Progressive Supranuclear Palsy
expanding the clinical and genetic spectrum

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Progressive Supranuclear Palsy
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Progressieve supranucleaire verlamming: verbreding van
het klinisch en genetisch spectrum

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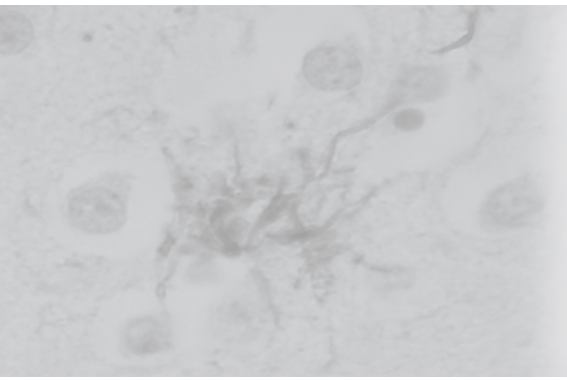

Chapter 1

Introduction

Chapter 1.1

General introduction to the thesis

Progressive Supranuclera Palsy (PSP) was first described by Steele, Richardson and Olszewski as a distinct entity.[1] Richardson recognized this clinical syndrome among several patients and his colleagues, Steele and Olszewski, identified the similarities at neuropathological examination. They presented their work in 1964 and named the disorder PSP; some however still refer to it as 'Steel-Richardson-Olszewski' syndrome. The disorder has now been recognised as the second most common type of parkinsonism after Parkinson's disease (PD), with a prevalence of 5 per 100.000. Frequent falls, vertical gaze palsy, pseudobulbar dysarthria and cognitive decline comprise a characteristic symptom complex of which its full-blown picture can be easily recognized by clinicians. With neuropathological examination as golden standard, the clinical heterogeneity is however increasingly being demonstrated during the last decades. Besides its clinical overlap with other atypical parkinsonian disorders, like MSA and LBD, much overlap is seen in pathological and genetic fields with disorders in the FTD spectrum. Familial cases with variable modes of inheritance are no longer anecdotal reports, but increasingly being reported. Besides a few mutations in MAPT, the majority of these familial cases await the identification of the genetic defect.

In 2003, a genetic-epidemiologic study on PSP was started at the Erasmus Medical Center in Rotterdam. Patients were ascertained nation-wide and over the last 8 years more than 200 patients are included into the study.
The aim of this thesis was to study the clinical presentation and the hereditary aspects of PSP. Furthermore, the neuropathological picture was examined in detail in a subset of patients who came to autopsy during follow-up.

In chapter 1.2 I will give a general overview of the disorder, covering clinical, genetic and pathological aspects of PSP. The next chapter presents the frontal subtype of PSP (chapter 2.1), which was identified by reviewing the clinical symptoms during the first two years of disease onset. A clinimetric comparison between PSP and PD patients (chapter 2.2) was performed to identify differences in motor, cognitive and autonomic symptoms between these two related disorders. A comparative study on survival in two large cohorts of PSP and FTD patients is described in chapter 2.3. In chapter 3.1 we present a case-control study on family history in PSP, showing an increased odds ratio for parkinsonism among first degree relatives of patients with PSP. This chapter contains the description of several familial PSP cases with pathological confirmation, in whom genetic testing was performed. In the next chapter (chapter 3.2), we describe a large family in which the proband presents with a PSP-like phenotype and other affected relatives with variable neurological

features, all having mild gait ataxia and prominent cognitive decline in common. A genome wide linkage study was performed in this family. After brain autopsy was carried out in one affected deceased relative, neuropathological examination showed 1C2 positive inclusions, suggestive for a polyglutamine disorder. In chapter 4, the main findings of the study are presented in light of the current knowledge about the disease and suggestions for future research are made.

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Chapter 1.2

Recent Advances in Progressive Supranuclear Palsy: A Review

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Abstract

Progressive Supranuclear Palsy (PSP) has been used to denote a unifying disorder with progressive parkinsonism with early falls, vertical supranuclear gaze palsy, pseudobulbar dysfunction and cognitive decline. Over the last decade, heterogeneity of the disease into different clinical subtypes has been recognized in clinicopathological studies. Although neuroimaging features and laboratory findings may support the diagnosis, true biomarkers are still lacking in the clinical setting. Neuronal and glial tau positive aggregates are predominantly found in basal ganglia and brainstem, and the significant association of PSP with the common H1 tau haplotype likely points to a pathophysiological role of the tau protein in the disease process. Future genetic studies of familial cases and an ongoing genome-wide association study of large series of pathological-proven cases may reveal additional genetic factors in the near future.

Introduction

In 1964 Steele et al. described an uniform clinical and neuropathological picture in 9 patients, and designated it with the term progressive supranuclear palsy (PSP).[1] Since its discovery, much work has been conducted on clinical, pathological and genetic aspects. The disorder has now been recognised as the second most common parkinsonian neurodegenerative disorder after Parkinson's disease (PD). Although clinical criteria for PSP have proven to be very useful and have been widely accepted,[2] recent studies have emphasized that clinical presentations do not always fulfil the criteria for possible or probable PSP. An important contribution to the clinical setting and future therapeutic interventions is the PSP Rating Scale which can be used to semi-quantitatively evaluate disease progression.[3] PSP has classically been considered a sporadic disease, but recent studies have reported families with PSP and increased familial aggregation.[4] Characteristic neuroimaging features have been demonstrated and a recent study has reported a potential biomarker in cerebrospinal fluid.[5] PSP is classified as a tauopathy because of the neuropathological aggregates which consist of hyperphosphorylated Microtubule Associated Protein Tau (MAPT). The genetic association of PSP with the H1 *MAPT* haplotype has been known for more than a decade, but it has been delineated in more detail in recent years. The type of tau pathology has been further characterized by the development of isoform-specific antibodies,[6] and a scoring system for tau severity has proven to be useful for the morphologic assessment of PSP tau pathology.[7] Several trials with different agents have been carried out, of which Coenzyme Q10 appears to improve cerebral energy metabolism.[8] In light of these recent developments, a review on recent advances in clinical, pathological and genetic research is warranted.

Epidemiology

PSP accounts for approximately 5% of all parkinsonian disorders.[9] The age-adjusted prevalence of PSP has been estimated at 5-6.4 per 100.000,[10-11] and the incidence of PSP increases with age, from 1.7 (per 100.000 person-years) for those aged 50 to 59, to 14.7 for those aged 80 to 99.[12] Lower incidence rates have recently been reported in Russia (0.14/100.000) and Sweden (1.2/100.000).[13-14] The disease might affect men more frequent than women.[15]

In Guadeloupe, a French Caribbean island, an unexpectedly high frequency (75%) of atypical parkinsonism unresponsive to levodopa has been reported; only 25% fulfilled the Brain Bank criteria for PD.[16] Half of the patients with atypical parkinsonism had a PSP like syndrome with oculomotor disturbances and postural instability with falls. However, only a small subset fulfilled the criteria for PSP, whereas the majority differed from classic PSP because of the presence of hallucinations (unrelated to

medication), dysautonomia and tremor.[17] Among these patients with atypical parkinsonism, a strong association was found with the consumption of herbal tea and tropical fruits containing acetogenins, which are potentially toxic inhibitors of the mitochondrial respiratory chain.[16] This has given impetus to investigate the role of annonacin (the major acetogenin in these tropical fruits) as a strong mitochondrial complex I inhibitor, which induces neurodegeneration with GABAergic cell loss in the striatum and cholinergic and dopaminergic cell loss of the substantia nigra in animal models.[18] In case-control series of Caucasian origin, no significant association has been found with environmental factors, although some evidence has been revealed for lower education levels in PSP patients compared to controls.[19-20]

Familial aggregation in PSP is an issue of controversy. Although classically considered a sporadic disease, a non-significant trend towards a positive family history was found and several families with clustering of PSP-like disorders have been reported in literature.[19, 21-24] More recently, a large case-control study has shown that the occurrence of parkinsonism in first-degree relatives of PSP patients (12%) is higher than in controls (3%), whereas equal frequencies of dementia were found in both groups (25% versus 23%).[4]

Clinical features and diagnosis

Progressive parkinsonism starting in the seventh decade with prominent disequilibrium problems and falls, is the typical presentation of the disease. Other less frequently reported symptoms at onset are memory impairment, personality change, pseudobulbar problems, blurred vision or diplopia.[25] Vertical gaze palsy, the most characteristic feature of PSP is usually absent in the initial phase, whereas slowing of vertical saccades can often be observed at neurological examination at this stage.[2]

High predictive values have been found for possible and probable PSP based on the international consensus criteria (NINDS-SPSP, Table 1).[2, 26-27] A few shortcomings of these criteria have to be mentioned. First of all, a considerable number of patients with a full-blown clinical picture of PSP did not have frequent falls in the first year, which classifies them as possible PSP and excludes the diagnosis probable PSP; Secondly, the existence of a parkinsonism subtype (PSP-P) in a large clinicopathological study[28] has broadened the clinical spectrum, but has therefore lowered the sensitivity of the criteria. In contrast to the classical picture of PSP with falls, gaze palsy and cognitive dysfunction (the so-called Richardson's syndrome), PSP-P is characterized by an asymmetric onset, tremor, a good response to levodopa, and longer disease duration.[28] This latter presentation accounts for 8-32% of all PSP patients and is often mistaken for PD.[28-29] Another clinical presentation of PSP is the

syndrome of Pure Akinesia with Gait Freezing (PAGF), which includes a gradual onset with early freezing of gait or speech, without rigidity, tremor, dementia, or eye movement abnormalities during the first 5 years of the disease and without benefit from levodopa therapy.[30]

NINDS-SPSP criteria for PSP
Possible PSP Gradually progressive disorder; onset age 40 or later; *either* vertical supranuclear palsy or both slowing of vertical saccades and postural instability with falls within a year of disease onset; no evidence of other diseases that could explain the foregoing features
Probable PSP Gradually progressive disorder; onset age 40 or later; vertical supranuclear palsy *and* prominent postural instability with falls within a year of disease onset; no evidence of other diseases that could explain the foregoing features
Definite PSP Clinically probable or possible PSP *and* histopathological evidence of typical PSP

Table 1. Clinical consensus criteria for PSP (Litvan et al. 1996)

A frontal presentation with prominent cognitive dysfunction and behavioural changes has been identified in 20 percent of a large population based cohort, which may suggest an alternative neurological or psychiatric diagnosis, for example FTD or depression in the initial phase.[29] Apart from mental slowness and apathy, executive dysfunction is one of the characteristic cognitive features in PSP and includes reduced verbal fluency, impaired abstract thinking, and difficulty planning and set shifting. The Frontal Assessment Battery (FAB) is a simple test of executive function and helps to differentiate PSP from MSA and PD (cut off score of 15).[31-32] The applause sign is frequently present in PSP patients and demonstrates the reduced motor control which is thought to be mediated by frontal and/or basal ganglia dysfunction.[33] Its specificity for PSP, however, is a current subject of debate, as it is present in several other neurodegenerative disorders.[34] In a recent paper, recognition of negative emotions appears to be impaired in PSP patients, but this has to be replicated.[35]

The fixed or surprised facial expression characteristic for PSP is presumed to result from focal dystonia of facial muscles.[36] Blepharospasm, limb dystonia and retrocollis are other dystonic features which may evolve during the disease.[37] The rapidly progressive nature of the disease is reflected by an average interval of five to six years between onset and a wheelchair-requirement stage.[38] Dysarthria and dyphagia develop much earlier in PSP than in PD, and an unintelligible speech occurs after a mean disease duration of six years.[38-39] Patients with the Richardson type of PSP

have a survival of seven to eight years, whereas PSP-P patients tend to have a much longer survival.[40-41] The early occurrence of falls, dementia, and oculomotor dysfunction as well as male gender and older age at onset is associated with increased mortality risk.[25, 41] Also, a high score on the PSP rating scale has proven to be a good independent predictor of survival and may be helpful to determine the prognosis in individual patients.[3, 41]

Differential diagnosis

PSP is often misdiagnosed in the early phase of the disease. This is reflected by a mean interval of 4 years between onset and time of correct diagnosis, often because ophtalmoplegia is lacking in this stage.[29, 42] PD or unspecified parkinsonism, balance disorder, cerebrovascular disease and dementia are the most common misdiagnoses.[25] On the other hand, in approximately 80 percent, pathological examination confirms the clinical diagnosis PSP established over the course of the disease.[27, 43] Vertical gaze palsy occasionally occurring in PD, multiple system atrophy (MSA), corticobasal degeneration (CBD) and dementia with Lewy Bodies (LBD), may have misled the clinician in false-positive cases.[27, 44-46] Usually, there is an isolated upward gaze limitation in these disorders (or even in normal aging) and therefore downward gaze palsy may be more discriminative for PSP. Tremor, psychosis, dementia and asymmetry are supportive findings against the diagnosis PSP.[43] Also, drug induced dyskinesia, late autonomic dysfunction and visual hallucinations are more supportive of PD, LBD or MSA than for PSP-P.[47] Patients with MSA are usually younger, commonly show signs of severe autonomic dysfunction and develop falls, unintelligible speech and cognitive impairment later in the disease course than in PSP.[40] Vascular Parkinsonism is characterized by more asymmetric signs, lower body involvement and a later occurrence of falls.[48-49] Differentiating PSP from CBD can be very challenging, as both disorders show considerable overlap in clinical features suggesting that both disorders represent different points of single disease spectrum. In a few published case series, PSP may present with CBS, including asymmetrical features, apraxia and alien limb phenomena (PSP-CBS subtype).[50] Finally, PSP (and CBD) may present with a progressive apraxia of speech, nonfluent aphasia (PNFA), or a combination of these.[51-52] It has been suggested as a new variant within the clinical spectrum of PSP, designated as PSP-PNFA (Table 2).

PSP- Subtypes	Clinical presentation
Richardson's syndrome	Early falls and postural instability; early vertical gaze palsy; early cognitive decline
PSP-parkinsonism	Asymmetric onset; levodopa response; tremor
PSP-PAGF	Gradual onset of freezing of gait or speech; no tremor; no sustained response to levodopa; and no dementia, rigidity and ophthalmoplegia in the first 5 years of disease.
PSP-PNFA	Difficulty with speech production (progressive apraxia of speech, nonfluent aphasia (PNFA), or a combination of these)
PSP-CBS	Asymmetrical features; apraxia; alien limb phenomena
PSP-FTD	Early cognitive and behavioural symptoms

Table 2. Clinical subtypes of PSP

Investigations

Characteristic neuroimaging features may improve the diagnostic accuracy in individual PSP patients, although visual interpretations are highly influenced by radiological expertise. Prominent midbrain atrophy is often present in PSP and is visualized as a "penguin" or "hummingbird" sign on midsagittal MRI[53-55], and "morning glory sign" on axial MRI,[56] although this feature may also be seen in MSA.[57] A significantly smaller anterior-posterior midbrain diameter measured in axial view has been found in some, but not all studies. [55, 58-59] Other quantitative studies of the midbrain include two- or three dimensional measurements,[60-61] which can be useful to rapidly differentiate PSP from other parkinsonian syndromes and to follow up disease progression.[61-63] MRI may also show atrophy of the superior cerebellar peduncle (SCP) in PSP, although its measurement has shown overlap with MSA.[60, 63-64] Recently, a so-called MR parkinsonism index has been proposed that combines measurements of structures mainly involved in PSP (midbrain and SCP) and MSA (pons and MCP), and could accurately differentiate PSP from PD and MSA.[60] The diagnostic value of diffusion-weighted MRI is relatively limited, as apparent diffusion coefficient (ADC) in basal ganglia has found to be higher in PSP compared to Parkinson's disease in some,[65-66] but not all studies.[67] In contrast, the superior cerebellar peduncle has shown higher ADC values in PSP than in PD and MSA, which indicates that demyelination and gliosis occur early in the course of the disease.[59] Finally, lower volumes of frontal cortex and subcortical nuclei in PSP patients have been correlated with executive deficits.[68-70]

Hypometabolism of the brainstem and anterior cingulate cortex (ACC) on PET scan is a disease-specific pattern of PSP with a high sensitivity and specificity,[71-72] and is in accordance with regional neuropathological changes. An interesting question is whether specific loss in neurotransmitter receptors accompanies this pattern of hypometabolism in ACC, as has been demonstrated in the neocortex of PD patients. PET scanning with an *in vivo* marker of peripheral benzodiazepine site expression (the radioactive ligand [^{11}C] PK11195), has visualized activated microglia in brainstem, cerebellum, basal ganglia, and frontal cortex, probably reflecting the glial response to the degenerative process.[73] However, this ligand causes a great amount of non-specific binding with considerable variation across individual patients. [74]

Reduced binding of striatal pre-synaptic dopamine transporters (DAT) is found in several parkinsonian disorders, including PSP, but is not helpful in differentiating them.[75] There is some evidence that statistical parametric mapping applied to [(123) I]beta-CIT SPECT in midbrain can enhance differentiation between PD and atypical parkinsonism.[76] Reduction of postsynaptic D2 receptors is suggestive for MSA or PSP, but cannot discriminate between them, whereas a normal postsynaptic D2 receptor status cannot exclude atypical parkinsonism.[77] Moreover, some late stage PD patients can show low striatal postsynaptic radiotracer binding as well.[78]

Iodine-123-meta-iodobenzylguanidine ([^{123}I]-MIBG) is a radio-iodinated analogue of norepinephrine and used to visualize the myocardial sympathic nerve terminals. The uptake is significantly lower in PD patients compared to PSP patients.[79] However, in a more recent study, nearly 70% of the patients without PD (including 7 PSP patients) had decreased uptake, with considerable overlap between PD patients, indicating that MIBG cannot necessarily distinguish PD from PSP patients.[80] Mitochondrial dysfunction in the pathophysiology has been suggested by the observation that high-energy metabolites on phosphorus MR spectroscopy are significantly reduced in basal ganglia and frontal cortex of patients with early-stage PSP.[81] This mitochondrial role is consistent with experimental studies with annonacin, which is linked to a PSP-like syndrome on Guadeloupe.[82]

A potential biomarker to improve the diagnostic accuracy of PSP may be the quantitative analysis of cerebrospinal fluid on tau products or isoforms. Although total and phospho-tau levels in PSP have proven to be similar to controls,[83] Borroni et al. have recently developed an immunoprecipitation assay recognizing proteolytic tau products and has found a significantly lower ratio (33kDa/55 kDa) in the CSF of PSP patients compared to that of other neurodegenerative disorders, like AD, FTD and MSA.[5] However, a recent study was not able to confirm the presence of these tau forms in CSF.[84]

Sandwich ELISAs for quantification of three-repeat and four-repeat tau isoforms have recently been developed, and have successfully shown increased 4R-tau in brain homogenates from frontal cortex and caudate nucleus of PSP brains.[85] The next step will be to use these assays to study tau isoform changes in CSF.

Neuropathology

In contrast to PD, LBD and MSA where the accumulation of alpha-synuclein is the prominent neuropathological feature, PSP belongs to the "tauopathies": a group of neurodegenerative disorders characterized by aggregates of hyperphosforylated tau protein. Globoid neurofibrillary tangels (NFTs), neuropil threads (NT), tufted astrocytes (TA) and oligodendroglial coiled bodies (CB) can be visualized with antibodies against tau and are found in basal ganglia, diencephalon and brainstem.[86] The insoluble aggregates of tau protein in PSP are made up of ultramicroscopic straight filaments in contrast to the paired helical filaments seen in AD. The severity of tau pathology varies considerably between different brain regions and between individual cases, and cortical tau pathology has been associated with cognitive impairment.[87] The subthalamic nucleus, globus pallidus and substantia nigra are the most severely affected brain regions. The motor cortex and anterior cingulate cortex are often involved with variable neuron loss and NFTs, whereas parietal and temporal cortex shows no neuron loss and only sparse NFTs. The presence of TA, commonly found in motor cortex and striatum, is highly specific for PSP and may represent a central degenerative process rather than a reactive change to gliosis.[88] Spinal cord may also be involved in the disease process, although this structure has not been routinely investigated.[89-90] The overall tau lesion severity in all brain regions has been significantly correlated to the CB + NT score in substantia nigra, caudate and dentate nuclei using a five-point grading system.[7] Interestingly, this PSP-tau score has shown a negative correlation with disease duration.[7, 41]

Through alternative splicing of the *MAPT* gene, six tau isoforms are generated. The in-or exclusion of exon 10 results in tau isoforms with four repeat (4R) or three repeat (3R) mictrotubule binding sites respectively. In normal situation, the level of 3R and 4R tau is equal, whereas in PSP there is an increased 4R/3R ratio. The concept of PSP as a 4R tauopathy has been confirmed by immunoblotting, where abnormal insoluble tau migrates as two bands (68 and 64kDa, which comprise 4R tau),[91] and positive immunohistochemical staining of tau aggregates with specific antibodies against 4R tau isoforms, and negative staining with antibodies against 3R tau isoforms.[92] Under normal conditions, tau is bound to microtubules and regulates the assembly and stabilization of microtubules which is essential for intraneuronal vesicle and organelle transport.[93] Unbound phosphorylated tau (particularly 4R tau)

has a tendency to aggregate, causing a toxic gain of function. Furthermore, the lack of tau to stabilize the microtubules leads to loss of the physiological function of microtubules.

Recent studies have shown differences in pathological severity in clinical subtypes in PSP. PSP-P showed relatively more 3R tau isoforms in the insoluble tau fraction and significantly less tau burden compared to Richardson's syndrome. In atypical PSP syndromes as PSP-PNFA, PSP-CBS and PSP-FTD, greater tau pathology in the cortical areas are found (cortical predominat atypical PSP), while PSP-P and PSP-PAGF show more tau burden in the globus pallidus, diencephalon and brainstem (brainstem predominat atypical PSP). [94]

Cholinergic deficits are thought be responsible for motor and cognitive symptoms in PSP, which is confirmed by the observation of reduced cholinergic receptors (M2 and M4 receptors) in the posterior striatum and thalamus,[95-96] but normal cholinergic receptor density in the frontal cortex.[97]

Concurrent pathologies in PSP have been reported and include AD,[98]Lewy bodies[99], argyrophilic grain disease[100] and CBD[101-103] and is thought to occur independently of PSP pathology. Their clinical relevance has been difficult to determine due to the limited number of cases. Increased age, female sex and apoe ε4 carrier status are risk factors for AD pathology in PSP.

Genetics

The involvement of *tau* in the pathogenesis of PSP is further supported by results from genetic studies. The initial association of PSP with the dinucleotide repeat (A0) in intron 9 of *MAPT* has been subsequently extended to other polymorfisms in linkage disequilibrium with the A0 polymorfism.[104-106] The high degree of linkage disequilibrium in the *MAPT* region is thought to result from an inversion of 900 kb occurring 3 million years ago, producing the two extended *MAPT* haplotypes H1 and H2.[107] Both the H1 haplotype (including the A0 polymorfism) and the H1/H1 genotype are found significantly more often in PSP patients.[106-108] Fine-mapping of this region has revealed a subhaplotype (H1c) with a variation in intron 0 of *tau*, which seems to influence the expression of tau.[109] Very recently, another subhaplotype including a variant 5' upstream of *MAPT* and *CRHR1* genes has been associated with an earlier age at onset and its location suggests a *cis* element regulating gene expression.[110]A single genome wide association study has found a second major locus on chromosome 11 containing several interesting candidate genes,[111] but this has to be replicated by other groups. An interesting finding from a recent genome wide association study in PD patients, revealed besides an association in the gene encoding alpha-synuclein, a second locus with strong association at the *MAPT*

locus.[112] These data suggest a link between molecular pathways between both disorders.

Mutations in *MAPT* are commonly associated with frontotemporal dementia with parkinsonsim linked to chromosome 17 (FTDP-17). In some families, the clinical phenotype is consistent with PSP including supranuclear gaze palsy, saccadic eye movements and axial rigidity, although age at onset is usually much younger than classical PSP.[113-117] Screening of large cohorts of sporadic and familial PSP cases, however, do not reveal these mutations.[118] In two series, ~7% of all PSP patients fulfilled the criteria for an autosomal dominant mode of transmission.[4, 119] The phenotype varied among PSP, dementia, tremor, and parkinsonism within these pedigrees. For one large family with an autosomal dominant form of PSP, linkage to the chromosome 1q31.1 region has been found and awaits identification of the causal gene defect.[120] Pathological examination in one affected family member within this pedigree confirmed the clinical diagnosis PSP, but the occurrence of action or postural tremor with facial tics or synkinesias in others, suggests independent segregation from the PSP phenotype. Familial clustering has also been described in several other studies, sometimes with pathological confirmation of PSP in affected relatives, but most of these families were too small for linkage analysis.

Management

To date, no effective therapy to delay or stop the progression of PSP is available. Levodopa may have a moderate, but transient response and it is worthwhile to attempt in the early stages. Amitriptyline has shown a beneficial effect on motor and bulbar problems in a few case reports.[121] Neurotransmitter replacement approaches are unsuccessful in PSP,[122] possibly due to the widespread neuronal loss. Riluzole has also proven to be unsuccessful as a disease-modifying agent in a recent multicenter double-blind randomized placebo-controlled trial.[123]

Mitochondria are the major source for the generation of reactie oxygen species and several studies provide evidence for mitochondrial dysfunction in PSP.[93] Imaging studies with proton en phosphorus MR spectroscopy showed decreased concentrations of high energy phosphates in basal ganglia and frontal lobes, which was unlikely due to neuronal death only. Furthermore, oxidative stress and reactive oxygen species activate tau kinases which causes tau to hyperphospforylate and aggerate more easily. Finally, annonacin (the toxic substance associated with PSP on Gouadeloupe) inhibits complex 1 which reduces ATP levels and induces tau redistribution from the axons to the cell body and leads to cell death. A recent phase II clinical trial with Coenzyme Q10, a physiological cofactor of complex I, showed significant improvement of cerebral energy metabolism and mild clinical

improvement in the short term.[8] However, more research is required to confirm these findings and to investigate long term effects. Results from other clinical studies with tau-kinase inhibitors, tau-aggregation inhibitors and microtubule stabilizers like davunetide will be awaited in the near future.
Relief of symptoms with palliative therapies remains the keystone of disease management and includes different aids (shoes with heels, weighted walker, angled glasses) and forms of rehabilitation programs for balance, gait, speech, swallowing and vision problems. In later stages however, insertion of PEG may be necessary. Botulinum toxin injections may lead to functional improvement in all forms of dystonia, especially blepharospasm.[124] The burden for caregivers is related to the disease severity and disability; this increases during the first 18 months after diagnosis and then stabilizes.[125] Psycho-educational programmes and supportive care can help lighten the burden for caregivers.

Future research
Over the last decade, clinical advancements have been achieved by refining the clinical spectrum of PSP into different subtypes. In this context, it is an intriguing question whether these subtypes represent pathophysiological hetereogeneity or only reflect the effect of a modulating factor. One of the challenges in the field of PSP research will be to develop biomarkers to establish the diagnosis of clinically typical and atypical PSP during life. A considerable number of PSP cases present with non-classical symptoms and for future trials, it is important to identify these patients. A second challenge will be to identify genetic networks involved in PSP, starting from the coming results of a genome-wide association study. For AD and PD, recent studies have demonstrated the early synaptic changes in mouse models of presenilin 1- , Pink1 gene mutations and overexpression of alpha-synuclein.[126-128] As PSP lacks a transgenic mouse model, an alternative approach might be to carry out proteomics of the synaptosome on fresh-frozen brain samples of patients died from PSP. Finally, the identification of annonacin as toxic agent in a PSP-like disorder may give further impetus to research on the possible role of mitochondrial dysfunction in PSP pathophysiology. Trials with Coenzyme Q10 and davunetide are hopefully the first steps in the strategy to delay the progression of this devastating and disabling disease.

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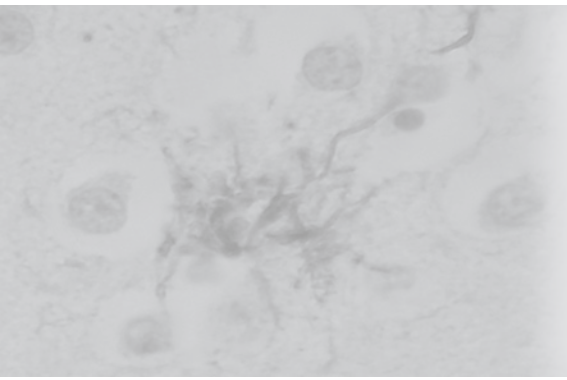

Chapter 2

Clinical heterogeneity

Chapter 2.1

Frontal presentation in Progressive Supranuclear Palsy

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Abstract

Background: Progressive Supranuclear Palsy (PSP) is a progressive hypokinetic rigid disorder with supranuclear gaze palsy and frequent falls. Although clinical consensus criteria are available, an atypical presentation may lead to clinical misdiagnosis in the initial phase. In the present study we investigated the clinical presentation of PSP and its relationship to initial clinical diagnosis and survival.
Methods: We ascertained PSP patients in a prospective cohort by nation-wide referral from neurologists and nursing home physicians. All patients underwent a structural interview and clinical examination before entering the study. Medical records were reviewed for the presence of symptoms during the first two years.
Results: 152 patients ascertained between 2002 and 2005 fulfilled the international consensus criteria for PSP. Categorical principal component analysis of clinical symptoms within the first two years showed apart from a cluster of typical PSP symptoms, the clustering of cognitive dysfunction and behavioural changes. Further analysis showed that 20 percent of patients had a predominant frontal presentation with less than two typical PSP symptoms. Survival analysis showed that this subgroup had a similar prognosis to that of the total group of PSP patients.
Conclusions: There exists a subgroup of PSP patients with a predominant frontal presentation, who progressed into typical PSP over the course of the disease.

Introduction

Progressive Supranuclear Palsy (PSP) is clinically characterized by parkinsonism, supranuclear gaze palsy, and cognitive decline.[1-2] Globose neurofibrillary tangles, tufted astrocytes and coiled bodies in basal ganglia and brainstem are characteristic for PSP.[3-5] Although most patients are sporadic, several studies showed a significant association with H1 *tau* haplotype.[6] To improve the diagnostic accuracy during life, international clinical consensus criteria have been established, including frequent falls in the first year and vertical supranuclear gaze palsy.[2] Despite these criteria, considerable clinical heterogeneity in PSP has proven to result into an incorrect initial diagnosis in 70 percent and into a misdiagnosis at final visit before death in 20 percent.[7-9] A distinct clinical phenotype called *PSP-Parkinsonism* is characterized by asymmetric onset, tremor, levodopa response and longer disease duration.[7] At the other end, behavioural changes and impaired executive functions frequently occur in PSP patients,[9-11] which may show considerable clinical overlap with frontotemporal dementia (FTD).[12]

In the present study we investigate the initial clinical symptoms in a large prospective cohort of clinically diagnosed PSP patients in The Netherlands, to determine clinical profiles and their relationship to the initial diagnosis and survival.

