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RESEARCH ARTICLE

Population-based analysis of pathological correlates of dementia in the oldest old

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Abstract

Objective: The aim of this study was to analyze brain pathologies which cause dementia in the oldest old population. **Methods**: All 601 persons aged ≥85 years living in the city of Vantaa (Finland), on April 1st, 1991 formed the study population of the Vantaa85 + study, 300 of whom were autopsied during follow-up (79.5% females, mean age-at-death 92 ± 3.7 years). Alzheimer's disease (AD) pathology (tau and beta-amyloid [Aβ]), cerebral amyloid angiopathy (CAA) and Lewy-related pathologies were analyzed. Brain infarcts were categorized by size (<2 mm, 2–15 mm, >15 mm) and by location. Brain hemorrhages were classified as microscopic (<2 mm) and macroscopic. **Results**: 195/300 (65%) were demented. 194/195 (99%) of the demented had at least one neuropathology. Three independent contributors to dementia were identified: AD-type tau-pathology (Braak stage V-VI), neocortical Lewy-related pathology, and cortical anterior 2–15 mm infarcts. These were found in 34%, 21%, and 21% of the demented, respectively, with the multivariate odds ratios (OR) for dementia 5.5, 4.5, and 3.4. Factor analysis investigating the relationships between different pathologies identified three separate factors: (1) AD-spectrum, which included neurofibrillary tau, Aβ plaque, and neocortical Lewy-related pathologies and CAA (2) >2 mm cortical and subcortical infarcts, and (3) <2 mm cortical microinfarcts and microhemorrhages. Multipathology was common and increased the risk of dementia significantly. **Interpretation**: These results indicate that AD-type neurodegenerative processes play the most prominent role in twilight cognitive decline. The high prevalence of both neurodegenerative and vascular pathologies indicates that multiple preventive and therapeutic approaches are needed to protect the brains of the oldest old.

Introduction

People aged ≥85 years old represent the fastest growing age group in Western countries, projected to increase 3.45-fold in the US by 2050 (The U.S. Census Bureau 2012, http://www.agingstats.gov/Main_Site/Data/2012_Documents/Population.aspx). Because of the consequences of aging – such as dementia – these people are likely to need help of the health and social care services. Care of people with dementia is a major economic burden in Western societies. Its total cost in the US was 604 billion US$ in 2010.[1] Therefore, prevention of dementia should be one of the main aims of the health care service.

The most common dementing disorder is Alzheimer's disease (AD), with estimated prevalence of 45% among the US citizens aged ≥85 in 2012.[2] Vascular dementia (VaD) and dementia with Lewy bodies (DLB) were estimated to comprise about one-third of all new dementia

cases in the US.[3] However, various pathologies may coexist in the brains of elderly individuals. For example, two-thirds of elderly AD patients have concomitant other brain pathologies such as cerebrovascular disease or Lewy-body-related[4,5,6] potentially resulting in "mixed" dementia.[7]

Previous neuropathological studies of mixed dementia have mostly focused on hospital-based series on ≤85 years old. To develop preventive measures against major components of dementia one must determine in which relation they prevail and coexist in the general old population, when clinically diagnosed dementia is present or absent. Recently, community-based studies[8,9,10] have added knowledge on the effect of multiple pathologies on dementia in the very old. We have addressed this question by performing a population-based and prospective neuropathological study on very elderly subjects (Vantaa 85+ study), to analyze the frequency and co-occurrence of common old-age-associated neuropathologies and their impact on the development of clinical dementia.

Materials and Methods

Vantaa 85+ study protocol and participants

Vantaa 85+ Study included all individuals aged ≥85 years living in the city of Vantaa in Southern Finland on April 1, 1991. The clinical follow-up studies were carried out in 1994, 1996 and 1999 for the survivors. Of the 601 eligible subjects, 552 participated at least in the first clinical follow-up study. Postmortem neuropathological examinations were performed in 304/601 (50.9%). Four were excluded due to the insufficient clinical data, resulting in 300 subjects comprising the autopsied subpopulation of this study (Table S1).

Clinical examination

The diagnosis of dementia followed the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 3th edition, revised (DSM III-R; American Psychiatric Association, 1987). The diagnosis was based on the Mini-Mental State Examination (MMSE), required the consensus opinion by two neurologists[11] and had to be set earlier than 3 months before death. Median time between the diagnosis of dementia and death was 16 months, SD 13 months. There were 313 demented subjects, of whom 195 were autopsied. *Apolipoprotein E* (*APOE)* genotyping was carried out as previously described.[12]

Macroscopic examination of the brain

The brains were fixed for at least 2 weeks in 4% phosphate-buffered formaldehyde. After external examination, the cerebrum, brain stem, and cerebellum were separated from each other. The cerebrum was cut in 1-cm-thick coronal and the brain stem and cerebellum in 5-mm-thick sagittal slices. The presence, location, and number of macroscopic small (2–15 mm) or large (>15 mm) infarcts in the cerebral cortex, white matter, basal ganglia region, cerebellum and brainstem were categorized as hemispheric and deep subcortical.[13]

