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RESEARCH ARTICLE

TDP-43 pathology in Alzheimer's disease, dementia with Lewy bodies and ageing

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ageing, Alzheimer's disease, dementia with Lewy bodies, hippocampal sclerosis, Lewy body diseases, mixed Alzheimer's disease and dementia with Lewy bodies, TDP-43.

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Abstract

Intracellular inclusions consisting of TAR DNA binding protein-43 (TDP-43 pathology) are present in up to 57% of Alzheimer's disease (AD) cases and follow a distinct topographical pattern of progression described in the TDP-43 in AD staging scheme. This scheme has not been applied to the assessment of TDP-43 pathology in dementia with Lewy bodies (DLB) and aged controls. We investigated TDP-43 pathology prevalence and severity in AD, DLB, mixed AD/DLB (Mx AD/DLB) and aged controls. One hundred and nineteen human *post-mortem* brains were included, neuropathologically diagnosed as AD: 46, DLB: 15, Mx AD/DLB: 19 and aged controls: 39. Paraffin sections inclusive of the amygdala, hippocampus, striatum and neocortex were immunohistochemically stained with antibodies against phosphorylated TDP-43 and staged according to the TDP-43 in AD staging scheme. TDP-43 pathology was present in all groups: AD: 73.9%, DLB: 33.3%, Mx AD/DLB: 52.6% and controls: 17.9%. Prevalence of TDP-43 pathology was significantly higher in AD and Mx AD/DLB compared to controls. In controls, higher age at death was associated with prevalence of TDP-43 pathology and higher TDP-43 in AD stage, suggesting that this type of TDP-43 pathology may partly be an age-associated phenomenon. Significantly higher prevalence of TDP-43 pathology in the AD group indicates that AD pathology possibly triggers and aggravates TDP-43 pathology. The validity of the TDP-43 in AD staging scheme is not limited to AD and should be applied to assess TDP-43 pathology in *post mortem* brains of aged individuals to further elucidate the role of TDP-43 pathology in age associated neurodegeneration.

INTRODUCTION

Transactive response DNA-binding protein 43 (TDP-43) is a ubiquitously expressed, highly conserved RNA- and DNA-binding nuclear protein (32). Under pathological conditions TDP-43 can be sequestered into the cytoplasm and cleaved into C-terminal fragments that are abnormally hyperphosphorylated and subsequently aggregate forming intracellular inclusions. These inclusions are observed as neuronal cytoplasmic inclusions (NCIs), neuronal intranuclear inclusions (NIIs) and/or dystrophic neurites (DNs) and are collectively referred to as TDP-43 pathology.

Although TDP-43 pathology is a hallmark pathology associated with a subtype of fronto-temporal lobar degeneration (FTLD)-TDP (4) and motor neuron disease (30), it is also present in Alzheimer's disease (AD) (1). The neuropathological hallmark lesions of AD include intracytoplasmic neurofibrillary tangles (NFTs) and dendritic and axonal neuropil threads (NTs) that are composed of aggregated hyperphosphorylated microtubule associated tau (HP-τ) (14, 26), extracellular depositions of amyloid-β protein (Aβ) and neuritic plaques consisting of Aβ and HP-τ in DNs (9). Prevalence rates of TDP-43 pathology in AD vary between 29% and 72% (1, 2, 8, 19–22, 38, 39), however, it is generally accepted that TDP-43 pathology in AD is frequent (22) and contributes to the AD neurodegenerative process (21). The presence of TDP-43 pathology in AD has previously been shown to modify the clinical and radiological phenotype: AD subjects with TDP-43 pathology had more severe cognitive impairment (21), with specific deficits in episodic and working memory (38, 39) and language domains (22) and greater hippocampal atrophy, as seen on MRI (19), compared to AD subjects lacking TDP-43 pathology.

The distribution of TDP-43 pathology differs between AD and FTLD-TDP, with the amygdala being the first and most frequently affected region in AD (12, 13, 20). The progression of TDP-43 pathology in AD follows a stereotypical pattern of deposition that has been reported and validated by Josephs and colleagues (20) who proposed a TDP-43 in AD staging scheme: TDP-43 pathology initially manifests in the amygdala (stage I), followed by the entorhinal cortex and/or subiculum (stage II), dentate gyrus and/or occipitotemporal cortex (OTC) (stage III), inferior temporal cortex (ITC) (stage IV) and finally the mid-frontal cortex and/or striatum (stage V). Here, the term TDP-43 pathology refers only to TDP-43 deposition in AD and not to the hallmark pathology of FTLD-TDP and motor neuron disease.

