RESEARCH ARTICLE

Copathology in Progressive Supranuclear Palsy: Does It Matter?

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ABSTRACT: Background: The influence of concomitant brain pathologies on the progression rate in PSP is unclear.
Objectives: To analyze the frequency and severity of copathologies and their impact on the progression in PSP.
Methods: We analyzed clinic-pathological features of 101 PSP patients. Diagnoses and stages of copathologies were established according to standardized criteria, including Alzheimer's disease–related pathology, argyrophilic grains, Lewy-related pathology, transactive response DNA-binding protein 43 pathology, fused in sarcoma pathology, cerebral amyloid angiopathy, and small vessel disease. Demographic data and major clinical milestones (frequency and latency to onset) were extracted from patients' files.
Results: Only 8% of 101 patients presented with pure PSP pathology without any copathology. Alzheimer's disease–related pathology was the most frequent (84%), followed by argyrophilic grains (58%), both occurring as single copathology or in combination with other proteinopathies or cerebrovascular disease. Lewy-related and transactive response DNA-binding protein 43 copathology occurred rarely (8% and 6%, respectively). Fused in sarcoma–positive cases were not found. While being common, copathology was mostly mild in severity, with the exception of frequently widespread argyrophilic grains. Small vessel disease was also frequent (65%). Cerebral amyloid angiopathy occurred only in the presence of Alzheimer's disease–related changes (25%). The copathologies did not have major impact on prevalence and time frame of major disease milestones.

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Conclusions: In PSP, concomitant neurodegenerative proteinopathies or cerebrovascular diseases are frequent, but generally mild in severity. Our data confirmed that four repeat tau is still the most relevant target for PSP, whereas the impact of copathologies on progression rate appears to be of less importance. This is relevant information for the development of disease-modifying therapies.

Key Words: clinical phenotype; clinic-pathological correlation; copathology; progressive supranuclear palsy

Understanding the mechanism underlying disease progression, as well as measuring the progression rate of neurodegenerative disorders (NDDs), is central to defining the effects of therapeutic intervention. Progression of NDDs, including PSP, is thought to be largely driven by specific protein aggregations and their cellular and regional transmission within the central nervous system. However, given that NDDs typically manifest later in life, it is not surprising that additional proteinopathies and vascular copathologies may coexist and may also contribute to the progression rate of such diseases. In line with this view, copathologies, such as Alzheimer;s disease (AD)-related pathology in synucleinopathies, as well as transactive response DNA-binding protein 43 (TDP-43) copathology in AD, were found to shape the clinical presentations of these NDDs.[1-4]

PSP is a primary tauopathy, defined by accumulation of the four repeat (4R) isoforms of the microtubule-associated protein tau in neurons and glial cells, with tufted astrocytes as the diagnostic hallmark lesion of this disease.[5,6] A recent study indicated that, among tauopathies, PSP has the highest prevalence of additional NDD-related proteins.[3] However, no data exist about the influence of such findings on the disease course in PSP.

Given that the tempo of disease progression is mainly highlighted through the latency to reach major clinical milestones of PSP, we aimed to analyze the possible influence of concomitant brain pathology on occurrence of such milestones, in a well-characterized clinicopathological cohort of 101 autopsy-confirmed PSP patients.

Materials and Methods

Collection of Data

All brain samples of consequently diagnosed PSP patients, available at the start of this study, were collected from four European brain banks and sent to the Center for Neuropathology and Prion Research, Munich (the German national reference center for neurodegenerative disorders and the coordinating center of the BrainNet Europe), where standardized central verification of the pathological diagnosis of PSP and analysis of concomitant brain pathology were performed. Patients were removed from analysis for a neuropathological copathology assessment, if they did not have paraffin-embedded materials available from all the neuroanatomical regions required for analysis of PSP pathology and copathology, as defined in our analysis protocol. In addition to this, cases were subsequently excluded, if they had insufficient clinical data regarding the presence and onset of the characteristic symptoms analyzed in the current study. A total sample of 101 PSP cases were finally included: 55 cases from Neurobiobank Munich, Center for Neuropathology and Prion Research, Ludwig-Maximilians-University, Munich, Germany; 12 cases from MRC London Neurodegenerative Diseases Brain Bank, King's College, London, United Kingdom; 23 cases from Netherlands Brain Bank, Amsterdam, Erasmus Medical Center, Rotterdam, The Netherlands; and 11 cases from Neurological Tissue Bank of the Biobanc–Hospital Clinic–IDIBAPS, Barcelona, Spain.

All donors or their next of kin had given written informed consent according to the Declaration of Helsinki for the use of brain tissue and medical records for research purposes. Usage of the material was in accordance with the directives of the local ethics commission regarding the use of archive material for research purposes.

