Concomitant LATE-NC in Alzheimer’s disease is not associated with increased tau or amyloid-β pathological burden

K. E. McAleese, L. Walker, D. Erskine, M. Johnson, D. Koss, A. J. Thomas and J. Attems

Translation and Clinical Research Institute, Newcastle University, Newcastle Upon Tyne, UK

Aims: Limbic-predominant age-related TDP-43 encephalopathy neuropathological change (LATE-NC) is present in approximately 50% of Alzheimer’s disease (AD) cases and is associated with accelerated cognitive decline. Studies indicate a potential synergistic relationship between LATE-NC and hyperphosphorylated tau. It is unknown if LATE-NC is an independent driver of cognitive impairment or exerts its influence through synergistic relationships with tau. This cliniconeuropathological study investigated the impact of LATE-NC on quantified measures of AD-associated pathology and its impact on clinical measures. Methods: A total of 61 AD cases underwent neuropathological assessment for LATE-NC and quantitative assessment [area covered by immunoreactivity (IR)] for early conformational tau (MC-1), late-stage hyperphosphorylated tau (AT8) and amyloid-β in the amygdala and five neocortical regions. Clinical measures included age of disease onset, final Mini-Mental State Examination (MMSE) score and rate of cognitive decline. Results: LATE-NC was present in 41 AD cases (AD/LATE-NC: 67.2%). No significant differences in MC-1-IR, AT8-IR or 4G8-IR were observed in any region between AD/LATE-NC and AD without LATE-NC, indicating no accelerated aggregation or hyperphosphorylation of tau proteins in the AD/LATE-NC cases. Final MMSE was significantly lower in AD/LATE-NC cases and was significantly associated with LATE-NC score even when controlled for the presence of both MC-1-IR and AT8-IR (P = 0.009). Conclusion: The presence of LATE-NC in AD is not associated with an increase in the burden of early or late tau or Aβ pathology. LATE-NC is associated with a lower final MMSE score independent of tau pathology.

Keywords: Alzheimer’s disease, LATE-NC, TDP-43, tau pathology

Introduction

Alzheimer’s disease (AD) is the most common age-related neurodegenerative dementia subtype and neuropathologically characterized by intracellular inclusions of aggregated hyperphosphorylated tau [1,2], in the form of neurofibrillary tangles (NFTs) and dendritic/axonal neunopil threads (NTs), extracellular depositions of amyloid-β protein (Aβ) and neuritic plaques, consisting of an Aβ core surrounded by hyperphosphorylated tau containing dystrophic neurites [3]. Approximately 50% (range 29–74%) of neuropathologically confirmed AD cases exhibit additional intracellular inclusions of phosphorylated transactive response DNA-binding protein 43 (TDP-43) [4–13]. Under
pathological conditions, the TDP-43 protein is phosphorylated and cleaved to generate c-terminal fragments that aggregate in the cytoplasm of neurones. Neuronal TDP-43 inclusions and dystrophic neurites are the neuropathological hallmark lesions of a subtype of frontotemporal lobar degeneration (FTLD)-TDP [14] and motor neurone disease (MND) [15] distributed throughout the neocortex, hippocampal dentate gyrus, deep grey matter and brain stem, as well as the spinal cord in MND [16]. However, such TDP-43 pathology has also been shown in ageing and AD, where it initially manifests in the amygdala and then spreads to other limbic, cortical and subcortical regions [17]. This type of TDP-43 pathology was recently termed limbic-predominant age-related TDP-43 encephalopathy neuropathological change (LATE-NC) [18]. The presence of LATE-NC in AD is associated with accelerated rates of cognitive decline [10], more pronounced deficits in episodic and working memory [11] and language domains [9], as well as greater hippocampal atrophy [6,19] compared to individuals with AD without LATE-NC.

A pathogenic link between TDP-43 and hyperphosphorylated tau was suggested by a study by Davis and colleagues that revealed the overexpression of phosphorylated TDP-43 in an APP/presenilin 1 mutation transgenic mouse model of AD resulted in increased hyperphosphorylated tau immunoreactivity (IR) [20]. Furthermore, a recent in vitro study revealed that physiological TDP-43 may have a suppressive role in the regulation of tau mRNA [21]; therefore, the phosphorylation of TDP-43 may lead to a ‘loss of function’ resulting in increased production of tau mRNA and subsequent translation of tau proteins available for hyperphosphorylation. Human neuropathological studies employing confocal and electron microscopy have indicated the co-localization of NFTs and TDP-43 cytoplasmic inclusions in neurones [22,23], specifically in the entorhinal cortex [4] and dentate gyrus [24]. Recently, two distinct subtypes of non-FTLD TDP-43 pathology were reported, namely TDP type-α inclusions, which are not associated with NFTs, and TDP type-β inclusions that are associated with NFTs within the same neurone, of which the latter was associated with AD pathology, specifically hyperphosphorylated tau pathology [25]. NFT/NT burden has been shown to correlate with the cognitive deficits and clinical progression of AD [26], as well as hippocampal atrophy [19,27,28]. Therefore, one may speculate that the interaction of TDP-43 with hyperphosphorylated tau within the neurones may result in synergistic interactions that accelerate the production and aggregation of hyperphosphorylated tau pathology, accounting for the accelerated cognitive decline observed in AD with LATE-NC.

