

## 5 Progressive Supranuclear Palsy Update

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### **Introduction**

Tauopathies constitute a group of neurodegenerative conditions characterised by the deposition of tau protein-containing neurofibrillary tangles. Cognitive impairment and extrapyramidal features are features common to the majority of tauopathies. Mutations in the tau gene (*MAPT*) or alterations in the expression of tau protein isoforms have been implicated in the molecular pathophysiology of these disorders. This, in turn, has led to a nosology of tauopathies, defined according to the nature of the predominant tau isoform, and explained in more detail below. Progressive supranuclear palsy (PSP, or Steele-Richardson-Olszewski syndrome), corticobasal generation and argyrophilic grain disease are thus classified as “four-repeat” tauopathies.

In its full-blown form, the clinical picture of PSP is highly characteristic. The patient has a fixed “Mona Lisa” stare, with a markedly reduced blink frequency. The head is retracted and the voice is reduced to a distinctive slurred growl. The patient walks clumsily and unsteadily (like a “drunken sailor”), with a notable tendency to topple backwards. Motor recklessness is often an early feature, leading to the highly distinctive “rocket sign” on rising from a chair. Clothes are soiled with spilled food, due an inability to look down at the plate and difficulties swallowing (the “messy-tie” sign). The time taken to respond to a question is prolonged, because of slow cognitive processing (bradyphrenia). There is sometimes palilalia or echolalia

This review will concentrate predominantly upon PSP, but it is important to recognise that both clinical phenotype and molecular mechanisms may overlap with other tauopathies.

### **Historical Perspective**

Charles Dickens may have been first to describe a subject with classical PSP in 1857 in his novel *The Lazy Tour of Two Idle Apprentices*:<sup>1</sup> “A chilled, slow, earthy, fixed old man. A cadaverous man of measured speech. An old man who seemed as unable to wink, as if his eyelids had been nailed to his forehead. An old man whose eyes - two spots of fire - had no more motion that [sic] if they had been connected with the back of his skull by screws driven through it, and riveted and bolted outside, among his grey hair.”

“He had come in and shut the door, and he now sat down. He did not bend himself to sit, as other people do, but seemed to sink bolt upright, as if in water, until the chair stopped him.”

From two daguerreotypes published in 1889 in the *Nouvelle Iconographie de la Salpêtrière* by Dutil, one of Charcot’s interns, a case of PSP may have been described as a “hemiplegic paralysis agitans with unusual postures of the trunk and head”.<sup>2</sup> Retrocollis and an eye movement disorder were prominent components of the clinical picture. Charcot’s celebrated case history and sketches of Bachere, reported as a case of Parkinson’s in extension, may be another missed early example.

It is of note that although MND and PSP were described within 20 years of each other, MND, with its accompanying distinctive pathology, was rapidly accepted by neurologists as a discrete morbid entity, while a full clinicopathological description of PSP had to wait almost another century. Mis-diagnosis of many cases of PSP as post-encephalitic parkinsonism (Von Economo’s disease) or inappropriate “lumping” with other atypical causes of Parkinsonism such as arteriosclerotic Parkinson’s Syndrome may be a partial explanation for this paradox.

In 1963, Dr J Clifford Richardson described eight patients with “a common syndrome of ocular, motor and mental symptoms” at the American Neurological Association (ANA) in Atlantic City. Richardson drew an analogy to this seemingly new condition with Von Economo’s disease and Asao Hirano, one of the discussants, was struck by its similarity to a new disorder being found amongst the indigenous Chamorros on the Mariana Islands (lytico-bodig).

More recently, Lees' group have described three distinct clinical phenotypes of PSP, based upon pathologically confirmed cases and retrospective notes review: Richardson's syndrome, PSP-parkinsonism and pure akinesia with gait freezing, thereby extending the clinical spectrum of the disorder and also increasing the challenge to clinicians for an accurate ante-mortem diagnosis.<sup>3,4</sup>

### ***Descriptive Epidemiological Studies***

Bower and colleagues studied the incidence of PSP over a 14 year period in Olmsted County, Minnesota.<sup>5</sup> Sixteen incident cases were identified and none had an age of onset before 50 years of age. The average annual incidence rate for ages 50 to 99 years was 5.3 per 100,000. There is no gender difference in susceptibility to PSP, while a recent study did not find any modifying influence of gender upon clinical features, including age at onset.<sup>6</sup>

Only three studies have directly addressed the prevalence of PSP.<sup>7-9</sup> Table 1 summarises these studies, together with other estimates of the prevalence of PSP. In the latter, standard diagnostic criteria were not used and the primary aim of the work was to determine the prevalence of Parkinson's disease. Seventeen cases of PSP were identified within the community study (population 259,998) of Nath *et al*, yielding an age-adjusted prevalence figure of 5.0 (95% CI 2.5-7.5) per 100,000, standardized to the hypothetical European population. When the Schrag data are standardized to the same population, an identical prevalence figure of 5.0 is obtained.

