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Late-onset temporal lobe epilepsy: insights from brain atrophy and Alzheimer's disease biomarkers

Alice Ballerini,[1] Niccolò Biagioli,[1] Chiara Carbone,[1] Annalisa Chiari,[2] Manuela Tondelli,[1,2] Giulia Vinceti,[2] Roberta Bedin,[1] Marcella Malagoli,[3] Maurilio Genovese,[3] Simona Scolastico,[1] Giada Giovannini,[4] Matteo Pugnaghi,[4] Niccolò Orlandi,[1,4] Louis Lemieux,[5] Stefano Meletti,[1,4] Giovanna Zamboni[1,2,†] and Anna Elisabetta Vaudano[1,4,†]

[†]These authors contributed equally to this work.

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Considering the growing age of the world population, the incidence of epilepsy in older adults is expected to increase significantly. It has been suggested that late-onset temporal lobe epilepsy (LO-TLE) may be neurodegenerative in origin and overlap with Alzheimer's disease (AD). Herein, we aimed to characterize the pattern of cortical atrophy and CSF biomarkers of AD (total and phosphorylated tau and amyloid-β) in a selected population of LO-TLE of unknown origin.

We prospectively enrolled individuals with temporal lobe epilepsy onset after the age of 50 and no cognitive impairment. They underwent a structural MRI scan and CSF biomarkers measurement. Imaging and biomarkers data were compared to three retrospectively collected groups: (i) age-sex-matched healthy controls; (ii) patients with mild cognitive impairment (MCI) and abnormal CSF AD biomarkers (MCI-AD); and (iii) patients with MCI and normal CSF AD biomarkers (MCI-noAD).

From a pool of 52 patients, 20 consecutive eligible LO-TLE patients with a mean disease duration of 1.8 years were recruited. As control populations, 25 patients with MCI-AD, 25 patients with MCI-noAD and 25 healthy controls were enrolled. CSF biomarkers returned normal values in LO-TLE, significantly different from patients with MCI due to AD. There were no differences in cortico-subcortical atrophy between epilepsy patients and healthy controls, while patients with MCI demonstrated widespread injuries of cortico-subcortical structures.

Individuals with LO-TLE, characterized by short disease duration and normal CSF amyloid-β and tau protein levels, showed patterns of cortical thickness and subcortical volumes not significantly different from healthy controls, but highly different from patients with MCI, either due to AD or not.

1 Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy
2 Neuroscience Department, Neurology Unit, OCB Hospital, AOU Modena, 41126 Modena, Italy
3 Neuroscience Department, Neuroradiology Unit, OCB Hospital, AOU Modena, 41126 Modena, Italy
4 Neuroscience Department, Neurophysiology Unit and Epilepsy Centre, AOU Modena, 41126 Modena, Italy
5 Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, London WC1N 3BG, UK

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Correspondence to: Stefano Meletti
Ospedale Civile Baggiovara (OCB)
Azienda Ospedaliero Universitaria of Modena
Via Giardini 1355, 41126 Modena, Italy
E-mail: stefano.meletti@unimore.it

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Introduction

The incidence of epilepsy can be well described by a U-shape curve, with its highest prevalence of new-onset cases occurring during childhood and in individuals aged 50 years and older.[1,2] Considering the increasing average age of the world population, late-onset epilepsies (LOEs) are expected to rise significantly, emerging as a prominent healthcare concern.[3] The majority of LOEs result from structural causes, primarily cerebrovascular disease (30%–50%),[4,5] traumatic brain injuries (≤25%)[6,7] and brain tumours (10%–30%).[8,9] However, up to 20% of LOEs lack identifiable structural damage and/or defined aetiology.[10,11] These cases are termed late-onset epilepsy of unknown aetiology (LOEU).[11] The features of LOEU seizures are predominantly described with a focal origin and a focal semeiology,[12] with only up to 15% of LOEU described as generalized seizures.[10,13-15] Electroclinical and metabolic studies have localized epileptiform discharges and hypometabolism in either unilateral or bilateral temporal lobes,[16] similar to those observed in common temporal lobe epilepsy (TLE) with onset earlier in life. Several studies have proposed a link between LOEU and neurodegenerative diseases, especially Alzheimer's disease (AD). While 10%–20% of AD patients report a history of seizures occurring years before the diagnosis, LOEU has a 7% to 38% chance of developing dementia within 4.5 years after the epilepsy onset.[10,13,17] Previous studies have proposed the temporal forms of LOEU (i.e. late-onset TLE; LO-TLE) as possible neurodegenerative diseases with a greater propensity to develop AD.[13,17] CSF pathological changes typical of AD, i.e. amyloid-β (Aβ) and tau protein, have been observed in some patients with LOEU. Specifically, recent studies have found decreased Aβ levels and increased phosphorylated tau ($pTAU_{181}$) levels in the CSF of people with LOEU compared with age-matched controls without epilepsy.[18-20] Interestingly, the pathological level of CSF Aβ at baseline was associated with a 3.4 times higher chance of progression to AD at the follow-up.[10,21] Additionally, brain atrophy and cognitive dysfunctions have been suggested as shared features between LO-TLE and mild cognitive impairment (MCI)[22]—in some cases, the prodromal phase of AD. Recently, Keastner *et al.*[22] demonstrated that older adults with TLE exhibit a similar pattern and magnitude of medial temporal lobe atrophy to amnestic MCI (aMCI), and similar profiles in memory and language impairment. However, so far, no study has characterized brain atrophy patterns coupled with AD biomarkers in LO-TLE. In this study, we compared cortico-subcortical morphometric features in a well-characterized population of LO-TLE taking into account the CSF Aβ and tau loads, with respect to healthy older adults and two cohorts of retrospectively collected patients with (i) MCI and pathologic CSF AD biomarkers (i.e. MCI due to AD[23]; MCI-AD); and (ii) MCI with normal CSF AD biomarkers (i.e. MCI not due to AD; MCI-noAD). We hypothesize that biological phenotyping of LO-TLE throughout CSF biomarkers for AD is crucial for a correct interpretation of related patterns of cortical and subcortical atrophy.

Materials and methods

Participants

Epilepsy cohort

From January 2020 to August 2023, we prospectively enrolled TLE patients aged 50 years and over, hospitalized at the Epilepsy Monitoring Unit (EMU) of the OCB Hospital (Modena, Italy) for clinical diagnostic workup in the context of a new onset seizure disorders of unknown origin. Patients underwent a dedicated epilepsy MRI protocol[24,25] and a lumbar puncture according to clinical needs, which includes, among other tests, the measurement of Aβ ($Aβ_{1–42}$, $Aβ_{1–42}/Aβ_{1–40}$ ratio), $pTAU_{181}$ and total tau (tTAU) as biomarkers for AD.[26] This clinical assessment follows a local diagnostic clinical pathway dedicated to patients with late-onset epilepsy of unknown aetiology.

For the specific purposes of the present study, we recruited patients with an epilepsy onset at >50 years of age; this age cut-off was chosen based on an inflection point in epilepsy incidence around the age of 50.[27] The TLE diagnosis was made by neurologists with expertise in epileptology (G.G., M.P., N.O., S.M. and A.E.V.) and according to the criteria defined by the International League Against Epilepsy (ILAE).[28] The evidence of unilateral or bilateral temporal lobe pathological activity was captured by long-term video-EEG (LT-VEEG) monitoring during the hospitalization at the EMU, and the high-resolution 3D T_1-weighted sequences (3D-T_1) with 1 mm voxel size performed on a 3 T scanner were collected for each patient. The MRI images were visually inspected by neuroradiologists with expertise in epileptology (M.M. and M.G.), and only those patients without brain tumours or lesions that would directly disrupt the cortical mantle were enrolled in this study. Patients with a medical history of other significant neurological and/or psychiatric diseases, a pre-existing diagnosis of dementia and/or use of acetylcholinesterase inhibitors, and a Mini-Mental State Examination (MMSE) raw score lower than 26[29] were excluded from the study. Additional exclusion criteria were a history of obstructive sleep apnoea syndrome, encephalitis/meningitis, head trauma and a past occurrence of ischaemic (i.e. lacunar stroke and transient ischaemic attack; TIA) and/or haemorrhagic events. A history of cardiovascular disorders or related risk factors (i.e. diabetes, hypertension) was not considered an excluding criterion. Patients with abnormal CSF inflammatory biomarkers (i.e. biochemical, cytology and microbiological evaluation) as well as with antineuronal antibodies suggestive of autoimmune paraneoplastic or non-paraneoplastic encephalitis [i.e. anti-NMDAR, GABABR, AMPAR 1 and 2, LGI1, CASPR2, DPPX, Amphiphysin, CV2, PNMA2, Ri, Yo, Hu, Recoverin, Titin, Zic4, GAD65, Tr (DNER) and AntiSOX1] were also excluded.

