

# Pathological tau burden and distribution distinguishes progressive supranuclear palsy-parkinsonism from Richardson's syndrome

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**Clinical syndromes associated with progressive supranuclear palsy-tau pathology now include progressive supranuclear palsy-parkinsonism (PSP-P), in addition to classic Richardson's syndrome (RS) and pure akinesia with gait freezing (PAGF). Although pathological heterogeneity of progressive supranuclear palsy (PSP) has also been established, attempts to correlate this with clinical findings have only rarely provided conclusive results. The aim of this study was to investigate whether regional variations in the types of tau lesions or differences in overall tau load may explain the clinical differences between the RS, PSP-P and PAGF. Quantitative tau pathology assessment was performed in 17 brain regions in 42 cases of pathologically diagnosed PSP (22 RS, 14 PSP-P and 6 PAGF). Neurofibrillary tangles, tufted astrocytes, coiled bodies and thread pathology were quantitated and a grading system was developed separately for each region. Using these grades the overall tau load was calculated in each case. To establish a simplified system for grading the severity of tau pathology, all data were explored to identify the minimum number of regions that satisfactorily summarized the overall tau severity. The subthalamic nucleus, substantia nigra and globus pallidus were consistently the regions most severely affected by tau pathology. The mean severity in all regions of the RS group was higher than in PSP-P and PAGF, and the overall tau load was significantly higher in RS than in PSP-P ( $P=0.002$ ). Using only the grade of coiled body + thread lesions in the substantia nigra, caudate and dentate nucleus, a reliable and repeatable 12-tiered grading system was established (PSP-tau score: 0, mild tau pathology, restricted distribution;  $>7$ , severe, widespread tau pathology). PSP-tau score was negatively correlated with disease duration (Spearman's rho  $-0.36$ ,  $P=0.028$ ) and time from disease onset to first fall (Spearman's rho  $-0.49$ ,  $P=0.003$ ). The PSP-tau score in PSP-P (median 3, range 0–5) was significantly lower than in RS (median 5, range 2–10, Mann–Whitney U,  $P<0.001$ ). The two cases carrying the tau-H2 protective allele had the two lowest PSP-tau scores. We have identified significant pathological differences between the major clinical syndromes associated with PSP-tau pathology and the restricted, mild tau pathology in PSP-P supports its clinical distinction from RS. The grading system we have developed provides an easy-to-use and sensitive tool for the morphological assessment of PSP-tau pathology and allows for consideration of the clinical diversity that is known to occur in PSP.**

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

**Abbreviations:** CB = coiled body; NFT = neurofibrillary tangle; PAGF = pure akinesia with gait freezing; PSP = progressive supranuclear palsy; PSP-P = progressive supranuclear palsy-parkinsonism; RS = Richardson's syndrome; TA = tufted astrocyte

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## Introduction

Progressive supranuclear palsy (PSP) is a degenerative disorder of the central nervous system with protean clinical manifestations. The classic clinical picture, originally described by Richardson is the most common clinical form (Richardson's syndrome or RS), with an insidious onset and relentlessly progressive postural instability and falls, gait disturbance, supranuclear vertical gaze abnormalities, pseudobulbar palsy, rigidity in extension and a dysexecutive syndrome (Richardson *et al.*, 1963; Steele *et al.*, 1964; Litvan *et al.*, 1996a; Williams *et al.*, 2005). We have recently defined a further clinical phenotype, PSP-parkinsonism (PSP-P), in which disease duration is longer and parkinsonism dominates the early clinical picture (Williams *et al.*, 2005). In contrast to RS these patients may show a moderate initial therapeutic response to levodopa, falls are delayed and if gaze palsy or dementia occur at all, they develop late in the course of the disease. PSP-tau pathology may also present as pure akinesia with gait freezing (PAGF), corticobasal syndrome or, exceptionally, with an isolated dementia (Imai *et al.*, 1993; Williams *et al.*, 2005; Tsuboi *et al.*, 2005; Josephs *et al.*, 2006).

The post-mortem diagnosis of PSP is dependent on the identification of neurofibrillary tangles (NFTs) and neuropil threads in basal ganglia and hindbrain structures (Litvan *et al.*, 1996b); tufted astrocytes (TAs) and coiled bodies (CBs) are other highly characteristic findings (Dickson, 1999). PSP is a primary tauopathy, in which tau dysfunction is regarded as central to the pathogenesis (Goedert, 2005). The predominance of 4-repeat tau isoforms in the neuronal and glial tau inclusions is also characteristic, although this is not included in the current operational diagnostic criteria (Komori *et al.*, 1998; Hauw, 2003; de Silva *et al.*, 2003). Pathological heterogeneity of PSP has also been reported (Braak *et al.*, 1992; Hof *et al.*, 1992; Mizusawa *et al.*, 1993; Verny *et al.*, 1996; Halliday *et al.*, 2000; Piao *et al.*, 2002; Williams *et al.*, 2005; Tsuboi *et al.*, 2005; Josephs *et al.*, 2005), but attempts to correlate this with clinical findings in PSP have only rarely revealed definitive correlations (Daniel *et al.*, 1995; Litvan *et al.*, 1996b; Tsuboi *et al.*, 2005). Unlike Parkinson's disease and Alzheimer's disease, cases of 'incidental' PSP-tau pathology are unusual and pathological examples of 'early' PSP are rare, hampering any attempts to develop a pathological staging system. It is unknown whether the pathology in PSP follows a consistent topographical progression or whether a disease 'footprint' of regional susceptibility is established early with subsequent uniform progression of pathology in all affected structures. However, serial imaging studies confirm that the brainstem, in particular the midbrain, and frontal lobes bear the brunt of the disease (Paviour *et al.*, 2006).