**Table 1 Prevalence Data for Progressive Supranuclear Palsy**

<b>Author</b>	<b>Year of Report</b>	<b>PSP Prevalence Primary</b>	<b>Geographical Area Studied</b>	<b>Population Denominator</b>	<b>Crude Prevalence (per 100,000)</b>
Golbe	1988	yes	New Jersey, USA	799,022	1.39
De Rijk	1995	no	Rotterdam, Netherlands	6969	14.3 *
Wermuth	1997	no	Faroe Islands	43,709	4.6
Chio	1998	no	Northwest Italy	61,830	3.2
Schrag	1999	yes	London & Kent, UK	121,608	4.9
Nath	2001	yes	Newcastle, UK	259,998	6.5

\*Only persons aged 55 years of age or older were included

A high prevalence of PSP has recently been reported in the French Antilles, with a minimum prevalence of 14 per 100,000 on the island of Guadeloupe.<sup>10</sup> Of 220 consecutive patients with Parkinson's syndrome examined, 58 had probable PSP, a further 96 had undetermined Parkinsonism (many of whom closely resembled the incomplete or atypical bradykinetic presentation of PSP), 50 had Parkinson's disease and 15 had an ALS-Parkinsonian syndrome. Pathological confirmation of PSP, with a major doublet of pathological tau at 64 and 69 kilodaltons in brain tissue homogenates (see below), has been found in all three of the probable PSP cases coming to post-mortem.

### ***Diagnostic Accuracy for PSP***

Age at disease onset for PSP characteristically occurs between 60 and 65 years, with no significant difference in the sex ratio. The median duration from disease onset to death is 5.8 to 5.9 years.<sup>11</sup> Mean interval from symptom onset to diagnosis ranges from 3.6 to 4.9 years, indicating that many patients with PSP may remain mis-diagnosed for much of their disease course.<sup>7</sup>

Primary care diagnoses on hospital referral are protean, and include Parkinson's disease (30%), "balance disorders" (20%), stroke (10%) and depression (7%).<sup>12</sup> Combining the studies of Schrag and the methodologically similar community-based component of the Nath study, a total of 23 PSP cases

were identified.<sup>8, 13</sup> Of these, only ten patients (43%) carried a primary referral diagnosis of PSP. In the remainder, Parkinson's disease and cerebrovascular disease accounted for all but one of the misdiagnosed cases.

A recent study of the diagnostic accuracy for PSP in the Society for PSP brain bank indicated that, of 180 cases referred with a clinical diagnosis of PSP, 137 had this confirmed pathologically, while 43 (24%) had other pathological diagnoses.<sup>14</sup> Corticobasal degeneration (CBD), multiple system atrophy (MSA) and dementia with Lewy bodies accounted for 70% of misdiagnosed cases. A history of tremor, psychosis, dementia and asymmetric findings were more frequent in misdiagnosed cases. In another clinicopathological study, the clinical diagnosis of PSP was confirmed in 78% of 60 patients, with MSA, DLB and PD making up the majority of mis-diagnoses.<sup>15</sup>

### ***Diagnostic Criteria and Clinical Heterogeneity***

Postural instability, leading to falls (typically backwards) within the first year of disease onset, coupled with a vertical supranuclear gaze paresis have good discriminatory diagnostic value when PSP is compared with other degenerative parkinsonian syndromes.<sup>16</sup> The National Institute of Neurological Disorders and Stroke and Society for Progressive Supranuclear Palsy, Inc., NINDS-SPSP diagnostic criteria (Table 2) are heavily reliant upon these clinical features, together with the fact that no pathologically confirmed PSP case has had a disease onset below the age of 40.<sup>17</sup> When applied retrospectively to a case mix comprising various parkinsonian syndromes the NINDS-SPSP criteria have high diagnostic sensitivity and specificity.<sup>18</sup> These parameters have not, however, yet been determined for prospective series with pathological correlation, nor have they been applied retrospectively to an independent clinicopathological series. One area where the NINDS-SPSP criteria would be predicted to have lower sensitivity is when the development of "core" diagnostic features is delayed.

PSP patients display significantly more apathy and disinhibition than PD cases. A "frontal" presentation is recognised in approximately 20% of PSP cases, with increased latency to diagnosis compared with other presentations and reduced initial diagnostic accuracy.<sup>19</sup> Neuropsychological assessment in the early stages may assist the accurate clinical diagnosis of a parkinsonian disorder, as may the time course and pattern of progression of cognitive and behavioural decline.<sup>20</sup> In particular, patients with PSP show a greater decline in attention, set-shifting and categorization abilities, compared with PD and MSA. Patients with PSP also show greater impairment in both phonemic and semantic fluency than patients with MSA or PD. Using discriminant function analysis, variables derived from four verbal fluency tasks (simple and alternate semantic and phonemic fluency) were able to correctly classify over 90% of PSP patients.<sup>21</sup>

Retrospective clinicopathological studies suggest that there are at least three main phenotypic variants of PSP: Richardson's syndrome is the classic disorder, described above. A clinical phenotype of PSP, labelled PSP-P has been described in which the disease duration is longer (9.2 years compared with 5.9 years for classic "Richardson"-type PSP).<sup>3</sup> Falls and supranuclear gaze paresis are by no means invariable in PSP-P and their appearance may be delayed, adding to the diagnostic conundrum. Furthermore, asymmetric onset and L-dopa responsiveness, previously considered highly atypical for PSP, occurred in 81% and 52%, respectively, of PSP-P cases.<sup>3</sup> Pure akinesia with gait freezing is an uncommon third phenotype of PSP characterized by difficulty initiating gait and "freezing" during walking, writing and speaking. Although not specific for PSP, this phenotype has a high predictive value for tau pathology.<sup>4</sup>

