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# Early Stage of Progressive Supranuclear Palsy: A Neuropathological Study of 324 Consecutive Autopsy Cases

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Diagnosing clinical progressive supranuclear palsy (PSP) is challenging. We hypothesize that there are more cases of pathological PSP than have been clinically identified, but its diagnosis is challenging because the initial lesions and progression of PSP have not yet been clarified. The purpose of our study was to clarify the incidence of PSP in consecutive autopsy cases and identify pathological characteristics of early PSP. We investigated 324 consecutive autopsy patients from a general geriatric hospital (age, mean±SD=82.5±8.7 years). Paraffin sections of the midbrain were immunostained with anti 4-repeat tau antibodies (RD4). We selected cases showing RD4-positive neurofibrillary tangles and tufted astrocytes in the midbrain sections. Then, we used anti-phosphorylated tau antibody to immunostain sections from the basal ganglia, subthalamic nucleus, midbrain, pons, medulla, and cerebellum. Of the 324 patients, 35 had RD4-positive structures in the midbrain. From these 35 cases, we excluded those for which autopsies confirmed definite PSP (n=5) and cases of corticobasal degeneration (n=1), Alzheimer's disease (n=11), dementia of grain (n=10), and neurofibrillary tangles predominant forms of senile dementia (n=2), leaving 8 cases. We diagnosed these 8 cases as pure PSP-type tauopathy.

Pure PSP-type tauopathy was detected in 2.5% of the consecutive autopsy cases, and this incidence was 1.6 times greater than that of neuropathologically definite PSP. This pure PSP-type tauopathy likely indicates preclinical stages of PSP. Furthermore, the novel neuropathological finding, which we term "preclinical PSP," is unique and has not previously been reported. In order to elucidate the causes and pathological mechanisms of PSP, preclinical PSP should be investigated further.

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**Key words:** 4-repeat tau, progressive supranuclear palsy, preclinical stage, tau

## Introduction

Progressive supranuclear palsy (PSP) was first described as a distinct disorder from Parkinson's disease (PD) in 1964[1]. Patients with PSP have clinical indicators such as opthalmoplegia affecting primarily vertical gaze, pseudobulbar palsy, dystonic neck rigidity, and mild dementia. At postmortem examination, these patients show neurofibrillary degeneration with neuronal loss, as well as gliosis affecting the striatum, globus pallidus, subthalamic nucleus, substantia nigra, locus ceruleus, tectum, tegmentum, basis pontis, and several cranial nerve nuclei.

PSP was first termed PSP-Richardson clinicopathology, prior to identification of various clinical subtypes such as PSP-Parkinsonism[2,3], pure akinesia with gait freezing[4], corticobasal syndrome[5], progressive non-fluent aphasia[6],

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PSP with primary lateral sclerosis[7], and PSP with cerebellar ataxia[8]. This variability in clinical phenotypes created challenges in diagnosing PSP.

Daniel SE et al. reported seventeen cases of pathologically confirmed PSP. Of the seventeen autopsy-proven PSP cases, only 7 cases were diagnosed clinically as PSP, and the remaining 10 cases were not diagnosed as PSP clinically because of their lack of ophthalmoplegia. The average age at death was higher for the patients with pathological PSP without opthalmoplegia than for those with opthalmoplegia[9]. This finding emphasizes the fact that diagnosis of clinical PSP is particularly difficult in elderly patients.

We previously reported 9 cases of diagnosed neuropathological PSP among 1,241 consecutive autopsy cases[10], which represents a prevalence of 0.7% for our study sample. This was much higher prevalence than those reported by previous studies, which indicated expected rates of 1–5 cases per 100,000 people in the general population, based upon community epidemiological studies[11,12].

The pathological process of neurodegenerative diseases is thought to begin many years before diagnosis, with clinical symptoms presenting when the rate of disease progression exceeds a threshold. This "preclinical" phase of disease progression is hypothesized to provide an opportunity for therapeutic intervention. There have been many reports on preclinical Alzheimer's disease (AD) and preclinical Parkinson's disease (PD)[13,14]. We previously reported 58 cases of PD with dementia (PDD) and dementia with Lewy bodies (DLB) in a sample of 1,241 consecutive autopsy cases, from which 196 cases had alpha synuclein-positive structures, but did not fulfill the diagnostic criteria[10]. These cases may represent preclinical PD, PDD, and DLB. We hypothesize that there are a considerable number of preclinical PSP cases, similar to preclinical AD and PD. However, few neuropathological reports on patients at the preclinical stage of PSP are found in the literature[15].

