Unravelling the tangled web of atypical parkinsonism

Thesis submitted in fulfilment of the degree of Doctor of Philosophy

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I, Helen Ling confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signed:

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Abstract

This thesis focuses on sporadic parkinsonian syndromes that are associated with neurofibrillary degeneration and the accumulation of abnormal tau protein in the brain.

The classic clinical presentation of corticobasal degeneration is a specific constellation of cortical and extrapyramidal signs, collectively termed corticobasal syndrome. The evaluation of all the archival cases with corticobasal degeneration in the Queen Square Brain Bank for Neurological Disorders reveals the high frequency of other phenotypic presentations. The result indicates that corticobasal degeneration commonly presents with a clinical picture, closely resembling progressive supranuclear palsy (PSP) or Richardson's syndrome. On the other hand, cases with typical PSP pathology may occasionally present with a corticobasal syndrome. A quantitative assessment of the severity of tau pathology in different brain regions of the two phenotypic presentations of PSP reveals topographical differences that are closely linked with their respective clinical features.

The features of repetitive finger tapping and handwriting in patients with PSP and Parkinson's disease are compared and a distinct abnormality is identified in PSP which may be useful in differentiating PSP-parkinsonism from Parkinson's disease.

Twelve cases clinically presenting with a levodopa-responsive parkinsonian syndrome and post-mortem findings of nigral degeneration and predominant tau inclusions, which could not be readily classified into any recognised clinicopathological entity are also studied.

Publications related to this thesis

1. **Ling H**, O'Sullivan SS, Holton JL, Revesz T, Massey LA, Williams DR, Paviour DC, Lees AJ. Does corticobasal degeneration exist? A clinicopathological re-evaluation. Brain. 2010 Jul;133:2045-57. **(Chapter 2)**

2. **Ling H**, de Silva R, Massey LA, Courtney R, Hondhamuni G, Bajaj N, Lowe J, Holton JL, Lees A, Revesz T. Characteristics of progressive supranuclear palsy presenting with corticobasal syndrome: a cortical variant. Neuropathology Applied Neurobiology. 2013 Feb 22. **(Chapter 3)**

3. **Ling H**, Massey LA, Lees AJ, Brown P, Day BL. Hypokinesia without decrement distinguishes progressive supranuclear palsy from Parkinson's disease. Brain. 2012 Apr;135:1141-53. **(Chapter 4)**

4. **Ling H**, Holton JL, Lees AJ, Revesz T. TDP-43 pathology is present in most postencephalitic parkinsonism brains. Neuropathology and Applied Neurobiology. 2013 Jun 13. **(Chapter 5)**

5. Kara E, **Ling H** (co-first author), Pittman AM, Shaw K, de Silva R, Simone R, Holton JL, Warren JD, Rohrer JD, Xiromerisiou G, Lees A, Hardy J, Houlden H, Revesz T. The MAPT p.A152T variant is a risk factor associated with tauopathies with atypical clinical and neuropathological features. Neurobiology of Aging. 2012 Sep;33(9):2231. **(Chapter 5)**

6. **Ling H**, Kara E, Bandopadhyay R, Hardy J, Holton J, Xiromerisiou G, Lees A, Houlden H, Revesz T. TDP-43 pathology in a patient carrying G2019S LRRK2 mutation and a novel p.Q124E MAPT. Neurobiology of Aging. 2013 May 9. **(Chapter 5)**

Publications during the degree period

1. Kiely AP, Asi YT, Kara E, Limousin P, **Ling H**, Lewis P, Proukakis C, Quinn N, Lees AJ, Hardy J, Revesz T, Houlden H, Holton JL. α-Synucleinopathy associated with G51D SNCA mutation: a link between Parkinson's disease and multiple system atrophy? Acta Neuropathologica. 2013 May;125(5):753-69.
2. Massey LA, Jäger HR, Paviour DC, O'Sullivan SS, **Ling H**, Williams DR, Kallis C, Holton J, Revesz T, Burn DJ, Yousry T, Lees AJ, Fox NC, Micallef C. The midbrain to pons ratio: a simple and specific MRI sign of progressive supranuclear palsy. Neurology. 2013 May 14;80(20):1856-61.
3. Magdalinou NK, **Ling H**, Smith JD, Schott JM, Watkins LD, Lees AJ. Normal pressure hydrocephalus or progressive supranuclear palsy? A clinicopathological case series. Journal of Neurology. 2013 Apr;260(4):1009-13.
4. Lee W, Simpson M, **Ling H**, McLean C, Collins S, Williams DR. Characterising the uncommon corticobasal syndrome presentation of sporadic Creutzfeldt-Jakob disease. Parkinsonism and Related Disorders. 2013 Jan;19(1):81-5.
5. Massey LA, Micallef C, Paviour DC, O'Sullivan SS, **Ling H**, Williams DR, Kallis C, Holton JL, Revesz T, Burn DJ, Yousry T, Lees AJ, Fox NC, Jäger HR. Conventional magnetic resonance imaging in confirmed progressive supranuclear palsy and multiple system atrophy. Movement Disorders. 2012 Dec;27(14):1754-62.
6. **Ling H**, Petrovic I, Day BL, Lees AJ. Smoking-induced transient motor deterioration in a levodopa-treated patient with Parkinson's disease. Journal of Neurology. 2012 Nov;259(11):2419-23.
7. Petrovic IN, **Ling H**, Asi Y, Ahmed Z, Kukkle PL, Hazrati LN, Lang AE, Revesz T, Holton JL, Lees AJ. Multiple system atrophy-parkinsonism with slow progression and prolonged survival: a diagnostic catch. Movement Disorders. 2012 Aug;27(9):1186-90.

8. **Ling H**, Braschinsky M, Taba P, Lüüs SM, Doherty K, Hotter A, Poewe W, Lees AJ. Decades of delayed diagnosis in 4 levodopa-responsive young-onset monogenetic parkinsonism patients. Movement Disorders. 2011 Jun;26(7):1337-40.

9. **Ling H**, Polke JM, Sweeney MG, Haworth A, Sandford CA, Heales SJ, Wood NW, Davis MB, Lees AJ. An intragenic duplication in guanosine triphosphate cyclohydrolase-1 gene in a dopa-responsive dystonia family. Movement Disorders. 2011 Apr;26(5):905-9.

10. **Ling H**, Lees AJ (2010). How can neuroimaging help in the diagnosis of movement disorders? Neuroimaging Clinics of North America. 2010 Feb;20(1):111-23.

Declaration of collaborative work

Chapter 5:

The majority of this study was undertaken by Dr Helen Ling. Genetic sequencing was performed by Dr Eleanna Kara, Department of Molecular Neuroscience, Institute of Neurology, University College London, Queen Square, London.

List of abbreviations

3R	Three-repeat
4R	Four-repeat
AD	Alzheimer's disease
AGD	Argyrophilic grain disease
ALS	Amyotrophic lateral sclerosis
ANOVA	Analysis of variance
CAA	Cerebral amyloid angiopathy
CB	Coiled body
CBD	Corticobasal degeneration
CBS	Corticobasal syndrome
CERAD	Consortium to Establish a Registry for Alzheimer's disease
CI	Confidence interval
C-SPOND	Cervical spondylosis
CST	Corticospinal tract
CTE	Chronic traumatic encephalopathy
CV	Coefficient of variation
CVD	Cerebrovascular disease
DEG	Degree
EL	Encephalitis lethargica
ESP	Exome Sequencing Project
F	Female
FAB	Frontal assessment battery
FTD	Frontal temporal dementia
FTDbv	Behavioural variant of frontotemporal dementia
FTDP-17	Frontotemporal dementia with parkinsonism linked to chromosome 17
GPi	Globus pallidus interna
GWAS	Genome-wide association study
H&Y	Hoehn and Yahr Parkinson's disease staging
LED	Levodopa equivalent dose
LRRK2	Leucine-rich repeat kinase 2

M	Male
MAP	Microtubule-associated protein
MAPT	Microtubule-associated protein gene
MND	Motor neuron disease
MSA	Multiple system atrophy
NA	Not applicable
NFT	Neurofibrillary tangle
NHLBI	National Heart Lung Blood Institute
NIEHS	National Institute of Environmental Health Sciences
NK	Not known / features not recorded
NPV	Negative predictive values
NS	Not statistically significant
NT	Neuropil threads
PAGF	Primary akinesia with gait freezing
PD	Parkinson's disease
PDC	parkinsonism-dementia complex of Guam
PD-OFF	Parkinson's disease during 'Off' state
PD-ON	Parkinson's disease during 'ON' state
PEP	Postencephalitic parkinsonism
PKAN	Pantothenate kinase-associated neurodegeneration
PND	Parkinsonism with neurofibrillary degeneration
PNFA	Progressive non-fluent aphasia
PNLA	Pallidonigroluysian atrophy
PPA	Primary progressive aphasia
PPV	Positive predictive values
PreT	Pretangle
PSP	Progressive supranuclear palsy
PSP-P	Progressive supranuclear palsy-parkinsonism
QSBB	Queen Square Brain Bank for Neurological Disorders
RS	Richardson's syndrome
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SMA	Supplementary motor area

SN	Substantia nigra
SPSS	Software package used for statistical analysis
SSPE	Subacute sclerosing panencephalitis
STN	Subthalamic nucleus
SVD	Small vessel disease
TA	Tufted astrocyte
TDP-43	Transactive response DNA-binding protein 43
UPDRS	Unified Parkinson's Disease Rating Scale
VSGP	Vertical supranuclear gaze palsy

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Chapter 1: Introduction to tau pathology and neurodegeneration

Tau aggregation, phosphorylation and propagation

Neurofibrillary tangles (NFTs) formed within the neurons are made up of filamentous materials, of which the major structural component is hyperphosphorylated tau (Goedert *et al.*, 1992). Ultrastructurally, they contain either paired helical (Kidd, 1963) or straight filaments (Goedert *et al.*, 2001).

Tau is the most abundant microtubule-associated protein (MAP) in the brain. Microtubules in neurons are important for intracellular transport. Tau promotes tubule formation through assembly of the tubulin subunits and, once formed, it stabilizes the microtubules. In adult human brain, there are six tau isoforms, differing from one another by the presence or absence of 29 or 58 amino acid inserts located in the amino-terminal half and an additional 31-amino acid repeat in the carboxy-terminal half (Figure 1). Inclusion of the latter, which is encoded by exon 10, the spliced products give rise to three tau isoforms with four repeats (4R) each, while the remaining three isoforms have three repeats (3R) each. 3R and 4R-tau isoforms are expressed in roughly equal amounts in the normal adult human brain, but this ratio may be altered in some neurodegenerative diseases.

As a result of abnormal hyperphosphorylation, tau is unable to bind to microtubules in axons (Bramblett *et al.*, 1993) and, instead, is redistributed to the cell body, where it accumulates to form insoluble filamentous inclusions and leads to neurodegenerative disease. Filamentous tau tends to aggregate in a stereotypic manner as the disease progresses. It is proposed that once tau aggregates are formed in discrete brain regions, intercellular transfer of aggregated tau occurs and, via the mechanism of ‘permissive templating’ (Hardy, 2005), triggers a self-propagating process and prion-like spread of the abnormal tau throughout the central nervous system (Clavaguera *et al.*, 2009).

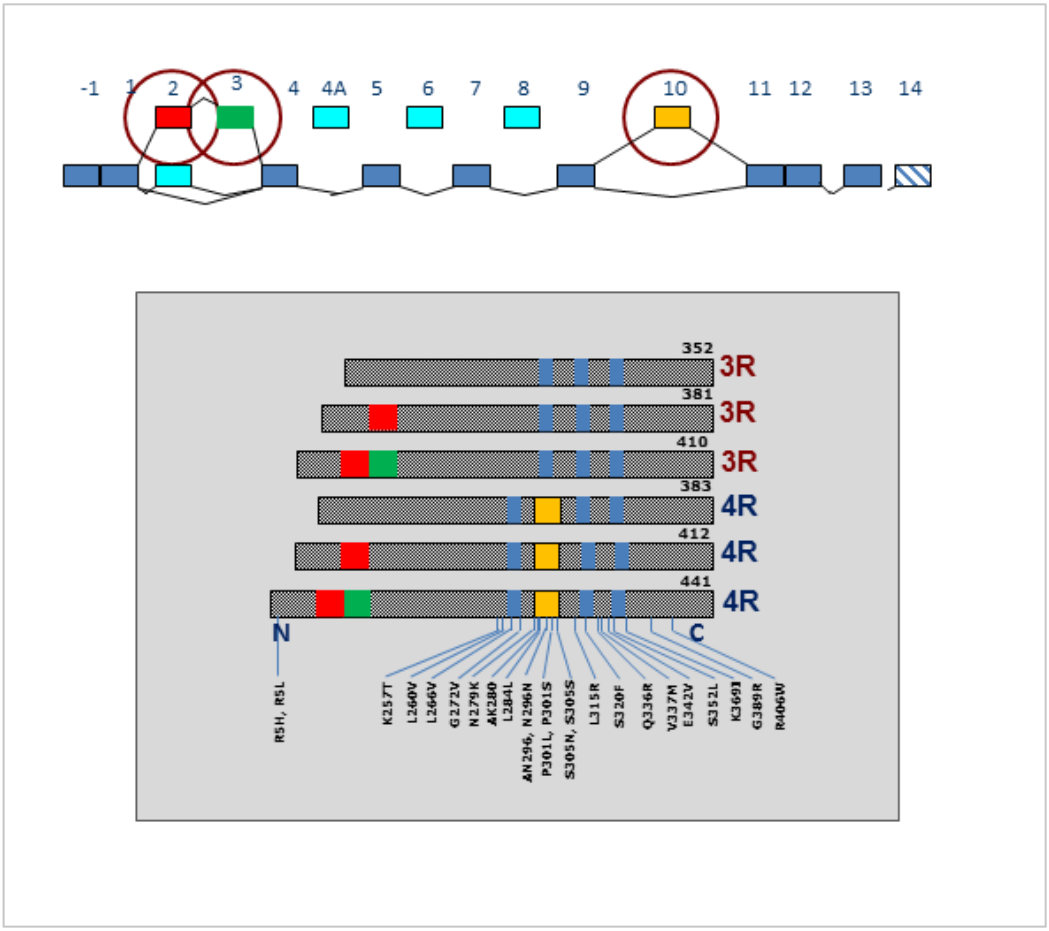

Figure 1. *Human MAPT gene and the six tau isoforms expressed in the brain.*

The six tau isoforms are expressed in the adult human brain by alternative pre-mRNA splicing of exons 2 (red), 3 (green) and 10 (yellow) of the MAPT gene located on chromosome 17. They differ from each other by the presence or absence of 29 or 58 amino acid inserts (red & green) located in the amino-terminal half and an additional 31-amino acid repeat (yellow) in the carboxy-terminal half. Inclusion of the latter produces the three isoforms with four repeats each, whereas the other three isoforms have three repeats each.

MAPT consists of 16 exons; exon 0 is part of the promoter, exon 14 is non-coding, exon 6 and 8 are not transcribed in human brain and exon 4a is only expressed in the peripheral nervous system. Blue bars indicate the microtubule-binding repeats of tau.

The diagram is kindly provided by Dr Rohan de Silva.

Tauopathies and isoform compositions

Diseases associated with insoluble tau aggregates in the brain are known as tauopathies, which comprise of more than 20 different conditions (Table 1)(Spillantini *et al.*, 2013). Tau is one of the two most common misfolded proteins that form intracellular inclusions in human neurodegenerative diseases, with the other being α-synuclein, the main component of Lewy bodies in Parkinson's disease. Most tauopathies either cause dementia or parkinsonism, and in some conditions, a combination of both. Tauopathies are commonly categorized by their predominant tau isoforms. Tau pathologies in PSP and CBD contain predominantly 4R-tau and are referred as 4R tauopathies, whereas Pick's disease contains predominant 3R-tau and is known as a 3R tauopathy. In conditions such as PSP, CBD, Pick's disease and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), tau inclusions are the predominantly pathology. Unlike, PSP and CBD, which are primary tauopathies, Alzheimer's disease is a tauopathy that is characterised by another predominant type of pathology, namely amyloid-β plaques.

Differences in tau isoform composition in these conditions are reflected by the different electrophoretic migration patterns on tau immunoblotting. Western blots of filamentous tau extracted from PSP and CBD brains shows a doublet pattern with 64kDa and 68kDa band, whereas filamentous tau from Pick's disease demonstrates a major 60 and 64kDa bands. Alzheimer's disease, postencephalitic parkinsonism, parkinsonism-dementia complex of Guam (PDC) and chronic traumatic encephalopathy (CTE) are characterised by a major triplet with 60, 64 and 68kDa bands (Buee-Scherrer *et al.*, 1997).

	Parkinsonism	Predominant tau isoforms
Predominant tau pathology		
Progressive supranuclear palsy	+	4R
Corticobasal degeneration	+	4R
Argyrophilic grain disease	-	4R
Pick's disease	+/-	3R
FTDP-17	+	variable
Postencephalitic parkinsonism	+	mixed 3R & 4R
Parkinsonism dementia complex of Guam	+	mixed 3R & 4R
Guadeloupean parkinsonism	+	4R
Tangle-only dementia	-	mixed 3R & 4R
Globubar glial tauopathy	+	4R
Associated with other pathologies		
Alzheimer's disease	+/-	mixed 3R & 4R
Down's syndrome	-	mixed 3R & 4R
Chronic traumatic encephalopathy	+	mixed 3R & 4R
Familial British dementia	-	mixed 3R & 4R
Familial Danish dementia	-	mixed 3R & 4R
PKAN	+	nk
Myotonic dystrophy	-	3R
Niemann Pick type C	+	nk
Subacute sclerosing panencephalitis	+	nk

Table 1. *Common tauopathies with or without parkinsonism*

+: yes, +/-: rare, -: no; 3R=3-repeat tau; 4R=4-repeat tau; FTDP-17=frontotemporal dementia with parkinsonism linked to chromosome 17; nk=not known; PKAN=pantothenate kinase-associated neurodegeneration,

Progressive supranuclear palsy

In 1963, a distinct syndrome of postural instability, supranuclear gaze palsy, mild dementia, progressive axial rigidity and bulbar palsy was described in nine patients (Richardson *et al.*, 1963). The consistent pathological findings in these cases led to the identification of progressive supranuclear palsy (PSP) as a distinct clinicopathological entity (Steele *et al.*, 1964). The neuropathological diagnosis of PSP is defined by the accumulation of NFTs and neuropil threads (NTs) mainly in the globus pallidus, subthalamic nucleus and substantia nigra (Dickson *et al.*, 2011, Hauw *et al.*, 1994, Ince *et al.*, 2008)(Figure 2). Other affected regions are the red nucleus, striatum, oculomotor nucleus, locus coeruleus, pontine tegmentum, medulla and dentate nucleus. Globose NFT is a typical but non-specific feature observed in the brainstem nuclei and nucleus basalis of Meynert, while in other regions, the NFTs are more frequently flame-shaped. Diffuse or granular neuronal tau cytoplasmic immunoreactive lesions, labelled pretangles (PreTs) are frequently encountered. Tufted astrocytes, a characteristic feature in PSP, are star-like tufts of tau-immunoreactive filaments located mostly in proximal astrocytic processes. They are frequently found in the motor cortex and striatum. Oligodendroglial lesions, called coiled bodies (CBs), are observed in the white matter. Neuronal loss in PSP is most severe in the substantia nigra and subthalamic nucleus, and less consistently, in the globus pallidus.

The original description of the clinical picture of PSP is now referred as the classic presentation of PSP or Richardson's syndrome (RS), which is seen in at least half of all pathologically confirmed cases, with a mean disease duration of five to eight years (Williams *et al.*, 2009). In 2005, a variant of PSP known as PSP-parkinsonism (PSP-P), with a longer disease duration and predominant parkinsonism in the early stage, was characterised and is observed in up to a third of all PSP cases (Williams *et al.*, 2005). PSP-P is frequently misdiagnosed as Parkinson's disease in the early stage due to the presence of asymmetrical limb bradykinesia, rigidity and, in some cases, tremor and transient response to levodopa (Williams *et al.*, 2005, Williams *et al.*, 2010). The pathologies of PSP-P are less severe and more restricted in distribution than in PSP-RS (Williams *et al.*, 2007). Another group of patients present with early gait disturbance, micrographia and hypophonia, followed by gait freezing are collectively termed PSP-pure akinesia with gait freezing (PSP-PAGF)(Williams *et al.*, 2007). The disease

progression in PSP-PAGF can be insidious and may have a prolonged disease course of more than 10 years in some cases. Neuronal loss in PSP-PAGF is restricted to the globus pallidus, substantia nigra and subthalamic nucleus (Ahmed *et al.*, 2008). PSP-P and PSP-PAGF subtypes are sometimes referred as the brainstem variants of PSP in view of the milder tau pathology and restriction of tau pathology in the basal ganglia and brainstem (Dickson *et al.*, 2010).

Another PSP subtype, with clinical presentation of progressive asymmetric limb dystonia, rigidity, ideomotor apraxia and cortical sensory loss, are collectively referred as PSP-corticobasal syndrome (PSP-CBS)(Tsuboi *et al.*, 2005). The subgroups with predominant features of apraxia of speech or behavioural disturbance and frontotemporal dementia, are known as PSP-progressive non-fluent aphasia (PSP-PNFA)(Josephs *et al.*, 2006) and PSP-frontotemporal dementia behavioural variant (PSP-FTDbv)(Hassan *et al.*, 2011), respectively. The subgroups of PSP-CBS, PSP-PNFA and PSP-FTDbv have been shown to have more severe cortical tau pathology than PSP-P and PSP-PAGF and are labeled as the cortical variants of PSP (Dickson *et al.*, 2010). The clinical features of all phenotypes are most distinct in the early disease stages, however, as the disease progresses, the symptoms evolve and after several years, most patients develop a clinical picture compatible with RS with supranuclear gaze palsy, especially on downward gaze, severe axial rigidity, postural instability, executive dysfunction and pseudobulbar palsy (Williams *et al.*, 2009).

Despite the heterogeneous phenotypic presentation, the classic RS and the variants are linked under the generic term of PSP by their rapid progressive course leading to death within a decade and post-mortem findings of NFT accumulation, predominantly in the globus pallidus, subthalamic nucleus and substantia nigra, atrophy of the subthalamic nucleus and presence of tufted astrocytes, fulfilling the established neuropathological diagnostic criteria of PSP (Dickson *et al.*, 2011, Hauw *et al.*, 1994, Ince *et al.*, 2008). The regional differences in the severity of tau pathology are linked with the distinct clinical features in the subtypes (Dickson *et al.*, 2010).

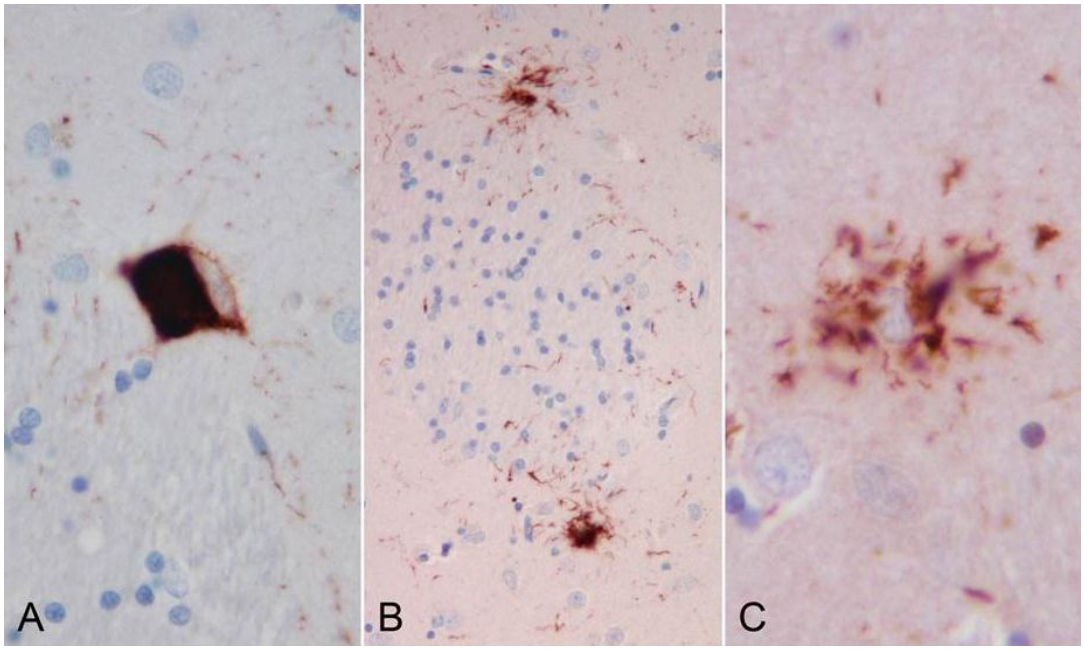

Figure 2. *Tau-immunohistochemistry of PSP.*

A: Tau-positive neurofibrillary tangle and fine neuropil threads in the caudate; B: Tufted astrocytes and neuropil threads; C: Tau-positive tufted astrocyte containing 4R-tau isoform; A & B: AT8 immunohistochemistry, C: 4R-tau immunohistochemistry

Corticobasal degeneration

In 1967, Rebeiz and colleagues reported a clinicopathological entity termed ‘corticodentatonigral degeneration with neuronal achromasia’ (Rebeiz *et al.*, 1967, Rebeiz *et al.*, 1968). Since the description, several other terms were used in the case reports of the same entity. In 1989, Gibb and colleagues coined the abbreviated term of corticobasal degeneration (CBD) and proposed it as a distinct clinicopathological entity that had pathological similarities to Pick’s disease (Gibb *et al.*, 1989). Molecular studies subsequently established the distinction by demonstrating that PSP and CBD are 4R tauopathies while 3R-tau predominates in Pick’s disease (Arai *et al.*, 2001).

The classic clinical presentation of CBD is corticobasal syndrome (CBS) with progressive clumsiness and loss of function of one hand due to a combination of frontoparietal and basal ganglia sensorimotor dysfunction. The mean disease duration of CBD is seven years (Kouri *et al.*, 2011). A similar constellation of signs has also been linked with cerebrovascular disease, Alzheimer’s disease, PSP and other rarer pathologies (Wadia *et al.*, 2007). Conversely, the pathological diagnosis of CBD can give a clinical picture of RS, PNFA, apraxia of speech and progressive posterior cortical atrophy syndrome (Kouri *et al.*, 2011).

The neuropathological diagnosis of CBD is based on the findings of numerous NTs in the frontal and parietal cortices, their adjacent white matter, striatum and midbrain (Dickson *et al.*, 2002)(Figure 3). Neuronal lesions in CBD are pleomorphic, including small sized NFTs or Pick body-like inclusions in superficial cortical neurons, PreTs, globose NFTs or corticobasal bodies. Astrocytic plaques, the pathological hallmark of CBD, are structures formed by annular clusters of short, stubby tau-positive structures located in the distal segments of astrocytic processes. They are numerous in the affected cortices, but are also found in the caudate nucleus, putamen and, sometimes, thalamus and midbrain tectum. It is of considerable diagnostic help that astrocytic plaques in CBD are morphologically distinct from tufted astrocytes in PSP. Morphological studies indicate that astrocytic plaques and tufted astrocytes do not coexist in the same brain (Komori *et al.*, 1998). Oligodendroglial tau accumulation and ballooned neurons are also characteristic features of CBD.

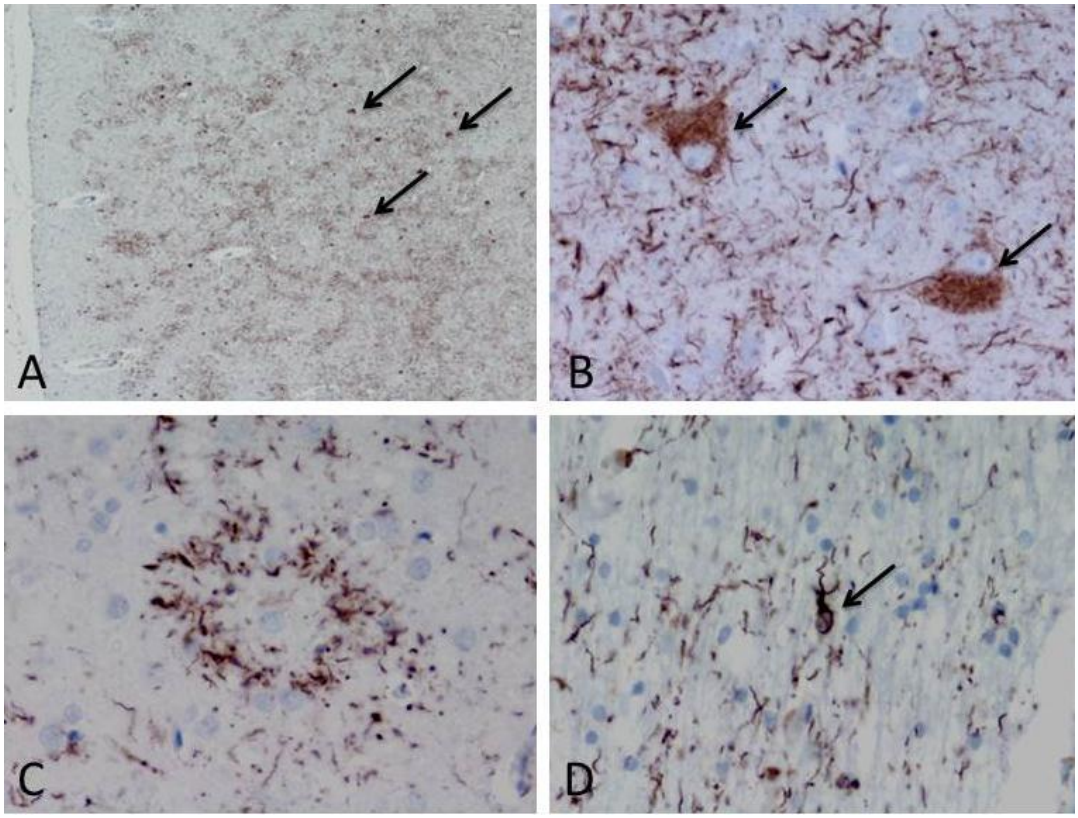

Figure 3. *Tau-immunohistochemistry of CBD.*

Tau-positive inclusions (A), pretangles (arrows) and threads (B), astrocytic plaque (arrow) in frontal cortex (C) and CBs (arrow) in the cortical white matter (D), are characteristic pathological lesions in CBD.

Tau genetics

In 1998, the first mutations in the *MAPT* gene on chromosome17q21-22 were reported in families with an autosomal dominantly inherited form of frontotemporal dementia with parkinsonism, which is now known as FTDP-17T (Hutton *et al.*, 1998, Poorkaj *et al.*, 1998, Spillantini *et al.*, 1998). The finding of this gene confirms the notion that dysfunction of tau protein is directly linked with the pathogenesis of disease and that the presence of tau aggregates in the brain is not merely an epiphenomenon. To date, over 50 pathogenic mutations of this gene have been identified, most of which are located in exons 9-12 and the adjacent introns. At a functional level, mutations reduce the ability of tau to interact with microtubules, leading to tau aggregation. Some mutations also promote the assembly of tau into filaments or enable the hyperphosphorylation of tau. Others alter the ratio of 3R and 4R-tau isoforms, resulting in the overproduction of 4R-tau, which is sufficient to cause disease (Spillantini *et al.*, 2013). It is now known that mutations in the progranulin gene can cause a tau-negative type of FTDP-17 (Baker *et al.*, 2006, Cruts *et al.*, 2006). On the other hand, mutations in the *MAPT* gene can be associated with phenotypic presentations and pathologies identical to those of PSP, CBD, argyrophilic grain disease and Pick's disease (Spillantini *et al.*, 2013). Phenotypic heterogeneity is associated with different *MAPT* mutations. Interfamilial and intrafamilial variability may be observed for the same mutation.

MAPT in populations of European descent is characterised by the H1 and H2 haplotypes, resulting from a 900-kb inversion (H1) or non-inversion (H2) polymorphism (Stefansson *et al.*, 2005). The H2 lineage is found in 20% of the Europeans, but is rare in Africans and almost absent in east Asians.

The H1 haplotype is a risk factor of PSP, CBD and PDC, but it does not contribute to risk for Alzheimer's disease. Notably, genome-wide association studies (GWAS) also confirm an association between H1 haplotype and Parkinson's disease, a disease characterised by α-synuclein pathology rather than tau (Simon-Sanchez *et al.*, 2009). A mechanism of cross-talk between molecular pathways of different aggregating proteins is thought to be implicated. Conversely, the H2 haplotype, is thought to be protective against PSP, CBD and Parkinson's disease (Caffrey *et al.*, 2008). H2 is associated with

increased expression of exon 3 of *MAPT* in grey matter (Trabzuni *et al.*, 2012), which has an inhibitory role in tau aggregation, unlike exons 2 and 10 which promote aggregation (Zhong *et al.*, 2012).

A GWAS study also identified three novel signals associated with risk for PSP; *EIF2AK3*, a gene that encodes for PERK, which is a component of unfolded protein response, *STX6*, that encodes syntaxin 6, which is a protein in vesicle trafficking and, *MOBP*, which encodes myelin oligodendrocyte basic protein, a structural component of myelin in the central nervous system (Hoglinger *et al.*, 2011). These loci provide insights into the additional pathways in the pathophysiology of PSP.

Chapter 2: Does corticobasal degeneration exist? A clinicopathological re-evaluation

Introduction

The earliest description of CBS dates back to 1925 when Jean L'Hermitte and colleagues reported the case of a 72-year old carpenter presenting with a clumsy useless arm with rigidity, ideomotor apraxia, abnormal flexed posture, 'jerky' contractions, alien limb phenomenon and cortical parietal sensory dysfunction (Ballan *et al.*, 1997, Lhermitte *et al.*, 1925). There is also speculation that the French composer Maurice Ravel (1875-1937) who developed aphasia, apraxia and loss of musical creativity had a CBS (Alajouanine, 1948).

In 1967, the first comprehensive clinicopathological description of CBD was published by Rebeiz and colleagues, who described three patients with progressive clumsy, slow and awkward movements of one limb for which no cause could be found (Rebeiz *et al.*, 1967, Rebeiz *et al.*, 1968). Other features such as alien hand, jerky tremor, dystonia, parkinsonism, pyramidal features and gait difficulty were also noted. Cortico-dentato-nigral degeneration with neuronal achromasia and swollen cortical cells identical to those found in Pick's disease led to the proposal that this was a hitherto unreported distinctive nosological clinicopathological entity (Rebeiz *et al.*, 1967, Rebeiz *et al.*, 1968). In the 1980's, several further small series of similar cases were described but the disorder received little interest and was considered to be a rare neurological curiosity (1985, Watts *et al.*, 1985). In 1989, Gibb and colleagues coined the abbreviated term corticobasal degeneration. They highlighted the pathological similarities to Pick's disease but concluded that the clinical picture and distribution of neuronal loss and neurofibrillary degeneration supported the view that this was a distinct disease (Gibb *et al.*, 1989). Molecular pathological studies have further supported this distinction by demonstrating that CBD, in common with PSP is a

predominantly 4R isoform tauopathy; whereas 3R tau accumulation predominates in Pick's disease (Arai *et al.*, 2001).

The classic description of CBD includes clumsiness and loss of function of one hand due to a combination of fronto-parietal and basal ganglia sensorimotor dysfunction. Ideomotor and limb-kinetic apraxia, cortical sensory loss leading to an alien limb, limb dystonia, focal action or stimulus-sensitive myoclonus and levodopa-unresponsive rigidity and bradykinesia may all be found on examination and contribute to the asymmetric limb dysfunction. A similar constellation of signs has been linked with cerebrovascular disease (Kreisler *et al.*, 2007), Alzheimer's disease (Imamura *et al.*, 2009), PSP (Tsuboi *et al.*, 2005), Pick's disease (Boeve *et al.*, 1999), dementia with Lewy bodies (Horoupian *et al.*, 1999), Creutzfeldt-Jakob disease (Vandenberghe *et al.*, 2007), neurofilament inclusion body disease (now known as FTLD-FUS)(Josephs *et al.*, 2003) and argyrophilic grain disease (Rippon *et al.*, 2005). Genes associated with frontotemporal dementia (FTD) including *MAPT*, *progranulin* and *C9orf72* are other rare causes (Casseron *et al.*, 2005, Lesage *et al.*, 2013, Masellis *et al.*, 2006, Snowden *et al.*, 2012, Tartaglia *et al.*, 2009). The term CBS has been used to embrace this distinctive collection of symptoms and signs.

Conversely, pathologically diagnosed CBD can masquerade as PSP (Boeve, 2005), FTD (Josephs *et al.*, 2006), PNFA and apraxia of speech (Josephs *et al.*, 2006, McMonagle *et al.*, 2006) and progressive posterior cortical atrophy syndrome (Renner *et al.*, 2004, Tang-Wai *et al.*, 2004). Cases carrying mutations in the *MAPT* (FTDP-17T) may have a similar pathological signature as CBD and it may be reasonable to look on these cases as examples of monogenetic CBD. Marked early cognitive dysfunction which was once considered an exclusion criteria for the diagnosis of CBD, is now accepted as a clinical variant (Grimes *et al.*, 1999). Kertesz and colleagues have suggested that the phenotypes of CBS, PSP, primary progressive aphasia (PPA) and FTDbv are closely related and frequently overlap leading them to propose that all of these different diagnoses should be lumped together and referred to as the FTD/Pick complex (Kertesz *et al.*, 2005).

Previous studies have indicated that between 25 and 56% of CBD cases are diagnosed correctly in life (Boeve, 2005, Grimes *et al.*, 1999, Hughes *et al.*, 2002, Murray *et al.*,

2007). Pathological prediction of a primary tauopathy is, however, much more accurate (Josephs *et al.*, 2006). Josephs et al, for example, indicated that the clinical presentation of CBS or a PSP-like syndrome was 100% specific for a primary tauopathy (Josephs *et al.*, 2006). Of 83 cases presenting with CBS studied in four large clinicopathological series, 69 (83%) had a tauopathy with pathological inclusions made up predominantly of 4R tau in CBD, PSP and 3R tau in Pick's disease (Wadia *et al.*, 2007).

The aim of the this study was to review the archival cases with either clinical diagnosis of CBS or pathological diagnosis of CBD examined at the Queen Square Brain Bank for Neurological Disorders (QSBB) between 1989 and 2009.

Materials and methods

Patients

Patients with either a final clinical diagnosis of CBS or pathological diagnosis of CBD were identified from the archive of 1440 cases referred to the QSBB over a 20-year period (1989 - 2009). Some cases have been included in previous QSBB series, previously known as the United Kingdom Parkinson's Disease Society Brain Research Centre (Houlden *et al.*, 2001, Jendroska *et al.*, 1995, Litvan *et al.*, 1996, O'Sullivan *et al.*, 2008, Pittman *et al.*, 2005, Williams *et al.*, 2005). Most of the cases were registered as donors while under the care of physicians at the National Hospital for Neurology and Neurosurgery, Queen Square, London. Prospective brain donors were required to fill in annual assessment forms which provided clinical information related to their neurological condition. Consent for brain donation was obtained from the patients prior to death and consent for post-mortem examination was obtained from the next of kin after death. The protocols for the retention and access to human tissue and clinical records at the QSBB have been approved by the London Multi-Centre Research Ethics Committee. The QSBB restricted itself to the collection of abnormal movement disorders until five years ago when it also started to collect brains of patients with presumed FTD, who had presented to the Dementia Research Centre at the Institute of Neurology, University College London.

Medical record review

A retrospective review was performed of all medical records, including the primary care medical notes, the correspondence between the medical specialists and general practitioners, medical files from the National Hospital for Neurology and Neurosurgery and the QSBB annual assessment data. All patients, except one who had incidental CBD pathology, had been assessed by hospital specialists (general neurologists, movement or cognitive disorders specialists) throughout the course of their illness. The information from the case notes was assessed by a neurologist who was blinded to the pathological diagnosis.

Symptoms were recorded as being absent if they were not reported in the case notes. Clinical signs were recorded as unknown if they were not specifically mentioned. The time of onset of selected clinical features was noted. When the onset was not documented, the onset was recorded as the time when the particular clinical feature was first mentioned in the chart. In cases with conflicting clinical features, the findings of the hospital specialist were used.

The clinical research definitions used in the study were as follows: (i) age of onset, approximated to the specific month when the first symptom considered to be attributable to the neurological disorder was reported, (ii) duration of illness, time between the age of onset and the age at death, (iii) initial clinical diagnosis, the first diagnosis made by neurologist, (iv) final clinical diagnosis, the last diagnosis recorded before death; where more than one possible clinical diagnosis was listed in the clinical records, an attempt was made to ascertain which diagnosis was thought by the neurologist to be most likely, (v) initial presenting symptoms, first symptoms considered to be attributable to the neurological disorder, (vi) alien limb, defined as a feeling that one limb is 'foreign' or 'has a will of its own', together with observable 'involuntary motor activity', including grasping of nearby objects or 'intermanual conflict'; where the reported symptoms fulfilled this definition or when 'alien limb phenomenon' was documented by neurologists, this feature was recorded as present, and limb levitation alone was not considered as alien limb phenomenon (Joseph *et al.*, 2004), (vii) falls, reported as present when an unprovoked fall was mentioned, (viii) backward falls, more than 50% of the falls were in the backward direction, (ix) pyramidal signs, when there was documentation of a Babinski's sign and pathologically brisk reflexes and (x) response to levodopa, a reported improvement of >30% coinciding with the introduction of levodopa was recorded as being a positive response: a 4-point scale of improvement post-levodopa was used as follows: 1 = nil, or <30% improvement; 2 = moderate response (30-50% improvement); 3 = good response (51-70% improvement); and 4 = excellent response (71-100% improvement). A transient response to levodopa was defined as a reported benefit lasting less than 2 years of commencing on levodopa.

Neuropathological methods

Cases were selected from the archival collection of the QSBB where brain donation takes place according to ethically approved protocols and tissue is stored under a license from the Human Tissue Authority. In all cases the neuropathological diagnosis was reviewed by two neuropathologists using established pathological criteria. The minimal pathological features required for the diagnosis of CBD were tau-positive neuronal and glial lesions, including astrocytic plaques and extensive thread pathology in both grey and white matter of cerebral cortex and striatum along with focal neuronal loss in cortical regions and in the substantia nigra (Dickson *et al.*, 2002, Ince *et al.*, 2008). Other neuropathological diagnoses established for cases in the present series included PSP (Ince *et al.*, 2008, Litvan *et al.*, 1996), FTLD (Cairns *et al.*, 2007), Alzheimer-type or α-synuclein pathologies (Ball *et al.*, 1997, Ince *et al.*, 2008) and Parkinson's disease (Oppenheimer, 1984).

Statistical methods

Four diagnostic parameters were calculated for CBD cases in the QSBB database: (i) sensitivity: the percentage of pathologically diagnosed CBD cases, which had been diagnosed clinically as CBS in life, (ii) specificity: the percentage of cases which did not have CBD pathology which had been clinically diagnosed as not having that diagnosis in life, (iii) positive predictive value (PPV): the percentage of cases which had been diagnosed clinically as CBS and were pathologically confirmed as CBD and (iv) negative predictive value (NPV): the percentage of cases which were not clinically diagnosed with CBS and were confirmed post-mortem not to have CBD pathology. The calculations of PPV and NPV depend on the prevalence of disease in a targeted population, whereas sensitivity and specificity are constant measures of clinical diagnostic acumen. Confidence intervals at 95% for each diagnostic parameter were calculated by Stata 10.0 statistical programme. Final clinical and neuropathological diagnoses were cross-tabulated using a two-by-two contingency table. Clinical features from patient subgroups were compared using the Student's *t* test for continuous variables and the χ^2 or Fisher exact tests for categorical variables by SPSS 17.0 statistical programme.

Results

Of the 1440 cases in the QSBB collected over the 20-year period, there were 179 cases with a pathological diagnosis of PSP, 117 multiple system atrophy and 608 Parkinson's disease. There were 35 patients (16 female, 19 male) with either a final clinical diagnosis of CBS or pathological diagnosis of CBD (Figure 4). Twenty-one of these had been followed up regularly at the National Hospital for Neurology and Neurosurgery (nine under the movement disorders team, six under the cognitive disorders team, four jointly by both teams, one followed up by a general neurologist and the other by a neuropsychiatrist). Of the 14 cases seen in other hospitals, nine were under the care of general neurologists and five followed by movement disorders specialists.

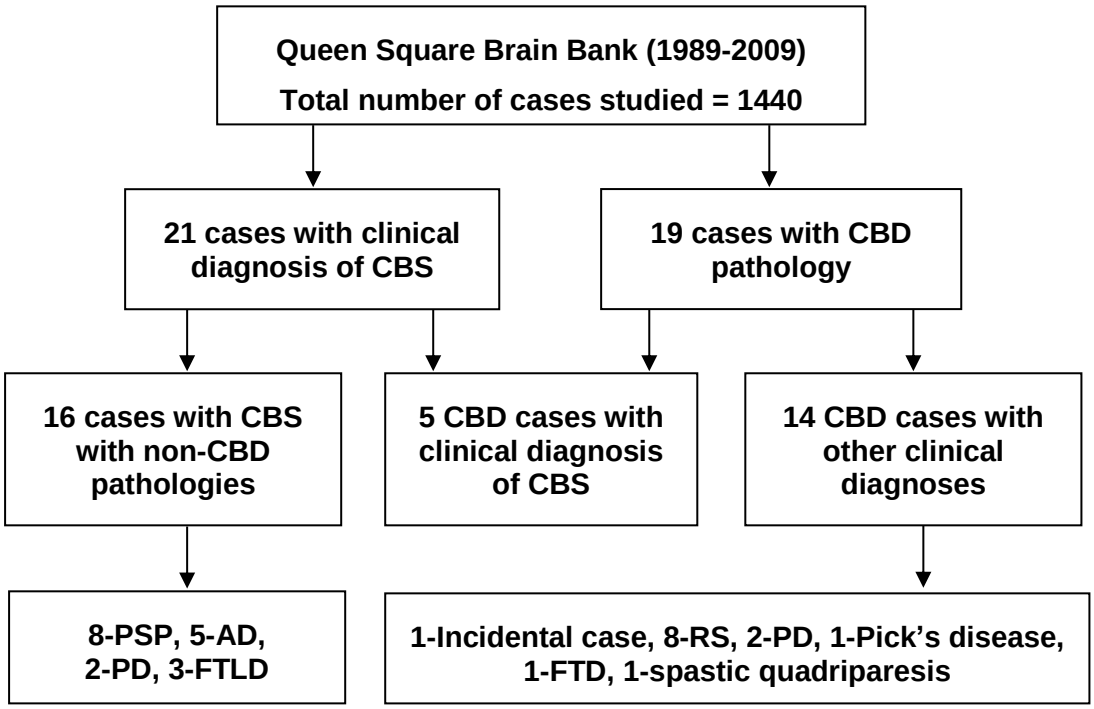

Figure 4. *Inclusion criteria of the study.*

Cases with either a clinical diagnosis of CBS or pathological diagnosis of CBD from the archival cases of the Queen Square Brain Bank were included in the study.

