Longitudinal association of shorter leukocyte telomere length with CSF biomarker dynamics across early Alzheimer's disease stages in at-risk individuals

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Abbreviations: Aβ, Amyloid-β; AD, Alzheimer's disease; ALFA, Alzheimer's and families; APOE, Apolipoprotein E; AT, Amyloid-β-tau staging; CAIDE, Cardiovascular risk factors, Aging, and incidence of dementia; CDR, Clinical dementia rating; CSF, Cerebrospinal fluid; CU, Cognitively unimpaired; CV, Coefficient of variation; DNA, Deoxyribonucleic acid; FDR, False discovery rate; GFAP, Glial fibrillary acidic protein; IL-6, Interleukin 6; LTL, Leukocyte telomere length; MCI, Mild cognitive impairment; MMSE, Mini-mental state examination; MRI, Magnetic resonance imaging; NfL, Neurofilament light; PET, Positron emission tomography; p-tau181, Phosphorylated tau181; RT-qPCR, Meal-time polymerase chain reaction-based assay; sTREM2, Soluble triggering receptor expressed on myeloid cells 2; TL, Telomere length; t-tau, Total tau; YKL-40, Chitinase-3-like protein 1

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Summary

Background Short telomere length (TL), a hallmark of biological ageing, has been associated with an increased risk of Alzheimer's disease (AD), but its pathophysiological role remains unclear. This study explored the relationship between blood leukocyte TL (LTL), cerebrospinal fluid (CSF) AD biomarkers changes, and brain structure across early stages of the AD *continuum*.

Methods We included 346 cognitively unimpaired participants (aged 49–71) from the ALFA cohort, enriched for AD risk (53.2% *APOE*-ε4 carriers; 34% amyloid-positive). LTL was measured at baseline (visit 0) using quantitative PCR. Associations were assessed between baseline LTL and CSF biomarkers at visit 1 (mean follow-up from baseline = 3.98 years, SD = 1.02), and with changes in CSF biomarkers between visits 1 and 2 (mean interval = 3.45 years, SD = 0.58). Cortical thickness in ageing- and AD-vulnerable brain regions was evaluated by magnetic resonance imaging (MRI) at visit 1. Analyses were stratified by *APOE*-ε4 status and amyloid-tau (AT) profiles. Mediation models tested whether CSF biomarkers mediated LTL-cortical thickness associations.

Findings Shorter LTL was associated with higher astrocytic reactivity at visit 1 and with increased synaptic dysfunction over time. Among *APOE*-ε4 carriers and AT-positive individuals, shorter LTL was associated with higher p-tau181 and neurodegeneration markers. Shorter LTL was associated with greater cortical thickness in ageing- and AD-vulnerable regions, partially mediated by astrocytic reactivity biomarkers.

Interpretation These findings suggest that shorter telomeres are associated with early AD-related biological changes, potentially via mechanisms involving astrocytic reactivity and brain structural alterations. LTL may serve as an early marker of vulnerability to neurodegenerative processes in at-risk populations.

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Introduction

Advancements in fluid and neuroimaging biomarkers have demonstrated that AD pathology begins decades before symptom onset.[1,2] AD is now conceptualised as a biological and clinical *continuum*, beginning with a prolonged asymptomatic stage. Core cerebrospinal fluid (CSF) biomarkers of AD, including amyloid-β42 (Aβ42), the Aβ42/40 ratio, phosphorylated tau (p-tau), and total tau (t-tau), enable early disease detection, reflecting amyloid deposition, tau pathology, and neurodegeneration. These biomarkers form the basis of the AT (Amyloid/Tau) classification framework, which allows staging of individuals along the Alzheimer's *continuum* and enhances biological precision in preclinical and prodromal phases.[3–5]

Beyond Aβ and tau, additional mechanisms such as neuroinflammation, glial reactivity, neuronal injury, and synaptic dysfunction emerge early in the disease course and are closely linked to its progression.[6–8] Incorporating biomarkers of these processes provides a more comprehensive characterisation of AD pathophysiology and may facilitate development of early therapeutic interventions.[9] Importantly, many of these pathological alterations are also detected during normal brain ageing.[10,11] This overlap has led to growing recognition that ageing and AD may share common and interacting biological mechanisms, collectively shaping disease progression.[12]

In this context, biological ageing is increasingly recognised as a more accurate indicator of physiological and functional status than chronological age.[13] Telomere length (TL) is a well-known hallmark of cellular ageing, reflecting the replicative history and the cumulative exposure to cellular stress.[14] Telomeres, composed of repetitive DNA sequences and associated proteins, protect chromosome ends and shorten progressively with cell division, a process accelerated by factors such as oxidative stress or environmental exposures.[15] This attrition leads to genomic instability and cellular senescence, contributing to ageing at cellular, tissue and organismal levels.[16,17]

Research in context

Evidence before this study

We reviewed the literature using PubMed and other scholarly databases. Ageing is the main risk factor for Alzheimer's disease (AD), but chronological age alone does not fully explain the biological processes contributing to AD. Telomeres, protective chromosome-end structures, shorten with each cell division. While telomere length (TL) is primarily age-dependent, genetic, lifestyle, and environmental factors can accelerate its attrition. Shorter leukocyte TL (LTL) has been associated with increased dementia risk and brain structural changes, but its role in AD pathology remains unclear. Longitudinal studies integrating multimodal biomarkers in cognitively unimpaired (CU) individuals at risk for AD are limited.

Added value of this study

This study examines how LTL relates to brain health and AD progression in CU individuals at increased risk. Half carried the *APOE*-ε4 risk allele, and 34% showed CSF amyloid-β (Aβ) positivity. We investigated associations between LTL and change in CSF AD-related biomarkers over 3.5 years, including Aβ, tau, neurodegeneration, synaptic dysfunction, glial reactivity, and inflammation. Inclusion of interaction terms and stratified analyses by *APOE*-ε4 status and core AD biomarker profiles further help elucidate specific relationships between LTL and AD pathophysiology beyond "normal" brain ageing mechanisms. A unique aspect of our study is the use of structural equation modelling to test the mediating role of CSF biomarkers in the association between LTL and brain structure.

Implications of all the available evidence

In CU individuals at higher risk of AD, shorter LTL was associated with elevated AD-related CSF biomarkers, including phosphorylated tau (p-tau181), neurodegeneration, synaptic dysfunction, and glial reactivity. These associations were more pronounced or exclusively encountered in at-risk individuals, i.e., *APOE*-ε4 carriers and individuals with positive AD core biomarker profiles. Astrocytic reactivity partially mediated the relationship between shorter LTL and structural brain integrity. These findings suggest that LTL shortening may exacerbate early AD pathological changes, before the onset of cognitive symptoms, by modulating neuroinflammation and brain homoeostasis, especially in individuals at increased risk of AD. This emphasises the potential of targeting TL, its determinants, and related pathways as part of strategies to prevent or delay the onset of AD symptoms.

Leukocyte telomere length (LTL) serves as a surrogate for TL in other tissues,[18] and has been associated with increased mortality and higher risk of age-related diseases, including all-cause dementia and AD.[19–22] Shorter LTL has also been associated with poorer cognitive performance and smaller brain volumes and cortical thickness[23–25] Mendelian Randomisation (MR) studies support a causal relationship between genetically predicted shorter LTL and increased risk of cognitive impairment, AD, and structural brain changes.[23,25,26] These findings suggest longer telomeres may contribute to greater brain reserve since early development, influencing cognitive resilience later in life.[27] However, some observational studies have reported paradoxical associations, such as longer LTL predicting greater cognitive decline in individuals with established AD pathology.[28] In a previous MR study involving cognitively unimpaired (CU) individuals at increased risk of AD, we encountered unexpected associations between genetically predicted LTL and core AD CSF biomarkers, which differed according to genetic predisposition to AD.[29] These findings suggest that the influence of LTL on AD pathophysiology may be both stage-specific and modulated by genetic background.

