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***Unraveling biomarkers in Parkinson's disease: the role of Insulin-like
growth factor-1 (IGF-1) and DAT imaging***

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List of abbreviations

BJLOT: Benton Judgment of Line Orientation test

DAT: dopamine transporter

GDS-15: 15-item Geriatric Depression Scale

HC: healthy controls

HVLT-DLRY: Hopkins Verbal Learning Test Delayed Recall

HVLT-DR: Hopkins Verbal Learning Test discrimination recognition

HVLT-IR: Hopkins Verbal Learning Test immediate recall

HVLT-R: Hopkins Verbal Learning Test – Revised

IGF-1: insulin-like growth factor-1

JOLO: Benton Judgment of Line Orientation

LNS: Letter Number Sequencing

MCI: mild cognitive impairment

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

MDS-UPDRS-III: Movement Disorders Society version of the Unified Parkinson's disease rating scale part III

MoCA: Montreal Cognitive Assessment

PD: Parkinson's disease

PPMI: Parkinson's Progression Markers initiative

SBRs: striatal binding ratios

SD: standard deviation

SDMT: Symbol Digit Modalities test

SF: Semantic fluency

SPECT: single positron emission computerized tomography

STAI: State-Trait Anxiety Inventory

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer's disease, affecting up to 10 million individuals worldwide [1]. Although symptomatic treatment ameliorates motor symptoms, currently there are no disease-modifying treatments.

A biomarker is defined by the National Institutes of Health as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention” [2]. Thus, biomarkers include clinical data, measurements of biological samples (e.g., plasma, serum, cerebrospinal fluid) and application of brain imaging techniques to detect changes in brain structure and function.

As for PD, biomarkers represent tools potentially suitable for either clinical or research settings and useful in predicting onset, confirming diagnosis, detecting progression and evaluating the response to disease-modifying treatments. In addition, biomarkers' trends in different stages of disease may reflect the widespread neurochemical and neuroanatomical changes that occur throughout the course of PD and, thus, possibly suggest new insights in the pathophysiological mechanisms underlying disease progression [3,4] (Figure 1). The range of available biomarkers in PD is fast expanding and includes an increasing number of laboratory, clinical and imaging data [5]. Indeed, the latter two represent the cornerstones of the diagnostic criteria for PD recently proposed by the International Parkinson and Movement Disorders Society (MDS) task force on the definition of PD [5-7]. As for imaging biomarkers, the largest amount of evidence is available for SPECT DAT Scan which is currently used in both clinical and research settings [8,9]. On the contrary, scant of data are

available for laboratory biomarkers. As such, although serum biomarkers would represent a fascinating, cost-effective and easy to collect source of information in PD, no valid and reliable serum biomarkers have been identified so far.

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Figure 1. Clinical and imaging biomarkers tackling pathological changes in Parkinson's disease from prodromal to middle/advanced phase.

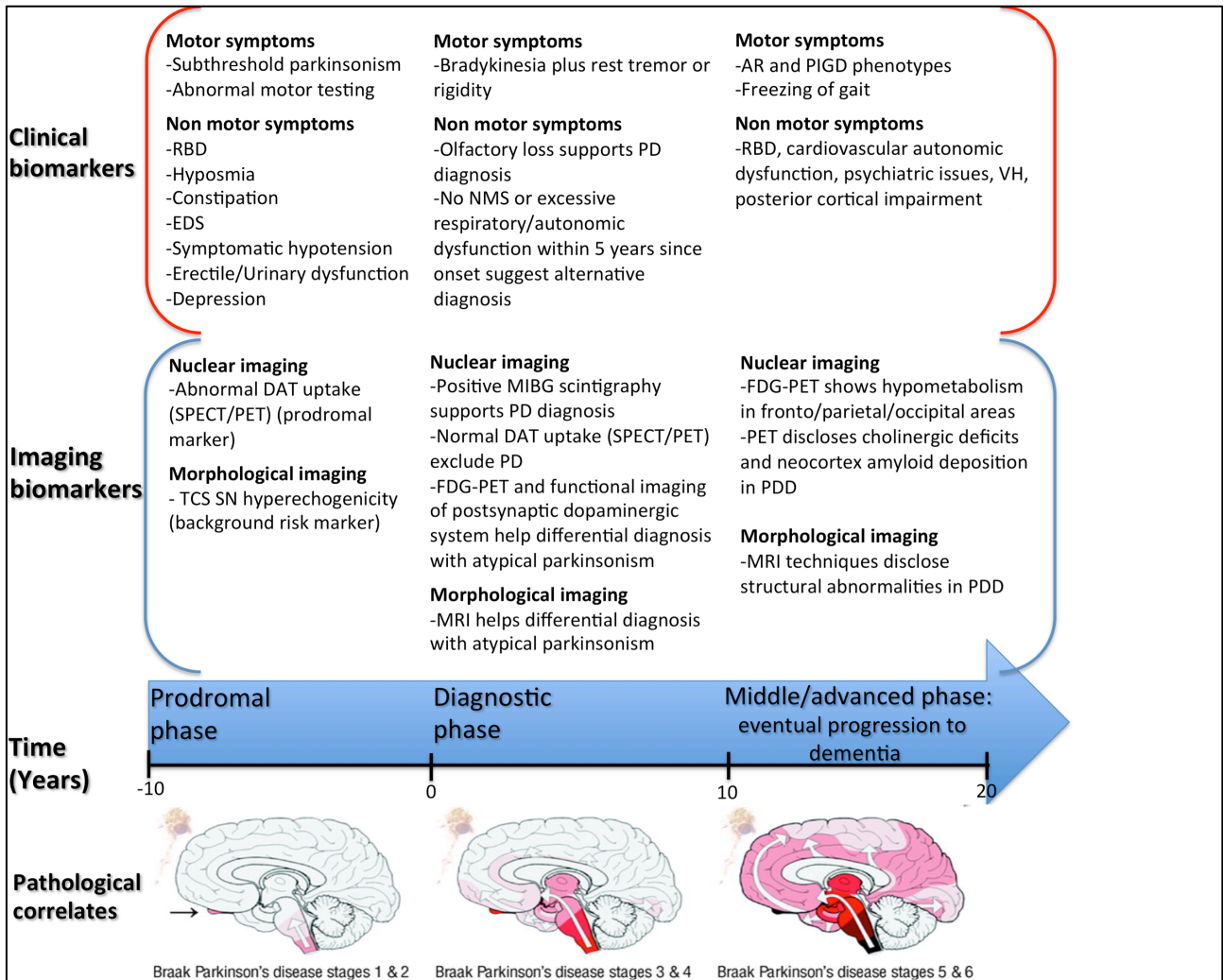


Figure 1 legend

AR: akinetic-rigid; EDS: Excessive daytime sleepiness; MIBG: iodine-123-meta-iodobenzylguanidine; MRI: magnetic resonance imaging; MSA: multiple system atrophy, NMS: non motor symptoms; PD: Parkinson's disease; PDD: Parkinson's disease with dementia; PET: positron emission tomography; PIGD: postural instability and gait disorders; RBD: REM-Sleep Behavior Disorders; SN: substantia nigra; SPECT: single photon emission computed tomography; TCS: transcranial sonography; VH: visual hallucinations. The inferior part of the figure (Pathological

correlates) has been taken from: Olanow CW, Stern MB, Sethi K. The scientific and clinical basis for the treatment of Parkinson disease (2009). *Neurology* 2009;72 (Suppl 4):S1-S136.

Scope of the thesis

The scope of this thesis is to explore the relationship between specific laboratory and imaging biomarkers and cognitive as well as behavioral features in de novo, drug-naïve PD patients.

In Part III of the thesis, we explored the role of serum Insulin-Growth Factor 1 (IGF-1) at diagnosis as a marker of worse cognitive performances.

In Part IV and V, we focused on the SPECT DAT Scan and aimed at demonstrating a direct relationship between nigro-striatal denervation and specific cognitive and behavioral features.

Indeed, we already reached such results in our small, single-center de novo PD population [1-4].

Thus, the main goal of the studies proposed in the present thesis were to replicate our previous findings in the largest cohort of de novo, drug-naïve PD patients existing worldwide, the Parkinson's Progression Markers Initiative (PPMI) study promoted by the Michael J Fox Foundation for Parkinson's research and following prospectively 424 de novo PD patients and 196 Healthy Controls (<http://www.ppmi-info.org>). Indeed, we took advantage of being an active part of the PPMI study and obtained the approval from the Steering Committee for dosing the IGF-1 serum levels of all the PPMI participants. The remaining data used for this thesis have been downloaded from the PPMI website (<http://www.ppmi-info.org/access-data-specimens/download-data>). The whole statistical design as well the execution for the three studies proposed have been performed entirely by the PhD candidate.

