





Accumulation of Lewy-Related Pathology Starts in Middle Age: The Tampere Sudden Death Study

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When effective treatments against neurodegenerative diseases become a reality, it will be important to know the age these pathologies begin to develop. We investigated alpha-synuclein pathology in brain tissue of the Tampere Sudden Death Study—unselected forensic autopsies on individuals living outside hospital institutions in Finland. Of 562 (16–95 years) participants, 42 were positive for Lewy-related pathology (LRP). The youngest LRP case was aged 54 years, and the frequency of LRP in individuals aged ≥ 50 years was 9%. This forensic autopsy study indicates LRP starts already in middle age and is more common than expected in the ≥ 50 years-of-age non-hospitalized population.

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Introduction

Neurodegenerative diseases are one of the most common causes of illness and death in older adults, continuing to burden social and health systems with the increase in the aging population. Knowledge of the prevalence of prodromal phases of these diseases will increase understanding of the pathobiology and assist in unravelling the etiology of disease mechanisms. Alpha-synuclein pathology accumulates in the nervous system of patients with synucleinopathies, such as Lewy body diseases including Parkinson's disease and dementia with Lewy bodies, pure autonomic failure, and multiple system atrophy (MSA).¹ Lewy body diseases are characterized by alpha-synuclein accumulation in the form of Lewy-related pathology (LRP) consisting of Lewy bodies (LBs) and/or Lewy neurites in the nervous system of individuals.²

Studies investigating the prevalence of LRP involving non-hospitalized populations are rare and usually limited by

size or selection criteria.^{3–14} When effective treatments against these brain diseases become a reality, it will be especially important to know the age at which pathology in the brain begins to develop, as this, or even before, will be the most successful time point to administer therapies. Thus, the main aim of the present study was to investigate the presence of alpha-synuclein pathology in the brains of the Tampere Sudden Death Study—an unselected forensic autopsy series.

Methods

The Tampere Sudden Death Study consists of 700 forensic autopsy cases collected from 2010 to 2015, encompassing individuals aged 16 to 97 years of that lived and died outside hospital institutions in a region of southwest Finland, with brain tissue samples collected from 586 of these (postmortem interval average 1.5 days, range 1–15 days). This covered approximately 20% of deaths in the region during the timeframe of the study collection, with institutional approval for the study from the ethical committees of Tampere University Hospital and Pirkanmaa hospital district, and research permission from Tampere University. Limited medical histories for cause of death determination were available. Individual informed consent was not required from autopsy cases or their next of kin in a forensic study setting in Finland. Brain tissue samples were collected from cerebellar cortex, frontal cortex, hippocampus, insula-putamen, pons, and substantia nigra during autopsy, fixed in formalin for up to a week, and processed into paraffin embedded tissue blocks.

Immunohistochemistry on 5- μ m thick tissue sections with 5G4 antibody against alpha-synuclein used the DAKO Envision FLEX staining kit (Glostrup, Denmark)

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according to manufacturer's instructions. Briefly, samples underwent deparaffinization, 20 minutes of 95°C in low-pH buffer pretreatment, followed by 5 minutes of formic acid pretreatment, before LabVision autostainer (60 minutes) with 5G4 antibody concentration at 1:5,000. Cases were included if representative brainstem (substantia nigra/pons) regions were available. Microscopic assessment was undertaken by one individual (E.K.), confirmed separately by two neuropathologists (L.M. and A.P., with consensus agreed on cases with different grading), and considered positive if at least 1 alpha-synuclein positive LB or threadlike Lewy neurite¹⁵ was observed in brainstem tissue sections alongside the presence of neuromelanin-positive stained cells. Positivity in the hippocampal region was defined by the presence of LRP in the hippocampus (CA2/3) and/or entorhinal cortex. Scoring used the McKeith system at $\times 10$ magnification with mild (1 LB or Lewy neurites), moderate (2 or 3 LBs), severe (≥ 4 LBs), very severe (extensive LB pathology).¹⁶ Positivity in the brainstem and hippocampal sections was confirmed with a phosphorylated alpha-synuclein antibody (Fujifilm Wako; pSyn#64).

