Shared hippocampal abnormalities in sporadic temporal lobe epilepsy patients and their siblings

Lili Long MD PhD,[1,2,] Marian Galovic MD,[2,3,4,*] Yayu Chen MD,[1] Tjardo Postma MD,[2] Sjoerd B Vos PhD,[2,3,5] Fenglai Xiao MD,[2,3] Wenyue Wu MD,[1] Yanmin Song MD PhD,[6] Sha Huang MD,[1] Matthias Koepp MD PhD, [2,3,#] Bo Xiao MD PhD [1,#]*

1. Department of Neurology, Xiangya Hospital, Central South University, China
2. Department of Clinical and Experimental Epilepsy, UCL Queen Square Institute of Neurology, London, United Kingdom
3. MRI Unit, Chalfont Centre for Epilepsy, Chalfont St Peter, United Kingdom
4. Department of Neurology, University Hospital Zurich, Switzerland
5. Centre for Medical Image Computing, University College London, London, United Kingdom
6. Department of Emergency, Xiangya Hospital, Central South University, China

[*] Lili Long and Marian Galovic contributed equally to this work

Corresponding Authors:
Bo Xiao, MD, PhD,
Xiangya Hospital, Central South University,
87 Xiangya Road,
Changsha, Hunan 410000, China.
E-mail: xiaobo_xy@126.com
Phone +86-731-84327216
Fax +86-731-84327401

Matthias Koepp, MD, PhD,
Institute of Neurology, University College London,
Box 29, Queen Square,
London, WC1N 3BG, UK.
E-mail: m.koepp@ucl.ac.uk
Phone +44 (0) 20 3448 8612
Fax +44 (0) 20 3448 8615

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ORCID number: Lili Long, 0000-0001-5078-8770; Marian Galovic, 0000-0002-2307-071X; Matthias Koepp, 0000-0002-4277-8000; Bo Xiao, 0000-0001-5204-1902

Summary

Objective: To examine the shared familial contribution to hippocampal and extrahippocampal morphological abnormalities in sporadic temporal lobe epilepsy (TLE) patients and their unaffected siblings.

Methods: We collected clinical, electrophysiological, and T1-weighted MRI data of 18 sporadic patients with TLE without lesions other than hippocampal sclerosis (12 right, 6 left), their 18 unaffected full siblings, and 18 matched healthy volunteers. We compared between-group differences in cortical thickness and volumes of five subcortical areas (hippocampus, amygdala, thalamus, putamen, pallidum). We determined the subregional extent of hippocampal abnormalities using surface shape analysis. All our imaging results were corrected for multiple comparisons using random field theory.

Results: We detected smaller hippocampal volumes in patients (right TLE: median right hippocampus 1.92ml, interquartile range [IQR] 1.39-2.62, $p<0.001$; left TLE: left hippocampus 2.05ml, IQR 1.99-2.33, $p=0.01$) and their unaffected siblings (right hippocampus 2.65ml, IQR 2.32-2.80, $p<0.001$; left hippocampus 2.39 ml, IQR 2.18-2.53, $p<0.001$) compared to healthy controls (right hippocampus 2.94ml, IQR 2.77-3.24; left hippocampus 2.71ml, IQR 2.37-2.89). Surface shape analysis showed that patients with TLE had bilateral subregional atrophy in both hippocampi (right > left). Similar but less pronounced subregional atrophy was detected in the right hippocampus of unaffected siblings. Patients with TLE had reduced cortical thickness in bilateral premotor/prefrontal cortices and the right precentral gyrus. Siblings did not show abnormalities in cortical or subcortical areas other than the hippocampus.

Significance: Our results demonstrate a shared vulnerability of the hippocampus in both patients with TLE and their unaffected siblings, pointing to a contribution of familial factors to hippocampal atrophy. This neuroimaging trait could represent an endophenotype of TLE, which might precede the onset of epilepsy in some individuals.

Key words: temporal lobe epilepsy; hippocampus; surface shape analysis; cortical thickness; endophenotype

Abbreviations: CAT = Computational Anatomy Toolbox; IQR = interquartile range; MMSE = Mini Mental State Examination; TLE = temporal lobe epilepsy; TIV = total intracranial volume.

