Department of Neurosurgery
and
Department of Pathology, Laboratory of Neuropathology
Helsinki University Central Hospital
University of Helsinki
Finland

α-Synuclein pathology in very elderly Finns

A population-based clinico-neuropathologic and molecular genetic study of Dementia with Lewy bodies

Minna Oinas

Academic Dissertation

To be publicly discussed, with the permission of the Faculty of Medicine of the University of Helsinki, in the Lecture Hall 1 of the Haartman Institute, Haartmaninkatu 3, Helsinki on October 2nd 2009 at 12 o’clock noon.

Helsinki 2009
Supervisors
Tuomo Polvikoski, MD, PhD
Clinical Senior Lecturer and Honorary Consultant Neuropathologist
Institute for Ageing and Health
Newcastle University
Newcastle upon Tyne, U.K.

Anders Paetau, MD, PhD
Docent of Neuropathology
Department of Pathology
University of Helsinki and Helsinki University Central Hospital
Helsinki, Finland

Reviewers
Irina Alafuzoff, MD, PhD
Professor of Neuropathology
Department of Genetics and Pathology
Uppsala University
Uppsala, Sweden

Juha Rinne MD, PhD
Professor of Neurotransmission
Turku PET Centre
University of Turku and Turku University Central Hospital

Opponent
Paul Ince, MD, PhD
Professor of Neuropathology
Royal Hallamshire Hospital
Sheffield, United Kingdom

ISBN 978-952-10-5707-6 (PDF)
http://ethesis.helsinki.fi/

Helsinki University Press
Helsinki 2009
To Jape, Stella and Kiara
Author’s contact information

Minna Oinas, MD
Neurosurgeon

Department of Neurosurgery
Helsinki University Central Hospital
Topeliuksenkatu 5
00260 Helsinki
Finland

Department of Pathology
P.O.Box 21 (Haartmaninkatu 3)
FI-00014 University of Helsinki
Finland

Mobile: +358 50 427 0394
E-mail: minna.oinas@helsinki.fi; minna.oinas@hus.fi
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ABBREVIATIONS

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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>αS</td>
<td>Alpha-synuclein</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
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<tr>
<td>Apo E</td>
<td>Apolipoprotein</td>
</tr>
<tr>
<td>CA 2-3</td>
<td>CA 2-3 subfields of the hippocampus</td>
</tr>
<tr>
<td>CBD</td>
<td>Corticobasal degeneration</td>
</tr>
<tr>
<td>CERAD</td>
<td>Consortium to establish a registry for Alzheimer’s Disease</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<tr>
<td>DAT</td>
<td>Dopamine transporter</td>
</tr>
<tr>
<td>DLB</td>
<td>Dementia with Lewy bodies</td>
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<tr>
<td>dmV</td>
<td>Dorsal motor nucleus of Vagus</td>
</tr>
<tr>
<td>DSM-IIIR</td>
<td>Diagnostic and Statistical Manual of Mental Disorders, third edition - revised</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
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<tr>
<td>EPS</td>
<td>Extrapyramidal symptom</td>
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<tr>
<td>FTD</td>
<td>Frontotemporal dementia</td>
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<tr>
<td>GCI</td>
<td>Glial cytoplasmic inclusions</td>
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<tr>
<td>H&amp;E</td>
<td>Hematoxylin and eosin</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
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<tr>
<td>LB</td>
<td>Lewy body</td>
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<tr>
<td>LBD</td>
<td>Lewy body disease</td>
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<tr>
<td>LC</td>
<td>Locus coeruleus</td>
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<td>LD</td>
<td>Linkage disequilibrium</td>
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<td>LNs</td>
<td>Lewy neurites</td>
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<td>LRP</td>
<td>Lewy-related pathology</td>
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<tr>
<td>MAPT</td>
<td>Microtubule-associated protein tau gene</td>
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<tr>
<td>MIBG</td>
<td>Metaiodobenzylguanidine</td>
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<tr>
<td>MMSE</td>
<td>Mini-Mental state examination</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>MSA</td>
<td>Multiple system atrophy</td>
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<tr>
<td>MTA</td>
<td>Medial temporal lobe atrophy</td>
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<tr>
<td>NAC</td>
<td>Non-amyloid component</td>
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<tr>
<td>nbM</td>
<td>Nucleus basalis of Meynert</td>
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<tr>
<td>NFT</td>
<td>Neurofibrillary tangle</td>
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<tr>
<td>NIA-RI</td>
<td>National Institute on Aging – Reagan Institute</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>PAF</td>
<td>Pure autonomic failure</td>
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<tr>
<td>PD</td>
<td>Parkinson’s disease</td>
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<tr>
<td>PDD</td>
<td>Parkinson’s disease with dementia</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>PSP</td>
<td>Progressive supranuclear palsy</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single photon emission computed tomography</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SN</td>
<td>Substantia nigra</td>
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<tr>
<td>SNCA</td>
<td>α-Synuclein gene</td>
</tr>
<tr>
<td>SNP</td>
<td>Single-nucleotide polymorphism</td>
</tr>
<tr>
<td>VCI</td>
<td>Vascular cognitive impairment</td>
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by their Roman numerals:


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ABSTRACT

Populations in developed countries are ageing fast. The elderly have the greatest incidence of dementia, and thus the increase in the number of demented individuals, increases the immediate costs for the governments concerning healthcare and hospital treatment. Attention is being paid to disorders behind cognitive impairment with behavioural and psychological symptoms, which are enormous contributors to the hospital care required for the elderly. The highest dreams are in prevention; however, before discovering the tools for preventing dementia, the pathogenesis behind dementia disorders needs to be understood. Dementia with Lewy bodies (DLB), a relatively recently discovered dementia disorder compared to Alzheimer's disease (AD), is estimated to account for up to one third of primary degenerative dementia, thus being the second most common cause of dementia in the elderly. Nevertheless, the impact of neuropathological and genetic findings on the clinical syndrome of DLB is not fully established.

In this present series of studies, we aimed to evaluate the frequency of neuropathological findings of DLB and its relation to the clinical findings in a cohort of subjects with primary degenerative dementia (I) and in a population-based prospective cohort study of individuals aged 85 years or older (II). We also compared the impact of α-synuclein (αS) immunoreactive neuropathological findings of DLB and the impact of AD-type pathology on the clinical symptoms (I-II), since both of these pathologies are commonly found in elderly subjects. In addition, the contribution of genetic variation in the αS gene (SNCA) to the extent of αS pathology and AD-type pathology (III) was investigated. Further, the relevance and temporal course of the substantia nigra (SN) degeneration (II) and of the spinal cord αS pathology were studied (IV) in relation to αS pathology in the brain.

αS pathology classifiable according to the DLB consensus criteria was found in one fourth of the primary degenerative dementia subjects (I). In the population-based study, the corresponding figure was one third of the population, 38% of the demented and one fifth of the non-demented very elderly Finns (II). However, in spite of the frequent discovery of αS pathology, its association with the clinical symptoms was quite poor. Indeed, the common clinical features of DLB, hypokinesia and visual hallucinations, associated better with the severe neurofibrillary AD-type pathology than with the extensive (diffuse neocortical) αS pathology when both types of pathology were taken into account (II). The severity of the neurofibrillary AD-type pathology associated with the extent of αS pathology in the brain (II). In addition, the genetic study showed an interaction between tau and αS; common variation in the SNCA gene associated significantly with the severity of the neurofibrillary AD-type pathology (Braak stage) and nominally significantly with the extensive αS pathology (III). The linear association between the extent of αS pathology in the brain and the neuron loss in SN (II) suggests that in DLB the degeneration of SN proceeds as the αS pathology extends from SN to the neocortex instead of early destruction of SN seen in Parkinson’s disease (PD) (II). Furthermore, the extent of αS pathology in the brain associated with the severity of αS pathology in the thoracic and sacral autonomic nuclei of the spinal cord (IV). The thoracic αS pathology was more common and more severe compared to sacral cord, suggesting that the progress of αS pathology proceeds downwards from the brainstem towards the sacral spinal cord (IV).
1 INTRODUCTION

Due to the increasing proportion of the elderly in the populations of developed countries, research on dementia has become essential. The need for social and health services expands with the aging of the population since the incidence of neurodegenerative diseases tends to increase with aging. For example, in Finland, the population of 5.3 million has been estimated to grow moderately up to 5.7 million by the year 2030 (Stakes OSF 2008). During the same period, however, the proportion of people aged 65 years and older has been estimated to increase from 17% to 26%, and the number of demented individuals from 80,000 to 128,000 (Stakes OSF 2008). In addition, the financial costs rise due to the need for institutional or housing services for the demented elderly. In Finland, the financial costs for the long-term hospital care and housing services have been growing steadily since the 1990s, leading to immediate costs of 1.7 billion euros to the Finnish government in the year 2005. With the prolongation of home care by just a single year instead of hospitalisation for that period, the Finnish government would save 168 million euros per year (Viramo and Sulkava 2006).

Since the shape of the population pyramid cannot be changed in a short period of time, considerable effort has been directed at recognising the signs of dementia earlier and at promoting the ability of those affected to manage at home for longer. In particular, the correct diagnosis of the dementia disorder and the correct medication with other treatment modalities has been noted to prolong the time that those affected can manage at home. However, dementia covers a large range of symptoms, and there have been difficulties in clinically differentiating dementia disorders such as Alzheimer's disease (AD) and Dementia with Lewy bodies (DLB), especially in elderly subjects (McKeith 2002, Jellinger 2004). To understand the clinical symptoms and signs, we need the neuropathological research. The pathological hallmarks of both AD and DLB were discovered as long ago as the early 1900s (Alzheimer 1907, Lewy 1912). AD has been researched extensively for almost forty-years (Tomlinson et al. 1970). Compared to this, the history of substantial DLB research is still relatively young (Kosaka 1978, Yoshimura 1983, McKeith et al. 1996). Research on AD stepped forward in the 1990s with the discovery of the progression of the AD-type changes in the brain (Braak and Braak 1991). In DLB, the time has come now. There is an urgent social and economic need to resolve crucial questions concerning DLB. First: How common a cause of a degenerative central nervous system (CNS) disorder is it in reality? Second: What is the clinical relevance of the neuropathological findings of DLB? Third: How does the α-synuclein (αS) pathology progress in the central nervous system, and what are the exacerbating or protective factors behind this progression?

The present study was designed to clarify these aspects in patients with clinically diagnosed primary degenerative dementia and in the population-based study of very elderly Finns.
2 REVIEW OF THE LITERATURE

From Lewy bodies to DLB

In 1817, a British physician, James Parkinson (1755-1824), described six individuals with shaking palsy, and named the disease paralysis agitans (Parkinson 1817). In 1877, a French neurologist Jean-Martin Charcot (1825-1893) described a patient with rigidity in the absence of tremor or paralysis and suggested the disease be referred to as Parkinson's disease (PD; la maladie de Parkinson, Charcot 1877). Almost a hundred years after Parkinson's "An Essay on the Shaking Palsy" was published, a German-American neurologist, Friedrich Heinrich Lewy (1885-1950), described eosinophilic inclusions in the neurons of the dorsal motor nucleus of vagus (dmV) and the nucleus basalis of Meynert (nbM) in patients with PD (Lewy 1912). Seven years later, the intracytoplasmic inclusions were named after Lewy by a Russian neuropathologist, Konstantin Tretiakoff (1892-1958). He described the Lewy bodies (LBs) in the substantia nigra (SN) which, accompanied by the neuronal loss, associated with clinical PD (Tretiakoff 1919). For the next forty years, LBs were thought to be confined to the brainstem and to be responsible for the neuronal degeneration in PD (Greenfield and Bosanquet 1953, Lipkin 1959, Eadie 1963).

In 1961, the first cortical LBs were identified in patients with dementia by Okazaki, Lipkin and Aronson (Okazaki et al. 1961). In addition to the brainstem, they found LBs in the cerebral cortex and spinal cord in two male patients presenting with a progressive dementia without parkinsonian signs (Okazaki et al. 1961). A neurodegenerative disorder with moderate to large numbers of LBs in the brainstem and cerebral cortex in the context of dementia and parkinsonism was first described by Kosaka (Kosaka et al. 1976, Kosaka 1978). Only a few additional case reports were published, in the late 1970s (Forno et al. 1978, Kosaka and Mehran 1979). Demented patients with LBs in the brainstem and cortex had been thought to represent a combination of atypical PD and AD, until in 1983 Yoshimura concluded that the pathological findings were a new disorder (Yoshimura 1983). Around 1990, this neuropathological disorder became more widely recognised due to the discovery of ubiquitin as a sensitive immunohistochemical marker for the identification of LBs (Lennox et al. 1989a, Lennox et al. 1989b, Dickson et al. 1991). Various terms have been applied to it, including “diffuse type of Lewy body disease” (Kosaka et al. 1984), “Lewy body dementia” (Gibb et al. 1987), “the Lewy body variant of AD” (Hansen et al. 1990), “senile dementia of Lewy body type” (Perry et al. 1990a), and “dementia associated with cortical Lewy bodies” (Byrne et al. 1991). Eventually in 1995, the consortium of the first international workshop recommended the term “dementia with Lewy bodies” (DLB) to be used. The First consensus guidelines for the clinical and pathological diagnosis of DLB were published the following year (McKeith et al. 1996). In 2005, the third workshop of the DLB consortium revised the clinical and pathological criteria for diagnosing DLB. In addition to assessing LBs and LNs, the newest guidelines proposed a probability statement of neuropathological findings associating with the DLB clinical syndrome (McKeith et al. 2005). This statement takes into consideration the extent of Lewy-related pathology (LRP) but also the often co-occurring Alzheimer type pathology.
Clinical findings

The most recent clinical diagnostic criteria for DLB were published in 2005 by the DLB consortium (McKeith et al. 2005). The criteria include guidelines for probable and possible DLB. The essential and central feature for DLB is the presence of dementia, defined as progressive cognitive decline resulting in significant social and occupational functional impairment (Table 1). In addition to this, the diagnostic criteria for probable DLB require at least two, and for possible DLB at least one, of the three core features: fluctuating cognition, recurrent well-formed visual hallucinations and spontaneous features of parkinsonism (McKeith et al. 2005). In the absence of two core features, the diagnosis of probable DLB can also be made if at least one suggestive feature is present with one core feature. One or more suggestive feature is sufficient for possible DLB even without the core features, but the diagnosis of probable DLB should not be done on the basis of suggestive features alone. Supportive clinical features may be commonly present but lack the diagnostic specificity (McKeith et al. 2005).

Table 1 The clinical features needed when assessing the diagnosis of probably or possible DLB. (Modified from McKeith et al. 2005)

<table>
<thead>
<tr>
<th>Central feature</th>
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<tr>
<td>Dementia</td>
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<tr>
<td>Core features</td>
</tr>
<tr>
<td>Fluctuating cognition</td>
</tr>
<tr>
<td>Recurrent visual hallucinations (typically well formed and detailed)</td>
</tr>
<tr>
<td>Spontaneous features of parkinsonism</td>
</tr>
<tr>
<td>Suggestive features</td>
</tr>
<tr>
<td>Rapid eye movement (REM) sleep behaviour disorder</td>
</tr>
<tr>
<td>Severe neuroleptic sensitivity</td>
</tr>
<tr>
<td>Low dopamine transporter uptake in basal ganglia demonstrated by SPECT or PET imaging</td>
</tr>
<tr>
<td>Supportive features</td>
</tr>
<tr>
<td>Repeated falls and syncope</td>
</tr>
<tr>
<td>Transient loss of consciousness</td>
</tr>
<tr>
<td>Severe autonomic dysfunction</td>
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<tr>
<td>Depression,</td>
</tr>
<tr>
<td>Systematized delusions</td>
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<tr>
<td>Hallucinations in other (sensory and perceptual) modalities</td>
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<tr>
<td>Relative preservation of medial temporal lobe structures on CT/MRI scan</td>
</tr>
<tr>
<td>Generalised low uptake on SPECT/PET perfusion scan with reduced occipital activity</td>
</tr>
<tr>
<td>Abnormal (low uptake) MIBG myocardial scintigraphy</td>
</tr>
<tr>
<td>Prominent slow wave activity on EEG</td>
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</table>
Subjects with described symptoms are, however, more prone to manifest the relatively rare pure form of DLB rather than combined (AD-type and αS) pathologies in the autopsy. Thus, the clinical criteria for probable DLB have had a good specificity (low false positive rate) but suboptimal sensitivity (high false negative rate) (Litvan et al. 2003, McKeith et al. 2004).