Genotype data for *APOE* were extracted from genome-wide assessments, and underwent quality control and imputation according to Hernesniemi et al.¹⁷

Results

Of the Tampere Sudden Death Study brains, 562 individuals (95.9%) aged 16 to 95 years (mean age 63.7 years) had representative tissue sections for alpha-synuclein pathology assessment (substantia nigra/pons, collectively: brainstem). Additionally, hippocampal regions were available in 476 cases of those with brainstem tissue (84.7% of brainstem available cases). Men (74%) are overrepresented in the population—417 versus 145 women. Of these 562, there were 46 (8.2%) cases found to be positive for alpha-synuclein pathology (Table, Fig 1, Supplementary data). The youngest, a 42-year-old woman, had clinical records of parkinsonism and neuropathologically determined as MSA, and was excluded from further analyses. An additional 3 cases were excluded from LRP analyses due to neuropathological assessment showing MSA-type glial cytoplasmic inclusions, although no reports of clinical history were apparent (Supplementary Fig S1). Of those without known neurological symptoms, or MSA-type pathology, the youngest case with LRP was a man aged 54 years with no reported neurodegenerative issues. LRP cases amounted to 9% of those aged >50 years, and had an average age of 75 years (range 54–90 years). The highest percentage of affected individuals occurred in

the 80 to 89 years age group (19.3%, Fig 2), and the lowest in the 50 to 59 year old age group (3.4%).

No cases were positive in the hippocampal region without also being positive in brainstem regions in our cohort. One positive brainstem case was missing a representative hippocampal region sample, but of the remaining 41 LRP cases, 25 (61%) were also positive in the hippocampal region. One-third of LRP cases showed at least one positive structure in the frontal cortex (13/39), and 76% in insula putamen regions (32/42).

Cases with LRP showed varying levels of amyloid beta and tau copathologies (see Table and Supplementary data), and approximately 12% had alcohol abuse issues or depression. Weakly trending associations were seen between Thal phase ($p = 0.035$) and Braak stage ($p = 0.037$), with alpha-synuclein pathology in hippocampus as assessed in χ^2 linear-by-linear tests. Consortium to Establish a Registry for Alzheimer's Disease score was not associated with hippocampal alpha-synuclein pathology. Substantia nigra alpha-synuclein pathology was not significantly associated with any of the Alzheimer's disease (AD) pathologies.

Of 501 cases with valid *APOE* genotyping results, 1 had genotype $\epsilon 2/\epsilon 2$ (0.2%), 35 $\epsilon 2/\epsilon 3$ (7.0%), 306 $\epsilon 3/\epsilon 3$ (61.1%), 12 $\epsilon 2/\epsilon 4$ (2.4%), 135 $\epsilon 3/\epsilon 4$ (26.9%), and 12 $\epsilon 4/\epsilon 4$ (2.4%). No associations were found between the presence of LRP and *APOE* $\epsilon 4$ allele carriership. When comparing LRP cases with varying levels of AD pathology, a trend was seen for those with moderate or high AD pathology having a higher frequency of *APOE* $\epsilon 4$ than low AD pathology cases, but did not reach significance ($p = 0.064$), possibly due to low numbers. Additionally, LRP cases with moderate or high AD pathology were statistically significantly more likely to be shorter ($p = 0.018$) and weigh less ($p = 0.011$) than those with low AD pathology.

Discussion

This is the largest autopsy-based study of out of hospital deaths as far as the authors are aware. Of 562 individuals aged from 16 to 95 years, 46 (8.2%) were found to have the presence of alpha-synuclein pathology in brainstem tissue sections. LRP was detected in 9% of individuals aged >50 years ($n = 462$), which is higher than expected. Previous reports focused on LRP in asymptomatic individuals have only included older cases (aged >62 years) with ranges of 8 to 17%,^{11,13} whereas to our knowledge, other studies have not investigated a non-hospitalized population including individuals aged <60 years. The youngest case was aged 54 years, suggesting LRP occurs already in people aged in their 50s.