Given these complexities, there is a critical need to integrate longitudinal and multimodal biomarkers, beyond Aβ and tau, alongside neuroimaging endophenotypes, to better elucidate the role of LTL in brain ageing and AD risk. As the global number of individuals living with AD is projected to triple to 152 million by 2050, primarily due to population ageing,[30,31] investigating these associations in preclinical stages could may help uncover biological mechanisms through which ageing contributes to AD pathology and identify resilience mechanisms that support cognitive health.[12,32]

In this study, we examined associations between LTL, measured at baseline, and CSF biomarkers assessed 3.98 and 7.43 years later, in CU middle-aged individuals at increased risk for AD. The CSF panel included biomarkers covering core pathogenic processes in AD: Aβ pathology (Aβ42/40 ratio), tau pathology (phosphorylated tau at threonine 181 [p-tau181]), neurodegeneration (t-tau and neurofilament light chain [NfL]), synaptic dysfunction (neurogranin and alpha-synuclein [α-synuclein]), astrocytic reactivity (glial fibrillary acidic protein [GFAP], chitinase-3-like protein 1 [YKL-40], and s100 calcium-binding protein B [s100B]), microglial reactivity (soluble TREM2 [sTREM2]), and inflammation (interleukin-6 [IL-6]). We also investigated LTL associations with cortical thickness in AD-sensitive brain regions and tested for effect modification by *APOE*-ε4 status and amyloid-tau (AT) profiles, aiming to clarify the biological mechanisms linking shorter LTL to AD

vulnerability. Finally, mediation models were applied to assess whether CSF biomarkers mediated the association between LTL and brain structure.

Methods

Study population

The present study was performed in a subset of individuals from the Alzheimer's and Families (ALFA) study, who were invited to participate in a longitudinal study based on their specific AD risk profile (ALFA+ study).[33,34] Inclusion criteria considered participants' AD parental history, *APOE*-ε4 status, verbal episodic memory score and Cardiovascular Risk Factors, Aging, and Incidence of Dementia (CAIDE) score. A comprehensive characterisation was performed in ALFA participants, including demographic characteristics, anthropometric measurements, a lumbar puncture for the measurement of CSF biomarkers and imaging (i.e., MRI and positron emission tomography (PET)) biomarker acquisition. ALFA + inclusion criteria were: (1) individuals who had previously participated in the ALFA study; (2) age between 45 and 65 years at the moment of inclusion in ALFA; and (3) long-term commitment to the study: inclusion and follow-up visits and agreement to undergo all tests and study procedures (MRI, PET and lumbar puncture). ALFA + exclusion criteria were: (1) cognitive impairment (Clinical Dementia Rating (CDR) > 0, Mini-Mental State Examination (MMSE) < 27 or semantic fluency < 12); (2) any systemic illness or unstable medical condition that could lead to difficulty complying with the protocol; (3) any contraindication to any test or procedure; and (4) a family history of monogenic AD.[35] Sex was self-reported by study participants using a binary classification (*i.e.,* female or male). Of the ALFA+ participants included in this study, 99.5% self-identified as white, based on responses to a combined race/ethnicity category collected at baseline.[36]

Leukocyte telomere length measurements

A total of 1660 participants were selected for LTL determinations based on the availability of biological samples (already stored at the biobank) and cognitive assessment (available at in-house databases).[37] Samples were sent to the Harvard Cancer Center Genotyping & Genetics for Population Sciences Facility for LTL determination using a high throughput version of the quantitative real-time polymerase chain reaction (qPCR)-based telomere assay.[38]

Genomic DNA was extracted from peripheral blood leukocytes using the QIAmp 96-spin blood protocol (Qiagen, Chatsworth, CA, USA). Pico-Green quantification of genomic DNA was performed using a Molecular Devices 96 well spectrophotometer (Sunnyvale, CA, USA). The quantitative real time polymerase chain reaction telomere assay was run on the Applied Biosystems 7900HT Sequence Detection System (Foster City, CA, USA). LTL was measured in a single batch for all samples. Laboratory personnel were blinded to participants' characteristics, and all assays were processed in triplicate by the same technician and under identical conditions. The average relative LTL (*i.e.*, Exp ddCt) was calculated as the exponentiated ratio of telomere repeat copy number to a single gene (36B4) copy number corrected for a reference sample. This reference sample consists of pooled buffy coat genomic DNA at a concentration of 5 ng/μl. It is run on all 384-well reaction plates to normalise 'Relative T/S ratio' for plate-to-plate variation. Quality control samples were interspersed throughout the plates to assess inter-plate and intra-plate variability of Ct values.

Sample triplicates coefficient of variation (CV) ranged between 0.01% and 1.97%. The intra-set CVs ranged between 0.93% and 1.15%. A combined inter- and intra-assay CV calculated from quality control samples was 8.36%, which passes the internal standard quality controls. Forty-five samples failed the assay (triplicate CV > 2%), of which 21 were expected to fail due to low concentration of DNA after the DNA quantification. A total of 48 samples did not fail but presented higher cycle threshold (Ct) values than they should be (Ct > 26 for telomere and Ct > 29 for 36B4) and were excluded from the analyses (N = 48). Additionally, *APOE*-ε2ε4 individuals (N = 30) were removed from the analyses. Outliers were detected and removed based on the Grubbs test (N = 5), leaving reliable data for a total of 1532 after quality control and 1520 after merging with genetic data.

Fluid biomarkers assessment

CSF biomarkers Aβ42, Aβ40, neurofilament light (NfL), soluble triggering receptor expressed on myeloid cells 2 (sTREM2), chitinase-3-like protein 1 (YKL40), glial fibrillary acidic protein (GFAP), S100B, neurogranin, α-synuclein, and interleukin 6 (IL-6), were measured using NeuroToolKit, a panel of exploratory robust prototype assays (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) on either the Cobas® e 411 or the Cobas e 601 analyser (Roche Diagnostics International Ltd). CSF phosphorylated tau181 (p-tau181) and total tau (t-tau) were quantified using the electrochemiluminescence Elecsys® Phospho-Tau (181P) CSF and Total-Tau CSF immunoassays (Roche Diagnostics International Ltd), respectively, on the fully automated Cobas e 601 analyser (all Roche Diagnostics International Ltd., Rotkreuz, Switzerland), as previously described in Milà-Alomà and colleagues.[35] All fluid biomarkers were measured at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. Amyloid groups were defined with the CSF Aβ42/40 ratio (Aβ+: <0.071). Participants were tau positive (T+) if CSF p-tau181 > 24 pg/mL or tau

negative (T–) if CSF p-tau181 ≤ 24 pg/mL. Fluid biomarkers were measured at two time points: at baseline (V1) and at follow-up visits (V2). CSF biomarker values were log-transformed to base 10. Mean (SD) follow-up time was 3.45 (0.58) years.

Ageing and AD cortical thickness signatures

The acquisition of neuroimaging data was performed for a subset of the participants through MRI. MRI scans were obtained with a 3-T scanner (Ingenia CX, Philips, Amsterdam, Netherlands). The MRI protocol was identical for all participants and included a high-resolution 3D T1-weighted turbo field echo (TFE) sequence (voxel size 0.75 × 0.75 × 0.75 mm, TR/TE: 9.90/4.6 ms, flip angle = 8°). Structural T1-weighted images were segmented using FreeSurfer version 6.0.[39] The average of the cortical thickness between hemispheres of specific brain regions was used to calculate the AD and ageing brain signatures. AD brain signature was calculated as the average cortical thickness of AD-vulnerable brain regions: entorhinal, inferior temporal, middle temporal, and fusiform.[40] Ageing brain signature was calculated as the average cortical thickness of ageing-vulnerable brain regions: calcarine, caudal insula, cuneus, caudal fusiform, dorsomedial frontal, lateral occipital, precentral, and inferior frontal.[41] We used AD and ageing brain signatures as the main outcomes to assess the association between LTL and brain structure. Higher values in these signatures represent a thicker cortex in the areas included in the signature.

Statistics

The final study sample was drawn from the ALFA parent cohort (baseline visit, 2013–2014), composed of cognitively unimpaired middle-aged individuals. A total of 1520 participants had valid LTL measurements at the baseline (V0) visit. For the present analyses, LTL data were merged with CSF biomarker and MRI data available from the ALFA+ longitudinal study (2016–2022). Biomarkers exhibiting right-skewed distributions were log10-transformed prior to analysis to approximate normality and improve model fit. As these variables were used as outcomes in linear regression models, the transformation was applied to better satisfy key statistical assumptions, particularly the normality of residuals.

Extreme LTL values were identified and excluded based on a threshold of 1.5 times the interquartile range (N = 27; Figure S1). In contrast, no extreme values were removed from log10-transformed CSF biomarkers, as they were considered potentially reflective of clinically meaningful biological variation (Figure S2 in Supporting Information).