Material and methods

Study design and diagnosis

Between 2002 and 2005 all hospital-based neurologists (n=520) and physicians in psychogeriatric hospitals or nursing homes (n=1154) received an annual postal enquiry to refer patients whom they suspected of PSP. Clinical history of each patient was obtained from both the patient and independently from a close relative or caregiver. All patients were examined at least once by either the research physician (LDK) or by a neurologist (AB and JvS), and videotaped according to a standardized protocol consisting of the examination of eye movements, speech, limb movements, postural reflexes and gait. The diagnosis was made according to the National Institute for Neurological Diseases and Stroke-Society for PSP (NINDS-SPSP) criteria.[2] In a consensus meeting medical records, including available neuropsychological evaluation, videotapes and neuroimaging of all suspected PSP patients were reviewed and the clinical diagnosis of each patient was made. The study was approved by the Medical Ethics Committee of the Erasmus University Medical Centre Rotterdam and all patients or first-degree relatives signed informed consent.

Assessment of clinical variables

The age at onset was defined as the age at which the first symptoms attributable to PSP appeared according to the patient's caregiver at ascertainment and from medical records. In case of discrepancies, data from medical records were used. Information on the clinical symptoms (including behavioural changes and cognitive decline) present in the first two years after onset was collected by reviewing medical records as described previously.[7] The following symptoms and clinical signs were recorded: falls, bradykinesia or motor slowing, tremor, vertical supranuclear gaze palsy, other visual symptoms not explained by gaze palsy, limb rigidity, dysphagia or any swallowing abnormality, speech disturbances or any alteration of speech quality, evident left-right asymmetry, extra-axial dystonia, pyramidal signs, cerebellar signs, cortical sensory loss and dyskinesia. These symptoms were considered absent if not mentioned in the clinical notes. Behavioural changes were defined as an alteration in behaviour (apathy, disinhibition or aggressiveness), which distinctively differed from patient's premorbide character (excluding affective disorders). Cognitive functioning was considered impaired if any decline (loss of concentration, mental slowing or forgetfulness) was reported by the patient, close relative or doctor. Impaired postural reflexes, impaired saccadic or pursuit movement and autonomic dysfunction were considered as present if deliberately mentioned in the clinical notes. The response to levodopa therapy at any time during the disease was recorded as absent or present. At ascertainment, cognitive functioning of patients was assessed by the Mini-Mental State Examination (MMSE). The recording of Frontal Assessment Battery (FAB) was introduced after the inclusion of the first 33 patients in the study, and was carried out in all consecutive patients.[13] The three clap test (or applause sign) was used as a simple test of motor control.[14] In this test the patient is asked to clap three times as quickly as possible. It is considered abnormal when he or she claps more then three times and discriminates PSP from PD and FTD. Disease severity was assessed by the Unified Parkinson Disease Rating Scale (UPDRS) III. Neuropsychological test results were obtained either by neuropsychological evaluation at our out-patient clinic or from reviewing medical records. Five cognitive domains were assessed in each patient: 1) language and speech 2) attention and concentration 3) memory 4) executive functions, and 5) visuoperception and construction. Family history was defined positive if at least one first degree relative suffered from parkinsonism, dementia or amyotrophic lateral sclerosis (ALS). Imaging of the brain was carried out in every patient by either CT or MR.

Statistical analyses

Software package SPSS (version 11.0) was used for the statistical analysis. The method of multiple regression analysis was used to identify to which degree the FAB scores could be estimated by the following determinants simultaneously: UPDRS-III scores, age at ascertainment, MMSE and disease duration.

Clustering of symptoms was performed by Categorical Principal Component Analysis (CATPCA). This analysis enables reduction of an original set of variables into a smaller set of components that represents the relationship of the original variables. Missing values were imputed by "mode" strategy. Only those symptoms which were present in more than 5% of the patients were entered in the analysis, otherwise the structure to be identified would be unstable.

Survival analysis was performed using the Cox proportional hazard model. Entry date was set as time of first symptoms. Censoring date was either date of death or date of end of follow-up (either last contact or end of study-date: November 23, 2005). The assumption of proportionality of hazards was examined by Log-Log plots. Age at onset was categorized into four groups: ≤ 62 years (n=38), 63-66 years (n=38), 67-72 years (n=37) and >72 years (n=39). Hazard ratios (HR) and 95% Confidence Intervals (CI) were calculated for clinical features adjusted for age and gender.

In addition, data were analysed by independent sample t-test or Chi-squared test. When appropriate, non-parametric tests were used. Correlation between variables was tested with the method of multiple linear regression. All statistical testing took place at a 0.05 level of significance (two-tailed).

Results

Demographic and clinical data

Of the 182 referred patients, 152 patients (81 men, 71 women) fulfilled the clinical diagnostic criteria of PSP. In the remaining 30 patients the diagnosis was Lewy body dementia (n=7), corticobasal degeneration (n=4), Parkinson's disease (n=3), multiple system atrophy (n=3), vascular parkinsonism (n=3) and undetermined (n=10). The clinical diagnosis was probable PSP in 80 and possible PSP in 57. Autopsy carried out in 15 patients confirmed the clinical diagnosis PSP in all, with concomitant Lewy bodies in the amygdala, substantia nigra and locus coeruleus in two patients. The most common diagnoses at first neurological visit were Parkinson's disease (29%), PSP (26%), and dementia (16%). The remaining group consisted of: neuropsychiatric diagnosis (6%), undetermined (12%) and a miscellaneous group of diagnoses (11%). A total of 125 patients entered the study after the first two years of the disease, whereas 27 patients were ascertained within the initial phase of two years. Demographic characteristics are summarized in table 1.

Features	Mean ± SD (range) or n (%)
Age at onset, y	66.8 ± 7.9 (46-91)
M/F	81/ 71
Duration disease at ascertainment, y	5.4 ± 2.7 (1-15)
Deceased (n=76)	
duration disease, y	6.9 ± 2.7 (2-16)
age at death, y	74.8 ± 7.9 (49-91)
Latency to diagnosis, y	3.9 ± 2.4 (0-14)
Presence of falls	147 (97)
< first year	96 (63)
> first year	51 (34)
Presence of vertical supranuclear gaze palsy	146 (96)
Latency to gaze palsy, y	3.9 ± 2.5 (0-14)
Positive family history	47 (31)
FAB (n=85)	9.7 ± 3.4 (3-17)
MMSE (n=118)	24.3 ± 4.2 (11-30)
UPDRS-III (n=136)	42.4 ± 18.4 (7-90)
Hoehn & Yahr stage at ascertainment	
II	2 (1)
III	21 (15)
IV	34 (23)
V	88 (61)

Table 1. Demographics and clinical features at ascertainment of 152 PSP patients. Missing data of FAB scores in first 33 ascertained patients. Recording of FAB and MMSE not possible in 34 patients due to severe disease disability. Missing data due to incomplete assessment of UPDRS-III (n=16) and Hoehn and Yahr (n=7).
FAB = Frontal Assessment Battery, MMSE = Mini-Mental Sate Examination, UPDRS = Unified Parkinson Disease Rating Scale, PSP = progressive supranuclear palsy.

The latency to supranuclear gaze palsy correlated with the latency to diagnosis of PSP (Pearson correlation 0.95, $p<0.001$). Scores on FAB questionnaire were obtained from 85 consecutive patients, whereas in 34 patients recording was impossible due to severe disease disability. The mean total score was 9.7 (maximum score = 18), with a score < 15 in 76 (89%) patients. Patients performed worst on Word Fluency (normal score > 9 words), with less then three words in a 1-minute trial in 50% and less then five words in 80% of all tested patients. Multiple regression analysis showed a correlation between the FAB scores and UPDRS-III scores (ß = -0.09, $p = 0.004$), age at onset (ß = -0.25, $p = 0.02$) and MMSE (ß = 0.19, $p=0.05$), but not with disease duration (ß = 0.19, $p=0.12$). Presenting symptoms in patients with higher scores (FAB > 10) did not differ from those in patients with lower scores (FAB < 10). An "applause sign" was present in 71 out of 98 patients (72%), and patients showing this sign performed

worse on FAB scores (independent t-test 8.6 versus 10.7, p=0.013) but not on MMSE (Mann-Whitney, p=0.42).

Fifty-seven of the 67 patients (85%) who underwent formal neuropsychological testing showed cognitive deficits, whereas cognitive evaluation was normal in the remaining 10 patients. The presence of cognitive deficits was not related to the duration of illness at testing. Mental slowing (77%), memory disturbances (63%), executive dysfunctions (88%) and changes in personality (77%) were the most common features in those patients with abnormal cognitive evaluation.

Symptom clustering and clinical subtypes

Data about symptoms within the first two years were analysed in 141 patients, whereas 11 patients were excluded from this analysis because of insufficient information as the first visit occurred after the first two years. Table 2 summarizes the frequencies of the symptoms in the three most common initial diagnoses.

	Initial diagnosis			
Clinical symptoms	PSP (n=31)	Dementia (n=21)	Parkinson (n=40)	p-value
Falls	90	71	56	<0.01
Bradykinesia	84	55	75	ns
Speech alterations	71	32	54	<0.05
Cognitive decline	65	81	25	<0.01
Behavioural changes	53	90	25	<0.01
Limb rigidity	63	9	67	<0.01
Vertical supranuclear gaze palsy	90	17	16	<0.01
Visual disturbances	48	21	15	<0.01
Tremor	19	14	38	ns
Dysphagia	32	10	12	ns
Asymmetry	19	5	24	ns
Pyramidal syndrome	33	5	8	<0.01
Urge-incontinence	35	16	5	<0.01
Levodopa response	29	17	31	ns

Table 2. Frequencies of clinical symptoms present within the first two years of the disease in three groups with most common initial diagnoses (n=92). P-values based on Chi-squared comparisons. PSP = progressive supranuclear palsy, ns = not significant.

The presence of behavioural changes and cognitive decline was supported by aberrant neuropsychological test results in 93% and 89% of tested patients. The relationship between the symptoms is visualized by the loading plot from the CATPCA, as shown in figure 1. Two individual clusters were identified: 1. behavioural changes and cognitive dysfunction clustered together; 2. parkinsonian symptoms including asymmetry, tremor, levodopa response and rigidity clustered together.

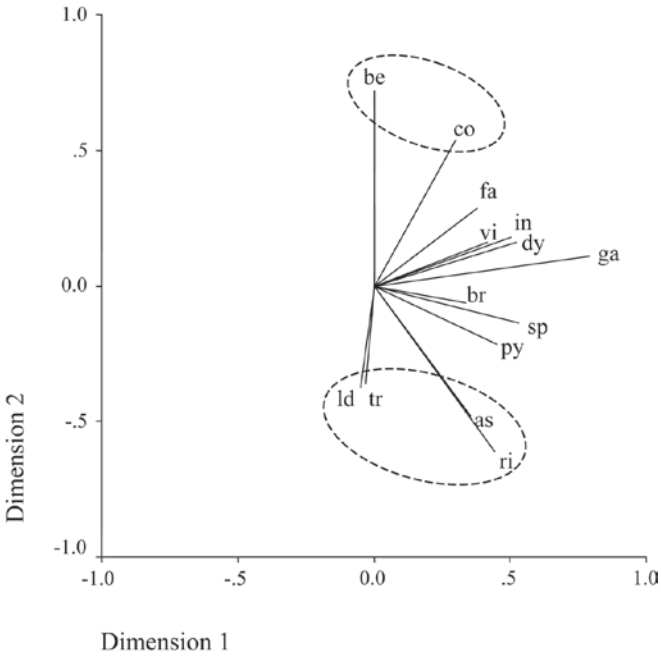

Figure 1. Loading plot from Categorical Principal Components (CATPCA). ri= rigidity, br=bradykinesia, fa=falls, sp=speech problems, ga=vertical supranuclear gaze palsy, co=cognitive decline, be= behavioural changes, vi=visual disturbances, dy= dysphagia, in= urge incontinence, py=pyramidal syndrome, as=asymmetry, tr=tremor, ld= levodopa response.

Dyskinesia, cortical sensory loss, dystonia and cerebellar signs were infrequent (present in < 3 percent) and therefore excluded from this analysis, whereas slowed saccades, postural instability and autonomic failure had many (>60%) missing values, making their analysis unreliable. Instead of autonomic failure, we scored the absence or presence of urge-incontinence.

Twenty percent of PSP-patients (n=28) presented with behavioral changes and/ or cognitive dysfunction, and less than two other symptoms. This group had a younger age at onset than in the total cohort (Table 3).

	Frontal subgroup (n=28)	Other PSP patients (n=113)	P value
Gender (% male)	64	53	0.29
Age at onset (yrs)	64.1 ± 6.1	67.7 ± 8.2	0.01
Duration at ascertainment (yrs)	6.2 ± 1.9	5.3 ± 2.7	0.10
Latency to diagnosis (yrs)	4.9 ± 1.9	3.7 ± 2.5	0.02
Deceased n	17	53	
Disease duration	7.1 ± 1.6	6.8 ± 2.9	0.69
Initial diagnosis n (%)			
- PSP	2 (7)	31 (27)	<0.001
- PD	3 (11)	40 (35)	
- Dementia*	13 (46)	11 (10)	
- Neuropsychiatric disorder	6 (21)	3 (3)	
- Other	4 (14)	28 (25)	

Table 3. Demographics and initial diagnosis of PSP-patients according to the clinical profile in the first two years. Frontal subgroup = behavioral changes or cognitive decline with ≤ 2 other symptoms. PSP = progressive supranuclear palsy; PD= Parkinson's disease. * frontotemporal dementia in 9 patients from frontal subgroup.

Presenting symptoms were behavioral changes (96%), cognitive dysfunction (71%) or both, accompanied by falls (68%), bradykinesia (36%) or tremor (17%). Other symptoms were infrequent (<5%). The most common initial misdiagnosis was dementia, in particular FTD (n=9). The percentage of positive family history did not differ between the frontal and non-frontal group. Neuropsychological testing was performed in 19 (68%) patients with frontal presentation which was more frequent than the total cohort (p=0.01). Executive dysfunction (95%), personality changes (90%), reduced mental speed (84%) and attention deficits (79%) were the most frequent findings, whereas memory problems (53%), language problems (37%) and visuospatial dysfunction (37%) were less frequent. Of the remaining 9 patients without neuropsychological testing, the presence of cognitive decline and behavioural changes was based on the evaluation of neurologists obtained form medical records and supplemented by reportment of family-members.

Survival

Sixty-five patients had died by the end of the follow-up period, whereas 71 were still alive; five patients were lost to follow-up. The mean follow-up duration of the total group in the study was 6.6 years, with disease duration of 6.8 years in the deceased group. None of the patients died within two years of symptom onset. Survival analysis on different clinical features within the first two years showed an increased mortality risk for urge-incontinence (HR 4.40, 95% CI 2.00 to 9.68), vertical

supranuclear gaze palsy (HR 2.74, 95% CI 1.52 to 4.94), dysphagia (HR 2.84, 95% CI 1.51 to 5.34), falls (HR 2.34, 95% CI 1.17 to 4.68) and cognitive decline (HR 1.99, 95% CI 1.17 to 3.38), all adjusted for sex and age at onset. Older age at onset was only associated with poorer survival in patients with age at onset > 72 years. Survival in the group with frontal presentation (described above) did not significantly differ from the group with non-frontal presentation (median survival time 7.9 versus 8.2 years).

Discussion

The present study shows that the 20 percent of PSP patients had predominant behavioural and cognitive presentation, often resulting into an incorrect initial diagnosis of dementia. Patients with this presentation were younger at onset, but did not differ in their disease progression into typical PSP. An old age at onset, the early presence of supranuclear gaze palsy, urge incontinence, dysphagia, as well as cognitive decline and falls were all associated with a reduced survival.

The clinical presentation of predominant behavioural or cognitive changes in 20 percent of the present cohort of PSP patients confirms the findings in previous studies. However, these studies reported that motor signs usually preceded the neuropsychiatric features.[10-11, 15] Interestingly, in the original paper of Steele, Richardson and Olszewski, several patients presented with behavioural and personality changes.[1] Most of the present patients with predominant behavioural and cognitive changes showed a clinical presentation similar to FTD. This probably reflects the early damage of striato-frontal pathways due to basal ganglia pathology, or the direct prefrontal cortical involvement.[16] Other arguments for the involvement of the frontal cortex and striato-frontal connections in PSP are the high frequency of the applause sign (71%) and low FAB scores in the present cohort.[13, 17] The observed worse performance in the verbal fluency in the present study emphasizes the importance of this sign, which may differentiate PSP from MSA and PD. Two recent studies showed that the severity of frontal atrophy in PSP patients correlated with the degree of executive dysfunction in PSP patients.[18-19] Another interesting finding is the correlation between the FAB-and UPDRS-scores in our study. This is in line with observed association between behavioural changes and increased motor disability in one study,[20] and between decline in FAB scores and increased midbrain atrophy in another study.[18] It suggests that in PSP behavioural and cognitive changes occur in parallel with motor impairment. Why PSP patients with frontal presentation have an earlier age at onset is unclear. Future studies are needed to confirm our observations and additional data on educational level may clarify this issue.

A correct initial diagnosis PSP found in only 26 percent of the present cohort is similar to that found in other studies,[8-9, 11] which may largely be explained by the absence of supranuclear gaze palsy in the initial phase. It has frequently led to the misdiagnosis of dementia in patients with neuropsychiatric features and to the misdiagnosis of Parkinson 's disease in patients with bradykinesia and rigidity. Josephs et al emphasized in a clinico-pathological study that moderate to severe dementia early in the disease should raise suspicion about the diagnosis PSP.[5] However, patients with this initial misdiagnosis in the present study fulfilled the clinical diagnostic criteria for PSP at ascertainment and eventually did not differ in their disease progression from those without dementia in terms of UPDRS, FAB and MMSE scores. Our observations support the findings of Osaki that the inclusion of frontal lobe signs and personality changes into diagnostic criteria may improve the positive predictive value of PSP.[9] The results from neuropsychological testing should be interpreted with caution since only 43% of the total cohort underwent neuropsychological testing, and results were collected from different centres. Nevertheless, 86% (n=56) showed substantial decline in cognitive functioning, which supports the importance of additional neuropsychological testing in PSP patients and may help to establish the correct diagnosis.

Principal component analysis of the present PSP cohort visualized also the clustering of parkinsonian features, although patients with these symptoms were in much lower frequency (8%) than in a recently described cohort of PSP-patients (23%).[7] As their patients with PSP-parkinsonism were clinically diagnosed as Parkinson's disease even at the last clinical visit, it is quite clear that these patients did not fulfil the clinical criteria of PSP and therefore never could have entered our study.

The observation that PSP patients with frontal presentation did not differ in survival from other PSP patients implies that frontal presentation is not predictive for prognosis. Although a higher percentage of patients with frontal presentation died during follow-up, this can be explained by a longer duration of illness at ascertainment in this group. The reduced survival for vertical gaze palsy, urge-incontinence, dysphagia, falls and cognitive decline in the first two years in the present study is in agreement with other studies, in which the early occurrence of typical PSP symptoms was also associated with a worse prognosis.[11, 15, 21]

One major limitation of our study is a selection bias towards typical cases, as atypical cases without oculomotor signs could not enter into the present study. Moreover, as prevalent PSP-cases were ascertained, this study may be biased towards patients with a longer survival. The assessment of FAB and MMSE in a subset of PSP patients might reflect selection bias as all untestable patients were in the advanced disease stage with Hoehn and Yahr score 5. However, the mean FAB and MMSE scores in our

study are similar to that found in other studies.[13, 17] Another limitation is that there was pathological verification in only 10 percent of patients. However, the NINDS-SPSP criteria show a good positive predicted value for probable PSP (100%) and possible PSP (83%) in patients presenting with parkinsonism,[2] but also in patients presenting with dementia (96% for combined possible and probable PSP).[22] Future pathological studies of the present cohort may help to determine the extent of cortical pathology in patients with and without frontal presentation in PSP. Additionally, PET studies on metabolism of the frontal lobes may elucidate the contribution of the frontal lobes to the clinical phenotype in PSP.

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Chapter 2.2

Clinimetric characterization of patients with progressive supranuclear palsy and comparison to patients with Parkinson's disease

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Submitted

Abstract

Background: Progressive supranuclear palsy (PSP) is an atypical parkinsonism with early falls and vertical gaze palsy. It is frequently misdiagnosed as Parkinson's disease (PD), especially early in the course. Besides the motor symptoms, non-motor symptoms are increasingly being recognized in both disorders. In this study we investigate differences in motor, autonomic and cognitive features in patients with PSP and PD.

Methods: Patients with PSP were ascertained through national wide referral and fulfilled the National Institute for Neurological Diseases and Stroke- Society for Progressive Supranuclear Palsy criteria. The assessment of motor, cognitive and autonomic functioning was performed by standardized rating scales and the results were compared to patients with PD, matched for age, sex and disability.

Results: Patients with PSP showed a significant shorter disease duration at similar disability compared to patients with PD and showed significantly more impairment on the items speech, swallowing and rise from a chair, while significantly less impairment was observed on the items rest and postural tremor, gait and arm rigidity. Furthermore, patients with PSP had significantly more problems with executive and visuospatial tasks. Autonomic dysfunction was more frequent compared to controls, but less compared to patients with PD.

Conclusions: The observed differences in motor, cognitive and autonomic dysfunction between PSP and PD may contribute to the differentiation of both disorders.

Introduction

Progressive supranuclear palsy (PSP) is a progressive hypokinetic-rigid disorder with aggregates of hyperphosphorylated tau protein in basal ganglia and brainstem. In general, PSP is distinguished from Parkinson's disease by the presence of vertical gaze palsy, equilibrium problems with frequent falls, more impaired cognitive function, and a more rapidly progressive course.[1-3] The recent recognition of a parkinsonian subtype in PSP (PSP-P) urges clinicians to examine non-motor domains in search of distinguishing features between PSP and idiopathic PD.[4] Although not extensively investigated, the presence of severe autonomic dysfunction is uncommon in PSP whereas it is an important early feature in MSA.[5-7] In PD, autonomic dysfunction is increasingly recognized as an important component of its clinical spectrum and autonomic symptoms increase with ongoing disease severity, age and medication use.[8] In both PD and PSP, cognitive deficits are common, although the extent and decline is usually greater in PSP patients. Impairment in attention, executive and visuopatial function are specific cognitive deficits seen in PD and PSP.[9]

To assess the various domains affected in hypokinetic rigid disorders, several rating scales have been developed with the Unified Parkinson Disease Rating Scale (UPDRS) as most commonly used. Recently, the SCOPA project (SCales for Outcomes in PArkinson's disease) has developed scales to assess amongst others motor, cognitive and autonomic function in PD. These rating scales have been demonstrated to be reliable and valid instruments to measure functional decline in PD.[10-12] A specific rating scale for PSP patients has recently been developed (PSP-RS) and has shown to be sensitive to disease progression.[13] The aim of the present study was to investigate clinimetric differences in motor, autonomic and cognitive features between PSP patients and PD patients.

Methods

Case ascertainment

PSP patients were ascertained through nation-wide referral from neurologists and nursing home physicians between 2002 and 2007, as described previously.[14] The diagnosis was made according to the National Institute for Neurological Diseases and Stroke-Society for PSP (NINDS-SPSP) criteria.[15] In a consensus meeting (LDK, AB, JvS) medical records, including available neuropsychological evaluation, videotapes and neuroimaging of suspected PSP patients were reviewed and the clinical diagnosis of each patient was established. The study was approved by the Medical Ethics Committee of the Erasmus University Medical Centre Rotterdam and all patients or caregivers signed informed consent. The PSP rating scale (PSP-RS),

the Short Parkinson's Evaluation Scale (SPES/ SCOPA) section motor impairment, Hoehn and Yahr (H&Y) stages and the Schwab and England (SE) disability scale were undertaken in all PSP patients who were included in the study. Additionally, SCOPA-COG and -AUT were administered later during our study period which resulted in a smaller subset of consecutively ascertained PSP patients. When severe dysarthria, anarthria or severe cognitive deterioration was present in PSP patients, the assessment op SCOPA-AUT and SCOPA-COG was not feasible.
The results from the SCOPA scales were compared to PD patients who were randomly selected from the database of more than 400 patients of the SCOPA project. This project has been described previously.[10-12] The PD patients were matched for age, sex and disability (H&Y stage 3 or 4). These patients visited the outpatient clinic of the Department of Neurology of the Leiden University Medical Center between 2003 and 2005, and fulfilled the United Kingdom Parkinson's Disease Society Brain Bank criteria for idiopathic PD. The SCOPA project was approved by the Medical Ethics Committee of the Leiden University Medical Center. Control subjects were selected from the SCOPA-project, matched for age and sex to the PSP patients and were used to compare the results from the SCOPA-AUT. The ascertainment of these controls was part of the SCOPA project and is described elsewhere.[8]

Clinimetric measurements
The PSP-RS comprises 28 items in six categories:[13] daily activities, behaviour, bulbar, ocular motor, limb motor and gait/midline. Scores range from 0 to 100, each item graded 0-2 (six items) or 0-4 (22 items). Motor impairment assessed by the SPES/ SCOPA was used to compare PSP patients and PD patients.[11] This rating scale is a shortened version of the UPDRS, but has a similar content. All items are rated on a 4-point scale, ranging from 0 (no impairment) to 3 (severe). The section includes the following items: rest and postural tremor assessed on both arms, rapid alternating hand movements, limb rigidity of the arms, rise from chair, postural instability, gait, speech, freezing of gait and swallowing. Autonomic dysfunction was assessed with the SCOPA-AUT,[12] a self reported symptoms questionnaire, which consists of the following domains: gastrointestinal, urinary, cardiovascular, thermoregulatory and pupillomotor. The response options grade the frequency of the problem from 0 (never) to 3 (often), with a maximum total score of 63. The domain of sexual dysfunction (2 items) was excluded, as most PSP patients showed considerable disease disability (physical and mental), resulting in missing values or inapplicable answers. Cognitive function was assessed with the SCOPA-COG, which is a bedside test battery with four domains of cognitive functioning, especially developed for PD patients.[10] The domains include memory, attention, executive functioning and

visuospatial functioning. The maximum total score is 43 and higher scores reflect better cognitive functioning. Finally, the MMSE scores were recorded. In order to compare PSP and PD patients at similar disease severity, we restricted the analyses on the SCOPA motor, autonomic and cognitive scores to patients from H&Y stages 3 and 4, because few PSP patients were available in H&Y stage 2 and few PD patients in H&Y stage 5.

Statistical analysis

Software Package of Social Sciences (SPSS) version 16.0 was used. Differences of PSP-RS scores between three H&Y groups within the PSP cohort, were analyzed by ANOVA with post hoc Bonferroni correction. Correlations between PSP-RS, SPES/SCOPA and SE disability scale were analyzed by Pearson correlation coefficient. Differences in frequencies between PSP and PD patients were analyzed using Chi-square distribution. Mean differences were analyzed with independent Students T test and medians with the Mann Whitney U test, when appropriate. Significance level for all tests took place at α=0.05.

Results

PSP cohort

Demographics and (sub)scores of the PSP-RS and SPES/SCOPA of 166 PSP patients (62 possible, 97 probable and 7 definite) according to H&Y stages, are summarized in Table 1.

	H&Y 3 (n=29)	H&Y 4 (n=44)	H&Y 5 (n=93)
Age, y	65.9 (6.9)	72.6 (6.0)*	73.4 (8.3)‡
Disease duration, y	3.9 (1.9)	4.7 (2.3)	6.0 (2.7)*‡
PSP-rating scale (total score)	28.3 (7.1)	39.7 (7.1)*	60.3 (11.4)*‡
daily activities	6.3 (2.6)	8.9 (2.8)*	13.1 (3.6)*‡
mentation	3.6 (2.2)	4.5 (2.1)	6.9 (2.8)*‡
bulbar	1.9 (1.1)	2.4 (1.0)	4.4 (2.0)*‡
ocular motor	7.7 (2.2)	8.2 (2.8)	11.3 (2.8)*‡
limb motor	2.5 (1.5)	3.7 (2.0)	6.8 (3.0)*‡
gait/ midline	6.3 (3.0)	11.9 (2.8)*	17.7 (2.2)*‡
SPES/SCOPA	10.1 (2.7)	14.3 (3.4)*	21.4 (4.4)*‡
SE disability scale	81.0 (7.2)	57.3 (12.3)*	30.7 (14.2)*‡

Table 1. Demographics and rating scale (sub)scores of PSP patients according to H&Y stage. Numbers represent mean (SD).

*Different compared to H&Y 3 with p≤0.01. ‡different compared to H&Y 4, with p≤0.01. H&Y= Hoehn and Yahr. y= years, SE= Schwab and England

According to the results from the PSP-RS, almost all patients (90%) had falls with a frequency of more than once per month. Besides a gaze paresis upward in 87% of the PSP patients, a downward gaze paresis was found in 69% and in 27% also limitation in horizontal movement was present. Mild to moderate blepharospasm was present in 39% and limb dystonia in 16%. Mean score on neck rigidity was significantly higher than limb rigidity ($p<0.001$), with partial or no passive neck movement in 40% of the cases. Withdrawal behaviour was present in 85% and emotional incontinence in 46%. The (sub)scores increased with ongoing disease severity reflected by H&Y stages. Most subscores showed a significant difference between H&Y stage 4 and 5, but not between H&Y stage 3 and 4. A good correlation (Pearson correlation 0.89, $p<0.001$) was observed between the scores of the SPES/SCOPA and PSP-RS, between the PSP-RS and SE disability scale (Pearson correlation -0.89, $p< 0.001$) and between the SPES/SCOPA and SE disability scale (Pearson correlation -0.84, $p<0.001$).

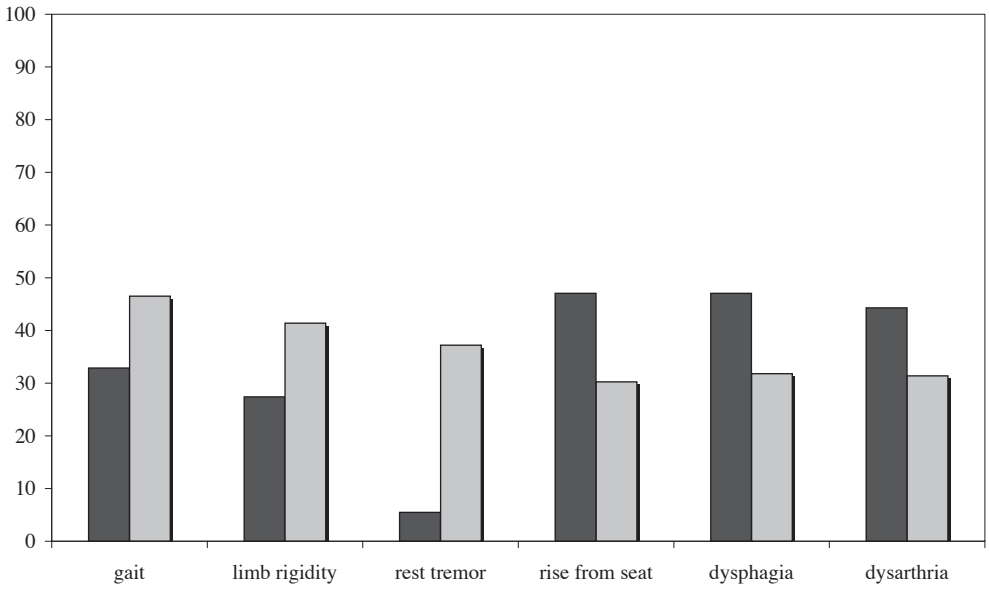

Figure 1. Impairment of motor function in 73 PSP (black) and 87 PD (grey) patients using the SPES/SCOPA at similar H&Y stage. Y axis indicates percentage of impairment (100 indicating maximal impairment). All items show a significant different between PD and PSP patients (Mann Whitney U test $p<0.001$).

