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Parkinson's disease without nigral degeneration: a pathological correlate of scans without evidence of dopaminergic deficit (SWEDD)?

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ABSTRACT

Objective: To describe 5 cases of Parkinson's disease lacking any detectable histopathology.

Background: The diagnosis of Parkinson's disease is supported histologically by the findings of α-synuclein immunopositive Lewy bodies and neurites and severe substantia nigra cell loss. Bradykinesia as defined by slowness of initiation of movement and a progressive reduction in speed and amplitude on finger tapping is a clinical correlate of pars compacta nigral degeneration. There are very few published cases of Parkinson's disease in which no pathological abnormality was found, and some of these cases were in hindsight thought to have probably been cases of indeterminate senile tremor or dystonic tremor.

Methods: Retrospective case notes review of the Queen Square Brain Bank archival collection and detailed neuropathological analysis of the selected cases.

Results: Five cases considered to have Parkinson's disease by neurologists throughout the entirety of their illness that lacked any histopathological findings known to be associated with Parkinson's syndromes were identified out of a total number of 773 brains with a final clinical diagnosis of Parkinson's disease in the Queen Square Brain Bank. Retrospective case note analysis did not suggest dystonic tremor or indeterminate tremor in any of them. There was a reduction in tyrosine hydroxylase (TH) density in the striatum in these cases when compared to healthy controls, but not in the substantia nigra.

Conclusions: Striatal dopamine deficiency without nigral cell loss is the most likely explanation for the clinical findings; other possible explanations include slowness due to co-morbidities misinterpreted as bradykinesia, a tardive syndrome related to undisclosed previous neuroleptic exposure, or 'soft age-related' parkinsonian signs. These cases emphasise the need to regularly review the diagnosis in cases of suspected Parkinson's disease and highlight the need for precision in the neurological examination particularly of elderly patients. These cases may represent a distinct entity of diagnostic exclusion and may be considered one explanation for the radiological phenomenon of SWEDD (scans without evidence of dopaminergic deficit).

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INTRODUCTION

The diagnosis of Parkinson's disease is supported at autopsy by the finding of severe loss of neurons in the pars compacta of the substantia nigra associated with α-synuclein immunopositive Lewy bodies and neurites. [1] There are a few case reports of Parkinson's disease in which no pathological abnormality was found despite detailed histological examination.[2-6] We now report 5 patients who were considered to have Parkinson's disease throughout the entirety of their illness,[7-9] but at post-mortem lacked any histopathological findings associated with any recognised neurodegenerative or toxic cause of Parkinsonism.

PATIENTS AND METHODS

Protocols used for brain donation in the QSBB were approved by a London Multi-Centre Research Ethics Committee and written consent was obtained from all cases. Tissue is stored at the QSBB under a license from the Human Tissue Authority.

Clinical data

Between 1989 and end of 2014, there were 773 brain donors diagnosed with Parkinson's disease received at the Queen Square Brain Bank (QSBB). Five cases were identified in which the detailed clinical documentation was highly suggestive of Parkinson's disease and in which the pathological diagnosis of Parkinson's disease, other neurodegenerative parkinsonian syndromes and vascular parkinsonism had been excluded at autopsy. All 5 selected cases had correspondence between neurologists and general practitioner (GP), full GP record and the patient's full prescription record obtained from the GP practice. Cases with drug-induced parkinsonism, whose clinical manifestations were considered to be directly related to medications such as dopamine receptor antagonists, were excluded from this study. Two of the selected cases (Cases 2 & 3) had a history of short-lived prochlorperazine use for 'giddiness'.

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Two cases were pre-registered brain donors and had received standardized prospective annual clinical assessments (Cases 4 & 5), one attended a regional neurological centre (Case 3) and three (Cases 1, 2 & 5) were followed up regularly throughout their illness in movement disorder clinics.

Complete clinical notes were obtained on all patients and retrospective case notes review was undertaken by three QSBB associated clinicians (HL, SK, HY) who formulated independent clinical summaries. Consensus opinion on each case was reached by two other clinicians (AJL, LSM). None of the patients had dopamine transporter scans and no video recordings of the patients were available.

Neuropathological methods

Immediately after post-mortem, the brains were divided in the mid-sagittal plane. One half, chosen randomly, was sliced and tissue blocks were frozen and stored, while the other half was immersed and fixed in 10% neutral formalin for three weeks before neuropathological examination. Tissue blocks were taken using standard QSBB protocols. 8-µm thick histological sections were stained with haematoxylin and eosin method. Immunohistochemistry with antibodies to α-synuclein (frontal, parietal and temporal cortices, hippocampus, amygdala, midbrain, pons), Aβ (frontal, parietal and temporal cortices, hippocampus, caudate, putamen, globus pallidus, midbrain, pons, cerebellum), phospho-tau (AT8; frontal, parietal and temporal cortices, hippocampus, amygdala, caudate, putamen, globus pallidus, subthalamic nuclei, midbrain, pons, cerebellum), p62 (frontal cortex, hippocampus, caudate, putamen, globus pallidus, cerebellum) and TAR DNA-binding protein 43 (TDP-43; hippocampus, amygdala) was carried out in selected brain regions using standard avidin-biotin method. Immunohistochemistry with tyrosine hydroxylase antibody (TH, caudate, putamen, midbrain) antibody was performed in 5 age- and disease duration-matched Parkinson's disease cases and 5 age-matched healthy controls.

Systematic histological analysis of neuronal loss in the substantia nigra and examination of the immunohistochemistry sections in search of inclusions known to be associated with clinical parkinsonism were performed by a neuropathologist (TR). Neuronal loss in the substantia nigra

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was determined using a four-tier semi-quantitative grading (0=absent, 1=mild, 2=moderate, 3= severe). Additional pathologies including cerebrovascular disease and cerebral amyloid angiopathy and Alzheimer's disease pathologic change were carefully assessed. [10]

Image analysis of TH-immunoreactivity

Using coded slides, quantitative assessment of TH-immunoreactivity was performed in 4 subregions of the striatum (dorsal putamen, ventral putamen, dorsal caudate and ventral caudate) and 4 subregions of the substantia nigra pars compacta (dorsal lateral, ventral lateral, dorsal medial and ventral medial). The images of the striatum and substantia nigra pars compacta using x20 objective were captured by a high resolution digital scanner and processed with image analysis software (Definiens TissueMap image analysis software Version 3.0, Definiens AG, Germany). Threshold was adjusted to capture the two-dimensional area of all TH-immunoreactivity and the same threshold setting was used for all the subregions in all cases included in the analysis. 'Areal fraction', defined by a ratio of the TH-immunoreactive pixels to the total number of pixels of the whole field was computed by the image analysis software and was expressed as percentage (areal fraction x 100%). [11]

Statistical analysis

The Mann-Whitney U-test was used to compare areal fraction percentage between cases in the present series and control groups. In this exploratory study, statistical significance was set at $p < 0.05$ and results were not adjusted for multiple comparisons. Chi square/Fisher's exatct test, the Student's t-test or oneway ANOVA was used to compare semi-quantitative grading or demographic data using p value of 0.05. The SPSS 22.0 program (IBM Corporation, New York, USA) was used for statistical analysis.

