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RESEARCH ARTICLE

Plasma TDP-43 levels are associated with neuroimaging measures of brain structure in limbic regions

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Abstract

Introduction: We evaluated the relationship between plasma levels of transactive response DNA binding protein of 43 kDa (TDP-43) and neuroimaging (magnetic resonance imaging [MRI]) measures of brain structure in aging.

Methods: Plasma samples were collected from 72 non-demented older adults (age range 60–94 years) in the University of Kentucky Alzheimer's Disease Research Center cohort. Multivariate linear regression models were run with plasma TDP-43 level as the predictor variable and brain structure (volumetric or cortical thickness) measurements as the dependent variable. Covariates included age, sex, intracranial volume, and plasma markers of Alzheimer's disease neuropathological change (ADNC).

Results: Negative associations were observed between plasma TDP-43 level and both the volume of the entorhinal cortex, and cortical thickness in the cingulate/parahippocampal gyrus, after controlling for ADNC plasma markers.

Discussion: Plasma TDP-43 levels may be directly associated with structural MRI measures. Plasma TDP-43 assays may prove useful in clinical trial stratification.

KEYWORDS
aging, biomarker, cortical thickness, entorhinal cortex, limbic-predominant age-related TDP-43 encephalopathy, neuroimaging, plasma transactive response DNA binding protein of 43 kDa

HIGHLIGHTS

- Plasma transactive response DNA binding protein of 43 kDa (TDP-43) levels were associated with entorhinal cortex volume.
- Biomarkers of TDP-43 and Alzheimer's disease neuropathologic change (ADNC) may help distinguish limbic-predominant age-related TDP-43 encephalopathy neuropathologic change (LATE-NC) from ADNC.
- A comprehensive biomarker kit could aid enrollment in LATE-NC clinical trials.

1 | BACKGROUND

Transactive response DNA binding protein of 43 kDa (TDP-43) proteinopathy is observed in > 25% of autopsies of older adults,[1–4] and the most prevalent TDP-43 proteinopathic condition has been termed limbic-predominant age-related TDP-43 encephalopathy (LATE).[1] LATE neuropathological change (LATE-NC) may be seen either with or without comorbid Alzheimer's disease (AD) neuropathological changes (ADNC[1,2,5,6]). In addition, LATE-NC and ADNC both show a predilection for medial temporal lobe (MTL) structures and share impairment of episodic memory as an early clinical feature in their disease course.[7,8] Consequently, LATE is considered an "AD mimic."[1,6,9]

Increasing levels of TDP-43 pathology have been consistently associated with poorer cognition for given levels of other pathologic changes (such as ADNC and cerebrovascular pathologies), providing evidence of the clinical significance of TDP-43 proteinopathy.[1,10,11] This has led to calls for in vivo biomarkers to identify individuals at high risk for LATE-NC for use in emerging LATE clinical trials.[1,10,12] LATE-NC biomarkers could also serve to sharpen existing biomarkers for ADNC, given the overlap in both the location of pathology and cognitive features between these diseases.

At present, no positron emission tomography (PET) tracers of cerebral TDP-43 exist. However, candidate plasma markers of TDP-43 have been developed.[13–15] Previous biomarker work has indicated that plasma TDP-43 (total and/or phosphorylated TDP-43 [pTDP-43]) levels are higher in patient groups such as AD, frontotemporal lobar degeneration (FTLD), and amyotrophic lateral sclerosis (ALS), compared to control groups.[13,15,16] In addition, plasma pTDP-43 levels have been found to correlate with TDP-43 brain pathology in FTLD,[15] although not in ADNC.[15] It should also be noted that plasma total TDP-43 levels did not discriminate significantly between brains with or without TDP-43 in either FTLD or AD.[15] Thus, plasma TDP-43 measures are currently limited as stand-alone diagnostic tools. However, plasma TDP-43 levels may offer promise as an adjunct measure used in clinical trial stratification, particularly if they can be linked with in vivo patterns of neurodegeneration in limbic brain regions where TDP-43 is known to accumulate.

Here, we sought to identify relationships between plasma TDP-43 levels (total TDP; section 2.2) and magnetic resonance imaging (MRI)-based measures of neurodegeneration in older adults, after controlling for established plasma markers of ADNC. Most participants in this study were cognitively normal and a small subset had mild cognitive impairment (MCI). Thus, our initial hypothesis-driven region of interest (ROI) analyses focused on the volume of three MTL regions (amygdala, entorhinal cortex, [ERC], and subiculum of the hippocampus) suggested to be affected by TDP-43 pathology in early stages of LATE-NC (Stages 1 or 2), according to detailed TDP-43 immunohistochemical staging schemes.[17–20] Additional exploratory cortical thickness analysis, unconstrained to specific ROIs, was also performed to identify potential neurodegenerative effects of TDP-43 within the neocortex.