Clinical differential diagnosis

The clinical differentiation between DLB and AD, PD, progressive supranuclear palsy (PSP), multiple system atrophy (MSA) or corticobasal degeneration may be problematic, particularly in elderly patients. DLB is, indeed, frequently misdiagnosed as AD (McKeith 2002), or PD (Gomez-Tortosa et al. 1998, Lopez et al. 2002). In spite of the difficulties in diagnosing DLB, an accurate clinical diagnosis is crucial. DLB patients often have a distinct clinical course (Del-Ser et al. 1996), rapid progression rate (Olichney et al. 1998), and a high incidence of neuroleptic sensitivity (McKeith et al. 1992, Ballard et al. 1998) compared to patients suffering from the other dementing diseases. Of the neuroleptic-treated DLB patients, 40% to 50% present with severe side effects, such as generalised rigidity, sedation, immobility, neuroleptic malignant syndrome with fever and muscle breakdown, which are associated with a two- to three-fold increase in mortality (McKeith 2002). Thus, an early and accurate DLB diagnosis ensures that physicians can avoid medication which may aggravate the clinical picture and also allows families and caregivers more time to prepare for the expected clinical decline.

According to the consensus guidelines DLB can be distinguished from PD dementia (PDD) only with the temporal appearance of extrapyramidal symptoms; in PDD, the motor symptoms precede the onset of dementia by at least one year, and in DLB, dementia occurs before or at the same time as parkinsonism (McKeith et al. 2005). However, this rough distinction is recommended to be used only in research studies, while in clinical practice a generic term such as LB disease is preferred as it covers both DLB and PDD (McKeith et al. 2005). In line with this, a recent study failed to find any association between the duration of parkinsonism and αS pathology distribution in the autopsy (Ballard et al. 2006).

The absence of radiological and biological markers that can reliably aid in the specific diagnosis of DLB has led to research for clinical measures that can serve as markers for pathology. Functional imaging of the dopamine transporter (DAT) reveals the integrity of the nigrostriatal dopaminergic system, where low uptake is associated with tremor (Marshall and Grosset 2003). Imaging with specific ligands for DAT has been used in connection with diseases such as PD (Rinne et al. 1997), MSA, PSP and DLB (Walker et al. 2002). Although this type of imaging does not help in distinguishing DLB from PD/PDD, it does provide a distinction between DLB and AD, since in the latter the striatal dopamine transporter uptake, as shown by SPECT or PET imaging, is normal (Walker et al. 2002, McKeith et al. 2007). However, the global cortical amyloid burden assessed by Pittsburgh Compound B (PIB) PET imaging is higher in the DLB and AD than in the PDD (Gomperts et al. 2008). Glucose hypometabolism demonstrated with PET (Ishii et al. 1999) and hypoperfusion demonstrated with SPECT (Lobotesis et al. 2001) in the occipital lobe appear to be helpful in discriminating DLB from other types of neurodegenerative disorders, but even these methods do not recognise DLB specifically. Myocardial scintigraphy with [I-123] radiolabelled metaiodobenzyl guanidine (MIBG) is reduced in DLB, which has been reported to help in distinguishing DLB from AD (Taki et al. 2004). The degree of generalised brain atrophy does not differentiate DLB from other disorders (Barber et al. 2000). Instead, medial temporal...
lobe atrophy (MTA) seen in MRI differentiates AD from DLB and vascular cognitive impairment (VCI; Burton et al. 2009). The neurofibrillary Braak AD stage has been reported as a significant predictor for MTA (Burton et al. 2009), but a previous study of the very elderly showed an MTA association with hippocampal sclerosis and combined brain pathology (Barkhof et al. 2007). The increase of γ and α-synuclein in the ventricular cerebrospinal fluid (CSF) is a novel finding (Mukaetova-Ladinska et al. 2008). However, it needs to be established whether these results can aid in distinguishing the LB disease from other types of neurodegenerative and vascular dementias. Particularly, because the elevated protein concentrations were detected in all demented subjects (Mukaetova-Ladinska et al. 2008).

Course of DLB

With a slight excess of males in the data, the age at onset of clinical DLB ranges from 50 to 83 years, and age at death from 68 to 92 years (McKeith 2002). There is a high variation in the disease duration from the onset of symptoms to death ranging from 1.5 to 24 years (Parkkinen et al. 2001, Stern et al. 1987, Kosaka 1990). Poorer prognosis and more aggressive disease course have been demonstrated to associate with earlier onset of disease and with the presence of AD-type pathology. Although the initial symptoms may vary between the most common dementia and less common parkinsonism, psychiatric disorder and autonomic failure (Byrne et al. 1989, McKeith et al. 1992), in the end stage most DLB subjects have at least mild dementia accompanied by one extrapyramidal symptom (Burkhardt et al. 1988). There is no therapy that has been proven to modify or to delay the disease progression. So far, the best treatments available are cholinesterase inhibitors, which have demonstrated modest control of fluctuating cognitive impairment and an impact on global function (Aarsland et al. 2004). Aspiration pneumonia is the most common cause of death (Burkhardt et al. 1988).

Neuropathological findings in DLB

Lewy bodies

When, almost a hundred years ago, F.H. Lewy found round, eosinophilic intraneuronal inclusions, he had detected for the very first time classical brainstem LBs, the characteristic hallmark of DLB and PD (Lewy 1912). In DLB, brainstem LBs are most often observed, according to their name, in brainstem nuclei such as SN, locus coeruleus (LC), and dmV (McKeith et al. 1996) but are also found in nbM (Dickson et al. 1987). Brainstem LBs are round or oval in shape, and their size is around 5 to 25 µm in diameter depending on the configuration of the neuronal cytoplasm, i.e. perikaryon, they occupy. Larger neurons, such as those in dmV, often contain bigger LBs or occasionally several LBs (Gibb et al. 1991). The terms “brainstem” or “classic” LB apply to inclusions with a hyaline core and pale halo. The eosinophilic core, consisting of an amorphous electron-dense material, is surrounded by a halo of radially-orientated filament fibrils of 5-20nm in diameter (Forno et al. 1986, Jellinger 1990, Pollanen et al. 1992, Spillantini et al. 1998). Amongst the filaments, the basophilic halo consists of scattered granules of lipofuscin and neuromelanin, mitochondria and vesicles with a dense circular outline of filaments (Yoshimura 1983).

Cortical LBs are most often detected in small to medium-sized non-pyramidal neurons in the deeper part of cerebral cortex, particularly in the fifth and sixth layer (Lennox et al. 1989b, Gibb et al. 1987). Cortical LBs are predominantly present in the entorhinal
cortex, cingulate gyrus, insula, amygdala and temporal cortex (Lennox et al. 1989a, Perry et al. 1990a, Gomez-Tortosa et al. 2000a), while in the parietal and occipital cortex and in hippocampus LBs are less frequent (Yoshimura 1983, Kosaka et al. 1984, Gibb et al. 1987, Gomez-Tortosa et al. 2000a, Gibb et al. 1989). Association cortices are more vulnerable than primary cortices, and monoaminergic (noradrenergic, dopaminergic and serotonergic) neurons are suggested to be intrinsically vulnerable to LB formation (Pollanen et al. 1993). The shape of cortical LBs is less well defined in that they are not always round and usually they are smaller than brainstem LBs, having a diameter range from 5 to 15 µm. Cortical LBs consist of similar fibrillar, granular and vesicular material to brainstem LBs, but the filaments are randomly orientated and loosely packed, and thus cortical LBs are less eosinophilic and lack the separate core and halo (Jellinger 1990, Pollanen et al. 1993, Forno 1996).

In addition to neuronal cytoplasm, LBs have been described in connection with nerve cell processes (intraneuritic) and lying free in the neuropil (extracellular) (Yoshimura 1983, Gibb et al. 1991, Alafuzoff et al. 2009).

**Lewy neurites**

Lewy neurites (LNs) constitute an important part of the neuropathological findings of DLB and PD/PDD. They correspond to abnormal neurites that contain filaments similar to those found in LBs, but in LNs they present as diffuse aggregates which do not contain crystallin (Dickson et al. 1991). The CA 2-3 hippocampal region, amygdala, nBM, dmV, and other brainstem nuclei are the predilection site for LNs. Together LBs and LNs have been termed Lewy-related pathology (LRP; McKeith et al. 2005) or αS pathology based on their immunoreactive profile (Spillantini et al. 1998).

**Ubiquitin**

Initially, LBs were detected with the conventional hematoxylin and eosin (H&E) histological technique. However, cortical LBs are difficult to identify with the H&E technique since they have a diffuse and less prominent morphology (Lennox et al. 1989b). LBs are not detectable with periodic acid Schiff (PAS), and thus are distinguishable from lipofuscin, age-related accumulation in neurons and glia, and from corpora amylacea (Forno et al. 1986). In the late 1980s, thanks to more specific methods provided by immunohistochemistry, LBs were recognised as being composed of two kind of proteins; functional i.e. cytoskeletal and incorporated proteins. Functional proteins such as neurofilament proteins form the cytoskeleton of the inclusion, and ubiquitin is involved in the cytosolic proteolysis (Lowe et al. 1988). Most LBs were ubiquitin-positive when stained immunohistochemically, but only some could be immunolabelled with antibodies against neurofilaments (Dale et al. 1992). Moreover, some studies showed LBs to be composed of ubiquitinated neurofilament proteins accumulating in response to cellular stress and abnormal phosphorylation, possibly a means of removing the damaged protein in a cytoprotective manner (Pollanen et al. 1993, Lowe et al. 1988). Thus, the use of ubiquitin immunohistochemistry was recommended as the most reliable method for the detection of LBs by the consortium on DLB in their First guidelines (McKeith et al. 1996). Since incorporated proteins such as tubulin and microtubule associated proteins, synaptic proteins and the β-amyloid precursor protein are present in LBs, tubulin and parkin were described to label LBs. However, α-synuclein (αS) was soon revealed to be the major functional protein component of LBs (Spillantini et al. 1997, Spillantini et al. 1998, Baba et al. 1998). αS immunohistochemistry (IHC) distinguished LBs from non-staining globose
neurofibrillary tangles (NFTs) (Gomez-Tortosa et al. 2000b), while the use of ubiquitin did not because ubiquitin is also present in NFTs (Lowe et al. 1988, Dickson et al. 1989). Thus, the second workshop of the DLB consortium recommended the use αS-IHC, the most specific and sensitive method for detecting LBs and the previously under-recognised LNs (McKeith et al. 1999).

Synuclein protein family

Synuclein protein was described for the very first time in 1988, when it was cloned from the electric organ of the fish Torpedo californica (Maroteaux et al. 1988). Initially in 1993, a protein sequence was purified from the amyloid plaque of an Alzheimer’s disease brain and named the non-amyloid component (NAC) (Ueda et al. 1993). A year later, this was found to be identical to the 61-95 amino acid sequence of αS protein (Jakes et al. 1994). It has been proposed that the sequence comprising residues 61-95 of αS (NAC) has self-aggregating properties and can form amyloid aggregates (Iwai et al. 1995). However, further studies did not detect NAC/αS in the β-amyloid plaques (Bayer et al. 1999, Culvenor et al. 1999).

The human synuclein proteins, including α-, β- and γ-synuclein and synoretin, constitute up to 1% of the total protein content in cytosolic brain fractions. They are small proteins (19-20kDA, 112-140 amino acids) with similar amino acid sequence of three modular domains (Figure 1) but they arise from distinct genes (Goedert 2001). Their amino-terminal (N-terminal) region (residues 1-60) contains five to six imperfect amino acid repeats and thus share a structural similarity with apolipoproteins. The N-terminal may facilitate protein-protein binding and as an α-helical secondary structure lipid binding. The middle hydrophobic NAC part is characteristic for αS only. The C-terminal tail contains acidic amino acids, remains free and unfolded, and protects cellular proteins from denaturation (Mukaetova-Ladinska and McKeith 2006).

<table>
<thead>
<tr>
<th></th>
<th>N-terminal amphipathic region (residues 1-60)</th>
<th>NAC (residues 61-95)</th>
<th>C-terminal acidic tail (residues 96-140)</th>
</tr>
</thead>
<tbody>
<tr>
<td>αS-140aa</td>
<td><img src="image1" alt="Diagram" /></td>
<td></td>
<td><img src="image2" alt="Diagram" /></td>
</tr>
<tr>
<td>αS-112aa</td>
<td><img src="image3" alt="Diagram" /></td>
<td><img src="image4" alt="Diagram" /></td>
<td><img src="image5" alt="Diagram" /></td>
</tr>
<tr>
<td>β-synuclein</td>
<td><img src="image6" alt="Diagram" /></td>
<td><img src="image7" alt="Diagram" /></td>
<td><img src="image8" alt="Diagram" /></td>
</tr>
<tr>
<td>γ-synuclein</td>
<td><img src="image9" alt="Diagram" /></td>
<td><img src="image10" alt="Diagram" /></td>
<td><img src="image11" alt="Diagram" /></td>
</tr>
</tbody>
</table>

Figure 1 Structure of synucleins. The 112 isoform of αS protein lacks 28 amino acids (aa 103-130) from the acidic tail. β-synuclein lacks 11 aa from the NAC region. Synuclein mutation sites are indicated with arrows, whereas the black boxes show the post-translationally modified sites. (Modified from Mukaetova-Ladinska and McKeith 2006).
The natively unfolded and soluble αS protein was termed ‘synuclein’ based on its localization to presynaptic nerve terminals and portions of the nerve cell nuclear envelope (Baba et al. 1998, Hsu et al. 1998, Irizarry et al. 1998). Mainly as an isoform of 140 but also as an isoform of 112 amino acids, αS is widespread in the CNS, including the anterior horns of the spinal cord, normal Schwann cells, cultured oligodendrocytes, platelets and CSF in both fetal and adult brain (Mukaetova-Ladinska and McKeith 2006). Native αS binds to synaptic vesicles and has a role in neurotransmission (Jensen and Gai 2001, Dev et al. 2003, Vekrellis et al. 2004). Transgenic expression of αS has been shown to protect the nerve terminal from neurodegeneration by co-operating with another synaptic vesicle protein, cysteine-string protein-alpha (CSPalpha) (Chandra et al. 2005).

β-synuclein is expressed axonally throughout the CNS, and in addition to presynaptic nerve terminals, it is also found in normal astrocytes. The amino acid sequence of β-synuclein lacks a portion of the NAC region (corresponding residues 73-83 of αS) and may effect on its inability to form fibrils. γ-synuclein is most abundant in the peripheral nervous system, but is also expressed in a subset of neurons and glial cells in the cerebral cortex and spinal cord (Mukaetova-Ladinska and McKeith 2006) and CSF (Mukaetova-Ladinska et al. 2008). γ-synuclein protein is also known as breast cancer specific gene-1 (BCSG1; Ji et al. 1997) or persyn (Buchman et al. 1998, Ninkina et al. 1999). Synoretin has 84% similarity with γ-synuclein and is expressed predominantly in the outer nuclear retinal layer.

In neurologically intact subjects, the highest level of synuclein expression in the brain has been detected within the neocortex, followed by the hippocampus and cerebellum, whereas the lowest levels have been seen in the basal ganglia and SN (Rockenstein et al. 2001). β-synuclein has the most abundant mRNA message of the synucleins (75-80%), most detected in neocortex and cerebellum, followed by γ-synuclein (10-15%) in hippocampus, whereas basal ganglia predominant αS message is the least detected (8-10%). This balance among synuclein proteins is important to maintain normal brain function (Rockenstein et al. 2001).

α-Synuclein aggregation

The site of initial αS aggregation has been proposed to be in the presynaptic terminals (Kramer and Schulz-Schaeffer 2007), in the axonal compartment (Marui et al. 2002), or in the soma (Katsuse et al. 2003). The aberrant interaction of αS with neurofilaments has been suggested to lead to the formation of insoluble inclusions, i.e. LBs and LNs (Trojanowski and Lee 1998). The reasons behind the αS aggregation are not clear. Apoptosis, inflammation and oxidative stress damage, neurotoxicity of protofibrils and oligomers, and aggresome-related cytoprotective processes have been suggested as mechanisms (Jellinger 1999, Hashimoto et al. 1999, Conway et al. 2000, McNaught et al. 2002, Norris and Giasson 2005). The αS protein of LBs may be ubiquitinated, indicating a role in the aggresomal response (Irizarry et al. 1998). However, the ubiquitination is not required for protein aggregation (Sampathu et al. 2003). The inhibition of the αS aggregation could provide a therapeutic potential against diseases presenting with αS aggregates, i.e. α-synucleinopathies. Some studies have already found protective endogenous protein molecules which inhibit αS aggregation, such as β-synuclein (Hashimoto et al. 2001, Park and Lansbury 2003) and gangliosides (Wei et al. 2009).
As pathological aggregates of αS form not only LBs but also LNs, the antibodies against the αS protein strongly immunostain both LBs and LNs as seen in Figure 2 (Spillantini et al. 1998). LNs are not detectable with the H&E technique, and with ubiquitin immunohistochemistry the staining intensity is not as high as with αS. Moreover, antibodies directed against β-synuclein and γ-synuclein failed to stain LBs and LNs (Spillantini et al. 1998). Thus, there is no evidence of LBs and LNs including other kind of synuclein in addition to αS.

