Original Article
Diversity of pathological features other than Lewy bodies in familial Parkinson's disease due to *SNCA* mutations

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Abstract: The clinical features of the genetically determined forms of familial Parkinson's disease (PD) have been described in multiple reports, but there have been few comparative neuropathologic studies. Five familial PD cases, with mutations in SNCA, were matched for age, sex, and Alzheimer type pathology with sporadic PD cases. Immunohistochemistry for phospho-tau and α-synuclein was performed in 8 brain regions. The frequency of tau pathology and the morphologic features of α-synuclein pathology in familial PD were compared with sporadic PD using semi-quantitative methods. In familial PD, there were significantly more tau positive extra-perikaryal spheroid-like and thread-like lesions than in the sporadic PD. There was no significant difference in the amount of α-synuclein positive neuronal perikaryal pathology between familial PD and sporadic PD, but α-synuclein positive oligodendroglial and neuritic lesions were significantly greater in familial PD compared to sporadic PD. In the substantia nigra, familial PD had more marked neuronal loss and fewer residential neurons with Lewy bodies than the sporadic PD, suggesting a close relationship between the severity of neuronal loss and Lewy body formation. The results show a diversity of pathological features of genetically determined familial PD, and they draw attention to the possible role of tau protein in neurodegeneration. Moreover, the presence of oligodendroglial inclusions at the light and electron microscopic levels in familial PD suggests that PD and multiple system atrophy form a continuum of α-synuclein pathology.

Keywords: Pathological features, Lewy bodies, familial Parkinson's disease, SNCA mutations

Introduction

Parkinson's disease (PD) is pathologically characterized by neuronal loss and the presence of Lewy bodies (LBs) in the substantia nigra [1] and other subcortical nuclei [2]. The main component of LBs is α-synuclein [3], which was discovered after a mutation in its gene (*SNCA*) was found in a large kindred with familial PD [4]. In addition to gene multiplication, there are several missense mutations in *SNCA* that cause PD. The best characterized are A30P, A46K and A53T, but several new mutations have also been reported [5-7].

Microtubule-associated protein tau, the major component of neurofibrillary tangles (NFT), has also been suggested to be a component of LBs. Presence of tau in LBs has been reported in immunohistochemical and electron microscopic studies [8-10]. Since tau pathology is frequent in aging, it is difficult to interpret tau pathology found concurrently in late-onset PD. In contrast, early-onset familial PD due to mutations or triplications in *SNCA* should be largely free of age-related tau pathology and offers an opportunity to explore the role of tau in PD.

The present study was undertaken to investigate the frequency of tau pathology and the morphologic features of α-synuclein pathology in familial PD with mutations or triplications in *SNCA* as compared to sporadic PD.

Table 1. Demographics of cases

Case	Sex	Age	Pathological Diagnosis	Braak NFT stage	CERAD plaque score	SNCA mutation
Familial PD						
1	M	42	DLBD	I-II	None	Triplication
2	M	48	DLBD	I	None	Triplication [15]
3	M	39	DLBD	IV	None	A53T + PRKN [11]
4	F	57	DLBD	0-I	None	A53T
5	F	71	DLBD/AGD/HpScl	I	Sparse	A53T [11]
Sporadic PD						
6	F	54	TLBD	I	None	None
7	M	50	TLBD	II	None	None
8	M	60	TLBD	II-III	Sparse	None
9	M	61	TLBD	I-II	None	None
10	F	58	BLBD	0	None	None

Demographics and pathological and genetic findings. BLBD = Brainstem Lewy body disease, TLBD = Transitional Lewy body disease, DLBD = diffuse Lewy body disease; NFT = neurofibrillary tangle; CERAD = Consortium to Establish a Registry for Alzheimer's Disease.

Material and methods

Subjects

We studied 5 cases of familial PD with *SNCA* mutations. One case also had Parkin gene (*PRKN*) mutation [11]. The cases were matched for age, sex, the severity of amyloid deposits and Braak NFT stage [12] with 5 sporadic PD cases (**Table 1**). The severity of amyloid deposits was assessed by using the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria [13]. The average age of death (± standard deviation) was 51±12 years for familial PD and 56±4 years for sporadic PD. The average Braak NFT stage (± standard deviation) was 1.6±1.3 for familial PD and 1.4±0.9 for sporadic PD.