Microscopic analyses

Neurodegenerative lesions: Alzheimer's disease and Lewy-related pathologies

The analysis of AD-related pathologies was performed following the original criteria.[14,15] Density of the neuritic senile plaques (CERAD score) in sections stained with modifed Bielschowsky silver method was scored according to the Consortium to Establish Registry for Alzheimer's Disease (CERAD) criteria.[14] Neuritic senile plaques were detected as silver-positive neocortical aggregations which may or may not have surrounded an intensively staining central core, and also included at least one intensively staining elongated structure orientating from the periphery toward the centre of the aggregate. Braak stages for the AD-related neurofibrillary tangles were defined using Gallyas silver-stained 80-μm-thick sections cut from polyethylene glycol-embedded samples from the entorhinal cortex at the level of the mammillary bodies, from the hippocampus at the level of the lateral geniculate body, and from the occipital lobe, so that the striate area, parastriatale field, and peristriate region were all represented in the sample.[15] The quantities of the plaques and tangles were counted in four specimens (middle frontal, superior temporal, and middle temporal gyri and inferior parietal lobule). The amount of β-amyloid protein was analyzed by quantifying the fraction of the cortical specimens covered by methenamine silver-stained plaques. Contiguous cortical microscopical fields were examined, extending from the pial surface to the white matter, at a magnification of 100 with a square microscopical graticule.[17] The tangles were counted in the modifed Bielschowsky silver-stained sections in contiguous cortical microscopical fields, extending from the pial surface to the white matter, at a magnification of 200 with a square microscopical counting frame, 0.55 mm in width, along a line perpendicular to the pial surface.[17] The Lewy-related pathology was assessed according to the consensus criteria[18] using histological staining methods and IHC against α-synuclein clone 42.[19]

Vascular lesions: cerebral amyloid angiopathy, microscopic ischemic lesions, and hemorrhages

The diagnosis of cerebral amyloid angiopathy (CAA, the deposition of amyloid β (Aβ) -derived amyloid in the

subarachnoid and intracortical small and middle-sized blood vessels) in six brain regions (frontal, parietal, temporal and occipital lobes, hippocampus, and cerebellum) was based on Congo red, confirmed using IHC against Aβ peptide clone 4G8.[20] The severity of CAA was assessed by counting the percentage of the Congo Red-positive and Aβ immune reactive small and middle-sized vessel profiles of all vessel profiles seen in samples obtained from each region. The counts were combined to create the median value for all six regions. The values higher than the upper quartile (Q3) value (5.3% for all six brain regions and 6% for the frontal lobe) were defined as severe forms of CAA.

The presence and count of old cortical microinfarcts and microhemorrhages were investigated in six brain regions (above). The microinfarcts, defined as focal lesions $\leq$2 mm and invisible to the naked eye showing neuronal loss, glia cell, and macrophage reaction and/or cystic tissue necrosis, were evaluated in the Hematoxylin–Eosin stained 6-μm-thick tissue sections. The presence of iron containing macrophages, reflecting the microhemorrhage, was verified using the Prussian blue stain. These microscopic diagnoses were set by at least two pathologists (AP, MT, or MM).

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics version 20 (IBM, New York, USA) software. Logistic regression was used to study the associations between different neuropathologies and dementia (Tables 1,2). Reciprocal correlations between the neuropathological and genetic variables were analyzed using Spearman bivariate correlation analysis (Table 3). The variance in eight quantitative variables was analyzed using factor analysis with the principal component analysis and rotation method (Fig. 1, Table S2). Statistical analyses were performed by two investigators (MT, I-LN).

Approval for the study

The Vantaa 85+ study was approved by the Ethics Committee of the Health Centre of the City of Vantaa. The Finnish Health and Social Ministry approved the use of the health and social work records and death certificates. The National Authority for Medicolegal Affairs (VALVIRA) has approved the collection of the tissue samples at autopsy and their use for research. Blood samples were collected after having obtained an informed consent from the subjects or their relatives. The consent for autopsy was obtained from the nearest relative.

Results

Prevalence of different types of neuropathologies in the population

194/195 of the demented and 105/105 of the nondemented subjects had at least one type of neuropathology (any severity, any location) of the five categories shown in Table 1: (1) AD-type (2) Lewy-related, (3) brain infarct, (4) CAA, and (5) brain hemorrhage. Thus, with the exception for one demented subject, all 300 subjects had at least one type of neuropathology. The prevalence of each neuropathology type/subtype in the demented and nondemented subjects is presented in Table 1.

Neuropathological variables and dementia

Dementia was diagnosed in 195/300 (65%). The associations between individual neuropathological categories and dementia were first tested with univariate analysis (Table 1). Among the evaluated parenchymal AD-type pathologies, Braak stage V–VI had the strongest association with dementia (OR: 10.76, 95% CI: 4.23–27.35). Lewy-related pathology, when spread to the neocortex had also a strong and significant association with dementia (OR: 4.78; 95% CI: 1.94–11.78), whereas Lewy-related pathology in the brainstem and limbic regions was not associated with dementia. Of the brain infarcts, 2–15 mm cortical infarcts (highest OR: 2.84, 95%, CI: 1.32–6.10 for cortical infarcts in the estimated territory of the anterior circulation) or thalamic infarcts (OR: 2.45, 95% CI: 1.03–5.79) associated with dementia. CAA in any brain region was associated with dementia (OR: 2.27, 95% CI: 1.37–3.77) but the association was strongest for severe CAA in the frontal lobe (OR: 3.67, 95% CI: 1.84–7.08). CERAD scores C and B were also associated with dementia in the univariate analysis (OR: 7.83 (2-75-22.34) and 2.78 (1.56–4.96).