TABLE. Characteristics of the Tampere Sudden Death Study Cohort (Population Aged ≥50 years)^{a,b}

	All ≥50 years (n = 465)	Asyn- negative (n = 420)	Asyn-positive LRP (n = 42)	Asyn LRP low AD pathology (n = 23)	Asyn LRP mod- high AD pathology (n = 19)	Asyn positive MSA ^b (n = 4)
Age	69.5 yr (50–95)	68.9 yr (50–95)*	75.1 yr (54–90)*	72.8 yr (54–87) ^d	77.9 yr (58–90) ^d	64.8 yr (42–83)
Sex	342 F, 123 M	308 M, 112 F	31 M, 11 F	19 M, 4 F	12 M, 7 F	3 M, 1 F
Height	170.43 cm (77–199)	170.52 cm (77–199)	169.69 cm (155–186)	172.48 cm (155–186)**	166.32 cm (157–181)**	165.0 cm (158–175)
Weight	83.8 kg (32–169)	83.9 kg (32–169)	82.38 kg (42–143)	89.87 kg (56–143)**	73.32 kg (42–130)**	82.5 kg (61–98)
Brain weight	1,415 g (1053–2,240)	1,418 g (1053–2,240)	1,386 g (1124–1,645)	1,413 g (1124–1,645)	1,352 g (1151–1,565)	1,433 g (1301–1,554)
COD						
Disease	388 (83.4%)	351 (83.6%)	34 (81.0%)	19 (82.6%)	15 (78.9%)	4 (100%)
Accident	52 (11.2%)	45 (10.7%)	7 (16.7%)	4 (17.4%)	3 (15.8%)	
Suicide	18 (3.9%)	17 (4.0%)	1 (2.4%)		1 (5.3%)	
Undetermined	7 (1.5%)	7 (1.7%)				
Apoe4 +ve ^c	132/419 (31.5%)	117/378 (30.9%)	14/38 (36.8%)	5/21 (23.8%) ^e	9/17 (52.9%) ^e	2/4 (50%)

Note: The bolding values are done to help differentiate the different columns and what ones are compared against each other.

AD = Alzheimer's disease; Apoe4 +ve = apolipoprotein E ε4 carriers; Asyn = alpha-synuclein; COD = cause of death, LRP = Lewy-related pathology; MSA = multiple system atrophy; TSDDS = Tampere Sudden Death Study.

*Refers to significantly different values between LRP-positive and Asyn-negative cases in those aged >50 years ($p \geq 0.001$).

**Refers to significantly different values between low and moderate/high AD pathology cases with LRP and aged >50 years ($p = 0.018$ for height and $p = 0.011$ for weight).

^aSupplementary Table S2 shows characteristics for the full cohort that was neuropathologically examined.

^bMSA cases included the 42-year-old diagnosed while alive.

^cGenotyping data were missing for 61 cases of those with brain tissue samples. The genotyping was carried out according to Hernesniemi et al.¹⁷

^dA trend was seen, but significance was not reached ($p = 0.090$) between low and moderate/high AD pathology cases with LRP and aged >50 years.

^eA trend was seen, but significance was not reached ($p = 0.064$) between low and moderate/high AD pathology cases with LRP and aged >50 years.

The presence of mild pathology in >1 brain region was interesting, showing that very modest alpha-synuclein pathology can be seen simultaneously throughout the brain also at the very early stages of accumulation. Early mild pathology was found to occur in sparse locations across a region and not uniformly, highlighting the need for comprehensive sampling to fully capture all pathology, and favoring the need for complete tissue blocks for assessment.

The presence of four MSA cases (0.71%) in such a cohort suggests this disease may be under- or misdiagnosed, and highlighting that forensic cohorts can include undiagnosed MSA cases, who may be prone to sudden death, as seen previously.¹⁸

An increase in LRP-affected individuals was seen with increasing age—in concordance with others'

findings^{5,13} (Fig 2), with those aged 80 to 89 years the most highly affected age group, after which numbers dropped off considerably. The drop and small number of cases in the oldest age group is likely because individuals more often die of natural causes or suffer from some kind of disease and are institutionalized; therefore, were more likely to be excluded from the cohort.

