

## Diagnosing Progressive Supranuclear Palsy: Role of Biological and Neuroimaging Markers

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Received date: Aug 04, 2014, Accepted date: Nov 05, 2014, Published date: Nov 12, 2014

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### Abstract

Progressive Supranuclear Palsy (PSP) is a neurodegenerative disorders characterised by a kinetic-rigid syndrome with ocular motor dysfunction, postural instability, and frontal lobe and bulbar dysfunction. In most of the cases, especially at early disease stages, diagnosis is still challenging. PSP signs and symptoms may indeed overlap with both dementing neurodegenerative syndromes and movement disorders. In the last few years, a better definition of clinical picture along with the identification of biological and neuroimaging markers have increased diagnostic accuracy. In the present work, we reviewed the current literature on PSP diagnosis and the usefulness of potential diagnostic markers.

**Keywords:** Progressive supranuclear palsy; Biological markers; Neuroimaging

### Introduction

Progressive Supranuclear Palsy (PSP) is an adult-onset progressive neurodegenerative disorder leading to an akinetic-rigid syndrome with ocular motor dysfunction, postural instability, and frontal lobe and bulbar dysfunction [1]. PSP usually occurs in the sixth decade, with clinical features that are often subtle and difficult to distinguish from other neurodegenerative disorders such as Parkinson's Disease (PD), Dementia with Lewy Bodies (DLB), Multiple System Atrophy (MSA) and Cortico Basal Syndrome (CBS) [2]. Early diagnosis can hence be challenging and the definite diagnosis is frequently deferred for many months. Neuropathologically, PSP is characterized by accumulation of Tau protein, neurofibrillary tangles and tufted astrocytes [3] in subcortical areas with a variable involvement of brain cortex.

The aetiology still remains elusive, but genetic background has a key-role in disease pathogenesis. Although the majority of PSP cases are sporadic, recent studies have reported high familial aggregation in PSP patients, and it has been widely demonstrated that Microtubule Associated Protein Tau (MAPT) gene mutations are causative of rare monogenic autosomal dominant PSP [4,5]. In sporadic cases, genetic advances have further confirmed the role of MAPT in increasing disease risk, and the H1 MAPT haplotype has been consistently associated with PSP, while the H2 haplotype seems protective [6]. Conversely, no major environmental risk factors have been reported so far [7]. A proper evaluation of known susceptibility factors related to PSP pathogenesis may help in defining neuroprotective therapeutic approaches [8].

In most cases PSP presents clinically as Richardson's syndrome (RS), or Steele-Richardson-Olszewski syndrome, with prominent postural instability, supranuclear vertical gaze palsy and frontal dysfunction [9,10]. The classical description of RS, although, did not include all neuropathologically confirmed cases of PSP. To justify this

heterogeneity, PSP was recently divided clinically and pathologically into two main phenotypes: classical RS and PSP-parkinsonism (PSP-P), the latter characterised by bradykinesia, rigidity and sometimes tremor, which tends to be asymmetric and modestly responsive to levodopa therapy [11-13].

Clinical heterogeneity is furthermore augmented by PSP presenting as CBS (PSP-CBS), as pure akinesia with gait freezing (PSP-PAGF), or, rarely, as PSP associated to bvFTD (PSP-FTD) or progressive non-fluent aphasia (PSP-PNFA)[14]. These subtypes are not included in the currently used diagnostic criteria [9,15].

The National Institute of Neurological Disorders and Stroke and the Society for PSP (NINDS-SPSP) clinical diagnostic criteria were compiled to reliably identify patients who had underlying PSP-tau pathology [9,15]. Briefly, the NINDS-SPSP criteria depend on the identification of a progressive disorder with onset after age 40 combining the two cardinal features (i.e. postural instability with falls during the first year of the disease and slow vertical saccades or supranuclear gaze palsy). A list of exclusion criteria aims to sort out look-alike disorders [1,16]. The sensitivity, specificity, and positive predictive value of the NINDS-SPSP criteria have been evaluated retrospectively in a pathologically confirmed series of patients, showing specificity and sensitivity of clinician diagnosis, which can be as high as 90% [17]. Because the specificity and PPV of the probable NINDS-SPSP clinical criteria have been found to be near perfect, and the specificity and PPV of the possible criteria to be high, a redefinition of this set of criteria has been proposed [15].

