University of Catania

Ph.D. IN NEUROBIOLOGY
25th CYCLE

EXPERIMENTAL THESIS

Dementia in Parkinson's Disease: relationship between clinical and neurobiological aspects

Ph.D. Coordinator: Prof. Roberto Avola

Dr. Maria Elena Di Battista
University of Rome "Sapienza"
Tutor: Prof. Giuseppe Meco

Background

Parkinson’s disease (PD) is the one of the most common neurodegenerative disorders. The clinical motor syndrome of PD have been classically associated with neuronal loss in the substantia nigra and inclusions containing the synaptic protein α-synuclein (α-syn) in the cell bodies and processes of surviving neurons (known as Lewy bodies and Lewy neurites, respectively) in this region.

However, PD is now recognized as being a more complex clinicopathological entity: a “proteiform” multiorgan disease resulting from synergistically convergent multi factorial biomolecular substrates.

Consistent with this expanded view of the malady, Dementia and Cognitive Impairment in Parkinson’s Disease are now going to be depicted as the resultant of complex connections between variable neurobiological “aetiologies” rather that the expression of a linear pathological process.

Clinically, fairly subtle cognitive disturbances may be characteristically present at the initial stages of Parkinson's disease; these deficits may progress in a subgroup of patients to a

more consistent cognitive impairment or, in some cases, worsen to the clinical picture of dementia.

At earliest stages, symptoms of cognitive impairment are mainly related to frontal–striatal dysfunctions and involve executive defects in planning, initiation, monitoring of goal-directed behaviors and working-memory (Pagonabarraga J, 2012). Other initial symptoms, which era not necessarily related to a frank dementia, may also include visuospatial deficit as expression of posterior cortical malfunctioning. However, major differences in the overall rate of cognitive decline among Parkinson patients, supporting the existence different pattern of progression. At least two differentiating patterns of decline have been described: a relatively slow decline of fronto-striatal deficits and a more rapid decline of posterior–cortical deficits (Williams-Gray, 2009). However, Parkinson dementia likely presents even a more remarkably variability, probably due to the interaction of pathophysiological, neuropathological and genetic substrates.

Understanding the basis of this heterogeneity and the correlation with the overall clinical picture of the disease is the purpose of the study. Starting from the “nosological” question of Parkinson Dementia and proceeding to analysis of neurobiological substrates

contributing not only to cognitive impairment, but also impact the motor phenotype of the disease.

Dementia in Parkinson's disease is a crucial determinant of reduced life expectancy in patients with this movement disorder.

Studies from the prelevodopa era include Lewy, who in 1923 reported that 54 of 70 (77%) developed dementia and Monroe, who reported that approximately one third of patients develop dementia.

A recent review accounted for a prevalence of PDD of 40% among 4336 patients included from 27 studies. However, applying different methodological criteria, other authors found a prevalence of PDD of 31.3% (Aarsland, 2005).

How common dementia is in PD still remain a matter of debate.

Two longitudinal studies, are now available: the Sydney study in 149 denovo PD patients, the prevalence of dementia was of 28% after 5 year, 48% at 15 years and 83% after 20 years (Hely, 2008). In the Stavanger study the prevalence of dementia increased up to 78% after 17 years of observation (Aarsland et al., 2003).

Clinical characteristics

Cognitive impairment in PD is present since the earliest stages and can be detected even in untreated ‘de novo’ patients although is not generally apparent to the patient or clinician.

Muslimovic at al. (Neurology 2005) assessed the neuropsychological profile in a cohort of patients with newly diagnosed PD, the group of 129 patients was characterized with an extensive neuropsychological battery and well matched with a group of healthy control. The authors found that 23.5% of PD patients had cognitive deficits: PD group displayed alterations on variables of psychomotor speed, language, attention and executive functions, memory, and visuospatial abilities. Impairment was observed in the domain of language was relatively rare (22%), whereas deficit in the domain of attention/executive functions was found in the entire subgroup of patients with cognitive impairment (100%).

The same authors found that older age at onset is an independent predictor of cognitive impairment in patients with PD. This finding is in accordance with a number of study indicating that the time of onset of the disease, rather than the disease duration, promote cognitive decline in PD patients.

Interestingly, a study carried out by Marini P. el al (Neurol Sci, 2003) on a small de-novo and levodopa naïve group of PD patients found that, at least a specific component of executive dysfunctions, reverse after administration of levodopa.

A recent study (Pfeiffer HC, Acta Neurol Scand. 2013) investigated the association between cognitive impairment and motor symptoms in a cohort of patients without a diagnosis of dementia demonstrated that cognitive dysfunction in non demented PD patients robustly correlate with *Levodopa 'non-responding'* symptoms, such as impairment of gait, posture abnormalities and dysarthria, underlying the contribution of non-dopaminergic systems.

Initial deficits, in non-demented PD patients include:

- Alternating and phonemic verbal fluency.

- Speech fluency: "tip-of-the-tongue phenomenon".

- Sustained attention.

- Working memory: backward digit span.

- Set-shifting, set-acquisition, conceptualization: Trail-Making Test,

- Wisconsin Card Sorting Test, Tower of London, WAIS similarities.

- Cognitive processing speed: Digit Symbol Test.

- Free-recall verbal and visual memory.

- Unprompted clock or figure drawing.

- Visuospatial skills: mental figure rotation, line orientation.

With the progression of the disease can be observed, in conjunction with a worsening of the symptoms above mentioned, the occurrence of deficiency regarding the following domains:

- Semantic verbal fluency.

- Confrontation naming.

- Recognition (cued-recall) memory.

- Constructional apraxia and figure copying

Cognitive impairment remain however highly heterogeneous, regarding both temporal progression as well dysfunctional profile.

Importantly, early deficits on frontostriatally based tasks are not necessarily related to subsequent development of dementia (Williams-Gray 2009).

Neuropathology of Parkinson Dementia

a-Synucleinopathy

The presence of neuronal intracytoplasmic inclusions known as LBs and inclusions confined to neuronal processes known as Lewy neurites (LN) is the classical neuropathological feature of Parkinson's Disease. The involvement of the substantia nigra with the destruction of dopaminergic neurons in the pars compacta is unanimously considered the PD "hallmark". A study undertaken to examine the neuropathological substrates of cognitive dysfunction and dementia in Parkinson disease (Irwin DJ, Ann Neurol 2012) indicates that CLB/LN, the neuropathological correlate of the so called

“synucleinopathy”, is the most significant correlate of dementia in PD. This results are in line with the Braak hypothesis of alpha-synuclein progression.

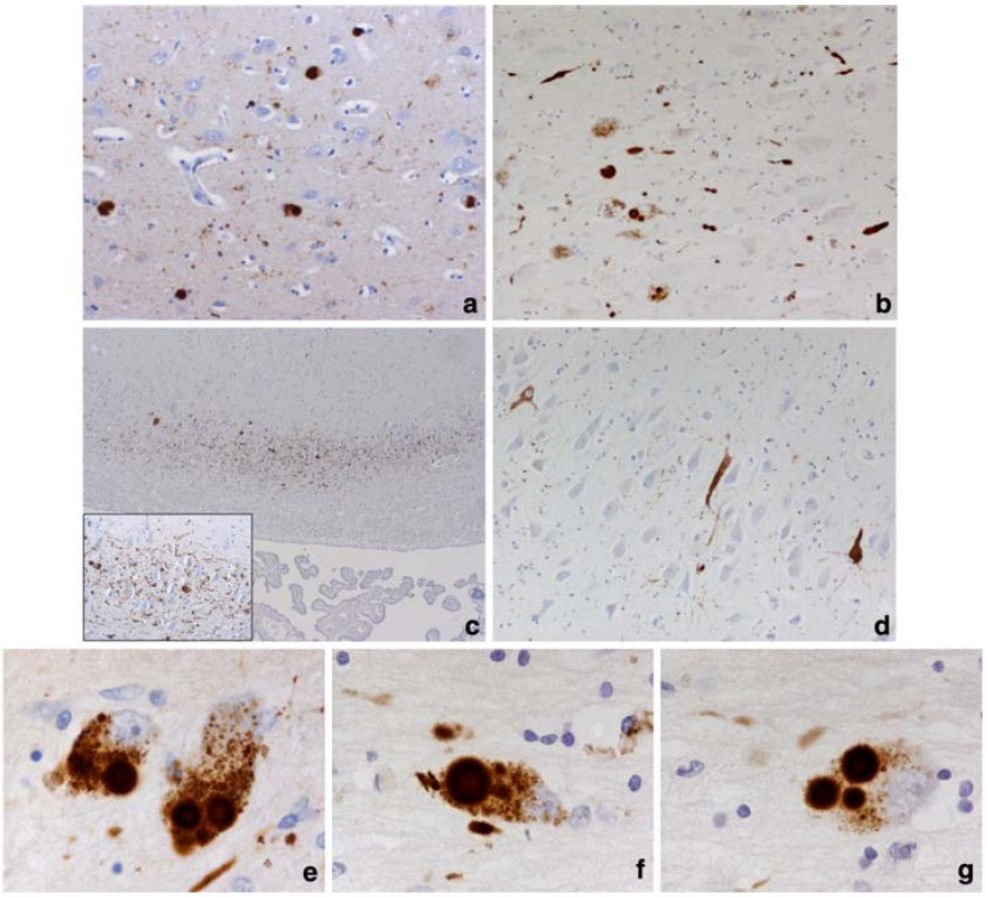


Figure from Kalaitzakis ME (Acta Neuropathol 2009). Pathology in cortical and subcortical regions.
a Cortical LB pathology in the cingulate gyrus of a demented PD case. b LB and LN pathology in the NBM of a demented PD case. c Selective vulnerability of the CA2 sector of the hippocampus to LN-type pathology. Inset higher magnification showing the web of LNs. d Neurofibrillary pathology in the CA2 sector of the hippocampus of a demented PD case. e Multiple ‘classical’ LB in a pigmented neuron of the medial substantia nigra. A single LB in a pigmented neuron is also evident. A single (f) and multiple (g) LB in a neuron of the NBM magnification: a and b 920, c 94 and for inset 910 and d 920, e, f and g 9100. PD Parkinson’s disease, LB Lewy bodies, LN Lewy neurites, NBM nucleus basalis of Meynert, CA Cornu Ammoni.

AD Pathology

Although several studies indicate cortical AD pathology as an important determinant for the development of dementia in PD, the significance of cortical plaques in AD and PD is

questionable (Kalaitzakis ME, Acta Neuropathol 2009), and there is no consistent correlation between cortical Ab burden and various measures of clinical deficit in patient with Parkinson Dementia.

A recent study (DJ Irwin, Ann Neurol 2012) based on analysis of a large cohort of PD patients from shows that the most robust correlate of dementia in PD is the severity of CLBs/LNs (in association with APOE4 genotype). However, in the same study, the authors observed that subanalysis of the PDD group designed to examine variables predictive of a comorbid AD diagnosis and demographics of PDD patients suggests that plaque and tangle pathology may influence cognitive status and the course of disease progression in a subset of PDD patients.

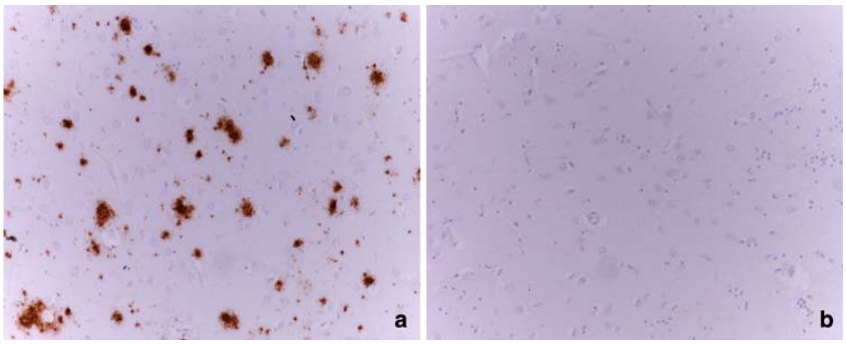


Figure from Kalaitzakis ME (Acta Neuropathol 2009) Immunostaining for b-amyloid deposits in the caudate nucleus. a A large number of Ab deposits in the caudate nucleus of a PD case with dementia. b Caudate nucleus without Ab deposits from a non-demented PD case. Magnification 920. PD Parkinson's disease, A*b* amyloid b peptide.

White Matter Lesions

White matter lesion are classically associated with motor disability and cognitive symptoms in non-demented and non-PD elderly individuals, therefore, comorbid vasculopathy is expected to contribute to clinical symptoms in PD, included cognitive deficits. An important review by Bohnen NI (Nat Rev Neurol 2011) highlights this concept.

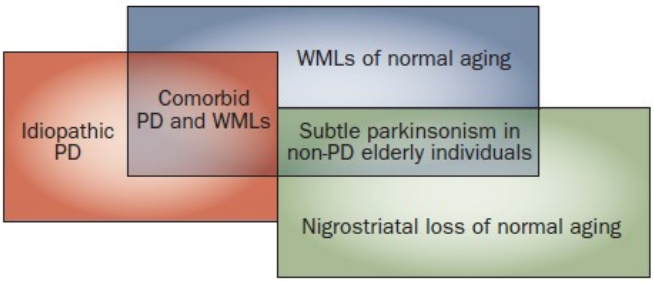


Figure from Bohnen NI, Nat Rev Neurol, 2011.

However, the impact of vascular pathology on cognitive dysfunctions in Parkinson's disease has not been jet commonly accepted, a recent study by Gonzalez-Redondo R. (Eu J Neurol 2012) analyzed WMHs in cerebral magnetic resonance images (MRI) from a large cohort of patients with PD using a cross-sectional and longitudinal design, to assess the possible relationship between silent vascular lesions and dementia or MCI in this

population. The author found that white matter hyperintensities do not influence the cognitive status of patients with PD. A specific pattern of Frontal WMHs have a negative impact on a single cognitive domain, specifically semantic fluency, but no effect of global cognitive functioning. Nevertheless , the mean follow-up period of the study was 30 months, given that vascular burden may have an effect on cognitive impairment in patients with PD as WMHs increase overtime there is need further studies with larger series of patient with relevant follow-up period.

At the state of the art, the elective brain changes yielding to dementia in PD have not been yet adequately characterized to permit a “consensus definition” from a neuropathological point of view. Although some studies indicate LB-type pathology in cortical and limbic structures as the main histological substrate of PDD, it is now evident that the mere spreading of alpha-Synuclein pathology cannot reliably account for the presence of dementia and the co occurrence of concomitant AD-type changes and/or vascular pathology underlie the complex phenomenology of the Dementia in PD.

Genetics

Carriers of mutations in the alpha-synuclein gene (SNCA) tend to develop dementia at an early stage of the disease, although these are rare conditions (Multiplication of the SNCA locus and the three known missense mutations: A30P, A53T, and E46K). SNCA dosage has been found to influence disease progression directly including development of dementia [56,57]. Functional studies on brain tissue revealed that SNCA genomic copy number and gene expression are related, as increasing number of SNCA copies lead to increase in alphasynuclein expression as well as Lewy body formation.

The apolipoprotien (APOE) ε4 allele is associated with a higher risk and earlier disease onset of Alzheimer's disease. Several studies have explored the role of APOE in PD, with inconsistent results (Aarsland D., 2010).

Recent findings from Tsuang D. (JAMA Neurol, 2013) indicate that The *APOE* ε4 allele is a strong risk factor across the LBD spectrum, specifically in Dementia with Lewy Body (DLB) and Parkinson Dementia (PDD). Interestingly, the high ε4 frequency in the PD dementia patients groups, id not correlate with overall brain neuritic plaque burden, indicating a contribute of apoE on neurodegeneration through mechanisms unrelated to amyloid

processing. At this regard, an association of the ε4 allele with the severity of Lewy body pathology has been reported.

In a recent prospective study based on an incidence cohort, an association between the H1 haplotype and increased risk for dementia was reported (Williams-Gray, 2009).

An association between COMT genotype and performance on a measure of attention has been recently reported (Morley JF, Mov Dis 2012), as has been described by others (Williams-Gray, 2009), and thought to reflect modulation of a frontostriatal network. On the other hand, the same authors found that H1 MAPT haplotype is related to the progression of deficits with respect to measures of the domains of memory and attention, over the entire follow-up period, but not with the overall rate of cognitive decline and the progression to the condition of Parkinson Dementia.