![Figure 2 Lewy bodies (LBs; arrows) and Lewy neurites (LNs; arrow heads). a) H&E staining of the Substantia nigra showing couple of LBs. αS immunohistochemistry b) of the neocortex showing few LBs and LNs (Zymed antibody) and c) of the hippocampal CA2-3 region showing numerous LNs and few LBs (Transduction antibody).](image)

**Immunohistochemistry**

The expression of αS pathology can be visualised with αS-IHC. Since the 1980s, IHC has been a good but capricious diagnostic tool, where the results depend on the antibody and the antigen retrieval method used, the fixation time and postmortem delay of the tissue and the expertise of the evaluator (Alafuzoff et al. 2008). The most commonly used antibodies and pretreatments are described in Table 2.
Review of the literature

Table 2 Summary of common α-synuclein immunohistochemistry protocols (Croisier et al. 2006, Alafuzoff et al. 2008, Beach et al. 2008).

<table>
<thead>
<tr>
<th>Antibody/clone</th>
<th>Source</th>
<th>Epitope</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synuclein-1/Syn42</td>
<td>BD Transduction Laboratories</td>
<td>aa 15-123 of rat αS</td>
<td>80% FA</td>
</tr>
<tr>
<td>α-synuclein /LB509</td>
<td>Zymed</td>
<td>aa 115-122 of human purified LBs</td>
<td>Proteinase K</td>
</tr>
<tr>
<td>α-synuclein /15G7</td>
<td>Alexis</td>
<td>aa 116-131 of synthetic peptide</td>
<td>MW + 80% FA</td>
</tr>
<tr>
<td>α-synuclein /4D6</td>
<td>Signet</td>
<td>Full length of human recombinant αS</td>
<td>MW + 80% FA</td>
</tr>
<tr>
<td>NCL-ASYN/KM51</td>
<td>Novocastra</td>
<td>Full length of human recombinant αS</td>
<td>AC + 80% FA</td>
</tr>
<tr>
<td>α-synuclein /Syn211</td>
<td>SantaCruz</td>
<td>aa 121-125 of human αS</td>
<td>100% FA</td>
</tr>
<tr>
<td>α-synuclein /polyclonal</td>
<td>Chemicon</td>
<td>aa 116-131 of human αS</td>
<td>80% FA</td>
</tr>
</tbody>
</table>

aa, amino acid; AC, autoclave; FA, formic acid; MW, microwave

Lewy-related/α-synuclein pathology

Pathological, biochemical and genetic studies have together resulted in retaining a substantial role for αS in the pathogenesis of DLB and PD/PDD. Presence of αS pathology in these and other clinical syndromes is described in Table 3. αS is the major component of LBs (both intra- and extraneuronal) and LNs, *i.e.* LRP (Spillantini et al. 1998). The terms LRP and αS pathology are used in the same context. However, in addition to LBs and LNs, αS-IHC also detects pale bodies (Irizarry et al. 1998, Kuusisto et al. 2003), grain-like intraneuronal inclusions, aggregates and threads in the neuropil (Alafuzoff et al. 2009), glial cytoplasmic inclusions seen in MSA (Dickson et al. 1999), aggregates in the astroglia and Schwann cells of the anterior horn of the spinal cord in amyotrophic lateral sclerosis (Mezey et al. 1998), and also granular aggregates seen in the peripheral autonomic plexuses (Minguez-Castellanos et al. 2007).

The different morphological αS-staining structures have been proposed to emphasise the stages of the aggregation process from punctate and diffuse cytoplasmic staining to pale bodies, and finally to compact LB inclusions (Kuusisto et al. 2003). Pale bodies are rounded and palely eosinophilic inclusions, which consist of sparsely arranged fibrils, homogenous or uniform granular matter and vacuoles (Gibb et al. 1991). Pale bodies are predominantly detected in SN and LC (Gibb et al. 1991).

In addition to neuronal structures, some αS-positive glial cytoplasmic inclusions (GCIs) have been reported in astrocytes and oligodendrocytes in PD subjects (Wakabayashi et al. 2000). More commonly, GCIs are seen in MSA, which covers the earlier terms of olivopontocerebellar atrophy, striatonigral degeneration and Shy-Drager syndrome.
Hallmarks of MSA are neuropathologically glial cytoplasmic inclusions, but also some neuronal cytoplasmic inclusions can be detected, predominantly in the amygdala, hippocampus, SN, putamen and pontine nuclei and in the intermediolateral column of the spinal cord, but not in the cerebral cortex (Dickson et al. 1999). Moreover, neuronal loss (in the myelin fibres of the external capsula, striatum, globus pallidus, cerebellar white matter, and pontis base), web-like fibrils in neuronal and glial nuclei, neuropil threads without tau-positivity, and astrocytic gliosis are seen with variable distribution and severity (Ellison and Love 2004, Dickson et al. 1999, Lin et al. 2004).

Diseases with αS pathology as the cause of neurodegeneration are called α-synucleinopathies (Spillantini and Goedert 2000). These include DLB, PD, PDD, MSA, and PAF (Table 3). Thus, not all diseases with the αS immunoreactive pathology are assigned under the family name α-synucleinopathies.

### Table 3 Clinical conditions associated with α-synuclein pathology. (Modified from Mukaetova-Ladinska and McKeith 2006)

<table>
<thead>
<tr>
<th>Disorders characterised by αS pathology (synucleinopathies)</th>
<th>Conditions with αS pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dementia with Lewy bodies</td>
<td>Ageing</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>PD dementia</td>
<td>Down’s syndrome</td>
</tr>
<tr>
<td>Multiple system atrophy</td>
<td>Tauopathies:</td>
</tr>
<tr>
<td>Pure autonomic failure</td>
<td>Pick’s disease</td>
</tr>
<tr>
<td></td>
<td>Progressive supranuclear palsy</td>
</tr>
<tr>
<td></td>
<td>Corticobasal degeneration</td>
</tr>
<tr>
<td></td>
<td>Frontotemporal lobar degeneration</td>
</tr>
<tr>
<td></td>
<td>Motor neuron disease:</td>
</tr>
<tr>
<td></td>
<td>Amyotrophic lateral sclerosis</td>
</tr>
<tr>
<td></td>
<td>Progressive muscular dystrophy</td>
</tr>
<tr>
<td></td>
<td>Neuroaxonal dystrophy</td>
</tr>
<tr>
<td></td>
<td>Traumatic brain injury</td>
</tr>
</tbody>
</table>

### Spinal cord α-synuclein pathology

In addition to the αS pathology in the brain, the spinal cord and especially its autonomic nuclei are often damaged in PD and DLB (Wakabayashi and Takahashi 1997, Kaufmann and Biaggioni 2003). However, up to 17% of the neurologically unimpaired subjects have been demonstrated to manifest at least some LBs and/or LNPs in the autonomic nuclei of the spinal cord (Bloch et al. 2006). αS pathology in the spinal cord autonomic nuclei has also been suggested to provide the pathological basis for autonomic failure, which earlier was assumed to occur only in the later stages of PD (Probst et al. 2008), or with the concept of pure autonomic failure (PAF; Kaufmann et al. 2001). So far concerning the spinal cord and peripheral nervous system, the neuropathological criteria for staging/typing αS pathology do not exist.
Progression of the $\alpha$-synuclein pathology

Several studies have discovered $\alpha$S pathology in the lower brainstem nuclei and in autonomic nuclei of the spinal cord in neurologically intact subjects (Klos et al. 2006, Dickson et al. 2008, DelleDonne et al. 2008). These incidental lesions, and also the fine granular $\alpha$S aggregates found in peripheral autonomic plexuses, have been suggested to represent a pre-symptomatic phase in the development of the PD or LB disorders (Minguez-Castellanos et al. 2007, Dickson et al. 2008, DelleDonne et al. 2008). Further, the lower brainstem nuclei, such as dmV, olfactory bulb and autonomic nuclei of the spinal cord, have been proposed to be the induction site or among the earliest affected regions in the pathological process of $\alpha$S pathology (Probst et al. 2008, Del Tredici et al. 2002). The sites of $\alpha$S pathology detected are presented in Table 4.

### Table 4: Discovery of $\alpha$-synuclein pathology.

<table>
<thead>
<tr>
<th>Anatomical region</th>
<th>Symptoms related</th>
<th>Reference for the determination/staging</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous nerves</td>
<td>Parkinsonism and/or dementia</td>
<td>Ikemura et al. 2008</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>Intact neurology or parkinsonism and/or dementia</td>
<td>Fumimura et al. 2007</td>
</tr>
<tr>
<td>Abdominopelvic plexuses</td>
<td>Neurologically intact</td>
<td>Minguez-Castellanos et al. 2007</td>
</tr>
<tr>
<td>Spinal Cord</td>
<td>Neurologically intact</td>
<td>Bloch et al. 2006, Klos et al. 2006</td>
</tr>
<tr>
<td>Olfactory bulb</td>
<td>Intact neurology or parkinsonism and/or dementia</td>
<td>Braak et al. 2003, Beach et al. 2009</td>
</tr>
<tr>
<td>Medulla (dmV, irx)</td>
<td>Neurologically intact</td>
<td>Del Tredici et al. 2002, Braak et al. 2003</td>
</tr>
<tr>
<td>Midbrain (SN)</td>
<td>Parkinsonism</td>
<td>Braak et al. 2003</td>
</tr>
<tr>
<td>Hippocampus (CA2-3)</td>
<td>Dementia</td>
<td>Dickson et al. 1991</td>
</tr>
<tr>
<td>Amygdala</td>
<td>Dementia, depression</td>
<td>Hamilton 2000, Lopez et al. 2006, Uchikado et al. 2006</td>
</tr>
<tr>
<td>Cortex</td>
<td>Dementia, parkinsonism</td>
<td>McKeith et al. 2005</td>
</tr>
</tbody>
</table>

dmV, Dorsal motor nucleus of vagus; irx, Intermediate reticular zone; SN, substantia nigra

In PD, after the first progression stages in the medulla and pons, $\alpha$S pathology accumulates in the dopaminergic projection cells of the SN pars compacta, resulting in the devastation of the neurons and causing parkinsonian motor symptoms such as rigidity and bradykinesia (Braak et al. 2006). When this pathological process extends to limbic and finally to neocortical areas, it associates with the clinical syndrome of PDD. Thus, the distribution of $\alpha$S pathology in PD is assumed to follow a topographic sequence in which the pathology proceeds to the rostral upper stages after the brainstem has become completely affected (Braak et al. 2003).
In DLB, at least some αS pathology in the brainstem is required for the neuropathological diagnosis, but limbic and diffuse neocortical distribution of αS pathology associates better with the clinical syndrome of DLB (McKeith et al. 2005). The temporal course of αS pathology in the spinal cord and peripheral nervous system in relation to the αS pathology in the brain is unknown.

**Neuropathological criteria for DLB**

The current diagnostic gold standard for diagnosing DLB is the neuropathological autopsy. Already at an early stage of the DLB history, the first attempts were made to standardise the assessment of brain pathology regarding the DLB (Kosaka et al. 1984). They divided the neuropathological findings into brainstem, limbic and neocortical subtypes based on the distribution of LBs. The First guidelines by the DLB consortium were published in 1996 (McKeith et al. 1996). The detailed instructions the DLB consortium recommended for brain sampling are still in use. Areas for sampling include three sections recommended for the frontal, temporal, and parietal cortex. These are compatible with the recommended CERAD sections for the neocortex (middle frontal gyrus, superior and middle temporal gyri, and inferior parietal lobule). The other samples needed are the anterior cingulate region, the transentorhinal section with parahippocampal gyrus, SN, LC and dmV (McKeith et al. 1996). In these recommendations, the load of LB burden on each sample area was assessed by counting every detected LB.

The most recent neuropathological guidelines by the DLB consortium were published in 2005 (Table 5; McKeith et al. 2005). In addition to advice for categorising αS pathology (into brainstem-predominant, limbic and diffuse neocortical types) based on density and regional distribution of LBs and LNs detected with αS-IHC, they recommend a semiquantitative assessment of the burden of αS pathology. The Third DLB consortium guidelines also proposed a probability statement of neuropathological findings associating with the DLB clinical syndrome, which takes into account the extent of αS pathology and the often co-occurring Alzheimer-type pathology (Table 6; McKeith et al. 2005). The category/type of αS pathology can be assigned according to the Third DLB consortium guidelines in many cases, but at the same time a considerable number of cases remain non-assignable (Fujimi et al. 2008, Parkkinen et al. 2008). Thus, other staging modalities have also been proposed.

The Braak staging strategy was initially used for staging αS pathology in PD (Braak et al. 2003). When assessing a large number of LB disorder brains, many have indicated that αS pathology is not always presented as is predicted according to the protocol of McKeith categorization or Braak staging of αS pathology. Thus, if these assessment recommendations are strictly followed, at least some and perhaps even half of the cases remain unclassifiable (Fujimi et al. 2008, Parkkinen et al. 2008). Recently, Leverenz and colleagues reported that they were able to increase the number of classifiable cases from 51% to 96% by modifying the classification strategy of McKeith’s categorization (Leverenz et al. 2008). They reduced the number of regions to be examined (using only the frontal of the neocortical samples), allowed more variability in the assessment of the severity of LB pathology, and added an amygdala-predominant category of LB disorder (Table 5). Only a few reports have been published assessing the usability of these recommended staging strategies (Alafuzoff et al. 2009, Leverenz et al. 2008, Müller et al. 2005).
Table 5 Categorization and staging of α-synuclein immunoreactive (IR) pathology (McKeith et al. 2005, Braak et al. 2003, Leverenz et al. 2008)

<table>
<thead>
<tr>
<th>Category/Stage</th>
<th>Brainstem regions</th>
<th>Basal regions</th>
<th>Forebrain</th>
<th>Limbic</th>
<th>Neocortical regions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IX-X</td>
<td>LC</td>
<td>SN</td>
<td>nbM</td>
<td>AC</td>
</tr>
<tr>
<td>McKeith</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brainstem</td>
<td>1-3</td>
<td>1-3</td>
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<td>0-2</td>
<td>0-2</td>
</tr>
<tr>
<td>Limbic</td>
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<td>1-3</td>
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<td>2-3</td>
</tr>
<tr>
<td>Diffuse NC</td>
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<td>1-3</td>
<td>1-3</td>
<td>2-3</td>
<td>3-4</td>
</tr>
<tr>
<td>Braak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1-2</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
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<td>0</td>
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<td>6</td>
<td>3</td>
<td>2-3</td>
<td>3</td>
<td>3</td>
<td>IR</td>
</tr>
<tr>
<td>Leverenz</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brainstem</td>
<td>1+</td>
<td>1+</td>
<td>0-2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Limbic</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
<td>1-3</td>
<td>0-1</td>
</tr>
<tr>
<td>Neocortical</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
</tr>
<tr>
<td>AC predom</td>
<td>0-1</td>
<td>0-1</td>
<td>1+</td>
<td>0-1</td>
<td>0-1</td>
</tr>
</tbody>
</table>

IX-X: cranial nerve nucleus of glossopharyngeus and vagus; LC, locus coeruleus; SN, substantia nigra; nbM, nucleus basalis of Meynert; AC, amygdala; Trans, transentorhinal cortex; Cing, Cingulate gyrus; Temporal, Frontal and Parietal cortex.

Table 6 Assessment of the likelihood that αS pathology is associated with the DLB clinical syndrome (McKeith et al. 2005).

<table>
<thead>
<tr>
<th>Category of αS pathology</th>
<th>Braak stage 0-II</th>
<th>Alzheimer type pathology Braak stage III-IV</th>
<th>Braak stage V-VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brainstem predominant</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Limbic transitional</td>
<td>High</td>
<td>Intermediate</td>
<td>Low</td>
</tr>
<tr>
<td>Diffuse neocortical</td>
<td>High</td>
<td>High</td>
<td>Intermediate</td>
</tr>
</tbody>
</table>

The most recent recommendations for staging αS pathology have been described by the BrainNet Europe (BNE) consortium (Alafuzoff et al. 2009). The main target was to achieve higher inter-observer agreement compared with the agreements achieved when using earlier assessment protocols. They also incorporated an amygdala-predominant category beside the earlier brainstem, limbic and neocortical types. They recommended a nine-block sampling strategy including: medulla with dmV, pons with LC, midbrain with SN, basal forebrain with nbM, striatum, hippocampus with transentorhinal cortex, cingulate gyrus, and temporal-, frontal- and parietal cortex (Alafuzoff et al. 2009). Furthermore, in the BNE-protocol, the αS pathology is assessed as being present or absent (dichotomized assessment) whereas the previous staging protocols are semiquantitative (Braak et al. 2003, McKeith et al. 2005, Leverenz et al. 2008).