TDP-43 pathology has also been identified in dementia with Lewy bodies (DLB), which is neuropathologically characterized by the presence of α-synuclein (α-syn) as intracytoplasmic aggregates, that is, Lewy bodies (LB) and dendritic and axonal Lewy neurites (LN) (33); although different studies have reported highly divergent prevalence rates, ranging from 0% (27) to 56% (2). The neuroanatomical distribution of TDP-43 pathology in DLB has been shown to be similar to that seen in AD, as it predominantly affects the amygdala and hippocampal structures (12).

Multiple pathological lesions are frequently seen in *post-mortem* brains of nondemented and demented elderly subjects, and in the latter group mixed pathology is rather the rule than the exception (16, 34, 35). Mixed dementia, conversely, is less frequent and should neuropathologically only be diagnosed if criteria for more than one full-blown disease are met as in mixed AD/DLB (Mx AD/DLB), which fulfills neuropathological criteria for both AD (26) and DLB (25). One study found TDP-43 pathology in 31% of Mx AD/DLB (27). However, it is not known whether Mx AD/DLB cases show the same severity and topographical progression pattern of TDP-43 pathology as "pure" AD cases, which exhibit no/minimal concomitant neurodegenerative or cerebrovascular pathology.

Ageing renders the brain vulnerable to pathological insults and accumulation of HP-τ, Aβ and α-syn pathology frequently occurs in normal aged individuals without compromising cognitive function (5, 15, 18). TDP-43 pathology has been shown to occur in cognitively normal aged subjects with prevalence rates ranging between 10.5% and 36.4% (3, 28). TDP-43 pathology increased with age in control cases, while it was absent in subjects below the age of 65 (11), suggesting that TDP-43 pathology occurs partly as consequence of ageing.

It is indeed conceivable that both ageing and neurodegenerative processes are independently associated with the presence and severity of TDP-43 pathology, but also may have a synergistic effect with advancing age rendering neurons vulnerable to TDP-43 pathology that may be exacerbated by other concomitant neurodegenerative diseases. However, data from previous studies on TDP-43 pathology in age-associated neurodegeneration cannot be compared as these studies have employed different methodologies.

Here, we investigate and compare the prevalence, severity and topographical distribution of TDP-43 pathology in subjects with AD, DLB, Mx AD/DLB and cognitively normal aged controls using a consistent staging scheme (20) and evaluate the relationship between stage of TDP-43 pathology and age at death, the severity of HP-τ, Aβ and α-syn pathology, as well as clinical measures of cognition.

MATERIALS AND METHODS

For this study we used a consecutive series of 119 human *post-mortem* brains from demented and nondemented elderly (mean age 81.39 ± 9.6 years; male: 65, female: 54) in whom AD pathology was assessed according to the National Institute on Aging-Alzheimer's Association (NIA-AA) criteria (26). Of note, cases with familial or rare neurodegenerative diseases (eg, motor neuron disease, progressive supranuclear palsy) were excluded. Brain tissue was obtained at autopsy and stored within the Newcastle Brain Tissue Resource (NBTR) in accordance with Newcastle University Ethics Board (The Joint Ethics Committee of Newcastle and North Tyneside Health Authority, reference: 08/H0906/136). After autopsy the right hemisphere, brainstem and cerebellum were immersion fixed in 4% buffered aqueous formaldehyde solution for 6 weeks. Irrespective of clinical diagnoses, all brains underwent neuropathological assessment and were stratified by neuropathogical diagnosis. Fourty-six cases fulfilled neuropathological criteria for high AD neuropathological change according to NIA-AA criteria (26), 15 for DLB (inclusive of DLB and Parkinson's disease dementia clinical phenotypes) according to McKeith Lewy body criteria (25) [limbic/neocortical Lewy body disease; Braak Lewy body stage, 4 or higher (6)], 19 for Mx AD/DLB [high AD neuropathologic change (26) and limbic/neocortical Lewy body disease (25)] and 39 clinically nondemented cases showed no to moderate degrees of neurodegenerative pathology. The presence and type of cerebral amyloid angiopathy (CAA) (36) and presence of hippocampal sclerosis were noted. For neuropathological details see Table 1.