Recently, the Neuroprotective and Natural History in Parkinson Plus Syndromes (NNIPPS) consortium has proposed modified clinical diagnostic criteria, aiming to facilitate the clinical diagnosis of PSP. Briefly, the NNIPPS criteria allow an age of onset after 30 and postural instability or falls within 3 years from disease onset [16,18].

A recent study has evaluated the efficacy of both criteria for the diagnosis of PSP. The authors conclude that the total NINDS-SPSP criteria, accepting both possible and probable for diagnosis, yielded the

highest sensitivity and might thus be most useful for routine clinical care. For scientific clinical trials, the NINDS-SPSP probable criteria might be preferred, possessing the highest positive predictive value and allowing, at present, the earliest, most specific diagnosis, thus allowing to include correct positives early on and to reliably exclude false positives [16].

Diagnosis according to clinical criteria is still hindered by low levels of specificity and sensitivity, because of clinical overlap with other neurodegenerative disorders characterised by Parkinsonism and dementia. These results should encourage the development of reliable biomarkers to increase diagnostic accuracy and improve early detection of disease, when neuropathologic burden of disease is not so severe as to prevent the action of upcoming disease-modifying therapies.

### Genetics in the Diagnosis of PSP

Both environmental and inherited factors contribute to the risk of developing PSP [19,20]. Large clinical series have observed a positive

family history for either parkinsonism or dementia in about a third of PSP patients[21,22], about 10% of which had an autosomal dominant inheritance pattern, thus genetic screening need to be considered in selected cases. Mutations within MAPT gene have been associated with PSP and other phenotypes within the FTL spectrum, such as frontotemporal dementia, PPA or CBS [4,5]. Tau is involved in microtubule assembly and stabilization, which is altered in the presence of known mutations. Tau protein is translated from a single gene named microtubule associated protein tau (MAPT) located on chromosome 17 q21.31. Its expression is regulated by an alternative splicing mechanism resulting in six isoforms in the human brain [23]. Disease-specific deposition of Tau isoforms has been identified, with either 3-repeat (3R) or 4-repeat (4R) Tau inclusions on the basis of exon 10 skipping [24-26].

Of the 44 pathogenic mutations in MAPT currently described (<http://www.molgen.ua.ac.be/FTDMutations/>) only 9 have been associated with a PSP phenotype [27-36] (see Table 1). Intriguingly, all MAPT mutations associated with PSP affect exon 10, increase the ratio of 4R Tau isoforms (Table 1) [4,5,25,31].

MAPT region	Mutation	Clinical features	Origin	Age at onset	Age at death	References
Exon 1	R5L	Falls, dysarthria, micrographia	U.S.A	62	67	
Exon 10	N279K	*1 bradyphrenia, parkinsonism, apathy	France	40	47	
		*2 apathy, attentive deficits		41	-	
		*3 parkinsonism with tremor, personality change, ocular apraxia, postural instability	Japan	42	54	
		*4 parkinsonism, gaze palsy, postural instability		44	56	
		*5 Parkinsonism, gaze palsy, falls		46	-	
		*6 Cognitive impairment, gaze palsy, postural instability, pyramidal signs		41	51	
		*7 Parkinsonism, gaze palsy, falls	42	54		
		*8 Parkinsonism, gaze palsy, falls, pyramidal signs	43	51		
	** 4 families with a common founder, 39 affected members; parkinsonism or dementia associated with behavioural changes	U.S.A (Caucasian)	32-58	6-9 y of duration		
	** 1 family, 3 affected members; parkinsonism or dementia associated with behavioural changes	France	38-45	6-8 y of duration		
L284R		*1 multiple falls with progressive dementia	UK (South England)	early-40s	48	
		*2 personality change, falls, progressive aphasia		mid-40s	52	
		*3 personality change, apathy, falls, dysarthria		43	47	
S285R		*1 Sporadic, supranuclear gaze palsy, bradykinesia, falls	Japan	46	49	

