or changes in cognitive measures (data not shown). Statistical parametric maps and change plots of VBM measures of cortical and hippocampal

**Table 2** Parameters of linear mixed effect models

	Numbers			Unstandardized fixed effects								Standardized effect size, PD change difference	
				HC baseline		PD baseline difference		HC change		PD change difference			
	BL	24FU	SD in PD, BL	Effect	95% CI	Effect	95% CI	Effect	95% CI	Effect	95% CI	Effect	95% CI
<b>Nonmotor symptoms</b>													
MDS-UPDRS I <sup>a,b</sup>	229	228	4.27	2.57	1.66 to 3.51	3.58	2.31 to 4.83	1.61	0.64 to 2.55	1.65	0.38 to 2.97	0.39	0.09 to 0.7
NMS Questionnaire	229	228	4.03	3.78	3.12 to 4.53	3.77	2.70 to 4.65	0.25	-0.32 to 0.79	0.45	-0.24 to 1.15	0.11	-0.06 to 0.29
NMS Scale <sup>a,b</sup>	227	228	30.89	13.04	8.61 to 17.37	23.92	18.07 to 29.86	1.90	-2.34 to 5.61	-5.99	-11.20 to -0.62	-0.19	-0.36 to -0.02
Assessment of autonomic dysfunction (Scopa-AUT) <sup>a,b</sup>	229	229	6.7	8.25	6.97 to 9.52	4.45	2.71 to 6.06	0.48	-0.52 to 1.4	1.69	0.38 to 3.07	0.25	0.06 to 0.46
<b>Depression and impairment of quality of life</b>													
Parkinson's Disease Questionnaire	229	229	18.62	9.42	6.14 to 12.48	18.88	14.68 to 22.97	1.18	-1.18 to 3.58	2.19	-1.05 to 5.49	0.12	-0.06 to 0.29
Beck Depression Inventory <sup>a,b</sup>	229	229	6.67	3.4	2.4 to 4.45	5.59	4.19 to 6.99	0.19	-0.7 to 1.07	-1.22	-2.44 to -0.02	-0.18	-0.36 to 0
Montgomery-Åsberg Depression Rating Scale <sup>a,b</sup>	227	225	7.23	4.05	2.87 to 5.17	4.89	3.32 to 6.47	-1.88	-3.09 to -0.67	-1.87	-3.41 to -0.31	-0.26	-0.47 to -0.04
<b>Cognitive tests</b>													
<b>Screening instruments</b>													
Mini-Mental State Examination	226	225	1.29	28.87	28.6 to 29.13	-0.28	-0.61 to 0.07	-0.33	-0.63 to -0.03	-0.07	-0.5 to 0.32	-0.06	-0.38 to 0.25
Montreal Cognitive Assessment	188	226	3	26.01	25.42 to 26.56	-1.03	-1.82 to -0.26	-0.45	-0.97 to 0.11	-0.3	-1.07 to 0.44	-0.1	-0.36 to 0.15
Clock Drawing Test	227	225	0.93	1.27	1.12 to 1.43	0.35	0.14 to 0.57	0.12	-0.05 to 0.29	-0.15	-0.37 to 0.07	-0.16	-0.4 to 0.08
<b>Domain-specific tests</b>													
<b>Attention and working memory</b>													
Trail-Making test (B/A)	223	222	0.89	2.72	2.5 to 2.94	0.02	-0.28 to 0.32	0.2	-0.03 to 0.44	0.05	-0.27 to 0.35	0.06	-0.31 to 0.4
Digit Span Backward	228	226	1.78	6.12	5.77 to 6.47	-0.51	-1 to -0.04	-0.15	-0.44 to 0.13	-0.06	-0.47 to 0.34	-0.04	-0.27 to 0.19
<b>Executive function</b>													
Verbal Fluency (RWT)	224	222	5.61	19.17	18.06 to 20.28	-2.61	-4.15 to -1.05	0.64	-0.27 to 1.54	-0.02	-1.26 to 1.2	0	-0.22 to 0.21
Stroop Test	218	221	26.07	79.31	74.84 to 83.72	17.35	11.36 to 23.2	-2.06	-5.1 to 1.1	-0.72	-5.18 to 3.78	-0.03	-0.2 to 0.15
<b>Language</b>													
Similarities (WIE)	227	224	5.65	24.8	23.73 to 25.8	-1.76	-3.14 to -0.37	0.65	-0.13 to 1.41	-0.05	-1.13 to 0.95	-0.01	-0.2 to 0.17
<b>Memory</b>													
Verbal Learning and Memory Test	220	224	4.8	10.02	9.13 to 10.94	-0.93	-2.25 to 0.28	-0.25	-1.08 to 0.62	-0.21	-1.33 to 0.95	-0.04	-0.28 to 0.2
Digit Span Forward	228	226	1.94	7.76	7.42 to 8.11	-0.58	-1.04 to -0.07	-0.28	-0.58 to 0.03	0	-0.38 to 0.4	0	-0.2 to 0.21