Additional rare "atypical" phenotypic variants of PSP add to the difficulty of accurate diagnosis. Unilateral limb dystonia, arm levitation, ideomotor apraxia, and palatal myoclonus, have all been described in PSP, sometimes early in the disease course. Conversely, cases of fronto-temporal dementia linked to chromosome 17 (tau exon 10+16 mutation,<sup>22</sup> Whipple's disease,<sup>23</sup> neurosyphilis,<sup>24</sup> CADASIL<sup>25</sup> and primary antiphospholipid antibody syndrome<sup>26</sup> may present with a PSP phenotype. In a clinicopathological study, based in a specialist movement disorders service, 19 of 143 cases of parkinsonism were pathologically confirmed as PSP. Ante-mortem clinical diagnosis was correct in 16 of these cases, while MSA, PD and "parkinsonism undetermined" constituted the three misdiagnoses.<sup>27</sup>

**Table 2 NINDS-SPSP Diagnostic Criteria for PSP<sup>17</sup>**

<b>PSP</b>	<b>Mandatory inclusion criteria</b>	<b>Mandatory exclusion criteria</b>	<b>Supportive criteria</b>
<b>Possible</b>	<p>Gradually progressive disorder</p> <p>Onset age 40 or later</p> <p><i>Either</i> vertical supranuclear palsy <i>or</i> both slowing of vertical saccades &amp; postural instability with falls &lt; 1 year disease onset</p> <p>No evidence of other diseases that could explain the foregoing features, as indicated by exclusion criteria</p>	<p>Recent history of encephalitis</p> <p>Alien limb syndrome, cortical sensory deficits, focal frontal or temporoparietal atrophy</p> <p>Hallucinations or delusions unrelated to dopaminergic therapy</p> <p>Cortical dementia of Alzheimer type</p> <p>Prominent, early cerebellar symptoms or unexplained autonomic dysautonomia</p>	<p>Symmetric akinesia or rigidity, proximal more than distal</p> <p>Abnormal neck posture, especially retrocollis</p> <p>Poor or absent response of parkinsonism to levodopa</p> <p>Early dysphagia &amp; dysarthria</p> <p>Early onset of cognitive impairment including &gt; 2 of: apathy, impairment in abstract thought, decreased verbal fluency, utilisation or imitation behaviour, or frontal release signs</p>
<b>Probable</b>	<p>Gradually progressive disorder</p> <p>Onset age 40 or later</p> <p>Vertical supranuclear palsy <i>and</i> prominent postural instability with falls &lt; 1 year disease onset</p> <p>No evidence of other diseases that could explain the foregoing features, as indicated by exclusion criteria</p>		
<b>Definite</b>	<p>Clinically probable or possible PSP <i>and</i> histopathological evidence of typical PSP</p>		

### ***Clinical Assessment***

A PSP Rating Scale (PSPRS) produces a score of 0 to 100, with 0 representing “normal”.<sup>28</sup> In this scale 28 items are sub-divided into six categories (daily activities, behavioural symptoms, bulbar symptoms, oculomotor deficits, limb motor deficits, gait and midline deficits). The rating takes approximately 10 minutes to perform and appears to have excellent inter-rater reliability. Scores increased at a mean rate of one point per month in a sample of 162 PSP cases, while the baseline score was a robust predictor of survival. The validity of the PSPRS needs to be established across multiple centres and in different clinical settings, but it represents a welcome addition to standardising the clinical assessment of PSP.

In addition to the PSPRS, a disease-specific quality of life scale, the PSP-QoL is also now available.<sup>29</sup> This comprises a 45-item self-completed questionnaire, sub-divided into mental and physical health domains. The PSP-QoL appears to be valid, with high reliability and small ceiling and floor effects. Its psychometric properties were similar in clinic and community-based samples. Further evaluation

to assess the utility of this measure in longitudinal studies, and its sensitivity to change will be of value.

## ***Investigations***

The diagnosis of PSP still rests on the clinical history and examination. Attempts have been made, however, to improve diagnostic accuracy through cerebrospinal fluid analysis and protein biomarkers, structural and functional imaging and neurophysiological techniques.

### **Cerebrospinal Fluid Analysis (CSF)**

Studies have attempted to identify biomarkers in the CSF to achieve early and accurate diagnosis, as well as monitoring response to treatment. CSF amyloid A- $\beta$ 42 levels are normal in PSP and do not discriminate this condition from PD.<sup>30</sup> Tau has potential as a candidate protein, although may lack specificity unless isoform analysis can also be performed (see below). Significantly higher tau protein levels in CSF have been reported in corticobasal degeneration, compared with PSP, yielding sensitivities and specificities of 100% and 87.5%, respectively.<sup>31</sup> Other proteins in the CSF, including neurofilament protein (NFL) and glial fibrillary acidic protein (GFAP) have been studied. Whereas no difference was found in CSF GFAP levels between PD, MSA and PSP, high NFL concentrations differentiated typical from atypical parkinsonian disorders.<sup>32</sup> The overlap in ranges, however, limit the sensitivity of this technique, and it was not possible to differentiate MSA from PSP cases. The concomitant use of a levodopa test in combination with CSF NFL assay may improve diagnostic accuracy for atypical parkinsonism to 90%.<sup>33</sup> Major products of lipid peroxidation are selectively increased in PSP midbrain tissue, suggesting that a CSF assay for these products could provide a specific biomarker.