For each patient who met the inclusion criteria, the following clinical variables were collected: age of first seizure, epilepsy duration, seizure frequency, number and type of anti-seizure

medications (ASMs) taken at the time of the MRI, family history of epilepsy and history of febrile seizures during childhood. Seizure frequency was assessed by modifying the So *et al.*[30] classification to a 1 to 7 continuum scale as follows: 1, less than one seizure per year; 2, one to three seizures per year; 3, four to eleven seizures per year; 4, one to three seizures per month; 5, one to six seizures per week; 6, one to three seizures per day; or 7, more than four seizures per day. The response to ASMs was evaluated according to the ILAE classification of drug resistance[28] and was collected at follow-up visits regularly scheduled at our department. The first visit was scheduled 6 months after hospital discharge, followed by annual follow-ups. Temporal lobe seizure onset—mesial or lateral—was determined based on LT-VEEG monitoring ictal recordings,[31-33] interictal epileptiform activity localization and clinical features. Finally, MRI images were reviewed for cerebral small vessel disease (SVD) and white matter hyperintensities. The 3D fluid attenuated inversion recovery (FLAIR) sequences were assessed using the visually rated Fazekas scale[34] by expert neuroradiologists (M.M. and M.G.) as follows: 0, none or a single lesion; 1, multiple punctate lesions; 2, beginning of confluency; and 3, large confluent lesions.

Mild cognitive impairment cohort

Two cohorts of patients with a MCI diagnosis stratified according to AD biomarkers profile were retrospectively recruited from the Cognitive Neurology Center at OCB Hospital (Modena, Italy) and included as control groups. The diagnosis was established by neurologists with expertise in neurodegenerative and cognitive disorders (A.C., M.T., G.V. and G.Z.) according to the Petersen criteria.[35] For the purposes of the present study, additional inclusion criteria for individuals with MCI were: (i) availability of structural MRI data including a high-resolution 3D-T_1 on the same scanner as LO-TLE; (ii) a CSF analysis including the dosage of AD biomarkers; and (iii) no history of epilepsy/seizures. All patients with a clinical diagnosis of dementia and the presence of other neurological diseases that would impair cognition (e.g. hydrocephalus, multiple sclerosis) were excluded.

Healthy controls

Finally, a population of age- and sex-matched healthy volunteers who were enrolled in previous MRI study protocols were retrospectively selected as a comparison group for both epilepsy and MCI patient groups. The inclusion criteria applied to healthy participants were: (i) no history of neurological disease, including subjective cognitive symptoms; and (ii) no psychotropic medications intake at the time of the MRI. MMSE raw scores were collected when available.

CSF Alzheimer's disease biomarkers

The CSF samples were collected during patients' hospitalization and measured with the CLEIA method (i.e. Lumipulse G600II) following manufacturer instructions (Fujirebio Inc.) by a biologist (R.B.) with expertise in AD diagnosis. From the CSF samples, the following biomarkers were analysed: $A\beta_{1–42}$, $A\beta_{1–40}$, tTAU and $pTAU_{181}$, and the $A\beta_{1–42}/A\beta_{1–40}$ ratio was calculated. Biomarkers cut-off for normal values, established according to literature, manufacturing and our specific laboratory data[36,37] were: $A\beta_{1–42} > 600$ pg/ml, $A\beta_{1–42}/A\beta_{1–40}$ ratio > 0.069, tTAU > 400 pg/ml and $pTAU_{181} < 56.5$ pg/ml.

According to Tondelli *et al.*,[38] and based on the above cut-off values, the MCI subjects were classified as MCI-AD if $A\beta_{1–42}/A\beta_{1–40}$ ratio, $pTAU_{181}$ and tTAU levels were abnormal, or MCI-noAD if the three markers were within normal limits.

Image acquisition, cortical thickness and volumetry quantification

The MRI evaluation was performed on a 3 T GE Healthcare MRI scanner, and the 3D-T_1 sequences were collected for patients and controls (repetition time = 7.7 ms, echo time = 3.1 ms, flip angle = 8°, field of view = 256 mm, matrix = 256 × 256, slices = 178, slice thickness = 1.0 mm).

FreeSurfer v7.3.2[39] software was adopted to obtain cortical thickness estimates and subcortical volumes. Regarding the cortical thickness quantification, a subject-specific cortical surface model was generated from each 3D-T_1 scan. Then, the cortical surface mantle was reconstructed and registered to the Conte69 template surface (32k vertices per hemisphere) using workbench tools[40-42] and spatially smoothed using a Gaussian kernel, full-width at half-maximum (FWHM) = 10 mm.[43] Cortical thickness was estimated as the distance between the white matter and the pial surfaces for each vertex. Each subject's cortical surface was parcellated based on the Glasser atlas[44] into 180 regions of interest (ROIs) per hemisphere using the ENIGMA-Toolbox's functions 'surface to parcel'[45] in MATLAB (R2021b).

Sixteen subcortical volumes were calculated from the automatic FreeSurfer segmentation. A dedicated pipeline for FreeSurfer was used to perform the automatic parcellation of the hippocampus and the amygdala subfields.[46,47] The following hippocampal subfields' volumes were thus obtained for each hemisphere: hippocampal body, hippocampal head, hippocampal tail, hippocampal fissure, subiculum, presubiculum, parasubiculum, CA1, CA2/3, CA4, molecular layer, granule cell and molecular layer of the dentate gyrus (GC-ML-GD), fimbria and hippocampal-amygdala transition area (HATA). Each amygdala was segmented into nine nuclei according to previous studies from our group[48-50]: anterior amygdaloid area (AAA), corticoamygdaloid transition area (CAT), basal nucleus (Ba), lateral nucleus (La), accessory basal nucleus (AB), central nucleus (Ce), cortical nucleus (Co), medial nucleus (Me) and paralaminar nucleus (PL). As previously performed by our group,[48-50] the amygdalae nuclei were clustered based on their cytoarchitectonics, histochemistry and connections[51] into major regions (or 'complexes'): (i) the basolateral amygdala (BLA), the deepest complex which includes La, Ba, AB and PL nuclei; (ii) the superficial group named cortical amygdala (CA), which include the Co nucleus only; (iii) and the central-medial amygdala (CMA) composed of the Me and Ce nuclei.

A quality check of cortical parcellation and subcortical segmentations was conducted following standardized ENIGMA protocols (http://enigma.usc.edu).

Statistical analysis

Demographic and clinical variables

One-way ANOVAs and chi-squared tests were conducted to assess differences in demographic and clinical variables, respectively, quantitatively and qualitatively, between groups. Statistical significance for all tests was set at $P < 0.05$.

CSF Alzheimer's disease biomarkers analysis

One-way ANOVA was conducted to assess differences in the concentration of each CSF AD biomarker across patients' groups only. Statistical significance was set at $P < 0.05$. All *P*-values were adjusted with false discovery rate (FDR) multiple comparisons correction, and only those that survived to the 5% FDR correction were considered significant.[52]

To assess the influence of the seizure frequency and the chronicity of epilepsy, we performed a non-parametric Spearman correlation between these clinical features and the CSF AD biomarker concentrations in the LO-TLE population.

Cortical thickness and subcortical volumetry analysis

The brain features were normalized into z-scores based on the controls' mean and standard deviation. To address the lateralization of the seizure focus, volumetric and thickness values of right-lateralized LO-TLE were flipped[48,53,54] to ensure that all morphometric data were considered ipsilateral to the epileptic focus on the left hemisphere. Thus, all the following analyses and results are presented as ipsilateral or contralateral concerning the LO-TLE's seizure focus. The same approach was not applied to the MCI cohorts due to the lack of a specific lateralizing pattern in neurodegenerative diseases.

After the normalization and flipping processes, the cortical thickness was analysed in two steps: a vertex-wise analysis was applied to the surface maps, then an ROI-based analysis was performed on the parcellated data. The vertex-wise statistical analysis was performed using BrainStat[55] on MATLAB (R2021b) exploring the differences between each patient's group (i.e. LO-TLE, MCI-AD, MCI-noAD) and the healthy controls, with age and sex included as covariates. Thereafter, the Glasser parcellation obtained from the surface-based maps with the ENIGMA-Toolbox[45] on MATLAB (R2021b) was analysed with a Multivariate Analysis of Covariance (MANCOVA) between all four groups using SPSS software 28 (IBM, Chicago, IL). Age and sex have been included in the model as covariates. A *post hoc* analysis was performed to assess the effect of each pairwise comparison.