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RESULTS

All five patients had presenting features and a clinical course highly suggestive of Parkinson's disease. Four of them presented with a levodopa responsive asymmetrical rest tremor. The mean age at onset was 70.8 years (range: 55-83) and the mean disease duration was 11 years (range: 4-18). None of the cases had a positive family history of movement disorder. Other clinical demographics are summarized in Table 1. Only 1 case (Case 5) had neuroimaging study, CT of the head was performed two years before death following a fall and no structural abnormalities were reported.

Case reports

Case 1

This 86-year-old man presented to his GP in 2006 with a 3-year history of gradually worsening right hand tremor and a 6 month history of poor balance and 'giddiness' on standing up. A pill rolling tremor of the right hand, bilateral cogwheel rigidity and shuffling gait were observed by his GP and a diagnosis of Parkinson's disease was made. He was subsequently referred to a specialist movement disorder clinic and one year after his first symptoms he was noted to have had a sustained marked improvement following treatment with levodopa/carbidopa 62.5mg four times daily. A festinant gait, reduced arm-swing and bilateral, asymmetrical cogwheel rigidity were now noted. Ropinirole 1mg three times daily was added and a further symptomatic improvement in stiffness and mobility occurred but he developed light-headedness due to orthostatic hypotension. At this stage he was recruited to a Parkinson's disease trial. He remained under regular review with slow but progressive motor deterioration until he died aged 88 from a pulmonary embolism secondary to lung carcinoma, 5 years after his clinical diagnosis of Parkinson's disease.

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Case 2

In 2001, a 78-year-old woman was diagnosed with Parkinson's disease by a consultant neurologist at a movement disorder clinic after presenting with a 10-month history of progressive worsening of general slowness and a tremor at rest. On examination, a marked bilateral rest tremor of the hands was observed in addition to a head tremor, bilateral bradykinesia, cogwheel rigidity, reduced blink rate and impaired postural reflexes. She had difficulty rising from a chair and her gait was of reduced stride length, with episodes of freezing. Levodopa/carbidopa 125mg three times daily was started but at review 2 months later, she was thought to have deteriorated. She started to have occasional falls backwards. The medication records from the general practitioner showed she was prescribed prochlorperazine 5mg for a few weeks for vertigo during the period of reported rapid deterioration. The prochlorperazine prescription was not repeated and her levodopa dose was gradually increased to 750mg daily and over the following 2 years some of the doctors who saw her considered her to have improved, while others commented that her parkinsonism had stabilized. She reported subjective worsening of her symptoms at the end of each levodopa dose, but she did not experience clear benefit from individual doses and on one occasion she inadvertently decreased her dose and did not notice any worsening. She died 4 years after the diagnosis of Parkinson's disease from bronchopneumonia, aged 81.

Case 3

In 1992, at age 59, this woman with a previous history of several non-specific symptoms was diagnosed with 'parkinsonism' by her GP after finally presenting with limb stiffness and a right sided tremor. She was taking prochlorperazine, which was withdrawn and she was subsequently seen by a consultant neurologist who documented 'parkinsonism with a right-sided emphasis' and started on levodopa/carbidopa 62.5mg twice daily. She complained of slowing of her handwriting but a letter written by her to the GP did not reveal micrographia (Figure 1). After several years of follow up she was considered to have 'benign tremulous parkinsonism'. Her levodopa dose was gradually increased, reaching a peak of 1250mg daily when, she reported unilateral involuntary facial movements that were thought to be levodopa-induced dyskinesias, but were later diagnosed as hemifacial spasm. A dispersible formulation of levodopa/benserazide was prescribed to treat nocturnal rigidity. Progressive deterioration in her

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mobility due to a combination of Parkinson's disease and lumbar degenerative disease led her to be wheelchair dependent 8 years after her diagnosis of Parkinson's disease. In the last year of her life, there were reports in her notes of involuntary wild 'twitching' movements following the intake of levodopa. She continued to be treated for Parkinson's disease for 12 years until she died from bronchopneumonia and heart failure aged 71.

Case 4

This man was diagnosed with Parkinson's disease in 1976 by his GP at the age of 55 after presenting with limb stiffness. Examination two years after the initial diagnosis revealed a 'mask-like face', a positive glabellar tap response and rigidity in the limbs with a right-sided predominance. He was started on levodopa/carbidopa 110mg twice daily two years after diagnosis. At his first assessment by a consultant neurologist, the patient reported his initial response to levodopa to be excellent. Seven consecutive annual QSBB prospective assessments all reported mild to moderate, usually symmetrical, bradykinesia and rigidity with no deterioration in his Hoehn and Yahr stage from the baseline level of 2. His levodopa dose was gradually titrated up to 750mg per day but he did not experience motor fluctuations or dyskinesias. He died from bronchopneumonia complicating myeloma after 18 years of disease.

Case 5

This woman was diagnosed with Parkinson's disease by her GP in 1987 at the age of 84 after presenting with a 4-year history of progressive bilateral rest tremor of the hands. An initial QSBB assessment form filled in by an affiliated neurologist in 1993 reported a Hoehn and Yahr stage of 2. Examination by a neurologist revealed mild bilateral limb rigidity, severe tremor in the hands and legs. She was commenced on levodopa/carbidopa 125mg which was gradually titrated up to four times daily. She reported a good response to levodopa therapy. Three years later, she noted mild choreiform movements of her head and neck on reaching the maximum levodopa dose and transient worsening of her motor symptoms coincided with the end of each dose of levodopa. Between 1990 and 1997, she was regularly followed up in a Parkinson's disease clinic and progressive deterioration in the tremor of her head and hands, mobility and

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gait freezing was documented. At the age of 93, she developed marked postural instability, freezing of gait and falls. She could only walk a few steps using a walking frame. She complained of drooling of saliva and difficulty in swallowing. She died after 15 years of disease and the cause of death was recorded as ‘end-stage Parkinson’s disease’.

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Table 1: Clinical demographics of the five cases included in this case series.

	Case 1	Case 2	Case 3	Case 4	Case 5
Gender	Male	Female	Female	Male	Female
Age at onset	83	77	59	55	80
Age at death (Disease duration)	88 (5)	81 (4)	72 (13)	73 (18)	95 (15)
Initial presentation	Rest tremor (Right hand)	Rest tremor (Left hand), parkinsonian gait	Rest tremor (Right hand)	Generalized slowing	Bilateral hand tremor
Other symptoms and signs	Asymmetric rest hand tremor, rigidity & reduced armswing, festinant gait, falls, postural hypotension, abnormal glabellar response	Asymmetric rest hand tremor, head and leg tremor, bradykinesia, general slowness, rigidity, gait freezing, falls, hypomima	Asymmetric rest hand tremor, rigidity, slow handwriting, general slowness, rigidity, postural instability (wheelchair use at age 69)	Asymmetric bradykinesia and rigidity, occasional rest hand tremor, hypomimia, abnormal glabellar response, hypomimia	Head and leg tremor, rigidity, postural instability & recurrent falls (age 93), dysphagia (age 94)
Predominant tremor?	Yes	Yes	Yes	No	Yes
Initial levodopa response	Excellent (improved all motor symptoms)	Mild	Moderate	Good	Good