	ΔN296	*1 gaze palsy, cognitive deficits, emotional lability	Spain	38	-	
		*2 personality change, parkinsonism		39	-	
		*3 Personality change, cognitive impairment, gaze palsy, falls	Japan	44	-	
		*4 Dysarthria, anterocollis, falls, parkinsonism	Italy	39	-	
	P301L	*1 Parkinsonism not further characterized	Nederland	-	-	
	G303V	*1 Parkinsonism, falls, gaze palsy, dysarthria	Spain	37	45	
		*2 Not well characterized parkinsonism		41	44	
		*3 Not well characterized parkinsonism		late-30s	-	
		*4 Parkinsonism with tremor and rigidity	France	41	45	
		*5 Parkinsonism, gaze palsy, eye apraxia		37	40	
*6 Parkinsonism with dystonia, eye apraxia		51		57		
*7 Parkinsonism with dystonia, gaze palsy		39		44		
*8 Parkinsonism, gaze palsy, dysphagia		42		46		
*9 Parkinsonism with dystonia, gaze palsy		32	36			
S305S	*1 Apathy/cognitive deficits	Australia (Caucasian)	55	-		
	*2 Dementia, apathy, aphasia		48	51		
	*3 Clumsiness, dysarthria, rigidity		49	56		
Intron 10	10+3	*1 "atypical PSP-presentation" not further characterized	U.S.A.	47	48	
		*2 "atypical PSP-presentation" not further characterized		52	58	
	10+16	*1 Sporadic parkinsonism with fatigue, apathy, micrographia, falls	UK	40	45	

**Table 1:** MAPT mutation associated with clinical diagnosis of PSP. \*: studies with single patients descriptions; \*\*: studies with families descriptions

Despite the fact that gene mutations are absent in most patients, since 1997 a large number of studies showed a significant association of PSP with the MAPT locus [6,37-40]. The MAPT locus exists as two major haplotype groups termed as "H1" and "H2" in European populations, defined by at least 100 SNPs that are inherited in strong linkage disequilibrium with each other. Inheritance of two copies of the H1 haplotype (H1/H1) has been associated with a major genetic risk factor for PSP [6]. A large collection of pathologically confirmed PSP samples have also been used to fine map PSP risk on H1 chromosomes [40,41]. The most likely explanation of the association with the MAPT H1 haplotype and PSP is that variants in the H1/H2 haplotypes confer risk/protection against disease by altering

expression at the locus, with the H1 haplotypes expressing higher levels of MAPT [40,42].

Projections of population-attributable risk suggest that only about 60% of the risk of developing PSP can be accounted for by the MAPT H1 haplotype, suggesting there may be additional risk genes involved in PSP. In 2007 the first GWAS analysis on PSP patients identified a new locus on chromosome 11, starting from a small cohort of pathologically proven PSP patients [39]. This locus contains the DNA damage-binding protein 2 (DDB2) and lysosomal acid phosphatase 2 (ACP2) genes. Since DNA damage and lysosomal dysfunction have been implicated in ageing and neurodegenerative processes, both genes were viable candidates for conferring disease risk. Searching for

new candidate genes, Höglinger et al. performed a world-wide GWAS analysis in 2011 considering two different PSP groups of more than 1000 individuals each with pathologically and clinically-diagnosed PSP patients [43]. After confirming the MAPT locus, the study identified previously unidentified signals associated with PSP risk at STX6, EIF2AK3 and MOBP. These genes encode proteins for vesicle-membrane fusion at the Golgi-endosomal interface, for the endoplasmic reticulum unfolded protein response and for a myelin structural component, generating testable translational hypothesis, further extending the complex pathophysiology of PSP [43].