PSP versus PD

The results of the SPES/SCOPA of 73 PSP patients in H&Y stages 3 and 4 were compared to 87 PD patients, matched for age, sex and H&Y stage. The disease duration of PSP patients was much shorter in PSP patients compared to PD patients (4.4 versus 13.0 years, p <0.001). Mean total score of SPES/SCOPA was significantly higher in PD patients compared to PSP patients. However, PSP patients showed significantly more impairment on the items speech, swallowing and rise from a chair, while significantly less impairment was observed on the items rest and postural tremor, gait and arm rigidity (Figure 1).

Rapid alternating hand movements, freezing of gait and postural instability did not differ significantly between both groups. Asymmetry in tremor and alternating hand movements was significantly less frequent in PSP patients compared to PD patients. The performances on the SCOPA-COG by PSP (n=25) and PD patients (n=84) are summarized in table 2.

	PD (n=84)	PSP (n=25)	p-value
Age, y	71.9 (8.0)	69.9 (7.5)	ns*
Gender (% male)	43	54	ns
H&Y (3/4)	47/37	13/12	ns
Disease duration, y	12.8 (7.0)	3.8 (1.6)	<0.001*
Mean MMSE	26.1	27.2	0.04
Education, y	11.3 (4.2)	11.4 (3.6)	ns
SCOPA-COG total score	22.1 (6.1)	19.2 (7.2)	ns
Memory subscore	7 (5-10)	7 (4.5-10)	ns
Executive subscore	8 (6-9)	6 (4.5-8)	<0.01
Attention subscore	3 (2-4)	4 (2-4)	ns
Visuospatial subscore	4 (3-5)	3 (1.5-4)	0.01

Table 2. Differences in (sub)scores of SCOPA-COG between PD and PSP patients.
Higher scores indicate less impairment. Numbers represent mean (SD) or median (IQR). P-values based on Mann-Whitney U test or Student T test (*).
H&Y= Hoehn and Yahr, SD=standard deviation, IQR=interquartile range, y= years

Although the mean total SCOPA-COG score did not significantly differ between the two groups, PSP patients had significantly more problems with executive and visuospatial tasks. Especially semantic fluency was significantly more disturbed in PSP patients (Figure 2).

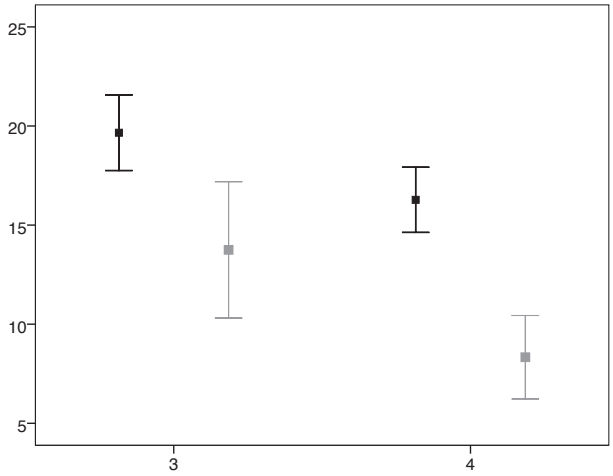

Figure 2. Verbal fluency in PSP (grey) and PD patients (black). Y-axis: mean number of animals produced in a one minute trial. X-axis: Hoehn and Yahr stage 3 and 4. Squares represent mean values, with their 95% confidence interval (vertical lines).

Mean total score on autonomic dysfunction was higher (p<0.01) in PD patients (n=87) compared to PSP patients (n=39), particularly for the gastrointestinal, cardiovascular and urinary domains (Table 3).

	PD (n=87)	PSP (n=39)	p-value
Age, y	72.2 (7.9)	69.7 (7.0)	ns*
Gender (% male)	44	49	ns
H&Y 3/4	47/40	17/22	ns
Disease duration, y	13.0 (6.9)	4.3 (2.3)	<0.001*
SCOPA-AUT total score	19.3 (7.1)	16.0 (8.6)	<0.01
Gastrointestinal subscore	6 (4-7)	4 (2-7)	0.01
Urinary subscore	7 (5-10)	6 (3-9)	0.05
Cardiovascular subscore	2 (0-2)	0 (0-2)	<0.01
Thermoregulation subscore	3 (2-4)	2 (0-4)	ns
Pupillomotor subscore	1 (0-1)	2 (1-3)	<0.01

Table 3. Differences in (sub)scores of SCOPA-AUT between PD and PSP patients.
P-values based on Mann-Whitney U test or Student T test (*). Numbers represent mean (SD) or median (IQR). Higher scores indicate more impairment. H&Y= Hoehn and yahr, SD= standard deviation, IQR= interquartile range, y= years.

Particularly the scores on the following separate questions were significantly higher in PD patients: "early abdominal fullness"($p<0.01$), "food become stuck in throat"($p<0.01$), "constipation" ($p=0.02$), "straining for defecation"($p<0.01$), "incomplete bladder emptying" ($p<0.01$), "weak stream of urine" ($p=0.01$), "light-headed when standing for some time" ($p<0.01$) and "heat intolerance" ($p=0.02$). In contrast, PSP patients showed significant higher median scores on "pupillomotor dysfunction" ($p<0.01$) and "swallowing/choking" ($p<0.001$). The remaining separate items from the questionnaire were higher in the PD group, but did not reach significance.

The results from the SCOPA-AUT of the PSP patients (n=39) were also compared to the results from 63 healthy control subjects, matched for age and sex. This revealed a significant higher total score in the PSP group ($p<0.001$) and also higher scores on the subdomains gastro-intestinal ($p<0.001$), cardiovascular ($p<0.001$), urinary ($p<0.01$) and pupillomotor ($p<0.001$), but not on the domain thermoregulatory ($p=0.11$).

Discussion

In the present comparative study we systematically examined the motor, autonomic and cognitive features with standardized rating scales and demonstrated characteristic differences between PSP and PD at similar disability. PSP patients showed more autonomic dysfunction compared to controls, but less compared to PD patients. Executive and visuospatial functions were significantly more impaired in PSP patients, but memory and attention deficits are similarly affected in PSP and PD.

Recently, a specific rating scale for PSP patient has been developed which can be used as a global measurement of clinical disability and progression. The present study showed a clear increase in PSP-RS scores with ongoing disease severity. The subscore gait/midline, showed a significant increase over all H&Y stages, in contrast to most other subscores with a significant increase between H&Y 4 and 5. This suggests that H&Y stages may less reflect the decline in oculomotor, bulbar, limb and mental functioning in PSP patients. In our population based cohort, all PSP patients except two were in H&Y stage 3 or more. Because many PSP patients present with equilibrium problems and falls, motor disability can already be classified as H&Y stage 3. In the study by Muller, a four times longer latency to H&Y stage 5 in PD patients was found compared to atypical parkinsonian syndromes, including PSP.[16] In their study, the observation of H&Y stage 3 within one year after disease onset, predicted an atypical parkinsonian syndrome with 72% sensitivity. H&Y stages are typically designed for motor progression in PD patients and may be less suitable for

staging PSP patients. It focuses on motor symptoms, while in PSP, the early presence of bulbar and cognitive dysfunction may also lead to serious disability. However, to date there is no other generally accepted scale available to compare different parkinsonian syndromes. In our analyses on motor, autonomic and cognitive items of the SCOPA scales, we chose to compare patients with similar H&Y stage. In this way, the differences in clinical profiles are attributable to the underlying nature of the diseases instead of to the level of impairment.

At similar disease disability, the disease duration in PSP was generally three times shorter compared to PD patients. Consistent with earlier reports, we found less tremor and more bulbar involvement in PSP.[17-19] Although tremor is an infrequent feature in PSP, the identification of a parkinsonisan subtype of PSP (PSP-P) probably underestimates its frequency in PSP. Interestingly, we demonstrate a difference in gait impairment: PD patients showed higher scores on the SPES/SCOPA, indicating more shuffling, slowing and festination in their gait compared to PSP patients. A difference in gait has also been observed by Cordato, who showed in PSP patients a more "rigid", "broad based" and "untidy" gait.[20] Furthermore, the present study showed more limb rigidity in PD compared to PSP patients. A previous study did not find a difference in limb rigidity compared to PD patients,[20] which might be explained by the smaller size of the study population and because these patients were not matched for disease disability, with more PSP patients in advanced H&Y stages. Finally, we found PSP patients to show more problems with arising from a chair, probably resulting from the prominent balance problems often with a backward direction. This is inline with the observation of shorter latency to falls in PSP patients compared to PD.[21]

In contrast to PD, involvement of autonomic dysfunction in PSP is controversial as far as it is investigated. Several functional studies have failed to demonstrate orthostatic hypotension in PSP,[5-6, 22] in contrast to some earlier studies.[23-24] The current study used a self-reported symptom questionnaire, which captures the full spectrum of autonomic dysfuntion and showed more cardiovascular, gastro-intestinal, urinary and pupillomotor dysfunction in PSP patients compared to controls. Only one study used a similar method with a structured questionnaire and could demonstrate autonomic dysfunction in PSP, although no difference was found with PD patients except for a cardiovascular autonomic test. The present study, however, showed less cardiovascular dysfunction in PSP patients compared to PD. A possible explanation might be that our PSP and PD patients were matched for disease disability. In most studies, PSP patients tend to be more disabled compared to PD patients. This may also explain why two previous studies show equal high frequencies of micturitional problems in PD and PSP,[7, 25] while we demonstrate more dysfunction in PD. The

micturition disturbances in PSP might be attributed to central lesions in the rostral brainstem tegmentum, nigrostriatal system, the frontal lobe, and the spinal cord but also to more peripheral lesions with proven pathological changes in Onuf's nucleus.[26-28]

The oversensitivity to bright light was the only autonomic dysfunction more present in PSP patients compared to PD and may suggest an inadequate constriction of the pupil. This is supported in a recent study by significantly smaller pupil diameters after dark adaptation in PSP compared to PD patients, although pupillary light reflexes were similar for both groups.[29] The pathophysiology of this impaired function is not yet clear, but a significant decrease in the number of neurons with immunoreactivitiy for choline acetyl transferase in the nucleus of Edinger Westphal has been reported in PSP patients.[30]

The significant lower scores on semantic fluency in PSP from this study are in line with the findings from previous studies[2-3, 31-32] and may contribute to the diagnosis. Impairment in verbal fluency is related to dysfunction of the frontal lobes directly or by the disruption of striato-frontal projections, which are suggested to be more impaired in PSP patients. Our observation of significant lower scores on visuospatial function in PSP patients compared to PD is supported by other studies.[2, 33] Bak et al. reported visuospatial dysfunction in PSP and patients with corticobasal degeneration, in contrast to normal performances in MSA patients. Because the impairment in the visual memory task from the SCOPA-COG, in which the patient has to remember a correct sequence of squares, did not show a difference between PD and PSP patients, it is unlikely that oculomotor dysfunction rather than cognitive decline is the cause of the visuospatial disturbances. The parietal cortex is suggested to contribute to impaired visuospatial function in CBD, which might also account for PSP patients as pathological changes in the parietal cortex has recently been demonstrated.[34]

MMSE scores did not fully correspond with SCOPA-COG scores, as total scores of the SCOPA-COG did not differ between both groups, whereas a higher mean MMSE score was found in PSP patients. This discrepancy has also been found in the study of Verbaan et al, where normal MMSE score were found in 58% of the PD patients who showed impaired cognition defined by the SCOPA-COG.[9] Thus, the MMSE may substantially underestimate the degree of cognitive impairment. Higher MMSE scores in PSP compared to PD patients were also found in the study of Aarsland, when these patients were matched for overall severity of dementia.[35]

There are a few potential limitations of the present study. First, in the majority of PD and PSP patients there is no pathological confirmation. However, the international criteria for PSP and PD show a good positive predictive value.[15, 36] Secondly,

we administered a motor rating scale, which is typically designed for the motor symptoms of PD patients and therefore lack some of the typical symptoms involved in PSP. Therefore, some interesting clinical features could not be compared between PD and PSP patients. Although the SCOPA-scales have not been validated in PSP patients, the UPDRS (which has a similar content and scoring system) has been shown to be a reliable and applicable scale for PSP patients.[37] Furthermore, there was a good correlation between the SPES/SCOPA and PSP-RS and between the SPES/SCOPA and SE disability scale, which suggests that the SPES/SCOPA is a reliable instrument for rating the severity of most parkinsonian symptoms in PSP. Finally, we did not adjust for co-morbidity, nor for medication use as many antiparkinonian drugs may give autonomic side-effects. Previous studies however suggest that autonomic effects of dopaminergic drugs may be minor.

In conclusion, this systematic study demonstrates several clinical features which differ between PSP and PD when matched for age, sex and disease severity. Compared to PD patients the disease progression is much faster in PSP patients, the fluency is strikingly reduced and there are less self reported autonomic disturbances.

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Chapter 2.3

Survival in progressive supranuclear palsy and frontotemporal dementia

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Abstract

Objective: To compare survival and to identify prognostic predictors for progressive supranuclear palsy and frontotemporal dementia.

Background PSP and FTD are related disorders. Homozygosity for H1 haplotype is associated with PSP, whereas several *MAPT* mutations have been identified in FTLD-tau. Survival duration probably reflects underlying pathophysiology or disease.

Material/design: Patients with PSP and FTD were recruited by nation-wide referral. Survival of 354 FTD patients was compared to that of 197 PSP patients. Cox regression analysis was performed to identify prognostic predictors. FTLD-tau was defined as Pick's disease and FTDP-17 with *MAPT* mutations. Semiquantitative evaluation of tau-positive pathology was performed on all pathologically proven cases.

Results: Survival of PSP patients (8.0 years; 95% CI 7.3-8.7) was significantly shorter than of FTD patients (9.9 years; 95% CI 9.2-10.6). Corrected for demographic differences, PSP patients were still significantly more at risk of dying than FTD patients. In PSP, male gender, older onset-age, and higher PSP Rating Scale score were identified as independent predictors for shorter survival, whereas in FTD a positive family history and an older onset-age were associated with a poor prognosis. The difference in hazard rate was even more pronounced when comparing pathologically proven cases of PSP with FTLD-tau.

Conclusion: Survival of PSP patients is shorter compared to FTD patients, and probably reflects a more aggressive disease process in PSP. Independent predictors of shorter survival in PSP were male gender, older onset-age and higher PSP rating scale score, whereas in FTD a positive family history and higher onset-age were predictors for worse prognosis.

Introduction

Progressive supranuclear palsy (PSP) is clinically characterized by parkinsonism, supranuclear gaze palsy and cognitive decline.[1-2] PSP shows clinical, pathological and genetic overlap with frontotemporal dementia (FTD), and is considered to be part of the spectrum of frontotemporal lobar degenerations.[3-5] A frontal presentation has been identified in 20 percent of PSP cases,[6] whereas FTDP-17 associated with microtubule associated protein tau (*MAPT)* mutations may present with the clinical picture of PSP.[7-8] Neuronal and glial tau-positive inclusions are found in PSP and consist mainly of hyperphosphorylated four-repeat tau isoforms. In contrast to this, a subset of FTLD with Pick bodies (so-called Pick's disease) is characterized by the accumulation of three-repeat tau isoforms,[9] whereas inclusions in FTDP-17 with *MAPT* mutations variably consist of three- and four-repeat tau isoforms, depending on the location of the mutation.[10] The relevance of clinical and pathological overlap is further emphasized by the strong association between *MAPT* H1/H1 genotype and PSP.[11] Determining survival within this FTLD-PSP spectrum is of important clinical relevance and may give insight into the underlying disease process. However, only a few small studies compared survival between PSP and FTD and did not find any differences.[12-13] Small pathological series of PSP and tau-positive and tau-negative FTLD patients have shown conflicting results regarding the effect of tau pathology on survival.[14-16] In a recent study, specific neuropsychological profiles in FTLD have been correlated to disease duration, whereas onset-age or positive family history were not.[12] Retrospective studies on PSP have consistently identified the early falls and gaze palsy as being of prognostic significance.[17-20] Very recently, the PSP Rating Scale (PSPRS) has also proven to be of predictive value in survival,[21] although this still has to be replicated. An inverse correlation of tau severity with prognosis in PSP was found,[22-23] whereas in FTLD conflicting results were reported with respect to the prognostic significance of tau pathology.[14-16, 24]

The aim of this study is to prospectively investigate the survival in two large cohorts of PSP and FTD patients in relationship to demographic and clinical features, and to the presence and severity of tau pathology in a subset of patients who underwent brain autopsy.

Material and methods

Patients with PSP and FTD were recruited by nation-wide referral from neurologists and by visiting patients in nursing homes.[6, 25] Detailed clinical history, including the first presentation of symptoms, was obtained from patients and their family members, and by reviewing medical records. The onset-age was defined as the age

at which the first symptom attributable to PSP and FTD appeared according to the patient's caregiver and from medical records. In case of discrepancies, data from medical records were used. Data on family history were obtained using a structured questionnaire provided by spouse or first-degree relative. Family history was defined positive if at least one first-degree relative suffered from dementia, parkinsonism or motor neuron disease. All available hard copies of neuroimaging of both PSP and FTD patients were reviewed by the investigators in order to exclude other structural causes of both conditions and to semiquantitatively measure the severity of lobar atrophy.

PSP patients were neurologically examined, videotaped and the severity of their cognitive and motor functioning was scored by means of Mini-mental State Examination (MMSE), Frontal Assessment Battery (FAB), Unified Parkinson's Rating Scale-III (UPDRS-III) and PSPRS.

FTD patients underwent neurological examination, neuropsychological evaluation and neuroimaging (CT, MRI or SPECT with 99mTc-hexamethyl propyleneamine oxime (HMPAO)). The clinical diagnosis of all patients was established in a consensus meeting according to the National Institute for Neurological Diseases and Stroke-Society for PSP (NINDS-SPSP) criteria [2] and the Lund and Manchester criteria for FTD.[26] PSP patients were subdivided according to phenotype as described by Williams *et al*.[27] 119 patients were classified as Richardson's syndrome (RS), but only 7 cases of PSP-parkinsonism (PSP-P) were identified in our cohort. Of 18 patients there was insufficient data available of the first two years after onset. The remainder of the patients (n=51) could not be subdivided into a phenotype. Both studies on PSP and FTD patients were approved by the Medical Ethics Committee of the Erasmus Medical Centre of Rotterdam. Informed consent for participation (including blood collection) was obtained from the spouse or a first-degree relative of each patient. *MAPT, CHMP2B* and *GRN* genes were sequenced in all familial FTD patients, as has been previously described.[28-30] In PSP patients with a positive family history, screening of *MAPT*, *GRN,* and *LRRK2* was performed according to previously described methods.[29, 31-32]

The possibility of post-mortem examination was discussed with patients and their relatives during follow-up. Brain autopsy of patients who gave consent and who died during follow up was conducted by the Netherlands Brainbank according to their Legal and Ethical Code of Conduct. All brains that became available for autopsy were processed for routine staining and immunohistochemistry with AT8 (1:40, Innogenetics, Ghent, Belgium), ubiquitin (1:500, Dako, Glostrup, Denmark), three-repeat tau isoform (RD3) and four-repeat tau isoform (RD4), p62 (BD Biosciences Pharmingen, San Diego, CA, USA; 1 : 200, following 80° C antigen retrieval), TDP-43

(Proteintech, Chicago, IL, USA; 1 : 100, following pressure-cooking), β-amyloid (anti-β-amyloid, DAKO, Glostrup, Denmark, 1: 100, following formic acid pre-treatment), α-synuclein (anti-α-synuclein, Zymed Laboratories, San Francisco, California, USA; undiluted, following formic acid pretreatment). These were incubated overnight at 4 °C. Endogenous peroxidase activity was inhibited by 30 min incubation in PBS–hydrogen peroxide–sodium azide solution (100 ml 0.1M PBS, 2ml 30% H2O2,1ml natriumazide). The Histostain-Plus broad-spectrum kit DAB (Zymed, San Francisco, CA, USA) was used as a detection system. Slides were counterstained with Mayer's haematoxylin and mounted in Entellan.

The neuropathological diagnosis FTLD was classified into FTLD-tau and FTLD-U (with or without TDP-43-positive inclusions).[33] FTLD-tau was defined as Pick's disease and FTDP-17 with *MAPT* mutations. Cases with FTD-MND were excluded from this study. In FTLD-tau cases, neuronal loss and tau-staining reactive neurons and glial cells were visually quantified (none, mild, moderate and severe) in the following regions: frontal lobe, temporal lobe, hippocampus, parietal lobe, caudate nucleus and substantia nigra.

The neuropathological diagnosis PSP was established according to international criteria,[34] and a semiquantitative assessment of neurofibrillary tangles (NFT), tufted astrocytes (TA), oligodendroglial coiled bodies (CB) and thread pathology (Th) in all regions was carried out by two raters (WK, JvS) using a five-point grading scale according to Williams *et al*.[23] The PSP-tau score was calculated from the combined grade of coiled bodies and thread lesions in the substantia nigra and caudate and dentate nucleus.

Follow-up of PSP and FTD patients was performed by visits to the outpatient department of the Erasmus Medical Centre or by telephone interview with relatives up to August 1, 2008.

Statistical analysis

SPSS 15.0 for Windows (SPSS, Chicago, IL) was used for analysis. Onset-age, gender and family history were analyzed by independent sample *t*-test or Chi-square test. Actuarially corrected median survival was calculated, as well as mean survival in deceased cases. Survival analysis was performed using the Cox proportional hazard model, using a backward selection procedure model. Only results of multivariate analyses are shown, with variables that were significant in the univariate analysis. As the early occurrences of clinical symptoms are incorporated into the PSPRS, these symptoms were not analyzed together with the PSPRS in one model. However, different sections of the PSPRS (history, mentation, bulbar, ocular, limb and gait sections) were analyzed separately. Entry date was set as time of first symptoms.

Censoring date was either date of death or end of follow-up (August 1st, 2008). The assumption of proportionality of hazards was examined by Log-Log plots. Hazard ratios (HR) and 95% confidence intervals (CI) were calculated. Onset-age and PSPRS score were categorized into quartiles. Correlation between tau pathology and disease duration and onset-age was examined using Spearman's calculation. All statistical testing took place at a 0.05 level of significance (two-tailed).

Results

The demographic data of patients with PSP and FTD are summarized in table 1. FTD-MND patients (n=30) were excluded, due to their known shorter disease duration. Two PSP patients died of non-natural cause and have not been included in the survival analyses. The mean onset-age and age at death of PSP patients were significantly higher than that of FTD patients. During follow-up, 133 of 197 patients with PSP died at a mean disease duration of 7.2 ± 2.6 years, whereas 242 out of 354 FTD patients died at the end of follow-up with mean disease duration of 9.2 ± 4.1 years.

	PSP	FTD	p value
N	197	354	
Age at symptom onset, years*	66.2 ± 8.1	57.5 ± 8.9	<0.001
Male gender, n (%)	102 (51.8)	164 (46.3)	0.220
Presence of family history, n (%)	62 (31.5)	169 (47.7)	<0.001

Table 1. Demographic characteristics of patients with progressive supranuclear palsy (PSP) and frontotemporal dementia (FTD).
*Mean ± SD

Survival and hazard analysis of PSP and FTD

The median disease duration in PSP patients (8.0 years; 95% CI 7.3 to 8.7) was significantly shorter than in FTD patients (9.9 years; 95% CI 9.2 to 10.6) (Chi-square 17.1, p<0.001) (Figure 1).

This worse prognosis for PSP patients compared to FTD patients in a univariate analysis (HR 0.634; 95% CI 0.509 to 0.788) remained significant after adjustment for gender, onset-age and family history (HR 0.766; 95% CI 0.603 to 0.975). Looking into the PSP phenotypes, RS (6.8 years; 95% CI 6.3 to 7.4) was found to have shortest median survival compared to PSP-P (10.9 years; 95% CI 7.5 to 14.2) and the non-conclusive group (8.8 years; 95% CI 8.2 to 9.3).

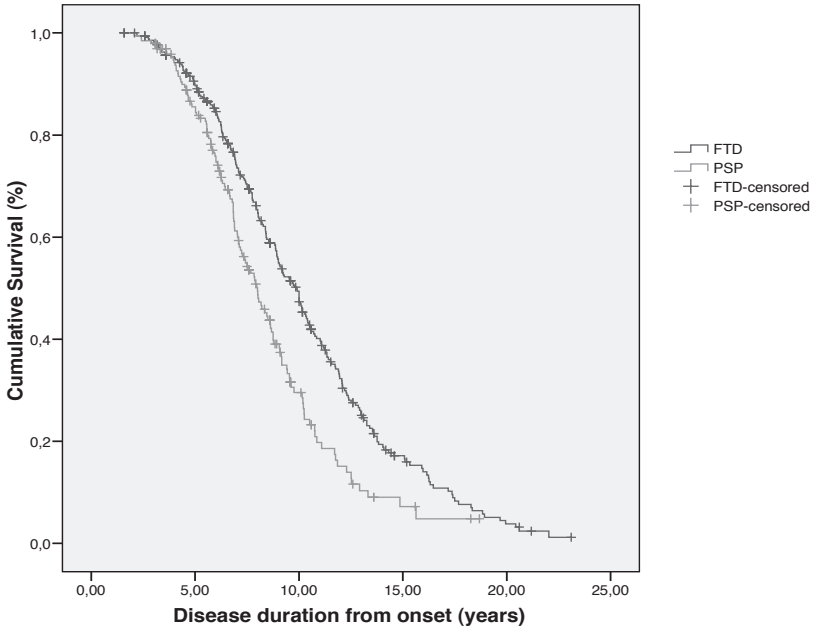

Figure 1. Kaplan Meier survival curve for PSP and FTD patients

When entering separate sections of the PSPRS in the model, only supranuclear ocular motor exam (HR 1.195; 95% CI 1.090 to 1.310) remained significant, whereas bulbar exam (HR 1.144; 95% CI 0.997 to 1.312) and gait exam (HR 1.063; 95% CI 0.999 to 1.131) were near significant.

	HR (95% CI)	p value
Gender	0.63 (0.43-0.92)	0.02
Positive family history		ns
Estimated onset-age		
<62	1 (reference)	
62-66	0.88 (0.52-1.50)	0.63
66-72	1.45 (0.85-2.50)	0.18
>72	2.03 (1.21-3.40)	0.01
PSP rating scale*		
0-35	1 (reference)	
35-48	1.96 (1.07-3.58)	0.03
48-62	2.99 (1.65-5.44)	<0.001
>62	8.55 (4.48-16.34)	<0.001

Table 2. Multivariate Cox models in progressive supranuclear palsy patients (HR with 95% CI). *10 patients had missing values on the PSP rating scale. ns, not significant

In FTD patients, positive family history (HR 1.438; 95% CI 1.114 to 1.858) and onset-age >64 years (HR 1.656; 95% CI 1.160 to 2.363) were significantly associated with poor survival. Looking into family history in FTD in more detail, mean disease duration of deceased FTD patients with a negative family history (9.9 years; 95% CI 9.1 to 10.6) was significantly longer than that of FTD patients with a positive family history (8.4 years; 95% CI 7.7 to 9.1; p=0.006). In this latter group a trend towards longer mean disease duration of patients with a *MAPT* mutation (n=36 from 10 families; 9.3 years; 95% CI 7.8 to 10.8) was found compared to patients without a *MAPT* mutation (n=83, including 17 patients with a *GRN* mutation from 3 families; 8.1 years; 95% CI 7.3 to 8.8; p=0.105). Of the *MAPT* mutations, L315R had the shortest mean disease duration (n=5; 5.7 ± 1.9 years), followed by P301L (n=20; 8.2 ± 3.0 years), whereas R406W had the longest mean disease duration (n=4; 17.5 ± 3.2 years). The remaining *MAPT* mutations, S320F (n=1), G272V (n=5), and ΔK280 (n=1) all had a mean disease duration of just above 10 years. Patients with a *GRN* mutation had a survival of 7.7 ± 2.8 years.

Pathology

Pathological examination was available for 24 PSP patients (all RS) and 61 FTLD patients (FTLD-tau n=32 and FTLD-U n=29). Men were over-represented (70.8%) in the PSP series, and the FTLD series showed a higher percentage of a positive family history (57.6%) and younger onset-age (55.3 years) compared to the total group, due to significant lower onset-age for cases with *MAPT* mutations (50.9 years).

After adjustment for gender, onset-age, and family history, FTLD-tau patients remained less at risk than PSP patients (HR 0.524; 95% CI 0.282 to 0.974), and a trend towards longer survival was found compared to FTLD-U patients (HR 0.608; 95% CI 0.361 to 1.024).

The FTLD-tau group consisted of 15 sporadic cases, all of which showed pure three-repeat tau pathology, and 17 cases with *MAPT* mutation, with pure three-repeat (G272V and ΔK280), pure four-repeat (P301L), or a mix of three-repeat and four-repeat (S320F, R406W and L315R) tau pathology depending on the location of the mutation. All PSP cases showed four-repeat tau pathology. The mean disease duration of sporadic Pick's disease cases was 12.1 years and was similar to that in *MAPT* cases with three-repeat tau pathology (n=6) of 10.2 years, whereas a trend (p=0.098) could be observed towards shorter survival in *MAPT* cases with 4-repeat tau pathology (n=7) of 8.6 years. Disease duration in *MAPT* mutations with a mix of three-repeat and four-repeat tau pathology varied considerably.

The FTLD-U cohort consisted of 4 *GRN*, 9 type 1 and 16 type 2 cases. Survival of pathological *GRN* cases did not significantly differ from the total group of deceased

GRN cases or FTLD-U type 2 cases (8.4 ± 3.1 years), but was significantly shorter than survival of FTLD-U type 1 cases (11.6 ± 5.0 years).

Tau pathology quantification

Neuronal loss in PSP cases was most prominent in subthalamic nucleus, globus pallidus, dentate nucleus and substantia nigra. Tau pathology consisting of globoid neurofibrillary tangles, tufted astrocytes and glial coiled bodies varied considerably between cases, with the subthalamic nucleus, thalamus, substantia nigra, basal pontine nuclei, locus coeruleus and dentate nucleus regions most severely involved. The severity of tau pathology, expressed in the tau-score, showed a significant negative correlation with disease duration (Figure 2), but was not correlated with onset-age.