### **Magnetic Resonance Imaging (MRI)**

Schrag reported that over 70% of patients with clinically diagnosed PSP could be correctly classified on the basis of 0.5-T or 1.5-T MRI brain scanning.<sup>34</sup> Criteria used for the diagnosis of PSP included midbrain diameter on axial scans of less than 17 mm, signal increase in the midbrain, atrophy or signal increase of the red nucleus and signal increase in the globus pallidus. No PSP patient was misclassified. Other studies have also suggested that reduced midbrain diameter on routine MRI may be of value in discriminating PSP from PD and MSA-P, although values may overlap with MSA-P and do not clearly correlate with disease duration or severity.<sup>35</sup> Atrophy or abnormal signal of the superior cerebellar peduncle on proton-density-weighted MRI, postulated to represent demyelination and gliosis, may help in differentiating PSP from PD.<sup>36, 37</sup> Pathological data indicate that atrophy of this structure is a relatively early feature of PSP and correlates with disease duration.<sup>38</sup> In all radiological studies, clinically “typical” PSP cases were selected, rendering the discriminatory value of MRI something of a tautology. The value of routine MRI scanning in longitudinal clinicopathological studies, particularly in early indeterminate cases, has not been investigated.

Magnetic resonance imaging-based volumetry (MRV) has also been used to differentiate PSP from other parkinsonian syndromes, by examining atrophy of the caudate nucleus, putamen, brainstem and cerebellum.<sup>39</sup> Voxel-based morphometry has demonstrated regions of reduced grey matter in PSP compared with controls, with specific involvement of the colliculae, hippocampal structures, precentral-premotor cortex, thalamus and orbitofrontal cortex.<sup>40</sup> Using longitudinal MRI imaging, the annualised rate of whole brain atrophy in PSP is three times the rate seen in healthy age-matched controls, while the midbrain atrophy rate is seven times more rapid in PSP.<sup>41</sup> Diffusion tensor MR imaging also suggests that changes in fractional anisotropy affecting white matter tracts may be help in differentiating PSP from other parkinsonian disorders.<sup>40, 42</sup>

Proton magnetic resonance spectroscopy may provide an indirect measure of neuronal loss *in vivo*. There have been a number of reports of the use of this technique in PSP, concentrating mainly upon spectral changes in the lentiform nucleus. In general, whilst lentiform N-acetylaspartate/choline and/or N-acetylaspartate/creatine ratios may be reduced in PSP, the discriminatory value of this technique on an individual basis remains unproven. A recent systematic review of proton magnetic resonance spectroscopy in parkinsonian syndromes concluded that the heterogeneity of the results to

date precludes the use of any of these findings in differential diagnosis at the present time.<sup>43</sup>

Apparent diffusion coefficient measurements using diffusion-weighted magnetic resonance imaging (DWI) may discriminate PSP from PD with a sensitivity of 90% and a positive predictive value of 100%, significant increases in regional apparent diffusion coefficients being noted in striatum and globus pallidus in PSP cases.<sup>44</sup> Importantly, DWI could not discriminate PSP from MSA-C in this study.

## Functional Imaging

Blood flow and oxygen or glucose metabolism positron emission tomography (PET) studies in PSP subjects have demonstrated relative frontal lobe hypometabolism, although bi-frontal hypoperfusion, using the more widely available <sup>99m</sup>Tc-HMPAO single photon emission computed tomography technique (SPECT) is not a robust finding in PSP. Evaluation of the nigrostriatal dopaminergic system using cocaine analogues and functional imaging (PET or SPECT) can reliably show presynaptic dopaminergic degeneration in PSP and also demonstrate progression of this degeneration. Unfortunately, the pattern of abnormality is non-specific and cannot differentiate PSP from other parkinsonian syndromes,<sup>45</sup> even when used in combination with a dopamine D<sub>2</sub> receptor ligand.<sup>46</sup>

The lack of specificity in functional imaging of the dopaminergic system has led to the study of other neurotransmitter systems. [<sup>11</sup>C]flumazenil and PET has been used to image benzodiazepine receptors in PSP.<sup>47</sup> Other than a modest reduction in binding in the anterior cingulate gyrus when compared with normal controls, no other regional abnormality was detected. Although benzodiazepine binding is reduced in the globus pallidus of post mortem tissue samples from PSP patients and preserved in the striatum, the low level of the receptors in the pallidum and the proximity of the putamen indicate functional imaging of the benzodiazepine receptor is too insensitive to detect these differences.

Analogues of vesamicol, an inhibitor of the acetylcholine vesicular transporter, demonstrate loss of intrinsic striatal cholinergic neurons,<sup>48</sup> while the use of *N*-methyl-4-[<sup>11</sup>C]piperidyl acetate and PET to determine acetylcholinesterase activity has demonstrated a preferential loss of cholinergic innervation to the thalamus in PSP, compared with PD and controls.<sup>49</sup>

*In vivo* imaging of activated microglia, using [<sup>11</sup>C]PK11195 and PET in two PSP cases has revealed increased binding in the lentiform nucleus and pons, in particular, although dorsolateral prefrontal cortex, caudate, substantia nigra and thalamus were also involved.<sup>50</sup> The significance of this preliminary finding is uncertain, although it raises the possibility of being able to monitor disease activity.