Sustained levodopa response?	Yes	Yes	Yes	Yes	Yes
Max levodopa equivalent dose	320mg/day	1200mg/day	1250mg/day	1200mg/day	400mg/day
Motor fluctuation	No	Mild wearing off	Generalised twitching movements at peak dose, wearing off	No	Mild choreiform movements of head at peak dose, wearing off
Visual hallucinations	No	No	No	No	No
Memory impairment	No	No	No	Mild cognitive impairment (MMSE scores: 23-30/30)	No
Disease progression	Slow progression only before levodopa with subsequent plateau	Initial rapid progression with subsequent plateau	Gradual deterioration	No progression	Gradual deterioration
Co-morbidities	Ischaemic heart disease	Depression, COPD	Anxiety, depression, hemifacial spasm, osteoarthritis, osteoporosis, cervical spondylosis,	Anxiety, migraine, ischaemic heart disease	Ischaemic heart disease, pulmonary tuberculosis

			chronic back pain, Sjogren's syndrome, asthma		
Features against Parkinson's disease	No record of bradykinesia	Head tremor, mild levodopa response, transient use of prochlorperazine (coincided with worsening of parkinsonism)	No record of bradykinesia, intermittent use of prochlorperazine (stopped when first diagnosed with Parkinson's disease)	Mild motor symptoms, lack of progression	Head tremor, lack asymmetry or record of bradykinesia

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Pathological Findings

None of the cases was deemed to have a pathological explanation for parkinsonism. No cases met the criteria for the pathological diagnosis of Parkinson's disease, vascular parkinsonism or other neurodegenerative parkinsonian syndromes. Staining for α-synuclein was negative in the medulla and substantia nigra in all cases confirming the absence of Lewy body pathology. Macroscopic examination was unremarkable with normal pigmentation of the substantia nigra and absence of cortical atrophy or ventricular dilatation. Mild nigral cell loss compatible with aging was found in three cases (1, 3 & 4) with evidence of negligible free pigment (1 & 3). The substantia nigra in the remaining two cases was normal (2 & 5; Figure 2). The locus coeruleus was normal in four cases (cases 1, 2, 4 & 5) but was unavailable for examination in case 3. The subthalamic nucleus, corpus striatum and thalamus were normal in all five cases with no cell loss or pathological inclusions.

No cases exhibited extra-nigral pathology deemed sufficient to cause clinical parkinsonism. In two cases (cases 1 & 3) there was mild mineralisation of blood vessels in the globus pallidus. Case 1 also had mild hyaline arteriolar thickening in the deep white matter and mild cerebral amyloid angiopathy, with the extent of vascular change being slightly more than expected for the patient's age. Mild perivascular accentuation associated with a mild degree of gliosis in the striatum was observed in 4 cases (1-4). These findings are common in older individuals and were not of the degree observed previously in vascular parkinsonism cases [12 13] and judged not severe enough to account for parkinsonism. Mild age-related Alzheimer's type tau pathology was identified in all cases at Braak and Braak stage II or below. [10] Immunohistochemistry with TDP-43 was negative in all cases.

Neuropathological examination including immunohistochemistry excluded any structural causes and confirmed the absence of any distinctive histological hallmarks for any of the neurodegenerative entities listed in Table 2. Genetic analysis performed in all five cases using DNA obtained from frozen brain tissue excluded mutation of the Huntington's disease gene (repeat size: 18-26).

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Image analysis of TH-immunoreactivity

TH synthesises dopamine from tyrosine and TH immunohistochemistry is used as a marker of catecholaminergic fibres including dopaminergic neurons.

Quantitative assessment of the density of TH-immunoreactivity was performed in these 5 cases (mean age of death: 81.8 years, mean disease duration: 11 years), 5 age- and disease duration-matched Parkinson's disease controls (mean age of death: 79.8 years, $p = 0.32$; mean disease duration: 14.2 years, $p = 0.76$) and 5 age-matched healthy controls (mean age of death: 77.0 years, $p = 0.16$). [14]

In all subregions of the substantia nigra pars compacta, there was reduction in the TH density in Parkinson's disease when compared with the present cohort (dorsolateral: $p = 0.009$, ventrolateral: $p = 0.009$, dorsomedial: $p = 0.028$, ventromedial: $p = 0.009$) and the healthy controls (dorsolateral: $p = 0.014$, ventrolateral: $p = 0.014$, dorsomedial: $p = 0.014$, ventromedial: $p = 0.027$). The TH density was the same between the present cohort and healthy controls (dorsolateral: $p = 0.806$, ventrolateral: $p = 0.462$, dorsomedial: $p = 0.624$, ventromedial: $p = 0.806$; Figure 3).

In the striatum, the TH density in the dorsal putamen ($p = 0.034$), ventral putamen (borderline significance; $p = 0.077$) and ventral caudate ($p = 0.034$) of the present cohort was less than that of the healthy controls, but not in the dorsal caudate ($p = 0.289$). The TH density in the present cohort was numerically greater than Parkinson's disease controls but it did not reach statistical significance (dorsal putamen: $p = 0.149$, ventral putamen: $p = 0.149$, dorsal caudate: 0.248, ventral caudate: 0.386; Figure 4 & 5).

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Table 2: Potential explanations for Parkinson's disease mimics without nigral atrophy and Lewy body pathology.

Neurodegenerative causes:
Huntington's disease
Fragile X tremor ataxia syndrome (FXTAS)
Chronic traumatic encephalopathy
C9orf72 expansion
Spinocerebellar ataxia (SCAs)
Non-neurodegenerative causes:
Mild extrapyramidal signs of the elderly
Indeterminate or senile tremor
Dystonic tremor
Tardive parkinsonism
Vascular parkinsonism
Psychomotor retardation due to depression
Rheumatological or orthopaedic conditions (e.g. arthritis, spondylosis)

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DISCUSSION

It came as a surprise that with the use of dopamine transporter SPECT scans, up to 15% of patients in trials for early Parkinson's disease had no evidence of presynaptic nigrostriatal dopamine denervation. [15-17] The radiological acronym SWEDD (standing for scans without evidence of dopaminergic deficit) was used to describe these patients, and there is ongoing debate regarding their true diagnosis. Repeat dopamine transporter scans often remain normal with only 8-13% converted to have an abnormal scan up to five years later.[18-20] Almost half of the SWEDD patients (40/90) in the PRECEPT Parkinson's disease clinical trial (N=799) had their diagnosis changed to a condition not associated with dopamine denervation at 22-month follow-up by study investigators blinded to the dopaminergic scan status with the most common revised diagnosis being tremor, followed by 'no neurological diagnosis' and vascular parkinsonism,[19] highlighting the diagnostic difficulties in the early disease stage, and even in Parkinson's disease patients with SPECT-confirmed dopaminergic deficits (N=707), 5 cases had been re-evaluated as having a tremor syndrome not associated with dopamine denervation and 13 had their diagnosis revised to vascular parkinsonism.[19] SWEDD patients generally had milder motor disability as measured by UPDRS,[19] their clinical symptoms tended to be stable with no deterioration after withdrawal of levodopa therapy[19] and they present with less non-motor features,[21 22] all of which might be useful clinical pointers. Most neurologists now consider that these patients do not have Parkinson's disease. [18 19] Some of the tremulous patients with SWEDD can present with dystonic features[23] and abnormal cortical plasticity[21] suggesting that dystonic tremor could be the underlying diagnosis. Patients with dystonic and indeterminate senile tremor may have reduced armswing, small stride length, asymmetric jerky rest and postural tremor, hypomimia, increased limb tone and slow repetitive finger movements and it has been claimed that 3% of dystonic tremor are misdiagnosed with Parkinson's disease.[23-25] Nevertheless, many SWEDD patients do not fall into this phenotype and pathological findings in SWEDD have not been published.