We have already demonstrated an increased contribution by 3-repeat tau isoforms to the total insoluble-tau fraction

in PSP-P, raising the possibility that biological differences influencing tau pathology may exist between PSP-P and RS (Williams *et al.*, 2005). In this study, we carried out a quantitative survey of different brain regions looking for regional differences in the tau load or variations in the type of tau lesions between the clinical PSP phenotypes. We have also applied a newly developed PSP-tau staging system in order to gain insight into the dynamics of the development of PSP-tau pathology.

## Material and methods

### Patients

We selected 42 cases from 102 pathologically diagnosed as PSP that were archived at the Sara Koe PSP Research Centre in the Queen Square Brain Bank for Neurological Disorders between 1992 and 2002. A systematic case note review was performed and the clinical features were recorded in a standardized fashion and cases were divided into three groups based on previously defined clinical criteria to allow for categorical analysis (Williams *et al.*, 2005). When falls, cognitive dysfunction, supranuclear gaze palsy, abnormalities of saccadic eye movements and postural instability were the predominant clinical features in the first 2 years of illness, RS was retrospectively diagnosed, whereas those without these features and with features including asymmetric bradykinesia of the limbs, a positive initial levodopa response, tremor or limb dystonia, were labelled as PSP-P. PAGF was defined when there was a history of gradual onset of freezing of gait or speech, absent limb rigidity and tremor, no sustained response to levodopa and no dementia or ophthalmoplegia in the first 5 years of disease. We aimed to select approximately equal numbers of cases classified as RS and PSP-P with additional representation of PAGF cases. In the most recent 21 brains, comprising 13 cases of RS, 7 of PSP-P and 1 of PAGF tissue blocks had been selected following a standardized protocol and these were therefore analysed first. A further 21 cases in which as many as possible of the appropriate anatomical areas were available for examination were randomly selected without reference to neuropathological details from a pool of 81 cases separated into RS, PSP-P and PAGF. This second group comprised nine cases of RS, seven cases of PSP-P and five cases of PAGF. In 26 cases tau haplotype data were available, and in 13 there were data regarding tau isoform profile in the pontine base (Williams *et al.*, 2005; Pittman *et al.*, 2005). The protocols for the retention and access to human tissue and clinical records at the Queen Square Brain Bank have approval from the London Multi-Centre Research Ethics Committee.

### Pathological methods

#### Diagnostic procedures

Consent for brain donation was obtained from the patients prior to death and consent for post-mortem examination was obtained from the next of kin after death. The diagnosis of PSP was made using standard methods including immunohistochemical analysis using the AT8 anti-tau antibody (tau phospho-epitope Ser202/Thr205). The preliminary PSP pathological diagnostic criteria were applied which insist on the presence of NFTs, neuropil threads and glial tau pathology (Litvan *et al.*, 1996b).

For this study further neuropathological evaluation with a standardized approach was carried out to document Alzheimer pathology using Bielschowsky's silver impregnation and CERAD (Consortium to Establish a Registry for Alzheimer's Disease) criteria (Mirra *et al.*, 1991). A $\beta$  immunohistochemistry was also performed using an anti-A $\beta$  antibody to document cerebral amyloid angiopathy (Revesz *et al.*, 2003). Tau immunohistochemistry with the AT8 antibody was also used in the hippocampal formation to identify the presence of tau-positive grains in the hippocampal formation (Togo *et al.*, 2002). The frontal lobe, lentiform nucleus and pons were examined for vascular pathology including small vessel atherosclerosis, lipohyalinosis, microaneurysm and arteriolosclerosis and their sequelae, such as lacunes, perivascular rarefaction and diffuse white matter attenuation of Binswanger's disease-type. Vascular pathology was graded as mild (occasional vessels affected), moderate (a significant proportion of the small vessels affected with few or no sequelae noted) or severe (a significant proportion of the small vessels affected with obvious sequelae). The presence of associated Lewy body pathology in the substantia nigra was assessed using  $\alpha$ -synuclein immunohistochemistry.

### Immunohistochemistry

Seven micrometres thick tissue sections were cut from tissue blocks of the anterior and posterior frontal cortex, parietal and temporal cortices, midbrain, pons and cerebellum for immunohistochemical analysis. Immunohistochemical staining for tau,  $\alpha$ -synuclein and A $\beta$  peptide (AT8, AutogenBioclear, 1 : 600;  $\alpha$ -synuclein, Novocastra, 1 : 50; A $\beta$  peptide, Dako, 1 : 100) was performed using a standard avidin-biotin method.

### Regional pathological examination and quantification

In each case quantitative assessment of the tau pathology was carried out by one rater (DRW), blinded to the clinical features, in 17 brain regions that have previously been documented to show variability in PSP or were predicted to contribute to the clinical features of disease. The cortex and white matter in anterior and posterior frontal lobes, parietal lobe and temporal lobe, internal and external globus pallidus, putamen, caudate nucleus, subthalamic nucleus, substantia nigra, pontine nuclei, dentate nucleus and cerebellar white matter were used. In each region AT8 positive NFTs, TAs and oligodendroglial CBs were counted separately in seven randomly placed microscopic fields using a  $\times 20$  objective. Thread-like positivity was counted within a  $10 \times 10$  graticule in the same field ( $\times 20$  objective) and added to the CB score to give a CB plus thread (CB+Th) score. This enabled the classification of all immunoreactive lesions that were not obviously part of NFTs or TAs. Absolute counts were converted to a five-point grading scale that was developed for each region after the distribution of lesion counts was examined. Counted values were plotted along the *x*-axis and ranges for grades 0 to 4 were assigned on the *y*-axis. Grade 0 was reserved for when tau-positive pathology was absent. If the distribution of counts fitted a logarithmic curve the upper limit of grade 4 was set as the highest number counted, grade 1 the lowest count above zero and grades 2, 3 and 4 evenly spaced between these markers on a log scale along the *y*-axis. If the distribution of counts suggested that a linear model was more appropriate the upper limit of grade 4 was set at the highest value counted and grades 1–3 were set at 25, 50 and 75% of this

highest value. In regions where the range included less than four values, absolute counts were used.