Key points

We detected hippocampal abnormalities in unaffected siblings of patients with TLE using two methods (volumetry and surface shape analysis), pointing to a contribution of familial factors to hippocampal atrophy.

Hippocampal atrophy could reflect an imaging endophenotype of TLE, which might precede the onset of epilepsy in some individuals.

Cortical thinning observed in our patients with TLE could be driven by seizures or environmental factors rather than genetic factors.

Introduction

Hippocampal sclerosis is the primary neuropathological feature of temporal lobe epilepsy (TLE), the most common focal epilepsy syndrome in adults.[1] Whether hippocampal atrophy is the cause or consequence of seizures remains controversial. Previous studies widely considered that hippocampal sclerosis might be an acquired phenomenon secondary to postnatal injury such as prolonged febrile seizures.[2,3] Patients with prolonged febrile seizures in early childhood have smaller, more asymmetric, or malrotated hippocampi.[4-6] Moreover, the evolution of hippocampal changes has been documented on magnetic resonance imaging (MRI) after an emergency event.[7,8] Several large prospective studies of children with febrile seizures failed to show a convincing association between a precipitating injury and hippocampal sclerosis.[9-12]

The genetic contribution to sporadic TLE is another topic of ongoing debate. Relatives of people with focal epilepsy have a 2.6-times higher risk for epilepsy compared to the general population.[13] Two large multicentre studies, however, failed to define common genetic variants associated with the risk of focal epilepsy.[14,15] Using more detailed phenotyping, a subsequent study described a common risk variant of temporal lobe epilepsy with hippocampal sclerosis and febrile seizures,[16] demonstrating that well-defined disease phenotypes combined with specific neuroimaging traits lend themselves to the study of genetic variation. In this regard, the concept of imaging endophenotypes might be helpful to define potentially heritable neuroimaging traits. An endophenotype (or intermediate phenotype) is a quantitative biological trait that is reliable and reasonably heritable, i.e. shows greater prevalence in unaffected relatives of patients than in the general population.[17] Hippocampal atrophy, due to its high prevalence and relative specificity to epilepsy arising from the temporal lobes,[18] represents an excellent candidate for such an imaging trait.

Studies including subjects with familial TLE indicated that structural abnormalities within the hippocampus may be determined by a strong genetic predisposition and thus represent a

risk factor for TLE.[19,20] Alhusaini and colleagues [21] reported alterations of cortical morphology in anteromedial regions of the ipsilateral temporal lobe in unaffected siblings of patients with sporadic TLE, and these findings were recently replicated by another group of investigators.[22] There is, however, little evidence for heritability of hippocampal traits in sporadic TLE. Previous studies used an automated segmentation pipeline that might be insensitive to small or sclerotic hippocampi,[23,24] and thus did not find significant volumetric changes in siblings of sporadic patients with TLE, although some observed a trend for bilaterally reduced hippocampal volumes.[21,25,26]

Here, we analysed the shared familial contribution to cortical and subcortical brain morphology in patients with TLE and their unaffected siblings with the aim to determine a neuroimaging endophenotype of TLE. We specifically focused on the hippocampus, the hallmark of structural changes in TLE, and were interested whether hippocampal abnormalities can be observed in unaffected siblings in the absence of precipitating injuries, seizures, or medication intake.

Methods

Subjects

We screened consecutive patients with temporal lobe epilepsy (TLE) under follow-up at the Outpatient Clinical Neurology Department of Xiangya Hospital, Central South University (Hunan Province, China) from March 2015 to March 2018. We included patients with a diagnosis of sporadic TLE who had an asymptomatic full sibling. Recruitment was complicated by the one-child policy in China, which is less strictly adhered to in rural China. TLE was diagnosed by certified neurologists specializing in epilepsy based on clinical history, seizure semiology, long-term video-electroencephalography (EEG), and magnetic resonance imaging (MRI). We excluded subjects with brain lesions other than hippocampal sclerosis and people

with concomitant hereditary, psychiatric, or neurologic conditions other than epilepsy.