(123)I-metaiodobenzylguanidine (MIBG) scintigraphy visualizes catecholaminergic terminals *in vivo* and is used as a biomarker to detect cardiac sympathetic degeneration. Cardiac MIBG uptake in PSP is significantly higher than in PD. Normal MIBG uptake in PSP is not invariable, however, as there can be slight reductions compared with normal controls.<sup>51</sup>

## Neurophysiological Techniques

A variety of neurophysiological techniques have been used to study PSP, both with the aim of improving diagnostic accuracy and also improving understanding of the underlying pathophysiological process. A longitudinal oculomotor study of patients with PSP and other parkinsonian syndromes suggested that electro-oculography may help diagnose PSP earlier. PSP patients display decreased saccadic velocity throughout their disease course, and have less specific findings of frequent square wave jerks and increased error rate on anti-saccade tasks.<sup>52</sup> Significant orthostatic hypotension is rare in PSP, in contrast to MSA, with only minor and inconsistent abnormalities on formal assessment of sympathetic and parasympathetic functions. There is a normal rise in growth hormone following clonidine administration in PSP patients.<sup>53</sup> Sphincter electromyography may differentiate atypical akinetic-rigid syndromes from Parkinson's disease, but fails to reliably discriminate between PSP and multiple system atrophy.<sup>54</sup> Neuronal loss in Onuf's nucleus of the sacral spinal cord in PSP could explain this lack of specificity.<sup>55</sup> The auditory startle response is delayed or absent in PSP and without habituation although there is overlap with values obtained from other akinetic-rigid syndromes. Abnormalities in visual event-related potentials (P300

amplitude and reaction times to rare target stimuli), somatosensory evoked potentials (enlarged cortical SEPs), and pattern of facial reflexes (EMG activity in mentalis but not orbicularis oculi muscles following electrical stimulation of the median nerve at the wrist) have all been reported in PSP patients. The clinical utility of these investigations remains uncertain, as does their pathophysiological significance. Computerized posturography testing may differentiate early PSP from early PD and age-matched controls.<sup>56</sup> Seventy-five per cent of the 20 PSP patients met “all optimal criteria” for PSP, according to the NINDS-SPSP criteria, implying early postural instability and falls within the first year of disease onset. The sensitivity and specificity of this technique in a group of prospectively followed “indeterminate” parkinsonian cases would be of interest.

## ***Pathology***

PSP is characterised pathologically by the destruction of a number of sub-cortical structures including the substantia nigra, globus pallidus, subthalamic nucleus and midbrain and pontine reticular formation.<sup>57</sup> Deeper cortical layers, especially around the pre-central gyrus, may also be affected to a lesser degree. Large numbers of neurofibrillary tangles (NFTs, made up of ultramicroscopic straight filaments), neuropil threads and tufted astrocytes are also found within these brain regions. These distinctive histopathological inclusions are made up of insoluble aggregates of tau phosphoprotein. Tau-positive glial inclusions are also a consistent feature in the brain of patients with PSP. They are classified according to their cellular origin: astrocytic (fibrillary or protoplasmic) and oligodendrocytic. “Coiled bodies” are small round cells of oligodendrocytic origin found in white matter, underlining the widespread nature of the pathology in PSP.<sup>57</sup>

Williams and colleagues have proposed a simplified system for grading the severity of tau pathology in PSP.<sup>58</sup> The mean severity of pathology in all regions examined of the Richardson syndrome group was higher than in PSP-P and pure akinesia with gait freezing groups, while the overall tau load was significantly higher in RS than in PSP-P. Using only the grade of coiled body plus thread lesions in the substantia nigra, caudate and dentate nucleus, a reliable and repeatable 12-tiered grading system was established. PSP-tau score negatively correlated with disease duration and time from disease onset to first fall.

## **Tau Aggregation and Cell Death**

Tau is critically important for the dynamic behaviour and stabilisation of microtubules in the cytoskeleton. In normal neurons, tau is soluble and binds reversibly to microtubules, while in PSP the protein loses its affinity for microtubules and becomes resistant to proteolysis. A number of post-translational processes may be involved in the aggregation of tau in PSP, but glycation and transglutamination have been principally implicated. The former process leads to the formation of advanced glycation end-products (AGEs), detected histochemically in NFTs.<sup>59</sup> Tissue transglutaminase (TGase) is a calcium activated enzyme which cross-links substrate proteins into insoluble, protease-resistant complexes, potentially initiating NFT formation. By altering the conformation of tau, TGase may render digestion sites inaccessible to proteases. In support of a pathogenic role for TGase, high levels of epsilon-(gamma-glutamyl) lysine cross-linked tau, together with increased TGase and mRNA levels for TGase 1 and 2 have been found in the pallidum and pons in PSP.<sup>60</sup> Antibodies capable of detecting nitrated tau have also shown labeling in the glial and neuronal tau of PSP, in addition to other tauopathies, implying that nitrative injury might also be involved.<sup>61</sup>

The mechanism leading to cell death in PSP is unknown but is likely to be multifactorial, with both environmental (toxic) and genetic influences playing a role. Microglial activation is greater in PSP than in control brains, and microglial activation correlates with tau burden in most areas. There is also accumulating evidence for oxidative stress and mitochondrial dysfunction in PSP.<sup>62</sup> In trans-mitochondrial cytoplasmic hybrid (cybrid) cell lines expressing mitochondrial genes from persons with PSP, complex I activity was significantly reduced compared with controls. It is not yet known whether the mitochondrial dysfunction is of toxic or genetic origin. In addition, tau-positive astrocytes may exert neurotoxicity through the overproduction of nitric oxide, in excess of the detoxification capacity of superoxide dismutase. The formation of AGE-tau, detectable in NFTs, is

also associated with the generation of oxygen free radicals and the induction of oxidative stress. Most recently, it has been proposed that the phosphorylation of tau in PSP and Pick's disease is a direct consequence of the oxidative-stress induced activation of mitogen-activated protein kinases, including the p38 pathway (phosphor-MKK6 and phosph-p38).<sup>63</sup>

