Lewy-related pathology in the adrenal gland is dependent on the progression pattern and stage of pathology in the central nervous system

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Helsingin yliopisto, Lääketieteellinen tiedekunta

Helsinki 3.1.2022

Tutkielma

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HELSINGIN YLIOPISTO

Lääketieteellinen tiedekunta

Tiivistelmä

Tiedekunta: Lääketieteellinen tiedekunta

Koulutusohjelma: Lääketieteen koulutusohjelma

Opintosuunta: Suomenkielinen opintolinja

Tekijä: Ville Kivistö

Työn nimi: Lewy-related pathology in the adrenal gland is dependent on the progression pattern and stage of pathology in the central nervous system

Työn laji: Syventävien opintojen tutkielma

Kuukausi ja vuosi: 1/2022

Sivumäärä: 18

Avainsanat: synucleinopathies, adrenal gland, Parkinson’s disease, dementia with Lewy bodies, neuropathology

Säilytyspaikka: HELDA

Muita tietoja:

Tiivistelmä:

Synukleinopatiat ovat joukko neurodegeneratiivisia sairauksia, joita yhdistää alfa-synukleiinin kertyminen hermostoon, ja johon kuuluvat muun muassa Parkinsonin tauti, Lewyn kappale -tauti sekä monisysteemiatrofia. Klassisesti alfa-synukleiinin aiheuttaman Lewyn kappale -patologian leviämisen on ajateltu Parkinsonin taudissa olevan alemmilta aivoalueilta kohti neokorteksia suuntautuva prosessi, mutta viimeaikaiset tutkimukset ovat luoneet pohjaa hypoteeseille kahdesta eri etenemismallista, joista toinen alkaa keskushermoston ulkopuolelta ja toinen aivoista. Neuropatologisissa tutkimuksissa tämä näkyy Lewyn kappale -patologian kaudo-rostraalisena tai amygdala-pohjaisena etenemisenä.

Tässä tutkimuksessa selvitettiin Vantaa 85+ -kohortin 163 tutkimuskohteelta saatujen 174 näytteen perusteella Lewyn kappale -patologian esiintyvyyttä lisämunuaisessa immunohistokemiallisesti kahdella alfa-synukleiini-vasta-aineella. Tutkittavat määriteltiin joko positiivisiksi tai negatiivisiksi mikroskooppilöydösten perusteella.

Tämän jälkeen negatiivista ja positiivista ryhmää verrattiin demografisten ominaisuuksiensa, DLB-konsortion määritelmän mukaisen aivopatologian tason sekä neuropatologisen etenemismallin perusteella. Ryhmien demografisissa ominaisuuksissa ei ollut tilastollisesti merkittäviä eroja, mutta naisilla lisämunuaispatologiaa esiintyi kuitenkin selvästi vähemmän. Lisämunuaispatologiaa ei esiintynyt tapauksissa, joissa ei ollut minkäänlaista Lewyn kappale -patologiaa keskushermostossa, vaan lisämunuaisen patologia liittyi lähes yksinomaan kaudo-rostraaliseen etenemismalliin, ja sen esiintyvyys riippui lisäksi aivopatologian tasosta. Amygdala-pohjaisten tapausten joukossa lisämunuaisen patologia oli harvinaista.

Havaittu ero lisämunuaisen patologian esiintyvyydessä etenemismallien välillä vahvistaa käsitystä niiden olemassaolosta. Tämän lisäksi lisämunuaispatologian esiintyvyyden riippuvuus kaudo-rostraalisesta etenemisestä sekä aivojen patologian tasosta viittaavat siihen, että lisämunuaisten alfa-synukleiinipatologian alkuperä on keskushermostossa.

Abstract

Faculty: Faculty of Medicine

Study programme: Medicine

Subject: Study orientation in Finnish

Author: Ville Kivistö

Title: Lewy-related pathology in the adrenal gland is dependent on the progression pattern and stage of pathology in the central nervous system

Level: Thesis

Month and year: 1/2022

Number of pages: 18

Keywords: synucleinopathies, adrenal gland, Parkinson's disease, dementia with Lewy bodies, neuropathology

Where deposited: HELDA

Additional information:

Abstract:

Synucleinopathies are a group of neurodegenerative diseases characterised by accumulation of the protein alpha-synuclein in the nervous systems, including diseases such as Parkinson's disease, dementia with Lewy bodies and multiple system atrophy. Classically, the progression of Lewy-related pathology caused by alpha-synuclein in Parkinson's disease has been thought to be an ascending process from the lower brain structures towards the neocortex, but recent studies have given credence to hypotheses regarding two distinct progression patterns, with one beginning outside the central nervous system and one in the brain. In neuropathological studies, this is seen as the caudo-rostral and amygdala-based progression patterns of Lewy-related pathology.

In this study, 174 samples from 163 subjects of the Vantaa 85+ cohort were used to determine the prevalence of Lewy-related pathology in the adrenal gland using immunohistochemistry with two anti-alpha-synuclein antibodies. The subjects were scored either negative or positive based on microscopic findings in the adrenal glands.

Afterwards, the positive and negative groups were compared based on demographic characteristics, as well as their stage of brain pathology according to DLB consortium staging and neuropathological progression pattern. There were no statistically significant differences in demographic characteristics, although women had a considerably lower prevalence of adrenal gland pathology. There were no positive cases without any Lewy-related pathology in the central nervous system, instead adrenal gland pathology was almost exclusively associated with the caudo-rostral progression pattern, and the prevalence was further dependent on the stage of brain pathology. Adrenal gland pathology was rare among the amygdala-based cases.