Likewise, a series of MANCOVAs, and relative *post hoc* analyses, were conducted to explore the volume differences of the subcortical structures and sub-segmentations across groups as well. Age, sex and intracranial volume (ICV) were included as covariates in all comparisons.

Statistical significance for all tests was set at $P < 0.05$, *P*-values were adjusted with FDR, and those who survived the 5% FDR correction were considered significant.[52]

Standard protocol approval, registration and patient consent

The study was approved by the local Ethical Committee of Area Vasta Emilia Nord (N. 155/14 and N. 238/23 for LO-TLE patients, N. 832/18 for MCI patients, N. 134/14 and N. 679/22 for healthy participants). Patients gave written informed consent for the use of their clinical records for this study. The study was conducted following the World Medical Association Declaration of Helsinki.

Results

Patients

From an original population of 52 patients with LO-TLE, 20 patients fulfilled all the reported inclusion/exclusion criteria and were enrolled (Fig. 1). Specifically: 14 patients did not have a lumbar puncture, six patients were excluded because of the lack of 3D-T_1 sequence, five LO-TLE had other neurological comorbidities (two patients had brain tumours, two cerebral amyloid angiopathy, and one Parkinson's disease), five had positive CSF results for autoimmune diseases. Finally two patients were excluded because of a MMSE score lower than 26.[29]

The clinical characterization of the 20 LO-TLE enrolled in the study is summarized in Table 1. The mean age of onset was 61.30 years (±8.8, range 51–79) and the disease duration was 1.80 years (±2.4, range 0–8). None of the patients presented febrile seizures during childhood, and only two patients (10%) had a positive family history of epilepsy. According to seizures' frequency classification, 8 of 20 patients presented less than one seizure per year (40%), two patients had yearly seizures (10%), seven patients presented monthly seizures (35%) and two patients had weekly seizures (10%); only one patient presented daily seizures (5%). All the LO-TLE enrolled were classified as mesial TLE except for Subject 2 who had focal-to-bilateral tonic-clonic seizures arising from sleep only. LT-VEEG monitoring for this patient showed interictal anterior temporal spikes (the electrode more involved was T3), although no seizures were recorded. Most of the patients (75%) showed a good response to ASMs, with only three cases of drug resistance. The mean follow-up for this cohort of patients was 21.95 (±9.185) months. Twelve out of 20 patients (60%) were on monotherapy at the time of the MRI scan. Nearly all MRIs (90%) from this cohort have been referred as negative for potentially epileptogenic lesions; the only exceptions were two cases with hippocampal sclerosis (HS). The mean Fazekas score in the LO-TLE cohort was 0.6 (±0.503, range 0–1).

Fifty MCI patients with no epilepsy history were enrolled; according to the results of the CSF biomarker evaluation, 25 were classified as MCI-noAD and 25 as MCI-AD. Finally, a population of 25 age-sex-matched controls was recruited and the relative 3D-T_1 images were collected.

Control and patient populations did not significantly differ in age, sex and ICV. Patients showed lower education compared to controls ($P = 0.036$), particularly MCI-noAD ($P_{FDR} = 0.032$) and LO-TLE ($P_{FDR} = 0.021$). LO-TLE did not differ statistically from both MCI groups in years of disease duration and/or onset age. The main difference between LO-TLE and MCI patients regarded the MMSE raw scores ($P < 0.001$): specifically, the *post hoc* comparison highlighted lower MMSE performances in MCI-AD compared to both MCI-noAD ($P_{FDR} = 0.005$) and LO-TLE ($P_{FDR} < 0.001$). Finally, both MCI populations showed lower MMSE scores when compared to healthy controls (MCI-AD: $P_{FDR} < 0.001$; MCI-noAD: $P_{FDR} = 0.036$), while the cognitive performance of LO-TLE did not statistically differ from the control population ($P_{FDR} = 0.725$).

Demographic and clinical variables are summarized in Table 2.

CSF Alzheimer's disease biomarkers

For all the LO-TLE patients, the lumbar puncture was performed at least 24 h apart (with a range of 24–120 h) from the latest seizure recorded or referred. All LO-TLE have shown normal values of the $A\beta_{1-42}/A\beta_{1-40}$ ratio and $pTAU_{181}$ (Fig. 2 and Table 3). Four LO-TLE patients (i.e. Subjects 8, 13, 90 and 94) demonstrated an isolated abnormal value of $A\beta_{1-42}$, while three (i.e. Subjects 7, s9 and 96) presented an isolated tTAU burden above the cut-off (Supplementary Table 1).

The concentration of CSF AD biomarkers was not statistically different in LO-TLE compared to MCI-noAD, whereas both groups presented levels of markers significantly different from the MCI-AD cohort ($P_{FDR} < 0.01$; Fig. 2 and Table 3). Correlation analyses did not return any relationship between the CSF AD biomarkers assay and the frequency of the seizures and/or the years of disease duration in the LO-TLE cohort (Supplementary Figs 1 and 2).

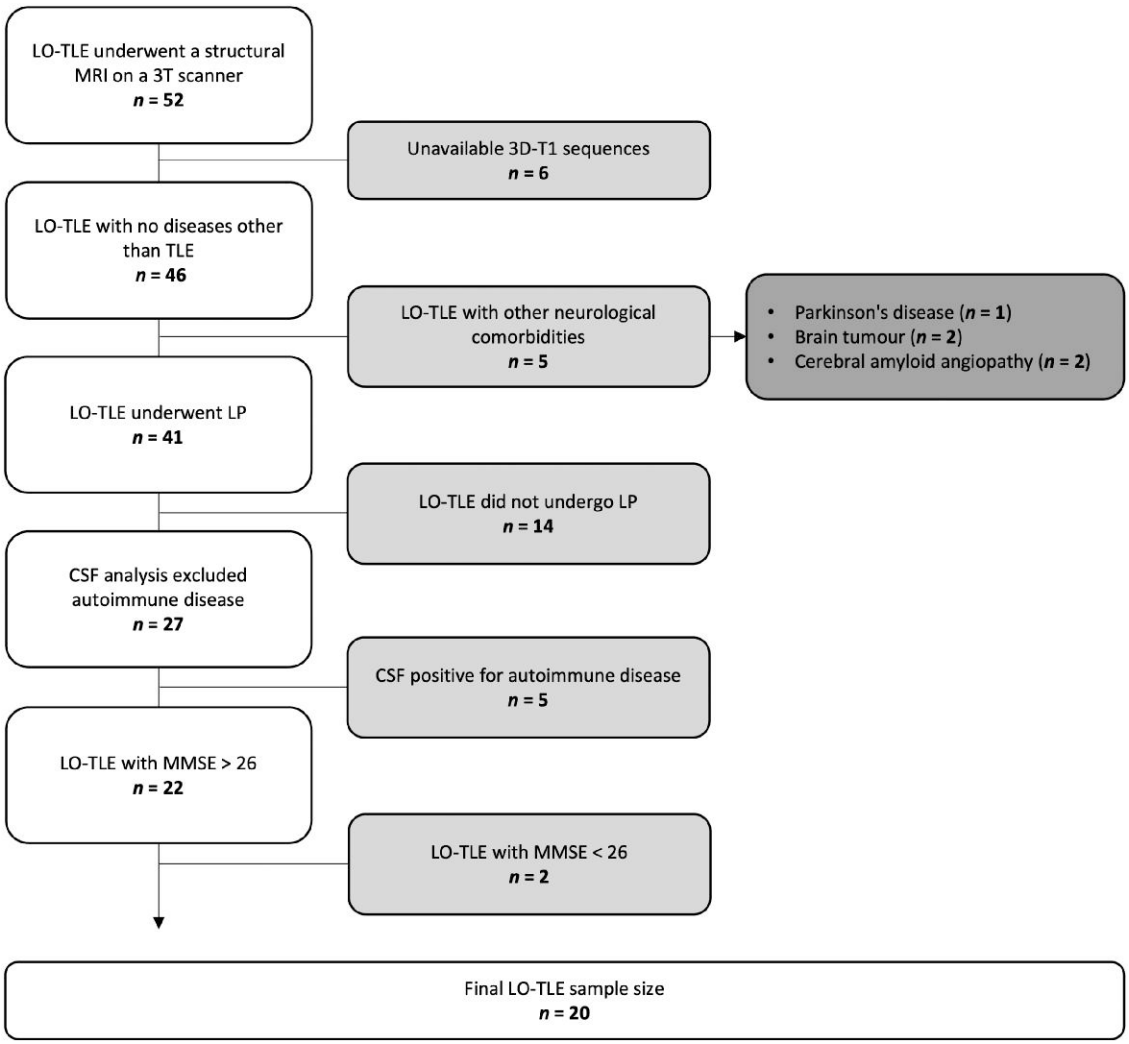

Figure 1 Selection of late-onset temporal lobe epilepsy patients. The flow chart illustrates the selection process for the late-onset temporal lobe epilepsy (LO-TLE) population, displaying all inclusion criteria and presenting a step-by-step section to demonstrate which patients met the criteria for being included in the present study. 3D-T1 = 3D T_1-weighted sequence; LP = lumbar puncture; MMSE = Mini-Mental State Examination.

