

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

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Running title: Neuropathology of Familial PD

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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: tau, α -synuclein, SNCA, Parkinson's disease, immunohistochemistry, electron microscopy

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 p.A30P, p.A46K and p.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.

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 (\pm standard deviation) was 51 ± 12 years for familial PD and 56 ± 4 years for sporadic PD. The average Braak NFT stage (\pm standard deviation) was 1.6 ± 1.3 for familial PD and 1.4 ± 0.9 for sporadic PD.

Immunohistochemistry

Eight consecutive 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 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 pathologic evaluation

The number of α -synuclein- and phospho-tau-immunoreactive neuronal perikaryal and glial inclusions was counted on microscopic fields at $\times 200$ 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 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 nigra [1], the average of three highest numbers of pigmented neurons at $\times 200$ 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 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

α -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 1A, B**). All sporadic and familial PD cases had a wide range of oligodendroglial inclusions as in previous reports [18-20] (**Figure 2A, B**). Some of the oligodendroglial inclusions were similar to glial cytoplasmic inclusions (GCI) that are found in multiple system atrophy (MSA) [21].

Immunoelectron microscopy

Glial inclusions in Case 1 were shown to be in cells that had ultrastructural features consistent with oligodendrocytes, including small cells with dense nuclear heterochromatin and scant cytoplasm [22]. Inclusions found in oligodendrocytes consisted of 10 nm wide filaments and associated dense granular material, labeled with antibody to α -synuclein (**Figure 3**). 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 (Fig. 1, 4). These results were similar to previous findings of patients with *SNCA* mutations that there are more structures 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 (Fig. 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 (Fig. 2d), while none of sporadic PD cases had this finding, where neuritic pathology was mostly limited to CA2 [26].

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 and a small amount of extraneuronal neuromelanin (**Figure 4**). 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 extra-perikaryal 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 (intraneuritic LBs, as well as Lewy neurites and dots) α -synuclein pathology compared to cortical LB, as well as prominent spongiform changes (**Figure 4C, D**). 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 5**). Case 3 had sparse and patchy NFTs in the neocortex, consistent with Braak NFT stage IV (**Figure 5A**). In this case, the thioflavin S fluorescent microscopy and A β 42 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 6**). 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 (Fig. 5). Extensive tau immunoreactivity was present 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 (Fig. 6C, D).

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 (intra-neuritic 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 may 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.