Immunohistochemistry

Eight standardized brain sections, including frontal and temporal cortex, cingulate gyrus, hippocampus, amygdala, midbrain, pons and medulla, were used for this study. The deparaffinized and rehydrated sections were steamed in distilled water for 30 minutes. Before staining for α-synuclein the slides were pretreated with 95% formic acid for 30 minutes, a sensitive and specific method of pathologic α-synuclein deposits [14]. Serial sections were immunostained with a monoclonal antibody to phospho-tau (CP13 [8]; 1:1000, Peter Davies, Albert Einstein College of Medicine, Bronx, NY) and a polyclonal antibody to α-synuclein (NACP

[15]. 1:3,000), using 3, 3'-diaminobenzidine (DAB) as the chromogen. After immunostaining, the sections were counterstained with hematoxylin.

Immunoelectron microscopy

For immunoelectron microscopy, small pieces of putamen from formalin-fixed brain from *SNCA* triplication (Case 1) were processed for post-embedding immunogold labeling as previously described [9]. Ultrathin sections collected on Formvar-coated nickel grids were incubated in primary antibodies overnight at 4°C, followed by secondary antibodies conjugated with colloidal gold particles. The rabbit polyclonal antibody to α-synuclein (NACP) was used.

Semi-quantitative histopathological evaluation