Patterns of cortical atrophy

Whole brain cortical thickness pattern of atrophy in LO-TLE, MCI-noAD and MCI-AD is shown in Fig. 3A and Supplementary Fig. 3. LO-TLE did not appear significantly different in cortical thickness when compared to controls, except for slight atrophy in the ventromedial visual cortex contralateral to the seizures focus [$F(1,88) = 5.938$, $P_{FDR} = 0.020$, $\eta^2 = 0.275$]. Cortical thickness brain maps related to uncorrected *P*-values are reported in Supplementary Fig. 4.

Conversely, MCI populations showed larger patterns of cortical thinning compared to controls. Specifically, MCI-noAD patients were characterized by bilateral widespread centro-frontal cortical atrophy. The most affected ROIs were the frontal polar area [$F(1,88) = 11.501$, $P_{FDR} < 0.001$, $\eta^2 = 0.392$] and the inferior frontal cortex [$F(1,88) = 8.445$, $P_{FDR} = 0.003$, $\eta^2 = 0.259$] on the left hemisphere, and the dorsal-transitional-visual area [$F(1,88) = 18.907$, $P_{FDR} = 0.005$, $\eta^2 = 0.340$] and the intraparietal sulcus [$F(1,88) = 21.672$, $P_{FDR} = 0.005$, $\eta^2 = 0.284$] on the right side. As for the MCI-AD, a widespread bilateral posterior pattern of cortical atrophy was detected, particularly at the temporal and parietal lobes with a higher magnitude of thinning in bilateral precuneus [left: $F(1,88) = 13.165$, $P_{FDR} < 0.001$, $\eta^2 = 0.482$; right: $F(1,88) = 17.135$, $P_{FDR} < 0.001$, $\eta^2 = 0.532$], bilateral medial [left: $F(1,88) = 18.922$, $P_{FDR} < 0.001$, $\eta^2 = 0.467$; right: $F(1,88) = 12.346$, $P_{FDR} < 0.001$, $\eta^2 = 0.414$] and lateral [left: $F(1,88) = 14.621$, $P_{FDR} < 0.001$, $\eta^2 = 0.428$; right: $F(1,88) = 18.397$, $P_{FDR} < 0.001$, $\eta^2 = 0.537$] intraparietal cortices and in the bilateral parietal-occipital sulcus [left: $F(1,88) = 11.036$, $P_{FDR} < 0.001$, $\eta^2 = 0.405$; right: $F(1,88) = 13.863$, $P_{FDR} < 0.001$, $\eta^2 = 0.488$]. The cortical atrophic pattern of MCI-AD was greater in magnitude compared to MCI-noAD.

When compared to LO-TLE, MCI-noAD showed a bilateral pattern of atrophy of the frontal regions, mainly on the left hemisphere (Fig. 4A). The ROIs most involved were the left orbital prefrontal cortex [$F(1,88) = 11.501$, $P_{FDR} < 0.001$, $\eta^2 = 0.363$], the left ventrolateral prefrontal cortex [$F(1,88) = 9.077$, $P_{FDR} < 0.001$, $\eta^2 = 0.372$] and the bilateral medial prefrontal cortex [left: $F(1,88) = 9.515$, $P_{FDR} < 0.001$, $\eta^2 = 0.341$; right: $F(1,88) = 11.940$, $P_{FDR} < 0.001$, $\eta^2 = 0.408$]. The MCI-AD showed bilateral and multilobar atrophy when compared to LO-TLE (Fig. 4A), especially in bilateral precuneus [left: $F(1,88) = 13.165$, $P_{FDR} < 0.001$, $\eta^2 = 0.277$; right: $F(1,88) = 17.135$, $P_{FDR} < 0.001$, $\eta^2 = 0.328$], lateral intraparietal cortices [left: $F(1,88) = 14.621$, $P_{FDR} < 0.001$, $\eta^2 = 0.377$; right: $F(1,88) = 18.397$, $P_{FDR} < 0.001$, $\eta^2 = 0.403$] and

Table 1 Clinical characterization of late-onset temporal lobe epilepsy patients

Subject	1	2	4	6	7	8	9	10	12	13	14	15	90	91	92	93	94	95	96	97
Age	75	51	51	65	69	70	78	55	66	51	62	58	69	60	51	72	79	52	68	60
Sex	F	F	M	F	M	M	M	F	M	F	F	M	F	M	F	M	F	F	M	F
Age of onset	75	51	51	63	68	64	74	51	65	51	62	57	69	59	51	64	79	51	62	59
Duration	0	0	0	2	1	6	4	4	1	0	0	1	0	1	0	8	0	1	6	1
MMSE	29/30	29/30	30/30	28/30	30/30	30/30	27/30	29/30	28/30	30/30	27/30	28/30	29/30	30/30	30/30	27/30	30/30	30/30	27/30	28/30
sMRI	Neg	Neg	Neg	Neg	Left HS	Neg	Neg	Left HS	Ne	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Fazekas	1	0	0	0	1	1	1	0	1	0	1	1	0	0	0	1	1	1	1	1
Interictal EEG	Left A-T (T3)	Left F-T (T3)	Left A-T (T3)	Left A-T (T3)	Left F-T (T3)	Left A-T (T3)	Bi A-T (T3/T4)	Left A-T (T3)	Left F-T (T3)	Left A-T (T3)	Left A-T (T3)	Left F-T (T3)	Bi F-T (T3/T4)	Right A-T (T4)	Left A-T (T3)	Bi F-T (T3/T4)	Left A-T (T3)	Left F-T (T3)	Left F-T (T3)	Right A-T (T4)
Ictal EEG onset	Left A-T (T3)	N/A	Left A-T (T3)	Left A-T (T3)	N/A	Left A-T (T3)	Left A-T (T3)	Left A-T (T3)	Left F-T (T3)	Left A-T (T3)	N/A	N/A	Left F-T (T3)	N/A	Left A-T (T3)	Left F-T (T3)	Left A-T (T3)	Left F-T (T3)	N/A	N/A
ASM type	LEV	LCS CLB	LCS	LCS PER CNZ	LCS	BRV PGB	LCS	BRV VPA	LCS	LCS VPA	LCS	LMG	LCS CBZ	LCS	LEV	CBZ	BRV CNZ	LCS VPA LEV	LCS	LCS
ASM response	Yes	Yes	Yes	D-R	Yes	Yes	Yes	D-R	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	D-R	Yes	Yes
Frequency	5	1	1	4	4	6	3	5	1	3	2	1	4	1	1	3	1	3	1	2
Family history	No	No	Yes	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No
Febrile seizures	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No

Age, age of onset, and disease duration are expressed in years. The Fazekas score was expressed as follows: 0 = if none or single lesion; 1 = multiple punctate lesions; 2 = beginning of confluency; 3 = large confluent lesions.[55] Frequency scale is adapted from So *et al.*[30] and expressed as follows: 1 = <1 seizure per year; 2 = 1–3 seizures per year; 3 = 4–11 seizures per year; 4 = 1–3 seizures per month; 5 = 1–6 seizures per week; 6 = 1–3 seizures per day; 7 = more than 4 seizures per day. ASM = antiseizure medication; A-T = anterior temporal; Bi = bilateral; BRV = brivaracetam; CBZ = carbamazepine; CLB = clobazam; CN = clonazepam; D-R = drug-resistant; F = female; F-T = frontotemporal; HS = hippocampal sclerosis; LCS = lacosamide; LEV = levetiracetam; LMG = lamotrigine; M = male; MMSE = Mini-Mental State Examination; N/A = no recorded seizure was available; Neg = negative MRI; PER = perampanel; PGB = pregabalin; sMRI = structural MRI outcome; VPA = valproate.