LBs and LNs are also the hallmarks in PD. Of the PD patients, up to 31% eventually develop dementia (Aarsland et al. 2005). Patients with PDD harbour αS pathology in the brainstem in addition to the cerebral cortex. To date, criteria to neuropathologically distinguish between the diffuse neocortical DLB and PDD do not exist. The only
Review of the literature

division method is clinical, based on the 1-year-rule of the temporal sequence of motor symptoms and dementia. There are no diagnostic histopathological criteria for PDD. If the neuropathological findings and clinical symptoms are in conflict (DLB/PDD), the term α-synucleinopathy and verbally describing the findings is recommended instead of assessing only the McKeith category, Braak stage or BNE type of αS pathology distribution. Similarly, clinically it is suggested to use the term Lewy body disease (LBD) instead of DLB or PDD in clinically problematic cases (McKeith 2009).

Other findings

Neuropathological diagnosis for diffuse neocortical DLB requires only the presence of brainstem and cortical LBs (McKeith et al. 1996). However, also neuron loss (especially in SN, LC and nbM), co-existing AD-type pathology (plaques and neurofibrillary tangles), microvacuolation (mainly restricted to medial temporal cortex and amygdala), and vascular pathology may be detected in varying manners (McKeith et al. 1996).

Abnormal protein accumulation also characterises other common age-related neurodegenerative diseases, such as AD. In AD, β-amyloid deposition leads to the formation of neuritic plaques, and accumulation of the hyperphosphorylated microtubule-associated protein tau leads to the formation of intraneuronal neurofibrillary tangles (NFTs). Conventionally, the AD-type and αS pathologies have been thought to be separate neurodegenerative processes. However, these pathologies are commonly found simultaneously in the brains of elderly people, and it has been recognised that the underlying neurodegenerative processes most likely share certain pathogenetic mechanisms (McKeith et al. 2005). The process of αS pathology has been suggested to be triggered by AD pathology (Saito et al. 2004). Jellinger and colleagues have found a correlation between the Braak Parkinson stage and the Braak neurofibrillary AD stage (Wenning and Jellinger 2005, Jellinger and Attems 2008). These findings suggest an interaction between αS and tau, as has also been suggested based on the genetic studies (Duda et al. 2002). Although some cases of DLB have no, or only some, AD-type pathology, it is a common feature of most cases. Actually, pure DLB is less frequent than DLB with AD pathology (Hansen and Samuel 1997). This explains the difficulties in finding a correct clinical diagnosis (Jellinger 2004). According to the CERAD protocol, plaque types in DLB should be subclassified as neuritic plaques with tau-positive neurites, neuritic plaques with tau-negative ubiquitin-positive neurites, or optionally further categorised according to their β-amyloid immunoreactivity (McKeith et al. 1996).

Assessment of vascular pathology should follow CERAD recommendations for vascular disease, particularly those related to the distribution and size of infarcts. CERAD protocols should also be followed for other pathologies if needed for the evaluation of periventricular white matter, for example (McKeith et al. 1996).

The grade of neuronal loss in the SN has been demonstrated to be one of the distinguishing factors between PD and pre-symptomatic PD (Dickson et al. 2008, DelleDonne et al. 2008). It has been estimated that neuronal loss in SN becomes symptomatic when 50% of the ventrolateral (VL) tier neurons have been lost. In normal aging, the ventromedial tier of the SN pars compacta remains intact, and the estimated rate of neuron loss in SN VL tier is 7% over a decade. At this rate, 40-50% of neurons have been devastated by the age of 65 (Ellison and Love 2004). In DLB, this subject has not been researched.
Epidemiology

Neurodegenerative diseases affect a large proportion of the general population over 65 years of age. Dementia affects 7% of the general population older than 65 and 30% of those over 80 years of age. DLB is a common symptom of brain neurodegeneration and cause of dementia in old age. Many factors influence the prevalence of DLB, and thus varying figures have been stated. Clinical studies may show different prevalences compared to neuropathological studies, since some subjects with αS pathology do not present with symptoms (Parkkinen et al. 2005a, Zaccai et al. 2008), but it may also be problematic to recognise DLB clinically (Jellinger 2004, Merdes et al. 2003). As the prevalence rate of dementia rises exponentially with age after 65 years, higher frequencies of dementia-related DLB are seen in elderly (Parkkinen et al. 2001). The prevalence of DLB increases with age (Fujimi et al. 2008), even between the eighth and tenth decades (Wakisaka et al. 2003). DLB is reported to affect men slightly more than women (McKeith et al. 1994). The prevalence also depends on the case selection, i.e. is the study based on demented subjects or the general population and what is the age range of the study group. If only demented subjects are studied and the neuropathological DLB frequency in the population estimated is based on the proportion of the demented in the population, the estimated frequency of pathological findings would be lower than the real rate since some non-demented also have αS pathology (Parkkinen et al. 2005a).

Estimates for clinical DLB prevalence in population-based studies have ranged from 2% to 31% of demented subjects over 65 years of age (Herrera et al. 2002, Stevens et al. 2002, Rahkonen et al. 2003). In the autopsy studies the estimate was up to 36% of the demented subjects (Perry et al. 1990b, McKeith et al. 2000). However, there are only two population-based studies of neuropathological DLB assessed by the recent DLB consortium guidelines (Fujimi et al. 2008, Zaccai et al. 2008): In the Hisayama study, αS pathology was detected in 10% of non-demented and 31% of demented elderly Japanese subjects (Fujimi et al. 2008). In the MRC CFAS study from the U.K., the prevalence of αS pathology was 37% in a population of the elderly (Zaccai et al. 2008). Main frequency and prevalence studies of αS pathology are described in Table 7.

Compared to the other disorders of the elderly, DLB is the most frequent synucleinopathy before PD, and the second most common cause of primary neurodegenerative dementia following AD (Perry et al. 1990b). DLB is estimated to affect up to 5% of the general population older than 65 (Rahkonen et al. 2003, Zaccai et al. 2005), while PD affects 1.6% of those aged over 65 years (de Rijk et al. 1997). In the same age group, the estimated prevalence of PDD is up to 0.5% of the population (Aarsland et al. 2005, Burn 2006). Patients with PD have a two- to sixfold higher probability of developing dementia than their age-matched peers (Emre 2003). Duration of PD is a risk factor for dementia (Aarsland et al 2003) On average one of four PD patients has dementia (Aarsland et al 2005). Over all, most of the dementia burden is known to be due to AD, which accounts for approximately 60% of dementia in the population. The recent population-based study showed 16% of dementia to be due to DLB and 15% due to DLB+AD (Fujimi et al. 2008). PDD is estimated to cause 3% of dementia (Aarsland et al. 2005). Pick’s disease, Huntington’s disease, Creutzfeldt-Jakob disease, Steele-Richardson syndrome and PSP together account for 6% of all dementia (Ritchie 2003). The sum of the previous causes cover 100% of dementia. However, the previous percentages do not include the major secondary
cause of dementia, vascular pathology. Significant vascular pathological causes have been detected in up to 34% of dementia cases (Hachinski et al. 2006). Thus, the exact frequencies of different causes for dementia are not known.

Table 7 Autopsy studies estimating the frequency and prevalence of \( \alpha \)S pathology.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study population (autopsy rate)</th>
<th>n</th>
<th>Dementia</th>
<th>Gender F/M</th>
<th>Age</th>
<th>( \alpha )S pathol of all</th>
<th>( \alpha )S pathol of demented</th>
<th>( \alpha )S pathol of non-demented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ince et al. 1995</td>
<td>Random sample of nursing homes (41%)</td>
<td>92</td>
<td>75%</td>
<td>67/25</td>
<td>85</td>
<td>20%</td>
<td>20%</td>
<td>17%</td>
</tr>
<tr>
<td>Esiri et al. 2001</td>
<td>Prospective population-based</td>
<td>209</td>
<td>48%</td>
<td>119/90</td>
<td>85</td>
<td>11%</td>
<td>12%</td>
<td>9%</td>
</tr>
<tr>
<td>Parkkinen et al. 2001</td>
<td>Consecutive autopsies</td>
<td>774</td>
<td>27%</td>
<td>384/390</td>
<td>72</td>
<td>14%</td>
<td>23%</td>
<td>11%</td>
</tr>
<tr>
<td>Saito et al. 2004</td>
<td>Consecutive autopsies</td>
<td>1241</td>
<td>43%</td>
<td>578/663</td>
<td>81</td>
<td>21%</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Fujimi et al. 2008</td>
<td>Prospective population-based (71%)</td>
<td>102</td>
<td>33%</td>
<td>51/51</td>
<td>80</td>
<td>23%</td>
<td>47%</td>
<td>10%</td>
</tr>
<tr>
<td>Fujimi et al. 2008</td>
<td>Prospective population-based (64%)</td>
<td>205</td>
<td>100%</td>
<td>127/78</td>
<td>86</td>
<td>31%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zaccai et al. 2008</td>
<td>Prospective population-based (46%)</td>
<td>208</td>
<td>50%</td>
<td>85/123</td>
<td>na</td>
<td>37%</td>
<td>na</td>
<td>na</td>
</tr>
</tbody>
</table>

F/M, number of females/males; Age, mean age at death; pathol, pathology; na*, not announced, range 70-105 and >80% older than 80 years at death.

Clinico-pathological correlation

Studies of the impact of \( \alpha \)S pathology on dementia and other clinical symptoms have provided discrepant results (Lennox et al. 1989a, Perry et al. 1990a, Harding and Halliday 2001, Samuel et al. 1996, Gomez-Isla et al. 1999). Some have proposed that the degree of cognitive impairment correlates with the density of dystrophic LNs in the CA2 region of the hippocampus (Dickson et al. 1991, Churchyard and Lees 1997) and some with the cortical LB density (Haroutunian et al. 2000, Mattila et al. 2000). One study has proposed that the cause of neurodegeneration and clinical symptoms in DLB is small presynaptic \( \alpha \)S aggregates (Kramer and Schulz-Schaeffer 2007).

Many demented subjects have combined pathologies, such as \( \alpha \)S and AD-type pathology (Figure 3; Parkkinen et al. 2001, Fujimi et al. 2008) or AD and vascular pathology (White et al. 2005). In cases with combined pathologies the actual cause of clinical symptoms may be problematic to determine. Several studies have attempted to estimate the effect of \( \alpha \)S pathology on dementia (Perry et al. 1990a, Galasko et al. 1994, Kalra et al. 1996). This estimate became more structured in 2005, when the diagnostic criteria for DLB were revised to take into account both \( \alpha \)S and AD-type pathology in assessing the the likelihood for DLB clinical syndrome (Table 6, p.22; McKeith et al. 2005).
The high, intermediate and low likelihood categories assigned based on neuropathological findings (McKeith et al. 2005) have shown moderate accuracy for dementia and extrapyramidal symptoms (EPS) in unselected autopsy studies (Parkkinen et al. 2008). It appears that the clinical DLB syndrome has a positive association to the extent of αS pathology and a negative association to the severity of AD-type pathology (McKeith et al. 2005, Fujishiro et al. 2008), i.e. subjects with pure DLB present more often with DLB core symptoms compared to those with severe concomitant AD-type pathology. Especially those with extensive neurofibrillary pathology have been reported to show fewer clinical features of DLB, such as visual hallucinations (Merdes et al. 2003, Ballard et al. 2004).

The competing effects of DLB and AD may well explain why the association between the EPS and αS pathology in a population-based study did not correlate with the likelihood categories convincingly (Fujimi et al. 2008, McKeith et al. 2005). Although the criteria for the likelihood categories created by the Third DLB consortium workshop are focussed on revealing the "pure" DLB behind the clinical features, the likelihood statement guidelines help enormously in determining the impact of the pathology behind the clinical syndrome (McKeith et al. 2005).

![Figure 3](image)

*Figure 3* A simplified diagram to show that certain pathology does not cause only one type of clinical syndrome, and on the other way around, what looks like one clinical syndrome may be manifested with multiple pathologies. PD, Parkinson’s disease; MSA, Multiple system atrophy; DLB, Dementia with Lewy bodies; AD, Alzheimer’s disease, PSP, Progressive supranuclear palsy.

In unselected studies, there have been a number of neurologically intact subjects with αS pathology. Moreover, some elderly with a high likelihood for DLB clinical syndrome do not present with cognitive impairment (Harding and Halliday 2001, Parkkinen et al. 2005b). Even 10-15% of those 65 years of age and older with mild neuron loss and LBs in SN die without any clinical evidence of neurological symptoms (Gibb and Lees 1988). The age-related prevalence of LBs has been estimated to vary between 3.8% and 12.8% of the population aged 60 to 90 years (Ellison and Love 2004).

Results of case-control studies, *i.e.* autopsies of those with clinical symptoms, seem to support the proposed probability statement (McKeith et al. 2005), whereas studies based on unselected cases have indicated a weaker association between the αS
pathology and clinical features of DLB (Parkkinen et al. 2008, Braak et al. 2005, Fujishiro et al. 2008). These discrepant results thus challenge the probability statement and the significance of αS pathology. In addition to αS and AD-type pathology, the occurrence of clinical features in DLB may also depend on other factors, such as neuronal loss and vascular changes.

For vascular dementia, histopathological criteria do not exist. Guidelines for neuropathological analysis of vascular cognitive impairment (VCI) published in 2006 recommend the documentation of the extent of co-morbidity of the AD-type and αS pathology but do not specify their contribution to the clinical syndrome (Hachinski et al. 2006). Thus, it is difficult to determine whether it is the pathological consequences of stroke or the frequently co-existing degenerative changes in the elderly that cause the cognitive decline. The understanding of VCI, which includes post-stroke dementia and vascular dementia, continues to evolve. The latest point of view is that VCI and AD interact strongly (Petrovitch et al. 2005), and thus VCI cannot be separated from AD (Gorelick and Bowler 2008). However, among the causes of dementia, only the VCI risk factors are treatable and or even preventable, and thus constitute a major factor contributing to the prevalence figures of cognitive impairment in the elderly (Erkinjuntti and Gauthier 2009).

The presence of LBs has been noticed in several neurodegenerative conditions (Table 3, p.19). In addition to α-synucleinopathies/LB diseases (DLB, PD, PDD, MSA, and PAF), where the neurodegeneration and thus the clinical symptoms are thought to be due to αS pathology (Spillantini and Goedert 2000, Ellison and Love 2004), αS-IHC - positive inclusions can also be detected in neuroaxonal dystrophy, in various amyloidoses and tauopathies (Jellinger 2003) such as sporadic and familial AD (Lippa et al. 1998, Hamilton 2000), PSP and Down’s syndrome (Lippa et al. 1999). In these cases out of the α-synucleinopathies the impact of αS pathology to the clinical symptoms is unclear.