Pathological Evaluation and Immunohistochemistry

After fixation in 10% formalin, 5-µm-thick paraffin sections were mounted, deparaffinized, hydrated, and stained. Hematoxylin and eosin (Hoffmann-LaRoche, Basel, Switzerland) staining was performed according to the manufacturer's guidelines. For antigen retrieval, slides were heated in 10 mM of sodium citrate buffer (Sigma-Aldrich, St. Louis, MO) at 90°C for 20 minutes. All immunohistochemical stainings were performed semiautomatically on a BenchMark device (Ventana, now Hoffmann-LaRoche). Nuclear counterstaining was performed with hematoxylin (Hoffmann-LaRoche). The antibodies that were used in our study, according to standard protocols, are presented in Supporting Information Table S1. Slides were analyzed on a Leica CTR 6000 microscope (Leica Microsystems, Wetzlar, Germany).

The neuropathological diagnosis of PSP was defined in analogy to National Institute of Neurological Disorders and Stroke neuropathological criteria[7] and specifications in Dickson and colleagues.[6] For this study, the major criterion for the neuropathological diagnosis of PSP was the presence of tufted astrocytes in the

striatum (preferably in the head of the caudate nucleus) and/or in the cortex of the middle or superior frontal gyrus at the level of the anterior striatum.

Tau pathology was assessed on AT8-stained paraffin sections. Different immunopositive structures of tau-pathology (neuronal cytoplasmatic inclusions, threads in neuropil or white matter, tufted astrocytes, grains in neuropil, and neuritic plaques [NPs]) were analyzed separately.

Diagnoses and stages of copathologies were established according to standardized criteria, as follows:

- AD-related pathology (Thal phases of amyloid beta [Aβ] plaques[8]; Consortium to Establish a Registry for Alzheimer's Disease [CERAD] NP frequency scores[9]; and Braak and Braak stages of anti-Tau 3-repeat isoform antibody [RD3]-positive neurofibrillary tangles [RD3-NFTs],[10] which are further transformed into the ABC scores[9]);
- Argyrophilic grains (AGs; Saito satges)[11];
- Lewy-related pathology (LRP; Braak stages)[12];
- Check for the presence of TDP-43 and fused in sarcoma (FUS)-positive inclusions;
- Cerebral amyloid angiopathy (CAA; Thal stages)[13]; and
- Small vessel disease (SVD; stages 0–3).[14]

Details on the evaluation of copathology are given as Supporting Information.

Apolipoprotein E Status

Apolipoprotein E (APOE) polymorphism had been determined by restriction enzyme analysis. The target DNA was amplified by polymerase chain reaction (PCR) and subsequently analyzed by digestion with the restriction enzyme, Hha I, followed by polyacrylamide gel electrophoresis of the cleavage products. Primer sequences and the detailed PCR protocol are available upon request

Clinical Data

The following clinical data were operationalized and extracted from the patient's medical records, as reported previously[15]: clinical diagnoses (first and the last), sex, age at onset, age at death, disease duration, occurrence, and latency from disease onset to onset of following clinical disease milestones, considered to be of utmost importance for PSP[15-17]: falls, SNGP, FLD, cognitive dysfunction, axial rigidity, bradykinesia, dysarthria, and dysphagia (for the details on the definition of each investigated milestone, please see Supporting Information Table S2).

These features were considered present if specifically mentioned in the clinical notes. They were considered absent if they were specifically mentioned as absent or if they were not mentioned ("not available"). The onset of features relative to disease onset was recorded. If the onset of a symptom or sign could not be abstracted from the files, the year of onset was excluded from the analysis of their temporal evolution.

Statistical Analysis

The IBM SPSS statistical software package (version 23.0; IBM Corp., Armonk, NY) was used in the statistical analysis.

The proportional distribution of categorical variables was analyzed with the chi-squared test.

To identify differences between two and multiple group means, *t* test (with Bonferroni correction) and analysis of variance (corrected for multiple testing with the Games-Howell post-hoc test) were used, respectively.

The association between onset of clinical PSP milestones and presence of additional NDDs or cerebral vessel copathology were further investigated through the multi- and binominal logistic regression analyses, controlled for age and sex.

Statistical significance was defined as a P value <0.05.

Results

Demographic and Clinical Data

Of the 101 cases included in the analysis, 43.6% were females. Average age at disease onset and at death was 65.5 and 73.1 years, respectively. Disease duration, from symptom onset to death, ranged from 2 to 27 years, with a mean of 7.6 years (Table 1).

Correct clinical diagnoses of PSP were made in 9.9% at first clinical visit and in 72.3% the final examination (Table 1).