We matched the patients' asymptomatic full siblings and unrelated healthy volunteers for age and sex. Siblings and healthy controls did not have a history of epileptic seizures, neuropsychiatric, or genetic disorders and they presented with a normal neurological examination and structural MRI. Excluded were those with a history of illicit drug abuse, febrile seizures, or other precipitating injuries. In healthy volunteers, there was no history of seizures or epilepsy in up to three generations of relatives. One sibling showed occasional asymmetrical generalised atypical sharp wave activity with frontal and temporal maximum on EEG but had no history of detectable seizures or epilepsy on detailed questioning. All other siblings and healthy volunteers had an unremarkable routine EEG.

The Ethics Committee of Xiangya Hospital of Central South University approved this study, and all participants gave written informed consent.

Demographic characteristics

In total, we included 18 patients with TLE (10 female; age 29y, interquartile range IQR 20-41y), 18 unaffected full siblings (11 female; age 30y, IQR 24-42), and 18 healthy volunteers (10 female; age 28y, IQR 22-43y). Demographic characteristics are displayed in Table 1. Visual MRI assessment detected hippocampal sclerosis in seven patients with TLE (6 with right TLE, 1 with left TLE) and in one unaffected sibling (not the same person as the sibling with abnormal EEG). Individual hippocampal volume in patients with TLE and their siblings are displayed in the online supplement, section 4. No healthy volunteers had hippocampal sclerosis on visual evaluation.

MRI data acquisition and processing

Three-dimensional (3D) brain anatomical images were acquired on a 3.0T GE Signal HDx

scanner, by using a T1-weighted MP-RAGE sequence according to the following parameters: TE=2.98 ms; TR=7792 ms; TI=800 ms; field of view=256×256 mm; number of slices=188; slice thickness=1.0 mm; flip angle=7 degrees; voxel size= 1×1×1 mm3.

Hippocampal volumetry and subcortical segmentation

We used Hipposeg (*http://niftyweb.cs.ucl.ac.uk/program.php?p=HIPPOSEG*) to automatically extract the initial hippocampal segmentations.[27] Hipposeg delineates the hippocampus with no more variability than seen between expert human raters and is robust to atrophic hippocampi. Next, one blinded rater (LL) received anonymized hippocampal masks and corrected segmentation errors according to a well-established protocol.[28]

To assess intra-rater variability of this combined manual-automated approach, one blinded rater manually corrected Hipposeg segmentation in randomly selected 10 patients with TLE on two different occasions 3 months apart and compared the resulting masks using Dice coefficients.[29] To determine inter-rater variability, a second blinded rater corrected Hipposeg segmentations of 10 randomly selected patients with TLE using the same segmentation protocol. A high intra-rater (0.98 ± 0.01) and inter-rater (0.96 ± 0.02) reliability demonstrated a high consistency of the combined manual-automated method, exceeding the reliability reported for an entirely manual method (intra-rater 0.89 ± 0.02, inter-rater 0.83 ± 0.02).[27]

Volumes of other subcortical structures relevant in epilepsy (thalamus, amygdala, putamen, pallidum)[30] and the total intracranial volume (TIV) were extracted using a parcellation algorithm based on Geodesic Information Flows (GIF)[31] freely available within NiftyWeb (*http://cmictig.cs.ucl.ac.uk/niftyweb*, UCL Centre for Medical Image Computing, UK).

Additionally, an experienced neuroradiologist visually assessed all MRI scans. Hippocampal sclerosis was defined visually as reduced hippocampal volume overall and in

comparison to the contralateral side (i.e. increased asymmetry) with loss of internal architecture and signal increase on T2-weighted imaging as supporting signs.[32]

Hippocampal shape analysis

Final binary hippocampal segmentations were converted to 3D surface meshes and parametrised with a spherical harmonics point distribution model (SPHARM-PDM).[33] In short, to ensure spherical topology of hippocampal segmentations uneven boundaries were minimally smoothed while the original binary surface was used as a constraint ensuring marginal loss of ± 3 voxels of the original surface. Subsequently, these surfaces were represented by spherical harmonics (SPHARM), which were then sampled onto triangulated surfaces (SPHARM-PDM), producing detailed surface information across 1002 vertices. We generated a mean mesh template from 18 healthy volunteers and all hippocampal surfaces were aligned to this mean mesh. Hippocampal shapes were visually checked for both surface mesh and alignment failures. Displacement values were generated using a point to mesh approach calculating the normal distance between the mean template surface and each point on an individual's hippocampal surface mesh. An inward displacement (indicated by a negative displacement value) typically corresponds to atrophy, outward displacement (positive displacement value) to hypertrophy. All preprocessing was done separately for left and right hippocampi.