RESEARCH IN CONTEXT

1. **Systematic Review**: The authors reviewed publicly available literature with a focus on transactive response DNA binding protein of 43 kDa (TDP-43) neuropathology in limbic-predominant age-related TDP-43 encephalopathy (LATE) neuropathologic change (LATE-NC), with or without comorbid Alzheimer's disease neuropathological change (ADNC). Relevant articles to this article are cited.
2. **Interpretation**: Our findings demonstrate that plasma TDP-43 concentration is associated with neurodegeneration in specific limbic system regions when controlling for plasma biomarkers of ADNC. We highlight the utility of using fluid biomarkers of TDP-43 and ADNC together to help identify individuals at risk of LATE-NC.
3. **Future Directions**: This article proposes that the established amyloid/tau/neurodegeneration framework may be expanded to include a fluid biomarker of TDP-43. Additional development of a comprehensive biomarker kit could aid in the identification of individuals at risk for LATE-NC for enrollment in (or exclusion from) future clinical trials. Replication studies with larger sample sizes are required.

2 | METHODS

2.1 | Participants

Seventy-two older adults participated in this study (47 women, age range 60–94 years). Participants were enrolled in an existing longitudinal cohort at the University of Kentucky Alzheimer's Disease Research Center (UK-ADRC). Inclusion criteria for enrollment in the UK-ADRC longitudinal cohort have been described in detail elsewhere.[21] These criteria include absence of medical, neurological, and psychiatric conditions that affect cognition including dementia, significant cerebrovascular disease (e.g., documented stroke), history of encephalitis, meningitis, or epilepsy; major head injury or initial cognitive status examination scores below standard clinical cut points for dementia; and a willingness to consider brain donation at death. Neuropsychological testing included the Uniform Data Set (UDS) Version 3.[22]

Additional inclusion criteria for the present study were the availability of plasma TDP-43 data from banked blood collected within 1 year of a T1-weighted MRI scan. The TDP-43 kit was added to our ADRC Biomarker Core plasma measures in March 2021. Characteristics of the final participant cohort meeting inclusion criteria are reported in Table 1.

TABLE 1 Group demographics and MoCA[23] scores.

	Mean (SD)	*N*
Age (years)	75.7 (7.3)	72
Sex ratio (F:M)	47:25	72
Education (years)	16.7 (2.7)	71
MoCA	26.2 (3.0)	72

Notes: The table lists the female/male ratio and mean (SD) age, years of education, and MoCA scores for the full sample.
Abbreviations: F:M, female to male; MoCA, Montreal Cognitive Assessment; SD, standard deviation.

2.1.1 | Consent statement

All participants provided informed consent under a protocol approved by the University of Kentucky Institutional Review Board.

2.2 | Plasma sample analysis

The general procedure for plasma sample analysis has been previously described.[24] Briefly, fasting ethylenediaminetetraacetic acid-plasma was collected at the annual clinical visit, stored on ice, processed within 4 hours of collection, and then aliquoted into 500 µl aliquots. The samples were stored at −80°C until retrieved and thawed on ice immediately prior to biomarker assessment. Thawed plasma samples were centrifuged at 4°C for 10 minutes at maximum speed (≈21,000 × g). Samples were then diluted and assayed using the Quanterix HD-X instrument (Table 2).

Quanterix Simoa immunoassays for markers of ADNC (amyloid beta 42 [Aβ42], Aβ40, and phosphorylated threonine-181 tau [p-tau181])[24] and for total TDP-43 were measured in singlicate and quantified (pg/mL) for each participant using the Quanterix Simoa HD-X instrument. The Aβ42/Aβ40 and the p-tau/Aβ42 ratios were calculated for every participant as these ratios may be more informative than individual measures.[25,26] Plasma values were not normally distributed, as is typical,[13,24] and were log-transformed (log10) for all analyses.[24]

2.3 | Image acquisition

Participants were scanned with a 3 Tesla Siemens Magnetom Prisma MRI scanner using a 64-channel head coil at the University of Kentucky's Magnetic Resonance Imaging and Spectroscopy Center (MRISC). A 3D T1-weighted, magnetization prepared rapid gradient echo (MPRAGE) sequence was acquired in the sagittal plane using a generalized autocalibrating partial parallel acquisition acceleration factor (factor 2), which had 1 mm^3 spatial resolution and covered the entire brain. Other parameters varied slightly depending upon whether the participant's T1-weighted image (collected within 1 year of their plasma draw) came from our UK-ADRC Biomarker Core Longitudinal MRI battery or from our UK-ADRC Supplement from the Standardized Centralized Alzheimer's Related Neuroimaging (SCAN) Initiative (UK-ADRC Longitudinal battery Multiecho MPRAGE [(MEMPRAGE): 256 × 256 × 176 mm acquisition matrix (176 slices), repetition time (TR) = 2530 ms, first echo time (TE1) = 1.69 ms, echo time spacing echo spacing (ΔTE = 1.86 ms), flip angle = 7°, scan duration = 5.88 minutes (n = 21)]; UK-ADRC SCAN Study MPRAGE: [256 × 240 × 208 mm acquisition matrix (208 slices), TR = 2300 ms, (TE = 2.98 ms) flip angle = 9°, scan duration = 5.20 minutes (n = 51)]).

