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Neocortical and hippocampal amyloid-β and tau measures associate with dementia in the oldest-old

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The emergence of longevity in the modern world has brought a sense of urgency to understanding age-related neurodegenerative diseases such as Alzheimer's disease. Unfortunately, there is a lack of consensus regarding the correlation between the pathological substrates of neurodegeneration and dementia status, particularly in the oldest-old. To better understand the pathological correlates of dementia in the oldest-old, we characterized the topographical spread and severity of amyloid-β, tau, TDP-43 and α-synuclein pathologies in the 90+ Study, a prospective longitudinal population-based study of ageing and dementia. Neuropathological analysis with immunohistochemically labelled sections was carried out blind to clinical diagnosis on the first 108 participants of the 90+ Study who came to autopsy including participants with dementia ($n = 66$) and without dementia ($n = 42$). We used quantitative and/or semi-quantitative measures to assess the burden of amyloid-β, tau, TDP-43 and α-synuclein pathologies as well as hippocampal sclerosis. Amyloid-β and tau were the predominant pathologies in the 90+ Study cohort and both amyloid-β area and tau area occupied measures were strongly associated with the presence of dementia, as was Braak staging but semi-quantitative plaque scores were not. Notably, TDP-43 pathology also correlated with dementia, while α-synuclein distribution did not. In addition, hippocampal sclerosis was specific to participants with dementia and correlated with the presence of limbic TDP-43. In contrast to previous reports, we found that tau and amyloid-β continue to be robust pathological correlates of dementia, even in the oldest-old. While individuals with no dementia had limited hippocampal tau and neocortical amyloid-β pathology, dementia associated with an expansion in pathology, including increased neocortical tau and hippocampal amyloid-β plaques, more abundant neocortical amyloid-β deposition and hippocampal sclerosis with its attendant TDP-43 pathology.

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Abbreviation: CERAD = Consortium to Establish a Registry for Alzheimer's Disease

Introduction

Current models of Alzheimer's disease suggest that dementia results when a neuropathological threshold is crossed (Vemuri *et al*., 2011). Several recent studies have suggested that this threshold between cognitively normal and individuals with Alzheimer's disease narrows with age, especially the oldest-old, those aged ⩾85–90 years (Giannakopoulos *et al*., 1994; Riley *et al*., 2002; Imhof *et al*., 2007; Haroutunian *et al*., 2008; Savva *et al*., 2009). As the extensive pathological build up of tau and amyloid-β may precede the emergence of clinical Alzheimer's disease by decades (Braak and Braak, 1997; Sperling *et al*., 2011), the threshold of neuropathology that defines Alzheimer's disease is challenging to define due to the differential effects of age, individual resilience or plasticity and other unknown factors. Since by 2050, the US population of oldest-old living with Alzheimer's disease is expected to outnumber the total number of patients with Alzheimer's disease today (Hebert *et al*., 2003), there is an urgent need to better understand the pathological correlates of dementia in the oldest-old. For these reasons, the study described here characterized the topographical spread and severity of Alzheimer's disease and related neurodegenerative disease pathology in the 90+ Study (Kawas, 2008), a prospective longitudinal population-based study of ageing and dementia. Briefly, we show that dementia in the 90+ Study cohort associated with neocortical tau, hippocampal amyloid-β plaques, more abundant neocortical amyloid-β deposition and hippocampal sclerosis with TDP-43 pathology.

Materials and methods

Study subjects

Study participants were the first 108 individuals to come to autopsy from the 90+ Study, a longitudinal study of ageing and dementia in people aged ⩾90 years. Although it is not standardized to the whole US population, the 90+ Study is a population-based study because its cohort is composed of survivors of a study conducted in the early 1980s in a geographically defined area (The Leisure World Retirement Community). In addition, recruited participants lived at home as well as institutions, lived across the country, represent the full spectrum of health and cognitive abilities, and are representative of the oldest-old population in Orange County, Califonia (Corrada *et al*., 2011).

All 90+ Study participants agreed to neurological examinations by a trained physician or nurse practitioner every 6 months, including a full neuropsychological battery that incorporated the Mini-Mental State Examination. Relevant medical history, medication use and brain imaging evaluations were obtained from the participant's physicians. Cognitive and functional abilities were obtained from informants in frequent contact with the participants using the Dementia Questionnaire (Kawas *et al*., 1994). After death, the Dementia Questionnaire was repeated to inquire about the participant's condition after the last evaluation. The Institutional Review Board of the University of California, Irvine, approved all procedures and all participants or their surrogates gave written informed consent. After a participant's death, all information available was reviewed and discussed during a multidisciplinary consensus diagnostic conference led by the 90+ Study principal investigator (C.K.). Participants were classified as either 'dementia' or 'no dementia'. Dementia diagnosis was established using Diagnostic and Statistical Manual of Mental Disorders 4th edition criteria (American Psychiatric Association, 1994). Participants with no dementia included individuals with a mild cognitive or functional impairment not severe enough for dementia. If a participant met dementia criteria, the primary and secondary aetiologies of dementia were specified using standard criteria. All cognitive diagnoses were made blinded to pathological evaluations.

