

Characteristics of two distinct clinical phenotypes in pathologically proven progressive supranuclear palsy: Richardson's syndrome and PSP-parkinsonism

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Summary

The clinical diagnosis of progressive supranuclear palsy (PSP) relies on the identification of characteristic signs and symptoms. A proportion of pathologically diagnosed cases do not develop these classic features, prove difficult to diagnose during life and are considered as atypical PSP. The aim of this study was to examine the apparent clinical dichotomy between typical and atypical PSP, and to compare the biochemical and genetic characteristics of these groups. In 103 consecutive cases of pathologically confirmed PSP, we have identified two clinical phenotypes by factor analysis which we have named Richardson's syndrome (RS) and PSP-parkinsonism (PSP-P). Cases of RS syndrome made up 54% of all cases, and were characterized by the early onset of postural instability and falls, supranuclear vertical gaze palsy and cognitive dysfunction. A second group of 33 (32%) were characterized by asymmetric onset, tremor, a moderate initial therapeutic response to levodopa and were frequently confused with Parkinson's disease (PSP-P). Fourteen cases (14%) could not be separated according to these criteria. In RS, two-thirds of cases were men, whereas

the sex distribution in PSP-P was even. Disease duration in RS was significantly shorter (5.9 versus 9.1 years, $P < 0.001$) and age at death earlier (72.1 versus 75.5 years, $P = 0.01$) than in PSP-P. The isoform composition of insoluble tangle-tau isolated from the basal pons also differed significantly. In RS, the mean four-repeat: three-repeat tau ratio was 2.84 and in PSP-P it was 1.63 ($P < 0.003$). The effect of the H1,H1 PSP susceptibility genotype appeared stronger in RS than in PSP-P (odds ratio 13.2 versus 4.5). The difference in genotype frequencies between the clinical subgroups was not significant. There were no differences in apolipoprotein E genotypes. The classic clinical description of PSP, which includes supranuclear gaze palsy, early falls and dementia, does not adequately describe one-third of cases in this series of pathologically confirmed cases. We propose that PSP-P represents a second discrete clinical phenotype that needs to be clinically distinguished from classical PSP (RS). The different tau isoform deposition in the basal pons suggests that this may ultimately prove to be a discrete nosological entity.

Keywords: progressive supranuclear palsy; PSP; Richardson's syndrome; PSP-P; tau

Abbreviations: apoE = apolipoprotein E; FTDP-17 = frontotemporal dementia with parkinsonism linked with chromosome 17; MAPT = microtubule-associated protein, tau; NFT = neurofibrillary tangle; PSP = progressive supranuclear palsy; PSP-P = PSP-parkinsonism; RS = Richardson's syndrome; 3R-tau = tau containing three microtubule-binding domains; 4R-tau = tau containing four microtubule-binding domains

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Introduction

In June 1963, Dr Clifford Richardson presented a clinical report of eight cases of 'heterogenous system degeneration' with supranuclear ophthalmoplegia, pseudobulbar palsy, nuchal dystonia and dementia to The American Neurological Association (Richardson *et al.*, 1963). This hitherto unrecognized disorder presented in the seventh and eighth decades of life and was relentlessly progressive, with death occurring within 9 years. The pathological findings in six of those cases were presented by Dr Jerzy Olszewski to the American Association of Neuropathologists in the same year, where he described extensive subcortical neurofibrillary degeneration in the globus pallidus, subthalamic nucleus, substantia nigra and dentate nucleus.

The following year, together with Dr John Steele, they published a seminal paper entitled 'Progressive supranuclear palsy' and in it predicted that 'further observations may (in the future) broaden the clinical spectrum of the disease' (Steele *et al.*, 1964). In 1965, Barbeau proposed that the disorder be called Steele-Richardson-Olszewski syndrome. Subsequently, cases presenting with pure akinesia without rigidity (Matsuo *et al.*, 1991; Verny *et al.*, 1996a), gait freezing (Matsuo *et al.*, 1991), rest tremor (Verny *et al.*, 1996a; Birdi *et al.*, 2002), isolated dementia (Davis *et al.*, 1985; Masliah *et al.*, 1991), parkinsonism without dementia (Davis *et al.*, 1985; Verny *et al.*, 1996a; Birdi *et al.*, 2002), limb apraxia and asymmetric parkinsonism (Motoi *et al.*, 2004) as well as a number of cases dying without recorded evidence of the distinctive supranuclear ophthalmoplegia (Dubas *et al.*, 1983; Davis *et al.*, 1985; Daniel *et al.*, 1995) have been reported. Despite these observations, the current operational criteria for the diagnosis of progressive supranuclear palsy (PSP) include very few clinical features that were not recognized in the original description (Litvan *et al.*, 2003).

Attempts have been made to embrace the broader clinical features that are now well documented in pathologically confirmed PSP, but are not part of the operational clinical diagnostic criteria. An imprecise classification has evolved which includes typical and atypical clinical subgroups. The arbitrary definitions of these subgroups have varied and have usually been applied retrospectively. They include: the presence or absence of supranuclear gaze palsy (Daniel *et al.*, 1995; Birdi *et al.*, 2002); the presence or absence of a diagnosis of PSP in life (Morris *et al.*, 2002; Gibb *et al.*, 2004); the application of retrospective diagnostic criteria (Morris *et al.*, 2002); and the presence or absence of early bulbar signs or falls (Nath *et al.*, 2003). Several authors have found genetic, prognostic or pathological differences between these putative clinical groups (Daniel *et al.*, 1995; Morris *et al.*, 2002; Nath *et al.*, 2003).

That significant prognostic factors in a progressively debilitating neurodegenerative condition have been found is not surprising. However, the clinical features that have been purported to have the greatest prognostic significance are also the classic clinical hallmarks of the disease described in Richardson's original report (Richardson *et al.*, 1963) and

are included in the accepted diagnostic criteria (Litvan *et al.*, 2003). Accordingly, the absence of supranuclear gaze palsy, early falls and early bulbar dysfunction, with a positive response to levodopa, convey a good prognosis, but patients presenting in this way are much less likely to be diagnosed as PSP (Daniel *et al.*, 1995), and one suspects would have been excluded by the astute eye of Richardson.

