SUPPORTING INFORMATION

PRECUNEAL THICKNESS AND DEPRESSION IN PARKINSON'S DISEASE

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SUPPLEMENTARY METHODS 1

The BoProPark study aimed to prospectively characterize a sample of motor and non-motor features in patients with a progressive neurodegenerative disease starting with parkinsonian signs (Parkinson's disease, PD; PD with dementia, PDD; Multiple system atrophy, MSA; Lewy body dementia, LBD; Progressive Supranuclear Palsy, PSP; Corticobasal degeneration, CBD) within three years of disease motor onset and to evaluate their diagnostic and prognostic role in the differential diagnosis of these diseases.

Patients

We enrolled all consecutive patients with a progressive neurodegenerative disease starting with parkinsonian features (tremor, bradykinesia, rigidity or postural instability) and disease duration up to 3 years referred from September 2007 until the present to the IRCCS Institute of the Neurological Sciences of Bologna and to the Department of BioMedical and NeuroMotor Sciences (University of Bologna). Only patients with pathological single-photon emission computerized tomography for imaging the dopamine transporter (DaTscan SPECT) were included in the study. Secondary causes of parkinsonism were also excluded before enrollment by means of appropriate investigations including brain magnetic resonance imaging.

Other exclusion criteria to enter the study were concurrent clinically severe medical or psychiatric disease that could have interfered with study results.

The study was approved by the local ethics committee of Bologna (AUSL of Bologna). Patients gave their written informed consent to participate in the study and to publish the data.

Study protocol

Each patient underwent the following evaluations:

1) history taking and neurological examination;
2) quantification of motor impairment and disease severity (Unified PD Rating Scale-part III-UPDRS-III- and Hoehn & Yahr -H&Y- score) (Fahn et al 1987; Hoehn and Yahr 1967);
3) quantification of motor response to levodopa (LD) through a subacute challenge test with a low (100/25 mg) oral dose of LD plus carbidopa or benserazide, based on a kinetic-dynamic approach consisting of objective computerized assessment of motor effect through alternate finger tapping test and simultaneous serial blood venous samples to analyze drug plasma concentrations (Contin et al 2001);
4) evaluation of autonomic control of the cardiovascular system through cardiovascular reflex tests (Tilt test, Valsalva's maneuver, Handgrip test, Deep breathing test) (Ewing 1981; Corazza et al 2014) and presence of autonomic dysfunctions through the questionnaire "Scales for Outcomes in Parkinson's Disease (PD) – Autonomic (SCOPA-AUT)" (Visser et al 2004);
5) assessment of sleep disturbances by means of a whole-night video-polysomnographic (VPSG) study and through the following sleep questionnaires: PD sleep scale 1 (PDSS-1) (Chaudhuri et al 2002), REM sleep behavior disorder (RBD) questionnaire (Scaglione et al 2005), Bologna questionnaire on sleepiness-related symptoms (BQS) (Rinaldi et al 2001), Restless Legs Syndrome (RLS) criteria and rating scale (Walters 1995; Walters et al 2003), Epworth sleepiness scale (ESS) (Vignatelli et al 2003). The whole night VPSG included electroencephalogram (EEG: C3-A2, O2-A1, Cz-A1), right and left electrooculogram (EOG), surface electromyogram (EMG) from submental, intercostalis, right and left extensor carpi radialis and tibialis anterior muscles, tracheal microphone, oro-nasal airflow, thoracic and abdominal respirogram, electrocardiogram (ECG), oxyhaemoglobin saturation (SaO2) by means of finger oxymeter and continuous video recording (Vetrugno et al 2004);

6) evaluation of quality of life by means of 39-item PD questionnaire (PDQ-39) (Peto et al 1998);
7) comprehensive cognitive and behavioral assessment evaluating global cognition, verbal and visual memory, attention, executive and visuospatial function, language, depression and anxiety. Neuropsychological evaluation included the following tests corrected for age, sex and education according to Italian standardizations: Mini-Mental State Examination (Measso et al 1993), Simple Copy Design Test (Carlesimo et al 1996), Selective Visual Attention Test (Stroop Test) (Caffarra et al 2002), Phonemic and Semantic Verbal Fluency Test (Novelli et al 1986; Carlesimo et al 1996) and the Brief Mental Deterioration Battery, consisting of Rey's Auditory Verbal Learning Test (immediate and delayed recall) (Carlesimo et al 1996), Visual Search Test (Barrage test) (Carlesimo et al 1996), Immediate Visual Memory Test (Carlesimo et al 1996) and Simple Verbal Analogies Test (Gallassi et al 2014). The Battery outcomes in a measure of global cognitive functioning, called Final Result (Gallassi et al 1986, 2002). All patients also fulfilled the 21-items Beck's Depression Inventory (Beck et al 1961) and the State-Trait Anxiety Inventory (Spielberger et al 1980).