Neuropathology

All autopsies were performed at the University of California, Irvine. After weighing the whole brain and gross inspection, one hemisphere was dissected as previously described (Berlau *et al*., 2009). Six-micrometre thick, coronal sections of mid-frontal cortex, superior temporal cortex, anterior hippocampus, amygdala, substantia nigra and medulla oblongata were cut. All histological staining, immunohistochemistry and microscopic analyses were performed at the University of Pennsylvania. Sections were subjected to immunohistochemistry using the avidin–biotin complex detection method (VECTASTAIN® ABC kit; Vector Laboratories) with ImmPACT™ diaminobenzidine peroxidase substrate (Vector Laboratories) as the chromogen using antibodies to tau (mouse antibody PHF1; 1:1000, gift from Dr Peter Davies), α-synuclein (mouse antibody Syn303; 1:4000, generated in Center for Neurodegenerative Disease Research) and TDP (rat antibody p409/410, 1:1000, gift of Dr Manuela Neumann) (Uryu *et al*., 2008), in addition to amyloid-β (mouse antibody NAB228; 1:15 000; generated in CNDR) (Lee *et al*., 2006).

Topographical Braak staging (Stages I–VI) was assigned, with Stage VI distinguished from Stage V by the presence of a large number of neurofibrillary tangle-bearing granule cells in the fascia dentata (Braak and Braak, 1991). Consortium to Establish a Registry for Alzheimer's Disease (CERAD) plaque scores (0, A, B, C) were obtained using thioflavin-s stained mid-frontal cortex and temporal cortex sections (Mirra *et al*., 1991). Anterior hippocampus slices were used to determine topographical amyloid phases (Thal *et al*., 2006): phases 1–4 as per Thal *et al*. (2006) and phase 0 as the complete absence of amyloid-β plaques. All sections were stained for TDP-43 whose pathology was scored as absent, minor, limbic (anterior hippocampus- or amygdala-positive) or neocortical (mid-frontal cortex- or temporal cortex-positive) similar to Geser *et al*. (2010). All sections were stained for α-synuclein and the distribution of pathology was determined as absent,

brainstem (substantia nigra- or medulla oblongata-positive), limbic (anterior hippocampus- or amygdala-positive) or neocortical (mid-frontal cortex- or temporal cortex-positive) similar to Leverenz *et al*. (2008). Hippocampal sclerosis was assessed based on haematoxylin and eosin staining using a 3-point scale (absent = no observed gliosis and/or neuronal loss, minor = mild gliosis and/or neuronal loss, definite = severe gliosis and neuronal loss). Amyloid-β and tau were the predominant pathological burdens in this study. Since the topographical amyloid phase and Braak stage schemes do not directly quantify the severity of the pathology, image analysis of neocortical amyloid-β burden and hippocampal CA2/CA1 tau burden was performed to generate amyloid-β and tau area occupied measures. To do this, sections were scanned (Media Cybernetics Evolution QE camera, gain 2.0, exposure 80 ms) at ×5 on a Leitz DMRB microscope (Leica) using Image Pro software 6.2 (Media Cybernetics). ImageJ 1.41o (National Institutes of Health) was used for analysis (Morrison, 2006). The amyloid-β area occupied measure was obtained from the maximum of mid-frontal cortex or superior temporal cortex values obtained as follows: selected NAB228 stained cortical grey matter had the multi-thresholder maximum entropy algorithm applied; particle analysis on 50 × 50 pixels or greater including holes (1500 μm^2) was applied. In 65 cases, both the frontal and temporal cortex gave similar values; in 37 cases, the frontal value was used; in six, the superior temporal cortex. Tau area occupied was obtained from selecting the PHF1-stained CA2/CA1 region; applying the maximum entropy algorithm, and performing particle analysis on 10 × 10 pixels or greater including holes (60 μm^2). Representative images of high and low, amyloid-β and tau area occupied measures are shown in Supplementary Fig. 1. Finally, we assessed the extent of cerebrovascular lesions via haematoxylin and eosin, and cerebral amyloid angiopathy via thioflavin S.

Statistical analysis

We compared characteristics of dementia and participants with no dementia using chi-square tests and Fisher's exact tests for categorical variables and *t*-tests for continuous variables. Logistic regression models determined the association between each individual neuropathological measure (as categorical variables) with the odds of dementia. For some measures, the categories were collapsed due to small numbers of individuals. To determine the independent contribution of each measure to the odds of dementia, we performed multiple logistic regression analyses with the neuropathological measures as continuous variables. We used likelihood score chi-square statistics to judge whether variables contributed significantly to the fit of a model. All logistic regression models were adjusted for age at death, gender and education. All statistical analyses were performed using SAS version 9.2 and SPSS version 17 (SPSS, Inc.).