Falls were the earliest and most frequently reported among the clinical milestones of PSP, occurring on average 2 years after disease onset. Bradykinesia, FLD, and cognitive impairment occurred on average 3 years after disease onset, followed by SNGP after 3.5 years on average. Axial rigidity and dysarthria occurred approximately after 4 years and dysphagia after 4.4 years, on average (Table 1).

Copathology

Frequencies of the investigated copathologies are presented in Figure 1A. Severity distributions (stages) for the observed copathologies are presented in Figure 1B to 1I.

Neurodegenerative Copathology Findings

Only 8% of patients had pure PSP-specific pathology, without any other neurodegenerative copathology. The following copathologies were observed (Fig. 1).

AD-Related Copathology. The most frequently observed copathology (84% of our PSP patients) was AD related (RD3-NFT and/or Aß deposits). Among these, 23 patients had only RD3-NFT in the absence of Aß pathology, 7 patients exhibited Aß pathology in the

TABLE 1. Demographic and clinical data of 101 definitive PSP patients

Female:male, no. (%)	44:57 (43.6:56.4)
Age at disease onset	65.5 ± 7.9 [41–91]
Age at death	73.1 ± 7.0 [55–93]
Disease duration	7.6 ± 4.2 [2–27]
Initial clinical diagnosis	
Correct, no. (%)	10 (9.9)
Not reported, no. (%)	49 (48.5)
Final clinical diagnosis	
Correct, no. (%)	73 (72.3)
Not reported, no. (%)	1 (1.0)
Falls	
Time latency from onset [years]	2.2 ± 2.4 [1–18]
Frequency [%]	83.2
Bradykinesia	
Time latency from onset [years]	2.9 ± 2.5 [1–15]
Frequency [%]	84.2
Cognitive dysfunction	
Time latency from onset [years]	3.0 ± 3.4 [1–18]
Frequency [%]	83.2
Frontal lobe dysfunction	
Time latency from onset [years]	3.1 ± 3.5 [1–18]
Frequency [%]	73.3
Supranuclear gaze palsy	
Time latency from onset [years]	3.5 ± 2.8 [1–14]
Frequency [%]	78.2
Dysarthria	
Time latency from onset [years]	3.9 ± 3.3 [1–19]
Frequency [%]	79.2
Axial rigidity	
Time latency from onset [years]	4.1 ± 2.3 [1–15]
Frequency [%]	65.4
Dysphagia	
Time latency from onset [years]	4.4 ± 3.4 [1–19]
Frequency [%]	78.2

Values are presented in years, as means ± standard deviations [range], unless noted otherwise. Frequencies indicate percentages of patients developing the respective symptoms. For calculation of latencies, only patients for which the symptoms and time of onset were reported were considered.

absence of RD3-NFT, whereas the majority (54 cases) had both RD3-NFT and Aß changes (Fig. 1A–D). In most cases, Aß deposits were restricted to cortical and subcortical areas (Thal phases from 1 to 3), whereas only in 7 cases these changes were also found in the brainstem and cerebellum (phases 4 and 5; Fig. 1B). According to the Consortium to Establish a Registry for Alzheimer's Disease modified by the National Institute on Aging–Alzheimer's Association guidelines (CERAD) NP frequency score, 95% of patients belong to the group of zero or mild frequency (score 1; Fig. 1C). Pathology of RD3-NFT was mainly restricted to the (trans)enthorhinal area (Braak & Braak stage I and II), rarely extending into the cortex of the fusiform gyrus (stage III), without further progression to other neocortical regions (Fig. 1D). Although AD-related copathology was a frequent finding in this PSP cohort, none of these cases showed the full neuropathological picture of an AD case with Braak & Braak stage IV or higher and frequent NPs according to a modified CERAD stage C. None of the cases fulfilled criteria for "intermediate" or "high" AD neuropathological change, according to ABC scores (Supporting Information Fig. S1).

Given that APOE ε4 was found to be the major genetic risk factor for AD-related pathology,[18] we further investigated the influence of APOE status on the occurrence of such pathology in PSP. Frequency of APOE status was statistically similar across investigated groups (RD3-NFT in the absence of Aß pathology vs. Aß pathology in the absence of RD3-NFT vs. both RD3-NFT and Aß pathology vs. no RD3-NFT and no Aß pathology; chi-square test, $P > 0.05$ for all comparisons).

AGs Copathology. AGs were identified in 58% of patients (Fig. 1A), mostly at Saito stage III (72% of AGs positive cases), whereas in 28% of these patients, changes were restricted to temporomesial structures (Saito stages I and II; Fig. 1E). Most of the cases (81%) revealed AGs in the presence of AD-related pathology (57% in the presence of both RD3-NFT and Aß, 21% in the presence of only RD3-NFT, and 3% in the presence of only Aß).