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Exploring the association between serum IGF-1 and cognitive functions in early, drug-naïve Parkinson's disease

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Abstract

Objective: Cognitive deficits are common in Parkinson's disease (PD) since the early stages and many patients eventually develop dementia. Yet, occurrence of dementia in PD is unpredictable. Evidence supports the hypothesis that insulin-like growth factor-1 (IGF-1) is involved in cognitive deficits. Our aim was to evaluate the relationship between serum IGF-1 levels and neuropsychological scores in a large cohort of drug-naïve PD patients during the earliest stages of the disease.

Methods: Serum IGF-1 levels were determined in 405 early, drug-naïve PD patients and 191 healthy controls (HC) enrolled in the Parkinson's Progression Markers Initiative (PPMI). The association between serum IGF-1 levels and neuropsychological scores was evaluated with linear regression analysis.

Results: IGF-1 levels were similar in PD and HC. In PD patients the lowest IGF-1 quartile was a predictor of lower performances at the Semantic Fluency task ($\beta=-3.46$, 95%CI: -5.87 to -1.01, $p=0.005$), the Symbol Digit Modalities Score ($\beta=-2.09$, 95%CI: -4.02 to -0.15, $p=0.034$), and Hopkins Verbal Learning Test Retention ($\beta=-0.05$, 95%CI: -0.09 to -0.009, $p=0.019$).

Conclusions: Lower serum IGF-1 levels are associated to poor performances in cognitive tasks assessing executive function, attention and verbal memory in a large cohort of early PD patients. Follow-up studies are warranted to assess if IGF-1 is related to the development of dementia in PD.

Introduction

Cognitive disturbances are common findings in Parkinson's disease (PD) since the earliest stages, manifesting with executive as well as with visuospatial deficits [1]. Cognitive function typically decline over time, with many patients eventually developing dementia [2]. Evolution to dementia is a key milestone in PD progression as denotes a subgroup of patients with worse prognosis and represents a heavy burden for both patients and caregivers. Among all, age, male gender and presence of subtle cognitive impairment have been identified as the most consistent predictors of dementia [2]. Despite considerable efforts of the research community, valid biomarkers of cognitive impairment for PD have yet to come. As minimally invasive and economically affordable, serum biomarkers for neurodegenerative diseases gained increasing attention in recent years.

Both preclinical and clinical evidence support the hypothesis that Insulin-like growth factor 1 (IGF-1) is involved in neuroprotection and plays a role in determining cognitive deficits: a) animal and in vitro studies show that IGF-1 is critical for neuronal cell functioning, enhancing neuronal survival and inhibiting apoptosis and B-amyloid deposition [3-5]; b) studies disclose cognitive impairment in children with growth hormone (GH) deficit [6]; c) cross-sectional epidemiologic studies report an association between low IGF-1 levels and reduced cognitive performance in healthy, elderly subjects [7,8], with particular regard to executive function, working memory and attention tasks [9];

d) prospective studies demonstrate that higher serum IGF-1 levels are associated with less cognitive decline in the elderly [10-13].

In keeping with this evidence, we reported a significant association between low serum IGF-1 levels and poor performances on executive tasks at PD diagnosis and on attention/executive and verbal memory tasks after two-years since diagnosis in a cohort of 65 drug-naïve, early patients, suggesting a link between IGF-1 and early cognitive impairment in PD [14].

In the present study, we sought to analyze the relationship between serum IGF-1 levels and cognitive scores at diagnosis in a larger cohort of drug-naïve PD patients. Based on previous evidence, lower serum IGF-1 levels were expected to be associated with worse cognitive performances. Furthermore, the association between IGF-1 levels and cognition was explored in a large cohort of healthy controls (HC).

Methods

Data used for this study were obtained from the Parkinson's Progression Markers Initiative (PPMI) database (www.ppmi-info.org/data). PPMI-a public-private partnership-is funded by the Michael J. Fox Foundation for Parkinson's Research and funding partners (www.ppmi-info.org/fundingpartners). The aims and methodology of the study are available at www.ppmi-info.org/study-design. The study was approved by the institutional review board at each site, and participants provided written informed consent. For up-to-date information on the study visit www.ppmi-info.org.

PD patients were enrolled in the PPMI study provided they were drug-naïve, had less than 2-years disease duration and evidence of dopamine transporter (DAT) deficit on DAT Scan imaging. Enrolled subjects underwent scheduled assessments to collect clinical data and biospecimens every 3 months for the first year and then every 6 months up to 5 years. Disease severity has been assessed by means of the Movement Disorders Society version of the Unified Parkinson's disease rating scale part III (MDS-UPDRS-III).

Baseline serum samples for the IGF-1 analysis were obtained from the PPMI (Cognition biomarkers, 2013; Grant ID 8800; www.ppmi-info.org/access-data-specimens/request-specimens/). Demographic, clinical and neuropsychological data were gathered from the PPMI database according to guidelines for data access and use (<http://www.ppmi-info.org/data>).

According to PPMI Laboratory Manual, venous blood samples from PD patients and HC were drawn at baseline visit in the morning after an overnight fast. Blood samples were centrifuged and serum was frozen (-80°C). Serum samples from 405 PD patients and 191 HC collected at the baseline assessment of the PPMI were shipped on dry ice and analyzed at the Department of Clinical Medicine and Surgery, Federico II University, Naples.

IGF-1 Measurement

We performed the IGF-1 measurement using the Quantikine Human IGF-1 Immunoassay (R&D Systems, Minneapolis, MN, USA), that is a solid-phase ELISA designed to measure human IGF-1 in serum and plasma. The immunoassay is calibrated against a highly purified Escherichia coli-expressed recombinant human IGF-1. To reduce analytical variance, samples were batch analyzed

using the same assay lot. Serum was pretreated to release IGF-1 from IGF-binding proteins with acid-ethanol extraction. Each sample was tested twice in the same assay and the mean of these two values was considered for statistical analysis. The NIBSC/WHO IGF-1 International Reference Reagent 02/254 was evaluated in this assay. The minimal detectable dose (MDD) of IGF-1 in this assay ranged from 0.007 to 0.056 ng/ml and the mean MDD was 0.026 ng/ml. The intra-assay coefficients of variation (CVs) were 3.5%, 4.3% and 4.3%, and the inter-assay CVs were 8.1%, 8.3% and 7.5% for low, medium and high points of the standard curve, respectively.

Cognitive evaluation

Global cognition was screened with the Montreal Cognitive Assessment (MoCA) [15]. An extensive battery of neuropsychological tests was administered to evaluate different cognitive domains. In detail, memory was tested with the Hopkins Verbal Learning Test-Revised (HVLT-R) [16]; visuospatial function with the Benton Judgment of Line Orientation (JOLO) 15-item version [17]; processing speed-attention with the Symbol-Digit Modalities Test (SDMT) [18]; and executive function and working memory with Letter-Number Sequencing (LNS) [19] and semantic fluency (SF) [20]. With regard to HVLT-R, Immediate Recall (IR), Delayed Recall (DLRY), Retention and Discrimination recognition (DR) were analyzed. Language domain was not assessed.

Statistical analysis

Differences in the distribution of categorical variables among groups were assessed by the chi-square test. After Kolmogorov-Smirnov testing for normal distribution, parametric testing was performed for group comparisons (independent sample t-test or one-way ANOVA with post-hoc

Bonferroni test, as applicable). Descriptive statistics are given as means and standard deviation (SD) with range.

Those variables of interest assessing specific cognitive domains (ie, all except the MoCA score) reaching a significance threshold ≤ 0.1 further underwent linear regression analysis to evaluate the association between serum IGF-1 levels and cognitive scores. Based on the observation that only the lowest quartile of IGF-1 tended to present worse cognitive outcomes compared to the other three quartiles (Figure 1 and Table 1), further analysis were performed comparing the lowest IGF-1 quartile to all other quartiles combined. Visual inspection of graphs also confirmed the lowest IGF-1 quartile tended to present worse scores. Linear regression analysis included age, education, gender as covariates with no interaction terms. Education years were divided into 2 groups based on the median (16 years); group 0: ≤ 16 years, group 1: >16 years. Results were considered statistically significant at $p < 0.05$.

Results

Demographic and clinical data of PD patients are reported in Table 1. Serum IGF-1 and age were not different between PD and HC (136.6 ± 56.1 vs 134.45 ± 56.13 ng/ml; $p = 0.64$). Serum IGF-1 levels were divided in quartiles as follows: lowest quartile ≤ 97.9 ng/ml; second quartile = $97.9001 - 124$ ng/ml; third quartile = $124.0001 - 162.200$ ng/ml; highest quartile ≥ 162.2001 ng/ml.

Linear regression analysis showed that in PD patients the lowest IGF-1 quartile was associated to worse performances in several cognitive tasks compared to all the others quartiles (Table 2). In detail, the lowest IGF-1 quartile was a predictor of lower scores at the SF task ($p = 0.005$), along

with age ($p < 0.001$), male gender ($p < 0.001$) and education ($p = 0.001$) (Figure 1A). Lower performances at the SDMT were associated to the lowest IGF-1 quartile ($p = 0.034$), age ($p < 0.001$) and male gender ($p = 0.001$) (Figure 1B). Finally, the lowest IGF-1 quartile was a predictor of lower performances at the HVLT Retention ($p = 0.019$), along with age ($p = 0.001$) and male gender ($p = 0.003$) (Figure 1C).