The number of α-synuclein- and phospho-tau-immunoreactive neuronal perikaryal and glial inclusions was counted on microscopic fields at ×200 magnification in the amygdala, nucleus basalis of Meynert, striatum (nucleus accumbens, putamen and caudate), hippocampus (5 regions; subiculum, CA1, CA2/3, endplate and dentate fascia), limbic cortex (parahippocampal and cingulate gyrus) and neocortex (superior temporal and middle frontal cortex), midbrain (substantia nigra and tegmentum), pons (pontine tegmentum and base), medulla (medullary tegmentum and inferior olive). The average number of the largest three values obtained for each region was calculated and scored from
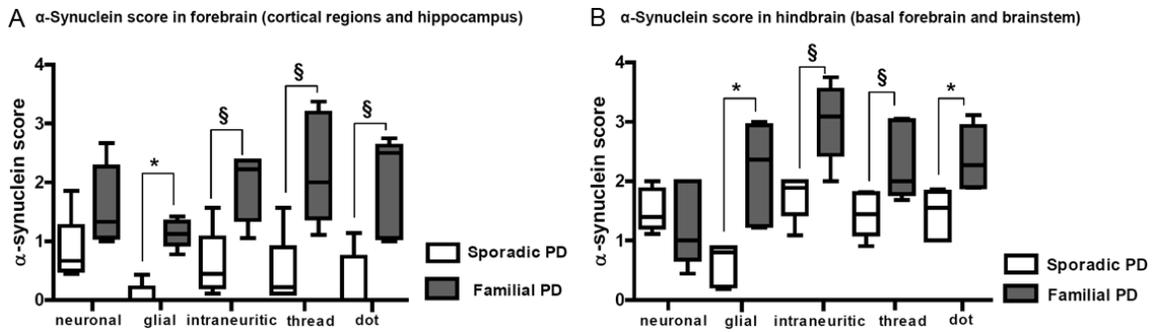
Figure 1. Comparison of α-synuclein scores between familial and sporadic Parkinson's disease with data grouped by forebrain and hindbrain regions. *; p<0.01, §; p<0.05 by Mann-Whitney Rank Sum Test.
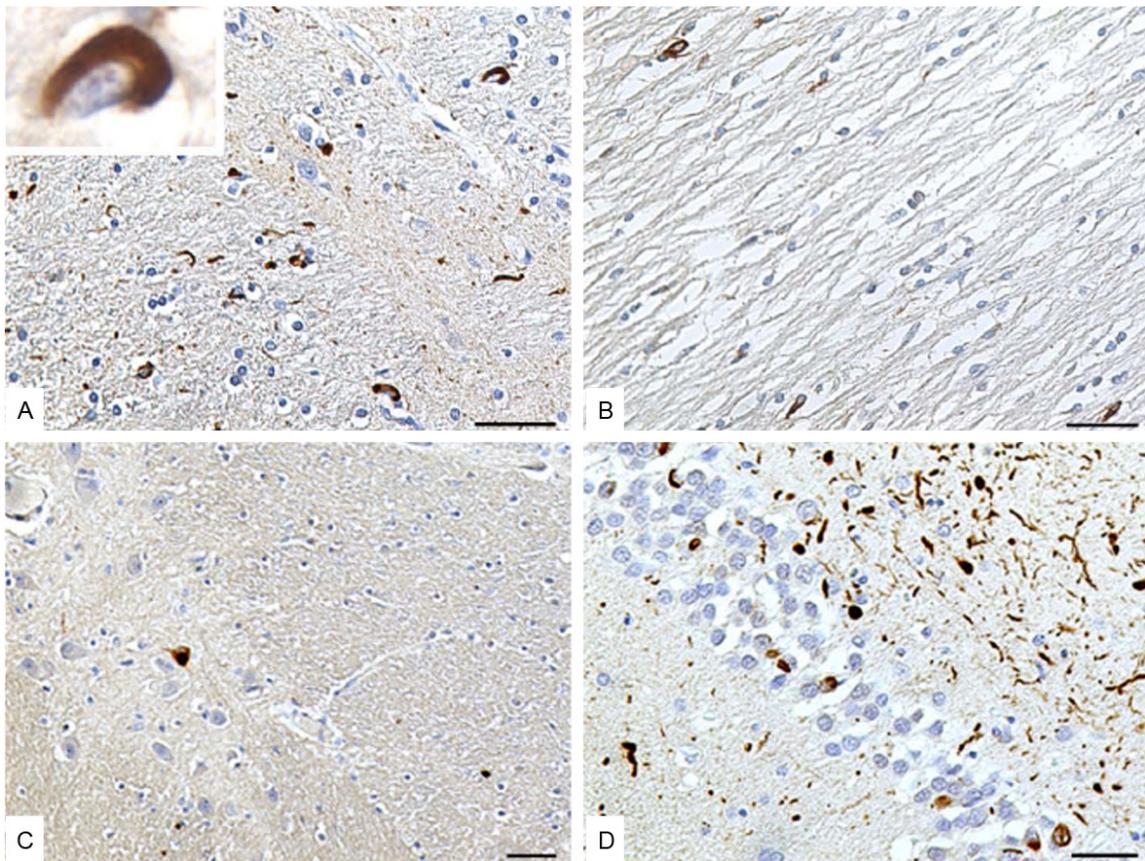
Figure 2. Alpha-synuclein immunoreactive pathology in familial PD. A, B: Many α-synuclein immunoreactive glial inclusions are noted in the pencil fibers in the striatum. Higher magnification (upper left corner) shows GCI-like pathology. C: A few α-synuclein immunoreactive lesions were seen in pontine base. D: Extensive neuritic pathology and neuronal inclusions were detected in the endplate and dentate fascia of hippocampus. Bar = 50 μm.