Biomarkers of Parkinson Dementia

Biological markers

While the failure of ancient studies (Molina 1997, Jensen 1998) on CSF biomarkers, subsequent publication have given rise to a wide research . In 2006 Mollenhauer reported

a specific biomarker profile on patients with PD, especially those patients with cognitive deficits and worse motor performances, present CSF τ higher and CSF Aβ lower. Alves G (2010) demonstrated that CSF Aβ levels are altered in a subset of patients with early PD and relate to memory impairment. This results study suggests that alterations in Aβ protein metabolism may contribute to the heterogeneity in pattern and course of cognitive decline associated with PD. T-tau increases in CSF in AD and other degenerative and destructive diseases of brain and is widely thought to signify damage to neurons. The same group (Montine TJ, 2011) found that CSF T-tau levels trended to lower values in the PD-D group, these were not significant and thereby concordant with the results of some14 but not others who observed an increase in average CSF T-tau in patients with PD-D.13 In contrast, we observed that average P181-tau concentrations in PD and PD-CIND groups were significantly 20% lower than age-matched controls, and this result differs from others who have reported no difference or increased average CSF P181-tau in these g groups. CSF P181-tau is more difficult to interpret than T-tau as its levels presumably reflect at least two potentially related mechanisms, cellular processes that lead to phosphorylation and release from damaged neurons. The reasons for these discrepant results among

studies are not clear. However, one interpretation of our results is that patients with PD, PD-CIND, and PD-D may have less neuron damage than patients with aMCI.

Neuroimaging

STRUCTURAL MAGNETIC RESONANCE IMAGING: Structural magnetic resonance imaging (MRI) is a validated biomarker for Alzheimer's disease (AD); it is capable of correlating the topographic pattern of cerebral atrophy with clinical progression identifying changes associated with MCI, and stratifying those patients with MCI at highest risk for AD. Recent research demonstrates that the topographic patterns of neurodegeneration underlying cognitive impairment in PD are also detectable with MRI. On the other hand, the heterogeneity and non linear progression of cognitive deficits in PD, as opposed to the progression of Mild Cognitive Impairment (MCI) to Alzheimer disease, is a significant confounding factor. A wide variations in cognitive profiles, disease duration, and age (that reflect the complex phenomenology of cognitive impairment in PD) give inconsistent and difficulty comparable results.

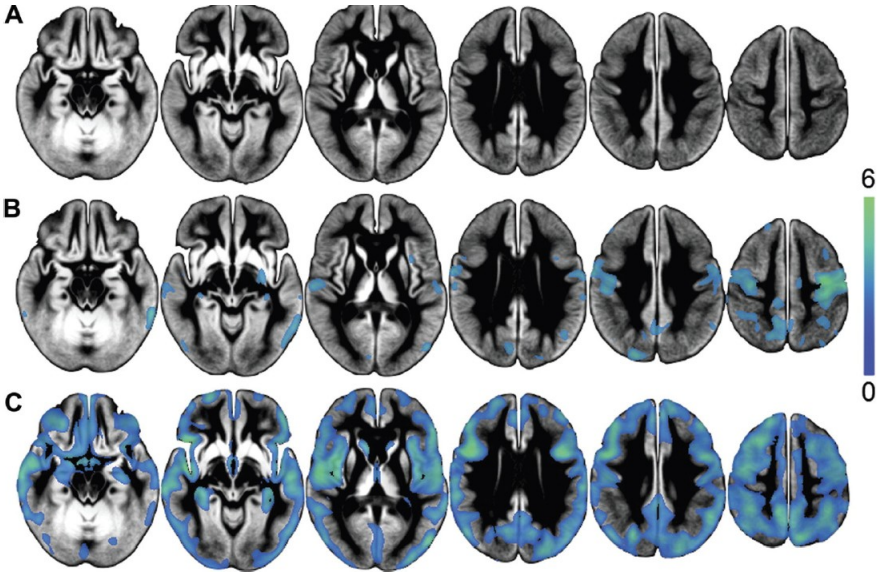
Cerebral Atrophy Associated with PD In established PDD, cortical atrophy may present a widespread pattern similar, although generally less severe, to that observed in DLB

(Burton, 2004; Beyer 2007). Burton et al. (2004), using Voxel Based Morphometry (VBM, automated and unbiased MRI technique), observed a relative gray matter (GM) atrophy in right superior, middle, and inferior frontal lobe in PD patients compared with control group; while a GM loss was reported in temporal hippocampus and parahippocampal gyrus, in occipital lobes; frontal lobes and left parietal lobe, atrophy of right caudate tail and of putamen and thalamus bilaterally in PDD (vs control group). These data have been substantially replicated in other subsequent PDD studies using VBM (Duncan 2013).

Although not a universal finding and less severe than in AD, using various MRI methodological approach (visual rating scales, ROI analyses and VBM) hippocampal atrophy is frequently reported in PDD (for rev. Duncan 2013).

In a recent study, Melzer et al. (2012), using VBM, showed that PD-MCI present a limited GM atrophy in the temporal, parietal e frontal cortex as well as the bilateral caudal hippocampus, amygdala and right putamen; interestingly specific cognitive impairments have been observed in relation to the relevant neuroanatomic structure in this study. Anyway, these changes are far less extensive and evident than that observed in PDD.

Author concluded that some gray matter atrophy may precedes the development of dementia but may be accelerated once frank dementia begins.



Regions that display areas of significant grey matter atrophy in patients who had (A) Parkinson's disease (PD) with normal cognition (PD-N), (B) PD with mild cognitive impairment (PD-MCI), and (C) PD with dementia (PD-D), relative to controls, are displayed on conventional, study-specific average grey matter neurological images (the left in each image is the left brain). (Melzer TR. J Neurol Neurosurg Psychiatry 2012) .

In contrast with these findings, other authors failed to detect significant correlations between cognitive domain test performance and GM loss in PD-MCI (Dalaker TO 2010).

Substantia innominata, another structure potentially involved in cognitive decline of PD, has been investigated using MRI protocols. A volume reduction of substantia innominata has been reported across all stage of PD, and degree of atrophy has been correlated with cognitive performance (Choi SH 2012).

White Matter Imaging White Matter Hyperintensities (WMH), also referred to as leukoaraiosis, are commonly observed on brain imaging studies in older adults. WMH have been linked with general cognitive decline, in particular with impairment of attention, executive functions and processing speed; these cognitive dysfunction are frequently reported among PD patients. Studies of WMH severity and association with cognitive decline or motor impairment in PD yielded inconsistent results (Lee, 2010; Dalaker TO,2009; Shin J, 2012; Bohnen NI,2011; Herman T,2013). Different WM analysis methods, study population and concomitant vascular burden are some confounding factors, that contribute to these conflicting results.

Diffusion Tensor Imaging Diffusion Tensor Imaging (DTI) represents a post-processing evaluation of MRI imaging, used to evaluate white matter tract integrity. In a recent paper, Hattori T (2012) identified decreased Fractional Anisotropy (FA, directionality of water molecules movement and then index of structure integrity) in many major tracts in PD-MCI and PDD patients, but not in PD without cognitive impairment, compared with control subject (the superior longitudinal fasciculus, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus, uncinate fasciculus, cingulum, and corpus callosum). Interestingly, FA values in bilateral parietal white matter were significantly correlated with MMSE scores in

PD patients. These data are partially consistent with a previous study of Matsui (2007), that observed a reduction of FA in posterior cingulated bundles, frontal, temporal, and occipital white matter in PDD patients compared with PD control group.

PET-STUDIES Several PET studies have suggest that striatal dopaminergic depletion presents a positive correlation with cognitive dysfunction. Jokinen P (2009) observed an impairment of cognitive performance (assessing visual memory and verbal memory) in PD patients with a more pronounced reduction of [^{18}F]-Fluorodopa uptake in the caudate nucleus. In another study PET study, Cropley VL (2008) illustrated that striatal dopamine denervation contributes to frontostriatal cognitive impairment in PD. Right caudate, ventral striatum and the anterior cingulate cortex are other structures potentially implicated in cognitive impairment in PD, as emerged in a study of Ito (2002) who compared PD

patients with and without dementia. PET studies of brain metabolism with FDG have shown decreased resting metabolic activity in various cortical regions: PDD showed a an impairment of glucose metabolism in frontal, in parietal and in occipital cortices and in posterior and anterior cingulate cortex (Jokinen P,2010). PET studies have also provided evidence that cognitive decline in PD is associated with significant degeneration of the

cholinergic system: cortical cholinergic denervation (assessed using [^{11}C]-PMP-AChE PET) were associated with decreased performance on test of executive functioning but not motor symptoms (Bohnen NI, 2006). In another study, PD patients with dementia had significantly lower parietal [11C]-MP4A uptake (used to assess cortical AChE activity) than PD patients without cognitive impairment (Hilker R, 2005). In a multitracer studies, demonstrating the interdependence of imaging biomarkers, Klein et al verified that cholinergic degeneration in PDD patients was spatially congruent with the metabolic changes measured with [18F]FDG.

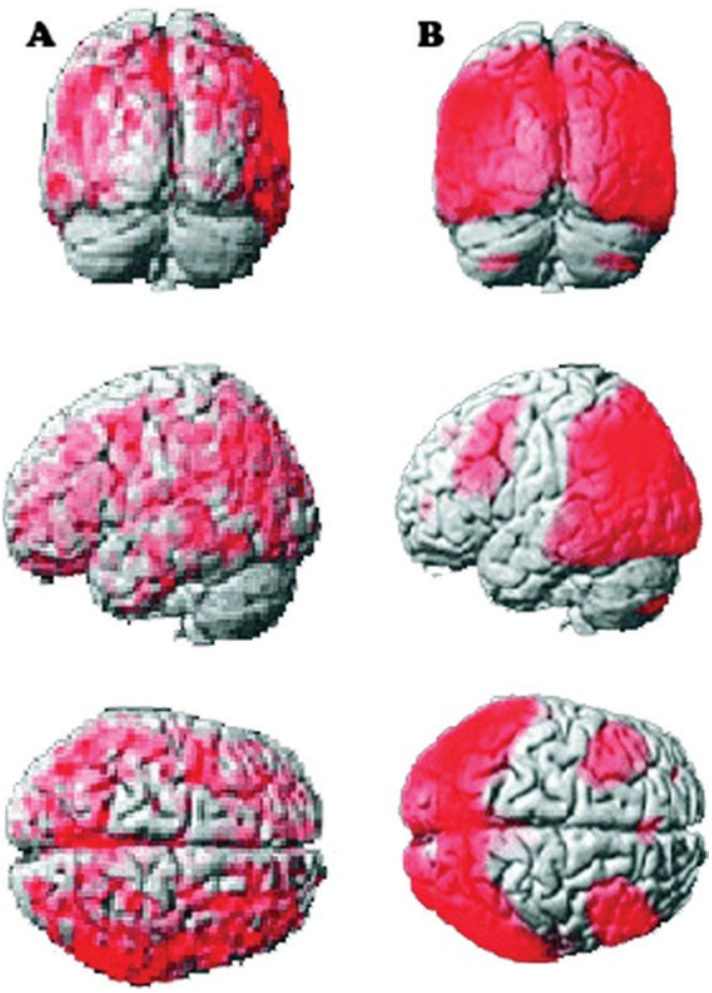


Image adapted from Klein et al. (2010).44 (A) Brain areas with significant reduction of [11C]MP4A uptake in PDD versus PD. (B) Brain areas with significant reduction of [18F]FDG uptake PDD versus controls. CMRglc, cerebral metabolic rate of glucose.(Ray and Stafella, Mov Dis, 2012).

The b-amyloid imaging, using [11C]-PIB, has been used to assess the amyloid load associated in Lewy-bodies disease spectrum. The Authors (Edison P,2008) observed that, whereas the majority of DLB patients showed an increased agent binding, only a small number of PDD patients (and none of the PD patients investigated) had PET images compatible with high brain amyloid load.

Progression of cognitive deficit in PD

The existence of a cognitive continuum that goes to from normal aging to frank dementia (Alzheimer Disease) and passing for a phase of mild impairment was postulated in 1995 by Petersen. The *"transposition"* of the MCI notion to the dyscognition area of PD was made in the last years (Caviness JN, 2007), with aprioristic assumptions and applying the same methodological criteria used for Alzheimer Disease. A *'critical review'* of the concept of Mild Cognitive Impairment in PD has been published on 2011 by the Movement Disorders Society (MDS). This condition emerges as a frequent (27% prevalent) and heterogeneous in terms of number and type of cognitive domains involved, and is considered to be a risk factor for the development of dementia. However, this criteria will require validation, future refinement and additional research.

TABLE 1. Criteria for the Diagnosis of PD-MCI

I. Inclusion criteria

- Diagnosis of Parkinson's disease as based on the UK PD Brain Bank Criteria[20]
- Gradual decline, in the context of established PD, in cognitive ability reported by either the patient or informant, *or* observed by the clinician
- Cognitive deficits on either formal neuropsychological testing or a scale of global cognitive abilities (detailed in section III)
- Cognitive deficits are not sufficient to interfere significantly with functional independence, although subtle difficulties on complex functional tasks may be present

II. Exclusion criteria

- Diagnosis of PD dementia based on MDS Task Force proposed criteria[18]
- Other primary explanations for cognitive impairment (e.g., delirium, stroke, major depression, metabolic abnormalities, adverse effects of medication, or head trauma)
- Other PD-associated comorbid conditions (e.g., motor impairment or severe anxiety, depression, excessive daytime sleepiness, or psychosis) that, in the opinion of the clinician, significantly influence cognitive testing

III. Specific guidelines for PD-MCI level I and level II categories

A. Level I (abbreviated assessment)

- Impairment on a scale of global cognitive abilities validated for use in PD[a] *or*
- Impairment on at least two tests, when a limited battery of neuropsychological tests is performed (i.e., the battery includes less than two tests within each of the five cognitive domains, or less than five cognitive domains are assessed)

B. Level II (comprehensive assessment)

- Neuropsychological testing that includes two tests within each of the five cognitive domains (i.e., attention and working memory, executive, language, memory, and visuospatial)[b]
- Impairment on at least two neuropsychological tests, represented by either two impaired tests in one cognitive domain or one impaired test in two different cognitive domains
- Impairment on neuropsychological tests may be demonstrated by:
  - o Performance approximately 1 to 2 SDs below appropriate norms *or*
  - o Significant decline demonstrated on serial cognitive testing *or*
  - o Significant decline from estimated premorbid levels

IV. Subtype classification for PD-MCI (optional, requires two tests for each of the five cognitive domains assessed and is strongly suggested for research purposes)[c]

- PD-MCI single-domain—abnormalities on two tests within a single cognitive domain (specify the domain), with other domains unimpaired *or*
- PD-MCI multiple-domain—abnormalities on at least one test in two or more cognitive domains (specify the domains)

Diagnostic Criteria for PD-MCI, from Litvan I (Mov Dis, 2012)

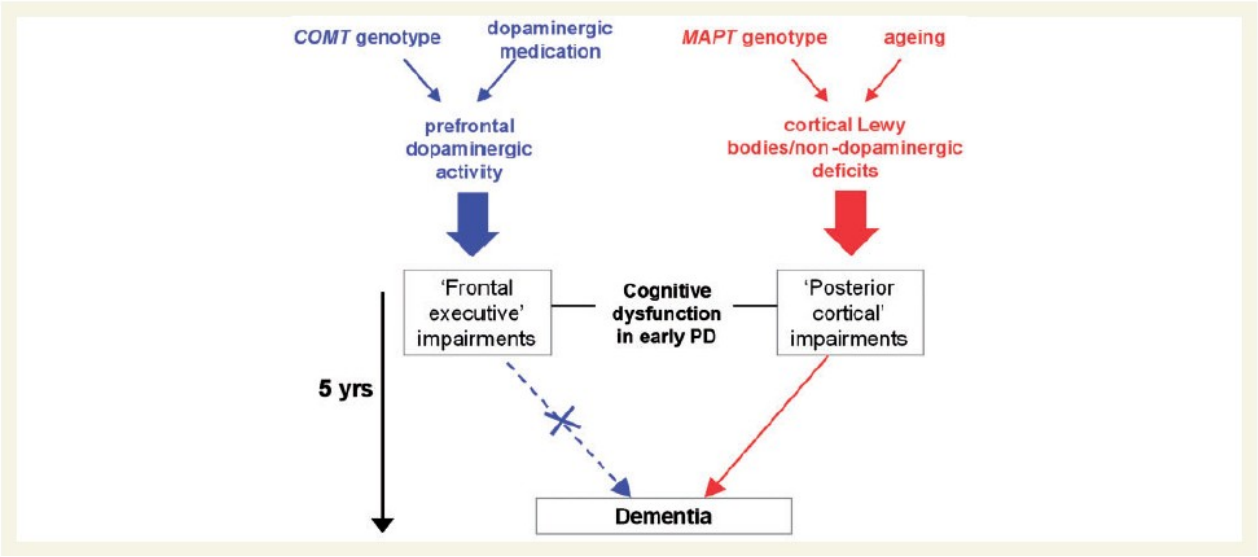
The construct of Mild Cognitive Impairment in Parkinson has received increased attention from clinicians and the term is now widely accepted, however, prospective longitudinal studies using formal MCI criteria are few and often inconsistent.