The marked difference between the progression patterns regarding the prevalence of adrenal gland pathology provides further evidence for their existence. Furthermore, the dependency of adrenal gland pathology on caudo-rostral progression and the stage of brain pathology suggests that alpha-synuclein pathology in the adrenal gland originates from the central nervous system.

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1 Introduction

Neurodegenerative diseases are a group of diseases characterised by progressive and often selective neuronal loss. This selectivity produces distinct anatomical patterns of pathology, which in turn have a major role in the clinical presentation. These features have been commonly used to classify neurodegenerative diseases. However, as the molecular mechanisms underlying these diseases have become better known, classification based on the molecular pathology has become more relevant. (1)

Synucleinopathies are a group of neurodegenerative diseases defined by the misfolding of the protein alpha-synuclein (α-syn), which then forms the main component of the histologically visible aggregates called Lewy bodies and Lewy neurites (2,3). Despite sharing the same pathological molecule, the group's primary clinical manifestations range from the movement disorder in Parkinson's disease (PD), to dementia in dementia with Lewy bodies (DLB) and autonomic dysfunction in multiple system atrophy (MSA). Furthermore, the various subtypes of each disease may, in part, result from physiological variation between individuals, but evidence is pointing towards these disease designations harbouring multiple distinct pathological processes. Particularly in the context of the diseases with Lewy bodies, PD and DLB, the origin and spread of α-syn have been a subject of research, with recent hypotheses suggesting two main disease patterns: one arising from outside the central nervous system, and one beginning in the brain (4,5).

1.1 Biochemistry and physiology of alpha-synuclein

α-syn is a soluble protein consisting of 140 amino acids. The soluble form is thought to be unfolded, unstructured and monomeric, with binding to lipid membranes causing the formation of α-helices in its secondary protein structure and promoting multimerisation. (6) However, some evidence points towards the non-membrane-bound structure being a tetramer with an α-helix structure resistant to aggregation (7,8).

α-syn is widely expressed in the brain, where it is enriched in the presynaptic terminals of neurons. The physiological significance of α-syn is not clearly understood, but based on protein and lipid interactions and animal models, a variety of functions have been suggested. These include roles in lipid transport and membrane formation, vesicle trafficking, synaptic plasticity, and synaptic monoamine regulation. In particular, overexpression of α-syn seems to inhibit the release of dopamine both by inhibiting its synthesis by tyrosine hydroxylase and the release of synaptic vesicles containing it. (6,9)

1.2 Parkinson's disease

1.2.1 Clinical manifestation

The defining motor symptoms of Parkinson's disease, referred to as parkinsonism, were described by James Parkinson in 1817. In his patients, he recognised a pattern of progressively worsening tremor, rigidity, bradykinesia, and postural instability. (10) These symptoms are to this day the foundation for a clinical diagnosis of PD. Modern guidelines are based on the patient exhibiting bradykinesia in combination with one or both of tremor and rigidity (11,12). Patients with PD often also develop non-motor symptoms, including hyposmia, depression, constipation, and REM-sleep behaviour disorder. Non-motor symptoms may appear several years before the motor symptoms and tend to be progressive. (13)

1.2.2 Epidemiology

Parkinson's disease is the second most common age-related neurodegenerative disease after Alzheimer's disease (AD). A systematic review of the studies on the prevalence and incidence of PD in the European population found that estimates of the prevalence ranged from 108 to 257/100 000. Similarly, the incidence ranged from 11 to 19/100 000 per year. When only the over 60-year-old population was considered, the prevalence was 1280 to 1500/100 000, and the incidence was 346/100 000 per year. (14) In a global meta-analysis, the overall prevalence was estimated to be 315/100 000, ranging from 41/100 000 in ages

40-49 to 1903/100 000 in ages 80+ (15). In general, the incidence of PD rises sharply after the age of 60, and the disease is rare before the age of 50. Young-onset PD is defined as PD diagnosed at under 40-years-old, and onset of PD at under 21-years-old is called juvenile PD; compared to PD occurring in older people, these forms are often familial. (16–18) It has been estimated that 6.1 million people worldwide had PD in 2016, showing a significant increase from 2.5 million in 1990. During this period, the age-standardised prevalence also rose by 21.7%, indicating that this change is not fully explained by the increased amount of older people. (19)

1.2.3 Pathophysiology and pathology

The pathological hallmarks of PD are intraneuronal Lewy body inclusions in the somas of neurons and Lewy neurites, representing affected cell processes (20). Lewy bodies represent abnormal localisation and aggregation of α-syn, which is normally localised mainly in the pre-synaptic terminals (6). However, whether Lewy bodies are the causal agent in neuronal loss or not is unclear (21). The damage to different brain structures is not random, instead showing a pattern that seems to begin in select areas in the nervous system, such as the dorsal motor nucleus of the vagus nerve, and progresses predictably through lower-level brain structures towards the neocortex. This pattern is the basis for classifying PD according to Braak's staging. (22) The characteristic bradykinesia and rigidity are thought to occur as the disease progresses to the dopaminergic neurons of the substantia nigra, disturbing the modulation of a corticostriatal circuit involved in the generation of movement (23).

1.3 Dementia with Lewy bodies

1.3.1 Clinical manifestation

Dementia is the foundational symptom in DLB, which can make differentiating between DLB and other dementias such as Alzheimer's disease difficult (24). In a meta-analysis of the accuracy of clinical diagnoses of DLB, it was found that on pathological examination, roughly 20% of diagnoses were incorrect (25).