Table 2 Demographic and clinical characteristics between patients and control populations

	LO-TLE	MCI-noAD	MCI-AD	Controls	Stat.	Sign.
n	20	25	25	25	–	–
Age, years	63.10 (±9.375)	61.08 (±4.582)	61.36 (±8.346)	60.08 (±7.158)	0.617[a]	0.606
Sex, female/male	11/9	13/12	14/11	15/10	0.331[b]	0.954
Education, years[c]	10.58 (±3.254)	11.16 (±3.934)	11.56 (±3.513)	13.50 (±2.614)	2.980[a]	0.036*
MMSE[d]	28.80 (±1.196)	27.50 (±2.993)	25.63 (±2.795)	29.05 (±0.921)	10.972[a]	<0.001***
ICV, mm^3	1.52×10^6 ($\pm 1.63 \times 10^5$)	1.50×10^6 ($\pm 1.81 \times 10^5$)	1.51×10^6 ($\pm 1.25 \times 10^5$)	1.53×10^6 ($\pm 1.34 \times 10^5$)	0.233[a]	0.873
Age of onset, years	61.30 (±8.779)	58.44 (±5.075)	58.40 (±7.911)	–	1.108[a]	0.336
Disease duration, years	1.80 (±2.441)	2.64 (±1.604)	2.96 (±2.371)	–	1.683[a]	0.194

Data are presented as mean (±standard deviation). AD = Alzheimer's disease; ICV = intracranial volume; LO-TLE = late-onset temporal lobe epilepsy; MCI = mild cognitive impairment; MCI-noAD = MCI not due to AD; MCI-AD = MCI due to AD; MMSE = Mini-Mental State Examination.

[a]ANOVA.

[b]Chi-squared.

[c]Years of education were not available for three healthy controls.

[d]MMSE scores were not available for four healthy controls, one MCI-AD patient and one MCI-noAD patient.

*P < 0.05, ***P < 0.001.

in the left middle temporal gyrus [$F(1,88) = 13.455$, $P_{FDR} < 0.001$, $\eta^2 = 0.382$].

Supplementary Table 2 summarizes all results from each comparison.

Patterns of subcortical atrophy

The volume-based analysis on subcortical structures reported no difference between controls and both LO-TLE and MCI-noAD (Fig. 3B). For MCI-AD, atrophy was observed in the hippocampus [left: $F(1,88) = 9.674$, $P_{FDR} < 0.001$, $\eta^2 = 0.349$; right: $F(1,88) = 15.998$, $P_{FDR} < 0.001$, $\eta^2 = 0.370$] and amygdala [left: $F(1,88) = 9.729$, $P_{FDR} < 0.001$, $\eta^2 = 0.345$; right: $F(1,88) = 11.727$, $P_{FDR} < 0.001$, $\eta^2 = 0.346$)] bilaterally. The analysis of the hippocampal subfields confirmed the previous analyses, showing no significant differences between controls' hippocampi and both LO-TLE and MCI-noAD, while the MCI-AD population reported bilateral atrophy, especially on the right side: hippocampal body [left: $F(1,88) = 7.855$, $P_{FDR} = 0.001$, $\eta^2 = 0.304$; right: $F(1,88) = 14.740$, $P_{FDR} < 0.001$, $\eta^2 = 0.326$)], hippocampal head [left: $F(1,88) = 7.779$, $P_{FDR} = 0.001$, $\eta^2 = 0.300$; right: $F(1,88) = 12.411$, $P_{FDR} < 0.001$, $\eta^2 = 0.295$] and hippocampal tail [left: $F(1,88) = 7.852$, $P_{FDR} < 0.001$, $\eta^2 = 0.289$; right: $F(1,88) = 15.788$, $P_{FDR} < 0.001$, $\eta^2 = 0.389$]. As for the amygdala subnuclei segmentation, the results highlighted a loss of volume in medial [$F(1,88) = 8.844$, $P_{FDR} = 0.042$, $\eta^2 = 0.177$] and cortical [$F(1,88) = 9.799$, $P_{FDR} = 0.042$, $\eta^2 = 0.149$] nuclei ipsilateral to the seizure focus in LO-TLE (Fig. 3C). The sub-segmentation confirmed no amygdale damage for MCI-noAD and a bilateral whole structure injury for MCI-AD (Fig. 3C).

When compared with LO-TLE, both MCI groups reported global atrophy of the bilateral hippocampi and amygdalae (Fig. 4B and C) more pronounced in MCI-AD [left hippocampus: $F(1,88) = 9.674$, $P_{FDR} < 0.001$, $\eta^2 = 0.331$; right hippocampus: $F(1,88) = 15.998$, $P_{FDR} < 0.001$, $\eta^2 = 0.496$; left amygdala: $F(1,88) = 9.729$, $P_{FDR} < 0.001$, $\eta^2 = 0.318$; right amygdala: $F(1,88) = 11.727$, $P_{FDR} < 0.001$, $\eta^2 = 0.429$], with respect to MCI-noAD [left hippocampus: $F(1,88) = 9.674$, $P_{FDR} = 0.027$, $\eta^2 = 0.116$; right hippocampus: $F(1,88) = 15.998$, $P_{FDR} = 0.004$, $\eta^2 = 0.193$; left amygdala: $F(1,88) = 9.729$, $P_{FDR} = 0.018$, $\eta^2 = 0.113$; right amygdala $F(1,88) = 11.727$, $P_{FDR} = 0.024$, $\eta^2 = 0.112$].

Supplementary Table 3 summarizes all results from each comparison.

Discussion

The present study aimed at expanding the characterization of LO-TLE and gaining insight into its potential predisposition to progress into AD. Herein, we present a thoroughly characterized cohort of LO-TLE patients in terms of clinical features, brain morphometry and pattern of CSF AD biomarkers. The main novelty of this study is the deep characterization of LO-TLE patients based on CSF AD biomarkers and brain morphometry. We found normal CSF AD biomarkers and slight grey matter atrophy in LO-TLE patients, suggesting that when LO-TLE is associated with normal CSF Aβ and tau loads, the cortico-subcortical pattern is similar to that observed in healthy older adults and significantly differs from that observed in patients with MCI, either due to AD or not.

Late-onset epilepsies: a growing concern

With the global population's advancing age, LOEs are expected to become increasingly prevalent in the coming decades.[2,56] While the majority of LOEs are caused by vascular disease, neoplasms,