Clinical parameters	All cases (N=19)	CBD-CBS (N=5)	CBD-RS (N=8)
Female : Male	9 : 10	2 : 3	4 : 4
Age of symptom onset	66.3 (56.6–77.2)	69.0* (64.3-76.3)	62.8* (56.6-69.5)
Duration of illness	6.0 (4.0–9.3)	6.3 (4.8-8.8)	5.6 (4.0-9.3)
Age of death	71.7 (63.8–81.6)	75.3* (70.9-81.0)	68.1* (65.1-73.8)
Duration from final diagnosis till death	2.3 (0.1-6.3)	3.2* (1.0-6.3)	1.5* (0.8-2.3)
Cases where final clinical diagnosis was different from initial clinical diagnosis:	14 cases	4 cases	7 cases
Disease duration at final clinical diagnosis	4.2 (1.0-8.0)	3.6 (0.1-5.5)	4.1 (2.4-8.0)
Duration from final diagnosis till death	1.9 (0.0-3.8)	2.5 (1.0-3.8)	1.5 (0.8-2.3)

Table *2. Demographic parameters of 19 CBD cases.*

*Data are mean in years (range); *=p < 0.05 comparing between CBD-CBS and CBD-RS subgroups using Student's t-test.*

CBD-CBS=corticobasal degeneration-corticobasal syndrome; CBD-RS=corticobasal degeneration-Richardson's syndrome

Cases with pathological diagnosis of CBD

Clinical diagnoses

Of the 35 patients included in the present study, 19 (9 female, 10 male) had CBD pathology (Figure 4). Five of these had been diagnosed with CBS at the time of death. The diagnoses in the other 14 cases were PSP (8), Parkinson's disease (2), FTD (1), Pick's disease (1), spastic quadriparesis with myoclonus (1) and Gilles de la Tourette's syndrome (1). At the time of first neurological specialist review, eight had been initially diagnosed with Parkinson's disease, two with PSP and the rest with other movement disorders or dementia syndromes.

Incidental finding of CBD pathology

One patient had the incidental finding of CBD pathology at post-mortem. The patient developed motor tics at age eight followed by vocal tics several years later and was followed-up with the psychiatrists at the National Hospital for Neurology and Neurosurgery, during which he joined the QSBB brain donor programme. Four generations of his family had also had tics. At age 63, he died of metastatic carcinoma of the prostate. Before his death, there was no documentation of any neurological symptoms or signs suggestive of a progressive neurodegenerative disorder.

Clinical features

The demographic features of all pathologically confirmed CBD cases are summarized in Table 2 and are similar to those reported in previous studies (Gibb *et al.*, 1989, Grimes *et al.*, 1999, Litvan *et al.*, 1997, Murray *et al.*, 2007).

Nine CBD cases (47%) had a markedly asymmetrical presentation. Seven patients (37%) had delayed initiation of horizontal saccadic eye movements. Majority of cases with features suggestive of CBS were correctly diagnosed in life. Twelve (63%) were noted to have a vertical supranuclear gaze palsy (VSGP). Initial clinical presentation of

parkinsonism was observed in nine (47%), aphasia in three (16%) and memory decline characterised by retrieval difficulties in seven (37%), four of whom also had executive dysfunction. Six of 12 patients with initial gait difficulty also had symmetrical bradykinesia. Sixteen patients (84%) had been prescribed levodopa, nine of whom (56%) had transient mild to moderate improvement and three (17%) had developed reversible and dose-related dystonic or choreiform movements of the limbs. Focal asymmetrical cortical atrophy was found in only three out of the 14 cases (21%) who had had a brain MRI performed.

CBD subgroups with different clinical diagnoses

Clinical diagnosis of CBS

Four of the five pathologically confirmed CBD cases who had been eventually considered to have CBS (CBD-CBS) had received an alternative earlier diagnosis: Parkinson's disease (2), PSP (1) and spastic paraparesis (1). All had presented with a clumsy useless limb and had subsequently developed symptoms of ideomotor apraxia and action myoclonus (Table 3). Focal limb dystonia (4), cortical sensory loss (4), alien limb (3) and stimulus-sensitive myoclonus (2) were also noted. Delayed initiation of horizontal saccades was observed in three cases. Of the four patients who had received levodopa, only one had a positive levodopa challenge test with transient improvement in mobility and subsequently developed a reversible levodopa induced foot dystonia.

None of the three cases who had a brain MRI performed showed focal or asymmetrical cortical atrophy. One patient had decreased speech fluency at presentation which later manifested as PNFA with preserved comprehension.

Clinical features	CBD-CBS (N=5)	CBD-RS (N=8)	*P*-values
Classical CBS phenotype:			
Initial useless limb	5	0	0.001*
Asymmetrical features	5	2	0.02*
Alien limb	3	0	0.04*
Limb dystonia	4	1	0.03*
Cortical sensory loss	4	0	0.002*
Limb apraxia	5	1	0.005*
Action myoclonus	5	2	0.02*
Delayed initiation of saccade	3 (nk: 1)	3	ns
Latency from first symptom onset to onset of delayed initiation of saccade	4.7years (2.5-7.7)	3.7years (2.5-5.8)	ns
Classical PSP phenotype:			
Symmetrical bradykinesia	0	6	0.02*
VSGP	2 (nk: 1)	8	0.04*
Predominant downgaze abnormalities	0	3	ns
Duration from first symptom to onset of VSGP	5.9years (4-7.7)	3.4years (1.2-7.3)	ns
Duration from first symptom to onset of falls	2.4years (1.0-3.0)	1.0years (0.0-3.0)	0.04†
Early falls within 2 years	1	7	0.03*
Predominant backward falls	2	5	ns
Frontal release signs	2	7	ns
Apraxia of eyelid opening	0	5	ns
Frontalis hyperactivity	0	4	ns
Neuropsychiatric features:			
Initial behavioural change	0	4	ns
Initial memory decline	1	3 (nk: 1)	ns
Initial aphasia	1	1 (nk: 1)	ns
Orobuccal apraxia	1	0 (nk: 1)	ns
Hallucination	0	1	ns
Other motor disabilities:			
Initial parkinsonism	2	4	ns
Tremor	2	2	ns
Pyramidal signs	2	4	ns

Table 3. *Clinical features of CBD-CBS and CBD-RS subgroups.*

*nk=not known; ns=not statistically significant; *=$p < 0.05$ comparing between CBD-CBS and CBD-RS subgroups using χ^2; †=$p < 0.05$ comparing between CBD-CBS and CBD-RS subgroups using Student's t-test.*

Clinical diagnosis of PSP

Among the eight CBD cases who had been diagnosed with PSP, seven were initially considered to have had a different disorder: Parkinson's disease (3), unclassifiable akinetic rigid syndrome (1), multiple system atrophy (1), Alzheimer's disease (1) and FTD (1). All of them had a VSGP (8)(Table 3). Seven had presented with gait difficulty and five had prominent postural instability with backward falls in the first year. Other symptoms and signs pointing in hindsight towards a clinical diagnosis of PSP were apraxia of eyelid opening (5), frontalis hyperactivity (4), frontal and subcortical cognitive impairment (4). All patients were prescribed levodopa, six of whom experienced transient mild to moderate improvement and two of whom developed levodopa induced dyskinesia. The MRI brain of one patient showed unequivocal midbrain atrophy. One patient had aphasia at presentation characterised by fluent phonemic paraphasia.

Not surprisingly, the clinical features of this PSP-lookalike subgroup (CBD-RS) were very different from the CBD-CBS subgroup. There were more CBD-CBS patients with initial presentation of a useless limb, limb apraxia and dystonia, action myoclonus, cortical sensory loss, alien limb and asymmetrical clinical features ($p < 0.05$, Table 3). On the other hand, there were more CBD-RS patients with VSGP, early falls and symmetrical bradykinesia ($p < 0.05$, Figure 5).

One of the two CBD-RS patients with asymmetric presentation manifested clinical features of CBS at late stage, whereas the other had recorded absence of these features (Figure 5).

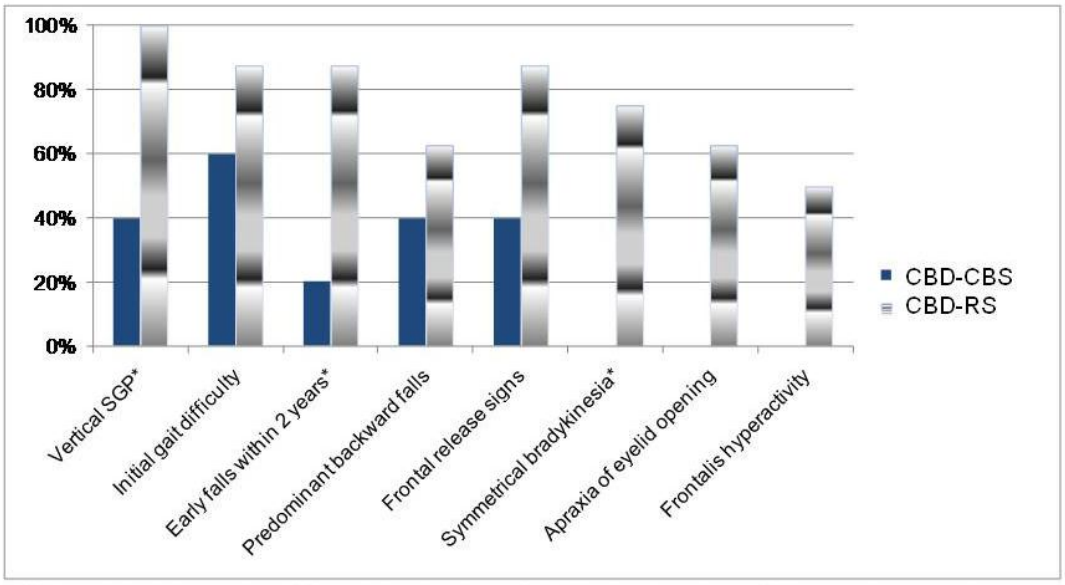

Figure 5. *Clinical features of CBD-CBS and CBD-RS subgroups.*

**=p < 0.05 comparing between CBD-CBS and CBD-RS subgroups using χ^2test; SPG=supranuclear gaze palsy*

Clinical diagnosis of Parkinson's disease

Two patients presented with strongly asymmetrical tremor-predominant parkinsonism and the presumptive diagnosis of Parkinson's disease remained unchanged throughout the disease course. Review of the notes, however, revealed a poor levodopa response, short duration of disease (4.5 and 5.7 years), early onset of falls (0.1 and 2.8 years) and early wheelchair dependence (4 and 5 years). The hand tremor was coarse, jerky and more obvious on movement than at rest and neither patient had a classical pill-rolling resting tremor. One patient had perseveration, apraxia of eyelid opening and developed visual hallucinations after an increase of levodopa to 800mg/day. Ocular movements were not recorded at late stage of disease in one patient.

Clinical diagnosis of Pick's disease

One patient presented with verbal communication and language difficulties (PNFA-phenotype) and was initially diagnosed with depression. Three years after the onset of her first symptoms, she was noted to have orofacial apraxia and dysphagia, and two years later, she developed prominent abulia and anhedonia (FTDbv-phenotype). In 2004, she was diagnosed with Pick's disease. Seven years into her illness, she developed limb apraxia, a symmetrical akinetic rigid syndrome, gait difficulty, falls and a VSGP with delayed initiation of saccades in all directions. She died at age 74 after a disease course of nine years.

Clinical diagnosis of FTD

This patient presented with an asymmetrical tremor-predominant parkinsonism in association with early striking behavioural change and memory impairment. He was initially diagnosed with Parkinson's disease although there was no response to therapeutic doses of levodopa. Four years into his illness, disinhibition, anti-social behaviour and excessive spending were striking clinical features and the diagnosis was revised to FTD. A few months before death, he was noted to have a VSGP with predominant restriction of downgaze. The patient died at the age of 69 years after a disease course of five years.

Clinical diagnosis of spastic quadriparesis with myoclonus

This patient presented with difficulty walking and recurrent falls and examination revealed bilateral pyramidal leg weakness with decreased vibration sense. She was first investigated for possible multiple sclerosis. In the following two years, the pyramidal weakness gradually involved her arms and she developed generalized marked action myoclonus and a symmetrical akinetic rigid syndrome without response to levodopa. She had pathological reflexes, equivocal plantar responses and normal cortical sensation. In 1995, despite extensive investigations, no underlying cause for her spastic quadriparesis was identified. Differential diagnosis shortly before death included spinal interneuronitis and primary lateral sclerosis. MRI brain showed symmetrical cerebral atrophy with focal atrophy in the peri-Rolandic area. The patient died at age 75 years after a disease course of eight years.

Secondary pathologies

One of the five clinically diagnosed CBS patients, who died aged 77 years, had Lewy body pathology in the neocortex and brain stem and moderate cerebral amyloid angiopathy (CAA). Another who presented with right hand apraxia and alien limb, had cerebral infarction of the left middle cerebral artery territory in addition to the characteristic pathological changes associated with CBD.

Two of the eight cases, who had been clinically diagnosed as PSP, had additional pathological findings. One patient who died at age 74, with a history of early falls, VSGP, apraxia of eyelid opening in association with frontal executive dysfunction and one recorded episode of hallucination, was found to have severe CAA and pathological ageing with Aβ deposition in the neocortical and medial temporal regions. Another patient, who initially presented with walking difficulties followed by the development of classical RS, was found to have small vessel cerebral vascular disease with Binswanger-type cerebral white matter changes.

The patient, who was clinically diagnosed with Pick's disease, had mild small vessel cerebral vascular disease with pathological ageing.

Cases with clinical diagnosis of CBS

There were 16 cases with CBS which did not have associated CBD pathology. There were six cases with PSP, five with Alzheimer's disease, two Parkinson's disease, two with FTLD-TDP and one with FTLD-FUS. The clinical features of the cases with CBS are summarized in Table 4.

Although five out of the six cases with pathological changes of PSP and CBS had asymmetrical clinical features, they also manifested some features which might have suggested PSP: VSGP was noted in three cases and early falls within two years of onset of first symptoms was also reported in three cases. Four had prominent frontal release signs including positive palmomental and grasp reflex, glabellar tap and utilization behaviour, and one of whom had frontal cognitive dysfunction. The mean onset of VSGP was 2.2 years from first symptom onset; this short duration had borderline statistical significance when compared to cases with CBD, Parkinson's disease and FTLD pathologies ($p = 0.09$). There were significantly more PSP (100%) and CBD cases (80%) with unprovoked falls when compared to the Alzheimer's disease cases (0%; $p = 0.005$). Apraxia of eyelid opening was noted in three PSP cases while none of the CBD or Alzheimer's disease cases had this feature ($p = 0.06$). Parkinsonism at presentation was observed in both the cases with Parkinson's disease, three of the PSP cases, but in none of the Alzheimer's disease cases ($p = 0.07$). One patient with Alzheimer's disease presented with word-finding difficulty followed by expressive aphasia.

Both cases with Parkinson's disease also had focal limb dystonia in late stage of disease and one had limb apraxia. The mean duration of illness of the cases with Parkinson's disease (17.3 years) was much longer than the rest of the cases ($p = 0.01$). Both had moderate response to levodopa for prolonged duration of 5.3 and 19.8 years respectively.

The patient with FTLD-TDP-MND presented with dyspraxia of the right hand followed by speech and swallowing difficulty. Nerve conduction studies revealed chronic partial denervation and fasciculations in the late stages of the illness. Another patient with FTLD-TDP subtype 2 presented with clumsiness of one arm followed by

behavioural changes suggestive of FTD-like syndrome. The patient had early marked delayed in initiation of horizontal saccades. The patient with FTLD-U presented with depression, limb apraxia and dystonia. Cognitive decline and behavioural changes followed.

Secondary pathologies

Of the cases with PSP pathology (N=6), one had mild CAA. Among the cases with Alzheimer's disease (N=5), one had incidental Lewy body pathology, one had CAA and one had small vessel cerebral vascular disease. One of the cases with Parkinson's disease had minor limbic cortical Lewy body involvement while the other had moderate neocortical Lewy bodies. The patient with FTLD-TDP with MND pathology also had Alzheimer pathology (Braak and Braak stage II) and small vessel cerebral vascular disease.

	Cases with CBS (N=21)				
Clinical features	**CBD pathology (N=5)**	**PSP pathology (N=8)**	**AD pathology (N=5)**	**PD pathology (N=2)**	**FTLD pathology (N=3)**
CBS phenotype:					
Initial useless limb	5	2	2	0	1
Asymmetrical features	5	5	2	1	2
Alien limb	3	1	2	0	0
Limb dystonia	4	4	0	2	2
Cortical sensory loss	4	2	5	0	0
Limb apraxia	5	4	5	1	2
Action myoclonus	5	3	5	0	0
Latency from first symptom onset to onset of myoclonus	2.7years (1.3-4)	4.1years (1.5-6.8)	4.0years (2.0-6.5)	na	na
Delayed initiation of saccade	3	3 (nk: 1)	1	0 (nk: 1)	1
PSP phenotype:					
VSGP	2	3	1	0	2
Predominant downgaze abnormalities	0	2	1	1	0
Duration from first symptom onset to VSGP	5.9years (4-7.7)	2.2years (1.7-3.1)	2.0years (2.0-2.0)	na	na
Early falls within 2 years	1	3	1	0	1
Unprovoked falls	4	6	0	0	2
Frontal release signs	2	4	1	2	2
Apraxia of eyelid opening	0	3	0	0	2
Neuropsychiatric features:					
Initial memory decline	1	1 (nk: 1)	4	0 (nk: 1)	1
Initial aphasia	1	0 (nk: 1)	1	0 (nk: 1)	0
Hallucination	0	0	1	1	0
Other motor disabilities:					
Initial parkinsonism	2	3	0	2	0
Pyramidal signs	2	4	1	0	1

Table 4. *Clinical features of cases with CBS*

na=not applicable; nk=not known; VSGP=vertical supranuclear gaze palsy

Diagnostic accuracy of CBD

Of the 19 pathologically diagnosed CBD cases, only five were accurately diagnosed as CBS in life, giving a sensitivity of 26.3% (Table 5). The positive predictive value (PPV) of the clinical diagnosis of CBS was 23.8%, with five cases confirmed to have CBD pathology out of 21 cases clinically diagnosed with CBS. The high specificity for CBD (98.9%) indicated that cases without CBD pathology were correctly identified in life as not having the condition.

The PPV for a clinical diagnosis of Parkinson's disease in the QSBB is 82.7% (564 out of 682), for PSP 69% (131 out of 190) and multiple system atrophy 70.1% (82 out of 117), figures which contrast strikingly with those obtained for CBD in this study (Table 5).

The sensitivities for other major parkinsonian disorders were also much higher than in CBD (Table 5). The sensitivity for Parkinson's disease was 92.8% (564 out of 608), indicating the high proportion of cases with Parkinson's disease pathology that had been correctly diagnosed with Parkinson's disease in life. The sensitivity for PSP was 73.2% (131 out of 179) and for multiple system atrophy was 70.1% (82 out of 117). In agreement with previous studies, the specificity for Parkinson's disease was lower than other parkinsonian disorders, suggesting Parkinson's disease was over-diagnosed (Litvan *et al.*, 1998).

Disorder	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
CBD	26.3% (9.1-51.0)	98.9% (98-99.4)	23.8% (8.2-47.2)	99.0% (99-99.5)
PSP	73.2% (66-79.5)	95.3% (94-96.4)	69.0% (61.8-75)	96.2% (95-97.2)
MSA	70.1% (60.9-78)	97.4% (96-98.2)	70.1% (60.9-78)	96.2% (95-97.2)
PD	92.8% (90-94.7)	85.8% (83.3-88)	82.7% (80-85.5)	94.2% (92.3-96)

Table 5. *Diagnostic accuracy for CBD and other parkinsonian disorders*

Data was generated from 1440 archival cases in the QSBB over a 20-year period; CI=confidence interval

Studies	CBD cases	Cases accurately diagnosed in life	Sensitivity (%)
Grimes *et al.* (1990)	13	4	31
Boeve *et al.* (2005)	32	18	56
Murray *et al.* (2007)	15	6	40
	Total = 60	Total = 28	Overall sensitivity = 47%

Table 6. *Summary of sensitivity in predicting CBD pathology in life*

Discussion

A previous study of 143 cases of parkinsonism followed by movement disorder specialists at the National hospital for neurology and neurosurgery reported that of four cases with CBD pathology, only one had been diagnosed correctly in life; whereas in the three cases with clinical CBS, only one had CBD pathology at post-mortem (Hughes *et al.*, 2002). Diagnostic accuracy of CBD does not appear to have improved in the decade since this paper was published, suggesting that the clinical spectrum of CBD remains poorly delineated and that the nosological entity first described by Rebeiz and colleagues is extremely difficult to diagnose accurately in life. The low sensitivity in predicting CBD reported in this study is consistent with the findings of other published clinicopathological series (Boeve, 2005, Grimes *et al.*, 1999, Litvan *et al.*, 1997, Murray *et al.*, 2007). It is also evident that CBS is much more likely to be caused by a pathology other than that described as characteristic of CBD which is reflected by the low PPV of 23.8% in the present series. Five of the 11 cases with CBS presentation followed by movement disorder specialists were confirmed to have CBD pathology. The PPV for movement specialists is 45.5%, which is almost twice as high as the overall PPV. The remaining 10 cases diagnosed with CBS, had been followed by general neurologists or cognitive neurologists, none of which had CBD pathology.

CBD cases correctly identified in previous published series manifested all the classical features reported in the seminal description by Rebeiz and colleagues, but together they constituted less than half of all cases with CBD pathology (28 out of 60, Table 6). Most of the cases in this series had been followed by movement disorder specialists, which differs from some of the other reported series where referral came primarily from cognitive disorder clinics (Boeve, 2005, Murray *et al.*, 2007, Shelley *et al.*, 2009). The difference in referral source almost certainly accounts for the marked overlap between CBD and PSP identified in this series whereas in the publications coming from cognitive disorder clinics, confusion between CBD and FTD, PNFA and Alzheimer's disease was more frequent (Alladi *et al.*, 2007, Hodges *et al.*, 2004, Josephs *et al.*, 2008). Hodges and colleagues reported nine cases with a clinical diagnosis of CBS, of whom seven had been confirmed to have CBD pathology and the other two cases had FTLD (Hodges *et al.*, 2004). In a series of 100 patients with

pathologically confirmed focal cortical syndromes 12 presented with CBS and six of them had CBD pathology while the other six fulfilled pathological criteria for Alzheimer's disease (Alladi *et al.*, 2007). Conversely, of the 12 cases with CBD pathology, on autopsy, six had presented with CBS, four with FTDbv and two with PNFA.

Some previous publications have reported overlap between CBD and PSP but to a significantly lesser extent than found in this study (Boeve, 2005, Josephs *et al.*, 2006, Murray *et al.*, 2007). In the Mayo Clinic series, seven out of 32 CBD cases (22%) had been diagnosed with PSP in life and six out of 36 cases (17%) with CBS were found to have PSP pathological changes (Boeve, 2005). In one study, where 10 pathologically diagnosed CBD cases were reconstructed as case vignettes and presented to neurologists, 42% of CBD cases received a clinical diagnosis of PSP (Litvan *et al.*, 1997). In the present study, eight out of 19 CBD cases had been diagnosed with PSP (42%) and of the 21 cases clinically diagnosed with CBS, six were found to have PSP pathology (29%).

In the present series, there are two main clinical variants which present to movement disorder specialists and are associated with the pathological changes considered characteristic of CBD. The term, CBD-CBS, has been provisionally used to refer to patients who present with the classical features first described by Rebeiz and colleagues and subsumed under the generic rubric of the CBS. The more problematical and more numerous group are patients who closely resemble classical PSP and which have been termed CBD-RS. These cases are characterised by VSGP, early falls within two years and symmetrical bradykinesia and, therefore, had been given a clinical diagnosis of PSP (Figure 5). Four of eight cases in the CBD-RS subgroup would have fulfilled the probable NINDS-PSP diagnostic criteria, the other four being excluded on the grounds of amnesia and aphasia (Litvan *et al.*, 1996). A previous QSBB study identified VSGP, early falls and fronto-limbic cognitive dysfunction as the characteristic features of RS (Williams *et al.*, 2005). Symmetrical motor handicap has been considered a helpful diagnostic pointer in distinguishing PSP from CBD, and represented one of the reasons why some of the CBD-RS cases had been clinically diagnosed with PSP. Post-mortem studies showed that aphasia and dementia can be presenting features in both CBD and PSP (Grimes *et al.*, 1999, Josephs *et al.*, 2008,

Masliah *et al.*, 1991). Of interest, the age of symptom onset and age of death were significantly younger in the CBD-RS subgroup (62.8 years and 68.1 years) compared to the CBD-CBS subgroup (69 and 75.3), although considerable overlap makes this an unreliable distinguishing criterion in the individual case (Table 2).

Delayed initiation of saccades may be helpful as a feature suggestive of underlying parietal lobe dysfunction and this ocular abnormality has been shown to correlate with the degree of limb apraxia in cases with CBS (Vidailhet *et al.*, 2000), however, this can be a difficult clinical sign to elicit with certainty. A supranuclear gaze paresis in both the vertical and horizontal plane can also be observed in CBD but when present it is usually in the terminal phase of the illness. In PSP, vertical saccades are slow and hypometric and are more affected than horizontal ones (Vidailhet *et al.*, 1994). Clinical diagnostic criteria for PSP have emphasised the importance of downgaze abnormalities (Lees, 1987, Litvan *et al.*, 1996, Williams *et al.*, 2005).

The present study identified RS as the most common phenotypic presentation of CBD, while the classical presentation of CBS was only found in a quarter of CBD cases. The clinical features in CBD-RS cases closely resemble classical cases with PSP pathology. The findings in this study indicated that some clinical features might point to underlying CBD pathology but further validation of these differences will be required. For instance, VSGP was detected at a later stage in CBD-RS (3.4years) than in cases with PSP pathology and CBS (2.2years). VSGP predominantly affecting downgaze was infrequent in CBD-RS (3 in 8). Delayed initiation of saccades noted in three CBD-RS cases (38%) can be helpful when present in distinguishing CBD-RS from PSP-RS. This feature, however, is not specific for CBD pathology and can be found in cases with PSP and Alzheimer's disease pathology presenting with CBS. Age of symptom onset in the CBD-RS subgroup (62.8years ±3.7) was younger than the archival QSBB cases with PSP pathology (66.4years ±12)(Williams *et al.*, 2005). Nevertheless, until more reliable diagnostic markers become available, the division of CBD-CBS and CBD-RS subtypes can only be confirmed retrospectively. The classical PSP phenotype was accountable for over a third of all CBD cases, whereas PSP was the most common pathology underlying cases with CBS presentation, emphasising the considerable diagnostic difficulties and significant overlap between these two primary 4R tauopathies. Conversely, among the QSBB archival collection of PSP cases, CBS is

a rare clinical presentation occurring in only six of the 179 pathologically diagnosed PSP cases (3%), and only five out of 160 PSP cases (3%) in the Mayo Clinic series were found to have CBS presentation (Tsuboi *et al.*, 2005).

Three other clinical presentations of CBD were also noted in this study: (i) a rapidly progressive levodopa unresponsive asymmetrical tremulous-parkinsonism with early postural instability, (ii) PNFA and (iii) FTDbv. The clinical presentation of PNFA and FTDbv are both recognised phenotypes of CBD (Geda *et al.*, 2007, Josephs *et al.*, 2006, Murray *et al.*, 2007). CBD can also present as an amnestic syndrome or posterior cortical atrophy, and is sometimes misdiagnosed as Alzheimer's disease (Boeve, 2005, Grimes *et al.*, 1999).

As few as one in four cases with clinical CBS who come to autopsy are associated with CBD pathology, suggesting that previous studies based on clinical diagnostic criteria and neuroimaging studies alone could be considered as unreliable. Post-mortem series prior to the publication of validated neuropathological criteria (Dickson *et al.*, 2002) may also have introduced error due to the overlapping pathological findings of CBD with other tauopathies such as PSP and Pick's disease. Secondary pathologies observed in 12 out of the 35 cases may in some cases have influenced the phenotype. Specific topographic distribution of tau pathology has been reported to correlate with clinical phenotypes in a small study which reported an increased tau burden in mid-frontal and inferior-parietal cortices in three PSP cases who presented with CBS (Tsuboi *et al.*, 2005).

Clinical presentation of CBS can be associated with a variety of underlying pathologies. If the disease course is very malignant with rapid clinical progression, the differential diagnoses of FTLD-TDP with progranulin mutation, FTLD-FUS and prion disease should be considered. If the symptom onset is acute followed by a static course, cerebrovascular disease is more likely whereas slow symptom progression with long disease duration along with sustained levodopa response are more suggestive of Parkinson's disease. *MAPT* or progranulin mutation should be considered in cases with at least one first degree relative with CBS, dementia or primary progressive aphasia (Cairns *et al.*, 2007, Le Ber *et al.*, 2008, Tartaglia *et al.*, 2009). A previous study identified initial episodic memory complaints as a specific predictor for Alzheimer's

disease with CBS presentation (Shelley *et al.*, 2009). In the present series, initial memory decline was also more common in Alzheimer's disease with CBS while none of the CBD-CBS cases had this feature. On the other hand, cortical sensory loss and action myoclonus were equally common in both Alzheimer's disease presenting with CBS and CBD-CBS subgroups, and it is interesting to note that action myoclonus was an early feature (within first 3 years of illness) in three of five cases of Alzheimer's disease presenting with CBS.

This study has identified three features which are likely to be helpful in excluding CBD in cases presenting with CBS, namely, a sustained initial levodopa response for over 2 years, early VSGP within 2 years of first symptom onset, and finally, a disease duration of over 10 years. By applying these criteria retrospectively, two cases with Parkinson's disease and two cases with PSP could be excluded, along with another case diagnosed with FTLD-TDP with MND supported by electrophysiological features. These more stringent criteria allow the exclusion of five false positive cases; accuracy in predicting CBD pathology in patients with CBS is improved from 23.8% to 31.3% (5 of 16 cases) and accuracy in predicting tau pathology in CBS reaches 69% (5 CBD, 6 PSP out of 16 cases). CBS is much more sensitive in predicting tau pathology than CBD pathology. Other post-mortem series had an overall 83% of CBS cases with tauopathy (Wadia *et al.*, 2007). PSP-like syndrome and PNFA are other clinical presentations that were identified to accurately predict underlying tau pathology (Hodges *et al.*, 2004, Josephs *et al.*, 2006). In the present series, 14 out of 19 CBD cases (73%) had a clinical presentation of either CBS, PSP or PNFA.

CBD and PSP are sporadic diseases sharing important biological and morphological features. In both conditions, the possession of H1 allele of the *MAPT* gene, and in particular, the H1/H1 genotype is a risk factor and the accumulation of 4R-tau in both neuronal and glial inclusions is characteristic (Baker *et al.*, 1999, Houlden *et al.*, 2001). These similarities have led some authors to propose that CBD and PSP are actually the same disorder with a wide spectrum of pathological and clinical manifestations (Scaravilli *et al.*, 2005). Nevertheless, their classical clinical presentations are strikingly different and the neuropathological diagnostic criteria of CBD and PSP were validated with high sensitivity and specificity (Dickson *et al.*, 2002, Litvan *et al.*, 1996). Although both conditions share extensive neuronal tau

pathology, astrocytic plaques of CBD are characteristic while in PSP tufted astrocytes are the hallmark glial lesion (Dickson, 1999). Furthermore Ishizawa et al have also demonstrated distinct patterns of microglial activation and tau distribution in pathologically diagnosed CBD and PSP cases (Ishizawa *et al.*, 2001). These findings support the notion that CBD and PSP are distinct if closely related clinicopathological entities which have a tendency to occur in patients sharing a similar genetic predisposition.

The greatest strength of this study is its size and the use of modern immunohistochemistry techniques to characterise the underlying pathology. All patients were reviewed by hospital specialists and the majority (60%) was followed-up at the National Hospital for Neurology and Neurosurgery, a tertiary referral centre, where atypical presentations, overlap syndromes and diagnostic conundrums are seen frequently. An over-inclusion of diagnostically more challenging cases occurs in any brain bank post-mortem series and the retrospective review of medical records has inherent limitations. The predominant referral from movement disorder clinics can also potentially lead to referral bias. Cognitive and language functions were not until recently consistently tested and recorded in those patients presenting with predominant motor handicap and could have resulted in underestimation of neuropsychiatric features. Six of 35 cases did not receive full neuropsychological evaluations. Nevertheless, the findings of this study are likely to be of relevance in neurological practice.

Conclusion

This study has identified a number of pathologically confirmed CBD cases whose clinical picture more closely resembled PSP than a CBS. CBD cases with PSP-like presentation often have delayed onset of VSGP after 3 years and infrequent observation of downgaze predominant abnormalities. Despite the clinical heterogeneity of CBD and the increasingly wide differential diagnosis of CBS, CBD should continue to be considered a distinct clinicopathological entity with an abnormality of tau aggregation which closely links it with PSP.

Chapter 3: Progressive supranuclear palsy presenting with corticobasal syndrome: a cortical variant

Introduction

The classic presentation of PSP, now known as PSP-RS, is characterised by early onset of postural instability with falls backwards, VSGP and frontal subcortical cognitive impairment (Steele *et al.*, 1964, Williams *et al.*, 2005, Williams *et al.*, 2008). Other well recognised clinical variants of PSP are PSP-P (Williams *et al.*, 2005, Williams *et al.*, 2010), PSP-PAGF (Ahmed *et al.*, 2008, Williams *et al.*, 2007), PSP-PNFA (Hodges *et al.*, 2004, Hu *et al.*, 2007, Josephs *et al.*, 2006, Kertesz *et al.*, 2005), PSP-FTDbv (Hassan *et al.*, 2011) and PSP-CBS (Tsuboi *et al.*, 2005). Clinicopathological studies have since demonstrated a close correlation between topographical severity of tau pathology and clinical phenotypes of PSP. For instance, severe tau pathology was identified in the inferior frontal gyrus in PSP-PNFA (Josephs *et al.*, 2006) and frontal and temporal cortices in PSP-FTDbv (Bigio *et al.*, 2001). Conversely, very mild cortical tau burden was observed in the PSP-PAGF subtype (Ahmed *et al.*, 2008, Williams *et al.*, 2007). Similar clinicopathological correlation was also identified in another closely related 4R tauopathy, CBD and its clinical phenotypes (Kouri *et al.*, 2011)

CBS commonly presents as progressive clumsiness and loss of function of one hand due to apraxia, an alien limb, cortical sensory loss, dystonia or levodopa-unresponsive rigidity, and it was initially described as the distinctive clinical presentation of CBD (Gibb *et al.*, 1989, Rebeiz *et al.*, 1967). Since its original description, multifarious other pathologies have been linked to a CBS presentation (Alladi *et al.*, 2007, Hodges *et al.*, 2004). From the QSBB archival cases, the most common underlying pathology for CBS is PSP (8 of 21) rather than CBD (5 of 21), however, only 4% of all pathologically diagnosed PSP cases (N=227) had a CBS presentation (PSP-CBS, Chapter 2). Previously, Tsuboi and colleagues quantified tau load in four selected

cortical regions, including cingulate gyrus, mid-frontal cortex, motor cortex and inferior parietal cortex in three PSP-CBS cases and eight randomly chosen PSP-RS cases (Tsuboi *et al.*, 2005). They reported an increased tau pathology in the mid-frontal and inferior-parietal cortices in PSP-CBS when compared to PSP-RS and concluded that the CBS presentation of PSP was either caused by a concurrent cortical pathology from a secondary process such as Alzheimer's disease or by the primary PSP-tau pathology involving the cortical regions (Tsuboi *et al.*, 2005). Nevertheless, it is uncertain if differences exist in the distribution of tau pathology in other brain regions or if the overall tau load is increased in the brains of PSP-CBS. It is noteworthy that imaging studies have identified predominant focal grey matter loss on voxel-based morphometry in the premotor cortex, posterior superior frontal lobe and supplementary motor area and relatively preserved brain stem grey matter in cases with PSP-CBS (Whitwell *et al.*, 2010). Therefore, this study was performed with the hypothesis that the distribution of tau pathology in PSP-CBS would resemble the distribution of grey matter loss identified by *in vivo* imaging in voxel-based morphometry.

The aims of this study were: (i) To validate the findings reported by Tsuboi and colleagues in a significantly larger cohort of PSP-CBS cases and to quantitatively assess tau distribution in more brain regions, (ii) To determine the cellular lesions which contribute to the tau pathology were characteristic of PSP pathology rather than Alzheimer-type NFT pathology and (iii) To assess the severity of neuronal loss in the substantia nigra and subthalamic nuclei and the extent of pathological involvement of the corticospinal tract.

Materials and methods

Case selection

Of the 227 PSP cases available in the QSBB archives between 1988 and 2010, nine had received a final clinical diagnosis of CBS during life (PSP-CBS, 3.9% of all PSP cases). An additional case, seen and diagnosed pathologically at the University of Nottingham, was also included. These ten PSP-CBS cases were matched with ten PSP-RS control cases for disease duration and age at death. The brain donor program of the QSBB was approved by a London Multi-Centre Research Ethics Committee and tissue is stored for research under a license from the Human Tissue Authority.

Medical record review

Systematic retrospective review of the medical records was carried out. All patients were assessed by at least one neurologist during life. Symptoms and clinical signs were recorded as being absent if they were not reported in the case notes. When the onset was not recorded, the onset was taken as the time when the particular clinical feature was first mentioned in the notes. If there were conflicting clinical features, the findings of the neurologist took precedence. The definitions for each selected clinical feature were described in Chapter 2.

Pathological material

The neuropathological diagnosis of PSP was confirmed in all 20 included cases. 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 at -80ºC, while the other half was immersed and fixed in 10% neutral formalin for three weeks before neuropathological examination. Tissue blocks were taken using standard protocols. Established pathological diagnostic criteria for PSP were used, requiring the presence of NFTs, NTs and glial tau pathology in different brain regions including the cerebral cortex, striatum, globus pallidus, subthalamic nucleus, midbrain, pons and

cerebellum together with neuronal loss and gliosis in basal ganglia and brainstem and cerebellar nuclei (Dickson *et al.*, 2011, Hauw *et al.*, 1994, Ince *et al.*, 2008, Litvan *et al.*, 1996). In each case, 8µm-thick tissue sections cut from the paraffin blocks and stained with haematoxylin and eosin (H&E) were used to assess neuronal loss and gliosis in the basal ganglia and substantia nigra. Immunohistochemistry with antibodies to phosphorylated tau (AT8; BioScience Life Sciences; 1:600), 3R and 4R tau (3R tau: RD3; 1:2000; 4R tau: RD4; 1:200)(de Silva *et al.*, 2003), microglia (CD68; Dako; PG-M1; 1:75), αB-crystallin (Novocastra; G2JF; 1:300), amyloid-β (Aβ) peptide (Dako; 6F/3D; 1:100) and α-synuclein (Vector Laboratories; KM51; 1:50) was performed using a standard avidin-biotin method. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol, followed by pressure cooker pre-treatment in citrate buffer pH 6.0. Sections were treated with 10% dried milk solution to block non-specific binding. Tissue sections were incubated with the primary antibody for one hour at room temperature, followed by biotinylated anti-mouse (Vector Laboratories, Burlingame, CA; 1:200) and ABC complex (Dako). Colour was developed with di-aminobenzidine/H_2O_2.

Additional pathologies were documented. Argyrophilic grains were identified by AT8 and αB-crystallin immunohistochemistry (Togo *et al.*, 2002) while Aβ cortical plaque pathology was characterised using the modified Consortium to Establish a Registry for Alzheimer's disease (CERAD) criteria (Mirra *et al.*, 1991). Alzheimer's type NFT pathology was determined using AT8 immunohistochemistry for Braak and Braak staging (Braak *et al.*, 2006). The presence of incidental Lewy body disease, cerebrovascular disease and CAA was documented. Only cases with limited Alzheimer-type NFT pathology of Braak and Braak stage III or less were recruited to avoid confounding the analysis of PSP-related tau pathology.

Regional tau quantitation with image analysis

Using coded slides, quantitative assessment of tau pathology, comprising of all tau-positive lesions including NFTs, PreTs, NTs, tufted astrocytes (TAs) and CBs was performed. Fifteen brain regions, which are known to be affected in PSP and whose involvement is predicted to contribute to the clinical features, were selected: the

posterior frontal cortex including the motor strip, cerebral cortex and subcortical white matter of the middle frontal gyrus (level: 1 cm behind the temporal pole), middle temporal gyrus (level: mammillary body) and parietal region (level: 1cm behind the splenium), caudate nucleus, putamen, globus pallidus, subthalamic nucleus, substantia nigra (level: emergence of the third cranial nerve), pontine base (including the pontine nuclei), cerebellar dentate nucleus and cerebellar white matter. The posterior frontal white matter was omitted from the analysis as the quantity was very small in some cases due to variability of routine sampling.

In each region, the images of ten random microscopic fields using a x20 objective were captured by a colour digital camera connected to the microscope (Nikon Microphot-FXA and Digit sight DS-L1) and processed with an image analysis software (Image Pro; MediaCybernetics®), converted to grey-scale images and labelling was measured in pixels. Threshold was adjusted to capture the two-dimensional area of all tau-positive lesions and the same threshold setting was used throughout the study. 'Areal fraction', defined by a ratio of the tau-positive immunoreactive pixels to the total number of pixels of the whole field was computed by Image Pro and tau load for each region, i.e. 'regional' tau load was expressed as percentage (areal fraction x 100%)(Gundersen *et al.*, 1988). 'Total' tau load was the sum of tau load in all fifteen brain regions. 'Cortical' tau load was the sum of tau load in seven regions, comprised of both grey and subcortical white matter in the anterior frontal, temporal and parietal regions and grey matter in the posterior frontal region. 'Basal ganglia' tau load was the sum of tau load in four structures: caudate nucleus, putamen, globus pallidus and subthalamic nucleus.

Quantitation of tau lesions

The different tau-positive lesions were quantified individually in ten random fields of three selected regions, posterior frontal cortex, anterior frontal cortex and caudate, where differences in regional tau load were found to be the most robust between PSP-RS and PSP-CBS. NFTs, PreTs, TAs and CBs were individually counted. NT pathology was quantified using a four-tiered semi-quantitative grading scale (0–3, with grade 0=no NT to grade 3=most severe NT).

Neuronal loss

Neuronal loss in the subthalamic nucleus and substantia nigra was determined using a four-tiered semi-quantitative grading system, while the case identity was blinded (0–3, with grade 0=no neuronal loss to grade 3=most severe neuronal loss). The substantia nigra was divided into five regions (medial, dorsomedial, dorsolateral, ventrolateral and lateral) for subregional assessment.

Corticospinal tract involvement

Microglial pathology of the corticospinal tract identified in the midbrain cerebral peduncles was assessed using CD68 immunohistochemistry, while the case identity was blinded. A semi-quantitative grade was used: grade 0=baseline microglial population to grade 3=most severe microglial pathology.

Tau biochemistry

Frontal cerebral cortex was used for tau biochemistry in two PSP-CBS, two PSP-RS cases and two pathologically diagnosed CBD cases with classic CBS presentation (CBD-CBS). Regional variation of phosphorylated tau species in PSP brains was previously reported (Puig *et al.*, 2005). However, tau protein extraction was limited to the frontal cortex in the present study.

Sarkosyl-Insoluble tau isolation

Isolation of sarkosyl-insoluble tau was carried out (Goedert *et al.*, 1992, Greenberg *et al.*, 1990). Brain tissue was homogenised in 10x volume (v/w) homogenisation buffer (10mM Tris–HCl pH 7.4, 0.8 M NaCl, 1 mM EGTA and 10% sucrose containing Complete protease inhibitor cocktail (Roche, Burgess Hill, UK). The suspension was then spun at 20,000 x *g* for 20 min at 4 ºC and the supernatant set aside. The pellet was re-suspended in 5x volumes of homogenisation buffer and re-centrifuged as above. The supernatants were combined and *N-lauryl* sarcosinate added to a concentration of

1% (w/v), and incubated at room temperature for one hour with shaking. The mixture was then centrifuged at 100,000 × *g* for 1 h at 4ºC. The sarkosyl-insoluble pellet was re-suspended in 50 mM Tris–HCL pH7.5 at 0.2 ml/g of starting material.

SDS-PAGE

Sarkosyl-insoluble tau was separated on 10% SDS-polyacrylamide gels and blotted onto nitrocellulose membranes using standard procedures. The blots were probed with a pan-tau rabbit polyclonal TP70 antibody that recognises the carboxy-terminus of tau (Brion *et al.*, 1993, Cairns *et al.*, 1997)(1/15,000; kind gift from Dr Diane Hanger, King's College, London) and IRDye 800CW Donkey Anti-Rabbit secondary antibody (Li-Cor Biosciences) followed by imaging on a Li-Cor Odyssey Infrared Scanner.

Haplotype analysis of the MAPT gene

Haplotype was determined by PCR typing of the 238 bp *MAPT* H2 deletion in intron 9 in seventeen cases (8 PSP-CBS, 9 PSP-RS) where frozen tissue was available for DNA extraction (Baker *et al.*, 1999, Hoglinger *et al.*, 2011).

Statistical analysis

The Mann-Whitney U test was used to compare tau load between PSP-CBS and PSP-RS. The null hypothesis (H0) was rejected if the *p*-value was <0.05 when 'total', 'cortical' and 'basal ganglia' tau load was assessed. For 'regional' tau load assessment, *p*-value of 0.0033 (0.05/15) was used to adjust for multiple comparisons; for tau-positive cellular lesion load, *p*-value of 0.01 (five different types of tau lesions: 0.05/5) was used. χ^2 / Fisher's exact test or the Student's t test was used to compare semi-quantitative grading or clinical data using *p*-value of 0.05. The intra-rater repeatability was assessed by repeating tau quantitation in four randomly selected cases (20%). The intraclass correlation coefficient was 0.80 ($p < 0.001$), indicating that the 'regional' tau load results were highly repeatable. The SPSS 17.0 programme was used for statistical analysis.

Results

Clinical features

PSP-CBS

All patients had been diagnosed with CBS/CBD by neurologists during life (Tables 7-8). Mean duration of first symptom onset to the final clinical diagnosis was 3.4 years. All cases had strikingly asymmetrical clinical features throughout the entire disease course; ten had ideomotor limb apraxia, eight had focal hand dystonia, five had distal myoclonus, three had an alien limb phenomenon, three had non-fluent aphasia, three had cortical sensory loss and two had hemisensory neglect. Delayed initiation of horizontal saccades was observed in three patients, two of whom also had head thrust at saccadic initiation (cases 2 and 5).

Seven patients developed ocular features suggestive of PSP including slow vertical saccades or VSGP but in most cases these occurred in the advanced stage of the illness. Two exceptions were cases 8 and 9, who developed VSGP within 4 years from symptom onset and an initial clinical diagnosis of PSP was considered, but was later revised to CBS, after the onset of asymmetrical cortical symptoms. Six patients developed postural instability or falls within the first year of symptom onset. Nevertheless, VSGP (χ^2, $p = 0.016$) and postural instability or early falls were still more frequent in PSP-RS than in PSP-CBS. Pyramidal signs were more frequent in PSP-CBS (N=5) than in PSP-RS (N=0)(χ^2, $p = 0.016$). Extensor plantars and hyper-reflexia were noted in five PSP-CBS patients, three of whom also had spasticity and one had pyramidal weakness, but none of these features was observed in PSP-RS.

PSP-RS

All PSP-RS patients had a final clinical diagnosis of probable PSP and had VSGP including downgaze abnormalities and early postural instability or falls (Tables 9-11). Three patients had cognitive decline and five had frontal type personality change characterised by apathy and abulia.