A recent study (Pedersen KN, Ann Neurol 2013, The Norwegian ParkWest Study), a prospective longitudinal cohort study designed to examine the course of MCI and its progression to dementia in an incident PD cohort, found that Mild cognitive impairment at PD diagnosis predicts a highly increased risk for early dementia. The authors also found that impairment in verbal memory and attention was associated with conversion from MCI to PDD. This is in line with previous studies that have shown frontal/executive and verbal memory deficits to be associated with the development of PDD although others have found that visuospatial based dysfunction were more prone to predict dementia in PD. These inconsistencies deserve further investigations but may reflect different definitions of cognitive impairment and PDD, differences in sample characteristics, or biological heterogeneity.

Research from the group of Williams-Gray C., conducted on an incident cohort of PD patient with a 10 years follow-up, indicate that cortical-posterior based deficit are predictive

to the development of Dementia in PD patients rather than deficit in executive dysfunction driven by fronto-striatal dopamine related circuitry.

The authors demonstrated that early deficits on frontostriatally based tasks are not related to subsequent dementia risk. The domain of “verbal fluency” is paradigmatic of the results and the subsequent discussion worked out by the authors. The dissociation between *semantic verbal fluency* (frontally driven) and *phonemic verbal fluency* (a posterior temporal lobe task) in terms of predicting dementia indicate that it is the semantic, temporal lobe component of the fluency task which is predictive of cognitive decline rather than the frontally based strategic retrieval common to both fluency tasks. Williams-Gray et al (Brain 2009).



Clinical Characterization of PD Dementia in our "Parkinson Centre".

The emerging importance of Parkinson Dementia, the lack of specific clinical diagnostic criteria as well necessity of a reliable work-up for diagnosis, follow-up and treatment led to start a comprehensive job of defining the epidemiological characteristics, clinical and neurobiological features of this condition, involving hundreds referred to our tertiary PD Centre.

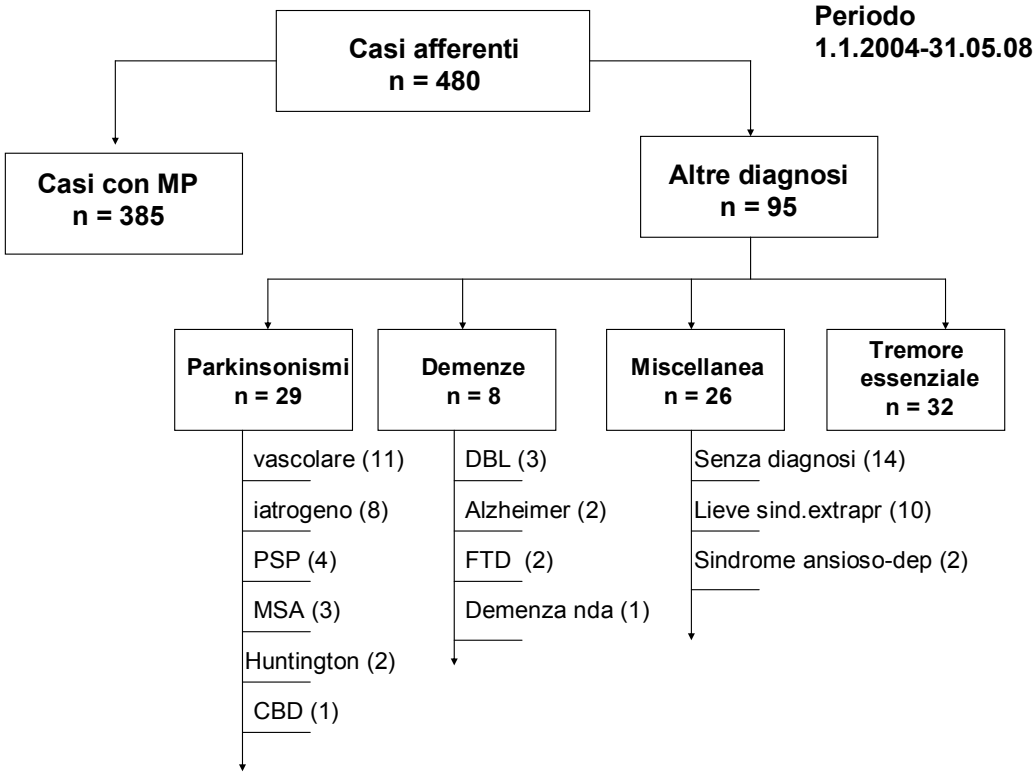
First, the epidemiology and the characteristics associated to progression to Dementia were assessed in our population in a recent retrospective study. Patient were diagnosed in accordance with the criteria defined by the United Kingdom Parkinson's Disease Society (UKPDS) Brain Bank (Gibb e Lees, 1988). The diagnosis of dementia was made from clinical notes reported or when a Cholinesterase-inhibitor was prescribed. The incidence of Dementia was calculated from the entire population of patient referring to our Parkinson Outpatients Ambulatory, patients were included in the analysis of Dementia incidence if they underwent at least two visits or three months of clinical observation.

Beside the epidemiological tool of prevalence, that indicate the proportion of subject suffering of a given condition, it is possible to assess the incidence of pathologic condition, which gives a dynamic assessment of the phenomenon inside the general population. In particular, density of incidence allows the calculation the frequency of the phenomenon inside the cohort of interest that are free from the disease at the baseline.

We calculated the incidence index for 1000 years person, a CI of 95% was fixed according to the following formula:

$$\pi \pm 1{,}96 \sqrt{\pi (1-\pi)/n}$$

We analyzed 480 clinical notes of the study period (January 2004-May 2008), patients were classified as:



Clinical characteristic of patients with PD [M= 200, (53,5%) ; F = 174, (46,5%)]

Variables	N	Mean	Standard deviation	Min	Max
Age at onset	353	61,80	10,16	29	84
Age at diagnosis	153	62,45	9,68	34	88
Age at first visit	374	65,90	9,61	33	90
UPDRS at first visit	237	16,95	9,78	2	50
Age at last visit	373	70,94	9,14	44	92
UPDRS at last visit	199	19,58	11,39	1	55
Levodopa equivalent dose	344	803,60	444,13	100	2370
Year-person	374	5,07	4,01	0	21

Estimated incidence. For our incidence value we considered 374 patients with a total amount of follow up of 1896 year-person (mean period of follow-up 5,07 ± 4,01). In the study period 35 new cases of Dementia occurred with an estimated incidence of 18.4 cases for 1000 year-person (IC95% 12,4-24,4) and 17 cases of cognitive impairment, with an estimated incidence of 9 cases for 1000 year-person (IC95% 4,8-13,2).

In order to ascertain possible predictive features for Dementia we compared clinical characteristic, at first and last evaluation, of our two groups of patients: patients with dementia (n=35) and patients without dementia (n=339).

Clinical features associated with Dementia. *Were compared to the clinical characteristics of the first and the last visit of the two groups of patients with (n = 35) and without dementia (n = 339) in order to envy possible predictive variables of the phenomenon under study and to estimate the progression of the disease during the period of observation of the patients in the study. The following table describes the demographic and clinical characteristics of patients with Parkinson's disease with and without dementia. The analysis of data documenting an age of onset of PD on average four years more advanced for patients who later developed dementia. In addition, it is noted that the male patients with Parkinson's disease have a probability of about 3 times higher than those of female to meet to dementia within five years. Patients who develop dementia have the same profile of schooling than those who did not develop.*

Variables	**PD-D (n=35)**	**PD-ND**	**OR**	**IC95%**	**P**
Age at onse	65,5±8,7	61,4±10,2			0.023
Age at diagnosis	70,4±5,2	61,8±9,7			0,006
Gender (M/F)	26/9	165/159	2,78	1,26-6,13	0,009
Formal education					
none	0	2 (1,1%)			n.s.
5 yrs	7(41,2%)	71 (40,8%)			
8 yrs	4(23,5%)	43(24,7%)			
13 yrs	5 (29,4%)	43 (24,7%)			
College degree	1 (5,9%)	15 (8,6%)			

This other table shows the clinical characteristics performed at the first visit at the outpatient clinic of the Department of Neurology. The analysis documents an older age of about 5 and a half years, statistically significant at the first visit for patients who develop dementia.

Variables	**PD-D (n=35)**	**PD-ND (n=339)**	**OR**	**IC95%**	**P**
Age at I visit	70,8±8,1	65,4±9,6			0,001
Familiarity for dementia	1/30	10/276	0,92	0,11-7,44	n.s.
Vascular risk factors (Y/N)	10/12	107/95	0,74	0,31-1,79	n.s.
Clinica subtype at disease onset					
Tremo	13 (46,4%)	157 (52,0%)			n.s.
Akinetic-rigid	11 (39,3%)	97 (32,1%)			
Mixed	4(14,3%)	48 (15,9%)			
Gait disordets (Y/N)	5/25	28/276	1,97	0,70-5,55	n.s.
Postural instability (Y/N)	5/24	30/273	1,90	0,67-5,33	n.s.
Hallucinations (S/N)	3/26	4/289	8,34	1,77-39,3	0,002
Hoehn-Yahr Stage					
I	7 (28,0%)	129 (44,9%)			n.s.
II	15 (60,0%)	135 (47,0%)			
III	3 (12,0%)	22 (7,7%)			
IV	0	1 (0,3%)			
UPDRS at I visit	22,11±10,36	16,57±9,62			0,021

The following summarizes the clinical features at the last visit in the clinic of the two groups of patients. Statistical analysis shows a significant difference in the frequency of disturbance of gait and postural instability (about two times greater for patients suffering from dementia), a clinical stage recorded at Hoehn-Yahr more advanced for people with dementia, a higher prevalence of hallucination (about 12 times more) and a greater motor deficit as measured in the UPDRS. Finally, the average amount of levodopa-equivalent expressed in mg is statistically reduced in patients with dementia compared to those without dementia.

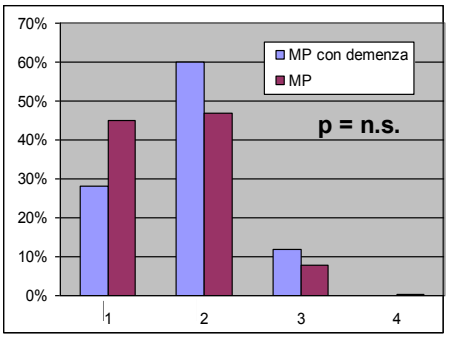
Variables	**PD-D (n=35)**	**PD-ND (n=339)**	**OR**	**IC95%**	**P**
Age at last visit	76,7±6,20	70,42±9,15			0,001
Gait disorders (Y/N)	12/16	65/228	2,63	1,18-5,84	0,014
Postural instability (Y/N)	12/15	65/224	2,76	1,23-6,18	0,011
Hallucination (Y/N)	16/10	34/258	12,14	5,1-28,9	0,001
Hoehn-Yahr Stage					
I	1 (5,9%)	51 (24,9%)			0.001
II	2(11,8%)	111 (54,1%)			
III	11 (64,7%)	27 (13,2%)			
IV	2 (11,8%)	15(7,3%)			
V	1(5,9%)	1(0,5%)			
UPDRS at last visit	31,83±13,60	18,81±10,59			0,001
Levodopa equivalent dose (last visit)	625,81±300,96	825,85±453,7			0,017

The histogram below shows the trend of Parkinson's disease observed in its motor component for the group of patients with dementia than those who did not report such occurrence. It is known that patients with dementia did not differ in the definition of the clinical stage of Hoehn-Yahr than other patients at the first visit, while the last visit reveals

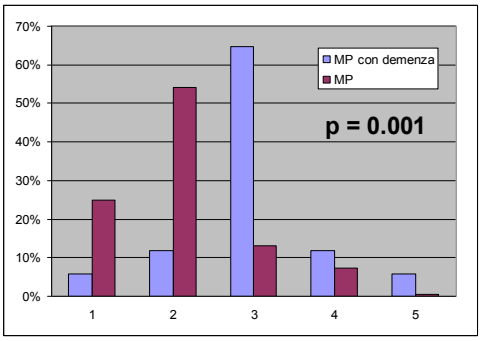
a statically significant difference. For an assessment of the mean differences observed in the two patient groups in scores on the UPDRS is noted that in the period of the study there was a decrease of about 44% for the group with dementia while the other group is evidence of worsening of motor 13.5%.

ANDAMENTO DI MALATTIA NEL CORSO DI CINQUE ANNI

PRIMA VISITA (STADIO HY)



ULTIMA VISITA (STADIO HY)



UPDRS

MP con demenza	MP
22,11±10,36	16,57±9,62

MP con demenza	MP
31,83±13,60	18,81±10,59

44% di aumento medio del punteggio UPDRS (MP con dem)
13,5% di aumento medio del punteggio UPDRS (MP)

The bivariate analyzes shown previously documented the identification of four predictor variables in the onset of dementia, such as age of onset age, the greater motor impairment as measured by the UPDRS, male sex, and the presence of hallucinations at the first visit in the clinic . The logistic regression model showed the greatest role of hallucinations and more advanced age of onset of parkinsonian symptoms in the onset of dementia than the other two variables identified in the bivariate analysis (male and a motor deficit with a higher score 15).

A logistic regression analysis for the identification of identification of the predictor variables for teh occurrence of Dementia.

Variables		B	E.S.	Sig.	OR	IC95,0%	
						Inferior	Superior
	Age at onset	1,336	0,601	0,026	3,804	1,171	12,358
	UPDRS	0,736	0,514	0,152	2,088	0,762	5,721
	Hallucinations	2,411	0,850	0,005	11,150	2,106	59,039
	Gender	-0,379	0,503	0,451	0,684	0,255	1,834

Dipendet variable: dementia

Indipendent variables:

- Age at onset
- UPDRS score
- Allucinations at first visit (Y/N)
- Gender (M/F)

Experimental procedures. Clinical Setting-Part 1.

Validation study of clinical criteria for PDD diagnosis

Introduction

The nosological classification of dementia in PD patients has been based on the DSM IV criteria (American Psychiatric Association 2000). These criteria define Parkinson's disease dementia as a clinical condition associated with memory loss and at least one of the following disorders: apraxia, agnosia, aphasia or executive dysfunctions; the cognitive deficits must cause a significant impairment in global functioning. The DSM criteria, which are only descriptive, have certain limitations: first, they are not specific to PD-D; second, memory or cortically based disturbances may not be prominent in PD even in the very late stages; lastly, it may be difficult to determine the specific effects of motor and cognitive impairment on the patients overall functioning. A PD-D Task Force was created to draw up specific diagnostic criteria for dementia in PD and to design tools that can be used to assess the cognitive state in parkinsonian patients. This group of specialists, named the Movement Disorders Society Task Force (MDSTF), proposed specific ''clinical criteria'' for the diagnosis of dementia in PD in a paper published in 2007 (Emre et al. 2007), in which

the authors described the core clinical features of dementia in PD and the behavioral features that closely correlate with PD-D and help to make a diagnosis. These criteria were not, however, validated. In a subsequent publication, the same authors provided a framework of the neuropsychological tests that could be used to assess cognitive deficits and to make a diagnosis, providing the ‘‘diagnostic criteria’’ (Dubois et al. 2007). Two levels of analysis were proposed for the diagnostic procedures: step I and the step II. Step I is based on simple tests that analyze various cognitive domains, such as: attention, executive functions, visuo-constructive abilities and memory, and rules out major depression and other possible cause of cognitive impairment (Vascular dementia, vitamin deficits, hypothyroidism). Once step I has been accomplished, the neurologist should be able to establish whether the patient has ‘‘possible’’ or ‘‘probable’’ Parkinson’s disease with dementia (Dubois et al. 2007). Step II is based on extensive neuropsychological tests designed to thoroughly explore different cognitive domains, thereby allowing the pattern and severity of the dementia in PD patients to be accurately defined. The MDSTF recommended that the second level of tests be used for pharmacological trials and research, though the authors of one paper suggested that step II should be applied even in cases in which the step I results are borderline or somewhat uncertain (Goetz et al. 2008).