Slowness of initiation of movement or reduced amplitude of movements without motor decrement may be seen in dystonia or pyramidal slowness,[24 26] but bradykinesia with motor decrement and fatiguing has been considered a clinical correlate of nigral deficiency. The QSBB

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diagnostic criteria stipulate that the presence of bradykinesia is obligatory for the diagnosis of parkinsonism.[7] On reexamination, some SWEDD subjects from clinical series exhibited slowness or reduction of amplitude, but no true motor decrement[21 23] highlighting the importance of repetitive finger tapping tests performed for 20 -30 seconds in the clinical differentiation between Parkinson's disease and SWEDD patients.[27] Nevertheless, a recent blinded video study showed that even experienced movement disorder specialists may have difficulty distinguishing slowness in SWEDD from bradykinesia in Parkinson's disease.[28] Our findings support this area of clinical uncertainty in that three of our patients were considered to have either bradykinesia (Cases 2, 4) or general slowness (Case 3). We cannot exclude the possibility that slowness due to aging or co-morbidities (depression, arthritis, spondylosis) was misinterpreted as criteria-defined bradykinesia in some of these cases.

One could argue that the slow rate of deterioration should have cast doubt on the clinical diagnosis of Parkinson's disease. Case 4 had Parkinson's disease for 18 years with little progression. Cases 3 and 5 had a gradual deterioration over 13 and 15 years. Case 2 initially had a rapidly progressive course, during which she was also receiving prochlorperazine, followed by a "stabilization of symptoms" after prochlorperazine withdrawal. In contrast, Case 1 exhibited a very typical course for Parkinson's disease, with a rest tremor at onset that progressed to a shuffling gait in the pre-treatment phase, but no further progression reported after commencing dopaminergic medication. Although Parkinson's disease is always progressive, heterogeneity exists in the rate of deterioration with some patients following a relatively mild course, remaining in stage 1 or 2 Hoehn and Yahr for up to 10 years.[29] Clinically, 4 of 5 of these cases had a tremor-predominant clinical picture and can mimic benign tremulous parkinsonism, a pathologically proven subtype of Parkinson's disease which is associated with a relatively slow rate of clinical progression at least in the early or mid-disease stage.[30] Our cases raise the possibility that a small proportion of those slowly progressive cases might in fact not have underlying nigral degeneration.

All the patients had some benefit from levodopa; Case 1 had adequate documentation of sustained levodopa responsiveness, while cases 2, 3 and 5 exhibited wearing off effects described in the notes. Occasional episodes of generalised twitching movements were reported at peak dose in case 3 in the last year of life and mild choreiform head movements were noted at peak dose of levodopa therapy in case 5. Marked initial and sustained levodopa responsiveness

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are features supportive of the diagnosis of Parkinson's disease, with 75% of pathologically confirmed Parkinson's disease exhibiting a good or excellent initial levodopa response.[7] The presence of severe levodopa-induced chorea has a high positive predictive value for the diagnosis of Parkinson's disease and is included as a supportive feature in Parkinson's disease diagnostic criteria.[7 31 32] However, in pathologically proven Parkinson's disease a poor or moderate levodopa response was noted in 17% cases, and no dyskinesias or motor fluctuations were reported in 34%.[7] Furthermore, dyskinesias are less common in poorly levodopa responsive patients or in the absence of motor fluctuations, with one recent clinic-pathological study reporting dyskinesia in only 14% of non-fluctuators.[33] The levodopa responses reported in our cases are clearly comparable to those seen in pathologically confirmed Parkinson's disease, highlighting further potential for diagnostic error.

Although not the primary emphasis of this paper, these cases provide a unique opportunity to observe clinical and pathological effects of long-term levodopa on healthy human substantia nigra. All patients received levodopa for at least 21 months (up to 216 months) in doses ranging from 200 to 1250 mg daily. Although three patients reported side effects, the lack of pathological change in our cases provides further reassurance that levodopa is not toxic to human substantia nigra.[4]

The severe loss of dopamine-containing neurons from the pars compacta of the substantia nigra is the pathological hallmark of Parkinson's disease and the prerequisite for the development of motor symptoms. This loss of dopaminergic neurons is accompanied by Lewy bodies of which the main component is α-synuclein [1]. Unlike neurofibrillary tangles in tauopathies, a constant proportion of nigral neurons of 3-4% contains Lewy bodies irrespective of the disease duration, supporting the notion that Lewy bodies are eliminated when the neurons that bear them die and that Lewy bodies are at the same time being constantly produced. [34] Based on current clinicopathological concepts of disease, the patients in the present series cannot be said to have had Parkinson's disease. In most of the previously reported cases of Parkinson's disease without pathological explanation, the phenotype was predominantly tremulous, there was absent or debatable bradykinesia and rigidity[3-5] and there was no significant response to levodopa with successful levodopa withdrawal in one case.[5] Furthermore, in retrospect, there were sufficient features to support a revised clinical diagnosis of essential tremor, atypical tremor or dystonic tremor retrospectively.[2 4 5] In contrast, the cases in this series had cardinal motor features of

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Parkinson's disease and had been followed by neurologists for prolonged periods. The clinical presentations in cases 1, 3 and 4 would be incompatible with essential tremor, atypical tremor or dystonic tremor. Although in cases 2 and 5, a head tremor was observed, the impaired postural response and gait freezing would be unusual for indeterminate or senile tremor, and head tremor has been reported in Parkinson's disease. [35]

TH density in the striatum in the cases in this series is reduced to a similar degree as that of Lewy body Parkinson's disease, but such reduction is not identified in the substantia nigra. In the absence of neuronal loss in the substantia nigra and striatum as shown by semi-quantitative assessment, the findings of TH-immunohistochemistry suggest a biochemical deficiency of dopamine. In dopa-responsive dystonia, there is selective nigrostriatal dopamine deficiency caused by genetic defects in the dopamine synthetic pathway without nigral cell loss and it is not considered a neurodegenerative disorder, but a biochemical disorder in which symptoms can be reversed by replacement of the depleted neurochemicals. [36] The reduction in TH density in cases in this series could only be identified by sensitive stereological image analysis rather than standard histopathological method, but we cannot exclude the possibility of mild loss of dopamine-containing terminals in these cases. Striatal dopamine deficiency is the most likely explanation for the clinical findings of parkinsonism and positive levodopa response in these cases. Its underlying cause however will warrant further study which is beyond the scope of this series. Other credible arguments can be proposed as to why these patients had parkinsonism and in some cases responded to levodopa. Undisclosed continued neuroleptic use or a tardive phenomenon (Cases 2 &3) following neuroleptic exposure in two of the cases could also be speculated. [37] All the patients were elderly and it may be that they had soft extrapyramidal signs related to aging that were confused with Parkinson's disease. [38 39]