In the present study, we conducted immunohistochemical analyses on brain tissue from a series of autopsied patients. We performed a midbrain screening test for all consecutive autopsy cases and then compared immunostaining for tau pathologies with anti 3-repeat tau (RD3) and anti 4-repeat tau (RD4) antibodies. The pathological finding of RD4-positive and RD3-negative neurofibrillary tangles (NFTs), pretangles, and tufted astrocytes in the midbrain was classified as PSP-type tauopathy. The purpose of this study was to describe the incidence of PSP-type tauopathy at autopsy and investigate propagation of this tauopathy.

## Materials and Methods

### Tissue Source

Brains and spinal cords were obtained from 340 consecutive autopsy patients at Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology between April 2006 and April 2011. We selected 324 cases for our study. Selected areas were sampled, and the remaining brain tissue was quickly frozen and stored at −80℃. Cases indicating Creutzfeldt-Jakob disease (n=5), widespread brain tumor (n=2), and severe bilateral cerebrovascular disease (n=9) were excluded. The ages of the patients at death ranged from 54 to 104 years (mean ± SD=82.5 ± 8.7 years), and there were 179 men and 145 women.

### Histology

Brains and spinal cords were examined as previously reported[10,16,17,40]. Briefly, the cerebral and cerebellar hemispheres, as well as the brainstem, were dissected in the sagittal plane at the time of autopsy. In each case, one hemisphere and brainstem was preserved at −80℃ for further biochemical and molecular analyses. The following anatomical areas were sampled: the frontal, temporal, and occipital poles parietal lobe including the intraparietal sulcus, anterior amygdala, posterior hippocampus, dentate nucleus, and midbrain. Samples were directly fixed in 4% paraformaldehyde for 48 h and prepared for immunohistochemical and ultrastructural analyses. The other hemisphere was fixed in 20% buffered formalin (WAKO, Osaka, Japan) for 7–13 days and sliced similarly to the contralateral hemisphere. Representative sections were embedded in paraffin. Serial sections (6 μm) were cut and stained with hematoxylin and eosin and Klüver-Barrera. Selected sections were further examined with modified methenamine silver[18] and Gallyas-Braak[19] staining for senile changes, Congo red for amyloid deposition, and elastica Masson trichrome stain for vascular changes.

### Immunohistochemistry

Selected sections were immunostained using an autostainer (Ventana 20NX; Ventana, Tucson, AZ, USA), as previously reported[10]. The antibodies included anti-phosphorylated tau (ptau; AT8, monoclonal; Innogenetics, Temse, Belgium), anti-4-repeat tau (RD4, monoclonal; Upstate, Lake Placid, NY, USA), anti-3-repeat tau (RD3, monoclonal; Upstate), anti-phosphorylated alpha-synuclein (psyn; monoclonal, psyn no. 64, a gift from Dr. Iwatsubo, The university of Tokyo, Tokyo, Japan), anti-beta-amyloid 11–28 (12B2, monoclonal; IBL, Maebashi, Japan), anti-ubiquitin (polyclonal; DAKO, Glostrup, Den-

mark), and anti-phosphorylated TAR DNA-binding protein of 43 kD (TDP43; PSer409/410; monoclonal, a gift from Dr. M. Hasegawa, Tokyo Institute of Psychiatry, Tokyo, Japan) antibodies, as previously reported[10].

**Clinical Data**

Clinical information was retrospectively obtained from medical charts as well as from interviews with the patients' attending physicians and caregivers. The Mini-Mental State Examination[20] or Hasegawa Dementia Screening scale (or its revised version)[21] and the Instrumental Activities of Daily Living scale[22] were used to evaluate cognitive function. Two independent board certified neurologists retrospectively determined clinical dementia rating scale[23] scores.

**Neuropathological Diagnosis**

NFTs were classified into 7 stages (from 0 to 6), and senile plaques were classified into 4 stages (0 and from A to C) according to the criteria of Braak and Braak[24]. The neuropathological diagnosis of AD was based on our definition[25], which proposes a modification of the National Institute on Aging-Reagan criteria. Diagnoses of dementia of grain (DG) and neurofibrillary tangle predominant forms of senile dementia (NFTD) were based on the definitions of Jellinger[26] and Jellinger and Bancher[27]. DLB diagnosis was based on the revised consensus guidelines of the National Institute on Aging-Alzheimer's Association[28] and PSP diagnosis was based on the National Institute of Neurological Disorders and Stroke criteria[29]. The diagnosis of corticobasal degeneration (CBD) was based on the National Institutes of Health criteria[30].