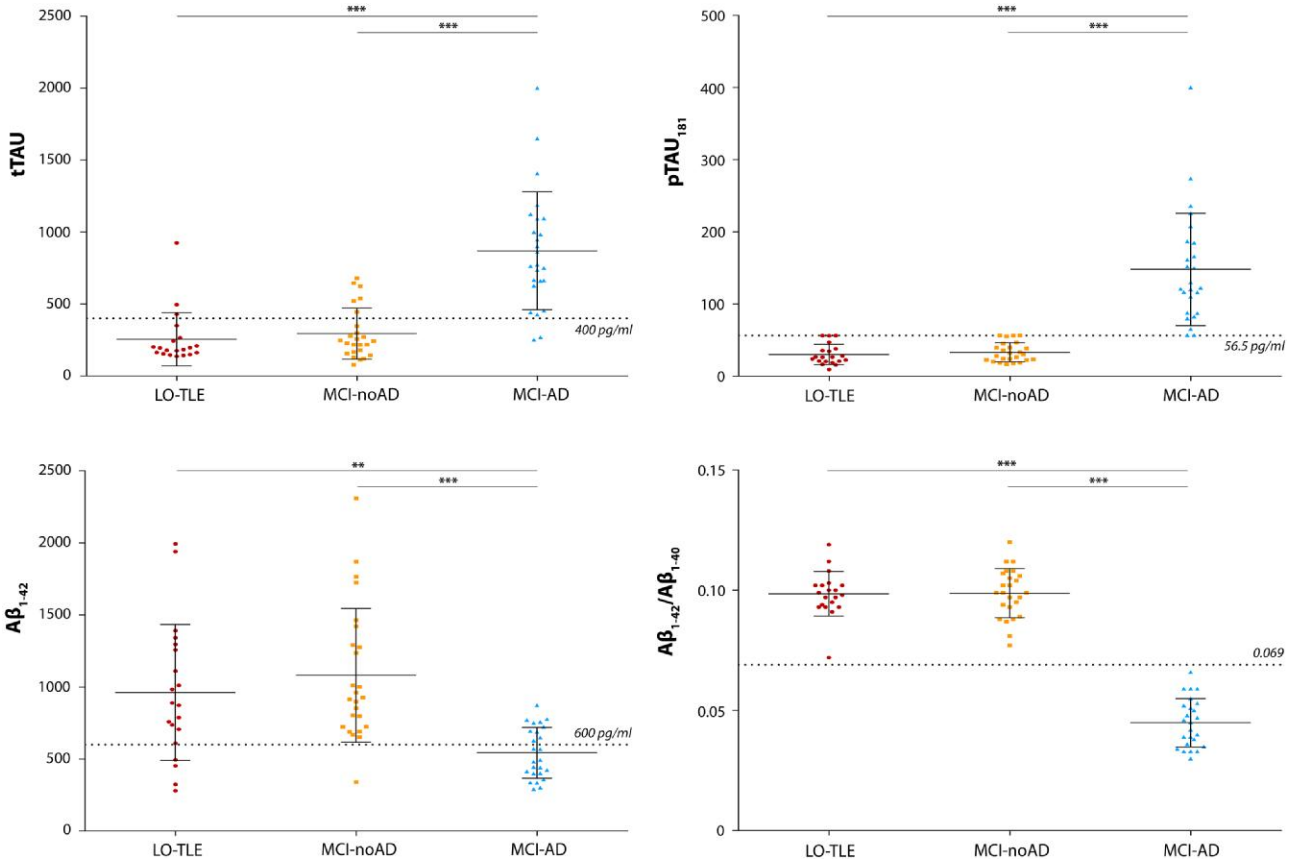

Figure 2 CSF biomarkers for Alzheimer's disease (AD) deposits in late-onset temporal lobe epilepsy, mild cognitive impairment (MCI) not due to AD and MCI due to AD. The box and whisker plots display the assay of total tau (tTAU), phosphorylated tau ($pTAU_{181}$) and amyloid-β (Aβ; expressed as $Aβ_{1–42}$ and $Aβ_{1–42}/Aβ_{1–40}$ ratio) in late-onset temporal lobe epilepsy (LO-TLE), mild cognitive impairment not due to Alzheimer's disease (MCI-AD) and MCI due to AD (MCI-AD). The central horizontal line of the distribution marks the median of the sample, and the upper and lower edges of the box mark the 25th and 75th percentiles. The black dashed line designates the cut-off of normal values for each biomarker. The asterisk on the box indicates the significant results of the multivariate analysis of covariance (MANCOVA) analyses, specifically when $^{**}P_{FDR} < 0.01$ and when $^{***}P_{FDR} < 0.001$. The plots were made using GraphPad Prism (www.graphpad.com). FDR = false discovery rate.

Table 3 CSF biomarkers deposits between late-onset temporal lobe epilepsy and mild cognitive impairment

	LO-TLE	MCI-noAD	MCI-AD	Stat.	Significance	Pairwise comparisons
tTAU	255.35 (±185.017)	294.64 (±177.271)	869.52 (±408.837)	34.984[a]	<0.001***	MCI-AD < LO-TLE ($P_{FDR} < 0.001$) MCI-AD < MCI-noAD ($P_{FDR} < 0.001$)
$pTAU_{181}$	30.04 (±14.133)	34.69 (±17.423)	147.94 (±77.870)	45.768[a]	<0.001***	MCI-AD < LO-TLE ($P_{FDR} < 0.001$) MCI-AD < MCI-noAD ($P_{FDR} < 0.001$)
$Aβ_{1–42}$	962.10 (±472.894)	1080.92 (±464.187)	542.92 (±175.762)	12.990[a]	<0.001***	MCI-AD < LO-TLE ($P_{FDR} = 0.002$) MCI-AD < MCI-noAD ($P_{FDR} < 0.001$)
$Aβ_{1–42}/Aβ_{1–40}$	0.10 (±0.009)	0.10 (±0.010)	0.04 (±0.010)	236.443[a]	<0.001***	MCI-AD < LO-TLE ($P_{FDR} < 0.001$) MCI-AD < MCI-noAD ($P_{FDR} < 0.001$)

Data are presented as mean (±standard deviation). Aβ = amyloid-β; AD = Alzheimer's disease; FDR = false discovery rate; LO-TLE = late-onset temporal lobe epilepsy; MCI = mild cognitive impairment; MCI-noAD = MCI not due to AD; MCI-AD = MCI due to AD; tTAU = total protein tau; $pTAU_{181}$ = phosphorylated tau.
[a]ANOVA.
***$P < 0.001$.

or pre-existing neurodegeneration, approximately 20% remain with unclear cause and unknown origin (i.e. LOEU).[10,11] In LOEU patients, epileptiform discharges and focal slowing commonly involve the temporal lobes,[12] sparking a rising interest in LO-TLE forms. In a large cohort study of more than 200 LOEU patients, nearly 90% presented epileptic activity arising from the temporal regions documented by routine scalp EEG acquired during a follow-up period.[57] In our cohort, the temporal lobe onset was defined based on ictal recordings, interictal epileptiform activity localization and clinical features, with nearly all cases presenting a mesial TLE phenotype. Overall, this evidence suggests a greater predisposition of the temporal regions to develop seizures in the elderly, as commonly observed in younger-aged subjects. Unlike LO-TLE, TLE onset in youth frequently follows febrile seizures[58] is often associated with structural anomalies[59] and has the highest drug resistance rates among focal epilepsy syndromes.[60,61] The cohort of

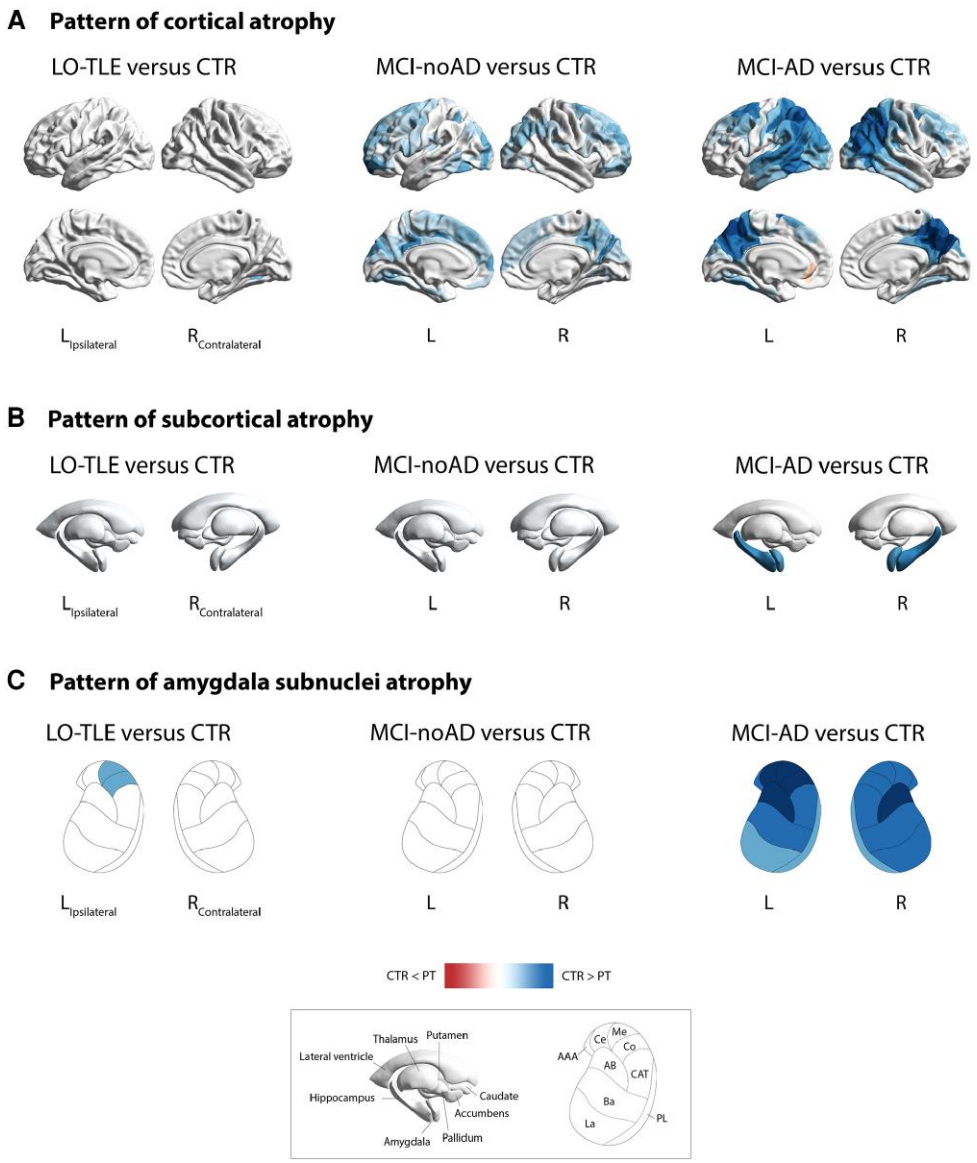

Figure 3 Whole brain comparison between late-onset temporal lobe epilepsy, mild cognitive impairment (MCI) not due to Alzheimer's disease (AD) and MCI due to AD versus controls. (**A**) Significant group differences in cortical thickness. (**B**) Visualization of significant group differences in subcortical volumes. (**C**) Illustration of the significant group differences in the amygdalae subnuclei volumes. Data for each comparison are presented as effect size (η^2). Only effect sizes associated with *P*-values that survived false discovery rate (FDR) correction (i.e. $P_{FDR} < 0.05$) are presented. Brain maps of **A** and **B** were created using the ENIGMA Toolbox.[45] AAA = anterior amygdaloid area; AB = accessory basal nucleus; Ba = basal nucleus; CAT = corticoamygdaloid transition area; Ce = central nucleus; Co = cortical nucleus; CTR = controls; L = left; La = lateral nucleus; LO-TLE = late-onset temporal lobe epilepsy; MCI = mild cognitive impairment; MCI-AD = MCI due to Alzheimer's disease; MCI-noAD = MCI not due to Alzheimer's disease; Me = medial nucleus; PL = paralaminar nucleus; PT = patients; R = right.