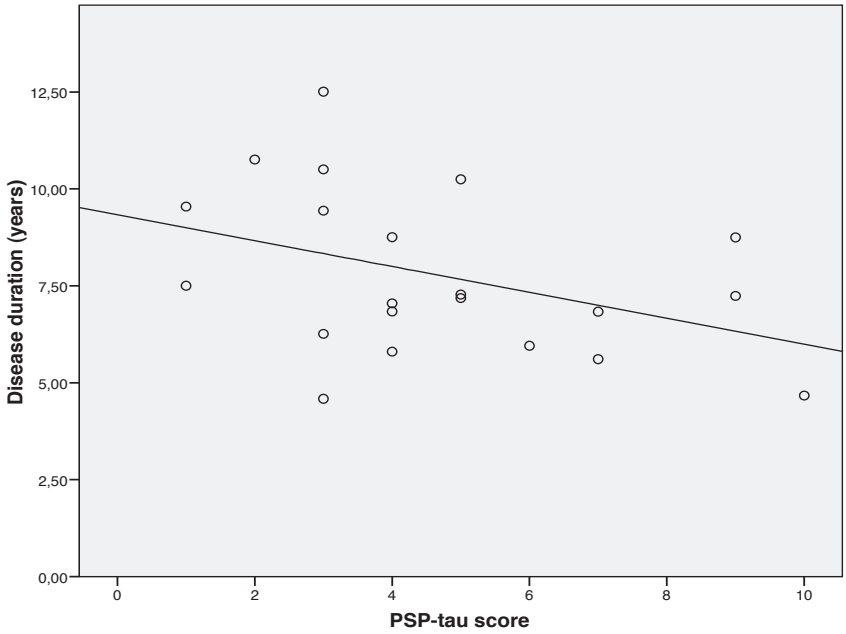

Figure 2. Disease duration of pathologically proven PSP cases according to PSP-tau score (Spearman's rho –0.44, p=0.045)

For the FTLD-tau group, neuronal loss in frontal, temporal and hippocampus regions was severe in most cases, whereas parietal, caudate nucleus and substantia nigra regions showed a variable neuron loss. Tau-positive inclusions showed a similar pattern of topographic distribution with severe tau pathology in frontal and temporal cortex and hippocampal regions, whereas the severity of tau pathology was

more variable in parietal cortex, caudate nucleus and substantia nigra. Astrocytic tau pathology was severe in the L315R mutation, but only mild in other *MAPT* mutations and sporadic Pick's disease. No significant correlation could be found between disease duration and either neuronal loss or tau-reactivity in any region.

Discussion

This study is the largest prospective population-based study comparing the survival between patients with PSP and FTD, and showed significantly shorter disease duration in PSP. This difference was even more pronounced when comparing pathologically proven cases of PSP with FTLD-tau. This study replicates, for the first time, the prognostic value of the PSPRS with a sharp increase of probability of death above a score >60. In PSP patients, male gender and older onset-age were also independent predictors for shorter disease duration, whereas a positive family history and an older onset-age were associated with a poor prognosis for FTD.

Our observation of a shorter disease duration for PSP compared to FTD contrasts with two other studies,[12-13] in which the small number of PSP patients may explain the lack of correlation. Our findings are probably close to true survival rates, as the patients were population-based ascertained. Looking into the natural history of PSP separately, the mean disease duration of deceased cases of 7.2 years in the present study comes very close to 6.8 years found in the only other large prospective study by Golbe *et al.*,[21] whereas a large retrospective study [20] showed a much shorter survival of 5.7 years. This was also true for RS cases in the clinicopathological study by O'Sullivan *et al.* (6.2 years),[35] whereas a much longer survival was found for PSP-parkinsonism patients (11.6 years). Although our PSP-P group consists of only 7 cases, due to the strict use of NINDS-SPSP criteria, the difference in survival compared to RS was striking as well. The effect of higher onset-age on survival in the present study was also found in retrospective studies,[20-21, 35] whereas our observed predictive value of gender contrasted to a weak or absent effect on survival in several other studies,[18, 20-21] but not all.[35] The prognostic significance of older onset-age in PSP resembles observations made in Alzheimer's disease [36] and Parkinson's disease (PD), whereas there is conflicting evidence regarding effect on prognosis of male gender in PD.[37] We do not have a good explanation for the reduced survival in men. Co-morbid clinical condition differences in gender at end stage may be associated to survival, but insufficient data was available to explore this more thoroughly.

The predictive value of the PSPRS score for survival in PSP patients confirms the first observations made in a tertiary referred cohort of Golbe *et al.*[21] and also proves its predictive value in a population-based cohort. In line with Golbe's observations,

a sharp rise in mortality risk was seen in patients with a PSPRS score above 60. In our study only the subsections supranuclear ocular motor exam, bulbar exam and gait exam were of prognostic value. The replication of Golbe's findings on the PSPRS has implications for its potential use in clinical trials.

Shorter survival in FTD patients with a positive family history in this study contrasts with other studies on the natural history of FTD,[12-13, 24, 38-39] and may suggest a more malignant disease process for hereditary forms. This is especially true for patients with *GRN* mutations and hereditary FTLD with an unknown genetic defect, both groups exhibiting ubiquitin pathology,[40] whereas *MAPT* mutations showed a trend towards longer disease duration. However, as several mutation carriers were related, we cannot exclude other familial genetic factors influencing the disease duration within the families. The absence of an association between positive family history and survival in other studies may be explained by a low number of patients or an unknown family history.

The longer survival of FTLD-tau group compared to pathologically proven PSP cases supports the hypothesis of a different disease process. The mean disease duration in the present series of 11.1 years is similar to that in the study by Hodges *et al.* (9.0 years).[24] The shorter survival of tau-positive cases (6 years) in the study by Xie *et al.*[16] can be explained by the inclusion of PSP and CBD cases. Our findings are very similar to the observations made by Hu *et al.*,[14] namely that three-repeat FTLD-tau have longest survival compared to four-repeat FTLD-tau and four-repeat controls, comprising PSP and CBD patients, and supports the idea that FTLD-tau patients tend to have a more indolent disease course than PSP.

The observed negative correlation between the severity of glial tau pathology and disease duration in PSP patients is in line with the study by Josephs *et al.*[22] The severity of oligodendroglial tau pathology in the substantia nigra and caudate and dentate nucleus represented the overall tau pathology reliably in the study by Williams *et al*, which again correlated negatively with disease duration,[23] and shown higher in RS than in PSP-P. Due to the absence of PSP-P in our pathological cohort, we could not replicate the latter finding. The correlation between the type and severity of tau pathology indicates that pathophysiological mechanisms determine the disease progression. Small sample size, a semiquantitative method of scoring, and different *MAPT* mutations with different functional effects may have hampered our analysis in FTLD. The association between shorter survival time and abundant tau pathology in basal ganglia in the study by Xie *et al.*[16] could not be confirmed by our study, and should probably be explained by the inclusion of PSP and CBD cases in their analysis. The best strategy would be to extend the survival analysis to a much larger series of pathologically proven FTD cases, which have been prospectively ascertained during

life in order to collect reliable clinical information.
A first drawback of the present study is a selection bias towards typical cases, and therefore missing cases with PSP-P, a subgroup that usually has a longer disease duration and an atypical presentation. Furthermore, as the population of the study consists of cases alive at the time of entry, there may be some degree of survival bias. A final limitation is that there was pathological confirmation in only 24 of 133 of the deceased patients. However the NINDS-SPSP criteria show a good positive predictive value for probable PSP (100%) and possible PSP (83%) in patients presenting with parkinsonism,[34] but also in patients presenting with dementia (96% for combined possible and probable PSP).[41] Also, no large differences were found between our clinical and our pathological cohort.
In conclusion, this large prospective study showed that survival in PSP is shorter than in FTD. This difference in prognosis was even more pronounced when comparing pathological PSP cases with FTLD-tau. Within the PSP group, male gender, older onset-age, and higher PSPRS score were independent predictors for shorter disease duration, whereas a positive family history and an older onset-age were associated with a poor prognosis in FTD. The significant effect of diagnosis on survival may suggest that the underlying pathophysiology in PSP is more aggressive than in FTD. This perspective should help clinicians anticipate disease progression of patients with PSP and FTD.

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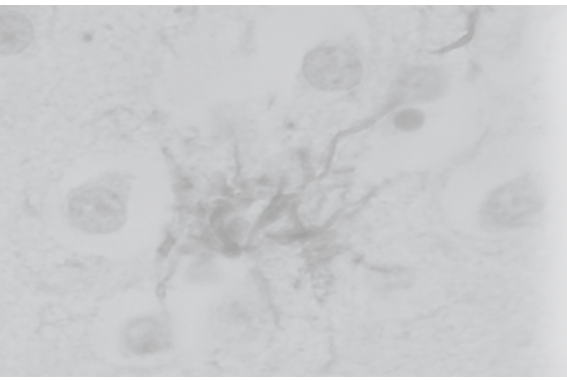

Chapter 3

Genetic heterogeneity

Chapter 3.1

Familial aggregation of parkinsonism in progressive supranuclear palsy

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Abstract

Background Progressive Supranuclear Palsy (PSP) is a progressive neurodegenerative disorder characterized by aggregates of the microtubule-associated protein tau (MAPT). A non-significant trend for positive family history has been observed in two case-control studies and several pedigrees with familial clustering of parkinsonism have been described. Occasionally, mutations in MAPT are found in patients with a clinical phenotype similar to PSP. In this case-control study we have compared the occurrence of dementia and parkinsonism among first degree relatives of PSP patients with an age and sex matched control group.

Methods Family history of dementia and parkinsonism was collected from all first degree relatives of PSP patients who fulfilled the international NINDS-criteria for PSP. Age and sex matched controls were selected from the Rotterdam Study. Genetic testing and pathological examination was performed in a subset of familial PSP cases.

Results Fifty-seven (33%) of the 172 PSP patients had at least one first degree relative who suffered from dementia or parkinsonism compared to 131 (25%) of the control subjects (OR 1.5, 95% CI 1.01-2.13). In PSP patients, more first degree relatives with parkinsonism were observed compared to controls, with an OR 3.9 (95% CI 1.99-7.61). Twelve PSP patients (7%) fulfilled the criteria for an autosomal dominant mode of transmission. The intrafamilial phenotype within these pedigrees varied between PSP, dementia, tremor and parkinsonism. Genetic studies revealed one patient with a P301L mutation in *MAPT*. Pathological examination of five familial cases confirmed the clinical diagnosis of PSP, with predominant four repeat tau pathology in affected brain areas.

Conclusion This study demonstrates familial aggregation of parkinsonism in PSP.

Introduction

Progressive supranuclear palsy (PSP) is a progressive neurodegenerative disorder characterized by frequent falls, pseudobulbar palsy, vertical gaze palsy and cognitive decline. Pathological examination shows deposits of abnormal phosphorylated tau protein as neurofibrillary tangles (NFT), tufted astrocytes (TA), coiled oligodendroglial inclusions and neuropil threads in basal ganglia and brain stem. PSP is considered to be a sporadic disorder, however several studies have suggested the involvement of genetic factors in the etiology of the disease. A non-significant trend towards positive family history for dementia and parkinsonism in PSP patients has been found in two case-control studies. [1-2] Several autopsy-proven cases of familial PSP have been reported, [3-5] and more recently, one family with autosomal dominant PSP has shown significant linkage to chromosome 1.[6] Moreover, mutations in the Microtubule Associated Protein Tau (*MAPT*) gene are occasionally associated with familial PSP. [7-10] A spectrum of clinical presentation varying from dementia, PSP, to CBD have also been described in *PGRN* and *LRRK* mutations. [11-12] Despite the fact that gene mutations are absent in most PSP patients, a large number of studies have shown a significant association with the *MAPT* H1 haplotype. [13]

In this case-control study we compared the occurrence of dementia and parkinsonism among first degree relatives of a large PSP cohort in the Netherlands with age and sex matched controls from the Rotterdam Study, and investigated the clinical, pathological and genetic aspects of several pedigrees with clustering of neurodegenerative disorders.

Material and methods

PSP patients were recruited by nation-wide referral from neurologists and nursing home physicians.[14] Patients were examined either by a research physician (LDK), a neurologist (AB and JvS) or both. The neurological examination was videotaped according to a standard protocol. The clinical diagnosis of all patients was established in a consensus meeting between the two neurologists and the research physician on the basis of history and neurological examination, including the videotapes. Diagnosis was made according to the National Institute for Neurological Diseases and Stroke-Society for PSP (NINDS-SPSP) criteria. [15] The study was approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam and all patients or first-degree relatives signed informed consent. Demographic data and the occurrence of dementia and parkinsonism in all first-degree relatives of patients were collected from the patient or caregiver. These items were collected from a structured questionnaire (Appendix E-1), and in case of a "yes" answer

further supplemented by thorough interview and, if available, medical records to support the diagnosis in affected relatives. Three levels of certainty of the diagnosis in relatives are distinguished according to a similar method described by Marder et al. [16]: 1. diagnosis based on medical record; 2. questionnaire with additional information from interview; parkinsonism was defined as the presence of two or more of the following symptoms: frequent falls, gait disturbance, speech problems, stiffness, slowness, tremor or medication use. Dementia was defined by the report of memory problems with or without behavioral changes, often requiring admission to a psychogeriatric nursing home; 3. report of dementia or Parkinson's disease by caregiver without further specification. In addition to the data collected from first degree relatives, we tried to gather as much information as possible about the grandparents of each PSP patient. Family history was considered to be positive when at least one first degree relative suffered from dementia (any form) or parkinsonism (Parkinson's disease or atypical parkinsonism). Autosomal dominance was defined as: at least three relatives affected with dementia or parkinsonism over two or more generations.

Control subjects came from a population based cohort study of people aged 55 years or over living in Ommoord, a district of Rotterdam.[17] As part of the baseline examination all participants were screened for presence of dementia and parkinsonism. Moreover, a structured interview was performed that included assessment of family structure regarding first degree relatives and presence and age at onset of major diseases including dementia and parkinsonism for all first degree relatives. For each patient, three control subjects were randomly selected, matched for age (within five years age-group) and sex, from participants without any signs of dementia or parkinsonism. Four PSP patients with a current age between 45-55 years were included in the age stratum 55-60 years.

Sequencing

Sequencing of *MAPT* (exons 1-3, 4, 5, 7, 9-13), *Progranulin* (all 13 exons) and *LRRK2* (exons 19, 31, 35 and 41) was performed according to previously described methods.[18-20] DNA was extracted from peripheral blood samples. The selected exons with flanking intronic sequences were amplified by PCR and directly sequenced on ABI 3730 using the BigDye terminator version 3.1.

Pathology

Brain autopsy was conducted by the Netherlands Brainbank according to their Legal and Ethical Code of Conduct. Formalin (10%) fixed and paraffin embedded tissue blocks were available for examination. Eight μm sections of all cortical regions, subcortical nuclei, brainstem and cerebellum underwent routine

staining. Immunohistochemistry was performed using the following antibodies: hyperphosphorylated tau (AT8, Innogenetics, Ghent, Belgium; 1 : 40), β-amyloid (anti-β-amyloid, DAKO, Glostrup, Denmark, 1: 100, following formic acid pre-treatment), α-synuclein (anti-α-synuclein, Zymed Laboratories, San Francisco, California, USA; undiluted, following formic acid pretreatment), three-repeat tau isoforms (RD3, Upstate, Charlottesville, VA, 1:3000) and four-repeat tau isoforms (RD4, Upstate, Charlottesville, VA, 1:100). [21]

The neuropathological diagnosis PSP was established according to international criteria. [22] A semi quantitative assessment of the tau pathology in all brain regions was carried out by two raters (WK and JvS). Neurofibrillary tangles (NFT), tufted astrocytes (TA), and oligodendroglial coiled bodies (CB) and threads (Thr) were separately scored on a five-point scale according to Williams et al. [23] The PSP tau score was calculated from the combined CB and Thr scores in the caudate nucleus, substantia nigra and dentate nucleus.

Statistical analysis

Software Package of Social Sciences (SPSS) version 15.0 was used for analysis. Mean differences were analyzed using student's T test. The Pearson Chi-squared test was used to compare frequencies between different groups. Family histories between cases and controls were compared by computing the Odds Ratio (OR) with 95% confidence intervals. All significance took place at α=0.05 (two-sided).

Results

A total of 176 patients fulfilled the criteria for PSP (65 possible, 91 probable and 20 definite). The mean age at PSP onset was 66.5 ± 8.1 years. Presenting symptom(s) were: gait disorder with falls (65%), behavioral changes (21%), slowness (20%), cognitive decline (16%), stiffness (14%), speech problems (9%) visual complaints (6%) and tremor (4%). Family history on first degree relatives was available for 172 of the 176 PSP patients. Four patients had incomplete information due to loss of family contact. Tremor at disease onset occurred significantly more frequently in PSP patients with a positive family history compared to PSP patients with a negative family history (9% versus 1%, p-value= 0.02).

A total of 57 (33%) PSP patients had *at least* one first-degree relative affected with dementia or parkinsonism. The certainty of the diagnosis in affected relatives was based on medical records in 28%, questionnaire plus additional information from interview in 67% and report from caregiver in 5% for parkinsonism, and for dementia 22%, 61% and 17% respectively. In 45 cases (26%) a single first-degree relative was affected , whereas two or more affected first-degree relatives was found in 12 patients.

Family	Relationship	Age at onset, y	Age at death, y	Clinical presentation and clinical diagnosis
Family 1	Proband (III:2)	54	63	Behavioral changes, followed by PSP symptoms*
	Father (II:4)	71	76	Dementia followed by parkinsonism
	Aunt (II:3)	NA	NA	Dementia
Family 2	Proband (II:3)	61	73	Bilateral tremor > 20 years, followed by PSP symptoms*
	Mother (I:2)	NA	75	Isolated tremor > 20 years, followed by parkinsonism
	Sister (II:11)	NA	54	Tremor
	Son (III:1)	NA	NA	Tremor
	Daughter (III:2)	NA	37	Tremor
	Grandson (IV:1)	NA	NA	Tremor
Family 3	Proband (III:4)	70	78	Memory problems, behavioral changes, followed by PSP symptoms*
	Sister (III:1)	NA	83	Dementia and tremor
	Sister (III:2)	76	78	Parkinsonism
	Mother (II:2)	NA	72	Dementia
	Grandmother (I:4)	NA	70	Dementia
Family 4	Proband (II:1)	67	73	PSP symptoms, followed by memory problems and behavioral changes*
	Sister (II:2)	63	71	Frequent falls, poor levodopa response and blepharospasm, PD
	Father (I:1)	NA	78	Dementia
Family 5	Proband (III:2)	62	67	Parkinsonian features, memory problems followed by PSP symptoms*
	Mother (II:2)	71	81	Chorea and behavioral changes
	Grandmother (I:4)	NA	79	Dementia

Table 1. Clinical features of five pedigrees of familial PSP patients. Parentheses represent corresponding generation number in the figure.
* pathological confirmed PSP patients. NA= not available

If we take second-degree relatives into account, 12 families (7%) fulfilled the criteria for an autosomal dominant mode of inheritance. Within these pedigrees the affected relatives (first or second degree) showed dementia (n=15), parkinsonism or PSP-like (n=8), dementia with parkinsonism (n=5). All families, except one, presented with a mixed phenotype of dementia or parkinsonism, with a PSP-like syndrome or parkinsonism in one or more affected relatives, and dementia in others. The proband of family 2 (see table 1) showed a similar phenotype as her mother, with isolated tremor over decades, followed by signs of progressive supranuclear palsy. Three other affected relatives presented with tremor at neurological examination, which strongly fits into the disease phenotype within this family. Another three families,

not fulfilling the criteria for autosomal dominance, contained multiple affecteds in one generation only, whereas in one family a patient with PSP had three third-degree relatives with FTD, in the absence of *MAPT* and *GRN* gene mutations (see below). The clinical diagnosis was neuropathologically confirmed in the proband of five pedigrees (autosomal dominance in four), which are described in Table 1 and the Figure.

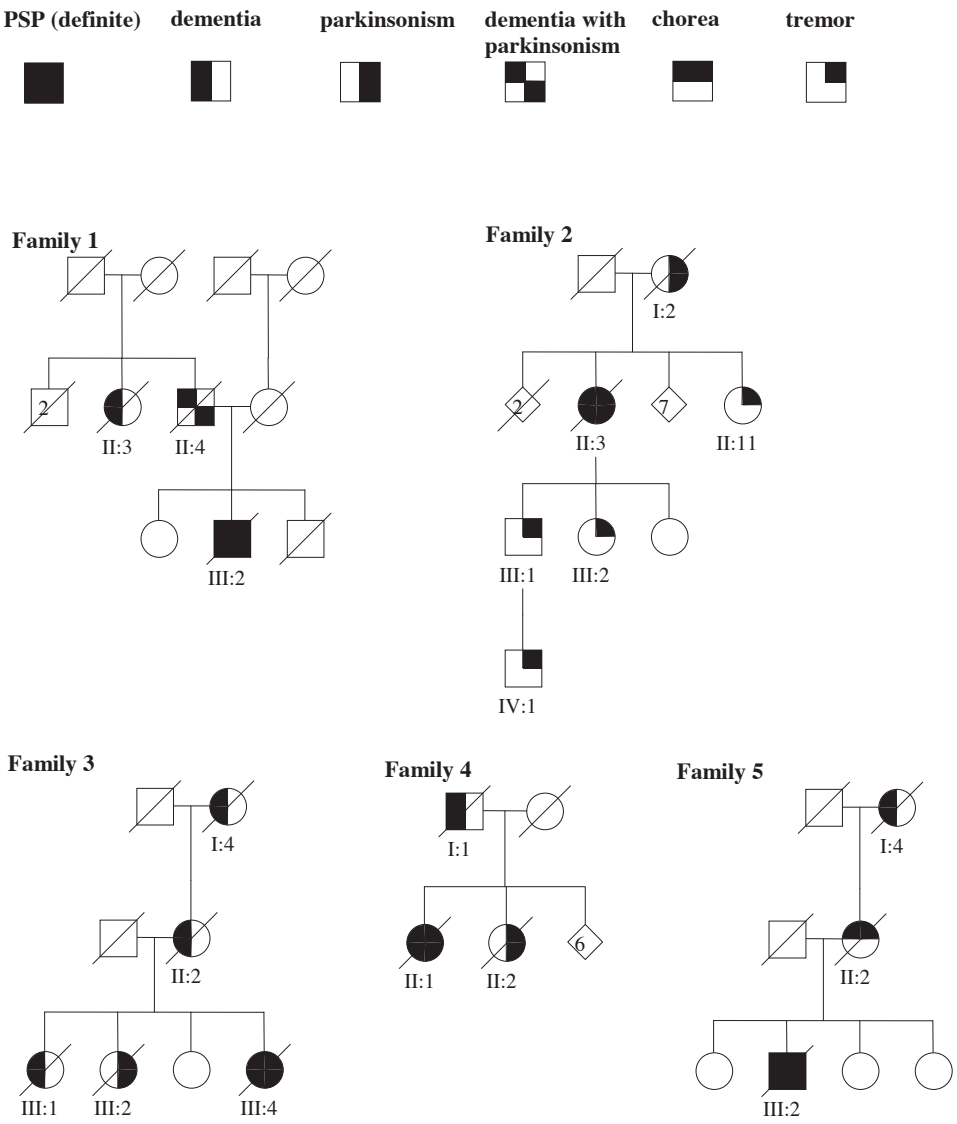

Figure. Pedigrees of 5 familial PSP cases with pathological confirmation of the proband. Autosomal dominant inheritance is defined as three or more affecteds in two generations

Cases versus controls

Information from a total of 3023 first-degree relatives (mean age 69.0) from controls and 986 (mean age 69.4 years) first degree relatives from PSP patients were collected. We observed more cases with positive family history than controls. Especially, parkinsonism occurred more frequently among first degree relatives of PSP patients, while dementia did not differ between the two groups (Table 2).

	PSP, n=172 (%)	Controls, n=519 (%)	OR [95% CI]
Family history			
Positive	57 (33)	131 (25)	1.5 [1.01-2.13]
Negative	115 (67)	388 (75)	
Women, family history			
Positive	28 (34)	65 (27)	1.4 [0.83-2.43]
Negative	54 (66)	178 (73)	
Men, family history			
Positive	29 (32)	66 (24)	1.5 [0.90-2.54]
Negative	61 (68)	210 (76)	
Affected first degree relative(s)			
0	115 (67)	388 (75)	
1	45 (26)	108 (21)	
2 or more	12 (7)	23 (4)	1.6 [0.79-3.32] *
Affected first degree relative with:			
Dementia	43 (25%)	119 (23%)	1.1 [0.75-1.67]
Parkinsonism	20 (12%)	17 (3%)	3.9 [1.99-7.61]

Table 2. Family history in 172 PSP patients compared to 519 control subjects.
* OR [95% CI] compared to the other two groups (0 or 1 affected).
OR= odds ratio; CI= confidence interval.

Mean number of first degree relatives did not differ between cases and controls (both mean n= 6). Positive family history did not differ between men and women in both cases and controls. First degree women were significantly more frequently affected than men of both PSP patients and controls.

Pathology

Neuropathological examination of the autopsies of 20 patients confirmed the clinical diagnosis PSP. Five brains of the familial cases mentioned above, are detailed in Table 3. Macroscopy (mean weight 1226 grams) showed mild frontal atrophy (three cases), depigmentation of the substantia nigra (four cases) and locus coeruleus (one case). Severe neuron loss and gliosis were found in the pallidum (two cases), subthalamic nucleus (two cases) and substantia nigra (all five cases). Methenamine-silver staining and immunostaining for β-amyloid of frontal, temporal and entorhinal cortex showed a variable number of diffuse plaques in three brains (cases 2, 3 and 4). The CA2 region of the hippocampus of all five brains contained a few to moderate number of NFTs.

Immunohistochemistry with AT-8 antibody of the striatum and caudate nucleus showed NFTs, TA and coiled bodies with threads in all cases. A variable number of tau-positive neurons (pretangles and NFTs), coiled bodies and threads were also found in the subthalamic nucleus and thalamus. Abundant tau-positive neurons were present in the substantia nigra, basal pontine nuclei and locus coeruleus, and lower brain stem. All five brains showed a few to a moderate number of tau-positive neurons in the dentate nucleus, with many tau-positive glial cells in the cerebellar white matter of two cases. In all five cases the CA2 region of the hippocampus was more strongly involved then the CA1 region. Severe involvement of enthorhinal cortex was observed in three cases. The neocortex showed a few pretangles and tufted astrocytes in the frontal, temporal and parietal cortex. In all regions of the cases, RD4 antibody gave similar staining of neuronal and glial inclusions. RD3 antibody gave negative or only faint staining of inclusions, except for a few NFTs in hippocampus. A moderate number of Lewy bodies was seen in the locus coeruleus and substantia nigra in case 3.

Sequencing

Sequencing of *MAPT*, *LRRK2*, and *Progranulin* genes in all probands from families with autosomal dominance and 14 sporadic PSP patients with onset < 55 years did revealed a *MAPT* mutation (P301L) in one patient only. Genealogical research revealed that this family was linked to a large Dutch pedigree with FTD described previously. [24] In the remaining cases no pathogenic variations were found in the coding exons of the selected genes.

	Family 1 (III:2)	Family 2 (II:3)	Family 3 (III:4)	Family 4 (II:1)	Family 5 (III:2)
Age at death	63	73	79	74	67
Sex	M	F	F	F	M
Clinical diagnosis (NINDS-SPSP)	Possible	Possible	Possible	Probable	Probable
Disease duration,y	9	12	9	7	5
Brain weight,g	1390	1135	1219	1180	1204
Neocortex (F,T,P)					
Neuronal	++	++	-	++	+
TA	-	+	+	++++	++
CB + Th	++	+	+	+++	++
Amygdala					
Neuronal	++	++	+	+++	++
TA	-	-	-	+++	++
CB + Th	+	+	+	+	+
Caudate nucleus					
Neuronal	+	++	+++	+	+
TA	+	++++	++++	+++	++++
CB + Th	-	+	+	++	+++
Striatum					
CB + Th	+	+++	++	+++	++++
Substantia nigra					
Neuronal	++	++++	++++	++	++
CB + Th	-	+++	+++	+++	+++
Tha + STN					
Neuronal	++	+++	++	+	++
TA	+	+	-	++	
CB + Th	+	++	+	++	+++
Dentate nucleus					
Neuronal	+	++	+++	++	++
CB + Th	+	+++	++	++++	++++
Pontine nuclei					
Neuronal	++	++	++	+++	++
CB + Th	-	+	++	++	+
Lower brainstem					
Neuronal	+	+++	++	+++	++
CB + Th	-	+	+	+	++
Total tau score*	1	7	6	9	10

Table 3. Pathological features of five cases of familial PSP.

* according to reference 23.

Neuronal: neurofibrillary tangles or pre-tangles; TA= tufted astrocytes; CB= coiled bodies; Th= threads; F = frontal; T = temporal; P = parietal; Tha= thalamus; STN= subthalamic nucleus.

Discussion

This study demonstrates a significant association of parkinsonism in first-degree relatives of PSP patients for the first time. In total, thirty-three percent of PSP patients had at least one first degree relative affected with dementia or parkinsonism, including autosomal dominance in seven percent of the cases. Pathological examination of five familial PSP patients showed the presence of tau pathology consistent with the diagnosis PSP.

A weakness of our study might be the uncertainty about the type of dementia and parkinsonism in affected relatives due to a lack of detailed clinical information. However, the information obtained from interviewing family members was checked as much as possible against their medical records. A strength of our study was the use of a structured family history questionnaire. It is unlikely that there is a selection bias towards referral of cases with a positive family history, as our study was population-based and not hospital-referred.

The observations of increased familial aggregation in PSP patients are in line with other smaller series investigating family history in PSP patients. One study of FTD and PSP patients showed a similar frequency of affected first-degree relatives (28 percent) and autosomal dominance (6 percent),[25] whereas in a previous case-control study a non-significant trend for a positive family history of dementia and parkinsonism was found. Although the latter could not be confirmed in a follow-up study, this might be due to the small sample size as stated by the authors themselves. [1-2]

The high frequency of a positive family history for dementia in the present study lies between 10 and 30 percent of other studies from Europe [26-27] and might be partly explained by the existence of relatively large families with high number of siblings per family in both cases and controls. As the prevalence of AD increase with higher age, a high current age of first-degree relatives in patients and age-matched controls may also contributed to a high positive family history. The higher frequency of positive family history for parkinsonism in PSP patients versus controls is unlikely to be due to differences in the study populations (nation-wide versus Rotterdam population), as the Netherlands is a small country with a relatively genetic homogeneous population except for a few well-documented genetic isolated populations. The use of less stringent criteria for dementia and parkinsonism in affected relatives of controls might have resulted into an overestimation of the percentage of positive family history in controls, and therefore into an underestimation of the observed effect.

The observed autosomal dominant inheritance in a subset of the present PSP cohort is supported by case reports with familial PSP cases. [28] One of these reported families had five siblings with clinical PSP or CBD, with pathological confirmation in two. [5]

A large study on familial clustering described the occurrence of PSP in 12 families as being suggestive for an autosomal dominant mode of inheritance with reduced penetrance. [3] Several of the affected family members also showed tremor, dystonia, dementia, gait disorders and tics, which were considered partially independent from PSP symptoms. [6] The uncertain mode of inheritance in four of the present families with affecteds in one generation only or affected third-degree relatives, might suggest a recessive disorder or autosomal dominance with reduced penetrance.

In the present study, affected first-degree relatives diagnosed as parkinsonism might have suffered from PSP for several reasons. Firstly, PD is the most frequent misdiagnosis in the initial phase of PSP, both in our series and in other reports described in the literature. [32] Secondly, a parkinsonism-form of PSP has been described in a subset of patients with pathological-proven PSP, who were diagnosed as having Parkinson's disease even later in the course of the disease. [33] Finally, of the 20 affected family members with parkinsonism in the present study, a PSP-like syndrome was reported in eight of them.