2.4 | Hypothesis-driven ROI, volumetric analyses

All participants' T1 images were visually examined for significant motion artifacts, poor contrast, or the presence of significant brain abnormalities that could reduce segmentation accuracy. All T1 images in this sample passed quality control criteria. FreeSurfer 6.0 was used with the recon-all option (with all available parcellations)[27] to automatically segment each participant's MPRAGE image or MEMPRAGE image (after averaging the four echoes into a single root mean square image), as described previously.[28]

For our initial hypothesis-driven a priori analyses, and to limit the number of statistical comparisons, we focused on potential relationships between plasma TDP-43 levels and volume of three MTL regions known to be early predilection sites of TDP-43 proteinopathy. Specifically, as the majority of our participants were cognitively normal, with a small subset having MCI (section 3.1), we focused on brain regions suggested to be affected by TDP-43 in Stages 1 or 2 LATE-NC, according to detailed TDP-43 immunohistochemical staging schemes[17–20]: the amygdala, ERC, and the subiculum of the hippocampus. The total volumes of the amygdala and ERC (in mm^3) were calculated per participant from ROI masks derived from the FreeSurfer segmentation and recorded for subsequent statistical analyses. FreeSurfer's estimated intracranial volume (eICV, in mm^3) was also extracted to control for participant head size in all ROI analyses. To compute the volume of the subiculum, the hippocampus was segmented into subfield ROIs as described previously.[29]

2.5 | Exploratory cortical thickness analysis

Cortical thickness analysis, unconstrained to specific ROIs, was performed to identify potential neurodegenerative effects of TDP-43 in the neocortex. Cortical thickness analyses are preferable to volumetric analyses of the neocortex given its folded structure, with most of its surface area being buried inside sulcal folds. Cortical thickness analyses were developed to address this issue using cortical inflation, flattening, and providing a surface-based coordinate system.[30] We used a vertex-wise, surface-based[31] general linear model (GLM) approach with FreeSurfer 6.0. After FreeSurfer segmentation (section 2.4), each participant's surface (cortical thickness) data was resampled to an "average" subject (fsaverage; https://surfer.nmr.mgh.harvard.edu/fswiki/FsAverage) in MNI305 space and concatenated

TABLE 2 Dilutions and performance analytics for each of the Quanterix Simoa immunoassays.

Assay	Dilution	Catalog number	LLOQ	LOD	Dynamic range
TDP-43	1:10	103293	8.23 pg/mL	2.48 pg/mL	0-8000 pg/mL
Aβ42	1:20	101995	0.142 pg/mL	0.045 pg/mL	0-240 pg/mL
Aβ40	1:20	101995	0.675 pg/mL	0.196 pg/mL	0-560 pg/mL
p-tau181	NEAT	103714	0.085 pg/mL	0.028 pg/mL	0-424 pg/mL

Notes: All immunoassays were carried out using the manufacturer's instructions. LLOQ and LOD were used to determine dilution.
Abbreviations: Aβ, amyloid beta; LLOQ, lower limit of quantification; LOD, limit of detection; p-tau181, phosphorylated threonine-181 tau; TDP-43, tar DNA binding protein of 43 kDa.

into a single file using mris_preproc (https://surfer.nmr.mgh.harvard.edu/fswiki/mris_preproc). Each participant's resampled data was then smoothed with a 10 mm full-width-at-half-maximum Gaussian kernel using mri_surf2surf (https://freesurfer.net/fswiki/mri_surf2surf) to improve the signal-to-noise ratio.

After conducting each GLM (section 2.6), surface clusters were corrected for multiple comparisons using a precomputed Z Monte Carlo simulation with 10,000 iterations provided with FreeSurfer[32] with the mri_glmfit-sim command (https://surfer.nmr.mgh.harvard.edu/fswiki/FsTutorial/GroupAnalysis). The cluster-forming threshold was set to $P < 0.001$, as recommended,[33] while the cluster-wise threshold was set to $P < 0.05$. The *P*-values were additionally adjusted for multiple comparisons in the initial GLM because both brain hemispheres (left and right) were explored. Only the left hemisphere was explored in the post hoc GLM because the initial GLM did not reveal any significant clusters in the right hemisphere.