Cortical thickness estimation

We estimated cortical thickness using a projection-based thickness method implemented in the Computational Anatomy Toolbox (CAT12, *www.neuro.uni-jena.de/cat/)* in Statistical Parametric Mapping (SPM12, *www.fil.ion.ucl.ac.uk/spm/software/spm12/*). This approach was validated using spherical and brain phantoms confirming accurate measurements under a wide set of parameters for several thickness levels.[34] CAT12 showed excellent test-retest reliability

(R^2 = 0.986) and was validated against other cortical surface reconstruction methods, showing fewer thickness measurement errors than comparable approaches.[34-36] After estimation of cortical thickness and the central surface, we carried out topology correction, spherical mapping, and spherical registration. Data were inspected visually and using the retrospective quality assurance protocol implemented in CAT12.

Statistical analysis

We first compared the overall group of patients with TLE and their siblings to healthy volunteers. We performed subsequent subgroup-analyses in left- and right-lateralized patients. We also split siblings into left- and right-lateralized groups based on the epilepsy lateralization of their relatives with epilepsy. The rationale behind this approach was to determine whether genetic factors contribute to morphological abnormalities in unaffected siblings in a lateralized manner, similar to the patients with TLE they are related to, as has been proposed before.[21,25]

Demographic characteristics were compared with the Chi-Square test or independent-sample T-test. Volumetric group-differences (patients vs. healthy controls; siblings vs. healthy controls) were analysed using a full-factorial general linear model with age, sex, educational level, Mini Mental State Examination (MMSE) scores, and TIV as covariates of no interest. Asymmetry indices were calculated as the difference of left and right hippocampal volumes divided by their mean (negative values indicating more left-lateralized atrophy). Data are presented as N (%) or median (interquartile range [IQR]). We also report bias-corrected p-values (p_{BS}) and effect sizes (ß) with 95% confidence intervals (CI) that were calculated for the volumetric results using 1000 bootstrapped random samples. These values are optimism-corrected and generalizable, because they are less dependent on sample composition. Calculations were performed in SPSS statistical analysis package, version 25.0 (IBM-SPSS,

Armonk, NY, USA).

We statistically compared vertex-wise cortical thickness and point-wise displacement values on hippocampal surfaces using fixed-effect linear models implemented in SurfStat (*http://www.math.mcgill.ca/keith/surfstat/*). We used age, sex, level of education, and MMSE scores as covariates of no interest. TIV was used as an additional covariate for hippocampal shape analysis.

We report all our findings thresholded at $p<0.05$ corrected for multiple comparisons using random field theory for nonisotropic images on a cluster level.[37]

Results

Overall group findings

There were no between-group differences in age or sex (Table 1). Patients had significantly lower MMSE scores compared to controls ($p=0.006$). There was a trend for lower MMSE scores in siblings ($p=0.07$) and lower level of education in patients ($p=0.12$) and siblings ($p=0.31$) compared to controls. All subsequent statistical analyses were adjusted for these variables.

Patients with TLE had reduced overall volume of both hippocampi, more on the right (TLE median 2.37 ml, IQR 1.79-2.67, vs. controls 2.94 ml, IQR 2.77-3.24, $p_{BS}=0.003$) than on the left (TLE 2.43 ml, IQR 2.07-2.67, vs. controls 2.71 ml, IQR 2.37-2.89, $p_{BS}=0.02$). Detailed hippocampal volume data are displayed in Table 2. Siblings had reduced overall volume of both right (2.65 ml, IQR 2.32-2.80, vs. controls $p_{BS}=0.001$) and left (2.39 ml, IQR 2.18-2.53, vs. controls $p_{BS}=0.002$) hippocampi.