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References

- [1] Dickson DW, Braak H, Duda JE, Duyckaerts C, Gasser T, Halliday GM, Hardy J, Leverenz JB, Del Tredici K, Wszolek ZK and Litvan I. Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. *Lancet Neurol* 2009; 8: 1150-1157.
- [2] Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN and Braak E. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol Aging* 2003; 24: 197-211.
- [3] Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R and Goedert M. Alpha-synuclein in Lewy bodies. *Nature* 1997; 388: 839-840.
- [4] Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI and Nussbaum RL. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* 1997; 276: 2045-2047.
- [5] Lesage S, Anheim M, Letournel F, Bousset L, Honore A, Rozas N, Pieri L, Madiona K, Durr A, Melki R, Verny C and Brice A. G51D alpha-synuclein mutation causes a novel parkinsonian-pyramidal syndrome. *Ann Neurol* 2013; 73: 459-471.
- [6] Appel-Cresswell S, Vilarino-Guell C, Encarnacion M, Sherman H, Yu I, Shah B, Weir D, Thompson C, Szu-Tu C, Trinh J, Aasly JO, Rajput A, Rajput AH, Jon Stoessl A and Farrer MJ. Alpha-synuclein p.H50Q, a novel pathogenic mutation for Parkinson's disease. *Mov Disord* 2013; 28: 811-813.
- [7] Hoffman-Zacharska D, Koziorowski D, Ross OA, Milewski M, Poznanski J, Jurek M, Wszolek ZK, Soto-Ortolaza A, Slawek J, Janik P, Jamrozik Z, Potulska-Chromik A, Jasinska-Myga B, Opala G, Krygowska-Wajs A, Czyzewski K, Dickson DW, Bal J and Friedman A. Novel A18T and pA29S substitutions in alpha-synuclein may be associated with sporadic Parkinson's disease. *Parkinsonism Relat Disord* 2013; published online 05 August 2013:
- [8] Ishizawa T, Mattila P, Davies P, Wang D and Dickson DW. Colocalization of tau and alpha-synuclein epitopes in Lewy bodies. *J Neuropathol Exp Neurol* 2003; 62: 389-397.
- [9] Fujishiro H, Tsuboi Y, Lin WL, Uchikado H and Dickson DW. Co-localization of tau and alpha-synuclein in the olfactory bulb in Alzheimer's disease with amygdala Lewy bodies. *Acta Neuropathol* 2008; 116: 17-24.
- [10] Duda JE, Giasson BI, Mabon ME, Miller DC, Golbe LI, Lee VM and Trojanowski JQ. Concurrence of alpha-synuclein and tau brain pathology in the Contursi kindred. *Acta Neuropathol* 2002; 104: 7-11.
- [11] Markopoulou K, Dickson DW, McComb RD, Wszolek ZK, Katechalidou L, Avery L, Stansbury MS and Chase BA. Clinical, neuropathological and genotypic variability in SNCA A53T familial Parkinson's disease. Variability in familial Parkinson's disease. *Acta Neuropathol* 2008; 116: 25-35.
- [12] Braak H and Braak E. Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol* 1991; 82: 239-259.
- [13] Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G and Berg L. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 1991; 41: 479-486.
- [14] Beach TG, White CL, Hamilton RL, Duda JE, Iwatsubo T, Dickson DW, Leverenz JB, Roncaroli F, Buttni M, Hladik CL, Sue LI, Nooragian JV and Adler CH. Evaluation of alpha-synuclein immunohistochemical methods used by invited experts. *Acta Neuropathol* 2008; 116: 277-288.