2.6 | Statistical analyses

All ROI linear regression analyses were conducted using SPSS 28 (IBM). We first explored whether plasma TDP-43 levels were negatively associated with volumes of the amygdala, the ERC, or the subiculum of the hippocampus. Separate linear regression models were run with plasma TDP-43 level as the independent variable and each ROI volume as the dependent variable. All models controlled for age, sex, and ICV. Multiple comparisons were corrected using a Bonferroni-corrected threshold for the three ROIs tested ($P = 0.05/3$ ROIs; $P < 0.0167$). The uncorrected *P*-values are reported using this threshold ($P < 0.0167$) to determine statistical significance. For significant models, additional analyses were run to control for fluid biomarkers of ADNC. Specifically, significant regression models were repeated using the plasma Aβ42/Aβ40 ratio and the p-tau/Aβ42 ratio as additional covariates. Separate models were required as these markers of ADNC are intercorrelated. All participants with plasma TDP-43 were included in all models using a pairwise deletion method to mitigate missing values. Statistical outliers were defined as values greater than 3 standard deviations from the group mean for any variable and were excluded from relevant analyses.

The initial vertex-wise GLM explored the association between plasma TDP-43 levels (independent variable) and cortical thickness (dependent variable), with age and sex as covariates (nuisance regressors) in the model. A second, post hoc vertex-wise GLM was then conducted adding the plasma Aβ42/Aβ40 ratio as an additional covariate, to statistically remove effects associated with markers of ADNC. Any participants who did not have plasma Aβ42/Aβ40 ratio values were excluded from this post hoc analysis, as the sole purpose of this analysis was to determine whether plasma TDP-43 was associated with cortical thickness after controlling for a plasma marker of AD.

3 | RESULTS

3.1 | Participant and data characteristics

Participant summary demographics are presented in Table 1. Of the 72 participants with available plasma TDP results, a subset of 59 had available plasma Aβ42 and Aβ40 results, while a subset of 60 had available p-tau181 results. Fifty-five participants were cognitively normal while the remaining 17 had MCI. There was one outlier value for plasma TDP-43, which was excluded from relevant analyses; no other outliers were present in any analyses.

3.2 | Associations between plasma TDP-43 levels and volume in ROIs

Plasma TDP-43 level was negatively associated with volume of the entorhinal cortex after controlling for multiple comparisons (Bonferroni-corrected threshold: $P < 0.0167$; ERC; Figure 1A; Table 3, $P = 0.009$), but was not associated with volume of the amygdala ($P = 0.796$) or the subiculum of the hippocampus ($P = 0.521$).

Follow-up analyses investigating the association between plasma TDP-43 level and ERC volume revealed that TDP-43 level was still associated with ERC volume after adding the plasma Aβ42/Aβ40 ratio to the model (Table 3, $P = 0.045$). Plasma TDP-43 level was also still associated with ERC volume when adding the plasma p-tau/Aβ42 ratio ($P = 0.037$) to the model instead of the plasma Aβ42/Aβ40 ratio.

To identify individuals at potential risk for LATE-NC + ADNC, versus those at risk for more "pure" LATE-NC, the scatterplot demonstrating a significant relationship between plasma TDP-43 level and ERC

TABLE 3 Associations between plasma TDP-43 levels and volume in regions of interest.

Predictor	β Estimate	95% CI	P value
Model 1: amygdala volume is DV			
Age	−0.418	−0.598 – −0.238	< 0.001
Sex	−0.216	−0.443 – 0.010	0.061
Estimated intracranial volume	0.441	0.214 – 0.668	< 0.001
Plasma TDP-43	−0.023	−0.202 – 0.155	0.796
Model 2: ERC volume is DV			
Age	−0.275	−0.475 – −0.076	0.007
Sex	−0.238	−0.489 – 0.014	0.063
Estimated intracranial volume	0.322	0.070 – 0.574	0.013
Plasma TDP-43	−0.267	−0.465 – −0.069	0.009
Model 3: subiculum volume is DV			
Age	−0.421	−0.603 – −0.239	< 0.001
Sex	−0.270	−0.499 – −0.040	0.022
Estimated intracranial volume	0.378	0.148 – 0.607	0.002
Plasma TDP-43	−0.058	−0.239 – 0.122	0.521
Post hoc Model 1: ERC volume is DV			
Age	−0.286	−0.509 – −0.064	0.013
Sex	−0.260	−0.543 – 0.023	0.071
Estimated intracranial volume	0.315	0.035 – 0.595	0.028
Plasma TDP-43	−0.236	−0.465 – −0.006	0.045
Plasma Aβ42/Aβ40 ratio	0.112	−0.123 – 0.346	0.343
Post hoc Model 2: ERC volume is DV			
Age	−0.267	−0.530 – −0.004	0.047
Sex	−0.226	−0.560 – 0.107	0.178
Estimated intracranial volume	0.325	0.005 – 0.645	0.047
Plasma TDP-43	−0.271	−0.525 – −0.17	0.037
Plasma p-tau/Aβ42 ratio	−0.030	−0.308 – 0.247	0.826

