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Age at onset influences neurodegenerative processes underlying PD with levodopa-induced dyskinesias

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ABSTRACT

Purpose: Recently, we demonstrated that PD patients with levodopa-induced dyskinesias are characterized by neuroanatomical and functional changes involving the prefrontal cortex. When compared with non-dyskinetic PD patients, dyskinetic PD patients showed increased volume of the inferior frontal cortex and a dysfunctional imbalance between this region and the supplementary motor area during motor task. In the current study, we investigated the impact of age at onset of the disease on the neuroanatomical characteristics of dyskinetic patients, because it is well known that early-onset PD patients usually develop dyskinesias sooner with respect to late-onset PD.
Methods: Whole-brain voxel-wise investigations of gray matter volume and cortical thickness were carried out in dyskinetic ($n = 33$), non-dyskinetic PD patients ($n = 33$) and in age-sex-matched healthy controls ($n = 40$). Neuroimaging analyses were performed separately according to the age at onset (early $< 50\ y >$ late).
Results: Independent of age at onset, dyskinetic PD patients showed altered morphology in the inferior frontal cortex when compared with non-dyskinetic patients. Moreover, additional significant abnormalities emerged in the early- and late-onset PD patients when compared to controls. In fact, early-onset dyskinetic patients showed increased volume in a large cluster of the midbrain encompassing substantia nigra and red nucleus, whereas late-onset dyskinetic patients were characterized by abnormal gray matter increase in the supplementary motor area.
Discussion: Our findings demonstrate different patterns of brain abnormalities in patients with LID according to age at onset, highlighting the role of the nigral pathology in early-onset and of the cortical pathology in late-onset patients with PD.

1. Introduction

The age of onset is a medical term referring to the age at which an individual acquires, develops or first experiences a symptom of a disease or disorder. In the neurological diseases, age at onset is one of the most important clinical variables, capable of influencing the severity of a disease. For instance, in multiple sclerosis, patients with early age at onset are characterized by a more favorable course of disease [1]. On the contrary, in Alzheimer disease, earlier age at onset yields a more progressive rate of symptoms [2]. Similarly, patients who experienced Parkinson's disease (PD) in early age (<40–50 ys) developed levodopa-induced dyskinesias (LID) earlier than those with later age of onset [3]. Indeed, in PD patients the risk of dyskinesias decreased with older age, reducing the risk by 20–30% for 10 years age differences. In several clinical trials [4,5] it was reported that the five-year LID incidence declined by decades of age: 50% frequency between the ages of 40 and 59 years, 26% frequency between the ages of 60 and 69 years.

The investigation of the neurobiological basis underlying early- and late-onset of LID has been restricted to the neurochemical changes (altered dopamine turnover) within the basal ganglia as assessed by positron emission tomography (PET) [6,7]. Our group has recently provided new fundamental evidence about neurodegenerative processes underlying LID [8–10], stimulating an interesting scientific debate [11,12]. In these works, we proposed that pathophysiological mechanisms underlying LID were associated

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with cortical morphological and functional alterations involving the inferior frontal cortex (IFC) and the supplementary motor area (SMA) [8–10], two fundamental regions taking part in the executive control system [11,13].

However, whether age at onset might influence these pathological mechanisms is still unknown. The purpose of the current study is to examine brain structural correlates of age at onset in PD patients with and without LID by combining two distinct morphologic whole-brain magnetic resonance (MR) measurements: voxel-based morphometry (VBM) [14] and cortical thickness (*Freesurfer*) [15] in a multi-method unbiased approach.