### Biological Markers in the Diagnosis of PSP

The CSF analyses represent the most direct and convenient means to study biochemical changes occurring in the central nervous system as they are directly related to specific pathogenetic mechanisms of neurodegeneration [44]. In Alzheimer's disease, CSF analyses show a typical decrease in amyloid  $\beta$ 42 (A $\beta$ 42), and an increase in tau (t-tau) and phospho-tau (p-tau) levels compared with controls and other neurodegenerative diseases [45-47]. Despite the fact that PSP is a primary tauopathy, most studies evaluating t-tau and p-tau CSF levels did not show any significant difference in PSP compared to controls or other parkinsonisms [47,48]. CSF A $\beta$  amyloid levels are also comparable between PSP and healthy controls in most but not all studies [47,49-52].

In addition, other CSF biological markers may be of help to exclude other neurodegenerative disorders. In PD and DLB, there is a consistent decrease in total alpha-synuclein levels and an increase in oligomeric or phosphorylated form [53,54], and the level of neurofilament light chain alone may differentiate between PD and other parkinsonisms [48], but cannot selectively recognise PSP [55,56]. Taken together, these results indicate that classical CSF levels have limited utility in distinguishing PSP from controls and other parkinsonian disorders [47,50,56-58].

To date, the most sensitive biomarker to detect PSP cases seems to be the evaluation of CSF full-length (55 kDa) and truncated (33 kDa) tau forms [59,60]. Using western blot and immuno-precipitation assays it has been demonstrated a reduction in 33 kDa/55 kDa in PSP, differentiating the condition from healthy controls, Alzheimer's disease and Parkinson's disease [59]. Interestingly, for the first time, the assay distinguished also PSP from bvFTD and Cortico-basal

degeneration, part of the complex FTL spectrum. These findings were reproduced by the same group in another larger cohort of patients [60]. More recently, by developing ELISA kit on tau fragments, Wagshal et al. [52] observed lower CSF N-terminal and C-terminal tau concentrations in PSP compared to controls and AD patients [61], thus confirming tau fragment abnormalities in PSP. Finally, Luk et al. showed a decrease in 4R-tau isoform in PSP and AD compared with CBS, PDD and healthy controls using an adapted immune-PCR procedure [62].

In the near future, providing a more reliable assay for tau fragments could be pivotal in order to extend these observations in larger clinical and pathological PSP sample.

### Neuroimaging in the Diagnosis of PSP

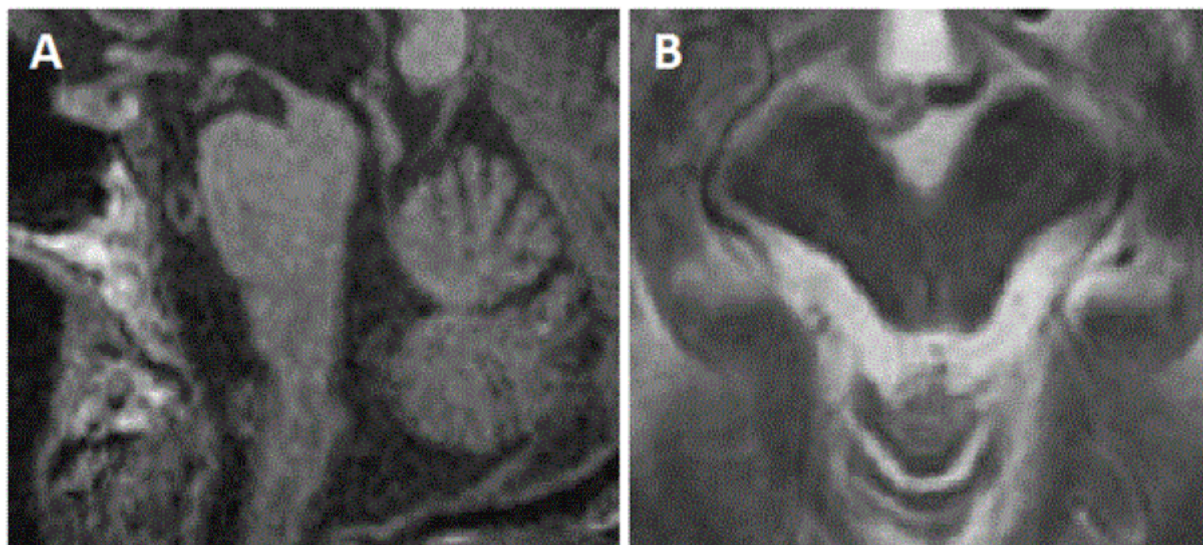
According to clinical research criteria, Magnetic Resonance Imaging (MRI) utility in PSP pathology is limited to the exclusion of relevant structural abnormalities, such as basal ganglia-brainstem infarcts or lobar atrophy [9]. However, different structural and functional techniques have been shown to be useful in PSP in vivo diagnosis, and could represent a first-phase approach when pathology exhibits an atypical presentation or in the first phases when differentiation from other movement disorders can be challenging.