There are no biological markers for the ante-mortem diagnosis of PSP (Litvan *et al.*, 2003), and the 'definite' diagnostic category has traditionally been reserved for cases that are pathologically confirmed (Litvan *et al.*, 1996a, b). The cases that remain undiagnosed in life make up at least 20% of the pathologically diagnosed cases of PSP (Hughes *et al.*, 2002) and most have unusual or atypical clinical pictures (Daniel *et al.*, 1995; Litvan *et al.*, 1999; Morris *et al.*, 2002). The pathological criteria for the diagnosis of PSP are well established (Litvan *et al.*, 1996b), and reflect many of the findings described by Olszewski, specifically neuronal loss with gliosis and neurofibrillary tangles (NFTs) in the subcortical and brainstem nuclei and the cerebellar dentate nucleus (Steele *et al.*, 1964; Jellinger 1971; Albert *et al.*, 1974). New immunohistochemical methods have allowed further characterization of the pathological changes (Hauw *et al.*, 1990; Braak *et al.*, 1992), and some regional pathological variation has been reported (Verny *et al.*, 1996a; Bergeron *et al.*, 1997; Bigio *et al.*, 1999; Morris *et al.*, 2002). The characteristics of the pathological accumulation of the microtubule protein tau into filamentous deposits of abnormally phosphorylated protein have also been examined in PSP (Morris *et al.*, 2002; Gibb *et al.*, 2004). Alternative splicing of exons 2, 3 and 10 of the tau gene transcript yields six different tau isoforms. Disease-specific deposition of these isoforms has been identified in Alzheimer's disease (Brion *et al.*, 1985; Grundke-Iqbal *et al.*, 1986; Flament *et al.*, 1989; Hanger *et al.*, 1991), Pick's disease (Lieberman *et al.*, 1998; Delacourte *et al.*, 1996), corticobasal degeneration (Ksiezak-Reding *et al.*, 1994), PSP (Vermersch *et al.*, 1994) and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) (Hutton *et al.*, 1998). The selective tau deposition in these clinically and pathologically distinct diseases differs by the relative amounts of tau containing four microtubule-binding domains (+exon 10, 4R-tau) and tau containing three microtubule-binding domains (–exon 10, 3R-tau) (Goedert *et al.*, 1998; Mailliot *et al.*, 1998). A range of different ratios of 4R-tau to 3R-tau have been reported in PSP, with some attempt to relate them to clinical and pathological phenotypes and to genetic background (Liu *et al.*, 2001; Gibb *et al.*, 2002).

The apparent clinical and pathological dichotomy has led us to examine the effect of different clinical factors on phenotype in a further attempt to identify distinct subgroups that might exist alongside the clinical entity originally described by Richardson. By examining the characteristics of these clinical subgroups, we attempt to clarify further the nosology of this condition and more clearly define prognostic factors and the biochemical hallmarks relating to tau pathology.

Material and methods

Patients

One hundred and three consecutive cases of pathologically diagnosed PSP, donated from all over the UK between 1988 and 2002 and stored in the Sara Koe PSP Research Centre (SKRC) at the Institute of Neurology, University College London, were reviewed. The diagnosis was made according to the National Institute for Neurological Diseases and Stroke-Society for PSP (NINDS-SPSP) criteria (Litvan *et al.*, 1996b), and was applied retrospectively to those cases acquired prior to the publication of the criteria. All patients had been assessed during life by a hospital specialist (neurologist or geriatrician). Some of these cases have been included in previous clinical and pathological reviews from the SKRC (Daniel *et al.*, 1995; Hughes *et al.*, 2002; Morris *et al.*, 2002; Gibb *et al.*, 2004).

Clinical data collection

A systematic chart review was performed on all the case notes. Specifically, the comprehensive case notes of the family doctor and all of the correspondence between the family doctor and the medical specialist were reviewed. When available, medical in-patient notes, in-patient consultations and emergency room admission notes were also scrutinized. A clinical data sheet was designed to record the presence or absence of clinical features either early in the disease course (within 2 years of first symptom onset) or at any time during the disease. Symptoms were recorded as being absent if not reported, and clinical signs were recorded separately as unknown if they were not specifically mentioned in the notes. Where conflicting clinical features were reported, the findings of the neurologist were used.

Definitions were as follows. (i) Age of onset: age, in years, at the time of the first reported symptom considered to be attributable to PSP or parkinsonism. (ii) Duration: time between the age of onset and the age at death. (iii) Falls: the presence of any report of falls. (iv) Bradykinesia: the presence of any mention of bradykinesia or motor slowing. (v) Cognitive decline: the presence of any perceived cognitive decline, by either the patient, the patient's relative or the treating doctor. This included annotation of difficulty in concentration, reports of intellectual functional decline and comments by the family of mental slowing, but did not include affective disorders. No patients underwent formal neuropsychological testing in the first 12 months of their disease. (vi) Speech disturbance: the recording of any alteration in speech quality compared with speech prior to disease onset. (vii) Dysphagia: a record of any swallowing abnormality. (viii) Asymmetric onset: if there was a clear difference between the signs on the left and the right, asymmetry was recorded as being present. This included asymmetry of tremor, rigidity, bradykinesia or functional decline. It did not include specific tasks such as writing and using tools. (ix) Tremor: the recording of any tremor. (x) Rigidity: the recording of axial or peripheral muscle rigidity; extra-pyramidal and pyramidal rigidity was not differentiated. (xi) Impaired postural reflexes: the presence of this sign was only recorded if specifically mentioned in the clinical notes. (xii) Supranuclear gaze palsy: the specific recording of restricted range of eye movement in the vertical plane. (xiii) Impaired saccadic or pursuit movements: the specific recording of abnormal saccadic or smooth pursuit eye movements. (xiv) Other visual symptoms: the recording of other visual symptoms not explained by the presence of gaze palsy or impaired saccadic or pursuit movements, which evolved during the disease course. Symptoms include painful eyes, dry eyes,

visual blurring, diplopia, blepharospasm and apraxia of eyelid opening. (xv) Extra axial-dystonia: the presence of dystonia in any body part apart from trunk and neck. (xvi) Pyramidal signs: pathologically brisk reflexes and/or extensor plantar response(s). (xvii) Autonomic dysfunction: either abnormal autonomic function testing or documentation of any two of urinary urgency, frequency and nocturia without hesitancy; chronic constipation; postural hypotension; sweating abnormalities; or erectile dysfunction. (xviii) Dyskinesia: the presence of chorea associated with levodopa therapy. (xix) Response to levodopa: the patient and clinician's interpretation of improvement was assessed from the case notes and in some cases from the completed Parkinson's Disease Society Brain Bank Annual Assessment (PDSBB) Forms. A self-reported improvement of >30% coincident with the introduction of levodopa was recorded as being a positive response. This degree of response was graded by a 4-point scale modified from the PDSBB annual assessment forms: 1 = nil, or slight response (<30% improvement); 2 = moderate response (30–50% improvement); 3 = good response (51–70% improvement); and 4 = excellent response (71–100% improvement).