Genetic findings

Being such a newly-discovered disease, the genetic studies on DLB lean heavily on the studies in PD, where 10-15% of cases run in families without a clear Mendelian pattern of inheritance (Bonifati 2006). The synuclein family arises from three distinct genes; αS is encoded by the SNCA gene on chromosome 4q21-q23, β-synuclein by the SNCB gene on chromosome 5q35and γ-synuclein by the SNCG gene on chromosome 10q23 (Goedert 2001). In vitro, mutations in the SNCA gene contribute to αS protein aggregation, which brings forth conformational changes, formation of protofibrils, fibrils, apoptosis and neurotoxicity (Mukaetova-Ladinska and McKeith 2006). The first monogenic PD form was mapped to the SNCA gene locus PARK1 in 1996, linking the αS protein to a rare autosomal dominant form of familial PD (Golbe et al. 1996). Altogether, three missense mutations in the SNCA gene (A53T, A30P, E46K) have been implicated in familial forms of PD (Polymeropoulos et al. 1997, Krüger et al. 1998, Zarranz et al. 2004). The genomic triplication of the SNCA locus has been associated with a hereditary, early-onset PDD (Goris et al. 2007, Singleton et al. 2003, Singleton et al. 2004). Although mutations in the SNCA gene are very rare, α-synuclein protein plays an essential role in PD. Thus, genetic variations in the SNCA and other genes, such as parkin, LRRK2 (leucin-rich repeat kinase) and DJ1, have been described to have a key role in the pathogenic process leading to PD. On the other hand, not all familial cases with PD due to LRRK2 mutation have LBs (Bonifati 2006).
In sporadic PD, an association with a common genetic variation in the SNCA gene has been reported (Farrer et al. 2001, Mueller et al. 2005, Mizuta et al. 2006, Parsian et al. 2007). In addition, H1 haplotype homozygosity of the microtubule-associated protein tau gene (MAPT) has been identified as a risk factor for sporadic PD and PDD (Goris et al. 2007). SNCA alleles and the MAPT H1/H1 haplotype may also have an interactive effect on PD risk (Goris et al. 2007).
3 AIMS OF THE STUDY

Since the First DLB consortium guidelines for diagnosing DLB were published in 1996, much has been written about the neuropathological findings in DLB. A large proportion of this, however, has been done from a clinico-pathological perspective, looking back and believing that the detected neuropathological findings in the brain caused the symptoms occurred before the death. On the other hand, many studies have shown similar neuropathological findings in the brains of neurologically unimpaired subjects. Thus, the primary aim of this study was to evaluate the frequency of neuropathological DLB and its relation to the clinical findings in a population-based prospective cohort study on very elderly Finns.

The specific study aims were as follows:

1. To assess the frequency of αS pathology and DLB in a cohort of subjects with primary degenerative dementia (I) and to investigate the load of αS pathology compared with AD-type pathology (I-II)

2. To evaluate the frequency of neuropathological DLB, categorised according to the revised consensus guidelines by the Third DLB Consortium (II)

3. To evaluate the association between DLB pathology and clinical symptoms, and to evaluate how valid the probability statement regarding the clinico-pathological associations was (II)

4. To determine the relevance of neuronal loss in SN to αS pathology and the clinical features (II)

5. To examine how genetic variation in the SNCA gene contributes to the extent of NFT (tau), β-amyloid and αS pathology (III)

6. To determine the frequency of αS pathology in the autonomic nuclei of the thoracic and sacral spinal cord and to evaluate its relations to the αS pathology in the brain (IV)

7. To evaluate how αS pathology progresses in the central nervous system (IV)
4 MATERIALS AND METHODS

Subjects

This study is based on two distinct study populations. The first study population consists of subjects with primary degenerative dementia (I), and the second of subjects in a prospective population-based study (II-IV).

Subjects with primary degenerative dementia

The study population of 71 consecutive, hospitalised subjects with primary degenerative dementia included 36 pre-senile cases (symptoms onset before the age of 65) and 35 senile cases (symptoms onset after the age of 65). All were patients in the Koskela Geriatric Hospital in Helsinki, Finland, and entered the study between June 1978 and June 1980. The criteria for the selection of subjects excluded aetiological factors other than primary degenerative dementia and were essentially those of Roth (Roth 1955) with some modifications (Sulkava et al. 1983). No clinical differentiation was made between the different primary degenerative dementias. However, patients with strokes and secondary dementias were excluded, so the study population was expected to represent mainly AD cases.

Subjects in the population-based study

The Vantaa 85+ study included all 601 residents of Vantaa, a small city in Southern Finland, who were at least 85 years of age on the 1st of April 1991. The study population consisted of 125 males (21%) and 476 females (79%). Of these, 11 refused to participate and one could not be contacted. Of the remaining 589 subjects, 553 underwent a structured general and neurological examination performed by a neurologist and a registered nurse; the remaining 36 died prior to the examination. Those still alive were re-examined in 1994, 1996, 1999 and 2001. During the ten-year follow-up to the 1st of April 2001, 565 of the eligible study subjects died. The results of autopsies (performed with consent) with neuropathological examinations were available in 304 cases (54%), which is the second highest autopsy rate among all population-based studies on dementia worldwide (Zaccai et al. 2006). The clinical data for the neuropathological subpopulation of 304 were based on examinations for 290 (95%) subjects and on medical records for those 14 (5%) who died prior to the examination. See also the flow chart in Figure 4. Peripheral blood (and DNA) samples were available for 272 (89%) of the autopsied subjects. Characteristics of the whole study population, the subpopulation of neuropathologically examined subjects and the subpopulation of autopsied subjects are described in Table 8.
Figure 4 Flow chart illustrating the Vantaa 85+ study population
**Materials and methods**

**Table 8** Characteristics of the whole study population, the subpopulation of neuropathologically examined subjects and the subpopulation of autopsied subjects with a useful DNA sample.

<table>
<thead>
<tr>
<th></th>
<th>Vantaa 85+ study population</th>
<th>Neuropathological subpopulation</th>
<th>with DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>565</td>
<td>304</td>
<td>273</td>
</tr>
<tr>
<td><strong>Sex (n, %)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>118 (21%)</td>
<td>52 (17%)</td>
<td>46 (17%)</td>
</tr>
<tr>
<td>Women</td>
<td>447 (79%)</td>
<td>252 (83%)</td>
<td>227 (83%)</td>
</tr>
<tr>
<td><strong>Dementia status (n, %)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Demented</td>
<td>326 (58%)</td>
<td>196 (64%)</td>
<td>177 (65%)</td>
</tr>
<tr>
<td>Non-demented</td>
<td>239 (42%)</td>
<td>108 (36%)</td>
<td>96 (35%)</td>
</tr>
<tr>
<td><strong>Frequency of dementia (n, %)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>63 (53%)</td>
<td>30 (58%)</td>
<td>29 (63%)</td>
</tr>
<tr>
<td>Women</td>
<td>263 (59%)</td>
<td>166 (66%)</td>
<td>147 (65%)</td>
</tr>
<tr>
<td><strong>Age at onset of dementia (m ± SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>85-89</td>
<td>86.8 (±4.5)</td>
<td>87.2 (±4.5)</td>
<td>87.3 (±4.4)</td>
</tr>
<tr>
<td>90-94</td>
<td>267 (47%)</td>
<td>146 (48%)</td>
<td>135 (49%)</td>
</tr>
<tr>
<td>&gt;95</td>
<td>110 (19%)</td>
<td>76 (25%)</td>
<td>70 (26%)</td>
</tr>
<tr>
<td><strong>Age at death (m ± SD)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>85-89</td>
<td>91.9 (±3.6)</td>
<td>92.4 (±3.7)</td>
<td>92.5 (±3.7)</td>
</tr>
<tr>
<td>90-94</td>
<td>188 (33%)</td>
<td>82 (27%)</td>
<td>68 (25%)</td>
</tr>
<tr>
<td>&gt;95</td>
<td>110 (19%)</td>
<td>76 (25%)</td>
<td>70 (26%)</td>
</tr>
<tr>
<td><strong>Hospitalisation status at death</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td>431 (76%)^*</td>
<td>238 (78%)</td>
<td>215 (79%)</td>
</tr>
<tr>
<td>Nursing home</td>
<td>99 (18%)</td>
<td>55 (18%)</td>
<td>51 (19%)</td>
</tr>
<tr>
<td>Home</td>
<td>26 (5%)</td>
<td>11 (4%)</td>
<td>7 (3%)</td>
</tr>
</tbody>
</table>

^* For nine subjects the hospitalisation status at death is not known

**Clinical examination**

**Clinical data**

In the study of subjects with primary degenerative dementia, the clinical data were collected on demographic, medical, behavioural, cognitive and therapeutic aspects as well as physical and neurological examination findings. An EEG was recorded in all cases once or twice a year. The detailed clinical information can be found in the original clinical publications from the year 1982 (Sulkava 1982, Sulkava and Amberla 1982).
In the population based study, cognitive functions were assessed by the Mini-Mental State Examination (Folstein et al. 1975), the Short Portable Mental Status Questionnaire and the Clinical Dementia Rating. Functional abilities were assessed by the Activities of Daily Living and Instrumental Activities of Daily Living Scale. Subjects were considered demented if they fulfilled the criteria in the Diagnostic and Statistical Manual of Mental Disorders (third edition, revised; DSM-III-R) and the duration of the dementia symptoms had been at least 3 months prior to the examination. All individual items of the criteria were assessed and recorded separately. Assessments were based on data obtained from interviews, physical examinations, previous health and social work records, and tests of cognitive function and functional capacity. The diagnosis of dementia was made by consensus between two physicians. Furthermore, all health and social work records were used for identifying new cases of dementia among those who were non-demented in the baseline study in 1991 or in the follow-up study in 1994, which was designed to detect moderate to severe cases of AD. The additional cases found in this way were had to fulfill the individual items of the DSM-III-R criteria. The information regarding the presence of rigidity and hypokinesia was derived from the last clinical examination prior to death, and that regarding the occurrence of visual hallucinations was obtained if reported at any time during the follow up. The mean time from the last clinical examination to death was 16 months (SD 13 months).

**Neuropathological examination**

**The cohort of primary degenerative dementia study**

Neuropathological autopsies were performed on 27 patients who had died by the year 1981 (Sulkava et al. 1983), and on 28 patients who had died during the years 1982 and 1997. After 1989, only one autopsy was performed. Thus, altogether 55 patients of the series of 71 patients were autopsied (77%). The remaining 16 patients were not autopsied or neuropathologically examined.

The brains were fixed in 4% phosphate-buffered formaldehyde. Samples from the temporal lobe including the hippocampus, frontal lobe, occipital lobe including area striata, basal ganglia, thalamus, substantia nigra, pons, medulla oblongata, and cerebellum were embedded in paraffin. Paraffin sections were stained with haematoxylin-eosin (H&E), Luxol fast blue/cresyl violet, Holmes’ silver stain, periodic acid/Schiff, and Congo red. Originally, 22 of the first 27 patients (82%) had neuropathological changes typical of AD, while 5 (18%) had another type of degenerative encephalopathy. Neuropathological criteria for AD required abundant neurofibrillary tangles and senile plaques in the hippocampal region in addition to moderate to large numbers of tangles and plaques in the neocortex, in accordance with the criteria of Tomlinson, Blessed, and Roth (Tomlinson et al. 1970) used at that time. These criteria actually quite closely correspond to a moderate or high likelihood of AD, according to the NIA-RI criteria (Hyman and Trojanowski 1997) used today.

Originally, all brains were processed and samplings were performed as above according to the scientific practice of that time. Amygdala was not included in the sampling protocols recommended at the time, and no formalin-fixed tissue was available for re-sampling during the re-evaluation. All 55 autopsied patients of the series of 71 were thus re-evaluated.
Materials and methods

Paraffin sections (6 µm) from SN, locus coeruleus, and medulla oblongata were stained with H&E. These, and the hippocampus including adjacent entorhinal and inferior temporal cortex were also immunostained with antibodies against αS. This was performed using standard methods; paraffin blocks were cut at 4 µm and mounted on glass slides. Deparaffinised sections were pretreated with pepsin for 40 minutes. After rinsing in phosphate buffered saline (PBS), a treatment with 0.5% H₂O₂ in methanol was performed to block endogenous peroxidase activity. The sections were first incubated with normal horse serum for 30 minutes, then with the primary αS antibody (Zymed, San Francisco, CA, USA, 18-0215, mouse monoclonal antibody, diluted 1:500) overnight in a humid atmosphere at room temperature. After rinsing in PBS, the bound antibody was visualised by incubation for 30 minutes at room temperature with biotinylated secondary horse anti-mouse antibody followed by an avidin-biotin complex for 30 minutes (Vectastain Elite ABC Kit, mouse IgG, Vector Laboratories, Burlingame, CA, USA). Finally, sections were developed in 3-amino-9-ethylcarbazol (AEC) - H₂O₂ and counterstained with Mayer's haematoxylin.

In cases with αS pathology detected in the screened areas, the immunohistochemical staining for α-synuclein was extended to samples from the temporal and frontal lobes, cingulate gyrus, and insula. The brain sampling and the cortical areas for LB and LN assessment were defined according to the consensus criteria (McKeith et al. 1996) with only one exception: parietal lobes were not included in the original sampling protocol; thus, the lacking parietal lobe samples were replaced by samples of insula. A semiquantitative grading of the severity of αS pathology into mild, moderate, severe and very severe was adopted according to the new recommendation (McKeith et al. 2005): Score 0 = none, 1 = mild (sparse LBs or LNs), 2 = moderate (more than one LB in a low power field and sparse LNs), 3 = severe (four or more LBs and scattered LNs in a low power field, 4 = very severe (numerous LBs and numerous LNs). Loss of pigmented cells in SN was scored as follows; score 0 = no or mild loss, + = moderate, ++ = severe neuronal loss. The αS-positive LNs in the hippocampal CA2/3 region were detected in every case and scored according to Dickson (Dickson et al. 1991). Individuals were defined as having extensive Lewy-related pathology if they had diffuse cortical Lewy-related pathology, according to the DLB consortium protocol (McKeith et al. 2005).

IHC with monoclonal antibodies against hyper-phosphorylated tau (Innogenetics Haven, Zwijndrecht, Belgium BR-03, clone AT8, diluted in 1:1000), the amyloid β-protein (Senetek PLC, Maryland Heights, MO, USA mAb 200, 4 G8, 1:1000), and ubiquitin (Senetek PLC mAb 500, 1:2000) was additionally performed on sections from the hippocampus as well as from frontal and temporal neocortex in order to detect AD, taupathies and frontotemporal lobar degeneration (FTLD). Amyloid β-protein antibodies were used with formic acid as a pretreatment of the sections, otherwise the sections were pretreated with pepsin. For the evaluation of the NFT Braak stage, a previously described simplified protocol (Harding et al. 2000) was used, including Bielschowsky silver stain of a hippocampal-inferior temporal large block in most cases. Similarly, the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) score of neuritic plaques was evaluated from this block supported by samples from occipital and frontal neocortex.
The population-based study

The brains of the autopsied subjects were fixed in phosphate-buffered 4% formaldehyde for at least 2 weeks before sampling. Specimens were obtained following the recommendations of the First DLB consortium international workshop (McKeith et al. 1996) for assessing αS pathology, following the recommendations of the neuropathologic staging of neurofibrillary changes (Braak and Braak, 1991) and according to the CERAD protocol for the diagnosis of AD (Mirra et al. 1991). Thus, the brain samples included the SN, middle frontal gyrus, superior and middle temporal gyri, inferior parietal lobule, anterior cingulate gyrus, left and right hippocampus (at the level of the lateral geniculate body), transentorhinal and entorhinal cortex (at the level of the mamillary bodies), and the primary and secondary visual cortices (the striate area, parastriate field and peristriate region all represented in the same specimen).

Tissue samples embedded in polyethylene glycol were cut at a thickness of 80 µm for free-floating staining with the Gallyas silver method (Polvikoski et al. 2001). The stage of neurofibrillary pathology was assessed according to the Braak staging protocol (Braak and Braak, 1991). NFTs were counted in five random columns of every sample of the middle frontal, superior temporal, and middle temporal gyri and inferior parietal lobule, and the average number of NFTs per column has been used for the analysis (Polvikoski et al. 2001).

Tissue samples embedded in paraffin were cut at a thickness of 10 µm for staining with a modified Bielschowsky method (Polvikoski et al. 2001). All four cortical ares, as used for staging neurofibrillary pathology, were screened to minimise the possible effect of variations in the β-amyloid load in different regions of the brain (Polvikoski et al. 2001). When an area with the maximum density of neuritic plaques found in each sample, this area was scored according to the CERAD protocol (Mirra et al. 1991).

Sections of SN stained with the haematoxylin and eosin (H&E) method and sections of SN and right hippocampus immunostained with antibodies against αS were used to screen for αS pathology. In one subject without a sample of SN, dmV and LC were used instead. If any αS pathology was detected in the screened areas, αS-IHC was performed on cortical samples obtained from the temporal, frontal, and parietal lobes and cingulate gyrus, as recommended by the First guidelines of the DLB consortium (McKeith et al. 1996). For immunohistochemistry, antigen retrieval was carried out by microwave heating in citrate buffer (pH 6) for the total of 20 minutes followed by immersion in 100% formic acid for 5 minutes. Sections were incubated overnight with the primary αS antibody (Transduction Laboratories, Lexington, KY, USA, clone 42, mouse monoclonal, diluted 1:800). Known positive and negative control tissues were included to ensure proper staining. The primary antibody and pretreatment used in the degenerative dementia study did not show any activity in these samples of the population based study, possibly based on different fixation times and post mortem delay, and thus were changed. Otherwise, immunohistochemistry was performed as described above in the degenerative dementia study.