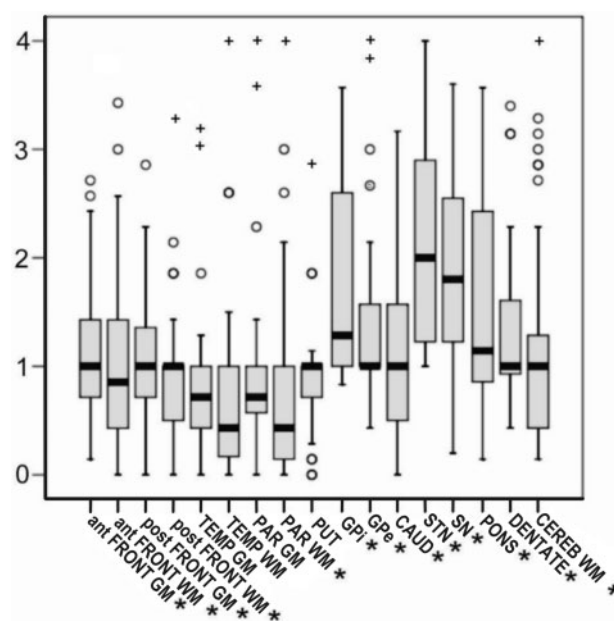
The repeatability of all counts was assessed by the same rater (DRW) by re-counting four (10%) cases. The intra-rater standard deviation (IRSD) was calculated as follows:  $IRSD = SD(R_{m1} - R_{m2})/\sqrt{2}$ , where SD is standard deviation,  $R_{m1}$  is median grade at first reading and  $R_{m2}$  is median grade at second reading.

### Regions representative of overall tau severity

All graded data were explored to identify the minimum number of regions that satisfactorily summarized the overall tau severity. Grades from the temporal lobe grey matter were excluded to remove the influence of Alzheimer-type pathology on the analysis. First, the overall tau severity was determined in each case, by adding the grades of all lesions in all regions together. Next, the median grades for NFT, TAs, CBs and CB+Th were calculated for each region in each case (see example, Fig. 1). After choosing regions with the greatest range of grades, different combinations of regions and lesion types were tested for correlation with overall tau severity. It was found that the sum of grades for CB+Th pathology in the substantia nigra, caudate and dentate nucleus best correlated with the overall tau severity (see section 'Results'). This number designated the PSP-tau score (substantia nigra CB+Th grade + caudate CB+Th grade + dentate CB+Th grade) had a range from 0 to 12. The Friedman test was used to assess whether increments in the PSP-tau scores were associated with statistically significant increases in overall tau severity.

### Neuropathological grading of tau severity

So that the repeatability of the pathological data used to produce the PSP-tau score could be tested, images representative of grades 1 to 4 of CB+Th pathology for the three regions of interest were



**Fig. 1** Coiled body + thread pathology grades in different brain regions; median, interquartile ranges;  $\circ$  outliers;  $+$  extreme outliers; \* regions used to test for correlation of overall tau.

obtained and used to produce a visual aid to grading (Fig. 2). This aid only, was used independently by two neuropathologists (TR and JH) as a guide to grading CB+Th pathology in the substantia nigra, caudate and dentate nucleus. While blinded from the clinical details, they were asked to grade the CB+Th pathology in the 34 cases of PSP with a complete data set (RS 21, PSP-P 12 and PAGF 1). The neuropathologists were asked to examine five to seven microscopic fields ( $\times 20$  objective) in the dentate nucleus, caudate and substantia nigra. They were instructed to consider all sub-regions of the substantia nigra represented in the section and informed that all tau-positive lesions except NFTs and TAs were to be evaluated to give the CB+Th grade. Grade 0 was given when there were no pathological lesions, and was not included on the visual aid. Finally PSP-tau scores were calculated using the sum of median scores from all the three sampled regions (substantia nigra CB+Th grade + caudate CB+Th grade + dentate nucleus CB+Th grade), giving a score with a minimum of 0 and maximum of 12. Agreement between these two raters was assessed by calculating the weighted kappa and applying Landis and Koch's categorization of responses (Landis and Koch, 1977).

Distribution of pathological tau

The regional distribution of PSP-tau pathology was compared between cases of different severity. Cases were separated according to their PSP-tau score (0–1, 2–3, 4–5, 6–7 and >7) and median values of CB+Th were calculated for each brain region in each group.

Statistical methods

Spearman's correlation calculation was used to determine the relationship between the PSP-tau score and disease duration, age of onset, age at death, time from disease onset to first fall and time from disease onset to supranuclear gaze palsy. The correlation between PSP-tau score and the severity of tau lesions in each region (grade and absolute counts) was also examined using Spearman's calculation. The significance level for this test was set at 0.01 because of the multiple comparisons. For other data univariable analyses using  $\chi^2$  for categorical and two-tailed  $t$  test or the Mann–Whitney U test, as appropriate, for continuous variables were applied with a significance level of 0.05. All statistical analysis was performed using SPSS for Windows (version 12.0.1).

Results

Forty-two patients (26 men, 16 women) were included in the study. The mean age of onset was 66.5 years (range 44.4–87.5), the mean age at death was 75.6 years (60.9–95.8) and the mean disease duration was 9.1 years (1.2–17.3). Additional pathological findings are recorded in Table 1. There was no difference between the RS and PSP-P groups in the prevalence or severity of the CERAD plaque score, small vessel pathology, cerebral amyloid angiopathy or prevalence of tau-positive grains.