Statistical methods

A complete data set of clinical variables each coded as present or absent was available from 29 cases only, and these were entered into a principal components analysis in order to summarize the information. Other cases had missing data on one or more variables and could not be included in this analysis. Statistical analysis was performed using SPSS for Windows (version 12.0.1). The principal components analysis was performed using clinical data from the first 2 years of disease. We selected the first two principal components and performed a 'Varimax' rotation on these components (this rotation maximizes the number of variables that have high loadings on each factor). The loadings of each variable on both of these components were plotted against each other. The plot was examined and two groups of variables in different areas of the plot were selected. A between-groups, hierarchical cluster analysis using squared Euclidean distance measures was performed to check that these sets of clinical features grouped together. Those cases that exhibited a greater number of the characteristics from set 1 than from set 2 were deemed to fall within group 1, and vice versa for group 2. A cross-tabulation was used to examine how many cases had a similar number of characteristics from the two variable sets. This was then applied to the cases that were excluded from the principal components analysis. Where there were missing data, we scaled up the number of positive results found (e.g. multiplied by 5/3 where status on three out of five characteristics was known). Clinical characteristics of the patients were compared between the two groups, and significance was calculated using Student's *t* test for normally distributed data, and χ^2 or Fisher's exact test for binary data.

Pathological material

In 69 cases, frozen brain tissue was available. In each case, a portion of the pontine base was taken for tau isoform analysis. The pontine base was selected for this analysis as it is considered to be free from Alzheimer NFTs and age-related tau pathology (Parvizi *et al.*, 2001). The tau that forms the pathological hallmark of PSP is deposited in glial and neuronal cells of the basal pons. The composition of the insoluble tau isolated from this region was analysed by dephosphorylation of the guanidine-solubilized deposits followed

by electrophoresis. The isoform profiles were compared with those of the soluble tau that is ubiquitous in the brain.

Isolation of soluble and guanidine-solubilized tau

Tau protein was extracted from the pontine base using a method described previously (Hanger *et al.*, 1998). Briefly, 0.5–0.7 g of brain tissue was homogenized in 0.1 M MES buffer, pH 6.5 containing 1 M NaCl and centrifuged at 27 000 g for 30 min at 4°C. The resulting supernatant was centrifuged at 100 000 g for 1 h at 4°C. This second supernatant, containing soluble tau, was exchanged into 50 mM Tris-HCl, pH 7.5. The 100 000 g pellet, containing insoluble tangle-tau, was solubilized in guanidine-HCl as described previously (Gibb *et al.*, 2004). The guanidine-solubilized tau was then dialysed into 50 mM Tris-HCl, pH 7.5 and centrifuged at 7000 r.p.m. for 1 min to remove precipitated material.

Tau dephosphorylation

Soluble tau and guanidine-solubilized tau extracted from the pontine base of all 69 PSP brains, guanidine-solubilized tau from the frontal lobe and pontine base of the Alzheimer brain and pontine base of the control brain were each dephosphorylated with lambda protein phosphatase (New England Biolabs) as previously described (Hanger *et al.*, 2002).

Purification of recombinant tau isoforms

The six isoforms of tau were each expressed in *Escherichia coli* BL21 cells and purified as described previously (Mulot *et al.*, 1994).

Analysis of tau on western blots

Proteins in the soluble, insoluble and guanidine-solubilized brain extracts, with and without phosphatase treatment, were separated by 10% (w/v) acrylamide SDS-PAGE (Hanger *et al.*, 1998). Resolved proteins were transferred onto PVDF membranes and probed with 1 : 20 000 TP70, a rabbit polyclonal antiserum that recognizes all forms of tau (a generous gift from Dr Diane Hanger) (Brion *et al.*, 1993). The immunoreactive bands were detected using standard enhanced chemiluminescence (Amersham Pharmacia Biotech). For semi-quantitative analysis, the detected bands of dephosphorylated guanidine-solubilized and soluble tau were scanned, and the density of each band was measured using 1D Image Analysis Software (Version 3.5, Kodak). For comparison, the band with the highest density was given a value of 1 and the other bands were given a value equal to the ratio of their density compared with this band.

Statistical analysis of banding patterns

Cases were separated according to clinical subtype. The values for each single band in all cases were added and the mean in each clinical group was calculated. A Mann-Whitney U test was performed using SPSS for Windows (version 12.0.1) to check for statistical significance of the difference between the two groups for each individual, tau isoform and the ratio of 4R-tau to 3R-tau.

Tau haplotype analysis and apolipoproteinE genotype

In the 72 cases where DNA was available, the tau haplotype was determined unambiguously using the 238 bp *MAPT* H2 deletion in

intron 9, as previously described (Baker *et al.*, 1999). In these cases, the apolipoproteinE (apoE) genotype was also determined using a restriction digest assay as previously described (Wenham *et al.*, 1991; Saunders *et al.*, 1993). Case-control allelic and genotypic association was calculated statistically in CLUMP software (Sham, 1995). The *P* values were derived by standard Pearson's χ^2 tests except in cases where cell counts in the contingency tables were <5. When cell counts were <5, *P* values were determined empirically by 10 000 simulations; the program uses a Monte Carlo approach that performs repeated simulations to generate random tables having the same marginal totals as the one under consideration and counting the number of times that a χ^2 value associated with the actual table is achieved by the randomly generated tables.

Results

Clinical features

Clinical data were available for 103 pathologically confirmed cases of PSP, of which 65 were male (63%). The clinical diagnosis of PSP was made during life in 71 cases (69%); 24 (23%) were diagnosed with idiopathic Parkinson's disease, two (2%) with atypical Parkinson's disease, two (2%) with multiple system atrophy, one (1%) with corticobasal degeneration and in three a final clinical diagnosis was not established. Neurologists made the final clinical diagnosis in 87% of cases and correctly diagnosed PSP in 72%. The mean age of onset was 66.4 years (SD 12), age at death 73.5 years (SD 7.5) and disease duration was 7.0 years (SD 3.7).

The clinical features of these patients are summarized in Table 1. There was an asymmetric onset in 28% of cases. Slowness of movement or bradykinesia was the most commonly reported feature early in the disease, occurring in 75% of cases. Falls (60%), tremor (20%), cognitive decline (29%), speech disturbance (39%) and non-specific visual symptoms (21%) were early features. Rigidity and impaired postural reflexes were found in nearly half of the patients early on in the disease course. Later in the disease, more clinical information was recorded. Bradykinesia was present in 98% of cases, rigidity in 98% and falls in 94%. Postural reflexes were impaired in 98% of cases, after the first 2 years of illness, and other signs included speech disturbance (87%), dysphagia (69%), cognitive decline (74%), tremor (23%), pyramidal signs (19%) and extra-axial dystonia (26%). Cerebellar signs (1%), autonomic dysfunction (2%) and cortical sensory loss (1%) were rare. The alien limb phenomenon was not reported.

Examination of the eye signs was not recorded in detail, beyond a statement of 'cranial nerve examination normal' in more than half of the cases early in the disease. Supranuclear gaze palsy was present early in the disease in 38%, and pursuit or saccadic eye movements were abnormal in 44% of cases where the examination was recorded completely. In contrast, later in the disease, 91% of cases were recorded as having supranuclear gaze palsy, though clinical examination was incompletely recorded in 21%.