**Case Selection for PSP-type Tauopathy**

Midbrain sections from consecutive autopsy cases were directly fixed in 4% paraformaldehyde for 48 hours[31]. Then, the blocks of midbrain tissue were stained by Immunohistochemistry with RD4 and RD3. We selected the cases having RD4-positive and RD3-negative NFTs and/or pretangles and RD4-positive and RD3-negative tufted astrocytes in the midbrain. Tufted astrocytes were detected as aggregates of fine tau-positive fibers with a concentric arrangement of tree-shaped branching[32]. Next, we used AT8 to immunostain sections of the basal ganglia, subthalamic nucleus, midbrain, pons, medulla, and cerebellum from the selected cases. These sections were fixed in 20% buffered formalin. At a magnification of 100×, we counted the number of NFTs, pretangles, and tufted astrocytes with AT8 immunohistochemistry in the putamen, globus pallidus, subthalamic nucleus, substantia nigra, oculomotor nucleus, pontine nucleus, inferior olive nucleus, and dentate nucleus. Samples were classified according to the following grading system: Grade 0, AT8-positive structures were not observed; Grade 1, 1–3 AT8-positive structures were observed; Grade 2, 4–9 AT8-positive structures were observed; Grade 3, more than 9 AT8-positive structures were observed (**Fig. 1**).

## Results

**Neuropathological Diagnoses**

Neuropathological diagnoses included AD (n=49); DG (n=20); NFTD (n=18); PD, PDD, and DLB (n=9); PSP (n=3); spinocerebellar degeneration (n=3); motor neuron disease (n=2); multiple system atrophy (n=2), CBD (n=1); and frontotemporal lobar degeneration (n=1). Comorbid pathologies included AD with DLB (n=11), AD with DG (n=7), DG with NFTD (n=6), AD with PSP (n=1), PDD with PSP (n=1), AD with motor neuron disease (n=1). The remaining patients did not fulfill both clinical and pathological criteria for any single neurodegenerative disease (**Table 1**).

**PSP-type Tauopathy**

Thirty-five of the 324 patients had RD4-positive structures in the midbrain, indicating a combination of NFTs, pretangles, and tufted astrocytes. We defined these pathological changes as PSP-type tauopathy. The neuropathological diagnoses of this tauopathy consisted of PSP (n=3), AD (n=9), AD with DG (n=10), CBD (n=1), PSP with AD (n=1), PSP with PD (n=1), DG with NFTD (n=1), and AD with DLB (n=1). We excluded 5 cases of autopsy confirmed definite PSP and 1 case of CBD, and the remaining 29 cases were selected as the focus of the present study (**Fig. 2**).

**Neuropathological Findings**

RD4 immunostaining

RD4-positive NFTs and pretangles were observed in the substantia nigra of all 29 cases. Tufted astrocytes were observed frequently in each case.

AT8 immunostaining

AT8-positive NFTs were observed in the substantia nigra of all cases. Of the 29 cases, 20 and 18 showed AT8-positive NFTs in the subthalamic nucleus and globus pallidus, respectively. AT8-positive tufted astrocytes were observed in the putamen of 8, in the substantia nigra of 6, and in the subthalamic nucleus of 5 of the 29 cases. There were few AT8-positive tufted astrocytes outside of these described regions.

We compared PSP-type tauopathy (n=8) without other tauopathy (**Table 2**), PSP-type tauopathy complicated by AD (n=10), and PSP-type tauopathy complicated by DG