After these quality control procedures, three nested datasets were established (Fig. 1): (1) CSF V1 dataset: 346 individuals with LTL at baseline and cerebrospinal fluid (CSF) biomarker measurements collected during the first ALFA+ visit (2016–2019). (2) MRI V1 dataset: 325 individuals with LTL, CSF, and structural MRI data from the same ALFA+ visit (2016–2019). (3) CSF longitudinal dataset: 237 individuals with LTL and CSF data available at both ALFA+ visits (V1: 2016–2019, V2: 2019–2022), enabling assessment of biomarker change over time.

To assess the external validity of LTL measurements, we examined their associations with age, sex, and body mass index (BMI) in both the full baseline sample (N = 1520) and the nested ALFA+ subsample. A significant inverse association between LTL and age was observed only in the full cohort, while females exhibited higher LTL than males in both samples. No significant associations were found with BMI. These results are detailed in Table S1 and Figure S3 in Supporting Information. The absence of association with age in the ALFA+ subsample likely reflects reduced statistical power and narrower age variability (see Figure S4).

The associations between LTL, CSF biomarkers at visit 1, and cortical thickness were assessed in the whole sample by using multiple linear regression models. All models were adjusted by age, sex, *APOE*-ϵ4 status, as well as firmware MRI version for neuroimaging outcomes. Further, linear models exploring the association between LTL and the 3-year rate of change in CSF biomarker were performed. These models were additionally adjusted by baseline CSF biomarker at visit 1 and the time differences between lumbar punctures. All regression β coefficients were standardised. Two-sided t-tests were used to assess the significance of regression coefficients. Interactions and stratified models by *APOE*-ϵ4 and AT status were run to test differential effects by genetic AD risk and pathology.

A multiple-comparison correction was applied following the Benjamini-Hochberg procedure at 5% to control for the false discovery rate (FDR). FDR was applied separately for models assessing the association with CSF biomarkers at visit 1 and CSF biomarkers change, and by pre-defined CSF biomarkers pathways: amyloid pathology (i.e., Aβ42/40), neurofibrillary tangles pathology (p-tau181), neurodegeneration (i.e., NfL), synaptic dysfunction (α-synuclein, neurogranin), astrocytic response (GFAP, S100B, YKL40), microglial reactivity (sTREM2) and inflammation (IL6). A FDR-adjusted P value < 0.05 was considered statistically significant; unadjusted P value < 0.05 was considered nominally significant and unadjusted P value = 0.05 was considered borderline significant.

Causal mediation analyses were conducted using a linear structural equation modelling approach with quasi-Bayesian confidence intervals, estimated through 1000 simulations using the "*mediation*" package in R. This approach was used to investigate the potential mediating role of CSF biomarkers in the association

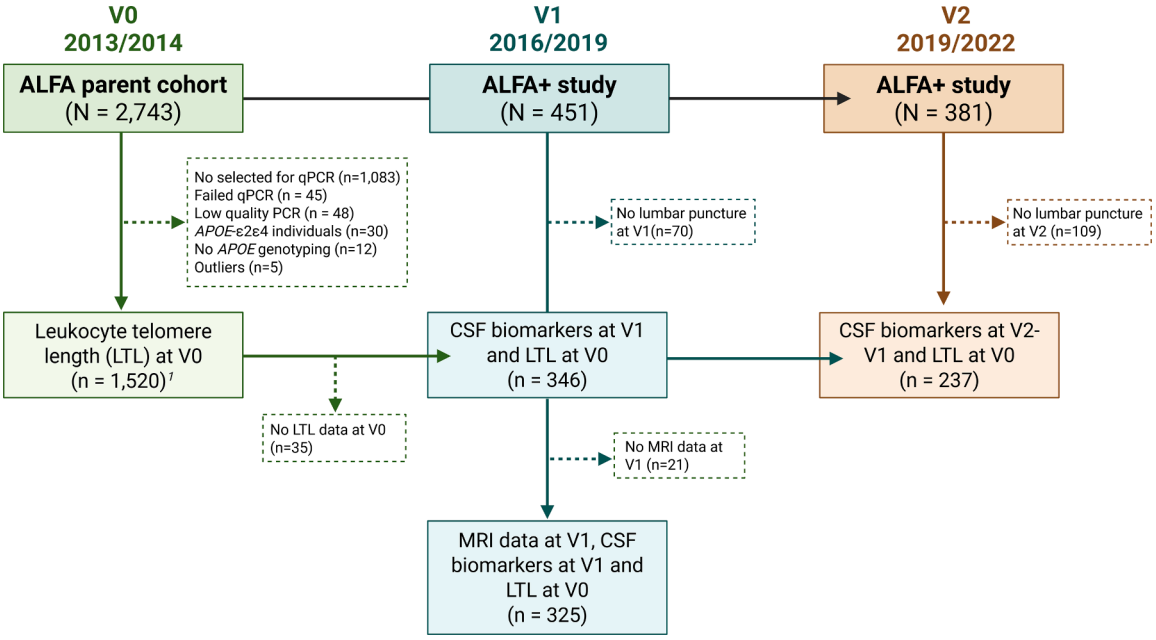

Fig. 1: **Flowchart of participant selection and dataset construction**. From 1520 cognitively unimpaired individuals with baseline LTL data (ALFA parent, visit 0, 2013–2014), three nested datasets were derived after quality control: (1) CSF V1 (N = 346), with CSF biomarkers at visit 1 (2016–2019) and LTL data at visit 0; (2) MRI V1 (N = 325), with CSF and MRI data at visit 1, and LTL data at visit 0; and (3) CSF longitudinal (N = 237), with CSF data at both visit 1 and visit 2 (2019–2022) and LTL data at visit 0. [1]Detailed quality control of LTL measurements can be found in Rodríguez-Fernández et al., 2024. [2]LTL outliers were removed once merged with CSF data.

between LTL and neuroimaging outcomes.[42,43] In this model, LTL was designated as the independent variable, CSF biomarkers reflecting astrocytic reactivity were considered the mediator, and cortical thickness was the dependent variable. To establish temporal precedence, LTL was measured in 2013/2014, whereas CSF biomarkers and cortical thickness were measured in 2016/2019. All analyses were conducted using R software (version 4.3.3).[44]

Ethics

The ALFA+ study (ALFA-FPM-0311) was approved by the Independent Ethics Committee of Parc de Salut Mar Barcelona and has been registered as a clinical trial (identifier: NCT02485730). All study participants provided written informed consent for study participation. The informed consent included authorisation to store biological samples for subsequent analyses related to the ALFA+ study, as well as for other research on AD and related disorders.

Role of funders

The funding sources were not involved in the study design, the analysis and interpretation of the data, the writing of this manuscript, or in the decision to submit this manuscript for publication. Roche Diagnostics provided NTK reagents in-kind to perform biomarkers measurements. A few Roche Diagnostics employees, listed as co-authors, made direct contributions to this research (see the Contributors section). In brief, CQR, GK helped in acquiring the biomarker data. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Characteristics of the study participants

Overall, no significant differences in LTL, sex, or BMI were observed across AT stages (Table 1). However, individuals in the A+T+ group were older and had lower levels of education compared to those in the A–T– or A+T– groups. A higher proportion of *APOE*-ε4 carriers was identified in the A+T– group compared to A–T– or A+T+, while no significant differences in *APOE*-ε4 status were observed between the A–T– and A+T+ groups.