In order to identify the independent contributions of each pathology to the prediction of dementia, we applied a multivariate analysis (Table 1). In the multivariate model the variable with the strongest association with dementia from each pathology category was selected as a covariate and these were tested together in the same analysis. From the AD-type pathology group, however, we included both Braak stage and CERAD score, both as categorical variables (Table 1), and in total there were five strong pathologies that were analyzed in multivariate analysis, in addition to gender, age at death, and the carriership of the *APOE* ε4. Three of these five pathological variables associated independently with dementia in the multivariate analysis (Table 1): (1) Braak stage V–VI, (2) diffuse neocortical type of Lewy-related pathology, and

Table 1. Frequency of various neuropathologies and association of such pathologies with dementia in the autopsied subpopulation in the Vantaa 85 + study.

	Autopsied population *N* = 300		Dementia	
Neuropathology present	Demented *N* = 195	Nondemented *N* = 105	OR (95% CI) *P* -value[1] Univariate	OR (95% CI) *P* -value[1] Multivariate[2]
Alzheimer type				
Braak stage				
Braak stage[3] 0–II	45 (23.1)	44 (41.9)	Reference	
Braak stage III–IV	84 (43.1)	55 (52.4)	1.493 (0.873–2.555) 0.143	1.161 (0.590–2.284) 0.666
Braak stage V–VI	66 (33.8)	6 (5.7)	10.756 (4.230–27.351) <0.001	5.554 (1.546–19.958) 0.009
CERAD score				
CERAD score[4] 0	33 (17.0)	38 (36.2)	Reference	
CERAD score A	17 (8.7)	16 (15.2)	1.223 (0.535–2.797) 0.633	1.545 (0.570–4.189) 0.393
CERAD score B	111 (56.9)	46 (43.8)	2.779 (1.557–4.959) 0.001	1.744 (0.821–3.705) 0.148
CERAD score C	34 (17.4)	5 (4.8)	7.830 (2.745–22.337) <0.001	2.366 (0.512–10.928) 0.270
Lewy-body-related: DLB category[5]				
Lewy bodies absent (brainstem)	120 (61.6)	84 (80.0)	Reference	NI
Lewy bodies present				
Brainstem predominant/limbic type	34 (17.4)	15 (14.3)	NS	NI
Diffuse neocortical type	41 (21.0)	6 (5.7)	4.78 (1.94–11.78) 0.001	4.486 (1.578–12.752) 0.005
Infarct[6]				
Absent	71 (36.4)	49 (46.7)	Reference	NI
Present	124 (63.6)	56 (53.3)	NS	NI
Superficial total	85 (43.6)	32 (30.5)	1.76 (1.07–2.91) 0.027	NI
Superficial > 15 mm	40 (20.5)	15 (14.3)	NS	NI
Superficial 2–15 mm	65 (33.3)	22 (21.0)	1.87 (1.08–3.29) 0.025	NI
Superficial cortical 2–15 mm	45 (23.1)	11 (10.5)	2.56 (1.26–5.2) 0.009	NI
Superficial cortical anterior 2–15 mm	41 (21.0)	9 (8.6)	2.84 (1.32–6.10) 0.008	3.400 (1.268–9.12) 0.015
Superficial cortical posterior 2–15 mm	31 (15.9)	8 (7.6)	2.29 (1.01–5.19) 0.047	NI
Deep subcortical total	76 (39.0)	32 (30.5)	NS	NI
Deep subcortical >15 mm	10 (5.1)	6 (5.7)	NS	
Deep subcortical 2–15 mm	72 (36.9)	29 (27.6)	NS	NI
Thalamic 2–15 mm	29 (14.9)	7 (6.7)	2.45 (1.03–5.79) 0.042	NI
Microscopic cortical <2 mm	34 (17.4)	16 (15.2)	NS	NI
Cerebral amyloid angiopathy (CAA)				
Absent in all 6 brain regions[6]	47 (24.1)	44 (41.9)	Reference	NI
Present				
In any 6 [7] brain regions	148 (75.9)	61 (58.1)	2.27 (1.37–3.77) 0.002	NI
In the frontal lobe	124 (63.6)	45 (42.9)	2.33 (1.43–3.78) 0.001	NI
Severe CAA				
Severe CAA in 6 brain regions[8]	60 (30.8)	15 (14.3)	2.67 (1.43–4.98) 0.002	NI
Severe CAA in the frontal lobe[9]	62 (31.8)	12 (11.4)	3.61 (1.84–7.08) <0.001	2.614 (0.973–7.024) 0.057
Cerebral hemorrhage				
Absent	78 (40.0)	34 (32.4)	Reference	
Present	117 (60.0)	71 (67.7)	NS	NI
Macroscopic	4 (2.1)	3 (2.9)	NS	NI
Cortical microscopic	115 (59)	70 (66.7)	NS	NI
Cortical microscopic with >5 siderophages	10 (5.1)	6 (5.7)	NS	NI

(*Continued*)

(3) cortical 2–15 mm infarcts in the anterior parts of the brains. Braak stage V–VI was the most significant risk factor for dementia both in the univariate and multivariate analyses (Table 1). Severe frontal CAA showed a borderline association with dementia in the multivariate analysis ($P = 0.057$), whereas CERAD score and *APOE* ε4-carrier status were not independently associated with dementia in the multivariate model ($P < 0.1$).