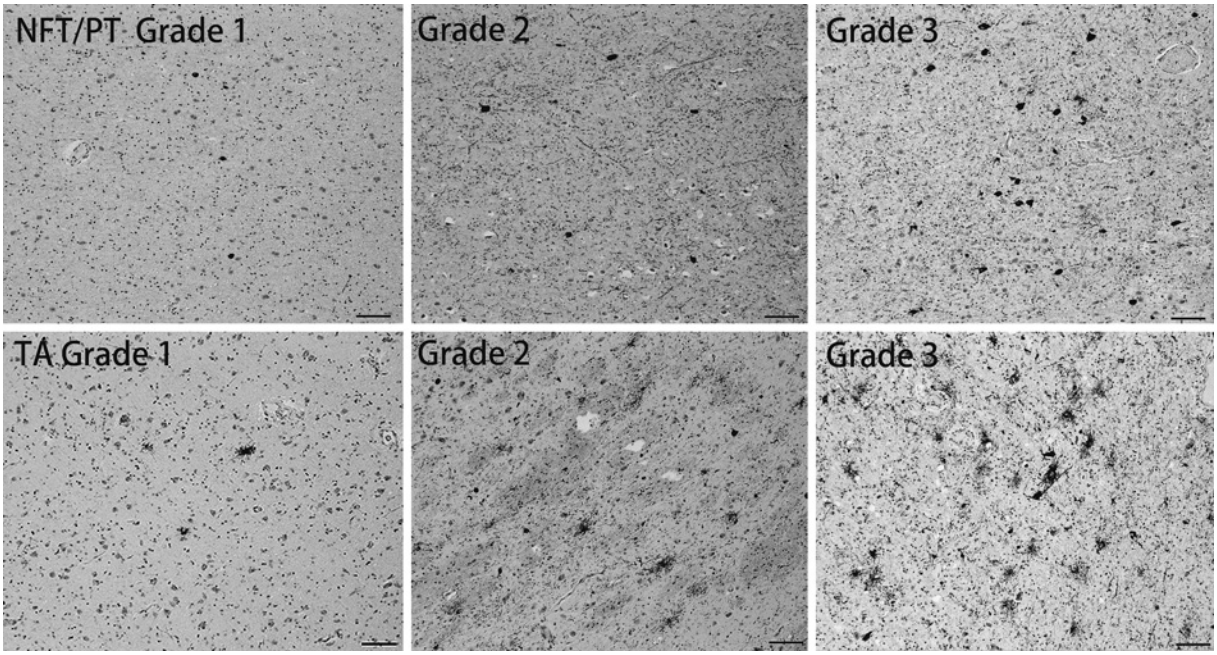


Fig. 1 Semi-quantitative evaluation of tau-positive structures.
The number of tau-positive NFTs, pretangles, and Tufted astrocyte were classified into the following grades: Grade 0, AT8-positive structures were not observed; Grade 1, 1–3 AT8-positive structures were observed; Grade 2, 4–9 AT8-positive structures were observed; Grade 3, more than 9 AT8-positive structures were observed. NFT, neurofibrillary tangle; PT, pretangle; TA, tufted astrocyte. Scale bar=100 μm

Table 1 Neuropathological diagnosis of neurodegenerative diseases in 324 cases

| Neuropathological diagnosis | n |
|---|---|
| Alzheimer's disease | 49 |
| Dementia of grain | 20 |
| Neurofibrillary tangle predominant form of senile dementia | 18 |
| Parkinson's disease/Parkinson's disease with dementia/Dementia with Lewy body | 9 |
| Progressive supranuclear palsy | 3 |
| Spinocerebellar degeneration | 3 |
| Motor neuron disease | 2 |
| Multiple system atrophy | 2 |
| Corticobasal degeneration | 1 |
| Frontotemporal lobe degeneration | 1 |
| Alzheimer's disease+Dementia with Lewy bodies | 11 |
| Alzheimer's disease+Argyrophilic grain disease | 7 |
| Argyrophilic grain disease+Neurofibrillary tangle predominant form of senile dementia | 6 |
| Alzheimer's disease+Progressive supranuclear palsy | 1 |
| Parkinson's disease with dementia+Progressive supranuclear palsy | 1 |
| Alzheimer's disease+Motor neuron disease | 1 |
| Non neurodegenerative disease | 189 |
| Total | 324 |

(n=10). NFTs and pretangles were more frequently observed in the dentate nucleus in cases of PSP-type tauopathy without other tauopathy. Additionally, more NFTs and pretangles were observed in the putamen in cases of PSP-type tauopathy with AD. There were more NFTs in the subthalamic nucleus and pontine nucleus in cases of PSP-type tauopathy with DG. The density of tufted astrocytes did not differ depending on whether there was other accompanying tauopathy.

From these 29 cases, we excluded those for which

autopsies confirmed definite AD (n=9), AD with DG (n=10), DG with NFTD (n=1), and AD with DLB (n=1), leaving 8 cases.

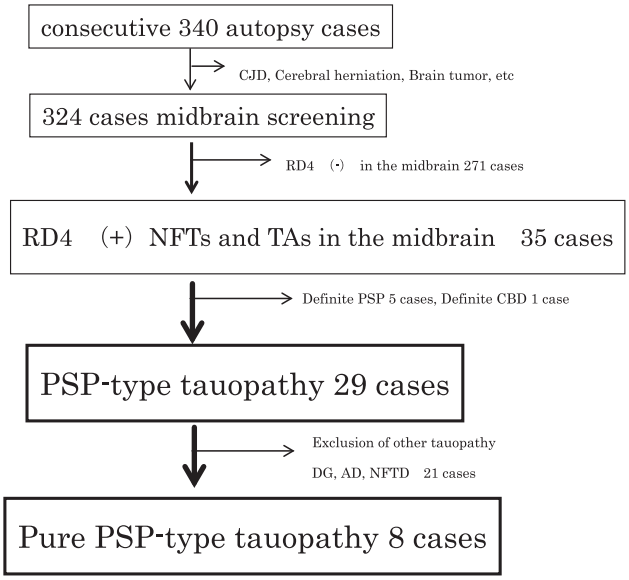


Fig. 2 Flow chart for selecting cases with "PSP-type tauopathy."
CDJ, Creutzfeldt-Jakob disease; PSP, progressive supranuclear palsy; DG, dementia of grain; AD, Alzheimer's disease; NFTD, neurofibrillary tangle predominant dementia