Using a cohort of post mortem brain tissue from individuals neuropathologically confirmed as AD, we aim to determine, (i) if the pathological burdens of early conformational and late hyperphosphorylated tau or Aβ differ between AD cases with LATE-NC (AD/LATE-NC) and AD cases without LATE-NC; (ii) if LATE-NC stage is associated with early/late tau or Aβ pathology burdens; and (iii) if the presence of LATE-NC, tau or Aβ pathological burdens impact the clinical measures of cognition.

Materials and methods

Study cohort

Our study cohort consisted of 61 consecutive human post mortem brains (mean age 84.79 ± 7.81 years; male: 30, female: 31), which were donated to the Newcastle Brain Tissue Resource (NBTR) between January 2009 and January 2018 and had a cliniconeuropathologically confirmed diagnosis of AD. During life, all subjects underwent clinical assessments and were clinically diagnosed by board-certified Old Age Psychiatrists or Neurologists. All subjects underwent a review of clinical records after death at NBTR (AJT). Brain tissue was obtained at autopsy and stored within the 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 10% buffered aqueous formaldehyde solution for 4 weeks and subsequently dissected in coronal planes at approximately 0.7 cm intervals and paraffin-embedded. All brains underwent neuropathological assessment according to the National Institute on Aging-Alzheimer’s Association (NIA-AA) criteria [2] that included assessment of Thal phases of Aβ deposition [29], Braak staging of neurofibrillary pathology [30] and scoring of neuritic plaques according to the Consortium to Establish a Registry for Alzheimer’s Disease (CERAD) [31]. In addition, cases were assessed for
Lewy body pathology [32], the contribution of vascular pathology to cognitive impairment [using the vascular impairment neuropathological guidelines (VCING) [33]] and the absence/presence of hippocampal sclerosis, defined as severe pyramidal cell loss and gliosis in the CA1 and subiculum of the hippocampal formation that is out of proportion to AD neuropathological change in the same structures [2].

**Tissue preparation and immunohistochemistry**

For the assessment of LATE-NC, 6 μm paraffin-embedded sections were cut that included the amygdala, subiculum and entorhinal cortex (BA 36, 28), dentate gyrus of the posterior hippocampus, occipitotemporal cortex (OTC) (BA 36), inferior temporal cortex (ITC; BA 20) and basal ganglia, that is, putamen, globus pallidus and caudate with associated insular cortex, substantia nigra, midbrain tectum and mid-frontal cortex (BA 8, 9). Sections were mounted onto 4% 3-aminopropyltriethoxysilane (APES)-coated glass slides, and antigen retrieval by microwaving slides in 0.01 ml EDTA for 10 min was performed prior to immunohistochemistry with phosphorylated TDP-43 (antibody phospho-TDP-43; pS0409/410-2; dilution 1:10,000; Cosmo Bio Ltd, Bicester, UK). For assessment of early tau, late tau and Aβ pathology, 6 μm sections were cut from a preconstructed tissue microarray (TMA) paraffin block (see below for details) [34], which contains 40 × 3 mm tissue core punches inclusive of frontal, entorhinal, temporal, parietal and occipital cortices and amygdala. Immunohistochemistry was performed for the antibodies MC-1 (discontinuous epitope; amino acid 7-9 and 312-342; P. Davies lab: mouse monoclonal; 1:1000) to visualize ‘early stage’ changes in the adopted conformation of tau [35,36], AT8 (pSer199/202/thr205; Innogenetics, Belgium: mouse monoclonal; 1:4000), to detect hyperphosphorylated tau in the form of ‘intermediate/late-stage’ NFT/NT and extracellular NFT, that is ‘ghost tangles’ [37] and 4G8 for the detection of Aβ pathology (Amyloid 17-24; Signet Labs, Dedham, MA, USA: mouse monoclonal; 1:15 000). Prior to immunostaining, antigen retrieval was performed by microwaving slides in 0.01 ml citrate buffer for 10 min for MC-1 and AT8 and immersion in concentrated formic acid for 1 h for 4G8. Immunopositivity for all sections 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 LATE-NC**

All cases underwent neuropathological semi-quantitative assessment for the presence of phosphorylated TDP-43 inclusions according to the updated TDP-43 in AD staging scheme [17] as recommended for research by the LATE consensus working group report [18]. TDP-43 IR is collectively observed as neuronal cytoplasmic inclusions (NCI), neuronal intranuclear inclusions (NII) and/or dystrophic neurites (DN). Classification of the six-tiered stages of TDP-43 deposition was as follows: stage I, scant to sparse deposition in the amygdala; stage II, moderate to frequent deposition in the amygdala and entorhinal cortex and/or subiculum; stage III, + dentate gyrus and/or OTC; stage IV, + ITC and/or insular cortex; stage V, + substantia nigra and/or midbrain tectum; and stage VI, + mid-frontal cortex and/or basal ganglia. The TDP-43 in AD score for each case was determined by consensus between three assessors (K.E.M, L.W and D.E).