No significant associations were found between IGF-1 and any of the neuropsychological tasks in HC.

Discussion

Confirming our hypothesis, we demonstrated that lower serum IGF-1 levels are associated with lower performances on cognitive tasks in a large cohort of early, drug-naïve PD patients. In detail, lower serum IGF-1 levels are linked to poor performances in cognitive tasks assessing executive function, attention and verbal memory. Deficits in such tasks are driven by a dysfunction in the prefrontal and temporal cortex. In detail, neuroimaging studies suggest that while the capability of holding information and sustaining attention (evaluated with the retention and delayed recall of words list and the SDMT) are both ensured by the prefrontal cortex [21], the ability to access the conceptual knowledge stores (evaluated by the Semantic fluency task) is provided by the temporal cortex [22]. Therefore, our data suggest that low IGF-1 levels are linked to impaired cognitive functions (i.e. cognitive flexibility, attention, devising a search strategy) mediated by prefrontal and temporal cortex.

In spite of using different neuropsychological tests, these results are consistent with our previous study in a cohort of 65 drug-naïve, early PD patients showing a significant correlation between low serum IGF-I levels and poor performance on both executive tasks at baseline and attention/executive and verbal memory tasks at 2-year follow-up [14]. Therefore, taken together the findings from the current and previous study support the link between IGF-1 and cognitive impairment in PD patients even in the earliest stages of disease.

Although explored in a spoonful of studies [14,23], the relation between IGF-1 and cognitive performance in PD is not surprising. Indeed, a relevant number of IGF-1 receptors have been reported in several brain areas essential for cognitive performance, with highest concentrations in the hippocampus and the frontal cortex [3,24,25]. Preclinical evidence also suggest, IGF-1 plays out an active defence mechanism against degeneration of the brain [26]. As a matter of fact, executive functioning, memory, attention and verbal fluency present specific improvement in GH-deficient populations treated with hormone replacement therapy [27].

Several preclinical and clinical studies highlighted a link between IGF-1 and neurodegeneration and demonstrated that it may play an important neuroprotective role by various mechanisms [28]. Indeed, animal and in vitro studies showed that IGF-1 enhances neuronal survival and inhibits apoptosis. In both Alzheimer's disease (AD) models and patients, IGF-1 has been suggested to increase B-amyloid clearance and protect neurons against B-amyloid toxicity [4,5]. Interestingly, a recent study including more than three thousands subjects from the Framingham community showed that healthy subjects with IGF-1 in the lowest quartile had a 51% greater risk of developing AD and, among persons without dementia, higher levels of IGF-1 were associated with greater

brain volumes, strongly suggesting that higher levels of IGF-1 may protect against subclinical and clinical neurodegeneration [29]. As regards relationship between IGF-1 and α -synuclein pathology, IGF-1 can rescue α -synuclein toxicity and suppress α -synuclein aggregation through the activation of Akt pathway in cultured cells [30,31].

Indeed, the pathophysiology of cognitive impairment in PD is complex. It involves multiple neurotransmitter systems and diffuse neurodegeneration, and significant gaps still remain in our knowledge of cognitively impaired, non-demented PD patients [32,33]. We acknowledge the range of neuropsychological findings in patients attributed to different IGF-1 quartiles is remarkable with a great overlap. Although our analysis took into account several additional factors possibly influencing cognitive performances in PD patients as age, gender and education, still several aspects were omitted. However, as there is a dearth of consistent serum biomarkers in PD, our intent was to focus on the role of serum IGF-1 as possible marker of cognitive impairment in a large cohort of early stage PD. Future studies should clarify the strength of the relationship between IGF-1 and cognitive performances when considering other clinical, genetic and neuroimaging markers.

In contrast with previous findings [34,35], we found IGF-1 levels similar in PD and HC, implying this is not a valid biomarker for PD diagnosis. Godau et al first detected higher IGF-1 levels in PD compared to healthy subjects and proposed IGF-1 as a marker for PD diagnosis [36,37]. Then, we replicated their findings in our small cohort of de novo PD patients [14,38]. On the other hand, Numao et al already reported no difference when comparing IGF-1 levels between Japanese PD patients and healthy subjects [39]. Indeed, the present study is the largest conducted so far and the sample size may in part account for the discrepancy with previous evidence.

Several confounding factors may affect IGF-1 levels including body mass index (BMI), diabetes, cancer, thyroid dysfunction, inflammatory diseases and medications as corticosteroids [14,35,38].

We identified such subjects in the PD PPMI cohort (diabetes: 17; cancer: 11; thyroid dysfunction: 59; on corticosteroids: 4) and repeated all the analysis after their exclusion. However, results were largely confirmed. In addition, such subjects did not show different IGF-1 values. Finally, the different IGF-1 quartiles did not display significant differences in terms of BMI (Table 1).

Our study has some limitations. First, our analysis focused only on the baseline assessments of the PPMI study and, thus, included patients with relatively preserved cognitive performances. Being the cognitive performances of recruited patients relatively stable over the first 2-year follow up, it is challenging nowadays to establish the real significance of any biomarker of cognitive impairment. However, the PPMI cohort is currently being followed and the next step is to verify if baseline biomarkers levels can predict the onset of PD with dementia on the long-term follow up. Second, in spite of being the largest existing cohort of de novo PD patients, subjects enrolled in the PPMI study may not completely represent the respective counterparts in standard clinical settings. Differently from a previous study analyzing preliminary data from the PPMI cohort (63 PD and 39 HC), no major differences in cognitive performances have been observed between PD and HC [40,41]. It should be noted that the PPMI PD cohort comprises predominantly high-educated volunteers committed to clinical examinations, neuroimaging and longitudinal follow-ups. As such, PD patients are generally more educated and present better cognitive performances as compared to previous, smaller cohorts of PD patients. Thus, our results may be not unhesitatingly generalizable to all PD patients. On the other hand, PPMI database ensures high data quality collection. Finally,

future studies evaluating the impact of a combined set of biomarkers (eg, IGF-1 and ApoE4 carrier status) are warranted.

In conclusion, this is the first large study to indicate a relationship between IGF-1 and specific cognitive functions in early PD patients. Long-term follow up of the same cohort will clarify if serum biomarkers represent useful tools for the early detection of dementia in PD.

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Table 1. Characterization of the PD cohort according to IGF-1 quartiles

	Total cohort (405)	The lowest quartile (110)	The second quartile (86)	The third quartile (100)	The highest quartile (109)	P
IGF-1, ng/ml	33.800-412.200	≤ 97.9	97.9001-124	124.0001-162.200	≥ 162.2001	NA
Demographic and motor variables						
Age, years	61.20 (9.76) (33, 84)	63.33 (9.04) (33, 85)	60.62 (11.63) (33, 82)	62.14 (11.91) (33, 83)	61.07 (10.44) (33, 82)	0.278
Gender (M/F)	264/141	58/52 (52.70/47.30)	62/24 (72.10/27.90)	65/35 (65/35)	79/30 (72.50/27.50)	<0.05*
BMI	27.20 (5.10) (16.80, 45)	26.92 (6.62) (18.18, 45)	28.01 (4.64) (19.65, 41.57)	27.21 (4.61) (17.72,41.05)	26.95 (3.91) (16.80, 36.85)	0.437
Education, years	15.56 (2.98) (5, 26)	15.25 (3.35) (5, 22)	15.84 (3.37) (8, 26)	15.39 (2.67) (8, 24)	15.76 (2.53) (11, 23)	0.443
Disease duration, months	6.33 (6) (0, 35)	5.8 (4.31) (0, 28)	5.92 (5.71) (4, 35)	6.21 (5.81) (6, 30)	6.51 (5.72) (8, 28)	0.499
MDS- UPDRS- III	20.25 (8.93) (4, 51)	21.03 (9.38) (6, 51)	20.87 (7.42) (6, 39)	18.44 (8.52) (6, 45)	20.55 (9.81) (4, 49)	0.140
Cognitive variables						
MoCA, total score	27.10 (2.34) (17, 30)	27 (2.37) (19, 30)	27.02 (2.32) (17, 30)	27.62 (2.08) (21, 30)	26.86 (2.51) (17, 30)	0.078
JOLO	12.69 (2.23) (5, 15)	12.81 (2.41) (5,15)	12.76 (2.28) (5,15)	12.96 (2.08) (7,15)	12.3 (2.13) (6, 15)	0.164
HVLT IR	24.45 (4.99) (9, 36)	24.11 (4.88) (11, 32)	24.52 (5.15) (14, 34)	25.49 (4.89) (9, 35)	24.86 (5.02) (13, 36)	0.097 [§]
HVLT DRLY	8.36 (2.52) (0, 12)	7.95 (2.72) (0, 12)	8.56 (2.14) (3, 12)	8.79 (2.57) (0, 12)	8.22 (2.51) (0, 12)	0.084 [^]
HVLT retention	0.85 (0.20) (0, 1.29)	0.81 (0.22) (0, 1.22)	0.89 (0.16) (0.44, 1.29)	0.86 (0.19) (0, 1.22)	0.85 (0.21) (0, 1.29)	0.074 [°]
HVLT DR	9.65 (2.54) (-2, 12)	9.51 (2.52) (-1, 12)	9.73 (2.52) (-1, 12)	9.89 (2.54) (0, 12)	9.55 (2.61) (-2, 12)	0.683
LNS	10.56 (2.66) (2-20)	10.15 (2.92) (4, 20)	10.41 (2.56) (2, 16)	10.68 (2.71) (3, 20)	10.98 (2.38) (5, 17)	0.120
Semantic fluency	48.52 (11.72) (20, 103)	46.31 (10.73) (20, 75)	49.15 (12.19) (26, 83)	50.13 (11.37) (25, 91)	48.77 (12.36) (24, 103)	0.100 [§]
SDMT	41.17 (9.78) (7, 82)	38.89 (9.88) (7, 61)	41.76 (10.74) (20, 82)	41.77 (8.9) (16, 70)	42.54 (9.35) (17, 66)	0.036 [^]

Table 1 legend

Data are in mean (SD) (range), unless otherwise specified. Significance level ≤ 0.1 are shown in italics.