During life, all dementia subjects underwent clinical assessments by board certified Old Age Psychiatrists or Neurologists and most were assessed in prospective research studies with repeated cognitive evaluation including Mini-Mental State Examination (MMSE; AD: 37, DLB: 15, Mx AD/DLB: 14) (10). Aged control subjects were assessed clinically and the absence of dementia confirmed but did not have repeated cognitive evaluation. The rate of cognitive decline (31) was determined for dementia cases with more than one MMSE score available. Age of onset of cognitive decline was also recorded in 76 patients (AD: 40, DLB: 14, Mx AD/DLB 17, aged controls: 5).

Tissue preparation

6 μm paraffin-embedded sections were taken from the amygdala, subiculum and entorhinal cortex (BA 36, 28), dentate gyrus of the posterior hippocampus, OTC (BA 36), ITC (BA 20), mid-frontal cortex (BA 8, 9) and basal ganglia, that is, putamen, globus pallidus and caudate. Sections were mounted onto 4% 3-aminopropyltriethoxysilane (APES)-coated glass slides and immunohistochemistry was performed for phosphorylated TDP-43 (antibody phospho-TDP-43 (pS0409/410-2; dilution 1:10 000; Cosmo Bio Ltd, Bicester, UK). Prior to immunostaining, antigen retrieval was performed by microwaving slides in 0.01 mL EDTA for 10 minutes. Immunopositivity was detected using the Menarini X-Cell-Plus HRP Detection Kit (Menarini Diagnostics, Winnersh-Wokingham, UK) with 3,3-diaminobenzidine (DAB) as a chromagen and haematoxylin as a counter stain. Sections were subsequently dehydrated through a series of alcohols, cleared and mounted using DPX (CellPath, Powys, UK).

Assessment of TDP-43 pathology

Neuropathological assessment of TDP-43 pathology was performed according to the TDP-43 in AD staging scheme (20). Briefly, TDP-43 pathology was examined by assessing TDP-43 immunoreactivity of NCIs, NIIs and DNs in sections of the amygdala, entorhinal cortex, subiculum, dentate gyrus, OTC, ITC, mid-frontal cortex and basal ganglia. The severity of TDP-43 pathology in the amygdala was graded on a five-tiered scale (0, absent; 1, scant; 2, sparse; 3, moderate; 4, frequent). Classification of stages of TDP-43 deposition was as follows: stage I, scant to sparse

Table 1. Characteristics of study cohort.

	AD	DLB	Mx AD/DLB	Controls
Case n	46	15	19	39
Age at death (mean ± years)	83.5 (± 8.4)	81.0 (± 5.3)	78.7 (± 9.3)	80.4 (± 12)
Sex (M : F)	23:23	9:6	13:6	20:19
Braak NFT stage (5)	NFT stage 5, n = 3 NFT stage 6, n = 43	NFT stage 1, n = 1 NFT stage 2, n = 1 NFT stage 3, n = 10 NFT stage 4, n = 3	NFT stage 5, n = 2 NFT stage 6, n = 17	NFT stage 0, n = 7 NFT stage 1, n = 7 NFT stage 2, n = 14 NFT stage 3, n = 10 NFT stage 4, n = 1
Thal Abeta phase (37)	Phase 4, n = 5 Phase 5, n = 41	Phase 0, n = 1 Phase 1, n = 1 Phase 2, n = 1 Phase 3, n = 5 Phase 4, n = 5 Phase 5, n = 2	Phase 4, n = 4 Phase 5, n = 15	Phase 0, n = 11 Phase 1, n = 13 Phase 2, n = 7 Phase 3, n = 4 Phase 4, n = 3 Phase 5, n = 1
CERAD score (26)	B, n = 3 C, n = 43	negative, n = 7 B, n = 8	C, n = 19	negative, n = 34 A, n = 4 B, n = 1
Alzheimer's disease neuropathologic change (NIA-AA) (26)	High, n = 46	Not, n = 1 Low, n = 4 Intermediate, n = 10	High, n = 19	Not, n = 12 Low, n = 25 Intermediate, n = 2
Cerebral amyloid angiopathy (36)	Absent, n = 1 Type 1, n = 21 Type 2, n = 24	Absent, n = 5 Type 1, n = 0 Type 2, n = 10	Absent, n = 1 Type 1, n = 6 Type 2, n = 12	Absent, n = 21 Type 1, n = 3 Type 2, n = 15
Braak LB stage (6)	LB stage 0, n = 41 LB stage 1, n = 1 LB stage 2, n = 2 LB stage 3, n = 1 LB stage 4, n = 1	LB stage 4, n = 2 LB stage 5, n = 3 LB stage 6, n = 10	LB stage 4, n = 3 LB stage 5, n = 2 LB stage 6, n = 14	LB stage 0, n = 35 LB stage 1, n = 1 LB stage 2, n = 1 LB stage 3, n = 2
McKeith criteria (25)	No LBD, n = 37 Brainstem, n = 2, Limbic, n = 7	Limbic, n = 3 Neocortical, n = 12	Limbic, n = 5 Neocortical, n = 14	No LBD, n = 36 Brainstem, n = 2, Limbic, n = 1
Hippocampal Sclerosis	Absent, n = 38 Present, n = 8	Absent, n = 15 Present, n = 0	Absent, n = 18 Present, n = 1	Absent, n = 37 Present, n = 2