**Clinical Data of Pure PSP-type Tauopathy**

One patient had dementia and Parkinsonism. One patient had mild dementia. There were no patients with vertical opthalmoplegia (**Table 2**).

## Discussion

Of our 324 autopsy patients, 35 cases had RD4-positive and RD3-negative NFTs, pretangles, and tufted astrocytes. We termed these pathological changes as PSP-type tauopathy. Of the 35 cases, only 5 fulfilled the diagnostic criteria of definite PSP. One case was diagnosed as CBD, but the remaining 29 cases did not fulfill the pathologic diagnostic criteria for PSP or CBD. These cases indicate a prevalence of 9% in our sample of consecutive autopsy cases, and represented 5.8 times the number of cases with clinicopathologically definite PSP.

The prevalence of clinical PSP was previously reported as 1–5 cases per 100,000 people in the general population, as indicated by community-based studies[11,12]. The prevalence of PSP-type tauopathy in the present study was much higher than previously reported estimates and indicates a greater number of pathological PSP cases than clinical PSP cases. Clinical PSP presents with various phenotypes, such as PSP-Parkinsonism[2,3], pure akinesia with gait freezing[4], corticobasal syndrome[5], or progressive

Table 2 Neuropathological findings in "pure PSP-type tauopathy" with AT8 immunostaining and clinical presentations

| Case | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| Neurofibrillary tangle/pretangle | Substantia nigra | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 1 |
| | Oculomotor nucleus | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 |
| | Pontine nucleus | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| | Inferior olive nucleus | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | Dentate nucleus | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 0 |
| | Subthalamic nucleus | 0 | 1 | 1 | 1 | 0 | 0 | 2 | 0 |
| | Globus pallidus | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| | Putamen | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 |
| | Opercular cortex | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| | Motor cortex | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Tufted astrocyte | Substantia nigra | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | Oculomotor nucleus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Pontine nucleus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Inferior olive nucleus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Dentate nucleus | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Subthalamic nucleus | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | Globus pallidus | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | Putamen | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | Opercular cortex | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | Motor cortex | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Clinical dementia rating scale | | 1 | 0 | 0 | 0 | 0 | 0 | 0.5 | 3 |
| Parkinsonism | | – | – | – | – | – | – | – | + |

non-fluent aphasia[6], in addition to the original prototype of PSP (PSP-Richardson). Moreover, elderly patients with PSP tend to not experience typical opthalmoplegia, gait disturbance, or dementia[9]. Pathological PSP without neurological or psychiatric signs has been reported previously[33]. Therefore, PSP cases other than those with PSP-Richardson types of presentations may not be diagnosed as PSP. There are a considerable number of neuropathological PSP cases that are misdiagnosed as PD, CBD, AD, or cerebrovascular disease.

Our observed prevalence of 9% for PSP-type tauopathy that did not fulfill the diagnostic criteria of PSP represents a rate 5.8 times that of neuropathological definite PSP. We previously reported a prevalence rate of 15.8% for presence of alpha synuclein-positive structures in consecutive autopsy patients who did not fulfill the diagnostic criteria of DLB[10]. This study indicated PD, PDD, and DLB prevalence rates 3.4 times the rate in consecutive autopsy cases in the study population[10]. The prevalence of PSP-type tauopathy was more frequent than neuropathological definite PSP, similar to the previous findings that the prevalence of alpha synuclein-positive structures that did not fulfill the diagnostic criteria for DLB was much greater than the prevalence of PD, PDD, and DLB.

In the present study, NFTs and pretangles were most prevalent in the substantia nigra, subthalamic nucleus, and globus pallidus. There were more NFTs and pretangles in the subthalamic nucleus and pontine nucleus in PSP-type tauopathy complicated by DG compared to that without other tauopathy and that complicated by AD. Neurofibrillary tangles in the subthalamic nucleus are observed in DG and advanced AD[34]. The complications of DG impact the expression of NFTs and pretangles in PSP-type tauopathy.