A trial of levodopa or dopamine agonist medications was undertaken in 91 of 103 cases (88%). The trial was not

Table 1 Clinical features in 103 pathologically confirmed cases of PSP

Clinical feature	First 2 years				Throughout disease			
	Present early	Absent early	Not recorded	Present in total cohort %	Present ever	Absent ever	Never recorded	Present in total cohort %
Falls	61	40	2	60	93	8	2	94
Bradykinesia	74	25	4	75	99	0	4	98
Tremor	20	79	4	20	23	76	4	23
Cognitive decline	28	68	7	29	72	25	6	74
Non-specific visual symptoms	20	77	6	21	48	49	6	50
Rigidity	43	53	7	42	95	2	6	98
Dysphagia	6	89	8	6	64	29	10	69
Speech disturbance	38	60	5	39	83	12	8	87
Impaired postural reflexes	49	30	24	62	84	2	17	98
Supranuclear gaze palsy	19	31	53	38	75	7	21	91
Abnormal pursuit or saccades	21	27	55	44	70	6	27	92
Extra-axial dystonia	7	89	7	7	25	70	8	26
Pyramidal signs	8	88	7	8	18	79	6	19
Cerebellar signs	0	10	3	0	1	98	3	1
Autonomic dysfunction	0	100	3	0	2	97	3	2
Cortical sensory loss	0	97	4	0	1	97	4	1
Asymmetric onset	26	67	10	28				
Levodopa response					29	62	12	32
Levodopa-induced dyskinesia					4	92	7	4

recorded or was not performed in the remainder. In 32% of cases, there was a >30% improvement in symptoms coincident with the initiation of the medication. The duration of treatment, and continued efficacy were not measured. In four cases, dyskinesia developed (4% of those treated).

Statistical analysis

The first two components from the principal component analysis performed on data from the first 2 years of disease explained 42% of the variance seen in all cases. The loading of each variable on one component was plotted against its loading on the other component (Fig. 1). This suggested that the variables fell into two approximate groups (characteristic set 1 with high positive loadings on component 1 and characteristic set 2 with positive loadings on component 2 and negative loadings on component 1). The variables grouped together in each characteristic set were: set 1 = falls, cognitive decline, supranuclear gaze palsy, abnormal saccadic/pursuit movements, postural instability; set 2 = tremor, bradykinesia, asymmetrical onset, extra axial dystonia, response to levodopa. A cluster analysis of clinical variables confirmed this grouping of variables as reasonable (Fig. 2). This suggests that sufficient information was incorporated into the first two components of variance in order to determine groupings of characteristics between patients.

The frequency of the two sets of characteristics in the 29 cases with a complete data set is summarized in Table 2A. Fourteen cases (48%) had more features in characteristic

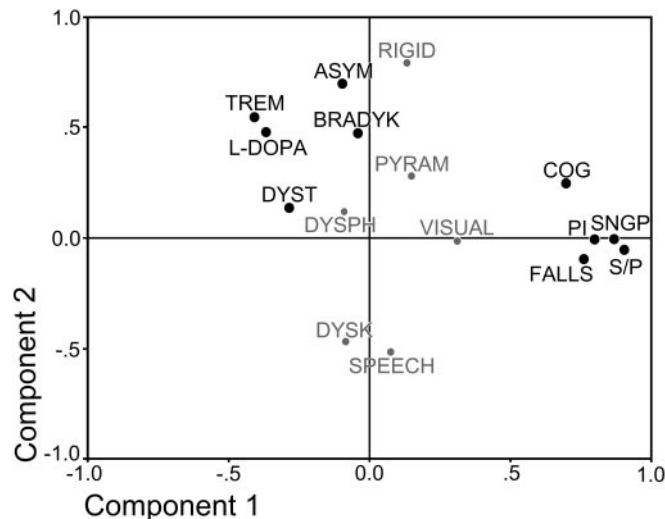


Fig. 1 Plot of factors for components 1 and 2 derived from factor analysis. ASYM = asymmetric onset; TREM = tremor; L-DOPA = response to levodopa; BRADYK = bradykinesia; DYST = extra-axial dystonia; DYSYPH = dysphagia; PYRAM = pyramidal tract signs; COG = cognitive dysfunction; DYSK = dyskinesia; VISUAL = non-specific visual symptoms; PI = postural instability; SNGP = supranuclear gaze palsy; S/P = abnormal saccadic or pursuit movements; SPEECH = speech disturbance.

set 1 than set 2, and were deemed to belong to group 1. Nine cases (31%) had more features in characteristic set 2, and were deemed to belong to group 2. Six cases (21%) had an equal number of features in each characteristic set.

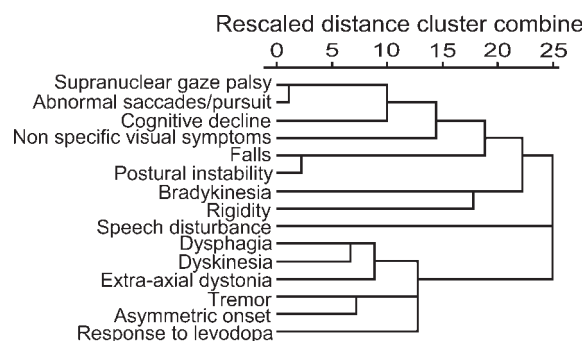


Fig. 2 Hierarchical cluster analysis of clinical variables: dendrogram using average linkage (between groups).

The frequency of the two sets of characteristics in the 71 cases with incomplete data is shown in Table 2B. Five cases (7.4%) had an equal number of features in each characteristic set. Three cases were excluded from the analysis because of insufficient clinical information. The low percentage of cases that were unclassified according to this division suggests that the identified characteristics are reasonable at distinguishing two groups. The two groups share few variables from each characteristic set, suggesting that they are clinically distinct.

The 66 cases that were not included in the principal components analysis, and were able to be separated into two groups with the cross-tabulation, were compared according to the presence or absence of early clinical features (Table 3). The co-occurrence of clinical features appeared to be similar to the cases with a complete data set, included in the principal components analysis. Early bradykinesia occurred in around three-quarters of cases in both groups and did not differentiate them.

All 89 cases that could be separated into groups were compared according to profile (Table 4) and the late clinical features that they displayed (Table 5). There was a difference in sex distribution between the two groups. Men were over-represented in group 1 [64.3%, 95% confidence interval (CI): 52–77%], but sex distribution was equal in group 2 (51.5%, 95% CI 33–67%). The age of disease onset was not different between the two groups. The ages at death and disease duration were significantly different: those in group 1 died at a younger age, and after shorter disease duration. Falls, cognitive decline and supranuclear gaze palsy continued to be significantly associated with group 1 later in the disease ($P < 0.001$). Tremor was the only clinical feature significantly associated with group 2 later in the disease though extra-axial dystonia was also more frequent in that group. There was no significant difference in the frequency of bradykinesia, speech disturbance, postural instability, pyramidal tract signs or levodopa-induced dyskinesias.