Notes: The table displays the standardized β estimates, the 95% confidence intervals (CI) for β estimates, and the *P* values for each predictor in the linear regression models investigating plasma TDP-43 and volumes of the amygdala, the ERC, and the subiculum of the hippocampus.
Abbreviations: Aβ, amyloid beta; CI, confidence interval; DV, dependent variable; ERC, entorhinal cortex; p-tau, phosphorylated threonine-181 tau; TDP-43, tar DNA binding protein of 43 kDa.

volume (Figure 1B) was split by the plasma Aβ42/Aβ40 ratio using a median split (median value = −1.20; Figure 1C). The bottom right quadrant in Figure 1C (highlighted by the black oval), represents individuals with low ERC volume and high plasma TDP-43 values; individuals at high risk for LATE-NC. However, splitting this group based on plasma Aβ42/Aβ40 ratio values (using a median split) reveals two subgroups: Participants with low plasma Aβ42/Aβ40 ratio values (red circles within the black oval) are at high risk for ADNC + LATE-NC, while a smaller subgroup with high plasma Aβ42/Aβ40 ratio values (blue circles within the black oval) are at low ADNC risk but are at high risk for "pure" LATE-NC. The small subgroup of individuals at risk for "pure" LATE-NC consisted of the following participants: S09 (female, 81 years old, Montreal Cognitive Assessment [MoCA] = 21), S47 (male, 70 years old, MoCA = 27), and S70 (female, 94 years old, MoCA = 26).

3.3 | Exploratory cortical thickness results

Plasma TDP-43 level was negatively associated with two surface cortical clusters in the left hemisphere (Figure 2A: left inferior/ventral temporal cluster [inferior temporal gyrus/fusiform gyrus]; peak t-value: −5.154, peak Montreal Neurological Institutes [MNI] right/anterior/superior [RAS] coordinates: [X = −49.2, Y = −48.7, Z = −16.0], cluster area: 355.00 mm^2; left inferior parietal cluster (angular gyrus): peak t-value: −3.835, peak MNI [RAS] coordinates: [X = −36.8, Y = −80.2, Z = 28.7], cluster area: 232.87 mm^2). No significant clusters were identified in the right hemisphere.

Repeating the same analysis but adding plasma Aβ42/Aβ40 ratio values to the model revealed a unique, negative association between TDP-43 values and 1 surface cortical cluster in the left hemisphere

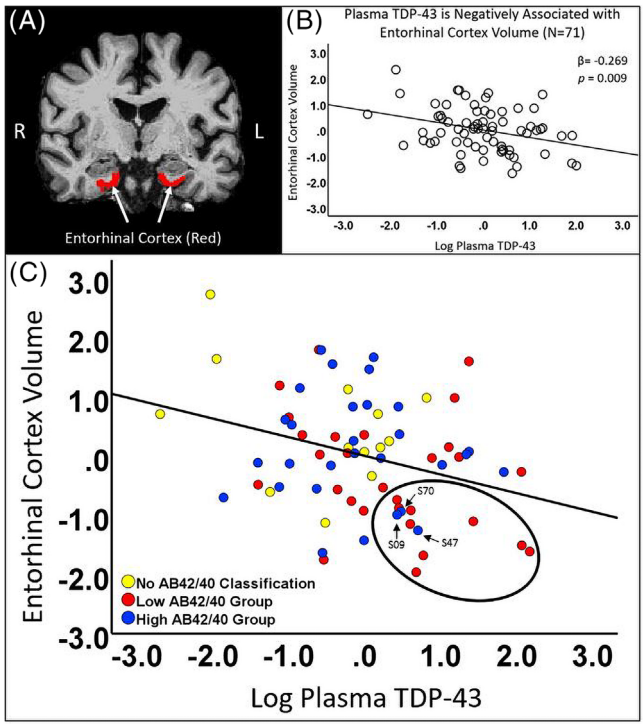

FIGURE 1 Association between plasma TDP-43 levels and ERC volume. The figure displays the ERC ROI mask on a representative participant from this study (A; red) and a scatterplot displaying plasma TDP-43 levels against ERC volume (B). There was a significant negative association between plasma TDP-43 level and ERC volume after controlling for age, sex, and ICV. Furthermore, a median split (C) was used to divide participants shown in (B) into low plasma Aβ42/Aβ40 ratio (red circles) and high plasma Aβ42/Aβ40 ratio (blue circles) groups to help identify those individuals at high risk for "pure" LATE-NC versus those at high risk for mixed pathologies of LATE-NC + ADNC (C). Participants in the bottom right quadrant (within the black oval) had both high plasma TDP-43 levels and low ERC volume. While most of these participants had a low plasma Aβ42/Aβ40 ratio (red circles: high risk for both ADNC + LATE-NC), a small subgroup had a high plasma Aβ42/Aβ40 ratio (blue circles: low ADNC risk, high risk for "pure" LATE-NC). Aβ, amyloid beta; ADNC, Alzheimer's disease neuropathologic change; ERC, entorhinal cortex; ICV, intracranial volume; LATE-NC, limbic-predominant age-related TDP-43 encephalopathy neuropathological change; ROI, region of interest; TDP-43, tar DNA binding protein of 43 kDa.