Step I of the MDSTF only lasts a few minutes and has been proposed to clinicians as a tool for the diagnosis of PD-D that obviates the need for a formal neuropsychological assessment. The aim of our study was to validate step I proposed by the MDSTF and assess its accuracy as a screening instrument for Parkinson's disease dementia. For this purpose, we decided to measure the diagnostic sensitivity and specificity of step I when compared with the diagnosis made on the basis of an extensive neuropsychological examination. We rigorously applied the DSM IV criteria for Parkinson's disease dementia which are, despite certain limitations, validated and represent the current gold standard for the diagnosis of this pathology.

Methods

Patients and recruitment. Patients were randomly recruited from June 2009 to April 2010 from the outpatient service of the Parkinson's disease and Extrapyramidal disorders Unit at the Department of Neurology and Psychiatry of the ''Sapienza'' University of Rome. The study population consisted of 76 patients. Table 1 summarizes the demographic and clinical characteristic of the study population. Informed consent was obtained from all the patients or their relatives. The local ethics committee approved the study.

Inclusion and exclusion criteria Inclusion and exclusion criteria. The inclusion criteria were: idiopathic Parkinson's disease according to the United Kingdom Parkinson's Disease Society Brain Bank criteria. No other inclusion criteria, such as age, disease duration or motor subtypes, were applied in order to minimize a possible selection bias.

	Total ($n = 76$)	No-Dem-PD ($n = 67$)	PD-D ($n = 9$)	P value
Age (M ± SD)	69.3 ± 8.5	68.2 ± 8.3	77 ± 5.4	0.003
Gender M/F	44/32	38/29	6/3	n.s.
Disease duration (M ± SD)	7.1 ± 3.7	7.1 ± 3.7	7.4 ± 3.7	n.s.
Yrs of education (M ± SD)	8.8 ± 4.8	9.1 ± 4.9	6.8 ± 4.4	n.s.
HY				0.001
I	13	13	0	
II	52	48	4	
III	10	6	4	
IV	1	0	1	
UPDRS (M ± SD)	17 ± 6.8	16 ± 6.3	24.8 ± 6.1	0.0001
Frequency of antidepressant use	17/76	13/54	4/5	n.s.

Table 1 Demographic and clinical characteristics of the study population

The exclusion criteria were:

• Definite vascular dementia according to the NINDS AIREN criteria;

• Major depression according to the DSM IV-TR criteria.

Patients taking antidepressants for moderate depression were not excluded from the study. Fifteen of the patients enrolled were on antidepressant treatment during the study: eight patients with Duloxetine, three patients with Venlafaxine, two patients with Sertraline, one patient with Escitalopram and one patient with Paroxetine.

Study design. The patients were evaluated by a first neurologist, as recommended by the MDSTF, who administered the tests and applied the recommended cut-off values (Dubois et al. 2007). At the end of step I, the first neurologist attributed a score to each patient according to the 8-point rule. Thereafter, the patients underwent the second part of the examination, i.e. step II, which yielded a neuropsychological report. Lastly, the patients underwent a neurological examination and a second neurologist, who was blind to the scores obtained in step I, made the diagnosis of PD-D according to the current gold standard DSM IV criteria.

The patients underwent the following neuropsychological tests:

- Global efficiency: Mattis DRS
- Working memory: Digit span, Spatial span (CANTAB), Digit ordering test
- Conceptualization: Similarities (WAIS-III), Wisconsin CST

- Set activation: Verbal fluency
- Set shifting: TMT
- Memory: RAVLT
- Language: Boston naming test
- Visuo-constructive: Copy of the clock
- Visuo-spatial Benton: line orientation test
- Visuo-perceptive: Benton face recognition test

Neuropsychiatric complains were assessed with the following tests:

- Apathy: Apathy scale
- Depression: Beck depression inventory
- Psychosis: NPI

The second neurologist used the neuropsychological reports (in step II) to appraise the patient's cognitive status and make a diagnosis of PD-D. We assessed the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of step I. Moreover, we calculated the kappa index of step I versus that of step II. A kappa index of 0.70 was considered satisfactory.

Results

Applying the DSM IV criteria after a full battery of neuropsychological tests, a standard neurological examination and an interview with the caregivers, we diagnosed nine patients with PD-D (12% of our cohort). In our setting, step I had a sensitivity of 78% and a specificity of 95.5%, while its positive predictive value was 70%, and its negative predictive value was 97%; moreover, step I displayed an accuracy of 93.4% (Table 2).

	DSM IV		Total
	PD-D	No-Dem-PD	
Step I			
PD-D	7 (true positive)	3 (false positive)	10
No-Dem-PD	2 (false negative)	64 (true negative)	66
Total	9	67	76

Table 2 A contingency table showing the diagnosis of PD-D according to the DSM IV following a neuropsychological examination (column 1) and the diagnosis of PD-D by means of step I (line 1)

The kappa index stood at 70%. Two patients with PD-D according to the DSM IV criteria were not demented according to the step I diagnostic scheme (Table 2). In one false negative case (MMSE = 26, normal at serial-7 and 3-word recall), step I did not capture

substantial memory and attention deficits that did not escape detection by the psychometric tools; moreover, this patient had but slight, though detectable, cortically based deficits such apraxia and anomia (below cut-off in the Boston naming test). A diagnosis of dementia was, however, made in this patient in view of his high educational level and his performances in the premorbid condition. The other false negative case also yielded normal results in tests regarding attention and memory, while his MMSE value was just above the cut-off. Worthy of note is the fact that both false negative patients displayed abnormalities in the copy of pentagons and design of the clock tests. Ten patients were diagnosed as having PD-D according to the step I procedure. Three of these ten patients did not fulfil the criteria of PD-D for the DSM IV (Table 2).

Memory deficits were not detected in the neuropsychological tests of the three false positive cases; also they were not a subjective impression or reported by the caregiver; these three patients proved to have a considerable dysexecutive syndrome though no cortical disturbances.

In accordance with the current literature, we found that PD-D patients were not only older than those without dementia (No-Dem-PD) but even had a more severe motor form of parkinsonism (Table 1). We observed a statistically significant difference in age, UPDRS

and HY. There was a difference between the two groups in the disease duration values, though this difference did not reach statistical significance. None of the patients with PD-D according to both the DSM IV criteria and step I checklist had significant neuroimaging or laboratory abnormalities.

Discussion

We assessed the screening properties of step I of the MDSTF for the diagnosis of PD-D. Our results indicate an average match between the cases diagnosed by means of step I and those diagnosed by means of a neuropsychological examination.

Dujardin and colleagues recently assessed the validity of step I for the diagnosis of PD-D in a group of 188 PD patients (Dujardin et al. 2010). The estimated clinical parameters reported in their paper were: specificity of 95%, sensitivity of 66%, PPV of 77% and NPV of 91%.

Step I is burdened, at least in our setting, by defects in specificity (1) and sensitivity (2):

1. A PPV of 70% indicates that 30% of the patients may be misdiagnosed as PD-D. We found three false positive cases in our cohort; these three patients had a long disease duration, moderate-to-severe motor symptoms and serious executive dysfunctions,

resulting in performances below the cut-off in memory, attention and global efficiency tests at the screening level, not accompanied, however, by cortical disturbances or memory deficits. The analysis conducted by Dujardin

and colleagues yielded a PPV value (77%) that is comparable to ours (70%).

2. Sensitivity was 78% in our study and 66% in the study conducted by Dujardin and colleagues. In keeping with the results of their paper, our findings raise doubts regarding the sensitivity of the MMSE cut-off, particularly as regards highly educated patients. In (2008) O'Bryant et al. also reported that the discriminative power of MMSE was lower for college graduates.

Since tests designed to assess memory and attention as well as visuo-constructive abilities all adopt the MMSE cut-off values, the use of such tests in highly educated individuals should be assessed in further studies.

The pill questionnaire appears to be a consistent indicator of the global functioning and organization of daily living in PD patients. In our cohort, PD-D had an impact on the activities of daily living in all the patients according to the pill questionnaire; however, numerous No-Dem-PD patients, including the three false positive cases, were unable to describe their therapies. Nonetheless, the DSM IV criteria specify that the decline in

activities of daily living must represent a decline from a previously higher level of functioning, as the authors themselves point out. Indeed, this highlights the need for formal validation of the pill questionnaire. Dujardin and colleagues believe that with an easy-to use, short battery of tests that are commonly used in routine clinical practice, it is possible to diagnose PDD in accordance with reference criteria and with the same sensitivity and specificity as in a more extensive evaluation (Dujardin et al. 2010). We affirm the contrary that while diagnosing dementia and cognitive impairment has become part of the clinical practice for neurologists, the use of step I as only diagnostic tool for PD-D may lead to a significant proportion of misdiagnoses as a consequence of both type I (false positive) and type II (false negative) diagnostic errors.

However, step I, which has a NPV of 97%, may be considered an otherwise valid screening test. Indeed, future fine-tuning of the cut-off values combined with more sensitive domain-related psychometric tests will maximize the overall screening properties of step I. Once step I has been optimized, it may be effectively be used as a screening test, while reserving step II for step I-positive individuals and borderline cases. Further studies on larger series of patients are warranted to assess the validity of step I and to draw up consensus criteria for the definition of PD-D and other forms of cognitive

impairment associated with PD. In the meantime, we suggest that the diagnosis of PD-D should still be based on a formal neuropsychological examination and longitudinal follow-up.

Experimental procedures. Clinical Setting-Part 2.

Follow-up analysis for cognitive decline in our cohort

Background

Cognitive impairment is one of the most common and important aspects in Parkinson Disease (PD), and greatly affects functioning and quality-of-life. The spectrum of cognitive dysfunction in PD has been lately coded in terms of mild cognitive impairment (PD-MCI) and dementia (PDD) with clinical and diagnostic criteria provided by specialists of the Movement Disorders Society (MDS). This nosological effort has been structured in order to support the identification of cognitive decline trajectories in PD population. Indeed, the term of MCI, initially conceptualized as a prodrome of Alzeheimer disease and subsequently extended to others degenerative disorders including PD, is generally codified as a transitory stage prior to the development of a full-blown dementia. However,

identify this intermediate stage of cognitive impairment presents a relative clinical relevance given that a large proportion of PD presents cognitive deficits (classified as MCI) even in earliest disease stage and the rate of cognitive decline remain largely unpredictable. Considering that cognitive disturbances are integral to the course of PD and this nosological characterization do not always demonstrate a reliable prognostic value, we evaluated the phenomenon of “cognitive conversion” at five years, focusing on clinical and neuropsychological features that characterized patients who worsen their cognitive abilities with respect to cognitively steady-staying patients, independently from baseline cognitive asset.

Methods

Patients. Patients were randomly recruited from June 2009 to November 2009 from the outpatient service of the Parkinson’s disease and Extrapyramidal disorders Unit at the Department of Neurology and Psychiatry of the “Sapienza” University of Rome; 54 patients of 67 of the initial cohort enrolled for a previous published study were revaluated about five years later. The inclusion criteria were: idiopathic Parkinson’s disease according to the United Kingdom Parkinson’s Disease Society Brain Bank criteria. No other inclusion criteria, such as age, disease duration or motor subtypes, were applied in order to

minimize a possible selection bias. The exclusion criteria were 1) PDD according to the MDS – Task force (MDS-TF) 2) Definite vascular dementia according to the NINDS-AIREN criteria 3) Major depression according to the DSM IV-TR criteria.

Clinical and neuropsychological evaluation. All patients underwent a detailed neuropsychological evaluation including all tests of level II diagnostic criteria for PD-D of the Movement Disorders Society. Motor examination included: H&Y, UPDRS part III and axial score. Clinical characteristics regarding "milestones " of PD progression were also reported, including: presence of levodopa induced dyskinesia, and occurrence of falls; presence of motor fluctuation at baseline were also recorded. Patients were classified in PD-normal cognition and PD-MCI at baseline (the diagnosis of MCI was made in accordance with MDS-TF criteria on the base of neuropsychological evaluation performed in 2009).

At follow-up patients were classified as:

a) Non-converter patients group (PD-NC): patients with normal cognition or MCI either at baseline or follow-up

b) Converter patients group (PD-C): patients normal cognition at baseline who worsened their cognitive performances developing a MCI and PD-MCI at baseline that developed a PDD at follow-up.

Statistical Analysis. We used the t-test for continuous variables and chi-square test for categorical variables. We performed a logistic regression analysis to assess the association between cognitive conversion adjusted for variables statistically relevant to univariate analysis . P values < 0.05 were considered as statistically significant. All statistical analyses were performed using SPSS software (version 20.0, SPSS inc. Chicago, IL, USA).

Results

At follow-up visit, 33 of 54 patients (61%, 19 with normal cognition and 14 with MCI) presented a stable cognitive assessment and were classified as non-converter, while 21 patients (11 normal cognition at baseline who developed a MCI, 8 MCI and 2 normal cognition at baseline who developed incident dementia meeting the criteria of probable PDD) worsened their cognitive performance and were classified as converter.

	non-converter	Converter	Statistical sign.
Patients	33	21	
Average Age	69,7 ± 8,1	75,4 ± 7,4	<0.05
Gender (m/f)	20/10	10/11	n.s.
Disease Duration	11,7 ± 4,1	12,0 ± 3,6	n.s.
Educational attainment	10,2 ± 4,5	8,2 ± 4,8	n.s.
LED (mg)	653,2 ± 331,0	706,2 ± 281,1	n.s.
H&Y >2 (y/n)	6/27	7/14	n.s.
UPDRS-pat III	15,0 ± 5,4	15,9 ± 6,2	n.s.
Axial score	6,4 ± 2,0	7,0 ± 3,2	n.s.
Motor Fluctuation (y/n)	13/20	10/11	n.s.
LID (y/n)	6/27	8/13	n.s.
Fall (y/n)	3/30	3/18	n.s.
CS: TD/NTD	16/17	7/14	n.s.
Neuropsychological evaluation			
Global efficiency	8/33	10/21	n.s.
Set activation	5/33	2/21	n.s.
Working memory	6/33	5/21	n.s.
Conceptualization	14/33	13/21	n.s.
Set Shifting	15/33	14/21	n.s.
Set maintenance	4/33	8/21	<0,05
Behavioral control	3/33	4/21	n.s.
Memory	1/33	4/21	n.s.
Language	4/33	5/21	n.s.
Visuo-constructive	4/33	6/21	n.s.
Visuo-spatial	14/33	18/21	<0,05
Visuo-perceptive	1/33	6/21	<0,05
Apathy	9/33	9/21	n.s
Depression	2/33	2/21	n.s
Visual Hallucination	2/33	5/21	n.s
Psychosis	1/33	4/21	n.s.

Table 1. univariate analysis of demographic and clinical characteristics.

Patients that converted cognitive status were older at baseline whoever, disease duration “per se” was not associated with a significant risk of cognitive conversion. No other differ significantly in demographic feature emerged. Non statistically difference were observed in motor performance at baseline, included clinical motor symptoms subtype at onset. There were no significant differences in the use of dopaminergic therapy (table 1).

At univariate analysis patients that converted cognitive status showed statistically significant deficits on measures of set maintenance, visual-spatial and visual-perceptive function; moreover a trend of significance were observed in test that explore memory function. Not other differ in neuropsychological variables emerged. Concerning neuropsychiatric complains, in converters group visual hallucinations and psychosis were more frequent without reaching, however, a statistical significance (table 1).

After logistic regression, test assessing visuospatial functions was the only variable that survived; a trend of significance was observed for the variables age and test assessing memory functions (Table 2).