Other subcortical areas (amygdala, thalamus, putamen, pallidum) showed no differences between patients, siblings and controls.

In the overall group of patients with TLE, hippocampal surface morphology detected focal atrophy in the lateral rim (224 points, 8.7 resels, p<0.00001), inferior medial rim (75 points, 4.2 resels, p=0.0003) and superior medial head (36 points, 2.5 resels, p=0.006) of the right hippocampus and medial body of the left hippocampus (44 points, 2.1 resels, p=0.01, Table 3). Hippocampal shape analysis in unaffected siblings demonstrated focal atrophy in the lateral tail (48 points, 1.9 resels, p=0.02) and head (37 points, 1.6 resels, p=0.04) of the right hippocampus.

Compared to healthy volunteers, the overall group of patients with TLE (Figure 1A; n=18; 12 right, 6 left) had reduced cortical thickness in the right superior frontal and precentral gyri (932 vertices, 4.7 resels, p=0.0005) and bilateral middle frontal gyri (right, 722 vertices, resels > 2.2, p<0.04; left, 359 vertices, 2.3 resels, p=0.03). Unaffected siblings (Figure 1B; n=18) did not show abnormal cortical thinning compared to healthy volunteers.

Patients with left TLE and their siblings

Patients with left TLE (Figure 2A; n=6) showed significantly smaller overall volume of the left hippocampus (left TLE 2.05 ml, IQR 1.93-2.33, vs. controls 2.71 ml, IQR 2.37-2.89, p_{BS}=0.03) and a trend for smaller volume of the right hippocampus (left TLE 2.68 ml, IQR 2.49-2.71, vs. controls 2.94 ml, IQR 2.77-3.24, p_{BS}=0.06). Unaffected siblings had reduced overall volume of both left (2.44 ml, IQR 2.31-2.66, vs. controls p_{BS}=0.009) and right (2.71 ml, IQR 2.50-2.89, vs. controls p_{BS}=0.02) hippocampi.

Hippocampal volumes of patients with left TLE were asymmetric with smaller volumes on the left (-16%, IQR -30 to 4, p=0.048), but no asymmetry was observed in their relatives (-8%, IQR -10 to 1, p=0.79).

In patients with left TLE, focal atrophy was detected in the left inferior hippocampal head (39 points, 2.2 resels, p=0.01) and tail (30 points, 1.7 resels, p=0.03). There was cortical

thinning in the bilateral superior frontal, middle frontal, and precentral gyri (left, 1029 vertices, 6.6 resels, p=0.00004; right, 1334 vertices, 6.7 resels, p=0.00003) and the left postcentral and superior parietal gyri (680 vertices, 4.7 resels, p=0.0005). Unaffected siblings of patients with left TLE (Figure 2B; n=6) did not show focal abnormalities of the hippocampal surface or abnormal cortical thinning.

Patients with right TLE and their siblings

Hippocampal volumetry showed reduced overall volume of the right (right TLE 1.92 ml, IQR 1.39-2.62, vs. controls 2.94 ml, IQR 2.77-3.24, p_{BS}=0.007) but not the left (right TLE 2.55 ml, IQR 2.29-2.91, vs. controls 2.71 ml, IQR 2.37-2.89, p_{BS}=0.26) hippocampus.

Unaffected siblings had reduced overall volume of both left (siblings 2.31 ml, IQR 2.09-2.51, vs. controls p_{BS}=0.009) and right (siblings 2.57 ml, IQR 2.28-2.78, vs. controls p_{BS}=0.009) hippocampi.

Hippocampal volumes of patients with right TLE were asymmetric, pointing to smaller volumes on the right (median asymmetry index 30%, IQR 0 to 68, p<0.001), whereas there was no asymmetry in their relatives (-1%, IQR -7 to 1, p=0.23) when compared to healthy volunteers (-4%, IQR -12 to -1).