Features	**PSP-CBS**				
Case no.	**1**	**2**	**3**	**4**	**5**
Gender	M	F	M	M	M
Age at onset, years	55	63.8	60.4	60.5	79.3
Age at death, years	64.3	70.2	66.3	68.8	82.8
Disease duration, years	9.3	6.4	5.9	8.3	3.5
Initial clinical diagnosis	PD	CBS	C-Spond	CVD	PD
Final clinical diagnosis	CBS	CBS	CBS	CBS	CBS
Duration from onset to final diagnosis, years	6.3	3.5	5.2	2.1	1.5
Presenting symptom(s)	Balance difficulty	Useless arm	Useless arm	Jerky arm	Falls
Asymmetrical features	+	+	+	+	+
Limb apraxia	+	+	+	+	+
Alien limb	-	+	-	-	-
Cortical sensory loss	-	+	+	-	-
Hemi-neglect	-	+	-	-	-
Aphasia	-	+	-	-	-
Hand dystonia	+	+	+	+	-
Clenched fist	+	+	+	+	-
Myoclonus	-	-	+	+	+
Tremor	-	-	-	-	+
Delayed initiation of saccades	nk	+	nk	+	+
Slow vertical saccades	nk	-	nk	+	+
VSGP	+	-	nk	-	+
Postural instability within first years	-	+	-	-	+
Cognitive decline	-	+	-	+	+
Personality change	-	-	-	+	-
Pyramidal signs	+	+	-	+	+
Akinetic rigidity in first two years	+	-	-	-	+
Dysarthria in first 2 years	+	-	-	+	-
Dysphagia in first 2 years	-	-	-	nk	-
Levodopa response	-	-	-	-	mild
MAPT genotype	H1/H1	H1/H1	H1/H1	nk	H1/H1

Table 7. *Features of PSP-CBS (cases 1 - 5).*

C-Spond=cervical spondylosis; CVD=cerebrovascular disease

Features	**PSP-CBS**				
Case no.	**6**	**7**	**8**	**9**	**10**
Gender	F	F	F	M	F
Age at onset, years	66.3	60	64	77	73
Age at death, years	77.9	70.8	72.5	81	79.2
Disease duration, years	11.6	10.8	8.5	4	6.2
Initial clinical diagnosis	Depressed	CBS	PSP	PSP	CBS
Final clinical diagnosis	CBS	CBS	CBS	CBS	CBS
Duration from onset to final diagnosis, years	2.6	7	6	4	3
Presenting symptom(s)	Gait difficulty & cognitive slowing	Useless arm & balance difficulty	Falls	Balance difficulty & slurred speech	Useless arm & falls
Asymmetrical features	+	+	+	+	+
Limb apraxia	+	+	+	+	+
Alien limb	-	-	-	+	+
Cortical sensory loss	-	-	-	-	-
Hemi-neglect	-	-	+	-	-
Aphasia	+	-	-	+	-
Hand dystonia	-	+	+	+	+
Clenched fist	-	+	-	+	+
Myoclonus	-	+	-	+	-
Tremor	-	-	-	+	-
Delayed initiation of saccades	nk	nk	-	-	nk
Slow vertical saccades	+	nk	+	nk	+
VSGP	+	nk	+	+	-
Postural instability within first years	+	-	+	+	+
Cognitive decline	+	-	+	-	+
Personality change	+	-	-	-	-
Pyramidal signs	-	+	-	-	-
Akinetic rigidity in first two years	+	-	+	+	+
Dysarthria in first two years	-	-	-	+	-
Dysphagia in first two years	-	nk	nk	+	-
Levodopa response	-	-	-	-	Mild
MAPT genotype	H1/H2	H1/H1	H1/H1	nk	H1/H2

Table 8. *Features of PSP-CBS (cases 6 - 10).*

VSGP=vertical supranuclear gaze palsy

Features	**PSP-RS**				
Case no.	**1**	**2**	**3**	**4**	**5**
Gender	F	F	M	M	F
Age at onset, years	62	65.2	63	74.3	66
Age at death, years	69.8	71.3	69.5	79.5	81.7
Disease duration, years	7.8	6.1	6.5	5.2	15.7
Initial clinical diagnosis	Depressed	PD	Depressed	PD	PD
Final clinical diagnosis	PSP	PSP	PSP	PSP	PSP
Duration from onset to final diagnosis, years	4	4	2.5	3	7
Presenting symptom(s)	Falls & cognitive slowing	Falls	Falls & cognitive slowing	Falls	Slow up
Asymmetrical features	-	-	-	-	-
Limb apraxia	-	-	-	-	-
Alien limb	-	-	-	-	-
Cortical sensory loss	-	-	-	-	-
Hemi-neglect	-	-	-	-	-
Aphasia	-	-	-	-	-
Hand dystonia	-	-	-	-	-
Clenched fist	-	-	-	-	-
Myoclonus	-	-	-	-	-
Tremor	+	-	-	-	-
Delayed initiation of saccades	nk	-	-	nk	-
Slow vertical saccades	+	nk	+	nk	+
VSGP	+	+	+	+	+
Postural instability within first years	+	+	+	+	+
Cognitive decline	+	-	+	-	-
Personality change	+	-	+	+	-
Pyramidal signs	-	-	-	-	-
Akinetic rigidity in first two years	+	+	+	+	+
Dysarthria in first two years	+	nk	-	-	-
Dysphagia in first two years	+	-	-	-	-
Levodopa response	-	-	-	-	-
MAPT genotype	H1/H1	H1/H1	nk	H1/H1	H1/H1

Table 9. *Features of PSP-RS (cases 1 - 5).*

Features	**PSP-RS**				
Case no.	**6**	**7**	**8**	**9**	**10**
Gender	M	M	F	F	M
Age at onset, years	61	52.1	67	72	76
Age at death, years	78.3	61.3	73	79.1	80.7
Disease duration, years	17.3	9.2	6	7.1	4.7
Initial clinical diagnosis	CVD	PSP	PSP	PSP	PSP
Final clinical diagnosis	PSP	PSP	PSP	PSP	PSP
Duration from onset to final diagnosis, years	2.2	3	3	3	2
Presenting symptom(s)	Slurred speech	Balance difficulty	Falls	Falls	Falls
Asymmetrical features	-	-	-	-	-
Limb apraxia	-	-	-	-	-
Alien limb	-	-	-	-	-
Cortical sensory loss	-	-	-	-	-
Hemi-neglect	-	-	-	-	-
Aphasia	-	-	-	-	-
Hand dystonia	-	+	-	-	-
Clenched fist	-	-	-	-	-
Myoclonus	-	-	-	-	-
Tremor	-	-	-	-	+
Delayed initiation of saccades	-	-	-	-	nk
Slow vertical saccades	+	nk	+	+	nk
VSGP	+	+	+	+	+
Postural instability within first years	+	+	+	+	+
Cognitive decline	-	-	+	-	-
Personality change	-	+	+	-	-
Pyramidal signs	-	-	-	-	-
Akinetic rigidity in first two years	+	+	+	+	+
Dysarthria in first two years	+	+	nk	+	-
Dysphagia in first two years	-	+	nk	+	-
Levodopa response	-	-	-	-	-
MAPT genotype	H1/H1	H1/H1	H1/H1	H1/H1	H1/H1

Table 10. *Features of PSP-RS (cases 6 - 10).*

VSGP=vertical supranuclear gaze palsy

	PSP-CBS	**PSP-RS**	***P*-values** (Student's t test)
Mean age of symptom onset (mean years ± S.D.)	65.9 ± 8.0	65.9 ± 7.1	0.98
Mean age of death (mean years ± S.D.)	73.4 ± 6.4	74.4 ± 6.5	0.72
Mean disease duration (mean years ± S.D.)	7.5 ± 2.7	8.6 ± 4.4	0.51

Table 11. *Comparison of features between PSP-CBS and PSP-RS groups.*

Neuropathological findings

Both PSP-CBS and PSP-RS groups met established pathological criteria of PSP (Dickson *et al.*, 2011, Hauw *et al.*, 1994, Ince *et al.*, 2008). All inclusion types were immunoreactive for 4R tau by differential immunohistochemistry but negative for 3R tau in all cases, which was characteristic for PSP.

Regional tau load

The median 'regional' tau load in the posterior frontal cortical grey matter (PSP-CBS: 0.59; PSP-RS: 0.05), anterior frontal cortical grey matter (PSP-CBS: 0.06; PSP-RS: 0.03) and parietal subcortical white matter (PSP-CBS: 0.06; PSP-RS: 0.01) was significantly greater in PSP-CBS than in PSP-RS ($p < 0.0033$ in all). The median 'regional' tau load in the caudate (PSP-CBS: 0.14; PSP-RS: 0.49; $p < 0.001$), subthalamic nucleus (PSP-CBS: 0.21; PSP-RS: 0.40; $p < 0.001$) and cerebellar white matter (PSP-CBS: 0.02; PSP-RS: 0.06; $p = 0.007$ with borderline significance) was greater in the PSP-RS than in PSP-CBS (Figures 6-7).

The presence of delayed initiation of horizontal saccades in PSP-CBS had a moderate correlation with an increased total parietal tau load (Spearman's correlation coefficient=0.59; $p < 0.001$). However, other cortical features such as cortical sensory loss, alien limb phenomenon or hemisensory neglect did not correlate with the parietal tau load ($p > 0.05$) or other regional tau load.

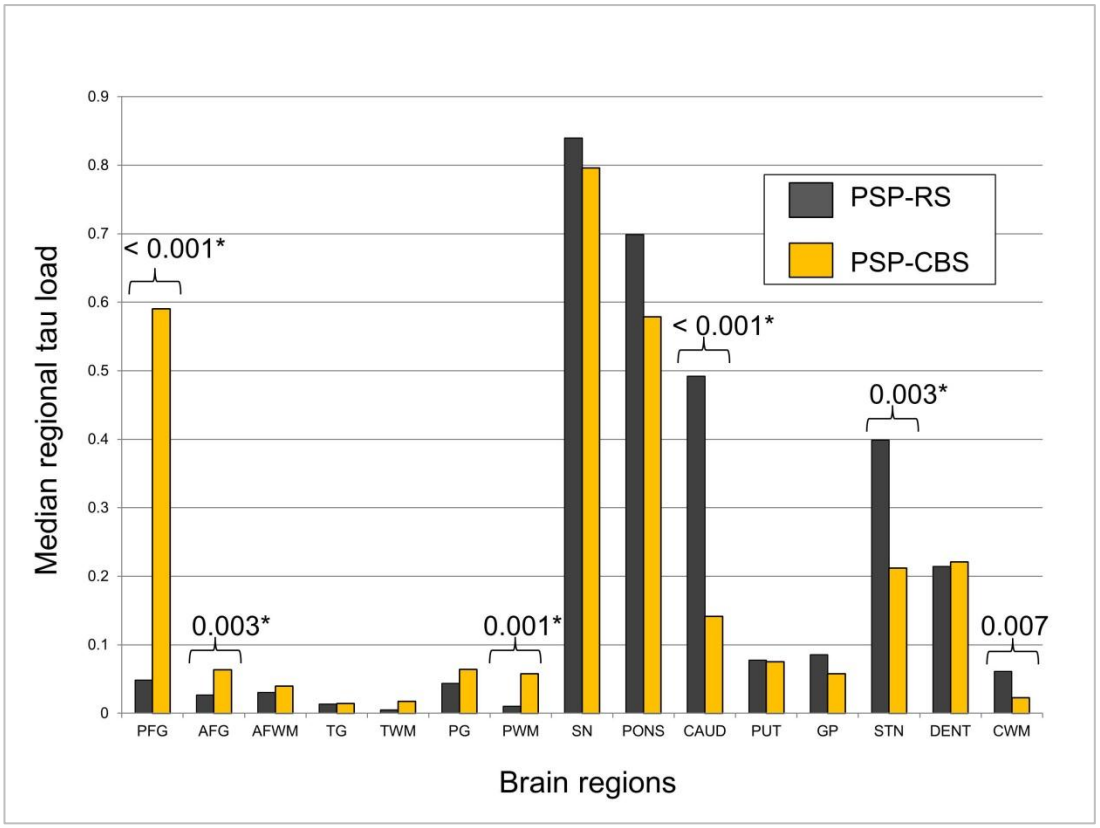

Figure 6. *Median regional tau load in PSP-CBS and PSP-RS.*

*Quantitative data illustrating median regional tau load in PSP-RS (black) and PSP-CBS (yellow) in fifteen selected brain regions. *= statistical significance adjusted for multiple comparisons, p < 0.0033 using the Mann-Whitney U test.*

PFG=posterior frontal grey matter; AFG=anterior frontal grey matter; AFWM=anterior frontal white matter; TG=temporal grey matter; TWM=temporal white matter; PG=parietal grey matter; PWM=parietal white matter; SN=substantia nigra; PONS=pons; CAUD=caudate; PUT=putamen; GP=globus pallidus; STN=subthalamic nucleus; DENT=dentate nucleus; CWM=cerebellar white matter.

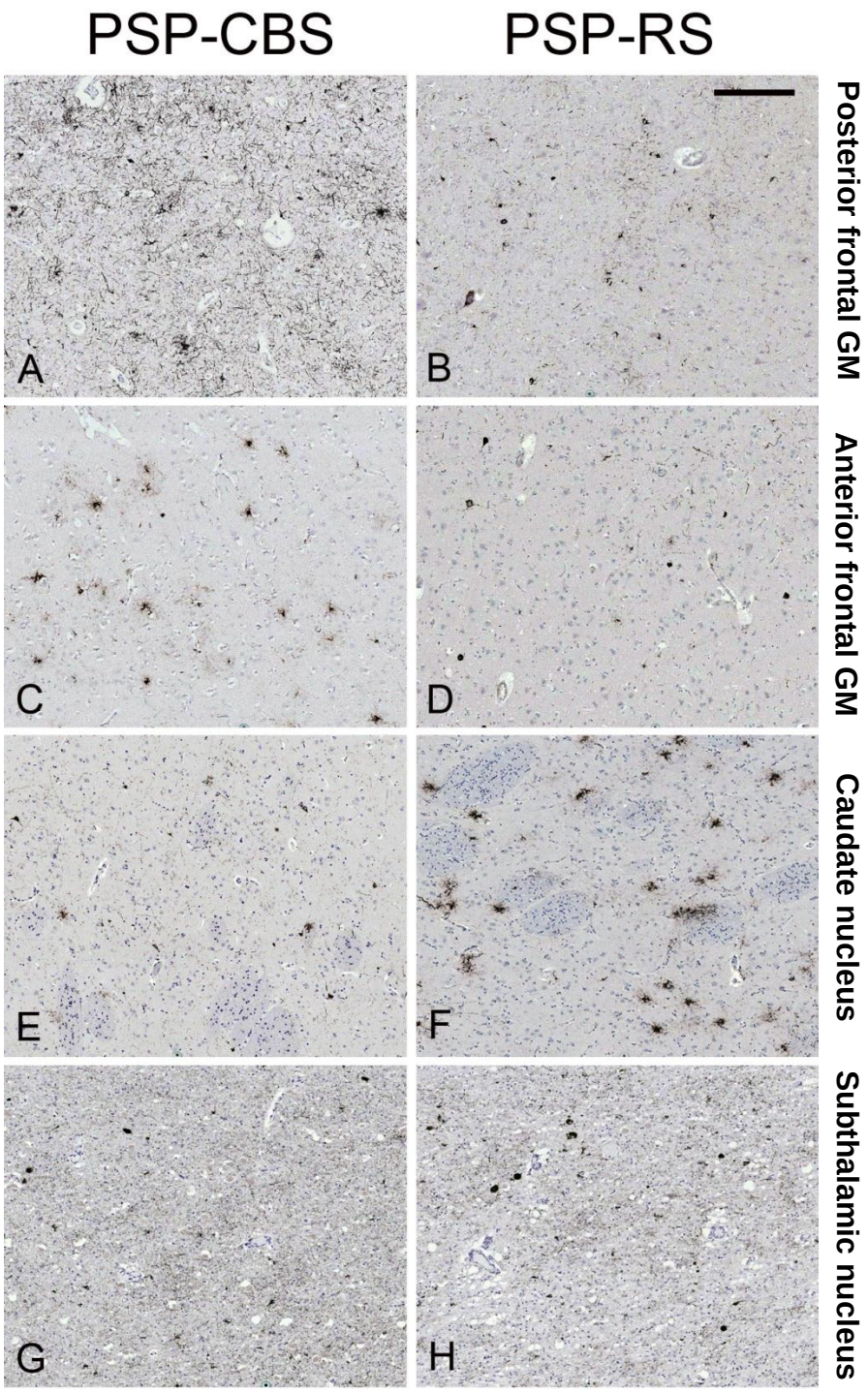

Figure 7. *Tau immunohistochemistry in selected brain regions.*

PSP-CBS has greater tau load in the posterior frontal and anterior frontal grey matter (GM) when compared with PSP-RS; whereas PSP-RS has greater tau load in the caudate and subthalamic nuclei than PSP-CBS. AT8 immunohistochemistry; scale bar in panel B=225microns in all the panels.

Total, cortical and basal ganglia tau load

There was no difference in 'total' tau load between the PSP-CBS and PSP-RS groups (p = 0.176, Figure 8). However, PSP-CBS had an increased 'cortical' tau load when compared with PSP-RS (p < 0.001), and the 'basal ganglia' tau load was greater in PSP-RS than in PSP-CBS (p = 0.003; Figure 8).

In five PSP-CBS cases, the half brains examined were contralateral to the side with the more predominant clinical symptoms and signs. The median 'total' and 'cortical' tau load were numerically, but not statistically, greater in these five cases (total tau load = 5.3; cortical tau load = 1.4) compared to the remaining PSP-CBS cases (total tau load=4.0; cortical tau load=1.0).

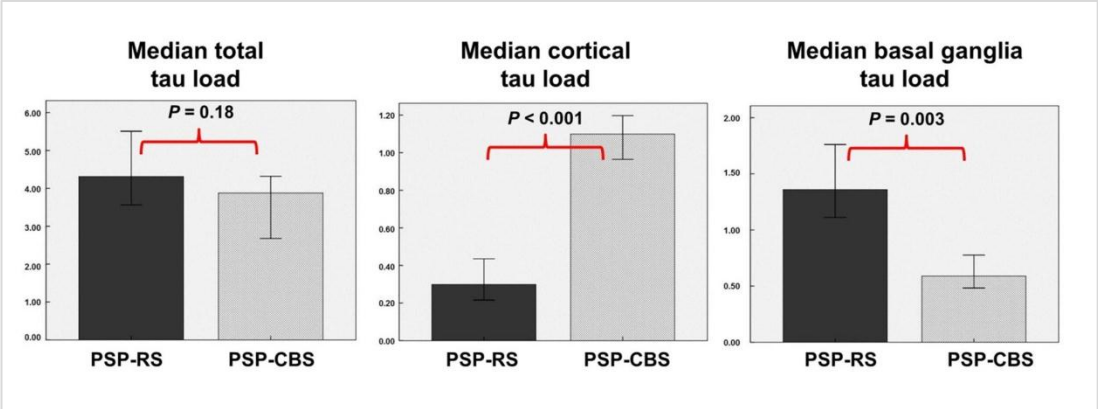

Figure 8. *Total, cortical and basal ganglia tau load in PSP-CBS and PSP-RS.*

Total tau load is the same between the two groups, but PSP-CBS has greater cortical tau load and less basal ganglia tau load than PSP-RS.

Mann-Whitney U test; Error bars represent 95% confidence interval.

Tau-positive cellular lesions

In the posterior frontal cortical grey matter, all types of tau lesions were more numerous in PSP-CBS than in PSP-RS (NFTs, TAs, CBs and NTs: $p < 0.001$; PreTs: $p = 0.005$). In the anterior frontal grey matter, there were numerically, but not statistically, more NFTs, CBs and NTs in PSP-CBS than in PSP-RS ($p > 0.01$ in all). In the caudate, there were more TAs, NTs and NFTs in PSP-RS than in PSP-CBS (TAs and NTs: $p < 0.001$, NFTs: $p = 0.01$).

Neuronal loss

In the subthalamic nucleus, the median semi-quantitative rating score for neuronal loss was moderate (grade 2) and there was no difference between the two groups (χ^2; $p \geq 0.05$). In the substantia nigra, neuronal loss was more severe in the dorsolateral (χ^2; $p = 0.033$) and ventrolateral (χ^2; $p = 0.018$) subregions in PSP-RS than in PSP-CBS (Figure 9).

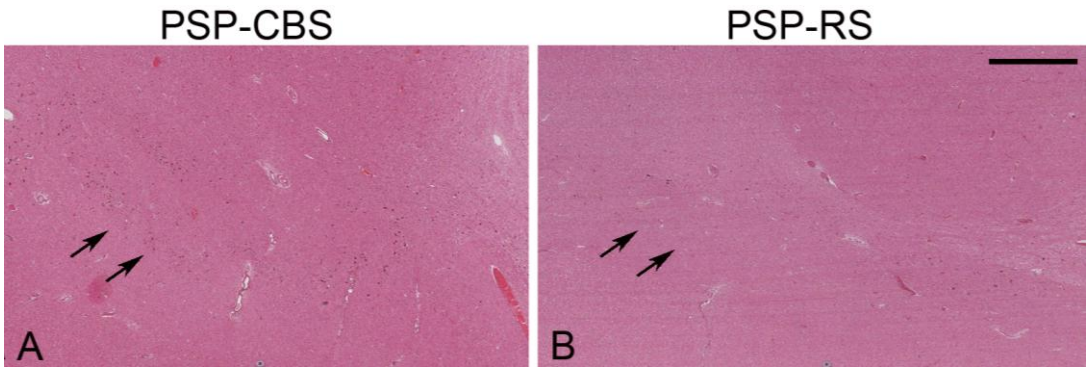

Figure 9. *Neuronal loss of substantia nigra in PSP-CBS and PSP-RS.*

Ventrolateral and dorsolateral subregions of the substantia nigra are relatively preserved from cell loss in PSP-CBS when compared with PSP-RS.

Haematoxylin and eosin method; scale bar in panel B=1135microns in both panels.

Corticospinal tract involvement

There was a more severe microglial response in the corticospinal tract in PSP-CBS, ranged from mild to severe, than in PSP-RS (χ^2; p = 0.035, Figure 10).

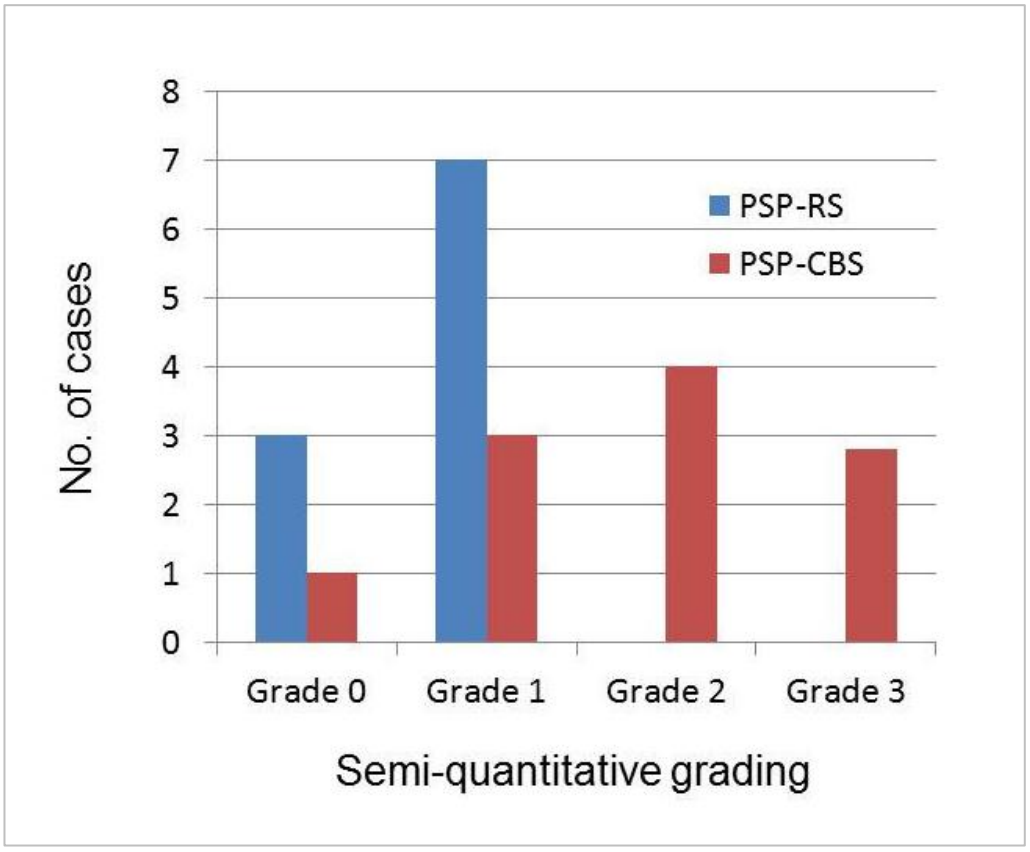

Figure 10. *Microglial pathology in the corticospinal tract of PSP-CBS and PSP-RS.*

More severe microglial pathology in the corticospinal tract of the PSP-CBS group is identified when compared with the PSP-RS group. A semi-quantitative grading scale was used to characterise the severity of microglial pathology; grade 0=baseline microglial population; grade 1=mild microglial pathology; grade 2=moderate microglial pathology; grade 3=severe microglial pathology.

Additional pathologies

The CERAD Aβ plaque score ranged from 'absent' to 'sparse', except for two PSP-CBS and one PSP-RS cases, which had a 'moderate' score (Mirra *et al.*, 1991). Small vessel cerebrovascular disease was noted in 1 PSP-CBS and 1 PSP-RS case. Other additional pathological findings are summarised in Table 12.

	PSP-CBS	**PSP-RS**
CERAD: Negative	1	1
CERAD: Infrequent	7	8
CERAD: Moderate	2 (cases 5, 7)	1 (case 15)
CERAD: Frequent	0	0
Argyrophilic grain disease	4 (cases 3, 5, 8, 9)	4 (cases 11, 14, 16, 18)
Incidental Lewy body disease	1 (Braak stage 3)	0
Cerebrovascular pathology	1 (mild)	1 (severe, case 19)
Cerebral amyloid angiopathy	1 (mild)	0

Table 12. *Additional pathological findings of PSP-CBS and PSP-RS.*

CERAD=Consortium to Establish a Registry for Alzheimer's disease

Tau biochemistry and haplotype analysis

Western blots of the sarkosyl-insoluble tau fractions from the frontal cortical homogenates showed the characteristic doublet at 64 and 68 kDa, indicating predominant 4R tau in PSP-CBS, PSP-RS and CBD-CBS cases (Figure 11). PSP-CBS and PSP-RS showed a single band at ~33kDa, whereas CBD-CBS had a doublet at ~ 37kDa, consistent with previous findings of the molecular differences in the low molecular weight proteolytic fragments between CBD and PSP (Arai *et al.*, 2001). No association was identified between H1/H1 genotype and either of the two PSP subgroup (χ^2 test; $p = 0.21$, Tables 7-10).

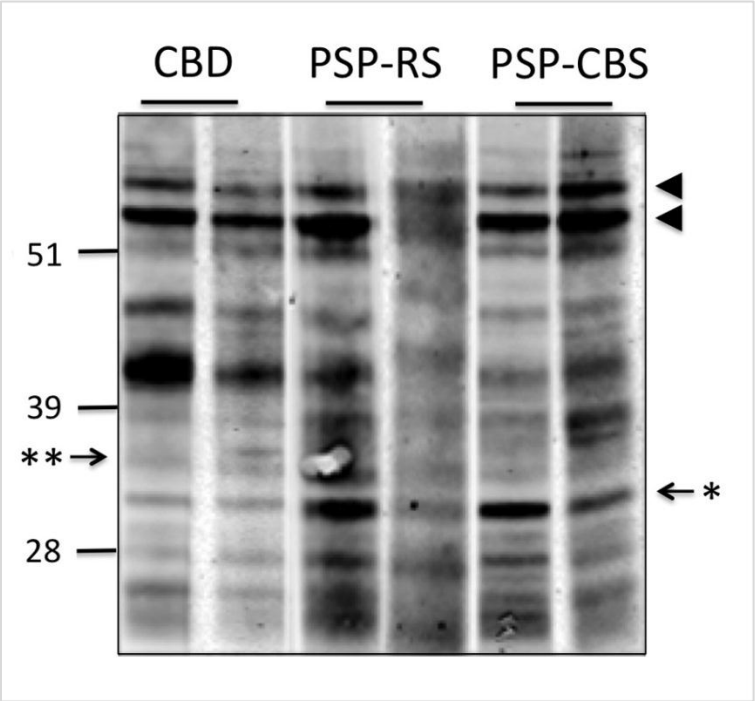

Figure 11. *Tau immunoblotting of CBD-CBS, PSP-CBS and PSP-RS.*

Western blot analysis of sarkosyl-insoluble tau from frontal cortex homogenates. The characteristic doublet of predominant 4R-tau in both CBD and PSP cases (arrowheads). CBD-CBS has a lower molecular weight doublet consisting of proteolytic fragments at ~37kDa (**). PSP-RS and PSP-CBS have a single band at ~33kDa (*). Numbers on the left indicate positions of molecular weight markers (kDa).

Discussion

The present study compared the morphological, biochemical and genetic characteristics of ten clinically well-characterised PSP-CBS cases and ten PSP-RS controls, matched for age and disease duration. Irrespective of the clinical presentation, all 20 included cases met established neuropathological diagnostic criteria of PSP, with the presence of 4R tau-positive neuronal and glial inclusions, including TAs in a characteristic distribution (Dickson *et al.*, 2011, Hauw *et al.*, 1994, Ince *et al.*, 2008). Biochemical studies of sarkosyl insoluble tau confirmed 4R tau as the main protein species in both PSP-RS and PSP-CBS groups and the presence of a smaller, faster migrating carboxy-terminal fragment, which has been previously reported in PSP (Arai *et al.*, 2001, Wray *et al.*, 2008). No difference in the distribution of the H1/H1 and H1/H2 genotypes was identified between the two PSP groups.

Morphometry was used to compare tau load, defined as the sum of all tau-positive lesions in the brain regions studied, between the two PSP groups and to determine the contribution of different neuronal and glial lesions to the overall tau pathology. A previous study at the Mayo Clinic established that PSP-CBS was associated with a greater tau burden in the mid-frontal and inferior-parietal cortices than in PSP-RS (Tsuboi *et al.*, 2005). By including a larger number of cases and evaluating more brain regions, this study found increased tau load in the cortical regions predominantly in the posterior frontal grey matter, anterior frontal grey matter and parietal white matter in PSP-CBS, validating the findings reported by Tsuboi and colleagues. The inclusion of other non-cortical regions in the quantitative assessment of tau load enabled the identification of a lower tau load in the caudate, subthalamic nucleus and cerebellar white matter in the PSP-CBS variant. It is noteworthy that the increased cortical tau load in PSP-CBS is compensated by the lower basal ganglia tau load, resulting in the total tau load, determined as the sum of the all regional tau load, being similar in the two PSP groups.

Increased cortical tau pathology has also been reported in PSP variants with clinical presentation of cortical symptoms, including PSP-PNFA (Josephs *et al.*, 2005) and PSP-FTDbv (Hassan *et al.*, 2012). Together with PSP-CBS, these clinical phenotypes

are collectively referred as the 'cortical' PSP variants (Dickson *et al.*, 2010). On the other hand, PSP-P and PSP-PAGF, which are considered as the 'brainstem' variants of PSP, have less severe overall tau pathology when compared to PSP-RS (Ahmed *et al.*, 2008, Williams *et al.*, 2007). Interestingly, these 'brainstem' variants are associated with a more benign disease course and a longer disease duration when compared to the classic PSP-RS (Ahmed *et al.*, 2008, Williams *et al.*, 2007, Williams *et al.*, 2007). The mean disease duration of the PSP-CBS in this series was similar to that of PSP-RS as previously reported (Williams *et al.*, 2005). It is plausible that the total tau pathology is inversely correlated with the disease duration in PSP variants. While the 'brainstem' PSP variant appears to be a more 'benign' form of PSP, the 'cortical' PSP variant represents a deviation from the classic presentation determined by a shift of tau pathology from the basal ganglia to the cerebral cortex. By selecting only cases with limited Alzheimer-type NFT pathology and assessing co-existing secondary pathologies, it is clear that the clinical presentation in the PSP-CBS cases was closely associated with the topographical severity of tau pathology which could not otherwise be explained by secondary pathologies. This study also showed that the regional differences in tau load between the two PSP groups were contributed by neuronal and glial lesions characteristic of PSP pathology rather than Alzheimer-related tau pathology (Oshima *et al.*, 2009).

A clinicopathological study from the Mayo Clinic compared the characteristics of CBS and RS clinical phenotypes in pathologically confirmed CBD cases (Kouri *et al.*, 2011). Their study on CBD also demonstrated significant differences in the topographical severity of tau pathology between the two CBD subtypes, which correlated with the different clinical presentations. Similar to the findings in the PSP-CBS cases in the present series, their study showed that CBD-CBS had more severe tau deposition in the cortical regions and less severe tau pathology in the lower brainstem and cerebellum when compared to the CBD-RS cases. However, total tau load and the contribution by different neuronal and glial lesions to the tau pathology were not assessed (Kouri *et al.*, 2011).

The subthalamic nucleus is one of the regions characteristically targeted by the PSP disease process (Hardman *et al.*, 1997, Hauw *et al.*, 1994), while this nucleus is better preserved in cases with pathologically confirmed CBD. The extent of neuronal loss in

the subthalamic nucleus was similar in both PSP-CBS and PSP-RS groups. However, there was less tau pathology in the subthalamic nucleus in PSP-CBS than in PSP-RS, indicating that it was probably glial rather than neuronal tau lesions that have significantly contributed to the differences in regional tau load. The tau pathology in the subthalamic nucleus has also been reported to be less severe in other PSP variants such as PSP-PNFA (Josephs *et al.*, 2005).

In PSP, cell loss in the substantia nigra affects both the pigmented neurons of the pars compacta and non-pigmented neurons in the pars reticulata and neurons in the medial nigra are relatively preserved (Oyanagi *et al.*, 2001). In the present study, neuronal loss was less severe in the ventrolateral and dorsolateral subregions in PSP-CBS when compared to PSP-RS (Figure 9). This regional difference may, in part, influence the clinical features due to the resulting selective damage to the dopaminergic and GABAergic neuronal nigral projections (Oyanagi *et al.*, 2001).

Pyramidal signs were documented in half of the PSP-CBS cases in the present series, but they were absent in the ten PSP-RS cases. Pyramidal signs are relatively uncommon in PSP and in one series they were present in only one fifth of all pathologically confirmed PSP cases (Williams *et al.*, 2005). On the other hand, 60% of pathologically confirmed CBD cases had pyramidal signs (Tsuchiya *et al.*, 2005). In CBD, the pathological involvement of the primary motor cortex including loss of Betz cells is a common finding, explaining the presence of pyramidal signs (Dickson *et al.*, 2010, Tsuchiya *et al.*, 2005). The common occurrence of pyramidal signs in the present PSP-CBS series can be explained by the abundant tau pathology in the primary motor cortex, which was 12-fold greater in PSP-CBS than in PSP-RS. There was also more severe microglial pathology in the corticospinal tract in PSP-CBS than in PSP-RS. Corticospinal tract degeneration and significant tau pathology in the motor cortex are prominent in globular glial tauopathy, previously referred as atypical PSP, which occasionally presents with a clinical picture of CBS (Ahmed *et al.*, 2013).

All PSP-CBS cases had received a final clinical diagnosis of CBS and they all had markedly asymmetrical cortical and extrapyramidal features, including unilateral limb clumsiness with a progressively maladroit and functionally useless hand. In the past, the presence of marked asymmetrical clinical signs would exclude the clinical

diagnosis of PSP, but this concept has been challenged in recent years with the findings of clinicopathological series confirming asymmetrical presentations in some PSP variants (Williams *et al.*, 2005). A post-mortem report of a Japanese patient who had focal limb dystonia and levitation has identified significantly more tau pathologies in the frontal cortices, basal ganglia and brain stem in the contralateral half brain than the ipsilateral half brain (Oide *et al.*, 2002). In the present PSP-CBS series, there was a numerically greater total tau load and cortical tau load in five cases where the contralateral half brain was available for evaluation when compared to the other five cases where the ipsilateral half brain was examined. However, this finding cannot confirm if tau load is greater in the clinically more manifested hemisphere within an individual as only half brains were used.

Asymmetrical limb apraxia and delayed initiation of horizontal saccades are clinical features suggestive of underlying parietal lobe dysfunction and are characteristic signs in CBS (Vidailhet *et al.*, 2000). The present data showed that regional tau load in the parietal white matter was five-fold greater in PSP-CBS than in PSP-RS and that PSP-CBS patients who had delayed initiation of horizontal saccades also had greater regional tau load in the parietal cortex and white matter.

Three patients had delayed initiation of horizontal saccades and, interestingly, half of them also had VSGP in the later stage of the illness, with involvement of downgaze, a diagnostic prerequisite for the diagnosis of PSP-RS (Lees, 1987, Rivaud-Pechoux *et al.*, 2000). VSGP is rare in CBD with classic CBS presentation (CBD-CBS), and was noted in only 18% of cases in the Mayo Clinic series (Kouri *et al.*, 2011) and was not observed in the QSBB series (Chapter 2). Six PSP-CBS patients had recurrent falls in the first year of their illness, whereas early falls were less frequent in CBD-CBS cases and were recorded in only 20% and 18% in the QSBB (Chapter 2) and Mayo Clinic series, respectively (Kouri *et al.*, 2011). The findings of the present study support the postulation that early postural instability, falls and supranuclear downgaze palsy in patients with CBS are clinical clues which, when present, suggest an underlying PSP pathology even in the presence of clinical signs of CBS. Notably, three cases in the PSP-CBS group (cases 2, 7, 10) had a pure CBS presentation throughout the disease course and did not have any tell-tale signs of PSP. This is in concordance with clinicopathological evaluation of cases in the QSBB that some pathologically

confirmed PSP and CBD cases present with a pure clinical syndrome such as CBS or RS irrespective of the underlying pathology, whereas some cases manifest overlap clinical features such as RS or CBS at the same time and occasionally, the clinical syndromes temporally evolve from one to another throughout the disease course as previously described (Kertesz *et al.*, 2005).

Conclusion

Data from transgenic animal studies indicate that soluble rather than fully aggregated tau species may ultimately be responsible for neuronal degeneration and cell death. However, the findings in this study support the notion that neuronal and glial inclusions composed of fibrillar pathological tau are useful and clinically valid pathological markers of the underlying neurodegenerative process. This study has provided comprehensive evidence that the topographical severity of tau pathology in PSP is closely associated with its clinical manifestation (Ahmed *et al.*, 2008, Hassan *et al.*, 2012, Josephs *et al.*, 2005, Williams *et al.*, 2007, Williams *et al.*, 2007). This is comparable to the findings in Alzheimer's disease where cognitive deficit shows a far better correlation with tau lesions than with Aβ plaques (Bennett *et al.*, 2004). A better understanding of the factors that influence the selective pathological vulnerability in different PSP variants will provide further insights into the underlying mechanistic pathways.

Chapter 4: Hypokinesia without decrement distinguishes progressive supranuclear palsy from Parkinson's disease

Introduction

Bradykinesia is a sine qua non for the diagnosis of Parkinson's disease. In clinical practice, the term bradykinesia is often used interchangeably with the terms akinesia and hypokinesia. Nevertheless, bradykinesia literally describes slowness in movements, akinesia means absence or poverty of expected spontaneous voluntary movement including slow reaction time (Golbe *et al.*, 2007), and hypokinesia refers to small amplitude movements. Bradykinesia, akinesia and hypokinesia are closely related but not necessarily well correlated in individual patients and each component of motor abnormality probably has a different underlying mechanism (Berardelli *et al.*, 2001). Both bradykinesia and hypokinesia in Parkinson's disease improve with levodopa therapy whereas reaction time is thought to be related to non-dopaminergic deficit (Berardelli *et al.*, 1986, Jahanshahi *et al.*, 1992, Velasco *et al.*, 1973). Bradykinesia is explicitly defined in the QSBB criteria for the diagnosis of Parkinson's disease as 'slowness of initiation of voluntary movement with progressive reduction in speed and amplitude of repetitive action' (Gibb *et al.*, 1988). The term sequence effect is used to describe the progressive reduction in amplitude and speed of sequential movements which is a key feature of Parkinson's disease. If the amplitude and speed of sequential movements progressively decline until the movement ceases, this is known as motor arrest (Iansek *et al.*, 2006, Kim *et al.*, 1998, Marsden, 1989). The pathophysiology and levodopa response of the sequence effect are unclear.

This study investigates whether bradykinesia, as defined above, is also a feature of PSP. PSP is characterised by VSGP, early gait instability with falls characteristically in a backwards direction, axial rigidity and bulbar dysfunction. In the seminal paper with

descriptions of the nine original PSP cases, Richardson and his colleagues provided brief accounts of elements of bradykinesia in only two cases, one of whom had slowness in walking and the other had awkwardness in performing rapid repetitive movements (Steele *et al.*, 1964). As a consequence of their findings, the authors concluded that PSP was a distinct clinicopathological entity which was unlikely to be confused with Parkinson's disease. However, much of the more recent literature closely links bradykinesia and parkinsonism with PSP and many movement disorder specialists consider PSP to be an example of atypical parkinsonism. In two large post-mortem series, early bradykinesia was reported in 88% and 75% of patients with pathologically confirmed PSP (Litvan *et al.*, 1996, Williams *et al.*, 2005). In line with this view, about 6% of cases with a clinical diagnosis of Parkinson's disease turn out to have tau pathology compatible with PSP at post-mortem examination (Hughes *et al.*, 2002). These and other findings (Morris *et al.*, 2002) have led to the delineation of two common clinical phenotypes: classical PSP-RS and PSP-P (Williams *et al.*, 2005). PSP-P closely resembles Parkinson's disease and is characterised by asymmetric symptoms at onset, tremor and a moderate initial therapeutic levodopa response.

Nevertheless, it is unclear whether the movement disorder described in the above literature adheres to the QSBB definition of bradykinesia. Clinical observations suggest that most PSP patients do not exhibit slowness or progressive reduction in amplitude and speed during finger tapping or handwriting.

Micrographia or small handwriting was first noted by Pick in 1903 (Pick, 1903) and has been associated with focal cerebral lesions (Derkinderen *et al.*, 2002, Kim *et al.*, 2005, Kuoppamaki *et al.*, 2005, Pick, 1903, Scolding *et al.*, 1994), postencephalitic parkinsonism (Snowden *et al.*, 2012), Parkinson's disease (McLennan *et al.*, 1972) and Huntington's disease (Iwasaki *et al.*, 1999). Micrographia characterised by small handwriting with further progressive reduction in size can be observed in 15% of patients with Parkinson's disease (McLennan *et al.*, 1972). The relationship between micrographia and bradykinesia remains controversial (McLennan *et al.*, 1972). It is also not known if the handwriting in Parkinson's disease differs from PSP.

This study investigated differences in the form of bradykinesia and handwriting between Parkinson's disease and PSP. Importantly, repetitive finger tap movements

were objectively studied. Repetitive finger tapping was selected as it is more severely impaired in patients with Parkinson's disease than hand opening and closing and hand pronation and supination elements of the motor section of Part III of the Unified Parkinson's Disease Rating Scale (UPDRS)(Agostino *et al.*, 1998, Agostino *et al.*, 2003). Both finger tapping and writing are simple and commonly used bedside assessments and any distinctive features identified for each condition would provide helpful diagnostic clinical clues.

Materials and methods

Participants

Fifteen patients with Parkinson's disease, nine with PSP and 16 healthy controls, matched for age range and gender, participated in this study (Table 13). Patients were recruited from the movement disorder clinic in the National Hospital for Neurology and Neurosurgery, Queen Square, London, United Kingdom. All patients with Parkinson's disease fulfilled the United Kingdom QSBB diagnostic criteria (Hughes *et al.*, 1992). All patients with PSP fulfilled the National Institutes of Neurological Disorders and Stroke (NINDS) Society for PSP diagnostic criteria (Litvan *et al.*, 1996). Parkinson's disease patients were included in the study if they were taking levodopa treatment with predictable motor fluctuations but were excluded if they had hand dystonia or if their tremor or dyskinesia were severe enough to interfere with their motor performance in the experiments. Exclusion criteria which applied to all subjects included significant medical co-morbidity, cognitive impairment (Mini Mental State Examination score < 28), depression (Beck depression score ≥ 21)(Beck *et al.*, 1961) and disabilities that might restrict finger movements. All participants were assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). The UPDRS was performed in all patients (Fahn *et al.*, 1987). The PSP Rating Scale (Golbe *et al.*, 2007) and the Frontal Assessment Battery (FAB)(Dubois *et al.*, 2000) were performed in patients with PSP. Patients' daily intake of anti-parkinsonian medications including levodopa, dopamine agonist, monoamine oxidase type B inhibitor, catechol-O-methyl transferase inhibitor and amantadine was recorded. Total daily levodopa equivalent dose (LED) was calculated for each patient according to published conversion formulae (Tomlinson *et al.*, 2010). The study was conducted with the understanding and written consent of all participants and was approved by the Camden and Islington Community Research Ethics Committee of the National Research Ethics Service.

	Controls (N=16)	**PSP (N=9)**	**PD (N=15)**	***P*-value**
Age, years	68.9±4.5	70.9±8.3	65.0±9.2	0.14**
Gender	9M : 7F	5M : 4F	9M : 6F	0.97$
Handedness	13R : 3L	7R : 2L	14R : 1L	0.51$
Edinburgh handedness inventory	61.9±56.4	55.6±77.3	78.0±50.9	0.62**
Disease duration, years	NA	4.5±3.3	10.8±7.4	0.01*
Total daily levodopa equivalent dose (mg/day)	NA	255.6±194.4	874.8±323.9	<0.001*
UPDRS	NA	I=NA II-ON=NA II-OFF=NA III-ON=NA III-OFF= 41.6±14 IV=NA	I=3.1±2.5 II-ON=7.7±3.9 II-OFF=17.2±9 III-ON=24.4±9 III-OFF= 36.3±9.7 IV=6.7±4.3	NA
H&Y-ON H&Y-OFF	NA	NA	2.1±0.4 2.8±0.6	NA
PSP rating scale	NA	39.4±2.4	NA	NA
Frontal assessment battery	NA	14.4±2.4	NA	NA

Table 13. *Features of control, PSP and PD groups.*

**=Student t-test; **=ANOVA; $= χ^2 test; NA=not applicable; UPDRS=Unified Parkinson's Disease Rating Scale*

Finger tapping task

Participants were instructed to repeatedly tap their index finger and thumb as rapidly and as widely as possible for 15 seconds. The participants were instructed to relax the 3rd, 4th and 5th digits in a semi-extended position so that the index finger-thumb movements were not restricted. The beginning and the end of the 15-second finger-tapping trial were signalled by a buzzer. Infrared-emitting diodes were fixed to eight designated regions on digits and the back of the hand, and motion was recorded in 3D (Coda Cx1, Charnwood Dynamics, Rothley, UK; Figure 12). Three 15-second trials were performed consecutively by each hand with 60 seconds rest in-between. Hand order was pseudo-randomised across participants. Parkinson's disease patients were tested during 'OFF' in the morning after 12 hours of overnight withdrawal of levodopa therapy, followed by a second experiment during 'ON' in the afternoon one hour after taking levodopa. Only two PSP patients were receiving levodopa treatment and both underwent overnight withdrawal of medication for 12 hours prior to testing.