	T	E.S.	Wald	gl	Sign	Exp	95% C.I. EXP(B)	
							Sup	Inf
Age	0,10	,064	2,39	1	0,12	1,10	0,97	1,25
Gender	0,67	0,74	0,81	1	0,37	1,96	0,45	8,48
Global efficiency	-1,72	1,08	2,53	1	0,11	0,18	0,02	1,50
Set maintenance	-0,99	1,32	0,57	1	0,45	0,37	0,03	4,89
Memory	3,05	2,27	1,82	1	0,18	21,25	0,25	1804,01
Visuo-spatial	2,02	0,87	5,41	1	0,02	7,58	1,37	41,73
Visuo-perceptive	1,44	1,65	0,77	1	0,38	4,24	0,17	106,84
Visual Hallucination	,45	1,81	0,06	1	0,80	1,57	0,04	54,80
Psychosis	1,46	1,56	0,87	1	0,35	4,31	0,20	92,48
Constant	-9,07	4,63	3,84	1	0,05	0,00		

Table 2. Logistic regression analysis.

Discussion

The aim of our study was to intercept a possible disease profile, including motor, neuropsychological and neuropsychiatric complains, that is associated with cognitive involution.

Regardless of their nosological characterization at baseline, namely if they are MCI or normal cognition, a proportion of 61% of PD patients remained overall cognitive stable over the observational period of five years, while 39% of patients consistently reduced their cognitive reserve during the follow-up period. Focusing on demographic

characteristics, age was the only variable significantly associated with cognitive conversion; these data are in line with the view of the *ageing effects* as the overall weakening of metabolic reserve, increasing the susceptibility to biochemical insults that are proper of the disease process. In our setting, clinical motor characteristics at baseline, including motor phenotype at onset (Tremor dominant vs akinetic-rigid), the presence of milestone of PD progression as well as LED assumption were not associated with cognitive conversion; this data may suggest that cognitive decline do not necessarily share the exactly alike pathophysiologic mechanism observed for motor disability progression.

With regard to neuropsychological profile we found that tests assessing the domains of visuo-spatial functions, visuo-perceptive functions, attention and, to a lesser extent, memory deficits and neuropsychiatric complains, were associated with cognitive conversion at univariate analysis. However, after logistic regression, only visuo-spatial deficits survived at a level of statistical significance and a trend of significance was observed for memory deficit.

Our findings may suggest that patients with “posterior” dysfunctions (visuo-spatial deficits and likely memory) are more prone to consistently worsen their cognitive abilities (whether they are cognitively preserved or MCI). Nevertheless, we have to underline that

visuospatial dysfunctions were presented in a consistent proportion of non converter, thus resulting in poor specificity. On the other hand, the measures of set maintenance, although associated with cognitive conversion at univariate analysis, showed a poor relevance after logistic regression; this may indicate that, although frequently represented in converter group, set maintenance as discrete variable has a limited predictive value for cognitive decline. Taken together these data are consistent with previous line of evidence, that hypothesized at least two pattern of discognitive progression. Indeed, other authors found a relative benign progression of fronto-striatal cognitive deficit when compared with posterior cortical dysfunction, that predispose to the development of dementia. Therefore, the seminal concept of different progression for cognitive patterns is replicable in a study design that assess the phenomenon of cognitive involution *per se*, across groups with different cognitive performance at baseline (normal-cognition or MCI). Moreover, although neuropsychiatric symptoms tended to be more present in converter group, no statistical difference emerged. Taking in to account the combined analysis of demographic, motor, neuropsychiatric and neurosychological profiles we can speculate that, according to our results, the presence of a MCI (dysexecutive-type) associated with motor complication, along with depressive symptoms and a long disease duration is not necessarily associated

to the collapse of cognitive function. On the other hand, older patients with cortical posterior dysfunction and memory impairment are more likely prone to cognitively deteriorate, in spite of the overall motor impairment.

The study present some limits: first, our approach is merely *interpolative* since our evaluation at baseline was performed in patients with a mean disease duration of about ten years and not at disease onset; second, the limited numerosity of our cohort do not allow generalization and translation in clinical practice; third: the categorization in the two groups (converter and non-converter), although original, is arbitrary and include patients consistently difference with regard to cognitive asset. Even though these limits, the notion of PD cognitive-conversion was not conceptualized to provide diagnostic criteria or clinical guidelines: the construct of cognitive-conversion represent an observational analysis focused on the phenomenon of cognitive breakdown in a cohort of patient followed for five years and assessed with a comprehensive neuropsychological battery. In this terms, the observation of an overall cognitive stability in a consistent proportion of patients, even when tagged as MCI, raises the question of the clinical utility and relevance (including therapeutic strategies) of the diagnosis of PD-MCI.

In conclusion, tracking with confident accuracy the profile of patients with risk of cognitive conversion remains difficult to obtain, partially ascribable to the complexity of the phenomena; however the role of visuospatial and memory deficit deserves attention and further analysis.

Experimental Procedures. Laboratory Setting-Part 1

The impact of MAPT haplotype on PD motor phenotype

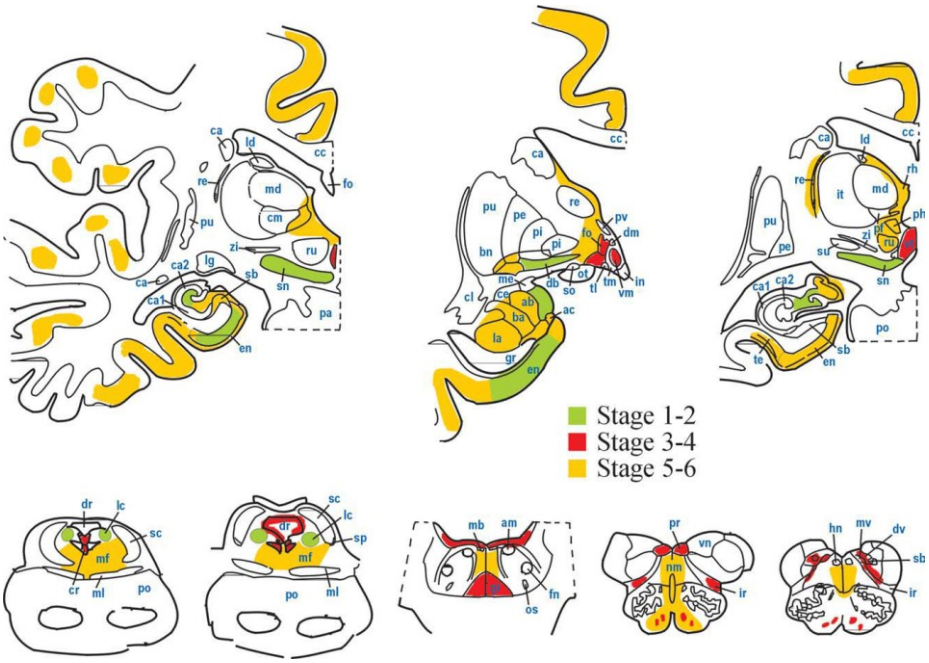
Introduction

Parkinson's disease (PD) is a multiorgan disease resulting from degeneration in different populations of neurons. Although the extent and progression of the degenerative process may to some extent be predictable (Braak et al 2003), PD patients present a wide range of clinical phenotypes, including symptoms in the pre-motor phase, motor signs and disabilities and, most importantly, a varying severity and rate of progression of motor and non-motor features. The clinical heterogeneity of PD is likely to reflect the contribution of different environmental and genetic factors, which in turn lead to different neuropathological substrates (Selikhova et al. 2009). Neither environmental factors nor individual genetic traits are currently recognized as determinant of clinical or pathological subtype of PD; for example, it has been observed that for LRRK2 patients (the most common autosomal dominant gene for PD) the extent of LB cortical involvement differs across patients and, interestingly, also inclusion of tau protein were present in a variable distribution and severity of brain autopsies (Poulopoulos et al. 2012). The microtubule-

associated protein tau (MAPT or tau) is being widely investigated in the field of neurodegeneration as there is a well-established genetic link between the *MAPT* gene locus and neurodegenerative diseases. There are two major haplotypes: H1 and H2, resulting from a common genomic inversion of approximately 900 kb in chromosome17q21of the *MAPT* gene. Recent studies have revealed haplotype-specific differences in expression and alternative splicing of MAPT transcripts in neurodegenerative diseases. One of the issues believed to underlie the heterogeneity of PD motor features is the interaction of pathogenic proteins involved in neurodegeneration, and tau protein is likely involved in this process. The association between *MAPT* haplotypes and motor features of PD has, however, not yet been clearly characterised. To evaluate the role of the *MAPT* gene in motor phenotype in PD, we performed a phenotype-genotype association study to assess the possible influence of H1 and H2 haplotypes.

The human *MAPT* locus lies on chromosome 17q21, and six isoforms of the encoded tau protein are expressed in the adult human CNS. The isoforms are generated via alternative splicing of exons 2, 3 and 10. Alternative splicing of exon 10 results in a protein containing either three tandem repeats (3R; exon 10–) or four tandem repeats (4R; exon 10+) of a microtubule-binding motif. Alternative splicing of exons 2 and 3 generates a protein with no

N-terminal inserts or with one or two N-terminal inserts (Wade-Martin, Nat Rev Neurolo 2012).



Progress and distribution pattern of PD-related neuronal pathology (ab, accessory basal nucleus of amygdala; ac, accessory cortical nucleus of amygdala; ad, anterodorsal nucleus of amygdala; am, anteromedial nucleus of thalamus; an, abducens motor nucleus; ba, basal nucleus of amygdala; bn, basal nucleus of Meynert; ca1, first Ammon's horn sector; ca2, second Ammon's horn sector; ca, caudate nucleus; cc, corpus callosum; ce, central nuclei of amygdala; cg, central gray of mesencephalon; cl, claustrum; co, cortical nuclei of amygdala; cr, central nucleus of raphe; db, nucleus of the diagonal band; dm, dorsomedial hypothalamic nucleus; dr, dorsal nucleus of raphe; ds, decussation of superior cerebellar peduncles; dv, dorsal nuclear complex of vagal nerve; en, entorhinal region; fn, facial motor nucleus; fo, fornix; gi, gigantocellular reticular nucleus; gr, granular nucleus of amygdala; hn, hypoglossal motor nucleus; in, infundibular nucleus; ir, intermediate reticular zone; lc, locus coeruleus; ld, laterodorsal nucleus of the thalamus; lg, lateral geniculate body; li, nucleus limitans thalami; lt, lateral nuclei of the thalamus; md, mediodorsal nuclei of thalamus; me, medial nuclei of amygdala; mf, medial longitudinal fasciculus; mg, medial geniculate body; ml, medial lemniscus; mm, medial mamillary nucleus; ms, medial septal nucleus; mt, mamillothalamic tract, mv, dorsal motor nucleus of vagal nerve; oi, oliva inferior; os, oliva superior; ot,optic tract; pe, external pallidum; pf, parafascicular nucleus; ph, posterior hypothalamic nucleus; pi, internal pallidum; po, pontine gray; pr, praepositus nucleus; pu, putamen; pv, paraventricular nucleus; re, reticular nucleus of the thalamus; rm, nucleus raphes magnus; ru, nucleus ruber; sb, subiculum; sc, superior cerebellar peduncle; sf, solitary fascicle; so, supraoptic nucleus; sn, substantia nigra; sp, subpeduncular nucleus; st, nucleus of the stria terminalis; su, subthalamic nucleus; te, transentorhinal region; tl, lateral

tuberal nucleus; tm, tuberomamillary nucleus; tp, tegmental pedunculopontine nucleus; vl, ventrolateral nuclei of thalamus; vm, ventromedial hypothalamic nucleus; vn, vestibular nuclei; vt, dopaminergic nuclei of ventral tegmentum (paranigral nucleus and pigmented parabrachial nucleus); zi, zona incerta. (Jelligr KA, Mov Dis 2012)

Methods

Population. A total of 184 patients were consecutively recruited from March 2011 to July 2012 from the Parkinson outpatient centre of the Sapienza University of Rome. All the patients gave their written informed consent. All patients were Caucasian and originating in the central-south regions of Italy. The patients fulfilled the UK Brain Bank criteria for PD. Patients with signs of atypical parkinsonism, doubtful response to dopaminergic replacement therapy or dementia (Mini-Mental State Examination score < 24), were not included. The sample's clinical records were reviewed and patients were assessed by neurologists who are experts in movement disorders; the patients' clinical and demographic data are shown in Table 1. The variables collected included: demographic data, age at onset, disease duration, familiarity for PD, total levodopa equivalent dose (TED) and UPDRS III at the time of last visit. The patients were finally classified in two clinical subtypes, by using criteria published in previous studies (Selikhova et al. 2009):

(a) tremor dominant (TD), i.e. patients with tremor as the only motor sign at onset or tremor as the prominent motor symptom according to the UPDRS part III

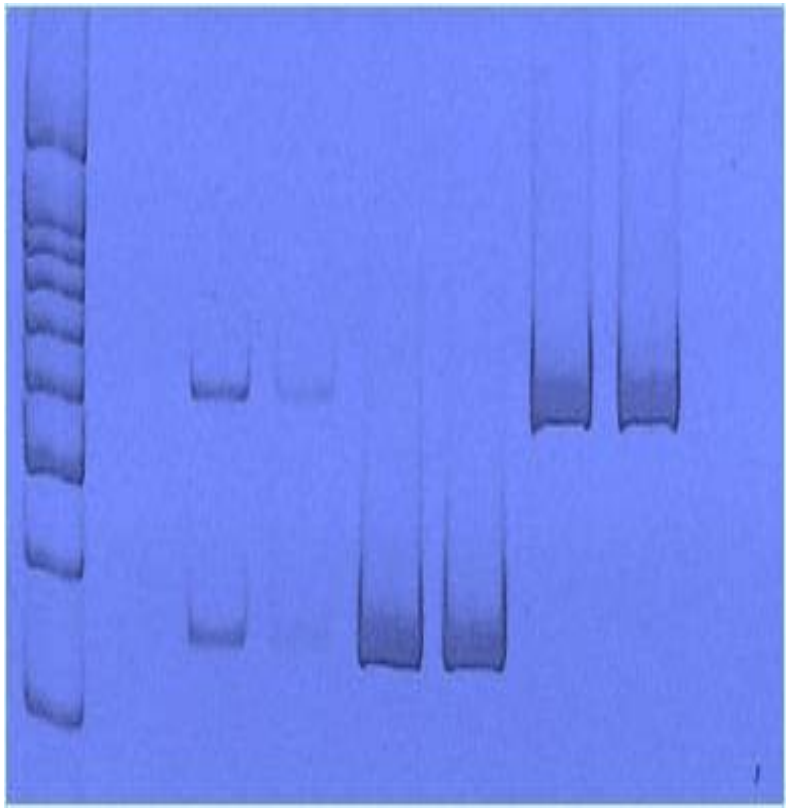
(b) non-tremor dominant (NTD), i.e. patients with predominant rigidity and bradykinesia but no tremor or only mild tremor at rest.

The process of clinical characterization was possible for 181 out of 184 patients and it was based on the use of medical history notes, symptoms reported by patients and on direct examination of motor disturbances at the time of study (akinetc/rigid score vs tremor score of UDPRS III). Moreover, only patients with at least three years of disease duration were enrolled for the study in order to obtain a reasonable depiction of the clinical subtype.

Genetic analysis. Genomic DNA was extracted from a peripheral blood sample by standard methods. The *MAPT* haplotype was determined by testing for the presence of a 238-base pair deletion between exons 9 and 10 (del-In9), which is characteristic of the H2 haplotype, figure 4, (Refenes et al. 2009). The del-In9 polymorphism was PCR amplified and separated on 6% native polyacrylamide gel and visualized after ethidium bromide staining by UV transillumination.

The primer sequences employed for PCR amplification were: 5'-GTTTCCACTGTTTCCAGAGTTCC-3' (F) and 5'-TTTTTACAATCTCAGCCCCTAGC-3' (R), corresponding to nucleotide positions 122640 to 122662 (F) and 123213 to 123191 (R), respectively, of the human MAPT gene (GenBank accession number AC091628.2). PCR amplification was carried out in a final volume of 50□l containing 50ng genomic DNA, 1.5mM $MgCl_2$, 200□M dNTP, 50mM KCl, 10mM Tris-HCl (pH8.3), 0.25□M of each primer and 1 U of Promega Taq DNA polymerase. Amplification conditions were 35 cycles of 94°C for 30 sec, 64°C for 20 sec, 72°C for 20 sec.