Abbreviations: AD = Alzheimer's disease; DLB = dementia with Lewy bodies; Mx AD/DLB = mixed Alzheimer's disease and dementia with Lewy bodies; Case n = case number; M = male; F = female; NFT = neurofibrillary tangle; Aβ = amyloid-beta; LB = Lewy body.

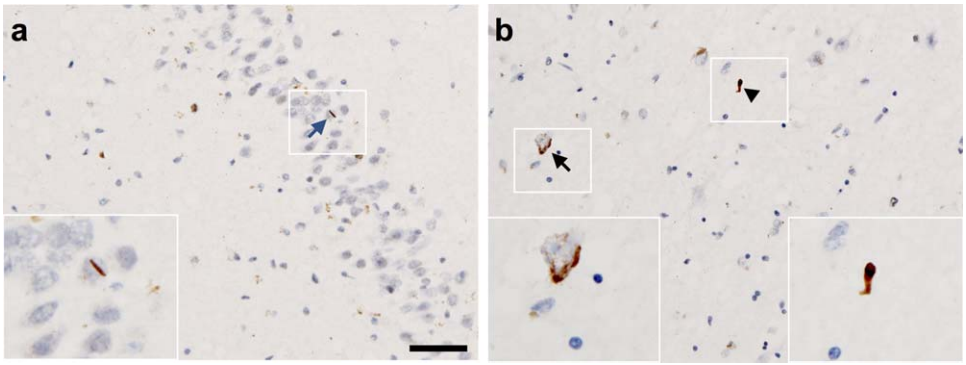

Figure 1. Photomicrograph illustrating types of TDP-43 pathology observed in the study, taken from a mixed AD/DLB case. **A.** Highlights a neuronal intranuclear inclusion (NII)—blue arrow, seen in the dentate gyrus of the hippocampus (stage III). **B.** Demonstrates a neuronal cytoplasmic inclusion (NCI)—black arrow, and dystrophic neurite (DN)—black arrowhead seen in the amygdala (stage I). Scale bar in (A) represents 50 μm and is valid for (B).

deposition in the amygdala; stage II, moderate to frequent deposition in the amygdala and entorhinal cortex/subiculum; stage III, deposition in the amygdala, entorhinal cortex/subiculum and dentate gyrus or OTC; stage IV, deposition in the amygdala, entorhinal cortex/subiculum, dentate gyrus/OTC and ITC; stage V, deposition in the amygdala, entorhinal cortex/subiculum, dentate gyrus/OTC, ITC and mid-frontal cortex or basal ganglia. The TDP-43 in AD stage for each case was determined by consensus between two assessors.