LRP. LRP-positive cases were rarely observed in our cohort (8%; Fig. 1A). Three percent of PSP patients had alpha-synuclein deposits restricted to the brainstem (Braak stages I and II), whereas 5% had widespread deposits, with affection of neocortex (Braak stages V and VI; Fig. 1F).

TDP-43 and FUS. Cases with intracytoplasmic aggregates of phosphorylated TDP-43 were rarely observed in our cohort (6 cases), and in 4 cases restricted to a few neurons and neuropil threads of temporomesial structures and in 1 case to few neurons and threads of the cortex of the middle frontal gyrus. In 1 case, both glial and neuronal cytoplasmic inclusions were found in the brainstem at levels of midbrain, pons, and medulla oblongata (including motoneurons of the hypoglossal nerve), and in the cerebellum showing features of an additional motoneuron disease. Cases with intracellular FUS aggregates were not identified (Fig. 1A,G).

Cerebral Vessel Copathology

In addition to proteinopathies, SVD was frequently (64%) found in PSP patients (Fig. 1A), mostly at stages I and II (Fig. 1H). Distribution of SVD stages did not differ between the neurodegenerative copathology disease groups (chi square test, $P > 0.05$; Fig. 3A).

CAA occurred in only 25% of PSP patients (Fig. 1A), mainly restricted to vessels of the neocortex (stage I; Fig. 1I), but exclusively occurred in the presence of AD-related copathology (Fig. 3B). Twenty-four of 25 patients exhibited CAA only in the presence of Aß plaques, whereas in 1 case CAA was present with RD3-NFT, but without Aß plaques.