CSF concentrations of NfL and t-tau progressively increased across AT stages at V1 (*i.e.*, from A–T– to A+T– to A+T+). At V2, no significant differences in CSF NfL concentrations were found between A–T– and A+T–. CSF concentrations of neurogranin, α-synuclein, GFAP, S100B, YKL-40, and sTREM2 were significantly higher in A+T+ compared to A–T–, with similar elevations (excluding S100B) noted in A+T+ compared to A+T–. In contrast, no significant differences in these biomarker concentrations were observed between A–T–

Characteristics	N	Overall	A−T− N = 215	A+T− N = 92	A+T+ N = 26	A−T+ N = 13	P value[a]
LTL at V0	346	1.03 (0.92, 1.15)	1.03 (0.92, 1.17)^{A}	1.02 (0.90, 1.15)^{A}	1.04 (0.99, 1.12)^{A}	1.02 (0.92, 1.06)^{A}	0.812
Age at V0 (y)	346	57.2 (53.5, 61.2)	56.3 (52.6, 60.9)^{A}	57.8 (55.8, 60.8)^{A}	57.2 (53.5, 61.6)^{B}	61.3 (57.4, 63.3)^{AB}	0.005
Age at V1 (ye)	346	61.4 (57.9, 64.6)	60.2 (57.0, 64.0)^{A}	61.9 (58.2, 65.2)^{A}	64.1 (62.5, 66.6)^{B}	62.6 (60.4, 63.2)^{A}	0.004
Sex	346						0.539
Females		215 (62%)	136 (63%)^{A}	52 (57%)^{A}	18 (69%)^{A}	9 (69%)^{A}	
Males		131 (38%)	79 (37%)	40 (43%)	8 (31%)	4 (31%)	
Education (y)	346	12 (11, 17)	12 (11, 17)^{A}	14 (11, 17)^{A}	11 (8, 17)^{B}	17 (12, 17)^{AB}	0.082
BMI (kg/m^2)	346	26.4 (24.3, 29.3)	26.4 (24.5, 30.1)^{A}	26.2 (23.9, 28.8)^{A}	26.3 (24.3, 28.6)^{A}	26.2 (22.9, 28.0)^{A}	0.654
***APOE*-ε4 carriers**	346	184 (53%)	88 (41%)^{A}	76 (83%)^{B}	15 (58%)^{A}	5 (38%)^{A}	<0.001
CSF biomarkers at V1, (pg/mL)							
Aβ42	346	1212 (847, 1678)	1397 (1085, 1819)^{A}	801 (669, 992)^{B}	842 (720, 1189)^{B}	2575 (2113, 2803)^{C}	<0.001
Aβ40	346	16,735 (13,760, 20,620)	16,530 (13,380, 20,060)^{A}	15,220 (13,490, 17,370)^{B}	22,505 (19,080, 25,030)^{C}	26,010 (25,980, 27,920)^{D}	<0.001
Aβ42/40 ratio	346	0.080 (0.061, 0.089)	0.085 (0.081, 0.093)^{A}	0.054 (0.044, 0.064)^{B}	0.042 (0.031, 0.051)^{C}	0.098 (0.091, 0.104)^{D}	<0.001
p-tau181	346	14 (11, 20)	13 (10, 17)^{A}	15 (12, 18)^{B}	29 (27, 33)^{C}	25 (24, 29)^{D}	<0.001
NfL	346	78 (61, 95)	74 (59, 88)^{A}	80 (63, 95)^{B}	111 (93, 138)^{C}	95 (85, 100)^{C}	<0.001
t-tau	346	181 (148, 237)	169 (139, 209)^{A}	183 (153, 218)^{B}	323 (302, 364)^{C}	303 (287, 325)^{C}	<0.001
Neurogranin	346	717 (570, 975)	680 (540, 895)^{A}	686 (571, 868)^{A}	1314 (1,178, 1443)^{B}	1356 (1283, 1419)^{B}	<0.001
α-synuclein	346	182 (142, 240)	173 (138, 230)^{A}	170 (146, 207)^{A}	295 (244, 352)^{B}	311 (298, 356)^{B}	<0.001
GFAP	346	7315 (5840, 9140)	6940 (5700, 8700)^{A}	7685 (5800, 8720)^{A}	10,755 (9050, 11,910)^{B}	7510 (6310, 9900)^{A}	<0.001
S100B	346	985 (854, 1160)	966 (852, 1120)^{A}	1030 (832, 1210)^{AB}	1130 (892, 1260)^{BC}	1120 (869, 1210)^{AC}	0.009
YKL-40	346	137,450 (107,900, 174,000)	133,300 (105,400, 159,300)^{A}	137,400 (100,950, 166,750)^{A}	200,850 (174,800, 234,100)^{B}	201,800 (150,000, 217,600)^{B}	<0.001
sTREM2	346	7575 (6390, 9280)	7550 (6380, 8950)^{A}	7185 (5960, 8740)^{A}	9875 (8700, 11,480)^{B}	10,250 (9930, 13,480)^{B}	<0.001
IL-6	346	3.56 (2.84, 4.43)	3.57 (2.91, 4.41)^{A}	3.54 (2.85, 4.44)^{A}	3.40 (2.77, 4.63)^{A}	2.98 (2.42, 4.65)^{A}	0.933
CSF biomarkers at V2, (pg/mL)							
Aβ42	237	1260 (862, 1795)	1424 (1,104, 1893)^{A}	755 (676, 989)^{B}	828 (661, 1128)^{B}	2249 (1,855, 3382)^{C}	<0.001
Aβ40	237	17,070 (14,560, 21,470)	16,740 (13,690, 20,860)^{A}	16,250 (14,360, 19,350)^{A}	22,410 (18,830, 25,780)^{B}	25,675 (23,565, 28,975)^{B}	<0.001
Aβ42/40 ratio	237	0.078 (0.057, 0.093)	0.088 (0.077, 0.098)^{A}	0.048 (0.040, 0.060)^{B}	0.038 (0.032, 0.049)^{C}	0.098 (0.073, 0.121)^{A}	<0.001
p-tau181	237	17 (13, 22)	15 (12, 19)^{A}	17 (15, 21)^{B}	32 (29, 39)^{C}	26 (22, 29)^{D}	<0.001
NfL	237	98 (78, 122)	93 (73, 117)^{A}	101 (78, 123)^{AB}	138 (119, 180)^{C}	104 (97, 126)^{BC}	<0.001
t-tau	237	196 (155, 255)	175 (145, 225)^{A}	201 (169, 260)^{B}	336 (292, 443)^{C}	300 (260, 337)^{D}	<0.001
Neurogranin	237	835 (621, 1158)	730 (566, 1018)^{A}	837 (724, 1092)^{B}	1443 (1347, 1623)^{C}	1412 (1199, 1521)^{C}	<0.001
α-synuclein	237	205 (147, 275)	181 (131, 243)^{A}	212 (158, 251)^{A}	322 (275, 403)^{B}	312 (281, 360)^{B}	<0.001
GFAP	237	10,200 (8160, 12,630)	9790 (7840, 12,040)^{A}	10,320 (8250, 12,550)^{A}	15,080 (14,070, 16,870)^{B}	11,320 (8805, 12,855)^{A}	<0.001
S100B	237	994 (849, 1150)	964 (834, 1090)^{A}	1010 (867, 1150)^{AB}	1160 (898, 1320)^{B}	1155 (1,000, 1195)^{B}	0.004
YKL-40	237	158,800 (123,000, 201,400)	149,300 (117,800, 186,100)^{A}	163,100 (135,300, 195,900)^{A}	229,000 (198,200, 272,500)^{B}	218,300 (167,750, 283,500)^{B}	<0.001
sTREM2	237	9290 (7510, 11,120)	8900 (7480, 10,530)^{A}	9280 (7390, 10,420)^{A}	10,830 (9,200, 14,000)^{B}	12,945 (11,950, 16,090)^{B}	<0.001
IL-6	237	4.21 (3.48, 5.28)	4.26 (3.58, 5.29)^{AB}	4.40 (3.56, 5.25)^{A}	4.03 (3.38, 5.07)^{AB}	3.76 (3.12, 3.89)^{B}	0.221
Cortical thickness							
Ageing signature	325	2.28 (2.23, 2.34)	2.28 (2.23, 2.35)^{AB}	2.28 (2.25, 2.34)^{A}	2.26 (2.18, 2.31)^{B}	–	0.099
AD signature	325	2.53 (2.47, 2.58)	2.53 (2.46, 2.58)^{AB}	2.54 (2.48, 2.60)^{A}	2.47 (2.42, 2.57)^{B}	–	0.026

Amyloid groups were defined with the CSF Aβ42/40 ratio (Aβ+: <0.071). Participants were tau positive (T+) if CSF p-tau181 > 24 pg/mL or tau negative (T−) if CSF p-tau181 ≤ 24 pg/mL. Mean (SD) follow-up time between visits 0 and 1 was 3.98 (1.02) years and between visits 1 and 2 was 3.45 (0.58) years. Abbreviations: A+, Aβ positive; A−T−, Aβ negative & tau negative; A+T−, Aβ positive & tau negative; A+T+, Aβ positive & tau positive; APOE-ε4: Apolipoprotein E ε4; BMI, Body Mass Index; CSF, cerebrospinal fluid; LTL, Leukocyte telomere length; N, sample size; V1, ALFA+ visit 1; V2, ALFA+ visit 2; y, years. Note: Continuous variables are summarised as median (Q1, Q3); categorical variables as n (%). [a]Comparisons across AT status groups were performed using the Kruskal–Wallis test for continuous variables and Fisher's exact test or Pearson's chi-squared test for categorical variables. Superscript capital letters indicate results from pairwise comparisons: groups sharing at least one letter are not significantly different (p > 0.05), whereas groups without a common letter differ significantly (p < 0.05). Pairwise comparisons between groups were conducted using Wilcoxon rank-sum tests or Fisher's exact tests, as appropriate.