Dopamine transporter locates in the presynaptic membrane on the terminals of dopaminergic projections from the substantia nigra to the striatum and it provides a marker for dopamine terminal innervation. [40] Dopamine transporter SPECT is normal in dopa-responsive dystonia.[41 42] We speculate that the result of the scan in these 5 cases would also have been reported as normal on visual assessment but semi-quantitative assessment might have revealed subtle reduction in tracer uptake in the striatum. We conclude that these 5 cases probably represent a subgroup of SWEDD cases and it may be reasonable to assume that SWEDD is an entity of diagnostic exclusion with several distinct causes.[19] Our cases serve to highlight the need for ongoing

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clinico-pathological research and highlight the need to review the clinical diagnosis including the judicious use of dopamine transporter scans, even in patients with longstanding disease if atypical features exist.

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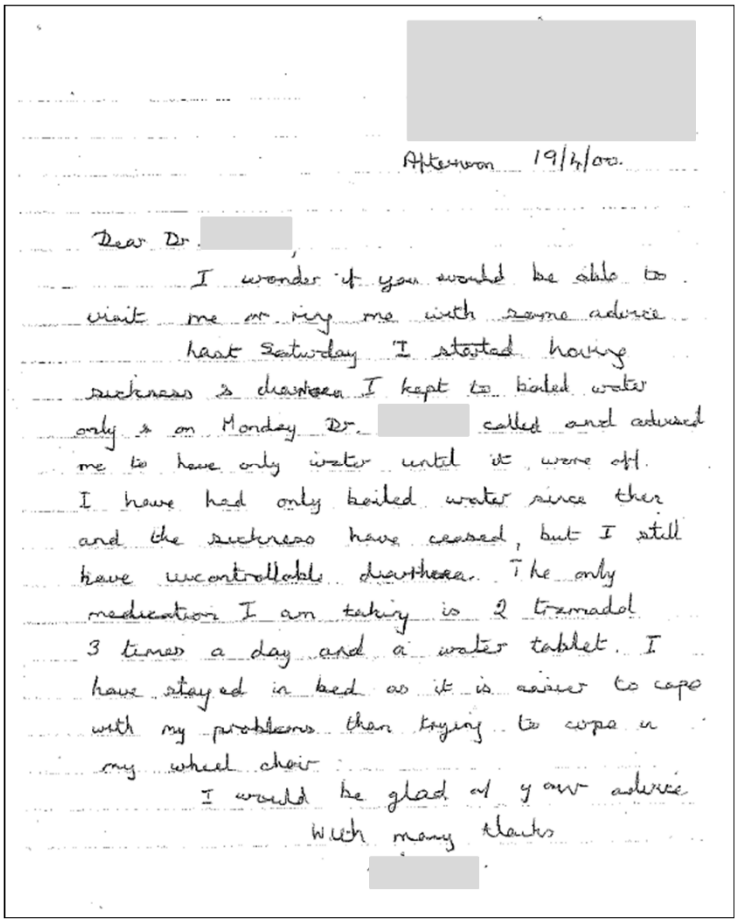
FIGURE LEGENDS

Figure 1:

An example of the handwriting of case 2. A letter written by the patient at age 68 to her GP eight years after the onset of her initial symptoms of asymmetric rest tremor of the hands. There is no evidence of micrographia but patient complained of slowness.



Afternoon 19/4/00.

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I would be glad of your advice.

With many thanks

[redacted]

Figure 2:

Substantia nigra of a case example of Parkinson's disease and case 2. In the Parkinson's disease case, severe degree of cell loss of pigmented neurons and gliosis (A-1, x4 objective) and Lewy bodies and Lewy neurites containing α-synuclein (α-syn, A-2, x10 objective) in the substantia nigra are evident. In case 2, the substantia nigra is well preserved without evidence of neuronal loss or gliosis (B-1, x4 objective) and no Lewy bodies or Lewy neurites are identified using α-synuclein immunohistochemistry (B-2, x4 objective).

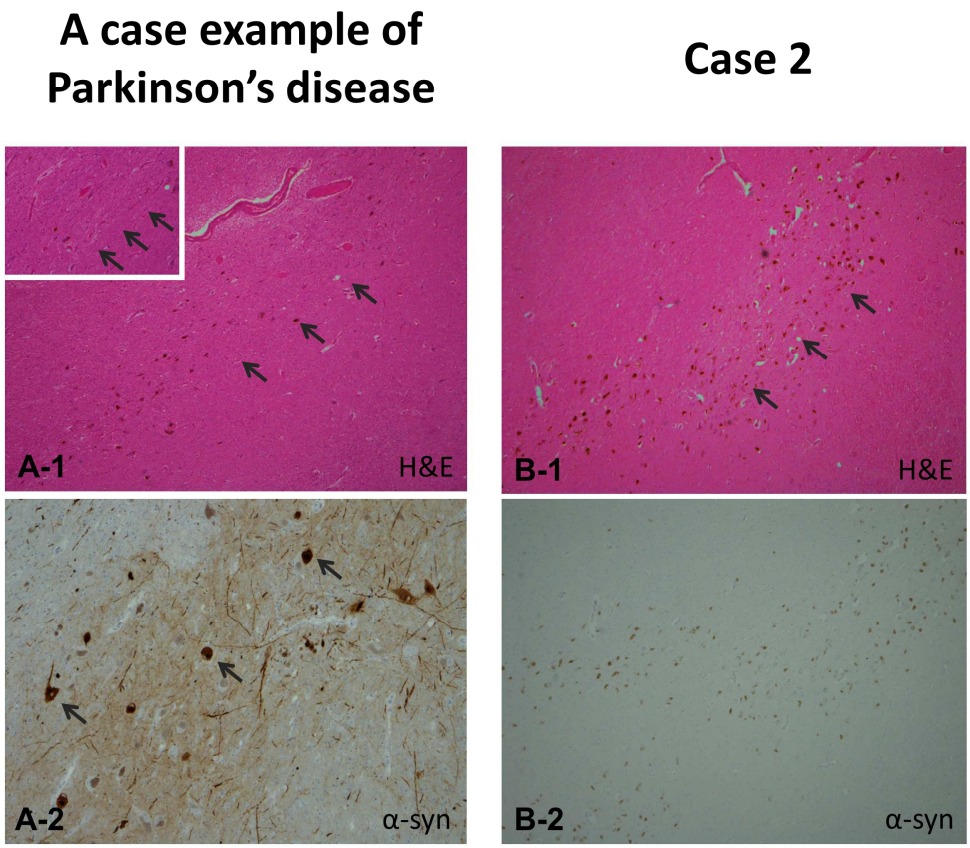

Figure 3: Substantia nigra of a healthy control, case 1 and a Parkinson's disease (PD) control (x4 objective). Severe neuronal loss, gliosis and markedly reduced TH density can be observed in the PD case. TH density in the substantia nigra is preserved in Case 1 and healthy control.