- [15] Gwinn-Hardy K, Mehta ND, Farrer M, Maraganore D, Muenter M, Yen SH, Hardy J and Dickson DW. Distinctive neuropathology revealed by alpha-synuclein antibodies in hereditary parkinsonism and dementia linked to chromosome 4p. *Acta Neuropathol* 2000; 99: 663-672.
- [16] Saito Y, Kawashima A, Ruberu NN, Fujiwara H, Koyama S, Sawabe M, Arai T, Nagura H, Yamanouchi H, Hasegawa M, Iwatsubo T and Murayama S. Accumulation of phosphorylated alpha-synuclein in aging human brain. *J Neuropathol Exp Neurol* 2003; 62: 644-654.
- [17] Kosaka K, Yoshimura M, Ikeda K and Budka H. Diffuse type of Lewy body disease: progressive dementia with abundant cortical Lewy bodies and senile changes of varying degree--a new disease? *Clin Neuropathol* 1984; 3: 185-192.
- [18] Arai T, Ueda K, Ikeda K, Akiyama H, Haga C, Kondo H, Kuroki N, Niizato K, Iritani S and Tsuchiya K. Argyrophilic glial inclusions in the midbrain of patients with Parkinson's disease and diffuse Lewy body disease are immunopositive for NACP/alpha-synuclein. *Neurosci Lett* 1999; 259: 83-86.
- [19] Wakabayashi K, Hayashi S, Yoshimoto M, Kudo H and Takahashi H. NACP/alpha-synuclein-positive filamentous inclusions in astrocytes and oligodendrocytes of Parkinson's disease brains. *Acta Neuropathol* 2000; 99: 14-20.
- [20] Hishikawa N, Hashizume Y, Yoshida M and Sobue G. Widespread occurrence of argyrophilic glial inclusions in Parkinson's disease. *Neuropathol Appl Neurobiol* 2001; 27: 362-372.
- [21] Dickson DW, Liu W, Hardy J, Farrer M, Mehta N, Uitti R, Mark M, Zimmerman T, Golbe L, Sage J, Sima A, D'Amato C, Albin R, Gilman S and Yen SH. Widespread alterations of alpha-synuclein in multiple system atrophy. *Am J Pathol* 1999; 155: 1241-1251.
- [22] Lin WL, Lewis J, Yen SH, Hutton M and Dickson DW. Filamentous tau in oligodendrocytes and astrocytes of transgenic mice expressing the human tau isoform with the P301L mutation. *Am J Pathol* 2003; 162: 213-218.
- [23] Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, Shen J, Takio K and Iwatsubo T. alpha-Synuclein is phosphorylated in synucleinopathy lesions. *Nat Cell Biol* 2002; 4: 160-164.
- [24] Obi T, Nishioka K, Ross OA, Terada T, Yamazaki K, Sugiura A, Takanashi M, Mizoguchi K, Mori H, Mizuno Y and Hattori N. Clinicopathologic study of a SNCA gene duplication patient with Parkinson disease and dementia. *Neurology* 2008; 70: 238-241.
- [25] Yamaguchi K, Cochran EJ, Murrell JR, Polymeropoulos MH, Shannon KM, Crowther RA, Goedert M and Ghetti B. Abundant neuritic inclusions and microvacuolar changes in a case of diffuse Lewy body disease with the A53T mutation in the alpha-synuclein gene. *Acta Neuropathol* 2005; 110: 298-305.
- [26] Dickson DW, Schmidt ML, Lee VM, Zhao ML, Yen SH and Trojanowski JQ. Immunoreactivity profile of hippocampal CA2/3 neurites in diffuse Lewy body disease. *Acta Neuropathol* 1994; 87: 269-276.
- [27] Mochizuki A, Komatsuzaki Y and Shoji S. Association of Lewy bodies and glial cytoplasmic inclusions in the brain of Parkinson's disease. *Acta Neuropathol* 2002; 104: 534-537.
- [28] Sikorska B, Papierz W, Preusser M, Liberski PP and Budka H. Synucleinopathy with features of both multiple system atrophy and dementia with Lewy bodies. *Neuropathol Appl Neurobiol* 2007; 33: 126-129.
- [29] Spira PJ, Sharpe DM, Halliday G, Cavanagh J and Nicholson GA. Clinical and pathological features of a Parkinsonian syndrome in a family with an Ala53Thr alpha-synuclein mutation. *Ann Neurol* 2001; 49: 313-319.
- [30] Pouloupoulos M, Levy OA and Alcalay RN. The neuropathology of genetic Parkinson's disease. *Mov Disord* 2012; 27: 831-842.

- [31] Giasson BI, Forman MS, Higuchi M, Golbe LI, Graves CL, Kotzbauer PT, Trojanowski JQ and Lee VM. Initiation and synergistic fibrillization of tau and alpha-synuclein. *Science* 2003; 300: 636-640.
- [32] Guo JL, Covell DJ, Daniels JP, Iba M, Stieber A, Zhang B, Riddle DM, Kwong LK, Xu Y, Trojanowski JQ and Lee VM. Distinct alpha-synuclein strains differentially promote tau inclusions in neurons. *Cell* 2013; 154: 103-117.
- [33] Nalls MA, Plagnol V, Hernandez DG, Sharma M, Sheerin UM, Saad M, Simon-Sanchez J, Schulte C, Lesage S, Sveinbjornsdottir S, Stefansson K, Martinez M, Hardy J, Heutink P, Brice A, Gasser T, Singleton AB and Wood NW. Imputation of sequence variants for identification of genetic risks for Parkinson's disease: a meta-analysis of genome-wide association studies. *Lancet* 2011; 377: 641-649.