patients enrolled in our study exhibited clinical profiles consistent with recent literature on LO-TLE.[16] The majority displayed negative MRI findings, with none reporting febrile seizure comorbidities during infancy. Two patients out of the entire cohort had HS at MRI. To date, it is unclear whether the evidence of HS may indicate an aetiology of LO-TLE as it normally represents the structural cause of TLE occurring earlier in life while in older it may be the results of different pathological processes, including AD.[12] Occult cerebrovascular diseases (e.g. SVD) are considered a possible underlying explanation of LOEU.[26] Indeed, several studies indicate that patients with LOEU present a higher load of vascular risk factors and periventricular hyperintensity compared to community-based studies in the elderly.[26,57,62] However, the mechanisms behind the association between LOEU and SVD are not well established and there is no clear consensus on the epileptogenic role of this abnormality. In our sample, the Fazekas score for all patients ranged from 0 to 1, consistent with previous evidence.[62] Interestingly, Abraira *et al.*[62] pointed out that the pattern of leukoaraiosis in LOEU is milder compared to TIA/lacunar stroke patients without epilepsy, suggesting that the aetiology of LOEU cannot be entirely attributed to cerebrovascular diseases. According to the LOEU literature, epilepsy onset later in life tends to be more responsive to pharmacological treatments, with a higher likelihood of achieving seizure freedom at follow-up.[4,63] In a previous study on a large cohort of patients with LOEU, 92% responded to a single ASM.[57] Our enrolled population reaffirmed previous observations of the favourable

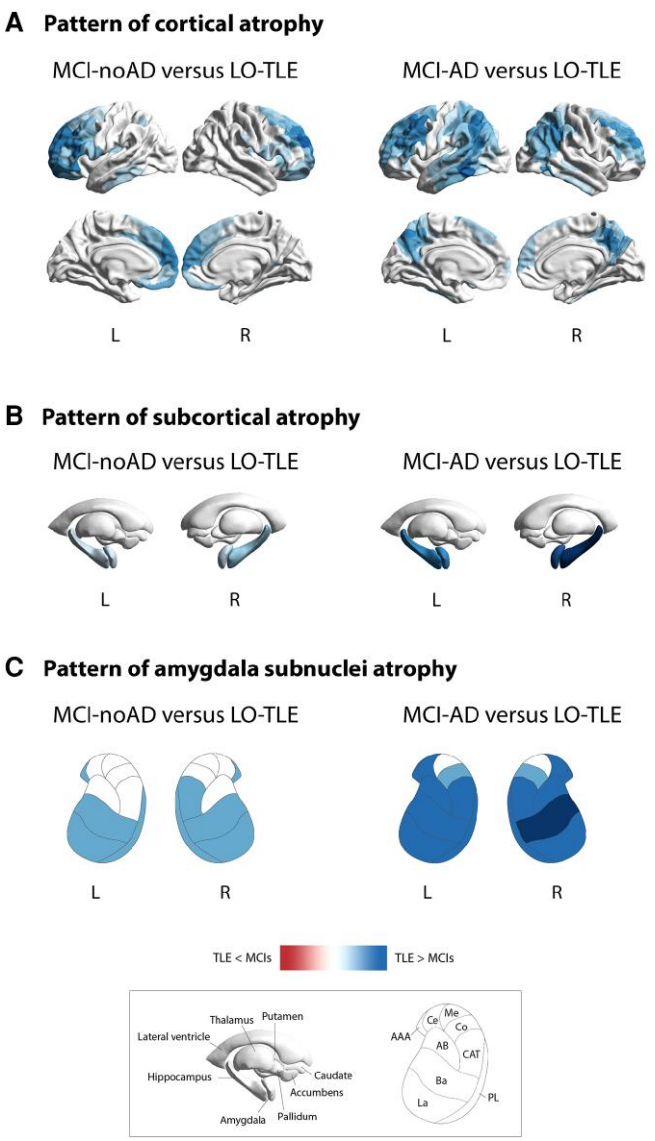

Figure 4 Whole brain comparison between mild cognitive impairment (MCI) due to Alzheimer's disease (AD) and MCI not due to AD versus late-onset temporal lobe epilepsy. (**A**) Significant group differences in cortical thickness. (**B**) Visualizaation of significant group differences in subcortical volumes. (**C**) Illustration of the significant group differences in the amygdalae subnuclei volumes. Data for each comparison are presented as effect size (η^2). Only effect sizes associated with *P*-values that survived false discovery rate (FDR) correction (i.e. $P_{FDR} < 0.05$) are presented. Brain maps of panels **A** and **B** were created using the ENIGMA Toolbox.[45] AAA = anterior amygdaloid area; AB = accessory basal nucleus; Ba = basal nucleus; CAT = corticoamygdaloid transition area; Ce = central nucleus; Co = cortical nucleus; L = left; La = lateral nucleus; MCI-AD = MCI due to AD; MCI-noAD = MCI not due to AD; Me = medial nucleus; PL = paralaminar nucleus; R = right.

seizure outcome in LOEs,[4,63,64] with 75% achieving seizure remission with ASMs and the majority requiring only monotherapy. Seizure frequency in our sample was sporadic in most cases, with only seven patients (35%) experiencing monthly or weekly ictal events. So far, the reason for this 'benign course of seizures in LOEU remains to be clarified. Krumholz *et al.*[65] proposed that the absence of a structural lesion on MRI might reduce the risk of recurrence.[65] However, seizures in older patients might not be easily recognized and correctly reported, as they often present with subtle symptoms, including non-specific periods of confusion.[66]

Despite the documented positive epilepsy-related outcomes, pathological brain aging remains a significant concern in LO-TLE, particularly due to its frequent cognitive implications. A recent study by Kaestner *et al.*[22] described shared cognitive impairments, such as memory encoding and language deficits, between LO-TLE and aMCI, exhibiting similar patterns of cortico-subcortical atrophy. According to Nardi Cesarini *et al.*,[15] 59% of LOEU cases present with MCI status at the time of epilepsy diagnosis; however, all LOEU cases with MCI displayed CSF findings with typical biomarkers profile of AD.[15,22] Thus, our findings may suggest a favourable prognosis of LO-TLE, especially if coupled with normal CSF AD biomarkers and cortical grey matter integrity. Nevertheless, the LO-TLE CSF biomarkers profile was alike MCI-noAD, therefore, the risk of a progression to a neurodegenerative disease different from AD that may occur in the future cannot be excluded.

The central role of CSF amyloid-β and tau protein levels in late onset-temporal lobe epilepsy

Characterizing AD biomarkers in TLE is crucial not only for excluding AD-like pathogenesis but also for comprehending the underlying chronic epileptogenic process, as Aβ and tau deposits appear to play a role in epileptogenesis and disease prognosis regardless of age or epilepsy onset. Compelling evidence supports the involvement of tau protein in TLE, indicating that seizures can induce abnormal tau phosphorylation,[67-70] while medications that reduce pTAU levels have demonstrated both anti-seizure and anti-epileptogenic effects in rodent models.[71-74] Pathological specimens from middle-aged to elderly individuals with TLE who underwent temporal lobectomy for epilepsy surgery have revealed deposits of pTAU proteins in the absence of Aβ-positive plaques.[20] Although tau deposits strongly correlate with poorer cognitive performance, according to the authors this may not necessarily signify AD pathology but could instead reflect other tau-related conditions.[20] In our cohort of LO-TLE, tTAU was found to be altered in just three subjects, while all the patients demonstrated normal values of $pTAU_{181}$. The increased values of tTAU likely reflect neuronal damage due to seizure activity. Interestingly, previous reports documented alteration of tTAU in patients with status epilepticus coupled with normal values of $pTAU_{181}$,[75] which is considered the strongest signature of AD.[76] Additionally, at a group level, both tTAU and $pTAU_{181}$ burdens were significantly different compared to MCI-AD (Fig. 2 and Table 3).