Continued

Table 2 Continued

	Numbers		SD in PD, BL	Unstandardized fixed effects								Standardized effect size, PD change difference	
				HC baseline		PD baseline difference		HC change		PD change difference			
	BL	24FU	Effect	95% CI	Effect	95% CI	Effect	95% CI	Effect	95% CI	Effect	95% CI	
<b>Social cognition</b>													
Social cognition (MASC)	221	218	5.98	28.16	26.87 to 29.33	-1.92	-3.49 to -0.3	0.4	-0.46 to 1.31	-0.86	-2.09 to 0.31	-0.14	-0.35 to 0.05
<b>Sleep/RBD and PSG</b>													
Parkinson's Disease Sleep Scale	228	228	8.79	10.17	8.58 to 11.77	5.34	3.25 to 7.54	0.2	-1.06 to 1.48	0.94	-0.77 to 2.71	0.11	-0.09 to 0.31
Epworth Sleepiness Scale <sup>a,b</sup>	228	229	3.93	6.93	6.18 to 7.68	0.42	-0.58 to 1.43	-0.59	-1.27 to 0.08	1.83	0.93 to 2.78	0.47	0.24 to 0.71
RBD Screening Questionnaire <sup>a,b</sup>	229	229	2.86	2.29	1.81 to 2.81	1.24	0.58 to 1.93	-0.18	-0.59 to 0.2	1.27	0.72 to 1.82	0.44	0.25 to 0.64
RBD by PSG <sup>a,b</sup>	228	215	0.44	0.02	-0.05 to 0.09	0.24	0.14 to 0.33	0.02	-0.04 to 0.08	0.16	0.08 to 0.24	0.37	0.19 to 0.55
<b>MRI and voxel-based morphometry</b>													
Gray matter volume (VBM), % <sup>a,b</sup>	175	175	2.31	43.38	43.02 to 43.77	-0.35	-0.84 to 0.14	-0.14	-0.31 to 0.02	-0.46	-0.71 to -0.22	-0.2	-0.3 to -0.09
Hippocampal volume (VBM), % <sup>a,b</sup>	171	171	2.61	64.54	64.10 to 65.01	-0.78	-1.46 to -0.17	-0.24	-0.46 to -0.03	-0.38	-0.69 to -0.07	-0.15	-0.27 to -0.03
<b>Laboratory marker in serum or CSF<sup>c</sup></b>													
Serum uric acid, mg/dL	229	229	1.38	5.53	5.27 to 5.79	-0.25	-0.61 to 0.11	0.13	-0.04 to 0.30	0.15	-0.09 to 0.40	0.11	-0.06 to 0.29
CSF $\alpha$ -synuclein, pg/mL	136	73	594.93	1,632.36	1,473.91 to 1,793.23	-198.55	-408.58 to 12.27	-55.39	-156.85 to 44.03	79.14	-55.37 to 205.90	0.13	-0.09 to 0.35
CSF $\beta$ -amyloid 1-42, pg/mL	135	75	204.68	875.99	816.72 to 932.52	-17.94	-95.44 to 62.44	10.30	-37.57 to 58.44	37.77	-24.18 to 97.36	0.18	-0.12 to 0.48
CSF total-tau protein, pg/mL	135	75	103.03	271.26	236.49 to 304.84	-40.03	-85.32 to 3.33	4.40	-33.65 to 43.23	33.67	-16.38 to 84.38	0.33	-0.16 to 0.82
CSF phosphorylated tau protein, pg/mL	128	73	14.47	47.66	43.34 to 52.21	-6.19	-12.10 to -0.59	-1.68	-4.16 to 0.77	1.32	-1.82 to 4.38	0.09	-0.13 to 0.3
CSF neurofilament light chain, pg/mL	133	74	477.38	614.40	502.11 to 736.21	102.75	-53.64 to 255.38	148.89	64.79 to 231.18	-37.84	-145.49 to 79.78	-0.08	-0.3 to 0.17