Table 1: **Descriptives of study sample stratified by AT status.**

and A+T− at either visit. CSF IL-6 concentrations did not differ across AT stages at V1 or V2. Moreover, at V1, individuals in the A+T+ group exhibited thinner brain cortex compared to the A+T− group for both ageing- and AD-vulnerable brain regions, with a trend toward thinner cortices also observed in A+T− individuals compared to A-T-.

When stratified by *APOE*-ε4 status, a higher proportion of A+T− and A+T+ individuals was observed among ε4 carriers, whereas non-ε4 carriers showed higher CSF sTREM2 concentrations at both V1 and V2. Higher cortical thickness was observed among *APOE*-ε4 carriers in AD vulnerable regions (Table S2 in Supporting Information).

Association between LTL and AD-related CSF biomarkers

In the whole sample shorter LTL was associated with higher baseline GFAP (β = –0.11, P = 0.042) (Fig. 2a). This association remained statistically significant after adjusting for CSF Aβ42/40 (β = –0.11, P = 0.044). However, the association was no longer statistically significant after controlling for CSF p-tau181 (β = –0.08, P = 0.103), or Aβ40 levels which account for differences in CSF production and clearance rates (β = –0.08, P = 0.084).[45] Similarly, shorter LTL was associated with higher baseline levels of S100B (β = –0.13, P = 0.013) (Fig. 2b). This association persisted after adjusting for CSF Aβ42/40 ratio (β = –0.13, P = 0.014), p-tau181 (β = –0.12, P = 0.027), and Aβ40 levels (β = –0.12, P = 0.024).

Longitudinally, shorter LTL was associated with increased CSF α-synuclein levels over time (β = –0.12, P = 0.046) (Fig. 3). This association remained statistically significant after adjusting for CSF Aβ42/40 ratio (β = –0.14, P = 0.046), but was no longer significant after controlling for p-tau181 (β = –0.10, P = 0.054), and Aβ40 baseline levels (β = –0.09, P = 0.101).

No other statistically significant associations were found in the whole sample (Table S3).

Modification by APOE-ε4 status on CSF biomarkers

LTL showed statistically significant interactions with *APOE*-ε4 status on the association with CSF YKL-40 at baseline (β*int* = –0.2, P*int* = 0.047) and the change in CSF sTREM2 over time (β*int* = 0.27, P*int* = 0.036) (Figures S5 and S6 in Supporting Information). Specifically, shorter LTL was associated with higher baseline CSF YKL-40 (β = –0.19, P = 0.006) and decreasing CSF sTREM2 over time (β = 0.17, P = 0.049) only among ε4 carriers. No other statistically significant interactions between LTL and *APOE*-ε4 status were encountered (Table S4 in Supporting Information).

Nonetheless, when stratifying by *APOE*-ε4 status (Fig. 4), shorter LTL was associated with higher p-tau181 (β = –0.15, P = 0.033), NfL (β = –0.15, P = 0.026), t-tau (β = –0.17, P = 0.021), neurogranin (β = –0.15, P = 0.034), GFAP (β = –0.15, P = 0.040) and YKL-40 at baseline in carriers of the ε4 allele (Table S5, Figure S7). These associations were independent of Aβ status (Table S6 in Supporting Information). While trends were observed for CSF NfL, t-tau and GFAP, only the association with YKL-40 remained statistically significant after adjusting for T status (Table S7). In addition, shorter LTL was associated with longitudinal increases of α-synuclein (β = –0.20, P = 0.018) and decreases of sTREM2 over time (β = 0.17, P = 0.046) among *APOE*-ε4 carriers (Table S5 in Supporting Information). The association with longitudinal α-synuclein remained significant after adjustment for both Aβ and T status at baseline, while trends were observed for the longitudinal change in sTREM2 (Tables S6 and S7).

Modification by AT status on CSF biomarkers

LTL showed significant interactions with AT status in its association with baseline CSF Aβ42/40 (A–T+ vs. A–T–: β*int* = 3.89, P*int* = 0.002; A+T– vs. A–T–: β*int* = 0.57, P = 0.09) (Table S8; Figure S8 in Supporting Information). When stratifying by AT status (Fig. 4), trends were observed between LTL and CSF Aβ42/40 in both A–T+ individuals (β = 0.74, P = 0.080) and A+T– individuals (β = 0.18; P = 0.091). Regarding p-tau pathology, no significant interactions or associations were found in any AT groups. However, a trend was observed among A+T+ individuals with a suggestive negative association between LTL and CSF p-tau181 (β = –0.42; P = 0.085). Even though no other interactions between LTL and AT status were observed, statistically significant associations were encountered between LTL and CSF biomarkers

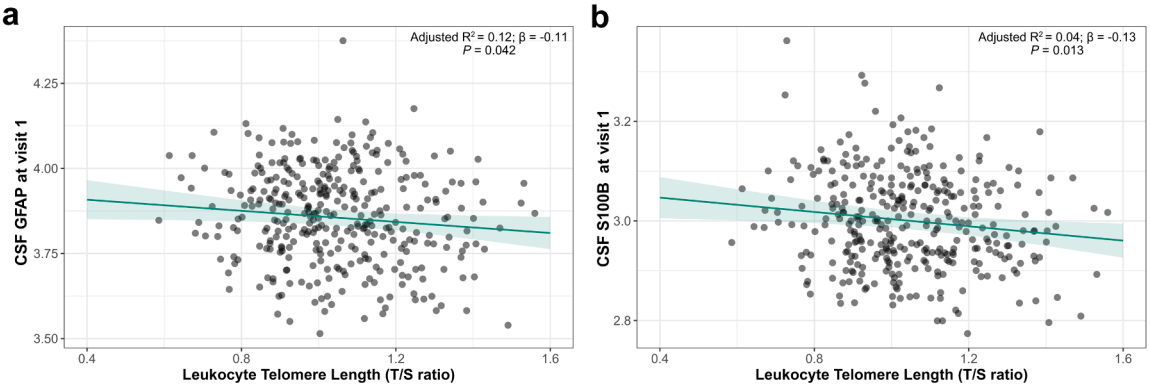

***Fig. 2:* Significant associations between LTL and CSF biomarkers at visit 1 in the global sample**. Scatter plot representing the linear association between LTL and log-transformed (a) CSF GFAP and (b) CSF S100B at visit 1 in the global sample (N = 346). Standardised regression coefficients (β) and P values (unadjusted; FDR-corrected values are provided in Supplementary Table S5) were calculated using a linear model adjusted for age, sex, and APOE-ε4 status.

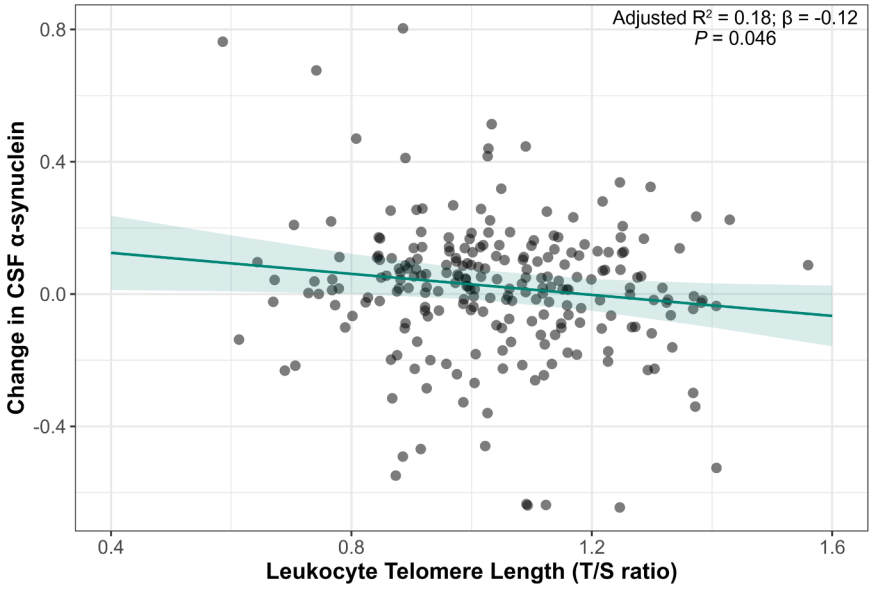

***Fig. 3:* Significant longitudinal association between LTL and change in CSF biomarkers over time**. Scatter plot representing the linear association between LTL and the change in log-transformed CSF α-synuclein over a mean follow-up period of 3.45 (SD = 0.58) years (N = 237). Standardised regression coefficients (β) and P values (unadjusted; FDR-corrected values are provided in Supplementary Table S5) were calculated using a linear model adjusted for age, sex, APOE-ε4 status, time difference between lumbar punctures, and CSF α-synuclein at visit 1.

when stratifying by AT status (Tables S5, S8 in Supporting Information).