The number of LBs in a single 20x objective/ 10x ocular field was counted. The highest count from the investigated region obtained was recorded for that brain region. The load of αS pathology was semiquantitatively scored (none =0, mild =1, moderate =2, severe =3 and very severe =4), and followed by the assignment of the type of αS pathology (none, brainstem-predominant, limbic, diffuse neocortical) for every subject according to the DLB consortium Third guidelines for diagnosis of DLB (McKeith et al.)
Since 45% (50/110) of the subjects with αS pathology did not precisely fit in any existing category of αS pathology, we modified the criteria by allowing score 1 for parietal lobe within the limbic type of αS pathology (17 subjects), and score 4 to any area within the diffuse neocortical type of αS pathology (8 subjects). Furthermore, 3 subjects with otherwise brainstem-predominant αS pathology but with score 1 in one neocortical area were kept in the brainstem-predominant type of αS pathology, and 9 subjects with otherwise limbic but with score 0 in transentorhinal cortex were included in the limbic type of αS pathology. Regardless of the modification, 13 subjects with score 1 αS pathology confined to the hippocampal-transentorhinal region (without αS pathology in SN, cingulate gyrus or neocortex) were not assignable to any of the existing αS pathology categories according to the DLB consortium guidelines (McKeith et al. 2005). Thus, these 13 were regarded as a category of their own, which was excluded from the association analyses. Based on the type of αS pathology and the Braak stage for neurofibrillary pathology, the likelihood that the observed neuropathology could be associated with the clinical syndrome of DLB was determined, as recommended, into low, intermediate and high (McKeith et al. 2005).

Additional investigations included a semiquantitative grading of the neuronal loss/atrophy in the ventrolateral tier of SN pars compacta. The grade, extending from none (0) to severe (3), was determined preferentially by assessing the loss of pigmented neurons, but in borderline cases the amount of extraneuronal pigment was also considered (Figure 5, p.44). Moreover, the semiquantitative assessment of the LNs in the hippocampal CA 2-3 region, as previously recommended (Dickson et al. 1991).

Tissue samples from the upper third of the thoracic spinal cord (T3-4) and from the sacral spinal cord (S1-2) of each subject were embedded in paraffin. Following conventional H&E staining, αS pathology was assessed in 4μm thick sections from thoracic and sacral spinal cord immunostained manually using mouse monoclonal αS antibody (Transduction Laboratories, Lexington, KY, USA, clone 42, diluted 1:800). IHC was performed as described above concerning the brain samples.

The presence and load of αS pathology were detected in the sympathetic intermediolateral column of thoracic spinal cord and in the sacral parasympathetic nucleus. The most severely affected side of the tissue sample was taken for the grading. αS pathology was assessed semiquantitatively in microscopic fields magnified at x200 and scored as none (negative), mild (+; grain- or dot-like neuropilic and/or cytoplasmic immunoreactive aggregates lacking focal LNs and LBs), moderate (1; few LNs and/or LBs), severe (2; some LNs and/or LBs), and very severe (3; many LNs and/or LBs) (Figure 6, p.45). Thus the "mild" category includes subjects who do not have unequivocal traditional αS pathology in the form of LNs and LBs, but do have granular deposits. The more severe forms have also traditional αS pathology.

**Molecular genetic methods**

DNA was extracted from peripheral blood leukocytes using standard methods. SNCA genotyping was performed using PCR-based standard methods (Taqman assay) as described by Clarimon and coworkers (Clarimon et al. 2007). Eleven single nucleotide polymorphisms (SNPs) within SNCA were genotyped using whole-genome-amplified DNA (Qiagen REPLI-g; Qiagen, Valencia, CA; www.qiagen.com). Six of the SNCA SNPs were selected as tagging SNPs from the linkage disequilibrium (LD) structure of
the SNCA gene, and five were selected from their association with PD. Intron 4 and the 3’ end of the SNCA gene were covered most thoroughly (Mueller et al. 2005). Genotyping of an insertion deletion polymorphism in intron 9 of MAPT that discriminates the common H1/H2 haplotype groups was performed as well.

The success rate of the genotyping was over 97%. The genotype distributions did not deviate from Hardy–Weinberg equilibrium. We did not obtain a blood sample from all of our subjects. Thus, of the 304 neuropathologically examined subjects the genotyping could be performed for a total of 273 subjects (90%). The Haploview program was used for the LD and haplotype analyses (http://www.broad.mit.edu/mpg/haploview/index.php).

Different groups were formed for the statistical analysis based on the extent of various neurodegenerative abnormalities. Individuals with absent or mild tau pathology were defined as group one, i.e. those with the Braak stage 0, I or II for NFTs (Braak and Braak 1991). They were compared with subjects with extensive tau pathology in group two, i.e. those with the Braak stage IV, V or VI. Correspondingly, to represent the varying extent of β-amyloid pathology, subjects without neuritic plaques were compared with those with the CERAD score “moderate” or “frequent” for the neuritic plaques (Mirra et al. 1991). Finally, subjects with extensive αS pathology, i.e. diffuse neocortical DLB, were compared with those without αS pathology.
Statistical analyses

Statistical analyses were conducted with the SPSS 15.0 and 16.0 program for Windows (SPSS Inc., Chicago, IL). For categorical variables, the differences between the groups were analysed with Chi-Squared or Fisher's exact tests, or with Chi-Squared or exact tests for a linear trend. A logistic regression analysis was used to assess association of several predictor variables with dichotomous dependent variables. A \( p \)-value <0.05 was considered significant.

Approval for the study

The prospective, population-based cohort study was approved by the ethics committee of the Health Centre of the City of Vantaa and by the National Authority for Medicolegal Affairs, Helsinki, Finland.

In the degenerative dementia study, which was carried out clinically between the years 1978 and 1980, all patients gave their permission for the materials and their family members gave their permission for autopsies.
5 RESULTS

Frequency of αS pathology and DLB
The primary degenerative dementia study

In the prospective Finnish series of 71 patients with primary degenerative dementia followed from the late 1970s until 1997, all but one of the 55 autopsies were performed before the neuropathological criteria for diagnosing DLB were proposed in 1995. In the reappraisal of 55 autopsies, αS pathology was found in 16 (29%) subjects. 14 (25%) were classifiable to the McKeith’s categories of αS pathology; three (5%) had AD with αS pathology limited to the brainstem (AD-bsDLB), and 11 (20%) had the limbic or diffuse neocortical type of DLB (Table 9). The αS pathology was more common in men (40%) than in women (20%) since 6 of 15 men and 8 of 40 women had classifiable αS pathology. Of the 11 DLB cases, 5 were men and 6 women. Taken together, of the 55 autopsied patients with primary degenerative dementia, 3 (5%) had AD with αS pathology limited to the brainstem (AD-bsDLB), 2 (4%) had AD with αS pathology limited to the limbic cortex, 11 (20%) had limbic–neocortical DLB, 32 (58%) had pure AD, and 7 (13%) had other types of neurodegenerative encephalopathy. LNs in the CA2/3 region were detected in 8 of the 11 DLB patients. LN counts were associated with the LB counts in the temporal lobe ($p=0.03$), but the association was not significant in other individual cortical areas. At the time of death, the degree of dementia among these cases varied from moderate (1/11) or severe (6/11) to very severe (4/11).

Table 9 Neuropathological findings of the 14 subjects with α-synuclein pathology in the primary degenerative dementia group.

<table>
<thead>
<tr>
<th>Case/ gender</th>
<th>LBs, αS</th>
<th>Neuron loss SN</th>
<th>LNs, αS</th>
<th>DLB type</th>
<th>CERAD</th>
<th>Braak</th>
<th>McKeith likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M</td>
<td>3*</td>
<td>0*</td>
<td>+++</td>
<td>nc</td>
<td>B/2</td>
<td>high</td>
<td></td>
</tr>
<tr>
<td>2/M</td>
<td>3</td>
<td>+</td>
<td>0</td>
<td>nc</td>
<td>C/5</td>
<td>intern</td>
<td></td>
</tr>
<tr>
<td>3/F</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>li</td>
<td>C/5</td>
<td>low</td>
<td></td>
</tr>
<tr>
<td>4/F</td>
<td>3</td>
<td>+</td>
<td>0</td>
<td>li</td>
<td>C/5</td>
<td>low</td>
<td></td>
</tr>
<tr>
<td>5/M</td>
<td>2</td>
<td>0</td>
<td>+</td>
<td>li</td>
<td>C/5</td>
<td>low</td>
<td></td>
</tr>
<tr>
<td>6/M</td>
<td>2</td>
<td>0</td>
<td>+</td>
<td>li</td>
<td>C/6</td>
<td>low</td>
<td></td>
</tr>
<tr>
<td>7/F</td>
<td>3</td>
<td>++</td>
<td>+</td>
<td>nc</td>
<td>C/5</td>
<td>intern</td>
<td></td>
</tr>
<tr>
<td>8/F</td>
<td>3</td>
<td>++</td>
<td>++</td>
<td>nc</td>
<td>C/6</td>
<td>intern</td>
<td></td>
</tr>
<tr>
<td>9/M</td>
<td>3</td>
<td>+</td>
<td>0</td>
<td>nc</td>
<td>C/5</td>
<td>intern</td>
<td></td>
</tr>
<tr>
<td>10/F</td>
<td>3</td>
<td>++</td>
<td>+++</td>
<td>nc</td>
<td>C/5</td>
<td>intern</td>
<td></td>
</tr>
<tr>
<td>11/F</td>
<td>3</td>
<td>++</td>
<td>++</td>
<td>nc</td>
<td>B/2</td>
<td>high</td>
<td></td>
</tr>
<tr>
<td>12/F</td>
<td>1</td>
<td>+</td>
<td>0</td>
<td>bs</td>
<td>C/6</td>
<td>low</td>
<td></td>
</tr>
<tr>
<td>13/M</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>bs</td>
<td>C/5</td>
<td>low</td>
<td></td>
</tr>
<tr>
<td>14/F</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>bs</td>
<td>C/6</td>
<td>low</td>
<td></td>
</tr>
</tbody>
</table>
The population-based study

Of the neuropathologically examined subpopulation (n=304), 83% were women, and 64% were demented. The mean age at death was 92.4 years (92.5 years for demented, and 92.1 years for non-demented). There were slightly more demented subjects in the neuropathologically examined subpopulation compared to the whole study population (64% vs. 58%), but regarding the other parameters the subpopulation and the whole-study population were essentially identical (Table 8, p.32).

αS pathology was present in the SN and/or hippocampal-transentorhinal region in 36% (110/304) of the autopsied subjects. Thirteen (4%) of the subjects had αS pathology confined to the hippocampal-transentorhinal region. The remaining 97 (32%, Table 10) were categorised as follows: in 8 subjects (3% of 304) the pathology was brainstem-predominant, in 42 (14%) it was limbic, and in 47 (15%) it was of a diffuse neocortical type according to the DLB consortium criteria with our modifications. The αS pathology was slightly more common in men (38%) than in women (31%), but the difference was not significant. Most men with αS pathology had the diffuse neocortical type (65%), while in αS-IHC -positive women the frequencies of the limbic (47%) and diffuse neocortical (44%) type were rather equal. In this very elderly population, neither the category of αS pathology nor the frequency of αS pathology was influenced by age.

Table 10 Autopsy studies, including the present studies, estimating the frequency of αS pathology.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study population (autopsy rate)</th>
<th>n</th>
<th>Dementia</th>
<th>Gender F/M</th>
<th>Age</th>
<th>αS pathol of all</th>
<th>αS pathol of demented</th>
<th>αS pathol of non-demented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ince et al. 1995</td>
<td>Random sample of nursing homes (41%)</td>
<td>92</td>
<td>75%</td>
<td>67/25</td>
<td>85</td>
<td>20%</td>
<td>20%</td>
<td>17%</td>
</tr>
<tr>
<td>Esiri et al. 2001</td>
<td>Prospective population-based</td>
<td>209</td>
<td>48%</td>
<td>119/90</td>
<td>85</td>
<td>11%</td>
<td>12%</td>
<td>9%</td>
</tr>
<tr>
<td>Parkkinen et al. 2001</td>
<td>Consecutive autopsies</td>
<td>774</td>
<td>27%</td>
<td>384/390</td>
<td>72</td>
<td>14%</td>
<td>23%</td>
<td>11%</td>
</tr>
<tr>
<td>Saito et al. 2004</td>
<td>Consecutive autopsies</td>
<td>1241</td>
<td>43%</td>
<td>578/663</td>
<td>81</td>
<td>21%</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Fujimi et al. 2008</td>
<td>Prospective population-based (71%)</td>
<td>102</td>
<td>33%</td>
<td>51/51</td>
<td>80</td>
<td>23%</td>
<td>47%</td>
<td>10%</td>
</tr>
<tr>
<td>Fujimi et al. 2008</td>
<td>Prospective population-based (64%)</td>
<td>205</td>
<td>100%</td>
<td>127/78</td>
<td>86</td>
<td>31%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zaccai et al. 2008</td>
<td>Prospective population-based (46%)</td>
<td>208</td>
<td>50%</td>
<td>85/123</td>
<td>na</td>
<td>37%</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Oinas et al. 2009a</td>
<td>Consecutive autopsies</td>
<td>55</td>
<td>100%</td>
<td>40/15</td>
<td>75</td>
<td>25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oinas et al. 2009b</td>
<td>Prospective population-based (46%)</td>
<td>304</td>
<td>64%</td>
<td>252/52</td>
<td>92</td>
<td>32%</td>
<td>38%</td>
<td>20%</td>
</tr>
</tbody>
</table>

F/M, number of females/males; Age, mean age at death; pathol, pathology; na*, not announced, range 70-105 and >80% older than 80 years at death.

Oinas et al. 2009a, the study of primary degenerative dementia; Oinas et al. 2009b, the population-based study of very elderly Finns. Frequency of αS pathology in the last two is based on cases classifiable to brainstem, limbic and diffuse neocortical types according to the DLB consortium guidelines.
When taking both αS pathology and the Alzheimer type neurofibrillary pathology into consideration, the likelihood that the observed neuropathology was presented with the DLB clinical syndrome was low in 19 (6%), intermediate in 39 (13%) and high in 39 (13%) subjects. Men had slightly lower Braak stages compared to women, but at the same time more extensive, αS pathology giving significantly higher likelihoods for men when comparing men vs. women ($p=0.006$).

Hippocampal CA2-3 LNs were detected in 73 (24%) subjects. Every subject with numerous LNs (n=14) had diffuse neocortical type of αS pathology. Thus, the grade of LNs associated with the extent of αS pathology ($p<0.001$).

**Co-existing AD-type pathology**

**The primary degenerative dementia study**

Of the 55 subjects, dementia in 5 whose αS pathology was confined to the limbic region only (n=2) or to the brainstem (n=3) was most likely related to their abundant AD-type pathology. Therefore, they were not included in the neuropathological DLB group of 11. Two of the eleven DLB cases had the neurofibrillary tangle pathology of Braak stage II, 6 of Braak stage V, and 3 of Braak stage VI. Nine of the eleven DLB cases had CERAD age-related plaque score C (all with frequent neuritic plaques), fulfilling the CERAD criteria for definite AD, and two DLB cases had plaque score B (one with infrequent and the other with moderate neuritic plaques) corresponding to CERAD's criteria for probable AD (Table 9, p.39).

**The population-based study**

Among the 304 neuropathologically examined subjects, there were 90 subjects (30%) with Braak neurofibrillary stage 0, I or II, 142 subjects (47%) with stage III-IV and 72 subjects (24%) with stage V-VI. When the subjects with αS pathology and Braak stage 0-II were considered pure DLB subjects, this group of 26 subjects (27% αS-IHC positive) included 2 with brainstem-predominant, 12 with limbic, and 12 with the diffuse neocortical type of αS pathology.

The Braak stage associated significantly with the extent of αS pathology ($p=0.011$, the chi squared test for a linear trend). Anatomically, there was a significant association between the regional αS pathology score in transentorhinal cortex and the increasing Braak stage when applying the stage in three groups (0-II, III-IV, V-VI, $p=0.027$), but there was no such association between the regional αS pathology score and Braak stage in any other brain area.

**Clinico-pathological correlations**

**The primary degenerative dementia study**

No significant association was seen between the severity of dementia and LB counts in any individual area (the strongest association: $p=0.64$). Nor was disease duration associated with LB counts in any area (the strongest association: $p=0.53$). Five of the eleven DLB patients (45%) had neuropsychiatric symptoms typically related to DLB, whereas these symptoms were present in only 10 (22%) of the 44 non-DLB patients.
Rigidity and hypokinesia were common in DLB patients (8 of 11; 73% and 100% of those with intermediate or high likelihood for DLB clinical syndrome), but also among the other patients with dementia (27 of 44; 61%). When the likelihood criteria were used, 7 (13%) of the patients had DLB+AD with a low likelihood of DLB clinical syndrome, and another 7 (13%) had an intermediate to high likelihood for clinical DLB syndrome. These groups matched the clinical symptoms slightly but not significantly better than the group of 11 patients with neuropathological DLB.