Statistical analyses. We used the t-test for continuous variables and chi-square test for categorical variables. We performed a logistic regression analysis to assess the association between clinical subtypes and *MAPT* haplotypes adjusted for sex, age and age at onset. P values < 0.05 were considered as statistically significant. All statistical analyses were performed using SPSS software (version 20.0, SPSS inc. Chicago, IL, USA).



Gel PCR for Haplotype determination , second column from left H1/H2, third column fron left H2/H2, fourth column from left H1/H1

Results

The H1/H2 genotype frequencies determined in our patients did not deviate from those predicted by Hardy Weinberg equilibrium ($P>0.05$). H1/H1 patients were comparable to H2 carriers with regard to age, sex, age at onset, disease duration and familiarity for PD. Neither total daily LED dose nor UPDRS III in on state were statistically different among groups. However, H1 homozygous patients presented a more severe clinical subtype (NTD) than H2 carriers (Table 1).

	Total n° = 184	H1/H1 n° = 114	H1/H2+H2/H2 n°= 70	*p*
Age (yrs)	70.93±8.40	71.28±8.48	70.37±8.30	n.s.*
Gender M/F	57.1% (105)/42.9% (79)	51.7% (59)/48.3% (55)	65.7% (46)/34.3% (24)	n.s.*
Age at onset	60.72%±9.06	60.83%±9.11	59.83%±8.45	n.s.*
Disease duration (yrs)	10.48±4.9	10.45±4.95	10.53±4.89	n.s.*
Familiarity for PD (Y/N)	15.4% (28)/84.6% (154)	15.2% (17)/84.8% (95)	15.7% (11)/84.3% (59)	n.s.*
UPDRS III score	16.6±8.6	17.8±9.2	14.6±7.1	n.s.*
Total LED mg/d	670.3±362.9	657.9±358.5	692.8±372.4	n.s.*
Clinical subtype (NTD/TD)	74.6% (135)/25.4% (46)	79.5% (89)/20.5% (23)	66.7% (46)/33.3% (23)	0.05

Table 1. Demographic and clinical characteristics of PD patients according to MAPT haplotypes.

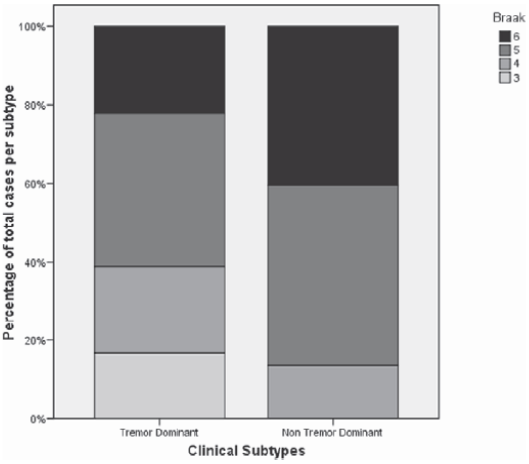
This result was confirmed by the multivariate analysis corrected for age, sex and disease duration (OR = 2.074, 95%CI 1.036- 4.152; p=0.039) (Table 2).

	B	E.S.	Wald	df	*p*	OR	95%CI	
							Lower	Upper
H1H1 vs other	.730	.354	4.246	1	.039	2.074	1.036	4.152
F vs M	-.371	.353	1.099	1	.294	.690	.345	1.380
Age onset	-.008	.037	.052	1	.819	.992	.923	1.066
Age	-.003	.039	.008	1	.931	.997	.924	1.075
Constant	1.569	1.520	1.066	1	.302	4.801		

Table 2. A regression logistic analysis on the association between non-tremor dominant clinical subtype of PD and MAPT haplotypes.

Discussion

The results of this study indicate that *MAPT* haplotypes influence motor features in PD by showing that H1/H1 PD patients are significantly more likely to present a NTD clinical phenotype characterized by widespread pathological degeneration (van de Berg et al. 2012) and worse clinical prognosis.



Distribution of Braak Lewy body stages at the time of death in 55 PD cases with extensive clinical reports. Eighteen patients had a tremor-dominant (TD) and 37 had a non-tremor dominant subtype (NTD). NTD patients had significant higher Braak stages than those with a TD type (Mann Whitney *U*; *P* = 0.03). The prevalence of dementia was higher in NTD patients compared to the TD group (Fisher's Exact; *P* = 0.01). Disease duration in the TD group (16.2±7.4) and NTD group (15.5±5.8) did not differ significantly [*t*(54) = 0.34, *P* >0.05].

The H1 variant has been found to be related to the risk of PD in numerous case-control and Genome-Wide-Association studies (GWAs), an association confirmed in a comprehensive metanalysis study (Zhang et al. 2004). Moreover, the H1 haplotype has recently been demonstrated to be a risk factor for PD in a series of brains with a pathologically-confirmed diagnosis of PD (Charlesworth et al. 2012), thereby helping to

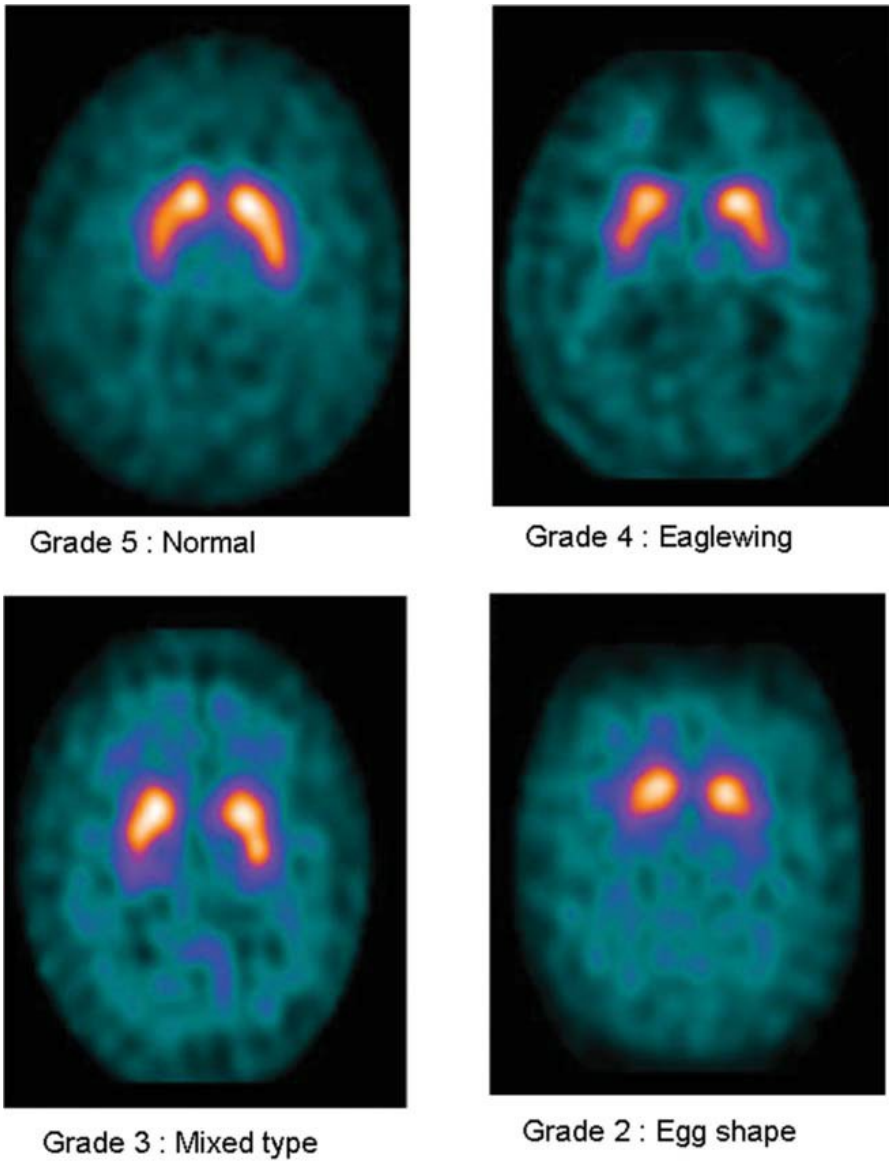
reduce the contamination with cases of other primary tauopathies; indeed, the H1 haplotype has previously been associated with various neurodegenerative conditions with tauopathy, such as PSP, corticobasal degeneration and frontotemporal dementia with parkinsonism. The haplotype associated with PD encompasses numerous genes whose underlying biological mechanisms have yet to be fully understood; however, tau remains the most likely underlying factor as variants in the *MAPT* gene increase tau expression, alter its splicing promoting aggregation and alter the 4/3 repeated transcript ratio.

The H1 haplotype does not only influence PD risk, but has also been related to the progression of cognitive deficits leading to PDD: in one longitudinal study (Williams-Gray et al. 2009), the H1 haplotype was recognized as a genetic risk factor for the development of dementia, a finding that was subsequently confirmed in other studies.

The underlying biological mechanisms that link the *MAPT* locus (and tau protein) to neurodegeneration are not yet adequately characterized; hypotheses include: (I) increased expression of tau in H1 subjects, particularly of the 4 repeat-containing transcripts; (II) interaction of tau with other misfolded pathogenic proteins, which induces aggregation; (III) the contribution of other loci whose function is unknown included in the haplotype.

The emerging concept of H1 pathogenicity points to the role of each of the various tau isoforms expressed rather than of the overall number of transcripts (Wade-Martins 2012). According to this model, the H1 haplotype is associated with an underexpression of the exon 3+ variant (protective) and an overexpression of the exon 10+ (detrimental) variant, which lead to a subtle neuronal dysfunction that accumulates over the years and induces or accelerates cellular degeneration.

In this perspective, PD motor phenotypes display different patterns of degeneration within the basal ganglia circuits (corresponding to different “visual patterns” of denervation on Dat-scan), and denervation progresses more rapidly in NTD-patients (Eggers et al. 2012).



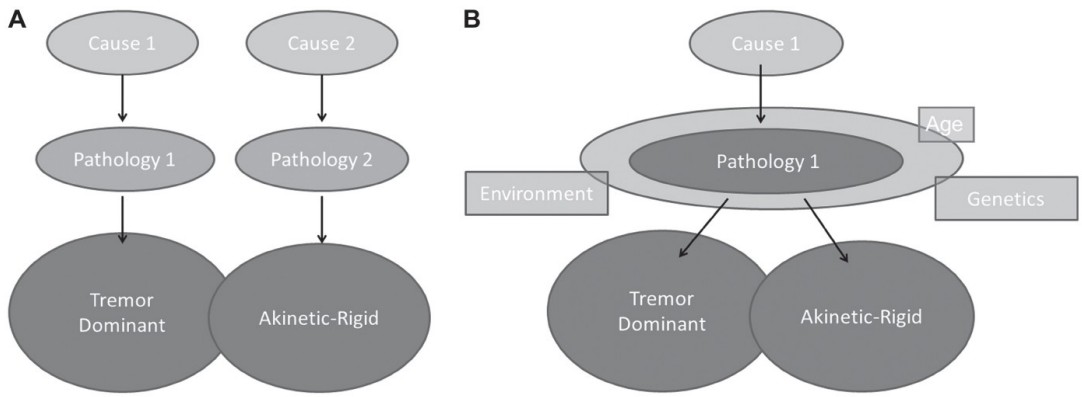
Eggers C, Mov Dis 2012. Grade 5 (normal): intense, symmetrical uptake in putamen and caudate nuclei. Grade 4 (eagle wing): uptake almost symmetrical with discrete reduction in one or both putamina. Grade 3 (mixed): asymmetric uptake with normal or almost normal uptake in the putamen in one hemisphere and severe reduction in the contralateral putamen. Grade 2 (egg shape): significant bilateral reduction of uptake in the putamen bilaterally and normal or almost normal uptake in the caudate nuclei. Grade 1 (burst striatum): severe bilateral reduction with almost missing uptake in both putamina and caudate nuclei.

A relationship between PD subtypes and tau protein has also recently been observed:

laboratory findings indicate that tau (as well as other CSF biomarkers) exhibits a different

expression profile in CSF depending on the motor phenotype, with NTD-patients displaying significantly higher levels of CSF tau than other PD patients (Jellinger 2012).

Clinical subtypes of PD likely result from the interaction of several pathophysiological factors (eg, age, environment, genetics) (Marras et al. 2013); here we observed that *MATP* H1 haplotype may be a genetic factor contributing to the expression of clinical features of PD, even before the onset of dementia.



Marras C., Lang A., JNNP 2013

Our findings may be consistent and complementary with the model of functional isoforms generated from H1 haplotype: tau pathogenicity, the *tauopathic wedge*, is related to dysfunctions acting over time so that pathological anomalies, as well clinical symptoms, are more severe in middle and later stages of the disease.

Wade-Martin (Nat Rev Neurol 2012) emphasize that "*there is now an increased appreciation that diseases such as Alzheimer disease and Parkinson disease are not so much disorders of protein aggregation, but are caused by earlier synaptic failure. A role for tau in cellular signalling is now emerging and, on the basis of recent findings it is tempting to speculate that, rather than tangle pathology, the real relevance of the different tau isoforms lies in the regulation of aspects of cell physiology, perhaps even those unrelated to microtubules*".

The cohort of patients enrolled for this study will be followed to assess the progression of motor disability and the severity and progression of non-motor symptoms in order to investigate the possible role of *MAPT* on the neuropathology accountable for non-motor features.

The results of this study need to be confirmed in larger series of patients and future researchers focusing on different tau isoforms are mandatory to confirm the possible role of this protein in the complex phenomenology of PD.

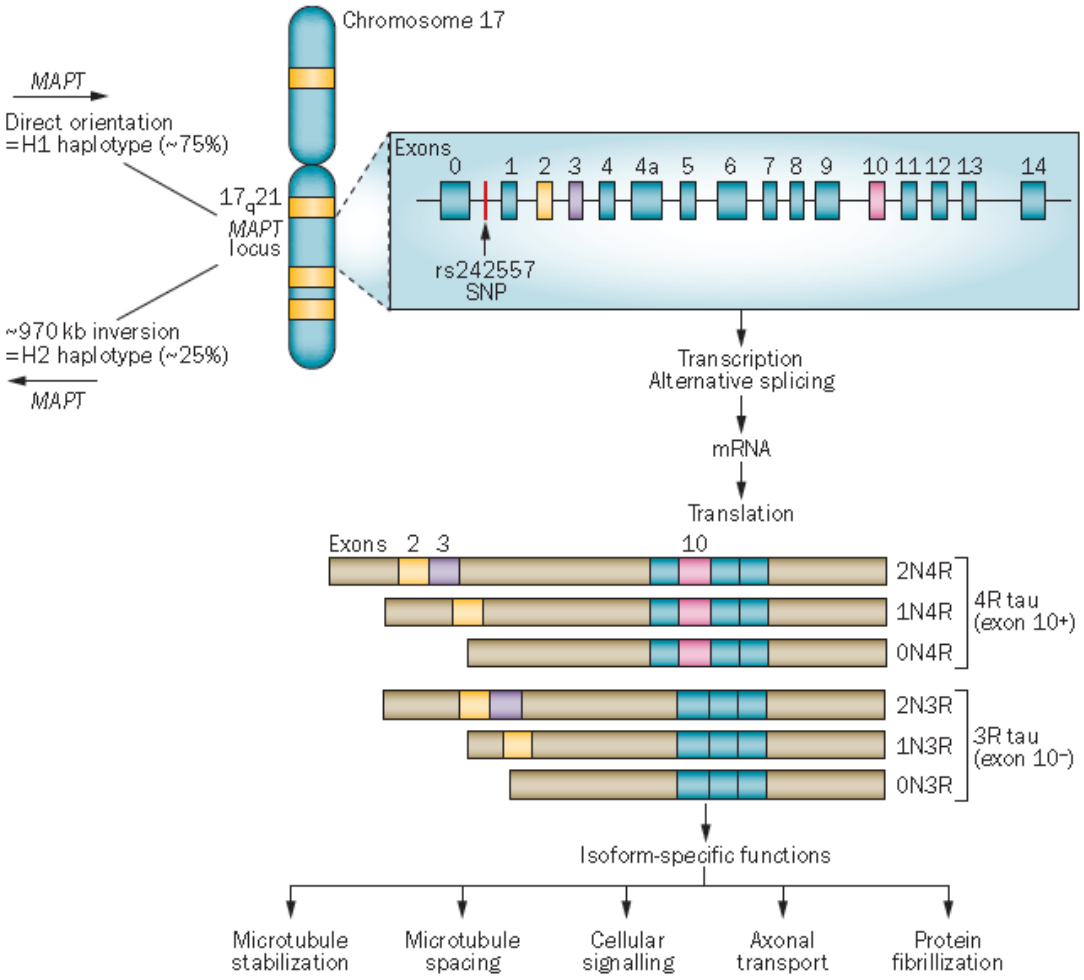


Figure 6. The *MAPT* gene locus and the tau protein isoforms. The *MAPT* locus has two haplotypes: the directly oriented H1 and the inverted H2. H1 gives relatively increased expression of exon 10, the exon found in protein tangles in several neurodegenerative diseases, whereas H2 is linked to increased expression of exon 3, which is proposed to be protective against the formation of tau tangles. Alternative splicing of exons 2, 3 and 10 of the *MAPT* gene gives rise to six protein isoforms, each of which may have distinct functions in cell biology. Abbreviation: MAPT, microtubule-associated protein tau.