Table 1. Case material

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

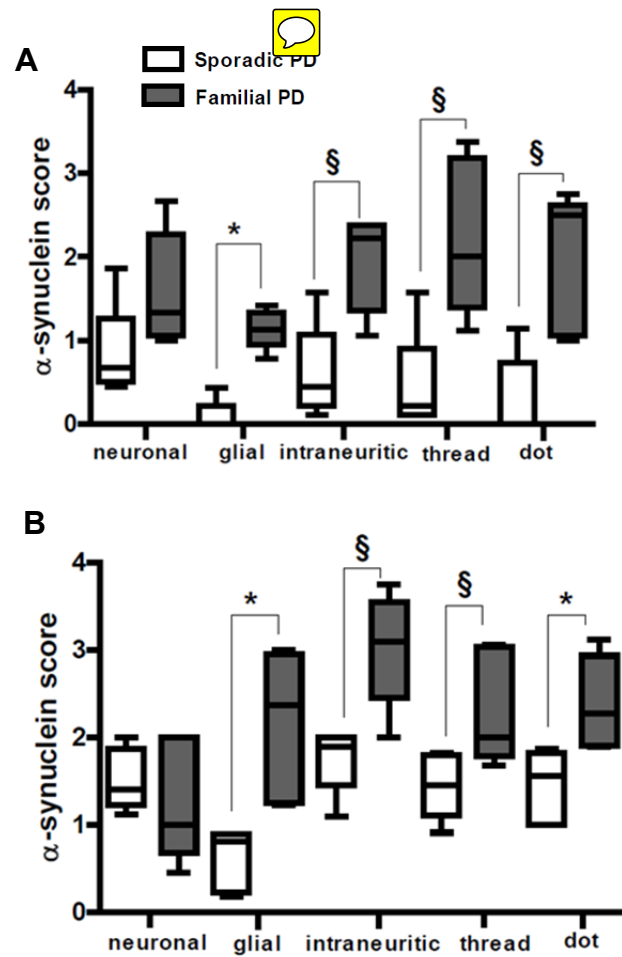


Figure 1. Comparison of α -synuclein scores between familial and sporadic Parkinson's disease with data grouped by forebrain (A) and hindbrain regions (B). *, $p < 0.01$, §, $p < 0.05$