Results

Subject characteristics

The demographics of the participants are summarized in Table 1. The first 108 individuals to come to autopsy from the 90+ Study were mostly females (74%) with a mean age at death of 97.5 years (range: 90–106) and the final Mini-Mental State Examination score obtained an average of 5 months before death. Autopsied participants did not differ from non-autopsy participants in the 90+ Study cohort in most regards including age at entry, gender, education, cognitive diagnosis or history of stroke or transient ischaemic attack. Sixty-six participants met criteria for dementia before death, with the majority (86%) diagnosed with clinical Alzheimer's disease with or without other possible dementias. Participants with dementia had a smaller brain weight ($P = 0.02$), a lower Mini-Mental State Examination score ($P < 0.001$), less education ($P = 0.04$) and were more likely to be APOEε4 allele carriers ($P = 0.04$) than participants with no dementia.

Pathological distribution

To begin to understand the pathological correlates of dementia in the oldest-old, we first focused on the topographical spread of the four major neurodegenerative proteinopathies: tau, amyloid-β, TDP-43 and α-synuclein. The results are presented in Table 2. When pathological tau spread beyond the hippocampus and into the neocortex, it clearly associated with dementia [Braak Stage IV; odds ratio (OR) 3.29; 95% confidence interval (CI) 0.74–14.53 or V and VI; OR 8.39; 95% CI 1.89–37.17). When the amyloid-β burden reached its highest levels in the hippocampus, it too associated with dementia (amyloid phase 4; OR 5.67; 95% CI 1.00–32.16). Surprisingly, although less widespread than either amyloid-β or tau, the presence of a significant number of limbic TDP-43 lesions also associated with dementia (limbic/neocortical; OR 3.81; 95% CI 1.41–10.24). Forty-one per cent of the participants with dementia had an appreciable amount of TDP-43 inclusions and neurites, an amount of pathology seen in only 17% of the subjects with no dementia. α-Synuclein pathology was even less widespread and failed to significantly associate with dementia (limbic/neocortical; OR 2.54; 95% CI 0.72–8.94). In total, only 10% of the subjects without dementia had limbic Lewy bodies and Lewy neurites, a burden that was limited to 20% of the participants with dementia.

The distribution patterns of the major proteinopathies in the 90+ brain led to the following conclusions. Tau was primarily hippocampal, although it was widespread in the cortex of participants with dementia. Amyloid-β deposits were primarily cortical, although they did occur in the hippocampus in some of the participants with dementia. In total, only six cases of dementia with Lewy bodies were diagnosed, along with two cases of frontotemporal lobar degeneration-TDP (Supplementary Table 1). The relatively sparse α-synuclein and TDP-43 levels suggest that in the majority of cases, both proteinopathies are co-morbid accumulations rather than the primary substrates of dementia.

Pathological severity

The amyloid phase and Braak staging protocols evaluate distribution but do not directly measure the severity of pathological amyloid-β and tau. To better understand the density of neocortical amyloid-β and hippocampal tau pathology, we employed three

Table 1 Characteristics of the 90+ Study participants

Characteristics	All subjects (n = 108)	No dementia (n = 42) n (%)	Dementia (n = 66)	P-value[a]
Gender				0.34
Male	28 (26)	13 (31)	15 (23)	
Female	80 (74)	29 (69)	51 (77)	
Education				0.04
⩽ High school	33 (31)	7 (17)	26 (39)	
Any college	51 (47)	24 (57)	27 (41)	
Any graduate school	24 (22)	11 (26)	13 (20)	
APOE ε4				0.04
0 alleles	81 (78)	37 (88)	44 (71)	
⩾1 alleles	23 (22)	5 (12)	18 (29)	
APOE ε2				0.54
0 alleles	87 (84)	34 (81)	53 (85)	
⩾1 alleles	17 (16)	8 (19)	9 (15)	
Clinical diagnosis				NA
Normal	22 (20)	22 (52)	–	
CIND	20 (19)	20 (48)	–	
AD	40 (37)	–	40 (61)	
VaD	5 (5)	–	5 (8)	
DLB	1 (1)	–	1 (1)	
AD/VaD	7 (6)	–	7 (11)	
AD/DLB	7 (6)	–	7 (11)	
AD/Other	2 (2)	–	2 (3)	
Other	4 (4)	–	4 (6)	
		Mean (SD)		
Age at death (yrs)	97.5 (3.5)	97.5 (3.1)	97.5 (3.8)	0.99
Last MMSE score[b]	17.0 (10)	25.8 (3.4)	11.3 (8.6)	<0.001
Interval from MMSE to death (months)[b]	5.1 (4.8)	5.0 (4.7)	5.2 (4.9)	0.85
Brain weight (g)[c]	1126 (123)	1162 (116)	1083 (123)	0.02

a P-values are for Fisher's exact tests for binary variables, χ^2 tests for categorical variables, t-tests for continuous variables and compare dementia versus not participants with dementia.
b excludes two participants with missing MMSE score.
c excludes four participants with missing brain weight.
AD = Alzheimer's disease; APOE = Apolipoprotein E; CIND = cognitive impairment no dementia; DLB = dementia with Lewy bodies; MMSE = Mini-Mental State Exam; Other = other neurodegenerative diagnoses; VaD = vascular dementia.

measures: amyloid-β area occupied to quantify the total cortical amyloid-β deposition, CERAD plaque scores to describe the severity of cortical neuritic plaques and tau area occupied to quantify the primarily neuritic, hippocampal tau pathology. In addition, because TDP-43 lesions may be specifically associated with hippocampal sclerosis (Amador-Ortiz *et al*., 2007), we also diagnosed the presence of hippocampal sclerosis.