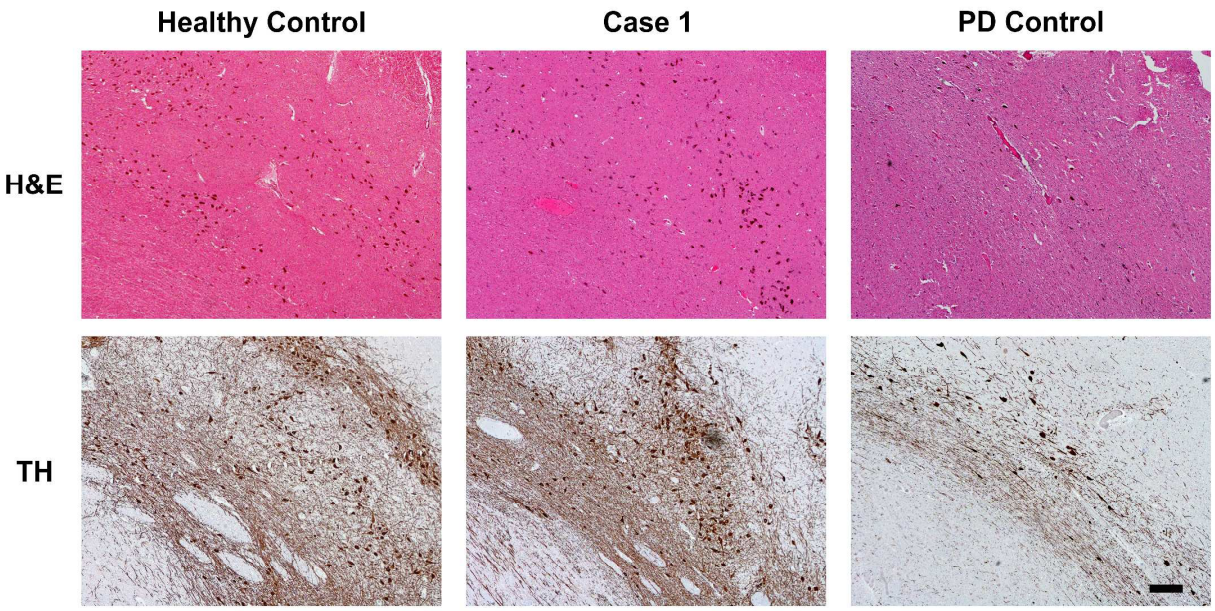

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Figure 4: Tyrosine hydroxylase (TH)-immunohistochemistry in the dorsal putamen (x4 objective) of a healthy control, case 1 and a Parkinson's disease (PD) control. TH density is markedly reduced in the PD control and mildly reduced in case 1 when compared with healthy control.

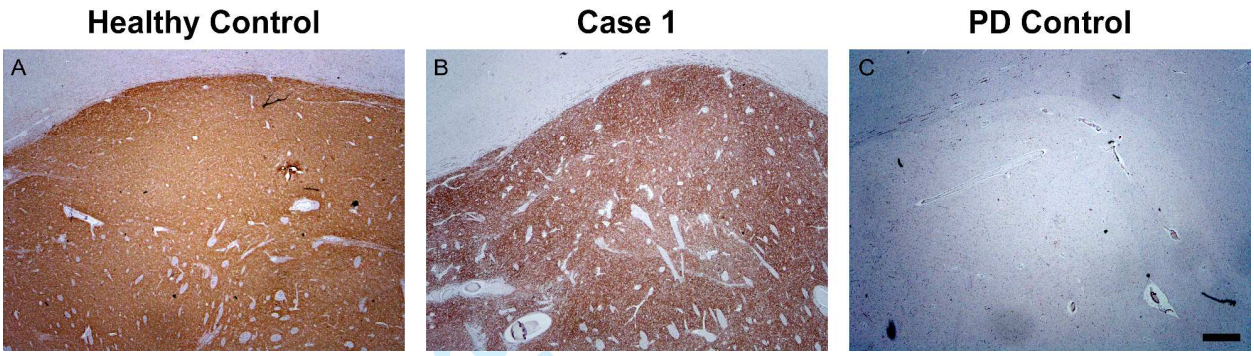

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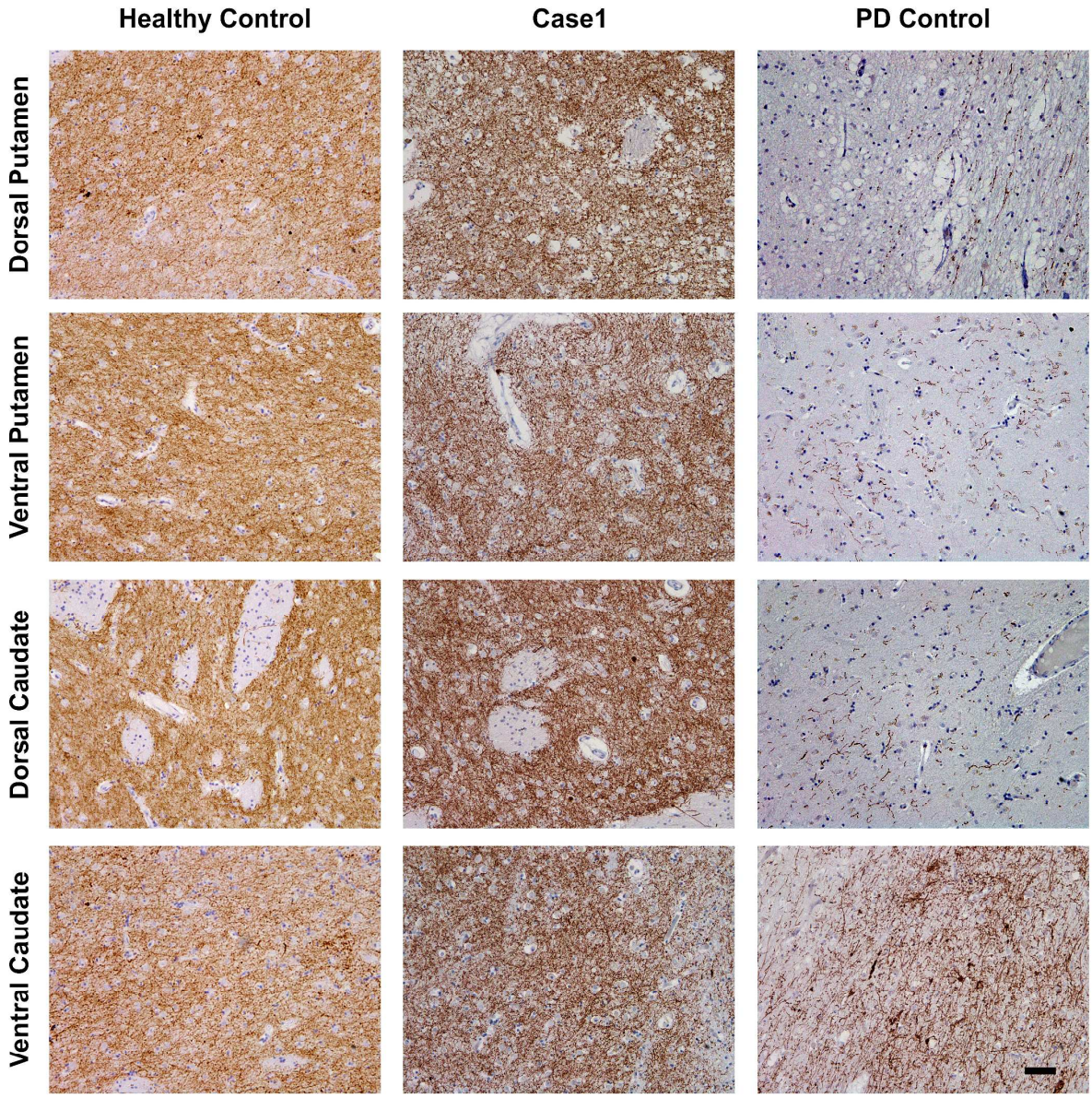
Figure 5: Tyrosine hydroxylase (TH)-immunohistochemistry in 4 subregions of the striatum (x20 objective) of a healthy control, case 1 and a Parkinson's disease (PD) control. When compared with healthy controls, TH density is reduced in the striatum in PD controls and, to a lesser extent, in the cases in this series.