2. Methods

2.1. Subjects

Our initial sample consisted of 162 case patients recruited from the Neurology Unit of the University "Magna Graecia" of Catanzaro. Inclusion criteria were: (1) clinical diagnosis of PD according to the United Kingdom Parkinson's Disease Society Brain Bank criteria [16]; (2) a minimum six-month duration of levodopa treatment; (3) presence or absence of peak-dose LID following an acute levodopa test observed by the examining neurologist on the occasion of the last visit; (4) no family history of PD extended to the first-degree relatives; (5) no evidence of structural abnormalities in the brain (6) no evidence of global cognitive impairment (MMSE $\geq$ 24); (7) no evidence of depression symptoms (as assessed by the Structured Clinical Interview for DSM-IV Axis I disorders). According to these criteria, 47 patients were excluded from the study. Among the cohort of the remaining 115 treated PD patients, 50 were dyskinetic and 65 were non-dyskinetic.

Before MRI examinations, patients underwent clinical assessment, including Hoehn–Yahr (HY) staging [17], Unified Parkinson's Disease Rating Scale (UPDRS) "OFF" scores and Abnormal Involuntary Movement Scale (AIMS) [18]. Each PD group was further coded in early- (age $<$ 50) and late-onset age of the disease (age $>$ 50), following previous published criteria [20]. Patients were then randomly assigned to four groups: early-onset PD with LID; late-onset PD with LID; early-onset PD without LID; late-onset PD without LID, using a computer-generated, site-stratified, randomization schedule. Randomization was stratified according to disease durations (±16 months). For each stratum, random numbers were assigned to the participants, which were put into envelopes. Participants were assigned to the groups by opening the envelopes. A total sample of 33 pairs (12 early PD and 21 late PD) for each PD group (dyskinetic and non-dyskinetic) was then available for the analysis. Thirty-eight PD patients from this study had also participated in our previous studies [8–10]. All PD patients were treated with levodopa. Additional treatments were ropinirol (6 diskinetic and 7 non-dyskinetic PD patients), pramipexol (5 diskinetic and 7 non-dyskinetic PD patients), amantadine (3 diskinetic).

Forty healthy volunteers with no previous histories of neurological or psychiatric diseases and with normal brain MRIs were matched for age and gender with PD patients. To ensure that healthy controls were perfectly matched with early-onset and late-onset PD groups we separated them into two groups. In the first group, we included controls (n = 15) age-/sex-matched with early-onset PD patients, whereas in the second group we included individuals age-/sex-matched with late-onset PD patients (n = 25). Demographical and clinical data are reported on Table 1.

All participants gave written informed consent, which was approved by the Ethical Committee of the University "Magna Graecia" of Catanzaro, according to the Helsinki Declaration.

2.2. Magnetic resonance imaging

Brain MRI was performed according to our routine protocol by a 1.5-T unit (Signa NV/I; GE Medical Systems, USA). Structural MRI data were acquired using a 3D T1-weighted spoiled gradient echo sequence with the following parameters: TR = 15.2 ms; TE = 6.7 ms; flip angle 15°; matrix size 256 × 256; FOV = 24 cm; slice thickness = 1.2 mm.

2.3. Voxel-based morphometry (VBM)

Data were processed and examined using VBM5 toolbox (http://dbm.neuro.uni-jena.de), which utilizes and extends the new unified segmentation approach implemented in Statistical Parametric Mapping (SPM5) [14]. Unified segmentation provides a generative model of VBM preprocessing that integrates tissue classification, image registration and MRI inhomogeneity bias correction. The VBM5 toolbox extends the unified segmentation model as it increases the quality of segmentation by applying a Hidden Markov Field model on the segmented tissue maps. The Montreal Neurological Institute (MNI) brain was used as a template for normalization. The normalized and segmented gray matter (GM) images were then modulated by the Jacobian determinants derived from the spatial normalization.

Table 1
Clinical and demographic characteristics.