Table 1. Continued.

Neuropathology present	Autopsied population *N* = 300 Demented *N* = 195	Nondemented *N* = 105	Dementia OR (95% CI) *P* -value[1] Univariate	OR (95% CI) *P* -value[1] Multivariate[2]
Covariates				
Age at death, mean (SD)	92.5 (3.7)	92.2 (3.8)	NS	NS
Male gender	30 (15.4)	22 (21.0)	NS	NS
Apolipoprotein E ε4 –carrier	72 (39.6)	16 (16.7)	3.27, 1.77–6.04 (<0.001)	1.096 (0.462–2.597) 0.836

OR, Odd's ratio; CI, confidence interval; NI, not included; NS, nonsignificant; DLB, dementia with Lewy bodies.
[1]Bivariate logistic regression analysis.
[2]Within each neuropathology category (Alzheimer, Lewy body, etc.) only the variable with strongest association with dementia was selected in the multivariate model. However, for AD-type pathology, both Braak stage and CERAD score were included and analyzed as categorized variables. Other included variables were diffuse neocortical type of the Lewy body -related pathology, cortical anterior infarcts 2–15 mm, severe CAA in the frontal lobe[10]. These selected variables were analyzed together with other covariates (age at death, gender, and the possession of *Apolipoprotein E ε4* allele).
[3]Subjects having neurofibrillary pathology in the neocortex versus subjects with such pathology in hippocampus, entorrhinal cortex, or limbic area or without such pathology (Braak[15]).
[4]Subjects with frequent plaques in the neocortex versus subjects with no to moderate plaques (Mirra et al.,[14]).
[5]McKeith et al.,[18]
[6]After: Troncoso et al.[13] We used the designation "superficial" for cortical and superficial subcortical lesions and the designation "deep subcortical" for lesions situated in the basal ganglia, brainstem, cerebellum, and capsula interna.
[7]Frontal, temporal, parietal and occipital lobe, hippocampus, cerebellum.
[8]Congo red positivity and Aβ immunoreactivity detected in 5.3% (Q3 value) of all vessel profiles in the 6six brain regions (Tanskanen et al.,[20]).
[9]Congo red positivity and Aβ immunoreactivity detected in >6% (Q3 value) of the vessel profiles in the frontal lobe (Tanskanen et al.,[20]).

Table 2. The effect of combined counts of four most significant neuropathologies on dementia.

Autopsied population *N* = 300			
Number of pathologies[2]	Demented *N* = 195	Nondemented *N* = 105	OR (95% CI)[3,4]
0	65 (33.3)	80 (76.2)	Reference
1	69 (35.4)	18 (18.4)	5.41 (2.75–10.62)[1]
2	44 (22.6)	6 (7.0)	9.91 (3.23–30.39)[1]
3	15 (7.7)	1 (1.2)	10.84 (1.26–93.03)[1]
4	2 (1.0)	0 (0.0)	n.a.
2–4	61 (31.3)	7 (8.0)	10.07 (3.57–28.28)[1]

OR, Odd's ratio; CI, confidence interval; n.a., not applicable.
[1]Significant.
[2]Pathologies associating significantly with dementia (Braak stages V–VI, the presence of diffuse neocortical type of Lewy-body-related pathology, frontal CAA > Q3 value (6%), and macroscopic infarcts 2–15 mm in the cortex supplied from anterior and middle cerebral arteries, see: Table 1).
[3]Bivariate logistic regression analysis.
[4]Adjusted for age at death, gender and the possession of *Apolipoprotein E ε4* allele.

Prevalence of multipathology

We next analyzed the co-occurrence of different types of neuropathologies. We included in the analysis the three independent neuropathological contributors of dementia (Braak stage V–VI, diffuse neocortical type of Lewy-related pathology, and anterior cortical 2–15 mm infarcts). In addition, as a representative of the Aβ pathology, severe frontal CAA was included, because the association between dementia and severe frontal CAA in the multivariate analysis was nearly significant, whereas the association between CERAD score and dementia was insignificant (Table 1). One-third of the demented had at least two and two-thirds had at least one of these most significant variables (Table 2). The risk for dementia of subjects with two or more of these four pathologies was almost two times higher than in those with only one of these pathologies (OR: 10.07, 95% CI: 3.57–28.28 vs. 5.41 95% CI: 2.75–10.62). On the other hand, one-third of the demented subjects did not have any of these pathologies. The distribution of these pathologies at an individual level is shown in Figure 2, highlighting that the distribution of these neuropathologies is random. The combination of tau- and neocortical Lewy-body-related pathologies produced the highest risk (Table S3).

Relationships between the various neuropathologies

Correlation analysis (Table 3) revealed correlations between the AD-type pathologies, neocortical Lewy-related pathology and CAA. Different types of brain

Table 3. Results of correlation analyses between the various quantitative neuropathological variables and the possession of *apolipoprotein E* ε4 allele in the whole autopsied subpopulation (N = 300) of the Vantaa 85 + Study.