The best pathological protein correlate of dementia was the neocortical amyloid-β area occupied measure (P = 0.004). Although a straightforward approach to measuring amyloid-β deposition compared with the topographical amyloid phase, amyloid-β area occupied proved surprisingly robust. The two highest amyloid-β area occupied quartiles accounted for less than one-third of the subjects with no dementia (28%), but almost two-thirds of the participants with dementia (65%). Importantly, amyloid-β area occupied had a consistent increase in the odds of dementia with an increasing burden of pathology (third quartile: OR 3.16, 95% CI 0.46–10.69; fourth quartile: OR 7.91, 95% CI 2.00–31.22). This increase was not seen with the CERAD plaque score. In fact, neither the CERAD plaque score nor amyloid phase were significantly associated with dementia (P = 0.41 and 0.11, respectively), whereas amyloid-β area occupied definitely was (P = 0.005).

The hippocampal tau area-occupied measure also significantly correlated with dementia (P = 0.005). Only 28% of the subjects with no dementia were in the two highest tau area quartiles compared with 64% of the dementia group. This compares favourably with the topographical staging of tau: 16% of the subjects with no dementia and 46% of the participants with dementia were graded Braak Stage IV or higher. Nonetheless, the odds of dementia increased linearly with higher Braak stages, but not with increasing quartiles of the tau area occupied measure.

The specific neuronal loss and gliosis of hippocampal sclerosis were limited to participants with dementia. Although confined to a subset of participants (n = 19; 29%), this was the only measure specific to dementia. Additionally, participants with hippocampal sclerosis correlated with a higher TDP-43 burden (Fig. 1F). Limbic TDP-43 pathology in particular, while relatively rare in subjects

Table 2 Association between neuropathology and dementia

Measure[a]	OR (95% CI)[b]		*P*-value[c]
Topographical distribution			
Braak stage			0.03
I and II	1.00 (reference)		
III	1.50 (0.55–4.12)		
IV	3.29 (0.74–14.53)		
V–VI	8.39 (1.89–37.17)		
Amyloid phase			0.11
0	1.00 (reference)		
1	1.56 (0.36–6.71)		
2	0.64 (0.13–3.31)		
3	1.80 (0.43–7.46)		
4	5.67 (1.00–32.16)		
TDP-43 score[d]			0.008
Absent/minor	1.00 (reference)		
Limbic/neocortical	3.81 (1.41–10.24)		
α-synuclein			0.15
Absent/brainstem	1.00 (reference)		
Limbic/neocortical	2.54 (0.72–8.94)		
Neocortex			
CERAD plaque score			0.41
0	1.00 (reference)		
A	2.40 (0.80–7.17)		
B	1.52 (0.46–4.96)		
C	2.25 (0.62–8.22)		
Amyloid-β area occupied quartiles			0.004
First (0.4–2.4)	1.00 (reference)		
Second (2.5–5.9)	0.89 (0.27–2.93)		
Third (6–14.5)	3.16 (0.46–10.69)		
Fourth (14.6–31.4)	7.91 (2.00–31.22)		
Hippocampus			
Tau area occupied quartiles			0.005
First (0.1–2.6)	1.00 (reference)		
Second (2.7–5.7)	0.45 (0.14–1.44)		
Third (5.9–9.2)	3.41 (1.00–11.58)		
Fourth (9.5–21.9)	2.74 (0.80–9.42)		
Hippocampal sclerosis[e]	No dementia	Dementia	NA
Absent/minor	100%	58%	
Definite	–	42%	

a People were combined into categories for analyses when categories had <3 individuals.
b ORs and 95% CIs were generated from logistic regression models where the dependent (outcome) variable was presence of dementia and the independent variables were the neuropathological measures as categorical variables. Age, gender and education were included as covariates and each neuropathological measure was analysed in a separate model.
c *P*-values refer to the significance of all the categorical variables simultaneously for each of the respective neuropathological measures. For example, the *P*-value for Braak stage represents the significance of a model with the four categorical variables representing Braak stage versus a model with only age, gender and education.
d excludes two participants.
e excludes one participant. Only participants with dementia had hippocampal sclerosis, therefore logistic regression analysis was not done.

with no dementia and participants with dementia without hippocampal sclerosis (17 and 26%, respectively), accounted for 79% of the dementia subgroup with hippocampal sclerosis. In fact, the dementia with hippocampal sclerosis subgroup seemed to account for the dementia group's overall high level of co-morbid TDP-43. Together, the robust correspondence between hippocampal sclerosis and TDP-43 imply a pathological substrate of dementia in participants with hippocampal sclerosis separate from the remaining participants with dementia. Nonetheless, most participants with dementia had significant Alzheimer's disease pathology, regardless of the presence of hippocampal sclerosis (Supplementary Table 2), suggesting that hippocampal sclerosis is a subset of pathological Alzheimer's disease and not a distinct diagnosis.