In A-T- individuals, shorter LTL was longitudinally associated with increased CSF α-synuclein (β = –0.17, P = 0.042) and IL-6 over time (β = –0.18, P = 0.029) (Figure S9 in Supporting Information). In the A+T– group, shorter LTL was associated with higher baseline CSF NfL (β = –0.21, P = 0.021), S100B (β = –0.22, P = 0.042) and YKL-40 (β = –0.22, P = 0.020), whereas no significant associations were found for the change in CSF concentrations over time (Figure S10). Among A+T+ individuals, shorter LTL was also associated with higher CSF t-tau (β = –0.58, P = 0.016), α-synuclein (β = –0.48, P = 0.039), sTREM2 (β = –0.49, P = 0.046) at baseline. In addition, shorter LTL was longitudinally associated with decreased CSF S100B levels over time among A+T+ individuals (β = 0.72, P = 0.049) (Figure S11). No significant associations were observed in the A–T+ group.

Imaging biomarkers and leukocyte telomere length

Shorter LTL was associated with a thicker brain cortex in regions vulnerable to AD-related neurodegeneration (β = –0.11, P = 0.046) (Fig. 5a). This association was independent of CSF Aβ42/40 (β = –0.11, P = 0.046), p-tau181 (β = –0.12, P = 0.035), and NfL (β = –0.13, P = 0.021). Shorter LTL was associated with thicker cortex in ageing-vulnerable brain regions (β = –0.13, P = 0.019) (Fig. 5b). This association was independent of CSF Aβ42/40 (β = –0.13, P = 0.019), p-tau181 (β = –0.13, P = 0.019), and NfL (β = –0.13, P = 0.017) (Table S9).

Previous studies have reported positive associations between CSF biomarkers of glial reactivity and inflammation with higher grey matter volumes and thicker cortical thickness.[46–48] Therefore, we investigated whether shorter LTL's association with increasing CSF glial and inflammatory biomarkers could mediate its association with cortical thickness. Specifically, we explored the putative mediating role of glial (i.e., GFAP, S100B, YKL-40 and sTREM2) and inflammatory biomarkers (i.e., IL-6) in the association between LTL and cortical thickness.

The association between shorter LTL and higher cortical thickness in AD-vulnerable regions was partially mediated by GFAP (i.e., 23.8% of the effect mediated) (P = 0.038). Specifically, shorter LTL was indirectly associated with thinner cortex in AD signature through its effect on GFAP (Indirect pathway: β = 0.03, P = 0.010). However, the direct negative association between LTL and AD signature was still significant after accounting for the GFAP pathway (Direct pathway: β = –0.14, P = 0.012) (Fig. 5c).

In addition, a significant indirect association between shorter LTL and thinner AD signature through YKL-40 was observed (Indirect pathway: β = 0.019, P = 0.040). The direct negative association persisted after accounting for the YKL-40 pathway (Direct

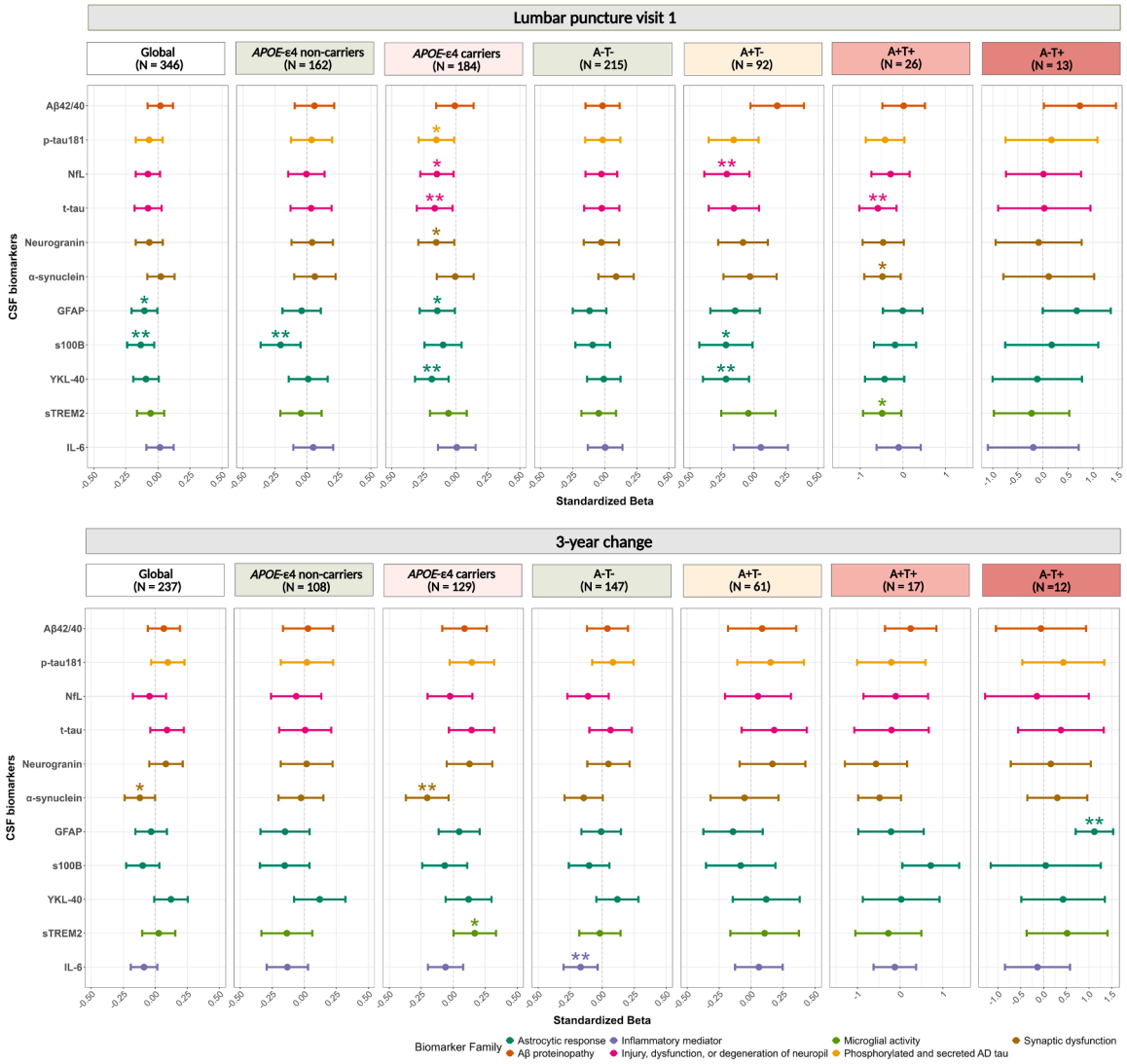

Fig. 4: **Forest plot illustrating the associations between LTL and CSF biomarkers at visit 1 and their longitudinal changes. Results are presented for the full sample and stratified by *APOE*-ε4 and AT status**. Each point represents the standardised coefficient for one association model, horizontal lines represent the 95% confidence intervals and colours represent the biomarker family for multiple comparison correction. Standardised regression coefficients (β) and P values were calculated using linear models for CSF biomarkers at visit 1 adjusted for age, sex, and *APOE*-ε4 status, while longitudinal models were further adjusted for time between lumbar punctures and CSF biomarker levels at visit 1.

pathway: β = –0.13, P = 0.018). A borderline mediating role of YKL-40 in the association between LTL and AD signature was observed (Mediation proportion: 16.94%; P = 0.064) (Fig. 5d).