Abbreviations: 24FU = 24 months follow-up; BL = baseline; CI = confidence interval; HC = healthy control; MASC = Movie for the Assessment of Social Cognition; NMS = nonmotor signs; PD = Parkinson disease; PSG = polysomnography; RBD = REM sleep behavior disorder; RWT = Regensburger Wortflüssigkeitstest; WIE = Wechsler Intelligenztest für Erwachsene; Scopa-AUT = Autonomic Scale for Outcomes in Parkinson's Disease; MDS-UPDRS = Unified Parkinson's Disease Rating Scale; VBM = voxel-based morphometry.

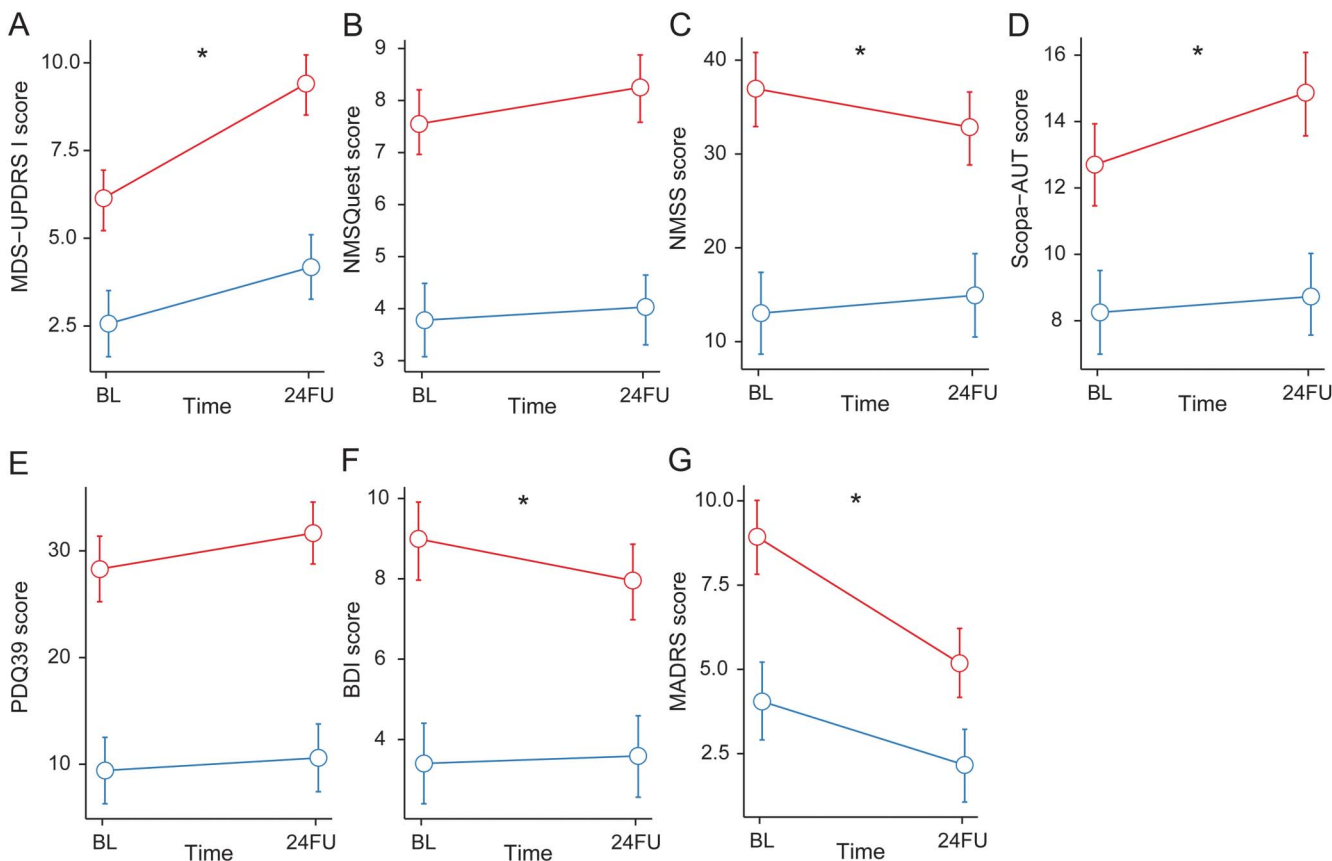
All estimates are unadjusted except for the neuroimaging measures, which are adjusted for age, sex, and total intracranial volume. Columns on the right indicate standardized effect size.