**Quantitative neuropathological assessment**

For the quantification of MC-1, AT8 and 4G8 IR aggregates, we employed our novel TMA system developed at Newcastle University [34,38–40] that allows for high-throughput quantitative neuropathological assessment. Our in-house constructed TMA block includes 40 × 3 mm punch biopsies from various diagnostically relevant regions from donor paraffin blocks from the same cases, which are placed into a recipient paraffin block. The TMA slides were stained with MC-1, and AT8 and 4G8 antibodies were quantitatively assessed using the automated image analysis protocol previously described [34]. Of note, TMA slides were not used for quantification of TDP-43 pathology as regions required for assessment according to the TDP-43 in AD staging scheme [17], that is, subiculum, dentate gyrus, OTC and brain stem nuclei were not incorporated into the TMA block. Briefly, using a Nikon Eclipse 90i microscope coupled with NIS...
Elements software v 3.0 (Nikon), each of the 40 tissue samples have 3 × 3 single images captured at 100x magnification and combined into one large image (total area of 1.7 mm²). If necessary, large images were subjected to manual setting of regions of interest to exclude residual white matter, large blood vessels, meningeal structures and anomalies. Standardized and bespoke Red Green Blue thresholds were applied separately for MC-1, AT8 and 4G8 positive pathology. In addition, size restriction threshold for the assessment of 4G8 was applied that excluded the measurement of IR signals with an area below 100 μm² to ensure that physiological APP, which is stained with the 4G8 antibody, was not included in the measurement. The percentage areas covered by MC-1, AT8 and 4G8 thresholds were measured per sample and the mean regional values for the frontal, entorhinal, temporal, parietal, occipital cortical and amygdala samples were calculated and are expressed as MC-1-IR, AT8-IR and 4G8-IR respectively.

Clinical measures
Age at disease onset was recorded and cognitive evaluation during life was inclusive of the Mini-Mental State Examination (MMSE: 30-point scale) [41]. If serial MMSE scores and time interval(s) were recorded, the rate of cognitive decline (MMSE points) per year was calculated: (first MMSE-last MMSE)/years [42].

Statistics
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. Group effects were assessed using either nonparametric (Kruskal–Wallis and Mann–Whitney U) or parametric (independent samples t-test) procedures and Fisher’s exact test was employed to assess independence of categorical variables (that is, the presence of LATE-NC and hippocampal sclerosis). Where appropriate, Spearman’s (p) correlation coefficients (one-tailed) were used to assess associations between variables, and partial Pearson’s (r') correlations (two-tailed) were used to assess the associations between LATE-NC severity with clinical measures while controlling for the influence of tau pathology.

Results
Frequency of LATE-NC in AD cohort
Cohort demographics and neuropathological characteristics are presented in Table 1. Lewy body pathology in all cases was restricted to the brainstem or amygdala and VCING scores were moderate or below, and therefore, no AD case was diagnosed as a mixed disease. LATE-NC (Figure 1A–Aiili), at any stage, was present in 41 cases (67.2%). No significant differences were observed in age at death between AD/LATE-NC and AD without LATE-NC (AD). The majority of LATE-NC was restricted to limbic or paralimbic areas with 92.7% of AD/LATE-NC cases at TDP-43 in AD stage V or below. Only 7.3% of AD/LATE-NC cases had LATE-NC pathology progressing to the brain stem, basal ganglia and/or mid-frontal cortex. Hippocampal sclerosis was present in AD/LATE-NC cases only (n = 8, 19.5%), of which one was classed at TDP-43 in AD stage II, six cases were stage IV and one case was stage VI. Hippocampal sclerosis was significantly associated with both presence (P = 0.034, Fisher’s exact test) and severity of LATE-NC (P = 0.001, Fisher’s exact test; TDP-43 in AD stage I-III vs. TDP-43 in AD stage IV-VI). No association between hippocampal sclerosis and VCING score was observed (P = 0.518, Fisher’s exact test).

Clinical differences between AD/LATE-NC and AD cases
Age at disease onset was available for 55 cases (AD/LATE-NC, n = 36; AD, n = 19), final MMSE score was available in 52 cases (AD/LATE-NC, n = 34; AD, n = 15) and 42 cases (AD/LATE-NC, n = 25; AD, n = 14) had serial MMSE score and time intervals allowing for the rate of cognitive decline to be calculated. No differences in age at dementia onset (P = 0.100) or rate of cognitive decline (P = 0.597) were observed. However, final MMSE was significantly lower in the AD/LATE-NC cases (P = 0.018) (Table 1).

Group differences in early and late tau and Aβ pathology
No significant differences were observed between AD cases with and without LATE-NC in any of the
amygdala and frontal, entorhinal, temporal, parietal and occipital cortical measurements of MC-1-IR or AT8-IR (Figure 2) or 4G8-IR (Figure 3). Examples of MC-1, AT8 and 4G8 positive pathology are shown in Figure 1B-D. We then further stratified the AD/LATE-NC cases into those that were TDP-43 in AD stage ≥ IV and exhibited hippocampal sclerosis (TDP-43 ≥ IV + HpSc; n = 7) and TDP-43 in AD stage ≤ III (TDP-43 ≤ III; n = 34). We compared the mean regional pathological measures of MC-1, AT8-IR and 4G8-IR between TDP-43 ≥ IV + HpSc, TDP-43 ≤ III and AD cases without LATE-NC. No significant differences were observed in any regional measure of MC-1-IR (P > 0.286), AT8-IR (P > 0.263) or 4G8 (P > 0.085) between the three groups (Figure 4).