Midbrain tufted astrocytes were frequently observed using RD4 immunostaining, but few AT8-positive tufted astrocytes were observed. Though sensitivity of immunostaining depends on fixation and antibody, RD4 is a more suitable antibody for assessment of tufted astrocytes than AT8. Tufted astrocytes expression was unaffected by coexisting tauopathy, as indicated by both RD4 immunostain and AT8 immunostain. Therefore, we hypothesized that tufted astrocytes must be specific pathologic changes associated with PSP and queried whether there is a relationship between the number of tufted astrocytes and degeneration. Previous reports have concluded that the number of tufted astrocytes is unrelated to the presence of NFTs in most brain regions[35]. There is no correlation between the distribution of gliosis and that of tufted astrocyte[36], indicating that Tufted astrocyte may not be directly involved in the progression of PSP-type tauopathy.

In cases with PSP-type tauopathy, NFTs were observed more frequently than Tufted astrocyte, using the AT8 immunostain. However the density of NFTs and pretangles, and that of ATs, were equal using the RD4 immunostain. The number of Tufted astrocyte did not correlate with the numbers of NFTs and pretangles in the brain areas that we examined. Therefore, in PSP-type tauopathy, tufted astrocyte are likely a separate type of pathology from NFTs. Although NFTs and Tufted astrocyte appear to be important diagnostic indicators of early PSP, the mechanisms of PSP lesion progression and origins of the characteristic neuropathological changes such as NFTs and Tufted astrocyte have not been clarified.

There have been 2 previous clinicopathological reports on early PSP. The observed patients were diagnosed with possible PSP and their clinical course was remarkably short; one clinical course was 3 months, and the other was 2 years and 5 months. In these 2 cases, NFTs, pretangles, and tufted astrocyte were confined mainly to the substantia nigra, subthalamic nucleus, and globus pallidus[37,38]. In the present study, most cases exhibited NFTs and pretangles in the substantia nigra, globus pallidus, and subthalamic nucleus. In the previous reports of early stage PSP cases, tau-positive NFTs and pretangles were not described in the dentate nucleus, putamen, and oculomotor nucleus. However, in the present study, NFTs and pretangles were observed not only in the substantia nigra, globus pallidus, and subthalamic nucleus, but also in the dentate nucleus, putamen, and oculomotor nucleus. These results suggest that NFTs may occur in the dentate nucleus, putamen, and oculomotor nucleus before they begin to occur in the globus pallidus and subthalamic nucleus. We found that PSP-type tauopathy affected several brain regions in early stage PSP.

Although the mechanisms by which abnormal proteins accumulate in the brain have been partially elucidated for several neurodegenerative diseases, it is not known how PSP-type tauopathy progresses. Clinical and experimental data suggest mechanisms for the pattern of increased intracellular abnormal protein accumulations in some neurodegenerative diseases. For example, in AD, the tau protein may spread from the entorhinal cortex to the neocortex[24]. In Lewy body disease, Braak has proposed that propagation of Lewy related pathology originates from the lower brainstem and olfactory bulb. The pathological changes may then ascend through the brain-

stem and basal forebrain, eventually reaching the cerebral cortex[39].

Our present results indicated that a unique and definite process of neurodegeneration does not exist for PSP-type tauopathy. However, NFTs were observed in the substantia nigra in all cases with PSP-type tauopathy, indicating that PSP-type tauopathy may begin with NFTs in the substantia nigra.

We found some cases with PSP-type tauopathy in this study. We assumed they might be preclinical PSP cases which was similar in nature to preclinical AD and PD. However, PSP-type tauopathy might be a part of nonspecific pathological changes related to aging.

We found a few cases compatible with early stage PSP in our sample of hundreds of consecutive autopsy cases. Our novel neuropathological findings are unique and have not been reported thus far. To elucidate the cause and pathological mechanisms of PSP, this new classification, which we have termed PSP-type tauopathy, should be investigated biochemically and molecular genetically further.