<sup>a</sup> $p < 0.05$ .

<sup>b</sup>The composite panel out of 10 measures as suggested.

<sup>c</sup>CSF samples with hemoglobin levels > 200 ng/mL were excluded from analysis (n=10).

**Figure 1** Longitudinal changes in nonmotor symptoms (NMS), impaired quality of life (QoL), and depression



Estimated means and 95% confidence intervals of Movement Disorder Society-Unified Parkinson's Disease Rating Scale I (MDS-UPDRS I) (A), NMS Questionnaire (NMSQuest) (B), NMS Severity Scale (NMSS) (C), Scopa-AUT (D), QoL measure (PDQ-39) (E), and the depression scales: Beck Depression Inventory (BDI) (F) and Montgomery-Åsberg Depression Rating Scale (MADRS) (G) at baseline (BL) and 2-year follow-up (24FU). Blue line represents healthy controls (HC); red line represents participants with Parkinson disease (PD). \*Significant change in PD vs HC ( $p < 0.05$ ).

volume for both groups indicate regions where atrophy rates differed significantly in patients with PD vs controls (figure 3, C and D).

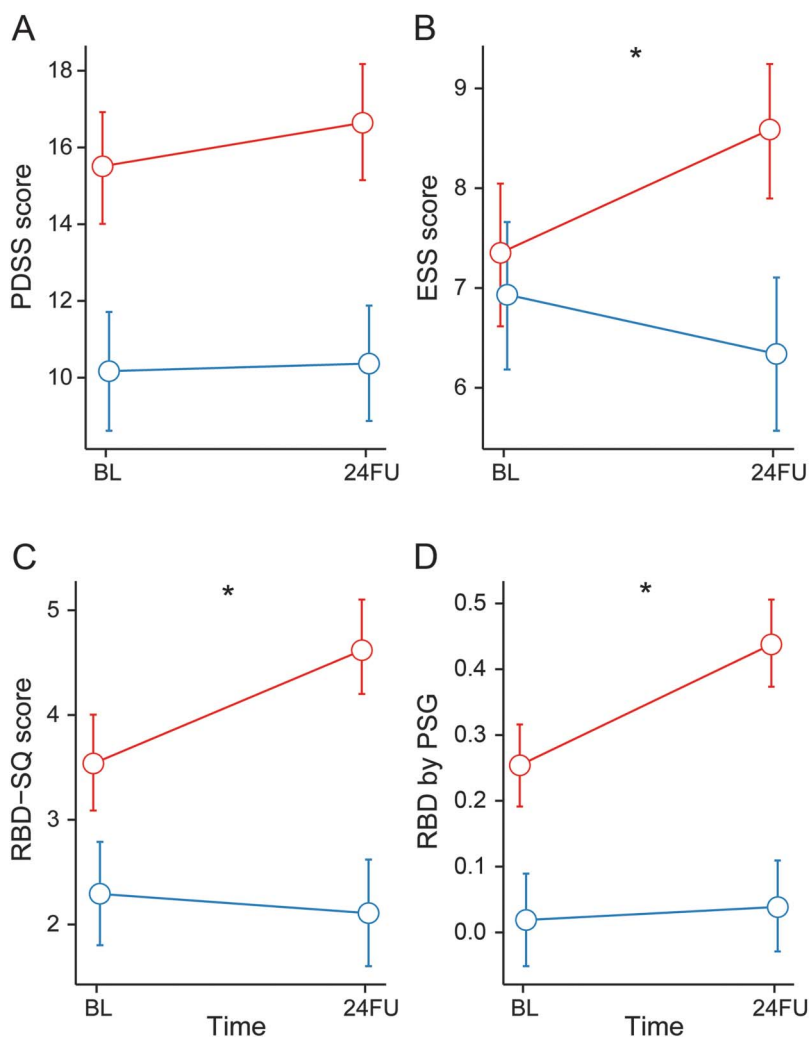
Serum uric acid levels did not significantly differ between PD and HC at baseline and at 24FU. Total CSF  $\alpha$ -syn levels were lower in patients with PD compared to HC at BL and 24FU, but the rates of changes were not significant. Furthermore, a nonsignificant increase in CSF for t-tau was observed (figure e-2). Results for CSF ratios were not significantly altered (data not shown).