(Figure 2B: left isthmus of the cingulate/parahippocampal gyrus; peak t-value: −4.998, peak MNI [RAS] coordinates: [X = −15.2, Y = −43.8, Z = −4.1], cluster area: 203.00 mm^2).

4 | DISCUSSION

We found that plasma TDP-43 levels were negatively associated with ERC volume in a cohort of community-dwelling older adults, after controlling for plasma markers of ADNC. Subsequent exploratory analyses demonstrated a negative relationship between plasma TDP-43 level and cortical thickness in the left cingulate/parahippocampal gyrus, after controlling for plasma markers of ADNC. Our findings indicate that plasma TDP-43 level is associated with neurodegeneration in specific limbic system brain regions, suggesting that it may be a useful in vivo biomarker of TDP-43-mediated neurodegeneration for those at risk for LATE-NC.

4.1 | Plasma TDP-43 level and ERC volume

Our results link plasma TDP-43 levels with volume of the ERC in nondemented older adults, after controlling for age, sex, and ICV. The ERC (Brodmann Area 28) is a critical hub of the episodic memory system, involved in relaying and integrating information between the hippocampus and neocortex.[34,35] The ERC is a very early site of ADNC[36] but also of LATE-NC.[17–20] Neuroimaging studies have reported reduced volume of the ERC, as well as other MTL structures, in individuals with presumed ADNC and amnestic MCI.[37–39] In addition, neuroimaging studies, including our own, have shown that low baseline ERC volume is a significant predictor of later decline in memory function and conversion to amnestic MCI.[40,41]

The assumption underlying most of these previous studies is that ERC volumetric reductions result from underlying ADNC. However, TDP-43 was not assessed in these previous studies and the present results suggest that TDP-43 is a contributor to ERC neurodegeneration, even after controlling for plasma markers of ADNC. Previous relevant studies have explored relationships between pathological TDP-43 load with either ex vivo MRI measures[42,43] or *ante mortem* MRI measures.[2,17,44–46] One of the reported patterns is that "pure" LATE-NC is associated with lower volume of specific MTL structures than "pure" ADNC, with volumes of MTL structures being lowest in mixed LATE-NC + ADNC.[2,17,42,44,45,47]

In the current study, our hypothesis-driven analyses focused on the volume of three MTL ROIs (amygdala, ERC, and subiculum of the hippocampus) affected by TDP-43 in early stages (Stages 1 or 2) of LATE-NC, according to TDP-43 immunohistochemical staging schemes.[17–20] Our results implicate low normalized ERC volume as a potential in vivo marker of LATE-NC. Nevertheless, while MRI-based patterns are a necessary component of the amyloid/tau/neurodegeneration (ATN) framework (i.e., the N component), the regional overlap between LATE-NC and ADNC established in *post mortem* studies suggests that other biomarkers will be needed for in vivo identification of individuals at risk for "pure" LATE-NC versus those at risk for "pure" ADNC or mixed pathology (i.e., LATE-NC + ADNC).

4.2 | Plasma TDP-43 level as a potential biomarker for LATE-NC

Our results suggest that the addition of plasma TDP-43 measures to the canonical ATN framework may aid in vivo differentiation of mixed ADNC + LATE-NC from more "pure" presentations (Figures 1 and 3). For example, as seen in Figure 1C, approximately 12 of our study participants appear to be at high risk for LATE-NC (those within the black