**Associations between LATE-NC and early and late tau and Aβ pathology**

We investigated whether TDP-43 in AD stage was associated with amygdala and frontal, entorhinal, temporal, parietal or occipital cortical values for MC-1-IR, AT8-IR and 4G8-IR. No correlations were observed between TDP-43 in AD stage with any of the six regional measures of MC-1, AT8-IR or 4G8-IR (Table 2).

**Table 1.** Cohort demographics and neuropathological and clinical characteristics

|                           | AD/LATE-NC | AD no LATE-NC | Statistics |
|----------------------------|------------|---------------|------------|
| Number of cases            | 41         | 20            |            |
| Mean age at death (years; SD) | 84.95 (7.11) | 84.45 (9.25) | t(59), P = 0.816 |
| Sex                        | 20M:21F    | 10M:10F       |            |
| Braak NFT Stage [29]       | IV, n = 1  | IV, n = 1     |            |
| Thal Phase [28]            | 0, n = 0   | 0, n = 0      |            |
| McKeith LB stage [31]      | Absent, n = 30 | Absent, n = 14 | Fisher’s, P = 0.466 |
| VCING [32]                 | Low, n = 37 | Low, n = 19   |            |
| CERAD score [30]           | B, n = 7   | B, n = 1      |            |
| TDP-43 in AD stage [17]    | I, n = 9   | 0, n = 20     |            |
| Hippocampal Scle. [2]      | Absent, n = 33 | Absent, n = 20 | Fisher’s, P = 0.034* |
| Mean age of dementia onset (years; SD) | (n = 36) | (n = 19) | t(53), P = 0.100 |
| Final MMSE score (SD)      | (n = 34)   | (n = 15)      | U(52), P = 0.018* |
| Cognitive decline (MMSE points per year) | (n = 25) | (n = 14) | U(52), P = 0.597 |

Abbreviations: AD, Alzheimer’s disease; LB, Lewy body; Scle, sclerosis. *P < 0.05.

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P = 0.049), frontal (ρ = -0.424, P = 0.001), temporal (ρ = -0.3361, P = 0.003), parietal (ρ = -0.416, P = 0.001) and occipital cortices (ρ = -0.392, P = 0.002) but not the entorhinal cortex (ρ = -0.045, P = 0.373). Furthermore, earlier age of disease onset correlated with amygdala (ρ = -0.268, P = 0.028) and occipital (ρ = -0.292, P = 0.016) 4G8-IR measures. No associations with TDP-43 in AD stage and any regional measure of MC-1-IR (P > 0.172) or any regional measure of 4G8-IR (P > 0.088).

We ran a partial correlation between TDP-43 in AD stage and final MMSE controlling for the effect of frontal, temporal, parietal and occipital MC-1-IR and frontal and entorhinal AT8-IR values; TDP-43 in AD stage...
remained significantly associated with final MMSE score ($r' = -0.449$, $P = 0.001$).

**Discussion**

In this clinicopathological study, we found that the presence of LATE-NC in AD does not increase the quantitative burdens of early or later stage tau or Aβ pathology in the amygdala or any neocortical regions, indicating that LATE-NC presence does not result in accelerated aggregation and phosphorylation of tau. In addition, the presence and severity of LATE-NC in AD were associated with lower final MMSE scores that are independent of both early and late tau pathology.

The prevalence of LATE-NC in our cohort of neuropathologically confirmed AD cases was 67%, which is in line with previous neuropathological studies [4–13]. The vast majority of the AD/LATE-NC cases had TDP-43 pathology restricted to the limbic or paralimbic region, with only three cases progressing to the middle frontal cortex or basal ganglia (TDP-43 in AD stage VI). Although not required for a diagnosis of LATE-NC [18], hippocampal sclerosis was present in eight of the 41 AD/LATE-NC cases. Human neuropathological studies
using large autopsy series have proposed a disease continuum of LATE-NC with hippocampal sclerosis associated with more severe LATE-NC stages [18,43], which is in agreement with our findings of an association between the presence of hippocampal sclerosis and more severe stages of the TDP-43 pathology. However, it must be noted that our study may have underestimated the presence of hippocampal sclerosis given approximately 50% present unilaterally [44,45] and only one hemisphere is routinely neuropathologically assessed.

Complex protein interactions within shared common pathways are implicated in both ageing and AD that can result in the pathologic phosphorylation, misfolding and aggregation of various proteins inclusive of TDP-43 and tau. Findings from in vitro models [46], human neuropathological studies [4,25,47] and transgenic C. elegans models [47] indicate a possible synergistic relationship between TDP-43 and tau. Therefore, we might expect to see differences in the pathological tau burden between AD/LATE-NC and AD cases, and an association between pathological tau load and LATE-NC stage. This study specifically investigated different stages of tau pathology, with MC-1 representing an early ‘paper clip formation’ [35,36,48] and AT8 signifying the intermediate/later stage deposition of tau phosphorylated at Serine 199/202 Threonine 205 residues [37] to determine if TDP-43 is associated with the tau protein at different stages of tau pathology development. We found that quantitative measures of both early and late tau pathology, as well as Aβ pathology, were not associated with LATE-NC stage in any of the six regions examined. In addition, no differences in regional measures of early or late tau pathology, as well as Aβ pathological burden, were observed between AD/LATE-NC and AD cases, nor between AD cases with higher TDP-43 in AD stages with hippocampal}