* $p=0.005$ for the lowest versus the second quartile; $p= 0.002$ for the lowest versus the fourth quartile.

§ -0.403 , $p=0.937$ for the lowest versus the second quartile; -1.370 , $p=0.286$ for the lowest versus the third quartile; -0.256 , $p=0.989$ for the lowest versus the highest quartile.

^ -0.615 , $p=0.543$ for the lowest versus the second quartile; -0.835 , $p=0.101$ for the lowest versus the third quartile; -0.266 , $p=0.787$ for the lowest versus the highest quartile.

° -0.075 , $p=0.060$ for the lowest versus the second quartile; -0.045 , $p=0.608$ for the lowest versus the third quartile; -0.040 , $p=0.811$ for the lowest versus the highest quartile

\$ -2.848 , $p=0.547$ for the lowest versus the second quartile; -3.827 , $p=0.109$ for the lowest versus the third quartile; -2.468 , $p=0.714$ for the lowest versus the highest quartile

& -2.866 , $p=0.247$ for the lowest versus the second quartile; -2.88 , $p=0.196$ for the lowest versus the third quartile; -3.560 , $p=0.042$ for the lowest versus the highest quartile

BMI: Body Mass Index; HVLTL DLR: Hopkins Verbal Learning Test Delayed Recall; HVLTL DR: Hopkins Verbal Learning Test Discrimination Recognition; HVLTL IR: Hopkins Verbal Learning Test Immediate Recall; JOLO: Benton Judgment of Line Orientation (15-item version); LNS: Letter Number Sequencing; MDS-UPDRS-III: Movement Disorders Society version of the Unified Parkinson's disease rating scale part III; MoCA: Montreal Cognitive Assessment; SDMT: Symbol Digit Modalities Test.

Table 2. Significant predictors of cognitive performances in PD patients at baseline.

	Parameter estimate (95% CI)	p
<i>Semantic Fluency</i>		
IGF-1 lowest quartile	-3.46 (-5.87, -1.01)	0.005
Gender	-7.08 (-9.31, -4.84)	<0.001
Age	-0.29 (-0.40, -0.18)	<0.001
Education	3.85 (1.61, 6.09)	0.001
<i>Symbol Digit Modalities Test</i>		
IGF-1 lowest quartile	-2.09 (-4.02, -0.15)	0.034
Age	-0.44 (-0.53,-0.36)	<0.001
Gender	-3.03 (-4.81, -1.24)	0.001
Education	0.92 (-0.87, 2.71)	0.313
<i>HVLT retention</i>		
IGF-1 lowest quartile	-0.05 (-0.09,-0.009)	0.019
Age	-0.004 (-0.006, -0.001)	0.001
Gender	-0.06 (-0.10, -0.02)	0.003
Education	0.04 (0.00, 0.08)	0.052

Table 2 legend

CI: confidence interval; HVLT: Hopkins Verbal Learning Test

Figure 1: Box plots showing differences in cognitive performances in different IGF-1 quartiles

(1A= Semantic fluency; 1B= Symbol-Digit Modalities Test; 1C= Hopkins Verbal Learning Test-Retention).

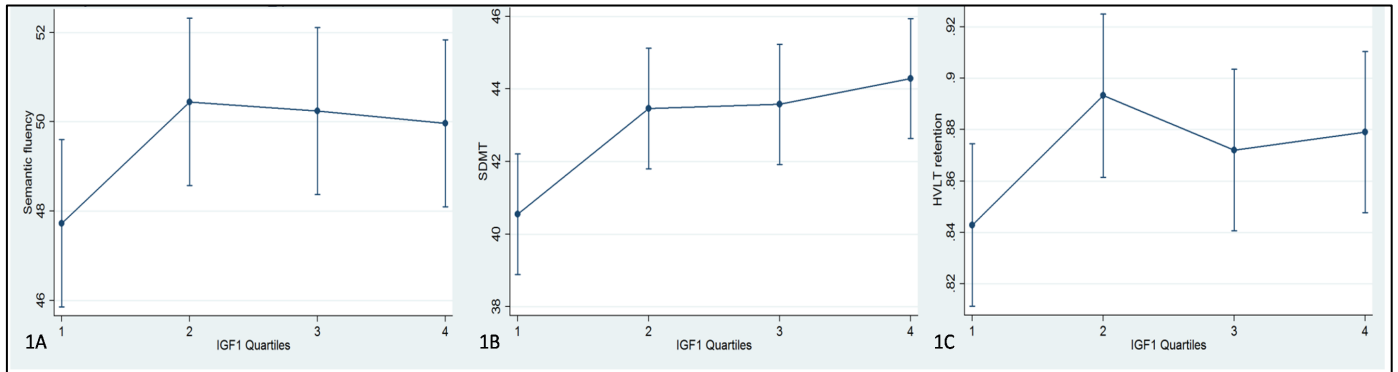


Figure 1 legend

Y axis shows cognitive scores adjusted for age, gender and education according to the regression analysis. According to one-way ANOVA, the lowest quartile presents worse performances as compared to the other quartiles. *: $p < 0.001$. Outliers are showed as circles.

Exploring the association between dopaminergic dysfunction and anxiety in de novo Parkinson's disease

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Abstract

Objectives: To explore the relationships between nigrostriatal dysfunction and neuropsychiatric symptoms (including anxiety, depression and apathy) in a large cohort of newly diagnosed, drug-naïve Parkinson disease (PD) patients compared to a cohort of healthy controls (HC).

Methods: This is a cross-sectional analysis of the Parkinson's Progression Markers Initiative (PPMI) cohort at baseline, including 405 PD patients and 187 HC. Nigrostriatal degeneration was evaluated by means of SPECT DAT scan. Relationships between neuropsychiatric symptoms and DAT uptakes were analysed by means of stepwise multiple regression analysis.

Results: In the PD group, lower DAT uptake in the right caudate was associated with higher STAI trait subscore ($\beta=-2.939$, 95%CI: -4.634 to -1.254, $p=0.001$). Depression and apathy scores were not related with DAT uptakes. No associations were found in the HC group.

Conclusions: Our cross-sectional analysis of the PPMI data shows that lower caudate DAT uptake is associated with higher level of anxiety. The data strengthens the relationship between dopaminergic dysfunction and neuropsychiatric symptoms in early PD.

Introduction

Neuropsychiatric symptoms are frequent complaints in patients with Parkinson disease (PD) with a negative impact on quality of life and associated caregiver burden [1]. Anxiety, depression and apathy are among the most common non motor symptoms, being present since the earliest stages of the disease [1]. Nigrostriatal dopamine depletion in PD contributes directly to motor symptoms, however there is increasing evidence that dopaminergic dysfunction spreading to the anteroventral part of the striatum has a major role in determining neuropsychiatric disturbance in PD patients, especially in the earliest stage of the disease [2-5]. Data from our single-center cohort of patients with newly diagnosed PD suggested an inverse correlation between both anxiety and apathy and dopamine transporter (DAT) availability in the right caudate [4,5]. However, previous studies have found conflicting results [2,3], likely due to the small sample size.

The aim of the present study is to investigate the relationships between nigrostriatal denervation and neuropsychiatric symptoms (including anxiety, depression and apathy) in a large cohort of newly diagnosed, drug-naïve PD patients compared to a cohort of healthy controls (HC). According to our initial hypothesis DAT availability would present an inverse correlation with neuropsychiatric symptoms.

Methods

Study Participants

The data used in this study was downloaded from the PPMI database, according to guidelines (<http://www.ppmi-info.org/data>, Grant ID 8800). PPMI is an ongoing, international multicenter study designed to identify biomarkers of PD progression.