We also assessed the extent of cerebrovascular lesions and noted the following cerebrovascular lesions pathology: a resolved micro-infarct in one case; another with acute hypoxic damage; in addition to minor hypoxic events, several cases had other focal abnormalities such as possible neurodevelopmental defects due to aberrant neuronal migration, but the majority of the cases displayed no evidence of infarcts, micro-infarcts or micro-bleeds in the sections examined here. Additionally, the presence of mild to moderate cerebral amyloid was detected in 52% (56/108) of the cases. The cerebrovascular lesion and cerebral amyloid angiopathy observations together imply a modest burden of vascular pathology for the majority of cases with a grade of 0 or 1+ as per Jellinger and Attems (2003); neither pathology was associated with dementia (data not shown).

Taking into account both the severity and distribution of pathology in the 90+ cohort, we propose a model of dementia where both hippocampal and neocortical lesions, primarily amyloid-β and tau, readily distinguish participants with dementia from those without (Fig. 1).

Neuropathological analysis

The best amyloid-β correlate of dementia was the severity of cortical amyloid-β plaques as quantified by amyloid-β area occupied (Fig. 1A). The subjects with no dementia mean of 5.6% nearly doubled to 10.7% in the participants with dementia. In contrast, the severity of cortical amyloid-β plaques as measured by the CERAD semi-quantitative plaque score did not significantly change from the no dementia mean of 1.2 to the dementia mean of 1.5 (Fig. 1C). The anatomical distribution of amyloid-β also associated with dementia, although the correlation was strongest only when amyloid-β deposition reached its highest medial temporal lobe severity as amyloid phase 4 (Fig.1D).

The distribution of pathological tau as measured by Braak stage (Fig. 1B) correlated with dementia status, as did the severity of tau pathology as quantified by tau area occupied (Fig. 1E). The subjects with no dementia Braak stage mean was 2.8, while participants with dementia averaged 3.6, a value that represents the spread of neurofibrillary tangles beyond the medial temporal lobe and a worsening of hippocampal tau pathology. Similarly, the mean hippocampal tau area occupied increased from 4.6% for subjects with no dementia to 7.2% for the participants with dementia. Nonetheless, the odds of dementia increased linearly