Studies on familial aggregation of dementia and parkinsonism have reported conflicting results. [29-31] One major criticism might be that the clinical phenotype in families of the present study varies between dementia, parkinsonism and tremor, and do not fit into the clinical spectrum of PSP. However, dementia, apraxia, tremor and orofacial dyskinesias have also been reported in other families with PSP. [3, 28] In addition, genotype-phenotype studies on causative genes for Parkinson's disease and dementia have repeatedly learned us that the clinical spectrum is always larger than initially expected. [10, 12] The occurrence of essential tremor in one proband and her mother (family 2) preceding the onset of PSP over 20 years deserves special attention. A concomitant postural tremor has also been reported in three other families in the literature, but was considered to segregate independently from PSP in two of these families. Although their co-occurrence in the present family might also be a matter of chance, the possibility of one specific phenotype or a risk factor contributing to the other phenotype could not be excluded, as suggested in a previous report. [4]

Frontotemporal dementia is the most common clinical presentation occurring in affected family members of the PSP patient with a P301L mutation, although PSP has occasionally been reported. [24, 34-35] Whether this intrafamilial variation is due to genetic or environmental factors is still unknown, although parkinsonism within *MAPT* mutations has been associated with homozygosity for the tau H1 haplotype. [36] In another family of the present study, FTD occurred in three third-degree relatives in the absence of mutations in one of the other candidate genes suggesting another genetic factor. Significant linkage to a 3.4 Mb region on chromosome 1 has been found for a family with PSP. [6] The present families were too small to determine

linkage to this candidate region. Together with homozygosity for H1/H1 haplotype of the MAPT gene as risk factor for PSP, several genetic factors may underlie both familial and sporadic PSP.

The present neuropathological findings in the five cases with a positive family history is consistent with the definite diagnosis PSP. NFT were consistently present in the subthalamic nucleus and substantia nigra, whereas the density of distinctive tau lesions in other regions differed between the five cases. The neuropathological diagnosis of PSP is further supported by the positive staining with the 4-repeat tau antibody and negative staining with 3-repeat tau antibody. The density of coiled bodies and threads in substantia nigra, caudate nucleus and dentate nucleus (PSP tau score) also reflected the overall lesion severity in the present cases relatively well. However, the number of our cases is too low to draw conclusions about a correlation between PSP tau score and disease duration as found by other investigators. [23, 37] The concomitance of Lewy Body pathology in the brainstem of one of the five cases is also found in another study as an independent phenomenon. [38]

Familial aggregation in PSP observed in this study supports the involvement of genetic factors and future studies that focus on identifying the genetic defect(s) underlying PSP and related disorders, may help to elucidate the pathophysiological process of this disease.

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Chapter 3.2

A novel hereditary late onset ataxia disorder with polyglutamine inclusions mimicking PSP

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To be submitted

Abstract

Introduction: Autosomal dominant spinocerebellar ataxias (SCAs) are clinically characterised by cerebellar ataxia often in combination with other signs like extrapyramidal symptoms, peripheral neuropathy and cognitive impairment. The polyglutamine SCAs are the most common group and encode for elongated glutamine sequences in the diseased protein. Besides the genetically defined SCAs, a subset shows significant linkage to a chromosomal region whereas the genetic locus is unknown for other families. In the present paper, we present a new Dutch SCA pedigree with late-onset progressive ataxia and prominent cognitive impairment.
Methods: The proband was referred with the clinical diagnosis PSP. All living affected family members were personally examined and their medical records and neuroimaging reviewed after obtaining informed consent. After mutational screen of a large number of candidate genes which did not reveal any mutation, a genomewide linkage scan was performed. During follow-up, brain autopsy was conducted in one patient.
Results: Fourteen affected individuals were identified, of whom nine personally examined, with a mean age at onset of 64.4 years. Clinical symptoms included gait ataxia, oculomotor problems, dysarthria, cognitive decline and parkinsonism. An affected only analysis on seven individuals revealed seven STR markers with LOD scores > 1.0, with highest for D18S59 (LOD score 1.64) on chromosome 18. Neuropathological examination of one case showed abundant 1C2 inclusions in multiple brain areas.
*Conclusions :*We present a new hereditary disorder with late-onset progressive cerebellar ataxia and prominent cognitive impairment. The neuropathological picture is strongly suggestive for a polyglutamine disorder. The genetic defect has still to be identified, possibly within a critical region on chromosome 18 with a LOD score of 1.6.

Introduction

Spinocerebellar ataxias (SCAs) are a heterogeneous group hereditary disorders characterised by pure cerebellar ataxia, or in combination with non-cerebellar signs, which include (extra) pyramidal features, peripheral neuropathy or cognitive impairment. At present, 27 distinct autosomal dominant forms are known.[1] Of the 19 identified gene defects, CAG repeat expansions have been found in coding regions of six different genes (SCA1, SCA2, SCA3, SCA6, SCA7 and SCA17), tri- or pentanucleotide repeat expansions in the non-coding regions of 4 genes (SCA8, SCA10, SCA12 and SCA31), and conventional mutations in nine other genes (SCA5, SCA11, SCA13, SCA14, SCA15/16, SCA20, SCA23, SCA27 and SCA28). The remaining eight SCA subtypes (SCA4, SCA18, SCA19, SCA21, SCA22, SCA25, SCA26 and SCA30) have shown significant linkage to a chromosomal region, and await the identification of the responsible gene defect. Besides these SCA subtypes, there are families where the genetic locus is unknown and around 44% of the patients with SCA do not show a genetic defect when the most prevalent genes are tested.

The polyglutamine SCAs are the most common group and encode for elongated glutamine sequences in the diseased protein. The threshold over which the disease develops varies between different genes, but is usually above 37-40 repeats. Genetic anticipation defined by younger age and increasing severity over successive generations is characteristic for these repeat disorders. The age of onset is usually in the third or fourth decade, but depends largely on length of repeat expansion. Gait impairment is the most common initial symptom. Cognitive impairment in varying severity occurs in several SCA types (SCA1, SCA2, SCA3, SCA6, SCA17 and SCA19), with executive dysfunction as the most consistent and prominent feature. Neuronal intranuclear inclusions (NII) are the morphological hallmark of most polyglutamine SCAs and are not found in SCAs with conventional mutations.[1-2] The involvement of affected regions is variable and intranuclear inclusions are sometimes seen beyond regions of neuronal loss.

The clinical, genetic and pathological research on SCA subtypes has been far from being complete. Several SCA subtypes await their neuropathological characterization and in several SCA families, the genetic defect still has to be identified. In the present paper, we describe a new Dutch SCA pedigree with late-onset progressive ataxia, oculomotor deficits, extrapyramidal signs and prominent cognitive impairment. The neuropathological picture is suggestive for a polyglutamine repeat disorder.

Methods

Ascertainment of patients

The proband (III:13) was suspected to suffer from progressive supranuclear palsy (PSP) and referred for inclusion in our nation wide study on PSP. Family history revealed that five out of nine sibs had signs and symptoms suggestive for a neurodegenerative disorder. Exploring a second branch of the family revealed multiple affected (most deceased) individuals (see Figure 1). Two neurologists (EB, JvS) and a resident (LDK) examined all affected family members who were still alive. Available medical records and hard copies of neuroimaging of patients were reviewed. The study was approved by the Medical Ethical Committee of the Erasmus Medical Center Rotterdam. Informed consent was obtained from all family members who participated in the study.

Genetic testing

DNA was extracted from venous blood samples using standard conditions. Diagnostic genetic testing of *MAPT*, *PSEN1*, *PRNP*, *HD*, *DRPLA* and *SCA3* was performed on individual III: 20, and *SCA1, SCA2, SCA6, SCA7, SCA17* and *FXTAS* on individual III:13.

Linkage analysis

A genomewide linkage scan was performed using 400 STR markers from the ABI PRISM Linkage Mapping Set MD-10 (Applied Biosystems). Markers were amplified in duplex and conditions were used according to methods specified in the protocol of the manufacturers. PCR products were loaded on an ABI 3100 automated sequencer (filter set D), and the data were analyzed with ABI GeneMapper (version 2.0) software.

For finemapping of the region of interest, additional markers were selected from the Marshfield integrated linkage map. SNP's used for finemapping were selected from the Hapmap database with minor allele frequencies > 0.2.

Two-point linkage analysis was performed with the MLINK program of the LINKAGE package (version 5.1) (Lathrop and Lalouel 1984). Maximum LOD were calculated for each marker with the following assumptions: autosomal dominant inheritance with 99% penetrance with a gene frequency of 1:10.000; phenocopies were allowed in 1%, and equal allele frequencies of the genotyped markers were used in the calculations. We performed an affected-only analysis on seven individuals (III:10, III:12, III:13, III:15, III:19, III:20, IV:1). Individuals III:7 and IV:2 were considered unknown as the clinical picture was less consistent with the other

affected individuals from the pedigree, suggesting the possibility of a phenocopy. The other individuals who were not clinically affected, were considered unknown in the linkage analysis. Power calculation of the pedigree was performed with SLINK program by simulating genotypes at one locus given the phenotypes at another locus. Through this analysis the maximum LOD score possible was 2.1 given the pedigree structure and using the conditions as described above.

Sequencing

The critical region on chromosome 18q contained seven genes: CETN1, CLUL1, TYMS, ENOSF1, YES1, ADCYAP1 and C18Orf56. All exons and exon-intron boundaries of these genes were amplified (conditions and primer sequences available upon request). Direct sequencing was performed using BigDye terminator chemistry (Applied Biosystems, Foster City, CA) and sequencing products were processed on an Applied Biosystems 3730 automated DNA sequencer and analyzed using SeqScape software version 2.1 (Applied Biosystems).

Neuropathology

One affected family member died during follow-up (individual III:20), and brain autopsy was performed within 4 hours after death. Brain autopsy was conducted by the Netherlands Brainbank according to their Legal and Ethical Code of Conduct. Formalin (10%) fixed and paraffin embedded tissue blocks were available for examination. Eight-micrometer sections of all cortical regions, subcortical nuclei, brainstem, and cerebellum underwent routine staining. Immunohistochemistry was performed using the following antibodies: hyperphosphorylated tau (AT8, Innogenetics, Ghent, Belgium; 1:40), beta-amyloid (anti- -amyloid, DAKO, Glostrup, Denmark; 1:100, following formic acid pretreatment), alpha-synuclein (anti- -synuclein, Zymed Laboratories, San Francisco, CA; undiluted, following formic acid pretreatment), TDP-43 (anti phospho TDP-43, Cosmo Bio, 1:100 and Proteintech Group, 1:100), p62 (mouse D3 Clone, Santa Cruz, 1:100) and 1C2 (mouse 5TF1-1C2-172 Clone, Chemicon, 1:3200).

Results

Mean age at onset of the 14 affected individuals (see figure 1) was 64.4 ± 8.4 years. Seven affected individuals had died after mean disease duration of 14.1 ± 3.1 years. The affected persons who were still alive (n= 7) had a mean disease duration of 9.6 ± 2.4 years.

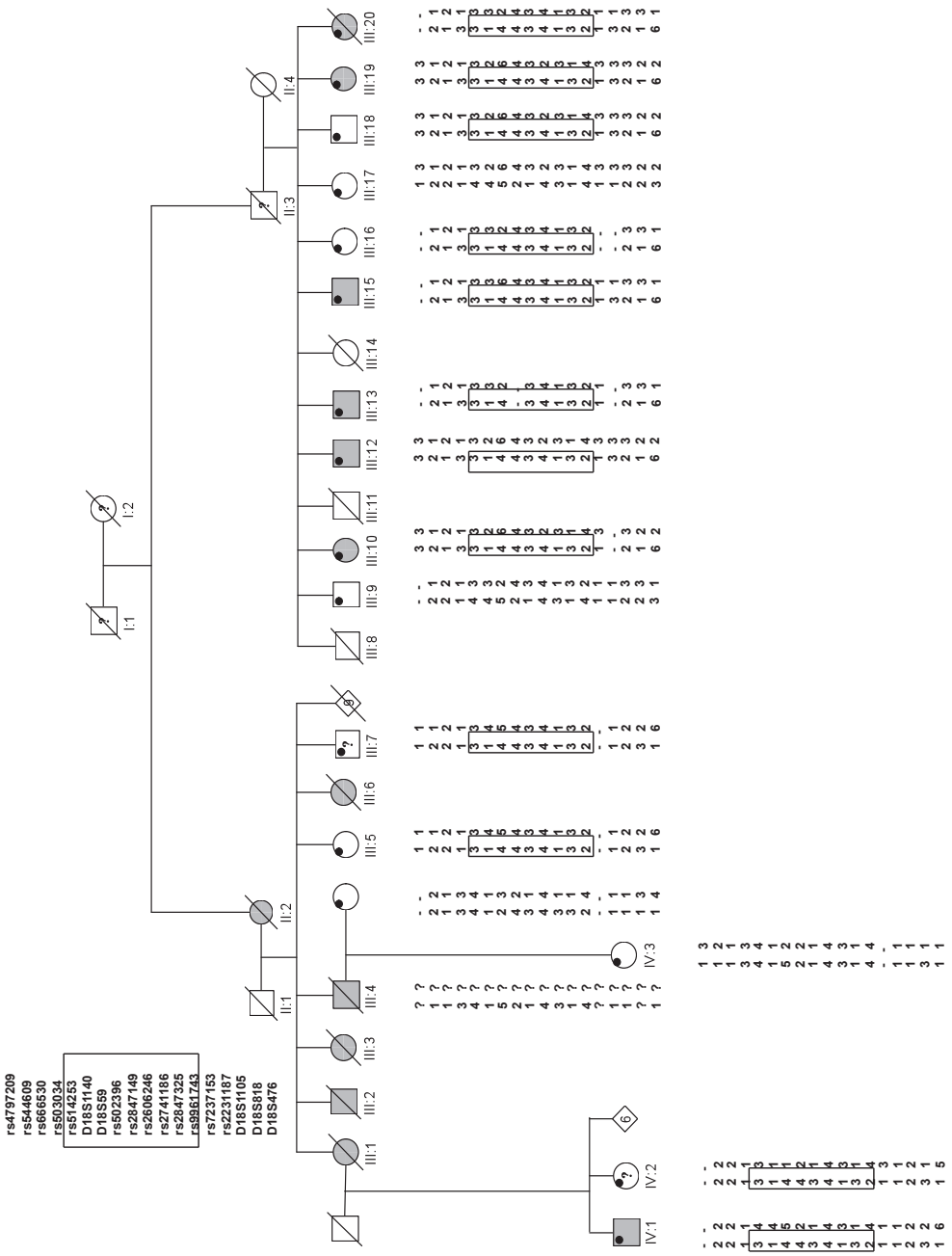

Figure 1. Pedigree structure with haplotypes on chromosome 18. Affected individuals are indicated with filled (grey) symbols. Open symbols represent unaffected individuals (but treated as unknown in the linkage analysis). Question marks within the symbols represent an unknown phenotype because the clinical picture was less consistent with the other affected individuals.
• = DNA available.

Description of the pedigree (see table 1 for overview and figure 1 for pedigree structure)

III:13 (proband) The patient, a 65-year old male, was treated with levodopa for gait problems and slowness in movements from the age of 65, but his symptoms did not improve. He had difficulty with dressing and turning in bed. Over the following years he increasingly experienced stiffness in the legs and had frequent falls. Neurological examination showed a short-stepped gait and diminished arm swing, without rigidity, tremor or altered hand writing. He had intact tendon reflexes, and absent vibratory sense on both feet. Subsequently he developed memory problems, behavioural changes with impulsiveness, disinhibition, loss of decorum and reduced insight of illness. He showed an upward gaze palsy at neurological examination and the diagnosis possible PSP was established. Neurological examination, seven years after onset, showed disinhibited behaviour, reduced abstract reasoning, and diminished spontaneous speech. His MMSE score was 24/30 and FAB score 7/18. He had dysarthria, an upward gaze palsy, saccadic pursuit eye movements without nystagmus, and bilateral mild bradykinesia without axial or limb rigidity, tremor, or ataxia at upper limbs. His postural reflexes were impaired and his gait showed festination and freezing. MRI scan showed generalized and cerebellar atrophy (figure 2).

II:2 the aunt of the proband developed cognitive decline, walking problems with "waddled" gait, and speech problems from the age of 55, and died at 67 years.

II:3 the father of the proband died from an acute myocardial infarction without any neurological symptoms at the age 64 years.

III:1 according to information from her family, she showed gait disturbance, motor restlessness, behavioural changes and disorientation over the last 10 years of her life, and died at age of 86 years. She was never investigated by a physician.

III:2 according to family history, this patient had problems of gait and motor restlessness over the last 20 years of his life, and died around the age of 70.

III:3 the medical record from this patient provided information about gait disturbance with ataxic-spastic component from the age of 60. CT scan was reported to show cerebellar atrophy and enlarged ventricles. She was diagnosed as chorea and died at age 75.

III:4. The patient developed uncontrolled movements and gait disturbance around the age of 70 years . Neurological examination at age 83 years showed choreatic movements, emotional bluntness, apathy, perseverations and broad-based walking pattern. No parkinsonian or pyramidal tract signs were present. No imaging was performed. He died at age 84.

III:6 This female with mild mental retardation from birth developed involuntary movements and speech problems at the age of 66. Neurological examination at

that time showed cerebellar dysarthria, involuntary movements at motion, ataxia of upper and lower limbs, and broad-based gait with abnormal tandem walking. Ankle-jerk reflexes were absent but sensory functions were intact. CT brain was reported as normal. She was diagnosed as having spinocerebellar degeneration, and she died at age 80.

III:7 This patient developed increasingly memory problems and disorientation from the age of 75. CT imaging showed mild generalized and cerebellar atrophy. He was diagnosed at a local hospital with Alzheimer's disease. Neurological examination at age 82 by the current investigators showed a MMSE score of 16/30 and FAB score of 8/18. Speech was slightly inarticulately and eye movements showed gaze palsy upward with saccadic pursuit eye movements. No parkinsonian signs were present nor limb ataxia. Gait pattern was normal but tandem walking was slightly impaired.

III:10 this woman developed a "waddled" gait with occasional falls from the age of 72. Concurrently, she showed cognitive decline with memory problems, delusions, and disorientation, and also behavioural changes with perseverations, aggressiveness and reduced insight. She was diagnosed as Alzheimer disease at the age of 76. At neurological examination one year later, the patient was highly distractible, did not understand simple instructions, showed perseverations and a positive applause sign, and had reduced insight in functioning. She had saccadic pursuit eye movements without gaze palsy or nystagmus, slight dysarthria, broad-based gait with short steps and impaired postural reflexes. Ataxia at upper extremities or rigidity was not found and tendon reflexes were intact. MRI scan of the brain showed generalized and cerebellar atrophy (figure 2).

III:12 This patient already suffered from gait problems due to a previous hip fracture, but developed a "waddled" walking pattern from the age of 69. He showed increasing difficulties with pronounciation, writing, dressing and memory functions. At age 70 the medical records note dysarthria and a waddled gait pattern, without any other abnormalities. CT imaging showed cerebellar atrophy. The clinical diagnosis of olivoponto-cerebellar atrophy was suspected. Our neurological examination four years later showed difficulty understanding simple instructions, apraxia, severe, perseverations, and reduced insight of illness. He had saccadic pursuit eye movements without gaze palsy, severe dysarthria and broad-based gait, without ataxia at upper extremities.

III:15 This brother of the proband developed difficulty of speaking (without dysphagia) and mental decline at the age of 67. Neurological examination three years later showed highly distractible behaviour, perseverations, difficulty following instructions, positive applause sign and no insight of illness. The patient had normal eye movements, severe dysarthria, positive snout and glabella reflexes, slightly impaired coordination at upper extremities and broadly based gait with intact postural

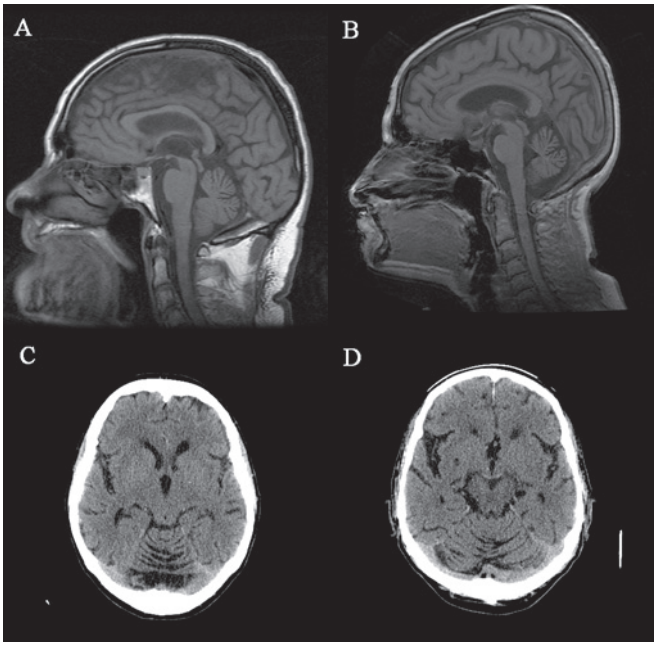

Figure 2. Neuroimaging features of affected individuals. Midsaggital T2 Magnetic Resonance imaging of individual III:13 (A) and individual III:10 (B). Axial Computed Tomography images of individual III:19 (C) and individual III:15 (D).

and tendon reflexes. CT imaging showed isolated cerebellar atrophy (figure 2).

III:19 This patient developed gait disturbance with occasional falling, cognitive decline and apathy from the age of 61. Neurological examination three years later showed highly distractive behaviour, disorientation, perseverations and reduced insight of illness. She had reduced spontaneous speech, had an impaired language comprehension, and a positive applause sign. Further neurological exam showed dysarthria, upward vertical gaze palsy, broad-based gait with mild shuffling, festination and reduced arm swing with intact postural reflexes. Isolated cerebellar atrophy was visible on CT imaging of the brain (figure 1).

III:20 This women presented with behavioral changes and gradual decline in cognitive functioning at age 50. Over the following 10 years, she developed distractible and childish behaviour with inadequate laughing, aggressiveness, perseverations, disorientation, memory problems and lack of insight into illness. The clinical diagnosis frontotemporal dementia was suspected. Our neurological examination at the age of 60 revealed above-mentioned cognitive dysfunctions, motor restlessness and stereotypic movements, upward gaze palsy, dysarthria,

ID	Sex	Age at onset	Disease duration	Current age / at death #	Clinical features							
					D	G	L	SP	GP	P	C	Imaging
II:2	F	55	12	67 #	+	+	?	?	?	?	+	na
II:3	M	-	-	64 #	?	?	?	?	?	?	?	na
III:1	F	76	10	86 #	?	+	?	?	?	?	+	na
III:2	M	50	20	70 #	?	+	?	?	?	?	?	na
III:3	F	60	15	75 #	?	+	?	?	?	?	?	CA§
III:4	M	70	14	84 #	?	+	?	?	?	–	+	na
III:6	F	66	14	80 #	+	+	+	?	?	?	+	N§
III:7 *	M	75	11	86	±	–	–	+	+	–	+	GA§
III:10*	F	72	10	82	+	±	–	+	–	–	+	CA, GA¶
III:12*	M	69	10	79	+	+	–	+	–	–	+	GA§
III:13*	M	65	13	78	+	–	–	+	+	+	+	CA, GA¶
III:15*	M	67	9	76	+	±	±	–	–	–	+	CA§
III:19*	F	60	9	69	+	±	–	–	+	±	+	CA§
III:20*	F	50	14	64 #	+	+	–	–	+	–	+	CA¶
IV:1 *	M	67	5	72	±	+	±	+	+	±	+	GA¶
IV:2 *	F	-	-	68	–	±	–	–	–	–	±	na

Table 1. Summary of demographics and clinical symptoms of the affected individuals.
Clinical symptoms: D= dysarthria, G= gait ataxia, L= upper limb ataxia, GP= gaze palsy, SP= saccadic pursuit, P= parkinsonism, C= cognitive impairment. *Imaging:* CA= cerebellar atrophy, GA= generalized atrophy, N= normal, na= not available. § CT imaging, ¶ MR imaging. – = absent; ± = subtle; + = present; ?= unknown. * personally examined by investigators.

and broad-based gait with small steps. MR imaging of the brain at age 59 showed cerebellar atrophy. She died from bronchopneumonia at age of 64 and brain autopsy was performed (see section pathology).

IV:1 Although the patient himself did not experience any complaints at the age of 63, his wife has noticed a slight behavioural change with loss of patience and increased impulsiveness, together with memory problems. Neurological examination five years later showed a MMSE score 22/30 and FAB score of 5/18. He had saccadic pursuit eye movements with slight upward gaze palsy, normal gait, but impaired tandem walking. In the next four years his cognitive functioning decreased. He showed chaotic behaviour and neuropsychological testing at age 72 showed impairment in executive and visuoconstructive functions and mild memory problems. Furthermore, he showed poverty of speech, motor relentlessness and a broad based walking pattern. No rigidity or tremor was observed and tendon reflexes were symmetrical low. MR imaging at ages 72 showed generalized atrophy. He was diagnosed as vascular dementia/Alzheimer's disease.

IV:2 This woman did not experience any physical or mental complaints. According to her daughter, she became forgetful and chaotic in conversations, and developed

walking instability with occasional side steps. Neurological examination showed an MMSE of 26/30 and FAB 11/18 and disturbed tandem walking, but no other abnormalities.

Linkage analysis

Diagnostic testing for *MAPT, PSEN1, PRNP, HD, DRPLA, FXTAS, SCA1, SCA2, SCA3, SCA6, SCA7* and *SCA17* did not reveal any pathogenic variations.

Two-point linkage analysis revealed LOD scores > 1.0 for 7 markers (Table 2).

Chromosome	Marker	Location (cM) according to Marschfield	LOD score at θ =0
2	D2S319	7.6	-1.70
	D2S2211	15.6	1.20
	D2S162	20.0	-1.88
2	D2S364	186.2	-1.33
	D2S117	194.5	1.46
	D2S325	204.5	-1.94
4	D4S405	56.9	-2.21
	D4S1592	69.5	1.34
	D4S329	80.4	-0.92
7	D7S516	41.7	0.57
	D7S484	53.5	1.31
	D7S510	59.9	0.60
8	D8S284	143.8	-1.39
	D8S272	154.02	1.11
13	D13S171	25.08	1.18
	D13S217	17.2	0.13
18	D18S59	0	1.64
	D18S63	8.3	0.67
	D18S452	18.7	0.37

Table 2. Summary of the STR markers (and surrounding markers) with LOD score > 1.0 from the genome wide linkage analysis.

Highest LOD score was obtained from marker D18S59, with a LOD score of 1.64 at theta 0.0. We choose further refinement of this region with seven additional STR markers (D18S1140, D18S476, D18S1105, D18S818, D18S1098, D18S481 and D18S976) and 14 SNPs (rs7244087, rs4797209, rs544609, rs666530, rs503034, rs514253, rs502396, rs2847149, rs2606246, rs2741186, rs2847325, rs9961743, rs7237153 and rs2231187). Reconstruction of the haplotypes was only possible in the right branch of the pedigree (individuals III:9 to III:20). However, two markers (18S1140 and D18S59) and eight SNPs (rs502396, rs2847149, rs2606246, rs2741186, rs2847325, rs9961743, rs7237153 and rs514253) showed a possible shared haplotype segregating through the pedigree with the disease (figure 1). Through recombination at rs503034 and

rs2231187, this region was narrowed down to ~500.000 bp, including 6 known genes: CETN1, CLUL1, TYMS, ENOSF1, YES1, ADCYAP1, and one predicted gene (C18Orf56). Direct sequencing of the coding regions and 3' and 5' regulatory regions of these genes revealed no pathogenic variations.
None of the markers with LOD score above 1.0 were in any of the loci known to be associated with SCA, except for the marker on chromosome 7, which is close by the SCA21 locus.[3] Through further finemapping of this region with markers D7S2496, D7S817 and D7S2251, we could reconstruct the haplotypes of the right branch of the pedigree (individuals III:9 to III:20). Although reconstruction at the left branch of the pedigree was not possible, one STR marker possibly co-segregated through the pedigree with the disease (D7S484), but this marker does not overlap with the SCA21 locus.