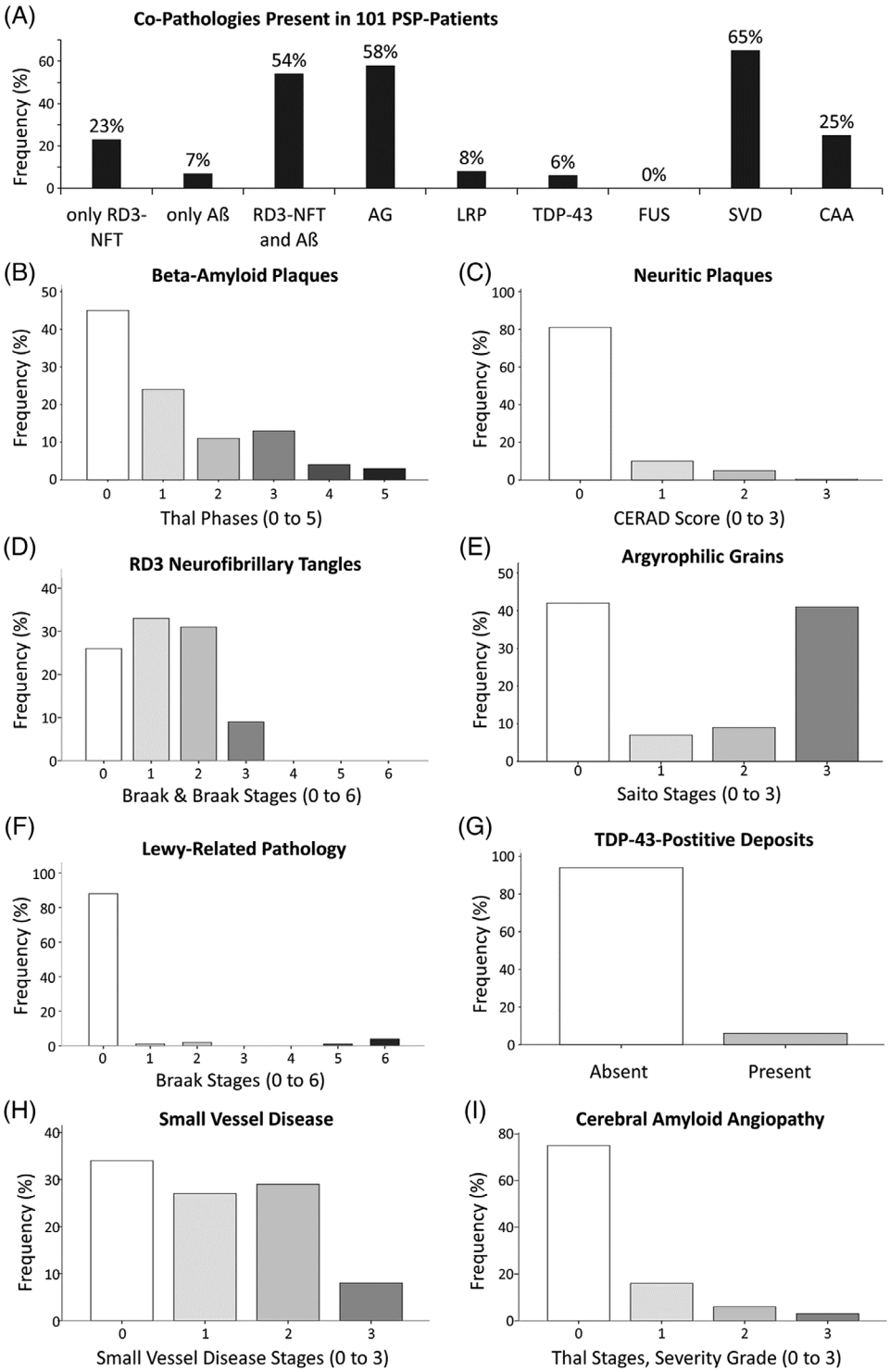

FIG. 1. Frequency (**A**) and distribution of severity (**B–I**) of concomitant proteinopathies or cerebrovascular disease observed in 101 definite PSP patients. Aß, amyloid beta; AD, Alzheimer's disease; AG, argyrophilic grains; CAA, cerebral amyloid angiopathy; FUS, fused in sarcoma protein pathology; LRP, Lewy-related co-pathology; PSP, progressive supranuclear palsy; RD3, NFT-RD3 positive neurofibrilary tangles; SVD, small vessel disease; TDP-43, transactive response DNA-binding protein 43 kDa.

Single and Multiple Neurodegenerative Disease Copathology

Forty-two percent of PSP patients had only a single copathology finding in addition to PSP-specific lesions: 34% with AD-related copathology and 8% with AGs (Fig. 2).

The most frequent dual copathology was the combination of AD-related plus AGs copathology, found in

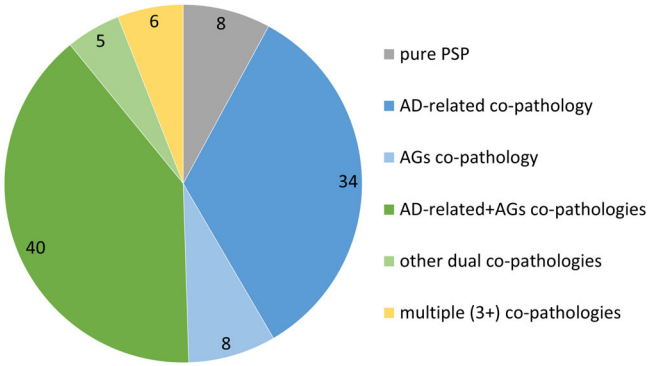

FIG. 2. Frequency of single and multiple neurodegenerative disease copathologies in 101 PSP patients. AD, Alzheimer's disease; AGs, argyrophilic grains; PSP, progressive supranuclear palsy. [Color figure can be viewed at wileyonlinelibrary.com]

40% of patients (Fig. 2). Other dual copathologies were present in only 5% (Fig. 2).

LRP and TDP-43 pathology were mainly observed in combination with multiple neurodegenerative disease copathologies (Fig. 2). Five of 6 cases with TDP-43-positive pathology, as well as 7 of 8 LRP-positive cases, had concomitant presence of AD-related copathology.

Influence of Copathology Findings on the Clinical Course of PSP

Association Between Copathology and Demographic Data

Higher stages of CAA, RD3-NFT, Aß, and AGs were associated with older age at onset and older age at death or both (Supporting Information Table S3, non-parametric Spearman correlation; these correlations were significant, but weak as shown by correlation coefficients). In addition to this, APOE ε4 carriers did not differ in age at onset or age at death than