Table 2. Correlations (Spearman’s) between TDP-43 in AD stage and regional measures of MC-1-IR, AT8-IR and 4G8-IR

| TDP-43 in AD stage | Amygdala | Frontal cortex | Entorhinal cortex | Temporal cortex | Parietal cortex | Occipital cortex |
|--------------------|----------|----------------|-------------------|-----------------|----------------|-----------------|
| MCI-IR             | rho = −0.12, P = 0.926 | rho = −0.2136, P = 0.297 | rho = −0.092, P = 0.482 | rho = 0.64, P = 0.626 | rho = −0.66, P = 0.615 | rho = −0.077, P = 0.554 |
| AT8-IR             | rho = −0.182, P = 0.175 | rho = 0.188, P = 0.147 | rho = −0.093, P = 0.478 | rho = −0.124, P = 0.341 | rho = 0.091, P = 0.485 | rho = −0.016, P = 0.901 |
| 4G8-IR             | rho = −0.214, P = 0.107 | rho = 0.084, P = 0.525 | rho = −0.129, P = 0.326 | rho = 0.144, P = 0.271 | rho = 0.051, P = 0.701 | rho = 0.023, P = 0.863 |
sclerosis, AD with lower TDP-43 in AD stages and AD without LATE-NC.

The absence of a modification of tau and its pathology when in the presence of TDP-43 pathology is further supported by a human proteomic study that found no difference in tau isoforms or total tau mRNA between AD/LATE-NC and AD human cases [49]. Therefore, the presence and severity of LATE-NC in AD does not appear to modify the tau protein via accelerating the conformational shift of the tau protein and, hence, do not accelerate tau aggregation and deposition and the disease progression.

It is well established that cortical tau burden is associated with cognitive impairment in AD as indicated by human neuropathological [26] and in vivo tau PET binding [50] studies. This study also revealed that higher burden of quantified cortical tau burden is associated with earlier age at disease onset which is in agreement with a previous quantification neuropathological study [51] as well as a tau PET binding study in autosomal dominant AD [52] that indicated that increased tau PET uptake was linked to the onset of cognitive dysfunction. Although the presence of LATE-NC has been implicated as a clinical modifier of AD, we could not find a relationship between severity of LATE-NC with age at onset or rate of cognitive decline between AD/LATE-NC and AD cases, further supporting the notion that TDP-43 pathology does not impact disease onset or progression of AD. However, this study did reveal that final MMSE scores were significantly lower in AD/LATE-NC cases compared to AD cases, and final MMSE scores were inversely correlated with LATE-NC stage independent of both early and later stage tau pathology. Given that pathologically only LATE-NC status differed between the two cohorts, this may suggest that additional LATE-NC may have an additive detrimental effect on cognitive impairment that is independent of tau pathology. This finding is in agreement with previous longitudinal clinicopathological studies that determined that TDP-43 pathology is associated with cognitive impairment independent of AD pathology and hippocampal sclerosis [53], and the clinical impact of TDP-43 and tau pathology is statistically independent [28] with their clinical effects exhibited at different time points in the disease progression [19]. On the other hand, cognitive decline was not significantly different between the AD/LATE-NC and AD groups indicating the rate of cognitive decline was not accelerated due to the presence of LATE-NC in contrast to a previous clinicopathological study [10]. Therefore, it is also possible that the AD/LATE-NC cohort exhibited greater cognitive impairment at baseline. However, in the absence of neuroimaging or biomarker data in this cohort, we are unable to determine if LATE-NC contributed to this or whether it is an artefact due to the nature of our retrospective neuropathological study. Further clinicopathological studies are warranted to elucidate the influence of LATE-NC on cognition in AD.