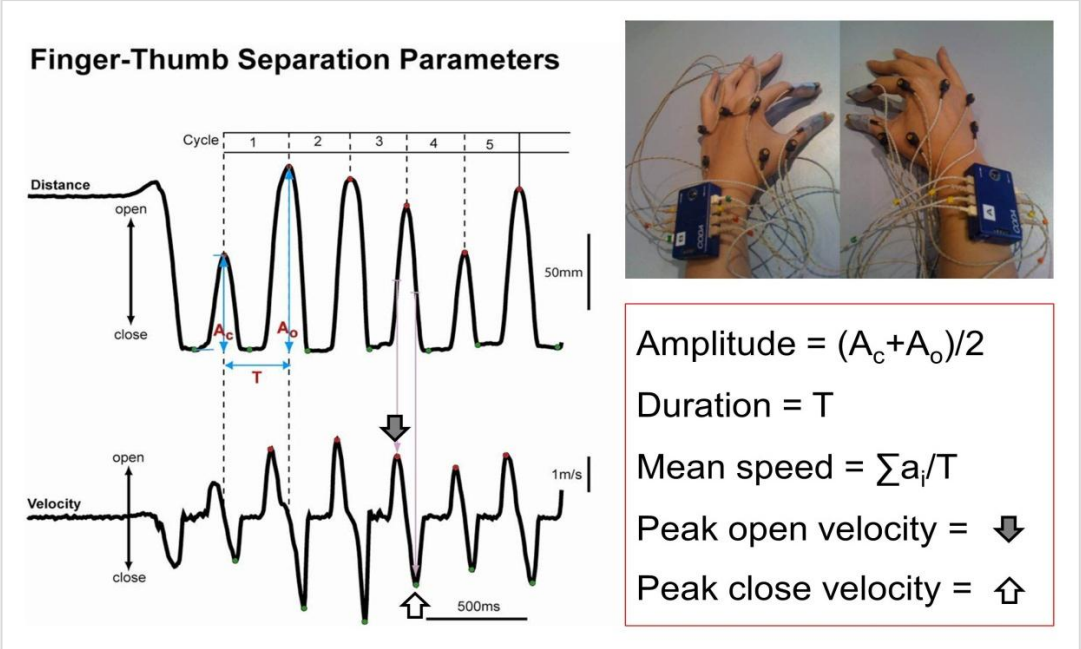

Figure 12. *Key parameters measured and infrared-emitting diodes.*

Motion was recorded in 3D (Coda) and key parameters were measured for each cycle of finger tap. Infrared-emitting diodes fixed to eight designated regions.

Handwriting task

Handwriting task was performed after the tapping experiments by all participants and was repeated during 'ON' by Parkinson's disease patients. The participant was asked to copy three times a standardized print of Times New Roman, 34 font size, eleven-word sentence on unlined A4 paper (Figure 13). No instructions were provided to the participants regarding the required size or speed of their script. The letters 'a' in the third (W3) and tenth words (W10) were selected and measurements were obtained using Microsoft Paint® programme. The script size (cm^2) of the selected letter was determined by the product of height and width outlined by the upper, lower, left and right margins of the loop in the letter. The size of W3 and W10 were plotted separately against successive sentence trials (1 to 3). Progressive reduction in size was represented by two slopes of the fitted linear regression line across the scatter-plots: script slope 1 from W3 and script slope 2 from W10.

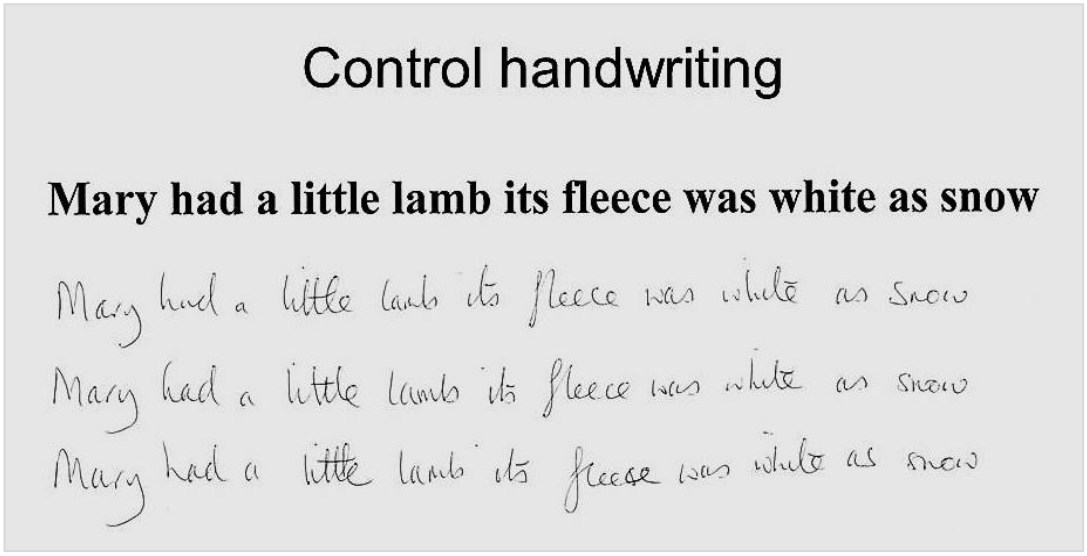

Figure 13. *Example of handwriting task.*

Handwriting performed by a healthy 65-year-old female. Mean script size=0.86cm^2, slope 1=0.28, slope 2=-0.06.

Kinematic parameters

Amplitude (mm), cycle duration (ms) and mean speed (mm/s) were measured for each cycle of finger tap from one finger-thumb separation to the next (Figure 12) using custom scripts written in Matlab. Mean speed, designed to be sensitive to both amplitude and cycle duration, was the mean rate of change in aperture regardless of whether the aperture was opening or closing. Thus, mean speed decreased when the cycle duration increased independently of amplitude, when amplitude decreased independently of duration, and when both occurred simultaneously. If amplitude increased at the expense of cycle duration, or vice versa, the mean speed tended to stay constant. Close and open velocities (mm/s) were the peak velocities of aperture closure and opening within a cycle. To eliminate potential confounding factors of different hand size and finger length across participants, distance (mm) measured was converted into the degree (deg) of angle separation between index finger and thumb. The conversion was obtained by the product of distance (mm) and k-value (deg/mm), calculated by the linear regression slope of maximum finger-thumb separation angle against maximum finger-thumb separation distance of each hand of the participant. The separation angle was calculated as the angle between the straight line segments joining the index fingertip marker and the thumb marker to the marker placed at marker 3.

Progressive changes in amplitude, duration and speed across a 15-second finger tap trial were represented by the slope of the fitted linear regression line across the scatter-plot of the kinematic parameter against the tap cycle. The slope of change in amplitude was used to assess progressive hypokinesia or ‘decrement’. The slope of change in speed which encompassed both amplitude and duration was used to assess progressive slowing of movement or ‘fatigue’ (Figure 15). Measurement of regularity of amplitude and speed across a tap trial was represented by the coefficient of variation (CV) which was computed by the residual standard deviation about the linear regression line divided by the mean value. High amplitude or speed CV values represent irregularities of these kinematic parameters.

Group parameters including amplitude, cycle duration, maximum close velocity, maximum open velocity, mean speed, slopes and CVs were summarized by computing

the mean parameter value for all tap cycles across three finger tap trials of both hands for all subjects.

Statistical analysis

Comparisons of continuous variables were carried out by univariate Analysis of Variance (ANOVA) with gender, age and disease duration as covariates. Student t-test was used to compare disease duration and total daily levodopa equivalent dose between the two patient groups. Tukey HSD post-hoc analysis was used to determine differences between groups (controls, PD-OFF and PSP). Paired t-tests were used to compare variables of Parkinson's disease patients in ON vs. OFF states. χ^2 test was used for discreet variables. Spearman's correlation was used to study correlation between group parameters and clinimetric scores. Statistical significance was determined when $p \leq 0.05$. SPSS version 17.0 was used for statistical analysis.

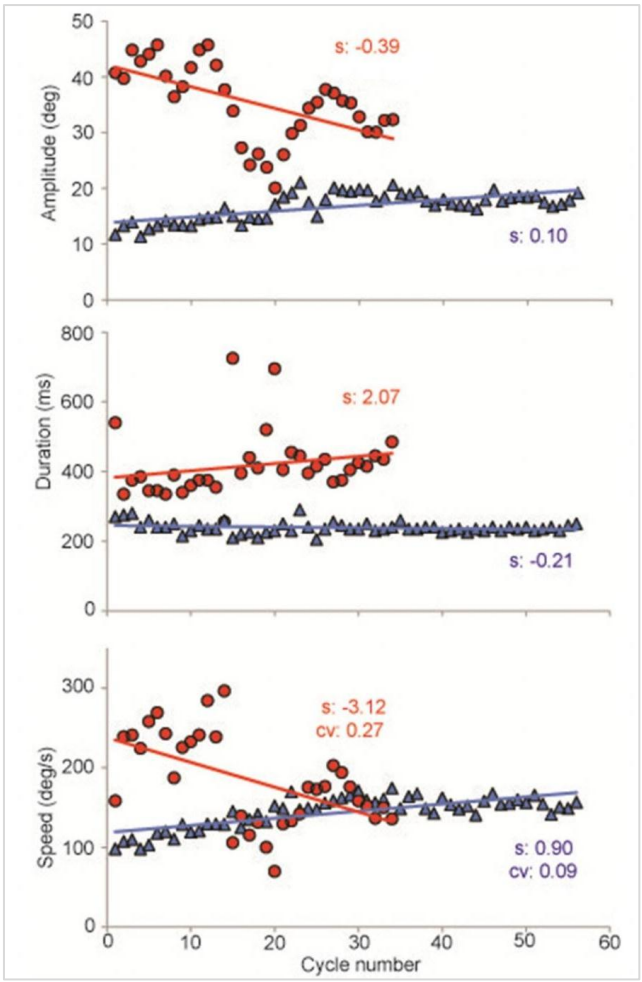

Figure 14. *Kinemetric parameters during 15-s finger tap trials.*

Kinematic parameters during the first 15-second right finger tap trial in a patient with Parkinson's disease during 'OFF', represented by red and circle plots (UPDRS-III-OFF=32), and a patient with PSP, represented by blue and triangle plots (UPDRS-III =69; Frontal assessment battery=14).

The slopes (S) for amplitude, duration and speed, and coefficient of variation (CV) for speed are shown. Lack of decrement and fatigue in PSP is reflected by the positive amplitude slope and speed slope. The speed CV in Parkinson's disease is three times greater than PSP, suggesting high irregularity in speed.

Results

The demographic features and clinimetric scores are listed in Table 13. Age was closely matched between groups. There were slightly more male participants compared to female in each group and the majority of participants were right-handed. All the patients with Parkinson's disease were receiving dopamine replacement therapies and all of them had derived good or excellent sustained therapeutic benefit. There were significant improvements in the Parkinson's disease patients' UPDRS-II, UPDRS-III, Hoehn and Yahr (H&Y) scores one hour after taking levodopa (OFF Vs. ON; $p <$ 0.001 in all). The mean total daily levodopa equivalent dose in Parkinson's disease was greater than that of PSP (t-test, $p < 0.001$). Eight PSP patients were taking amantadine but only two were receiving levodopa therapy. The PSP patients who were not receiving levodopa had failed to respond to levodopa and had a negative therapeutic response to an acute levodopa challenge (Steiger *et al.*, 1992).

Five of nine PSP patients had had evidence of midbrain atrophy on their most recent MRI. One PSP patient died six months after participating in this study and his pathological diagnosis was confirmed to be PSP at post-mortem. The mean disease duration in Parkinson's disease was longer than in PSP (t-test, $p = 0.01$).

The mean bradykinesia subscore, which included the sum of UPDRS motor scores for finger tap, hand opening and pronation/supination movements, also improved after levodopa therapy (OFF: 2.06 ± 0.54; ON: 1.76 ± 0.66; paired t-test, $p = 0.009$).

Repetitive finger tap movements

Spatial and temporal variables (amplitude, duration, peak velocities and mean speed) were measured for each tap cycle and used to characterise different aspects of motor performance (Figure 14). The analyses focused on mean performance, progressive changes in performance (slope of linear regression line of variable against cycle number), and regularity of performance (coefficient of variation; CV) achieved over a 15-s trial. As shown in Table 14, one-way ANOVA revealed significant differences between the three groups for all measures apart from slope of cycle duration. In the

following sub-sections, post-hoc analyses are used to study these group differences in order to describe the important performance characteristics of each group in turn.

Healthy subjects

Linear regression analysis did not show a significant correlation of any performance variables with age or gender. There was a modest effect of hand dominance in that mean cycle duration was longer for the non-dominant hand (dominant hand: 289.38 ± 64.6ms; non-dominant hand: 302.23 ± 67.5ms; $p = 0.003$) but no other performance parameters differed between the two hands.

The slope of the dominant hand's mean speed was significantly more negative in the third trial compared to the first (trial 1 speed slope = -1.03deg/s/cycle; trial 3 speed slope=-1.46deg/s/cycle; $p = 0.043$), indicating an increase in physiological fatigue. All other parameters showed a similar, but non-significant, slight decline in performance in progressive trials.

	Controls (N=16)	PSP (N=9)	PD-OFF (N=15)	F(1,19)	*P*-value
Average no. of tap cycles / 15s	50.0(10.7)	52.2(10.3)	41.5(9.7)	8.5	0.009*
Average parameters					
Amplitude (º)	45.9(8.7)	18.7(6.3)	37.8(916.0)	18.5	< 0.001*
Duration (ms)	295.8(65.6)	288.3(67.7)	356.5(80.6)	7.8	0.012*
Close velocity (º/s)	928.2(215.2)	386.3(134.4)	737.5(385.0)	11.4	0.003*
Open velocity (º/s)	788.9(167.9)	327.7(106.1)	584.4(297.0)	10.3	0.005*
Speed (º/s)	330.1(64.6)	142.9(49.8)	224.1(93.1)	8.3	0.009*
Average coefficients of variations					
Amplitude	0.09(0.03)	0.27(0.13)	0.41(0.08)	9.69	0.006*
Duration	0.09(0.03)	0.28(0.18)	0.17(0.10)	4.26	< 0.001*
Speed	0.09(0.02)	0.236(0.9)	0.167(0.06)	6.88	0.017*
Average slopes values					
Amplitude slope (º/cycle)	-0.12(0.12)	0.01(0.17)	-0.20(0.21)	4.45	0.048*
Duration slope (s/cycle)	0.77(0.75)	1.86(2.58)	1.49(2.39)	0.16	0.70
Speed slope (º/s/cycle)	-1.52(0.81)	-0.39(0.79)	-1.71(1.59)	7.81	0.012*

Table 14. *Comparison of parameter measurements.*

*Mean parameter measurements (SD) of control, PD-OFF and PSP subgroups and p-values from one-way ANOVA adjusting for age, gender and duration. Covariates appearing in the model above are evaluated at the following values: gender=0.58 (0-female; 1-male), age=67.87 years, disease duration=8.43 years; F(degrees of freedom)=f value from one-way ANOVA; *=p < 0.05 by one-way ANOVA*

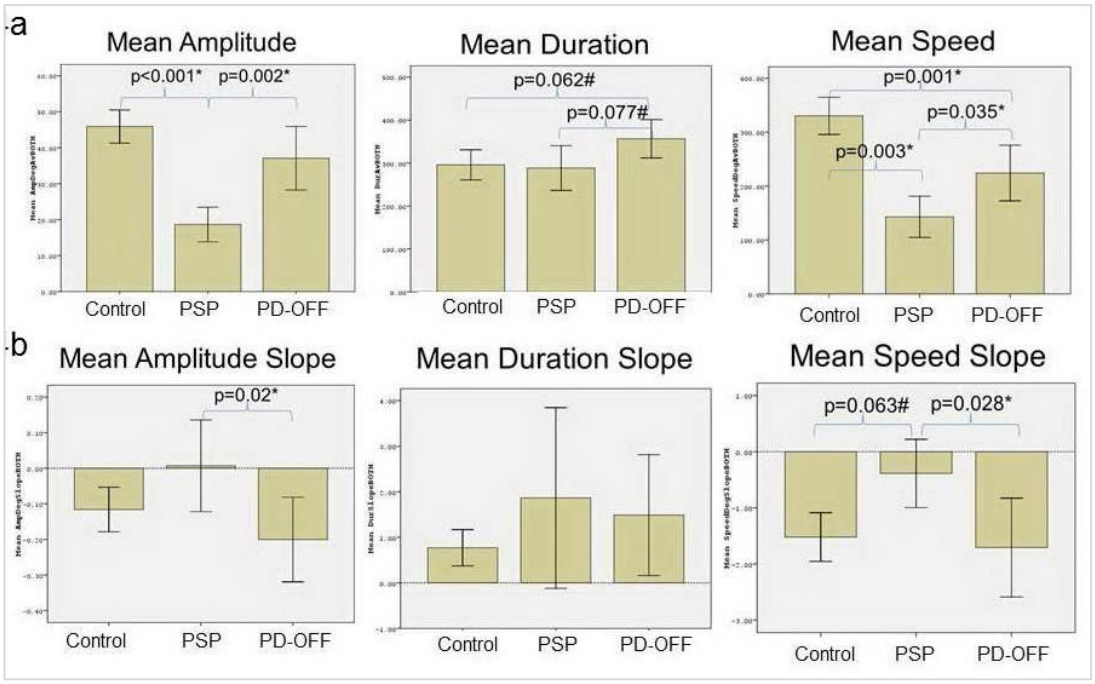

Figure 15. *Comparison of key parameters between groups.*

*a. Mean amplitude, duration and speed of controls, PSP and PD-OFF and p values by post-hoc analysis. Error bars represent 95% confidence intervals. *=p < 0.05 indicates statistical significance and #=p: 0.05-0.10 indicates borderline significance by Tukey HSD post-hoc analysis.*

*b. Mean slope values for amplitude, duration and speed of controls, PSP and PD-OFF and p values by post-hoc analysis. Error bars represent 95% confidence intervals. Dotted reference lines represent zero, values below which represent progressive downward negative slope across the 15-second finger tap trial. *=p < 0.05 indicates statistical significance and #=p = 0.05-0.10 indicates borderline significance by Tukey HSD post-hoc analysis.*

Patients with PSP

The performance of patients with PSP was characterised by strikingly small amplitudes of finger-thumb separation distance with a lack of performance decrement during a trial and with excessive variability of performance between cycles.

The small mean amplitude in PSP (mean = 18.65deg) was less than half that of healthy subjects (mean = 45.91deg) and PD-OFF (mean = 37.82deg; $p < 0.001$ in both cases; Table 14 & Figure 15a). The amplitude slope in PSP had a positive value of 0.01deg/cycle, indicating a lack of amplitude decrement throughout the 15-second finger tap trial. This value differed significantly from the negative slope in PD-OFF ($p = 0.002$; Table 14 & Figure 15b). The possibility that the very small tapping amplitude in PSP as a group might have masked the detection of small degree of decrement was further explored. After adjusting for mean amplitude, there was no difference in amplitude slope between PSP and controls, indicating an absence of decrement in PSP ($p = 0.36$, Table 15).

A greater number of tap cycles were achieved by patients with PSP during a 15s trial (mean=52.22 cycles) when compared to PD-OFF (mean = 41.54 cycles; $p = 0.046$), but not controls (50.03 cycles; Table 14).

Although PSP cycle duration was normal, the markedly reduced amplitude led to an overall reduction in close and open velocities and mean speed in PSP when compared to PD-OFF (close velocity: $p = 0.01$; open velocity: $p = 0.02$; mean speed: $p = 0.04$) and controls ($p < 0.001$ in all; Table 14 & Figure 15a). This probably does not indicate an intrinsic slowing of movement as such, but simply stems from the digits moving through a smaller amplitude with approximately the same cycle duration.

In PSP, there was greater variability of performance from one cycle to the next as reflected in the highest CV values. They were greater than control for amplitude CV, cycle duration CV and mean speed CV ($p < 0.001$ in all cases) and were also greater than PD-OFF for amplitude CV ($p = 0.001$) and mean speed CV ($p = 0.009$).

Among the PSP group, there was no correlation between mean amplitude and clinical markers of disease severity including disease duration ($p = 0.40$), total daily levodopa

equivalent dose (p = 0.72), UPDRS motor score (p = 0.64), H&Y (p = 0.57), PSP staging (p = 0.40) or Frontal Assessment Battery scores (p = 0.15). There was a positive correlation between the number of tap cycles/15-second and the FAB score in PSP (Spearman's coefficients: 0.64; p = 0.04).

	PD-OFF vs. Control		**PSP vs. Control**		**PSP vs. PD-OFF**	
	F (1, 26)	***P* values**	**F (1, 20)**	***P* values**	**F (1, 18)**	***P* values**
Amplitude slope (adjusted for mean amplitude)	4.41	0.046*	0.88	0.36	4.45	0.048*
Duration slope (adjusted for mean duration)	0.47	0.501	3.75	0.067#	3.20	0.09#
Speed slope (adjusted for mean speed)	2.89	0.070#	1.70	0.208	3.33	0.085#

Table 15. *Comparison of slope values between groups.*

Comparison of slope values between PD-OFF, PSP and controls after adjusting for mean amplitude, duration and speed respectively. General linear model univariate analysis (=significant, p < 0.05, #=borderline significant, p = 0.05-0.10); F (degrees of freedom) = f value from univariate analysis of variance.*

Covariates appearing in the model PD-OFF vs. controls are evaluated at the following values: gender=0.58 (0=female; 1=male), age=66.99 years, mean amplitude for amplitude slope model=42.34 deg, mean duration for duration slope model=325.18s, mean speed for speed slope model=278.81deg/s.

Covariates appearing in the model PSP vs. controls are evaluated at the following values: gender=0.56 (0=female; 1=male), age=69.6 years, mean amplitude for amplitude slope model=36.09 deg, mean duration for duration slope model=293.12s, mean speed for speed slope model=262.73deg/s.

Covariates appearing in the model PSP vs. PD-OFF are evaluated at the following values: gender=0.58 (0=female; 1=male), age=67.19 years, disease duration = 8.43 years, mean amplitude for amplitude slope model=31.08 deg, mean duration for duration slope model=330.95s, mean speed for speed slope model=193.64deg/s.

Patients with Parkinson's disease

When compared with controls, the main finding in PD-OFF was slowness of movement coupled with greater variability of speed between tap cycles. When compared with PSP, PD-OFF exhibited larger amplitude movements, a smaller number of tap cycles and greater decrement of performance during a trial.

PD-OFF amplitude ($p = 0.10$) tended to be smaller than that in healthy subjects while cycle duration ($p = 0.06$) tended to be more prolonged, but only with borderline significance. However, the combination of both these trends led to a highly significant lower mean speed of PD-OFF compared with controls ($p = 0.001$; Figure 15). Similarly, peak open velocity of PD-OFF was less than controls ($p = 0.033$), although there was no difference in peak close velocity between the two groups. In addition, CV of mean speed in PD-OFF was significantly greater than that of controls ($p = 0.004$), suggesting proportionally greater irregularities between cycles.

Both amplitude and speed slopes in PD-OFF, reflecting the progressive decrement in performance, were more strongly negative when compared to those of PSP (amplitude: $p = 0.002$; mean speed: $p = 0.028$). However, the negative amplitude and speed slopes of PD-OFF, were numerically, but not significantly, greater than healthy subjects. In Parkinson's disease patients with severe parkinsonism, slope measurements may be underestimated due to poor performance during the tap trial, rendering their slope values lower than patients with milder disease severity who do not exhibit a 'floor' effect. After adjusting for differences in mean amplitude, the amplitude slope in PD-OFF became significantly more strongly negative than PSP and healthy controls (PD-OFF vs. PSP, $p = 0.048$; PD-OFF vs. controls, $p = 0.046$, Table 15). There was a trend for a more negative speed slope in PD-OFF when compared to controls after adjusting for mean speed ($p = 0.07$, Table 15). These findings demonstrate progressive decrement in performance in PD-OFF and represents sequence effect in Parkinson's disease.

The UPDRS-III-OFF score was correlated with small amplitude (Spearman's coefficient: -0.79, $p < 0.001$), slow mean speed (Spearman's coefficient: -0.68, $p = 0.005$) and high irregularities in speed (Spearman's coefficient: 0.75, $p = 0.001$; Figure

16). There was no correlation between performance decrement, i.e. slopes for amplitude and speed, and disease duration, total daily levodopa equivalent dose, UPDRS motor scores or H&Y ($p > 0.05$ in all cases).

Levodopa therapy improved the total number of tap cycles (OFF = 41.5cycles/15s ± 9.7; ON = 45.9cycles/15s ± 9.5, $p = 0.04$), peak open velocity (OFF = 584.4deg/s ± 297.0; ON = 639.9deg/s ± 269.0; $p = 0.04$), mean speed (OFF = 224.1deg/s ± 93.1; ON = 255.6deg/s ± 86.4; $p = 0.006$) and speed CV (OFF = 0.167 ± 0.07; ON = 0.150 ± 0.08; $p = 0.014$). However, levodopa therapy did not significantly improve performance decrement (amplitude slope: OFF = -0.20deg/cycle ± 2.1; ON = -0.17deg/cycle ± 2.1; speed slope: OFF = -1.71deg/s/cycle ± 1.6; ON = -1.78deg/s/cycle ± 1.4).

When analysis of the effect of levodopa was limited to the Parkinson's disease patients' more affected hand, more robust ON vs. OFF differences were observed. In addition to the improvements described above, improvement was also observed in mean cycle duration (OFF = 370.8ms ± 103.6; ON = 321.1ms ± 93.2; $p = 0.005$) and there was a trend towards improvement in performance decrement (amplitude slope: OFF = -0.20deg/cycle; ON = -0.15deg/cycle; $p = 0.07$).

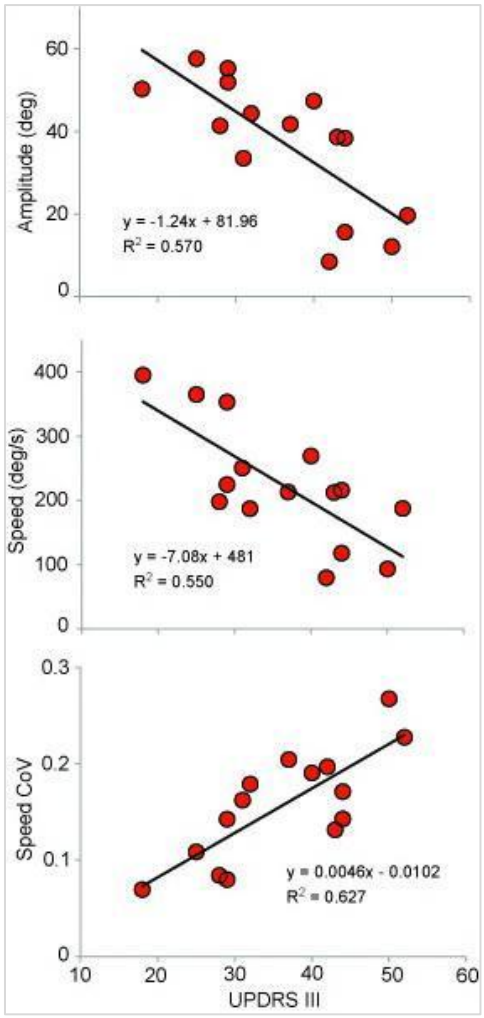

Figure 16. *Correlations between UPDRS motor scores and finger tap parameters.*

Among PD-OFF subgroup, a more severe UPDRS-III-OFF score was correlated with smaller mean amplitude, slower mean speed and greater variability in speed. Deg=degree; CoV=coefficient of variation; UPDRS=Unified Parkinson's Disease Rating Scale.

Hypokinesia without decrement

Each 15-second finger tap trials in the PSP and PD-OFF groups were analysed. Hypokinesia was defined as a mean amplitude of <23deg, i.e. 50% of the control group's mean amplitude. Hypokinesia was observed in 70% of the finger tap trials in the PSP group, 24% in the PD-OFF group and 2% of the control group. The remaining 30% of the PSP finger tap trials had a small mean amplitude of 27.8deg ±3.7 and a positive mean amplitude slope of 0.05deg/cycle. The 24% of PD-OFF finger tap trials with hypokinesia were performed by a small group of four Parkinson's disease patients who had severe parkinsonism with a mean UPDRS-III-OFF score of 46.4 and a long mean disease duration of 17.5 years. All four patients had good levodopa response and an average improvement in UPDRS motor score by 14.3 one hour after intake of levodopa therapy. Despite severe hypokinesia with a mean amplitude of 11.4deg ± 5.6, decrement was still evident with a negative mean amplitude slope of -0.037deg/cycle ± 0.1 (vs. control, $p = 0.05$). When lack of decrement, defined as a positive amplitude slope, was combined with hypokinesia, 87% of finger tap trials in the PSP group, 12% in the PD-OFF group and none in the control group were noted to exhibit both features.

Handwriting in Parkinson's disease and PSP

The scripts from one PSP and two Parkinson's disease patients were discarded from the analysis as they were written in capital letters. The mean script size of PSP ($0.50cm^2 \pm 0.46$) was numerically, but not statistically, smaller than PD-OFF ($0.78cm^2 \pm 0.38$; $p = 0.29$) and controls ($0.79cm^2 \pm 0.20$; $p = 0.07$). Progressive changes in script size were assessed from the slopes of the linear regression lines separately fitted for W3 (script slope 1) and W11 (script slope 2) across the three successive sentences. There was less of a decrement over successive sentences for W11 in PSP (mean script slope 2; 0.06 ± 0.09) than in PD-OFF (-0.08 ± 0.30) after adjusting for age, gender, disease duration and mean script size ($p = 0.02$). A similar trend was found in mean script slope 1 (PSP: -0.004 ± 0.21; PD-OFF: -0.103 ± 0.21; $p = 0.16$). After levodopa therapy, six Parkinson's disease patients exhibited a mean increase of $0.26cm^2$ in script

size, however, the overall script size did not achieve statistical significance between PD-OFF and PD-ON (p = 0.28, Figure 17). Decrements in script size persisted in Parkinson's disease patients despite levodopa therapy as shown by the negative script slope 1 (OFF: -0.10, ON: -0.05; p = 0.48) and script slope 2 (OFF: -0.08, ON: -0.09; p = 0.82).

Micrographia was determined as present when the mean script size was less than 0.40cm^2, i.e. half the mean script size of the control group, and the lack of progressive micrographia was defined by a positive mean script slope. Micrographia was more frequent in PSP (N=6, 75%) than in PD-OFF (N=2, 15.4%, p = 0.01) and control (N=1, 6.3%; p = 0.001) subjects. The script size numerically improved in the two Parkinson's disease patients with micrographia but their ON script size still did not exceed 0.40cm^2. A positive script slope 2 was more frequent in PSP (N=5, 62.5%) than in control (N=1, 6.3%; p = 0.007) and, possibly, in PD-OFF (N=3, 23.1%; p = 0.09) subjects. A positive script slope 1 was more frequent in PSP (N=6, 75%) than in PD-OFF patients (N=3, 23.1%; p = 0.03), but it did not differ from control subjects (N = 8, 50%; p = 0.23). The patients with the smallest script size in the PSP and PD-OFF were also noted to have the most severe UPDRS-III score in their group (smallest script size in PSP = 0.14cm^2, UPDRS-III = 69, Figure 17; smallest script size in PD-OFF = 0.11 cm^2, UPDRS-II I= 50).

There were more PSP patients (N=5, 62.5%) who had both hypokinesia (< 23deg) and micrographia (< 0.40cm^2) than controls (0; p = 0.001) and PD-OFF (N=1, 7.7%, p = 0.014). In PSP, the finger tap amplitude slope was strongly correlated with script slope 2 (Spearman's coefficient = 0.88, p = 0.004). No correlation was found between script findings and markers of disease severity in either Parkinson's disease or PSP groups.

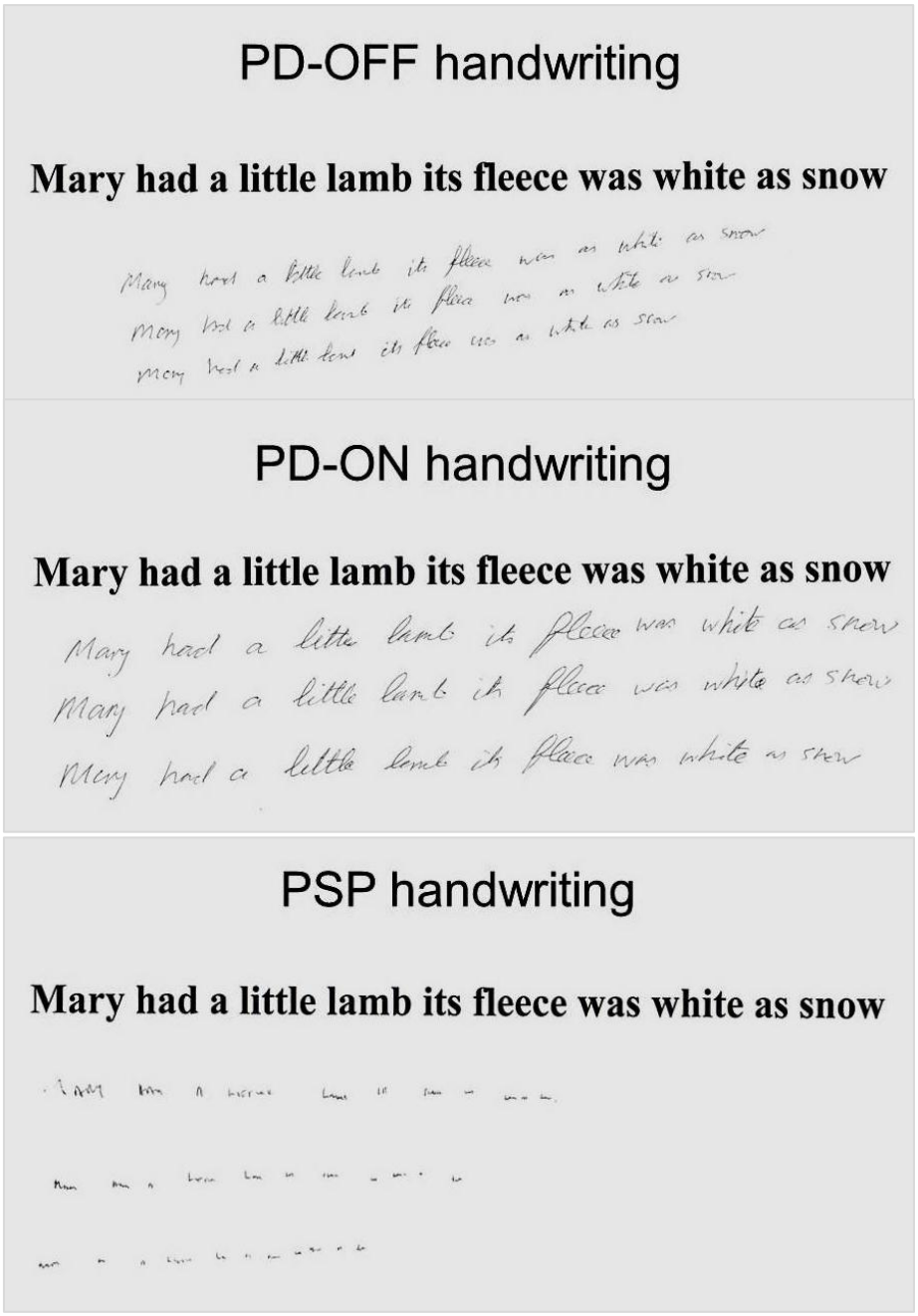

Figure 17. *Handwriting in PD and PSP.*

PD: Handwriting performed by a 58-year-old right-handed patient with PD during 'OFF' (UPDRS=18, mean script size=0.66cm^2, slope 1=-0.01, slope 2=-0.16) and 'ON' (UPDRS=13, mean script size=1.14cm^2, slope 1=-0.08, slope 2=-0.46).

PSP: Example of microscopic micrographia by a patient with advanced PSP who had the smallest script size in the PSP group (UPDRS-III=69, mean script size=0.14cm^2, slope 1=0.23, slope 2=-0.01). UPDRS=Unified Parkinson's Disease Rating Scale.

Discussion

Bradykinesia in Parkinson's disease

This study captured objective recordings of the sequence effect during repetitive finger tap movements and found that a progressive decrement in amplitude was present in Parkinson's disease but not in PSP. This study confirmed that the characteristic finger tap pattern in Parkinson's disease consists of slowness with variability in speed and progressive decrement in performance (Agostino *et al.*, 1998, Agostino *et al.*, 1994). Although levodopa improved most tapping parameters in Parkinson's disease, it did not improve the sequence effect of progressive deterioration in cycle duration and speed. However, there was a borderline improvement in decrement in treated Parkinson's disease when only the more affected hand was studied. The findings indicated that the sequence effect in Parkinson's disease may be relatively independent of dopaminergic regulation. A recent study using a Modified Purdue Pegboard Test showed that sequence effect in Parkinson's disease did not respond to levodopa medication (Kang *et al.*, 2010). In another study, reduced stride length (hypokinesia) improved with either levodopa or visual cues, but the progressive reduction of stride length (sequence effect) only improved with cueing (Iansek *et al.*, 2006). The present study showed that the variability of speed was significantly greater in PD-OFF when compared to controls, and that it improved with levodopa therapy, suggesting that the mechanisms underlying the temporal regularity of movements and the sequence effect are likely to be different.

Hypokinesia without decrement in PSP

The most striking finding in the present study was the very small index finger-to-thumb separation amplitude during repetitive finger tapping in PSP. The average amplitude of finger separation in PSP was less than half of that in controls and PD-OFF. PSP patients also had a greater number of tap cycles and higher variability in amplitude and speed when compared to PD-OFF. The greater number of tap cycles was most probably related to the small amplitude as the digits moved through a smaller

distance allowing more cycles to be performed within a given time. While small amplitude in PD-OFF was correlated with more severe UPDRS motor score, there was no correlation between amplitude and markers for disease severity in the PSP group. Thus, the differences in disease duration between Parkinson's disease and PSP could not account for the reduced mean amplitude in PSP. Furthermore, it could not be explained by medication status because all patients were tested after 12 hour-withdrawal of anti-parkinsonian medications.

The second key finding was the lack of progressive reduction in amplitude. This is compatible with general clinical observation that most PSP patients do not exhibit progressive reduction in performance during repetitive finger tapping. The positive amplitude slope of 0.01deg/cycle in PSP was similar to controls but differed significantly from the negative slope of -0.2deg/cycle in PD-OFF. It is possible that a lack of decrement in PSP might be due to a floor effect caused by severe hypokinesia. However, even among the subgroup of Parkinson's disease patients with severe hypokinesia (amplitude < 23deg), a mean negative amplitude slope of -0.037deg/cycle was found. Furthermore, when comparisons were performed after adjustment for any differences in mean amplitudes between groups, the mean amplitude slope in PD-OFF was shown to be more negative than PSP and controls, while there was no difference between PSP and controls (table 15). These findings support a minimal or lack of performance decrement and sequence effect in PSP that is incompatible with the QSBB definition of bradykinesia for the clinical diagnosis of parkinsonism.

Pathophysiological mechanisms

Severe neuronal loss in the substantia nigra pars compacta is observed in both Parkinson's disease and PSP with greater involvement in the ventromedial and dorsal tiers in PSP (Fearnley *et al.*, 1990, Hardman *et al.*, 1997). In PSP, substantial damage also occurs in the zona reticulata of the nigra (Hardman *et al.*, 1996), the internal segment of the globus pallidus (GPi), the subthalamic nucleus of Luys (STN), the dentate nucleus, superior cerebellar peduncle and, to a lesser degree, the striatum and thalamus (Bryant *et al.*, 2010, Demirci *et al.*, 1997).

In Parkinson's disease, the STN and GPi are affected functionally with increased neuronal discharges as a result of disruption of the basal ganglia circuit (Wichmann *et al.*, 2003). Functional compensatory change in the putamen has also been reported in Parkinson's disease, which has been proposed to contribute to the diminished levodopa response later in the disease course (Halliday, 2007). The cerebellum may play a role in motor sequencing (Garraux *et al.*, 2005). Greater activity of both cerebellar hemispheres was found in functional imaging in Parkinson's disease patients during automated movements when compared with healthy controls, suggesting that the cerebellum might contribute to the compensatory pathway in Parkinson's disease (Wu *et al.*, 2005).

It has been postulated that movement size is regulated by phasic signals from GPi to the supplementary motor area (SMA) and premotor cortex (Alexander *et al.*, 1990). Severe hypokinesia in PSP, therefore, might be due to the extensive pathological damage to the GPi and STN (Hauw *et al.*, 1994, Litvan *et al.*, 1996). There is also loss of cholinergic neurons in the putamen and loss of pyramidal neurons in the premotor cortex (Halliday, 2007) that may also influence the nature of the motor deficit. Finally, potential compensatory mechanisms via the cerebellar outflow pathway are cut off in PSP due to damage of the superior cerebellar peduncle (Tsuboi *et al.*, 2003, Whitwell *et al.*, 2011). The putamen appears to have a role in movement timing which may contribute to the variability in performance in PSP and Parkinson's disease (Garraux *et al.*, 2005).

Sequence effect is reflected by the impairment of scaling of motor sequences and contributes to prolonged movement time in Parkinson's disease (Agostino *et al.*, 1994, Benecke *et al.*, 1987). Its pathophysiology in Parkinson's disease is still poorly understood but it is likely to be independent of dopaminergic pathways. It appears that the sensorimotor apparatus in patients with Parkinson's disease is set smaller but the capacity to achieve the correct amplitude is intact and can be overcome by visual guidance (Hallett, 2003). These findings may not be relevant in PSP where the pathological lesion is more extensive and where visual cueing is an ineffective strategy to improve gait. The lack of levodopa response in sequence effect in Parkinson's disease was also supported by the findings in this study.

In Parkinson's disease, rigidity and tremor are thought to contribute to slowness in limb movements (Berardelli *et al.*, 2001, Quencer *et al.*, 2007). On the other hand, PSP patients who have more axial symptoms and sometimes no detectable rigidity of the limbs on examination might arguably manifest less degree of slowness on repetitive finger tapping.

Finger tapping assessments

'Hypokinesia without decrement' was identified in 87% of finger tap trials in the PSP group and only 12% in the PD-OFF group. This finding might be particularly useful in patients with PSP-P, where the physical signs can mimic Parkinson's disease. Other PSP finger tap trials also had a small mean amplitude of 27.8deg, not quite making the cut off value of 23deg for hypokinesia. Small finger tap amplitudes can be easily recognised by careful bedside examination. Small degrees of decrement may, however, be difficult to detect in Parkinson's disease patients with severe motor impairment who have small amplitude finger movements on initiation of finger movements. These patients are readily differentiated from PSP by their sustained levodopa response and relatively long disease duration. The Parkinson's disease patients with severe hypokinesia in the present study had a mean disease duration of 17.5 years, whereas the mean duration from diagnosis to death in PSP is 7 years (Williams *et al.*, 2005). In addition to decrement, delayed initiation of voluntary movements and motor arrests during repetitive finger tapping in Parkinson's disease may also have clinical usefulness (Fahn *et al.*, 1987, Marsden, 1989).

The average number of tap cycles performed in 15 seconds was 50 in controls, 52 in PSP, 42 in PD-OFF and 46 in PD-ON. Therefore, to detect the differences reported above would require a tap trial of approximately 50 finger-thumb tap cycles. The modified MDS-UPDRS (Goetz *et al.*, 2008) proposed a 10-tap trial, which would take an average of 3.8 seconds (15s/42taps x 10taps) for PD-OFF subjects to perform. It is likely that a tap trial consisting of only 10 taps would be too brief for the sequence effect to emerge in either treated or untreated Parkinson's disease. To investigate this, an additional analysis on the collected data was performed by arbitrarily assessing only the first 20 taps of the first trials performed by both hands after adjusting for disease

duration, age and gender. With a 20-tap trial, PSP can still be differentiated from both PD-OFF and controls by having amplitudes of less than half the expected size. Mean speed in PD-OFF was slower than controls ($p = 0.007$). However, after 20 taps, the amplitude slope (mean = +0.04) and speed slope (mean = +0.21) in PD-OFF group were both positive, indicating the lack of decrement and fatigue at that time point and the slope values did not differ between PD-OFF, PSP and control groups. This analysis indicated that 20-tap trials were not adequate to detect either decrement or fatigue in Parkinson's disease. Based on these findings, it is proposed that repetitive finger tapping with 50-tap cycles is required to detect criteria-defined bradykinesia in treated and untreated patients with Parkinson's disease.

Handwriting in Parkinson's disease and PSP

Micrographia was more common in PSP (75%) than in Parkinson's disease (15%). Decrement in script size was less common in PSP than in PD-OFF. These findings were similar to the 'hypokinesia without decrement' in repetitive finger tapping observed in PSP. Five of the six PSP patients with micrographia, also manifested hypokinesia on repetitive finger tapping. Despite the similarities in the findings of finger tapping and handwriting in PSP, the correlations between the parameters of these two kinematic tasks are inexact. 'Fast micrographia' characterised by microscopically small letters performed at a normal or slightly faster than normal speed may be a physical sign related to pallidal damage (Kuoppamaki *et al.*, 2005) and has been associated with some cases of PSP. General clinical impression suggests an increase in writing speed in some PSP patients. Nevertheless, it is uncertain if the 'fast' speed represents a shorter performance time due to the reduced stroke size or an intrinsic increase in writing speed. This cannot be verified as the handwriting task was not timed in the present study.

McLennan and colleagues reported micrographia in 15% of Parkinson's disease patients and, in 16 out of 30 cases, a significant and sustained improvement in script size was noted after levodopa therapy (McLennan *et al.*, 1972). The findings of this study also showed the same percentage of micrographia in Parkinson's disease and after levodopa therapy, six patients exhibited marked improvement in script size but

decrements in script size persisted. Copying scripts, as in the present study, writing on parallel lines and verbal reminders to write 'big' can serve as external cues to correct a reduction in script size (Bryant *et al.*, 2010, Kim *et al.*, 2005, Oliveira *et al.*, 1997). Abnormally increased dependence on external visual feedback has been noted in patients with Parkinson's disease (Demirci *et al.*, 1997). The mechanism of micrographia is poorly understood but the hypothesis of a 'tuned-down' sensorimotor apparatus might explain the reduction in motor scaling during sequential motor tasks such as finger tapping and handwriting (Demirci *et al.*, 1997).