0 to 4: 0=absent; 1=(>0-1); 2=(>1-5); 3=(>5-9); 4=(>9). α-synuclein- and phospho-tau-immunoreactive extra-perikaryal deposits were classified into three types - intraneuritic LBs (round or spheroid-like deposits in cell processes), thread-like (Lewy neurites) and dot-like (Lewy dots [16]), and graded from 0 to 4: 0=absent;

1=rare; 2=some; 3=many; 4=numerous in the same regions used to assess perikaryal deposits.

The degree of neuronal loss in the substantia nigra was evaluated on H&E stained sections. In the lateral and ventral region of substantia
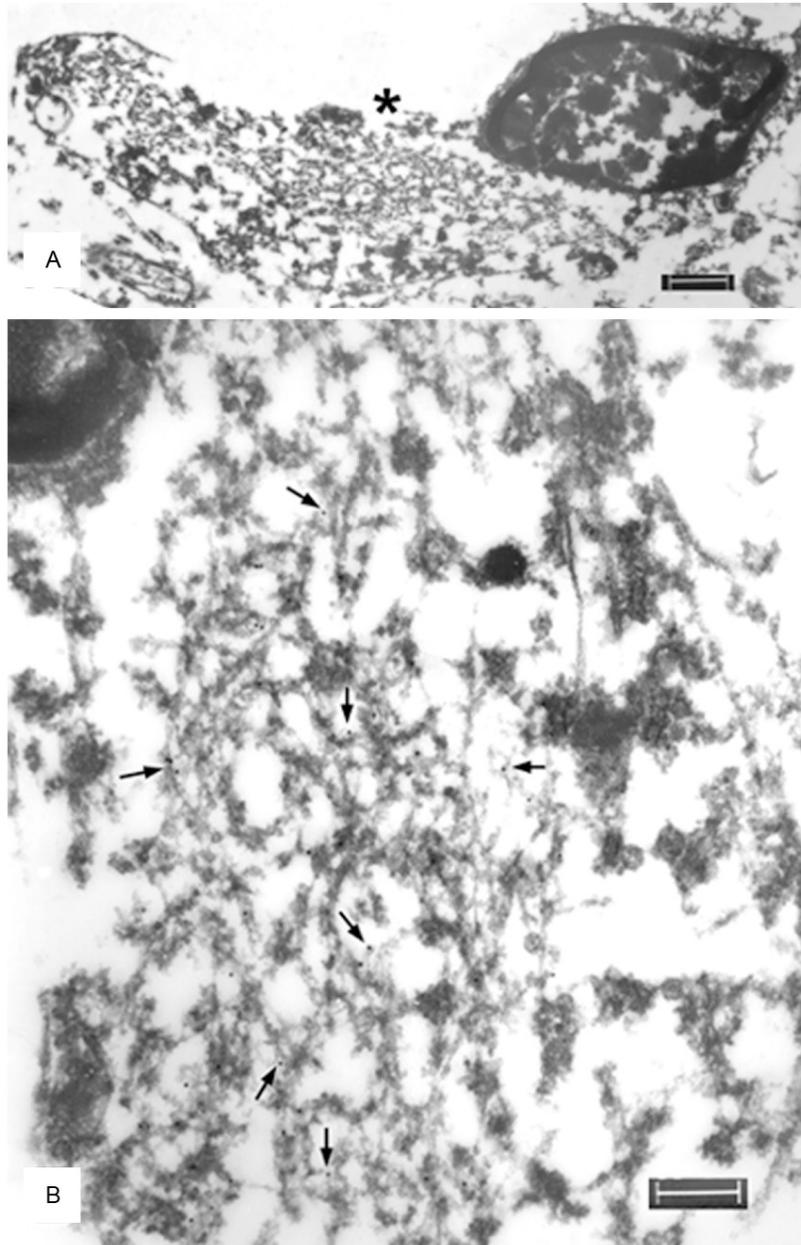
Figure 3. A: An oligodendrocyte with characteristic round nucleus and dense heterochromatin contains a cytoplasmic inclusion. *indicates area enlarged in B. Bar = 1 μm. B: The inclusion contains aggregated filaments and associated dense granular material; both have α-synuclein immunoreactivity (indicated by gold particles at arrows). Bar = 0.3 μm.

significance level was set at $p<0.05$. The results of semi-quantitative pathological evaluation were compared between sporadic and familial PD groups with Mann-Whitney Rank Sum Test.

Results

Alpha-synuclein immunoreactive pathology

All familial PD cases had distribution of Lewy related pathology consistent with diffuse Lewy body disease (DLBD), while the sporadic PD group had 4 transitional type Lewy body disease (TLBD) and 1 had brainstem type Lewy body disease (BLBD) [17] (**Table 1**). To adjust for the possible effect this might have had on observed differences between familial PD and sporadic PD, we compared separately the data from forebrain (cortex plus hippocampus) and hindbrain (subcortical areas plus brainstem). There was no difference in the score of perikaryal neuronal inclusions between familial and sporadic PD for forebrain and hindbrain regions, even though there was a different mix of Lewy body disease subtypes in familial and sporadic PD (**Figure 1**). All sporadic and familial PD cases had a wide range of oligodendroglial inclusions as in previous reports [18-20] (**Figure 2A**, **2B**). Some of the oligodendroglial inclusions were similar to glial cytoplasmic inclusions (GCI) that are found in multiple system atrophy (MSA) [21].

Immunoelectron microscopy of glial inclusions in Case 1 were shown to be in cells that had ultrastructural features consistent with oligo-

nigra [1], the average of three highest numbers of pigmented neurons at ×200 magnification was graded from 0 to 4: (0) ≤50; (1) >25-50; (2) >15-25; (3) >5-15; (4) ≤5.