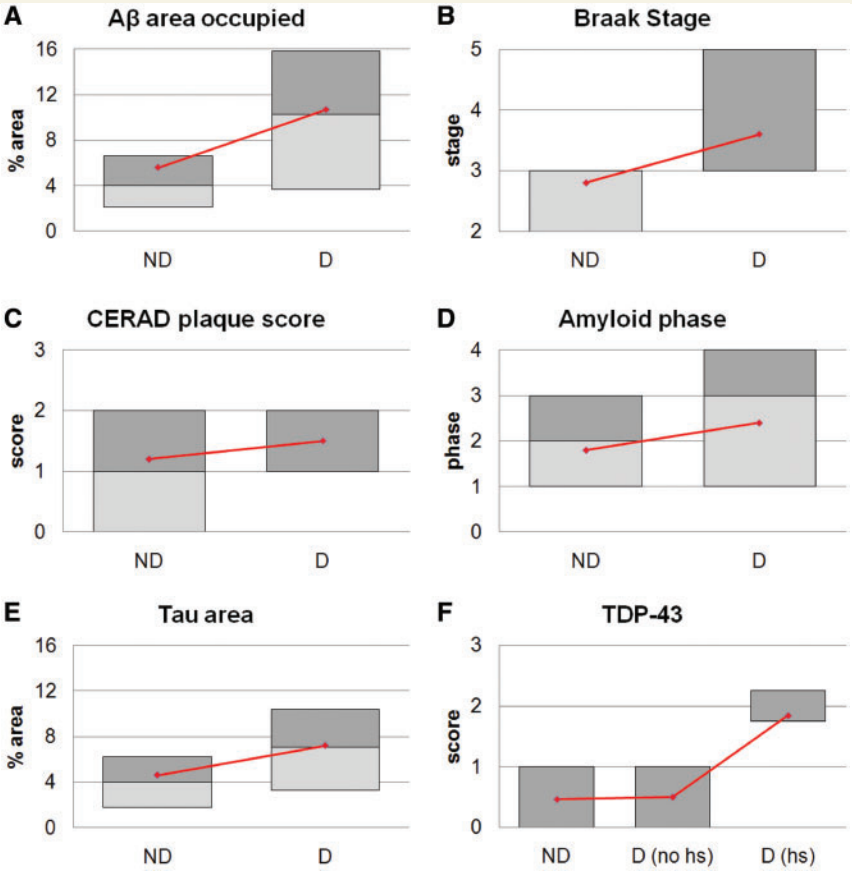

Figure 1 Neuropathology means according to dementia status. Dementia associated with (**A–C**) neocortical and (**D–F**) hippocampal pathology. Neocortically, dementia associated with (**A**) a severe total amyloid-β plaque burden and (**B**) an outward spread of tau neurofibrillary tangles. (**C**) The CERAD plaque scores were not significant. Hippocampal proteinopathies included (**D**) an increase in amyloid-β plaques in the CA/subiculum and (**E**) an increase in neuritic tau levels in CA2/CA1. (**F**) Hippocampal sclerosis was present in a subset of participants with dementia (29%; $n = 19$) and tightly correlated with limbic TDP-43 pathology. Box plots display the lower (light grey) and upper (dark grey) interquartile range overlaid with a model depicting mean values (red) for each group. D = dementia; hs = hippocampal sclerosis; ND = not dementia.

with higher Braak stages but not with increasing quartiles of tau area occupied, leading us to conclude that Braak stages are a better measure of pathological tau.

In conclusion, all measures of proteinopathy lesions, except CERAD plaque scores, were on average higher in dementia than in participants without dementia (Fig. 1) and by logistic regression analyses, several of the pathological measures significantly associated with dementia (Table 2). The most robust measures were topographical tau as captured by Braak stage and the severity of amyloid-β as quantified by amyloid-β area occupied. To determine if the other measures further improved this model of dementia, we ascertained the impact of each variable in a multiple logistic regression model that included Braak stage and amyloid-β area occupied. Both hippocampal sclerosis ($P < 0.001$) and TDP-43 ($P = 0.02$) significantly associated with dementia when individually added to the model. This was not the case for α-synuclein ($P = 0.13$) or the other tau and amyloid-β variables (data not shown). Furthermore, TDP-43 failed to improve a model that included Braak stage, amyloid-β area occupied and hippocampal sclerosis ($P = 0.98$).

Discussion

Our clinical pathological investigation of the 90+ Study cohort is the first to analyse the four major neurodegenerative disease protein pathologies in the oldest-old, i.e. tau, amyloid-β, α-synuclein and TDP-43 lesions. Although we report hippocampal tau and neocortical amyloid-β pathology in individuals without dementia, dementia in the oldest-old was associated with higher Braak staging, hippocampal amyloid-β plaques, more abundant neocortical amyloid-β deposition and hippocampal sclerosis with its attendant TDP-43 pathology.

These findings are significant since recent studies have raised questions about the extent to which Alzheimer's disease pathology accounts for dementia in the oldest-old (Haroutunian *et al*., 2008; Savva *et al*., 2009). While we are not the first study to report an association between dementia and Alzheimer's disease pathology, even above 90 years of age (Gold *et al*., 2000; Nelson *et al*., 2008; Dolan *et al*., 2010), our model of dementia in the oldest-old takes into account the severity and topographical distribution of both tau and amyloid-β.

Notably, the more extensive spread of tau pathology beyond Braak Stage III may be important in the development of clinical Alzheimer's disease. Consistent with a previous report (Giannakopoulos *et al*., 1994), we observed a substantial increase in clinical dementia at Braak Stages IV–VI (81%; $n = 30/37$). Not only does tau pathology extend more broadly into the frontal cortex in Stage IV, but also hippocampal lesions increase substantially (Braak *et al*., 2006), a result compatible with the increase in the tau area occupied measure. While Braak staging is a well-established correlate of dementia in this and most studies (Giannakopoulos *et al*., 1994; Riley *et al*., 2002; Sonnen *et al*., 2007; Nelson *et al*., 2008; Dolan *et al*., 2010), it is challenging to determine how the diverse morphologies of amyloid-β deposits (Thal *et al*., 2006) relate to neurodegeneration and dementia.

It has long been known that the exclusion of diffuse amyloid-β pathology from CERAD criteria may omit an important biomarker of Alzheimer's disease (Mirra *et al*., 1991) and the validity of the CERAD plaque score has been questioned previously (Prohovnik *et al*., 2006). The severity of the cortical amyloid-β area occupied measure had a stronger association with dementia than either the amyloid phase or CERAD plaque score. The amyloid phase scheme simplified the assessment of amyloid-β deposition, but this measure only correlated with dementia at phase 4, rendering it a binary variable in our hands. In contrast, the amyloid-β area occupied measure doubled in participants with dementia compared with subjects with no dementia. By implication, low levels of amyloid deposits can be tolerated, but a severe burden is not; a result not incompatible with a previous study (McKeel *et al*., 2004). Moreover, as a quantitative measure of a single region compared with the staging of amyloid phase or the multi-cortical CERAD plaque score, the amyloid-β area occupied measure has the advantage of being readily operationalized.