**Predictors of change during 24-month observation.** Multiple regression analysis in the PD group, which tested the joint statistical influence of 8 potentially confounding factors, showed only 24 (10%) significant effects regarding changes within the panel of 30 investigated markers (table e-1). For example, the presence of RBD at BL indicated worse cognitive decline on MoCA. The LED, as another surrogate marker for clinical severity, was significantly associated with decreased cognitive function (by the memory screening test MMSE and the attention/working memory

test Digit Span Backward) and increasing RBD as well as serum uric acid. The intake of dopamine agonists was associated with improvement of depression (BDI), worse performance on the memory task Verbal Learning and Memory Test, and increase on CSF p-tau but no other marker.

**DISCUSSION** We performed a 2-year follow-up investigation on clinical measures and biomarkers through clinical and technical assessment, including PSG, MRI, and serum/CSF measures in the largest single-center cohort of 123 clinically diagnosed early PD participants and 106 matched healthy controls. Overall, we found that 20 of 30 tests showed no statistically significant and no specific progression in our DeNoPa patients. The remaining 10 markers showed statistically significant changes reflecting either progression in PD or change due to treatment or both when compared to controls: these encompassed increasing sleep disturbances and worsening of NMS, whereas NMS severity (NMSS) and depression (by MADRS and BDI) improved. Regarding imaging markers, general gray matter

**Figure 2** Longitudinal changes in sleep parameters



Estimated means and 95% confidence intervals of the Parkinson's Disease Sleepiness Scale (PDSS-2) (A), Epworth Sleepiness Scale (ESS) (B), REM Sleep Behavior Disorder by Questionnaire (RBD-SQ) (C), and by video-supported polysomnography (PSG) (D), at baseline (BL) and 2-year follow-up (24FU). Blue line represents healthy controls (HC); red line represents participants with Parkinson disease (PD). \*Significant change in PD vs HC ( $p < 0.05$ ).

volume and hippocampal volume specifically and significantly decreased over the 2-year period.