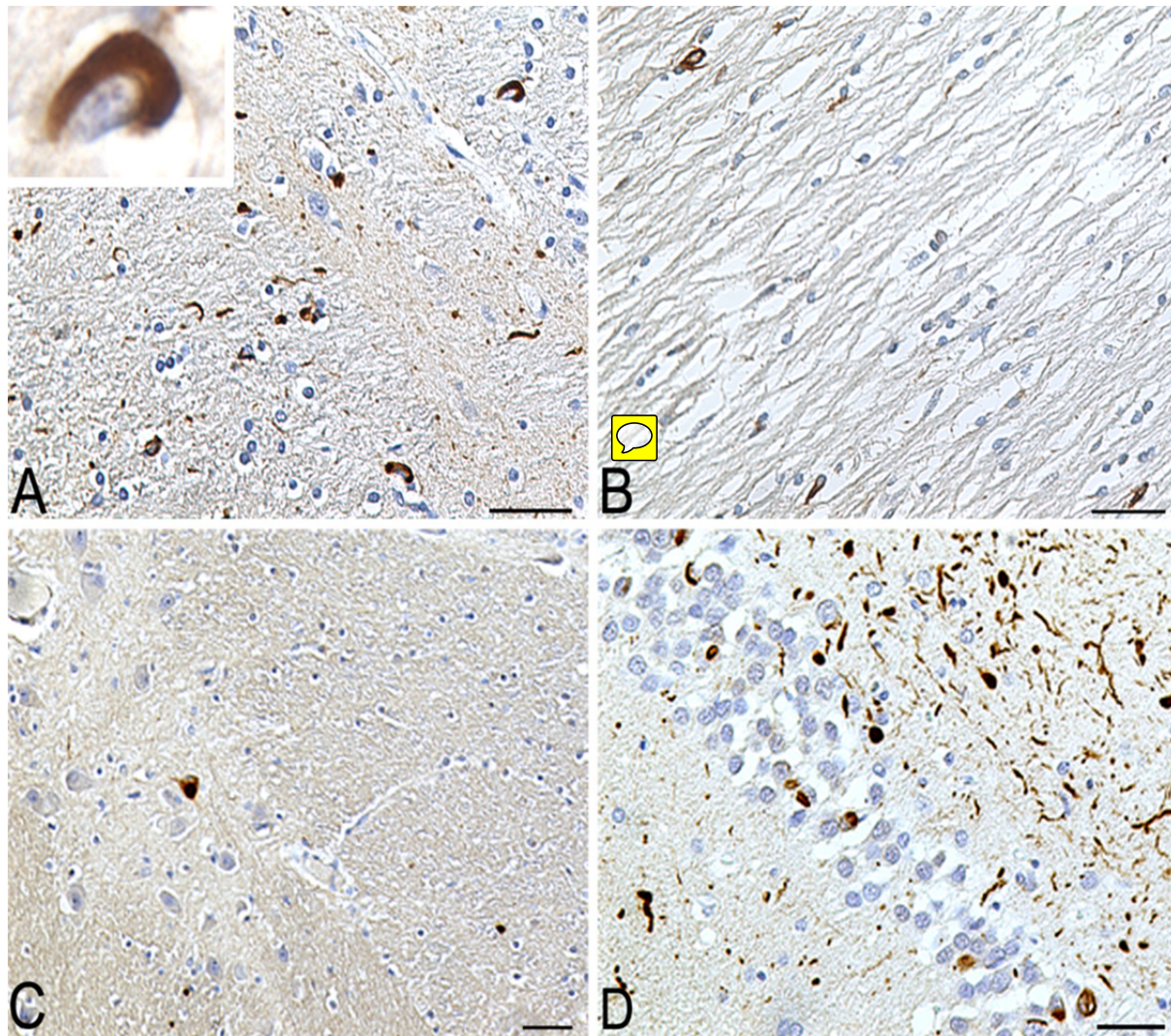


Figure 2 Alpha-synuclein immunoreactive pathology in familial PD. A, B. Many α -synuclein immunoreactive glial inclusions are noted in the pencil fibers in the striatum. Higher magnification (upper left corner) shows GCI-like pathology. C. A few α -synuclein immunoreactive lesions were seen in pontine base. D. Extensive neuritic pathology and neuronal inclusions were detected in the endplate and dentate fascia of hippocampus. Bar = 50 μ m.