All procedures are performed at baseline (T0), after 16 months (T1) and 5 years (T2) from baseline. Diagnoses for each patient are carried out based on the results of each evaluation (T0, T1 and T2) according to the international diagnostic criteria for PD (Gelb et al 1999), Parkinson' disease with dementia (PDD) (Emre et al 2007; Dubois et al 2007), MSA (Gilman et al 2008), DLB (McKeith et al 2005), PSP (Litvan et al 1996) and CBD (Riley and Lang 2000). Patients not fulfilling any of such criteria are diagnosed as unspecified atypical Parkinsonism (AP).

SUPPLEMENTARY METHODS 2

A semi-automatized threshold-based segmentation method has been applied to T2-w FLAIR images by using Jim software Version 7.0 (Xinapse Systems, Northants, UK, http://www.xinapse.com).

T2-w FLAIR images were then linearly co-registered to T1-w 3D images (FLIRT) (Jenkinson M et al., 2002) and the transformation matrix was used to register the lesion map onto the T1-w image, with a nearest-neighbor interpolation method. Then the FSL lesion filling tool (Battaglini M et al., 2012) was applied to the T1-w images using the co-registered lesion masks. This allowed to reduce intensity contrast within known lesion areas and therefore to improve the accuracy of volume measurements.

Cortical thickness analysis was performed by following the FreeSurfer 4.4.0 pipeline for image analysis. Briefly, this pipeline includes motion correction, removal of non-brain tissue, Talairach transformation and intensity normalization. Surface boundaries are then tessellated to model the whole brain by multiple vertices. Once the cortical models are complete, surface inflation and registration to a spherical atlas are performed. Cortical thickness is calculated as the closest distance from the grey/white boundary to the grey/CSF boundary at each vertex on the tessellated surface (Fischl B and Dale AM, 2000). Before statistical analysis, cortical maps were smoothed with a 10-mm FWHM Gaussian kernel.

SUPPLEMENTARY METHODS 3

To confirm that the population included in the present study was representative of the BoProPark PD cohort, we tested differences in age at evaluation and disease onset, gender, disease duration, UPDRS-III, Hoehn-Yahr scale, MMSE and BDI scores between our sample and the remaining 41 patients of the BoProPark cohort who did not perform brain MR at our Unit.

Considering the small sample size, we summarized clinical and demographic data with median and range and therefore we applied non-parametric tests to evaluate differences among groups: Kruskal-

Wallis test to compare age at evaluation and altered white matter volume among D-PD, ND-PD and HC, Mann-Whitney U test to compare continuous variables between D-PD and ND-PD and Pearson's χ^2-test to compare gender and Hoehn-Yahr's score between D-PD and ND-PD. Correlations among demographic, clinical and neuropsychological variables were performed by using Spearman's correlation test. A correction for multiple comparisons with Bonferroni's method was applied as appropriate. Statistical analyses were performed by using the program IBM® SPSS® v.21.

A vertex-wise ANCOVA was performed with a general linear model (GLM) within the FreeSurfer suite, considering the subgroup membership (D-PD, ND-PD and HC) as a factor and adding age, sex and total intracranial volume (TIV) as covariates of no interest.

Post-hoc comparisons were performed in a vertex-wise manner only within the areas that had shown a significant effect of different groups in ANCOVA.

The correlation between CT and BDI score was explored, for the vertices which showed a significant difference in CT among groups, by applying a GLM on the vertex base.

For both the post-hoc comparisons and the correlations, we controlled for the effect of age, sex and TIV.

When correcting for multiple comparisons at the vertex level through the Monte Carlo simulation provided in FreeSurfer's QDEC 1.5 (Query, Design, Estimate, Contrast) interface, no significant differences were observed.