		AD type		Lewy rel	Infarct (N)			CAA[3]	Hem (N)	*APOE*
		Tangle[4]	Plaque[5]	Neocortical[6]	Hemisph[7]	Deep sc[8]	Microsc. <2 mm[9]	6 regions[10]	Microsc. cortical	ε4-carrier
AD type	Tangle[4]	1.000	0.591[2]	0.128[1]	−0.028	0.008	0.121[1]	0.460[2]	−0.030	0.427[2]
	Plaque[5]	0.591[2]	1.000	0.124[1]	−0.066	−0.022	0.059	0.487[2]	−0.004	0.460[2]
Lewy rel	Neocortical[6]	0.128[1]	0.124[1]	1.000	0.026	−0.025	−0.003	0.052	−0.043	0.102
Infarct (N)	Hemisph[7]	−0.028	−0.066	0.026	1.000	0.214[2]	0.141[1]	−0.045	0.014	0.103
	Deep sc[8]	0.008	−0.022	−0.025	0.214[2]	1.000	0.010	−0.060	0.005	−0.038
	Microsc. <2 mm[9]	0.121[1]	0.059	−0.003	0.141[1]	0.010	1.000	0.130[1]	0.102	0.164[2]
CAA[3]	6 regions[10]	0.460[2]	0.487[2]	0.052	−0.045	−0.060	0.130[1]	1.000	−0.016	0.481[2]
Hem (N)	Microsc. cortical	−0.030	−0.004	−0.043	0.014	0.005	0.102	−0.016	1.000	−0.022
APOE	ε4−carrier	0.427[2]	0.460[2]	0.102	0.103	−0.038	0.164[2]	0.481[2]	−0.022	1.000

The counts represent correlation coefficients (Spearman's ρ).

AD, Alzheimer's disease; Lewy rel, Lewy-related pathology; CAA, cerebral amyloid angiopathy; Hem, hemorrhage; *APOE*, *Apolipoprotein E*; Tangle, neurofibrillary tangle, Plaque, methenamine-silver-positive plaque (any type); Hemisph, hemispheric; Deep sc, deep subcortical; Microsc, microscopic.

[1]Significant at the 0.005 level.

[2]Significant at the 0.001 level.

[3]Percentage of the amyloid laden blood vessels of all vessels (Tanskanen et al., NAN 2012; 38: 329-36).

[4]Average number of neurofibrillary tangles in the four cortical regions (frontal, temporal, parietal, occipital).

[5]Percentage of neocortex covered by methenamine silver-positive plaques (frontal, temporal, parietal, occipital, Polvikoski et al.,[16]).

[6]The presence or absence of neocortical Lewy bodies in the temporal, frontal and parietal lobes and cingulate gyrus (McKeith et al.,[18]).

[7]Macroscopic infarcts in the cerebral cortex and superficial subcortical white matter (Troncoso et al.,[13]).

[8]Macroscopic infarcts in the basal ganglia, brainstem, and cerebellum (Troncoso et al.,[13]).

[9]Microscopic cortical infarcts <2 mm.

[10]Frontal, temporal, parietal, occipital lobe, hippocampus, cerebellum.

infarcts correlated with each other and *APOE ε4* correlated with AD-type parenchymal pathology, CAA and microscopic (<2 mm) cortical infarcts. To investigate the relationships of different pathologies further we used factor analysis. This analysis identified three separate factors: (1) AD-spectrum, which included neurofibrillary tau pathology, Aβ plaque pathology, CAA, and neocortical Lewy-related pathology, (2) >2 mm cortical and subcortical infarcts, and (3) <2 mm cortical microinfarcts and microhemorrhages (Fig. 1, Table S2).

Discussion

We have performed a study on the spectrum of various neuropathologies underlying dementia in a population-based study on subjects aged ≥85 years. Our major findings were: (1) the prevalence of any age-associated brain pathology was extremely high (99.7%), (2) neurofibrillary AD-type pathology (Braak stage V–VI) was the strongest independent neuropathological correlate of dementia, although two other pathologies (neocortical type of Lewy-related pathology, and 2–15 mm cortical infarcts especially in the territory of the anterior circulation) also associated independently with dementia, (3) the different types of neuropathologies formed up into three separate, frequency-based "clusters". (4) multipathology is common in the very elderly demented individuals and increases the risk of dementia significantly.

Neuropathological and clinical diagnoses

Autopsy with neuropathological examination is often needed to determine the background of dementia, in addition to the clinical and radiological examinations. Today, neuropathological examination is a prerequisite for the definite diagnosis of AD.[21] However, in the very elderly, the differential diagnostics of dementia syndromes may be challenging.[17] Although the contemporary neuropathological diagnosis of AD notifies some other pathologies than the AD-related, such as DLB with consensus neuropathological criteria[18] there are conditions such as VaD that lack such criteria. Therefore, we used the clinical dementia diagnosis as a starting point, and tested the presence versus not presence of clinical dementia against the panel of neuropathological components. The criteria for the clinical diagnosis of dementia can be considered strict enough to reliably differentiate between the demented and nondemented. Our neuropathological