Neuropathology
Macroscopic inspection of the brain (weight 1090 gram) of individual III:20 showed slight atrophy, mostly at the temporal pole, and with slightly, dilated ventricles and a small size of the cerebellum. Both substantia nigra and locus coeruleus were normally pigmented. Microscopic examination with routine staining (HE, methenamine-silver stain, Congo, Gallyas silver stain) showed no abnormalities in cortical areas, except for focal neuron loss in the parietal and occipital cortex. Moderate numbers of neurofibrillary and extracellular tangles were visible in the parahippocampal gyrus (Braak stage 1). Basal ganglia, substantia nigra, locus coeruleus, amygdala, dentate nucleus and pons showed no abnormalities with routine staining. There was almost complete loss of neurons in inferior olives of the medulla, and of Purkinje cells with Bergmann gliosis in the cerebellum.
Immunohistochemistry with the p62-antibody showed few neuronal intranuclear inclusions (NII) in all cortical areas, striatum, brainstem and cerebellar granular cells (figure 3). No NII were found in the gyrus cinguli, dentate nucleus and cervical spinal cord. Hippocampus showed few NII in the cornu ammonis 1-4, while in the dentate gyrus p62 positive neuropil inclusions were seen. p62 positive neuropil inclusions were also visible in the cerebral cortex, gyrus cinguli, brainstem, dentate nucleus and spinal cord.
Immunostaining with 1C2-antibody showed abundant diffuse nuclear staining (DNS) in all cortical areas, sometimes accompanied by cytoplasmic inclusions (CI), while no NII were seen (figure 3). Interestingly, many 1C2 punctate CI and neuropil inclusions were seen in gyrus cinguli without nuclear staining. Abundant 1C2-positive DNS was seen in striatum, with many 1C2-punctate CI in striatum and brainstem. Moving through the Cornu Ammonis (from region 4 to 1), an increasing

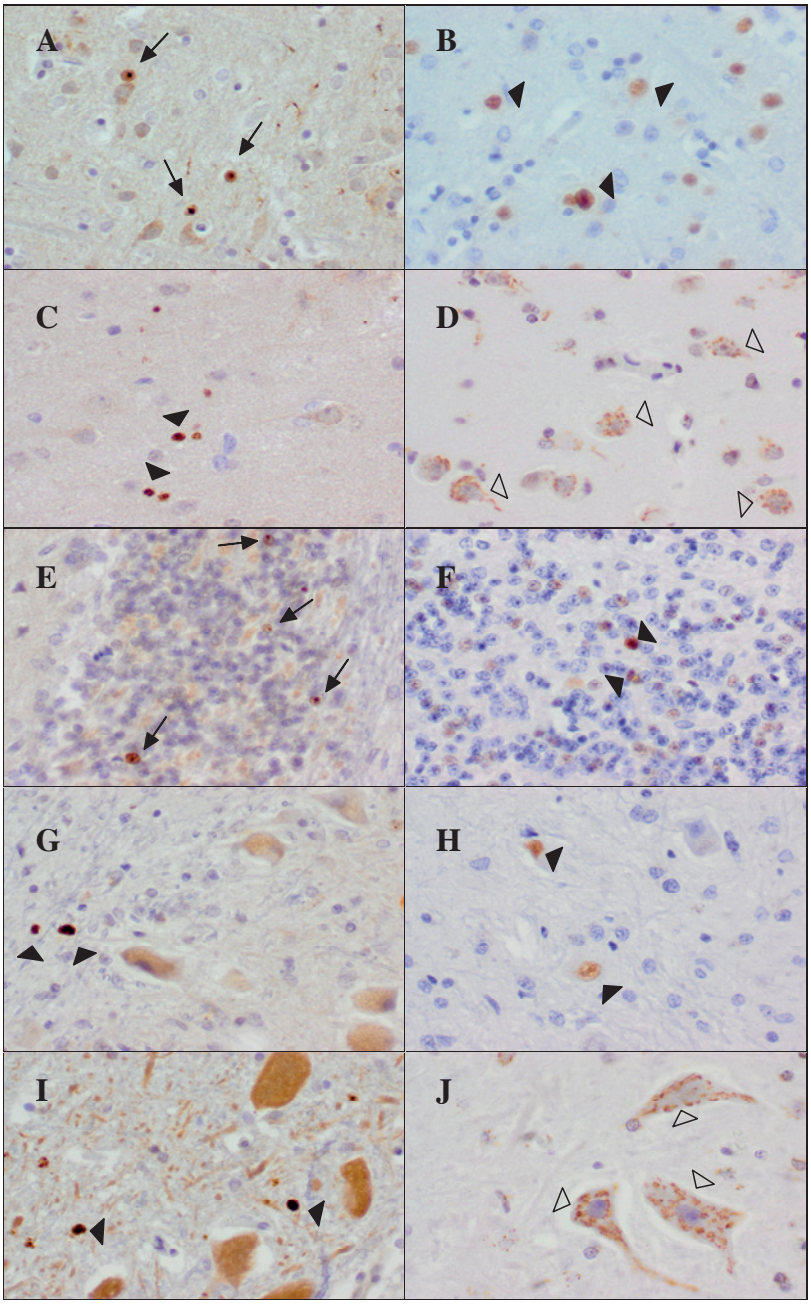

Figure 3. Microscopic examination of individual III:20.
Occipital cortex (A,B), gyrus cinguli (C,D), granular layer of cerebellum (E,F), dentate nucleus (G,H) and nucleus hypoglossus (I,J). Immunostaining with p62 (A,C,E,G and I) and 1C2 (B,D,F,H and J) shows intranuclear inclusions (arrows), diffuse nuclear staining (filled arrow heads) or cytoplasmic inclusions (open arrow heads)

number of punctate CI and DNS was seen. Dentate gyrus showed many punctuate CI and occasional DNS. In the few remaining Purkinje cells, no inclusions were found. Cerebellar granular cells showed abundant DNS, but few DNS were seen in dentate nucleus. The spinal cord showed few DNS and punctuate CI. Table 2 gives an overview of the polyglutamine inclusions of the present case in comparison with other polyglutamine SCAs.

Immunohistochemistry for alpha-synuclein and TDP-43 antibodies in selected brain regions were negative. Staining with AT8 tau-antibody showed a moderate number of neurofibrillary tangles and tau-positive threads in the parahippocampus, and a few of both in the Ammon's horn. Other brain regions did not show tau positive inclusions.

	Present case	SCA1	SCA2	SCA3	SCA6	SCA7	SCA8	SCA17	DRPLA
Cerebral cortex									
NII	+	+	+	+	–	+	–	+	+
DNS	++	+	–		–	–	–	++	+
CI	+	–	–	+	–	+	–	–	+
Basal ganglia									
NII	+	+	+	++	–	+	–	+	+
DNS	++	+	–		–	–	–	++	++
CI	++	–	–	+	–	+	–	–	++
Brainstem									
NII	+	++	+	++	–	+	+	+	+
DNS	–	+	–	+	–	–	+	++	++
CI	++	+	–	+	+	+	–	–	++
Purkinje cells									
Neuronal loss	++	++	++	+	++	++	+	+	–
NII	–	–	–	–	+	+	+	–	–
DNS	–	–	–	–	–	–	+	++	
CI	–	–	++	–	++	–	–	–	–
Dentate nucleus									
NII	–	+	–	++	–		+	+	+
DNS	+	+	–		–		+		++
CI	–	–	–	++	+		–	–	++

Table 3. Distribution and frequency of polyglutamine containing inclusions.[2, 8, 14-15, 23-25]
– absent, + present, ++ abundant. NII= neuronal intranuclear inclusions, DNS= diffuse nuclear staining, CI= cytoplasmic inclusions.

Discussion

This paper describes a new hereditary disorder with late-onset progressive cerebellar ataxia, parkinsonism, opthalmoplegia and prominent cognitive impairment. The neuropathological picture is strongly suggestive for a polyglutamine disorder, with widespread 1C2 positive inclusions. Although we could not demonstrate significant linkage, seven markers showed a possible locus of the genetic defect, with highest LOD score on chromosome 18q.

The occurrence of ataxia in multiple affected individuals in the present family fits best an autosomal dominant mode of inheritance. The unaffected status of the father (individual II:3) may reflect reduced penetrance, genetic anticipation in his offspring, or results from the relatively early age at death before the development of clinical signs. The number of affected individuals from successive generations in the present family is too small to draw firm conclusions about genetic anticipation. A recessive mode of inheritance cannot be ruled out for sure, although consanguinity could not be detected in the previous four generations (data not shown).

Cognitive impairment as the core clinical feature in the present family has also been found in other SCA subtypes, but is usually preceded by cerebellar symptoms.[4] The presence of frontal-like symptoms and executive dysfunction in the present family have also been reported in SCA1-3, SCA6, SCA10, SCA17, SCA19.[4-6] It is not surprising that the clinical diagnosis of frontotemporal dementia has been established in one individual of the present family (III:20). The observation of reduced cerebral blood flow in the prefrontal cortex in SCA6 reflects its involvement in cognitive dysfunction,[7] but an interesting question is whether its origin lies primarily in the cerebellum or in the disruption of cerebrocerebellar or cortico-striato-thalamo circuits, or in the direct involvement of the neocortex. A recent study demonstrates widespread neurodegeneration in SCA6 patients, which probably accounts for the variety of disease symptoms.[8] The patient who came to autopsy within the current pedigree showed intranuclear inclusions (NII and DNS) in the neocortex, but also many 1C2-positive inclusions in the hippocampus. Various diagnoses including Alzheimer's disease, FTD, Huntington's disease, ataxia and PSP have been considered in affected individuals of the present pedigree, and indicate the clinical heterogeneity of the present SCA type. The combination of parkinsonism with ophthalmoplegia in the proband have raised suspicion on the diagnosis PSP. This diagnosis has been described earlier in other SCA subtypes, including SCA2 and SCA17. [9] [10] This is in line with the fact that parkinsonism is part of the clinical phenotype of several SCA types, reflected by a prevalence of SCA2 gene mutations in 1.5 - 10% of patients with familial parkinsonism. It is usually accompanied by atypical features although,

but a PD phenotype with levodopa response is also possible. The occurrence of parkinsonism is often associated low-range repeat expansions.[11] Reduced uptake on FP-CIT scan has been found in an asymptomatic SCA2 carrier and marked neuronal loss in the substantia nigra (without Lewy body pathology) in another individual without clinical signs of parkinsonism, [12] indicating that additional factors contribute to parkinsonism in SCA. Evidence for neuropathy was found in two patients in the current family, but unknown in the other affected family members.

Generalized atrophy on MRI in some individuals of the present family is similar to other SCA types (SCA12, SCA17, SCA19 and DRPLA), while isolated cerebellar atrophy in most affecteds of the present family is the characteristic picture of SCA. The presence of 1C2-positive NII in the brain of a patient from the current pedigree is strongly suggestive for a polyglutamine SCA subtype, as it has been considered to be the pathological hallmark of CAG repeat disease.[2] Still, 1C2-positive inclusions have also been found in SCA8, which is caused by an intronic CTG repeat expansion. The distribution of neuronal loss and inclusions of the present case shows differences as well as similarities to other polyglutamine SCAs. The almost complete loss of Purkinje cells in the present case resembles that of most other polyglutamine SCAs (except in SCA3 and DRPLA).[2] In the few remaining Purkinje cells, no inclusions were found, which is in line with the pathological reports of SCA1. A possible explanation might be the more rapid degeneration of these cells (although one would expect to detect at least a few Purkinje cells with NII). Another explanation might be the presumed protective effect of NII and as a consequence Purkinje cells without NII being more vulnerable to neuronal death. [13].

In DRPLA and SCA17 a similar staining pattern with few NII but abundant DNS as the current case is seen. DNS with 1C2 antibody can be restricted to regions with NII formation as in SCA1, or only seen in Purkinje cells, medullary and dentate neurons as in SCA8, whereas it is rare or absent in SCA2, SCA3, SCA6 and SCA7. The widespread punctate cytoplasmic inclusions with 1C2 antibody in striatum, gyrus cinguli, Cornu Ammonis and brainstem of the present case, has also been found in DRPLA and SCA3, whereas its presence is restricted to a few regions in SCA1 and SCA7, and only found in Purkinje cells in SCA2 and SCA6. This observation in the latter is controversial as studies showing conflicting results.[14-15] The staining pattern of the hippocampus of the present case strongly resembles to what has been found in Huntington's disease.[16] Electron microscopic immunohistochemistry in SCA3 and DRPLA have shown that the cytoplasmic granules correspond to lysosomes, suggesting the involvement of lysosomal pathway for the degradation of mutant proteins with expandend polyglutamine stretches.[17-18] From a recent paper studying the frequencies of occurrence of the various staining patterns (NII, DNS and CI)

in SCA3, an order of events is suggested, in which CI are an initial event, whilst neurons with NII represent later stages of pathology.[18]

The detection of inclusions by the 1C2-positive antibody is strongly suggestive for an expansion of at least 38 CAG repeats, although 1C2-positive NII and CI have been found in a SCA6 case with 22-24 polyglutamine streches [14]. It is therefore possible that other proteins (such as TATA box proteins and transcript factors) containing longer polyglutamine stretches may interact with the disease protein and aggregate into these inclusions. Furthermore, some caution should be taken with the interpretation of 1C2 positive inclusions in the substantia nigra, as normal controls have demonstrated 1C2 immunoreactivity in the substantia nigra (Marinesco bodies), locus coeruleus, and pituitary gland.[16]

NII have been found in degenerated as well as in spared brain regions in polyglutamine SCAs, and the lack of significant association between the formation of inclusions and cell death,[19] suggests that NII are not directly pathogenic in affected nerve cells. In an animal model of Huntington's disease, the formation of inclusions in striate neurons has shown to be protective.[20] A recent hypothesis suggests that instead of the formation of inclusions, the development of polyglutamine oligomers may have an important role in cytotoxicity.[21] The negative staining with 1C2 of p62-positive NII in the present case contrasts to the observations in SCA3.[18] As the phosphoprotein p62 aggregates poly-ubiquitinated mutant proteins,[22] it might be possible that the latter becomes buried in the inclusions. Although the pathological findings of the present and single case does not fit well into any of the known polyglutamine SCAs, we should bear in mind that variation exists in pathological phenotype within SCA subtypes. Also, the selective vulnerability of specific brain areas among different SCA types may result from the (ab)normal function of the (mutated) gene products or its interaction with other proteins.

The exclusion of all known CAG repeat expansions in SCA genes and DRPLA encouraged us to search for a novel gene defect. The 1C2 positivity makes one of the other conventional SCA mutations very unlikely, and additionally, linkage analysis did not give an increased LOD score above 1.0 with any of these regions, nor in any of the SCA loci with significant linkage. Furthermore, the clinical and pathological features of the current pedigree does not fit into the picture of these SCAs: in SCA4, SCA18 and SCA25 no cognitive impairment has been reported and in SCA4 no 1C2 positivity is seen; the age at onset in SCA18 and SCA19/22 is strikingly younger, with a very slow progression in SCA19/22 not influencing life expectancy and in SCA18 the neuropathy is the clinical hallmark of the disease.

We choose to perform an affected-only analysis, which reduces the statistical power to detect linkage, but increases the probability to find the true genetic locus. The

highest LOD score was found on chromosome 18. We studied this region in more detail, but could not identify pathogenic variations in the genes of this (possible) critical region. The region on chromosome 7 is close to, but did not overlap with the SCA21 locus, and the clinical and pathological phenotype differ between both disorders.

In conclusion, we have identified a new late onset SCA subtype with prominent cognitive deficits. The pathological report is strongly suggestive for a polyglutamine repeat disorder. The next step will be the identification of the genetic defect, which will shed new light on the pathogenesis of CAG repeat disorders and hopefully lead to the development of therapeutic options.

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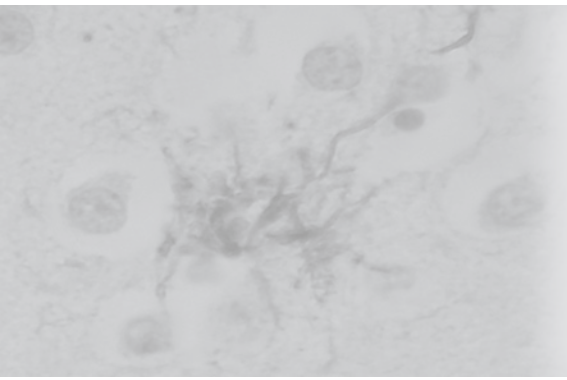

Chapter 4

General Discussion

Progressive supranuclear palsy (PSP) constitutes a separate group called 'atypical parkinsonism' together with multiple system atrophy (MSA), corticobasal degeneration (CBD) and dementia with Lewy bodies (DLB). In addition, PSP has clinical overlap with disorders in the spectrum of FTD. The disorder is relatively rare, with prevalence rates around 5 per 100.000. Many studies over the last two decades have enormously increased our knowledge about clinical, genetic and pathologic aspects of the disease. International clinical consensus criteria have helped its recognition in clinical practice and have proven to be useful in research studies. Clinicopathological studies have shown that the clinical presentation PSP is more heterogeneous than previously considered. Pathological and genetic studies have emphasized an important role of MAPT in the pathophysiology of the disease. This thesis describes the clinical and genetic aspects of a large collected cohort of PSP patients in the Netherlands.
In this chapter the main findings of our studies are discussed in the light of current literature and suggestions for future research are made

Clinical aspects in PSP

PSP and its subtypes
Determination of a correct clinical diagnosis of PSP is necessary for adequate management of the patient and caregiver; its progressive nature, additional cognitive impairment and early swallowing problems have a considerable impact on daily functioning of the patient. Also for therapeutic interventions in the future, it will be important to distinguish the disease from related disorders with different pathophysiological processes. According to the NINDS consensus criteria (see table 1 in chapter 1),[1] the presence of early falls and vertical gaze palsy / slowed vertical saccades have a high predictive value for the definite diagnosis PSP. Despite the use of these criteria false-positive diagnoses occur and include PD, AD, MSA, CBD and Pick's disease,[2] whereas false negative cases are mainly due to the absence of vertical gaze palsy during the disease course. Not surprisingly, all patients with probable or possible PSP in our cohort that came to autopsy had the definite PSP diagnosis, except for one case. One potential drawback of this high rate of true-positive cases is the lack of inclusion of atypical cases. Clinicopathological studies over the last decade have further broadened the clinical picture of PSP, resulting into a new nosology in PSP with Richardson syndrome as classical presentation, and PSP-P as the parkinsonian subtype.[3] This subtype was retrospectively identified by reviewing the clinical symptoms in the early (<2years) or late (>2 years) stage of the disease in pathological-proven cases of atypical PSP. By using a similar method, the current

thesis identifies the new clinical subtype with frontal presentation (cognitive and behavioural changes) into the spectrum of PSP, in which the core clinical features of PSP eventually develop and with a disease progression similar to classical PSP.[4] Patients with this subtype are difficult to be recognized in the early stage, as reflected by the high rate (93%) of false-negative diagnoses at first neurological visit. The cognitive and behavioural changes are frequently suggestive for FTD. The presence of apathy with relatively low scores on the mood subtest can not simply be accounted for by depression.[5] Disinhibition, aggression, stereotypic behaviour and alterations in eating habits are also observed in PSP, although less frequent compared to FTD patients. Also, deficits in recognizing negative emotions and loss of insight, characteristically for FTD, may sometimes occur in PSP.[6-7] The identification of this frontal subtype within the PSP spectrum is partly due to parallel research on FTD in our center at Rotterdam, whereas parkinsonian subtypes (i.e. PSP-P) have been reported from centers specialized in movement disorders. Excitingly, the existence of the first subtype has been confirmed in a recent study with cases from several brain banks in Europe and Canada.[8] The PSP-P subtype also occurred, although relatively infrequent, whereas the majority showed the classical phenotype of Richardson syndrome. This indicates that the NINDS-SPSP criteria need to be revised with the incorporation of atypical features in the clinical diagnostic criteria as their sensitivity has proven to be low. Interestingly, behavioural changes and cognitive decline have been described in several patients in the original description of Steele et al.[9] Also, cognitive problems were reported as the initial presenting complaint in 15% of a series of 187 prevalent cases,[10] whereas dementia defined by the DSM-IV criteria occurred in 52% of a series of 110 cases.[11] The cognitive profile usually denoted as subcortical dementia shows strong similarities to dementia in PD, though occurring earlier and more severe.[12] The cognitive domains most vulnerable in PD have recently been gathered in a short, practical test battery (SCOPA-COG) and includes memory, attention, executive and visuospatial functioning.[13] We found no difference in memory and attention problems between PD and PSP patients at equivalent disease course, whereas visuospatial dysfunction and verbal fluency were more impaired in PSP. Observations from several studies support the hypothesis that both frontal and subcortical damage contribute to the cognitive deficits in PSP. More cortical tau pathology is found in PSP patients presenting with dementia[14] and lower brainstem and frontal volumes are associated with cognitive impairment in PSP.[15] Correlations between tests on cognition and motor performance, suggest that in PSP cognitive decline occurs in parallel with motor impairment,[4, 12] with atrophy in one brain region running parallel with that in another.

The larger clinical spectrum of PSP leads inevitably to more overlap with other neurodegenerative diseases, as visualized in figure 1. The pathological distribution of tau lesions proves to be related to the clinical subtype, with restricted subcortical pathology in PSP-P and PAGF, abundant cortical pathology in PSP-FTD and PSP-CBS, and widespread involvement in the classical presentation (RS).[16] There is considerable clinical and pathological overlap between PSP and CBD, sometimes with vertical gaze palsy and falls in CBD, and vice versa, asymmetrical features, apraxia and alien limb phenomena in PSP. Progressive apraxia of speech, nonfluent aphasia (PNFA), or a combination of these may occur in both disorders. Both PSP and CBD belong to the 4R tauopathies and share the *MAPT* risk haplotype (see below). One may therefore argue that these disorders represent part of a spectrum, rather then two different disorders.

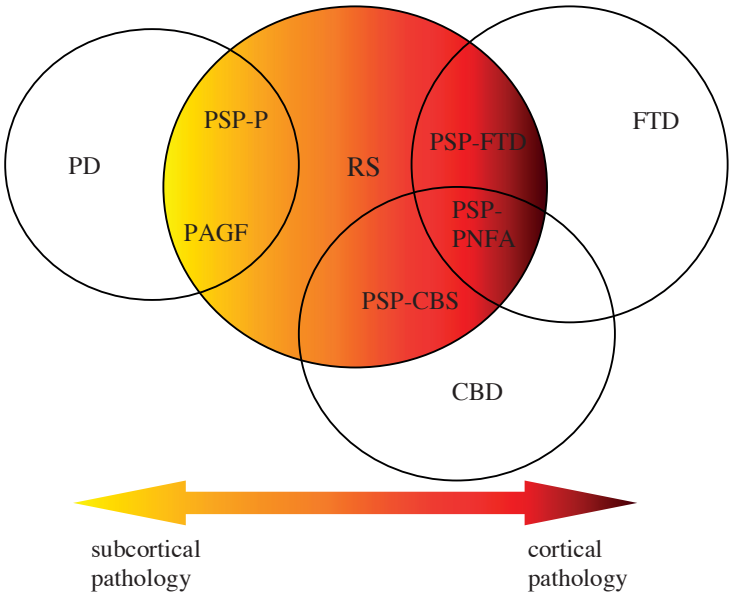

Figure 1. The clinical spectrum of PSP and its relationship to pathology.
RS= Richardson Syndrome; PSP-P= PSP-Parkinsonism; PAGF= Pure Akinesia of Gait Freezing; PSP-PNFA= PSP-Progressive Non-Fluent Aphasia; PSP-CBS= PSP-Corticobasal Syndrome; CBD= Corticobasal degeneration; PD= Parkinson's disease; FTD= frontotemporal dementia

PSP versus PD

PD is the most frequent misdiagnosis in PSP patients, occurring around 30% at first neurological visit,[4] especially when typical features of PSP are lacking. Patients with the misdiagnosis PD show predominantly bradykinesia and limb rigidity, and have tremor in a high percentage of cases. In chapter 2.2 we have investigated

the occurrence of motor features in PSP and PD by using validated rating scales and found more speech and swallowing difficulties in PSP, as well as problems in rising from a chair, while tremor, arm rigidity and gait problems were significantly less observed. These features showed however considerable overlap and none of these can fully discriminate between both disorders. This emphasized that pattern recognition beyond any formal set of diagnostic criteria remains essential for a correct diagnosis, as has been demonstrated for neurologists specialized in movement disorders.[17] Moreover, the more rapidly progressive nature in PSP remains an important hallmark of the disease. Although drug induced dyskinesias, late autonomic dysfunction and visual hallucinations help to distinguish PD patients from PSP-P,[18] finding diagnostic tests that discriminates both disorders is one of the major challenges for the next years. The relative percentage PSP-P in the total group of PSP is not exactly known, with estimates between 5% and 32% across studies. Whereas clinical series probably may underestimate its true frequency, pathological studies may be biased toward a higher percentage as more atypical cases come to autopsy.

Frequent falls occur in the early stage of PSP and are often a disabling symptom. Impaired balance in PSP has been associated with hypometabolism of the thalamus,[19] which receives cholinergic projections from the pedunculopontine tegmental and laterodorsal tegmental nuclei. Subcortical cholinergic activity measured on PET scans has been found more decreased in MSA-P and PSP than PD, which may account for the greater gait disturbances in the first two disorders.[20] Studies examining the clinical correlates of pathological changes across different disorders are important to understand which brain regions are involved in the clinical symptoms. In this way, an association was found between the absence of resting tremor and the resistance to L-dopa treatment to greater atrophy of the globus pallidus in PSP, MSA and PD patients. [21]

Autonomic dysfunction in PSP is controversial and studies showing conflicting results. From the results in chapter 2.2, we suggest that history taking on autonomic dysfunction should however be part of the examination of PSP patients, as autonomic symptoms occurred more frequent in PSP patients compared to normal controls. Whether other causes may account for this difference such as co-morbidity or medication use can not be ruled out. Autonomic symptoms were more frequent in PD patients compared to PSP which supports the increasing awareness of autonomic dysfunction in PD. There is substantial evidence that αsynuclein pathology beyond the nigrostriatal dopaminergic system is involved in non-motor features of PD.[22] Systematically examination of these structures in PSP patients may provide more information about a possible role for autonomic dysfunction.

Survival in PSP

Both PSP and FTD are rapidly progressive diseases. FTD has been found to progress faster then AD, and disease duration in PSP is much shorter compared to PD. Survival within the FTLD spectrum has not been extensively investigated, except for small series of patients. Depending on the definition of tau-positive and tau-negative cases within the FTLD spectrum, survival rates differed considerably between several studies. Not surprisingly, a reduced survival was found in FTD-MND cases (median survival of two years),[23] in the study of Hodges et al, as well as in other studies. As inclusion of these cases in the tau-negative group dramatically reduces the survival rate, we therefore excluded MND cases from our analysis and found 1) worse survival in PSP versus FTD patients and 2) a trend towards better survival in FTD-tau compared to FTLD-U. A better survival in tau-positive group has found to be even more pronounced in case of a higher percentage of sporadic Pick's disease.[23-25] Inclusion of more PSP and CBD cases in the tau-positive group has resulted into a shorter survival, as demonstrated in the study by Xie et al.[26] This implicates that the group with tau pathology is heterogeneous with respect to survival. The question is whether this is due to the different composition of tau isoforms or the more involvement of basal ganglia structures. We found a trend towards a better prognosis of 3R tau cases compared to 4R tau within the FTD cases, which has been observed before.[27] Another argument for better prognosis of 3R tauopathies, is the observation of longer survival for patients with PSP-P compared to classic RS by Williams et al.[28] In their clinicopathological series, PSP-P group consisted of more 3R tau pathology. The involvement of basal ganglia structures has also found to be associated with worse survival in PSP patients.[26] Furthermore, a trend towards reduced survival in FTD patients presenting with extrapyramidal signs has been observed and tests which depend on the integrity of frontal-subcortical circuits, such as reverse digit span and letter fluency, are associated with reduced survival in FTD.[24]

Within PSP and FTD patient groups, survival rates may still vary considerably, indicating that other genetic and /or environmental factors should play a role. Family history had a negative effect on survival in the FTD group but not in PSP, which suggest that genetic factors may play a more important role in survival of FTD than in PSP. On the other hand, the effect of older age at onset on survival in PSP was not found in FTD. It is possible that age related (environmental?) factors have an invigorating effect on the disease process in PSP, which leads to a shorter survival.

Tau pathology

The tau protein consists of six distinct isoforms in the brain, which are produced from the tau gene by alternative splicing (see figure 2). In physiological conditions, the tau protein binds and stabilizes microtubules, which are involved in intraneuronal vesicle and organelle transport. Neurofibrillary tangles,tufted astrocytes and neuropil threads consist mainly of hyperphosphorylated 4-repeat (4R) isoforms in PSP. The mechanism how normal non-phosphorylated tau protein assemblies into aggregates in PSP is not known. Under pathological conditions, the tau protein becomes hyperphosphorylated by kinases, and detached from microtubules and thereby destabilizing the microtubules; this results in a 'loss of function'. Furthermore, unbound tau (particularly the 4R isoform), is prone to form aggregates which results in a 'toxic gain of function'.

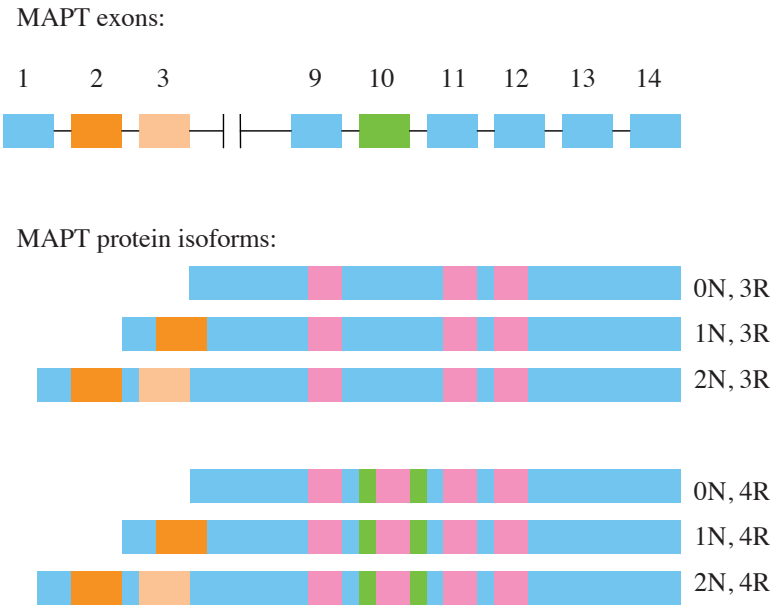

Figure 2. Structure of the MAPT splicing products.
Through alternative splicing of exon 2, 3 and 10, six isoforms are produced. The in-or exclusion of exon 10 (green) results in 3 or 4 microtubule binding domains (pink) respectively.

The neuropathological changes of the 20 PSP cases described in chapter 3.1 are consistent with the definite diagnosis of PSP. In line with Western blot findings in the literature, the aggregates stained positive to the 4R tau antibody and negative to 3R tau antibody. However, the density of tau lesions in distinct brain regions differed considerably among the cases. Such pathological heterogeneity has also been

reported in recent studies. In these studies, the subthalamic nucleus and substantia nigra were the most severely affected regions in PSP whereas considerable differences were observed within the cerebral cortex, pons, caudate, cerebellar dentate nucleus and cerebellar white matter.[28] Williams and colleagues developed a scoring system to assess the severity of tau pathology in PSP cases taking into account the tau burden in oligodendroglia and threads in three anatomical regions: the substantia nigra, caudate and dentate nucleus. They also showed that higher tau scores were associated with more widespread tau pathology and shorter disease duration. Using this scoring system, we confirmed the observation of a negative correlation between tau pathology and disease duration (survival chapter). An intriguing question is which factors may influence the severity and extent of tau pathology. It is possible that the type of tau isoform (3R or 4R) may play a role. The PSP-P subtype with a higher contribution of 3R tau isoforms, shows a significantly lower tau burden compared to classical Richardson syndrome.[28] Another possible explanation might be that the H1C risk haplotype may also account for the extent of tau pathology. Another intriguing question is how the aggregates spread throughout the brain. A recent hypothesis across distinct neurodegenerative disorders suggests that protein aggregates may be transmitted in a prion-like fashion.[29] This may unveil new therapeutic opportunities targeting the cell-to-cell aggregate propagation process.

Genetic studies

Familial aggregation

PSP is classically considered to be a sporadic disorder, although early case control studies described a non-significant trend towards positive family history.[30] We systematically investigated the family history in PSP (chapter 3.1) and found an increased frequency of parkinsonism in first degree relatives (odds ratio 3.9). Although there still exists uncertainty about the type of parkinsonism in affected relatives, these first degree relatives might have suffered from PSP. As PD is the most frequent misdiagnosis in the initial phase of PSP, a diagnosis of PD does not exclude PSP in these cases. Moreover, PSP-P subtype is frequently mistaken for PD during life. Interestingly, symptom-free first degree relatives of patients with PSP scored abnormally on the UPDRS in almost 40%[31] and abnormal striatal uptake on PET scan has also been found in symptom free relatives from two kindreds with familial PSP.[32] Whether this is due to an asymptomatic carrier state for PSP or a shared environmental exposure is, however, not clear.