Because of the exploratory nature of the study, we set the significance level at $p<0.001$, uncorrected, for the ANCOVA, the post-hoc and the correlations analysis. Moreover, a threshold over the number of contiguous vertices was also applied, considering as significant only clusters of 100 or more vertices.

SUPPLEMENTARY TABLE

Supplementary table 1. Cortical regions with significant thickening in Parkinson's disease patients with depression compared to those without depression.

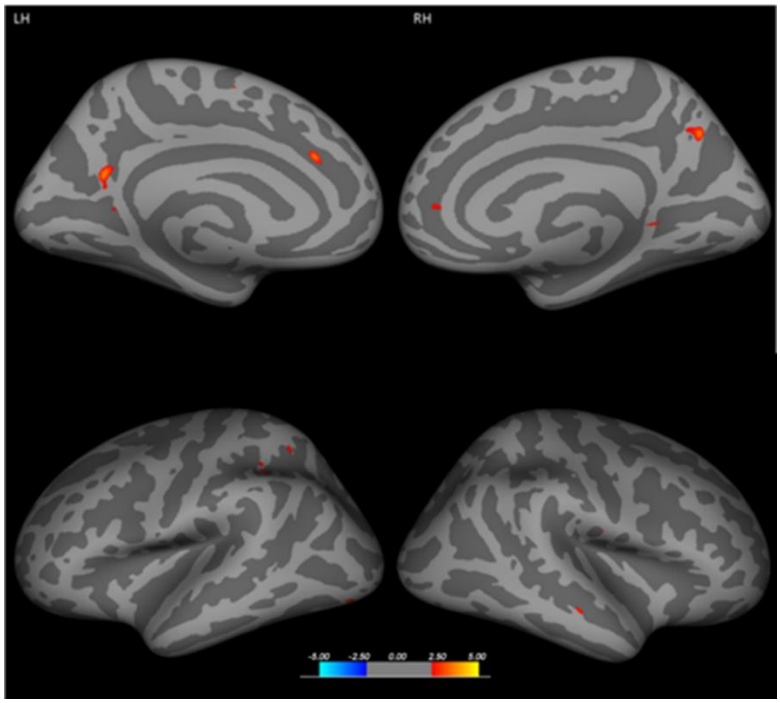
	Cluster n°	Cortical region	Z Max	Size (mm^2)	TalX	TalY	TalZ	NVtx
Right hemisphere	**1**	**precuneus**	**3.925**	**70.73**	**6.5**	**-64.6**	**35.6**	**149**
	2	isthmuscingulate	3.310	15.48	17.4	-44.6	1.5	44
	3	middletemporal	3.237	12.79	51.3	-21.1	-11.4	22
	4	rostralanteriorcingulate	3.081	8.19	14.4	39.5	2.8	20
	5	postcentral	3.080	2.21	59.9	-10.1	14.2	5
	6	superiorparietal	3.054	0.96	9.4	-62.3	53.3	2
Left hemisphere	**1**	**precuneus**	**4.049**	**73.60**	**-4.3**	**-58**	**19.5**	**167**
	2	superiorfrontal	3.918	23.47	-14	27.9	22.3	52
	3	supramarginal	3.386	30.06	-42	-41.5	37.8	79
	4	lateraloccipital	3.358	31.71	-37	-81	-8.6	41
	5	superiorparietal	3.296	15.08	-30	-49.8	51.4	35
	6	superiorparietal	3.165	10.68	-34	-39.6	39	31

	7	superiorfrontal	3.077	1.36	-6.9	-2.8	52.1	3
	8	isthmuscingulate	3.066	4.52	-16	-50.5	7.2	11

Legend. TalX: X coordinate in Talaraich space; TalY: Y coordinate in Talaraich space; TalZ: Z coordinate in Talaraich space; NVtx: number of vertices.

SUPPLEMENTARY FIGURES

Supplementary Figure 1. ANCOVA results of cortical thickness analysis displayed at $p<0.001$, uncorrected. Whole brain vertex-wise differences of cortical thickness among Parkinson's disease (PD) patients with depression, without depression and healthy controls are displayed with -log10(p) values. Areas of differences in grey matter thickness are overlaid on a reference grey matter surface.



Legend. LH: left hemisphere; RH: right hemisphere.

Supplementary references

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