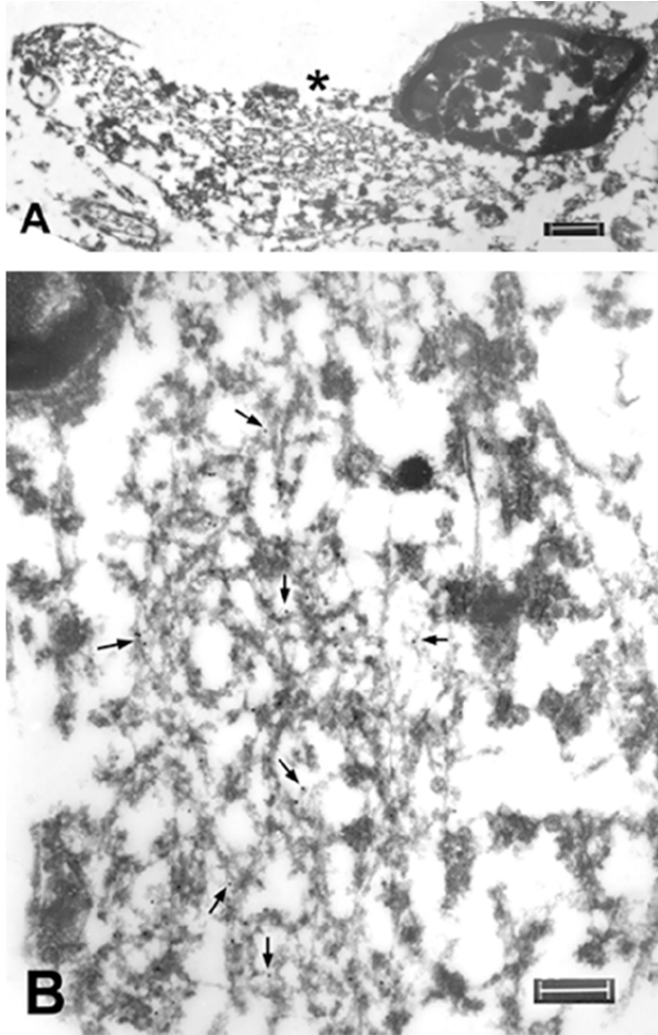


Figure 3. An oligodendrocyte with characteristic round nucleus and dense heterochromatin contains a cytoplasmic inclusion. * indicates area enlarged in B. Bar = 1 μ m. B. The inclusion contains aggregated filaments and associated dense granular material; both have α -synuclein immunoreactivity (indicated by gold particles at arrows). Bar = 0.3 μ m.