The growing number of papers describing familial PSP cases refutes the earlier assumption that these reports were 'anecdotal'. The type of inheritance in familial PSP

is not exactly clear; families with an autosomal recessive as well as dominant mode of inheritance have been described. There exist considerable clinical heterogeneity within these families and one may argue that this does not fit into the clinical spectrum of PSP. However, genotype-phenotype studies on causative genes for PD and dementia have taught us that the clinical spectrum is always larger than initially expected. Even the pathological picture may vary, as is observed in PD patients with *LRRK2* mutations.[33] Linkage analysis has been found to be a powerful method detecting genetic defects in familial cases of FTD. To date, it has not been successful in PSP. Collecting large PSP pedigrees for linkage analysis is difficult due to the late onset of the disease, which is often associated with deceased affected individuals, or death of carriers before clinical symptoms may arise. Another reason might be a failure to recognize atypical cases. One study has reported a single family with an autosomal dominant form of PSP and with significant linkage to chromosome 1, but the genetic defect has not been identified yet.[34] The phenotype within this pedigree consisted of the typical PSP presentation (with pathological verification in one), but also of tremor and facial tics, sometimes accompanied by abnormal ^{18}F-fluoro-dopa or ^{18}F-deoxyglucose positron emission tomography (PET) scans. There might be several reasons why the gene defect has not been identified so far. The critical region on chromosome 1 contains many candidate genes, but also phenocopies or wrong assumptions about the genetic model are possible explanations. An oligogenetic model with moderate contribution of two or three genes rather than a pure monogenic model might explain clinical diversity and variable penetrance. One pedigree described in chapter 3.1 of the current thesis shows similarities with the chromosome 1–family. The onset of clinical symptoms of PSP in the proband as well as in her mother has been preceded by decades of isolated essential tremor, whereas three younger family members were suffering from isolated essential tremor. This family is too small to draw conclusions about co-seggregation of the tremor with the disease. In the literature, essential tremor has shown an increased prevalence among first degree relatives of PD patients. However, the nature of this relationship is unknown,[35] as essential tremor proves to have another aetiology than PD in the first family with an α-synuclein gene defect.

Several studies have reported the occurrence of PSP in patients with *MAPT* mutations, although these mutations were not found in large series of PSP patients.[36] In chapter 3.1 we describe one single patient presenting with a PSP phenotype caused by the P301L *MAPT* mutation. The relatively young age at onset (45 years), the prominent behavioural changes and the isolated gaze palsy upward together with a positive family history for dementia were features that point towards the possibility of a *MAPT* mutation. The clinical diversity within a single *MAPT* mutation and even in

single families may be wide, which suggest other genetic factors to play a role. One of such genetic modifiers might be the H1 tau haplotype as homozygosity of this haplotype has been associated with parkinsonism in *MAPT* mutations.[37] Besides the identification of *MAPT* mutations in FTDP-17, a second gene defect in *Progranulin (GRN)* was found in familial FTDP-17 patients with ubiquitin/ TDP43 positive inclusions in 2006.[38] A CBS phenotype is frequently observed among mutation carriers, whereas a PSP presentation was much more rarely found. Moreover, screening the *GRN* gene in all familial PSP patients did not reveal any mutations (chapter 3.1). Interestingly, one PSP patient came from a family with hereditary FTD associated with TDP-43 pathology, which was not caused by *MAPT* or *GRN* mutations. This implicates that there is still another gene defect involved in the spectrum FTD – PSP. [39] After identification of the responsible genetic defect in this family, it will be very interesting to determine whether it may play a role in other familial PSP and FTD cases.

Association studies

There are strong arguments that genetic variants in or around the *MAPT* gene play an important role in the pathophysiology of PSP. The H1 *MAPT* haplotype has convincingly been associated with PSP and plays a predisposing role in the vast majority of sporadic and familial PSP patients. The initial association of the dinucleotide repeat (A0) in intron 9 of *MAPT* with the occurrence of PSP has been subsequently extended to other polymorphisms in linkage disequilibrium in the *MAPT* region. A large haplotype block around the MAPT gene results from an inversion of 900 kb occurring 3 million years ago.[40] Through finemapping, the subhaplotype H1c was found to be associated with PSP, containing one SNP in intron 0 which influences the expression of tau.[41-42] Functional studies show an increase in both total tau transcript as well as more 4R tau containing transcripts[43] and the risk for PSP may result from a lifelong higher level of 4R tau expression. The *MAPT* haplotype may also play a role in other neurodegenerative disorders, as a recent genome wide association study in PD also found this association. [44] The involvement of tau in PD is further supported by interactions between α-synuclein and tau promoting fibrillization, which may drive the formation of pathological inclusions.[45] Double immunostaining of Lewy bodies has suggested that tau may coaggregate with α-synuclein in Lewy bodies, especially in neuronal populations vulnerable to both neurofibrillary tangles and Lewy bodies.[46] Furthermore, the H1 *MAPT* haplotype is association with mild cognitive impairment[47-48] and although genome wide association studies of AD do not identify the *MAPT* locus, association studies with a candidate gene approach, did show an increased risk of AD with H1c haplotype.[49]

In search for other genetic factors involved in PSP, two genome wide association studies (GWAS) have been performed. The first study with a 500K SNP array in pooled DNAs from 288 PSP patients identified a second locus on chromosome 11, apart from the strong association with the H1 *MAPT* haplotype.[50] However, this chromosome 11 locus could not be replicated in a recently performed GWAS study of more than 1100 pathological-proven PSP patients.[51] Apart from SNPs in the MAPT region (odds ratio 5.5), this study revealed significant association with a number of SNPs in interesting genes as STX6, EIF2AK3 and MOBP. These associations were replicated in the joint analysis with additional 1051 clinically diagnosed PSP patients. EIF2AK3 is a gene that encodes PERK, which is a protein that sensors endoplasmic reticulum (ER)-stress and is involved in the unfolded protein repons (UPR). In PSP, activated PERK is detected in brain areas that contain abnormal tau protein and it is present in neurons, astrocytes and oligodendrocytes. Activation of UPR induces the activity of GSK-3beta, a major tau kinase, both in vitro and in postmortem AD brains.[52] In turn, phosphorylation of tau by kinases is one of the prerequisites of aggregate formation. Syntaxin 6 (STX6) belongs to the SNARE proteins which are involved in the fusion of vesicles with membranes and participates in vesicle transport in the endosomal pathway. Finally, Myelin-associated oligodendrocyte basic protein (MOBP) is produced by oligodenodrocytes and highly expresses in the white matter of the medulla, pons, cerebellum and midbrain; although its function remains unknown, it is thought to be involved in myelin formation.

A novel genetic disorder mimicking PSP

One patient from our cohort came from a family which was sufficiently large to conduct a genome wide linkage study. The clinical picture of the proband consisted of frequent falls, parkinsonism, vertical gaze palsy (upward) and cognitive decline, consistent with a clinical diagnosis of possible PSP, but additional family members within this pedigree were initially diagnosed with variable neurological disorders ranging from Huntington's disease, spinocerebellar ataxia, AD to FTD. All affecteds however had a slowly progressive waddling gait together with behavioural changes and cognitive decline in common. The cognitive decline showed overlapping features with the cognitive deficits found in PSP and FTD. It dominated the clinical picture which implies that his gene may play an important role in cognitive functioning. The presence of abundant 1C2 positive inclusions at neuropathological examination of the brain from one of the deceased affected relatives strongly suggests a polyglutamine disorder. This observation conflicts with the generally well-accepted correlation between the clinical presentation and tau pathology at neuropathological examination in PSP. Although, we have to await the neuropathological confirmation

in the proband with a PSP-line phenotype, it is likely that polyglutamine inclusions instead of tau inclusions will be found in this case. Still, from earlier genetic defects we have learned that the pathological variability may be wide, such as found in *LRRK2* mutations. Moreover, besides the few FTDP-17 cases with *MAPT* mutations, other genetic defects with a PSP-like phenotype have been described; a R1441C mutation in *LRRK2* showed clinically PSP-like features and tau inclusions at pathological examination[53] and more recently, a p.Thr272SerfsX23 mutation in *GRN* was associated with PSP phenotype.[54] These observations, together with our present family suggest that more genetic defects are associated with a PSP-like phenotype. The identification of the causative gene defect in this family will elucidate whether it plays a role in mendelian traits of familial PSP cases or as a predisposing genetic risk factor for PSP patients in general.

Tauopathies and polyglutamine diseases are considered as two distinct diseases, but a very recent paper shows a possible link between the two disorders.[55] Intermediate (31-34) polyglutamine repeats of the ataxin-2 gene (ATXN2) were found to be associated with PSP. Although the total number of PSP patients with expanded repeats was low (n=4, 0.8% of cohort), it was significantly higher (OR=5.8, p=0.004) compared to normal controls. The intermediate ATXN2 polyglutamine repeats have also been associated with ALS and in yeast, ATXN2 has shown to be a modulator of TDP-43 toxicity.[56] Expanded ATXN-2 glutamine stretches enhances the interaction of ATXN2 with TDP-43 and promotes TDP-43 mislocalization under situations of stress. Although PSP patients do not show TDP-43 pathology at autopsy, the hypothesis is that ATXN2 polyglutamine repeats also interact with tau and promote tau protein aggregation or mislocalization.

Further studies of the family with clinically PSP, waddling gait and cognitive decline associated with 1C2 pathology are needed to identify the genetic defect. First of all, the pedigree might be extended with additional affecteds, which will increase the power to detect the true linkage region and (hopefully) narrowing the candidate region. Secondly, RED (repeat expansion detection) analysis may be used to detect the gene defect for this polyglutamine repeat disorders, as has been proven to be successful in SCA8 and SCA12.[57] Finally, whole exome sequencing is a more recent and promising way to detect novel gene defects. However, we have to keep in mind that the trinucelotide repeat expansion may be localized in non-coding regions, which has been the case for a few SCA subtypes.

Future directions

Biomarkers

To date, a specific diagnostic test for PSP is lacking and pathological examination remains the gold standard for its diagnosis. Magnetic resonance imaging (MRI) may be helpful in the differential diagnosis of PSP, although overlap in patterns of atrophy exist with other related conditions. Although PET studies are being conducted with several ligands, a specific marker for PSP has to be developed yet. In AD, PET imaging with a specific ligand to amyloid (PIB, or 'Pittsburgh Compound-B') is widely investigated and it appears to be a sensitive and specific marker for underlying Amyloid-beta pathology.[58] Specific tau ligands are currently under development and will have to await its usefulness in PSP. The identification of new biomarkers in CSF remains a promising field because of its direct localization to dying neurons. At the same time, it may provide problems finding a specific biomarker for the disease due to the breakdown of neurons releasing several aspecific proteins. For instance, raised neurofilament light and heavy chain levels probably reflect the rapid progression of the disease in PSP rather then being a specific biomarker.[59] A decreased ratio of 33kDa /55kDa proteolytic tau products has been proposed as a very promising biomarker in PSP.[60] Unfortunately, these results in the original study could not be reproduced by a recent study, in which the 33 and 55kDa fragments might reflect the light and heavy chains of IgG used in the assay. A proteomic approach without prior assumptions has been applied in AD and PD patients and has identified eight potential biomarkers. Similarly, reproduction of these results is necessary to exclude false positive findings due to background contaminants.

Pathophysiology

There is increasing evidence that mitochondrial dysfunction plays an important role in PSP;[61] postmortem studies of PSP patients show oxidative damage and cell lines with mitochondria from patients with PSP showed reduced activity of complex I, ATP-production and oxygen consumption. Dysfunction of mitochondrial complex I decreases ATP levels and induces reactive oxygen species which have found to activate many tau-kinases in PSP-neurons and glial cells. In PSP brains there is also a failure in upregulating chaperones which may protect the cell from oxidative stress. The role of mitochondria is further supported by the association between PSP-like cases and the chronic consumption of acetogenins containing plants on Gouadeloupe.[62] Acetogenins are extremely potent inhibitors of complex I and when administered intravenously to rats, it induces neurodegeneration in basal ganglia and brainstem nuclei; immunohistochemical studies in cultured neurons of rat striatum showed redistribution of the tau protein from the axons to the cell body and finally cell death.

There exist several complex I inhibitors to which humans are potentially exposed. In foetal rat striatum, a large number of these compounds caused decreased ATP levels, induced neuronal cell death and caused somatodendritic tau redistribution.[63] These experiments support the hypothesis that environmental factors play a role in the pathophysiological process. However, epidemiological case–control studies have failed to demonstrate association with other agents. Living in more rural area has been related to PSP,[30] which might imply the potential involvement of pesticides in PSP as has also been found in PD.[64] This is also in line with animal studies, in which the neurotoxin MPTP causes inhibition of complex I in mitochondria and eventually nigral and striatal cell loss similar to PD.[65]

As most Mendelian diseases are caused by mutations in the coding regions or splice sites, whole exome sequencing might be a promising tool to identify a genetic defect in small families. [66] Specific plans are currently underway to carry out this technique in PSP patients with a positive family history. Identification of one or more genetic defect will certainly elucidate which factors other than the *MAPT* gene are involved in the disease process. Understanding its pathophysiology will probably provide leads to develop pharmacological interventions.

Therapeutic approaches

Earlier studies with neurotransmitter replacement therapies have been disappointing, probably due to the widespread involvement of dopaminergic and nondopaminergic neurotransmitter systems in PSP. There are however several potential mechanisms in PSP suitable for drug interference.[61] In line with the hypothesis of mitochondrial dysfunction in PSP, a recent trial with Co-enzyme Q10 (a physiological cofactor of complex I) showed significant increase in the ratio of high-energy metabolites to low-energy metabolites (ATP / ADP) measured by proton en phosphorus MR spectroscopy. [67] It also demonstrated an improvement on motor and cognitive functioning in PSP patients. Another therapeutic target is the inhibition of tau phosphorylation in order to decrease or prevent aggregate formation. Unfortunately, a multicenter study with lithium (GSK-3 inhibitor) has been aborted due to the appearance of serious side effects in PSP patients. Another tau-aggregation inhibitor, methylthioninium chloride, has shown a slowing in cognitive decline in AD patients. Davunetide is a peptide protecting microtubule function, which has resulted in a significant improvement in patients with mild cognitive impairment. Finally, stabilizing the stem loop of tau with stem loop stabilizers like neomycin and mitoxantrone, might result in a decreased inclusion of exon 10 and therefore an increment in 3R tau. These ongoing trials will learn us whether these strategies focusing on the tau protein might be effective in PSP. The time has come that better understanding of the disease process will lead us to specific pharmacological trials in PSP patients.

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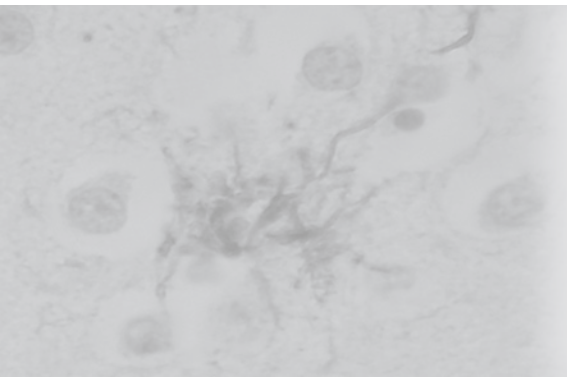

Chapter 5

Summary / Samenvatting

Summary

This thesis describes studies on clinical and genetic aspects of Progressive Supranuclear Palsy (PSP). The clinical diagnosis relies on the presence of characteristic symptoms: progressive parkinsonism with early falls, vertical supranuclear gaze palsy, pseudobulbar dysfunction and cognitive decline; reliable biomarkers are still lacking. The gold standard is the presence of neuronal and glial tau positive aggregates found at autopsy in basal ganglia and brainstem structures. Familial cases of PSP have been described in several recent studies and the strong association with the H1 *tau* haplotype convincingly emphasized the genetic basis of the disease.

The aim of this thesis is to study the variation in clinical presentation, the familial clustering of similar disorders and the identification of genetic factors. The PSP patients described in this thesis were ascertained by nation-wide referral from neurologists and nursing home physicians in the Netherlands. After informed consent, all patients underwent a structural interview, neurological examination, assessment of several rating scales and questionnaires. DNA was collected from peripheral blood samples and in a subset of patients who died brain autopsy was performed.

After a general introduction to the thesis in **chapter 1.1**, **chapter 1.2** gives a general overview of PSP covering clinical, epidemiological, pathological and genetic aspects of the disease. Subsequently, the thesis is divided into two parts. **Chapter 2** covers the clinical heterogeneity of PSP. In **chapter 2.1** we investigated the clinical presentation of PSP in the initial phase. By examining the early symptoms of the disease in 152 PSP patients, a cluster of symptoms with cognitive dysfunction and behavioural changes was identified. Further analysis shows that 20 percent of the cohort shows this presentation, i.e. the frontal subtype. These patients eventually develop all core features of PSP and do not differ in survival. PSP patients with frontal presentation are poorly recognized as PSP at initial neurological visit and are often misdiagnosed as dementia, in particular frontotemporal dementia.
In **chapter 2.2** we undertook a comparative study of motor, autonomic and cognitive symptoms between PSP and PD patients. Standardized rating scales were applied on PSP and PD patients, matched for age, sex and disability. At similar disease disability, disease duration is much shorter in PSP reflecting the rapid progression of the disease. Motor evaluation shows characteristic differences between PSP and PD: PSP patients have more bulbar and postural symptoms, while patients with PD show more tremor, limb rigidity and gait problems. Although autonomic

dysfunction in PSP patients was more frequent compared to controls, PD patients reported more autonomic symptoms on gastrointestinal, cardiovascular and urinary domains. Inline with previous studies, we observed arguments for pupil dysfunction in PSP reflected by more oversensitivity to bright light in PSP patients compared to PD patients. Finally, more problems with executive and visuospatial tasks were observed in PSP.
In the next chapter (**chapter 2.3**) we studied survival rates of PSP patients and FTD patients. Comparing 354 FTD and197 PSP patients shows worse prognosis in PSP patients, which remained significant after correction for onset age, gender and family history. The difference in survival is even more pronounced when comparing pathologically proven cases of PSP with FTLD-tau. This difference may suggest that the underlying pathophysiology in PSP is more aggressive than in FTD. Furthermore, in PSP, male gender, older onset-age, and higher PSP Rating Scale score are identified as independent predictors for shorter survival, whereas in FTD a positive family history and an older onset-age are associated with a poor prognosis. The severity of tau pathology in PSP cases, expressed in the tau-score, shows a significant negative correlation with disease duration.

Chapter 3 of the thesis describes the genetic heterogeneity of PSP. Familial aggregation of neurodegenerative disorders in PSP is presented in **chapter 3.1.** Family history of dementia and parkinsonism was collected from all first degree relatives of PSP patients and compared to age and sex matched controls from the Rotterdam Study. Fifty-seven (33%) of the 172 PSP patients has at least one first degree relative who suffered from dementia or parkinsonism compared to 131 (25%) of the control subjects (OR 1.5, 95% CI 1.01-2.13). In PSP patients, more first degree relatives with parkinsonism are observed compared to controls, with an OR 3.9 (95% CI 1.99-7.61). Data from family history fulfills the criteria for an autosomal dominant mode of transmission in twelve PSP patients (7%). The clinical phenotype within these pedigrees varies between PSP, dementia, tremor and parkinsonism. Genetic studies revealed one patient with a P301L mutation in *MAPT*. Pathological examination of five familial cases confirms the clinical diagnosis of PSP, with predominant four repeat tau pathology in affected brain areas.
In **chapter 3.2** we describe a novel hereditary late onset ataxia with polyglutamine inclusions mimicking PSP. The proband of this family was referred with the clinical diagnosis PSP. Additionally, fourteen affected individuals were identified, of whom nine personally examined. The mean age at onset is 64.4 years and the clinical symptoms include gait ataxia, oculomotor problems, dysarthria, cognitive decline and parkinsonism. After a mutational screen did not reveal any mutation in a large

number of candidate genes, a genomewide linkage scan was performed. An affected only analysis on seven individuals reveals seven STR markers with LOD scores > 1.0, with highest for D18S59 (LOD score 1.64) on chromosome 18. Neuropathological examination of the brain in one single patient deceased during follow-up shows abundant 1C2 inclusions in multiple brain areas, which is strongly suggestive for a polyglutamine disorder.

In **chapter 4**, the main findings of the study are presented in light of the current knowledge about the disease and suggestions for future research are made.

Samenvatting

Dit proefschrift beschrijft het onderzoek naar de klinische en genetische aspecten van progressieve supranucleaire verlamming (PSP). De diagnose PSP is gebaseerd op de aanwezigheid van karakteristieke klinische symptomen zoals een progressief parkinsonisme, houdingsinstabiliteit met vallen, verticale supranucleaire blikverlamming en cognitieve achteruitgang. Betrouwbare biomarkers ontbreken tot op heden. De gouden standaard is nog altijd de aanwezigheid van tau positieve inclusies in neuronen en glia cellen welke bij hersenobductie wordt gevonden in basale kernen en hersenstam structuren. Familiaire gevallen van PSP zijn recent in verschillende studies beschreven en de sterke associatie met het *tau* H1 haplotype ondersteunt de genetische basis voor de ziekte.

Het doel van dit proefschrift is het bestuderen van de variabiliteit in klinische presentatie, de familiaire clustering van verwante aandoeningen en het identificeren van genetische factoren. De PSP patiënten die beschreven worden in dit proefschrift zijn verzameld vanuit landelijke verwijzingen van neurologen en verpleeghuisartsen in Nederland. Na informed consent ondergingen alle patiënten een structurele anamnese, neurologisch onderzoek en evaluatie met behulp van schalen en vragenlijsten. DNA werd verzameld uit bloed monsters en bij een deel van de patiënten die overleden werd hersenobductie verricht.

Na een algemene introductie op het proefschrift in **hoofdstuk 1.1**, geeft **hoofdstuk 1.2** een algemeen overzicht over PSP welke de klinische, epidemiologische, pathologische en genetische aspecten van de ziekte bevat.
Vervolgens is het proefschrift verdeeld in twee onderdelen. **Hoofdstuk 2** omvat de klinische heterogeniteit van PSP. In **hoofdstuk 2.1** wordt de initiële klinische presentatie van PSP beschreven. Bestudering van vroege symptomen in 152 PSP patiënten leverde een cluster van symptomen op bestaande uit cognitieve achteruitgang en gedragsveranderingen. Nadere analyse toont dat 20 procent van het totale cohort deze presentatie, het zogenaamde frontale subtype, bezit. Uiteindelijk ontwikkelen deze patiënten alle karakteristieke kenmerken van PSP zonder verschil in prognose. PSP patiënten met het frontale subtype worden over het algemeen slecht als PSP herkend bij het eerste neurologische bezoek en worden vaak gediagnosticeerd als dementie, met name frontotemporale dementie (FTD).
In **hoofdstuk 2.2** worden de klinische symptomen tussen patiënten met PSP en met de ziekte van Parkinson met gestandaardiseerde schalen vergeleken. Bij gelijke leeftijd, geslacht en mate van invaliditeit blijkt de ziekteduur bij PSP patiënten vele

malen korter, hetgeen de snelle progressie van de ziekte weerspiegelt. Motorische evaluatie laat verschillende (sub)scores zien tussen PSP en Parkinson patiënten: PSP patiënten hebben meer bulbaire en houdingsgerelateerde klachten, terwijl Parkinson patiënten meer rigiditeit, tremor en loopstoornissen vertonen. Ondanks dat autonome klachten vaker bij PSP patiënten dan gezonde controles voorkomen, hebben patiënten met PSP minder klachten op gastrointestinaal, cardiovasculair en urogenitaal gebied dan patiënten met de ziekte van Parkinson. In overeenstemming met voorgaande studies zijn er aanwijzingen gevonden voor meer pupil dysfunctie bij PSP dan bij de ziekte van Parkinson, weergegeven door de overgevoeligheid voor fel licht. Tot slot, PSP patiënten hebben meer problemen met executieve en visuospatiele taken dan patiënten met de ziekte van Parkinson.
In **hoofdstuk 2.3** onderzoeken we de overleving bij PSP en FTD patiënten. Analyse van 197 PSP en 354 FTD patiënten laat een slechtere prognose bij PSP zien, zelfs na correctie voor leeftijd, geslacht en familie anamnese. Het verschil in overleving is zelfs meer uitgesproken wanneer pathologisch bevestigde PSP patiënten worden vergeleken met patiënten met FTD-tau. Dit verschil suggereert dat het onderliggende ziekteproces in PSP meer agressief verloopt dan in FTD. Daarnaast zijn mannelijk geslacht, oudere beginleeftijd en een hogere score op de 'PSP rating scale' onafhankelijk geassocieerd met een kortere overleving in PSP, terwijl in FTD een positieve familie anamnese en ouder beginleeftijd geassocieerd zijn met een slechtere prognose. Neuropathologische analyse toont verder een negatieve correlatie tussen de ernst van de tau pathologie in PSP patiënten en ziekteduur.

Hoofdstuk 3 van dit proefschrift omvat de genetische heterogeniteit in PSP.
Familiaire aggregatie van verwante aandoeningen in PSP wordt gepresenteerd in **hoofdstuk 3.1.** De aanwezigheid van dementie en parkinsonisme bij alle eerstegraads familieleden van PSP patiënten is vergeleken met controle gegevens uit de Rotterdam studie. Vijfenvijftig (33%) van de 172 PSP patiënten hebben tenminste één eerstegraads familielid met dementie of parkinsonisme in vergelijking met 131 (25%) van de controle personen (odds ratio 1.5, 95% CI 1.01-2.13). Bij PSP patiënten worden met name meer eerstegraads familieleden met parkinsonisme geobserveerd in vergelijking met controles (odds ratio 3.9, 95% CI 1.99-7.61). In twaalf gevallen (7%) voldoet de familie anamnese aan de criteria voor een autosomaal dominant overervingpatroon. Het klinisch fenotype binnen deze families varieert tussen PSP, dementie, tremor en parkinsonisme. Genetische analyse leverde één patiënt op met een P301L mutatie in het *tau* gen; in de overige gevallen is de mutatie screening op *tau*, *progranuline* en *LRRK2* negatief. Pathologisch onderzoek van vijf familiaire PSP patiënten bevestigt de definitieve diagnose PSP waarbij met name vier repeat (4R)

tau pathologie in aangedane hersenstructuren wordt gevonden.
In **hoofdstuk 3.2** beschrijven we een nieuwe erfelijke vorm van ataxie op late leeftijd en klinische gelijkenis met PSP. De proband van deze familie werd verwezen met een klinische diagnose PSP. Bestudering van de familie anamnese leverde vervolgens veertien aangedane familieleden op van wie negen neurologisch onderzoek ondergingen. De gemiddelde beginleeftijd van de ziekte is 64.4 jaar en de symptomen omvatten loop ataxie, oculomotore stoornissen, dysarthrie, cognitieve achteruitgang en parkinsonisme. Nadat mutatie analyse van verschilleden kandidaat genen niets opleverde, werd een genoom wijde linkage scan verricht. Met behulp van een 'affected only' analyse van 7 individuen zijn zeven STR markers gevonden met een LOD score > 1.0, waarbij de hoogste score werd gevonden voor marker D18S59 (LOD score 1.64) op chromosoom 18. Neuropathologisch onderzoek in één patiënt die tijdens follow-up was overleden, toonde uitgebreide 1C2 positieve inclusies in verscheidene hersenen gebieden, hetgeen sterk suggestief is voor een polyglutamine ziekte.

Tot slot worden in **hoofdstuk 4** de belangrijkste bevindingen van dit proefschrift besproken in het licht van de huidige kennis over de ziekte en worden suggesties voor toekomstig onderzoek gedaan.

List of abbreviations

4R	Tau isoforms with four repeat mictrotubule binding sites
3R	Tau isoforms with three repeat mictrotubule binding sites
AD	Alzheimer's disease
CB	coiled bodies
CBD	Corticobasal degeneration
CBS	Corticobasal syndrome
CI	Cytoplasmic inclusions
CSF	Cerebrospinal fluid
DNS	Diffuse nuclear staining
FAB	Frontal assessment battery
FTD	Frontotemporal dementia
FTD-MND	Frontotemporal dementia with motor neuron disease
FTLD	Frontotemporal lobar degeneration
FTDP-17	Frontotemporal dementia with parkinsonism linked to chromosome 17
GRN	*Progranulin*
H&Y	Hoehn and Yahr
LBD	Dementia with Lewy bodies
LRRK2	*Leucine-rich repeat kinase 2*
MAPT	*Microtubule associated protein tau*
MMSE	Mini-Mental state examination
MSA	Multiple system atrophy
NFT	Neurofibrillary tangels
NII	Neuronal intranuclear inclusions
NINDS-SPSP	National Institute for Neurological Diseases and Stroke-Society for PSP
NT	Neuropil threads
PD	Parkinson's disease
PET	Positron emission tomography
PGAF	Pure akinesia with gait freezing
PNFA	Progressive nonfluent aphasia
PSP	Progressive supranuclear palsy
PSP-P	Progressive supranuclear palsy -parkinsonism
PSP-RS	Progressive supranuclear palsy rating scale
SCA	Spinocerebellar ataxia
SCOPA-AUT	Scales for outcomes in Parkinson's disease- autonomic

SCOPA-COG	Scales for outcomes in Parkinson's disease - cognition
SNP	Single nucleotide polymorphism
SPECT	Single-photon emission computed tomography
SPES-SCOPA	Short Parkinson's Evaluation Scale/ Scales for outcomes in Parkinson's disease
TA	Tufted astrocytes
Th	Thread pathology
TDP-43	TAR DNA binding protein of 43 kDA
UPDRS	Unified Parkinson disease rating scale

Appendices

PSP-rating scale

SPES/SCOPA motor evaluation

SCOPA-AUT

SCOPA-COG

FAB (Frontal assessment battery)

Family history questionnaire

Appendix

Progressive Supranuclear Palsy Rating Scale

(Golbe et al. Brain 2007 Jun;130(Pt 6):1552-65)

I. HISTORY (from patient or other informant)

1. Withdrawal (relative to baseline personality) **0 1 2**

0 None
1 Follows conversation in a group, may respond spontaneously, but rarely if ever initiates exchanges.
2 Rarely or never follows conversation in a group.

2. Aggressiveness (relative to baseline personality) **0 1 2**

0 No increase in aggressiveness
1 Increased, but not interfering with family interactions
2 Interfering with family interactions

3. Dysphagia for solids **0 1 2 3 4**

0 Normal; no difficulty with full range of food textures
1 Tough foods must be cut up into small pieces
2 Requires soft solid diet
3 Requires pureed or liquid diet
4 Tube feeding required for some or all feeding

4. Using knife and fork, buttoning clothes, washing hands and face (rate the worst) **0 1 2 3 4**

0 Normal
1 Somewhat slow but no help required
2 Extremely slow; or occasional help needed
3 Considerable help needed but can do some things alone
4 Requires total assistance

5. Falls **0 1 2 3 4**

(average frequency if patient attempted to walk unaided)
0 None in the past year
1 <1 per month; gait may otherwise be normal
2 1-4 per month
3 5-30 per month
4 > 30 per month (or chairbound)

6. Urinary incontinence **0 1 2 3 4**

0 None or a few drops less than daily
1 A few drops staining clothes daily
2 Large amounts, but only when asleep; no pad required during day
3 Occasional large amounts in daytime; pad required
4 Consistent, requiring diaper or catheter awake and asleep

7. Sleep difficulty **0 1 2 3 4**

0 Neither 1° nor 2° insomnia (i.e., falls asleep easily and stays asleep)
1 Either 1° or 2° insomnia; averages >5 hours sleep nightly
2 Both 1° and 2° insomnia; averages >5 hours sleep nightly
3 Either 1° or 2° insomnia; averages <5 hours sleep nightly
4 Both 1° and 2° insomnia; averages <5 hours sleep nightly

II. MENTAL EXAM

Items 8-11 use this scale
0 Clearly absent
1 Equivocal or minimal
2 Clearly present, but not interfering with activities of daily living (ADL)
3 Interfering mildly with ADL
4 Interfering markedly with ADL

8. Disorientation **0 1 2 3 4**

9. Bradyphrenia **0 1 2 3 4**

10. Emotional incontinence **0 1 2 3 4**

11. Grasping/imitatative/utilizing behavior **0 1 2 3 4**

III. BULBAR EXAM

12. Dysarthria (ignoring palilalia) **0 1 2 3 4**

0 None
1 Minimal; all or nearly all words easily comprehensible (to examiner, not family)
2 Definite, moderate; most words comprehensible
3 Severe; may be fluent but most words incomprehensible
4 Mute; or a few poorly comprehensible words

13. Dysphagia **0 1 2 3 4**

(for 30-50 cc of water from a cup, if safe)

0 None

1 Fluid pools in mouth or pharynx, or swallows slowly, but no choking/coughing

2 Occasionally coughs to clear fluid; no frank aspiration

3 Frequently coughs to clear fluid; may aspirate slightly; may expectorate frequently rather than swallow secretions

4 Requires artificial measures (oral suctioning, tracheostomy or feeding gastrostomy) to avoid aspiration

IV. SUPRANUCLEAR OCULAR MOTOR EXAM

Items 14-16 use this scale. Rate by inspection of saccades on command from the primary position of gaze to a stationary target.