Statistical analysis

The Statistical Package for Social Sciences software (SPSS ver. 21) was used for statistical evaluation. Variables were tested for normality using the Shapiro-Wilk test and visual inspection of variable histograms. Our data were not normally distributed; therefore, non-parametric procedures were employed. X^2 and Fisher's exact test were employed to assess differences in TDP-43 prevalence (indicated by TDP-43 pathology in the amygdala) between the disease groups. Spearman's (ρ) correlation coefficient (two tailed) was used to assess associations between TDP-43 stage with age at death, Braak NFT stage, Thal Aβ phase and Braak Lewy body stage. Partial Spearman's (ρ') correlation coefficient (one tailed) was used to assess associations between TDP-43 stage with age at death (controlling for Braak NFT stage) and Braak NFT stage (controlling for age at death). Group effects on age were assessed using Kruskal-Wallis and clinical measurements were assessed using a Mann-Whitney U tests, respectively. *P*-values below 0.05 were accepted as statistically significant.

RESULTS

No significant differences in age at death were found between any of the disease groups. In the entire cohort, cases with TDP-43 pathology were significantly older at death compared to cases without TDP-43 pathology ($P = 0.008$). When stratified by neuropathopathological diagnosis, only the control group cases with TDP-43 pathology were significantly older compared to cases without TDP-43 pathology ($P = 0.005$), while no such differences were seen in AD ($P = 0.117$), DLB ($P = 0.099$), or Mx AD/DLB ($P = 0.720$) groups.

Prevalence of TDP-43 pathology

TDP-43 pathology (Figure 1) was observed in 34 AD cases (73.9%), 5 DLB cases (33.3%), 10 Mx AD/DLB cases (52.6%) and 7 aged controls (17.9%). The prevalence of TDP-43 pathology was significantly higher in AD and Mx AD/DLB compared to aged controls (AD vs. aged controls, $X^2 = 24.28$, $P = 0.0001$; Mx AD/DLB vs. aged controls, $X^2 = 5.838$, $P = 0.016$) and in AD compared to DLB ($X^2 = 6.414$, $P = 0.011$). However, no such significant differences were seen between AD and Mx AD/DLB ($X^2 = 1.9$, $P = 0.168$), Mx AD/DLB and DLB ($X^2 = 0.604$, $P = 0.437$) as well as DLB and aged controls ($P = 0.279$, Fisher's exact test).

Severity of TDP-43 pathology

TDP-43 in AD stages for each disease group are shown in Table 2. TDP-43 pathology reached highest stages in AD cases; of 34 AD cases exhibiting TDP-43 pathology, a considerable proportion

Table 2. Prevalence and severity of TDP-43 pathology.

TDP-43 in AD stage (21)	Negative	I	II	III	IV	V
AD n, (%)	12 (26.1)	10 (21.7)	6 (13)	4 (8.7)	13 (28.3)	1 (2.2)
DLB n, (%)	10 (66.7)	4 (26.7)	0	0	1 (6.6)	0
Mx AD/DLB n, (%)	9 (47.4)	2 (10.5)	2 (10.5)	5 (26.4)	1 (5.2)	0
Controls n, (%)	32 (82.1)	2 (5.1)	1 (2.5)	1 (2.5)	3 (7.8)	0

Abbreviations: AD = Alzheimer's disease; DLB = dementia with Lewy bodies; Mx AD/DLB = mixed Alzheimer's disease and demential with Lewy bodies.

Table 3. Correlations between TDP-43 in AD stage with age and cortical pathology stages.

	AD	DLB	Mx AD/DLB	Controls
	TDP-43 in AD stage (21)	TDP-43 in AD stage	TDP-43 in AD stage	TDP-43 in AD stage
Age at death	$\rho = 0.175$, $P = 0.245$	$\rho = -0.485$, $P = 0.067$	$\rho = 0.099$, $P = 0.688$	$\rho = 0.448$, $P = 0.004$**
Braak NFT stage (8)	$\rho = 0.139$, $P = 0.357$	$\rho = 0.372$, $P = 0.172$	$\rho = 0.100$, $P = 0.683$	$\rho = 0.370$, $P = 0.020$*
Thal Aβ phase (39)	$\rho = 0.051$, $P = 0.735$	$\rho = -0.139$, $P = 0.622$	$\rho = -0.378$, $P = 0.111$	$\rho = 0.145$, $P = 0.378$
Braak LB stage (7)	$\rho = -0.069$, $P = 0.651$	$\rho = 0.296$, $P = 0.304$	$\rho = 0.189$, $P = 0.437$	$\rho = -0.148$, $P = 0.376$

Abbreviations: NFT = neurofibrillary tangle; Aβ = amyloid-beta; LB = Lewy body; AD = Alzheimer's disease; DLB = dementia with Lewy bodies; Mx AD/DLB = mixed Alzheimer's disease and demential with Lewy bodies; ρ = Spearman's rank correlation coefficient; N/A = data not available. TDP-43 stage in AD determined as negative to stage V.