#### Structural Imaging

Atrophy of the upper brainstem, of the superior cerebellar peduncles and dilatation of third ventricle are the radiological and pathological features of PSP [10,63,64].

Two useful "easy to assess" structural signs typical of PSP pathology have been described, namely the hummingbird [65] or giant penguin sign [66] and the morning glory sign [67]. The hummingbird sign is the expression of atrophy of the rostral tegmentum on mid-sagittal view, with sparing of the pons (Figure 1, panel A). Morning glory sign can be seen on axial images and is due to midbrain atrophy with concavity of the lateral margin of the midbrain tegmentum (Figure 1, panel B). The latter sign has been related to supranuclear gaze palsy and is more frequently observed in the RS. The sensitivity, specificity, and accuracy of radiological diagnosis of PSP are 72.7%, 94.3%, and 86.0%, respectively [68], thus being of help in clinical practice.





**Figure 1:** Neuroimaging features of PSP. Panel A: Hummingbird sign. Atrophy of upper midbrain (head with beak extending anteriorly towards the optic chiasm) with sparing of the pons (body of the bird). Panel B: Morning glory sign. Concavity of the lateral margin of midbrain tegmentum.

Beside conventional MRI, different quantitative measures of atrophy, to assess an early diagnosis in patients with atypical presentation, have been studied. The area of the midbrain in patients with PSP is about half of the area of the midbrain in patients with PD, MSA-P and age-matched controls. However, patients with MSA-P showed some overlap of individual areas with values from patients with PSP [66]. On the other hand, pons area is smaller in MSA-P, and a reduced midbrain/pons ratio has been proposed as a more sensitive quantitative marker of PSP [66], even in combination with CSF markers [69].

Despite that, other studies failed in replicating the stability of midbrain/pons ratio [70,71], and suggested other quantitative approaches, such as the MR parkinsonism index (MRPI, calculated by multiplying the pons area-midbrain area ratio by the middle cerebellar peduncle width-superior cerebellar peduncle width ratio, sensitivity and specificity 100%) [71]. Interestingly, it has been reported that an abnormal MRPI ( $> 13.55$ ) could also predict evolution of clinically unclassifiable parkinsonisms. Morelli found that 78.5% of patients with an abnormal MRPI developed PSP after 2 year-follow-up [72].

Other work evaluated white matter damage in PSP, as neuropathological studies indicated this as hallmark of the disorder. Both fractional anisotropy and mean diffusivity reveal a diffuse cerebellar, brainstem, cerebral, thalamic involvement [73-75], with a more frontal spatial distribution in the RS [76]. In particular, it has been reported that corpus callosum and superior cerebellar peduncle abnormalities can discriminate PSP from PD patients with high accuracy [77]. Studies with diffusion tensor imaging have identified extensive white matter lesions in corticobasal degeneration and progressive supranuclear palsy, showing strong diagnostic marker potential for these diseases [73-75].