Strengths and limitations

The three-dimensional motion assessment used in this study proved particularly useful in tremulous patients who would have had difficulties maintaining their finger separation in a two-dimensional plane. This method captures accurate and diverse measurements of the finger tap trials. Pilot studies were conducted on healthy volunteers and it was determined that healthy elderly participants become tired after prolonged tap sequences of more than 15 to 20 seconds. In order to minimise the confounding factor of physiological fatigue, the trial duration was, therefore, limited to 15 seconds. The study was specifically designed to study motor execution, not processing or reaction time. Certain aspects of the quantitative measurements made here may not be applicable to subjective bedside observation. Further studies are warranted to apply the findings of this study in a clinical context. However, very small finger tapping amplitudes can be easily identified during neurological examination. The relative persistence of the sequence effect despite levodopa therapy in Parkinson's disease makes it an especially useful physical sign to help distinguish Parkinson's disease and PSP. Future prospective studies on patients with early PSP or PSP-P subtype will determine if this specific finger tap pattern can be used as a reliable early diagnostic sign to distinguish between Parkinson's disease and PSP and if the specific patterns in PSP applies to all PSP subtypes. Kinematic studies in PSP may also help our understanding of the mechanisms underlying the findings in this study.

An inherent limitation of clinical studies of this kind is the lack of pathological confirmation of diagnosis given that about 20% of suspected PSP cases and 10% of

suspected Parkinson's disease cases are found at post-mortem to have a different pathology (Snowden *et al.*, 2012). Finally, it should be noted that patients with prominent tremor were excluded from this study. In tremor-predominant Parkinson's disease, motor flurries can potentially interrupt normal self-paced movements, confounding clinical interpretation (Bajaj *et al.*, 2010).

Conclusion

Patients with PSP have small finger separation amplitude without progressive decrement on repetitive finger tapping and do not have criteria-defined limb bradykinesia. The severe hypokinesia irrespective of disease severity and the lack of a sequence effect help distinguish PSP patients from those with Parkinson's disease. Similarly, 'micrographia' and 'lack of decrement' in script size is also more common in PSP than in Parkinson's disease.

Chapter 5: Parkinson's syndrome associated with neurofibrillary tangle pathology

5.1: Parkinsonism with neurofibrillary degeneration: a new clinicopathological entity?

Introduction

Parkinson's disease is a progressive levodopa-responsive bradykinetic syndrome, which, in specialist centres, can be accurately diagnosed in life in the great majority of cases (Hughes *et al.*, 1992, Lees *et al.*, 2009). The salient histopathological findings are nigral cell loss and the accumulation of α-synuclein-positive Lewy bodies (Gibb *et al.*, 1988). A QSBB series identified 24 out of 100 cases, that were misdiagnosed as Parkinson's disease by United Kingdom hospital specialists and did not have Lewy body pathology at post-mortem (Hughes *et al.*, 1992). The pathological diagnoses in the misdiagnosed cases were PSP (6), multiple system atrophy (5), Alzheimer's disease (3), vascular parkinsonism (3) and essential tremor with normal neuropathological findings (1). Another five cases, which were considered unclassifiable had Alzheimer-type neurofibrillary tangle pathology (NFT) with striatal involvement (3), postencephalitic parkinsonism (PEP)-type pathology (1) and pure nigral degeneration (1).

The epidemic of encephalitis lethargica (EL) occurred between 1917 and 1928 and was first fully described by Constantin von Economo in Vienna (Von Economo, 1917, Von Economo, 1931). At least half of all EL survivors developed progressive parkinsonism after a latent interval that ranged from a few months to several decades (Duvoisin *et al.*, 1965, Geddes *et al.*, 1993, Howard *et al.*, 1987, Rail *et al.*, 1981). Von

Economo suspected the extensive neuronal loss in the substantia nigra in PEP brains was the pathological substrate for akinesia and rigidity in these patients (Ransmayr, 2007). The predominant pathological aggregates in PEP are Alzheimer-type NFTs (Geddes *et al.*, 1993, Jellinger, 2011, Torvik *et al.*, 1966, Wenning *et al.*, 1997). In the 1920's and 1930's, PEP accounted for half of all parkinsonism in Europe and up until the mid-nineteen sixties, it was common for all young onset cases of slowly progressive parkinsonism to be labeled as PEP even in the absence of a history of encephalitis or the presence of oculogyric crises. In some patients, trivial flu-like illnesses were over-interpreted as indicative of an antecedent mesencephalitis (Burr, 1925, Duvoisin *et al.*, 1965, Gibb *et al.*, 1987, Rail *et al.*, 1981, Vilensky *et al.*, 2011). Some of the first described cases of PSP were also erroneously attributed as PEP (Brusa *et al.*, 2004, Steele, 1994) and it was speculated that parkinsonism-dementia complex of Guam might be a PEP variant (Kurland *et al.*, 1954). Cases coming to autopsy with the classical pathological findings associated with PEP were also often considered to have had the disorder even when the clinical picture differed markedly from the recognised presentation (Geddes *et al.*, 1993, Gibb *et al.*, 1987). By the end of the twentieth century, virtually all the survivors of von Economo's disease had died but sporadic PEP cases presenting with a clinical picture identical to that reported during the pandemic have continued to be reported (Duvoisin *et al.*, 1965, Eadie *et al.*, 1965, Ghaemi *et al.*, 2000, Hu *et al.*, 2012, Rail *et al.*, 1981, Williams *et al.*, 1979).

Sporadic cases of Parkinson's syndrome with NFT pathology without a history of encephalitis were first highlighted by Stadlan, Duvoisin and Yahr (Stadlan *et al.*, 1965) and ten cases were subsequently described by Forno and Alvord (Forno *et al.*, 1971). Morris and colleagues reviewed clinically atypical PSP cases in the QSBB and found further support for a separate entity of NFT-related parkinsonian disorder with the deposition of Alzheimer-type mixed 3R and 4R tau and clinically more closely resembling Parkinson's disease than PSP (Morris *et al.*, 2002). This study reviewed all the archival QSBB cases in which the final clinical diagnosis was either Parkinson's disease or PEP without a history of encephalitis with severe nigral degeneration and NFT pathology that could not be ascribed to any recognised clinicopathological entity, and identified seven cases with clinicopathological findings that resembled the group of patients described by Forno and Alvord almost half a century earlier (Forno *et al.*,

1971). This group of cases will be referred as ‘slowly progressive parkinsonism with neurofibrillary degeneration’ (PND) in the rest of this chapter.

Materials and methods

Written consent was obtained from all the included cases. The protocols used for brain donation in the QSBB have been approved by a London Multi-Centre Research Ethics Committee and tissue is stored under a license from the Human Tissue Authority. This study has been approved by the Tissue Request Committee of the QSBB.

Case selection

Cases were selected from the QSBB archival collection on the basis of the following criteria: (i) they had a final clinical diagnosis of Parkinson’s disease or PEP, (ii) an absence of a history of definite encephalitis despite full clinical documentation, (iii) slowly progressive disease course of ten years or more and (iv) neuropathological findings of nigral cell loss, the absence of Lewy body pathology and predominant NFT pathology that did not readily fulfil pathological criteria for any known primary tauopathy.

Another five archival PEP cases were included in this study as comparative controls. These PEP cases fulfilled the diagnostic criteria of definite PEP with a history of EL which was followed by the development of progressive parkinsonism (Duvoisin *et al.*, 1972, Duvoisin *et al.*, 1965, Geddes *et al.*, 1993, Jellinger, 2011). All of these cases had been previously published in a previous QSBB PEP series (Geddes *et al.*, 1993). Only two of the PEP cases had frozen tissue available for genetic analysis.

Retrospective review of the medical records was carried out. All patients were assessed by at least one neurologist during life. Symptoms and signs were recorded as being absent if they were not reported in the case notes.

Neuropathological methods

Following the QSBB protocols, the brains were divided mid-sagittally post-mortem. One-half of the brain was immediately frozen and stored at -80ºC, and the other half was immersed and fixed in 10% neutral formalin for 3 weeks. After slicing and sampling the brain, tissue blocks were processed using standard protocols. Using on 8-μm thick sections histological slides were stained with haematoxylin and eosin method and modified Bielschowsky and Gallyas silver impregnations. Immunohistochemistry with antibodies to phospho-tau (AT8 clone recognizing Ser202/Thr205; BioScience Life Sciences; 1:600 and AT100 recognising Thr212/Ser214; BioScience Life Sciences; 1:200), 3R and 4R tau isoforms (3R tau: RD3; 1:2000; 4R tau: RD4; 1:200)(de Silva *et al.*, 2003), α B-crystallin (Novocastra; G2JF; 1:3000), Aβ (Dako; 6F/3D; 1:100), p62 (BD Transduction Laboratories; 1:200), TAR DNA-binding protein-43 (TDP-43, monoclonal; clone 2E2-D3; 1:3000), and α-synuclein (Vector Laboratories; KM51; 1:50) was carried out using a standard avidin-biotin method. Double immunofluorescence for AT8 with GFAP (Dako; Z0334 1:1000) was detected using isotype specific anti-rabbit IgG or anti-mouse IgG secondary antibodies conjugated with either Alexa 488 or 594 fluorescent dyes (Life technologies, Paisley, UK; 1:400) followed by mounting with glass coverslips using VECTA shield mounting media with 4',6-diamidino-2-phenylindole (DAPI) nuclear stain (Vector laboratories, Peterborough, UK). Images were visualised using confocal fluorescence microscopy (Leica DM5500 B).

Neuropathological analysis

A systematic neuropathological evaluation was carried out. Neuronal loss in the substantia nigra, subthalamic nucleus, locus coeruleus, caudate, putamen, globus pallidus and dentate nucleus were determined using a four-tiered semi-quantitative grading system (0-3, with grade 0=no neuronal loss to grade 3=most severe neuronal loss). The substantia nigra was divided into five regions for the grading (medial, dorsomedial, dorsolateral, ventrolateral and lateral). The different tau-positive cellular lesions, NFTs, PreTs, NTs, CBs and astrocytic lesions were quantified using a four-tiered semi-quantitative grading scale (0-3, with grade 0=absence to grade 3=frequent).

Additional pathologies were documented. Argyrophilic grain disease was identified by AT8, α B-crystallin and p62 immunohistochemistry (Saito *et al.*, 2004). Aβ cortical plaque pathology was characterised using the CERAD criteria (Mirra *et al.*, 1991). Alzheimer's type NFT pathology was determined using AT8 immunohistochemistry for Braak and Braak staging (Braak *et al.*, 2006). Alzheimer's type Aβ-deposition was assessed for a Thal phase score (Thal *et al.*, 2002). The ABC score according to the National Institute on Aging-Alzheimer's Association (NIA-AA) guidelines representing the level of Alzheimer's disease pathologic change was applied (Montine *et al.*, 2012). The presence of cerebrovascular disease and CAA were recorded (Revesz *et al.*, 2003).

Tau biochemistry

Isolation and guanidine solubilisation of sarkosyl-insoluble tau was performed in seven PND cases and two PEP cases with frozen brain tissue available using a standard protocol as described in Chapter 3 (Goedert *et al.*, 1992). Guanidine solubilized tau was then dephosphorylated using lambda protein phosphatase (New England Biolabs, Hitchin, UK) as previously described (Hanger *et al.*, 2002) and separated by standard SDS-polyacrylamide gel electrophoresis (Laemmli, 1970) on a 10% gel followed by Western blot detection with a rabbit polyclonal anti-tau antibody (1;60,000; rPeptide, Bogart, GA). Cases with the pathological diagnosis of Alzheimer's disease, PSP, CBD and Pick's disease were included on the blot as comparative controls. Brain samples were obtained from the frontal cortex, caudate, pontine tegmentum and cerebellar dentate nucleus. However, not all regional samples yielded sufficient tau for analysis.

Genetic analysis

Genomic DNA was extracted from the frozen brain tissue of 12 included cases. Exons and exon-intron boundaries for all exons of *parkin* and *MAPT*, and for exons 24, 25, 27, 29, 35, 36, 41, and 48 of *LRRK2* were screened through Sanger sequencing of transcripts with accession numbers NM_004562.2/ NP_004553.2, NM_005910.5/NP_005901.2 and NM_198578.3/NP_940980.3 respectively. These genes were chosen because they may cause a Parkinson's syndrome with absence of Lewy body pathology (Doherty *et al.*, 2013, Hoglinger *et al.*, 2011, Hutton *et al.*, 1998, Wszolek *et al.*, 2004, Zimprich *et al.*, 2004). *PINK1* and *DJ1* were sequenced in two cases, where heterozygous *parkin* mutations (c.1310C>T, p.P437E (rs149953814), c.823C>T, p.R275W (rs34424986))(Kay *et al.*, 2007, Lesage *et al.*, 2008) were identified, to exclude digenic mutations (Funayama *et al.*, 2008). All cases were also assessed for whole exon deletions or multiplications in the Parkinson's disease-related genes *parkin*, *SNCA*, *PINK1*, *PARK7*, *UCHL1*, *GCH1* and *LRRK2* through the Multiplex Ligation-dependent Probe Amplification kits P051 and P052 (MRC Holland). *MAPT* haplotypes (Hayesmoore *et al.*, 2009) and *APOE* genotype (Rail *et al.*, 1981) were determined through the H1/H2-tagging SNP rs1052553 and through sanger sequencing of exon 4 (Ghebranious *et al.*, 2005), respectively.

Results

Between 1989 and 2012, 750 cases were donated to the QSBB with a final clinical diagnosis of Parkinson's disease. Of these, 70 cases (9.3%) showed no evidence of Lewy body pathology, with 14 cases fulfilling the inclusion criteria of this study. Two cases were later excluded due to the lack of frozen brain tissue which was required for tau immunoblotting and DNA extraction for genetic analysis. The two excluded cases were previously reported as case 6 in a PEP series (Geddes *et al.*, 1993) and case 1 in a report of non-demented parkinsonian patients with Alzheimer-type pathology (Daniel *et al.*, 1991). Seven of the 12 included cases were classified as PND. The remaining five had other confirmed genetic and neuropathological findings which distinguished them from the PND group (Figure 18):

Two cases carried a rare p.A152T variant in exon 7 of the *MAPT* gene which was a risk factor for atypical neurodegeneration and may present with parkinsonism or dementia and abnormal tau accumulation in post-mortem (Coppola *et al.*, 2012, Kovacs *et al.*, 2011)(Chapter 5.2). One case carried the G2019S *LRRK2* mutation and a novel heterozygous p.Q124E variant in exon 4 of the *MAPT* gene and had nigral degeneration, Alzheimer-type tau, occasional TDP-43 pathology and absence of Lewy bodies (Chapter 5.3). One case, who had a ten-year history of parkinsonism with gait freezing, normal cognition, bilateral extensor plantar responses and transient levodopa response, came to post-mortem at the age of 76 in 1989, and was initially reported to have Alzheimer-type pathology (Daniel *et al.*, 1991). Re-evaluation using modern immunohistochemistry with AT8, 3R and 4R antibodies showed unequivocal 4R PSP-tau pathology, including tufted astrocytes and CBs in the caudate nucleus and globus pallidus. Concomitant extensive Alzheimer-type tau pathology was also observed (Braak and Braak IV and 'frequent' CERAD neuritic plaque score). The relatively restricted and sparse tau pathology was compatible with PSP-P (Williams *et al.*, 2007). Another case had early fall and subsequently developed progressive parkinsonism with moderate levodopa response, came to post-mortem in 1992. Re-evaluation confirmed the pathological diagnosis of PSP-P and the patient also carried a heterozygous p.P437E *parkin* mutation. However, the direct causative role of a single heterozygous *parkin* mutation in parkinsonism remains unclear (Klein *et al.*, 2007).

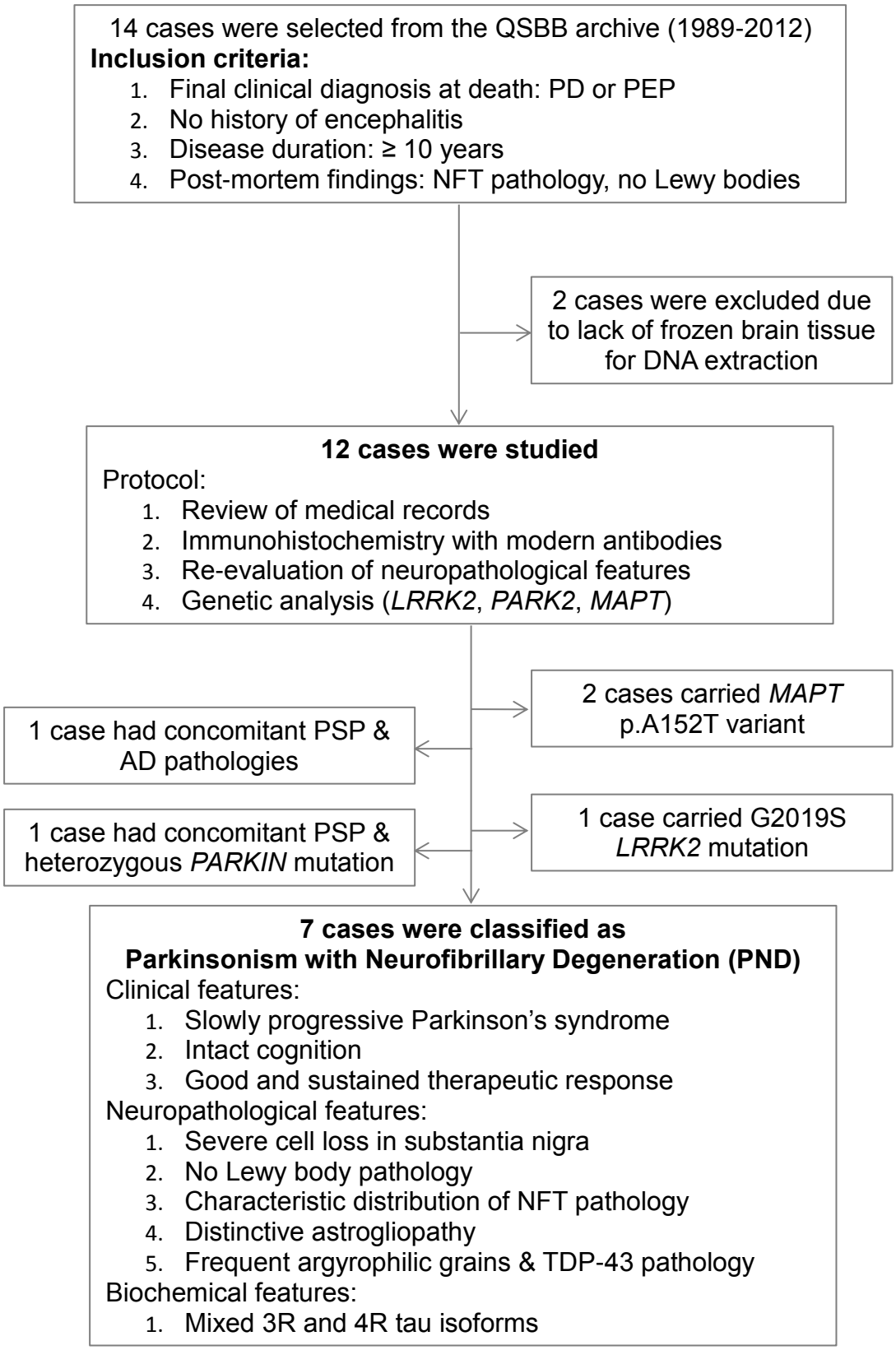

Figure 18. *Overview of 12 included cases with parkinsonism associated with neurofibrillary tangle pathology.*

Parkinsonism with neurofibrillary degeneration

Seven cases with distinct clinicopathological features were identified and collectively referred as PND. All patients had a slowly progressive levodopa-responsive Parkinson's syndrome with intact cognition and histopathological findings of severe nigral degeneration, Alzheimer-type NFT pathology in a characteristic distribution, distinctive astrogliopathy and high prevalence of argyrophilic grains and TDP-43 pathology (Tables 16-17).

Demographic and clinical features

All patients were born between 1910 and 1929 and came to post-mortem between 1989 and 2001. Cases 6 and 7 were previously reported (Geddes *et al.*, 1993). Three cases (cases 1, 5 and 6) had received a differential diagnosis of PEP because of their history of oculogyric crises (cases 5 and 6), young age of onset of parkinsonism in the early 30's (cases 1 and 5) and a history of Spanish flu during the epidemic in 1919 (case 1). None of the cases had a history of encephalitis, but one patient (case 7), at the age of 8 in 1927, spent two months in bed with what was labelled as 'nerves' and, at age 24, developed parkinsonism with good levodopa response and was clinically diagnosed as Parkinson's disease. One patient had influenza during the epidemic in 1919. There was no history of significant head injury, family history of movement disorders or dementing illnesses.

The mean age of onset of parkinsonism was 42.6 years (range: 24-63 years), mean disease duration was 36.4 years (range: 19-50 years) and mean age of death was 78.9 years (range: 67-87 years). Four patients were reported to have sustained asymmetry in their motor disability throughout the disease course (cases 1, 2, 6, 7). Four had a resting limb tremor (cases 1, 4, 6 and 7) and two underwent unilateral thalamotomy with complete resolution of tremor (cases 6 and 7). Six patients had received levodopa therapy with sustained good to excellent benefit and one patient (case 6) was never prescribed anti-parkinsonian medications because his parkinsonian features significantly improved after unilateral thalamotomy. Three developed levodopa-induced chorea. No patients had dementia and only one patient (case 4) reported mild

impairment in short term memory. Psychiatric symptoms were noted in three patients (cases 1, 2 and 7), including nervous breakdown after a stressful life event (N=1), depression (N=1) and anxiety (N=2). Two patients had a history of oculogyric crises (cases 5 and 6), two had restricted upgaze (case 2 at age 77, case 5 at age 67) and one had blepharospasm (case 2).

Case vignette (case 5)

In 1947, aged 32, this woman developed weekly episodes of tonic oculogyric spasms lasting for 30 minutes but their frequency gradually waned after a few years. She then developed a very slowly progressive akinetic rigid syndrome. In 1975, levodopa was administered with good response. Mild levodopa-induced chorea was noted. At age 73, examination revealed hypomimia, intermittent hand tremor and bilateral limb rigidity, corresponding to H&Y stage II. An Apomorphine challenge test was positive which confirmed a beneficial response to dopaminergic replacement therapy. Two years later, she had increasing gait and balance difficulties and started to fall. At age 77, her UPDRS motor score was 27 and H&Y stage was IV. She had restriction of upgaze, occasional jaw tremor, marked bilateral bradykinesia with minimal limb rigidity, festinant parkinsonian gait and poor postural stability. She suffered two hip fractures as a result of frequent falls. Her speech and swallowing deteriorated relentlessly necessitating tube feeding in the last year of life. She was taking 300mg/day of levodopa and 5mg/day of selegiline. She died of pneumonia at age 82. The final clinical diagnosis was Parkinson's disease but the diagnosis of PEP had also been suggested earlier in the course of the illness. The lack of Lewy body pathology in post-mortem prompted genetic analysis which identified a heterozygous p.R275W mutation in the *parkin* gene.

Features	Case 1	Case 2	Case 3	Case 4
Year of birth	1910	1929	1913	1917
Gender	F	M	M	M
Acute Illnesses (year)	Flu (1919)	-	-	-
Oculogyric crises	-	-	-	-
Onset of parkinsonism	34 (1944)	48 (1977)	63 (1976)	46 (1963)
Levodopa response	Excellent	Good	Good	Good
Dyskinesia	+	-	-	+
Duration of parkinsonism,year	48	19	25	35
Age at death	82	67	87	81
Year of post-mortem	1993	1997	2001	1998
Neurofibrillary tangles				
Frontal cortex	+	-	+	+
Temporal cortex	+	+	+	+++
CA1 hippocampal subregion	+++	-	+	+++
GCL hippocampal subregion	+++	-	-	+++
Amygdala	+++	+	+++	+++
Caudate	++	+	+	++
Putamen	+	+	+	++
Globus pallidus	++	+	-	++
Subthalamic nucleus	+	+	+	+
Substantia nigra	+	++	+	+
Oculomotor nucleus	NA	-	+	+
Pontine tegmentum	+	+	+	+
Pontine base	-	-	-	+/-
Locus coeruleus	+	+	+	+
Dentate nucleus	+	+/-	-	+
Astrocytic lesions				
Frontal cortex	++ B	-	-	+ G
Temporal cortex	++ B	-	-	-
Caudate	++ G	-	-	+ G
Putamen	++ G	-	-	+ G
Subthalamic nucleus	+ G	-	-	-
Midbrain tegmentum	++ F,G	-	-	+ F
Substantia nigra	-	-	-	+ F
Pontine tegmentum	++ F,G	-	+ G	+ G
Substantia nigra cell loss	3 (except DM-2, M-2)	3 (except M-2)	3 (except M-2)	3-all
Locus coeruleus cell loss	0	3	1	3
Subthalamic nucleus cell loss	0	0	0	0
Ghost tangles	-	-	-	+
Braak and Braak stage	II	I	III	IV
CERAD	None	Few	Few	Moderate
Thal Aβ phase score	3	1	5	4
Alzheimer-related ABC score: likelihood of AD change	A2B1C0: low	A1B1C1: low	A3B2C1: intermediate	A3B2C2: intermediate
Hippocampal sclerosis	-	-	-	+
Argyrophilic grain disease	+ (stage 3)	-	+ (stage 1)	1 (stage 2)
TDP-43 pathology	-	-	+	+
Cerebral amyloid angiopathy	-	-	+ (mild)	+ (moderate)
Cerebrovascular disease	+ (mild SVD)	-	+ (mild SVD)	-
Genetic findings (*MAPT, PARK2, LRRK2)*	-	*MAPT**	-	-
MAPT genotype	H1/H1	H1/H1	H1/H2	H1/H2
APOE	E2/E3	E3/E3	E3/E3	E3/E4

Table 16. *Features of PND (cases 1 - 4).*

Tau immunoreactive neurofibrillary tangles and astrocytic lesions were determined using a four-tiered semi-quantitative grading system; -: absent, (-/+: very sparse), +: few, ++: moderate, +++: frequent.

B=bush-like, F=filamentous, G=granular, NA=not applicable as the oculomotor nucleus was not included in tissue section; GCL=granule cell layer; CERAD= Consortium to Establish a Registry for Alzheimer's disease

+: present; -: absent; Substantia nigra was divided into five regions for the grading; M=medial, DM=dorsomedial, DL=dorsolateral, VL=ventrolateral and L=lateral.

**=MAPT intron 4A-5 was identified in case 2 (rs138482356) which is probably non-pathogenic.*

Cell loss of the substantia nigra, locus coeruleus and subthalamic nucleus were determined using a four-tiered semi-quantitative grading system; 0-3; grade 0=no neuronal loss to grade 3=most severe neuronal loss.

Features	Case 5	Case 6*	Case 7*
Year of birth	1915	1904	1919
Gender	F	M	F
Acute Illnesses (year)	-	-	'nerves' (1927)
Oculogyric crises	+ (32)	+ (26)	-
Onset of parkinsonism	32 (1947)	51 (1955)	24 (1933)
Levodopa response	Good	NA#	Good
Dyskinesia	+	NA	-
Duration of parkinsonism, years	50	33	45
Age at death	82	84	69
Year of post-mortem	1998	1989	1989
Neurofibrillary tangles			
Frontal cortex	++	+	+
Temporal cortex	+	+	+
CA1 hippocampal subregion	+++	+	+++
GCL hippocampal subregion	-	-	+
Amygdala	+++	+++	+++
Caudate	+	+	+++
Putamen	+	+	+
Globus pallidus	-	+	+
Subthalamic nucleus	+	+	+
Substantia nigra	++	+	+
Oculomotor nucleus	NA	+	NA
Pontine tegmentum	+	++	+
Pontine base	-	+/-	+
Locus coeruleus	++	+	+
Dentate nucleus	+/-	+	+
Astrocytic lesions			
Frontal cortex	+ G	-	+ B
Temporal cortex	-	-	-
Caudate	-	++ B, G	+ B, G
Putamen	+ G	-	-
Subthalamic nucleus	-	-	-
Midbrain tegmentum	+ F, G	++ G	+ F, G
Substantia nigra	+ G	-	++ G
Pontine tegmentum	-	++ G	+ F
Substantia nigra cell loss	3 (except M-1)	3-all	3-all
Locus coeruleus cell loss	0	0	2
Subthalamic nucleus cell loss	0	0	0
Ghost tangles	-	-	+
Braak and Braak stage	IV	III	III
CERAD	Few	None	None
Thal Aβ phase score	4	1	1
Alzheimer-related ABC score: likelihood of AD pathologic change	A3B2C1: low	A1B2C0: low	A1B2C0: low
Hippocampal sclerosis	-	-	-
Argyrophilic grain disease	-	-	1 (stage 1)
TDP-43 pathology	+	+	+
Cerebral amyloid angiopathy	-	-	-
Cerebrovascular disease	+ (mild SVD)	-	+ (lacunes)
Genetic findings (*MAPT, PARK2, LRRK2)*	*Heterozygous parkin***	-	-
MAPT genotype	H1/H2	H1/H1	H1/2
APOE	E3/E3	E3/E3	E2/E3

Table 17. Features of PND (cases 5 - 7).

Tau immunoreactive neurofibrillary tangles and astrocytic lesions were determined using a four-tiered semi-quantitative grading system; -: absent, (-/+: very sparse), +: few, ++: moderate, +++: frequent.

B=bush-like, F=filamentous, G=granular, NA=not applicable as the oculomotor nucleus was not included in tissue section; GCL=granule cell layer; CERAD= Consortium to Establish a Registry for Alzheimer's disease

+: present; -: absent; Substantia nigra was divided into five regions for the grading; M=medial, DM=dorsomedial, DL=dorsolateral, VL=ventrolateral and L=lateral; #Tremor and parkinsonian features were significantly improved following thalamotomy in 1960 and anti-parkinsonian medications were never prescribed.

**case 6 was reported as case 5 in Geddes et al, 1993; *case 7 was reported as case 7 in Geddes et al, 1993; **=A heterozygous p.R275W parkin mutation was identified in case 5 and additional sequencing of the PINK1 and DJ1 genes were negative.*

Cell loss of the substantia nigra, locus coeruleus and subthalamic nucleus were determined using a four-tiered semi-quantitative grading system; 0-3; grade 0=no neuronal loss to grade 3=most severe neuronal loss.

Neuropathological findings

Neuronal loss

Histological examination demonstrated severe neuronal loss, atrophy and gliosis in the substantia nigra with relative preservation in the medial region in some cases (cases 1-3 and 5; Figure 19). Neuronal loss in the locus coeruleus was observed in some cases, ranging from mild to severe (cases 2-4 and 7). The subthalamic nucleus was preserved without evidence of neuronal loss or atrophy. No cell loss was observed in the striatum and globus pallidus apart from occasional enlarged perivascular space noted in some cases (cases 1 and 3).

α-synuclein, AT8 and Aβ immunohistochemistry

There were no Lewy bodies, Lewy neurites or other abnormal structures labelled with the anti-α-synuclein antibody. AT8 immunohistochemistry demonstrated NFTs and NTs in variable amount and the severity of the tau pathology ranged from mild to severe in the frontal and temporal cortices. In some cases, neuritic plaques were also seen (cases 2-5; Figure 20). Only few NFTs and NTs were observed in the parietal cortex. Aβ-immunoreactive diffuse and mature parenchymal plaques were sometimes, but not always found in the frontal and temporal cortices. The amygdala was similarly affected by tau and Aβ pathologies. The severity of NFTs, PreTs, NTs ranged from moderate to severe in all hippocampal subregions; except in case 2, where tau pathology was very sparse. Aβ-positive diffuse and mature plaques were observed in the hippocampal subregions in some cases only (cases 1, 3-5). NFTs, PreTs, and NTs were invariably observed in the striatum, subthalamic nucleus, substantia nigra, pontine tegmentum, locus coeruleus and, in most cases, globus pallidus. Tau pathologies were sometimes seen in motor neurons of the oculomotor nucleus, but were rare in the pontine base and dentate nucleus. The tau lesions were both 3R- and 4R-tau positive.

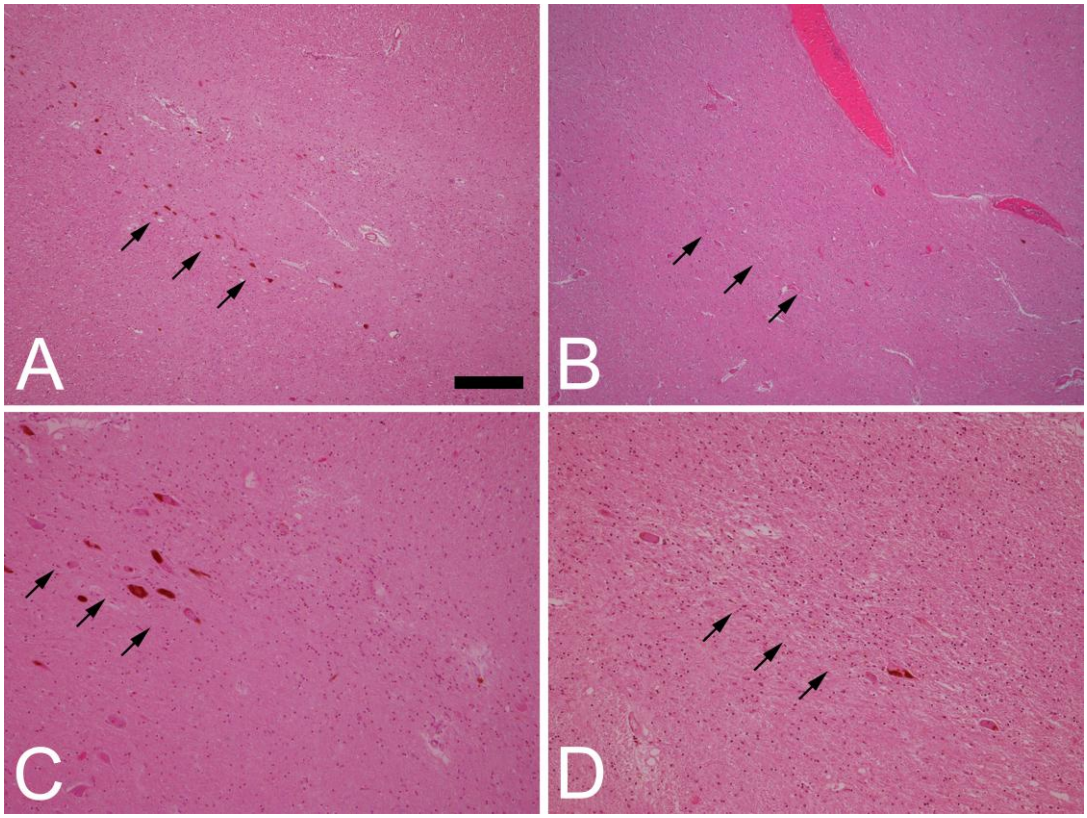

Figure 19. *Neuronal loss of substantia nigra in PND, PEP and PD.*

Neuronal loss in substantia nigra of cases with PD (A), PEP (B) and PND (C & D). Predominant neuronal loss in the ventral tier with remaining pigmented neurons in the dorsal tier in PD (A, arrows). Neuronal loss and gliosis in PEP (B=PEP-5) and PND (C=case 6, D=case 2) are more severe than in PD, with involvement of all parts of the pigmented pars compacta but sparing of the non-pigmented pars reticulata. Preservation of the medial tier of the pars compacta is occasionally observed in PND (C, arrows). H&E; scale bar in A=250μm in A & B, =100 μm in C and =20μm in D.

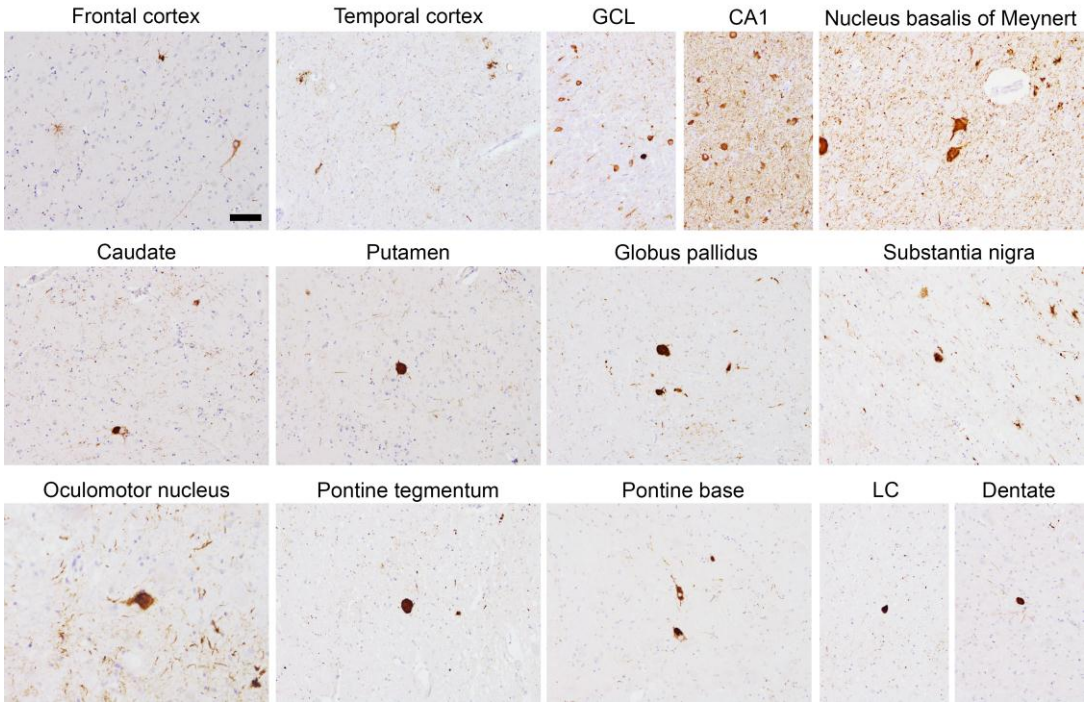

Figure 20. *Distribution of tau-positive lesions in different brain regions of PND.*

Tau immunoreactivity in different regions in parkinsonism with neurofibrillary degeneration (case 4). Neurofibrillary tangles, pretangles, neuropil threads and occasional astrocytic lesions, are observed. AT8; scale bar=50μm.

AT8 immunoreactive astrogliopathy

Astrocytic lesions characterised by bush-like, filamentous or granular tau immunoreactivity along with astrocytic processes were variably observed in the frontal and temporal cortices, striatum, subthalamic nucleus, midbrain tegmentum, substantia nigra and pontine tegmentum; except in case 2, where tau pathology was mild in all regions (Tables 16-17, Figure 22). The presence of an astroglial nucleus with perinuclear enhancement of tau immunopositivity in the centre of a meshwork of labelled processes was observed allowed these structures to be distinguished from the patchy and more widely dispersed NTs. The majority of the astrocytic lesions were AT100-positive and Gallyas-negative, with some resembling those in argyrophilic grain disease. Rarely, astrocytic processes of the filamentous type were both AT100- and Gallyas-positive (case 5). Analysis of double label immunofluorescence microscopy revealed co-localisation of AT8 immunoreactive inclusions with GFAP positive astrocytes, shown in the pontine tegmentum of case 1 (Figure 21).

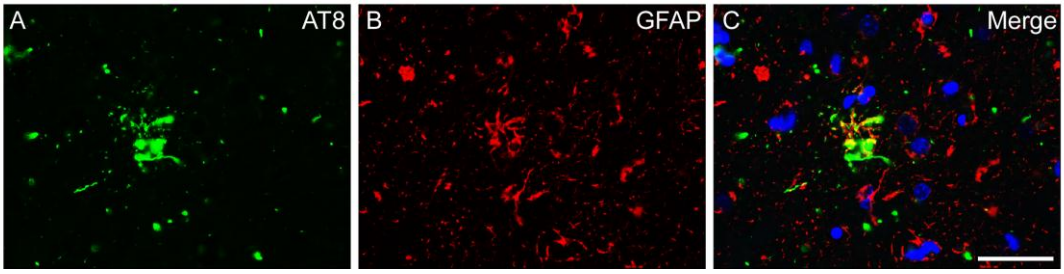

Figure 21. *Co-localisation of AT8 with GFAP-positive astrocytes.*

Representative double immunofluorescence images, from the pontine tegmentum of case 1, probed for AT8 (A, green; phospho-tau; Ser202/Thr205) and GFAP (B, red) show strong co-localisation in a subset of inclusions including fine neurites (C, yellow). DAPI (C, blue) stains the nuclei of surrounding neuronal and glial cells; scale bar=50μm.

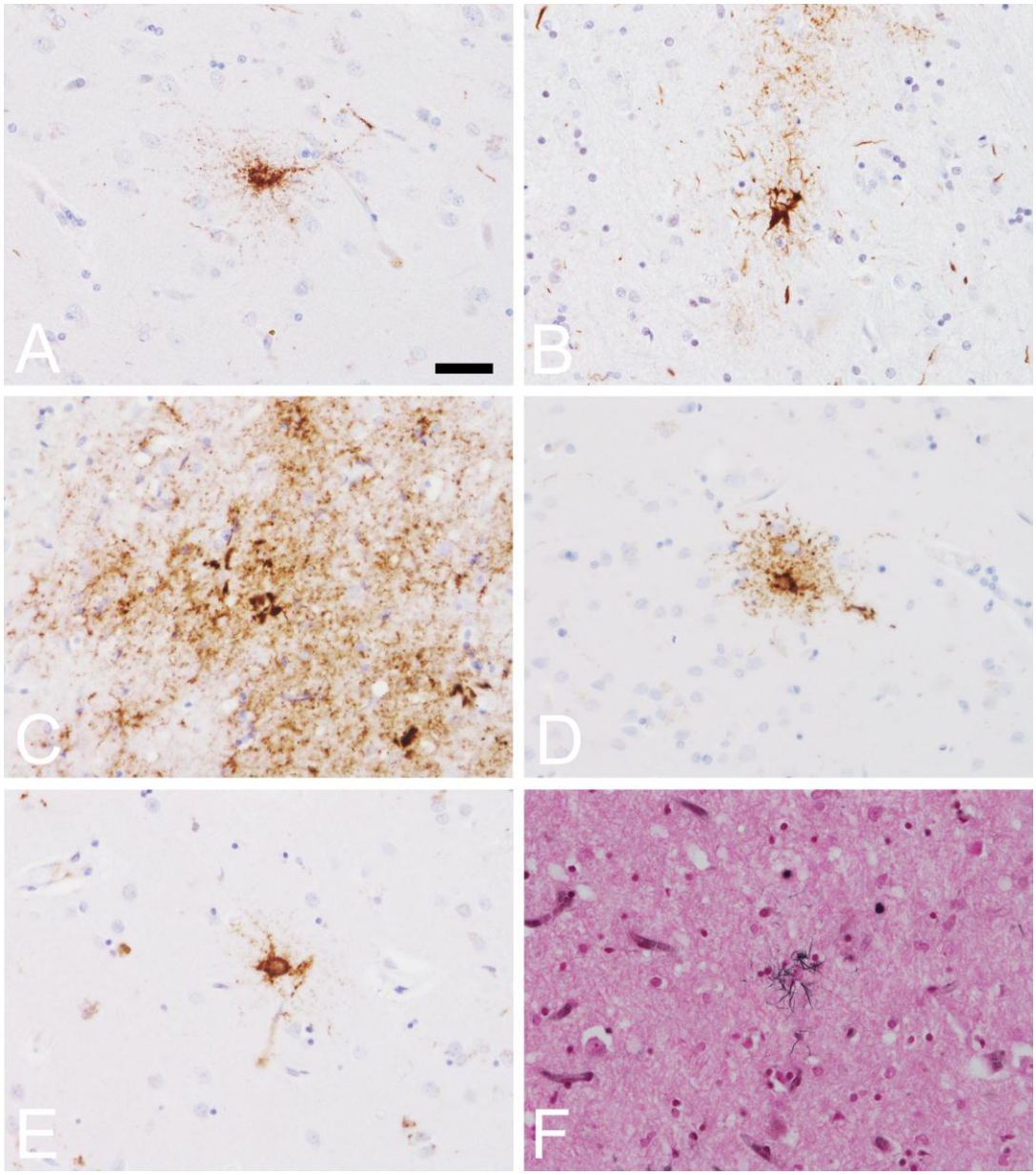

Figure 22. *Astrocytic lesions in PND.*

Characteristic astrocytic immunoreactivities in PND; granular lesion in the caudate (A=case 6), filamentous lesion adjacent to bush-like processes (B=case 7, midbrain), granular perinuclear accentuation and diffuse bush-like processes (C=case 1, midbrain). Bush-like astrocyte with perinuclear accentuation and thin process, most likely related to argyrophilic grain disease, in the temporal cortex (D=case 1). Most astrocytic lesions are AT100-positive (E=case 1; putamen) and Gallyas-negative. A rare Gallyas-positive filamentous astrocytic lesion in the midbrain tegmentum of case 5 (F). AT8: A-D; AT100: E; Gallyas: F; scale bar in A=25µm.

Ghost tangles

Ghost tangles, representing residual NFTs lying in the neuropil following neuronal death, were abundant in the CA1 hippocampal subregion, observed occasionally in the entorhinal and temporal cortices in two cases (cases 4 and 7), and were visible in H&E stained sections, Bielschowsky's silver impregnation and tau immunohistochemistry (Figure 23). Some of them were decorated with anti-Aβ antibody, indicating secondary Aβ deposition on extracellular tau filaments.

Additional pathologies

Alzheimer's disease related neuropathologic changes were identified as 'low' in five cases (cases 1, 2, 5-7) and 'intermediate' in two cases (cases 3 and 4) according to the NIA-AA guidelines (Tables 16-17)(Montine *et al.*, 2012). Argyrophilic grains were commonly observed (cases 1, 3, 4 and 7; Figure 23)(Saito *et al.*, 2004). Occasional TDP-43 immunoreactive neuronal cytoplasmic inclusions (NCIs) and threads (cases 3-7) and, rarely, neuronal intranuclear inclusions (NIIs) were identified (case 6; Figure 23). CAA, hippocampal sclerosis and cerebrovascular disease were occasionally seen (Tables 16-17). There were no p62-positive 'star-shaped' inclusions in the hippocampus or small 'dot-like' structures in the cerebellar granule cells of the type associated with *C9orf72* repeat expansion.

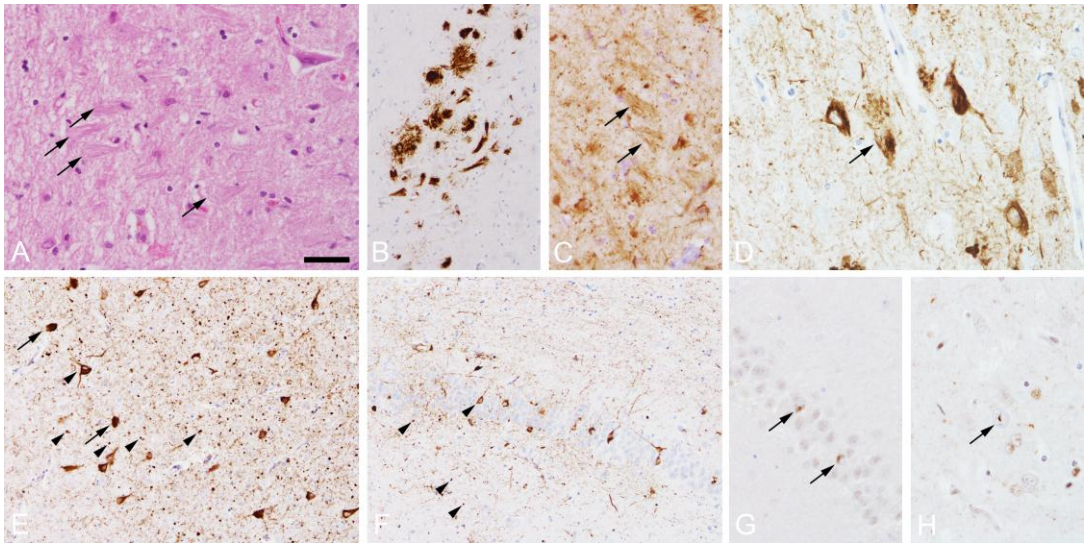

Figure 23. *Ghost tangles, argyrophilic grains and TDP-43 pathologies in PND.*

Extracellular ghost tangles (A-D, arrows) are visible in H&E (A=case 4, CA1 hippocampal subregion) and some are Aβ (B=case 4, CA1), representing extracellular protein aggregates and tau (C=case 4, CA1; D=case 7, CA1) immunoreactive. Pretangles and a neurofibrillary tangle are also seen in D. Tau immunoreactive argyrophilic grains (arrowheads) from the entorhinal cortex (E=case 1) and granule cell layer of the hippocampus (F=case 1) are observed. There are several pretangles (arrowheads) and neurofibrillary tangles (arrow) next to the grains. TDP-43 immunoreactive neuronal cytoplasmic inclusions (NCIs) in the granule cell layer of the hippocampus (G=case 3, arrows) and a rare neuronal intranuclear inclusion (NII; F=case 6, arrow) are identified. H&E: A, Aβ: B, AT8: C & D, TDP-43: G & H; scale bar in A=25μm in A, C, D, G & H, =50μm in B, E & F.