PD patients were enrolled in the study if they met the following criteria: a) an asymmetric resting tremor or asymmetric bradykinesia; or evidence of either bradykinesia or resting tremor and rigidity; b) diagnosed within two years; c) a Hoehn and Yahr stage of I or II; d) age 30 years or older at diagnosis; e) evidence of dopamine transporter deficit on SPECT DATscan imaging; and f) drug-naïve for PD. Demographically comparable healthy subjects were also recruited into the study if they had no current or active neurological disorder, no first-degree relative with PD and no detectable evidence of dopamine transporter deficit on the SPECT DAT scan imaging. All participants underwent a comprehensive clinical, neuropsychiatric, neuropsychological and imaging assessment at enrollment.

The current analysis includes 405 newly diagnosed, drug-naïve PD patients and 187 HC and their clinical, neuropsychiatric and SPECT DAT scan imaging data collected at enrollment (either at screening or baseline visit).

Clinical and neuropsychiatric evaluations

Clinical examination included the Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale part III (MDS-UPDRS-III) to evaluate motor dysfunction and disease severity [6].

Severity of anxiety was assessed by means of the State-Trait Anxiety Inventory (STAI) [7], while depressive symptoms were evaluated using the 15- item Geriatric Depression Scale (GDS-15) [8], with a cut off score of 5 or more indicating clinically significant symptoms [9]. STAI sub-scores can differentiate between the temporary condition of *state anxiety* (i.e., the temporary fear and nervousness due to a certain situation) and the more general and long-lasting *trait anxiety* (i.e., the stress, worry and discomfort perceived in typical situations on a daily basis) [9]. Apathy was assessed with the single item from the MDS-UPDRS part I (MDS-UPDRS-I) [6]. Any non-zero score was considered as an indicator of presence of apathy.

SPECT studies

SPECT studies were performed during the screening visit. All subjects received an intravenous injection of 185 MBq of [123I]FP-CIT (DAT scan). The acquisition started between 3.30 and 4.30 h after the radiotracer injection. This time window between 3 and 6 h allows stable measurement of specific-to-non-displaceable ratio of [123I]FP-CIT. Specific acquisition parameters were selected for each participating center during a preceding technical visit. Images were acquired with a 128 x 128 matrix in a step and shoot mode. Subsequently, the raw projection data was reconstructed using iterative reconstruction algorithm at a central SPECT Core lab in New Haven, Connecticut, USA.

Site-specific attenuation correction was applied to the reconstructed data by an automated ellipse drawing technique and corrected for attenuation using Chang's algorithm ($\mu = 0.06/\text{cm}$). The site-specific attenuation coefficient, μ , was based on an anthropomorphic distributed source phantom acquired during the preceding technical site visit. Hermes (Hermes Medical Solutions, Stockholm, Sweden) and Pmod (PMOD Technologies Ltd., Zurich, Switzerland) brain softwares for quantification were used. Spatial normalization was performed in Pmod software using a template image derived from the Hermes FP-CIT template based on a European multicenter database of healthy controls for [^{123}I]FP-CIT SPECT (ENC-DAT).

Regions of interest (ROI) were then placed on the left and right caudate, the left and right putamen, and the occipital cortex (reference tissue). Count densities for each region were extracted and used to calculate striatal binding ratios (SBRs) for each of the 4 striatal regions. SBR was calculated as $(\text{target region}/\text{reference region})-1$.

Statistic analysis

T tests and chi-squared tests were used for comparisons of demographic, clinical, neuropsychiatric and imaging variables between PD and HC. Descriptive statistics are given as means and standard deviation (SD). Stepwise multiple linear regression analysis evaluating the association between DAT scan uptakes (right and left caudate and putamen uptakes set as independent variables) and neuropsychiatric scores (GDS-15 and STAI sub-scores set as dependent variables) was performed both in PD and HC. As for apathy, multiple logistic regression analysis was used since it was evaluated with a dichotomous assessment. All the analyses included age, education, gender and

disease severity as assessed by the MDS-UPDRS-III as covariates with no interaction terms. Results were considered statistically significant at $p < 0.05$ (two-tailed). Analyses were performed with the Statistical Package for the Social Sciences (SPSS) statistics (version 19; SPSS, Inc., Chicago, IL).

Standard protocol approvals, registrations, and patient consents

Each participating PPMI site obtained written informed consent from all participants, and received approval from an ethical standards committee on human experimentation.

Results

Demographic, clinical, imaging and neuropsychiatric features of PD and HC are displayed in Table 1.

In the PD group, lower DAT uptake in the right caudate was associated with higher STAI trait sub-score ($\beta = -2.939$, 95%CI: -4.634 to -1.254, $p = 0.001$) along with younger age ($\beta = -0.187$, 95%CI: -0.282 to -0.092, $p < 0.001$) and female gender ($\beta = -2.139$, 95%CI: -4.051 to -0.226, $p = 0.029$) (Figure 1). STAI state sub-score and 15-GDS showed no relationship with DAT uptakes. Presence of apathy was predicted by MDS-UPDRS-III ($\beta = 0.047$, $p = 0.001$, OR=1.048, 95%CI=1.020 to -1.078) and younger age ($\beta = -0.033$, $p = 0.015$, OR=0.968, 95%CI=0.943 to -0.994), but not by any DAT uptakes. Given the well-known comorbidity between anxiety, depression and apathy [10-12], the regression analysis for anxiety was run again controlling also for the presence of apathy and severity of depression and the results were largely confirmed (lower right caudate uptake predicted higher STAI trait sub-score, $\beta = -1.536$, 95%CI: -2.687 to -0.384, $p = 0.009$).

When comparing DAT uptakes between PD patients with and without anxiety (according to the STAI total score cut off of 39 [9]), anxious patients (88 out of 405) presented a tendency towards significance for lower right caudate uptake (1.918 vs 2.01, $p=0.1$).

Finally, we did not detect any difference in executive and attentive functions between anxious and non-anxious PD patients (data not shown), further supporting the specificity of the relationship between anxiety and caudate uptakes.

No correlation between neuropsychiatric symptoms and DAT uptakes was found in the HC group.

Discussion

Confirming previous findings, a recent paper on PPMI data showed that neuropsychiatric symptoms are more common in early, drug-naïve PD compared to the general population [9]. Here, we sought to detect associations between striatal dopaminergic dysfunction and anxiety, depression and apathy in PPMI cohort. After controlling for possible confounding factors, anxiety severity was found to relate to lower right caudate uptake in PD. No such correlation was found in the control group. The relationship between dopaminergic dysfunction and anxiety disorders in PD is complex and still only partially understood. Neuropsychiatric symptoms in PD are not a mere reaction to motor symptoms and chronic disability, but are likely due to the combination of psycho-social factors and the underlying cortical-limbic-striatal dysfunction [1]. Regarding the association between nigrostriatal DAT availability deficits and anxiety symptoms in PD, previous studies have found conflicting results. Weintraub et al., by TRODAT-1 imaging, found left anterior putamen DAT availability to be negatively correlated with STAI scores [2]. Conversely, Moriyama et al. found an

increased DAT density in left striatum and right putamen in PD patients with social anxiety disorder [3]. Different methodologies and definition of anxiety may account for such discrepancies. Moreover, both studies enrolled smaller cohorts of PD patients with longer disease duration and under dopaminergic treatment [2,3]. Indeed, the results of the present study largely confirm our previous data on a smaller cohort of early drug-naïve PD patients suggesting that the association between anxiety and right caudate may be specific for drug-naïve patients in the earliest stages of the disease [5]. This relationship is not surprising and can be explained in light of the crucial non motor role played by the caudate in PD [13]. Indeed, this association suggests a more pronounced disruption of dopaminergic-frontal circuits in PD patients with anxiety. According to the dopaminergic theory, the neurodegeneration of mesolimbic and mesocortical dopaminergic projections observed as a primary neuroanatomical alteration in PD is strongly associated with anxiety [13]. Furthermore, the exclusive association between STAI trait sub-score and DAT binding suggests that dopaminergic dysfunction is specifically related to anxiety-related personality traits rather than the temporary condition of state anxiety. Although the role of other neurotransmitters (i.e. serotonin and noradrenalin) cannot be overlooked, the dopaminergic involvement in anxiety of PD is supported by clinical evidence, as the common relationship between anxiety and motor fluctuations and the occurrence of anxiety symptoms following the withdrawal of dopaminergic medications [14].

Indeed, anxiety is also recognized as part of the pre-motor symptoms of PD. A recent population-based cohort study found that the likelihood of developing PD was greater amongst patients with

anxiety than patients without anxiety, and the severity of anxiety correlated with the risk of PD [15].

From a practical standpoint, these results may have treatment implications. For instance, dopamine agonists, which help compensate for nigrostriatal dopamine deficiencies in PD [14], may be of particular use in PD patients with anxiety. Although ad hoc studies are lacking, we previously described a reduction in anxiety prevalence in de novo PD patients after the addition of the dopaminergic treatment [16,17].