Tau isoform composition

Homogenates of the pontine base of 69 cases were prepared; guanidine-solubilized tau extracts were examined in 68 cases

Table 2A Cross-tabulation of the number of characteristics in each of two sets exhibited by each case for the initial data set

	Set 2					Total
	0	1	2	3	4	
Set 1						
0	1	1	1	2	2	7
1	0	2	0	0	0	2
2	1	0	3	2	0	6
3	0	0	0	0	1	1
4	2	2	1	1	0	6
5	0	6	1	0	0	7
Total	4	11	6	5	3	29

Table 2B Cross-tabulation of the number of characteristics for the second data set where at least one variable was missing

	Set 2					Total
	0	1	2	3	4	
Set 1						
0	2	7	8	6	2	25
1	1	0	0	0	0	1
2	0	1	1	1	0	3
3	2	10	4	2	0	18
4	1	3	3	0	0	7
5	3	8	2	4	0	17
Total	9	29	18	13	2	71

Set 1: falls, cognitive decline, supranuclear gaze palsy, abnormal saccades/pursuit movements, postural instability. Set 2: tremor, bradykinesia, asymmetric onset, non-axial dystonia, response to levodopa.

Table 3 Early clinical features in cases with an incomplete data set

	Group 1 (% reported as present)	Group 2 (% reported as present)	P value
Symptoms			
Falls*	85.7 (36/42)	0% (0/24)	<0.001 [‡]
Cognitive decline*	50.0 (19/38)	0 (0/22)	<0.001 [‡]
Tremor*	9.8 (4/41)	39.1 (9/23)	0.007 [‡]
Speech disturbance	32.5 (13/40)	26.1 (6/23)	0.406 [‡]
Dysphagia	2.7 (1/37)	4.3 (1/23)	0.624 [‡]
Other visual symptoms	23.1 (9/39)	4.3 (1/23)	0.051 [‡]
Clinical signs			
Asymmetric onset*	17.9 (7/39)	45.0 (9/20)	0.030 [‡]
Bradykinesia*	75.6 (31/42)	73.9 (17/23)	0.880 [‡]
Rigidity	39.5 (15/38)	52.5 (12/23)	0.333 [‡]
Postural instability*	84.4 (27/32)	7.7 (1/13)	<0.001 [‡]
Extra-axial dystonia*	0 (0/38)	8.7 (2/21)	0.138 [‡]
Supranuclear gaze palsy*	70.0 (7/10)	0 (0/9)	0.002 [‡]
Abnormal saccades/pursuits*	63.6 (7/11)	0 (0/6)	0.017 [‡]
Pyramidal tract signs	7.9 (3/38)	4.3 (1/23)	0.513 [‡]
Response to levodopa ever*	14.3 (5/35)	50.0 (11/22)	0.005 [‡]

*Variables used in initial analysis; [‡] χ^2 analysis; [‡]Fisher's exact test. Group 1 = Richardson's syndrome; group 2 = PSP-P.

(Fig. 3) and soluble tau extracts were examined in 49 cases. In one case, guanidine-solubilized tau could not be detected in tissue homogenate. There was considerable heterogeneity in tau isoform profiles between individuals, including variation in all six tau isoforms. The 1N3R, 0N4R and 1N4R isoforms were most prominent in the guanidine-solubilized tau fractions, but other isoforms were present in only small

Table 4 Patient profiles

	Group 1	Group 2	<i>P</i> value*
Sex (male, %)	64.3	51.5	
Age at disease onset (years)	66.1	66.4	0.872
Age at death (years)	72.1	75.5	0.041
Disease duration (years)	5.94	9.12	<0.001

*Student's *t* test.

Table 5 Late clinical features in 89 cases separated into distinct groups

	Group 1 (% reported as present)	Group 2 (% reported as present)	<i>P</i> value
Symptoms			
Falls	100 (56/56)	80.6 (25/31)	0.001 [†]
Cognitive decline	90.7 (49/54)	51.6 (16/31)	<0.001 [†]
Tremor	13.0 (7/54)	43.8 (14/32)	0.002 [‡]
Speech disturbance	90.2 (46/51)	81.3 (26/32)	0.242 [†]
Dysphagia	75.5 (37/49)	56.3 (18/32)	0.070 [†]
Other visual symptoms	56.6 (30/53)	34.4 (11/32)	0.047 [†]
Clinical signs			
Bradykinesia	98.2 (55/56)	96.9 (31/32)	0.685 [†]
Rigidity	98.1 (53/54)	96.8 (30/31)	0.687 [†]
Postural instability	100 (51/51)	96 (23/24)	0.142 [†]
Extra-axial dystonia	21.2 (11/51)	42.4 (13/32)	0.054 ^b
Supranuclear gaze palsy	100 (50/50)	71.4 (15/21)	<0.001 [†]
Pyramidal tract signs	17.0 (9/53)	15.6 (5/32)	0.561 [‡]
Levodopa-induced dyskinesia	1.9 (1/52)	6.3 (2/32)	0.323 [‡]

[†] χ^2 analysis; [‡]Fisher's exact test. Group 1 = Richardson's syndrome; group 2 = PSP-P.

quantities and were frequently not detectable. The soluble tau fractions were more homogeneous, with all six isoforms represented in most individuals and 1N3R being present in large amounts.

To summarize these data, results from semi-quantitative analysis of western blots were pooled according to clinical group and tau isoform. Different patterns of guanidine-solubilized tau isoforms were detected in the two clinical subgroups (Fig. 4A) but the profile of soluble tau expression did not differ between them (Fig. 4B). In the guanidine-solubilized fractions, there were substantially more 4R-tau than 3R-tau isoforms in both groups. Pooled data from group 1 indicated that 1N4R and 0N4R were most prominent, 1N3R was present in smaller amounts and there were only small amounts of the other 3R-tau isoforms. In group 2, 1N4R and 0N4R were also most prominent, but an approximately equivalent amount of 1N3R was present, significantly more so than in group 1. The other 3R-tau isoforms were detected in higher amounts in group 2 than in group 1 (0N3R *P* = 0.015; 1N3R, *P* = 0.002; 2N3R, *P* = 0.002). Put another way, between the two clinical groups, there was no difference in relative amounts of pooled, guanidine-solubilized 4R-tau but there was 57% more 3R-tau in group 2 when compared with group 1 (*P* = 0.001) (Fig. 4C). The mean 4R-tau/3R-tau ratio in group 1 was 2.84 (SD, 1.64; SEM, 0.26) and in group 2 was 1.63 (SD, 0.88; SEM 0.19; *P* < 0.003) (Fig. 5). 3R-tau predominant deposits were identified in the guanidine-solubilized extract from the basal pons of the patient with Alzheimer's disease. No insoluble tau was identified in the basal pons of the control patient.