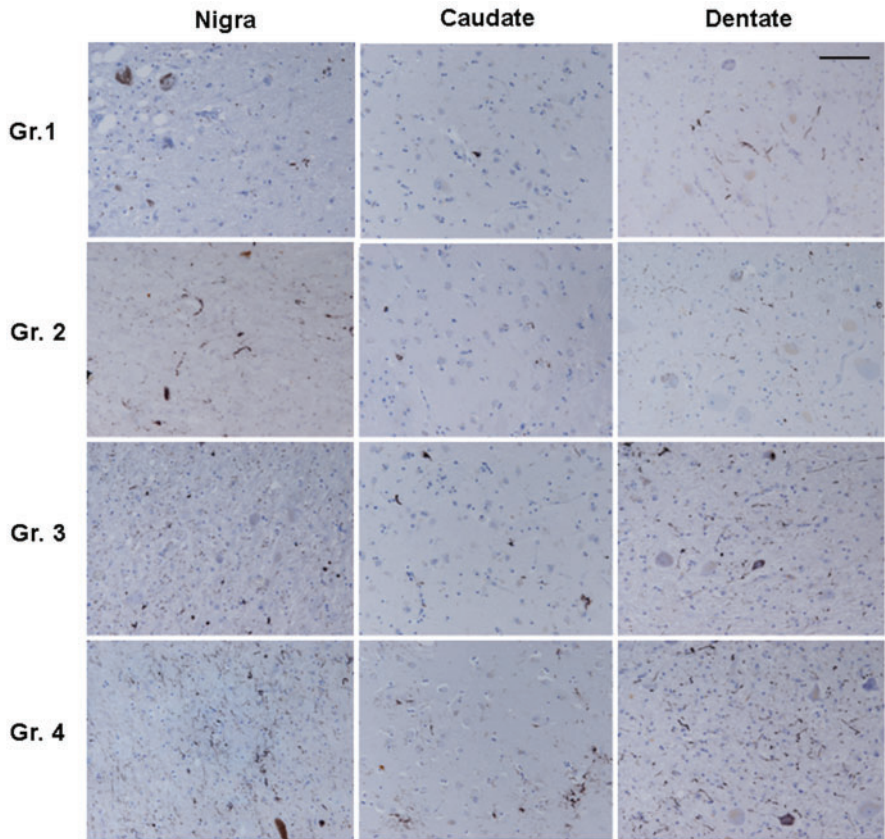


Fig. 2 Visual guide for scoring pathology severity in three regions of interest. Grade 0 was reserved for there was no AT8 positivity; bar represents 77  $\mu$ m on all panels ( $\times 20$  magnification).

## PSP-tau pathology

The diagnosis of PSP was confirmed in all the cases by the presence of typical PSP-tau lesions including NFTs, TAs, CBs and neuropil threads in the expected distribution. The intra-rater standard deviation of the regional analysis was 0.012, implying that the variability of counting was <2% and was thus highly repeatable.

The number of pathological lesions per microscopic field varied substantially between regions and between cases, although in no case was the subthalamic nucleus, substantia nigra or internal globus pallidus spared. NFTs were most abundant in the basal ganglia and brainstem structures and TAs in the cortices, putamen and caudate nucleus. CB+Th were the major contributor to the overall tau load and were most numerous in the subthalamic nucleus (median 283/microscopic field, range 8–1710), substantia nigra (median 186/microscopic field, range 0–1430) and internal globus pallidus (median 49/microscopic field, range 0–720), and least dense in the cortical regions.

**Table 1** Additional pathological findings by clinical group

Number	RS 23	PSP-P 13	PAGF 6
CERAD neuritic plaque score			
Negative	10 (45)	6 (46)	2 (33)
Positive	13 (55)	7 (54)	4 (67)
Sparse	5 (21)	3 (23)	2 (33)
Moderate	7 (30)	3 (23)	2 (33)
Frequent	1 (4)	1 (8)	0
Tau-positive grains	4 (18)	6 (46)	0
Lewy body pathology	1 (4)	0	0
Small vessel pathology	7 (30)	6 (46)	0
Mild	6 (26)	5 (38)	0
Moderate	0	1 (8)	0
Severe	1 (4)	0	0
Cerebral amyloid angiopathy	3 (13)	3 (23)	1 (17)

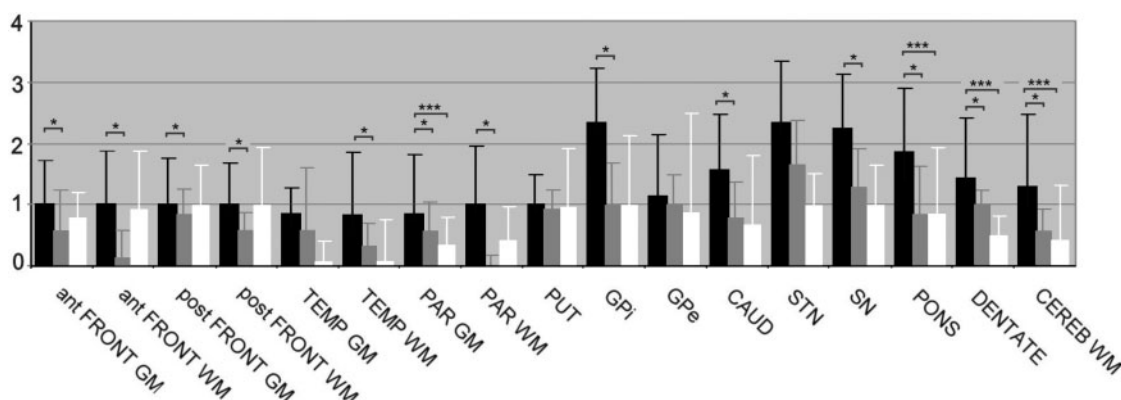
Note: Percentages are shown in brackets.

In the cases with the lowest density of lesions, tau pathology was limited to the subthalamic nucleus, substantia nigra and internal globus pallidus, with sparse tau immunoreactivity in the posterior frontal cortex and white matter, and few NFTs in these structures. As the severity of CB+Th pathology in the subthalamic nucleus, substantia nigra and internal globus pallidus increased, there was a concurrent increase in the density and severity of tau immunoreactivity in the pontine nuclei, dentate nucleus, cerebellar white matter and frontal cortex. The parietal lobe was not affected in cases with milder tau pathology, but in those with severe CB+Th pathology in the basal ganglia it was severely affected. Alzheimer changes lay within the expected range for the age groups, but Lewy body pathology was less frequent than previously reported (Braak *et al.*, 1992; Tsuboi *et al.*, 2001).