Commonly accepted core clinical features of DLB are fluctuating cognition (especially attention and alertness), recurring visual hallucinations, and REM-sleep behaviour disorder (RBD). Testing for difficulties in processing speed, alternating or divided attention, as well as visuoperceptual problems may aid in clinical decision making. Memory processes are often less affected in DLB. (24,26) Parkinsonism is also a common feature, and the timing of its onset is important for differentiating between Parkinson's disease with dementia (PDD) and DLB. To diagnose PDD, parkinsonism must precede symptoms of dementia by at least a year, whereas if dementia precedes or develops concurrently with parkinsonism, the diagnosis is DLB. (11,24)

1.3.2 Epidemiology

DLB is thought to be the second most common form of dementia after AD. A meta-analysis found the mean population prevalence of DLB in those aged 65-years-old and older to be 0.36%, although the variance between studies was large (0% to 21.9%). In patients diagnosed with dementia, the prevalence was 4.2%. The incidence was 0.87/1000 per year in the 65+ population, representing 3.8% of new dementia diagnoses. (27)

1.3.3 Pathophysiology and pathology

Similar to PD, the pathological hallmark in DLB are intraneuronal Lewy body inclusions and Lewy neurites, and due to their many shared features, some consider DLB, PD and PDD to represent different manifestations of a common disease spectrum. The distinguishing neuropathological factor between PD and DLB seems to be the distribution of Lewy pathology throughout the central nervous system. When classified using the DLB consortium staging, PD patients fall predominantly into the limbic transitional stage, whereas DLB and PDD are almost exclusively diffuse neocortical. (24,28)

1.4 Multiple system atrophy

1.4.1 Clinical manifestation

MSA can present with various motor and non-motor symptoms. Based on the dominant motor features, MSA is subdivided into the parkinsonian form (MSA-P), showing features such as rigidity and akinesia, and the cerebellar form (MSA-C), which typically presents with symptoms of cerebellar origin, such as gait ataxia and limb ataxia. In contrast to PD, the parkinsonism caused by MSA is poorly responsive to levodopa, a medication metabolised into dopamine in the brain and intended to counteract the loss of dopaminergic neurons. This lack of response is included in the criteria of the clinical diagnosis of probable MSA. (29–31) As the disease progresses, patients often develop symptoms of both forms, and the designation is based on the dominant motor feature at the time of evaluation (29). Autonomic failure is also at the core of MSA, most often resulting in genitourinary symptoms such as urinary incontinence and erectile dysfunction in men, as well as orthostatic hypotension (29). Autonomic dysfunction being present at the time of diagnosis or developing rapidly after the initial symptoms seems to be a strong predictor of a worse prognosis (32,33).

1.4.2 Epidemiology

Two studies on European and Japanese MSA patients found a mean age of onset of 56.2 and 55.4 years, respectively. The proportions of MSA-P and MSA-C were different between the studies; in the European study, 62% of the patients were classified as having MSA-P, whereas in the Japanese study 67% of the patients were classified as MSA-C. (32,33) Another study found the incidence of MSA in the USA to be 3 per 100 000 person-years between ages 50 to 99 (34). Overall, the prevalence of MSA has been estimated to be 1.94 - 4.4/100 000 (34–37).

1.4.3 Pathophysiology and pathology

In MSA, the primary driver of neurodegeneration is thought to be the pathological aggregation of α-syn in oligodendrocytes. This causes dysfunction of neuronal support, and the misfolded α-syn released by the affected oligodendrocytes may

also be taken up by nearby neurons, forming neuronal inclusions. (38) Neuropathologically, typical macroscopic findings include atrophy of the olivopontocerebellar and striatonigral systems. Common histological findings seen in these areas include features such as loss of neurons, gliosis, and axonal degeneration. (39)

1.5 Hypotheses on the propagation of α-synuclein

Multiple hypotheses have been put forward regarding the starting point and propagation of α-syn pathology. Braak's staging for PD presents the propagation of Lewy-related pathology (LRP) as ascending through the lower brain structures towards the neocortex, with the assumed starting point being the dorsal motor nucleus of the vagus nerve and possibly the olfactory bulb and related areas. (22) However, Braak also had the idea that the disease could originate from the enteric nervous system (ENS) and travel retrograde towards the central nervous system (CNS) (40). More recent studies on mouse disease models have demonstrated the retrograde spread of pathological α-syn from an intramuscular injection or from the ENS to brain structures, and secondary anterograde spread into the heart and back into the gastrointestinal system was also observed (41).

Since Braak's hypothesis, studies on patients with idiopathic RBD have provided evidence for two distinct phenotypes of Parkinson's disease: one where the disease begins in the peripheral nervous system (PNS-first) and one where the disease begins in the central nervous system (CNS-first). The PNS-first subtype is characterised by damage to the autonomic nervous system, such as cardiac sympathetic nerves, before damage is seen in the dopaminergic systems. It is also tightly linked to RBD presenting during the prodromal phase of the disease. Conversely, in the CNS-first subtype the dopaminergic system is damaged before the involvement of the PNS, and usually there is no prodromal RBD. (5)

Based on neuropathological studies on the elderly, it has been shown that the propagation of LRP can be divided into caudo-rostral and amygdala-based patterns. This division is based on the distribution and severity of LRP along several points of interest in the spinal cord and the brain. The amygdala-based pattern is also associated with concomitant AD pathology. (4) In addition,

pathology in the olfactory bulb (OB) and olfactory peduncles (OP) differs between these two progression patterns: all the amygdala-based patients were positive for OB/OP LRP and showed strong predominance of LRP in the anterior olfactory nucleus (AON), whereas 23% of the caudo-rostral patients were negative, and the predominance of AON pathology was less pronounced (42). Recently, it was proposed that these neuropathological findings correspond to the PNS-first/CNS-first-division, with caudo-rostral being equivalent to the PNS-first type, and amygdala-based being equivalent to the CNS-first type (43).