Variables	EO dyskinetic	EO nondyskinetic	Controls	*p*-values
No	12	12	15	
% Male	50%	50%	53%	
Age (years)	52.7 ± 6.9	52.6 ± 4.5	49.9 ± 3.6	0.29[a]
Age at onset (years)	44.9 ± 5.1	46.6 ± 4.2	–	0.35[b]
Disease duration (years)	6.5 ± 3.1	4.8 ± 3.1	–	0.15[b]
Hohen and Yahr stage	2.5 (1–4)	2 (2–3)	–	0.38[c]
Therapy duration (years)	6.5 ± 3.6	4.8 ± 3	–	0.21[b]
UPDRS (OFF-drug)	28.8 ± 13.4	20.6 ± 9.2	–	0.42[b]
Mean dose of levodopa (mg/die)	530.6 ± 170	492.7 ± 185	–	0.61[b]
AIMS	9.4 ± 3.5	–	–	–
MMSE	27.1 ± 1.5	27 ± 2.1	28.1 ± 2	0.77[a]
Variables	LO dyskinetic	LO nondyskinetic	Controls	*p*-values
No	21	21	25	
% Male	52%	55%	58%	
Age (years)	65.5 ± 6.1	67.7 ± 7.1	66.4 ± 6.7	0.51[a]
Age at onset (years)	58.1 ± 5.1	60.1 ± 6.4	–	0.33[b]
Disease duration (years)	6.1 ± 3.1	4.98 ± 3.9	–	0.58[b]
Hohen and Yahr stage	2.5 (2–3)	2.5 (1.5–4)	–	0.84[c]
Therapy duration (years)	6.2 ± 3.7	4.9 ± 3.8	–	0.39[b]
UPDRS (OFF-drug)	24 ± 8.1	25.6 ± 7.6	–	0.37[b]
Mean dose of levodopa (mg/die)	564.4 ± 310	560.7 ± 206	–	0.87[b]
AIMS	6.9 ± 3.4	–	–	–
MMSE	26.6 ± 2.3	26.2 ± 1.5	27.6 ± 1.1	0.63[a]

Data are given as mean values (SD) or median values (range) when appropriate.
EO: Early-Onset PD patients; LO: Late-Onset PD patients; UPDRS: Unified Parkinson's Disease Rating Scale; AIMS: Abnormal Involuntary Movement Scale; MMSE: Mini Mental State Examination.
[a] One-way ANOVA.
[b] Unpaired *t* test.
[c] Mann–Whitney test.

Finally, the modulated volumes were smoothed with a Gaussian kernel of 10 mm full width at half maximum.

The GM volume maps were statistically analyzed using the general linear model based on Gaussian random field theory. To assess the main effect of group (PD with LID versus PD without LID versus controls) on GM volume, the smoothed GM images were entered into a second-level ANCOVA model, with age, gender and total intracranial volume as covariates of no-interest. We performed distinct ANCOVA analyses for early- and late-onset PD groups. Based on previous findings, we decided to use the IFC (Broadmann's areas, BA 45–47), cingulate cortex (BA 32), SMA and pre-SMA (BA 6/8), basal ganglia, substantia nigra (SN), red nucleus, subthalamic nucleus and cerebellum as regions of interest (ROIs) given their involvement in the pathophysiological mechanisms of PD [8,9,20–22]. All ROIs were created with the "aal.02" atlas included in the Wake Forest University Pickatlas software version 1.04 (http://www.fmri.wfubmc.edu/download.htm). All analyses were thresholded by using correction for multiple comparisons (false-discovery rate (FDR) $p < 0.05$) within ROIs. Moreover, to evaluate co-variation between GM volume changes and clinical data, we performed a correlation analysis using the multiple regression function of SPM5. Specifically, we were interested in investigating the effects of the AIMS scores on the detected anatomical changes. Correlation analyses were performed within ROIs using a statistical threshold corrected for multiple comparisons (FDR < 0.05).

Considering that to set the boundary between early- and late-onset PD at 50 years of age might be arbitrary, we further perform additional AnCOVA analysis dividing the sample into tertiles. For more information see Supplementary Materials.

2.4. Cortical thickness

To corroborate voxel-based findings we further performed cortical thickness analysis of the cortical mantle by using Freesurfer [8,15]. This method has been