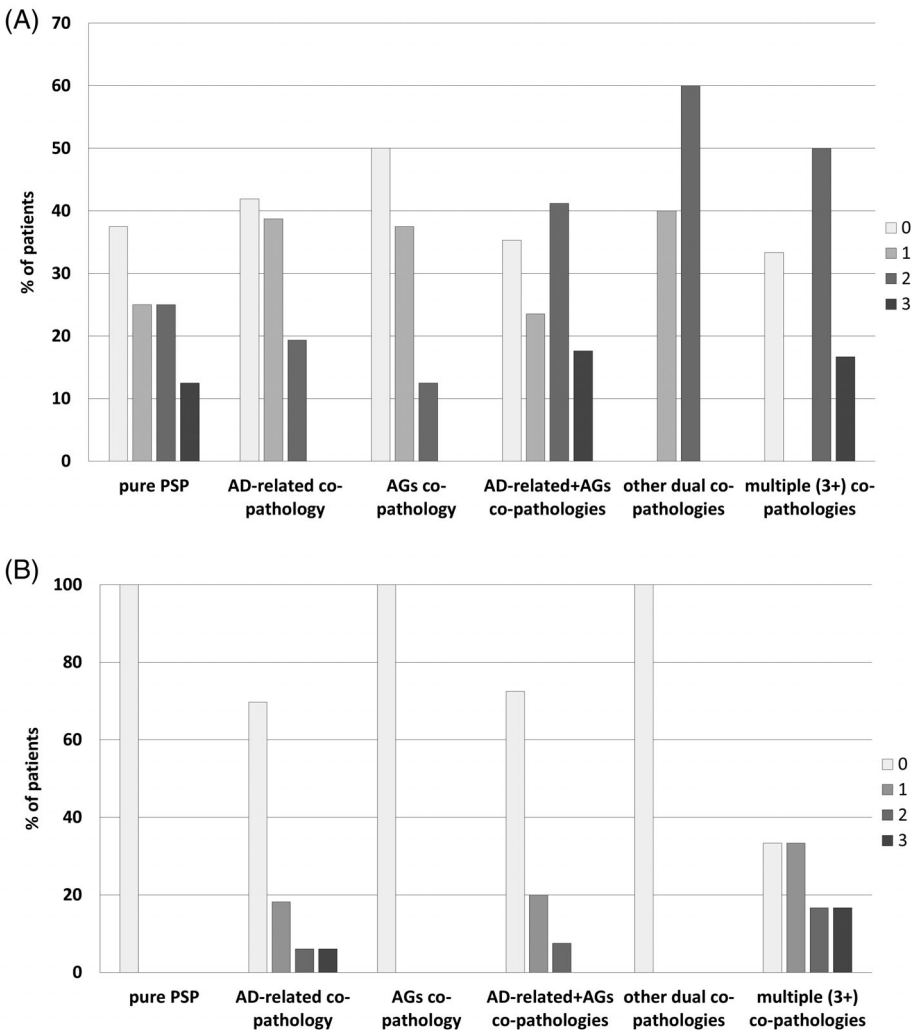

FIG. 3. (**A**) Distribution of SVD stages among neurodegenerative copathology PSP groups. (**B**) Distribution of CAA stages among neurodegenerative copathology PSP groups. AD, Alzheimer's disease; AGs, argyrophilic grains; CAA, cerebral amyloid angiopathy; PSP, progressive supranuclear palsy; SVD, small vassel disease.

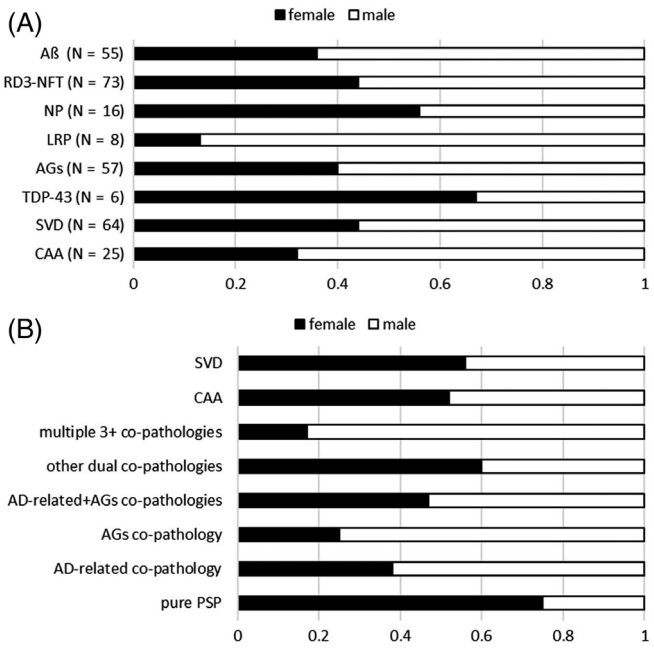

FIG. 4. (**A**) Sex-specific proportion of copathologies in PSP. (**B**) Sex-specific proportion of pure and mixed PSP groups. Aß, amyloid beta plaque; AGs, argyrophilic grains; CAA, cerebral amyloid angiopathy; LRP, Lewy-related co-pathology; NP, neuritic plaques; PSP, progressive supranuclear palsy; RD3-NFT, RD3 positive neurofibrilary tangles; SVD, small vessel disease; TDP-43, transactive response DNA - binding protein 43.

noncarriers ($P > 0.05$, two-independent t test). There were no significant differences in sex distribution in the groups defined by presence of individual single or mixed copathologies (Fig. 4A,B; chi-square test, $P > 0.05$).