Hippocampal shape analysis detected focal atrophy in patients with right TLE in a large area affecting mainly the lateral side, inferior surface, and superomedial head of the right hippocampus (404 points, 18.5 resels, p<0.00001). In unaffected siblings, focal atrophy was found in the lateral body/tail (83 points, 3.4 resels, p=0.001) and inferior head (35 points, 1.6 resels, p=0.04) of the right hippocampus.

We did not detect significant cortical thinning in patients with right TLE (Figure 3A; n=12), nor in their unaffected siblings (Figure 3B; n=12)

Other analyses

We conducted a sensitivity analysis excluding one sibling who had an abnormal EEG. Excluding this dataset did not alter the overall results (see online supplement, section 1). We also correlated clinical factors (duration of epilepsy, history of secondarily generalized seizures, seizure frequency, and number of antiepileptic drugs) with brain morphology in patients with TLE and provide the detailed results in the online supplement, section 2. Lastly, we found abnormalities of cortical surface area in patients with TLE but not in their siblings, as described in the online supplement, section 3.

Discussion

We performed detailed morphological analyses in a cohort of patients with sporadic temporal lobe epilepsy, their asymptomatic full siblings, and matched healthy controls. We found shared hippocampal abnormalities that were mostly asymmetric in patients and bilateral in siblings, and were detected using two independent methods (volumetry and surface shape analysis). Structural changes in siblings were restricted to the hippocampus and were not found in other subcortical or cortical areas. These shared traits point to a familial vulnerability of the hippocampus in sporadic patients with TLE and their siblings and could represent an imaging endophenotype of TLE.

Little is known about the genetic contribution to sporadic TLE and defining genetic risk factors has proven difficult.[14-16] Our findings stand out in this regard because they strongly suggest a familial contribution to hippocampal atrophy in sporadic TLE. We found hippocampal morphological abnormalities in siblings who never had seizures, did not take regular medication, and had no history of febrile seizures or neurologic disease. This is of interest because it shows that hippocampal abnormalities can develop in the absence of precipitating injuries and the effects of seizures or medication. Our results support the notion

that, in some cases, disruption of hippocampal structural integrity might precede the onset of seizures rather than being the consequence of epilepsy. They also indicate that genetic factors are likely to contribute to hippocampal atrophy observed in TLE and their siblings. Recently, common genetic variants associated with hippocampal volume were identified in the general population.[38,39] It will need to be determined whether these or other variants contribute to the imaging endophenotype of hippocampal atrophy in TLE and their siblings.

Another relevant observation is that siblings did not experience seizures despite presenting with a hippocampal imaging trait, which indicates that additional environmental and/or genetic factors are necessary to develop epilepsy. There are three plausible explanations. Firstly, mild hippocampal disturbances observed in siblings might not be severe enough to cause the emergence of seizures, whereas a more severe phenotype or genotype in patients with TLE could lead to epilepsy. Secondly, a combination of the genetic predisposition to hippocampal structural impairment[40] and additional environmental factors, e.g. HHV-6 viral infection, febrile seizures or other precipitating injuries, could be the cause of more severe hippocampal damage and, subsequently, seizures in TLE. Lastly, hippocampal atrophy could be an epiphenomenon of a genetic predisposition to epilepsy without a causative role in disease development. This is less likely because hippocampal atrophy is not prevalent in the general nonepileptic population,[18] surgical removal of the atrophic hippocampus frequently alleviates or stops seizures,[41] and a wealth of animal research proposes a central role of the hippocampal formation in epileptogenesis.[42]

Alhusaini and colleagues summarized the literature on familial traits in epilepsy before 2016, and found a probable familial component to hippocampal structural alterations particularly in patients with TLE with a strong family history for seizures.[43] Previous cohorts of sporadic TLE, including two recent studies,[22,44] did not find significant hippocampal changes in unaffected relatives of patients with sporadic TLE,[21,25,26] whereas one study