Tau haplotype analysis

Tau haplotype analysis was performed in 72 cases. The H1,H1 genotype was significantly associated with PSP, when compared with controls [odds ratio (OR) 5.6, 95% CI 3.7–8.5, *P* < 0.001]. The association was different between the two clinical subtypes (group 1 OR 13.2, 95% CI 3.0–57.2, *P* < 0.001; group 2 OR 4.5; 95% CI 1.3–16.0, *P* = 0.018). In total, five patients (7%) were heterozygous for the H2 allele and its overall frequency was 3.2%. The frequency of the minor allele was different between the groups, 2.4% in group 1 and 8.7% in group 2 (*P* = 0.108). The frequency of the H2 allele in both clinical groups was significantly less than in an unrelated control population (controls, 23.4%). There was no

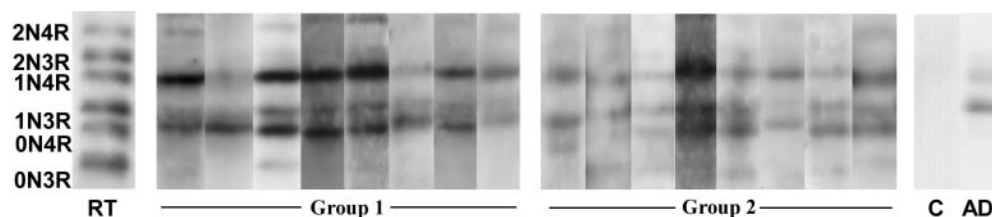


Fig. 3 Western blots of guanidine-solubilized, dephosphorylated tau from basal pons. RT = recombinant tau; C = control; AD = Alzheimer's disease.

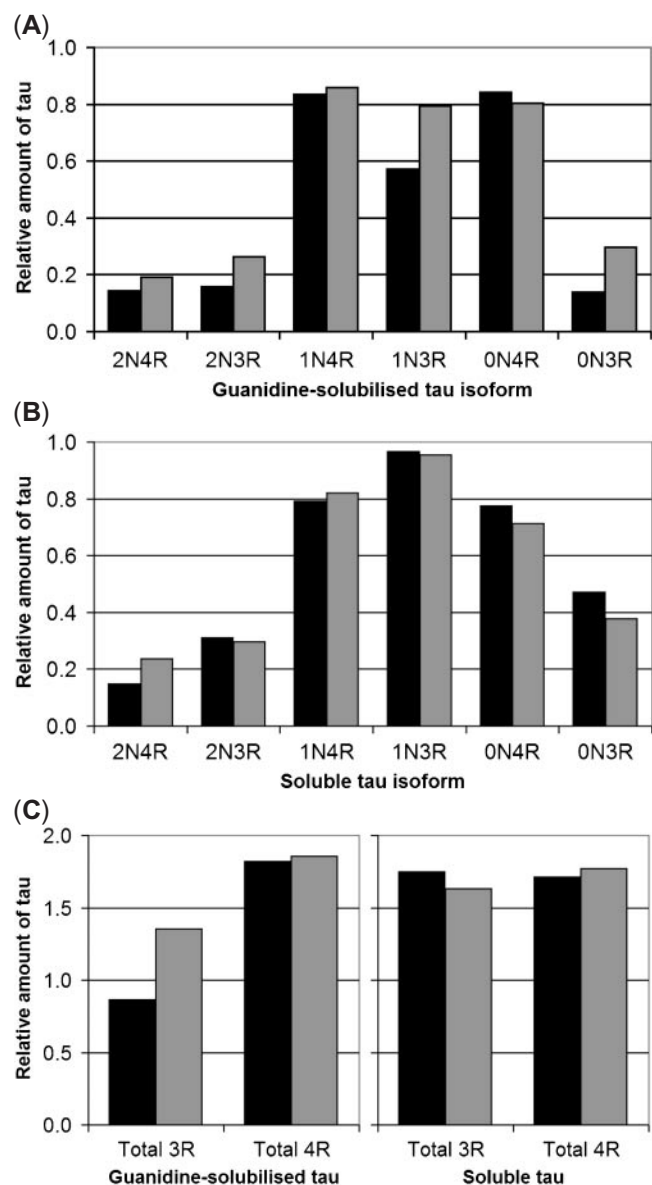


Fig. 4 Black = group 1; grey = group 2. (A) Guanidine-solubilized tau isoforms according to clinical phenotype; (B) soluble tau isoforms according to clinical phenotype; (C) total 3R-tau and 4R-tau according to clinical phenotype.

significant difference in the clinical features of carriers of the H2 allele. The single patient who was homozygous for H2,H2 was classified in clinical subgroup 2, and shared characteristics with that group. Haplotype did not influence the guanidine-solubilized tau isoform profile.

ApoE genotype

ApoE allelic and genotypic distributions were examined and were compared with 134 control cases with no evidence of abnormal brain histopathology. No association was found when comparisons were made between PSP and controls, group 1 and controls and group 2 and controls. There was no significant difference between groups 1 and 2.

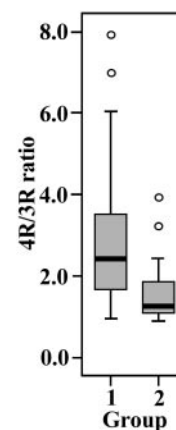


Fig. 5 Distribution, median, upper and lower quartiles of 4R/3R tau ratios according to clinical group.

Discussion

This study confirms that two distinct clinical phenotypes exist in patients with pathologically proven PSP. One is characterized by early falls, early cognitive dysfunction, abnormalities of gaze and postural instability, and the other by asymmetric onset, tremor, early bradykinesia, non-axial dystonia and a response to levodopa medications. The clinical characteristics present in this first group are similar to those first described in PSP by Richardson (Richardson *et al.*, 1963), whereas the clinical features in the second group resemble Parkinson's disease. We propose naming the first group 'Richardson's syndrome' (RS) and the second 'PSP-parkinsonism' (PSP-P). RS has a shorter duration of disease and the female to male ratio is 1 : 1.8, whereas the sex distribution is equal in PSP-P (see Table 4). Differences also exist in the tau isoform composition of pontine NFTs between RS and PSP-P. In PSP-P, there was significantly more 3R-tau deposition in the basal pons. There was no significant difference in the frequency of the H2 allele, apoE alleles or apoE genotype between these groups. The effect of the H1,H1 susceptibility genotype appeared to be stronger in RS.