The cellular mechanism of the propagation of α-syn pathology has been compared to that of prions. It is thought that pathological α-syn can be transferred through synapses and induce further aggregation in the target neuron. This kind of neuron-to-neuron transmission is also supported by the finding that among the caudo-rostral and amygdala-based progression patterns, a decreasing gradient of LRP could be observed in the brain areas adjacent to the main site of pathology (43).

2 Aim of the study

The aim of this study was to qualitatively assess the Lewy-related pathology in adrenal gland samples of elderly Finnish subjects using immunohistochemical staining and visual analysis with a light microscope, and to quantify the association of adrenal gland LRP with different characteristics of the study subjects. The subjects' brain and spinal cord LRP have been extensively studied to determine their progression pattern, but peripheral involvement has not yet been described. As a sympathetic ganglion, the adrenal gland is a point of interest in the propagation of α-syn. In this study, the existence of adrenal gland LRP was compared against the stage of LRP in the brain according to DLB consortium staging and the progression patterns as assessed by Raunio et al. to determine whether adrenal gland LRP was associated with either (4).

3 Materials and methods

3.1 Study sample

The sample set of this study was obtained from the material of the Vantaa 85+ study, a population-based study that includes 601 individuals aged 85 years or older living in Vantaa on April 1, 1991, of which 304 (54%) were neuropathologically examined. The full neuropathologically examined subset has been described in previous studies (4).

The material for this study comprises 174 adrenal gland samples from 163 subjects from the neuropathological subset of the Vantaa 85+ study. Data was not available for 3 subjects, who were excluded from analysis. The basic characteristics of the subjects included in this study are presented in table 1.

Table 1. Basic characteristics of the study sample.

		Sex		
		Male	Female	All
Dementia status	No dementia	10	44	54
	Dementia	15	91	106
	Total	25	135	160
Onset of dementia (age)	Mean	88.67	87.54	87.68
	SD	3.46	4.48	4.36
Duration of dementia	Mean	4.75	5.82	5.68
	SD	3.68	4.05	4.00
Age at death	Mean	94.11	93.34	93.46
	SD	2.22	3.76	3.56

Note: SD = standard deviation

3.2 Staining procedure

For a subset of 55 samples, three slides of each sample were stained using two different antibodies against alpha-synuclein (*Fujifilm Anti Phosphorylated α-Synuclein, Monoclonal Antibody (pSyn#64) and Sigma-Aldrich Anti-Aggregated a-Synuclein Antibody, clone 5G4 (MABN389))* and one against synaptophysin (*Dako Monoclonal Mouse Anti-Human Synaptophysin*). The remaining 119 samples were only stained with the pSyn#64-antibody. The staining was

performed using a LabVision LV-1 Autostainer with the Dako REAL™ EnVision detection system (*REF: K5007*).

A test set of slides were stained using different antibody concentrations and incubation times in order to optimise the staining protocol for the study sample. Figure 1 shows a visual comparison between two different incubation times for the MABN389 antibody, demonstrating the difference in background staining. The dilutions and incubation times used for the study set were as shown in table 2.

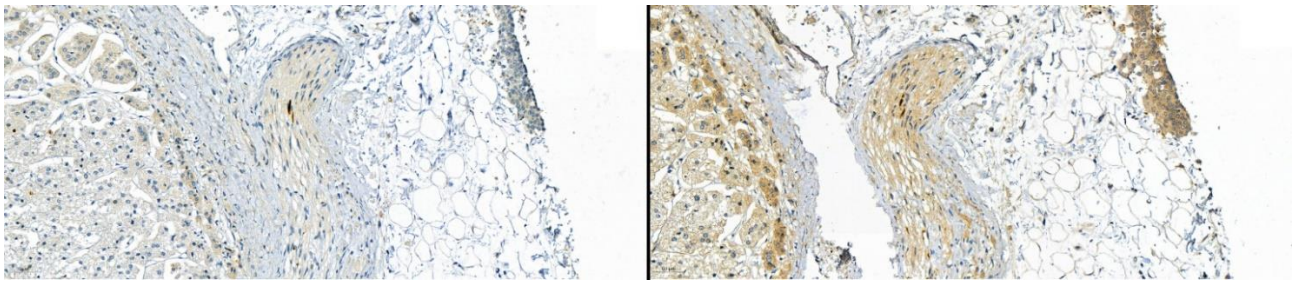

Figure 1. MABN389 antibody, dilution 1:2000, incubation time 1h on the left and overnight on the right.

Table 2. Dilutions and incubation times for the antibodies used for the study samples.

Antibody	Dilution	Incubation time
MABN389	1:2000	Overnight
pSyn#64	1:2000 or 1:1800	Overnight
Synaptophysin	1:500 or 1:400	Overnight

3.3 Analysis of samples

After staining, the samples were analysed using a light microscope. At 10x magnification, subjects were scored either positive or negative based on whether typical Lewy pathology was present in the adrenal gland or in the surrounding fat tissue in the slides stained with either anti-α-syn-antibody. Anti-synaptophysin-stained slides were used to help confirm the location of nervous structures. Figure 2 shows examples of positive findings.