In contrast to previous findings [2,4], we failed to detect any associations between either depression or apathy and DAT availability in PD patients enrolled in PPMI. This discrepancy may reflect the different methodologies used to evaluate depressive symptoms and apathy. As for depressive symptoms, Weintraub et al. [2] used the depression subscale of Profile of Mood States, whereas PPMI study used GDS-15, which disclosed only mild depressive symptoms in PD patients, with only 13.9% presenting with significant depression [9]. As for apathy, in contrast to our previous study on a smaller cohort of early, drug-naïve patients, where we found an association between apathy assessed by Apathy Evaluation Scale and right caudate uptake [4], here the occurrence of apathy was evaluated by one single item of the MDS-UPDRS-I which is not considered to be an adequate screening measure. Thus, we suggest that both the GDS-15 "floor effect" reflecting mild depressive symptoms and the lack of specific scales assessing apathy may in part account for the lack of associations with DAT availability in the present study.

Limitations of the study include the cross-sectional design, which precludes firm conclusions of causality, lack of adequate screening measures for apathy (e.g., Apathy Evaluation Scale) and

assessment of meso-limbic and meso-cortical dopaminergic dysfunction with appropriate imaging techniques. We acknowledge our data describe only in part the complex scenario underlying neuropsychiatric disturbances in PD patients. The mild severity of depressive symptoms and the low number of subjects defined depressed according to the GDS-15 cut-off may in part account for the lack of association between dopaminergic dysfunction and depression in the PD cohort. As such, our results on depression and apathy should be interpreted with caution and deserve further investigations. Furthermore, the DAT scan was performed during the screening visit while behavioral assessments during the baseline visit (within 45 days from the screening). We cannot exclude this timeframe has a role in the lack of association with depression and STAI-state anxiety. Despite these drawbacks, this is the largest controlled-study to date assessing the relationship between dopaminergic dysfunction and anxiety in PD.

In conclusion, our cross-sectional analysis of the PPMI data shows that high level of anxiety is associated with reduced DAT uptake in the right caudate.

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Table 1. Demographic, clinical, imaging and neuropsychiatric data of PD and HC.

	PD (n=405)	HC (n=187)	p
Age, years	61.20 (9.76)	60.24 (11.21)	0.31
Gender (M/W, %)	264/140 (65.3/34.7)	121/66 (64.7/35.3)	0.41
Education, years	15.56 (2.98)	16.12 (2.9)	<i>0.032</i>
MDS-UPDRS-III	20.25 (8.93)	NA	NA
Most affected side (R/L/B, %)	224/173/7 (55.6/42.7/1.7) ^a	NA	NA
Right Caudate uptake	1.98 (0.59)	2.95 (0.63)	<i><0.001</i>
Left Caudate uptake	1.99 (0.59)	3.0 (0.65)	<i><0.001</i>
Right Putamen uptake	0.84 (0.36)	2.15 (0.58)	<i><0.001</i>
Left Putamen uptake	0.81 (0.35)	2.14 (0.56)	<i><0.001</i>
STAI total score	65.35 (18.47) ^b	57.03 (14.33)	<i><0.001</i>
STAI state sub-score	32.93 (10.22) ^b	28.03 (8.14)	<i><0.001</i>
STAI trait sub-score	32.39 (9.56) ^b	29.01 (7.5)	<i><0.001</i>
GDS-15 score	2.29 (2.37) ^b	1.28 (2.08)	<i><0.001</i>
GDS-15 cut off (not depressed/depressed)	347/56 (85.9/13.9)	174/12 (93/6.4)	<i>0.005</i>
MDS-UPDRS-I Apathy score (negative/any positive, %)	80/324 (19.8/80.2)	178/9 (95.1/4.9)	<i><0.001</i>

Table 1 Legend

Data are expressed in mean (standard deviation) unless otherwise specified. Significant differences are in italic.

^a Data available for 404 subjects; ^b Data available for 400 subjects; B: bilateral; GDS-15: 15-item Geriatric Depression Scale; HC: healthy controls; L: left; M: men; MDS-UPDRS-I: the Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale part I; MDS-UPDRS-III: Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale part III; NA: not applicable; PD: Parkinson disease; R: right; STAI: State-Trait Anxiety Inventory; W: women.

Figure 1. Correlation between right caudate uptake and STAI trait sub-score according to the multiple linear regression model.

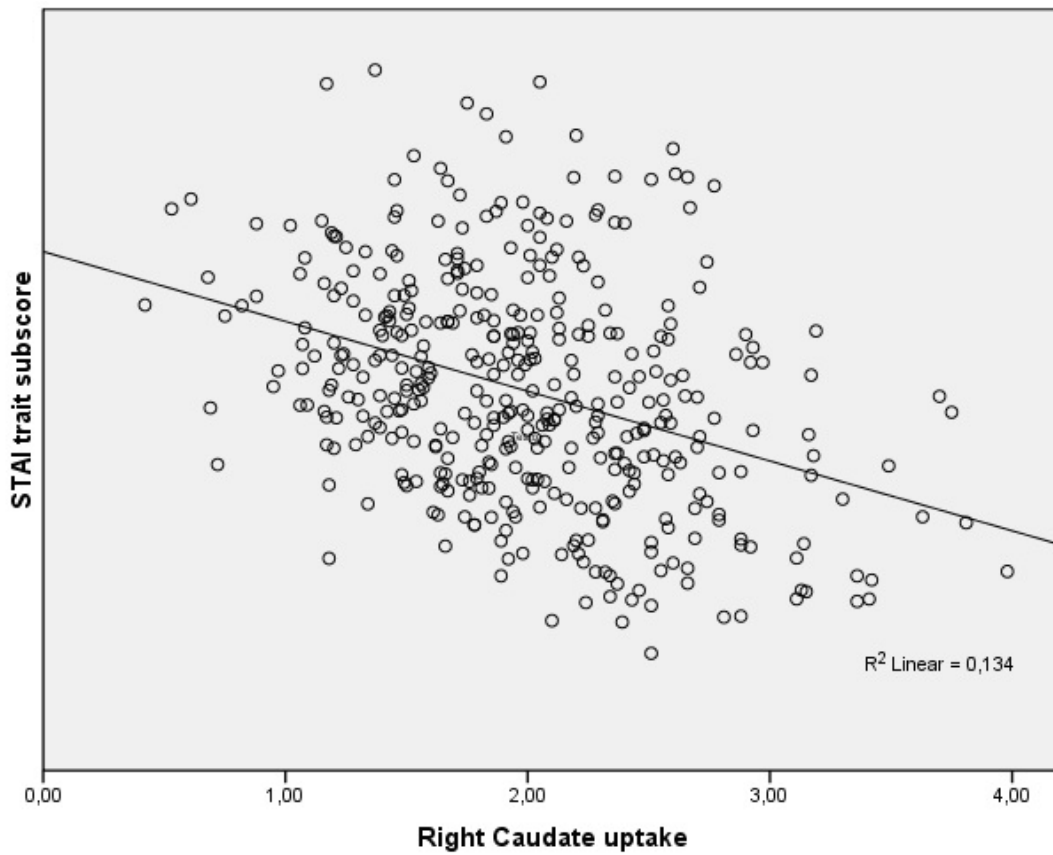


Figure 1 legend

STAI: State-Trait Anxiety Inventory.

Exploring the relationship between cognition and dopaminergic dysfunction in de novo Parkinson's disease: a longitudinal prospective study

Plos One (under review)

Abstract

Objectives: To explore the relationship between nigrostriatal dopamine uptake at diagnosis and both baseline and 2-year longitudinal cognitive scores in a large cohort of drug-naïve (at baseline) Parkinson's disease (PD) patients compared to a matched cohort of healthy controls (HC).

Methods: This is a longitudinal analysis of the Parkinson's Progression Markers Initiative (PPMI) cohort, including 423 PD patients and 196 HC at baseline. Nigrostriatal dopaminergic degeneration was evaluated at baseline by means of SPECT dopamine transporter (DAT) scan. Relationships between specific cognitive tests and DAT uptake were analysed by means of multivariate linear regression analysis controlling for age, gender, education and disease severity.

Results: At baseline, lower left caudate DAT availability was associated with worse performances in the processing speed/attention domain ($\beta=1.535$, 95%CI: 0.102 to 2.969, $p=0.036$), while lower right putamen uptake presented a trend towards significance for association with lower verbal recognition memory scores ($\beta=1.067$, 95%CI: -0.022 to 2.157, $p=0.055$). At 2-year follow up, lower left putamen DAT availability at baseline predicted greater decline in tests evaluating immediate ($\beta=-1.42$, 95%CI: -2.59 to -0.246, $p=0.018$) and delayed ($\beta=-0.869$, 95%CI: -1.53 to -0.210, $p=0.009$) verbal memory, while higher right caudate DAT availability at baseline presented a trend for greater improvement in visuo-spatial performances ($\beta=0.326$, 95%CI: -0.012 to 0.664, $p=0.059$).

Conclusions: This data strengthens the relationship between dopaminergic dysfunction and cognitive performances in early PD.