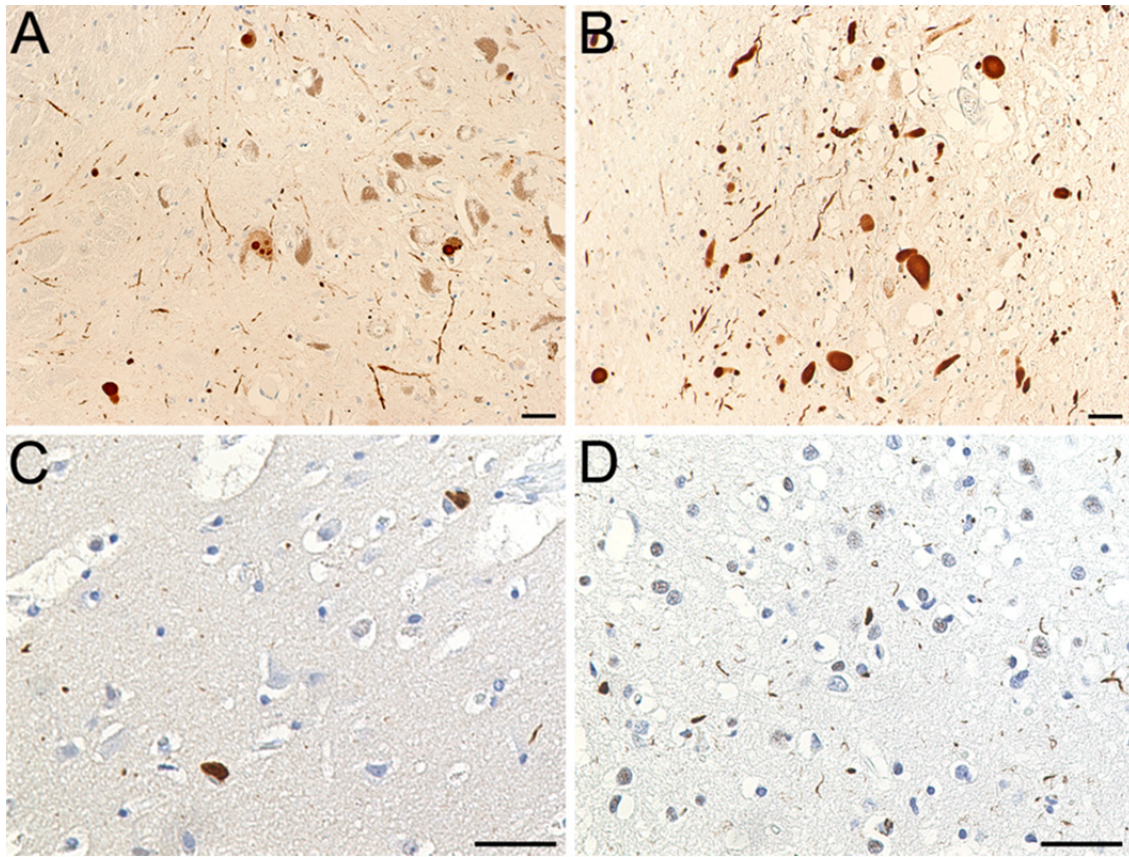


Figure 4 Comparison of α -synuclein pathology in the substantia nigra and temporal cortex. A. SN in sporadic PD has neurons with LBs. B. SN in familial PD has extensive of α -synuclein immunoreactive intraneuritic LBS and Lewy neurites, but only a few residual neurons with LBs. C. Sporadic PD had sparse cortical LBs in the temporal cortex. D. Familial PD had many Lewy neurites and spongiform change, but only a few cortical LBs. Bar = 50 μ m.