Author Contributions

Dr. Ling contributed to drafting the manuscript for content and collection, analysis and interpretation of data.

Dr. Kearney contributed to drafting the manuscript for content and analysis and interpretation of data.

Dr. Yip contributed to drafting the manuscript for content and analysis and interpretation of data.

Dr. Silveira-Moriyama contributed to revising the manuscript for content, study concept and design and analysis and interpretation of data.

Prof. Revesz contributed to revising the manuscript for content and analysis and interpretation of data.

Prof. Holton contributed to revising the manuscript for content and analysis and interpretation of data.

Ms Strand contributed to collection and analysis of data.

Ms. Davey contributed to collection and analysis of data.

Dr. Mok contributed to revising the manuscript for content, analysis and interpretation of data.

Dr. Polke contributed to analysis and interpretation of data.

Prof. Lees contributed to revising the manuscript for content, study concept and design and analysis and interpretation of data.

Competing interests

The authors report no competing interests.

Disclosures

Dr. Ling has received grants from CBD Solutions, PSP (Europe) Association and Reta Lila Weston Fellowship.

Dr Kearney has received travel grants from Teva-Lundbeck and Brittania pharmaceuticals. He has recieved honoraria from UCB and Abbvie pharmaceuticals in the past two years.

Dr. Yip reports no disclosures.

Dr. Silviera-Moriyama declares employment from Universidade Nove de Julho (Uninove). She has received grants from The Reta Lila Howard Foundation, FAPESP, FAEPEX-UNICAMP, Parkinson´s UK and CNPq-CAPES, and travel grants form Genus, Ipsen and Abbott. She has

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received no royalties or honoraria from any pharmaceutical company for the past 2 years.

Prof. Revesz is a consultant for MerckSerono pharmaceuticals and has received an honorarium from MerckSorono pharmaceuticals and grants from Alzheimer's Research UK, Mutiple System Atrophy Trust and Parkinson's UK.

Prof. Holton has received grants from Mutiple System Atrophy trust, Alzheimer's Research UK and Parkinson's UK

Ms. Strand reports no disclosures.

Ms. Davey reports no disclosures.

Dr. Mok has received grants from CBD Solutions.

Dr. Polke reports no disclosures.

Prof. Lees serves on the advisory board for Novartis, Teva, Meda, Boehringer Ingelheim, GSK, Ipsen, Lundbeck, Allergan, Orion, BIAL, Noscira and Roche pharmaceuticals and has received honoraria from Novartis, Teva, Meda, Boehringer Ingelheim, GSK, Ipsen, Lundbeck, Allergan, Orion, BIAL, Noscira and Roche pharmaceuticals and grants from the PSP Association, Weston Trust- The Reta Lila Howard Foundation.

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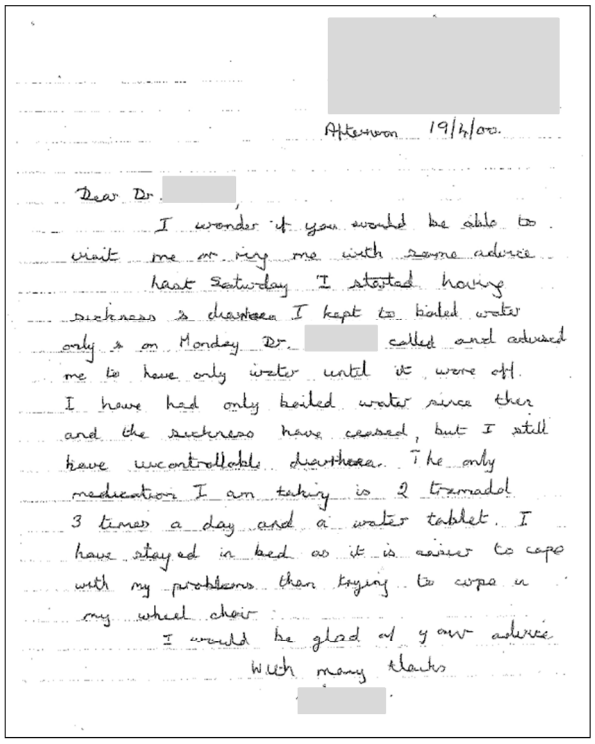

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An example of the handwriting of case 2. A letter written by the patient at age 68 to her GP eight years after the onset of her initial symptoms of asymmetric rest tremor of the hands. There is no evidence of micrographia but patient complained of slowness.
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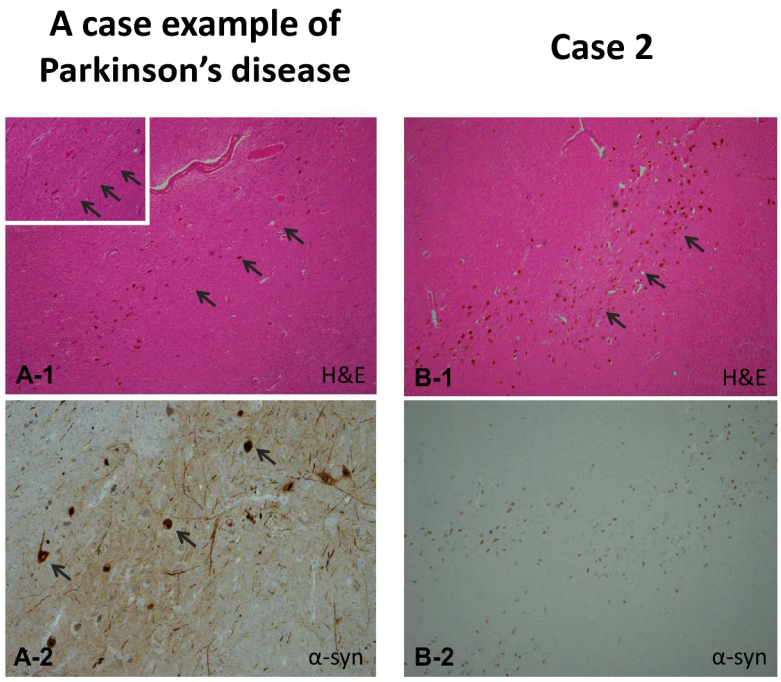