**Clinical measures.** Several smaller studies<sup>6,7,10</sup> and one longitudinal study<sup>8</sup> have previously suggested the use of NMS as candidate progression markers. NMS worsened in PD as measured by the MDS-UPDRS I in DeNoPa, while the severity of NMS measured by NMSS improved in our cohort. Autonomic dysfunction, as assessed by Scopa-AUT, also showed statistically significant worsening in PD compared to HC. The only systematic longitudinal investigation on NMS showed an increase in NMS domains after 24FU, but NMS severity and more specific autonomic features had not been addressed. Also, these patients were at different stages of disease and the majority had received symptomatic treatment already at BL.<sup>8</sup> While the NMS tools cover various

NMS, the MDS-UPDRS I captures a few key symptoms. Both the NMSS and MDS-UPDRS I show strong convergent validity.<sup>23</sup> The discrepancy of performance of self-reported vs investigator-reported NMS in DeNoPa could also be the result of different sensitivity of scales and questionnaires, and points towards the need for an improved and widely standardized assessment of NMS for longitudinal investigations.

We recorded an improvement of depression in our patients with PD on 2 independent scales and individual differences in improvement were not significantly associated with dopaminergic dosage. The improvement of depression and quality of life in the early honeymoon phase of PD (generally considered to represent the first 5–10 years after diagnosis) is in accordance with other studies,<sup>8</sup> and is also related to dopamine agonists.<sup>24</sup>

Following analysis of various domains of cognition, we identified clear differences in cognitive performance in PD compared to controls (see table 2; PD baseline differences), but only cross-sectionally and not with any significant change longitudinally. Most previous longitudinal studies did not include control groups.<sup>25</sup> Therefore previous studies in PD may have overinterpreted normal cognitive aging and we have thus corrected this deficit in our study design.

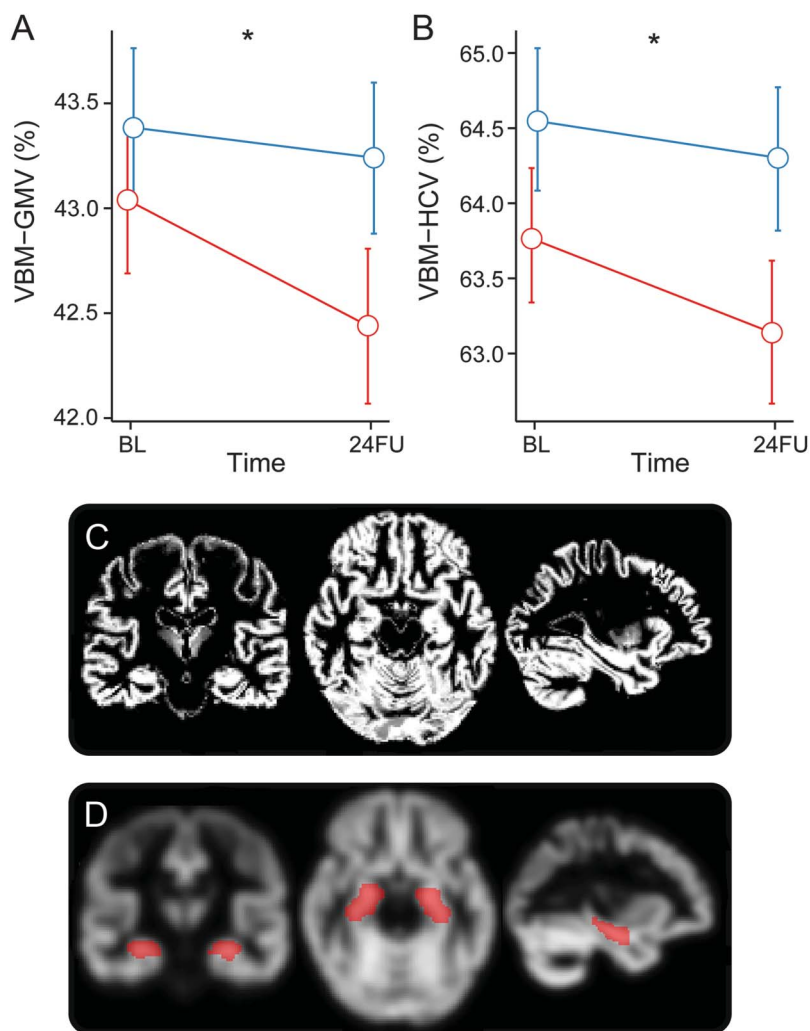
RBD and reduced subjective quality of sleep are recognized inherent symptoms during prodromal and fully developed motor PD.<sup>26</sup> Daytime sleepiness progressively worsens in PD, confirming that it may be dependent on dopaminergic therapy<sup>27</sup> or an intrinsic factor of disease progression. RBD may be a useful marker to monitor progression of PD either with PSG or with a similar effect size by a 10-item self-rating questionnaire (RBD-SQ). The presence of RBD indicates worse cognitive decline by MoCA, as has been shown previously.<sup>28</sup>