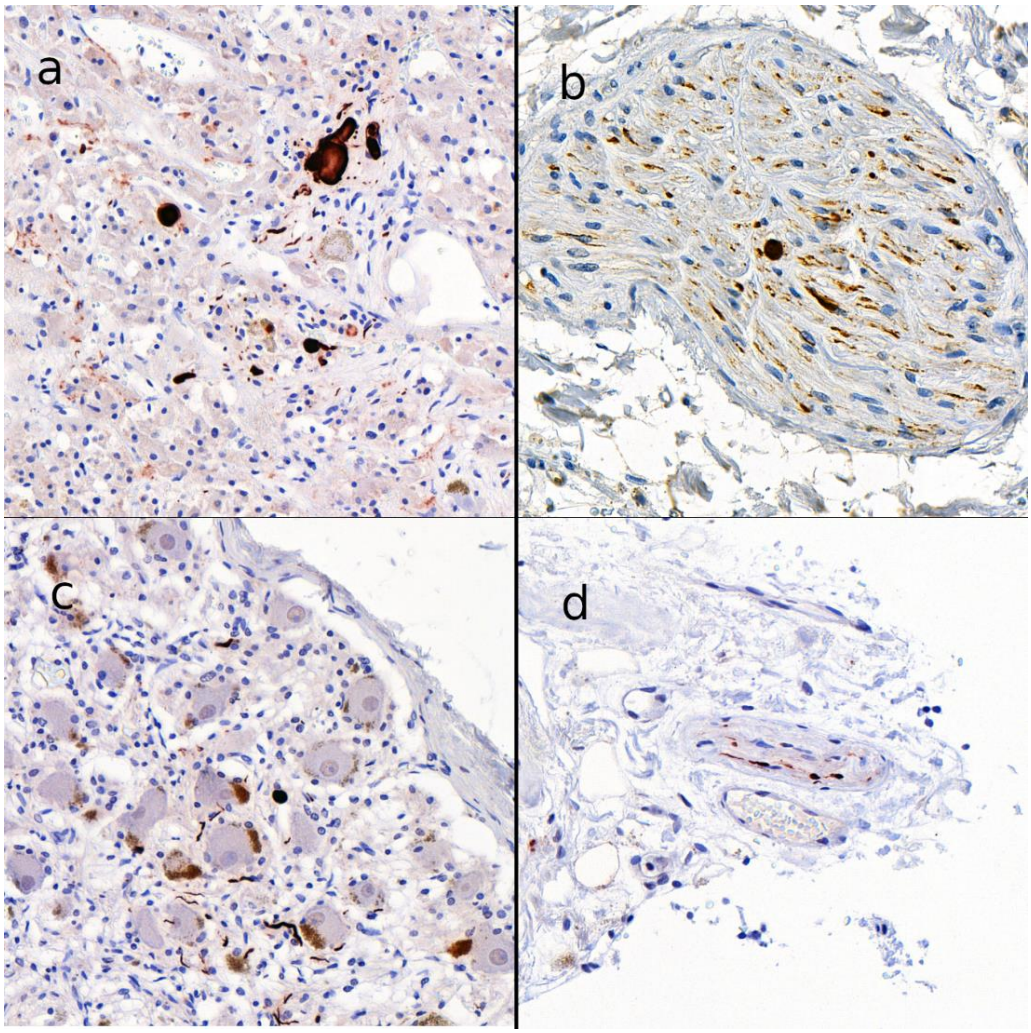

Figure 2. Adrenal gland α-syn pathology visualised using the pSyn#64-antibody, dilution 1:1800. a) Adrenal gland stroma with large Lewy bodies. b) Lewy-body along with neurites in a nerve. c) Lewy pathology among a cluster of ganglion cells. d) A smaller nerve with neurites.

The two anti-α-syn antibodies performed equally in visualising pathological α-syn aggregations, identifying the same subjects as positive. Figure 3 shows a comparison between all three antibodies in the same location.

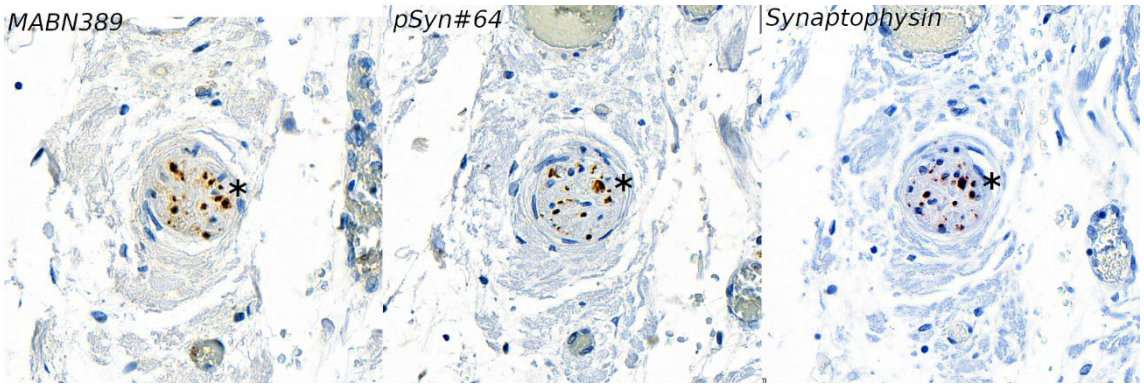

Figure 3. Lewy-body like inclusions (star) could be seen using all three antibodies in a nerve in the periadrenal fat tissue. From left to right: MABN389, pSyn#64, Synaptophysin.

Statistical analysis was conducted using RStudio (version 1.3.1056) with the *stats* package. For categorical data, Pearson's Chi-squared test with Yates' continuity correction and Fisher's exact test were used when appropriate. For comparing means, Welch's two sample t-test for unequal variances was used. Statistical significance was set at p-value < 0.05.

4 Results

Overall, 21 out of 160 (13.1%) subjects showed Lewy-related pathology (LRP) in the adrenal gland. Characteristics of the sample set according to adrenal gland pathology are summarised in table 3.

Table 3. Basic characteristics of the sample according to the adrenal gland pathology status.