*P < 0.05.

**P < 0.01.

reached higher stages IV and V (38.2% stage IV and 3% stage V) while 29.4% showed stage I, 17.6% stage II and 11.8% stage III. By contrast, one of five DLB cases with TDP-43 pathology showed stage IV (20%), and the remaining four cases (80%) reached stage I only. Of the 10 Mx AD/DLB cases with TDP-43 pathology, 20% were stage II, 20% were stage I, while 50% showed stage III and 10% stage IV. Finally, seven aged controls exhibited TDP-43 pathology with 28.5% at stage I, 14.3% in stage II and III each and 42.9% at stage IV.

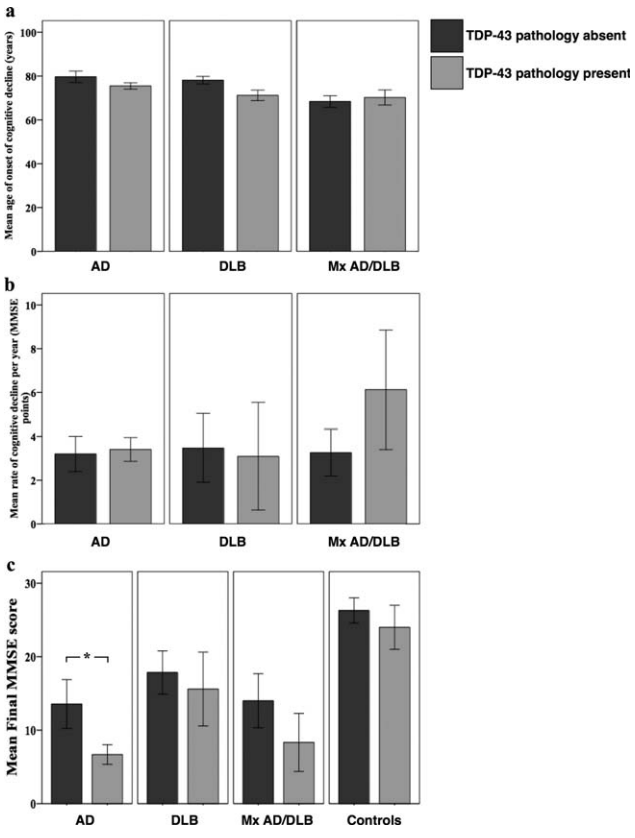

Figure 2. Age of onset of cognitive decline, rate of cognitive decline per year and final MMSE score in cases with and without TDP-43 pathology. **A.** No significant differences were seen in age of onset between cases with and without TDP-43 pathology in AD, DLB and Mx AD/DLB. **B.** No significant difference were seen in rate of cognitive decline per year between cases with and without TDP-43 pathology in AD, DLB and Mx AD/DLB. **C.** Final MMSE score was significantly lower in AD cases with TDP-43 pathology compared to AD cases without TDP-43 pathology. No significant difference was seen in DLB, Mx AD/DLB or controls. AD, Alzheimer's disease, DLB, dementia with Lewy bodies; Mx AD/DLB, mixed AD/DLB; *, $P < 0.05$.

Associations between TDP-43 in AD stage, age and severity of AD and DLB pathology

We investigated whether age at death, Braak NFT stage, Thal Aβ phase or Braak LB stage was associated with the TDP-43 in AD stages (absent—stage V). A higher TDP-43 in AD stage correlated with both age at death ($\rho = 0.448$, $P = 0.004$) and Braak NFT stage ($\rho = 0.370$, $P = 0.020$) in the control group only, while no such correlations were seen in any of the disease groups (Table 3).