Experimental Procedures. Laboratory Setting-Part 2

The impact of MAPT haplotype on non-motor symptoms profile

In the second part of our laboratory setting we focused on non-motor symptoms of PD.

Background

For each non-motor clinical feature there is strong evidence to suggest a role for a-synuclein pathology, lending further support for the notion that PD is a multisystem a-synucleinopathy.

Anatomical region	α-Synuclein pathology	Braak PD stage	Non-motor feature
Autonomic nervous system			
Sympathetic ganglia	LN, LB		Autonomic (orthostatic hypotension, impotence, urinary frequency, constipation)
Gastroesophageal	LN		
Cardiac	LN		
Adrenal	LN		
Olfactory bulb			
Anterior olfactory n.	LN	1	Hyposmia
Medulla			Autonomic (parasympathetic)
Dorsal motor n. vagus	LB, iLB	1	
Pons			
Locus ceruleus, raphe and lateral tegmental nuclei	LB, LN	2	Depression & RBD
Midbrain			
Substantia nigra	LB, LN	3	Extrapyramidal motor
Basal forebrain			
Basal nucleus	cLB, LN	4	Dementia
Amygdala & hippocampus	cLB, LN		
Neocortex			
Frontal cortex	cLB, LN	5	Dementia
Parietal cortex	cLB, LN	6	

Dickson W N, Neuropathology of non-motor features of Parkinson disease, 2009.

Starting from the our previous results, demonstrating an effect of MAPT haplotype on motor phenotype of PD, in the subsequent part of the experimental setting we focused on the evaluation of a possible influence of tau on the expression of non-motor symptoms profile in our cohort of PD patients. Assuming that the occurrence and severity of NMS is due to neural loss and Lewy body pathology similarly to that observed in the central nervous system, and taking in to account that MAPT haplotype influences Lewy body burden in cortical structures, we conducted a genotype-fenotype correlation study between NMS profile and MAPT haplotype.

Methods

Patients

From the original cohort of 184 patients, genotyped to establish the condition of H1 homozygousity or the presence of H2 haplotype we evaluated 63 patients with extensive and validated clinimetric instruments to assess the occurrence and severity of NMS; all the sphere of NMS expression were examined, from behavioral-affective symptoms to urinary disturbances. Patients with organic pathologies that could be responsible or interfere with

specific symptoms were not enrolled in the study (i.e. patients with severe cardiomyopathy or arrhythmias , urinary incontinence due to uterine prolapsed). Patients with dementia according to the MDS TF criteria were not considered eligible for the study.

Clinical evaluation

All patients were evaluated in ON, there is now a growing body of evidence that demonstrate the occurrence of fluctuation even in NMS. Scales and questionnaires were administered by Neurologists who are expert in Movement Disorders.

The patients were assessed with the following scales/questionnaires:

- Beck depression Inventory (BDI) for depressive symptoms
- Montreal Cognitive Assessment (MOCA) for the evaluation of cognitive status and in order to exclude patient with severe cognitive dysfunctions
- SCOPA-AUT, provided to assess the wide spectrum of dysautonomic dysfunctions, this scale investigate dysfunctions in cardiovascular, gastrointestinal, sudomotor, papillary, urinary and sexual domains.

- Non Motor Symptoms Scale (NMSS), concerning frequency and severity of a wide range of NMS; this scale is divided into 9 sub-items, the result for each of these items is given by the multiplication of the score for frequency and the score for severity; this scale is therefore a validated instrument to assess the overall impact of a specific NMS.

- Epworth Sleepiness Scale (EPSS), for sleep disturbances evaluation

- Neuropsychiatric Inventory Scale (NPI) to assess the presence of psychotic symptom

Statistical Analysis

Statistical analysis was conducted using SPSS for Windows (version 15.0; SPSS Inc., Chicago, IL). The chi-square test was used for the comparison independent categorical variables. For the comparison of independent numerical variables, the independent *t* test was used when data were normally distributed. The paired sample *t* test was used to determine differences between 2 dependent groups when data were normally distributed and the Wilcoxon test when they were not. $P \leq .05$ was considered statistically significant.

Results

The results are summarized in table in the following tables.

	H1H1 n	H2 carriers n	P value
Patients (n)	39	24	
Age	71,6±7,5	69,6±6,2	n.s.
Disease duration	9,8±3,8	11,1±3,9	n.s.

Table 1. age and disease duration of the two groups: 1.0 H1H1 homozygous, 2.0 H2 carriers.

	H1H1 n	H2 carriers n	P value
MOCA	21,4±4,0	21,2±5,1	n.s.
BDI	12,3±10,7	14,2±8,3	n.s.
NPI	13,4±14,3	14,4±14,5	n.s.
ESS	7,7±5,4	8,0±5,1	n.s.
SCOPA	14,1±9,4	13,9±6,1	n.s.
NMSS total	59,3±46,7	65,1±37,1	n.s.
NMSS cardio	2,7±3,0	1,25±1,9	<0,05

Table 2. Univariate analysis of NMS burden in the two groups.

Concerning to demographic features, the group of H1 homozygous and the group of H2 carriers did not present significant differences. No difference emerged in cognitive global efficacy (MOCA), nor neuropsychiatric complaints neither mood disorders were differently

distributed across the two groups, as well as sleep disturbances. No differences were observed in the overall burden of non motor symptoms assessed with scopa-aut and NMSS; however the score of item assessing cardiovascular domain of NMSS was statistically significant higher in H1H1 homozygous group. No other specific item regarding non-motor symptoms reached a statistical difference.

Discussion

Our preliminary results, derived from the partial analysis of non-motor symptoms profile in the initial cohort of 184 patients indicate a potential role for MAPT haplotype in the specific domain of cardiovascular symptoms; the global impairment in terms of non-motor symptoms was not different across groups of our analysis. Evidences derived from neuropathological studies suggest that cardiac sympathetic denervation correlates with clinical and pathologic stages of Parkinson's, furthermore, assessing the pattern of myocardial sympathetic denervation deficits in vivo (by using myocardial 123I-metaiodobenzylguanidine –MIBG- scintigraphy) has been reported that cognitive decline in patients with PD is associated with autonomic involvement. Finally, in a recent longitudinal

study, has been reported that symptomatic hypotension in one of the strongest predictors of cognitive decline in PD, together with sleep disturbances.

According to what we found, a biological issue linking motor severity of PD and, in a lesser extent, cardiac autonomic dysfunctions to the development of Dementia has emerged: it is now plausible theorize tau dysfunction as a primary target for research on pathophysiology and future neuroprotective strategies.

Nevertheless, these data should be considered with caution: first, the examination of non-motor profile has been conducted in a relatively small proportion of our initial cohort; second: a statistical significant emerged only in the *cardiovascular domain* of NMSS but not in the SCOPA-AUT questionnaire, indicating a potential poor reliability of the clinimetric instruments currently available.

Conclusion and future perspectives.

The preliminary retrospective study conducted in our PD Centre allows to confirm data reported from other researches: Dementia in PD is associated with older age but not disease duration; a clinical phenotype characterized by gait disturbances and postural instability is more common in patients with cognitive dysfunctions and psychotic phenomena such hallucinations are risk factors for the development of Dementia; most important, PD patients with Dementia present a more severe progression of motor disability (progression of H&Y and UPDRS scores of 44% in PD patients with dementia vs 13.5% in PD patients without dementia). Taken together, these data support the hypothesis that PD patients with Dementia have a more aggressive form of malady. It is worth to consider that most of the features associated with Dementia are not dopamine-related dysfunction.

The estimated incidence reported in our retrospective study was, however, 3-4 fold lesser than the incidence reported in literature (18 cases for 1000 year-person vs 71 cases year-

person). The underestimation of the phenomenon in our setting may be representative of the difficulty encountered in the condition of the standard ambulatory setting.

Conversely, the psychometric diagnostic tool for Parkinson Dementia proposed in 2007 do not always displays sensitivity and accuracy for those patients with subtle memory deficits and visuo-constructive deficits resembling a neuropsychological profile observed in demented patients with a cortically shaped dyscognitive profile. The results of this study underlies the need of more accurate psychometric tool to assess cognitive deficits in PD and encourage to work for a better nosological classification of Parkinson Dementia. The question of PD heterogeneity rise to a priority in the field of epidemiological research in PD (Foltynie, 2002), the concept has to be necessarily translated into the field of Parkinson Dementia. The considerable difference in incidence and prevalence estimations, the lack of reliable biomarker (both for laboratory and neuroimaging tools), and the perfectible diagnostic instruments currently available confirm this assumption. Following to what illustrated in this thesis, it is possible to propose the implementation of the current criteria for Parkinson Dementia with the adoption of different subtypes of Parkinson Dementia (for example a profile of Parkinson Dementia with prominent executive dysfunction and slowness of cognitive speed and a profile of cortical involution with major deficits

concerning cortical and superior associative related deficits). In our follow-up study, we found that a proportion of 61% of PD patients remained overall cognitive stable over the observational period of five years, while 39% of patients consistently reduced their cognitive reserve during the follow-up period. We observed that tests assessing the domains of visuo-spatial functions, visuo-perceptive functions, attention and, to a lesser extent, memory deficits and neuropsychiatric complains, were associated with cognitive conversion at univariate analysis. However, after logistic regression, only visuo-spatial deficits survived at a level of statistical significance and a trend of significance was observed for memory deficit.

Our findings may confirm that patients with “posterior” dysfunctions (visuo-spatial deficits and likely memory) are more prone to consistently worsen their cognitive abilities (whether they are cognitively preserved or MCI). Therefore, the seminal concept of different progression for cognitive patterns is replicable in a study design that assess the phenomenon of cognitive involution *per se*, across groups with different cognitive performance at baseline (normal-cognition or MCI).

The existence of several Parkinson subtypes probably reflect the contribution of different environmental and biological factor, these act synergically, with different influence and interference with each other, and finally result in one of the PD subtypes. Van Rooden et al (Mov Dis, 2012) identified, with an unbiased and aprioristic approach, four subtypes of the disease: based on a cluster analysis in two independent large cohort of PD patients. Studies on environmental or biological factors conditioning PD clinical characteristic or progression are complex and often with conflicting results; at to date, we do not know the significance and clinical weight of vascular burden to PD course, we even do not know what vascular PD is.

The results of this project, indicating an influence of MAPT haplotype on motor features of PD before the onset of dementia, needs to be confirmed on larger and independent cohort of patients. The study presents some limits; first, the number of patients in our cohort is not enough to allow robust conclusion in this type of genotype-phenotype association study and the statistical significance is weak; second, the evaluation of clinical characteristic is mostly based retrospective analysis, although patients were all re-evaluated by Neurologists; finally, no data on brain tissue with autopsies are programmed.

However, the contribution of a genetic trait along the course of the disease seems to be consistent with very recent lines of research focusing on the biological significance of protein tau alterations for neuron physiology and fit with a number of studies, including our epidemiological data, stating that PD patients with dementia have a more aggressive form of the malady.

The finding that tau protein related alterations can contribute to the clinical phenotype of the disease, although to be confirmed on larger cohort of patients, needs to be underlined.

Alpha-synuclein and the related Lewy Body and Lewy Neurites are the pathological hallmark of the disease and represent one of the molecular based hub of the research but, not the totality of PD related pathology can be attributed to alpha-synuclein dysfunction. At least three genetic conditions, namely Parkin, PINK 1 and DJ 1 cause clinical condition characterized by signs of parkinsonism, and these are not related to alpha-synuclein inclusions. Although clinically dissimilar (these form cause juvenile parkinsonism with a very young onset and a lesser extent of non-motor symptomatic compound, including a lesser degree of dementia), patients with Parkin mutation present a very good and long lasting response to levodopa. Furthermore, in patients with Parkinson and LRRK2 mutation, which closely resemble idiopathic Parkinson’s disease, the neuropathology is

highly variable: findings from 49 patients with LRRK2 mutation revealed a majority with alpha-synuclein positive pathology, but several other types of pathology also occurred, including tau pathology. Conversely, not all subjects with alpha-synuclein pathology show clinical signs or symptoms of parkinsonism, the so called “Lewy Body incidental pathology”; what is the meaning of such findings is not clear, it remains to understand if these alterations represent the pathological portrait of the premorbid phase or are not linked to the pathophysiology of the disease.

The effect magnitude effect of MAPT haplotype on PD clinical subtype is statistically significant although weak; whatever, the theoretical implications of this study tend to confirm a model of complex pathophysiology related to aetiology, phenotype and progression of idiopathic Parkinson’s disease: no single genetic or environmental factor as disease determinant but cases-specific modifying factors accounting for different manifestations. In this perspective, future researches focusing on non-motor symptoms of PD will shed light on this speculations. Actually, Lewy Body and Lewy Neuritis in different populations of neuron (central nervous system, peripheral and spinal cord structures) account, together with neural loss, for the presence of wide spectrum of symptoms, ranging from CNF nuclei-related disturbances (depression, hyposmia, sleep cycle

alteration) to peripheral neural circuitry dysfunction correlates (urinary disturbances, constipation). It will be important to determinate whether MAPT haplotype, influencing tau isoforms expression, contribute to degenerative process in neural circuits not related to motor control. The cohort of this study is now under investigation to assess, with specific clinimetric tools, the presence and severity of some of the most disabling non-motor symptoms. If future results will confirm this hypothesis, it will be possible to evaluate to extend future neuroprotective researches to the molecular pathways regulated by tau protein. Recently, growing body of evidences indicate that misfolded protein can behave in a manner that closely resemble that observed in the selfsustaining aggregation process of Prions, most of these evidence derive from Prion-like properties observed in amyloidogenic proteins. Briefly, proteins in the amyloid state are thermodynamically highly stable and, even if potentially all proteins can generate amyloid under the favorable conditions, protective mechanisms ensure that relatively few do so in physiologically functioning conditions. The molecular structure responsible for the toxicity of amyloid and amyloid intermediates is not fully characterized. Recent works suggest that a deviation from the energetically favourable and stable parallel and in-register b-sheet amyloid state is linked to toxicity. This model is non not unique for amyloid, accumulating experimental

evidences indicate that this *seeding principle* also concern other pathogenic proteins, many of which form amyloid-like inclusions within cells like tau and alpha-synuclein. Whether if tau dysfunctions, driven by H1 haplotype, promote or accelerate protein misfolding or the transcription of different isoforms, likewise due to H1 haplotype, harm neural physiology, remain to understand. Earlier reports have in fact shown an increased *MAPT* expression from H1 homozygous compared with H2 carriers. More recently, Trabzuni at al. suggest that this effect could represent an experimental artefact deriving from H1/H2 haplotype-specific sequence differences in target regions of probes used for *MAPT* gene expression analysis. More tuned investigation of *MAPT* expression from the H1 and H2 haplotypes did not evidence difference in *MAPT* expression between the two haplotypes. However, an increase in the expression of exon 3+ transcripts specifically from the H2 haplotype was observed. This finding confirms earlier studies of haplotype-specific expression in human postmortem brains that found no difference in total *MAPT* expression between H1 and H2 haplotypes, furthermore, underlie a robust increased expression of exon 3 from the H2 haplotype. The theoretical implications of this result are significant, in the way that the link between MAPT haplotype and degeneration relies not simply on accumulation of misfolded proteins but regards the regulation of specific aspects

of cell physiology, perhaps even those unrelated to microtubules. H1 carriers, according to this view, are prone to a wide spectrum of neurodegenerative disease due to relative underexpression of the protective exon 3+ splice variant of *MAPT* and overexpression of the disease-associated exon 10+ form, and this mechanistic model is confirmed by the observation that tau-positive tangles in other tau-related diseases, such as CBD and PSP, consist predominantly of exon 10+ (4R) tau protein lacking exon 3. Tau isoforms have specific cellular functions, including microtubule stabilization and spacing, cellular signalling, axonal transport and protein fibrillization, the harmful effect of a data isoform is related to the suboptimal long lasting functioning in that specific physiological process.