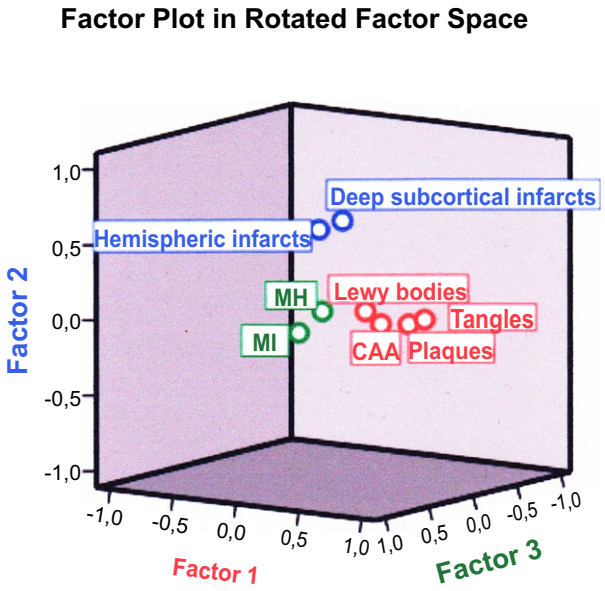

Figure 1. The variance in eight quantitative variables, as analyzed using factor analysis: The variables (components) investigated consisted of counts of neurofibrillary tangles (tangles), percentage of cortical thickness covered by methenamine silver-positive senile plaques (plaques), hemispheric and deep macroscopic infarcts, cortical micro infarcts <2 mm (MI), cortical microhemorrhages (MH), and the severity of cerebral amyloid angiopathy (CAA) in six brain regions, and the presence/absence of neocortical Lewy bodies, as illustrated in a three-dimensional form. Three factors explaining the variability between the eight components investigated were extracted (Table 2) showing that the cluster of four variables (Factor 1, red color), consisted of tangles, plaques, CAA and the neocortical type of Lewy-body-related pathology, explained the majority of the variability between the variables.

analyzes followed the traditional neuropathological parameters for AD[14,16] and LBD. These criteria have recently been refined and adjusted for the needs of clinical diagnostics, for example, by applying IHC-based methods but the original methods can still be judged reliable indicators of the severity of the pathology.[22,23,24] For the assessment of CAA several methods have been proposed. Here we have preferred the above described, allowing quantitative analyses based on whole tissue sections.[20]

The neuropathological spectrum of the aged brain

The extremely high prevalence of any neuropathology (99.7%) shows that the development of at least some type of neuropathology during aging seems inevitable. The extremely high frequency of the AD-related pathologies (291/300 [97%] with Braak stage >0 or CERAD score>0, strongly contributed to this count similarly as in another population-based study (Honolulu Asia Aging Study, HAAS) on elderly subjects.[25] Other population-based studies had found somewhat lower frequencies of AD-type pathologies[1,13,26–28], probably as those studies included younger subjects, with lower mean age at death, as compared to our study (92.4 years for the autopsied population, Table S1). Indeed, Strotzyk et al. reported AD in 55/190 of subjects in a community-based series with mean age at death was 84.4 years.[29] Other common pathologies were CAA (any severity 209/300 [70%]), cortical microhemorrhages (185/300 [62%]), and brain infarcts (180/300 [60%]). The significance of CAA in dementia has been somewhat unclear. Previous population-based studies[1,30,31] have demonstrated higher prevalence of CAA in the demented as compared with the nondemented, referring to an impact for CAA for the development of dementia. However, only one population-based study has demonstrated an independent association between CAA and dementia.[26] This discrepancy[28] may reflect the effect of variability in the selection of variables in the multivariate analyses. Some brain lesions, such as cortical microhemorrhages, although associated with age[32] did not associate with dementia. There are only few histological studies on MH and dementia. One previous study[33] found microhemorrhages to be frequent in AD brains in contrast to us (Table 3). Hemorrhage, also microhemorrhage, can be a manifestation of CAA[34] but here we did not find a statistical association between the presence of microhemorrhage and CAA (Table 3). One reason for these discrepancies may be that the presence of microhemorrhage was analyzed here in the cortical regions only.

The significant neuropathologies

In this study, the AD-related tau-pathology (Braak stages V–VI, Table 1) was the strongest single contributor to dementia, but the impact of the neocortical Lewy-related pathology was also strong. These findings are consistent with previous longitudinal studies investigating neuropathological correlates of dementia.[13,26,27,28,29,35,36,37] Several studies also have demonstrated a strong association between high CERAD score and dementia, but the results in the multivariate analyses have varied. Similar to our study, the association was lost in one study[27], in contrast to another[26] in which the strong association prevailed in the multivariate analysis. These discrepancies may reflect the differences in the variables selected in the multivariate analyses.

We confirmed the previous findings on the independent association between dementia and cerebral small macroscopic infarcts (2–15 mm)[13,36], emphasizing the role of vascular backgrounds in dementia in the very elderly. Indeed, not only cerebral infarcts but even

atherosclerosis of the cerebral large and small vessels have recently shown to increase the risk of AD-type dementia.[38] However, the association between dementia and the presence of 2–15 mm cortical infarcts in the anterior parts of the brain was even stronger (OR: 2.84, 95% CI: 1.32–6.10), and represented an independent determinant of dementia, thus referring to the role of lesion localization in the clinical outcome, independent from the background (an obstruction/decreased blood flow in either the local small blood vessels or larger arteries). The association between cerebral microscopic infarcts and dementia, found in several previous studies[1,13,27,36] was not confirmed here, most probably as we have not analyzed white matter lesion but cortical microscopic infarcts only. Although deep infarcts as a group did not associate with dementia, similar to another longitudinal study[13], we showed the significance of infarcts in the thalamic region in cognitive impairment (Table 1).