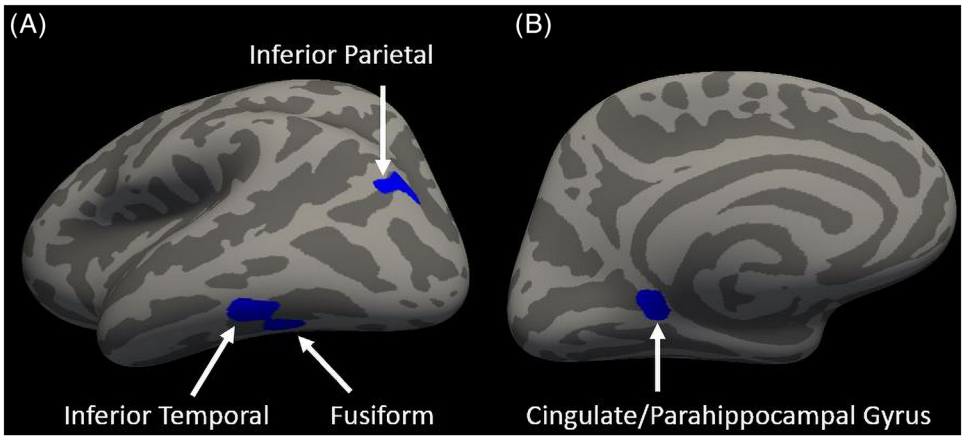

FIGURE 2 Relationship between plasma TDP-43 level and cortical thickness. Plasma TDP-43 level was negatively associated with cortical thickness in the left inferior/ventral temporal areas and in the left inferior parietal lobe (A; blue regions). However, after controlling for plasma Aβ42/Aβ40 ratio values, plasma TDP-43 level was only negatively associated with cortical thickness in the left inferior isthmus of the cingulate/parahippocampal gyrus (B; blue). Aβ, amyloid beta; TDP-43, tar DNA binding protein of 43 kDa.

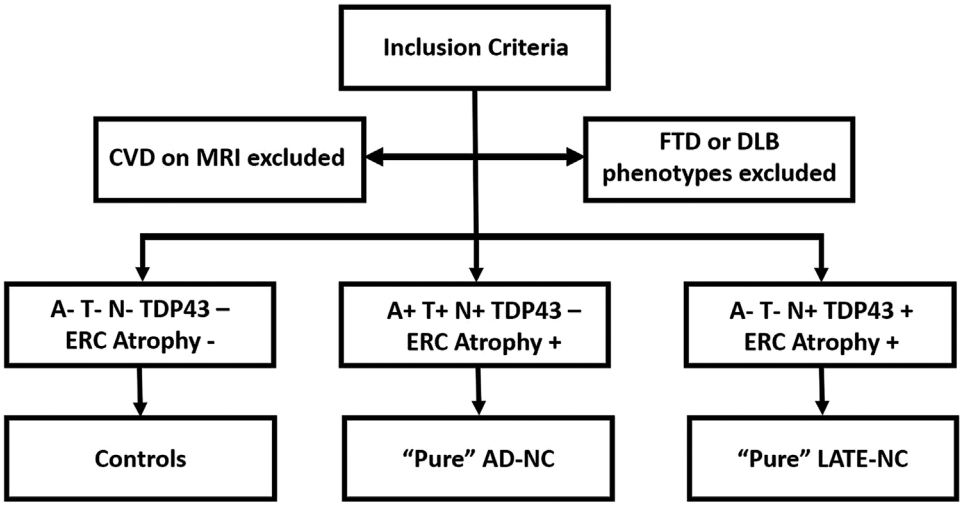

FIGURE 3 A schematic summarizing the potential implications of our results for future clinical trials. Our results suggest that integrating TDP-43 values and ERC volume within the ATN framework could aid in the differentiation of those at high risk for "pure" ADNC and those at risk for "pure" LATE-NC. Further validation of fluid biomarkers of TDP-43 will be necessary to ensure accurate quantification of TDP-43 levels in vivo. ADNC, Alzheimer's disease neuropathologic change; CVD, cerebrovascular disease; ERC, entorhinal cortex; DLB, dementia with Lewy bodies; FTD, frontotemporal dementia; LATE-NC, limbic-predominant age-related TDP-43 encephalopathy neuropathological change; MRI, magnetic resonance imaging; ROI, region of interest; TDP-43, tar DNA binding protein of 43 kDa.

oval in Figure 1C) based on their pattern of high plasma TDP-43 protein levels along with low normalized ERC volume. However, splitting this group based on the Aβ42/Aβ40 ratio (using a median split) shows that the majority ($N = 9$) of these participants appear to be at high risk for the more common mixed pathology of ADNC + LATE-NC, while a small subgroup ($N = 3$) appear to be at high risk for "pure" LATE-NC.

Interestingly, of all study participants with available fluid markers of ADNC, 3 out of 59 showed a pattern of low ERC volume, low risk for ADNC, and high risk for LATE-NC. This percent of participants (5%) is similar to the percent reported[10] to show the pathologic combination of LATE-NC (Stage > 1) and low/no ADNC (8.9%). The lower percentage in our study is not unexpected in that we included low ERC volume as an additional metric in our classification of high risk for LATE-NC. It may be possible to test the relationship between plasma TDP-43 level and TDP-43 pathology more directly in our sample, which consists of participants who have agreed to brain donation at death.