## Functional Imaging

Functional imaging techniques employ tracers or blood-oxygen contrasts to measure changes in brain metabolism. In clinical practice, post-synaptic dopamine tracers or dopamine transporter (DaT SCAN) may assist in differentiating PD or neurodegenerative parkinsonisms from other neurodegenerative disorders, but this is not useful in the differential diagnosis among parkinsonisms [78-80].

Brain  $^{18}\text{F}$  fluorodeoxy glucose (FDG)-PET allows the characterization of PSP hypometabolism [81], with brainstem, medial thalamus, caudate nuclei and the medial frontal cortex involvement [82,83]. Moreover, in PSP patients, it may be of help in discriminating RS from PSP-P: when comparing RS to PSP-P, a reduced uptake in frontal and thalamic regions in the former group, and putaminal in the latter, was found. These patterns are in line with clinical presentation, with frequent cognitive decline in RS and predominant motor symptoms in PSP-P [84]. Only few studies explored functional connectivity in PSP pathology. This technique is promising as a very early marker of pathology but it cannot be still applied in single subject analysis [85,86].

An interesting and modern approach is represented by support vector machine analysis. This approach is based on algorithms able to automatically extract multiple information from image sets without requiring an a priori hypothesis of where this information may be coded. By combining different techniques, the automated algorithm allowed to discriminate correctly between PSP and PD with 100% accuracy, 90% sensitivity and 96% specificity. These results could be encouraging for computer-based diagnosis in the near future [87,88].

## Combined Markers in PSP

Despite the efforts to establish reliable clinical criteria, PSP diagnosis still represents a clinical challenge, because of clinical overlap with either tau or synuclein-based neurodegenerative

disorders that lead to parkinsonism and dementia [89]. Several studies have attempted to identify reliable techniques for improving PSP diagnosis, especially in early disease stages. As a result, different biological markers, neuroimaging techniques, and sonography studies have been proposed.

Research on Alzheimer's disease has long been taking advantage of different biomarkers and using them in combination to obtain higher levels of diagnostic accuracy. Conversely, in PSP, combined biomarkers are currently lacking. A recent study by Borroni et al. has sought to improve diagnostic accuracy by combined evaluation of biological and neuroimaging biomarkers. The authors, employing the CSF tau 33 kDa/55 kDa ratio [60] and MRI midbrain to pons measure, obtain significantly improved sensitivity and specificity for PSP detection, achieving diagnostic accuracy ranging from 86.3% to 92.5% in different comparison groups [90]. The use of combined biomarkers seems to be a rational and promising approach for enhancing diagnostic accuracy in identifying PSP, especially in the early stages of disease when clinical diagnosis is still challenging.

## Conclusions

In spite of the recent advances in the comprehension of the underlying pathophysiology of PSP, combined with an improvement of neuroimaging techniques and genetic analyses, the diagnostic accuracy of PSP remains low in some clinical settings. Structural, functional and metabolic imaging techniques show great potential in refining the diagnostic accuracy in atypical cases. On the other hand, as sustained by the great body of literature, CSF biomarkers will be chief in the differential diagnosis of neurodegenerative diseases. However, most of promising PSP CSF biomarkers have been evaluated by western- and immune-blot and a validation of reliable clinical assays (e.g. ELISA) in larger cohort will be pivotal in order to further understand their clinical relevance [52, 56]. Furthermore, there is a crucial need to diagnose neurodegenerative diseases early in their progression, when therapeutic approaches are likely to be most effective, to monitor disease progression and response to future treatments. The precise recognition of a specific molecular pathology in the preclinical or prodromal stages of disease will provide a proper diagnostic and therapeutic approach in early phases of disease where neuronal loss is not so severe as to be irreversible.

A limitation of many studies is that they are cross-sectional and retrospective, frequently lacking post-mortem neuropathological assessment. Hence, there is a necessity for prospective longitudinal studies with neuropathological confirmation to establish a better estimate of the accuracy of clinical diagnosis, and to determine whether additional investigations can improve diagnostic reliability, both individually and in combination.

## Acknowledgements

This work was supported by grant from Ministero della Salute RF-FSL-2008-1281075 to A.P. and M.D.L.

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