Our study found no such pathological or clinical associations between LATE-NC and hyperphosphorylated tau pathology. As a result of the distinct limbic and neuronal preference, and human co-localization studies, it is inferred and often assumed that tau and TDP-43 pathology in AD are pathologically linked. Two distinct subtypes of non-FTLD TDP-43 pathology were recently reported [25], inclusive of non-NFT-associated type-α inclusions and the NFT-associated type-β inclusions. In this study, 99% of type-β inclusions were found to occur in cases with Braak stage IV–VI, the presence of type-β inclusions was associated with a higher Braak NFT stage and type-β was highly likely to be identified in cases with AD. Since all of our cases in this study were neuropathologically confirmed AD cases with a Braak stage V/VI, we did not employ this criterion as virtually all of our cases would be classified as type-β. However, TDP type-β was not found to be associated with the robust neuropathological and genetic correlates of AD, such as CERAD neuritic plaques or APOE ε4 genotype, which one may expect if TDP-43 and tau are pathologically linked [28]. Therefore, indicating the higher prevalence of type-β inclusions in AD is perhaps reflective of the simultaneous presence of more severe NFT burden. Furthermore, a human post mortem study investigating TDP-43 in AD found no role of TDP-43 in the regulation of tau splicing or expression [49]. Therefore, it is tempting to speculate that ‘co-localization’ studies reflect rather ‘co-existing’ or ‘co-deposition’ of TDP-43 and tau within the same neurone that are part of two independent age and/or disease spectra, as has been indicated in FTLD [54]. Double labelling and confocal examination of TDP-43 and tau filaments in human AD tissue have revealed two patterns of co-localization: an overlap of the two epitopes that was observed only occasionally, and an intermingling of IR of two distinctly separate structures, which was more frequently observed [4]. A second human tissue study also found that neurones...
with NFT and NT were mostly devoid of TDP-43 IR and occasionally NFT was overlapped with nuclear (physiological) TDP-43 IR [23]. LATE-NC is not exclusive to AD and is present in normal ageing, dementia with Lewy bodies (DLB) and mixed AD/DLB [13], as well as constituting its own progressive clinical amnestic disease entity termed LATE [18]. Therefore, LATE-NC is likely also reflecting pathological mechanisms independent of tau. It is possible that the presence of LATE-NC is indicative of a yet to be determined independent pathological event and/or complex pathological events between proteins, but it may also be representative of an age-related phenomenon associated with basic cellular functional decline. The presence of cytoplasmic TDP-43 inclusions has been linked to oxidative stress, kinase dysfunction and autophagy impairment independent of neurodegenerative diseases (please see [55] for review), and hence may constitute an indicator of cellular dysfunction and vulnerability to further insults that may subsequently lead to neurodegenerative disease. The high prevalence of LATE-NC in AD conceivably indicates a shared overlap of cellular mechanism involved in the hyperphosphorylation of both TDP-43 and tau; a human neuropathological study of NFT burden in LATE cases (TDP-43+, Braak NFT ≤ II) revealed a higher burden of NFT in LATE cases compared to matched TDP-43 negative cases (TDP-43-, Braak NFT ≥ II), indicating an initiator event of NFT pathology independent of AD, which is potentially shared with TDP-43 pathology [24].

In conclusion, the presence of LATE-NC in AD does not increase the pathological burden of tau, either in respect to altered conformation or hyperphosphorylation, or Aβ pathology and does not accelerate tau pathology aggregation into late-stage tau. Furthermore, LATE-NC presence and severity are associated with a lower final MMSE score independent of tau pathology indicating that the clinical impact of LATE-NC and tau pathology is independent and may have an additive influence on cognitive impairment. The presence of comorbid LATE-NC in AD may be an indicator of cellular vulnerability and/or dysfunction with the involvement of shared pathological mechanisms of TDP-43 and tau.

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Conflict of interest

Professor Johannes Attems is an active board member for the British Neuropathological Society. All other authors declare no conflict of interest.

Author contributions

K.E.M, L.W and D.E contributed to the study design, methodologies, data collection and interpretation. K.E.M performed the statistical analysis and wrote the manuscript. M.J provided technical assistance. J.A provided neuropathological expertise, A.J.T provided clinical assessment expertise and D.K provided biomolecular expertise and data interpretation. L.W, D.E, D.K, A.J.T and J.A provided critical revisions of the manuscript.

Peer Review

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Data availability statement

The data that support the findings of this study are available on request from the corresponding author.
References

1 Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, et al. National Institute on Aging-Alzheimer’s Association guidelines for the neuropathologic assessment of Alzheimer’s disease. *Alzheimers Dement* 2012; 8(1): 1–13

2 Montine TJ, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Dickson DW, et al. National Institute on Aging-Alzheimer’s Association guidelines for the neuropathologic assessment of Alzheimer’s disease: a practical approach. *Acta Neuropathol* 2012; 123(1): 1–11

3 Duycakaerts C, Delatour B, Potier MC. Classification and basic pathology of Alzheimer disease. *Acta Neuropathol* 2009; 118(1): 5–36

4 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. *Am Neurol* 2007; 61(5): 435–45

5 Josephs KA, Murray ME, Whitwell JL, Parisi JE, Petrucelli L, Jack CR, et al. Staging TDP-43 pathology in Alzheimer’s disease. *Acta Neuropathol* 2014; 127(3): 441–50

6 Josephs KA, Whitwell JL, Knopman DS, Hu WT, Stroh DA, Baker M, et al. Abnormal TDP-43 immunoreactivity in AD modifies clinicopathologic and radiologic phenotype. *Neurology* 2008; 70(19 Pt 2): 1850–7

7 Arai T, Mackenzie IR, Hasegawa M, Nomaka T, Niizato K, Tsuchiya K, et al. Phosphorylated TDP-43 in Alzheimer’s disease and dementia with Lewy bodies. *Acta Neuropathol* 2009; 117(2): 125–36

8 Davidson YS, Raby S, Foulds PG, Robinson A, Thompson JC, Sikkinik S, et al. TDP-43 pathological changes in early onset familial and sporadic Alzheimer’s disease, late onset Alzheimer’s disease and Down’s syndrome: association with age, hippocampal sclerosis and clinical phenotype. *Acta Neuropathol* 2011; 122(6): 703–13