Statistical analysis

Data were analyzed with SigmaStat 3.5 (Systat Software, Inc., Point Richmond, CA), and the
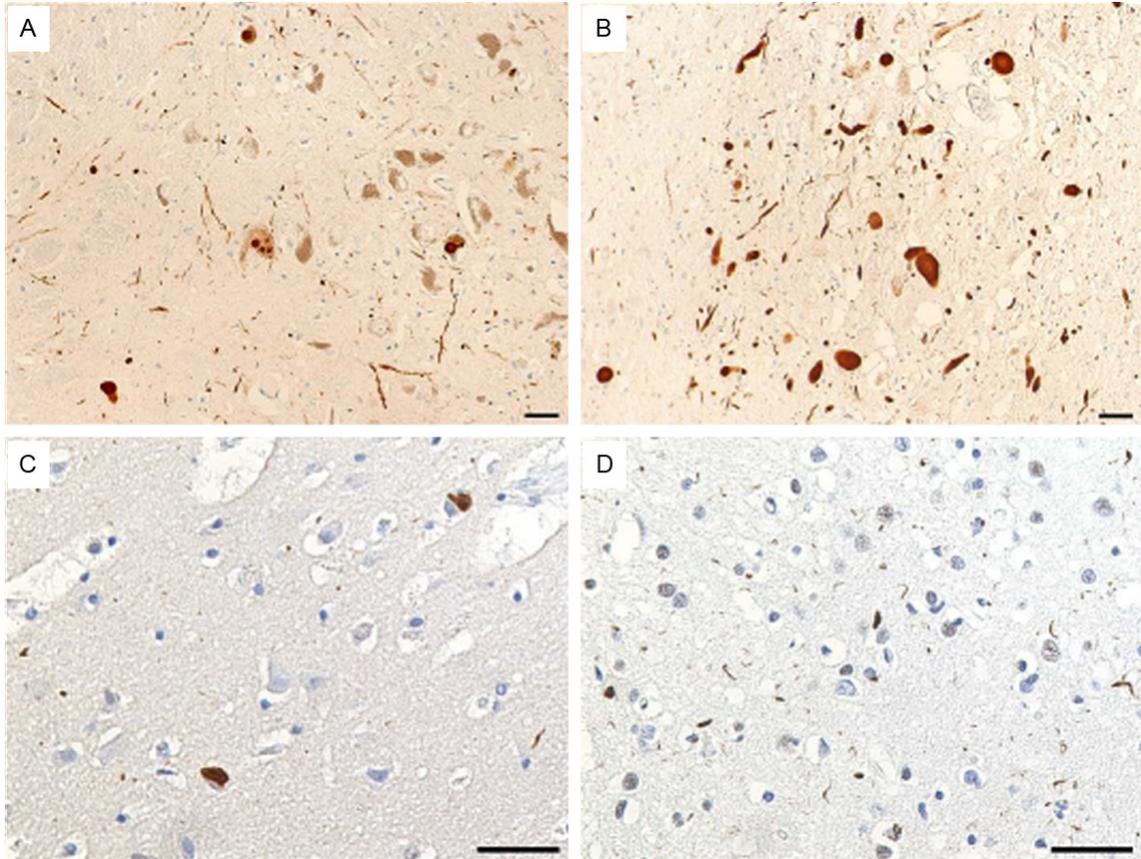
Figure 4. Comparison of α-synuclein immunoreactive pathology in the substantia nigra and temporal cortex. A: Sporadic PD had residual neurons with LBs. B: Extensive of α-synuclein immunoreactive extra-perikaryal deposits are detected in familial PD, but there are only a few residual neurons with LBs. C: Sporadic PD had sparse cortical LBs in the temporal cortex. D: Familial PD had many α-synuclein immunoreactive extra-perikaryal deposits with spongiform change, but only a few cortical LBs. Bar = 50 μm.

dendrocytes, including small cells with dense nuclear heterochromatin and scant cytoplasm (**Figure 3**) [22]. Inclusions found in oligodendrocytes consisted of 10 nm wide filaments and associated dense granular material, labeled with antibody to α-synuclein (**Figure 2**). The same structures were also labeled with an antibody to phosphorylated Serine 129 α-synuclein (gift of Dr. Iwatsubo, Tokyo University) (data not shown) [23]. This result confirmed our previous findings with double labeling immunofluorescence microscopy (Case 2) [15].