Tau biochemistry

Western blots of the non-dephosphorylated sarkosyl-insoluble tau fractions from the homogenates showed the characteristic triplet electrophoretic migration pattern in the PND, PEP and Alzheimer's disease cases indicating presence of paired helical filament-tau composed of both 3R and 4R-tau isoforms (not shown). This was confirmed after dephosphorylation of the sarkosyl-insoluble tau, where tau isoform profiles were comparable to that of Alzheimer's disease with similar levels 0N and 1N variants of 3R and 4R-tau. PSP and CBD controls showed the expected dominance of 4R-tau, mostly 0N4R and 1N4R, whereas Pick's disease showed almost exclusively 3R-tau (Figure 24).

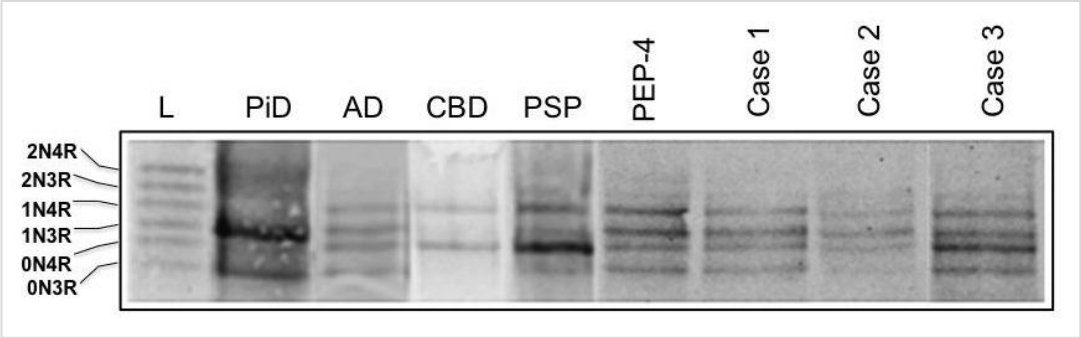

Figure 24. *Tau immunoblot of cases with PND, PDP and controls.*

Dephosphorylated sarkosyl-insoluble tau was analysed by Western blotting with detection by a rabbit anti-tau antibody. All six tau isoforms are observed in cases with parkinsonism with neurofibrillary degeneration (PND), postencephalitic parkinsonism (PEP) and Alzheimer's disease (AD). Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are 4R- tau predominant, mostly 0N4R and 1N4R. Pick's disease (PiD) showed almost exclusively 3R-tau. L-tau ladder (the relative positions of the six isoforms are indicated on the left); PND: case 1 (frontal cortex), case 2 (cerebellar dentate nucleus) and case 3 (frontal cortex).

Genetic analysis

Sanger sequencing of *LRRK2*, *parkin* and *MAPT* genes and dosage analysis of Parkinson's disease-related genes were negative in five of the seven cases with PND. A heterozygous p.R275W *parkin* mutation was identified in case 5; secondary sequencing of the *PINK1* and *DJ1* genes in this case was negative. A heterozygous rare variant in the *MAPT* intron 4A-5 was identified in case 2 (rs138482356). This mutation locates deeply within the intron (37 base pairs away from the end of exon 4A) and leads to a deletion of nine nucleotides (CCTCCCAGG) and is probably non-pathogenic. Four cases had H1/H2 and three cases had H1/H1 *MAPT* genotypes. The *APOE* genotype frequencies in the PND group were similar to those reported in the general population (Singh *et al.*, 2006) and in other tauopathies, where they probably do not confer to disease risk (Cairns *et al.*, 2007).

Postencephalitic parkinsonism

Five cases with a definitive clinical diagnosis of PEP were included in this study as comparative controls (Table 18). Four cases had a clear history of classical EL during the epidemic and one case (PEP-2) developed an EL-like illness in 1944. Two cases had a history of oculogyric crises.

The key neuropathological findings of our PEP control cases were consistent with those described in the literature (Geddes *et al.*, 1993, Haraguchi *et al.*, 2000, Jellinger, 2009, Jellinger, 2011, Torvik *et al.*, 1966, Wenning *et al.*, 1997). All subregions of the substantia nigra pars compacta was almost entirely devoid of pigmented neurons with atrophy and gliosis, but there was relative preservation of the non-pigmented pars reticulata (Gibb *et al.*, 1987). Moderate cell loss in the locus coeruleus was observed in most cases. The subthalamic nucleus was preserved.

Tau-immunoreactive NFTs, PreTs and NTs were found in the frontal and temporal cortices, hippocampus, amygdala, subthalamic nucleus, substantia nigra and, to a lesser extent, in the parietal cortex, striatum, globus pallidus, pontine tegmentum and locus coeruleus. Only minimal tau pathologies, if any, were observed in the pontine base and dentate nucleus in the cerebellum. Globose NFTs were identified in the residual neurons of the substantia nigra and locus coeruleus. These tau lesions were 3R- and 4R-tau positive. Bush-like and granular astrocytic tau immunoreactive lesions were common features (Haraguchi *et al.*, 2000, Ikeda *et al.*, 1993), some of which resembled the astrogliopathy in argyrophilic grain disease (Saito *et al.*, 2004). However, argyrophilic grains, frequently seen in PSP, were not found in these PEP cases (Togo *et al.*, 2002). Ghost tangles were abundant in three cases.

Few incidental α-synuclein-positive Lewy bodies were observed in the upper brain stem in one case (PEP-1) but α-synuclein pathology was absent in all other cases. Alzheimer-related Aβ parenchymal plaques and neuritic plaques with tau-positive abnormal neurites were sometimes observed in these elderly individuals (Table 18). Hippocampal sclerosis, CAA and TDP-43, were occasional features. Sequencing of the *LRRK2, parkin* and *MAPT* genes performed in two cases (PEP-4, PEP-5) was negative.

Features	PEP-1*	PEP-2	PEP-3	PEP-4	PEP-5
Year of birth	1907	1909	1912	1917	1922
Gender	F	M	M	F	M
Onset of EL: Age (year)	10 (1927)	35 (1944)	10 (1922)	10 (1927)	1 (1923)
OGC (age of onset)	-	-	+ (27)	-	+ (38)
Latency to parkinsonism[#]	<15	3	47	51	37
Onset of parkinsonism	<35(1942)	38 (1947)	57 (1969)	61 (1978)	38 (1960)
Levodopa response	Good	Good	NA	NA	Good
Dyskinesia	-	-	NA	NA	+
Duration of parkinsonism	>44	41	17	20	41
Age at death	79	79	74	81	79
Year of post-mortem	1987	1989	1986	1999	2001
Neurofibrillary tangles					
Frontal cortex	-	++	+	+	+
Temporal cortex	+/-	++	+	++	+
CA1 subregion	+++	+++	++	+++	+++
Amygdala	++	+++	+++	+++	+++
Caudate	+	+	+	+	++
Putamen	-	+	+	+	-
Globus pallidus	-	+	+	++	+
Subthalamic nucleus	+/-	+	+	++	+
Substantia nigra	+	+++	++	+	+++
Oculomotor nucleus	NA	NA	NA	NA	-
Pontine tegmentum	-	+	+	+	+
Pontine base	-	+	+	-	-
Locus coeruleus	-	+	+	+	-
Dentate nucleus	+	+	-	-	+
Astrocytic lesions					
Frontal cortex	-	-	-	-	-
Temporal cortex	-	-	-	-	-
Caudate	-	+ B	-	+ G	-
Putamen	-	-	-	-	-
Subthalamic nucleus	-	+ G	-	-	-
Midbrain tegmentum	-	+ G	+ G	+ B	+ G
Substantia nigra	-	+ G	-	-	+ G
Pontine tegmentum	-	+ B	+ G	-	-
Substantia nigra cell loss	3-all	3-all	3-all	3-all	3-all
Locus coeruleus cell loss	0	2	2	2	2
STN cell loss	0	0	0	0	0
Ghost tangles	+	+	-	-	+
Braak and Braak stage	II	IV	II	IV	III
CERAD	None	Moderate	None	Moderate	None
Thal Aβ phase score	0	3	0	5	2
Alzheimer-related ABC score: likelihood of AD	A0B1C0: not	A2B2C2: moderate	A0B1C0: not	A3B2C2: moderate	A1B2C0: low
Hippocampal sclerosis	-	+	-	+	+
Argyrophilic grain disease	-	-	-	-	-
TDP-43 pathology	-	+	-	-	+
CAA	-	+ (mild)	-	+ (mod)	-
Cerebrovascular disease	-	+ (mild)	+ (multiple infarcts)	-	-
Genetic findings (*MAPT, PARK2, LRRK2)*	NK	NK	NK	-	-
MAPT genotype	NK	NK	NK	H1/H2	H1/H2
APOE	NK	NK	NK	E3/4	E3/3

Table 18. *Features of five PEP control cases.*

Tau immunoreactive neurofibrillary tangles and astrocytic lesions were determined using a four-tiered semi-quantitative grading system (-: absent, +: few, ++: moderate, +++: frequent)

B=bush-like, F=filamentous, G=granular, NA=not applicable (as the oculomotor nucleus was not included in tissue section)

CERAD=Consortium to Establish a Registry for Alzheimer's disease, EL=encephalitis lethargica, Int=intermediate, NK=not known (no frozen brain tissue available for frozen tissue extraction), OGC=oculogyric crises, SVD=small vessel disease, +: present, -: absent

**These PEP cases were published in Geddes et al 1993: PEP-1 was reported as case 2, PEP-2 was reported as case 3, PEP-3 was reported as case 4. PEP-4 was reported as case 6, PEP-5 was reported as case 7*

#Latency from encephalitis lethargica to onset of parkinsonism

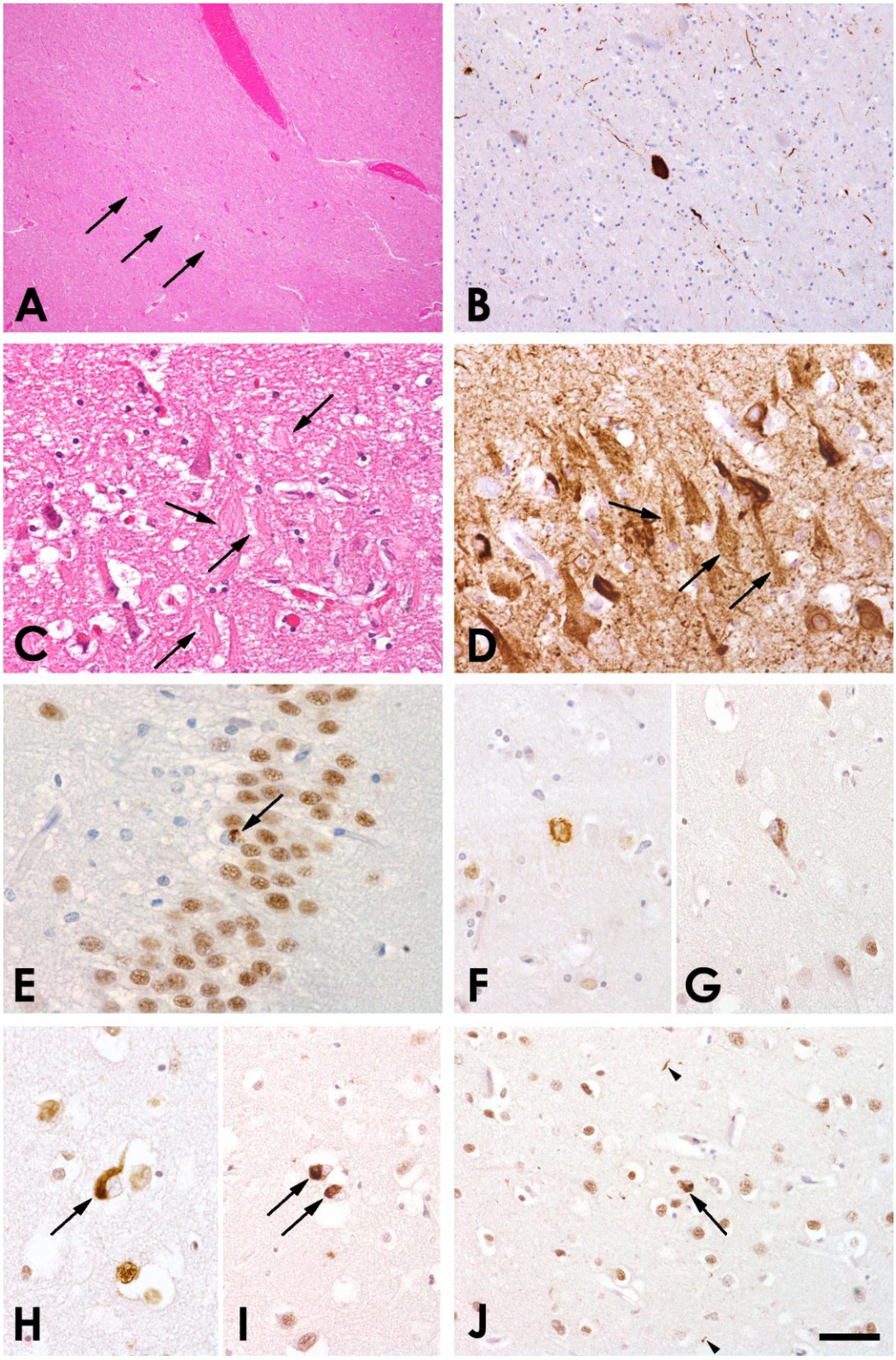

Figure 25. *Neuropathological features of PEP.*

Severe neuronal loss and gliosis in the ventral (arrows) and dorsal tier of the substantia nigra (SN) pars compacta with relatively sparing of the neurons in pars reticulala (A). Tau immunoreactive neurofibrillary tangle (NFT) and neuropil threads (NTs) in the SN (B). Many ghost tangles (arrows) are observed using H&E staining (C) and tau immunohistochemistry (D) in CA1 hippocampal subregion. TDP-43 immunoreactive neuronal cytoplasmic inclusions (NCI) in the dentate fascia of the hippocampus (arrow, E), NCI in the amygdala (left inset, F), tangle-like NCI in the subiculum (right inset, G), 2 NCIs (left inset) and a tangle-like NCI (right inset) in the entorhinal cortex (G), and a NCI as well as few neurites (arrows) in the inferior temporal neocortex (H).

A, C: H&E, B, D: tau immunohistochemistry and E-H: TDP-43 immunohistochemistry. A- D, E (left inset): case 7; E (right inset), F, G: case 2; scale bar in A=250µm, in B=50µm, in C, D, F-H=25 µm and in E=17µm.

Distinguishing features between PEP and PSP

Histopathological findings in PEP were thought to bear similarities to PSP (Geddes *et al.*, 1993). However, our re-evaluation provided some helpful histopathological distinctions (Table 19). Key histological features that distinguish PSP from PEP are atrophy of the subthalamic nucleus, cell loss in the dentate nucleus, cell loss in both the pigmented substantia nigra pars compacta and non-pigmented pars reticulata, frequent tau pathologies in the pontine base and dentate and the absence of ghost tangles (Dickson *et al.*, 2011, Hauw *et al.*, 1994, Oyanagi *et al.*, 2001). 4R-tau immunoreactive tufted astrocytes are frequently observed in the precentral gyrus, striatum and superior colliculus in PSP, and they differ from the mixed 3R and 4R-tau immunoreactive lesions in both PEP and PND in terms of morphology and distribution (Dickson *et al.*, 2011). The tau pathology is overall more severe in PSP than in either of these other two conditions. The *MAPT* H1/H1 genotype is over-represented in PSP but only three out of the seven PND cases are H1 homozygous. The remaining four cases as well as the two PEP cases that were analysed are H1/H2 heterozygous (Baker *et al.*, 1999, Houlden *et al.*, 2001).

Features	PND	PEP	AD	PSP
Substantia nigra cell loss	++	++	-/+	++
Subthalamic nucleus cell loss	-	-	-/+	++
Ghost tangles	-/+	+	+	-
Tau pathology				
Frontal cortex	+	++	+	+
Temporal cortex	++	++	++	-/+
Hippocampus	++	++	++	-
Amygdala	++	++	+	-
Caudate	++	+	-/+	++
Putamen	++	+	-/+	++
Globus pallidus	+	+	-/+	++
Subthalamic nucleus	++	++	-/+	++
Substantia nigra	++	++	-/+	++
Oculomotor nucleus	+	+	-/+	++
Pontine tegmentum	++	+	-/+	++
Pontine base	-/+	-/+	-	++
Locus coeruleus	++	+	+	++
Dentate nucleus	-/+	-/+	-	++
Astrocytic lesions				
Tufted astrocytes	-	-	-	++
Others	++ B,G,F	++ B,G	-	-
Tau isoforms	4R + 3R	4R + 3R	4R + 3R	4R>>

Table 19. *Neuropathological findings in PND, PEP, AD and PEP.*

Cell loss and tau immunoreactive lesions; -: not described, -/+: rare, +: commonly observed, ++: characteristic; B=bush-like, F=filamentous, G=granular, >> predominant.

Discussion

Of the 12 cases included in this study, seven cases were identified as PND who had slowly deteriorating levodopa-responsive Parkinson's syndrome and pathology compatible with that described in PEP with severe nigral degeneration and accumulation of Alzheimer-type tau pathology. The remaining five cases had other confirmed genetic and neuropathological findings, distinguishing them from the PND group, three of which are reported in Chapters 5.2 and 5.3.

The clinical features of PND resembled Parkinson's disease, but had a more benign course with normal longevity and a disease duration lasting up to 50 years. The deterioration in motor symptoms was very slow with preservation of cognition. Three patients (cases 3-5) had a more rapid worsening of gait, balance, speech and swallowing in the later stage. Another three patients (cases 1, 2 and 7), who died of cancer, had well controlled motor symptoms prior to death. Levodopa-induced dyskinesia observed in three PND cases was never a predominantly disabling feature as observed in patients with PEP or *parkin* disease. The mean age of onset was relatively young (42.6 years) and three patients developed their symptoms before the age of 40 (cases 1, 5, 7). A single heterozygous *parkin* mutation was identified in case 5. Although heterozygous carriers of *parkin* mutation is thought to have an increased generic risk for nigrostriatal dysfunction, its role in causing symptomatic parkinsonism is still unclear (Pavese *et al.*, 2009).

All PND cases were born before or during the EL epidemic but none of them had a history consistent with EL. Nevertheless, one cannot exclude the possibility that a history of mild encephalitis with rapid recovery may have been overlooked or dismissed as inconsequential by the patients. The time convergence of the EL epidemic and the Spanish influenza initially led to the hypothesis of a common aetiological agent (Foley, 2009, Foley, 2009, Ravenholt *et al.*, 1982), a hypothesis that was rejected by von Economo (Von Economo, 1931). Studies using polymerase chain reaction to successfully identify the H1N1 influenza virus responsible for the epidemic in 1918 failed to detect influenza RNA in archival EL and PEP brain tissue (Lo *et al.*, 2003, McCall *et al.*, 2001, McCall *et al.*, 2008). A recent study identified enterovirus in a

small number of EL and PEP brains (Dourmashkin *et al.*, 2012). Sporadic viral mesencephalitis presenting with an EL-like illness and extrapyramidal sequalae is well recognised (Duvoisin *et al.*, 1965). Viruses, such as the one responsible for Japanese B encephalitis, are identified in some cases of PEP (Casals *et al.*, 1998). There is some evidence to support an immunological role in the pathogenesis of EL (Dale *et al.*, 2009, Vincent, 2004). Studies on patients with sporadic EL have identified antineuronal antibodies, oligoclonal bands in the cerebrospinal fluid and N-methyl-D-aspartate (NMDA) receptor antibodies in some cases (Dale *et al.*, 2004, Dale *et al.*, 2009, Howard *et al.*, 1987). Furthermore, the findings of elevated anti-streptolysin-O titres and anti-basal ganglia antibodies have been described, suggesting a post-streptococcal autoimmune phenomenon (Dale *et al.*, 2004, Vincent, 2004).

Classic EL was an acute or subacute encephalitic illness, commonly associated with other major clinical features including somnolence, sleep inversion, ophthalmoplegia, oculogyric crises, extrapyramidal symptoms, obsessive-compulsive behaviour, akinetic mutism and central respiratory irregularities (Howard *et al.*, 1987). Nevertheless, the diagnostic label of 'EL' was sometimes retrospectively and erroneously applied in patients with parkinsonism who recalled a past history of a catarrh-like illness, headache or flu that had occurred between the late 1910's and late 1920's (Vilensky *et al.*, 2011). About a third of 'PEP' cases in the literature never had any antecedent encephalitic illness (Duvoisin *et al.*, 1965, Foley, 2009, Geddes *et al.*, 1993, Rail *et al.*, 1981, Vilensky *et al.*, 2011, Vilensky *et al.*, 2010). These cases were reported as 'post-encephalitic disorder without acute phase' (Foley, 2009) or 'sequelae of epidemic encephalitis without any preceding acute illness' (Burr, 1925) and were considered as *forme fruste* PEP. Whether neurological sequelae can occur following 'asymptomatic' EL remains unproven.

Cases with PEP in which parkinsonism developed more than 15 years after apparent recovery from EL have occasionally been reported (Duvoisin *et al.*, 1965). A long latent interval was also observed in two of the PEP control cases (PEP-4, PEP-5; Table 18). Nevertheless, latencies of more than a decade between acute EL and onset of parkinsonism are considered exceptional. Large series reported that the latent interval was five years or less in about half of the cases and less than ten years in more than 80% of patients (Duvoisin *et al.*, 1965), therefore, most survivors would have

developed parkinsonism by the 1940's. The onset of parkinsonism in three of the PND cases was in the 1960's and 1970's. If these PND cases did have indolent EL, which was missed clinically or in the history, the latent interval between the 'asymptomatic' EL and onset of parkinsonism would have to have been four decades or more.

Oculogyric crises was historically considered as pathognomonic for PEP and occurred in 30 to 60% of patients with PEP (Duvoisin *et al.*, 1965, Howard *et al.*, 1987). This phenomenon was observed in two PND patients and neither had a history of neuroleptic exposure. The two cases with oculogyric spasms in the present study seemed to improve with levodopa and the phenomenon has also been reported to remit spontaneously over time (Duvoisin *et al.*, 1972, Rail *et al.*, 1981). Dystonic spasms of the extraocular muscles, have also been described in other conditions such as dopa-responsive dystonia, *parkin* disease, tic disorders and brainstem encephalitis (Della Marca *et al.*, 2011). The phenomenon is associated with a dopaminergic deficient state and pathologies in the mesencephalic locomotor region of the dorsal midbrain (Clot *et al.*, 2009, Della Marca *et al.*, 2011, Wenning *et al.*, 1997). Other associated features historically served to distinguish PEP from Parkinson's disease were not observed in the PND group, notably behavioural change, psychosis, bulbar palsy, torticollis, facial tics, pallilalia, scoliosis, pyramidal signs and respiratory disturbances (Duvoisin *et al.*, 1965, Litvan *et al.*, 1998, Vilensky *et al.*, 2011). Ophthalmoplegia was common in PEP (Wenning *et al.*, 1997), but the restricted upgaze noted in the two PND patients were most likely age-related. Severe dyskinesias or neuropsychiatric sequelae from levodopa were not observed in the PND group (Duvoisin *et al.*, 1972). The gradual disease progression of the PND cases also differed from the rapid initial deterioration followed by a static disease for decades and late motor deterioration in old age described in PEP (Calne *et al.*, 1988, Duncan, 1924).

The pathological findings of PND are very similar to PEP (Geddes *et al.*, 1993, Haraguchi *et al.*, 2000, Jellinger, 2011, Torvik *et al.*, 1966, Wenning *et al.*, 1997)(Table 19), but differ considerably from PSP. Severe depletion of pigmented neuron in the substantia nigra pars compacta is characteristic in both PND and PEP cases, and was much more extensive than in Parkinson's disease (Gibb *et al.*, 1987)(Figure 19). Relative preservation in the medial region of the nigra is noted in some PND cases, whereas the damage affects all parts of the pars compacta in PEP

(Geddes *et al.*, 1993, Gibb *et al.*, 1987, Jellinger, 2011). There is no evidence of active cell breakdown or extraneuronal melanin as seen in Parkinson's disease (Gibb *et al.*, 1987), indicating a protracted degenerative process. The distribution of tau-immunoreactive NFTs, PreTs and NTs is similar in PND and PEP, predominantly affecting the frontal and temporal cortices, hippocampus, amygdala, striatum, subthalamic nucleus, remaining neurons of the substantia nigra, locus coeruleus and pontine tegmentum. Other similarities between PND and PEP are the preservation of the subthalamic nucleus and cerebellar dentate nucleus, the presence of ghost tangles and the frequent concomitant TDP-43 pathology. The findings of severe and extensive nigral atrophy and ghost tangles also underpin the prolonged disease duration in these cases. The acute markers of disease activity observed in active EL brains, such as leptomeningeal and parenchymal perivascular lymphoplasmocytic infiltrates with scattered foci of acute neuronal injury, are absent in both PND and PEP. However, the consistent findings of granular PreTs, a precursor of the final filamentous NFTs, support a continuing neuronal degenerative process. The tau lesions in both PND and PEP are immunoreactive to both 3R- and 4R-tau antibodies and immunoblotting confirms a tau-triplet pattern, which is identical to that observed in Alzheimer's disease, PDC of Guam and CTE, but it differs from the predominant 4R-tau lesions and tau-doublet pattern in PSP and CBD (Buee-Scherrer *et al.*, 1997).

Astrocytic lesions are a distinctive feature in PND. Most of these lesions are granular and are clearly different from the characteristic tufted astrocytes in PSP. Some can be bush-like with very fine processes or, rarely, filamentous. The fine bush-like lesions resemble those observed in argyrophilic grain disease (Saito *et al.*, 2004), however, the lesions in PND are more widely distributed and are also detected in cases without grains. Similar astrocytic lesions are also noted in the PEP controls and in other PEP series (Ikeda *et al.*, 1993), but have a more restricted distribution and are less abundant than those in PND. The astrogliopathy in both PND and PEP groups are predominantly AT100-positive and Gallyas-negative, unlike tufted astrocytes in PSP, which are Gallyas-positive. Tufted astrocytes, which were previously described in a single case of PEP, are not seen in the PEP controls in this series (Haraguchi *et al.*, 2000). Oligodendroglial CBs and astrocytic plaques, as seen in CBD, are also absent.

Lewy bodies, the morphological hallmark in Parkinson's disease, are absent in all PND cases. However, a few incidental Lewy bodies are found in one PEP case (PEP-1). Concomitant α-synuclein pathology, which can be observed in 12% of normal elderly, was reported to be characteristically absent in other PEP series (Jellinger, 2009, Josephs *et al.*, 2002). The NFTs seen in PND and PEP are visibly no different from those in Alzheimer's disease and tangle-predominant dementia (Jellinger *et al.*, 2007). However, considering the most severe Braak and Braak stage being IV in our cases, their NFT distribution exceeds those expected in moderate Alzheimer-related pathology (Table 19). Aβ pathology, which was also reported to be rare in PEP (Jellinger, 2011), was found in all PND and three elderly PEP cases. Despite the co-existence of amyloid plaques, dementia was not a feature in the PND and PEP groups, and the dissociation between pathology and dementia is now increasingly recognised in the elderly (Wharton *et al.*, 2011).

In 1971, Forno and Alvord referred to a group of parkinsonian cases without a history of EL but with NFTs as 'idiopathic parkinsonism with Alzheimer neurofibrillary degeneration' (Forno *et al.*, 1971). In 1989, Rajput and colleagues reported four patients with benign levodopa-responsive parkinsonism. Unlike cases in the present series, their tau lesions were restricted to the substantia nigra and locus coeruleus and extraneuronal melanin in the substantia nigra was observed in two cases, indicating active breakdown of pigmented neurons (Rajput *et al.*, 1989). Genetic analysis has not been performed on these cases (personal communication). Other case reports of unclassifiable neurofibrillary tangle parkinsonian disorders were all published more than two decades ago and all had some distinctive clinical or pathological differences to the PND cases (Daniel *et al.*, 1991, Lidov *et al.*, 1989, Mata *et al.*, 1983, Renkawek *et al.*, 1993). Some of these cases in the literature may belong to various genetic and neurodegenerative entities which are now better recognised, for instance, *parkin* disease (Mori *et al.*, 1998), *LRRK2* mutation (Zimprich *et al.*, 2004) , FTDP-17 (Hutton *et al.*, 1998), PSP-P (Williams *et al.*, 2007), PSP-PAGF (Williams *et al.*, 2007), cortical variants of PSP (Dickson *et al.*, 2010), globular glial tauopathies (Ahmed *et al.*, 2013), Niemann-Pick type C (Suzuki *et al.*, 1995), Perry's syndrome (Wider *et al.*, 2010) and pantothenate kinase-associated neurodegeneration (Li *et al.*, 2012). Likewise, five of the 12 cases included in this study had a genetic or

neuropathological explanation after evaluation (Figure 18). Unclassifiable sporadic tauopathy with cognitive presentation has also been reported. Kovacs and colleagues studied the post-mortem findings of seven elderly patients, age ranged from 77 to 94, with dementia and, in three cases, associated parkinsonism (Kovacs *et al.*, 2011). They all had a constellation of tau pathology and distinctive astrogliopathy, in a characteristic distribution, along with some Alzheimer-related changes, reminiscent of our PND cases. The authors referred them collectively as complex tauopathy which may have heterogeneous aetiologies.

Guamanian PDC is a rapidly progressive parkinsonian and dementia syndrome, affecting the indigenous Chamorro population in the Western Pacific (Hirano *et al.*, 1961). The disorder, referred by the locals as 'bodig', begins in the fifth or sixth decade, resulting in death after five years. Oculomotor abnormalities are sometimes observed. Guamanian PDC, PEP and PND share many neuropathological similarities, including severe nigral cell loss, NFT accumulation in the hippocampus, temporal and frontal cortices and striatum, ghost tangles and concomitant TDP-43 pathology (Geddes *et al.*, 1993, Hasegawa *et al.*, 2007, Hirano *et al.*, 1961). However, the typical Gallyas-positive hazy granular astrocytic lesions and tau-positive fine white matter granules described in PDC are notably absent in the PEP and PND cases in the present study (Oyanagi *et al.*, 1997, Yamazaki *et al.*, 2005). Like PEP, the cause of PDC remains enigmatic, although a combination of environmental and genetic factors is hypothesized (McGeer *et al.*, 2011). Two sites in the *MAPT* gene conferring risk for PDC of Guam have been identified, indicating a role in genetic susceptibility (Sundar *et al.*, 2007). Since 1965, the incidence of bodig has significantly decreased and, similarly, 'lytico' amyotrophic lateral sclerosis, which was once prevalent in Guam, has become much rarer (Plato *et al.*, 2003). There are only a few new cases of PDC born after 1951 (McGeer *et al.*, 2011). The intriguing change in epidemiology suggests a local exogenous insult, which most likely to have occurred in the first half of the twentieth century and has since declined significantly. A change in diet is proposed as the explanation but this remains controversial (McGeer *et al.*, 2011). Mulder and Kurland initially speculated links with von Economo's disease and the possibility of a viral pathogen was raised (Kurland *et al.*, 1954). The absence of new PND cases born

after 1930's in this series suggests that it may be an entity which is dying out spontaneously, in the same way as Guamanian PDC and PEP.

It is plausible that PND and PEP share a common aetiology based on the evidence that all PND cases were born before or during the EL epidemic posing opportunities of exposure, and that there is a striking resemblance of the neuropathology between the two groups. The lack of a history of encephalitis in PND, however, is a fundamental anomaly that should serve as a stimulus to question the paradigm of PEP (Vilensky *et al.*, 2010). A historical analysis has highlighted that EL and PEP had a complex rather than a direct relationship (Vilensky *et al.*, 2010). Nevertheless, the limited knowledge of the cause of EL and of its pathophysiological links with PEP poses difficulties in speculating whether the patients with PND could have had EL without clinical evidence. In both PEP and PND, there are extensive neuronal loss and NFT pathologies in the brainstem where the brunt of pathology in acute EL is also observed, supporting some causal relations (Jellinger, 2011). If indeed PND is *forme fruste* PEP, patients with PND and PEP would have had the same early insult, which may be an infective agent or an immunological response to an external or internal antigen, leading to acute injury of the central nervous system. It is hypothesized that in PEP the acute neuronal injury subsequently triggers a neurodegenerative process which presents clinically some time later and progresses slowly over many years (Geddes *et al.*, 1993).

Other examples of acute neuronal insult triggering progressive neuronal degeneration with NFT accumulation are subacute sclerosing panencephalitis (SSPE)(Cobb *et al.*, 1984, McQuaid *et al.*, 1994) and CTE (McKee *et al.*, 2013). Alzheimer-type NFT pathology occurs after a latent period in SSPE survivors and in individuals exposed to repetitive brain injury. The NFT pathology in these cases has a similar distribution as in PND and PEP. Nevertheless, the findings of neuronal measles viral genome in SSPE and subpial perivascular astrocytic tangles in CTE provide distinguishing markers for these conditions (Geddes *et al.*, 1999, McKee *et al.*, 2013, McQuaid *et al.*, 1994). It is proposed that once tau aggregates are formed in discrete brain regions, they undergo intercellular transfer, promoting a self-propagating process, which leads to the spread of abnormal tau throughout the brain (Clavaguera *et al.*, 2009, Hardy, 2005).

Genetic susceptibility as identified in other sporadic tauopathies, such as the H1 haplotype in PSP and CBD (Houlden *et al.*, 2001), may also exist for PND. The genome-wide association studies for Parkinson's disease, a disorder characterised by α-synuclein pathology, revealed a surprising *MAPT* locus, suggesting cross-talk between molecular pathways of different protein aggregates (Simon-Sanchez *et al.*, 2009). A search of genetic loci outside the *MAPT* gene in these PND and PEP cases might in the future also reveal unexpected hotspots.

This study identified a group of patients with NFT pathology resembling that found in PEP but with no history of encephalitis and a somewhat different clinical picture, leading to a reconsideration of the links between acute EL and presumed PEP and the mechanisms involved.

5.2: The MAPT p.A152T variant: a risk factor associated with atypical tauopathies

Introduction

Tau belongs to the family of the microtubule-binding proteins (Witman *et al.*, 1976) and is encoded by the *MAPT* gene located on chromosome 17 (17q21)(Andreadis *et al.*, 1992). *MAPT* mutations have been mainly linked to FTD (Hutton *et al.*, 1998), as well as to a variety of neurodegenerative diseases with abnormal tau accumulation, including PSP, CBD and other rarer tauopathies (Conrad *et al.*, 1997, Hoglinger *et al.*, 2011, Momeni *et al.*, 2009). Recently, an A152T variation in *MAPT* exon 7 was identified in a patient who had dementia and unclassifiable tauopathy (Kovacs *et al.*, 2011). With this background, primary sequencing of the *MAPT* gene was performed as part of the evaluation of the 12 cases with slowly progressive levodopa-responsive parkinsonism and unclassifiable tau accumulation as detailed in Chapter 5.1 (Figure 18). Of the 12 cases sequenced, two were found to carry the p.A152T variation. A secondary sequencing was subsequently carried out in a larger series of cases with PSP, CBD and Parkinson's disease to determine the nature of the phenotype associated with this variant and two additional cases with this variant were identified.

Materials and methods

Primary sequencing

Initial sequencing was performed on the entire *MAPT* gene open reading frame in 12 cases fulfilling the inclusion criteria as described in Chapter 5.1 (Figure 18).

Three of these cases have been previously reported (case 1 with the *MAPT* p.A152T variant=case 8 in Geddes' series, PND-6=case 5 in Geddes' series and PND-7=case 7 in Geddes' series (Geddes *et al.*, 1993).

Initial post-mortem study reported case 1 as PEP despite the absence of a history of encephalitis. The original neuropathological examination did not reach a conclusive diagnosis in case 2, which had been categorized as ‘parkinsonism associated with unclassifiable neurofibrillary tangle pathology’.

Genetic analysis

Genomic DNA was extracted from brain tissue of the 12 included cases. After the p.A152T mutation was found in exon 7 in two cases, additional screening of this exon was carried out in blood derived DNA of the three siblings of case 1 (two suffering from dementing illnesses and one unaffected), and in 150 neuropathologically defined controls and 133 1958 Wellcome Trust blood donor controls. At this stage, the occurrence and frequency of this variant in public databases was also assessed.

Statistical analysis

Fisher exact tests were conducted using an online tool: http://research.microsoft.com/enus/um/redmond/projects/mscompbio/FisherExactTest/ *P*-value below 0.01 was considered statistically significant.

Secondary sequencing

After identifying the variant in two of the 12 included cases and failing to find the variant in control samples, secondary screening was performed in DNA derived from the brains of the QSBB archival collection, including pathologically confirmed cases with PSP (N=114)(Pittman *et al.*, 2004), CBD (N=8)(Houlden *et al.*, 2001) and Parkinson’s disease (N=48).

Results

Primary sequencing

Genetic analysis

Among the 12 cases included in the primary genetic analysis, two cases were identified to carry a heterozygote non-synonymous variant in exon 7 (rs143624519, c.454G>A, p.A152T)(accession numbers NM_005910.5 and NP_005901.2 respectively)(Figure 26). This variant was also found in the two living sisters of case 2, who were both developing progressive cognitive impairment, but this variant was absent in the third unaffected sister. All patients are Caucasian and of northern European descent. This variant is present with a frequency of 19 in 3510 (0.54%) individuals of European American origin (19 in 7020 alleles) in the publicly available database from the National Heart Lung Blood Institute (NHLBI) GO-Exome Sequencing Project (Exome Variant Server, NHLBI GO-ESP, Seattle, WA; http://evs.gs.washington.edu/EVS/, accessed on January 2012). Additional screening was performed on 283 control cases but this variant was not identified. The Fisher exact test used to compare both allele and genotype frequencies between the unclassifiable tauopathy and the ESP cohort gave a two-tailed p-value of < 0.01 for allele and genotype frequencies. A search through two publicly available databases: 1000 genomes and The National Institute of Environmental Health Sciences Environmental Genome Project (NIEHS, Seattle, WA; http://evs.gs.washington.edu/niehsExome/, accessed on March 2012) revealed similarly low frequencies of the A152T variant (1000 Genomes Project Consortium)(2010). This variant has been reported more frequently in Alzheimer's disease than in controls (Cruchaga *et al.*, 2012).

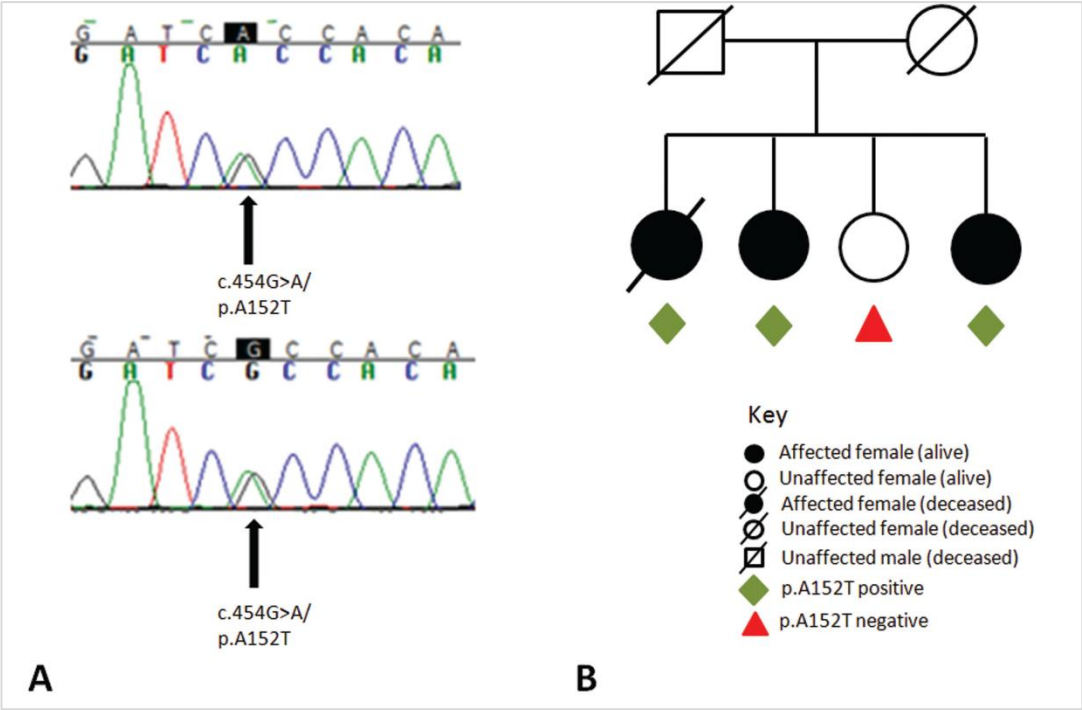

Figure 26. *Sequencing chromatograms and family tree of cases carrying the MAPT p.A152T variant.*

A: c.454>A/p.A152T variant in case 1 (upper panel) and case 2 (lower panel); B: family tree of case 2 showing segregation of the variant with affected family

Case 1

At the age of 26, this patient developed a slowly progressive levodopa-responsive Parkinson's syndrome with intact cognition. There was a long history of levodopa-induced orofacial dystonia. She never had any encephalitic illnesses prior to the onset of parkinsonism. The disease duration was 54 years and she died at the age of 80. A family history of dementia was noted, but not elaborated in her medical records.

Neuropathological examination showed a significant degree of neuronal loss in the substantia nigra, which was most severe in the ventrolateral tier. There were tau-immunoreactive NFTs and numerous NTs in the substantia nigra. Sparse NFTs and NTs were also seen in the midbrain tegmentum. Amyloid plaque-associated abnormal neurites were also observed in the midbrain tectum. Scattered NFTs and NTs were identified in the striatum, globus pallidus and subthalamic nucleus. There was a single NFT with sparse NTs in the cerebellar dentate nucleus. Aβ-positive diffuse and mature plaques in the frontal, parietal and temporal cortices were noted. Severe NFTs, NTs and plaque-associated neurites in the CA1 hippocampal subregion, entorhinal cortex and fusiform gyrus corresponded to Braak and Braak stage IV (Figure 27). The tau inclusions were both 3R and 4R-tau positive. Neither TDP-43-related pathology nor argyrophilic grain disease was observed. The tau-positive lesions observed in this case were consistent with age-related changes. However, the severe neuronal loss in the substantia nigra was as extensive as those in PEP and PND (Chapter 5.1). The neuropathological findings of this case were not compatible with any known entity.

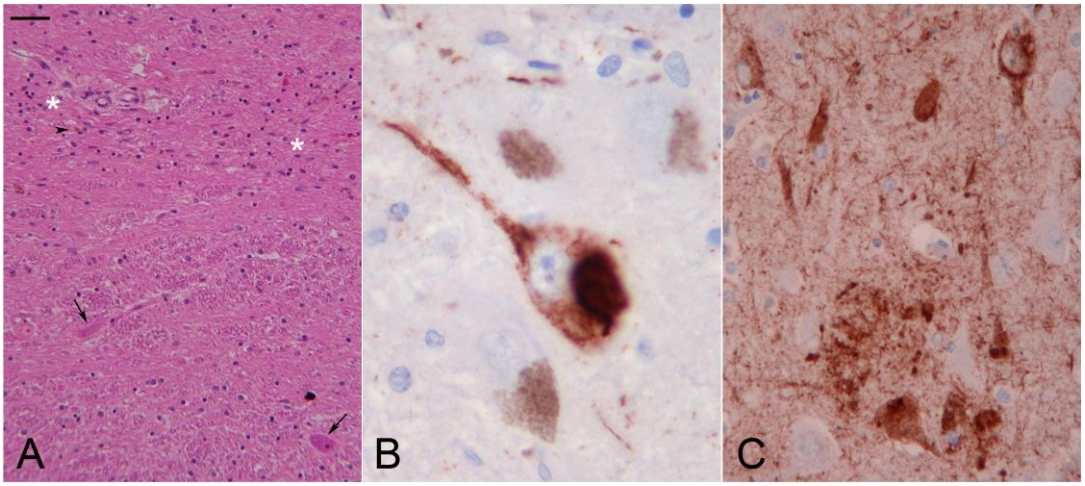

Figure 27. *Neuropathological features of a case carrying the MAPT p.A152T variant.*

A: Severe loss of pigmented neurons in ventral tier of substantia nigra (white asterisks) with gliosis and free pigment (arrow head). Non-pigmented neurons are still present in this region of the substantia nigra (arrows); B: A tau-positive neurofibrillary tangle in a nigra neuron; C: neurofibrillary tangles, neuropil threads and neuritic plaques in the CA1 hippocampal sub-region.

A: H&E; B & C: AT8 immunohistochemistry; scale bar on A=80microns on A, =20microns on B, =40microns on C.

Case 2

At the age of 57, this English woman began to develop a symmetrical akinetic rigid syndrome. Two years later, examination revealed hypomimia, drooling, predominant axial rigidity with mild symmetrical limb rigidity, brisk deep tendon reflexes and flexor plantar response. A diagnosis of Parkinson's disease was made, but she only had transient benefit from levodopa therapy. Her symptoms gradually deteriorated and seven years later, she developed dysarthria and dysphagia. In her mid-60's, she started to experience daily episodic dystonic spasms with an opisthotonus posture and oculogyric crises. Examination in-between these episodes revealed normal pursuit and saccadic eye movements, fixed retrocollis, slow tongue movement, anarthria and severe axial and limb rigidity and dystonic posturing of the feet. Her cognitive function remained intact. The presence of oculogyric spasms prompted the neurologist to revise the diagnosis to PEP in the last year of her life. She died after a disease duration of ten years.

This patient had three younger sisters (Figure 26). The second sister, now in her mid-80s, has developed dementia with short term memory impairment, executive dysfunction, disorientation to time and required assistance with dressing and housework. Her youngest sister, in her mid-70s, has experienced symptoms of forgetfulness but remains independent. These sisters were assessed in person and over the telephone. Her third sister remains well. Both affected sisters carried the mutation but the healthy sister did not.