We further investigated the correlations between TDP-43 in AD stage with age at death and Braak NFT stage in the control group using partial Spearman's correlation analysis (ρ'). The correlation between TDP-43 in AD stage and age at death remained statistically significant when controlled for Braak NFT stage ($\rho' = 0.297$, $P = 0.041$), while no correlation was seen between TDP-43 in AD and Braak NFT stages when the analysis was controlled for age at death ($\rho = 0.125$, $P = 0.236$).

In the seven control cases with TDP-43 pathology the TDP-43 in AD stage and AD neuropathologic change (NIA-AA criteria) were stage IV and "low" (n = 3), stage III and "intermediate" (n = 1) stage II and "low" (n = 1), stage I and "low" (n = 1) and stage I and "no" (n = 1).

TDP-43 pathology and clinical measures of cognitive impairment

In AD, DLB and Mx AD/DLB there was no significant difference between age of onset of cognitive decline (Figure 2A) as well as rate of cognitive decline between cases with and without TDP-43 pathology (Figure 2B). However, AD cases with TDP-43 pathology had significantly lower final MMSE scores compared to AD cases lacking TDP-43 pathology ($P = 0.041$: Figure 2C), while no such significant differences were seen in DLB, Mx AD/DLB and aged controls.

Additional pathological observations

CAA was observed in 45 AD cases (97.8%), 10 DLB cases (66.7%), 18 Mx AD/DLB cases (94.7%) and 18 aged control cases

(46.2%) (Table 1). Hippocampal sclerosis was present in 11 cases (AD, 8; Mx AD/DLB, 1; aged control cases, 2) which all exhibited TDP-43 pathology in the amygdala. TDP-43 pathology was present in the hippocampus in ten cases (stage III), of which eight progressed to ITC (stage IV: AD, 6; aged control cases, 2). In general, 17.9% of cases exhibiting TDP-43 pathology had hippocampal sclerosis.

DISCUSSION

Here, we investigated TDP-43 pathology in a cohort of AD, DLB, Mx AD/DLB and cognitively normal aged controls using the TDP-43 in AD staging scheme. This is the first study to record the prevalence of TDP-43 pathology in AD, DLB, Mx AD/DLB and aged control cases using a consistent staging scheme, that is, TDP-43 in AD staging scheme (20). We have confirmed that TDP-43 pathology is not exclusively found in AD, but is found across the spectrum of age-related neurodegenerative diseases and in aged controls, where its spread follows the same topographical pattern that has been described for TDP-43 pathology in AD. Our findings suggest that age has an independent role in the onset and development of TDP-43 pathology. However, development of TDP-43 pathology may also be exacerbated by the presence of AD pathology, as indicated by the higher prevalence of TDP-43 pathology in AD and Mx AD/DLB.

Previously reported prevalence rates of TDP-43 pathology in neurodegenerative diseases range from 29% to 72% in AD (1, 2, 8, 19–22, 38, 39), from 0% to 56% in DLB (2, 12, 27, 38, 39) and from 10.5% to 36.4% in controls (3, 11, 27, 28, 37, 38), while only one study has been conducted in Mx AD/DLB showing a respective prevalence rate of 31% (27). While our data support previous studies, our reported TDP-43 pathology prevalence rates for AD, DLB and aged controls are comparatively higher. We suggest that previously reported lower prevalence rates may be partly caused by inadequate sampling as some studies did not assess TDP-43 pathology in the amygdala (8, 27), which has been shown to be the first and most severely affected region (12, 13, 20). Furthermore, rather than using antibodies specific for phosphorylated TDP-43, some studies (12, 27) used antibodies that target the nonphosphorylated epitope and such an approach has been suggested to result in an underestimation of TDP-43 pathology (38, 39).

Concomitant TDP-43 pathology has been shown to modify the clinical phenotype of AD since AD subjects with TDP-43 pathology had more severe cognitive impairment compared to AD subjects lacking TDP-43 pathology (21). In our cohort, MMSE scores were lower in AD subjects with TDP-43 pathology compared to those lacking TDP-43 pathology, which is in agreement with findings from Josephs and colleagues (19). It is unclear whether the onset and evolution of TDP-43 pathology in AD is associated with the fundamental neurodegenerative process of AD, or merely reflects age associated changes and has to be regarded as part of cerebral multimorbidity, which is frequently seen in aged human brains (15, 17, 23, 24, 34).