observed a trend for bilaterally smaller hippocampi in unaffected siblings,[21] similar to our results. One possible reason for the failure to detect hippocampal atrophy in the relatives of sporadic patients in previous studies is the use of an automated segmentation pipeline implemented in Freesurfer, which is known to be unreliable in small, sclerotic or malrotated hippocampi.[23,24,45] A strength of our study is the implementation of a highly reliable (intra-rater reliability 0.98 ± 0.01, inter-rater reliability 0.96 ± 0.02) semi-automated segmentation approach based on Hipposeg, that has been developed specifically for epilepsy and remains robust when applied to sclerotic hippocampi. Hipposeg (Pearson correlation coefficient r = 0.93 - 0.94) performs better than Freesurfer (r = 30.69 - 0.79) when compared to manual segmentations.[23,24,27] The increased accuracy of hippocampal segmentations in our study assured a higher sensitivity to detect differences between siblings and healthy volunteers. In addition, we used hippocampal shape analysis, a novel sensitive approach to detect morphological abnormalities on a subregional level that allows to reveal focal atrophy that might be missed by volumetry alone.

One previous study included a mixed cohort of familial and sporadic patients with TLE and described reduced hippocampal volumes in their unaffected relatives, particularly in those related to people with familial TLE.[20] There are important differences between this previous study and our cohort and we expand on the previous findings. Because the inclusion of familial TLE cases might overestimate the genetic contribution to neuroimaging traits, we have only included sporadic TLE cases. We also excluded siblings who had a history of precipitating injuries or febrile seizures, to minimise the effect of these events on hippocampal morphology. We did not restrict our calculations to the hippocampus but performed whole-brain cortical and subcortical analysis. Lastly, we evaluated the subregional extent of hippocampal abnormalities with surface-shape analysis.

We found bilaterally smaller hippocampi in the overall group of unaffected siblings and in

both subgroups of siblings related to patients with left or right TLE. In contrast, visual assessment found definitive hippocampal sclerosis in only one sibling. This confirms that hippocampal changes in siblings are typically mild and usually do not fulfil the visual criteria for definitive hippocampal sclerosis. We did not observe hippocampal volumetric asymmetry in siblings, whereas focal hippocampal changes on surface shape analysis were more likely to be detected in the right hippocampus. This suggests that familial contribution to hippocampal damage is usually bilateral, potentially with a slight right-sided predominance. Focal hippocampal changes detected with surface shape analysis in patients with TLE and their siblings showed largely similar patterns. In both groups, surface abnormalities were most pronounced in the right lateral hippocampus mainly corresponding to the CA1 subfield,[46] in accordance with histological findings related to hippocampal sclerosis.[47] This spatial overlap might suggest that familial factors affect the hippocampus in a subregionally specific manner.

In contrast to unaffected siblings, patients with TLE had pronounced asymmetry of hippocampal volumes. This asymmetry might be the consequence of a precipitating injury predominantly affecting one hemisphere[5] or due to lateralized hippocampal damage by neuronal disconnection or seizures once unilateral epilepsy is established. The number of patients with right TLE was greater than those with left TLE. This may have driven the observations of a more significant reduction in the right hippocampal volume in the overall patient group.

Patterns of cortical thinning detected in patients with TLE were similar to those described in previous research, mainly affecting bilateral precentral and prefrontal areas.[30,48] In line with previous findings, patients with left TLE were more likely to show widespread cortical thinning than people with right TLE.[30,49] In contrast, unaffected siblings did not show morphological abnormalities outside of the hippocampus. This observation might suggest that genetic factors shared between patients with TLE and their siblings mainly affect the hippocampus and have

little influence on other subcortical or cortical areas. Cortical thinning observed in patients with TLE could be, thus, driven by the spread of epileptic discharges or ongoing seizures rather than genetic factors. In support of this, cortical thinning was previously associated with seizure frequency and duration of epilepsy[30,50] and we also found a correlation with duration of epilepsy (online supplement, section 2). On the other hand, two previous studies found altered cortical morphology driven by surface area contractions in siblings of patients with TLE,[21,22] but there were no changes in cortical thickness.[44] In our study, significant surface area abnormalities were found in patients with TLE but not in their siblings (online supplement, section 3). Further larger studies will be needed to address this issue.