Despite the importance of tau and amyloid-β lesions in Alzheimer's disease, it is clear from our data that other pathologies, such as concomitant α-synuclein and TDP-43 (Nelson *et al*., 2008), may also contribute to dementia. The McKeith *et al*. (2005) criteria formally address the problem of co-existent tau, amyloid-β and α-synuclein pathologies, but no guidelines exist for TDP-43 pathology. TDP-43 inclusions and neurites may be downstream co-morbidities in the brains of patients already affected by plaques, tangles or Lewy body pathology (Uryu *et al*., 2008). Indeed, a recent clinicopathological study found TDP-43 pathology more prevalent in dementia of the Alzheimer's-type patients than in patients with primary progressive aphasia or even non-aphasic frontotemporal dementia (Bigio *et al*., 2010). Intriguingly, the same study also tightly correlated limbic TDP-43 pathology with hippocampal sclerosis, as in our cohort and others (Amador-Ortiz *et al*., 2007). We also note that the distribution pattern of these TDP-43 lesions appears to develop according to a predictable sequence and may be age related (Geser *et al*., 2010) as is the case with pathological tau.

In vivo imaging studies have shown that a third of cognitively normal individuals harbour a significant amyloid-β load (Clark *et al*., 2011). While imaging methods differ from post-mortem measures, the results are not dissimilar from our own measurements, where 28% of the subjects with no dementia had a high number of amyloid-β lesions (amyloid-β area 3rd and 4th quartile). The transition to dementia could have occurred in the future, if these individuals lived longer (Giannakopoulos *et al*., 1994) or this may reflect cognitive reserve.

There are several limitations to this study that should be noted. For example, it is known that vascular lesions are particularly common at advanced ages (Xuereb *et al*., 2000) even in cognitively intact people. While the presence of cerebral amyloid angiopathy was noted in approximately half the participants ($n = 56$), lacunar infarcts, microinfarcts and cystic infarcts were limited in the regions sampled. A more comprehensive investigation including an evaluation of white matter and subcortical regions, combined with observations of arteriosclerosis and territorial haemorrhages at time of death will be the focus of future studies of the 90+ cohort. Finally, until the amyloid-β and tau area occupied measures are extended to larger numbers of the oldest-old, our results should be interpreted cautiously. It would also be important to employ these measures in other studies to define the full range of measurements and quartiles and to determine their applicability in a younger cohort.

In summary, we propose a model of Alzheimer's disease pathology that includes both the step-wise progression of pathological tau and amyloid-β deposition as correlates of dementia in the oldest-old. We found that the best measures associated with dementia were: Braak stage, neocortical amyloid-β area occupied and hippocampal sclerosis with attendant TDP-43 pathology. Replication of these findings may lead to improved criteria for the post-mortem diagnosis of Alzheimer's disease and a better understanding of the neuropathology underlying dementia in the oldest-old.

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Supplementary material

Supplementary material is available at *Brain* online.

References

Amador-Ortiz C, Lin WL, Ahmed Z, Personett D, Davies P, Duara R, et al. TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. Ann Neurol 2007; 61: 435–45.

American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders (DSM IV). Washington, DC, 1994.

Berlau DJ, Corrada MM, Head E, Kawas CH. APOE epsilon2 is associated with intact cognition but increased Alzheimer pathology in the oldest old. Neurology 2009; 72: 829–34.

Bigio EH, Mishra M, Hatanpaa KJ, White CL, Johnson N, Rademaker A, et al. TDP-43 pathology in primary progressive aphasia and frontotemporal dementia with pathologic Alzheimer disease. Acta Neuropathol 2010; 120: 43–54.

Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol 1991; 82: 239–59.

Braak H, Braak E. Frequency of stages of Alzheimer-related lesions in different age categories. Neurobiol Ageing 1997; 18: 351–7.

Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Stageing of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. Acta Neuropathol 2006; 112: 389–404.

Clark CM, Schneider JA, Bedell BJ, Beach TG, Bilker WB, Mintun MA, et al. Use of florbetapir-PET for imageing beta-amyloid pathology. JAMA 2011; 305: 275–83.

Corrada M, Berlau D, Kawas C. A population-based clinicopathological study in the oldest-old: The 90+ Study. Curr Alzheimer Res 2011 (in press).

Dolan D, Troncoso J, Resnick SM, Crain BJ, Zonderman AB, O'Brien RJ. Age, Alzheimer's disease and dementia in the Baltimore Longitudinal Study of Ageing. Brain 2010; 133: 2225–31.

Geser F, Robinson JL, Malunda JA, Xie SX, Clark CM, Kwong LK, et al. Pathological 43-kDa transactivation response DNA-binding protein in older adults with and without severe mental illness. Arch Neurol 2010; 67: 1238–50.

Giannakopoulos P, Hof PR, Mottier S, Michel JP, Bouras C. Neuropathological changes in the cerebral cortex of 1258 cases from a geriatric hospital: retrospective clinicopathological evaluation of a 10-year autopsy population. Acta Neuropathol 1994; 87: 456–68.