**Technical investigations.** To date, DeNoPa comprises the largest collection and interrogation of longitudinal data of PSG, MRI volumetry, and CSF markers.

Our MRI structural measures showed significant overall and hippocampal gray matter volume loss in PD. The hippocampal region was selected, since, according to the Braak staging scheme,<sup>29</sup>  $\alpha$ -syn pathology is expected to emerge there before affecting the neocortex, and hippocampal involvement has been suggested in PD dementia.<sup>30</sup> However, effect sizes were similar in both measures and correlations with changes in cognitive markers were nonsignificant in this early PD cohort (data not shown).

A longitudinal CSF study found increasing tau and tau/A $\beta$ 1-42 levels,<sup>9</sup> but significantly decreasing CSF  $\alpha$ -syn levels in PD.<sup>31</sup> In our study, none of the CSF markers (or their ratios) that were tested reached statistical significance for progression. Different

**Figure 3** Longitudinal changes in 1.5T volumetric MRI



Estimated means and 95% confidence intervals of voxel-based morphometry (VBM) in total gray matter volume (GMV) (A) and hippocampal volume (HCV) (B) at baseline (BL) and 2-year follow-up (24FU). Blue line represents healthy controls (HC); red line represents participants with Parkinson disease (PD). \*Significant change in PD vs HC ( $p < 0.05$ ). (C) Example of gray matter segmentation in 3 orthogonal cutplanes. For the analysis, native space maps were summed to generate an absolute volume per class and subject. (D) Example of smoothed gray matter maps (in Montreal Neurological Institute space) shown with the a priori defined hippocampal analysis region overlaid in red. Within this region, the mean average gray matter probability was calculated yielding one average value of hippocampal gray matter per subject.

demographics and sample preparation could account for this discrepancy. It could also be explained by the small number ( $n = 85$ ; 37%) of DeNoPa participants who consented to serial lumbar puncture (37%) at 24FU. A similarly small annual rate of change in CSF markers has also been observed in patients with established Alzheimer disease (AD). The stability of our markers in this early stage of motor PD could result from a slowing of the neurodegeneration process in the symptomatic phase similar to that seen in AD.<sup>32</sup> This underlines the need for more specific progression marker candidates for the early phase of the disease, which will emerge from this and other ongoing studies.<sup>33</sup>

We explored many obvious candidates in a large cohort of PD participants and looked for progression markers for future use in clinical trial cohorts and tried to exclude noninformative ones. The fact that we identified no more than 33% of the markers analyzed having significantly changed during our 24-month observational period highlights the mandate of finding reliable progression markers. These results have immediate consequences for clinical research in PD by restricting the potential usage of some markers, and will take more time to eventually change the clinical care delivered to patients with PD.

There are limitations of clinical practice-led exploratory studies, such as ours, which merit discussion. First, DeNoPa is a real-life observational survey study. Therefore, we symptomatically treated patients' motor symptoms. The effects of dopaminergic treatment on NMS vary depending up on the underlying mechanisms; i.e., a symptom influenced by dopaminergic deficit may respond positively to dopaminergic treatment (such as depression and NMS),<sup>24</sup> whereas a non-dopamine-responsive symptom may worsen with such treatment (such as daytime sleepiness, especially in the early titration phase; further discussion can be found in appendix e-1).<sup>27</sup> Therefore, we cannot remove any influence conferred by therapy on our investigational markers, but purposefully included 8 markers that are likely independent of any introduced symptomatic therapy,<sup>34</sup> such as MRI volumetry and blood and CSF markers.