Introduction

Cognitive impairment is a frequent complaint in patients with Parkinson's disease (PD) with a negative impact on quality of life and associated caregiver burden. [1-3] Nigrostriatal dopamine depletion in PD contributes directly to motor symptoms, however there is increasing evidence that dopaminergic dysfunction in the striatum has a major role also in determining cognitive dysfunction, especially in the earliest stages of the disease. [4-8] Recent studies focused their attention on PD patients with mild cognitive impairment (PD-MCI), suggesting that striatal dopamine depletion disrupts the cortico-striatal pathway and, thus, recruitment of cortical regions involved in cognitive tasks in this subgroup of patients. [9-11] Our single-centre experience suggests a direct relationship between reduced DAT availability in both caudate and putamen and impaired cognitive performances in frontal/executive and visuospatial tasks in newly diagnosed PD. [9] However, despite the evidence of a close relationship between nigrostriatal dysfunction and cognitive impairment in PD, the role of nigrostriatal deafferentation at disease onset in predicting subsequent worsening of specific cognitive performances has been poorly investigated. [7]

In the present study, we sought to explore the relationship between nigrostriatal uptake at diagnosis and 1) cognitive scores at diagnosis and 2) change in cognitive scores from diagnosis to 2-year follow up in a large cohort of drug-naïve PD patients compared to a cohort of healthy controls (HC).

Methods

Study Participants

The data used in this study was downloaded from the PPMI database, according to guidelines (<http://www.ppmi-info.org/data>, Grant ID 8800, last download January 4th 2016). PPMI is an ongoing, international multicenter study designed to identify biomarkers of PD progression. Detailed descriptions of the study have been published elsewhere. [12]

PD patients were enrolled in the study if they met the following criteria: a) an asymmetric resting tremor or asymmetric bradykinesia; or evidence of (either bradykinesia or resting tremor) and rigidity; b) diagnosed within two years; c) a Hoehn and Yahr stage of I or II; d) age 30 years or older at diagnosis; e) evidence of dopamine transporter deficit on SPECT DATscan imaging; and f) drug-naïve for PD; g) diagnosis of dementia based on site investigator's clinical impression.

Demographically comparable healthy subjects were also recruited into the study if they had no current or active neurological disorder, no first-degree relative with PD and no detectable evidence of dopamine transporter deficit on the SPECT DATscan imaging and a Montreal Cognitive Assessment (MoCA) battery score >26 [13]. All participants have been performing scheduled assessments to collect biospecimens, clinical, neuropsychological and imaging data from enrollment and up to 5-year follow up.

The current analysis includes 423 newly diagnosed, drug-naïve PD patients and 196 HC and their SPECT DAT scan imaging data collected at enrollment and clinical and neuropsychological data collected at both enrollment and 2-year follow up.

SPECT studies

SPECT studies were performed during the screening visit. All subjects received an intravenous injection of 185 MBq of [123I]FP-CIT (DAT scan). The acquisition started between 3.30 and 4.30 h after the radiotracer injection. This time window between 3 and 6 h allows stable measurement of specific-to-non-displaceable ratio of [123I]FP-CIT. [14] Specific acquisition parameters were selected for each participating center during a preceding technical visit. Images were acquired with a 128 x 128 matrix in a step and shoot mode. Subsequently, the raw projection data was reconstructed using iterative reconstruction algorithm at a central SPECT Core lab in New Haven, Connecticut, USA. Site-specific attenuation correction was applied to the reconstructed data by an automated ellipse drawing technique and corrected for attenuation using Chang's algorithm ($\mu = 0.06/\text{cm}$). The site-specific attenuation coefficient, μ , was based on an anthropomorphic distributed source phantom acquired during the preceding technical site visit. Hermes (Hermes Medical Solutions, Stockholm, Sweden) and Pmod (PMOD Technologies Ltd., Zurich, Switzerland) brain softwares for quantification were used. Spatial normalization was performed in Pmod software using a template image derived from the Hermes FP-CIT template based on a European multicenter database of healthy controls for [123I] FP-CIT SPECT (ENC-DAT). [15]

Regions of interest (ROI) were then placed on the left and right caudate, the left and right putamen, and the occipital cortex (reference tissue). Count densities for each region were extracted and used to calculate striatal binding ratios (SBRs) for each of the 4 striatal regions. SBR was calculated as (target region/reference region)-1.

Clinical and neuropsychological evaluations

Clinical examination included the Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale part III (MDS-UPDRS-III) to evaluate motor dysfunction and disease severity. [16]

All patients underwent the MoCA [13] to evaluate global cognitive functioning and a comprehensive battery of neuropsychological tests to assess the following cognitive functions: (i) verbal learning and memory, by means of the Hopkins Verbal Learning Test – Revised (HVLT-R), including the Immediate Recall (IR), Delayed Recall (DLRY), Retention, and Discrimination Recognition (DR); [17] (ii) executive function and working memory, with the Letter Number Sequencing (LNS) [18] and the Semantic Fluency (Animal, Vegetable, Fruit) tests; [19](iii) processing speed/attention with the Symbol Digit Modalities test (SDMT); [20] (iv) visuo-spatial functions with the short version of Benton Judgment of Line Orientation (BJLOT) (15-item version). [21]

Statistic analysis

T tests and Fisher's exact tests were used for comparisons of baseline demographic, clinical, neuropsychological and imaging variables between PD and HC and values with $p \leq 0.05$ were regarded as statistically significant. Descriptive statistics are given as means and standard deviation (SD). Multivariate linear regression was used to evaluate the association between baseline DAT uptakes (right and left caudate and putamen uptakes set as independent variables) and cognitive task scores at baseline and their change from baseline to 2-year follow up (dependent

variables). All the models adjusted for age, education, gender and disease severity as assessed by the MDS-UPDRS-III. In addition, the 2-year models also adjusted for levodopa equivalent daily dose (LEDD). Baseline DAT uptakes were assessed individually and if any were found to be significant at the $p < 0.2$ level they were assessed together in a single backwards regression model with a $p < 0.1$ inclusion level. All β -values and p -values are from the backwards selection models. Statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). Because this study was exploratory in nature, analyses were done without correction for multiple comparisons.

Standard protocol approvals, registrations, and patient consents

Each participating PPMI site obtained written informed consent from all participants, and received approval from an ethical standards committee on human experimentation.

Results

Baseline and 2-year follow up demographic, clinical, imaging and neuropsychological features of PD and HC are displayed in Table 1.

Relationship between DAT uptakes and baseline cognitive performances

In the PD group, lower left caudate uptake was associated with lower SDMT scores ($\beta=1.535$, 95%CI: 0.102 to 2.969, $p=0.036$) (Figure 1A), while lower right putamen uptake presented a trend towards significance for association with lower HVLT-DR score ($\beta=1.067$, 95%CI: -0.022 to 2.157, $p=0.055$) (Figure 1B). In the HC group, lower uptake in the right putamen was found to be

associated with reduced MoCA performances ($\beta=0.309$, 95%CI: 0.015 to 0.603, $p=0.040$). No other significant associations were detected.

Relationship between DAT uptakes and change in cognitive performances from baseline to 2-year follow up

In the PD group, lower left putamen uptake was associated with greater worsening in HVLT IR ($\beta=-1.42$, 95%CI: -2.59 to -0.246, $p=0.018$), HVLT DRLY ($\beta=-0.869$, 95%CI: -1.53 to -0.210, $p=0.009$) and HVLT retention ($\beta=-0.069$, 95%CI: -0.135 to -0.005, $p=0.035$). There was a trend towards significance for higher right caudate uptake and greater improvement in BJLOT ($\beta=0.326$, 95%CI: -0.012 to 0.664, $p=0.059$). No significant associations were detected in HC group.

Discussion

Regarding baseline analysis, and after controlling for possible confounding factors, we found a significant association between left caudate uptake and information processing speed and between right putamen uptake and memory task. Although with a different methodology, other analyses of the PPMI data already disclosed a relationship between striatal uptake and cognitive performances suggesting a role for DAT Scan as an imaging biomarker for cognitive impairment in PD. [6,7,22]

The present study, however, discloses for the first time the relationship between striatal uptakes and specific cognitive functions. Indeed, the association between executive function and caudate DAT uptake is well established in early untreated PD and relates to a disruption of the dopaminergic-frontal circuits. [4,5]

Conversely, the association between striatal dopaminergic depletion and memory has only recently gained attention. By studying patients with a mean disease duration of 6 years on dopaminergic treatment, Christopher et al. suggested that memory impairment in PD is associated with a loss of dopaminergic function in the salience network and right para-hippocampal gyrus. [23] They found significant differences in [11C] DTBZ binding in associative striatum according to cognitive status, with the greatest impairment in PD amnesic MCI. [23] The authors hypothesized that a disruption of dopaminergic modulation in the salience network could impact the switch between the central executive and default mode network required for mental flexibility and the coordination of memory, thus explaining poor executive and memory performances in PD. [23] Our data strongly support the role of the striatum in both executive and memory tasks in PD since the early stages of disease. Alternatively or complementarily, our findings may also support the hypothesis that PD memory impairment is secondary to impaired attention and executive dysfunction. [24] As the cornerstone of memory deficit in PD is the retrieval, [23] the lack of association between DAT uptakes and HVLT DRLY at baseline would support the latter hypothesis.