The results of this study lay for a role of the haplotype H1 *MAPT* throughout the course of the disease, acting with a degenerative pressure wedge over the years: risk factor for PD with an OR of ~ 1.5, a phenotype co-determining factor with a OR>2 and, finally, a robust genetic risk factor for dementia with an OR>10. To date, no pathological or functional studies have yet been made on the role of the different isoforms.

The initial results on the non-motor symptoms profile suggest a possible role of *MAPT* haplotype and tau in determining cardiac autonomic dysfunction, preceding the development of Dementia and without association of other non-motor symptoms. These

results represent a preliminary analysis and should be considered with caution. Nonetheless, this data are in line with pathological, clinical and experimental evidences that link cardiac denervation to PD severity and cognitive decline.

According to what we found, a biological issue has emerged linking motor severity of Parkinson's disease and, in a lesser extent, cardiac autonomic dysfunctions to the development of Dementia; it will be plausible theorize that dysfunction of tau can be considered as a primary target for research on pathophysiology and neuroprotective therapy.

In conclusion, the study of clinical and biological aspects of dementia in Parkinson's disease confirmed the complex phenomenology of this disease, but at the same time offered the possibility to extend the research on molecular aspects of the function of which is still poorly characterized.

The concept of "Parkinsonian Syndromes" in the term of a family of proteiform multiorgan degenerative diseases has reached increasing consistency.

The accomplishment of a good treatment strategy passes through the individuation of the different profile of motor, cognitive, psychiatric and medical impairment of the patients, the

so called tailored therapy. Finally, it is plausible to consider that researches for future neuroprotective treatment may be “adjusted” and refined in accordance to a possible role of tau protein in the overall weakening of metabolic reserve that increase the susceptibility to biochemical insults that are proper of the disease process.

References

Williams-Gray CH, Evans JR, Goris A, Foltynie T, Ban M, Robbins TW, Brayne C, Kolachana BS, Weinberger DR, Sawcer SJ, Barker RA.
The distinct cognitive syndromes of Parkinson's disease: 5 year follow-up of the CamPaIGN cohort.
Brain. 2009 Nov;132(Pt 11):2958-69

Aarsland D, Zaccai J, Brayne C.
A systematic review of prevalence studies of dementia in Parkinson's disease.
Mov Disord. 2005 Oct;20(10):1255-63.

Hely MA, Reid WG, Adena MA, Halliday GM, Morris JG.
The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years.
Mov Disord. 2008 Apr 30;23(6):837-44

Aarsland D, Andersen K, Larsen JP, Lolk A, Kragh-Sørensen P.
Prevalence and characteristics of dementia in Parkinson disease: an 8-year prospective study.
Arch Neurol. 2003 Mar;60(3):387-92

Irwin DJ, White MT, Toledo JB, Xie SX, Robinson JL, Van Deerlin V, Lee VM, Leverenz JB, Montine TJ, Duda JE, Hurtig HI, Trojanowski JQ.
Neuropathologic substrates of Parkinson disease dementia.
Ann Neurol. 2012 Oct;72(4):587-98

Kalaitzakis ME, Pearce RK.
The morbid anatomy of dementia in Parkinson's disease.
Acta Neuropathol. 2009 Nov;118(5):587-98

Bohnen NI, Albin RL.
White matter lesions in Parkinson disease.
Nat Rev Neurol. 2011 Apr;7(4):229-36

González-Redondo R, Toledo J, Clavero P, Lamet I, García-García D, García-Eulate R, Martínez-Lage P, Rodríguez-Oroz MC.
The impact of silent vascular brain burden in cognitive impairment in Parkinson's disease.
Eur J Neurol. 2012 Aug;19(8):1100-7

Aarsland D, Kurz MW.
The epidemiology of dementia associated with Parkinson disease.
J Neurol Sci. 2010 Feb 15;289(1-2):18-22

Tsuang D, Leverenz JB, Lopez OL, Hamilton RL, Bennett DA, Schneider JA, Buchman AS, Larson EB, Crane PK, Kaye JA, Kramer P, Woltjer R, Trojanowski JQ, Weintraub D, Chen-Plotkin AS, Irwin DJ, Rick J, Schellenberg GD, Watson GS, Kukull W, Nelson PT, Jicha GA, Neltner JH, Galasko D, Masliah E, Quinn JF, Chung KA, Yearout D, Mata IF, Wan JY, Edwards KL, Montine TJ, Zabetian CP.
APOE å4 increases risk for dementia in pure synucleinopathies.
JAMA Neurol. 2013 Feb;70(2):223-8

Morley JF, Xie SX, Hurtig HI, Stern MB, Colcher A, Horn S, Dahodwala N, Duda JE, Weintraub D, Chen-Plotkin AS, Van Deerlin V, Falcone D, Siderowf A.

Genetic influences on cognitive decline in Parkinson's disease.
Mov Disord. 2012 Apr;27(4):512-8

Mollenhauer B, Trenkwalder C, von Ahsen N, Bibl M, Steinacker P, Brechlin P, Schindehuette J, Poser S, Wiltfang J, Otto M.
Beta-amlyoid 1-42 and tau-protein in cerebrospinal fluid of patients with Parkinson's disease dementia.
Dement Geriatr Cogn Disord. 2006;22(3):200-8

Alves G, Brønnick K, Aarsland D, Blennow K, Zetterberg H, Ballard C, Kurz MW, Andreasson U, Tysnes OB, Larsen JP, Mulugeta E.
CSF amyloid-beta and tau proteins, and cognitive performance, in early and untreated Parkinson's disease: the Norwegian ParkWest study.
J Neurol Neurosurg Psychiatry. 2010 Oct;81(10):1080-6

Leverenz JB, Watson GS, Shofer J, Zabetian CP, Zhang J, Montine TJ.
Cerebrospinal fluid biomarkers and cognitive performance in non-demented patients with Parkinson's disease.
Parkinsonism Relat Disord. 2011 Jan;17(1):61-4

Caviness JN, Driver-Dunckley E, Connor DJ, Sabbagh MN, Hentz JG, Noble B, Evidente VG, Shill HA, Adler CH.
Defining mild cognitive impairment in Parkinson's disease.
Mov Disord. 2007 Jul 15;22(9):1272-7

Burton EJ, McKeith IG, Burn DJ, Williams ED, O'Brien JT.
Cerebral atrophy in Parkinson's disease with and without dementia: a comparison with Alzheimer's disease, dementia with Lewy bodies and controls.
Brain. 2004 Apr;127(Pt 4):791-800.

Beyer MK, Larsen JP, Aarsland D.
Gray matter atrophy in Parkinson disease with dementia and dementia with Lewy bodies.
Neurology. 2007 Aug 21;69(8):747-54.

Duncan GW, Firbank MJ, O'Brien JT, Burn DJ.
Magnetic resonance imaging: a biomarker for cognitive impairment in Parkinson's disease?
Mov Disord. 2013 Apr;28(4):425-38. doi: 10.1002/mds.25352. Epub 2013 Feb 28. Review

Melzer TR, Watts R, MacAskill MR, Pitcher TL, Livingston L, Keenan RJ, Dalrymple-Alford JC, Anderson TJ.
Grey matter atrophy in cognitively impaired Parkinson's disease.
J Neurol Neurosurg Psychiatry. 2012 Feb;83(2):188-94.

Dalaker TO, Zivadinov R, Larsen JP, Beyer MK, Cox JL, Alves G, Bronnick K, Tysnes OB, Antulov R, Dwyer MG, Aarsland D.
Gray matter correlations of cognition in incident Parkinson's disease.
Mov Disord. 2010 Apr 15;25(5):629-33.

Volumetric analysis of the substantia innominata in patients with Parkinson's disease according to cognitive status.
Choi SH, Jung TM, Lee JE, Lee SK, Sohn YH, Lee PH.
Neurobiol Aging. 2012 Jul;33(7):1265-72. doi: 10.1016/j.neurobiolaging.2010.11.015. Epub 2011 Jan 8.

Lee SJ, Kim JS, Yoo JY, Song IU, Kim BS, Jung SL, Yang DW, Kim YI, Jeong DS, Lee KS
Influence of white matter hyperintensities on the cognition of patients with Parkinson disease..
Alzheimer Dis Assoc Disord. 2010 Jul-Sep;24(3):227-33.

Chen-Plotkin AS, Hu WT, Siderowf A, Weintraub D, Goldmann Gross R, Hurtig HI, Xie SX, Arnold SE, Plasma epidermal growth factor levels predict cognitive decline in Parkinson disease.
Grossman M, Clark CM, Shaw LM, McCluskey L, Elman L, Van Deerlin VM, Lee VM, Soares H, Trojanowski JQ.
Ann Neurol. 2011 Apr;69(4):655-63.

Shin J, Choi S, Lee JE, Lee HS, Sohn YH, Lee PH.
Subcortical white matter hyperintensities within the cholinergic pathways of Parkinson's disease patients according to cognitive status.
J Neurol Neurosurg Psychiatry. 2012 Mar;83(3):315-21. doi: 10.1136/jnnp-2011-300872.

Bohnen NI, Müller ML, Zarzhevsky N, Koeppe RA, Bogan CW, Kilbourn MR, Frey KA, Albin RL.
Leucoaraiosis, nigrostriatal denervation and motor symptoms in Parkinson's disease.
Brain. 2011 Aug;134(Pt 8):2358-65.

Herman T, Rosenberg-Katz K, Jacob Y, Auriel E, Gurevich T, Giladi N, Hausdorff JM.
White matter hyperintensities in Parkinson's disease: do they explain the disparity between the postural instability gait difficulty and tremor dominant subtypes?
PLoS One. 2013;8(1):e55193. doi: 10.1371/journal.pone.0055193. Epub 2013 Jan 31

Hattori T, Orimo S, Aoki S, Ito K, Abe O, Amano A, Sato R, Sakai K, Mizusawa H.
Cognitive status correlates with white matter alteration in Parkinson's disease.
Hum Brain Mapp. 2012 Mar;33(3):727-39.

Matsui H, Nishinaka K, Oda M, Niikawa H, Kubori T, Udaka F.
Dementia in Parkinson's disease: diffusion tensor imaging.
Acta Neurol Scand. 2007 Sep;116(3):177-81

Jokinen P, Brück A, Aalto S, Forsback S, Parkkola R, Rinne JO.
Impaired cognitive performance in Parkinson's disease is related to caudate dopaminergic hypofunction and hippocampal atrophy.
Parkinsonism Relat Disord. 2009

Jokinen P, Scheinin N, Aalto S, Någren K, Savisto N, Parkkola R, Rokka J, Haaparanta M, Röyttä M, Rinne JO.
[(11)C]PIB-, [(18)F]FDG-PET and MRI imaging in patients with Parkinson's disease with and without dementia.
Parkinsonism Relat Disord. 2010 Dec;16(10):666-70. doi: 10.1016/j.parkreldis.2010.08.021.

Bohnen NI, Kaufer DI, Hendrickson R, Ivanco LS, Lopresti BJ, Constantine GM, Mathis ChA, Davis JG, Moore RY, Dekosky ST.
Cognitive correlates of cortical cholinergic denervation in Parkinson's disease and parkinsonian dementia.
J Neurol. 2006 Feb;253(2):242-7.

Hilker R, Thomas AV, Klein JC, Weisenbach S, Kalbe E, Burghaus L, Jacobs AH, Herholz K, Heiss WD.
Dementia in Parkinson disease: functional imaging of cholinergic and dopaminergic pathways.
Neurology. 2005 Dec 13;65(11):1716-2

Edison P, Rowe CC, Rinne JO, Ng S, Ahmed I, Kemppainen N, Villemagne VL, O'Keefe G, Någren K, Chaudhury KR, Masters CL, Brooks DJ.
Amyloid load in Parkinson's disease dementia and Lewy body dementia measured with [11C]PIB positron emission tomography.
J Neurol Neurosurg Psychiatry. 2008 Dec;79(12):1331-8.

Neumann J, Bras J, Deas E, et al. Glucocerebrosidase mutations in clinical and pathologically proven Parkinson's disease. *Brain* 2009; 132: 1783–94.

Muslimovic D, Post B, Speelman JD, Schmand B.
Cognitive profile of patients with newly diagnosed Parkinson disease.
Neurology. 2005 Oct 25;65(8):1239-45

Marini P, Ramat S, Ginestroni A, Paganini M.
Deficit of short-term memory in newly diagnosed untreated parkinsonian patients: reversal after L-dopa therapy.
Neurol Sci. 2003 Oct;24(3):184-5

Pfeiffer HC, Løkkegaard A, Zoetmulder M, Friberg L, Werdelin L.
Cognitive impairment in early-stage non-demented Parkinson's disease patients.
Acta Neurol Scand. 2013 Oct 11

Trabzuni, D. *et al. MAPT* expression and splicing is differentially regulated by brain region: relation to genotype and implication for tauopathies. *Hum. Mol. Genet.*

Rolinski M, Fox C, Maidment I, McShane R.
Cholinesterase inhibitors for dementia with Lewy bodies, Parkinson's disease dementia and cognitive impairment in Parkinson's disease.
Cochrane Database Syst Rev. 2012 Mar 14;3. Review

Seppi K, Weintraub D, Coelho M, Perez-Lloret S, Fox SH, Katzenschlager R, Hametner EM, Poewe W, Rascol O, Goetz CG, Sampaio C.
The Movement Disorder Society Evidence-Based Medicine Review Update: Treatments for the non-motor symptoms of Parkinson's disease.
Mov Disord. 2011 Oct;26 Suppl 3:S42-80. Review

Olde Dubbelink KT, Hillebrand A, Twisk JW, Deijen JB, Stoffers D, Schmand BA, Stam CJ, Berendse HW.
Predicting dementia in Parkinson disease by combining neurophysiologic and cognitive markers.
Neurology. 2013 Dec 18

Gao WJ, Krimer LS, Goldman-Rakic PS.
Presynaptic regulation of recurrent excitation by D1 receptors in prefrontal circuits.
Proc Natl Acad Sci U S A. 2001 Jan 2;98(1):295-300.

Cooper JA, Sagar HJ, Doherty SM, Jordan N, Tidswell P.
Sullivan EV. Diff erent eff ects of dopaminergic and anticholinergic therapies on cognitive and motor function in Parkinson's disease.
A follow-up study of untreated patients. *Brain* 1992; 115: 1701–25.

Tiraboschi P, Hansen LA, Alford M, et al. Cholinergic dysfunction in diseases with Lewy bodies. *Neurology* 2000; 54: 407–11.

Shimada H, Hirano S, Shinotoh H, et al. Mapping of brain acetylcholinesterase alterations in Lewy body disease by PET. *Neurology* 2009; 73: 273–78.

Kehagia AA, Murray GK, Robbins TW. Learning and cognitive flexibility: frontostriatal function and monoaminergic modulation. *Curr Opin Neurobiol* 2010; 20: 1–6.

Puschmann A.
Monogenic Parkinson's disease and parkinsonism: clinical phenotypes and frequencies of known mutations.
Parkinsonism Relat Disord. 2013 Apr;19(4):407-15. doi: 10.1016/j.parkreldis.2013.01.020. Epub 2013 Feb 23.Review.

DelleDonne A, Klos KJ, Fujishiro H, Ahmed Z, Parisi JE, Josephs KA, Frigerio R, Burnett M, Wszolek ZK, Uitti RJ, Ahlskog JE, Dickson DW.
Incidental Lewy body disease and preclinical Parkinson disease.
Arch Neurol. 2008 Aug;65(8):1074-80. doi: 10.1001/archneur.65.8.1074

Albert ML, Feldman RG, Willis AL.
The 'subcortical dementia' of progressive supranuclear palsy.
J Neurol Neurosurg Psychiatry. 1974 Feb;37(2):121-30