9 Josephs KA, Whitwell JL, Tosakulwong N, Weigand SD, Murray ME, Liesinger AM, et al. TAR DNA-binding protein 43 and pathological subtype of Alzheimer’s disease impact clinical features. *Ann Neurol* 2015; 78(5): 697–709

10 Josephs KA, Whitwell JL, Weigand SD, Murray ME, Tosakulwong N, Liesinger AM, et al. TDP-43 is a key player in the clinical features associated with Alzheimer’s disease. *Acta Neuropathol* 2014; 127(6): 811–24

11 Wilson RS, Yu L, Trojanowski JQ, Chen EY, Boyle PA, Bennett DA, et al. TDP-43 pathology, cognitive decline, and dementia in old age. *JAMA neurology.* 2013; 70(11): 1418–24

12 Uchino A, Takao M, Hatsuta H, Sumikura H, Nakano Y, Nagami A, et al. Incidence and extent of TDP-43 accumulation in aging human brain. *Acta neuropathologica communications.* 2015; 3: 35

13 McAleese KE, Walker L, Erskine D, Thomas AJ, McKeith IG, Attems J. TDP-43 pathology in Alzheimer’s disease, dementia with Lewy bodies and ageing. *Brain Pathol.* 2016; 27(4): 472–9

14 Bigio EH. TDP-43 variants of frontotemporal lobar degeneration. *J Mol Neurosci.* 2011; 45(3): 390–401

15 Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006; 314(5796): 130–3

16 Josephs KA, Stroh A, Dugger B, Dickson DW. Evaluation of subcortical pathology and clinical correlations in FTLD-U subtypes. *Acta Neuropathol* 2009; 118(3): 349–58

17 Josephs KA, Murray ME, Whitwell JL, Tosakulwong N, Weigand SD, Petrucelli L, et al. Updated TDP-43 in Alzheimer’s disease staging scheme. *Acta Neuropathol* 2016; 131(4): 571–85

18 Nelson PT, Dickson DW, Trojanowski JQ, Jack CR, Boyle PA, Arfanakis K, et al. Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report. *Brain* 2019; 142(6): 1503–27

19 Josephs KA, Dickson DW, Tosakulwong N, Weigand SD, Murray ME, Petrucelli L, et al. Rates of hippocampal atrophy and presence of post-mortem TDP-43 in patients with Alzheimer’s disease: a longitudinal retrospective study. *Lancet Neurol* 2017; 16(11): 917–924

20 Davis SA, Gan KA, Dowell JA, Cairns NJ, Gitcho MA. TDP-43 expression influences amyloidbeta plaque deposition and tau aggregation. *Neurobiol Dis.* 2017; 103: 154–62

21 Gu J, Wu F, Xu W, Shi J, Hu W, Jin N, et al. TDP-43 suppresses tau expression via promoting its mRNA instability. *Nucleic Acids Res* 2017; 45(10): 6177–93

22 Spires-Jones TL, Attijes J, Thai DR. Interactions of pathological proteins in neurodegenerative diseases. *Acta Neuropathol* 2017; 134(2): 187–205

23 Nakashima-Yasuda H, Uryu K, Robinson J, Xie SX, Hurtig H, Duda JE, et al. Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. *Acta Neuropathol* 2007; 114(3): 221–9

24 Smith VD, Bachstetter AD, Ighodaro E, Roberts K, Abner EL, Fardo DW, et al. Overlapping but distinct TDP-43 and tau pathologic patterns in aged hippocampi. *Brain Pathol.* 2018; 28(2): 264–73

25 Josephs KA, Murray ME, Tosakulwong N, Weigand SD, Serie AM, Perkerson RB, et al. Pathological, imaging and genetic characteristics support the existence of distinct TDP-43 types in non-FTLD brains. *Acta Neuropathol* 2019; 137(2): 227–38

26 Giannakopoulos P, Herrmann FR, Bussiere T, Bouras C, Kovari E, Perl DP, et al. Tangle and neuron numbers, but not amyloid load, predict cognitive status in Alzheimer’s disease. *Neurology* 2003; 60(9): 1495–500

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27 Dawe RJ, Bennett DA, Schneider JA, Arfanakis K. Neuropathologic correlates of hippocampal atrophy in the elderly: a clinical, pathologic, postmortem MRI study. *PLoS One* 2011; 6(10): e26286

28 Buciu M, Wennberg AM, Weigand SD, Murray ME, Senjem ML, Sypychall A, et al. Effect modifiers of TDP-43-associated hippocampal atrophy rates in patients with Alzheimer’s disease neuropathological changes. *J Alzheimers Dis* 2020; 73(4): 1511–23

29 Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. *Neurology* 2002; 58(12): 1791–800

30 Braak H, Alafuzoff I, Arzberger T, Kretzschmar H, Del Tredici K. Staging of Alzheimer disease-associated neurofibrillary pathology using paraffin sections and immunocytochemistry. *Acta Neuropathol* 2006; 112(4): 389–404

31 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(4): 479–86

32 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(12): 1863–72

33 Skrobout OA, Attems J, Esiri M, Hortobagyi T, Ironside JW, Kalaria RN, et al. Vascular cognitive impairment neuropathology guidelines (VCING): the contribution of cerebrovascular pathology to cognitive impairment. *Brain* 2016; 139(11): 2957–69