The clinical DLB consortium criteria could not be fully applied in these cases, because in the early 1980s the significance of the fluctuation of symptoms was not known and, as a result, not reliably registered in the clinical study. All but one DLB case had an abnormal EEG recording showing mainly slowing of the background activity. Two of the eleven patients (18%) had parents or siblings with a dementing disorder, equal to the frequency among the non-DLB patients (8 of 44 patients). Of the 11 DLB patients, the mean age at onset was 65.7 (range 59-74) years. The median illness duration of dementia was 7.1 years, and the mean age at death 72.8 (range 67–85) years. The mean age at death was 76.6 years (range 56–93) years in the pure AD group, in the AD-bsDLB group only 67.7 years (range 66–69), and in the AD with limbic αS pathology group 82 years (range 68–96). The most common immediate cause of death both in the DLB patients, and in the whole series, was bronchopneumonia.

The population-based study

Dementia

Both the αS pathology and Braak stage had an effect on the likelihood of dementia. All subjects with a diffuse neocortical type and Braak stage V-VI pathology were demented, but there was also an independent association between dementia and αS pathology ($p=0.021$), and between dementia and the Braak stage ($p<0.001$) when adjusted for gender and age at death. Among 26 pure DLB subjects (i.e. with αS pathology and Braak stage 0-II), 50% of both the brainstem-predominant and limbic type subjects, and 75% of the diffuse neocortical type subjects were demented. Subjects with diffuse neocortical disease were 4.3 times (OR, 95% CI 1.7-11.2) more likely to be demented than subjects without αS pathology when adjusted for gender, age at death, and Braak stage in the logistic regression analysis. Among the 194 subjects with neurofibrillary pathology only, 48% of those with Braak stage 0-II, 53% with stage III-IV, and 88% with stage V-VI were demented. The probability for being demented was 8.0 times (OR, 95% CI 3.1-20.9) higher in subjects with Braak stage V-VI compared to subjects with Braak stage 0-II pathology when adjusted for gender, age at death and type of αS pathology.

A significant association was also found between dementia and the density of dystrophic LNs in the hippocampal CA2-3 region ($p=0.009$, adjusted for gender and age at death). Although the overall significance of LNs was lost if adjusted for the Braak stage, the association of the highest score of LNs with dementia remained significant (OR 8.8; 95% CI 1.1-71.1; $p=0.041$).

Anatomically, the presence of dementia increased with the increase of the regional αS pathology score in the transentorhinal cortex independently of the Braak stage ($p=0.039$, adjusted also for gender and age at death). In the other brain areas, there was no significant association between dementia and the αS pathology score regardless of the Braak stage.
Results

Extrapyramidal symptoms (EPS) and visual hallucinations

The proportion of subjects with EPS (rigidity and/or hypokinesia) tended to increase along with the extension of aS pathology from the limbic to the diffuse neocortical type, but also along with the increasing Braak stage. Thus, of the subjects with pure diffuse neocortical type of DLB (Braak stage 0-II), 25% manifested rigidity and hypokinesia, while the corresponding figures of the subjects with Braak stage V-VI and without aS pathology were 34% and 24%. The type of aS pathology associated significantly with rigidity ($p=0.042$), as well with hypokinesia ($p=0.016$), but only the association with hypokinesia remained significant when adjusted, in addition to gender and age at death, for the Braak stage ($p=0.027$) and additionally for dementia ($p=0.022$). However, the association with hypokinesia was based on the brainstem-predominant type of aS pathology, a category which included only 8 subjects. At the same time, the Braak stage did not associate with hypokinesia ($p=0.11$) but did with rigidity ($p=0.031$), though the significance was lost if adjusted for dementia ($p=0.34$) or for the type of aS pathology ($p=0.13$). It is worth noting, however, that when both pathologies were taken into account, the subjects with the diffuse neocortical type and those with Braak stage V-VI both had a more or less borderline association with rigidity ($p=0.063$ and $p=0.075$), but only subjects with Braak stage V-VI showed nearly significant association with hypokinesia ($p=0.054$). The aS pathology score in SN was not associated with rigidity ($p=0.17$) or with hypokinesia ($p=0.33$).

Visual hallucinations were reported in 53 subjects, associated with dementia regardless of the Braak stage or the type of aS pathology (OR 4.1; 95% CI 1.7-10.0; $p=0.002$) and with Braak stage independently of the presence of dementia ($p=0.041$), but no association was seen with the type of aS pathology ($p=0.78$). The probability for presenting with visual hallucinations was more than three times higher in subjects with Braak stage III-IV or V-VI compared to subjects with Braak stage 0-II pathology when gender, age at death, type of aS pathology and dementia were all taken into consideration.

Likelihood categories and clinical symptoms

The likelihood, assessed according to the Third DLB consortium guidelines, that the observed neuropathology presenting with the DLB clinical syndrome was low in 19 (6%), intermediate in 39 (13%) and high in 39 (13%) subjects. When analysing the likelihood categories and the clinical symptoms, dementia was most common in the intermediate-likelihood category (85% demented), and least common in the high-likelihood category (69% demented). Rigidity, hypokinesia and visual hallucinations were most common in the low-likelihood category, within which 47% manifested rigidity, 37% hypokinesia, and 26% visual hallucinations. It is proposed that the subjects with intermediate and high likelihood of DLB are regarded as neuropathological DLB (Fujimi et al. 2008) Within this group of subjects, 77% were demented, 35% had at least one extrapyramidal symptom, and 15% had visual hallucinations.

Impact of SN degeneration

Only 7 (2%) of the 303 with SN available did not have any neuron loss in the pars compacta of SN; 161 (53%) had mild, 115 (38%) had moderate and 20 (7%) had severe neuron loss (Figure 5). There was a significant positive association between the
Results

grade of SN neuron loss and the αS pathology score in SN (p<0.001) and the type of αS pathology (p<0.001). These associations remained significant even if subjects with the Braak stage V-VI were excluded, most likely because SN neuron loss did not associate with Braak stage (p=0.37).

The grade of SN neuron loss associated with rigidity (p=0.036), most strongly in the severe neuron loss group compared to others (OR 3.27; 95% CI 1.30-8.27; p=0.012, adjusted for gender and age at death). However, rather unexpectedly, no association was seen between the grade of SN neuron loss and hypokinesia (p=0.57).

![Figure 5](image)

**Figure 5** Assessment of the neuron loss in the substantia nigra; A) none, B) mild, C) moderate and D) severe.

αS pathology in the spinal cord

αS pathology was detected in the thoracic sympathetic intermediolateral cell column in 231 (76%) subjects, and in the sacral parasympathetic nucleus in 191 (63%) of 304 subjects. There were only 5 subjects with mild to moderate sacral αS pathology without any thoracic pathology, but 45 subjects had mild to severe thoracic αS pathology without sacral pathology. Thus, αS pathology was found at least in one of the two autonomic nuclei in 236 (78%) subjects. The mean age at death of these 236 spinal cord αS pathology-positive subjects was 92.89 years (SD ±3.8 years). When the subjects were divided into three age groups (85-89, 90-94, ≥95 years), the spinal cord αS pathology was most frequently detected in the oldest age group, in which αS pathology was found five times more often compared to the youngest age group (OR 5.39; 95% CI 2.2-13.3; p<0.001). Of the oldest age group, only 9% were αS pathology-negative, compared with the 36% in the youngest age group (p<0.001). However, the association with age was based exclusively on the mildest positive αS pathology in the spinal cord, regardless of the αS pathology in brain. The occurrence of the most severe (severe to very severe) forms of αS pathology was similar among the age groups (20% of the oldest vs. 19% of the youngest). The spinal cord αS pathology had no gender association.

The most common form of αS immunoreactivity detected was “mild”, with diffuse sytoplasmic staining in the perikaryon and grain or dot-like neuropil staining lacking focal LN and LB aggregates (Figure 6). In the moderate, severe and very severe forms of spinal cord αS pathology, the most common structures were dot-like or short and
stubby LNs, whereas longer curvilinear LNs and LBs were infrequent and most often seen in a very severe form.

![Figure 6](image)

**Figure 6 αS pathology in the intermediolateral column of the thoracic spinal cord; (a) negative, (b) mild and (c) severe form.**

**Association between αS pathology in the brain and spinal cord**

The frequency of neuropathological DLB was discovered to associate with the Braak neurofibrillary stage and with the grade of SN degeneration (neuron loss). αS pathology was present at least in the SN and/or hippocampal-transentorhinal region in 36% of the autopsied 304 subjects, who could be categorised as follows: 8 (3%) had brainstem-predominant, 42 (14%) had limbic, and 47 (15%) had diffuse neocortical αS pathology. Thirteen (4%) subjects had αS pathology confined to the hippocampal-transentorhinal region, and were not included in the clinical association analyses. Only 7 (2%) of the 303 subjects with SN available did not have any neuron loss in the pars compacta. A significant positive association between the grade of SN neuron loss and the extending αS pathology \( (p<0.001) \) was observed. A similar positive association was noted between the extent of αS pathology and the Braak neurofibrillary stage.

The extent of the αS pathology in the brain associated significantly with the spinal cord αS pathology; subjects with the diffuse neocortical type of αS pathology had spinal cord αS pathology in the autonomic nuclei nearly 28 times more often compared to subjects without αS pathology in the brain \( (OR \, 27.83; \, 95\% \, CI \, 3.6-212.4; \, p=0.001, \, adjusted \, for \, gender \, and \, age \, at \, death) \). Of the 50 subjects with severe or very severe αS pathology in the thoracic intermediolateral column, 60% had the diffuse neocortical type. Of the 18 subjects with severe or very severe αS pathology in the sacral parasympathetic nucleus, 89% had the diffuse neocortical type of αS pathology. Of the 47 subjects with diffuse neocortical type of αS pathology, only one had no αS pathology in the spinal cord, and an additional four subjects without αS pathology in the sacral parasympathetic nucleus had, instead, αS pathology in the motor columns of the anterior horns and in the thoracic sympathetic nuclei. Subjects with the brainstem-predominant type of αS pathology had a similar, mostly negative or mild, αS pathology in the spinal cord autonomic nuclei compared to the 13 subjects with αS pathology confined to the hippocampal-transentorhinal region. Furthermore, the extent of thoracic and sacral αS pathology in the spinal cord autonomic nuclei showed a significant association with the severity of SN neuron loss, but this association was lost when adjusted for αS pathology in the brain. Thus, this association was based on the cerebral, not spinal cord αS pathology. The spinal cord αS pathology did not associate with the neurofibrillary Braak stage either \( (p=0.76) \).
Impact of genetic variation in SNCA gene

Five of the 11 SNCA polymorphisms genotyped associated with the extent of neurofibrillary Alzheimer pathology (Braak stages IV-VI). The strongest association was seen with intron 4 SNP marker rs2572324 ($p=0.0006$), which also retained significant after correction for multiple testing (corrected $p=0.004$ and $p=0.021$). The same variant of SNCA (rs2572324) showed an association with diffuse neocortical $\alpha$S pathology ($p=0.023$), but probably because of the low number of cases, this did not remain statistically significant on permutation ($p=0.127$). None of the SNCA markers were associated with the extensive $\beta$-amyloid pathology.

SNP marker rs2572324 occurred in 20% of the study population. Its genotype A/A was overrepresented in subjects with Braak stage IV-VI. Subjects with A/A genotype had almost three times more often Braak stage IV-VI compared with the A/G or G/G genotypes (OR 2.81; 95% CI 1.56-5.07). Allele G enriched in the subjects with low Braak stage, suggesting the protective genetic factor behind the pathologic-genetic association.

SNP Markers rs2572324 and rs2583985 were in LD with each other ($D'=0.72$, $r^2=0.44$), and most of the other markers also exhibited LD with each other. Genotyping of MAPT revealed that 89% of the study population had MAPT H1/H1 haplotype. Because of the small number of non-H1/H1 subjects, the interactive effect of SNCA and MAPT H1/H1 could not be adequately addressed.
6 DISCUSSION

Occurrence of $\alpha$S pathology and DLB

Reappraisal of the old series of 55 patients with primary degenerative dementia revealed 14 (25%) cases classifiable to the brainstem, limbic or diffuse neocortical type of $\alpha$S pathology. Three subjects with $\alpha$S pathology confined to the brainstem had abundant AD-type pathology and thus, exceptionally, were not included in the group of neuropathological DLB in this study. If considering only patients with an original neuropathological diagnosis of AD (n=48), this re-evaluation showed that 23% of the AD cases can actually be diagnosed as DLB. It is quite easy to understand that DLB cases were previously overlooked because the established DLB entity was not recognised more widely until the late 80s, and the consensus criteria were not published until 1996 (McKeith et al. 1996).

Applying the most recent DLB consortium criteria, the burden of neuropathological DLB classified as the brainstem-predominant, limbic or diffuse neocortical type, in the autopsy cohort of a very elderly age group in a large prospective, population-based study was as high as 32%. $\alpha$S pathology was detected in 38% of demented subjects, and was also common among non-demented subjects of whom 20% showed neuropathological DLB. Furthermore, it is of interest that a great majority (92%) of the $\alpha$S-IHC -positive subjects had a more extensive, i.e., the limbic or diffuse neocortical, type of $\alpha$S pathology distribution. Thus, if only limbic and diffuse neocortical $\alpha$S pathology considered neuropathological DLB, 29% of the population had this kind of pathology. When the likelihood categories were applied instead, 26% had an intermediate or high likelihood of suffering from the DLB clinical syndrome.

The frequency of overlapping AD-type pathology was high. In the primary degenerative dementia study, all 11 neuropathological DLB cases displayed some AD-type pathology, and as many as 82% of them had the most severe Braak stage V to VI (Braak and Braak 1991, Harding et al. 2000) with histological findings compatible with the most severe CERAD plaque score C, i.e., definite AD (Mirra et al. 1991). Of the definite AD subjects, 63% had the diffuse neocortical type of $\alpha$S pathology, and thus belonged to the intermediate likelihood group for having DLB clinical syndrome. However, the manner in which the plaques and NFTs affect the clinical picture is interesting. In pure AD patients, senile plaques are thought to have less effect on cognition than neurofibrillary tangles (Samuel et al. 1996). In addition, a low level of NFTs in DLB has been noticed earlier (Lennox et al. 1989a, Weiner, 1999). Thus, the impact of the AD-type pathology in most DLB subjects could be only minor (with a high CERAD score and low Braak score).

In spite of the common occurrence of $\alpha$S pathology, the neuropathological DLB did not really predict the clinical symptoms commonly related to DLB. In the population-based study, one fifth of the subjects with the limbic or diffuse neocortical type were not demented and nearly two thirds of them did not have a single EPS. Based on the likelihood categories (McKeith et al. 2005), 26% of our subjects had extensive $\alpha$-synucleinopathy corresponding to the intermediate and high likelihood, but these did not predict the DLB clinical syndrome: almost one fourth of the subjects were not demented and two thirds did not have a single EPS. In the primary degenerative study, no significant association between the severity of dementia or disease duration and neocortical LB counts was found, nor did the disease duration associate with LB...
Discussion

density in any individual area. The high prevalence of extensive AD-type pathology in these DLB patients and their relatively uniform dementia status are probably significant in this context. It is noteworthy that 3 patients (27%) with αS pathology did not have any features of parkinsonism, and 5 (45%) did not present with visual hallucinations, while all these clinically non-classical DLB patients had Braak stage V or VI. Only 2 patients (18%) – both with Braak stage V or VI – demonstrated both visual hallucinations and parkinsonism, i.e. two of the three clinical core features of DLB. Based on the knowledge currently available, it is impossible to say, which one of the two pathologies caused dementia in cases with abundant αS and Alzheimer-type pathology in subjects with primary degenerative dementia. Thus, while the clinical diagnosis of DLB remains problematic, a significant proportion of patients with clinical and neuropathological AD may have coexisting αS pathology, with a possibly unnoticed DLB clinical syndrome. If in these cases both pathologies are abundant, there is as yet no method of identifying the neuropathological cause of dementia. Thus, a more complete understanding of the corresponding patho-biology of combined pathologies, in this case AD and DLB, is needed.