Association Between Neurodegenerative Disease Copathology and Main Clinical PSP Milestones

No differences in age at onset and age at death were found in any neurodegenerative copathology group, when compared to pure PSP (Supporting Information Table S3). Age at onset and age at death were only higher in patients with multiple copathologies and with AGs plus AD-related copathology compared to the group with PSP plus AGs. There were no differences in disease duration between the groups.

No differences were found in the clinical course of main PSP symptoms between groups with different neurodegenerative disease copathologies (Supporting Information Table S4) or in prevalence of such symptoms across these groups (chi-square test, $P > 0.05$). These findings were further confirmed through the multinominal logistic regression analysis controlled for age and sex, where no significant association was found between onset of clinical symptoms and presence of additional NDD copathology (data not shown).

Association Between Cerebral Vessel Copathology and Main Clinical PSP Milestones

When we compared PSP patients with or without presence of SVD/CAA, no differences were found in demographic and clinical symptoms ($P > 0.05$, two-independent t test with Bonferroni correction; Supporting Information Table S5) or in prevalence of such symptoms across these groups (chi-square test, $P > 0.05$). Furthermore, binominal logistic models for SVD and CAA controlled for age and sex did not reveal any significant association between clinical symptoms and presence of additional cerebral vessel copathology (data not shown).

Discussion

Coexistence of major NDD proteins and vessel changes with primary PSP-specific pathological lesions is very prevalent in our cohort, where only 8% of patients were found to have pure PSP. AD-related pathology was the most frequent one, both in the form of single copathology findings or in the combination with other protein alterations and vascular changes. However, the copathologies observed in PSP were mild and rarely reached high neuropathological stages, with the exception of widespread AGs observed in our cohort. Consistently, the observed copathologies did not affect the frequency and timing of occurrence of main clinical disease milestones in PSP.

In our study, pure PSP represented a minority of cases (8%). This proportion was reported to be higher in previous studies (ranging from 21.8% to 29%),[3,19] where different methodological approaches had been used, including assessment strategy and the spectrum of evaluated copathologies. However, our data are in line with previous reports, where different NDD protein alterations and cerebrovascular changes were found to commonly coexist in addition to defining histopathology of PSP.[3,19,20] Moreover, the study by Robinson and colleagues revealed that the highest prevalence of copathology was found in PSP in contrast to other investigated NDD, and that the presence of copathology is rather the rule than the exception in PSP.[3]

As expected in the typical age of PSP patients, the most common concomitant finding was AD-related pathology, accounting for 84% of our PSP cohort. The main factor driving AD-related pathology was age, whereas examination of APOE status revealed no association with AD-related copathology in our PSP cases. Furthermore, APOEε4 carriers did not differ in age at onset and age at death. As in previous series, we found RD3-NFT restricted to temporomesial structures and to the cortex of the fusiform gyrus corresponding to Braak and Braak stages I to III, and Aß plaques mainly restricted neocortical and temporomesial structures with maximal moderate densities of NPs corresponding

to modified CERAD B in 5 cases never reaching the full neuropathological picture of AD.[19,21]

AGs were the second-most common concomitant finding in our cohort, observed in 58% of cases, which fits to the range of the previously reported frequencies from 18.8% to 80%.[22-24] Tatsumi and colleagues recently reported that grains pathology in PSP is rather restricted to the limbic region (Saito stage I).[25] However, our analysis revealed that the majority of patients presented with widespread grains (Saito stage III). This finding is more similar to the extensive grain distribution reported in corticobasal degeneration.[25] We have also observed that AGs commonly coexist with RD3-NFT, but with relatively low AD Braak and Braak score (≤ stage III). Further careful analyses are warranted to clarify the interplay of AGs with other NDDs, but these findings rather indicate that AGs are part of the disease-specific process of 4R tauopathies, such as corticobasal degeneration and PSP, and not merely the part of aging.[22,25]

The rare occurrence of LRP is comparable to previous reports on PSP series and does not exceed the frequency observed in the normal aging brain.[19,26] Interestingly, LRP is mainly found, in our study, in the presence of AD-related pathology, which supports the hypothesis that 3-repeat isoforms of tau favor alpha-synuclein deposition, whereas 4R-tau might do less so.[1]

We found a low proportion of cases exhibiting TDP-43-positive deposits. The combination of PSP and TDP-43 pathology has been described previously with various frequency, from 6% to 26%.[3,27,28] Most TDP-43-positive cases occurred in combination with AD and AGs, which has also been reported elsewhere.[29]

In addition to protein alterations, cerebrovascular pathologies are commonly observed in our PSP cohort. Vascular lesions, including SVD and CAA, have been thought to be associated with AD, but are also present in normal older individuals and have been reported in some types of parkinsonism.[20,30] Most of the studies concluded that any particular parkinsonism is neither protective nor a risk factor for cerebrovascular pathologies.[8] Our results indicate that CAA in PSP occurred exclusively in the presence of AD-related pathology, whereas SVD did not show any association with specific protein alterations.