34 Walker L, McAleese KE, Johnson M, Khudakar AA, Erskine D, Thomas AJ, et al. Quantitative neuropathology: an update on automated methodologies and implications for large scale cohorts. *J Neural Transm* 2017; 124(6): 671–83

35 Jicha GA, Bowser R, Kazam IG, Davies P. Alz-50 and MC-1, a new monoclonal antibody raised to paired helical filaments, recognize conformational epitopes on recombinant tau. *J Neurosci Res* 1997; 48(2): 128–32

36 Uboga NV, Price JL. Formation of diffuse and fibrillar tangles in aging and early Alzheimer’s disease. *Neurobiol Aging* 2000; 21(1): 1–10

37 Augustinack JC, Schneider A, Mandellkow EM, Hyman BT. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer’s disease. *Acta Neuropathol* 2002; 103(1): 26–35

38 McAleese KE, Firbank M, Dey M, Colloby SJ, Walker L, Johnson M, et al. Cortical tau load is associated with white matter hyperintensities. *Acta Neuropathol Commun.* 2015; 3: 60

39 McAleese KE, Walker L, Graham S, Moya ELJ, Erskine D, Johnson M, et al. White matter lesions in Alzheimer’s disease are associated with cortical neurodegenerative pathology but not with small vessel disease. *Acta Neuropathol* 2017; 134(3): 459–473

40 Yamamoto Y, Ibara M, Tham C, Low RW, Slade JY, Moss T, et al. Neuropathological correlates of temporal pole white matter hyperintensities in CADASIL. *Stroke* 2009; 40(6): 2004–11

41 Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; 12(3): 189–98

42 Olichney JM, Galasko D, Salmon DP, Hofstetter CR, Hansen LA, Katzman R, et al. Cognitive decline is faster in Lewy body variant than in Alzheimer’s disease. *Neurology* 1998; 51(2): 351–7

43 Dickson DW, Davies P, Bevona C, Van Hoeven KH, Factor SM, Grober E, et al. Hippocampal sclerosis: a common pathological feature of dementia in very old (> or = 80 years of age) humans. *Acta Neuropathol* 1994; 88(3): 212–21

44 Hokkanen SRK, Hunter S, Polvikoski TM, Keage HAD, Minett T, Matthews FE, et al. Hippocampal sclerosis, hippocampal neuron loss patterns and TDP-43 in the aged population. *Brain Pathol.* 2018; 28(4): 548–59

45 Kero M, Raunio A, Polvikoski T, Tienari PJ, Paetau A, Myllykangas L. Hippocampal sclerosis in the oldest old: a Finnish population-based study. *J Alzheimers Dis* 2018; 63(1): 263–72

46 Gu J, Chen F, Iqbal K, Gong CX, Wang X, Liu F. Transactive response DNA-binding protein 43 (TDP-43) regulates alternative splicing of tau exon 10: implications for the pathogenesis of tauopathies. *J Biol Chem* 2017; 292(25): 10600–12

47 Latimer CS, Burke BT, Liaicho NF, Currey HN, Kilgore MD, Gibbons LE, et al. Resistance and resilience to Alzheimer’s disease pathology are associated with reduced cortical pTau and absence of limbic-predominant age-related TDP-43 encephalopathy in a community-based cohort. *Acta Neuropathol Commun.* 2019; 7(1): 91

48 Jegannathan S, Hascher A, Chinnathambi S, Biernat J, Mandellkow EM, Mandellkow E. Proline-directed pseudo-phosphorylation at AT8 and PHF1 epitopes induces a compaction of the paperclip folding of Tau and generates a pathological (MC-1) conformation. *J Biol Chem.* 2008; 283(46): 32066–76

49 Niblock M, Hortobagyi T, Troakes C, Al-Sarraj S, Spickett C, Jones R, et al. Lack of association between TDP-43 pathology and tau mis-splicing in Alzheimer’s disease. *Neurobiol Aging* 2016; 37: 45–6

50 Johnson KA, Schulz A, Betensky RA, Becker JA, Sepulcre J, Rentz D, et al. Tau positron emission tomographic imaging in aging and early Alzheimer disease. *Ann Neurol* 2016; 79(1): 110–9

51 Hanna Al-Shaikh FS, Duara R, Crook JE, Lesser ER, Schaeverbeke J, Hinkle KM, et al. Selective vulnerability of the nucleus basalis of Meynert among...
neuropathologic subtypes of Alzheimer disease. JAMA Neurol. 2020; 77(2): 225

52 Gordon BA, Blazey TM, Christensen J, Dincer A, Flores S, Keefe S, et al. Tau PET in autosomal dominant Alzheimer’s disease: relationship with cognition, dementia and other biomarkers. Brain 2019; 142(4): 1063–76

53 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(1): 66–79

54 Robinson AC, Thompson JC, Weedon L, Rollinson S, Pickering-Brown S, Snowden JS. et al. No interaction between tau and TDP-43 pathologies in either frontotemporal lobar degeneration or motor neurone disease. Neuropathol Appl Neurobiol 2014; 40(7): 844–54

55 Chornenkyy Y, Fardo DW, Nelson PT. Tau and TDP-43 proteinopathies: kindred pathologic cascades and genetic pleiotropy. Lab Invest 2019; 99(7): 993–1007

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