		Lewy-related pathology of adrenal gland	
		No LRP	LRP
Sex (n, %)	Male	19 (76.0)	6 (24.0)
	Female	120 (88.9)	15 (11.1)
	Total	139 (86.9)	21 (13.1)
Dementia status (n, %)	No dementia	48 (88.9)	6 (11.1)
	Dementia	91 (85.8)	15 (14.2)
Onset of dementia (age)	Mean	87.59	88.30
	SD	4.14	5.73
Duration of dementia	Mean	5.72	5.47
	SD	4.08	3.60
Age at death	Mean	93.42	93.70
	SD	3.56	3.68

Note: SD = standard deviation

There were no statistically significant differences between the basic characteristics of the positive and negative groups, although women had a considerably smaller proportion of adrenal gland LRP positive subjects compared to men (Fisher's exact test, OR 0.40; 95% CI 0.13-1.41; p-value = 0.1035).

The LRP according to the DLB consortium staging and the caudo-rostral or amygdala-based progression patterns of these subjects have been assessed in a previous study (4,24). The distribution of the subjects among these stages and progression patterns according to their adrenal gland pathology is shown in table 4.

Table 4. The distribution of positive subjects according to the DLB consortium staging for Lewy-related pathology and the progression patterns.

		Lewy-related pathology of adrenal gland	
		No LRP (n, %)	LRP (n, %)
DLB consortium staging	None	100 (100.0)	0 (0.0)
	Brainstem-predominant	10 (90.9)	1 (9.1)
	Limbic	9 (69.2)	4 (30.8)
	Diffuse neocortical	11 (40.7)	16 (59.3)
	Amygdala-predominant	3 (100.0)	0 (0.0)
	Non-classifiable	6 (100.0)	0 (0.0)
Progression pattern	None	100 (100.0)	0 (0.0)
	Caudo-rostral	25 (56.8)	19 (43.2)
	Amygdala-based	14 (93.3)	1 (6.7)
	Unclassifiable	0 (0.0)	1 (100.0)

LRP in the adrenal gland was dependent on the existence of LRP in the CNS according to the DLB Consortium staging (Pearson's Chi-squared test, p-value < 0.001). There were no positives detected in subjects without LRP in the CNS. Additionally, LRP in the adrenal gland was not uniformly distributed among the four DLB consortium stages, excluding none and non-classifiable (Fisher's exact test, p-value = 0.0034). LRP in the adrenal gland appeared most frequently in the diffuse neocortical (59.3%) and less frequently in the limbic (30.8%) and brainstem-predominant (9.1%) cases, and not at all in the amygdala-predominant or non-classifiable cases. Using brainstem-predominant as the baseline, the odds ratio for the probability of adrenal gland LRP was 4.19 (Fisher's exact test, 95% CI 0.33 – 239.96, p-value = 0.3271) for the limbic cases and 13.62 (Fisher's exact test, 95% CI 1.55 – 667.06, p-value = 0.0099) for the diffuse neocortical cases. Overall, adrenal gland LRP was seen in 21 of the 60 (35.0%) subjects with some

form of LRP in the CNS. Figure 4 shows a chart of the distribution of adrenal gland LRP across the DLB consortium stages.

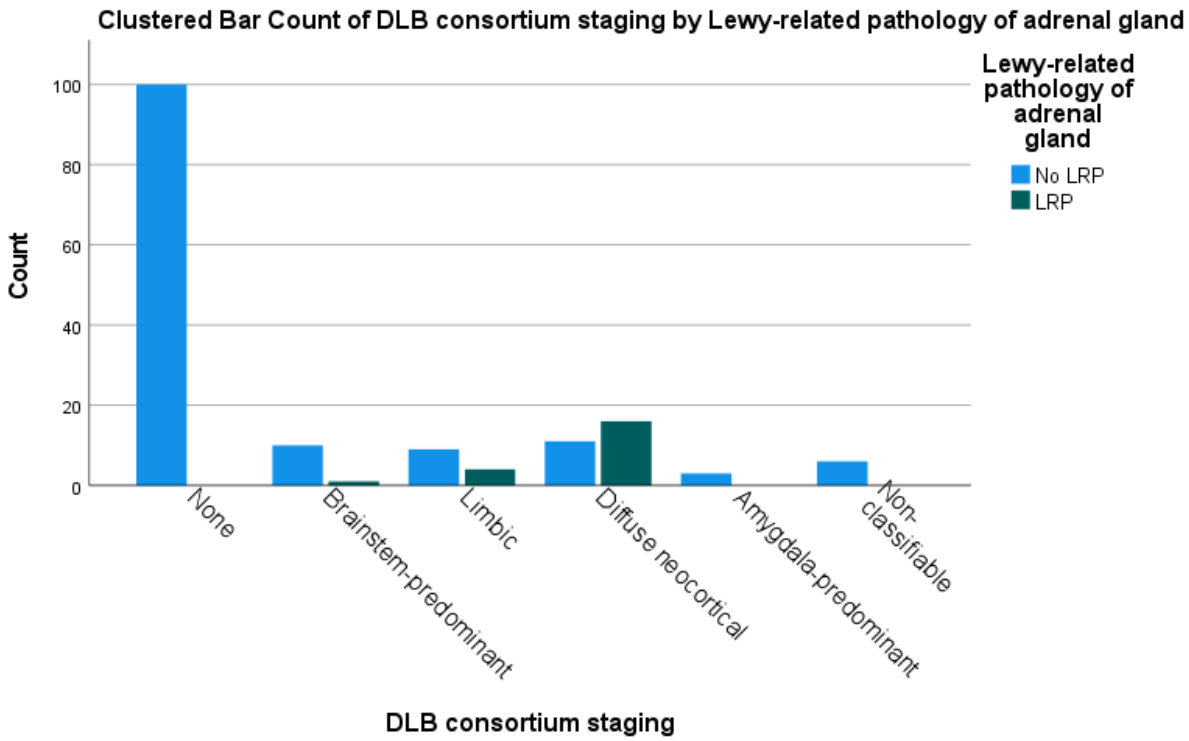

Figure 4. Clustered bar count showing the amount of adrenal gland positive and negative cases for each DLB consortium stage.