This cohort comprised 54% RS, 32% PSP-P, 11% with equal numbers of features of both subtypes and 3% lacking sufficient clinical data to be classified. These subgroups, defined by the clinical features present in the first 2 years of disease, represent two separate points on a spectrum of clinical features related to PSP tau pathology. The proportions of patients presenting with RS and PSP-P in this study may not reflect the proportions of these subtypes in the community because of ascertainment bias that is inherent in a brain banked population (Maraganore *et al.*, 1999). Furthermore, this study is limited by its retrospective nature. Despite this, the frequency of falls (94%), bradykinesia (98%), speech disturbance (87%) and dysphagia (69%) in the whole group was consistent with data reported in other clinical and clinicopathological studies (Maher *et al.*, 1986; Verny *et al.*, 1996a; Nath *et al.*, 2003). In contrast to some studies,

we found a higher incidence of levodopa responsiveness (32%), extra-axial dystonia (26%) and a longer duration of disease (Maher and Lees, 1986; Nath *et al.*, 2003). The inclusion of only pathologically proven cases of PSP partially accounts for this, and is a strength of this study, allowing us to identify atypical clinical features more clearly in cases that would have been automatically excluded from clinical reports relying on PSP clinical diagnostic criteria.

Previously, attempts have been made to define clinical subgroups in cohorts of PSP (Birdi *et al.*, 2002; Morris *et al.*, 2002; Nath *et al.*, 2003; Gibb *et al.*, 2004) and to apply this classification in small pathologically proven series (Braak *et al.*, 1992; Daniel *et al.*, 1995; Verny *et al.*, 1996a,b; Bergeron *et al.*, 1997). Daniel and co-workers found that in a subgroup without supranuclear gaze palsy, women were over-represented, age of onset was later and duration of disease was longer when compared with the group with supranuclear gaze palsy, where men were over-represented (Daniel *et al.*, 1995). No quantitative or qualitative pathological differences were distinguished, although a subsequent study on the same material showed greater neuronal loss in the nucleus interpositus in those with a supranuclear gaze palsy (Revesz *et al.*, 1996). Birdi and collaborators reported that patients with gait or balance difficulties at onset were less likely to improve on levodopa therapy, more likely to develop supranuclear gaze palsy and had a shorter duration of disease (Birdi *et al.*, 2002). Morris and colleagues classified pathologically confirmed cases of PSP according to the diagnosis of PSP in life and the retrospective application of diagnostic criteria for PSP (Morris *et al.*, 2002). All cases in their 'typical PSP' subgroup had a diagnosis of PSP in life, all retrospectively satisfied the diagnostic criteria and such cases were more likely to have the PSP susceptibility tau haplotype (H1,H1). In a series where only 60% of cases had pathological confirmation, Nath and co-workers found that the subgroup with early falls and bulbar dysfunction had a shorter survival, and those with a diagnosis of probable PSP according to the NINDS-SPSP criteria had a worse prognosis (Nath *et al.*, 2003). The variations in definition between these studies make it difficult to compare clinical groups and even more difficult to apply clinical characteristics objectively to pathological cohorts. By applying the clinical distinctions identified in our study, comparisons can be made between more homogeneous clinical groups, potentially enabling a better understanding of the spectrum of pathological changes in PSP.

We have demonstrated the strength of this methodology by identifying molecular heterogeneity in PSP that correlates with different clinical phenotypes. Classically, PSP has been considered a 4R tauopathy because of the predominance of 4R-tau isoforms in insoluble tau deposits (Buee and Delacourte 1999). Other tauopathies can also be defined by their pattern of insoluble tau isoform aggregation though the relationship between specific isoforms and aetiopathogenesis of these clinico-pathological entities is unclear (Buee and Delacourte, 1999). This classification of tauopathies has been

blurred by the demonstration of heterogeneity in aggregated insoluble tau in PSP (Morris *et al.*, 2002). Analysis using specific antibodies to 3R-tau and 4R-tau has shown that, to a variable degree, 3R-tau is a component of these insoluble deposits and that different 4R-tau/3R-tau ratios exist between cases (de Silva *et al.*, 2003b; Gibb *et al.*, 2004). The differences observed were not related to soluble tau expression (Gibb *et al.*, 2004) and have not previously been related to clinical phenotype. Variations in the ratio of 4R-tau to 3R-tau in PSP and other neurodegenerative conditions have been found by a number of authors using biochemical and immunohistochemical methods (Chambers *et al.*, 1999; Arai *et al.*, 2001; Liu *et al.*, 2001; Takanashi *et al.*, 2002; Gibb *et al.*, 2004). We found diversity in the ratio of 4R-tau to 3R-tau, and in four cases it was <1. Interestingly the clinical phenotypes of RS and PSP-P could be separated according to the ratio of 4R-tau to 3R-tau (Fig. 5). The expression of soluble tau was not a factor in these differences (see Fig. 4C). It is of interest that the most atypical tau profiles, with relatively more 3R-tau, were mainly from the PSP-P clinical subgroup. Another report found a 3-fold increase in the 4R-tau/3R-tau ratio in the lentiform nucleus of all cases of PSP, without concurrent Alzheimer's disease or neuropathological ageing in other brain areas (Liu *et al.*, 2001). Those cases were collected from an Alzheimer's and PSP brain bank, and selection bias may have limited the number of cases with PSP-P type presentations that would, according to our results, favour a higher 4R-tau/3R-tau ratio. The absence of Alzheimer pathology contributing to the banding patterns would also favour a higher 4R-tau/3R-tau ratio. The identification of cases with lower 4R-tau/3R-tau ratios in our series is unlikely to be due to co-existent Alzheimer pathology, as we carefully extracted brain tissue from the pontine base, a region where Alzheimer's disease NFTs do not occur and Alzheimer's disease tau immunostaining has been found to be weak (Parvizi *et al.*, 2001). Nevertheless we identified tau in guanidine-solubilized brain extract from a case with Alzheimer's disease which had a low 4R-tau to 3R-tau ratio. In the PSP cases, co-existent Alzheimer's disease was present in only three (9.1%) of the PSP-P group and in one (1.8%) of the RS group ($P = 0.1$), and is therefore unlikely to account for the difference in tau isoform deposition between the groups. Age-related diffuse plaques were present in 11 (19.6%) of the RS cases and in seven (21%) of the PSP-P cases ($P = 0.5$).

It has been suggested that overexpression of 4R-tau mRNA in brainstem regions of PSP, where the most severe gliosis and NFT deposition is found, plays an important role in the pathogenesis (Chambers *et al.*, 1999). The factors surrounding the pathogenicity of tau are still to be fully elucidated, although the degree to which tau accumulation is the primary event probably varies in different disease processes (Avila *et al.*, 2004; Forman, 2004; Schraen-Maschke *et al.*, 2004). The genetic association of PSP with the microtubule-associated protein, tau (*MAPT*) gene and rare reports of

families with more than one affected member suggest that tau accumulation in PSP is closely associated with the molecular pathways which cause the disease (Conrad *et al.*, 1997; de Silva *et al.*, 2003a; Pittman *et al.*, 2004). The pathogenic potential of *MAPT* dysfunction has been demonstrated by the identification of missense and splice site mutations causing FTDP-17. In most of these cases, tau accumulation is seen in association with neuronal loss and gliosis, but no clear genotype-phenotype correlation has been found (Forman, 2004). PSP and corticobasal degeneration remain the most likely candidates for *MAPT* to have a central role in the onset or evolution of disease, since both diseases are associated with the H1 tau haplotype and both exhibit predominant 4R-tau pathology. We have confirmed this association, demonstrating an OR of 5.6 for the H1,H1 genotype for PSP. Morris and colleagues reported that patients homozygous for H1 are more likely to present with clinically 'typical' PSP, and in their small cohort of 'atypical' PSP there was a higher frequency of the H2 allele (Morris *et al.*, 2002). We found a difference of H2 allele frequency between the two clinical subgroups, but the difference was not significant. The effect of the H1,H1 PSP susceptibility genotype was stronger in RS than in PSP-P (OR 13.2 versus 4.5). The difference in genotype frequencies between the clinical subgroups was not significant. We did not find any correlation between genotype and tangle-tau isoform profile.