The network of various neuropathologies

Factor analysis has mainly been applied in the social sciences, for instance, in the opinion polls. It measures the conceptual distance between different quantitative variables relative to each other. Here we applied the method for the first time on a neuropathological study, to identify the settling of the quantitative variables in relation to the others to form variable "clusters". The resulting perspective (Fig. 1, Table S3) recapitulated the results of the correlation analysis (Table 3). The AD-type pathology, CAA, and Lewy-related pathologies were separated into their own "cluster" (Factor 1, "AD-spectrum"). The placing of the Lewy-related pathology in the "AD-spectrum" is not unexpected, as the Lewy-related pathology – when present – tends to coexist with AD pathology.[39] This also explains the high association between the combinations of these pathologies and dementia. The natural background for the second "cluster" (Factor 2, the macroscopic >2 mm infarcts) is arteriosclerosis/thrombosis of the arterioles and larger arteries. The third "cluster" (Factor 3) consisted of cortical microinfarcts and microhemorrhages. Although both components of this "cluster" potentially represent a type of capillary injury neither was associated with dementia. Thus, apart from CAA, the various vascular pathologies in this study did not seem to be linked with the AD- or Lewy-related pathologies, suggesting that these vascular and primary neurodegenerative pathologies mainly are independent from each other, as suggested previously.[25]

Multipathology

We show the extent (Tables 1,2) and spectrum (Fig. 1,2 and Table S2, S3) of the concomitant pathologies in the brains of the oldest old, demonstrating the high frequency of multiple pathologies, as has been reported before.[37] The increase in the number of pathologies increased the OR for dementia (Table 2, Figure 2), similarly to other recent community-based studies.[8,9,10] It is of interest that the effect on multipathology on dementia was similar in spite of differences in the pathologies included in the "mixed" pathology group in the different studies. For example, in the study of Kawas et. al.[9], the subjects were categorized in four different groups, based on the presence and severity of AD pathology and the presence of other type of pathologies, whereas another study[10] focused on the presence of AD and TDP-43 pathologies. These results imply that the association between multipathology and dementia is not restricted to specific neuropathologies or their combinations but several different combinations of several different neuropathologies may increase the risk of dementia. Second, the clinical diagnosis and especially the determination of the type of dementia in the very old may be challenging,[40] as many patients fulfill more than one diagnostic criterion and more than one pathophysiology. Third, it is possible that the co-occurrence of multiple pathological processes in the brain and their additive effect in the risk of dementia may also have a deteriorating effect on the reliability of short term drug trials lacking neuropathological confirmation of the diagnoses.[37] On the other hand, probably as a result of this multipathology, many modifiable risk factors for dementia have been identified[41] and several of them are (1) shared with atherosclerotic cerebrovascular disease and (2) amenable to simple preventive measures of, for example, lifestyle and nutrition.

Even though we found that two-thirds of the demented subjects had at least one of the four pathologies that associated independently or nearly independently with dementia (Braak stage V–VI, diffuse neocortical type of Lewy-related pathology, anterior cortical 2–15 mm infarcts and frontal severe CAA), it is noteworthy that one-third of the demented did not have any of these pathologies. On the other hand, 99% of the demented had at least one neuropathology analyzed here, of which some, for example, the presence of any 2–15 mm cortical infarcts or CERAD score C may explain dementia in these individuals. It is also of note that our study lacked data on some important dementia-associated neuropathologies, for example, hippocampal sclerosis[42] and the other TDP-43-related pathologies as well as primary age-related tauopathy (PART)[43], the analysis of which are not completed yet in our study material. Further studies, including these and other, for example, vascular neuropathologies may further specify the neuropathological backgrounds of dementia in the very elderly.