Regarding progression patterns, adrenal gland LRP was almost exclusively seen in the caudo-rostral form; 19 of the 21 subjects with adrenal gland LRP had caudo-rostral progression. Only one of the amygdala-based cases had adrenal gland LRP, and the unclassifiable case, due to high amount of pathology in all brain areas, was also positive. The unclassifiable case was excluded from analysis concerning the progression patterns. Figure 5 shows the distribution of adrenal gland LRP according to progression pattern.

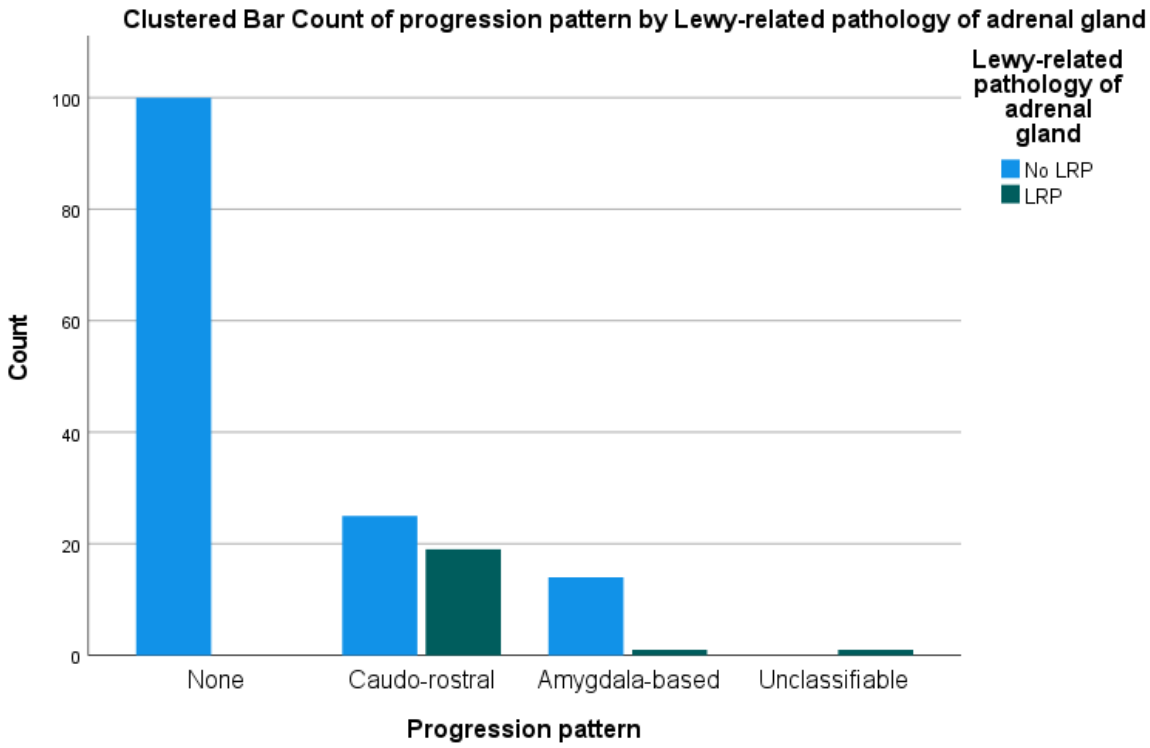

Figure 5. Number of adrenal gland positive and negative cases according to progression pattern

Table 5 shows the distribution of adrenal gland LRP according to the DLB staging and progression pattern. An unequal pattern of adrenal gland LRP similar to the one in table 4 and figure 4 is seen in the caudo-rostral cases excluding those with none and non-classifiable DLB consortium stage (Fisher's exact test, p-value < 0.001), with the limbic and diffuse neocortical forms now showing a stronger predominance, with proportions of 57.1% and 70.0% respectively. Using brainstem-predominant cases as the baseline, limbic cases had an odds ratio of 11.14 (Fisher's exact test, 95% CI 0.75 – 715.80, p-value = 0.0474) and the diffuse neocortical cases had an odds ratio of 20.77 (Fisher's exact test, 95% CI 2.16 – 1081.74, p-value = 0.0021) for adrenal gland LRP. There were no amygdala-predominant cases with caudo-rostral progression. Conversely, the DLB consortium stages in the amygdala-based cases did not show a relation to adrenal gland LRP (Fisher's exact test, p = 1.0).

Table 5. Adrenal gland pathology and DLB consortium staging according to progression pattern

		Progression pattern			
		Caudo-rostral		Amygdala-based	
		No LRP	LRP	No LRP	LRP
DLB consortium staging (n, %)	None	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Brainstem-predominant	10 (90.9)	1 (9.1)	0 (0.0)	0 (0.0)
	Limbic	3 (42.9)	4 (57.1)	6 (100.0)	0 (0.0)
	Diffuse neocortical	6 (30.0)	14 (70.0)	5 (83.3)	1 (16.7)
	Amygdala-predominant	0 (0.0)	0 (0.0)	3 (100.0)	0 (0.0)
	Non-classifiable	6 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)

Note: percentages add up to 100% on each row separately for both progression patterns.