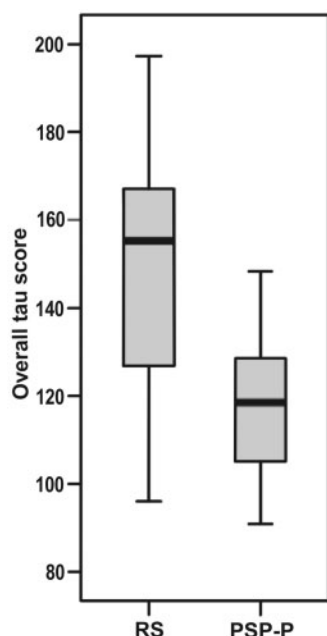
The mean regional tau-severity was higher in RS than PSP-P and PAGF in all brain regions. The difference with PSP-P was significant in all regions except the putamen (Mann–Whitney U test,  $P=0.15$ ) and subthalamic nucleus (Mann–Whitney U test,  $P=0.77$ ) (Fig. 3). Tau pathology was significantly more severe in RS than PAGF in the parietal cortex (Mann–Whitney U test,  $P=0.022$ ), pontine nuclei (Mann–Whitney U test,  $P=0.003$ ), dentate nucleus (Mann–Whitney U test,  $P=0.01$ ) and cerebellar white matter (Mann–Whitney U test,  $P=0.023$ ). Total tau load ( $\Sigma$  of grades for all lesions in all regions) was higher in the RS group (median 155) than in the PSP-P group (median 116, Mann–Whitney U test,  $P=0.002$ ) (Fig. 4). The PAGF group was not analysed further because of the small number of cases with a complete data set.

## Grading of PSP-tau severity

The PSP-tau score ( $\Sigma$  CB+Th grade in the substantia nigra, caudate and dentate nucleus) correlated significantly



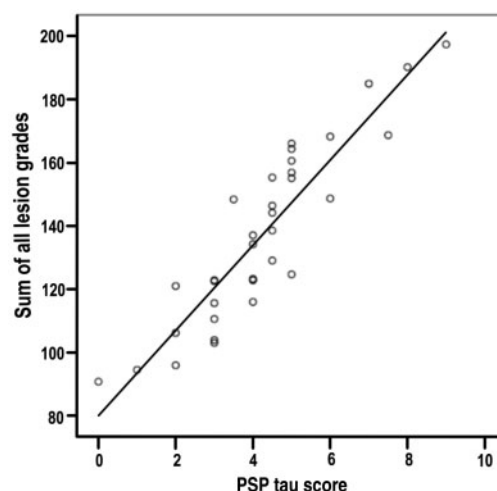
**Fig. 3** Median regional tau severity in RS (black), PSP-P (grey) and PAGF (white). 1 standard deviation indicated by error bars. \*RS versus PSP-P, Mann–Whitney U,  $P < 0.05$ ; \*\*\*RS versus PAGF, Mann–Whitney U,  $P < 0.05$ . ant, anterior; post, posterior; GM, grey matter; WM, white matter; FRONT, frontal lobe; TEMP, temporal lobe; PAR, parietal lobe; PUT, putamen; GPI, internal globus pallidus; GPe, external globus pallidus; CAUD, caudate; STN, subthalamic nucleus; SN, substantia nigra; PONS, pontine nuclei; DENTATE, dentate nucleus; CEREB, cerebellar.



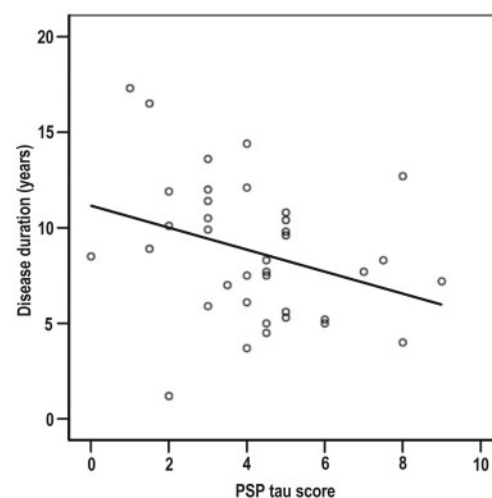
**Fig. 4** Overall tau severity (sum of grades for all tau lesions in all regions) according to clinical group. Median (Mann–Whitney U test,  $P = 0.002$ ) and interquartile ranges.

with the overall CB+Th severity ( $\sum$  CB+Th grades in all 17 regions, Spearman's rho 0.89,  $P < 0.001$ ) and also highly correlated with the overall lesion severity comprising all lesion types ( $\sum$  all lesion grades in all 17 regions, Fig. 5, Spearman's rho 0.93,  $P < 0.001$ ) in the 34 cases with a complete data set. There was a significant positive correlation (Spearman's rho,  $P < 0.01$ ) between the PSP-tau score and lesion grades in 70% of regions and lesion counts in 63% of regions (supplementary material). PSP-tau score correlated with NFT grades in 36% of brain regions, CB grades in 100% and CB+Th grades in 94% of regions (all, Spearman's rho  $P < 0.01$ ). No other combination of regional grades correlated with more regions. Each increment in the PSP-tau score (tau score 0 to 10—this latter being the highest observed) was significantly different from the previous according to the mean ordinal rank of tau severity (Friedman's  $\chi^2$ , 23.7,  $P = 0.005$ ). The weighted kappa measuring agreement between two independent pathologists using the visual aid and instructions for grading was 0.71, which is 'very' good inter-rater agreement (Landis and Koch, 1977).