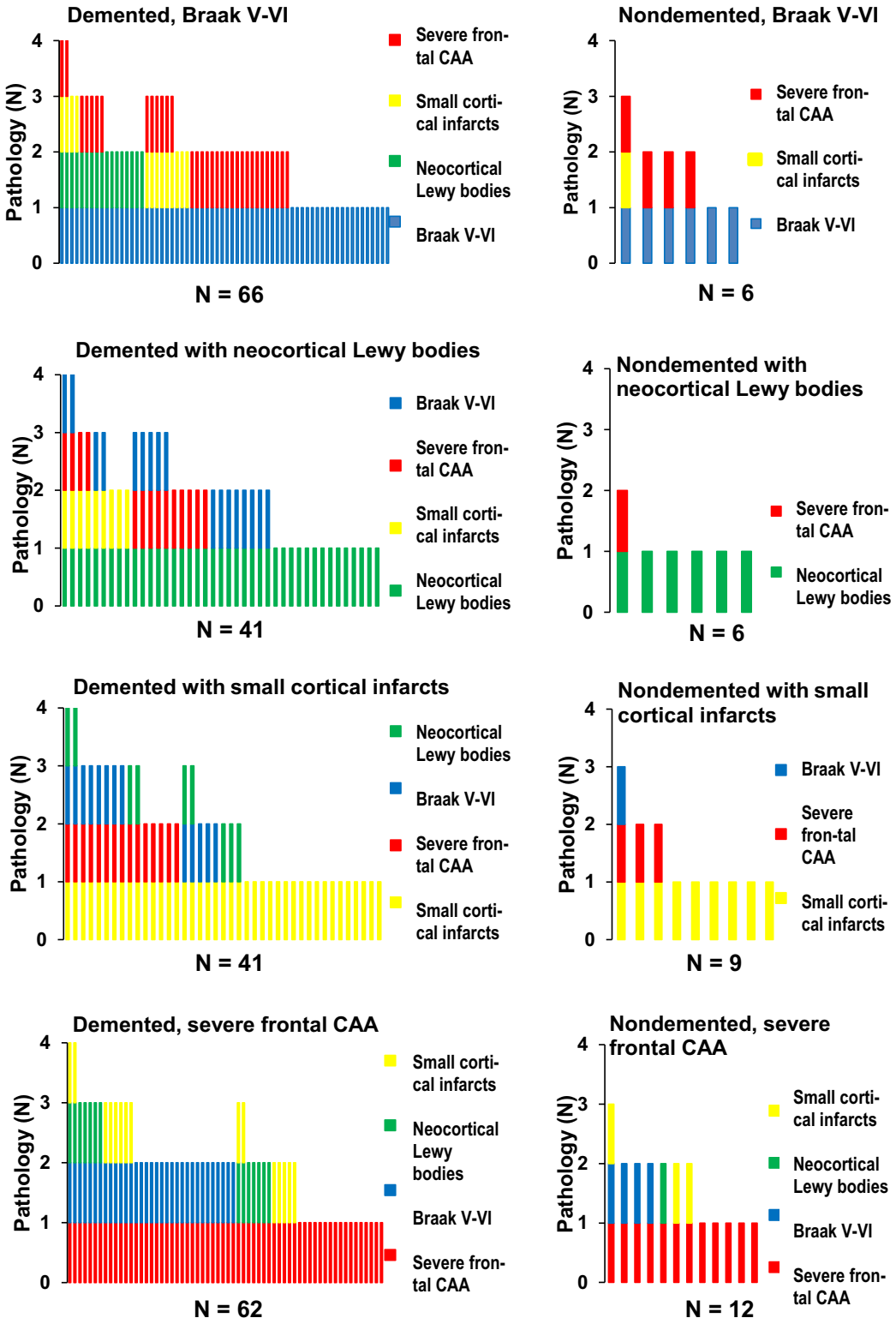

Figure 2. Distribution of four dementia-associated pathologies in demented and nondemented: Distribution and co-occurrence of the three neuropathologies which associate independently with dementia: Braak stages V-VI (blue color), neocortical Lewy-related pathology (green color), and the presence of 2–15 mm infarcts in the anterior cortical regions (yellow color), and severe cerebral amyloid angiopathy (CAA) in the frontal lobe (red color, borderline association). Individual level presentation, one bar represents one subject.

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Author Contribution

Dr. Maarit Tanskanen: Study conception and design, collection of data, preparation of manuscript, statistical analyses, obtaining funding. Dr. Mira Mäkelä: Collection of data, study design, preparation of manuscript. Dr. Irma-Leena Notkola: Study design, preparation of manuscript, statistical analyses. Dr. Liisa Myllykangas: Study design, collection of data, preparation of manuscript, funding. Dr. Sari Rastas: Collection of data, preparation of manuscript, study design. Dr. Minna Oinas: Collection of data, preparation of manuscript, study design. Dr. Perttu J Lindsberg: Study conception and design, preparation of manuscript. Dr. Tuomo Polvikoski: Collection of data, study design, preparation of manuscript. Dr. Pentti J Tienari: Study conception and design, preparation of manuscript. Dr. Anders Paetau: Collection of data, preparation of manuscript, obtaining funding, study design.

Conflict of Interest

Dr. Maarit Tanskanen reports no disclosures. Dr. Mira Mäkelä reports no disclosures. Dr. Irma_Leena Notkola reports no disclosures. Dr. Liisa Myllykangas reports no disclosures. Dr. Sari Rastas reports no disclosures. Dr. Minna Oinas reports no disclosures. Dr. Perttu J Lindsberg reports no disclosures. Dr. Tuomo Polvikoski reports no disclosures. Dr. Pentti J Tienari reports no disclosures. Dr. Anders Paetau reports no disclosures.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Demonstrates the demographic features of the whole Vantaa 85 + population compared with the neuropathologically examined (autopsied) subpopulation.
Table S2. Demonstrates the results of factor analysis investigating the variance in eight quantitative variables: the neurofibrillary tangles, senile plaques, Lewy-body-

related pathology, infarcts (macroscopic hemispheric and deep subcortical, microscopic, MI), cerebral amyloid angiopathy (CAA), and microscopic hemorrhages (MH).

Table S3. Shows the distribution of combinations of the four pathologies which showed the strongest association with dementia (Braak stages V–VI, presence of neocortical Lewy bodies, presence of cortical infarcts 2–15 mm in the anterior parts of the brains and presence of severe CAA in the frontal lobe) in the demented and nondemented subjects in the Vantaa 85 + Study population.