**Conflict of Interest:** The authors declare no conflict of interest.

## References


1. Steele JC, Richardson JC, OlszewskiI J: Progressive supranuclear palsy. A heterogeneous degeneration involving the brain stem, basal ganglia and cerebellum with vertical gaze and pseudobulbar palsy, nuchal dystonia and dementia. Arch Neurol 1964; 10: 333–359.
2. Williams DR, Holton JL, Strand C, Pittman A, de Silva R, Lees AJ, Revesz T: Pathological tau burden and distribution distinguishes progressive supranuclear palsy-parkinsonism from Richardson's syndrome. Brain 2007; 130 (Pt 6): 1566–1576.
3. Jellinger KA: Different tau pathology pattern in two clinical phenotypes of progressive supranuclear palsy. Neurodegener Dis 2008; 5: 339–346.
4. Williams DR, Holton JL, Strand K, Revesz T, Lees AJ: Pure akinesia with gait freezing: a third clinical phenotype of progressive supranuclear palsy. Mov Disord 2007; 22: 2235–2241.
5. Tsuboi Y, Josephs KA, Boeve BF, Litvan I, Caselli RJ, Caviness JN, Uitti RJ, Bott AD, Dickson DW: Increased tau burden in the cortices of progressive supranuclear palsy presenting with corticobasal syndrome. Mov Disord 2005; 20: 982–988.
6. Josephs KA, Duffy JR, Strand EA, Whitwell JL, Layton KF, Parisi JE, Hauser MF, Witte RJ, Boeve BF, Knopman DS, Dickson DW, Jack CR Jr, Petersen RC: Clinicopathological and imaging correlates of progressive aphasia and apraxia of speech. Brain 2006; 129 (Pt 6): 1385–1398.
7. Josephs KA, Katsuse O, Beccano-Kelly DA, Lin WL, Uitti RJ, Fujino Y, Boeve BF, Hutton ML, Baker MC, Dickson DW: Atypical progressive supranuclear palsy with corticospinal tract degeneration. J Neuropathol Exp Neurol 2006; 65: 396–405.
8. Iwasaki Y, Mori K, Ito M, Tatsumi S, Mimuro M, Yoshida M: An autopsied case of progressive supranuclear palsy presenting with cerebellar ataxia and severe cerebellar involvement. Neuropathology 2013; 33: 561–567.
9. Daniel SE, de Bruin VM, Lees AJ: The clinical and pathological spectrum of Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy): a reappraisal. Brain 1995; 118 (Pt 3): 759–770.
10. Saito Y, Ruberu NN, Sawabe M, Arai T, Kazama H, Hosoi T, Yamanouchi H, Murayama S: Lewy body-related alpha-synucleinopathy in aging. J Neuropathol Exp Neurol 2004; 63: 742–749.
11. Nath U, Ben-Shlomo Y, Thomson RG, Morris HR, Wood NW, Lees AJ, Burn DJ: The prevalence of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome) in the UK. Brain 2001; 124 (Pt 7): 1438–1449.
12. Golbe LI, Davis PH, Schoenberg BS, Duvoisin RC: Prevalence and natural history of progressive supranuclear palsy. Neurology 1988; 38: 1031–1034.
13. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR Jr, Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, Phelps CH: Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011; 7: 280–292.
14. DelleDonne A, Klos KJ, Fujishiro H, Ahmed Z, Parisi JE, Josephs KA, Frigerio R, Burnett M, Wszolek ZK, Uitti RJ, Ahlskog JE, Dickson DW: Incidental Lewy body disease and preclinical Parkinson disease. Arch Neurol 2008; 65: 1074–1080.
15. Evidente VG, Adler CH, Sabbagh MN, Connor DJ, Hentz JG, Caviness JN, Sue LI, Beach TG: Neuropathological findings of PSP in the elderly without clinical PSP: possible incidental PSP? Parkinsonism Relat Disord 2011; 17: 365–371.
16. Fumimura Y, Ikemura M, Saito Y, Sengoku R, Kanemaru K, Sawabe M, Arai T, Ito G, Iwatsubo T, Fukayama M, Mizusawa H, Murayama S: Analysis of the adrenal gland is useful for evaluating pathology of the peripheral autonomic nervous system in lewy body disease. J Neuropathol Exp Neurol 2007; 66: 354–362.
17. Saito Y, Kawashima A, Ruberu NN, Fujiwara H, Koyama S, Sawabe M, Arai T, Nagura H, Yamanouchi H, Hasegawa M, Iwatsubo T, Murayama S: Accumulation of phosphorylated alpha-synuclein in aging human brain. J Neuropathol Exp Neurol 2003; 62: 644–654.
18. Yamaguchi H, Haga C, Hirai S, Nakazato Y, Kosaka K: Distinctive, rapid, and easy labeling of diffuse plaques in the Alzheimer brains by a new methenamine silver stain. Acta Neuropathol 1990; 79: 569–572.
19. Gallyas F: Silver staining of Alzheimer's neurofibrillary changes by means of physical development. Acta Morphol Acad Sci Hung 1971; 19: 1–8.
20. Folstein MF, Folstein SE, McHugh PR: "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12: 189–198.
21. Hasegawa K, Inoue K, Moriya K: [An investigation of dementia rating scale for the elderly]. Seishin Igaku 1974; 16: 965–969.
22. Lawton MP, Brody EM: Assessment of older people: self-maintaining and instrumental activities of daily living.