In our study the prevalence of TDP-43 pathology in AD was almost 74%, which was significantly higher than in DLB and controls. Moreover, AD subjects showed highest stages of TDP-43 pathology (20). However, there was no significant difference regarding the prevalence of TDP-43 pathology between AD and Mx AD/DLB and the latter had more advanced TDP-43 pathology than DLB. This may suggest a synergistic relationship between classical AD pathology, (ie, HP-τ and Aβ) and TDP-43 pathology as the combination of AD and DLB pathology (ie, α-syn)—but not DLB alone—is associated with an increase in both prevalence and severity of TDP-43 pathology. HP-τ has previously been suggested as a possible risk factor for the development of TDP-43 pathology as a small number of NFTs have been shown to colocalise with cytoplasmic TDP-43 inclusions and were associated with reduced nuclear TDP-43 immunopositivity (27). We were unable to determine associations between TDP-43 in AD stage and Braak NFT stage in AD cases, likely due to a ceiling effect as all AD cases were classified as Braak NFT stage V/VI. Braak NFT stages are based on semi-quantitative assessment and, therefore, quantitative methods could potentially help to clarify if the development of TDP-43 pathology is directly influenced by HP-τ pathology.

Although we found a correlation between TDP-43 in AD stages and Braak NFT stages in aged controls this was not significant when controlled for age of death. Also, subjects with TDP-43 pathology were significantly older than those without TDP-43 pathology. However, when stratified by neuropathological diagnosis, only the difference in control subjects remained significant, suggesting that TDP-43 pathology is associated with increasing age and can occur independently of concomitant AD pathology. A neuropathological study by Davidson and colleagues showed that the prevalence of TDP-43 pathology was significantly higher in sporadic late onset AD (over 65 years of age) compared to sporadic early onset AD (under 65 years of age with no associated genetic mutations) (8), indicating an influence of age on the development of TDP-43 pathology in AD. Furthermore, in our study no independent association between AD pathology and TDP-43 pathology was seen in control cases, while age was positively correlated with TDP-43 pathology. Conversely, we found a high prevalence and severity of TDP-43 pathology in AD and Mx AD/DLB and therefore, we suggest that while age plays an important role in the onset of TDP-43 pathology it may be exacerbated by concomitant AD pathology.

Of the cases exhibiting hippocampal sclerosis, 91% were positive for TDP-43 in the hippocampus in agreement with previous studies indicating an association between the presence of hippocampal sclerosis and TDP-43 deposition (1, 8, 19, 29). Although hippocampal sclerosis likely influences cognitive decline and cortical atrophy when present in AD (7, 29), an association between TDP-43 pathology with cognitive decline, memory impairment and medial temporal atrophy have been shown to occur independent of hippocampal sclerosis (21). The presence of TDP-43 without hippocampal sclerosis was evident in our cohort as only 17.9% of cases that exhibited TDP-43 pathology had co-existing hippocampal sclerosis, indicating the development of TDP-43 pathology can be independent of hippocampal sclerosis.

In conclusion we employed a consistent methodology to evaluate the prevalence of TDP-43 pathology in AD, DLB, Mx AD/DLB and aged controls. We confirmed that TDP-43 pathology is not exclusively seen in AD, but is also present in DLB, Mx AD/DLB and aged controls, where its topographical progression follows the pattern described for TDP-43 pathology in AD (20). In addition, our data suggest that TDP-43 pathology is associated with age and exacerbated by the presence of concomitant AD pathology. Further studies are warranted to elucidate the interactions between

AD and TDP-43 pathology and to clarify the implications of additional TDP-43 pathology for patient stratification, the development of biomarkers and novel therapies against age associated neurodegeneration.

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AUTHOR CONTRIBUTIONS

Study concept and design was conceived by K.E.M, L.W, D.E and J.A. Data acquisition, collection, analysis and interpretation were carried out by K.E.M, L.W and D.E. Clinical data was collected by A.J.T and I.G.M. K.E.M drafted the manuscript with critical revisions from L.W, D.E, A.T, I.M and J.A. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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