Second, most of our proposed marker candidates showed promising results as early diagnostic markers, which does not necessarily qualify them as progression markers. Further marker candidates specific for progression of the disease need to be explored. Third, we did not control for multiple testing. Although this is an appropriate exploratory strategy for detecting true changes in patients with PD (i.e., reducing type II errors), it may lead to false-positive findings. However, the detection of 10 significant changes among 30 tested is clearly more than would be expected by chance alone, thus corroborating the significance of our results as a whole.<sup>35</sup>

The use of our proposed panel of outcome measures needs further validation before being used to rate progression across centers. One limitation is the responsiveness of depression and some NMS to therapy,<sup>36</sup> requiring new elaborated NMS scales. This should be undertaken independently by multicenter cohorts enrolling larger numbers of participants; this is currently underway.<sup>33</sup>

Future studies need to investigate the validity of these proposed markers, standardize the assessment of nonmotor features, and identify more sensitive and disease-specific marker candidates that reflect underlying biological processes (such as propagation



of  $\alpha$ -syn pathology, inflammation, and neuronal death). In this way, validated and improved markers will be used as clinically meaningful endpoints of progression that are essential in the development of treatments for PD.

### AUTHOR CONTRIBUTIONS

B.M., F.S.-D., and C.T. designed the study and were responsible for recruitment and data processing. J.Z., E.T., and T.F. oversaw all statistical analyses. T.W., J.E., E.T., M.S., E.L., and N.K.F. were responsible for performing investigations and collecting and entering data. H.Z. and P.T. were involved in sample analyses and data interpretation. C.T. oversaw patient recruitment, reassessed independently the clinical diagnosis and characterization, and assisted in the interpretation of data. B.M., C.T., and J.Z. wrote the manuscript. F.S.-D., T.F., N.K.F., P.T., and H.Z. coedited the manuscript. B.M., C.T., and F.S.-D. had full access to the clinical primary data. All authors had access to the data generated in the study including the statistical analysis and decided to submit the paper for publication.

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### DISCLOSURE

B. Mollenhauer has received speaker honoraria from Orion Corporation and GlaxoSmithKline; holds or has pending patents re: Method of differentially diagnosing dementias, Novel ELISA-based quantification of  $\alpha$ -synuclein proteins in cerebrospinal fluid and peripheral blood products using 384-well plates, and MicroRNA expression profiling of cerebrospinal fluid; serves as a consultant for Bayer Schering Pharma AG, Roche, Biogen, and GE Healthcare; and receives research support from Teva Pharmaceutical Industries Ltd., Desitin Pharmaceuticals, GmbH, Boehringer Ingelheim, GE Healthcare and the Michael J. Fox Foundation for Parkinson's Research as well as from the EU (within the Joint Programming for Neurodegenerative disorders). J. Zimmerman reports no disclosures relevant to the manuscript. F. Sixel-Döring serves/has served on scientific advisory boards for Orion Corporation and Medtronic Inc. and UCB; has received funding for travel from Boehringer Ingelheim, Cephalon, TEVA and MundiPharm; and has received speaker honoraria from Abbvie, Bayer Healthcare, Boehringer Ingelheim, Desitin, GSK, Meda Pharma, Medtronic Inc., Novartis, Orion Corporation, and UCB. N. Focke received funding from the Stifterverband für die Deutsche Wissenschaft (Dr. Werner Jackstädt-Stipend), served on an advisory board for GE Healthcare, and received honoraria from GlaxoSmithKline. T. Wicke reports no disclosures relevant to the manuscript. J. Ebentheuer has received speaker honoraria from Desitin and UCB. M. Schaumburg is supported by the Deutsche Parkinson Vereinigung (DPV). E. Lang, E. Trautmann, H. Zetterberg, and P. Taylor report no disclosures relevant to the manuscript. T. Friede serves as a consultant to Novartis, Biogen Idec, Grünenthal, AstraZeneca, Almirall, SGS, and Pharnalog. C. Trenkwalder serves on scientific advisory boards for Teva Pharma, Boehringer Ingelheim, UCB, Novartis, Mundipharma International

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