The mechanisms responsible for isoform-specific selective pathological deposition of tau in PSP are a matter for conjecture and probably relate to both environmental and genetic risk factors (Buee *et al.*, 2001). The differences that we have found in tangle-tau isoforms between RS and PSP-P may be due to a number of different mechanisms that ultimately account for the observed clinical and epidemiological differences. Genetic variations underlying the H1 haplotype affecting either the overall level of expression of tau or tau splicing at exon 10 in different neuronal subgroups may be a factor (Schraen-Maschke *et al.*, 2004). Alternatively, selective degradation of individual tau isoforms may be different between the two groups.

A number of authors have found pathological differences in cases of clinically 'typical' and 'atypical' PSP, without applying a standardized system for clinical classification. In one report, two subgroups were identified that could be divided on clinical and pathological grounds (Verny *et al.*, 1996a). A group with mild involvement of the pedunculopontine nucleus invariably had mild cortical involvement and a number of 'unusual' clinical signs including absence of oculomotor palsy and axial rigidity, severe early dementia and rest tremor. The subcortical structures involved had a preferentially 'pallido-luysio-nigral' distribution. The second group all had typical clinical signs with more severe involvement of the pedunculopontine nucleus and cortex, and more widespread involvement of subcortical structures. The sample size, however, was too small to come to definitive conclusions relating to differences. In other reports, patients with early severe dementia were found to have more cortical

pathology (Braak *et al.*, 1992; Bigio *et al.*, 1999), and pathological changes in the nucleus raphe interpositus which correlate with supranuclear gaze palsy in PSP have been reported (Revesz *et al.*, 1996). The variation of pathological involvement in PSP, and the identification of some clinical correlation with it, supports the notion that different clinical groups exist.

The identification of RS and PSP-P goes some way to reconcile the differences that exist between the pathological and clinical criteria for the diagnosis of PSP. This study was designed to synthesize a large body of clinical data with no *a priori* assumptions. Using this data-driven approach, we have identified two discrete syndromes in patients who have pathologically diagnosed PSP. What this strategy could not identify was clinical syndromes with characteristics that were not included in the data collection. In addition, it relies on consistent syndromes to occur in large enough numbers to impact on the principal components in the factor analysis. For example, gait ignition failure was not a clinical criterion that was extracted from the case notes, and cases where it may have been the presenting feature and other features of PSP were lacking, or developed later, were infrequent enough to have no significant impact on the factor analysis. Ascertainment bias inherent in a brain bank cohort will also inevitably skew the proportion of cases in each clinical group, and may not reflect proportions in the community. Therefore, primary progressive gait freezing may still be a third distinct clinical syndrome in PSP, but it occurs in sufficiently small numbers that it has not been a significant finding in this study. Prospective clinicopathological studies are now needed to confirm these proposed subgroups further and could potentially identify less common, but distinct clinical phenotypes.

We found no cases that exhibited any of the NINDS-SPSP mandatory exclusion criteria (Litvan *et al.*, 1996a). However, according to our data, some features that are included in the NINDS-SPSP 'supportive criteria' may be misleading. Early speech disturbance was found in 39% of cases, and 32% had a modest or good response to levodopa (PDSBB grade 2 or 3), though an excellent response to levodopa (PDSBB grade 4) was not recorded. While an asymmetric onset was a feature in 28%, persistent markedly asymmetric parkinsonism was not seen.

The core clinical features of PSP appear to be bradykinesia, rigidity and postural instability, and are almost always present later in the disease. Together with the supranuclear vertical ophthalmoplegia, dementia, dysarthria and pseudobulbar palsy, they form the classic features of PSP (Steele *et al.*, 1964). When these features appear in the first 2 years, a diagnosis of RS is most likely. We have confirmed a high frequency of non-specific visual complaints early in the course of the disease and a predominance of males in the RS subgroup (Richardson *et al.*, 1963).

The expanded clinical phenotype now includes a syndrome that, at least in the early stages, may closely resemble idiopathic Parkinson's disease. The presence of the PSP-P subgroup may account for the small proportion of cases in other

clinicopathological reports where an incorrect ante-mortem diagnosis of Parkinson's disease was made (Hughes *et al.*, 2001). The features which most clearly differentiate this syndrome from RS appear to be an asymmetric onset, extra-axial dystonia, tremor and benefit from levodopa. Early bradykinesia appears to be essential for the diagnosis, but does not adequately differentiate it from RS, especially later in the disease course. Disease duration in PSP-P is significantly longer than in RS (Table 4), and to our knowledge exceeds median survival in all clinicopathological PSP case series.

The clinical features in these two groups may give some insights into the pathological substrate for PSP. Axial rigidity, bradykinesia and postural instability are universal end-points of this heterogeneous degeneration and may be related to the invariable pathological findings which form the basis of the diagnostic criteria (Litvan *et al.*, 1996b). Though supranuclear gaze palsy is seen in the later stages of most cases (Table 6), it appears earlier in RS. This clinical finding is specifically associated with degeneration of some cholinergic brainstem structures (Juncos *et al.*, 1991) and the nucleus raphe interpositus (Revesz *et al.*, 1996). The current study suggests that early involvement of these areas predicts early dementia and poorer prognosis. The striatal cholinergic neurons that bear the dopamine receptors are affected in PSP either by degeneration of intrinsic striatal projection neurons or by altered regulation of D2 receptors (Ruberg *et al.*, 1985; Pierot *et al.*, 1988; Arnold *et al.*, 1994; Landwehrmeyer and Palacios 1994; Suzuki *et al.*, 2002). The partial response to levodopa in PSP-P may imply a different pattern of neurodegeneration where these projection neurons are relatively less affected. Differences in early pathological involvement of the striatum, pallidum or subthalamus may account for the observed marked clinical differences between RS and PSP-P, which appear to be two different points on a continuous spectrum of PSP tau pathology. The differences in tau deposition provide a tantalizing clue that the molecular processes that influence neurodegeneration in PSP may vary between these two clinical phenotypes.

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