The score of oligodendroglial inclusions was greater in familial PD than in sporadic PD (p<0.01), consistent with previous reports [15, 24] (**Figure 1**). The scores for all extra-perikaryal deposits were also greater in familial PD than in sporadic PD (**Figures 1**, **4**). These results were similar to previous findings of patients with *SNCA* mutations that there are more struc-

tures consistent with intraneuritic Lewy bodies than with inclusions in neuronal perikarya [10, 25]. Moreover, a few α-synuclein immunoreactive lesions were seen in pontine base (**Figure 2C**) and inferior olivary nucleus of familial PD cases, a distribution of α-synuclein pathology similar to that found in MSA. Three familial PD cases also had extensive neuritic pathology and neuronal inclusions in the endplate and dentate fascia of hippocampus (**Figure 2D**), while none of sporadic PD cases had this finding, where neuritic pathology was mostly limited to CA2 [26].

The relationship of α-synuclein pathology and neuronal loss

In all familial PD cases, the substantia nigra (SN) had marked neuronal loss with only a few residual neurons, consistent with our grade 4 neuronal loss score. There was diffuse gliosis
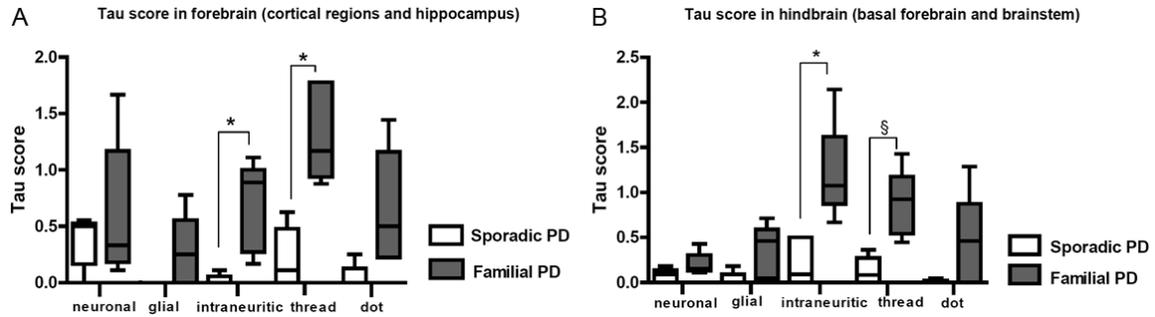
Figure 5. Comparison of tau scores between familial and sporadic Parkinson's disease with data grouped by forebrain and hindbrain regions. *; p<0.01, §; p<0.05 by Mann-Whitney Rank Sum Test.
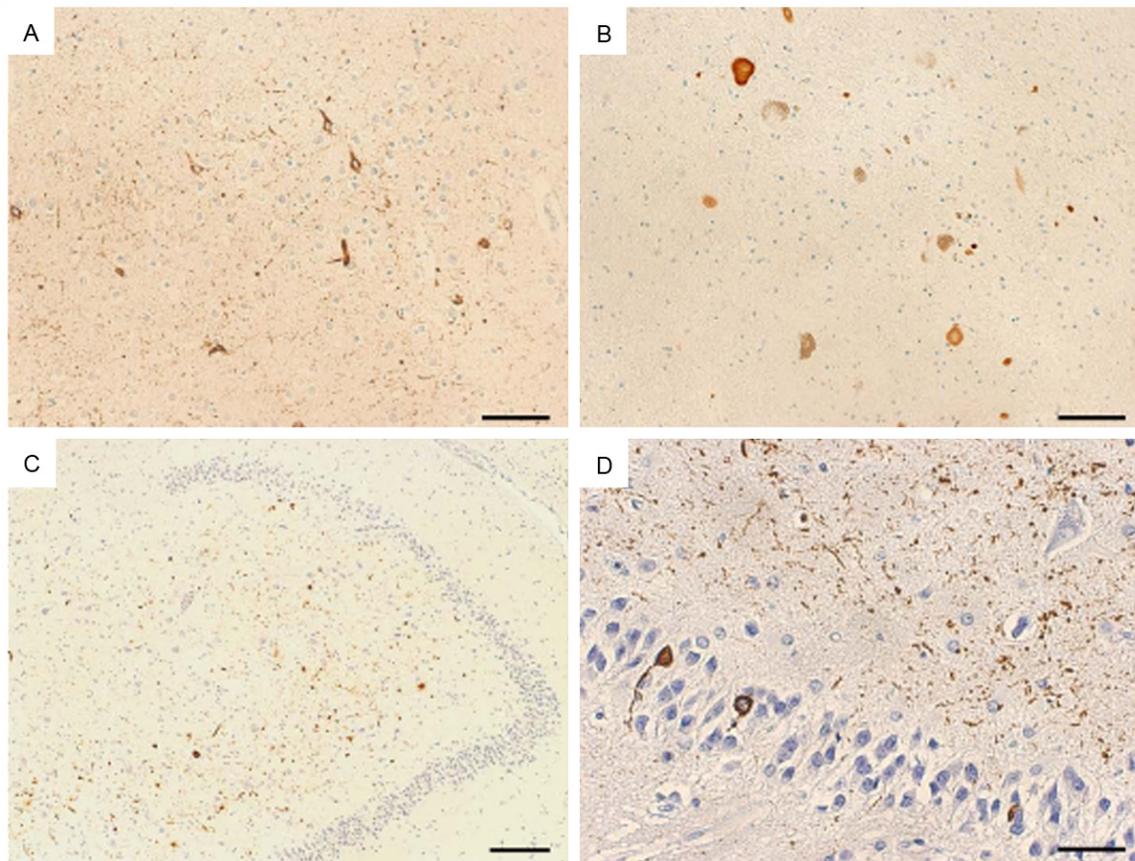
Figure 6. Tau immunoreactive pathology in familial PD. A: Tau immunostaining revealed the sparse and patchy NFT in the temporal cortex (Case 3). B: Tau immunoreactive round or spheroid-like structures in the locus ceruleus (Case 5). C: Many of tau immunoreactive dystrophic neuronal processes (similar to those in **Figure 2D**) are detected in the hippocampal endplate (Case 2). D: Case 3 had tau immunoreactive neuronal inclusions in the dentate fascia. Bar = 100 μm (A-C), 50 μm (D).