Neuropathological re-evaluation using modern immunohistochemistry staining indicated that the findings were consistent with the pallido-nigro-luysial atrophy (PNLA) variant of PSP (Ahmed *et al.*, 2008). There were moderate numbers of AT8 and 4R-positive and 3R-negative tufted astrocytes, NFTs, NTs and CBs in the caudate and, to a lesser severity, in the putamen. Severe gliosis and neuronal loss was noted in the substantia nigra. Significant reduction in size, cell loss and gliosis was observed in the globus pallidus and subthalamic nucleus. However, the cerebellar dentate nucleus and superior cerebellar peduncle were relatively well preserved, which differs from the typical findings in classic PSP-RS. Together with both neuronal and glial tau pathology in the basal ganglia, brainstem and cerebellar nuclei and the relatively mild

tau pathology in the cerebral cortex, pontine base and cerebellar dentate nucleus, these histopathological findings were in favour of the diagnosis of the PNLA variant PSP. The age-related tau pathology corresponded to Braak and Braak stage II. There was no evidence of argyrophilic grains.

Secondary sequencing

The secondary screening of exon 7 of the *MAPT* gene was subsequently carried out in 114 PSP, 8 CBD and 48 Parkinson's disease cases from the QSBB archival collection. The mutation was identified in one case with CBD. More surprisingly, it was also found in one case with Parkinson's disease.

Case 3

In the mid-60s, this patient developed word-finding difficulties, right-sided rigidity followed by difficulties in walking. His predominant symptoms evolved from non-fluent aphasia to mutism, increasing dependency and then death within 11 years. There was no documented family history of dementing illness.

Neuropathological findings were consistent with CBD, including achromatic, swollen neurons in the neocortex and limbic areas, numerous PreTs and NFTs, dense meshwork of tau-positive threads, astrocytic plaques in the frontal, temporal and parietal cortices and tau-positive threads and some CBs in subcortical white matter. Additionally, there were numerous TDP-43 positive NCIs and occasional NIIs in the globus pallidus, caudate and putamen. Severe neuronal loss in the substantia nigra was noted.

Case 4

In his late 40s, this patient developed an anxiety disorder and poor concentration which resulted in him getting dismissed from his job. In the following year, he started to stutter and was diagnosed with depression. Two years later, he had memory loss, executive impairment, slowness in movement. Following the subsequent findings of markedly reduced tracer reuptake in the basal ganglia on DAT-SPECT, a diagnosis of Parkinson's disease with dementia was made when he was in his early 50's. He was commenced on levodopa therapy with moderate response. His cognitive function deteriorated markedly in the following year with fluctuating consciousness, disorientation, urinary incontinent, severe dementia, visual hallucination, myoclonus and asymmetrical akinetic rigidity. He died seven years after the onset of his first symptoms.

Neuropathological findings were consistent with the diagnoses of Parkinson's disease with dementia and pathological ageing. Histopathological examination revealed moderate to severe cell loss in the substantia nigra in association with α-synuclein-positive Lewy bodies and Lewy neurites. Frequent cortical Lewy bodies were observed in the frontal, temporal, parietal, cingulate gyrus and transentorhinal cortices, corresponding to Braak stage 6 (Braak *et al.*, 2003) and diffuse neocortical Lewy body type pathology according to consensus criteria (McKeith *et al.*, 2005). NFTs were found in the hippocampus, entorhinal and transentorhinal cortices which were consistent with Braak and Braak II. There were few tau-positive NTs in the caudate nucleus, midbrain tegmentum and periaqueductal grey. A single NFT was noted in the locus coeruleus. A 'low' level of Alzheimer Disease pathologic change (A1, B1, C0) was identified according to the NIA-AA guideline (Montine *et al.*, 2012).

Discussion

Four cases were identified in this study to carry the p.A152T *MAPT* mutation in primary and secondary sequencing. This rare variant has only been reported in few cases in the literature, including one case with dementia and unclassifiable tauopathy (Kovacs *et al.*, 2011), one with a clinical picture of PSP (Coppola *et al.*, 2012), one with PNLA variant of PSP, akin to case 2 in this report (Graff-Radford *et al.*, 2013) as well as in few Alzheimer's disease cases (Cruchaga *et al.*, 2012, Jin *et al.*, 2012). These case reports, along with the segregation with affected family members in case 2, its absence in the 283 control samples and its rarity in the public databases all support the notion that the p.A152T variant contributes to disease risk. The finding that the mutation occurs in one of eight CBD cases sequenced may also be adduced to support this view.

The identification of the variant in a case of Parkinson's disease was a surprise. However, this case was unusual in view of the rapid disease course and the considerable burden of tau pathology exceeded the amount of age-related change which would have been expected in a case of a relatively young age. Additionally, the clinical and pathological findings vary in all four reported cases in this series. Despite some evidence for segregation in the single family of case 2, in which this analysis was possible, and a positive family history in case 1, there was no family history of dementia or movement disorders in cases 3 and 4. Unlike in classic cases with *MAPT* mutations, the tau pathology was variable, both in morphology and distribution; being 4R in cases 2 and 3 and a classic mixture of 3R and 4R in cases 1 and 4.

The position of the variant is interesting in that it creates an additional phosphorylation in the tau protein and tau hyperphosphorylation has been repeatedly suggested to contribute to NFT formation and cell death (Rademakers *et al.*, 2004). One plausible suggestion is that this mutation contributes to disease pathogenesis through the creation of an additional phosphorylation site but that this is not sufficient, of itself, to cause disease in the way that the *MAPT* splice and P301L mutations do. The mutated residue is next to Thr153 (T153), a phosphorylation site, which is a target for proline-directed kinases MAPK, cdk5 and GSK3α. Using an antibody against the

phosphorylated T153 (pT153), a study showed that although intra-neuronal and extra-neuronal tangles were labelled, the dominant staining was punctate and of PreTs within morphologically intact neurons, with normal cellular integrity and well preserved dendrites (Augustinack *et al.*, 2002). A similar staining pattern of PreTs was observed with the conformation-specific TG3 antibody (Jicha *et al.*, 1997) and a phospho-tau specific pS262 antibody, suggesting that these antibodies label an early stage of tangle formation characterised by punctate inclusions that precede fully-fledged fibrillar tangles (Augustinack *et al.*, 2002). It is possible that the additional Thr152 increases phosphorylation and, thus, further contributes to the early stages of the fibrillogenic pathway. A recent functional study has shown that the p.A152T *MAPT* variant plays a role in reducing tau binding to the microtubule and increases tau oligomer formation (Coppola *et al.*, 2012).

LRRK2 mutations offer a precedent for this complex relationship between mutations and disease. Some mutations (e.g. p.G2019S) cause disease in an almost fully penetrant autosomal dominant fashion (Paisan-Ruiz *et al.*, 2004), whereas others (e.g. p.G2385R) increase disease risk by a modest amount (Tan *et al.*, 2010). In the same way as in the p.A152T *MAPT* variant, *LRRK2* mutations also cause variable pathologies, with the majority having Lewy body pathology, but, tau, ubiquitin and TDP-43 pathologies have also been reported (Zimprich *et al.*, 2004). For the true role of this variant in neurological disease to be determined, very large number (in the thousands) of cases with various tauopathies and controls will need to be sequenced. Certainly until that time, the present data would not support this mutation becoming part of the clinical test in the way that the penetrant mutations are.

In conclusion, the rare *MAPT* p.A152T variant is likely to increase the susceptibility to the development of relatively common or atypical neurodegenerative diseases, associated with abnormal tau accumulation and with clinical presentation of either dementia or movement disorder. Further functional studies are necessary in order to fully dissect the functional consequences and precise pathogenic mechanisms associated with this mutation.

5.3: LRRK2 G2019S mutation with tau and TDP-43 pathologies

Introduction

Among the five identified pathogenic leucine-rich repeat kinase 2 (*LRRK2*) mutations, G2019S is the most common and accounts for 1% of sporadic Parkinson's disease and 4% of hereditary parkinsonism worldwide (Healy *et al.*, 2008). Clinical presentation of *LRRK2* mutation resembles idiopathic Parkinson's disease but may be associated with a more benign disease course (Healy *et al.*, 2008). Most cases with *LRRK2* mutation exhibit neuropathological features consistent with typical Lewy body Parkinson's disease (Gilks *et al.*, 2005, Khan *et al.*, 2005, Zimprich *et al.*, 2004), but 'pure' nigral degeneration, tau, α-synuclein or ubiquitin pathologies, resembling PSP, multiple system atorphy (Hasegawa *et al.*, 2009), and frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U) have also been reported (Dachsel *et al.*, 2007, Gaig *et al.*, 2008, Giasson *et al.*, 2006, Rajput *et al.*, 2006, Zimprich *et al.*, 2004). Ubiquitinated TAR DNA-binding protein-43 (TDP-43) is a major disease protein in FTLD and amyotrophic lateral sclerosis (ALS) and can occasionally be observed in Lewy body disorders and tauopathies (Mackenzie *et al.*, 2010, Neumann *et al.*, 2006, Sreedharan *et al.*, 2008). Recently, TDP-43 related pathology was reported in three cases carrying *LRRK2* mutations (p.R1441C, p.R793M and L1165P)(Covy *et al.*, 2009, Zimprich *et al.*, 2004).

As part of the evaluation of cases presenting with parkinsonism and unclassifiable tau accumulation in post-mortem (Chapter 5.1), sequencing of the *LRRK2*, *MAPT* and *parkin* genes was performed. Of the 12 cases included in the study, one case was found to carry the *LRRK2* G2019S mutation as well as a novel heterozygous variant c.370C>G, p.Q124E in exon 4 of the *MAPT* gene. This patient was clinically diagnosed with Parkinson's disease and had neuropathological findings of nigral degeneration with absence of Lewy body pathology.

Materials and methods

Case selection and genetic analysis

In addition to the methods described in Chapter 5.1, sequencing of the *TDP-43* gene was performed in this case with *LRRK2* G2019S mutation. In four other cases with proven *LRRK2* G2019S mutations and Lewy body pathology available in the QSBB archival collection (Gilks *et al.*, 2005), sequencing of the *MAPT* gene was performed.

Neuropathology

In addition to the neuropathological methods described in Chapter 5.1, a semi-quantitative assessment of nigral cell loss was performed in 12 randomly selected cases with confirmed pathological diagnosis of Alzheimer's disease. The aim of the assessment was to evaluate if the neuronal loss in the substantia nigra pars compacta identified in the *LRRK2* case was related to the genetic mutation or to its Alzheimer-related pathology.

Results

Genetic analysis

Of the 12 included cases, one was identified to carry both the *LRRK2* G2019S mutation and a heterozygous variant in *MAPT* exon 4 (c.370C>G, p.Q124E, Figure 28). The *MAPT* p.Q124E variant is absent in all control subjects in the public databases (N=6568, of which 3913 are Caucasians), including ENSEMBL (http://useast.ensembl.org/index.html), Exome Variant Server (NHLBI GO-ESP, Seattle, WA; http://evs.gs.washington.edu/EVS/, accessed in January, 2013)(Table 20). The pathogenicity is predicted to be 'possibly damaging' based on a web-based assessment on Polyphen2 (http://*genetics.bwh.harvard.edu/pph2/*). This variant is absent in the four archival *LRRK2* cases with Lewy body pathology. The *MAPT* genotype of this patient was H1/H2. Sequencing of the *parkin* and *TDP-43* genes in this case was negative. Dosage study using MLPA was negative for all analysed genes including *parkin* and *LRRK2*.

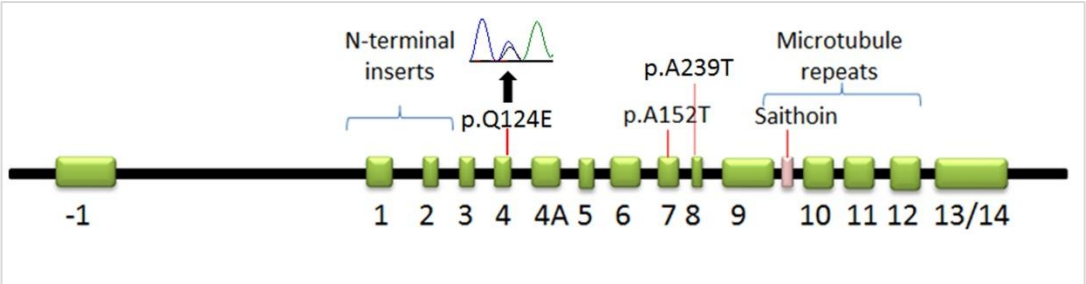

Figure 28. *Diagram of the MAPT gene and locations of three rare risk variants associated with tau pathology.*

The novel c.370C>G variant is indicated with a black arrow on the chromatogram above the p.Q124E variant.

Database	Ethnicity of subjects	No. of subjects included	Genotype frequency of the p.Q124E *MAPT* variant	Allele frequency of the p.Q124E variant
NHLBI GO-ESP	European American	3510	0/3510 (0%)	0/7020
NHLBI GO-ESP	African American	1869	0/1869 (0%)	0/3738
1000 genomes	Mixed American	181	0/181 (0%)	0/362
1000 genomes	European	381	0/381 (0%)	0/762
1000 genomes	African East Asian South Asian	532	0/532 (0%)	0/1064
NIEHS	Caucasian	22	0/22 (0%)	0/44
NIEHS	African American	14	0/14 (0%)	0/28
NIEHS	Asian	24	0/24 (0%)	0/48
NIEHS	Hispanic	22	0/22 (0%)	0/44
NIEHS	Yoruban	13	0/13 (0%)	0/26

Table 20. *Genotype frequency of the MAPT p.Q124E variant in public databases.*

The MAPT p.Q124E variant is not identified in any control subject. ESP=Exome Sequencing Project; NHLBI=National Heart Lung Blood Institute; NIEHS=The National Institute of Environmental Health Sciences

Case report

This British Caucasian woman, who carried the *LRRK2* G2019S mutation and the heterozygous p.Q124E *MAPT* variant, developed right hand tremor at the age of 73. She had mild bradykinesia and rigidity and had received a clinical diagnosis of Parkinson's disease. She was initially started on an anticholinergic medication and five years later, levodopa therapy was administered and titrated to 1000mg/day with good response. Her motor symptoms gradually deteriorated over the years. She reported wearing-off symptoms but she never had any dyskinesia. She had intact cognition throughout the disease course. In the last year of life, she had balance difficulty, multiple falls and required a rollator to mobilize. She died at the age of 85. There was no family history of any neurological disorders.

Neuropathological analysis confirmed a moderate degree of neuronal loss in the substantia nigra, except in the ventrolateral tier where severe neuronal loss was found (Figure 29). There were no Lewy bodies on α-synuclein or phospho-α-synuclein immunohistochemistry. There were sparse tau-positive NTs in the frontal and parietal cortices; sparse NTs, NFTs and PreTs in the CA1 and CA4 hippocampal subregions; mild PreTs, moderate numbers of neuritic plaques, NFTs and NTs in the subiculum; severe NFTs, NTs and PreTs in the entorhinal cortex and very sparse NTs in the striatum. All tau inclusions were both 3R and 4R-tau positive. The subthalamic nucleus was preserved and no tau pathology was noted.

Aβ immunohistochemistry demonstrated parenchymal deposition in the cerebral cortex, hippocampus and striatum, corresponding to a Thal phase score '3' (Thal *et al.*, 2002) and the distribution of tau pathology corresponded to Braak and Braak stage III (Braak *et al.*, 2006). Using the CERAD criteria (Mirra *et al.*, 1991), the neuritic plaque score was 'sparse' in the temporal cortex, 'moderate' in the frontal cortex and 'sparse' in the parietal cortex. These results gave a 'low' likelihood of Alzheimer's disease according to the NIA-Reagan criteria (Hyman *et al.*, 1997) and 'intermediate' level of Alzheimer-pathologic change (A2, C2, B2) according to the NIA-AA criteria (Montine *et al.*, 2012).

TDP-43 immunohistochemistry showed numerous fine thread-like processes and few coarser neurites in the CA1 hippocampal subregion and subiculum. In the amygdala, temporal and frontal cortices, there were occasional NCIs and few threads. Occasional NCIs, threads and NIIs were observed in the subiculum, striatum and substantia nigra. Skein-like NCIs were also found in the substantia nigra.

No hippocampal sclerosis, α-synuclein immunoreactive inclusions, argyrophilic grains, CAA or vascular pathology was observed. There were no p62-positive 'star-shaped' inclusions in the hippocampus or small 'dot-like' structures in the cerebellar granule cells of the type associated with *C9orf72* repeat expansion.

Findings in other *LRRK2* G2019S cases

In the other four archival cases with proven *LRRK2* G2019S mutation, no TDP-43 related pathology was observed in the hippocampus or amygdala. Sequencing of the *MAPT* gene was negative.

Substantia nigra in Alzheimer's disease

In the 12 randomly selected control cases with confirmed pathological diagnosis of Alzheimer's disease (NIA/Reagan 'high' likelihood of Alzheimer's disease), cell loss of the substantia nigra was at most mild as evidenced by regional pigment incontinence in the pars compacta.

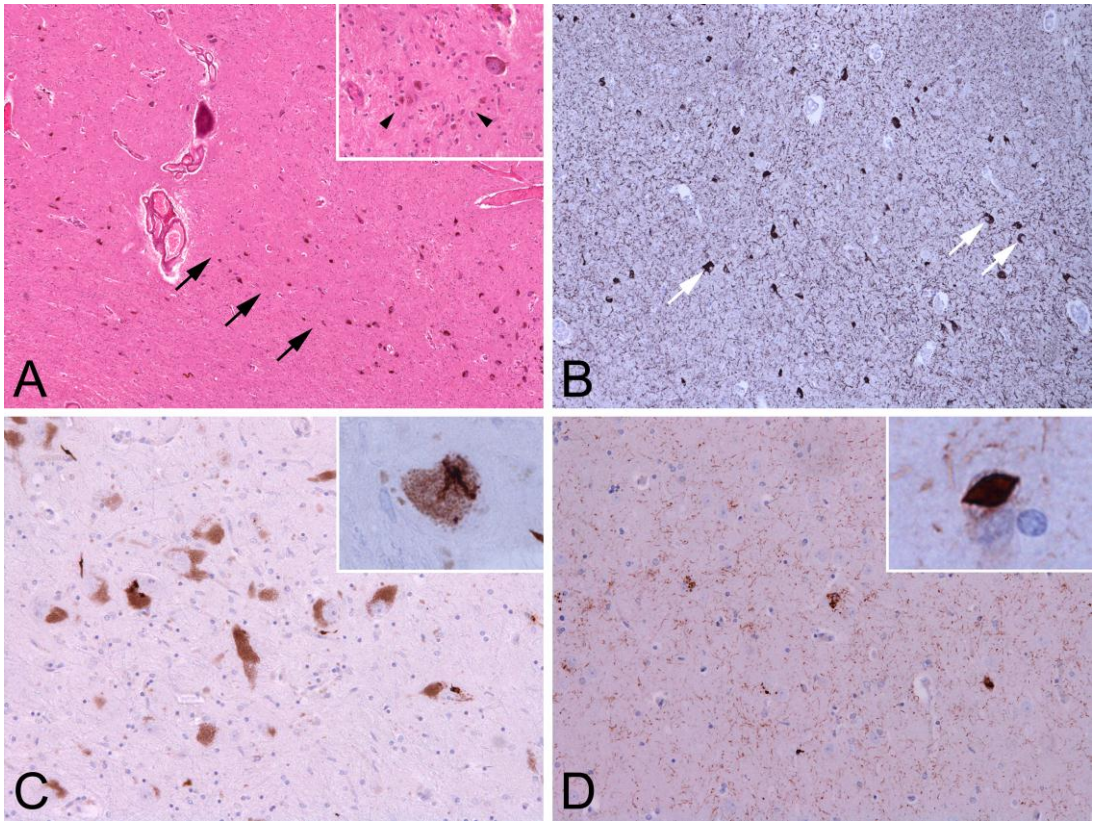

Figure 29. *Neuropathological findings of the LRRK2 G2019S case.*

A: Severe loss of neuromelanin-containing neurons in the ventrolateral tier of the substantia nigra (arrows) as well as gliosis and free pigment (arrowheads); B: abundant tau-positive neurofibrillary tangles, neuropil threads and pre-tangles in the entorhinal cortex; C: phospho-TDP-43 (p-TDP) -immunoreactive neuronal cytoplasmic inclusions (NCIs), neurites and a skein-like structure (inset) in the substantia nigra; D: numerous p-TDP-positive fine thread-like processes, few coarser neurites, round, dot-like structures and a 'cat-eye' neuronal intranuclear inclusion (NII, inset) in the subiculum.

A: H&E, B: AT8, C&D: Phospho-TDP-43 immunostaining.

Discussion

LRRK2 G2019S mutation is commonly associated with Lewy body pathology. Of the 22 published post-mortem cases with this mutation, only four cases had absence of Lewy bodies. Rajput et al reported a case with slowly progressive non-levodopa-responsive parkinsonism and tau-positive NFTs, resembling the neuropathology of PSP (Rajput *et al.*, 2006), Gaisson et al and Gaig et al each reported a case with classic tremor-dominant parkinsonism and pure nigral degeneration (Gaig *et al.*, 2008, Giasson *et al.*, 2006) and Dachsel et al reported a case with dementia and tremor and neuropathology consisted of FTLD with ubiquitinated neuronal inclusions (FTLD-U)(Dachsel *et al.*, 2007). On the other hand, pleomorphic pathologies, including α-synuclein, tau and ubiquitin, seem to be more commonly associated with other pathogenic *LRRK2* mutations (Hasegawa *et al.*, 1997, Hasegawa *et al.*, 2009, Santpere *et al.*, 2009, Wszolek *et al.*, 2004, Wszolek *et al.*, 1997, Zimprich *et al.*, 2004).

The present case, with *LRRK2* G2019S mutation, was clinically diagnosed as Parkinson's disease and had good levodopa response. Neuropathological analysis revealed nigral degeneration with Alzheimer-type tau and TDP-43 proteinopathy and absence of Lewy body pathology. Interestingly, this patient was also found to carry a novel p.Q124E *MAPT* variant. Prompted by the TDP-43 pathology, screening for TDP-43 mutations was carried out and was negative. A survey for TDP-43 pathology in another four archival QSBB cases with *LRRK2* G2019S mutation and Lewy body pathology did not identify any TDP-43-immunoreactive lesions and *MAPT* sequencing of these cases were also negative.

To date, only three cases with *LRRK2* mutation have been reported to have TDP-43 related pathology (p.R1441C, p.R793M and L1165P)(Covy *et al.*, 2009, Zimprich *et al.*, 2004), and none of which carried the G2019S mutation. Only three other published cases carrying the *LRRK2* G2019S mutation were evaluated for TDP-43 pathology (Giasson *et al.*, 2006), but no TDP-43-positive inclusions were observed (Covy *et al.*, 2009).

The discovery of TDP-43 in 2006 as a major disease protein in FTLD and ALS led to the introduction of TDP-43 immunohistochemistry into the routine diagnostic

protocols of brain banks. It is, therefore, likely that other reported *LRRK2* cases with ubiquitin pathology may also have TDP-43 inclusions (Dachsel *et al.*, 2007, Wszolek *et al.*, 2004, Wszolek *et al.*, 1997, Zimprich *et al.*, 2004) and will warrant comprehensive assessment. In addition to FTLD-TDP and ALS, TDP-43 inclusions can sometimes be detected in other neurodegenerative diseases, including Alzheimer's disease, Lewy body disorders, CBD, PSP, Guamanian PDC, and CTE (Arai *et al.*, 2009, Hasegawa *et al.*, 2007, Higashi *et al.*, 2007, King *et al.*, 2010, Lashley *et al.*, 2011, Mackenzie *et al.*, 2010, McKee *et al.*, 2013, Uryu *et al.*, 2008). The cause and mechanism of TDP-43 in tauopathies are not known but it has been postulated that tau aggregates may promote aggregation of TDP-43 through cross-seeding (Morales *et al.*, 2009, Uryu *et al.*, 2008). TDP-43 immunoreactivity may modify clinical features in Alzheimer's disease and other types of dementia (Josephs *et al.*, 2008, Lashley *et al.*, 2011) and is also closely associated with hippocampal sclerosis (Amador-Ortiz *et al.*, 2007, Josephs *et al.*, 2008). It remains to be determined if TDP-43 protein plays a role in influencing the clinical features in *LRRK2* cases.

As in Lewy-body Parkinson's disease, degeneration of the substantia nigra is a typical finding in *LRRK2* mutation and is considered the pathological substrate of clinical parkinsonism (Wider *et al.*, 2010). Although previous studies have shown a correlation between nigral pathology and extrapyramidal symptoms in Alzheimer's disease (Burns *et al.*, 2005), the screening of 12 randomly selected Alzheimer's disease controls in this present study did not reveal significant neuronal loss in the substantia nigra. It is, therefore, unlikely that the modest Alzheimer-type tau pathology in this case is directly contributory to the extent of the neuronal loss and atrophy of the substantia nigra.

The pleomorphic pathologies in *LRRK2* mutation supports the notion that *LRRK2* acts upstream from the pathway of other proteins implicated in neuronal death. It is likely that genetic and environmental factors then influence the type of proteinopathy that eventually develops in the individual, whether it is α-synuclein, tau or ubiquitin pathology (Wider *et al.*, 2010). The novel *MAPT* variant identified in this case is located in a region of the protein far from microtubule binding domains and does not have an obvious role in the molecule's function. The p.A152T *MAPT* variant in exon 7 described Chapter 5.2 and the p.A239T in exon 8 (NM_005910.5) found in a carrier of the *C9orf72* repeat expansion (King *et al.*, 2013) are another two rare variants recently

identified. Interestingly, these three variants all localize in an uncharacterised region of the *MAPT* protein in cases with unexpected tau pathology, suggesting that they may have a disease modifying role in predisposing the individual to tau pathology (Coppola *et al.*, 2012). Nevertheless, when encountering *LRRK2* cases with atypical clinical presentation or unusual pathologies, the possibility of a non-penetrant *LRRK2* mutation and coincidental neurodegenerative disorder should also be considered (Goldwurm *et al.*, 2011, Sierra *et al.*, 2011, Xiromerisiou *et al.*, 2012).

Summary of findings and future work

Traditionally, neurodegenerative diseases have been classified based on the basis of their combined clinical and neuropathological findings. Clinicopathological studies on CBD and PSP have recently widened the spectrum of clinical phenotypes far beyond their classic presentations, and the classic clinical picture of CBD and PSP is now known to be associated with heterogeneous pathologies. It has also been determined that the clinical features of CBD and PSP are closely linked with the severity and the topographical distribution of the underlying tau pathology and neuronal loss. Advances in molecular genetics have further complicated the taxonomy of neurodegenerative diseases and it is now clear that the same gene mutation may have different clinical manifestations and diverse pathology while identical clinical presentations can be seen with different genetic disorders. Disease entities, which were once considered as 'sporadic' are now suspected as being due to genetic abnormalities. This thesis used a combination of clinical, neuropathological, and genetic approaches to re-evaluate a group of disorders in which parkinsonism is seen in association with abnormal tau accumulation.

Review of archival cases in the QSBB over a 20-year period identified 19 cases with CBD; five had a CBS presentation and eight had a RS phenotype, all of whom had vertical supranuclear gaze palsy and seven had falls within the first 2 years. Of 21 cases who had a clinical diagnosis of CBS; only five had CBD pathology, giving a positive predictive value of 24%, six others had PSP pathology, five had Alzheimer's disease and the remaining five had other non-tau pathologies. CBD can present very commonly with a clinical picture closely resembling the classic RS, and the term CBD-RS for this subgroup was proposed. Cases with CBD-RS have delayed onset of vertical supranuclear gaze palsy and infrequent occurrence of predominant downgaze abnormalities, both of which can be helpful pointers to their underlying CBD pathology. Forty-two per cent of CBD cases presented clinically with a RS phenotype and 29% of CBS had underlying PSP pathology. In contrast, CBS is a rare clinical presentation of PSP, occurring in 4% of all pathologically diagnosed PSP cases. These

results indicate that CBD is a discrete clinicopathological entity but has a broader clinical spectrum than was originally proposed.

PSP-CBS was reported to have more tau pathology in the mid-frontal and inferior-parietal cortices than in PSP-RS. However, it was uncertain if differences exist in the distribution of tau pathology in other brain regions or if the overall tau load was increased in the brains of PSP-CBS. The clinical and pathological features of 10 PSP-CBS and 10 PSP-RS cases were compared and quantitative assessment of tau load in 15 cortical, basal ganglia and cerebellar regions were carried out. In addition to the similar age of onset and disease duration, the study demonstrated that the total tau load was the same between PSP-CBS and PSP-RS. However, there was a shift of tau burden towards the cortical regions and away from the basal ganglia, supporting the notion that PSP-CBS is a 'cortical' PSP variant. PSP-CBS also had less severe neuronal loss in the dorsolateral and ventrolateral subregions of the substantia nigra and more severe microglial response in the corticospinal tract than in PSP-RS, but neuronal loss in the subthalamic nucleus was equally severe in both groups.

Repetitive finger tapping is commonly used to assess bradykinesia in Parkinson's disease. The QSBB diagnostic criterion of Parkinson's disease defines bradykinesia as 'slowness of initiation with progressive reduction in speed and amplitude of repetitive action'. However, it is not known if patients with PSP have criteria-defined bradykinesia. Objective assessment of repetitive finger tap performance and handwriting in 15 patients with Parkinson's disease, nine patients with PSP and 16 healthy age- and gender-matched controls were carried out. The motion of the hand and digits was recorded in three-dimension during 15-second repetitive index finger-to-thumb tapping trials. The main finding was 'hypokinesia without decrement' in patients with PSP, which differed from the finger tap pattern in Parkinson's disease. Average finger separation amplitude in PSP was less than half of that in controls and Parkinson's disease. Change in tap amplitude over consecutive taps was computed by linear regression. The average amplitude slope in PSP was nearly zero, indicating a lack of decrement, which differed from the negative slope in patients with PD-OFF. 'Hypokinesia' and 'absence of decrement' were identified in 87% of finger tap trials in the PSP group and only 12% in the PD-OFF group. In Parkinson's disease, finger tap pattern was compatible with criteria-defined bradykinesia characterised by slowness

with progressive reduction in amplitude and speed and increased variability in speed throughout the tap trial. Analyses of handwriting showed that 'micrographia' was present in 75% of PSP and 15% of Parkinson's disease patients. Most scripts performed by patients with PSP did not exhibit decrements in script size. The distinct finger tap and handwriting pattern in PSP can be useful clinically to differentiate the PSP-P subgroup from Parkinson's disease.

A review of all the archival cases in the QSBB, who had a final clinical diagnosis of Parkinson's disease and extensive neuronal loss in the substantia nigra and predominant NFT pathology at post-mortem, was performed. Cases with a definite history of encephalitis or the presence of Lewy bodies at autopsy were excluded. Twelve of the 750 cases with unclassifiable NFT pathology were included in the study. Within this subgroup, seven cases with 'parkinsonism with neurofibrillary degeneration' (PND) were identified and they had presented with levodopa-responsive Parkinson's syndrome, normal cognition and a prolonged disease duration. In addition to severe cell loss in the substantia nigra and Alzheimer-type NFT, a distinctive astrogliopathy, with diffuse granular tau-immunoreactivity of astrocytic processes in a characteristic distribution, was identified. TDP-43 and argyrophilic grain pathologies were common associated pathologies. The other five cases were distinguished from the PND group because two carried the *MAPT* p.A152T risk variant, one carried the G2019S *LRRK2* mutation and two had pathological findings consistent with the PSP-P variant. The identification of the PND cases with NFT pathology resembling that found in PEP but with no history of encephalitis and a somewhat different clinical picture has led to a reconsideration of the links between acute EL and presumed PEP and the mechanisms involved.

In conclusion, despite the widening spectrum of clinical phenotypes of CBD and PSP, meticulous clinical observation continues to be of great importance if progress is to be made in understanding the diversity of the clinical and pathological presentation of these brutal diseases. It is hoped that new studies to analyse the spread of tau pathology in the brain will shed light on the mechanisms underlying the selective vulnerability of neurons in CBD and PSP. Future studies of the temporal progression of pathology in CBD and PSP and the identification of early molecular events preceding the spread of hyperphosphorylated tau will lead to new insights into the

disease biology. A search for genetic loci in sporadic CBD and PSP using genome wide association studies and exome sequencing will help to delineate the biochemical pathways involved in disease pathogenesis, which will underpin future therapeutic approaches. Prospective studies on patients with early PSP and PSP-P subtype will determine if the specific finger tap pattern of 'hypokinesia without decrement' can be used as a reliable early diagnostic sign to distinguish PSP-P from Parkinson's disease. Studies on viral RNA, immunological antibodies and more extensive genetic studies may shed light on the aetiology of PND and PEP.

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Bibliography

Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 38-1985. A 66-year-old man with progressive neurologic deterioration. N Engl J Med 1985; 313: 739-48.

A map of human genome variation from population-scale sequencing. Nature 2010; 467: 1061-73.

Agostino R, Berardelli A, Curra A, Accornero N, Manfredi M. Clinical impairment of sequential finger movements in Parkinson's disease. Mov Disord 1998; 13: 418-21.

Agostino R, Berardelli A, Formica A, Stocchi F, Accornero N, Manfredi M. Analysis of repetitive and nonrepetitive sequential arm movements in patients with Parkinson's disease. Mov Disord 1994; 9: 311-4.

Agostino R, Curra A, Giovannelli M, Modugno N, Manfredi M, Berardelli A. Impairment of individual finger movements in Parkinson's disease. Mov Disord 2003; 18: 560-5.

Ahmed Z, Bigio EH, Budka H, Dickson DW, Ferrer I, Ghetti B, et al. Globular glial tauopathies (GGT): consensus recommendations. Acta neuropathologica 2013; 126: 537-44.

Ahmed Z, Josephs KA, Gonzalez J, DelleDonne A, Dickson DW. Clinical and neuropathologic features of progressive supranuclear palsy with severe pallido-nigro-luysial degeneration and axonal dystrophy. Brain 2008; 131: 460-72.

Ahmed Z, Josephs KA, Gonzalez J, DelleDonne A, Dickson DW. Clinical and neuropathologic features of progressive supranuclear palsy with severe pallido-nigro-luysial degeneration and axonal dystrophy. Brain : a journal of neurology 2008; 131: 460-72.

Alajouanine T. Aphasia and artistic realization. Brain : a journal of neurology 1948; 71: 229-41.

Alexander GE, Crutcher MD. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 1990; 13: 266-71.

Alladi S, Xuereb J, Bak T, Nestor P, Knibb J, Patterson K, et al. Focal cortical presentations of Alzheimer's disease. Brain : a journal of neurology 2007; 130: 2636-45.

Amador-Ortiz C, Lin WL, Ahmed Z, Personett D, Davies P, Duara R, et al. TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. Ann Neurol. 2007; 61: 435-45.

Andreadis A, Brown WM, Kosik KS. Structure and novel exons of the human tau gene. Biochemistry 1992; 31: 10626-33.

Arai T, Ikeda K, Akiyama H, Shikamoto Y, Tsuchiya K, Yagishita S, et al. Distinct isoforms of tau aggregated in neurons and glial cells in brains of patients with Pick's disease, corticobasal degeneration and progressive supranuclear palsy. Acta Neuropathol. 2001; 101: 167-73.

Arai T, Mackenzie IR, Hasegawa M, Nonoka T, Niizato K, Tsuchiya K, et al. Phosphorylated TDP-43 in Alzheimer's disease and dementia with Lewy bodies. Acta Neuropathol. 2009; 117: 125-36.

Augustinack JC, Sanders JL, Tsai LH, Hyman BT. Colocalization and fluorescence resonance energy transfer between cdk5 and AT8 suggests a close association in pre-neurofibrillary tangles and neurofibrillary tangles. J Neuropathol Exp Neurol 2002; 61: 557-64.

Bajaj NP, Gontu V, Birchall J, Patterson J, Grosset DG, Lees AJ. Accuracy of clinical diagnosis in tremulous parkinsonian patients: a blinded video study. J Neurol Neurosurg Psychiatry 2010; 81: 1223-8.

Baker M, Litvan I, Houlden H, Adamson J, Dickson D, Perez-Tur J, et al. Association of an extended haplotype in the tau gene with progressive supranuclear palsy. Hum Mol Genet. 1999; 8: 711-5.

Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 2006; 442: 916-9.

Ball MJ, Murdoch GH. Neuropathological criteria for the diagnosis of Alzheimer's disease: are we really ready yet? Neurobiol Aging. 1997; 18: S3-12.

Ballan G, Tison F. A historical case of probable corticobasal degeneration? Mov Disord 1997; 12: 1073-4.

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961; 4: 561-71.

Benecke R, Rothwell JC, Dick JP, Day BL, Marsden CD. Disturbance of sequential movements in patients with Parkinson's disease. Brain 1987; 110 (Pt 2): 361-79.

Bennett DA, Schneider JA, Wilson RS, Bienias JL, Arnold SE. Neurofibrillary tangles mediate the association of amyloid load with clinical Alzheimer disease and level of cognitive function. Arch Neurol 2004; 61: 378-84.

Berardelli A, Dick JP, Rothwell JC, Day BL, Marsden CD. Scaling of the size of the first agonist EMG burst during rapid wrist movements in patients with Parkinson's disease. J Neurol Neurosurg Psychiatry 1986; 49: 1273-9.

Berardelli A, Rothwell JC, Thompson PD, Hallett M. Pathophysiology of bradykinesia in Parkinson's disease. Brain 2001; 124: 2131-46.

Bigio EH, Vono MB, Satumtira S, Adamson J, Sontag E, Hynan LS, et al. Cortical synapse loss in progressive supranuclear palsy. J Neuropathol Exp Neurol. 2001; 60: 403-10.

Boeve BF. Corticobasal degeneration: The syndrome and the disease. In: Litvan I, editor. Atypical parkinsonian disorders: Clinical and research aspects. New Jersey: Humana Press 2005.

Boeve BF, Maraganore DM, Parisi JE, Ahlskog JE, Graff-Radford N, Caselli RJ, et al. Pathologic heterogeneity in clinically diagnosed corticobasal degeneration. Neurology 1999; 53: 795-800.

Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol. 2006; 112: 389-404.

Braak H, Del Tredici K, Rub U, de Vos RA, Jansen Steur EN, Braak E. Staging of brain pathology related to sporadic Parkinson's disease. Neurobiol Aging 2003; 24: 197-211.

Bramblett GT, Goedert M, Jakes R, Merrick SE, Trojanowski JQ, Lee VM. Abnormal tau phosphorylation at Ser396 in Alzheimer's disease recapitulates development and contributes to reduced microtubule binding. Neuron 1993; 10: 1089-99.

Brion JP, Couck AM, Robertson J, Loviny TL, Anderton BH. Neurofilament monoclonal antibodies RT97 and 8D8 recognize different modified epitopes in paired helical filament-tau in Alzheimer's disease. J Neurochem 1993; 60: 1372-82.

Brusa A, Stoehr R, Pramstaller PP. Progressive supranuclear palsy: new disease or variant of postencephalitic parkinsonism? Mov Disord 2004; 19: 247-52.

Bryant MS, Rintala DH, Lai EC, Protas EJ. An investigation of two interventions for micrographia in individuals with Parkinson's disease. Clin Rehabil 2010; 24: 1021-6.

Buee-Scherrer V, Buee L, Leveugle B, Perl DP, Vermersch P, Hof PR, et al. Pathological tau proteins in postencephalitic parkinsonism: comparison with Alzheimer's disease and other neurodegenerative disorders. Ann Neurol. 1997; 42: 356-9.

Burns JM, Galvin JE, Roe CM, Morris JC, McKeel DW. The pathology of the substantia nigra in Alzheimer disease with extrapyramidal signs. Neurology 2005; 64: 1397-403.

Burr LW. Sequelae of epidemic encephalitis without any preceding acute illness (chronic encephalitis). Arch Neurol Psychiat 1925; 20-4.

Caffrey TM, Joachim C, Wade-Martins R. Haplotype-specific expression of the N-terminal exons 2 and 3 at the human MAPT locus. Neurobiol Aging. 2008; 29: 1923-9.

Cairns NJ, Atkinson PF, Hanger DP, Anderton BH, Daniel SE, Lantos PL. Tau protein in the glial cytoplasmic inclusions of multiple system atrophy can be distinguished from abnormal tau in Alzheimer's disease. Neurosci Lett 1997; 230: 49-52.

Cairns NJ, Bigio EH, Mackenzie IR, Neumann M, Lee VM, Hatanpaa KJ, et al. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. Acta neuropathologica 2007; 114: 5-22.

Cairns NJ, Bigio EH, Mackenzie IR, Neumann M, Lee VM, Hatanpaa KJ, et al. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. Acta Neuropathol 2007; 114: 5-22.

Calne DB, Lees AJ. Late progression of post-encephalitic Parkinson's syndrome. Can J Neurol Sci 1988; 15: 135-8.

Casals J, Elizan TS, Yahr MD. Postencephalitic parkinsonism--a review. J Neural Transm 1998; 105: 645-76.

Casseron W, Azulay JP, Guedj E, Gastaut JL, Pouget J. Familial autosomal dominant cortico-basal degeneration with the P301S mutation in the tau gene: an example of phenotype variability. J Neurol 2005; 252: 1546-8.

Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, et al. Transmission and spreading of tauopathy in transgenic mouse brain. Nat Cell Bio. 2009; 11: 909-13.

Clot F, Grabli D, Cazeneuve C, Roze E, Castelnau P, Chabrol B, et al. Exhaustive analysis of BH4 and dopamine biosynthesis genes in patients with Dopa-responsive dystonia. Brain : a journal of neurology 2009; 132: 1753-63.

Cobb WA, Marshall J, Scaravilli F. Long survival in subacute sclerosing panencephalitis. J Neurol Neurosurg Psychiatry 1984; 47: 176-83.

Conrad C, Andreadis A, Trojanowski JQ, Dickson DW, Kang D, Chen X, et al. Genetic evidence for the involvement of tau in progressive supranuclear palsy. Ann Neurol 1997; 41: 277-81.

Coppola G, Chinnathambi S, Lee JJ, Dombroski BA, Baker MC, Soto-Ortolaza AI, et al. Evidence for a role of the rare p.A152T variant in MAPT in increasing the risk for FTD-spectrum and Alzheimer's diseases. Hum Mol Genet 2012; 21: 3500-12.

Coppola G, Chinnathambi S, Lee JJ, Dombroski BA, Baker MC, Soto-Ortolaza AI, et al. Evidence for a role of the rare p.A152T variant in MAPT in increasing the risk for FTD-spectrum and Alzheimer's diseases. Human molecular genetics 2012; 21: 3500-12.

Covy JP, Yuan W, Waxman EA, Hurtig HI, Van Deerlin VM, Giasson BI. Clinical and pathological characteristics of patients with leucine-rich repeat kinase-2 mutations. Mov Disord 2009; 24: 32-9.

Cruchaga C, Chakraverty S, Mayo K, Vallania FL, Mitra RD, Faber K, et al. Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. PLoS One 2012; 7: e31039.

Cruchaga C, Haller G, Chakraverty S, Mayo K, Vallania FL, Mitra RD, et al. Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. PloS one 2012; 7: e31039.

Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 2006; 442: 920-4.

Dachsel JC, Ross OA, Mata IF, Kachergus J, Toft M, Cannon A, et al. Lrrk2 G2019S substitution in frontotemporal lobar degeneration with ubiquitin-immunoreactive neuronal inclusions. Acta Neuropathol 2007; 113: 601-6.

Dale RC, Church AJ, Surtees RA, Lees AJ, Adcock JE, Harding B, et al. Encephalitis lethargica syndrome: 20 new cases and evidence of basal ganglia autoimmunity. Brain : a journal of neurology 2004; 127: 21-33.

Dale RC, Irani SR, Brilot F, Pillai S, Webster R, Gill D, et al. N-methyl-D-aspartate receptor antibodies in pediatric dyskinetic encephalitis lethargica. Ann Neurol. 2009; 66: 704-9.

Daniel SE, Lees AJ. Neuropathological features of Alzheimer's disease in non-demented parkinsonian patients. J Neurol Neurosurg Psychiatry 1991; 54: 972-5.

de Silva R, Lashley T, Gibb G, Hanger D, Hope A, Reid A, et al. Pathological inclusion bodies in tauopathies contain distinct complements of tau with three or four microtubule-binding repeat domains as demonstrated by new specific monoclonal antibodies. Neuropathol Appl Neurobiol 2003; 29: 288-302.

Della Marca G, Frisullo G, Vollono C, Dittoni S, Losurdo A, Testani E, et al. Oculogyric crisis in a midbrain lesion. Arch Neurol 2011; 68: 390-1.

Demirci M, Grill S, McShane L, Hallett M. A mismatch between kinesthetic and visual perception in Parkinson's disease. Ann Neurol 1997; 41: 781-8.

Derkinderen P, Dupont S, Vidal JS, Chedru F, Vidailhet M. Micrographia secondary to lenticular lesions. Mov Disord 2002; 17: 835-7.

Dickson D, Hauw JJ, Agid Y, Litvan I. Progressive supranuclear palsy and corticobasal degeneration. In: Dickson D, Weller RO, editors. Neurodegeneration: The molecular pathology of dementia and movement disorders: Wiley-Blackwell; 2011. p. 135-55.

Dickson DW. Neuropathologic differentiation of progressive supranuclear palsy and corticobasal degeneration. J Neurol 1999; 246 Suppl 2: II6-15.

Dickson DW, Ahmed Z, Algom AA, Tsuboi Y, Josephs KA. Neuropathology of variants of progressive supranuclear palsy. Curr Opin Neurol 2010; 23: 394-400.

Dickson DW, Bergeron C, Chin SS, Duyckaerts C, Horoupian D, Ikeda K, et al. Office of Rare Diseases neuropathologic criteria for corticobasal degeneration. J Neuropathol Exp Neurol. 2002; 61: 935-46.

Doherty KM, Silveira-Moriyama L, Parkkinen L, Healy DG, Farrell M, Mencacci NE, et al. Parkin disease: a clinicopathologic entity? JAMA Neurol 2013; 70: 571-9.

Dourmashkin RR, Dunn G, Castano V, McCall SA. Evidence for an enterovirus as the cause of encephalitis lethargica. BMC Infect Dis 2012; 12: 136.

Dubois B, Slachevsky A, Litvan I, Pillon B. The FAB: a Frontal Assessment Battery at bedside. Neurology 2000; 55: 1621-6.

Duncan AG. The sequelae of encephalitis lethargica. Brain : a journal of neurology 1924; 79-108.

Duvoisin RC, Lobo-Antunes J, Yahr MD. Response of patients with postencephalitic Parkinsonism to levodopa. J Neurol Neurosurg Psychiatry 1972; 35: 487-95.

Duvoisin RC, Yahr MD. Encephalitis and Parkinsonism. Arch Neurol 1965; 12: 227-39.

Eadie MJ, Sutherland JM, Doherty RL. Encephalitis in Etiology of Parkinsonism in Australia. Arch Neurol 1965; 12: 240-5.

Fahn S, Elton RL. Unified Parkinson's disease rating scale. In: Fahn S, Marsden CD, Goldstein M, Calne DB, editors. Recent developments in Parkinson's disease. Florham Park: NJ: Macmillan Healthcare Information; 1987. p. 153-63, 293-304.