The consumption of tropical plants and herbal teas has been linked with an abnormally high frequency of a levodopa-resistant form of parkinsonism, clinically and pathologically resembling PSP, in Guadeloupe (French West Indies).<sup>64</sup> Stabilisation or even improvement in some symptoms has been reported after cessation of consumption of these fruits and infusions. Moreover, when mesencephalic dopaminergic neurons are exposed in culture to corexime and reticuline, the most abundant subfractions of *Annona muricata* (corossol, soursop), apoptotic cell death occurs.<sup>65</sup> Cell death in these cultures seems independent of excitotoxic mechanisms, although energy depletion has been implicated.

### ***Familial PSP***

Although PSP is considered to be a late-onset, sporadic neurodegenerative disease, a number of families with sometimes heterogeneous clinical presentation have been described. Post-mortem confirmation of diagnosis in at least one member of the family has been obtained in many of these reports. In a report of 12 pedigrees, the presence of affected members in at least two generations in eight of the families and the absence of consanguinity suggested autosomal dominant transmission with incomplete penetrance.<sup>66</sup>

In 2005 Ros reported the linkage of a large Spanish family with typical autosomal dominant PSP to a new locus in chromosome 1.<sup>67</sup> Four members of this family had typical PSP, confirmed by neuropathology in one case. At least five ancestors had similar disease. The condition was linked to an area on chromosome 1q31.1 containing at least three genes whose relevance in PSP is unknown.

One clinical study yielded the intriguing observation that 39% of 23 asymptomatic first degree relatives of patients with PSP scored abnormally on a Parkinson's disease test battery, compared with none of 23 age-matched normal controls.<sup>68</sup> The authors suggested that the test battery could have detected an asymptomatic carrier state or risk for PSP, or a subclinical effect of a shared environmental exposure. Further evidence for subclinical cases comes from PET studies using <sup>18</sup>F-dopa and <sup>18</sup>fluorodeoxyglucose.<sup>69</sup> Four of 15 asymptomatic relatives scanned from two kindreds with familial PSP had abnormal striatal <sup>18</sup>F-dopa uptake and a fifth subject showed significant reduction in cortical and striatal glucose metabolism.

The relative rarity of familial PSP cases may be due to a failure to recognize atypical cases, other diagnostic problems or death of the carriers before the appearance of clinical symptoms. The use of neurophysiological and/or imaging techniques to detect presymptomatic cases could assist in linkage analysis of potential PSP families, with a view to identifying causative genes. Conversely, families with phenotypically "characteristic" PSP may have been reported where molecular pathology would be indicative of another neurodegenerative condition. Frontotemporal dementia-parkinsonism (FTDP-17), for example, is clearly linked to mutations in the tau gene (see below) and may mimic PSP clinically.

Most recently, Kaat and colleagues compared the occurrence of dementia and parkinsonism among first-degree relatives of patients with PSP with an age- and sex-matched control group.<sup>70</sup> Fifty-seven (33%) of 172 patients with PSP had at least one first-degree relative who had dementia or parkinsonism compared to 131 (25%) of the control subjects (OR 1.5, 95% CI 1.01-2.13). In patients with PSP, more first-degree relatives with parkinsonism were observed compared to controls, with an OR 3.9 (95% CI 1.99-7.61). Twelve patients with PSP (7%) fulfilled criteria for an autosomal dominant mode of transmission. The intra-familial phenotype within these pedigrees varied among PSP, dementia, tremor, and parkinsonism. These results suggest familial aggregation of PSP.

### ***Molecular Pathology***

The human tau gene is located on chromosome 17q21 and contains 16 exons. Six different isoforms of tau are found in the human brain, generated by alternate splicing of exons 2, 3 and 10. These

isoforms can be divided into two groups of three, differing in the presence of three or four repeated microtubule-binding domains (three-repeat or four-repeat tau). The isoform is determined by whether the transcript of exon 10, a 31 amino acid repeat located in the C-terminal part, is spliced in or out of the final tau protein product. In normal brain, there is a slight preponderance of three-repeat tau, while in PSP the ratio is at least 3:1 in favour of four-repeat tau. This isoform ratio contrasts with Alzheimer's disease in which the paired helical filaments contain both three- and four-repeat tau and also with Pick's disease where only three-repeat tau is present.<sup>57</sup> Although many of the cases of bodig (parkinson-dementia complex of Guam) bear close clinical and biological similarity to PSP, a triplet band identical to Alzheimer's disease is found on immunoblotting, thereby distinguishing the two conditions. Some of the clinically "atypical PSP" cases recently reported by Morris and colleagues were also found to have a triplet band raising the possibility that bodig may not be a disorder restricted to a few geographic isolates.<sup>71</sup> These differences may help the neuropathologist to categorise and distinguish the tauopathies, since electrophoresis reveals two main protein bands of 64 and 68kDa in PSP and CBD, whereas Pick's disease has two lighter bands of 55 and 64kDa.