No evidence for other mediation was detected for CSF S100B, sTREM2 or IL-6 in the association between LTL and AD signature. Regarding the ageing signature, a borderline significant mediating effect was observed for YKL-40 (Mediation proportion 16.19%, P = 0.088). LTL was indirectly and positively associated with the ageing signature through YKL-40 (Indirect pathway: β = 0.02, P = 0.036). Nonetheless, shorter LTL remained associated with higher cortical thickness in ageing-vulnerable regions after accounting for YKL-40 indirect pathway (Direct pathway: β = –0.13, P = 0.018). No evidence for other mediating roles were detected for CSF GFAP, S100B, sTREM2 or IL-6 in the association between LTL and ageing signature (Figure S12 in Supporting Information).

Discussion

In this study, we examined the association between baseline LTL, AD-related CSF biomarkers (measured

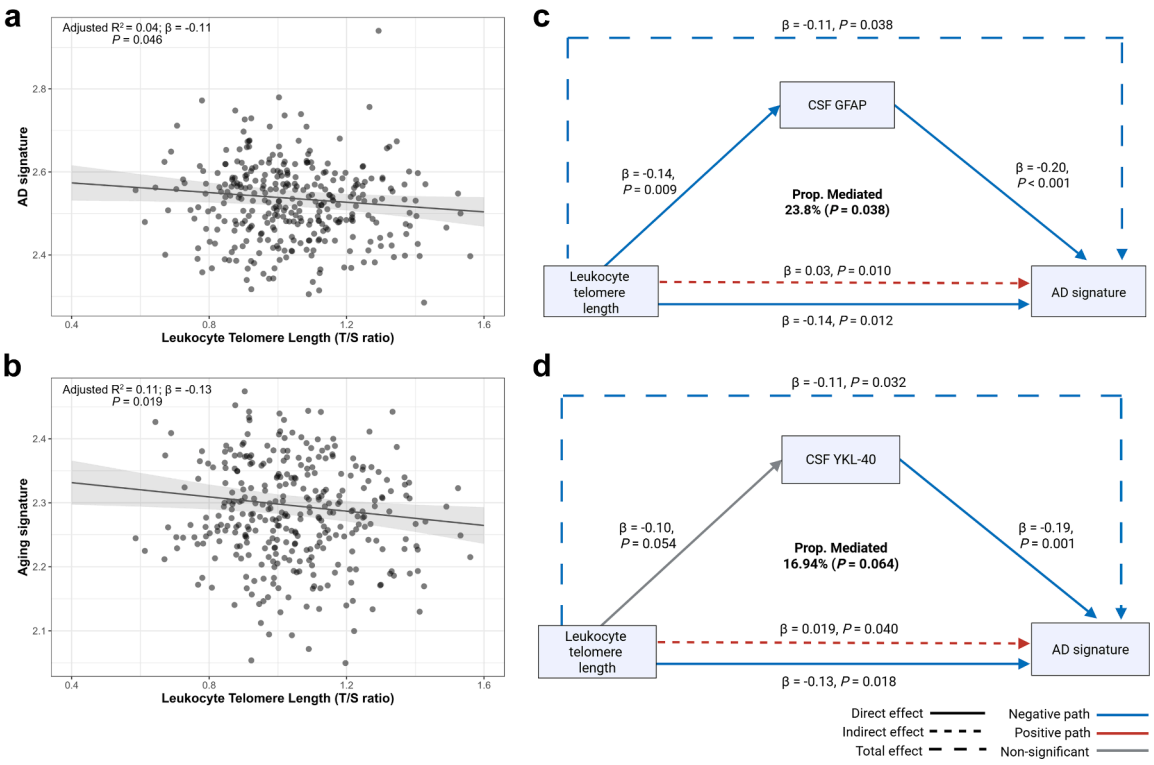

Fig. 5: **Associations between LTL and cortical thickness in AD- and ageing-vulnerable regions**. Scatter plot representing the linear association between LTL and (a) AD cortical thickness signature and (b) ageing cortical thickness signature. (c) Schema representing linear structural modelling results evaluating the mediator role of (c) CSF GFAP and (d) CSF YKL-40 on the association between LTL and AD cortical thickness signatures (N = 325). Models were corrected for age, sex, *APOE*-ε4 status, and firmware MRI version. All coefficients were standardised. AD signature represents average cortical thickness in entorhinal, inferior temporal, middle temporal, and fusiform. Ageing signature represents average cortical thickness in calcarine, caudal insula, cuneus, caudal fusiform, dorsomedial frontal, lateral occipital, precentral, and inferior frontal.

after 4 years and their longitudinal changes over 3 years), and cortical thickness in CU individuals at increased risk of AD. We found that shorter LTL was associated with biomarkers of tau pathology, neurodegeneration, synaptic dysfunction, and glial reactivity. These associations varied depending on *APOE*-ε4 carriership and AT biomarker status. Notably, shorter LTL was also associated with greater cortical thickness in brain regions vulnerable to ageing and AD-related neurodegeneration, with astrocytic reactivity biomarkers partially mediating the relationship.

Prior studies exploring the associations between LTL and AD-related CSF biomarkers focused on cognitively impaired patients with MCI or AD.[28,49] Our study extends these findings by incorporating genetic risk and disease stage during preclinical AD. Specifically, we observed that shorter LTL was associated with higher levels of p-tau181, t-tau, and NfL in *APOE*-ε4 carriers. Associations with NfL and t-tau were also detected in A+T– and A+T+ individuals, respectively. Although we did not observe significant associations between LTL and p-tau181 across AT-defined subgroups, we observed a trend toward increasingly negative effect estimates with disease progression, which may reflect stage-specific differences and limited power due to small sample sizes.

Shorter LTL was also associated with biomarkers of synaptic dysfunction. Among *APOE*-ε4 carriers, shorter LTL was associated with higher CSF neurogranin, a postsynaptic protein involved in memory formation[50] and previously associated with synaptic degeneration in patients with AD.[51–53] Additionally, shorter LTL was associated with higher CSF α-synuclein in A+T+ individuals and, longitudinally, with an increase in α-synuclein over time in the overall sample and among *APOE*-ε4 carriers. As we measured total CSF α-synuclein, this likely reflects the effect of shorter LTL on synaptic loss and neuronal injury.[35] This aligns with *in* vitro models in which telomere-shortened hiPSC-derived neurons show ageing-related phenotypes such as reduced neurite density and length.[54] Genes regulating telomere maintenance also impact gene expression and synaptic homoeostasis in the brain,[55] which

may underlie the observed associations. Given that LTL is highly heritable and established early in life, these findings may reflect developmental mechanisms with implications for brain ageing.[56]

LTL associations with biomarkers of tau pathology, neurodegeneration, and synaptic dysfunction were independent of amyloid status, suggesting a pathway through which telomere attrition may contribute to increased vulnerability to neurodegeneration and synaptic dysfunction via amyloid-independent pathways. These effects may be amplified under pathological conditions or in individuals with increased genetic risk, highlighting the importance of examining early-stage interactions between biological ageing mechanisms and AD pathology.

We also found that shorter LTL was associated with astrocytic reactivity, as reflected by higher CSF GFAP and S100B in the overall sample. These associations were independent of amyloid pathology but differed by *APOE*-ε4 carriership: the association between LTL and GFAP was primarily driven by *APOE*-ε4 carriers, whereas the association with S100B was observed only in *APOE*-ε4 non-carriers and in A+T– individuals. Although interaction models did not reveal significant modification by *APOE* or AT status, a significant interaction was observed for YKL-40. Specifically, shorter LTL was associated with higher YKL-40 exclusively among *APOE*-ε4 carriers.

These findings are consistent with previous evidence showing that increased glial activation and reactivity occurs early in the disease *continuum*.[35,57] CSF GFAP levels are elevated in patients with AD and have been associated with cognitive decline.[58,59] S100B, a protein primarily expressed by astrocytes and released under cell stress,[60] is found to be elevated in CSF across various neurodegenerative diseases, including AD, and correlates with brain atrophy and cognitive impairment.[61] Similarly, YKL-40 is an astrocyte-derived biomarker upregulated in response to pathology[62] which is highly expressed in astrocytes surrounding Aβ deposits and shows increased CSF concentrations since prodromal AD.[63]

Beyond astrocytic reactivity, our results suggest that shorter LTL is linked to dynamic changes in microglial activation and neuroinflammation. While CSF sTREM2 typically increases during early stages of AD following amyloid accumulation,[64] microglial dysfunction has also been documented in normal ageing and among *APOE*-ε4 carriers.[65,66] In our study, shorter LTL was associated with higher baseline sTREM2 in A+T+ individuals and with longitudinal decreases in sTREM2 among *APOE*-ε4 carriers. Furthermore, shorter LTL was associated with longitudinal increases in IL-6 in A-T-individuals. In our sample, higher baseline CSF sTREM2 has been previously associated with better memory and executive outcomes, independent of AD pathology.[67] The contrasting baseline and longitudinal associations between LTL and CSF sTREM2 suggest a complex and dynamic interplay between LTL, microglial activation, and neuroinflammation across the disease *continuum*, varying by *APOE*-ε4 status.