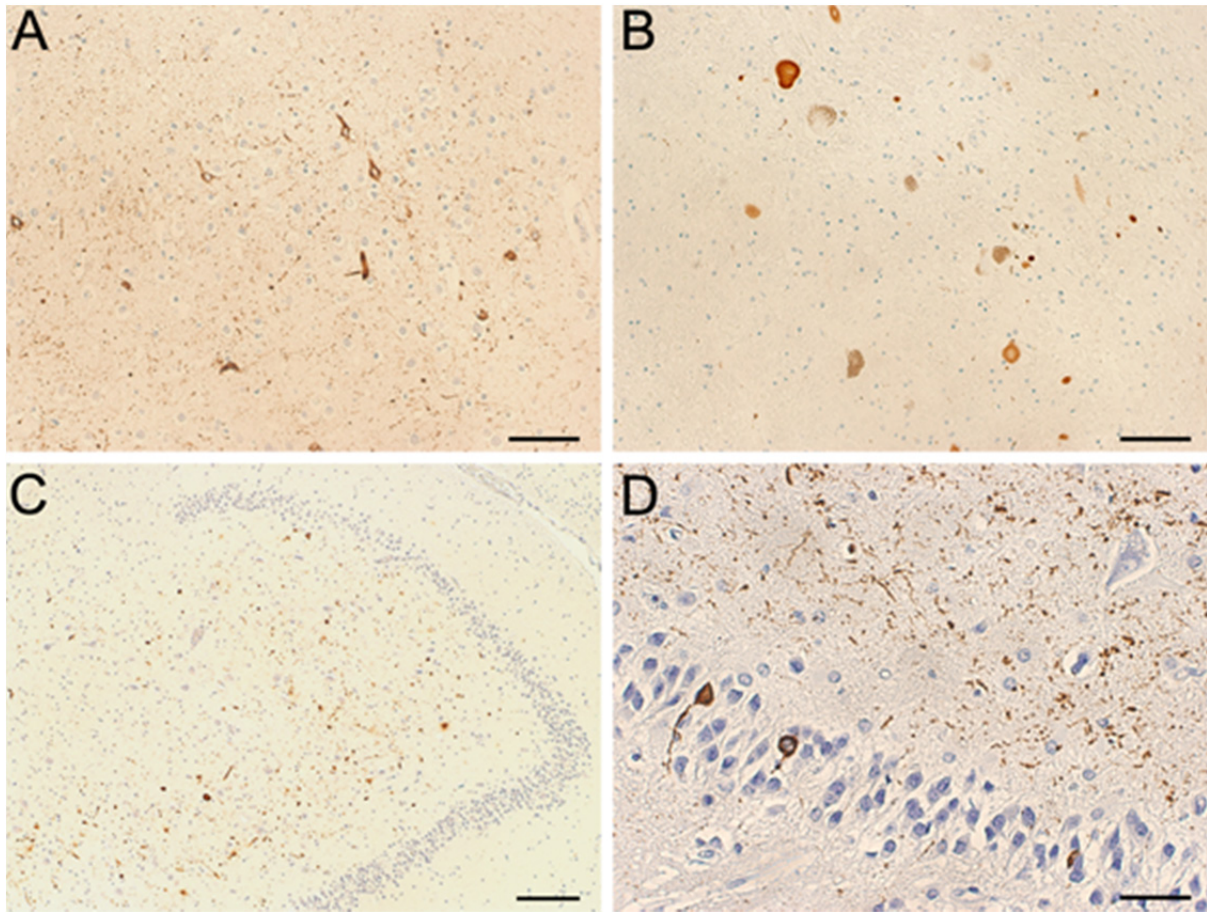


Figure 5. Tau immunoreactive pathology in familial PD. A. Tau immunostaining revealed the sparse and patchy NFT in the temporal cortex (Case 3). B. Tau immunoreactive round or spheroid-like structures in the locus ceruleus (Case 5). C. Many of tau immunoreactive dystrophic neuronal processes (similar to those in Fig. 2D) are detected in the hippocampal endplate (Case 2). D. Case 3 had tau immunoreactive neuronal inclusions in the dentate fascia. Bar = 100 μ m (A, B, C), 50 μ m (D).

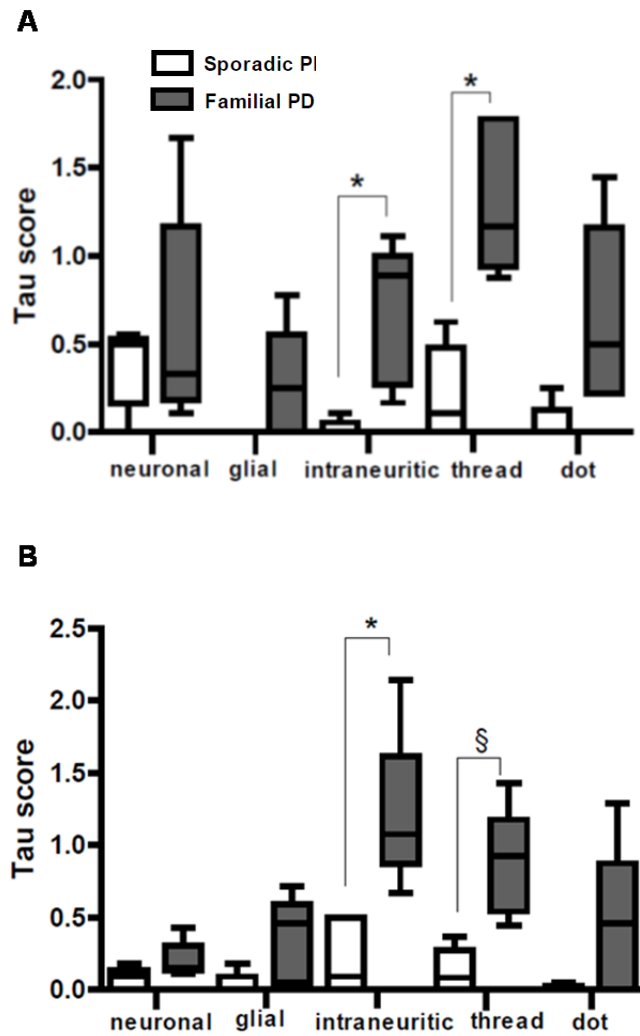


Figure 6. Comparison of tau scores between familial and sporadic D with data grouped by forebrain (A) and hindbrain regions (B). *. $p < 0.01$, §; $p < 0.05$