The discovery of mutations in the tau protein gene on chromosome 17 in some families with FTDP-17 confirmed that tau dysfunction can lead to neurodegeneration. In some of these families, the three to four-repeat tau ratio is similar to that found in PSP. A number of different mutations have now been found in FTDP-17 families, in and near the 5' splice site, downstream of exon 10.<sup>72</sup> Through disruption of a stem-loop structure formed in pre-mRNA, 5' splice site mutations increase recognition of exon 10 by U1 snRNP splicing factor, increasing the proportion of exon 10+ mRNA and thus four-repeat tau. Analysis of FTDP-17 families would support this "stem-loop hypothesis" and its pathogenicity, in that mutations with the greatest effect on splicing *in vitro* cause an earlier age of disease onset. Disruption of the stem-loop structure need not, of course be only genetic in origin and toxic causes could also be involved.

There is some evidence from analysis of tau mRNA in affected brain regions in PSP that selective four-repeat tau deposition in PSP may also involve disruption of exon 10 alternative splicing. Furthermore, there have now been two reported families with a clinical syndrome resembling PSP where mutations in the tau gene have been detected.<sup>73, 74</sup> In the first, Australian kindred, a "silent" mutation (S305S) was identified in the stem-loop structure. Although not producing an amino acid substitution (hence "silent"), functional exon-trapping experiments suggested that the mutation caused up to a 5-fold increase in splicing of exon 10, resulting in over-expression of four-repeat tau. In the second, Spanish kindred, two brothers born from a third degree consanguineous marriage were both affected by clinically atypical PSP. Both cases had an age of disease onset below the age of 40, a history of cocaine abuse asymmetric parkinsonism and reduced saccadic speed, while neither had an ophthalmoparesis. In one of the two cases, a homozygous deletion at codon 296 (delN296) was identified, lying within the sequence corresponding to the second tubulin repeat of tau protein. The clinical phenotype of these siblings closely resembled that described in a familial tauopathy with a N279K mutation, where cases developed parkinsonism, supranuclear gaze paresis and dementia in their fifth decade.<sup>75</sup> The nosology of these "familial PSP" mutations remains a matter of debate, and at this point it may be better to classify them as familial tauopathies. It is likely that further sporadic cases clinically resembling young onset PSP will be found to have tau mutations. However, a sequence analysis of tau exons 9-13 in two small families with PSP and seven clinically typical and atypical sporadic PSP cases with pathological confirmation of diagnosis has not identified coding or splice site mutations, suggesting that PSP or typical PSP-like syndromes are not due to mutations in tau.<sup>76</sup>

Conrad and colleagues first reported a polymorphic dinucleotide repeat sequence in intron 9 (between exons 9 and 10) of the tau gene in which the A0 allele (TG repeat number of 11), and in particular the A0/A0 genotype, were over-represented in PSP cases compared with controls in the white population.<sup>77</sup> These data were later extended to a haplotype, H1, including several polymorphisms in linkage disequilibrium with A0, spanning the tau gene.<sup>78</sup> During evolution of the two human *tau* haplotypes, H1 and H2, almost no recombination has occurred between the two alleles. Although the H1 allele has a frequency approaching 100% in pathologically confirmed patients with PSP it is also found in about 70% of controls. It is not known at the present time whether there is a rarer mutation

on the H1 haplotype predisposing to PSP, or whether it is the haplotype itself. The presence of an H1 haplotype or H1/H1 genotype may therefore be regarded as no more than a modest genetic predisposition towards developing PSP. Furthermore, almost all normal Japanese carry the H1/H1 genotype. The H1 haplotype seems to have no effect upon the tau or amyloid burden in the lentiform nucleus of PSP cases. Furthermore, the H1/H1 genotype does not influence age at disease onset, severity or survival of patients with PSP. The Saitohin gene (*STH*) Q7R polymorphism, nested within intron 9 of the tau gene, is in complete linkage disequilibrium with the extended H1/H2 haplotype.<sup>79</sup> This implicates the Q allele of this non-silent *STH* polymorphism as a potentially important candidate pathogenic variant in PSP. *STH* codes for a protein of unknown homologies and function that may play an important role in tau regulation.

Additional intriguing findings are of significant associations between the A0 polymorphism of tau and both PD and CBD, and between the extended tau gene haplotype H1 and CBD and clinically defined non-demented PD cases.<sup>80, 81</sup> For PD, it has been postulated that the H1 haplotype might interact with  $\alpha$ -synuclein, thereby influencing the propensity of  $\alpha$ -synuclein to aggregate. This would imply potential pathogenic synergism between “synucleinopathies” and “tauopathies”.