Collectively, these findings suggest that telomere length and homoeostasis may be associated with glial activity and neuroinflammation in an ApoE and pathology-associated manner, particularly in early AD stages. Interestingly, shorter telomeres in human iPSC-derived astrocytes from old donors were related to increased production of inflammatory cytokines, GFAP expression and elevated DNA damage when compared to younger donors.[54] In addition, distinct inflammatory and glial signatures have been found in relation to *APOE* genotype and cognitive performance stages in patients with AD,[68] suggesting that LTL could play a role in pathways associated with resilience to disease progression.

Interestingly, shorter LTL was associated with greater cortical thickness in AD-vulnerable regions. This finding contrasts with previous studies reporting positive associations between LTL and cortical thickness.[25,27,69] However, transient increases in cortical thickness have been described in CU individuals at risk for AD and are thought to reflect early glial activation and amyloid accumulation.[48,70–74] In our sample, the association between LTL and cortical thickness was independent of CSF Aβ42/40, p-tau181, and NfL, and was partially mediated by astrocytic biomarkers, including GFAP and YKL-40. The persistence of a direct effect even after accounting for these mediators suggests additional pathways linking peripheral telomere biology and brain structure.

The divergence between our observational findings and prior MR analyses in the ALFA cohort[29] may reflect differences in underlying biological processes captured by each approach. MR estimates the lifelong effects of genetically determined LTL and may reflect telomerase-related or developmental pathways contributing to brain reserve.[75] In contrast, our observational findings likely capture the impact of acquired telomere shortening on biological ageing. Notably, the MR study did not include astrocytic or inflammatory biomarkers, which were significantly associated with LTL in our analyses. Future MR studies incorporating broader biomarker panels, including glial and immune markers, and using bidirectional or multivariable designs will be essential to clarify these relationships.

The mechanisms by which telomere shortening impacts brain structure remain unclear,[27] but may involve cellular ageing, oxidative stress, and immune dysregulation.[76] LTL is inversely and strongly correlated with a blood proteomic age clock driven by immune and inflammatory cytokines, which predicts mortality, cognitive decline, and neurodegeneration.[77] LTL is also considered a marker of peripheral immune ageing, and is associated with increased risk of infection and

systemic inflammation.[14,78] Growing evidence suggests that peripheral immune activation may trigger microglial responses, neuroinflammation and astrocyte reactivity via the neuroimmune axis.[79] Based on our findings, we propose that peripheral immune ageing, as reflected by telomere shortening, may contribute to altered glial reactivity and cortical remodelling, potentially influencing vulnerability to neurodegeneration.[80,81] The dynamic and context-dependent effects of LTL on glial activation may underlie its non-linear associations with cognition and AD risk across disease stages.

Our study is not without limitations. The ALFA+ cohort includes middle-aged, CU individuals at increased risk of AD, which may limit generalisability to more advanced disease stages or to individuals with comorbidities. The causal mediation model is based on cross-sectional data for CSF and imaging biomarkers, which may limit causal inference; however, it aligns with biological models suggesting that astrocytic changes precede neurodegeneration. The average follow-up duration of 3.5 years may have restricted our ability to capture long-term biomarker dynamics. Additionally, the sample size limited our power to detect associations surviving correction for multiple comparisons. Future research should examine LTL associations with a broader range of neuroimaging phenotypes, including plasma biomarkers related to cellular senescence and peripheral immune ageing, and extend to larger, population-based samples with longer follow-up.

A key strength of this study is the use of a relatively young CU sample, which minimises the effects of survival and diagnostic bias and allows for the investigation of early mechanisms of disease vulnerability. By focussing on preclinical population, we are better positioned to capture the earliest biological changes linking biological ageing to central nervous system pathology.

In conclusion, our findings suggest that telomere shortening is associated with AD-relevant biomarker changes, particularly those related to synaptic dysfunction and glial reactivity. Astrocytic biomarkers partially mediated the relationship between LTL and cortical thickness, supporting a pathway that links peripheral biological ageing to brain structural integrity. Further research is warranted to elucidate the role of telomere dynamics in brain ageing and to explore their potential in identifying determinants of resilience or targets for dementia prevention.

Contributors

MC-B and NV-T designed the study and secured the funding to perform the research. BR-F analysed and interpreted data and wrote the manuscript. NV-T, MC-B, AG-E contributed to data interpretation and manuscript writing. NV-T, PG and AG-E contributed to statistical modelling. MC-B, MS-C, TEE, PO-R, GK, CM, JDG, NV-T, NJA, HZ, KB, AN, AS-V contributed to data collection or analysis. All authors critically revised the manuscript and approved its content before submission. BR-F, AG-E, PG, TEE, MC-B, and NV-T have accessed and verified the underlying data. The ALFA Study Group established the ALFA+ cohort and collected the clinical, biological, and imaging data used in this manuscript. All authors read and approved the final version of the manuscript.

Data sharing statement

All requests for raw and analysed data and materials will be promptly reviewed by the corresponding authors and the Barcelonaβeta Brain Research Center to verify whether the request is subject to any intellectual property or confidentiality obligations. Requests can be directed to: brodriguez@barcelonabeta.org, nvilor@barcelonabeta.org. Bulk Anonymised data can be shared by request from any qualified investigator for the sole purpose of replicating procedures and results presented in the article, provided data transfer is in agreement with EU legislation.

Declaration of interests

BR-F, AG-E, TEE, MS-C, PO-R, NA, AN, and AS-V have nothing to disclose. GK is a full-time employee of Roche Diagnostics GmbH. CM has received research funding from an EU-FINGERS JPND research grant and from an ADDF digital biomarkers research grant; both paid to the institution. JDG receives research funding from Roche Diagnostics, Hoffmann-La Roche, GE Healthcare, the Innovative Health Initiative (IHI) of the European Commission (Grant agreement No. 101112145), BrightFocus Foundation (A2022034S), Instituto de Salud Carlos III (PMP22/00022), and Fundació La Marató de TV3 (202318-30-31-32). He has given lectures in symposia sponsored by Biogen, Philips, and Life-MI; received consulting fees from Roche Diagnostics; and serves on a scientific advisory board at Prothena Biosciences. HZ has served on scientific advisory boards and/or as a consultant for: Abbvie, Acumen, Alector, Alzinova, ALZpath, Amylyx, Annexon, Apellis, Artery Therapeutics, AZTherapies, Cognito Therapeutics, CogRx, Denali, Eisai, LabCorp, Merry Life, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Quanterix, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave. He has given lectures sponsored by: Alzecure, BioArctic, Biogen, Cellectricon, Fujirebio, Lilly, Novo Nordisk, Roche, and WebMD. HZ is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is part of the GU Ventures Incubator Program (outside the submitted work). He is supported by the Swedish Research Council (#2023-00356, #2022-01018, and #2019-02397); the European Union's Horizon Europe research and innovation programme (grant agreement No. 101053962); Swedish State Support for Clinical Research (#ALFGBG-71320); Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862); the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C, #ADSF-21-831377-C, and #ADSF-24-1284328-C); the European Partnership on Metrology (NEuroBioStand, #22HLT07); the Bluefield Project; Cure Alzheimer's Fund; the Olav Thon Foundation; the Erling-Persson Family Foundation; Familjen Rönströms Stiftelse; Stiftelsen för Gamla Tjänarinnor; Hjärnfonden, Sweden (#FO2022-0270); the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 860197 (MIRIADE); the European Union Joint Programme—Neurodegenerative Disease Research (JPND2021-00694); the National Institute for Health and Care Research University College London Hospitals Biomedical Research Centre; and the UK Dementia Research Institute at UCL (UKDRI-1003). KB has served as a consultant, on advisory boards, or on data monitoring committees for: Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Prothena, Roche Diagnostics, and Siemens Healthineers. He is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), part of the GU Ventures Incubator Program. He is supported by the Swedish Research Council (#201700915); Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB2018092016615); the Swedish Alzheimer Foundation (#AF742881); Hjärnfonden, Sweden (#FO20170243); the Swedish state under the agreement between the Swedish government and the County

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2025.105886.

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