Braak's staging for neurofibrillary tangles related to Alzheimer's disease was not significantly associated with LRP in the adrenal gland when including all subjects (Fisher's exact test, p-value = 0.9565) or when considering only those with LRP in the CNS (Fisher's exact test, p-value = 0.7147).

5 Discussion

5.1 Previous studies on adrenal gland LRP

In a study of 783 consecutive autopsied brains by Fumimura et al., Lewy-body related alpha-synucleinopathy (LBAS) was seen in 207 (26.4%) subjects. 87 out of the 207 (43.3%) with LBAS had adrenal gland LRP, making up 11.1% of all subjects. There was also adrenal gland LRP in 1 out of 577 (0.2%) subjects with no LBAS. The results are not fully comparable to this study, as its subjects' ages at death ranged from 48 to 104, with a mean of 80.68 (SD 8.8) years, as opposed to all subjects being at least 85 years old, and with a mean age of 93.46 (SD 3.56) years in this study. Furthermore, the sample set in Fumimura et al. is not a population-based one, unlike the sample set used in this study. These factors could explain some of the differences between the proportions of adrenal gland LRP in the full sample sets (26.4% in Fumimura et al. and 13.1% in this study). Still, the proportions of adrenal gland LRP in those with CNS pathology are relatively similar at 43.3% and 35.0%, respectively. Another common factor is the

lack of adrenal gland LRP in those with Lewy pathology focused on the amygdala. The number of amygdala-predominant cases was low in this study, consisting of only 3 subjects, but Fumimura et al. also found no adrenal gland LRP in 12 cases with Lewy pathology in the brain presenting preferentially in the amygdala. (44)

Another study by Gelpi et al. with 15 patients with PD or DLB found adrenal gland LRP in 53.3% of the cases. They also reported a craniocaudal gradient of α-syn aggregates along the sympathetic chain, with the highest density of aggregates in the stellate ganglion and the cervical ganglia, reducing towards the thoracic and lumbar ganglia. (45) The low number of patients predisposes the observed prevalence to considerable variance, and as Gelpi et al. looked specifically at patients with a diagnosis of PD or DLB, the prevalence of adrenal gland LRP is not directly comparable to the one found in this study among those with any CNS LRP. A clinical diagnosis of PD or DLB could be associated with more severe pathology, which in turn could affect the observed prevalence of adrenal gland LRP. For example, in the case of DLB the sensitivity of a clinical diagnosis, confirmed by a pathological examination, increases sharply with more severe pathology, rising from 19.4% for probable diagnosis in early stages to 72.3% for possible diagnosis in later stages (25).

5.2 Association of adrenal gland LRP with the DLB consortium staging and progression patterns in CNS

The adrenal gland positive subjects showed a pattern of increasing proportions through the brainstem-predominant, limbic, and diffuse neocortical cases. Additionally, there were no subjects with LRP only in the adrenal gland. As the DLB consortium stages form an ascending anatomical continuum and represent the progression of Lewy pathology, this suggests that adrenal gland LRP develops after the disease has already reached the CNS, possibly through anterograde spread along the sympathetic innervation of the adrenal gland. Many of the premotor neurons located in the brainstem, in areas such as the locus coeruleus and the medullary raphe nuclei, controlling the activity of both the sympathetic and parasympathetic preganglionic neurons, become affected in PD

(46). This would also fit the craniocaudal gradient of α-syn pathology along the sympathetic chain described by Gelpi et al.

The pattern of increasing proportions becomes even more prominent when considering only the subjects with caudo-rostral progression pattern, and notably, 19 of the 21 cases with adrenal gland LRP were classified as caudo-rostral. On the other hand, adrenal gland LRP was only seen in 1 of 15 subjects with amygdala-based progression. Such a strong contrast between the groups supports the existence of these progression patterns. Conversely, the fact that adrenal gland LRP is nearly non-existent in the amygdala-based cases while showing a marked increase in prevalence as the pathology in the brain progresses past the brainstem-predominant stage in the caudo-rostral cases is consistent with the idea that adrenal gland LRP develops secondarily through anterograde propagation of pathological α-syn from the CNS.

5.3 Strengths and limitations of the study

The study has certain limitations. First, the sample contained a relatively small number of cases according to the different DLB consortium stages, especially the amygdala-predominant consisting of only 3 cases. Thus, rigorous statistical analysis could not be applied. Second, it is possible that the immunohistochemical methods and visual analysis applied are not sufficient to find cases with very early α-syn pathology, which could present with different findings than classical Lewy bodies and neurites. Due to this, the possibility that LRP develops independently in the adrenal gland cannot be disregarded. Third, there was notable heterogeneity within the adrenal gland samples in terms of how much surrounding fat tissue was included, and how many nervous structures were present overall. As noted by Fumimura et al., the nerve fascicles in the periadrenal fat tissue had α-syn pathology in 93.3% of the cases with any α-syn pathology in the adrenal gland, compared to only 26.4% in the cortical nerve fascicles. Ganglia in the adrenal medulla were involved in 66.7% of the cases. Therefore, the number of positive cases in this study could be an undercount.

This study was conducted on a population-based sample set, which provides a low-bias basis for assessing the proportions of different characteristics within a

specific population. The Vantaa 85+ study is unique even in an international context, being one of the few true population-based neuropathological cohorts for dementia and neuropathology around the world (47).

6 Conclusion

This study describes the prevalence of adrenal gland LRP in the population-based Vantaa 85+ sample set and shows that it is dependent on the progression pattern and stage of LRP in the brain. These results provide further support for the two progression patterns and suggest that α-syn pathology spreads to the adrenal gland from the CNS rather than developing independently.

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