The PSP-tau score did not correlate with age at symptom onset or age at death, but there was a negative correlation between PSP-tau score and disease duration (Spearman's rho  $-0.36$ ,  $P = 0.028$ ) (Fig. 6). Time from disease onset to first fall (Spearman's rho  $-0.49$ ,  $P = 0.003$ ) was negatively correlated with PSP-tau score. There was a modest, but significant correlation between tau isoform ratio (4-repeat tau:3-repeat tau) in the few cases with this data (Spearman's rho 0.56,  $P = 0.048$ ). The only two cases that



**Fig. 5** Correlation between PSP-tau score and sum of all tau grades (Spearman's rho 0.93,  $P < 0.001$ ).

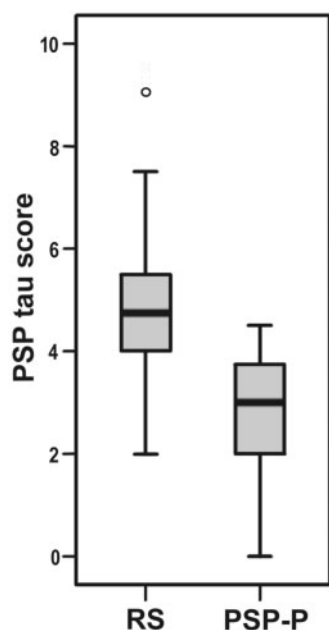


**Fig. 6** PSP-tau score versus disease duration (Spearman's rho  $-0.36$ ,  $P = 0.028$ ).

did not have the H1/H1 PSP susceptibility genotype (H2/H2 and H2/H1) had the lowest PSP-tau scores (0 and 1, respectively). The three cases homozygous for the H1c haplotype did not have a significantly higher tau score than cases with other genotypes. No cases classified as PSP-P had a PSP-tau score of more than 5 (median 3), and there was a significant difference between the median PSP-tau scores for RS (median 5, versus PSP-P, Mann–Whitney U test,  $P < 0.001$ ) (Fig. 7).

### Distribution of PSP-tau pathology

As our findings suggested that the PSP-tau score (sum of CB+Th grades in substantia nigra, caudate and dentate nucleus) was a reasonable surrogate marker for pathological



**Fig. 7** PSP-tau score according to clinical group, median (Mann–Whitney U test,  $P < 0.001$ ) and interquartile ranges.

disease severity with significant clinical correlations, the relationship between this surrogate marker for pathological severity and the distribution of lesions throughout all 17 brain regions was further examined. Cases were grouped according to PSP-tau score (PSP-tau score 0–1, 2 cases; 2–3, 9 cases; 4–5, 16 cases; 6–7, 3 cases; >7, 3 cases) and median values of CB+Th for each region were calculated (Fig. 8). Pathological features noted, in addition to the graphically displayed median data for CB+Th, are discussed according to PSP-tau score.

*Scores 0–1: involvement limited to pallido-luysio-nigral distribution with sparse involvement of the pre-motor cortex:* Regions caudal to the substantia nigra had little or no tau pathology, including the dentate nucleus, cerebellar white matter and the pontine nuclei. In these cases the parietal cortex was spared from tau lesions and the anterior frontal lobe had very few lesions.

*Scores 2–3: moderate involvement of basal ganglia, pontine nuclei and dentate nucleus in the absence of parietal lobe lesions:* The severity of the previously described lesions was increased and in particular the number of tau positive lesions in the subthalamic nucleus and globus pallidus was increased. Caudal regions, including the dentate nucleus and pontine nuclei, were affected and in some cases the cerebellar white matter was mildly involved. There were NFTs, TAs and thread pathology in the posterior frontal lobe, but the anterior frontal lobe was rarely involved.

*Scores 4–5: more severe involvement of the basal ganglia and dentate nucleus with involvement of the frontal and parietal lobes:* Brains with scores of 4 or 5 were most numerous in this study. The internal globus pallidus,