Recent evidence has also highlighted frequent Aβ burden in LOEU patients.[10,11,15] In a prospective longitudinal study, Costa *et al.*[10] showed that 37.5% of LOEU patients presented pathological CSF $Aβ_{1–42}$ levels. During a 3-year follow-up period, 17.5% of LOEU patients converted to AD. Demographic factors, epileptiform activities on EEG, and ASMs regimen did not influence progression to AD, while pathological CSF levels of $Aβ_{1–42}$ were associated with a hazard ratio of 3.4 for progression to AD. Similarly, other studies reported that lower CSF $Aβ_{1–42}$ levels at the baseline were associated with memory impairment at follow-up assessments in LOEU.[14] Notably, most of the previous studies included heterogeneous populations of LOEU, where only a proportion of patients presented temporal lobe origin. Interestingly, when explored as a subgroup, LO-TLE was more frequently characterized by normal CSF $Aβ_{1–42}$ levels.[10] In our cohort, while $Aβ_{1–42}$ resulted below normal values in 4 patients, the average value at the group level was statistically different compared to MCI-AD. In addition, the $Aβ_{1–42}$/

$A\beta_{1–40}$ ratio was above the cut-off in all LO-TLE (Fig. 2). It has been shown that the $A\beta_{1–42}/A\beta_{1–40}$ ratio reflects more accurately the presence of amyloid plaques compared to the $A\beta_{1–42}$ only, representing a more robust tool for the diagnostic and prognostic evaluation of patients with cognitive decline.[77] Overall, our results indicate a low rate of positive CSF AD biomarkers in LOEU with temporal lobe seizures at disease onset, which is supported by previous data.[10] Our results underscore the importance of a deep phenotypical characterization in LOEU for the correct interpretation of biological findings.

In a recent review, Hickman *et al.*[12] stratified LOEU based on the ATN classification,[78] considering CSF Aβ and tau levels, cognitive scores, and FDG-PET findings as risk factors. They proposed three major phenotypes: (i) LOEU as prodromal (when associated with MCI) or preclinical (when not associated with MCI) AD due to Aβ and tau abnormal concentrations; (ii) Aβ-related LOEU when only Aβ is altered; and (iii) LOEU with no abnormal Aβ biomarkers, where either both Aβ and tau are negative for AD-like CSF or when only tau is altered. Based on our findings, we support this classification scheme of LOEU and suggest expanding it by adding the knowledge that LO-TLE without AD-like biomarkers—particularly $pTAU_{181}$ and $A\beta_{1–42}/A\beta_{1–40}$ ratio—shows cortical and subcortical integrity as measured by MRI structural imaging.

Cortical atrophy in late onset-temporal lobe epilepsy

The literature examining cortical atrophy in TLE has frequently suggested a widespread, multilobar, and bilateral pattern of cortical thinning in many studied populations.[53,79-81] However, our results demonstrate substantial preservation of brain morphometry compared to controls, revealing only mild atrophy in the cortico-medial amygdala ipsilateral to the seizure focus (Fig. 3). This suggests distinct patterns of atrophy in LO-TLE compared to MCI. In contrast, the MCI-AD group exhibited greater cortical thinning and subcortical volume loss, particularly in bilateral precuneus, hippocampi, and amygdalae structures, consistent with the neurodegenerative cortical pattern observed in AD patients.[82,83] Moreover, our findings suggested a divergence in the nature of LO-TLE and MCI, even when not associated with AD, as evidenced by the distinct patterns of cortico-subcortical atrophy observed in the direct comparisons between TLEs and MCIs (Fig. 4). Our findings deviate from the study by Kastner *et al.*,[22] who reported a similar magnitude of cortical atrophy between TLE onset after 50 years and aMCI, particularly in the mesial temporal lobes. Nevertheless, in their study a formal biological characterization of the patients was unavailable, preventing from establishing the percentage of LO-TLE characterized by an AD-like pattern of Aβ and tau brain deposits. Herein, our findings were able to exclude potential AD pathogenesis in this LO-TLE population at the time of brain imaging. The lumbar puncture is not a standard procedure in most epileptic clinical settings, potentially obscuring early identification of LO-TLE patients in whom AD pathogenic processes are already present. Additionally, our LO-TLE population differed from the one described by Kaestner *et al.*[22] in terms of disease duration. This study population had a mean duration of epilepsy of 1.8 years at the time of MRI scans, while Kaestner *et al.*[22] evaluated patients with a mean of 6.6 years of epilepsy duration. A recent longitudinal study by Galovic and colleagues[84] reported greater cortical thinning in individuals with epilepsy older than 55 years, particularly within the first 5 years following the first seizure. Given this evidence and the disparities observed compared to the sole study exploring cortical thinning in LO-TLE,[22] we hypothesize that preserved grey matter in our epilepsy cohort may stem from two distinct factors: (i) normal CSF levels of AD biomarkers, suggesting overt absence of AD neuropathology at the time of this study; and (ii) the recruitment of patients with very short epilepsy duration could result in absent cortical atrophy, which is primarily driven by disease chronicity, particularly in the elderly.

Given the favourable clinical prognosis observed in LO-TLE, longitudinal studies are required to clarify disease progression, potential concurrent cognitive comorbidities, and the influence of disease duration on brain injury rates among the elderly with epilepsy.

Limitations and future directions

Our study has several limitations. Despite thorough clinical characterization, the sample size was limited due to the exclusion of patients who did not undergo the lumbar puncture, a procedure not routinely performed in epilepsy clinical settings (Fig. 1). Since the main purpose of this manuscript was to collect a clean and well-characterized LO-TLE population, we evaluated a relatively small sample preventing us from fully exploring the diversity of phenotypes within LOEU. Future studies on multicentric populations may help to address this limitation and maximize the number of patients included. Additionally, our study lacked cognitive assessments and comparisons between LO-TLE and MCIs due to differences in neuropsychological evaluation methods. Further studies should consider standardized cognitive assessment protocols to facilitate comparisons and improve understanding of cognitive impairment patterns associated with LO-TLE. Our study lacked a longitudinal evaluation of LO-TLE, which would provide valuable insights into changes in AD biomarker concentrations, cognitive function, and cortical neurodegeneration over time. Ethical constraints limit the feasibility of multiple consecutive lumbar punctures, underscoring the need for alternative biomarker assays in serum or blood samples.

Despite the present study willingly focusing on LO-TLE, it is important to acknowledge the lack of a subset of individuals with LOEU with extratemporal seizure focus.[10] Within this context, our findings may signify a first step towards better phenotyping of different subtypes of LOEU, proposing a characterization of the temporal lobe forms of LOEU. Herein, we underscore the significance of advanced neuroimaging assessment and the necessity for increasingly precise clinical characterization of individuals experiencing focal seizures after the age of 50. Indeed, the relationship between focal epilepsy with onset in the elderly and neurogenerative disease (particularly AD) is complex and bidirectional. Significant overlap has been found across several domains including epidemiology,[85] pathology,[86] neuroimaging[22] and CSF biomarkers.[10,12,15] Various factors contribute to this association, such as neuroinflammation and/or epigenetic factors,[26] which have not been considered in the present study. Even though we believe the present LO-TLE population is representative of the LO-TLE condition,[16] we recognize that the design of the study, along with the strict inclusion criteria and the short disease duration, prevents us from drawing more general conclusions about the underlying pathogenetic mechanisms that might link focal epilepsy and AD.

Taking these limitations into account, our study contributes new knowledge and represents a significant step towards better understanding the complex link between epilepsy and neurodegenerative conditions in the future.

Conclusions

Our study aimed to contribute to the literature on late-onset TLE of unknown origin by providing insights from both CSF biomarkers and neuroimaging. To our knowledge, this is the first study to explore AD biomarker concentrations through CSF analysis alongside cortico-subcortical injury in a population of LO-TLE, compared with groups of MCIs with or without underlying AD pathology. Furthermore, we support the potential phenotypic variability underlying epilepsy onsets in the elderly and underscore the necessity of a thorough clinical examination of LO-TLE patients, including advanced MRI assessments. Nevertheless, so far, it remains important to continue characterizing CSF in LO-TLE to further confirm our observations. Taken together, our results suggest that LO-TLE itself may not intrinsically represent a risk factor for developing AD. However, a longitudinal assessment is required to confirm this hypothesis as other potential underlying factors, not explored in this study, might drive the development of a neurodegenerative disease in LO-TLE.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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Competing interests

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Supplementary material

Supplementary material is available at *Brain* online.

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