Fearnley JM, Lees AJ. Striatonigral degeneration. A clinicopathological study. Brain 1990; 113 (Pt 6): 1823-42.

Foley PB. Encephalitis lethargica and the influenza virus. II. The influenza pandemic of 1918/19 and encephalitis lethargica: epidemiology and symptoms. J Neural Transm 2009; 116: 1295-308.

Foley PB. Encephalitis lethargica and the influenza virus. III. The influenza pandemic of 1918/19 and encephalitis lethargica: neuropathology and discussion. J Neural Transm 2009; 116: 1309-21.

Forno L, Alvord E. The pathology of parkinsonism: some new observations and correlations. Recent advances in Parkinson's disease. Oxford: Blackwell Scientific; 1971. p. 119-30.

Funayama M, Li Y, Tsoi TH, Lam CW, Ohi T, Yazawa S, et al. Familial Parkinsonism with digenic parkin and PINK1 mutations. Mov Disord 2008; 23: 1461-5.

Gaig C, Ezquerra M, Marti MJ, Valldeoriola F, Munoz E, Llado A, et al. Screening for the LRRK2 G2019S and codon-1441 mutations in a pathological series of parkinsonian syndromes and frontotemporal lobar degeneration. J Neurol Sci 2008; 270: 94-8.

Garraux G, McKinney C, Wu T, Kansaku K, Nolte G, Hallett M. Shared brain areas but not functional connections controlling movement timing and order. J Neurosci 2005; 25: 5290-7.

Geda YE, Boeve BF, Negash S, Graff-Radford NR, Knopman DS, Parisi JE, et al. Neuropsychiatric features in 36 pathologically confirmed cases of corticobasal degeneration. J Neuropsychiatry Clin Neurosci 2007; 19: 77-80.

Geddes JF, Hughes AJ, Lees AJ, Daniel SE. Pathological overlap in cases of parkinsonism associated with neurofibrillary tangles. A study of recent cases of postencephalitic parkinsonism and comparison with progressive supranuclear palsy and Guamanian parkinsonism-dementia complex. Brain : a journal of neurology 1993; 116 (Pt 1): 281-302.

Geddes JF, Vowles GH, Nicoll JA, Revesz T. Neuronal cytoskeletal changes are an early consequence of repetitive head injury. Acta Neuropathol. 1999; 98: 171-8.

Ghaemi M, Rudolf J, Schmulling S, Bamborschke S, Heiss WD. FDG- and Dopa-PET in postencephalitic parkinsonism. J Neural Transm 2000; 107: 1289-95.

Ghebranious N, Ivacic L, Mallum J, Dokken C. Detection of ApoE E2, E3 and E4 alleles using MALDI-TOF mass spectrometry and the homogeneous mass-extend technology. Nucleic Acids Res 2005; 33: e149.

Giasson BI, Covy JP, Bonini NM, Hurtig HI, Farrer MJ, Trojanowski JQ, et al. Biochemical and pathological characterization of Lrrk2. Ann Neurol 2006; 59: 315-22.

Gibb WR, Lees AJ. The progression of idiopathic Parkinson's disease is not explained by age-related changes. Clinical and pathological comparisons with post-encephalitic parkinsonian syndrome. Acta Neuropathol. 1987; 73: 195-201.

Gibb WR, Lees AJ. A comparison of clinical and pathological features of young- and old-onset Parkinson's disease. Neurology 1988; 38: 1402-6.

Gibb WR, Lees AJ. The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 1988; 51: 745-52.

Gibb WR, Luthert PJ, Marsden CD. Corticobasal degeneration. Brain : a journal of neurology 1989; 112 (Pt 5): 1171-92.

Gilks WP, Abou-Sleiman PM, Gandhi S, Jain S, Singleton A, Lees AJ, et al. A common LRRK2 mutation in idiopathic Parkinson's disease. Lancet 2005; 365: 415-6.

Goedert M, Spillantini MG, Cairns NJ, Crowther RA. Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. Neuron 1992; 8: 159-68.

Goedert M, Spillantini MG, Serpell LC, Berriman J, Smith MJ, Jakes R, et al. From genetics to pathology: tau and alpha-synuclein assemblies in neurodegenerative diseases. Philosophical transactions of the Royal Society of London Series B, Biological sciences 2001; 356: 213-27.

Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, et al. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. Mov Disord 2008; 23: 2129-70.

Golbe LI, Ohman-Strickland PA. A clinical rating scale for progressive supranuclear palsy. Brain 2007; 130: 1552-65.

Goldwurm S, Tunesi S, Tesei S, Zini M, Sironi F, Primignani P, et al. Kin-cohort analysis of LRRK2-G2019S penetrance in Parkinson's disease. Mov Disord. 2011; 26: 2144-5.

Graff-Radford J, Whitwell JL, Dickson DW, Josephs KA. Pallidonigroluysian atrophy associated with p.A152T variant in MAPT. Parkinsonism Relat Disord. 2013; 19: 838-41.

Greenberg SG, Davies P. A preparation of Alzheimer paired helical filaments that displays distinct tau proteins by polyacrylamide gel electrophoresis. Proceedings of the National Academy of Sciences of the United States of America 1990; 87: 5827-31.

Grimes DA, Lang AE, Bergeron CB. Dementia as the most common presentation of cortical-basal ganglionic degeneration. Neurology 1999; 53: 1969-74.

Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, et al. Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 1988; 96: 379-94.

Hallett M. Parkinson revisited: pathophysiology of motor signs. Adv Neurol 2003; 91: 19-28.

Halliday G. Clinicopathological aspects of motor parkinsonism. Parkinsonism Relat Disord 2007; 13 Suppl 3: S208-10.

Hanger DP, Gibb GM, de Silva R, Boutajangout A, Brion JP, Revesz T, et al. The complex relationship between soluble and insoluble tau in tauopathies revealed by efficient dephosphorylation and specific antibodies. FEBS Lett 2002; 531: 538-42.

Haraguchi T, Ishizu H, Terada S, Takehisa Y, Tanabe Y, Nishinaka T, et al. An autopsy case of postencephalitic parkinsonism of von Economo type: some new observations concerning neurofibrillary tangles and astrocytic tangles. Neuropathology 2000; 20: 143-8.

Hardman CD, Halliday GM, McRitchie DA, Cartwright HR, Morris JG. Progressive supranuclear palsy affects both the substantia nigra pars compacta and reticulata. Exp Neurol 1997; 144: 183-92.

Hardman CD, Halliday GM, McRitchie DA, Morris JG. The subthalamic nucleus in Parkinson's disease and progressive supranuclear palsy. Journal of neuropathology and experimental neurology 1997; 56: 132-42.

Hardman CD, McRitchie DA, Halliday GM, Cartwright HR, Morris JG. Substantia nigra pars reticulata neurons in Parkinson's disease. Neurodegeneration 1996; 5: 49-55.

Hardy J. Expression of normal sequence pathogenic proteins for neurodegenerative disease contributes to disease risk: 'permissive templating' as a general mechanism underlying neurodegeneration. Biochem Soc Trans. 2005; 33: 578-81.

Hasegawa K, Kowa H. Autosomal dominant familial Parkinson disease: older onset of age, and good response to levodopa therapy. Eur Neurol 1997; 38 Suppl 1: 39-43.

Hasegawa K, Stoessl AJ, Yokoyama T, Kowa H, Wszolek ZK, Yagishita S. Familial parkinsonism: study of original Sagamihara PARK8 (I2020T) kindred with variable clinicopathologic outcomes. Parkinsonism Relat Disord 2009; 15: 300-6.

Hasegawa M, Arai T, Akiyama H, Nonaka T, Mori H, Hashimoto T, et al. TDP-43 is deposited in the Guam parkinsonism-dementia complex brains. Brain : a journal of neurology 2007; 130: 1386-94.

Hassan A, Parisi JE, Josephs KA. Autopsy-proven progressive supranuclear palsy presenting as behavioral variant frontotemporal dementia. Neurocase 2011.

Hassan A, Parisi JE, Josephs KA. Autopsy-proven progressive supranuclear palsy presenting as behavioral variant frontotemporal dementia. Neurocase 2012; 18: 478-88.

Hauw JJ, Daniel SE, Dickson D, Horoupian DS, Jellinger K, Lantos PL, et al. Preliminary NINDS neuropathologic criteria for Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy). Neurology 1994; 44: 2015-9.

Hayesmoore JB, Bray NJ, Cross WC, Owen MJ, O'Donovan MC, Morris HR. The effect of age and the H1c MAPT haplotype on MAPT expression in human brain. Neurobiol Aging 2009; 30: 1652-6.

Healy DG, Falchi M, O'Sullivan SS, Bonifati V, Durr A, Bressman S, et al. Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. Lancet Neurol. 2008; 7: 583-90.

Higashi S, Iseki E, Yamamoto R, Minegishi M, Hino H, Fujisawa K, et al. Concurrence of TDP-43, tau and alpha-synuclein pathology in brains of Alzheimer's disease and dementia with Lewy bodies. Brain Res 2007; 1184: 284-94.

Hirano A, Kurland LT, Krooth RS, Lessell S. Parkinsonism-dementia complex, an endemic disease on the island of Guam. I. Clinical features. Brain : a journal of neurology 1961; 84: 642-61.

Hirano A, Malamud N, Kurland LT. Parkinsonism-dementia complex, an endemic disease on the island of Guam. II. Pathological features. Brain : a journal of neurology 1961; 84: 662-79.

Hodges JR, Davies RR, Xuereb JH, Casey B, Broe M, Bak TH, et al. Clinicopathological correlates in frontotemporal dementia. Ann Neurol. 2004; 56: 399-406.

Hoglinger GU, Melhem NM, Dickson DW, Sleiman PM, Wang LS, Klei L, et al. Identification of common variants influencing risk of the tauopathy progressive supranuclear palsy. Nat Genet 2011; 43: 699-705.

Hoglinger GU, Melhem NM, Dickson DW, Sleiman PM, Wang LS, Klei L, et al. Identification of common variants influencing risk of the tauopathy progressive supranuclear palsy. Nature genetics 2011; 43: 699-705.

Hoglinger GU, Melhem NM, Dickson DW, Sleiman PM, Wang LS, Klei L, et al. Identification of common variants influencing risk of the tauopathy progressive supranuclear palsy. Nat Genet 2011; 43: 699-705.

Horoupian DS, Wasserstein PH. Alzheimer's disease pathology in motor cortex in dementia with Lewy bodies clinically mimicking corticobasal degeneration. Acta Neuropathol. 1999; 98: 317-22.

Houlden H, Baker M, Morris HR, MacDonald N, Pickering-Brown S, Adamson J, et al. Corticobasal degeneration and progressive supranuclear palsy share a common tau haplotype. Neurology 2001; 56: 1702-6.

Howard RS, Lees AJ. Encephalitis lethargica. A report of four recent cases. Brain : a journal of neurology 1987; 110 (Pt 1): 19-33.

Hu SC, Hebb AO. Effect of deep brain stimulation on postencephalitic parkinsonism: a case report. Mov Disord 2012; 27: 1071-2.

Hu WT, Parisi JE, Knopman DS, Boeve BF, Dickson DW, Ahlskog JE, et al. Clinical features and survival of 3R and 4R tauopathies presenting as behavioral variant frontotemporal dementia. Alzheimer Dis Assoc Disord 2007; 21: S39-43.

Hughes AJ, Ben-Shlomo Y, Daniel SE, Lees AJ. What features improve the accuracy of clinical diagnosis in Parkinson's disease: a clinicopathologic study. Neurology 1992; 42: 1142-6.

Hughes AJ, Daniel SE, Ben-Shlomo Y, Lees AJ. The accuracy of diagnosis of parkinsonian syndromes in a specialist movement disorder service. Brain : a journal of neurology 2002; 125: 861-70.

Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J Neurol Neurosurg Psychiatry 1992; 55: 181-4.

Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 1998; 393: 702-5.

Hyman BT, Trojanowski JQ. Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. J Neuropathol Exp Neurol. 1997; 56: 1095-7.

Iansek R, Huxham F, McGinley J. The sequence effect and gait festination in Parkinson disease: contributors to freezing of gait? Mov Disord 2006; 21: 1419-24.

Ikeda K, Akiyama H, Kondo H, Ikeda K. Anti-tau-positive glial fibrillary tangles in the brain of postencephalitic parkinsonism of Economo type. Neurosci Lett 1993; 162: 176-8.

Imamura A, Wszolek ZK, Lucas JA, Dickson DW. Corticobasal syndrome with Alzheimer's disease pathology. Mov Disord 2009; 24: 152-3; author reply 3.

Ince PG, Clark B, Holton JL, Revesz T, Wharton S. Disorders of movement and system degenerations. In: Love S, Louis DN, Ellison DW, editors. Greenfield's Neuropathology. 8th ed. London: Arnold; 2008. p. 889-1030.

Ishizawa K, Dickson DW. Microglial activation parallels system degeneration in progressive supranuclear palsy and corticobasal degeneration. J Neuropathol Exp Neurol 2001; 60: 647-57.

Iwasaki Y, Ikeda K, Shindoh T, Suga Y, Ishikawa I, Hara M, et al. Micrographia in Huntington's disease. J Neurol Sci 1999; 162: 106-7.

Jahanshahi M, Brown RG, Marsden CD. The effect of withdrawal of dopaminergic medication on simple and choice reaction time and the use of advance information in Parkinson's disease. J Neurol Neurosurg Psychiatry 1992; 55: 1168-76.

Jellinger KA. Absence of alpha-synuclein pathology in postencephalitic parkinsonism. Acta Neuropathol. 2009; 118: 371-9.

Jellinger KA. Tauopathies: Postencephalitic Parkinsonism. In: Dickson DW, Weller RO, editors. Neurodegeneration: the molecular pathology of dementia and movement disorders. 2 ed. West Sussex: Willey-Blackwell; 2011.

Jellinger KA, Attems J. Neurofibrillary tangle-predominant dementia: comparison with classical Alzheimer disease. Acta Neuropathol. 2007; 113: 107-17.

Jendroska K, Rossor MN, Mathias CJ, Daniel SE. Morphological overlap between corticobasal degeneration and Pick's disease: a clinicopathological report. Mov Disord 1995; 10: 111-4.

Jicha GA, Lane E, Vincent I, Otvos L, Jr., Hoffmann R, Davies P. A conformation- and phosphorylation-dependent antibody recognizing the paired helical filaments of Alzheimer's disease. J Neurochem 1997; 69: 2087-95.

Jin SC, Pastor P, Cooper B, Cervantes S, Benitez BA, Razquin C, et al. Pooled-DNA sequencing identifies novel causative variants in PSEN1, GRN and MAPT in a clinical early-onset and familial Alzheimer's disease Ibero-American cohort. Alzheimers Res Ther. 2012; 4: 34.

Joseph KA, Rossor MN. The alien limb. Pract Neurol. 2004; 4: 44-5.

Josephs KA, Boeve BF, Duffy JR, Smith GE, Knopman DS, Parisi JE, et al. Atypical progressive supranuclear palsy underlying progressive apraxia of speech and nonfluent aphasia. Neurocase 2005; 11: 283-96.

Josephs KA, Duffy JR. Apraxia of speech and nonfluent aphasia: a new clinical marker for corticobasal degeneration and progressive supranuclear palsy. Curr Opin Neurol 2008; 21: 688-92.

Josephs KA, Duffy JR, Strand EA, Whitwell JL, Layton KF, Parisi JE, et al. Clinicopathological and imaging correlates of progressive aphasia and apraxia of speech. Brain : a journal of neurology 2006; 129: 1385-98.

Josephs KA, Holton JL, Rossor MN, Braendgaard H, Ozawa T, Fox NC, et al. Neurofilament inclusion body disease: a new proteinopathy? Brain : a journal of neurology 2003; 126: 2291-303.

Josephs KA, Parisi JE, Dickson DW. Alpha-synuclein studies are negative in postencephalic parkinsonism of von Economo. Neurology 2002; 59: 645-6.

Josephs KA, Petersen RC, Knopman DS, Boeve BF, Whitwell JL, Duffy JR, et al. Clinicopathologic analysis of frontotemporal and corticobasal degenerations and PSP. Neurology 2006; 66: 41-8.

Josephs KA, Whitwell JL, Knopman DS, Hu WT, Stroh DA, Baker M, et al. Abnormal TDP-43 immunoreactivity in AD modifies clinicopathologic and radiologic phenotype. Neurology 2008; 70: 1850-7.

Kang SY, Wasaka T, Shamim EA, Auh S, Ueki Y, Lopez GJ, et al. Characteristics of the sequence effect in Parkinson's disease. Mov Disord 2010; 25: 2148-55.

Kay DM, Moran D, Moses L, Poorkaj P, Zabetian CP, Nutt J, et al. Heterozygous parkin point mutations are as common in control subjects as in Parkinson's patients. Ann Neurol 2007; 61: 47-54.

Kertesz A, McMonagle P, Blair M, Davidson W, Munoz DG. The evolution and pathology of frontotemporal dementia. Brain : a journal of neurology 2005; 128: 1996-2005.

Khan NL, Jain S, Lynch JM, Pavese N, Abou-Sleiman P, Holton JL, et al. Mutations in the gene LRRK2 encoding dardarin (PARK8) cause familial Parkinson's disease: clinical, pathological, olfactory and functional imaging and genetic data. Brain 2005; 128: 2786-96.

Kidd M. Paired helical filaments in electron microscopy of Alzheimer's disease. Nature 1963; 197: 192-3.

Kim EJ, Lee BH, Park KC, Lee WY, Na DL. Micrographia on free writing versus copying tasks in idiopathic Parkinson's disease. Parkinsonism Relat Disord 2005; 11: 57-63.

Kim JS, Im JH, Kwon SU, Kang JH, Lee MC. Micrographia after thalamo-mesencephalic infarction: evidence of striatal dopaminergic hypofunction. Neurology 1998; 51: 625-7.

King A, Al-Sarraj S, Troakes C, Smith BN, Maekawa S, Iovino M, et al. Mixed tau, TDP-43 and p62 pathology in FTLD associated with a C9ORF72 repeat expansion and p.Ala239Thr MAPT (tau) variant. Acta Neuropathol. 2013; 125: 303-10.

King A, Sweeney F, Bodi I, Troakes C, Maekawa S, Al-Sarraj S. Abnormal TDP-43 expression is identified in the neocortex in cases of dementia pugilistica, but is mainly confined to the limbic system when identified in high and moderate stages of Alzheimer's disease. Neuropathology : official journal of the Japanese Society of Neuropathology 2010; 30: 408-19.

Klein C, Lohmann-Hedrich K, Rogaeva E, Schlossmacher MG, Lang AE. Deciphering the role of heterozygous mutations in genes associated with parkinsonism. Lancet Neurol. 2007; 6: 652-62.

Komori T, Arai N, Oda M, Nakayama H, Mori H, Yagishita S, et al. Astrocytic plaques and tufts of abnormal fibers do not coexist in corticobasal degeneration and progressive supranuclear palsy. Acta Neuropathol. 1998; 96: 401-8.

Kouri N, Murray ME, Hassan A, Rademakers R, Uitti RJ, Boeve BF, et al. Neuropathological features of corticobasal degeneration presenting as corticobasal syndrome or Richardson syndrome. Brain : a journal of neurology 2011.

Kouri N, Whitwell JL, Josephs KA, Rademakers R, Dickson DW. Corticobasal degeneration: a pathologically distinct 4R tauopathy. Nat Rev Neurol. 2011; 7: 263-72.

Kovacs GG, Molnar K, Laszlo L, Strobel T, Botond G, Honigschnabl S, et al. A peculiar constellation of tau pathology defines a subset of dementia in the elderly. Acta Neuropathol. 2011; 122: 205-22.

Kovacs GG, Wohrer A, Strobel T, Botond G, Attems J, Budka H. Unclassifiable tauopathy associated with an A152T variation in MAPT exon 7. Clin Neuropathol 2011; 30: 3-10.

Kovacs GG, Wohrer A, Strobel T, Botond G, Attems J, Budka H. Unclassifiable tauopathy associated with an A152T variation in MAPT exon 7. Clin Neuropathol 2011; 30: 3-10.

Kreisler A, Mastain B, Tison F, Fenelon G, Destee A. [Multi-infarct disorder presenting as corticobasal degeneration (DCB): vascular pseudo-corticobasal degeneration?]. Rev Neurol (Paris) 2007; 163: 1191-9.

Kuoppamaki M, Rothwell JC, Brown RG, Quinn N, Bhatia KP, Jahanshahi M. Parkinsonism following bilateral lesions of the globus pallidus: performance on a variety of motor tasks shows similarities with Parkinson's disease. J Neurol Neurosurg Psychiatry 2005; 76: 482-90.

Kurland LT, Mulder DW. Epidemiologic investigations of amyotrophic lateral sclerosis. I. Preliminary report on geographic distribution, with special reference to the Mariana Islands, including clinical and pathologic observations. Neurology 1954; 4: 355-78.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-5.

Lashley T, Holton JL, Revesz T. TDP-43 pathology may occur in the BRI2 gene-related dementias. Acta Neuropathol. 2011; 121: 559-60.

Le Ber I, Camuzat A, Hannequin D, Pasquier F, Guedj E, Rovelet-Lecrux A, et al. Phenotype variability in progranulin mutation carriers: a clinical, neuropsychological, imaging and genetic study. Brain : a journal of neurology 2008; 131: 732-46.

Lees AJ. The Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy). In: Marsden CD, Fahn S, editors. Movement Disorders 2. London: Butterworth-Heinemann; 1987. p. 272-87.

Lees AJ, Hardy J, Revesz T. Parkinson's disease. Lancet 2009; 373: 2055-66.

Lesage S, Le Ber I, Condroyer C, Broussolle E, Gabelle A, Thobois S, et al. C9orf72 repeat expansions are a rare genetic cause of parkinsonism. Brain : a journal of neurology 2013; 136: 385-91.

Lesage S, Lohmann E, Tison F, Durif F, Durr A, Brice A. Rare heterozygous parkin variants in French early-onset Parkinson disease patients and controls. J Med Genet 2008; 45: 43-6.

Lhermitte J, Levy G, Kyriaco N. Les perturbations de la representation spatiale chez les apraxiques. Rev Neurol (Paris) 1925; 2: 586-600.

Li A, Paudel R, Johnson R, Courtney R, Lees AJ, Holton JL, et al. Pantothenate kinase-associated neurodegeneration is not a synucleinopathy. Neuropathol Appl Neurobiol 2012.

Lidov HG, Duchen LW, Thomas PK, Thrush DC. Progressive medullary failure associated with neurofibrillary degeneration. J Neurol Neurosurg Psychiatry 1989; 52: 643-7.

Litvan I, Agid Y, Calne D, Campbell G, Dubois B, Duvoisin RC, et al. Clinical research criteria for the diagnosis of progressive supranuclear palsy (Steele-Richardson-Olszewski syndrome): report of the NINDS-SPSP international workshop. Neurology 1996; 47: 1-9.

Litvan I, Agid Y, Goetz C, Jankovic J, Wenning GK, Brandel JP, et al. Accuracy of the clinical diagnosis of corticobasal degeneration: a clinicopathologic study. Neurology 1997; 48: 119-25.

Litvan I, Hauw JJ, Bartko JJ, Lantos PL, Daniel SE, Horoupian DS, et al. Validity and reliability of the preliminary NINDS neuropathologic criteria for progressive supranuclear palsy and related disorders. J Neuropathol Exp Neurol. 1996; 55: 97-105.

Litvan I, MacIntyre A, Goetz CG, Wenning GK, Jellinger K, Verny M, et al. Accuracy of the clinical diagnoses of Lewy body disease, Parkinson disease, and dementia with Lewy bodies: a clinicopathologic study. Arch Neurol 1998; 55: 969-78.

Litvan II, Jankovic J, Goetz CG, Wenning GK, Sastry N, Jellinger K, et al. Accuracy of the clinical diagnosis of postencephalitic parkinsonism: a clinicopathologic study. Eur J Neurol 1998; 5: 451-7.

Lo KC, Geddes JF, Daniels RS, Oxford JS. Lack of detection of influenza genes in archived formalin-fixed, paraffin wax-embedded brain samples of encephalitis lethargica patients from 1916 to 1920. Virchows Arch 2003; 442: 591-6.

Mackenzie IR, Rademakers R, Neumann M. TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. Lancet Neurol 2010; 9: 995-1007.

Marsden CD. Slowness of movement in Parkinson's disease. Mov Disord 1989; 4 Suppl 1: S26-37.

Masellis M, Momeni P, Meschino W, Heffner R, Jr., Elder J, Sato C, et al. Novel splicing mutation in the progranulin gene causing familial corticobasal syndrome. Brain : a journal of neurology 2006; 129: 3115-23.

Masliah E, Hansen LA, Quijada S, DeTeresa R, Alford M, Kauss J, et al. Late onset dementia with argyrophilic grains and subcortical tangles or atypical progressive supranuclear palsy? Ann Neurol. 1991; 29: 389-96.

Mata M, Dorovini-Zis K, Wilson M, Young AB. New form of familial Parkinson-dementia syndrome: clinical and pathologic findings. Neurology 1983; 33: 1439-43.

McCall S, Henry JM, Reid AH, Taubenberger JK. Influenza RNA not detected in archival brain tissues from acute encephalitis lethargica cases or in postencephalitic Parkinson cases. J Neuropathol Exp Neurol. 2001; 60: 696-704.

McCall S, Vilensky JA, Gilman S, Taubenberger JK. The relationship between encephalitis lethargica and influenza: a critical analysis. J Neurovirol 2008; 14: 177-85.

McGeer PL, Steele JC. The ALS/PDC syndrome of Guam: potential biomarkers for an enigmatic disorder. Prog Neurobiol. 2011; 95: 663-9.

McKee AC, Stein TD, Nowinski CJ, Stern RA, Daneshvar DH, Alvarez VE, et al. The spectrum of disease in chronic traumatic encephalopathy. Brain : a journal of neurology 2013; 136: 43-64.

McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005; 65: 1863-72.

McLennan JE, Nakano K, Tyler HR, Schwab RS. Micrographia in Parkinson's disease. J Neurol Sci 1972; 15: 141-52.

McMonagle P, Blair M, Kertesz A. Corticobasal degeneration and progressive aphasia. Neurology 2006; 67: 1444-51.

McQuaid S, Allen IV, McMahon J, Kirk J. Association of measles virus with neurofibrillary tangles in subacute sclerosing panencephalitis: a combined in situ hybridization and immunocytochemical investigation. Neuropathol Appl Neurobiol 1994; 20: 103-10.

Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, et al. 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: 479-86.

Momeni P, Pittman A, Lashley T, Vandrovcova J, Malzer E, Luk C, et al. Clinical and pathological features of an Alzheimer's disease patient with the MAPT Delta K280 mutation. Neurobiol Aging 2009; 30: 388-93.

Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, et al. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol. 2012; 123: 1-11.

Morales R, Green KM, Soto C. Cross currents in protein misfolding disorders: interactions and therapy. CNS Neurol Disord Drug Targets. 2009; 8: 363-71.

Mori H, Kondo T, Yokochi M, Matsumine H, Nakagawa-Hattori Y, Miyake T, et al. Pathologic and biochemical studies of juvenile parkinsonism linked to chromosome 6q. Neurology 1998; 51: 890-2.

Morris HR, Gibb G, Katzenschlager R, Wood NW, Hanger DP, Strand C, et al. Pathological, clinical and genetic heterogeneity in progressive supranuclear palsy. Brain : a journal of neurology 2002; 125: 969-75.

Morris HR, Gibb G, Katzenschlager R, Wood NW, Hanger DP, Strand C, et al. Pathological, clinical and genetic heterogeneity in progressive supranuclear palsy. Brain : a journal of neurology 2002; 125: 969-75.

Murray R, Neumann M, Forman MS, Farmer J, Massimo L, Rice A, et al. Cognitive and motor assessment in autopsy-proven corticobasal degeneration. Neurology 2007; 68: 1274-83.

Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 2006; 314: 130-3.

O'Sullivan SS, Burn DJ, Holton JL, Lees AJ. Normal dopamine transporter single photon-emission CT scan in corticobasal degeneration. Mov Disord 2008; 23: 2424-6.

Oide T, Ohara S, Yazawa M, Inoue K, Itoh N, Tokuda T, et al. Progressive supranuclear palsy with asymmetric tau pathology presenting with unilateral limb dystonia. Acta Neuropathol. 2002; 104: 209-14.

Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971; 9: 97-113.

Oliveira RM, Gurd JM, Nixon P, Marshall JC, Passingham RE. Micrographia in Parkinson's disease: the effect of providing external cues. J Neurol Neurosurg Psychiatry 1997; 63: 429-33.

Oppenheimer. Diseases of the basal ganglia, cerebellum and motor neurons. In: Adams JH, Corsellis JA, Duchen LW, editors. Greenfield's neuropathology. 4th ed. New York: Wiley; 1984. p. 699-747.

Oshima K, Dickson DW. Cortical Alzheimer type pathology does not influence tau pathology in progressive supranuclear palsy. Int J Clin Exp Pathol 2009; 2: 399-406.

Oyanagi K, Makifuchi T, Ohtoh T, Chen KM, Gajdusek DC, Chase TN. Distinct pathological features of the gallyas- and tau-positive glia in the Parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam. J Neuropathol Exp Neurol 1997; 56: 308-16.

Oyanagi K, Tsuchiya K, Yamazaki M, Ikeda K. Substantia nigra in progressive supranuclear palsy, corticobasal degeneration, and parkinsonism-dementia complex of Guam: specific pathological features. J Neuropathol Exp Neurol 2001; 60: 393-402.

Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, et al. Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 2004; 44: 595-600.

Pavese N, Khan NL, Scherfler C, Cohen L, Brooks DJ, Wood NW, et al. Nigrostriatal dysfunction in homozygous and heterozygous parkin gene carriers: an 18F-dopa PET progression study. Mov Disord 2009; 24: 2260-6.

Pick A. Uber eine eigenthumliche schreibstorung, mikrographie, in folge cerebraler erkrankung. Prag Med Wochenscher 1903; 1-4.

Pittman AM, Myers AJ, Abou-Sleiman P, Fung HC, Kaleem M, Marlowe L, et al. Linkage disequilibrium fine mapping and haplotype association analysis of the tau gene in progressive supranuclear palsy and corticobasal degeneration. J Med Genet 2005; 42: 837-46.

Pittman AM, Myers AJ, Duckworth J, Bryden L, Hanson M, Abou-Sleiman P, et al. The structure of the tau haplotype in controls and in progressive supranuclear palsy. Hum Mol Genet 2004; 13: 1267-74.

Plato CC, Garruto RM, Galasko D, Craig UK, Plato M, Gamst A, et al. Amyotrophic lateral sclerosis and parkinsonism-dementia complex of Guam: changing incidence rates during the past 60 years. Am J Epidemiol 2003; 157: 149-57.

Poorkaj P, Bird TD, Wijsman E, Nemens E, Garruto RM, Anderson L, et al. Tau is a candidate gene for chromosome 17 frontotemporal dementia. Ann Neurol. 1998; 43: 815-25.

Puig B, Rey MJ, Ferrer I. Individual and regional variations of phospho-tau species in progressive supranuclear palsy. Acta Neuropathol. 2005; 110: 261-8.

Quencer K, Okun MS, Crucian G, Fernandez HH, Skidmore F, Heilman KM. Limb-kinetic apraxia in Parkinson disease. Neurology 2007; 68: 150-1.

Rademakers R, Cruts M, van Broeckhoven C. The role of tau (MAPT) in frontotemporal dementia and related tauopathies. Hum Mutat 2004; 24: 277-95.

Rail D, Scholtz C, Swash M. Post-encephalitic Parkinsonism: current experience. J Neurol Neurosurg Psychiatry 1981; 44: 670-6.

Rajput A, Dickson DW, Robinson CA, Ross OA, Dachsel JC, Lincoln SJ, et al. Parkinsonism, Lrrk2 G2019S, and tau neuropathology. Neurology 2006; 67: 1506-8.

Rajput AH, Uitti RJ, Sudhakar S, Rozdilsky B. Parkinsonism and neurofibrillary tangle pathology in pigmented nuclei. Ann Neurol 1989; 25: 602-6.

Ransmayr G. Constantin von Economo's contribution to the understanding of movement disorders. Mov Disord 2007; 22: 469-75.

Ravenholt RT, Foege WH. 1918 influenza, encephalitis lethargica, parkinsonism. Lancet 1982; 2: 860-4.

Rebeiz JJ, Kolodny EH, Richardson EP, Jr. Corticodentatonigral degeneration with neuronal achromasia: a progressive disorder of late adult life. Trans Am Neurol Assoc 1967; 92: 23-6.

Rebeiz JJ, Kolodny EH, Richardson EP, Jr. Corticodentatonigral degeneration with neuronal achromasia. Arch Neurol 1968; 18: 20-33.

Renkawek K, Horstink MW. Striatonigral degeneration with neurofibrillary tangles. Acta Neuropathol 1993; 86: 405-10.

Renner JA, Burns JM, Hou CE, McKeel DW, Jr., Storandt M, Morris JC. Progressive posterior cortical dysfunction: a clinicopathologic series. Neurology 2004; 63: 1175-80.

Revesz T, Ghiso J, Lashley T, Plant G, Rostagno A, Frangione B, et al. Cerebral amyloid angiopathies: a pathologic, biochemical, and genetic view. J Neuropathol Exp Neurol 2003; 62: 885-98.

Richardson JC, Steele J, Olszewski J. Supranuclear Ophthalmoplegia, Pseudobulbar Palsy, Nuchal Dystonia and Dementia. A Clinical Report on Eight Cases of "Heterogenous System Degeneration". Trans Am Neurol Assoc 1963; 88: 25-9.

Rippon GA, Staugaitis SM, Chin SS, Goldman JE, Marder K. Corticobasal syndrome with novel argyrophilic glial inclusions. Mov Disord 2005; 20: 598-602.

Rivaud-Pechoux S, Vidailhet M, Gallouedec G, Litvan I, Gaymard B, Pierrot-Deseilligny C. Longitudinal ocular motor study in corticobasal degeneration and progressive supranuclear palsy. Neurology 2000; 54: 1029-32.

Saito Y, Ruberu NN, Sawabe M, Arai T, Tanaka N, Kakuta Y, et al. Staging of argyrophilic grains: an age-associated tauopathy. J Neuropathol Exp Neurol. 2004; 63: 911-8.

Santpere G, Ferrer I. LRRK2 and neurodegeneration. Acta Neuropathol 2009; 117: 227-46.

Scaravilli T, Tolosa E, Ferrer I. Progressive supranuclear palsy and corticobasal degeneration: lumping versus splitting. Mov Disord 2005; 20 Suppl 12: S21-8.

Scolding NJ, Lees AJ. Micrographia associated with a parietal lobe lesion in multiple sclerosis. J Neurol Neurosurg Psychiatry 1994; 57: 739-41.

Shelley BP, Hodges JR, Kipps CM, Xuereb JH, Bak TH. Is the pathology of corticobasal syndrome predictable in life? Mov Disord 2009; 24: 1593-9.

Sierra M, Gonzalez-Aramburu I, Sanchez-Juan P, Sanchez-Quintana C, Polo JM, Berciano J, et al. High frequency and reduced penetrance of LRRK2 G2019S mutation among Parkinson's disease patients in Cantabria (Spain). Mov Disord. 2011; 26: 2343-6.

Simon-Sanchez J, Schulte C, Bras JM, Sharma M, Gibbs JR, Berg D, et al. Genome-wide association study reveals genetic risk underlying Parkinson's disease. Nat Genet. 2009; 41: 1308-12.

Singh PP, Singh M, Mastana SS. APOE distribution in world populations with new data from India and the UK. Ann Hum Biol 2006; 33: 279-308.

Snowden JS, Rollinson S, Thompson JC, Harris JM, Stopford CL, Richardson AM, et al. Distinct clinical and pathological characteristics of frontotemporal dementia

associated with C9ORF72 mutations. Brain : a journal of neurology 2012; 135: 693-708.

Spillantini MG, Goedert M. Tau pathology and neurodegeneration. Lancet neurology 2013; 12: 609-22.

Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. Proc Natl Acad Sci U S A. 1998; 95: 7737-41.

Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance C, Rogelj B, et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 2008; 319: 1668-72.

Stadlan EM, Duvoisin RC, Yahr MD. The pathology of parkinsonism. Proceedings of the Fifth International Congress of Neuropathology. Zurich; 1965. p. 569-71.

Steele JC. Progressive supranuclear palsy. Historical notes. J Neural Transm Suppl 1994; 42: 3-14.

Steele JC, Richardson JC, Olszewski J. PROGRESSIVE SUPRANUCLEAR PALSY. A HETEROGENEOUS DEGENERATION INVOLVING THE BRAIN STEM, BASAL GANGLIA AND CEREBELLUM WITH VERTICAL GAZE AND PSEUDOBULBAR PALSY, NUCHAL DYSTONIA AND DEMENTIA. Arch Neurol 1964; 10: 333-59.

Steele JC, Richardson JC, Olszewski J. Progressive supranuclear palsy. A heterogeneous degeneration involving the brain stem, basal ganglia and cerebellum with vertical gaze and pseudobulbar palsy, nuchal dystonia and dementia. Arch Neurol 1964; 333-59.

Stefansson H, Helgason A, Thorleifsson G, Steinthorsdottir V, Masson G, Barnard J, et al. A common inversion under selection in Europeans. Nat Genet 2005; 37: 129-37.

Steiger MJ, Quinn NP. Levodopa challenge test in Parkinson's disease. Lancet 1992; 339: 751-2.

Sundar PD, Yu CE, Sieh W, Steinbart E, Garruto RM, Oyanagi K, et al. Two sites in the MAPT region confer genetic risk for Guam ALS/PDC and dementia. Hum Mol Genet 2007; 16: 295-306.

Suzuki K, Parker CC, Pentchev PG, Katz D, Ghetti B, D'Agostino AN, et al. Neurofibrillary tangles in Niemann-Pick disease type C. Acta Neuropathol. 1995; 89: 227-38.

Tan EK, Peng R, Teo YY, Tan LC, Angeles D, Ho P, et al. Multiple LRRK2 variants modulate risk of Parkinson disease: a Chinese multicenter study. Hum Mutat 2010; 31: 561-8.

Tang-Wai DF, Graff-Radford NR, Boeve BF, Dickson DW, Parisi JE, Crook R, et al. Clinical, genetic, and neuropathologic characteristics of posterior cortical atrophy. Neurology 2004; 63: 1168-74.

Tartaglia MC, Sidhu M, Laluz V, Racine C, Rabinovici GD, Creighton K, et al. Sporadic corticobasal syndrome due to FTLD-TDP. Acta Neuropathol 2009.

Tartaglia MC, Sidhu M, Laluz V, Racine C, Rabinovici GD, Creighton K, et al. Sporadic corticobasal syndrome due to FTLD-TDP. Acta Neuropathol 2009; 30: 30.

Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology 2002; 58: 1791-800.

Togo T, Sahara N, Yen SH, Cookson N, Ishizawa T, Hutton M, et al. Argyrophilic grain disease is a sporadic 4-repeat tauopathy. J Neuropathol Exp Neurol 2002; 61: 547-56.

Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. Mov Disord 2010; 25: 2649-53.

Torvik A, Meen D. Distribution of the brain stem lesions in postencephalitic Parkinsonism. Acta Neurol Scand 1966; 42: 415-25.

Trabzuni D, Wray S, Vandrovcova J, Ramasamy A, Walker R, Smith C, et al. MAPT expression and splicing is differentially regulated by brain region: relation to genotype and implication for tauopathies. Hum Mol Genet. 2012; 21: 4094-103.

Tsuboi Y, Josephs KA, Boeve BF, Litvan I, Caselli RJ, Caviness JN, et al. Increased tau burden in the cortices of progressive supranuclear palsy presenting with corticobasal syndrome. Mov Disord 2005; 20: 982-8.

Tsuboi Y, Slowinski J, Josephs KA, Honer WG, Wszolek ZK, Dickson DW. Atrophy of superior cerebellar peduncle in progressive supranuclear palsy. Neurology 2003; 60: 1766-9.

Tsuchiya K, Murayama S, Mitani K, Oda T, Arima K, Mimura M, et al. Constant and severe involvement of Betz cells in corticobasal degeneration is not consistent with pyramidal signs: a clinicopathological study of ten autopsy cases. Acta Neuropathol 2005; 109: 353-66.

Uryu K, Nakashima-Yasuda H, Forman MS, Kwong LK, Clark CM, Grossman M, et al. Concomitant TAR-DNA-binding protein 43 pathology is present in Alzheimer disease and corticobasal degeneration but not in other tauopathies. J Neuropathol Exp Neurol. 2008; 67: 555-64.

Vandenberghe W, Sciot R, Demaerel P, Van Laere K. Sparing of the substantia nigra in sporadic Creutzfeldt-Jakob disease presenting as an acute corticobasal syndrome. Mov Disord 2007; 22: 1668-9.

Velasco F, Velasco M. A quantitative evaluation of the effects of L-dopa on Parkinson's disease. Neuropharmacology 1973; 12: 89-99.

Vidailhet M, Rivaud S, Gouider-Khouja N, Pillon B, Bonnet AM, Gaymard B, et al. Eye movements in parkinsonian syndromes. Ann Neurol. 1994 Apr;35(4):420-6.

Vidailhet M, Rivaud-Pechoux S, Rivaud S, Gouider-Khouja N, Pillon B, Bonnet AM, et al. Eye movement disorders in corticobasal degeneration. adv Neurol. 2000 Apr;82(4):161-7.

Vilensky JA, Duvosin RC, Gilman S. The diagnosis of postencephalitic parkinsonism at the neurological unit of Boston City Hospital, 1930-1981. Neurol Sci 2011; 32: 343-6.

Vilensky JA, Gilman S, McCall S. A historical analysis of the relationship between encephalitis lethargica and postencephalitic parkinsonism: a complex rather than a direct relationship. Mov Disord 2010; 25: 1116-23.

Vincent A. Encephalitis lethargica: part of a spectrum of post-streptococcal autoimmune diseases? Brain : a journal of neurology 2004; 127: 2-3.

Von Economo C. Encephalitis lethargica. Wien Klin Wochenschr 1917; 581-3.

Von Economo C. Encephalitis lethargica: its sequelae and treatment. London: Oxford University Press; 1931.

Wadia PM, Lang AE. The many faces of corticobasal degeneration. Parkinsonism Relat Disord 2007; 13 Suppl 3: S336-40.

Watts RL, Williams RS, Growden JD. Corticobasal ganglionic degeneration. Neurology 1985; 35(Suppl 1): 178.

Wenning GK, Jellinger K, Litvan I. Supranuclear gaze palsy and eyelid apraxia in postencephalitic parkinsonism. J Neural Transm 1997; 104: 845-65.

Wharton SB, Brayne C, Savva GM, Matthews FE, Forster G, Simpson J, et al. Epidemiological neuropathology: the MRC Cognitive Function and Aging Study experience. J Alzheimers Dis 2011; 25: 359-72.

Whitwell JL, Jack CR, Jr., Boeve BF, Parisi JE, Ahlskog JE, Drubach DA, et al. Imaging correlates of pathology in corticobasal syndrome. Neurology 2010; 75: 1879-87.

Whitwell JL, Master AV, Avula R, Kantarci K, Eggers SD, Edmonson HA, et al. Clinical correlates of white matter tract degeneration in progressive supranuclear palsy. Arch Neurol 2011; 68: 753-60.

Wichmann T, DeLong MR. Functional neuroanatomy of the basal ganglia in Parkinson's disease. Adv Neurol 2003; 91: 9-18.

Wider C, Dachsel JC, Farrer MJ, Dickson DW, Tsuboi Y, Wszolek ZK. Elucidating the genetics and pathology of Perry syndrome. J Neurol Sci 2010; 289: 149-54.

Wider C, Dickson DW, Wszolek ZK. Leucine-rich repeat kinase 2 gene-associated disease: redefining genotype-phenotype correlation. Neurodegener Dis 2010; 7: 175-9.

Williams A, Houff S, Lees A, Calne DB. Oligoclonal banding in the cerebrospinal fluid of patients with postencephalitic Parkinsonism. J Neurol Neurosurg Psychiatry 1979; 42: 790-2.

Williams DR, de Silva R, Paviour DC, Pittman A, Watt HC, Kilford L, et al. Characteristics of two distinct clinical phenotypes in pathologically proven progressive supranuclear palsy: Richardson's syndrome and PSP-parkinsonism. Brain : a journal of neurology 2005; 128: 1247-58.

Williams DR, Holton JL, Strand C, Pittman A, de Silva R, Lees AJ, et al. Pathological tau burden and distribution distinguishes progressive supranuclear palsy-parkinsonism from Richardson's syndrome. Brain : a journal of neurology 2007; 130: 1566-76.

Williams DR, Holton JL, Strand K, Revesz T, Lees AJ. Pure akinesia with gait freezing: a third clinical phenotype of progressive supranuclear palsy. Mov Disord 2007; 22: 2235-41.

Williams DR, Lees AJ. Progressive supranuclear palsy: clinicopathological concepts and diagnostic challenges. Lancet Neurol 2009; 8: 270-9.

Williams DR, Lees AJ. What features improve the accuracy of the clinical diagnosis of progressive supranuclear palsy-parkinsonism (PSP-P)? Mov Disord 2010; 25: 357-62.

Williams DR, Lees AJ, Wherrett JR, Steele JC. J. Clifford Richardson and 50 years of progressive supranuclear palsy. Neurology 2008; 70: 566-73.

Witman GB, Cleveland DW, Weingarten MD, Kirschner MW. Tubulin requires tau for growth onto microtubule initiating sites. Proc Natl Acad Sci U S A 1976; 73: 4070-4.

Wray S, Saxton M, Anderton BH, Hanger DP. Direct analysis of tau from PSP brain identifies new phosphorylation sites and a major fragment of N-terminally cleaved tau containing four microtubule-binding repeats. J Neurochem 2008; 105: 2343-52.

Wszolek ZK, Pfeiffer RF, Tsuboi Y, Uitti RJ, McComb RD, Stoessl AJ, et al. Autosomal dominant parkinsonism associated with variable synuclein and tau pathology. Neurology 2004; 62: 1619-22.

Wszolek ZK, Vieregge P, Uitti RJ, Gasser T, Yasuhara O, McGeer P, et al. German-Canadian family (family A) with parkinsonism, amyotrophy, and dementia - Longitudinal observations. Parkinsonism Relat Disord 1997; 3: 125-39.

Wu T, Hallett M. A functional MRI study of automatic movements in patients with Parkinson's disease. Brain 2005; 128: 2250-9.

Xiromerisiou G, Houlden H, Sailer A, Silveira-Moriyama L, Hardy J, Lees AJ. Identical twins with Leucine rich repeat kinase type 2 mutations discordant for Parkinson's disease. Mov Disord. 2012; 27: 1323.

Yamazaki M, Hasegawa M, Mori O, Murayama S, Tsuchiya K, Ikeda K, et al. Tau-positive fine granules in the cerebral white matter: a novel finding among the tauopathies exclusive to parkinsonism-dementia complex of Guam. J Neuropathol Exp Neurol 2005; 64: 839-46.

Zhong Q, Congdon EE, Nagaraja HN, Kuret J. Tau isoform composition influences rate and extent of filament formation. J Biol Chem. 2012; 287: 20711-9.

Zimprich A, Biskup S, Leitner P, Lichtner P, Farrer M, Lincoln S, et al. Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology. Neuron 2004; 44: 601-7.