To date, no linkage has been found between PSP and the candidate genes for Parkinson’s disease synuclein, synphilin or parkin, nor the ApoE4 allele, a risk factor for late onset Alzheimer’s disease. The common LRRK-2 G2019S mutation is not over-represented in PSP<sup>82</sup> and case of PSSP has yet been reported with a progranulin mutation. In addition, no association with polymorphisms in the CYP2D6 (which encodes for debrisoquine 4-hydroxylase cytochrome P450), CYP1A1, N-acetyltransferase 2, dopamine transporter (DAT1) and glutathione *s*-transferase M1 genes has been found for PSP. A genome wide study found, in addition to MAPT, an additional major locus on chromosome 11p12, which was narrowed to a single haplotype block containing genes encoding a DNA damage-binding protein and lysosomal acid phosphatase.<sup>83</sup>

## **Treatment**

Drug treatment for PSP is inadequate and fundamental breakthroughs in our understanding of the pathogenesis may be needed before real advances are seen.<sup>84</sup> The widespread neuronal loss in the disorder suggests that a neurotransmitter-specific approach, such as replacement or reuptake inhibition, is unlikely to succeed. A small review of 12 patients with pathologically proven PSP concluded that use of levodopa, dopamine agonists, amantadine, tricyclic antidepressants, anticholinergics and selective serotonin reuptake inhibitors was largely ineffective and frequently associated with side effects.<sup>85</sup> Despite this nihilism, many neurologists feel that trials of amantadine and amitriptyline are worth considering, since they may benefit some patients. Botulinum toxin to relieve pretarsal blepharospasm and neck rigidity may be useful.

Clinical trials of agents affecting a wide range of neurotransmitter systems have been undertaken (Table 3) with almost invariably negative results.<sup>86-94</sup> The small size of these studies does not exclude the possibility of type II error.

Cholinesterase inhibitors are not recommended for the treatment of the cognitive syndrome in PSP. Two trials in PSP (one Level 1b, one 2b) showed no significant cognitive benefits.<sup>92, 93</sup>, while ADL/mobility scores significantly worsened in one study<sup>92</sup>. Riluzole did not prolong survival in PSP in a large multicentre international RCT (n=362), nor did it influence rate of disease progression.<sup>95</sup> Coenzyme Q10, a physiological co-factor of mitochondrial complex I, was associated with an increased ratio of high-energy phosphates to low-energy phosphates (adenosine-triphosphate to adenosine-diphosphate, phospho-creatine to unphosphorylated creatine) on MRS in a small, short-term randomised trial.<sup>96</sup> These changes were significant in the occipital lobe and showed a consistent trend in the basal ganglia. The PSP rating scale and Frontal Assessment Battery also improved slightly but significantly with CoQ10 treatment compared to placebo.

Disease-modifying approaches to PSP and CBD include the inhibition of glycogen synthase kinase-3 (GSK-3), a key enzyme in the hyperphosphorylation of tau protein. Lithium inhibits GSK-3 but is poorly tolerated in people with PSP and CBD. An NIH-funded tolerability trial of lithium was recently halted prematurely as the drug was associated with unacceptable side-effects and led to

withdrawals that exceeded a pre-defined percentage of participants (Galpern, personal communication). A trial of valproate, another putative GSK-3 inhibitor, is ongoing in France, while the Noscira compound NP031112 is about to enter phase II trial.

As a vital part of therapeutic evolution, the rapid increase in our knowledge of the molecular pathology of PSP will hopefully lead to the development of transgenic mice and *Drosophila* models which can then be used to test new disease modifying treatments.

**Table 3 Drug Studies in PSP**

Agent	Author & Year	Design	Outcome	Comment
L-DOPS (noradrenaline precursor)	Yamamoto 1997	single case, open	transient benefit only	
Efaroxan (alpha-2 antagonist)	Rascol 1998	14 cases, DB, PC, crossover	no benefit on motor function	
Pramipexole	Weiner 1999	6 cases, open label	no benefit on motor function or ADLs	
Zolpidem (GABAergic agonist for BZ1)	Daniele 1999	10 cases, DB, PC, crossover, single dose	motor benefits after 40-60 mins lasting ~ 2 hours	drowsiness & ↑ postural instability
Zolpidem	Mayr 2002	single case, open label	improved eye movements & parkinsonism	benefits lasted only 4 weeks
Physostigmine	Frattali 1999	DB, PC, crossover	no benefit for dysphagia	
Donepezil	Litvan 2001	21 cases, DB, PC, crossover	modest benefit on some cognitive tasks	worsening of motor function/ ADLs on drug
Donepezil	Fabbrini 2001	6 cases, open label	no benefit on cognitive, motor features or ADLs	
Gabapentin	Poujois 2007	14 cases, single blinded, PC	reduced anti-saccade error rate	no change in motor score

L-DOPS=L-threo-3,4-dihydroxyphenylserine; DB=double-blind; PC=placebo-controlled; ADLs=activities of daily living; BZ1=benzodiazepine subtype receptor

## Conclusions

Despite greater awareness in recent years, many patients with PSP remain undiagnosed or misdiagnosed for much of their disease duration. The role of tau protein in the pathophysiological process, together with the establishment of a modest genetic predisposition has stimulated research. At the same time, studies on a cluster of a PSP-like condition in Guadeloupe have produced new clues for potential environmental toxins. If the fundamental pathophysiological process in PSP turns out to be an over-production of four-repeat tau, it will be crucial to determine how the function of the RNA stem-loop structure, a key regulator in the alternate splicing process, may be affected by various genetic influences and toxins.

As disease-modifying therapies emerge it will be vital to intervene as early in the pathological process as possible. There is therefore a need for a greater awareness of PSP and the development of robust diagnostic clinical and investigational markers that predict whether an individual with suspicious, but not “typical classical” features, will go on to develop PSP. Physicians should consider PSP when rigidity and bradykinesia co-exist with early falls. The patient should be examined carefully for slowing of downward saccadic eye movements and the presence of square wave jerks. The presence of frank vertical ophthalmoparesis is of significant diagnostic help but it should also be borne in mind that this physical sign may take several years to develop in some patients.

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