Based on our results, we provide a schematic (Figure 3) of how TDP-43 could be added to the canonical ATN framework for clinical trials focused on "pure" LATE-NC. First, inclusion criteria for future clinical trials focusing on "pure" LATE-NC or "pure" ADNC can exclude for cerebrovascular disease using clinical or MRI data and exclude for FTD and dementia with Lewy bodies clinical phenotypes. Individuals

meeting inclusion criteria would then be grouped based on both their ATN status and biofluid TDP-43 levels. Our results suggest that volumes of the ERC would be an appropriate measure of neurodegeneration (i.e., the N in ATN) for trials focused on LATE-NC. Further, grouping based on TDP-43 values and AT values would allow for differentiation between individuals at high risk of "pure" LATE-NC pathology and those at risk for "pure" ADNC.

It is important to point out that our schematic is intended simply as a working heuristic based on our findings. The optimal choice of biomarkers will need to evolve over time based on scientific and technological advancements. Continued refinement of biomarkers for LATE-NC is a worthwhile endeavor that has potential to aid the development of pharmacological interventions for use in future clinical trials focusing on LATE-NC. In addition, development of accurate biomarkers of LATE-NC will also implicitly sharpen/refine existing biomarkers of ADNC.

4.3 | Plasma TDP-43 level and cortical thickness

Our exploratory analyses focused on potential relationships between plasma TDP-43 and neocortical structure. Results indicated that high levels of plasma TDP-43 were associated with cortical thinning in the inferior temporal lobe and inferior parietal lobe. However, after controlling for plasma Aβ42/Aβ40 ratio values, TDP-43 pathology was associated with cortical thinning in the cingulate/parahippocampal gyrus. This suggests that "pure" LATE-NC may not be associated with neurodegeneration in the lateral, inferior temporal, or parietal lobes independent of ADNC.

In contrast, our cortical thickness results suggest that TDP-43 may uniquely contribute to neurodegenerative processes in portions of the limbic system. Specifically, plasma TDP-43 levels were associated with cortical thickness in the left inferior isthmus of the cingulate/parahippocampal gyrus, a region of the limbic system situated between the posterior, inferior portion of the cingulate gyrus, and the posterior portion of the parahippocampal gyrus. Gray matter reductions in this area have also been linked to early AD.[48,49] However, these studies did not also consider the potential association of TDP-43 with gray matter reductions in this area. Our exploratory cortical thickness results converge with our a priori volumetric findings, linking plasma TDP-43 with neurodegeneration in portions of the limbic system, although future research will be required to confirm this exploratory result.

4.4 | Limitations

Current plasma immunoassays for TDP-43 have not yet been shown to demonstrate clinical utility.[14,15,50] This study used Quanterix Simoa immunoassays and future studies are needed to establish which immunoassays are optimally sensitive and specific to detect total TDP-43 or pTDP-43 in human plasma.[14,50] Another potential limitation relates to the use of continuous measures of plasma values rather than defined cut points, as such cut points have yet to be established for plasma TDP-43. Further, we lack pathological confirmation related to those individuals identified for being at high risk for "pure" LATE-NC in this study (Figure 1C), although this may be possible in the future as most participants in our longitudinal cohort have agree to brain donation at death. Finally, this was a study of convenience in which participants were recruited based on the availability of plasma and neuroimaging data. This may limit generalizability to other aging populations.

In summary, this study demonstrates that plasma TDP-43 levels are associated with unique patterns of neurodegeneration in specific limbic system brain regions after statistically removing effects associated with ADNC. These results suggest that adding biofluid TDP-43 measures to the ATN framework may aid the identification of individuals at high risk for LATE-NC for enrollment in future clinical trials. We offer one possible set of criteria for future trials of individuals at high risk for LATE-NC that incorporates biofluid TDP-43 levels and ERC volume. These criteria remain a working heuristic that will need to be further developed based on results from studies with larger sample sizes.

AUTHOR CONTRIBUTIONS

Christopher E. Bauer: conceptualization, methodology, validation, formal analysis, investigation, writing—original draft, writing—review & editing, visualization. **Valentinos Zachariou**: methodology, software, data curation, writing—review & editing. **Tiffany L. Sudduth**: validation, formal analysis, investigation. **Linda J. Van Eldik**: writing—review & editing, resources, funding acquisition. **Gregory A. Jicha**: conceptualization, writing—review & editing. **Peter T. Nelson**: conceptualization, writing—review & editing. **Donna Wilcock**: conceptualization, writing—review & editing, supervision. **Brian T. Gold**: conceptualization, methodology, formal analysis, resources, data curation, writing—original draft, writing—review & editing, visualization, supervision, project administration, funding acquisition.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest. Author disclosures are available in the supporting information.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are freely available upon request from the senior author, Dr. Brian T. Gold, via FTP or Dropbox.

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SUPPORTING INFORMATION

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