In the second part of our study we tried to address the very important question on the clinical significance of such findings in PSP. The rate of correct clinical diagnosis of PSP at initial and final examination (Table 1) was low and comparable with previous studies[31-33] and highlights the difficulty to establish the diagnosis of this devastating disorder ante mortem in a real-world setting without systematic and prospective application of clinical diagnostic criteria.[17] However, the main aim of our study was to analyze the possible effects of copathologies on the frequency and temporal evolution of the main clinical milestones of PSP. We were not addressing the question whether copathologies might affect the ante-mortem diagnosis.

Our data indicate that the presence of NDD and vascular copathologies do not significantly influence the prevalence and time course (latency to onset) of main clinical milestones in PSP. Given that the tempo of disease progression is mainly highlighted through the latency to reach main clinical milestones of PSP, this information could be very important for future clinical trials on drugs with the disease-modifying effect. Thus, our data imply that 4R tau is still the most relevant target for PSP, whereas the impact of copathologies on clinical course appears to be of less importance.

Largely, studies which were dealing with similar problems in other NDDs showed that there could be both clinical and pathological synergisms mainly between AD-related pathology and synucleinopathies, as well as interactions between TDP-43 pathology and AD.[1-4] Our study raises the question why PSP tau pathology is not involved in these inter-relationships with Aß, LRP, and TDP pathology. If the copathology is very prevalent in PSP, why does it not reach the level of clinical significance? Recently, Irwin and colleagues demonstrated that AD-related tau pathology in Lewy body disorders with dementia independently predicted a higher progression rate, as well as shorter overall survival, clearly indicating that, if present, tau plays a crucial role in the disease process of Lewy body disorders.[4,34] One may speculate that 4R PSP-tau pathology is so strong that it over-rides the possible influence of other copathologies and is the main driver of disease progression in PSP. Future studies, both clinicopathological and molecular, are warranted to confirm this theory.

There are several advantages and limitations of our study that need to be emphasized here. Our data are based on a large number of clinically and pathologically well-defined PSP patients. Recruitment bias is reduced to a minimum by identifying neuropathological centers with special expertise in NDDs associated with both neurological and psychiatric clinics, by the central harmonization of all data and central sample readings, and by the inclusion of cases only with excellent clinical documentation available. At the same time, this study is dealing with a broad spectrum of NDDs and copathology findings.

Certainly, the important limitation is the retrospective extraction of clinical data from available documentations. Data were obtained from clinical charts and may be incomplete and underestimated as in any retrospective clinicopathological study. In order to minimalize this retrospective limitation, we relied on hard disease milestones (relevant to our analysis), which are commonly reported by patients, caregivers, and/or documented by treating physicians. In addition, we take into account only the prevalence and latency to onset of each investigated milestone. Therefore, this study

cannot address directly the influence of copathology on the severity of individual symptoms, given that such scales were not available at examination.

It is important to be aware that our copathology evaluation was based on staging recommendations of a given NDD or vascular alterations, which are defined mainly by the presence of specific lesions in specific anatomical areas, defined in the respective neuropathological diagnostic criteria, but not by the severity and the distribution of these lesions across the entire brain. Thus, our conclusions on incidence rates of copathology findings and their influence on clinical presentation and progression need to be interpreted with caution, given that the assessment may be carried out more widely and in a (semi-)quantitative manner to cover all anatomical sites or relevance for PSP symptoms and including lesion severities per region.

Taking into account all of the study limitations mentioned above and the high prevalence of copathology found in PSP in our cohort, as well as in previous reports,[3,19,20] we have to be very careful in making "strong" conclusions. We still cannot argue with certainty that copathologies do not influence the complex clinical presentation in PSP and severity of individual clinical features, but we may conclude that the tempo of disease progression is largely determined by PSP-specific pathology, whereas the copathology does not appear to have a major impact on progression rate. So far, these findings suggest that disease-modifying therapies under development to target the 4R-tauopathy[5] are unlikely to be compromised by copathology in PSP. Still, it may turn out, in the future, that it will be necessary to target specific copathologies therapeutically in PSP under specific circumstances. ■

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APPENDIX

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.