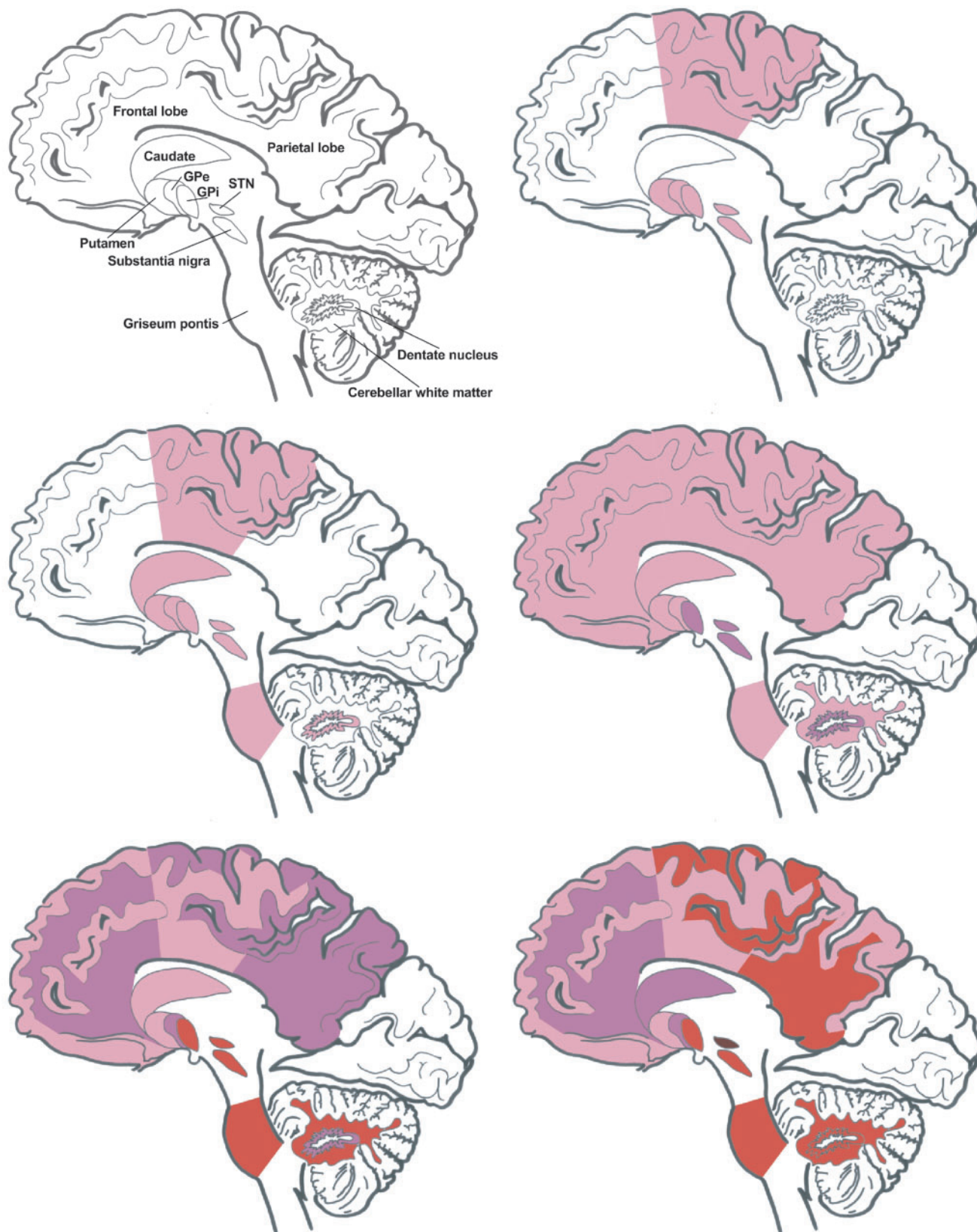
subthalamic nucleus, substantia nigra, pontine nuclei, dentate nucleus and cerebellar white matter were more severely affected, but there was no increase in CB+Th pathologies in the external globus pallidus. Cortical regions, including the frontal and parietal lobes, were consistently moderately affected.

*Scores 6–7: moderately severe pathology in the basal ganglia, pontine nuclei, parietal and frontal lobes:* In the internal globus pallidus numerous NFTs, threads and CBs were present, and the severity of all lesions, except NFTs, was equally severe in the external globus pallidus. The caudate and putamen remained only moderately affected, similar to the lower scored brains, but the involvement of caudal structures was more severe, in particular the cerebellar white matter. In these brains the neocortical regions, except the temporal lobe, had moderately severe NFTs, TAs, thread pathology and CBs.

*Score >7: severe involvement of the subthalamic nucleus, substantia nigra, internal globus pallidus as well as neocortical areas, pontine nuclei and cerebellar structures:* In the three cases in this category there was severe pathology throughout the regions examined, with the exception of the caudate, putamen and temporal lobe where there was very little increase in the numbers of tau lesions as the score increased above 2.

## Discussion

The higher contribution by 3-repeat tau isoforms to the insoluble tau fraction in PSP-P raised the possibility that the biological differences determining different clinical PSP sub-types might also affect the severity and distribution of the histopathological lesions (Williams *et al.*, 2005). We have indeed demonstrated pathological differences between the two major clinical sub-types, with the RS group having a significantly higher total PSP-tau burden than the PSP-P group. The quantitative data for the overall severity of PSP-tau pathology can be assessed reliably by using a 12-tiered scoring system that takes into account the tau burden in oligodendroglia and threads in three anatomical regions: the substantia nigra, caudate and dentate nucleus. This can also be reproduced consistently when only a visual aid, akin to those used for the CERAD diagnosis of Alzheimer's disease (Mirra *et al.*, 1991) and dementia with Lewy bodies (McKeith *et al.*, 2005), is used for grading. Having assigned a PSP-tau score to each case, an excellent correlation was found with the overall tau-load determined by the morphometric data. Further analysis also revealed important associations between PSP-tau scores and clinical, biochemical and genetic features of the cases. Those with RS had a significantly higher PSP-tau score than the PSP-P cases, and no PSP-P case had a score of more than 5. In addition, we established that with increments in PSP-tau scores tau deposition also becomes more widespread indicating that PSP-P is not only associated



**Fig. 8** Distribution of median coiled body + thread tau pathology, according to PSP-tau score. Colour/median grade per PSP-tau score: pink/grade 1; purple/grade 2; red/grade 3; brown/grade 4. A = legend; B = PSP-tau scores 0–1; C = PSP-tau scores 2–3; D = PSP-tau scores 4–5; E = PSP-tau scores 6–7; F = PSP-tau scores >7.

with a lower tau burden, but also that this takes place in a topographically more restricted pattern. Disease duration was shortest in patients with the most severe tau pathology, and therefore the highest PSP-tau score and topographically most extensive tau pathology. Tau severity, according to this score, correlated with 4-repeat tau:3-repeat tau ratio. Patients who carried the H2 PSP-protective allele had the lowest tau scores.

Although differences in the severity of pathology in some brain regions have previously been correlated with particular clinical features, heterogeneity in regional tau distribution has made qualitative and quantitative comparisons between pathology and clinical features problematic (Daniel *et al.*, 1995; Verny *et al.*, 1996; Revesz *et al.*, 1996; Bigio *et al.*, 1999; Halliday *et al.*, 2000; Tsuboi *et al.*, 2005; Josephs *et al.*, 2006). We used an unbiased, region-specific and lesion-specific grading system to overcome these difficulties and to allow for regional comparisons to be made. The severity of CB+Th pathologies were the most useful in separating cases of PSP and the PSP-tau score correlated well with the majority of other pathological lesions, but not NFTs in the striatum, substantia nigra or subthalamic nucleus (see supplementary material). Unlike NFTs, there is no convincing evidence that threads, representing accumulation of PSP-tau in both neuronal and oligodendroglial processes (Probst *et al.*, 1988; Ikeda *et al.*, 1994; Dickson, 1999), decrease in density in PSP as the disease progresses (Halliday *et al.*, 2000), making their post-mortem analysis more likely to reflect dynamic changes during the course of the disease.