0 Not slow or hypometric; 86-100% of normal amplitude

1 Slow or hypometric; 86-100% of normal amplitude

2 51-85% of normal amplitude

3 16-50% of normal amplitude

4 15% of normal amplitude or worse

14. Voluntary upward saccades **0 1 2 3 4**

15. Voluntary downward saccades **0 1 2 3 4**

16. Voluntary left and right saccades **0 1 2 3 4**

17. Eyelid dysfunction **0 1 2 3 4**

0 None

1 Blink rate decreased (< 15/minute) but no other abnorm.

2 Mild inhibition of opening or closing or mild blepharospasm; no visual disability

3 Moderate lid-opening inhibition or blepharospasm causing partial visual disability

4 Functional blindness or near-blindness because of involuntary eyelid closure

V. LIMB EXAM

18. Limb rigidity (rate the worst of the four) **0 1 2 3 4**
0 Absent
1 Slight or detectable only on activation
2 Definitely abnormal, but full range of motion possible
3 Only partial range of motion possible
4 Little or no passive motion possible

19. Limb dystonia **0 1 2 3 4**
(rate worst of the four; ignore neck and face)
0 Absent
1 Subtle or present only when activated by other movement
2 Obvious but not continuous
3 Continuous but not disabling
4 Continuous and disabling

20. Finger tapping (if asymmetric, rate worse side) **0 1 2**
0 Normal (>14 taps/5 sec with maximal amplitude)
1 Impaired (6-14 taps/5 sec or moderate loss of amplitude
2 Barely able to perform (0-5 taps/5 sec or severe loss of amplitude)

21. Toe tapping (if asymmetric, rate worse side) **0 1 2**
0 Normal (>14 taps/5 sec with maximal amplitude)
1 Impaired (6-14 taps/5 sec or moderate loss of amplitude
2 Barely able to perform (0-5 taps/5 sec or severe loss of amplitude)

22. Apraxia of hand movement **0 1 2**
0 Absent
1 Present, not impairing most functions
2 Impairing most functions

23. Tremor in any part **0 1 2**
0 Absent
1 Present, not impairing most functions
2 Impairing most functions

VI. GAIT/MIDLINE EXAM

24. Neck rigidity or dystonia **0 1 2 3 4**

0 Absent
1 Slight or detectable only when activated by other movement
2 Definitely abnormal, but full range of motion possible
3 Only partial range of motion possible
4 Little or no passive motion possible

25. Arising from chair **0 1 2 3 4**

0 Normal
1 Slow but arises on first attempt
2 Requires more than one attempt, but arises without using hands
3 Requires use of hands
4 Unable to arise without assistance

26. Gait **0 1 2 3 4**

0 Normal
1 Slightly wide-based or irregular or slight pulsion on turns
2 Must walk slowly or occasionally use walls or helper to avoid falling, especially on turns
3 Must use assistance all or almost all the time
4 Unable to walk, even with walker; may be able to transfer

27. Postural stability (on backward pull) **0 1 2 3 4**

0 Normal (shifts neither foot or one foot)
1 Must shift each foot at least once but recovers unaided
2 Shifts feet and must be caught by examiner
3 Unable to shift feet; must be caught, but does not require assistance to stand still
4 Tends to fall without a pull; requires assistance to stand still

28. Sitting down **0 1 2 3 4**

(may touch seat or back but not arms of chair)
0 Normal
1 Slightly stiff or awkward
2 Easily positions self before chair, but descent into chair is uncontrolled
3 Has difficulty finding chair behind him/her and descent is uncontrolled
4 Unable to test because of severe postural instability

SPES/SCOPA

Marinus J, Visser M, Stiggelbout AM, Rabey JM, Martínez-Martín P, Bonuccelli U, Kraus PH, van Hilten JJ. A short scale for the assessment of motor impairments and disabilities in Parkinson's disease: the SPES/SCOPA. J Neurol Neurosurg Psychiatry 2004;75:388- 395.

A. Motor evaluation

Clinical examination

1. **Rest tremor**
   assess each arm separately during 20 seconds; hands rest on thighs; if tremor is not evident at rest, try to keep the patient attentive, e.g. by having him/her count backwards with eyes closed
   0 = absent
   1 = small amplitude (< 1 cm) occurring spontaneously, or obtained only while keeping patient attentive (any amplitude)
   2 = moderate amplitude (1-4 cm), occurring spontaneously
   3 = large amplitude (≥ 4 cm), occurring spontaneously.
2. **Postural tremor**
   check with arms outstretched, pronated and semipronated, and with index fingers of both hands almost touching each other (elbows flexed); assess each position during 20 seconds
   0 = absent
   1 = small amplitude (< 1cm)
   2 = moderate amplitude (1-4 cm)
   3 = large amplitude (≥ 4 cm).
3. **Rapid alternating movements of hands**
   rapid alternating pronation/supination movements of upper hand, each time slapping the palm of the horizontally held lower hand during 20 seconds; each hand separately
   0 = normal
   1 = slow execution, or mild slowing and/or reduction in amplitude; may have occasional arrests
   2 = moderate slowing and/or reduction in amplitude or hesitations in initiating movement or frequent arrests in ongoing movements
   3 = can barely perform task.
4. **Rigidity**
   assess passive movements of elbow and wrist over full range, with the patient relaxed in sitting position; ignore cogwheeling; check each arm separately
   0 = absent
   1 = mild rigidity over full range, no difficulty reaching end positions

2 = moderate rigidity, some difficulties reaching end positions
3 = severe rigidity, considerable difficulties reaching end positions.

5. **Rise from chair**
   patient is instructed to fold arms across chest; use straight back chair
   0 = normal
   1 = slowly; does not need arms to get up
   2 = needs arms to get up (can get up without help)
   3 = unable to rise (without help).
6. **Postural instability**
   stand behind the patient and pull patient backwards, while s/he is standing erect with eyes open and feet spaced slightly apart; patient is <u>not</u> prepared
   0 = normal, may take up to 2 steps to recover
   1 = takes 3 or more steps; recovers unaided
   2 = would fall if not caught
   3 = spontaneous tendency to fall or unable to stand unaided.
7. **Gait**
   assess gait pattern; use walking aid or offer assistance, if necessary
   0 = normal
   1 = mild slowing and/or reduction of step height or length; does not shuffle
   2 = severe slowing, or shuffles or has festination
   3 = unable to walk.
8. **Speech** 0 = normal
   1 = slight loss of expression, diction and/or volume
   2 = slurred; not always intelligible
   3 = unintelligible always or most of the time.

Historical information

9. **Freezing during 'on'**
   Freezing is characterized by hesitation when trying to start walking or 'gluing' to the ground while walking.
   0 = absent
   1 = start hesitation only, occasionally present
   2 = frequently present, may have freezing when walking
   3 = severe freezing when walking.
10. **Swallowing**
    0 = normal
    1 = some difficulty or slow; does not choke; normal diet
    2 = sometimes chokes; may require soft food
    3 = chokes frequently; may require soft food or alternative method of food intake.

B. Activities of Daily Living

11. **Speech**
    0 = normal
    1 = some difficulty; may sometimes be asked to repeat sentences
    2 = considerable difficulty; frequently asked to repeat sentences
    3 = unintelligible most of the time.
12. **Feeding (cutting, filling cup, etc.)**
    0 = normal
    1 = some difficulty or slow; does not need assistance
    2 = considerable difficulty; may need some assistance
    3 = needs almost complete or complete assistance.
13. **Dressing**
    0 = normal
    1 = some difficulty or slow; does not need assistance
    2 = considerable difficulty; may need some assistance (e.g. buttoning, getting arms into sleeves)
    3 = needs almost complete or complete assistance.
14. **Hygiene (washing, combing hair, shaving, brushing teeth, using toilet)**
    0 = normal
    1 = some difficulty or slow; does not need assistance
    2 = considerable difficulty; may need some assistance
    3 = needs almost complete or complete assistance.
15. **Changing position (turning over in bed, getting up out of bed, getting up out of a chair, turning around when standing)**
    0 = normal
    1 = some difficulty or slow; does not need assistance with any change of position
    2 = considerable difficulty; may need assistance with one or more changes of position
    3 = needs almost complete or complete assistance with one or more changes of position.
16. **Walking**
    0 = normal
    1 = some difficulty or slow; does not need assistance or walking aid
    2 = considerable difficulty; may need assistance or walking aid
    3 = unable to walk, or walks only with assistance and great effort.
17. **Handwriting**
    0 = normal
    1 = some difficulty (e.g. slow, small letters); all words legible

2 = considerable difficulty; not all words legible; may need to use block letters
3 = majority of words are illegible.

C. Motor Complications

18. **Dyskinesias (presence)**
    0 = absent
    1 = present some of the time
    2 = present a considerable part of the time
    3 = present most or all of the time.
19. **Dyskinesias (severity)** 0 = absent
    1 = small amplitude
    2 = moderate amplitude
    3 = large amplitude
20. **Motor fluctuations (presence of 'off' periods)**
    What proportion of the waking day is patient 'off' on average?
    0 = none
    1 = some of the time
    2 = a considerable part of the time
    3 = most or all of the time.
21. **Motor fluctuations (severity of 'off' periods)** 0 = absent
    1 = mild end-of-dose fluctuations
    2 = moderate end-of-dose fluctuations; unpredictable fluctuations may occur occasionally
    3 = severe end-of-dose fluctuations; unpredictable on-off oscillations occur frequently.

SCOPA-AUT

Visser M, Marinus J, Stiggelbout AM, Van Hilten JJ. Assessment of autonomic dysfunction in Parkinson's disease: the SCOPA-AUT. Mov Disord. 2004;19:1306-12.

By means of this questionnaire, we would like to find out to what extent in the past month you have had problems with various bodily functions, such as difficulty passing urine, or excessive sweating. Answer the questions by placing a cross in the box which best reflects your situation. If you wish to change an answer, fill in the 'wrong' box and place a cross in the correct one. If you have used medication in the past month in relation to one or more of the problems mentioned, then the question refers to how you were while taking this medication. You can note the use of medication on the last page.

1. In the past month, have you had difficulty swallowing or have you choked?

☐ never ☐ sometimes ☐ regularly ☐ often

2. In the past month, has saliva dribbled out of your mouth?

☐ never ☐ sometimes ☐ regularly ☐ often

3. In the past month, has food ever become stuck in your throath?

☐ never ☐ sometimes ☐ regularly ☐ often

4. In the past month, did you ever have the feeling during a meal that you were full very quickly?

☐ never ☐ sometimes ☐ regularly ☐ often

5. *Constipation is a blockage of the bowel, a condition in which someone has a bowel movement twice a week or less.*
In the past month, have you had problems with constipation?

☐ never ☐ sometimes ☐ regularly ☐ often

6. In the past month, did you have to strain hard to pass stools?

☐ never ☐ sometimes ☐ regularly ☐ often

7. In the past month, have you had involuntary loss of stools?

☐ never ☐ sometimes ☐ regularly ☐ often

Questions 8 to 13 deal with problems with passing urine. If you use a catheter you can indicate this by placing a cross in the box "*use cathether*".

8. In the past month, have you had difficulty retaining urine?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *use catheter*

9. In the past month, have you had involuntary loss of urine?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *use catheter*

10. In the past month, have you had the feeling that after passing urine your bladder was not completely empty?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *use catheter*

11. In the past month, has the stream of urine been weak?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *use catheter*

12. In the past month, have you had to pass urine again within 2 hours of the previous time?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *use catheter*

13. In the past month, have you had to pass urine <u>at night</u>?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *use catheter*

14. In the past month, when standing up have you had the feeling of either becoming lightheaded, or no longer being able to see properly, or no longer being able to think clearly?

☐ never ☐ sometimes ☐ regularly ☐ often

15. In the past month, did you become light-headed after standing for some time?

☐ never ☐ sometimes ☐ regularly ☐ often

16. Have you fainted in the past 6 months?

☐ never ☐ sometimes ☐ regularly ☐ often

17. In the past month, have you ever perspired excessively during the day?

☐ never ☐ sometimes ☐ regularly ☐ often

18. In the past month, have you ever perspired excessively during the night?

☐ never ☐ sometimes ☐ regularly ☐ often

19. In the past month, have your eyes ever been over-sensitive to bright light?

☐ never ☐ sometimes ☐ regularly ☐ often

20. In the past month, how often have you had trouble tolerating cold?

☐ never ☐ sometimes ☐ regularly ☐ often

21. In the past month, how often have you had trouble tolerating heat?

☐ never ☐ sometimes ☐ regularly ☐ often

The following questions are about sexuality. Although we are aware that sexuality is a highly intimate subject, we would still like you to answer these questions. For the questions on sexual activity, consider every form of sexual contact with a partner or masturbation (self-gratification). An extra response option has been added to these questions. Here you can indicate that the situation described has not been applicable to you in the past month, for example because you have not been sexually active. Questions 22 and 23 are intended specifically for **men**, 24 and 25 for **women**.

The following 3 questions are only for men

22. In the past month, have you been impotent (unable to have or maintain an erection)?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *not applicable*

23. In the past month, how often have you been unable to ejaculate?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *not applicable*

23a. In the past month, have you taken medication for an erection disorder? (If so, which medication?)

☐ no ☐ yes: _____

Proceed with question 26

The following 2 questions are only for women

24. In the past month, was your vagina too dry during sexual activity?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *not applicable*

25. In the past month, have you had difficulty reaching an orgasm?

☐ never ☐ sometimes ☐ regularly ☐ often ☐ *not applicable*

The following questions are for everyone

The questions below are about the use of medication for which you may have or have not needed a doctor's prescription. If you use medication, also give the name of the substance.

26. In the past month, have you used medication for:

a. constipation? ☐ no ☐ yes: _____

d. urinary problems? ☐ no ☐ yes: _____

e. blood pressure? ☐ no ☐ yes: _____

f. other symptoms *(not symptoms related to Parkinson's disease)* ☐ no ☐ yes: _____

_____

Visser M, Marinus J, Stiggelbout AM, Van Hilten JJ. Assessment of autonomic dysfunction in Parkinson's disease: the SCOPA-AUT. Mov Disord 2004;**19**:1306-12.

For further information, please contact Dr. J. Marinus, Leiden University Medical Center, Department of Neurology (K5Q), P.O. Box 9600, NL-2300 RC Leiden (email: scopa@lumc.nl).

SCOPA-COG

Marinus J, Visser M, Verwey NA, Verhey FRJ, Middelkoop HAM, Stiggelbout AM, van Hilten JJ. Assessment of cognition in Parkinson's disease. Neurology 2003;61:1222-1228.

Memory and learning

1. *Verbal recall*

   Ten words are repeatedly shown for at least 4 seconds, get the patient to read them out loud, the time allowed for recall is unlimited. Underline each word that has been named. When words are named that were not shown, no penalty is given. When a false answer is changed (e.g. king into queen), it is correct.

   Instruction: "Read the following 10 words aloud and try to remember as many as possible. After reading them all, name as many words as possible, the order of the words is not important".

   10 words: Butter arm shore letter queen cabin pole ticket grass engine

   (10 correct = 5, 8-9 correct = 4, 6-7 correct = 3, 5 correct = 2, 4 correct = 1, ≤ 3 correct= 0)

   score ……../5

2. *Digit span backward*

   Ask the patient to repeat a series of numbers backwards; the numbers are read out separately, 1 second per number; if incorrectly repeated, the alternative in the second column is presented. Continue until both the first and the alternative series are repeated incorrectly. Make sure the time interval between numbers stays the same. Read the numbers calmly and make sure the time between numbers is equal. Record the highest series that is repeated correctly at least once; Give an example: "If I say 2-7-3, than you say (3-7-2)

backwards		score:
2-4	5-8	= 1
6-2-9	4-1-5	= 2
3-2-7-9	4-9-6-8	= 3
1-5-2-8-6	6-1-8-4-3	= 4
5-3-9-4-1-8	7-2-4-8-5-6	= 5
8-1-2-9-3-6-5	4-7-3-9-1-2-8	= 6
9-4-3-7-6-2-5-8	7-2-8-1-9-6-5-3	= 7

score ……../7

SCOPA-COG

3. Indicate cubes

Point to the cubes in the order given below; the patient should copy this; do this slowly; the patient decides for himself with which hand he/she prefers. Indicate the cubes in the order as indicated. Observe carefully if the patient copies the order correctly. When a patient wants to correct a mistake, let him/her do the complete order again. This is not counted as a mistake. However, if the patient forgets the order and would like to see the order a second time, the researcher does not repeat the order again but starts with the next order.

1 2 3 4

a. 1-2-4-2

b. 1-2-3-4-3

c. 3-4-2-1-4

d. 1-4-2-3-4-1

e. 1-4-2-3

score ……../5

Attention

4. Counting backwards (30 to 0)

Instruction: "Would you subtract three from 30, and subtract three again from the result and continue till zero?".

Mistakes can be: the order, missing or not knowing a number, or not finishing off the series. Record the order of numbers named by the patient. If the patient asks where to start or how much to subtract, the researcher repeats the instructions but counts that as one mistake. If the patient makes a mistake but continues from that point to subtract three, it is only one mistake. If the patient stops the order and starts all over again, it is one mistake.

(0 mistakes = 2, 1 mistake = 1, ≥ 2 mistakes = 0) score ……../2

SCOPA-COG

5. *Months backwards*

Instruction: "Name the months of the year in reverse order, starting with the last month of the year".

Mistakes are: the order, missing or not knowing the next month, or not finishing off the series. Underline the months that are named correctly. When a month is passed over, this is a mistake, even if the patient corrects it later on. If the patient stops the order and starts all over again, it is one mistake. If the patient starts naming the month forward, repeat the instructions and count it as one mistake.

Dec- Nov-Oct-Sept-Aug-July-June-May-April-March-Feb-Jan.

(0 mistakes = 2, 1 mistake = 1, ≥ 2 mistakes = 0) score ……../ 2

Executive functions

6. *Fist-edge-palm*

1. fist with ulnar side down, 2. stretched fingers with ulnar side down, 3. stretched fingers with palm down; Practice 5 times together with the patient, the patient chooses which hand he/she prefers. Do it slowly and tell the patient to watch carefully and repeat what you are doing. Practice first 5 rounds, with verbal help, e.g. FIST- STRETCH-PALM. Then tell the patient to make the movements alone.

Instructions: "Now it is your turn to make the three movements, fist-stretch-palm, 10 times in a row. You don't have to count, I will tell you when to stop".

Note the number of correct trios from a total of 10; Count carefully but not out loud. Every time a patient makes a wrong movement, count it as a mistake, even when the patient corrects it halfway.

(10 correct = 3, 9 correct = 2, 8 correct = 1, ≤ 7 correct = 0) score ……../3

7. *Semantic fluency*

Tell the patient to name as many animal as he/she knows in one minute. Note all answers that are given by the patient. No repetition or variations of words, such as lion-lioness, tiger-tigress; categories are allowed, bird and pigeon are both correct. Count the number of animals correctly named. The purpose is that the patient generates the animals actively, therefore no clues are allowed. When the patient asks whether, for instance, naming different types of birds is allowed, this may be confirmed. When the patient almost immediately says he/she does not know any more animals, try to

SCOPA-COG

stimulate the patient by saying "there is still a lot of time left", but do not give clues. When the patient starts naming other things than animals, do not correct the patient. Naming other things besides animals is not counted as an additional mistake.

(≥ 25 correct = 6, 20-24 = 5, 15-19 = 4, 10-14 = 3, 5-9 = 2, 1-4 = 1 0=0)

number of animals correct: ……. score ……../6

Write down all animals named:

8. Dice

Use 2 cards, one with YES = EVEN, NO = ODD; one with YES = HIGHER, NO = LOWER. Put the correct card face up next to the explanation of the test and make sure that the other, irrelevant card is out of sight. The first round (situation 1) is not scored, and the patient is corrected if necessary.

Situation 1: YES = EVEN

Put the card "YES=EVEN, NO=ODD" on the table and leave it there during the test. Instruction: "Say YES for an even number on a dice and NO for an odd number, when you see a picture of a dice with an EVEN number of pips, I would like you to say YES, and NO when the number of pips is ODD".

Show the first two examples (3 even and 3 odd dices) and ask the patient "If you see one of these dice, do you say yes or no?" Tell the patient if the answer is correct or not. If the answer is not correct, explain why. It is important that the patient says YES or NO and not EVEN or ODD. Show the next two examples (with only one dice) and ask the patient "if you see this dice, do you say yes or no?" Tell the patient if the answer is correct or not. If the answer is not correct, explain why.

Then show the patient the following 10 dices. Correct the patient if the answer is wrong.

SCOPA-COG

Situation 2: YES = HIGHER

With the card "example 1" (dice with 3 pips) the next condition starts. Put the card "YES=HIGHER, NO=LOWER" on the table and remove the former card.

Instruction: "Now, we change the test a little. When you see a picture of a dice that is higher than de dice on the page before, you say YES. When the dice is lower, you say NO".

Tell the patient you have an example (example 1). "Try to remember this dice" (turn the page) "Is this YES or NO?" Tell the patient whether the answer is correct or not. If the answer is not correct, explain why. Continue with example 2 and say "now remember this dice"(turn the page) "Is this YES or NO?" Tell the patient if the answer is correct or not. If the answer is not correct, explain why.

Then start the test and show all 10 dices one after another. The first response counts and corrections are not allowed. Do NOT correct when a wrong answer is given. If a patient corrects a wrong answer, it is still counted as a mistake. If the patient asks for the instruction, the researcher explains but that is counted as one mistake.

(10 correct = 3, 9 correct = 2, 8 correct =1, ≤ 7 correct = 0)

number correct: …./10

score ……../3

Visuo-spatial functions

9. Assembling patterns

The patient is shown 5 incomplete patterns and has to choose 2 or 3 shapes out of 4 to 6 possible alternatives in order to complete the pattern. First practice 2 figures.

Show the patient example A and give the instruction to choose the shapes that form the pattern. Tell the patient if the answer is correct or not. If the answer is not correct, explain why and give the correct solution. Repeat this with example B. Then show the 5 patterns. Do not tell the patient whether the answer is correct or not. There is no time limit. If the patient corrects a wrong answer, this is not counted as a mistake.

a. b. c. d. e.

score ……../5

SCOPA-COG

Memory

10. Delayed recall

Instruction: "Can you name as many as possible of the 10 words that you learned during the first test? "

Underline each word that has been named. When words are named that were not shown, no penalty is given. When a false answer is changed (e.g. king into queen), it is correct.

10 words: butter arm shore letter queen cabin pole ticket grass engine

(10 correct = 5, 8-9 correct = 4, 6-7 correct = 3, 5 correct = 2, 4 correct = 1, ≤ 3 correct= 0)

number of correct words: …. /10

score ……../5

Total COG score: … /43

FAB (Frontal Assessment Battery)

Dubois et al. Neurology 2000. Dec 12;55(11)1621-6

1. Similarities (conceptualization)

"In what way are they alike?"

- *A banana and an orange*

(In the event of total failure: "they are not alike" or partial failure: "both have peel," help the patient by saying: "both a banana and an orange are..."; but credit 0 for the item; do not help the patient for the two following items)

- *A table and a chair*
- *A tulip, a rose and a daisy (madeliefje).*

Score (only category responses [fruits, furniture, flowers] are considered correct)

0. None correct
1. One correct
2. Two correct
3. Three correct

2. Lexical fluency (mental flexibility)

"Say as many words as you can beginning with the letter 'S,' any words except surnames or proper nouns."

If the patient gives no response during the first 5 seconds, say: "for instance, snake." If the patient pauses 10 seconds, stimulate him by saying: "any word beginning with the letter 'S.' The time allowed is 60 seconds.

Score (word repetitions or variations [shoe, shoemaker], surnames, or proper nouns are not counted as correct responses)

0. Less than three words
1. Three to five words
2. Six to nine words
3. More than nine words

3. Motor series (programming)

"Look carefully at what I'm doing."

The examiner, seated in front of the patient, performs alone three times with his left hand the series of Luria "fist– edge–palm." "Now, with your right hand do the same series, first with me, then alone." The examiner performs the series three times with the patient, then says to him/her: "Now, do it on your own."

0. Patient cannot perform three correct consecutive series even with the examiner
1. Patient fails alone, but performs three correct consecutive series with the examiner
2. Patient performs at least three correct consecutive series alone
3. Patient performs six correct consecutive series alone

4. Conflicting instructions (sensitivity to interference)

"Tap twice when I tap once."

To be sure that the patient has understood the instruction, a series of three trials is run: 1-1-1. "Tap once when I tap twice."

To be sure that the patient has understood the instruction, a series of three trials is run: 2-2-2. The examiner performs the following series: 1-1-2-1-2-2-2-1-1-2.

0. Patient taps like the examiner at least four consecutive times
1. More than two errors
2. One or two errors
3. No error

5. Go–No Go (inhibitory control)

"Tap once when I tap once."

To be sure that the patient has understood the instruction, a series of three trials is run:1-1-1.

"Do not tap when I tap twice."

To be sure that the patient has understood the instruction, a series of three trials is run:2-2-2. The examiner performs the following series: 1-1-2-1-2-2-2-1-1-2.

0. Patient taps like the examiner at least four consecutive times
1. More than two errors
2. One or two errors
3. No error

6. Prehension behaviour (environmental autonomy)

"Do not take my hands."

The examiner is seated in front of the patient. Place the patient's hands palm up on his/her knees. Without saying anything or looking at the patient, the examiner brings his/her hands close to the patient's hands and touches the palms of both the patient's hands, to see if he/she will spontaneously take them. If the patient takes the hands, the examiner will try again after asking him/her: "Now, do not take my hands."

0. Patient takes the examiner's hand even after he/she has been told not to do so
1. Patient takes the hands without hesitation
2. Patient hesitates and asks what he/she has to do
3. Patient does not take the examiner's hands

Totaalscore (max. 18):……...

Family history questionnaire:

(separate form for each relative: father, mother, sibling)

Surname, first name	……..
Date of birth, place of birth	……..
Died	yes/ no/ don't know
Date of death	……..
Place of death	……..
Cause of death	…..……

Did the person suffer from any of the following diseases?

- PSP	yes/ no/ don't know
- Dementia	yes/ no/ don't know
- Parkinson's disease	yes/ no/ don't know
- other parkinsonian disorders	…….. ……

Did the person show any of the following symptoms?

- gait disturbances	yes/ no/ don't know
- frequent falls	yes/ no/ don't know
- stiffness of arms or legs	yes/ no/ don't know
- tremor of the hands	yes/ no/ don't know
- speech problems	yes/ no/ don't know
- memory problems	yes/ no/ don't know
- behavioral changes	yes/ no/ don't know

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About the author

Laura Donker Kaat was born on September 21st, 1978 in Haarlem, the Netherlands. After completing the Stedelijk Gymnasium in Haarlem in 1996, she started medical school at the Erasmus University in Rotterdam. In 2001 she participated in a research project on frontotemporal dementia at the Department of Neurology at the Erasmus Medical Centre Rotterdam (dr. S.M. Rosso and prof. dr. J.C. van Swieten). In the following year she started the project on the clinical and genetic aspects of progressive supranuclear palsy underlying this thesis. In 2005 she obtained her medical degree. During her PhD period she worked in the laboratory at the Department of Clinical Genetics (section medical genomics) at the VU Medical Centre in Amsterdam (prof. dr. P. Heutink). In 2007 she finished her Master in "Genetic epidemiology" at the Netherlands Institute for Health Sciences (NIHES).
From December 2007 onwards she works as a resident in Neurology at the Erasmus Medical Centre Rotterdam (prof. dr. P.A.E. Sillevis Smitt). Currently she lives in Rotterdam together with her husband and two children.

List of publications

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Books

Donker Kaat L, van Swieten JC. Progressive Supranuclear palsy, In: Wolters E.Ch., van Laar T., Berendse H.W., [editors]. Parkinsonism & related disorders. 2007. VU University Press

PhD portfolio-summary of PhD training and teaching activities

	Year	Workload (ECTS)
1. PhD training (research school: Nihes)		
Research skills		
Erasmus Summer Programme	2002	3.5
Study Design	2002	4.3
Classical Methods for Data-analysis	2005	5.7
Moderns Statistical Methods	2005	4.3
Genetic-Epidemiologic Research Methods	2006	5.7
Introduction to Clinical research	2007	0.7
In-depth courses		
Advances in Clinical Neuro-epidemiology	2007	0.7
Advances in Population-based Studies of Complex Genetic Disorders	2007	1.4
Genetic Linkage Analysis I: Model based Analysis	2003	1.4
Genetic Linkage Analysis: Model-free Analysis	2007	1.4
SNP's and Human Diseases	2005	1.4
International Conferences		
8th international congress of Parkinson's disease and movement disorders, Rome. Poster presentation	2004	1.0
5th International Conference on Frontotemporal dementias, San Fransisco. Poster presentation.	2006	1.0
60th Annual Meeting American Academy of Neurology, Chicago. Oral presentation.	2008	1.0
6th International Conference on Frontotemporal dementias, Rotterdam. Oral presentation.	2008	1.0
Seminars, workshops and other		
Clinical training in PSP, Birmingham.	2002	3.0
Fifth International PSP Medical Workshop, London.	2005	0.3
Vlaams-Nederlandse werkgroep bewegingsstoornissen, Leuven, oral presentation.	2006	0.3
Wetenschappelijke vergadering NVN, oral presentation.	2007	0.3
Genetica van bewegingstoornissen, NVN Werkgroep Bewegingsstoornissen, Amersfoort, oral presentation.	2010	0.3
2. Teaching		
Lecturing		
Landelijke parkinsonismen dag, Woerden.	2005	0.3
Dementie update, Amsterdam, IPC neurobrain.	2010	0.3
Supervising		
Master student	2007	2.0
TOTAL		41.3