AD pathology was weakly associated with alpha-synuclein pathology in the hippocampus, but not substantia nigra, corroborating previous reports of a relationship between LRP and AD pathology.¹⁹ Due to our small sample size, the implications of this are not possible to elucidate, but suggest a connection between these neuropathological markers in the hippocampal

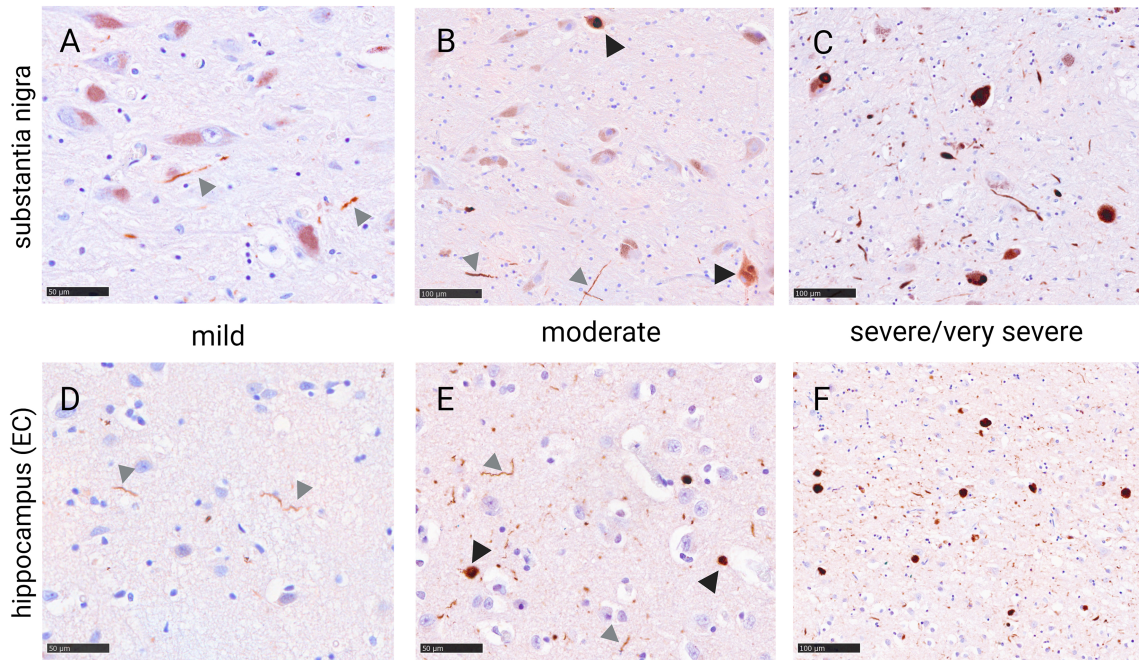


FIGURE 1: Immunohistochemical staining with 5G4 antibody against alpha-synuclein in the Tampere Sudden Death Study cohort. (A) Mild Lewy-related pathology (LRP; 1 Lewy body [LB] or Lewy neurites [LNs]) in substantia nigra at $\times 400$ magnification, (B) moderate LRP (2 or 3 LBs) in substantia nigra at $\times 200$ magnification, (C) severe LRP (≥ 4 LBs) in substantia nigra at $\times 200$ magnification, (D) mild LRP (1 LB or LNs) in the hippocampal region (entorhinal cortex [EC]) at $\times 400$ magnification, (E) moderate LRP (2 or 3 LBs) in the hippocampal region (entorhinal cortex) at $\times 400$ magnification, and (F) very severe LRP (extensive LB pathology) in the hippocampal region (entorhinal cortex) at $\times 200$ magnification. Black arrowheads highlight LBs and gray arrowheads point to LNs.