Gerontologist 1969; 9: 179–186.
23. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL: A new clinical scale for the staging of dementia. Br J Psychiatry 1982; 140: 566–572.
24. Braak H, Braak E: Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 1991; 82: 239–259.
25. Murayama S, Saito Y: Neuropathological diagnostic criteria for Alzheimer's disease. Neuropathology 2004; 24: 254–260.
26. Jellinger KA: Dementia with grains (argyrophilic grain disease). Brain Pathol 1998; 8: 377–386.
27. Jellinger KA, Bancher C: Senile dementia with tangles (tangle predominant form of senile dementia). Brain Pathol 1998; 8: 367–376.
28. McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, Cummings J, Duda JE, Lippa C, Perry EK, Aarsland D, Arai H, Ballard CG, Boeve B, Burn DJ, Costa D, Del Ser T, Dubois B, Galasko D, Gauthier S, Goetz CG, Gomez-Tortosa E, Halliday G, Hansen LA, Hardy J, Iwatsubo T, Kalaria RN, Kaufer D, Kenny RA, Korczyn A, Kosaka K, Lee VM, Lees A, Litvan I, Londos E, Lopez OL, Minoshima S, Mizuno Y, Molina JA, Mukaetova-Ladinska EB, Pasquier F, Perry RH, Schulz JB, Trojanowski JQ, Yamada M; Consortium on DLB: Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005; 65: 1863–1872.
29. Hauw JJ, Daniel SE, Dickson D, Horoupian DS, Jellinger K, Lantos PL, McKee A, Tabaton M, Litvan I: Preliminary NINDS neuropathologic criteria for Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy). Neurology 1994; 44: 2015–2019.
30. Dickson DW, Bergeron C, Chin SS, Duyckaerts C, Horoupian D, Ikeda K, Jellinger K, Lantos PL, Lippa CF, Mirra SS, Tabaton M, Vonsattel JP, Wakabayashi K, Litvan I; Office of Rare Diseases of the National Institutes of Health: Office of Rare Diseases neuropathologic criteria for corticobasal degeneration. J Neuropathol Exp Neurol 2002; 61: 935–946.
31. Akiyama H, McGeer PL: Brain microglia constitutively express beta-2 integrins. J Neuroimmunol 1990; 30: 81–93.
32. Komori T, Shibata N, Kobayashi M, Sasaki S, Iwata M: Inducible nitric oxide synthase (iNOS)-like immunoreactivity in argyrophilic, tau-positive astrocytes in progressive supranuclear palsy. Acta Neuropathol 1998; 95: 338–344.
33. Oshima K, Tsuchiya K, Iritani S, Niizato K, Akiyama H, Arai H, Ikeda K: [An autopsy case of "progressive supranuclear palsy" without psychiatric or neurological signs]. No To Shinkei 2004; 56: 157–161.
34. Mattila P, Togo T, Dickson DW: The subthalamic nucleus has neurofibrillary tangles in argyrophilic grain disease and advanced Alzheimer's disease. Neurosci Lett 2002; 320: 81–85.
35. Ito K, Arai K, Yoshiyama Y, Kashiwado K, Sakakibara Y, Hattori T: Astrocytic tau pathology positively correlates with neurofibrillary tangle density in progressive supranuclear palsy. Acta Neuropathol 2008; 115: 623–628.
36. Togo T, Dickson DW: Tau accumulation in astrocytes in progressive supranuclear palsy is a degenerative rather than a reactive process. Acta Neuropathol 2002; 104: 398–402.
37. Toru S, Uchihara T, Akashi T, Kobayashi T: Minimal cytological changes of progressive supranuclear palsy type in clinically possible progressive supranuclear palsy. Eur Neurol 2010; 63: 122–125.
38. Sakai K, Yamada M: Early-stage progressive supranuclear palsy with degenerative lesions confined to the subthalamic nucleus and substantia nigra. Neuropathology 2011; 31: 77–81.
39. Braak H, Del Tredici K, Rüb U, de Vos RA, Jansen Steur EN, Braak E: Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003; 24: 197–211.
40. Adachi T, Saito Y, Hatsuta H, Funabe S, Tokumaru AM, Ishii K, Arai T, Sawabe M, Kanemaru K, Miyashita A, Kuwano R, Nakashima K, Murayama S: Neuropathological asymmetry in argyrophilic grain disease. J Neuropathol Exp Neurol 2010; 69: 737–744.

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