We have previously shown that amongst the clinical features of patients with PSP-tau pathology, a number of distinct clinical syndromes can be identified (Williams *et al.*, 2005). The close association between the severity of the PSP-tau scores, as a measure of the PSP-tau load, and the clinical sub-types also suggests that they are a sensitive surrogate marker of the functional and structural changes that accompany the underlying neurodegenerative processes and determine the different PSP phenotypes. Others have also found that the severity of tau accumulation, and neuronal loss, in the substantia nigra pars reticulata (Halliday *et al.*, 2000), mesencephalon (Juncos *et al.*, 1991) and the pontine nucleus raphe interpositus (Revesz *et al.*, 1996), is higher in patients with gaze palsy (RS) compared to those without (mostly PSP-P). However, the current findings suggest these localized changes mirror neurodegeneration in a number of other susceptible regions, and are part of more severe and widespread disease in the brain. This notion is supported by several reports of an association between the severity of tau changes in cortical regions and cognitive dysfunction in PSP and by our finding of more severe cortical pathology in RS (Bergeron *et al.*, 1997; Tsuboi *et al.*, 2005; Josephs *et al.*, 2006). In contrast, patients who present with parkinsonism without gaze palsy, early falls and dementia have more

moderate pathological tau accumulation, in a more restricted pattern of distribution.

The general pattern of distribution of tau pathology in PSP appears remarkably consistent despite the broad spectrum of clinical features recorded during life. No case of 'incidental' PSP-tau has been included in this analysis, and, to our knowledge, definitive reports of such cases have not been made. In their absence, and without a substantial number of cases with early PSP, attempts to determine the dynamics of topographical evolution of tau pathology cannot be considered. However, we have constructed a model that may represent the disease 'footprint' of relative susceptibility to regional pathological tau accumulation in PSP. This model identifies the subthalamic nucleus, substantia nigra and globus pallidus as the most susceptible regions and that other regions lag behind in severity in a gradient from the posterior frontal lobe, dentate nucleus, cerebellar white matter, pontine nuclei, caudate, to the anterior frontal and parietal lobes. The significant negative correlation between the PSP-tau score and disease duration suggests that more fulminant disease affects more regions, more severely from disease onset and contributes to an earlier death. The factors that influence the severity and extent of PSP-tau pathology are unknown, but the genetic background and, in particular, possession of the H1c tau sub-haplotype, may be important (Baker *et al.*, 1999; Pittman *et al.*, 2005). Our observation that the patients with at least one H2 allele accounted for the two mildest cases of tau accumulation is of interest given recent suggestions that the H2 tau haplotype not only lacks an association with PSP, but also that it contributes to protection from the disease (Pittman *et al.*, 2005). The least severe pathology also tended to have the lowest ratio of 4-repeat tau:3-repeat tau in the pontine base. Recent findings suggest that the presence of the *MAPT* H1c sub-haplotype favours the increased production of 4-repeat tau in controls and possibly its accumulation in disease (Myers *et al.*, 2006). The small number of cases homozygous for H1c tau sub-haplotype in our study did not have a significantly higher severity of tau pathology implying that other modifying factors may influence the effect of genetic susceptibility.

Our data are also consistent with the notion that regions predicted to be most susceptible to PSP-tau neurodegeneration (subthalamic nucleus, substantia nigra and globus pallidus) have the lowest threshold for disease manifestation and in the context of protective factors, such as the H2 allele for example, may remain the only severely affected regions. Other related tauopathies, such as post-encephalitic parkinsonism and parkinsonism dementia complex of Guam have overlapping pathological features (Geddes *et al.*, 1993), and recent data suggest that genetic factors that contribute to the Guam neurodegenerative disease risk may also, at least in part, be similar to those in PSP (Sundar *et al.*, 2007). The differences in the type, ultrastructural and biochemical characteristics of the tau

pathologies of these diseases and PSP that are also documented are, however, consistent with the presumed different aetiologies of these conditions (Geddes *et al.*, 1993; Buee-Scherrer *et al.*, 1995; Litvan *et al.*, 1996b; Mawal-Dewan *et al.*, 1996; Buee-Scherrer *et al.*, 1997; Oyanagi *et al.*, 2001; Oyanagi, 2005; Williams, 2006). Nevertheless the similarities in the distribution of the pathological lesions in post-encephalitic parkinsonism and parkinsonism dementia complex of Guam that overlap with the range described in the present work, raise the possibility of similar factors contributing to regional tau susceptibility in these diseases particularly in the substantia nigra, subthalamic nucleus and pallidum (Geddes *et al.*, 1993; Oyanagi *et al.*, 1997, 2000, 2001).

The grading system used in this study is a practical and sensitive tool for the morphological assessment of PSP-tau pathology and takes into account the clinical, biochemical and genetic diversity that is known to occur in PSP. Although the relationship between neuronal loss and PSP-tau pathology remains to be determined, the proposed model provides a framework for further evaluating the cellular factors that contribute to PSP-tau neurodegeneration, and the clinical and biochemical manifestations of the disease. A pathological distinction between PSP-P and RS can be made, which together with the biochemical differences gives weight to previous attempts of separating these two PSP phenotypes clinically. Future research identifying genetic and other disease markers may also help to underpin the notion that PSP-P is a distinct clinicopathological entity, albeit with a close relationship with RS. In the meantime the pathological distinction between PSP-P and RS emphasizes the need to identify these patients in the clinic and that research in PSP should also take into account the biological differences that exist between these clinical phenotypes.

## Supplementary material

Supplementary material is available at *Brain* online.

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