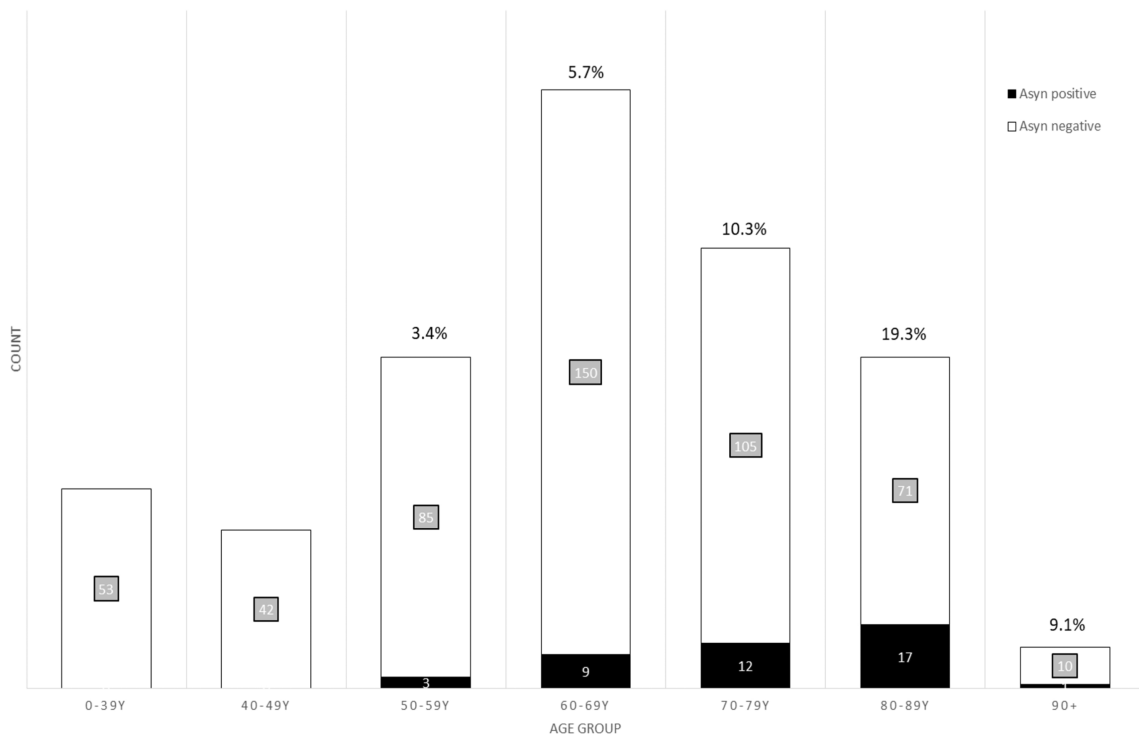


FIGURE 2: Lewy-related pathology prevalence according to age in the Tampere Sudden Death Study cohort. Asyn = alpha-synuclein.

region. Moderate and high AD pathology of LRP cases was associated with smaller height and weight, as is often seen in AD patients, with a trend for a higher

incidence of *APOE* $\epsilon 4$ carriership, compared with those with low AD pathology, although our small case numbers necessitate further study.

Of positive LRP cases, approximately 12% had alcohol abuse issues or depression. Although it is not possible to draw conclusions in the current cohort due to incomplete data, further studies should be undertaken to decipher whether psychiatric disorders associate with a higher incidence of alpha-synuclein pathology and what the underlying connection is.²⁰

The strength of the present study is the size and unselected forensic nature, including a large number of younger cases. Limitations due to the nature of the forensic autopsy series include limited medical history data and sampling differences compared with clinical neuropathological sampling, resulting in less comprehensive brain analyses. It is possible that sampling may have missed positive regions and may be underestimating the prevalence of alpha-synuclein pathology, and that variation in postmortem interval and fixation periods may influence immunohistochemistry results. There may be an overrepresentation of cases with drug abuse problems or psychiatric issues, leading to their inclusion. The high proportion of men is probably due to them being less likely to visit a doctor²¹ and also having a higher likelihood of dying due to risk-taking behavior.²²

The present study is the first investigation of a non-hospitalized population across the span of adult life showing prevalence of LRP in individuals aged as young as their 50s, with a frequency of 9% in those aged ≥ 50 years. Further studies and verification in other similar cohorts are warranted to corroborate these findings and substantiate the age at which treatments against these brain diseases would be most effectively administered.

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Author Contributions

E.K., L.M., P.K., L.P.L., and T.L. contributed to the conception and design of the study; E.K., L.M., A.P., P.K., L.P.L., and M.M. contributed to the acquisition and analysis of data; E.K. and L.M. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

Nothing to report.

Data Availability

Due to the nature of national laws and sensitive information, these data are not freely available. With sufficient ethical and data transfer permissions, some data may be provided on request.

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