and a small amount of extraneuronal neuromelanin (**Figure 4B**). The severity of neuronal loss in the SN of familial PD was significantly greater than in sporadic PD. The score of α-synuclein positive neuronal perikaryal pathology in the SN was ranged from 0 to 2 in familial PD, while in sporadic PD it ranged from 2 to 3, significantly greater than in familial PD (p<0.05). On the other hand, the average score of extraperikaryal deposits (Lewy neurites and dots) in the SN was greater in familial PD than in sporadic PD. These results may suggest that

marked neuronal loss in PD accounts for fewer residual neurons with LBs, while abundant intraneuritic process may be in part derived from neurons outside of the SN. In the cortex, sporadic PD had sparse cortical LBs, while most familial PD cases showed disproportionate extra-perikaryal (intra-neuritic LBs, as well as Lewy neurites and dots) α-synuclein pathology compared to cortical LB, as well as prominent spongiform changes (**Figure 4C**, **4D**). In the cortex of familial PD, Case 1 had the highest score for cortical LBs (2.7) and the least spongiform change, while the cases with fewer cortical LBs (average score 1.3; range 1.0 to 1.9) and more extra-perikaryal α-synuclein pathology and greater spongiform change, suggesting a relationship between extra-perikaryal α-synuclein pathology and spongiform change.

Tau immunoreactive pathology

Case 5 had tau pathology mostly in the medial temporal lobe, consistent with argyrophilic grain disease. None of the other cases had other concurrent tauopathy. Except for Case 3, all familial PD cases showed some tau positive extra-perikaryal lesions in most examined areas, while sporadic PD cases had very little tau positive pathology in any region (**Figure 6**). Case 3 had sparse and patchy NFTs in the neocortex, consistent with Braak NFT stage IV (**Figure 6A**). In this case, the thioflavin S fluorescent microscopy and Aβ42 immunohistochemistry failed to show any amyloid deposits in any section, even in regions with neurofibrillary pathology (data not shown). There was no significant difference in the score of intra-perikaryal deposits in the forebrain or hindbrain of familial PD and sporadic PD (**Figure 5**). The score of extra-perikaryal tau deposits within intraneuritic LBs and neuropil threads was significantly greater in familial PD than in sporadic PD (p<0.05). This difference remained, even if Case 5 with AGD was excluded for comparison. There was no difference in the score of tau positive dot-like extra-perikaryal deposits (**Figure 5**). Extensive tau immunoreactivity was presence in intraneuritic LBs and thread-like processes seen in the endplate (CA4) of the hippocampus in familial PD, in a distribution similar to that seen with α-synuclein immunohistochemistry (**Figure 6C**, **6D**).