Gold G, Bouras C, Kovari E, Canuto A, Glaria BG, Malky A, et al. Clinical validity of Braak neuropathological stageing in the oldest-old. Acta Neuropathol 2000; 99: 579–82; discussion 583–4.

Haroutunian V, Schnaider-Beeri M, Schmeidler J, Wysocki M, Purohit DP, Perl DP, et al. Role of the neuropathology of Alzheimer disease in dementia in the oldest-old. Arch Neurol 2008; 65: 1211–7.

Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA. Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol 2003; 60: 1119–22.

Imhof A, Kovari E, von Gunten A, Gold G, Rivara CB, Herrmann FR, et al. Morphological substrates of cognitive decline in nonagenarians and centenarians: a new paradigm? J Neurol Sci 2007; 257: 72–9.

Jellinger KA, Attems J. Incidence of cerebrovascular lesions in Alzheimer's disease: a postmortem study. Acta Neuropathol 2003; 105: 14–17.

Kawas CH, Segal J, Stewart WF, Corrada M, Thal LJ. A validation study of the Dementia Questionnaire. Arch Neurol 1994; 51: 901–6.

Kawas CH. The oldest old and the 90+ Study. Alzheimers Dement 2008; 4: S56–9.

Lee EB, Leng LZ, Zhang B, Kwong L, Trojanowski JQ, Abel T, et al. Targeting amyloid-beta peptide (Abeta) oligomers by passive immunization with a conformation-selective monoclonal antibody improves learning and memory in Abeta precursor protein (APP) transgenic mice. J Biol Chem 2006; 281: 4292–9.

Leverenz JB, Hamilton R, Tsuang DW, Schantz A, Vavrek D, Larson EB, et al. Empiric refinement of the pathologic assessment of Lewy-related pathology in the dementia patient. Brain Pathol 2008; 18: 220–4.

McKeel DW Jr, Price JL, Miller JP, Grant EA, Xiong C, Berg L, et al. Neuropathologic criteria for diagnosing Alzheimer disease in persons with pure dementia of Alzheimer type. J Neuropathol Exp Neurol 2004; 63: 1028–37.

McKeith IG, Dickson DW, Lowe J, Emre M, O'Brien JT, Feldman H, et al. Diagnosis and management of dementia with Lewy bodies: third report of the DLB Consortium. Neurology 2005; 65: 1863–72.

Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. Neurology 1991; 41: 479–86.

Morrison CM. Automated Identification of Beta-Amyloid Plaques by Image Analysis. Systems Biology Research Group; 2006. http://www.systemsbiology.ulster.ac.uk (18 November 2011, date last accessed).

Nelson PT, Abner EL, Schmitt FA, Kryscio RJ, Jicha GA, Smith CD, et al. Modeling the Association between 43 Different Clinical and Pathological Variables and the Severity of Cognitive Impairment in a Large Autopsy Cohort of Elderly Persons. Brain Pathol 2010; 20: 66–79.

Prohovnik I, Perl DP, Davis KL, Libow L, Lesser G, Haroutunian V. Dissociation of neuropathology from severity of dementia in late-onset Alzheimer disease. Neurology 2006; 66: 49–55.

Riley KP, Snowdon DA, Markesbery WR. Alzheimer's neurofibrillary pathology and the spectrum of cognitive function: findings from the Nun Study. Ann Neurol 2002; 51: 567–77.

Savva GM, Wharton SB, Ince PG, Forster G, Matthews FE, Brayne C. Age, neuropathology, and dementia. N Engl J Med 2009; 360: 2302–9.

Sonnen JA, Larson EB, Crane PK, Haneuse S, Li G, Schellenberg GD, et al. Pathological correlates of dementia in a longitudinal, population-based sample of ageing. Ann Neurol 2007; 62: 406–13.

Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Ageing-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 2011; 7: 280–92.

Thal DR, Capetillo-Zarate E, Del Tredici K, Braak H. The development of amyloid beta protein deposits in the aged brain. Sci Ageing Knowledge Environ 2006; 2006: re1.

Uryu K, Nakashima-Yasuda H, Forman MS, Kwong LK, Clark CM, Grossman M, et al. Concomitant TAR-DNA-binding protein 43 pathology is present in Alzheimer disease and corticobasal degeneration but not in other tauopathies. J Neuropathol Exp Neurol 2008; 67: 555–64.

Vemuri P, Weigand SD, Przybelski SA, Knopman DS, Smith GE, Trojanowski JQ, et al. Cognitive reserve and Alzheimer's disease biomarkers are independent determinants of cognition. Brain 2011; 134 (Pt 5): 1479–92.

Xuereb JH, Brayne C, Dufouil C, Gertz H, Wischik C, Harrington C, et al. Neuropathological findings in the very old. Results from the first 101 brains of a population-based longitudinal study of dementing disorders. Ann NY Acad Sci 2000; 903: 490–6.