Regarding the role of the dopaminergic dysfunction in predicting cognitive changes at 2-year follow up, we found an association between lower left putamen uptake at baseline and greater decline in both immediate and delayed recall of the HVLT. This finding is consistent with a recent analysis of the PPMI data showing that baseline age, olfaction and REM-sleep behavior disorders scores, CSF_{AB42} and DAT uptakes allowed the best prediction of 2-year cognitive impairment as assessed with the MoCA scores. [6] The present study, however, analyzes for the first time the relationship between baseline DAT uptakes and specific cognitive performances after 2-year follow up. Indeed,

a link between memory and activity in both the hippocampus and the putamen during encoding has been already shown by fMRI studies in healthy subjects. [25,26] In addition, the association between higher right caudate uptake and greater improvement in visuospatial task indicates a role for caudate nucleus in visuospatial abilities, as already reported in patients with dementia with Lewy bodies. [27] Again, these findings support the central role of frontal circuits in cognitive performances in early PD. As a matter of fact, visuospatial abilities in early PD are mainly driven by frontal circuits, which have wide connections with posterior visuo-spatial areas, rather than by primary posterior cortex. [11]

Finally, our analysis shows a positive association between MoCA scores and right putamen uptake in healthy controls. The MoCA is a screening test assessing multiple functions related to both cortical and subcortical areas and widely used in studies involving both subjects affected by diseases other than PD and healthy elderly. A recent study shows that MoCA total score is related with MRI measures evaluating subcortical atrophy in healthy subjects [28]. Thus, we can argue that dopaminergic circuitries have a role in determining MoCA performances in healthy subjects. However, the detailed examination of this relationship is outside the scope of the present study.

Indeed, in spite of being one of the largest existing cohort of de novo PD patients so far, both PD and HC subjects enrolled in the PPMI study may not completely represent their respective counterparts in standard clinical settings. In fact, although PD patients enrolled in PPMI showed lower cognitive performances when compared to healthy controls, [29] they were generally more educated and presented better cognitive performances as compared to previous, smaller cohorts of

PD patients. [30,31] Thus, our results may not be applied universally to PD patients and healthy subjects.

We acknowledge our data describe only in part the complex scenario underlying cognitive deficits in PD patients. Our study misses to consider the role of other neurotransmitters (e.g., acetylcholine) and the dysfunction of other neural networks (e.g., meso-limbic and meso-cortical dopaminergic dysfunction detectable with other imaging techniques), which are pivotal in the pathogenesis of cognitive impairment in PD. However, the present study was specifically focused on the relationship between DAT availability at diagnosis and cognition at baseline and its change after 2-year follow up, and we believe that the large cohort of PD patients and healthy controls involved in this analysis strengthens our results.

In conclusion, our longitudinal analysis of the PPMI data shows that: a) at diagnosis reduced processing speed is related to lower DAT availability in the left caudate and poorer memory scores present a trend towards significance for reduced right putamen uptake; b) lower left putamen uptake at diagnosis envisages greater decline in working memory after 2-year follow up; and c) higher right caudate uptake at diagnosis is associated with better visuo-spatial functions at 2-year follow up. This data strengthens the relationship between dopaminergic dysfunction and cognitive performances in early PD.

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Table 1. Demographic, clinical and cognitive data of PD and HC at baseline and 2-year follow

up.

	PD (n=423)	HC (n=196)	p
<i>Baseline</i>			
Age, years	61.65 (9.7)	60.80 (11.2)	0.33
Gender (M/W, %)	277/146 (65.4/34.5)	126/70 (64.2/35.7)	0.78
Education, years	15.54 (2.99)	16.04 (2.89)	0.05
Most affected side (R/L/B, %)	179/234/10 (42.32/55.32/2.36)	NA	NA
Right Caudate DAT uptake ^a	1.99 (0.59)	2.96 (0.63)	<0.001
Left Caudate DAT uptake ^a	2.00 (0.60)	3.0 (0.65)	<0.001
Right Putamen DAT uptake ^a	0.84 (0.36)	2.15 (0.58)	<0.001
Left Putamen DAT uptake ^a	0.81 (0.36)	2.14 (0.57)	<0.001
MDS-UPDRS-III	20.89 (8.86)	1.20 (2.19)	<0.001
MoCA ^b	27.13 (2.32)	28.23 (1.11)	<0.001
BJLOT ^c	12.77 (2.13)	13.12 (1.98)	0.051
HVLT-IR ^c	24.44 (4.98)	26.05 (4.50)	<0.001
HVLT-DRLY ^c	8.36 (2.52)	9.29 (2.32)	<0.001
HVLT retention ^c	0.86 (0.20)	0.90 (0.18)	0.003
HVLT DR ^c	9.63 (2.63)	10.07 (2.80)	0.062

LNS ^c	10.59 (2.66)	10.87 (2.57)	0.21
Semantic fluency ^c	48.67 (11.6)	51.80 (11.2)	<i>0.001</i>
SDMT ^c	41.18 (9.73)	46.78 (10.5)	<i><0.001</i>
<i>2-year follow up</i>			
	PD (n=380)	HC (n=174)	p
MDS-UPDRS-III	27.3 (11.3)	1.5 (2.82)	<i><0.001</i>
MoCA, total score	26.3 (3.16)	27.2 (2.34)	<i><0.001</i>
BJLOT	12.8 (2.26)	13.1 (2.08)	0.24
HVLT IR	23.7 (5.45)	25.9 (5.05)	<i><0.001</i>
HVLT DRLY	8.2 (2.95)	9.2 (2.53)	<i><0.001</i>
HVLT retention	0.9 (0.23)	0.9 (0.19)	<i>0.03</i>
HVLT DR	10.7 (2.41)	10.6 (3.0)	0.72
LNS	10.3 (2.81)	10.9 (2.51)	<i>0.01</i>
Semantic fluency	49 (13)	56.2 (11.7)	<i>0.002</i>
SDMT	40 (11.1)	46.5 (10.7)	<i><0.001</i>

Table 1 legend

Data is in mean (standard deviation) unless otherwise specified. Significant differences are in italic.

^aData available for 418 PD and 193 HC; ^bData available for 420 PD and 196 HC; ^cData available for 422 PD and 196 HC; B: bilateral; BJLOT: Benton Judgment of Line Orientation test; HC: healthy controls; HVLT-DLRY: Hopkins Verbal Learning Test Delayed Recall; HVLT-DR: Hopkins Verbal Learning Test discrimination recognition; HVLT-IR: Hopkins Verbal Learning Test immediate recall; L: left; LNS: Letter Number Sequencing; M: men; MDS-UPDRS-I: the Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale part I; MDS-UPDRS-III: Movement Disorder Society-sponsored revision of the Unified

Parkinson's Disease Rating Scale part III; MoCA: Montreal Cognitive Assessment; NA: not applicable; PD: Parkinson disease; R: right; SDMT: Symbol Digit Modalities test; W: women.

Figure 1: A) Correlation between left caudate uptake and SDMT according to the multiple linear regression model; B) Correlation between right putamen uptake and HVLТ-DR according to the multiple linear regression model.

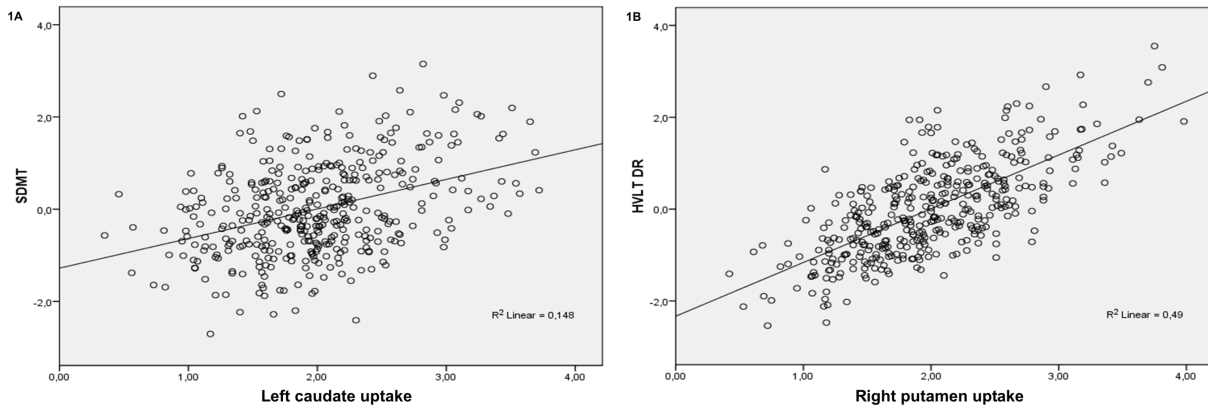


Figure 1 legend

Y axis shows standardized scores. Abbreviations: HVLТ DR: Hopkins Verbal Learning Test recognition discrimination; SDMT: Symbol Digit Modalities test.

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