Discussion

The present study showed two major findings based upon comparative neuropathological investigation of familial compared to sporadic PD. First, the severity of oligodendroglial and extra-perikaryal α-synuclein pathology (intraneuritic LBs, as well as Lewy neurites and Lewy dots) was greater in familial PD than in sporadic PD. Second, tau immunoreactive pathology was mild in familial PD, but more frequently than in sporadic PD. These results confirm diversity of pathological features beyond Lewy bodies and Lewy neurites in genetically-determined familial PD.

There was a difference in the distribution of subtypes of Lewy body disease between familial PD and sporadic PD. All familial PD cases had DLBD, while sporadic PD cases included BLBD and TLBD. On the other hand, there was no difference in the score of α-synuclein positive neuronal perikaryal pathology between familial PD and sporadic PD when data were grouped into forebrain and hindbrain regions, to adjust for this potential confounder. A weakness of this study is the small number of cases included in the analysis, but this is unavoidable given the difficulty to find sporadic cases that could be matched to the young age of familial cases. We feel that anatomical subgroup analysis obviates the possibility that differences were merely due to differences in distribution of Lewy body disease subtype between familial PD and sporadic PD. While there was a trend (p=0.06) for familial PD to have greater LB scores in the forebrain compared to sporadic PD, but the score of oligodendroglial and extra-perikaryal α-synuclein positive pathology was greater in familial PD than in sporadic PD in both regions.

In the substantia nigra, familial PD cases had marked neuronal loss and only a few LBs indicating a close relationship between the severity of neuronal loss and the density of observed LBs. These results raise the possibility that familial PD shows advanced ("end-stage") pathology whereby vulnerable neurons had formed inclusions and subsequently died, leaving behind only reactive gliosis and inclusions within neuronal cell processes possibly derived from surviving neurons in other brain regions projecting into the SN.

An interesting aspect of the present study was that the α-synuclein positive oligodendroglial

lesions in familial PD had features somewhat similar to GCI of MSA, with inclusions in brainstem regions usually resistant to Lewy-related pathology in sporadic PD, [2] such as the pontine base and the inferior olivary nucleus. The presence of oligodendroglial inclusions suggests that sporadic PD, familial PD and MSA form α-synucleinopathy disease spectrum. Infrequent reports of patients with combined pathological features of Lewy body disease and MSA may support this possibility [27, 28]. It is difficult to reconcile the current findings with reports showing no α-synuclein immunoreactive glial pathology in the familial PD with *SNCA* mutation [25, 29]. On the other hand (and similar to our findings), Obi and co-workers reported widespread α-synuclein immunoreactive oligodendroglial inclusions in familial PD with *SNCA* duplication [24]. Interestingly, they also found oligodendroglial inclusions in the pontine base and cerebellar white matter. In our study, all familial PD cases with SNCA mutation had α-synuclein positive oligodendroglial inclusions. A limitation of the present study was the small number of familial PD with SNCA mutations, a problem that has plagued even meta-analyses reviewing the pathology of genetically-determined familial PD [30].

Tau immunoreactive pathology in most familial PD cases was mild, consistent with previous reports [24, 25, 29]. On the other hand, the present study showed that familial PD had more tau pathology than sporadic PD. It is worth noting that Case 3 had some NFT in the neocortex without any amyloid deposits, consistent Braak NFT stage IV, indicating that this tau pathology cannot be attributed to concomitant Alzheimer's disease. Most tau immunoreactive pathology in familial PD was characterized by co-localization with α-synuclein in intraneuritic LBs, but also as thread-like lesions in the brainstem nuclei, nucleus of basalis of Meynert and hippocampus (especially CA2/3) and endplate, similar to a previous case report of familial PD due to A53T mutation in *SNCA* [10]. It is of interest that the distribution of greatest tau pathology follows the distribution of most severe α-synuclein pathology. The possibility that tau and α-synuclein my synergistically interact has been suggested by *in vitro* fibrillation studies [31] and more recently intracellular and animal models [32]. These results, as well as the identification of the tau gene (*MAPT*) as a genetic risk factor for PD with genome wide association studies [33] and our pathologic findings in familial PD due to *SNCA* mutation, draw attention to the role of tau protein in neurodegeneration in PD.

Acknowledgements

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