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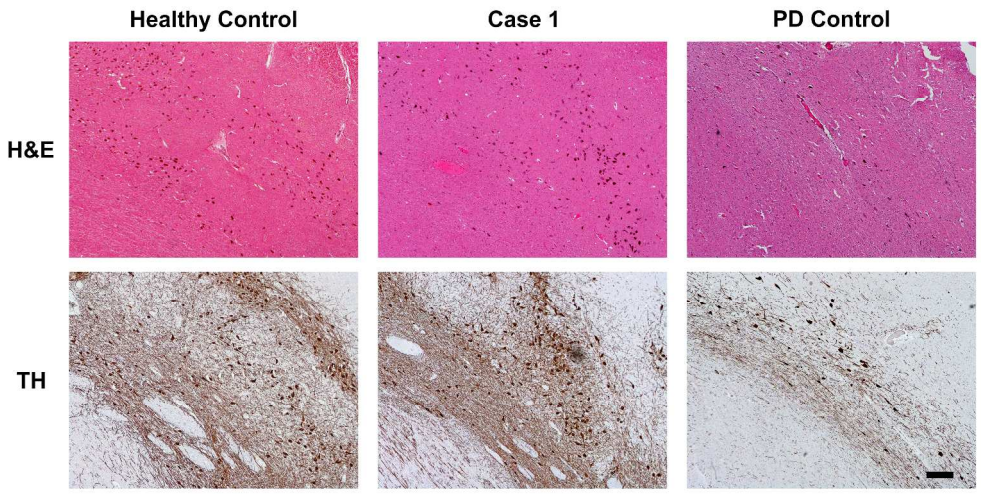

Substantia nigra of a healthy control case, case 1 and a Parkinson's disease (PD) control (x4 objective). Severe nigral cell loss and gliosis and markedly reduced TH-immunoreactivity can be observed in the PD case. The substantia nigra and its dopamine-containing terminal (shown by TH density) are preserved in Case 1 and healthy control.
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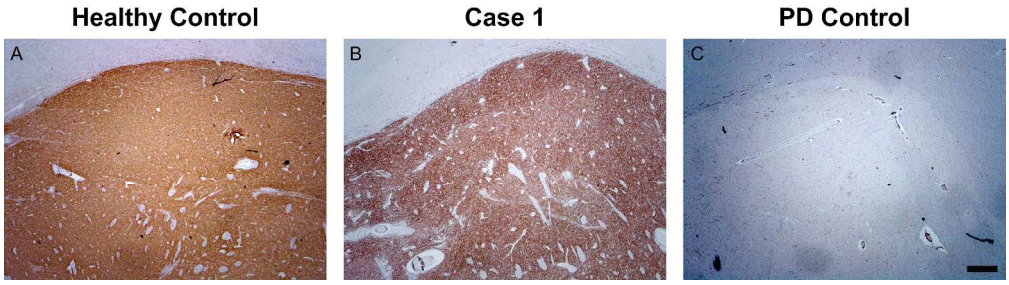

Tyrosine hydroxylase (TH)-immunohistochemistry in the dorsal putamen (x4 objective) of a healthy control, case 1 and a Parkinson's disease (PD) control. TH density is markedly reduced in the PD control and mildly reduced in case 1 when compared with healthy control.
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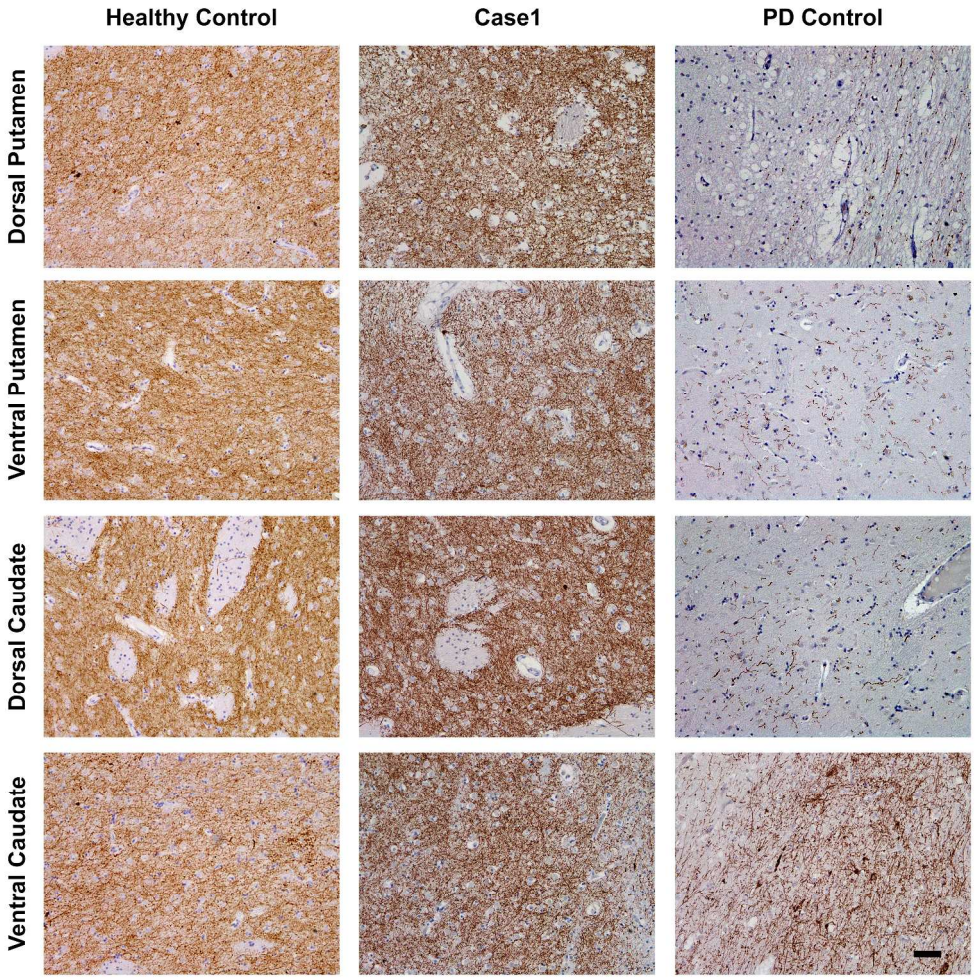

Tyrosine hydroxylase (TH)-immunohistochemistry in 4 subregions of the striatum (x20 objective) of a healthy control, case 1 and a Parkinson's disease (PD) control. When compared with healthy controls, TH density is reduced in the striatum in PD controls and, to a lesser extent, in the cases in this series.
367x367mm (300 x 300 DPI)

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