Comparisons with previous studies

In previous studies, the frequency of AD-type pathology in DLB cases has been high, too, varying between 54% and 91% (Gomez-Isla et al. 1999, Hansen and Samuel 1997, Gomez-Tortosa et al. 1998). Factors possibly explaining the differences are the variable severity of dementia, variations in patient age, and variations in the criteria for defining AD. The series of degenerative dementia subjects in this present research comprised hospitalised patients with severe or very severe dementia, with the exception of one case with moderate dementia. Therefore, the likelihood of an end-stage disease in these patients was high. In the population-based study, the frequency of αS pathology in all screened areas was 36%, which is almost identical to the previously reported prevalence (37%) in an elderly population (MRC CFAS study) from the U.K. (Zaccai et al. 2008). Our results are also consistent with those of a recently published population-based Hisayama study, in which 31% of demented elderly Japanese individuals had αS pathology; of these 23% had the limbic or diffuse neocortical type, and altogether 16% showed an intermediate or high likelihood that their neuropathology was related to the clinical DLB syndrome (Fujimi et al. 2008). The lower frequencies reported in the Hisayama study (Fujimi et al. 2008) may well be explained by the younger mean age of the subjects in that study (mean age at death for demented subjects in the Hisayama study was 86.2 years vs. 92.5 years in our study).

DLB is reported to affect men slightly more frequently (McKeith et al., 1994). Our results are consistent with that impression; however, it is important to note that only 17% of our population-based subpopulation were men. In the primary degenerative dementia study men were affected by αS pathology significantly more often than women. Nevertheless, also population-based study suggests that neuropathological DLB is also slightly more frequent in men among individuals aged 85 years and older, and men most often exhibit the diffuse neocortical type of αS pathology. On the other hand, the presence of αS pathology in men seemed to be less often associated with the clinical symptoms than in women.

Earlier studies have concluded that the frequency and extent of αS pathology increases with age (Fujimi et al. 2008, Gibb and Lees 1988), even as late as between the eighth
and tenth decades (Wakisaka et al. 2003). In our study, we could not find evidence – within this limited age range of very elderly subjects – for age being a risk factor for αS pathology in the brain. Instead, only the mildest form (fine granular aggregates in the neuropil) of αS pathology in the spinal cord αS pathology autonomic nuclei significantly associated with age. However, the LB and LN type of αS pathology in the spinal cord did not associate with age. It may be that the frequency of αS pathology (LBs and LNs) increases with aging only until a certain age.

We discovered that a marked number of neuropathologically diagnosed DLB patients with overlapping AD-type pathology showed only some, or even none at all, of the typical clinical features of DLB. Consistent with this, others have also reported that DLB cases with a high Braak stage show a more AD-like clinical pattern, and a concomitant AD-type pathology makes the recognition of DLB more difficult (Lopez et al. 2000, Merdes et al. 2003, Jellinger 2004) The most recent consensus statement reflects the difficulties in predicting the actual cause of dementia in these patients (McKeith et al. 2005). However, it is uncertain which one of these two neurodegenerative processes explained the dementia in those cases where both types of pathology were present. Thus far, we only know that in those cases the clinical picture is less likely to be the typical DLB syndrome (McKeith et al. 2005).

**Genetic findings**

The most striking genetic finding was the unexpected and highly significant association between the SNCA gene SNP and the high Braak stage for the NFT pathology. Interestingly, the same SNCA marker and haplotype which associated with the extent of tau pathology also associated nominally with the extent of αS pathology. In contrast, there was no association between the SNCA markers and β-amyloid pathology. Thus, the findings suggest that the SNCA polymorphism may have an effect on the intraneuronal aggregation of hyperphosphorylated tau and αS proteins, but it does not seem to affect the extraneuronal aggregation of the β-amyloid protein. There is also previous evidence for a link between synuclein and tau based on biochemical and gene interaction evidence. They have been shown to interact at the protein level, and they can seed each other’s aggregation (Giasson et al. 2003). Two recent genetic studies have suggested that SNCA alleles and MAPT haplotype H1 homozygocity may have an interactive effect on PD risk (Goris et al. 2007). Furthermore, there is increasing clinical and pathological evidence suggesting that synucleinopathies and tauopathies are linked (Hamilton et al. 2000).

These results provide the first evidence that SNCA variation dissects the two principal pathological features of AD, NFT and β-amyloid, since the SNCA variation only associates with NFTs in our population-based sample. We have previously found in the Vantaa 85+ series that the APOE ε4 allele is associated with both the β-amyloid and NFT pathologies (Polvikoski et al. 1995, Myllykangas et al. 1999). Now we know that both the SNCA variation and APOE ε4 contribute to the extent of the NFT pathology but only APOE ε4 contributes to the β-amyloid pathology. Conventionally, genetic association studies compare genotype distributions in case and control groups which have been clinically defined by man-made disease criteria, and these studies are often prone for selection bias. In contrast, in our population-based study, the genotype distributions were compared between subjects with neuropathologically-defined phenotype data. Careful neuropathological examination may be particularly important in the very elderly population, in which the clinical diagnostics of neurological disorders
and their exclusion based on clinical criteria is unreliable (Polvikoski et al. 2001, Polvikoski et al. 2006). Although the number of subjects in this study is relatively low, it is one of the largest among population-based studies in which neuropathological data are available (Zaccai et al. 2006). Until recently population-based studies have been under-emphasised, but at present it is recognised that they may provide unique insights in neurodegenerative disorders by giving unbiased information of the occurrence of diseases and pathology as well as genetics at the population level (Zaccai et al. 2006).

**Findings in the spinal cord**

This study reveals the common occurrence of αS pathology in the spinal cord. Almost 80% of the elderly in the general population exhibited αS pathology, either in the thoracic or sacral autonomic nuclei or in both. The frequency of the spinal cord αS pathology increased with age, but interestingly, only the mild type with αS pathology aggregates was age-related. Nevertheless, αS pathology in the spinal cord autonomic nuclei associated significantly with αS pathology in the brain. Of the subjects with the diffuse neocortical type of αS pathology, only one had no αS pathology in the spinal cord, and of the subjects with severe to very severe αS pathology in the autonomic spinal cord nuclei only three had no αS pathology in the SN or in the hippocampal-transentorhinal region. The αS pathology in the spinal cord did not associate with the Braak neurofibrillary stage, although the brain αS pathology did, suggesting that the cerebral Alzheimer’s disease pathology is one element of the pathological disease progression of αS pathology but not the main promoter of it.

The αS pathology distribution in the spinal cord autonomic nuclei is informative: 19% of αS pathology-positive subjects had thoracic cord pathology without sacral αS pathology and only 2% of αS pathology-positive subjects had sacral αS pathology without thoracic αS pathology. Given the higher frequency of αS pathology and more severe αS pathology in the thoracic compared to the sacral cord, this suggests that the progression of αS pathology proceeding in a descending pathway, i.e. affecting the thoracic spinal cord before the sacral one. The severity of αS pathology in the spinal cord in subjects without the brain αS pathology was similar to those with αS pathology in the SN (brainstem-predominant) or αS pathology only in the hippocampal-transentorhinal region, suggesting that the SN and autonomic nuclei of the spinal cord are affected by αS pathology simultaneously. This is concordant with an earlier study indicating that the autonomic nuclei of the spinal cord are among the earliest affected regions in the pathological process of αS pathology (Probst et al. 2008). Because our screening method for the αS pathology in the brain did not include lower brainstem nuclei, the precise induction site of αS pathology remains uncertain. From this induction site, potentially the lower brainstem nuclei as proposed in neurologically intact subjects (Del Tredici et al. 2002), the αS pathology seems to progress along a descending pathway towards the thoracic and sacral spinal cord, and at the same time move rostrally via an ascending pathway towards the neocortex. Then, after the αS pathology has reached the target, the load of αS pathology seems to proceed on all affected levels at the same time: the load of αS pathology in the SN was the most severe with an extended (i.e. diffuse neocortical distribution) αS pathology, and the most severe spinal cord αS pathology was seen with an extended brain αS pathology. Yet, there are some subjects with a moderate to severe spinal cord αS pathology without brain αS pathology, and vice versa. Some subjects with the diffuse neocortical type of αS pathology did not show any αS pathology in the spinal cord. Whether these subjects
with an αS pathology distribution diverging from the suggested pathways represent an incidental Lewy body disease (iLBD), and what the other factors needed are for the neuropathological findings to determine the clinical signs and symptoms, will hopefully be illuminated when the clinical findings are combined with our neuropathological results.

Several studies have discovered αS pathology in the autonomic nuclei of the spinal cord and brainstem in neurologically intact subjects (Klos et al. 2006, Dickson et al. 2008, DelleDonne et al. 2008). These incidental lesions have been suggested to represent a pre-symptomatic phase of PD (Dickson et al. 2008, DelleDonne et al. 2008). Based on the findings of these previous publications and the current work on the progression of the αS pathology in the nervous system, I would like to emphasise the paradigm that incidental lesions of αS pathology in the spinal cord and in the brainstem (without neuron loss in the SN) represent the pre-symptomatic phase of Lewy body disease (LBD), not PD nor DLB. Yet, to date, we cannot be certain whether incidental findings would have progressed to manifestations of PD or DLB. If neuron loss is detected in the SN (with no diffuse neocortical type of αS pathology) it is more probably a signal of nascent or symptomatic PD (Dickson et al. 2008, DelleDonne et al. 2008), whereas severe SN neuron loss in DLB has been seen most often in the later phase of the disease progression, i.e. with the diffuse neocortical type of αS pathology distribution and with severe to very severe αS pathology in the spinal cord.

Strengths and limitations of the study

The strength of the present study is the exceptional material; a consecutive autopsy serie of the patients with primary degenerative dementia as a reference group, and a population-based autopsied subpopulation of very elderly Finns as the main study group. Because of the age and gender (only 17% were men) of the subpopulation, I believe that the αS pathology detected in the population-based study represents mainly the neuropathological findings of DLB and/or iLBD instead of PD. Furthermore, only 9 subjects in our study population of 601 had the clinical diagnosis of PD. Therefore, these results bring the long-awaited information on the frequency and progression of αS pathology in the brain and spinal cord in the DLB, and also of the clinical features of the αS pathology in the elderly. The progression of αS pathology in iLBD/DLB seems to deviate from the topographically predictable six–stage progression of αS pathology in PD (Braak et al. 2003).

There are some limitations in the present study. We screened subjects for αS pathology in the brain with a sample of the SN and hippocampal-transentorginal region; thus, we have not investigated all the brain areas with immunohistochemistry. In this way, we might have missed some cases with positive αS pathology in the brain, and further, this form of screening system may create a bias towards the working hypothesis of the αS pathology progression. The null-hypothesis was that in DLB αS pathology proceeds from the brainstem towards the neocortex, and based on this, αS pathology was screened for using the sample of the brainstem. As a result, I conclude that there is no (significant for DLB) αS pathology in the neocortex without some brainstem αS pathology, and that, I have been able to find only cases with the “earlier stage”, the brainstem, affected as well.
Future challenges

It will be important to verify these neuropathological findings in other elderly subjects and also further explore the functional effects linked to the SNCA gene and other possible protective factors. To strengthen my suggestion for the progress of αS pathology, I also need to stain immunohistochemically all the cortical areas and amygdala of every subject.
7 CONCLUDING REMARKS

DLB was considered to be an uncommon cause of dementia until improved neuropathological staining methods for ubiquitin were developed in the late 1980s. Subsequent recognition of the fact that 10-15% of dementia cases in older people were associated with Lewy body pathology led to the publication of clinical and pathological diagnostic criteria by DLB Consortium in 1996. These have greatly raised the global awareness of DLB. In this series of studies, neuropathological DLB was a common finding. In a population-based study, aS pathology, classifiable according to the DLB consensus criteria, was found in one third of the brains of the very elderly subjects. 92% of them had more extensive, i.e. limbic or diffuse neocortical aS pathology. However, in spite of the frequent occurrence of the aS pathology, its association with the clinical symptoms was quite poor, and indeed, the common clinical symptoms of DLB associated better with the severe neurofibrillary AD-type pathology than with the extensive aS pathology when both types of pathology were taken into account. Although both pathologies had an independent significant impact on dementia, 15% of the subjects with extensive aS pathology and Braak stage III or higher were not demented. We found no explanation behind this, but some protective factors may play a role here. The most striking genetic finding made was the unexpected, highly significant association between an SNCA gene SNP and the high Braak stage for the NFT pathology. We also found a protective allele of the SNCA gene which is part of its common variation and associated with the low Braak stage. Our findings suggest that in DLB, the progression of aS pathology (after starting initially in the lower brainstem) proceeds simultaneously rostrally towards the neocortex and caudally towards the sacral spinal cord, and finally with the most extended aS pathology the destruction of the SN pars compacta is the most severe. More studies are needed to strengthen our results, and also to study further the corresponding patho-biology of aS.
ACKNOWLEDGEMENTS

This work was carried out at the Department of Neurosurgery, Helsinki University Central Hospital, and at the Department of Pathology, Laboratory of Neuropathology, University of Helsinki. I am most grateful to all those who have helped, guided and supported me during the long journey of this study at both departments. I would particularly like to thank Professors Veli-Pekka Lehto and Eero Saksela, the present and former head of the Department of Pathology, for providing excellent research facilities.

Furthermore, I wish to express my gratitude to:

Tuomo Polvikoski and Anders Paetau for the supervision over my work. Following your genuine enthusiasm for neuropathology I, too, fell in love with the Brain. I appreciate your patience and willingness to help in completing this work also when neurosurgery has occupied my time.

Hannu Kalimo for teaching me how to write in the scientific way. I am most grateful for your fatherly guidance, support and for pushing me forward at the times of despair.

Liisa Myllykangas, Terhi Peuralinna, Irma-Leena Notkola, Raimo Sulkava, Leena Niinistö, Kati Juva, Sari Rastas, and Pentti Tienari, my other co-authors, for their valuable contribution to this work.

Tuija Järvinen, for the skilful technical assistance and friendship over the years. From you and Eija I have learned priceless survival hints.

All the colleagues at the Department of Pathology for providing such friendly companionship during my time there.

Irina Alafuzoff and Juha Rinne, reviewers of this thesis, for their constructive comments to improve this work at short notice.

Juha Hernesniemi, the head of the Department of Neurosurgery, for patience and encouragement. I am extremely proud of being trained in your Department. I can never be too grateful.

My neurosurgical and anesthesiological colleagues and co-workers in the OR, ICU and at wards. This work would not have been possible if the daily clinical work had not been so enjoyable and easy as it was, and is, with you. Martin and Riku, for your help, friendship and support. I will never forget the EANS courses we went through together. In a good way. Mikka, for the technical support with the never-ending problems with my MacBook. Anna, for standing the mess in our office. Ville, for the help in editing the cover of this thesis book. Virpi and Eve, for being available to help when ever needed.

Our Head & Neck Surgery team: Mika, Jyrki, Pate, Timo, Lefa, Harri, Mari, and Antti for their patience and for creating a pleasant atmosphere during the long operative hours we share.

Our Craniofacial Surgery team: Atte, Riku, Pia, Jyri and Junnu for being so easy-going. It is always a pleasure to work with you.
I feel fortunate to have had the opportunity to spend 13 months in the Department of Neurosurgery, UAMS, Little Rock, Arkansas. I am grateful to Dr. Krisht, Dr. Al-Mefty and Professor Yasargil.

Sara Iglesias Moraño for sharing the time in the cadaver lab. I am sure that neither of us will ever forget the Delilah radio show. I am thankful for your genuine friendship.

Karen and Dusty Rhoades for making my stay in Little Rock warm and memorable. I am most grateful for keeping me in focus during the time of homesickness and confusion.

All my relatives and family friends for their interest in my work and support. Especially all the members of “Rantaremme” for the relaxing moments at Lake Kitka.

Anne, Lotta, Maaria, Miksu, Katja M and Katja P, my dear friends, for long-lasting friendship, splendid times spent together, and your positive attitude towards this work and also towards my constant complaints. I feel extremely lucky to have friends like you.

My parents and sisters for their love and belief in me. I am proud of my Laplander roots, and father, I promise to you not to change my last name if ever I get married.

Stella and Kiara for being such sunshines. My loving thanks to you both. You know I will always be here for you.

Jape for his love and understanding. You are the best possible companion I could ever wish for. You have gone through a lot during the years with me, but still you have the strength to care and support. I wish to be able to prove to you how much that means to me.

This study was financially supported by the Finnish Medical Foundation, Duodecim, Alzheimer Foundation of Finland, Maire Taponen Foundation, Finnish Cultural Foundation, Uulo Arhio Foundation, Pirjo Kuusakoski-Tikkanen and Helsinki University Central Hospital competitive research fund (EVO).

In Helsinki, September 2009
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