Our study has limitations. Firstly, the included cohort was small. Nevertheless, we obtained detailed phenotypic information and used robust and accurate methods to analyse the data, allowing us to detect shared hippocampal abnormalities in patients and siblings using two different approaches (volumetry and shape analysis) despite the small number of subjects. We report bias-corrected effect-sizes and overoptimism-corrected p-values (p_{BS}) that increase the generalizability of our results because the corrected metrics are independent of sample composition. Nevertheless, larger studies with higher statistical power might extend these findings in future. Secondly, the included subjects were all ethnic Chinese and replication in other ethnic groups or mixed cohorts will be necessary. Thirdly, this study included four patients with TLE with normal hippocampal volumes (TLE-NV), which may impact the detection of disease-specific endophenotypes. Separating patients with TLE into those without and with hippocampal atrophy (TLE-HA) based on the quantitative results will be relevant for follow-up studies. Fourthly, apart from hippocampal surface shape changes, it might be of interest to determine the intra-hippocampal alterations in siblings of patients with epilepsy. These could be explored using probabilistic hippocampal subfield mapping in future studies. Lastly, familial factors shared by siblings and patients with TLE include not only genetic but

also shared environmental factors. The impact of shared environmental factors on our results cannot be completely eliminated, but their contribution to brain morphology is likely smaller than that of shared genetic factors.

Conclusion

We found shared morphological abnormalities in the hippocampus of both patients with TLE and their unaffected siblings. Hippocampal atrophy occurred in unaffected siblings in the absence of precipitating injuries, seizures, or medication intake. This challenges the notion that hippocampal atrophy is an exclusively acquired phenomenon and rather lends support to the idea that, in some cases, hippocampal changes might precede the onset of epilepsy and could be the cause rather than the consequence of seizures. Shared familial contribution to hippocampal damage could reflect an imaging endophenotype of TLE, representing the underlying genetic or shared environmental risk, or their combination, of TLE in both disease-affected and -unaffected siblings. Such an imaging trait could be used as a potential biomarker for future genetic studies to identify common variants associated with risk of sporadic TLE. Early hippocampal morphological changes could also serve as prognostic markers for the development of epilepsy after an initial insult or a first seizure and this should be evaluated in future prospective trials.

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Disclosure of Conflicts of Interest

None of the authors has any conflict of interest to disclose.

Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Tables

	TLE patients (n = 18)	Unaffected siblings (n = 18)	Healthy volunteers (n = 18)
Sex			
Female	10 (56%)	11 (61%)	10 (56%)
Male	8 (44%)	7 (39%)	8 (44%)
Age and duration (*years*)			
Age at scan	29 (20-41)	30 (24-42)	28 (22-43)
Age at seizure onset	14 (11-26)		
Duration of epilepsy	11 (6-16)		
Level of Education (*years*)	9 (9-16)	12 (9-16)	16 (9-16)
MMSE	29 (25-30)	30 (29-30)	30 (30-30)
Handedness			
Right	18 (100%)	18 (100%)	18 (100%)
Left	0 (0%)	0 (0%)	0 (0%)
Seizures			
CPS	6 (33%)		
CPS+SGS	10 (56%)		
CPS +SPS+SGS	2 (11%)		
Status epilepticus	0 (0%)		
CPS frequency			
≤once per month	4 (22%)		
2~4 times per month	4 (22%)		
＞4 times per month	10 (56%)		
Epilepsy lateralization			
Right	12 (67%)		
Left	6 (33%)		
Hippocampal sclerosis (visual)	7 (39%)	1 (6%)	0 (0%)
History of febrile convulsion	5 (28%)	0 (0%)	0 (0%)
Number of antiepileptic drugs			
None	2 (11%)	18 (100%)	18 (100%)
1	10 (56%)	0 (0%)	0 (0%)
2	5 (28%)	0 (0%)	0 (0%)
3	1 (56%)	0 (0%)	0 (0%)

Table 1: Demographic and clinical characteristics. Data presented as N (%) or median (interquartile range). MMSE, Mini Mental State Examination; CPS, complex partial seizures; SGS, secondary generalized seizures; SPS, simple partial seizures; AED, antiepileptic drugs.

	Median (ml)	IQR (ml)	Statistical comparison vs. controls			
			ß	95% CI	p_{uncorr}	p_{BS}
All subjects						
Right hippocampal volume						
Patients	2.37	1.79-2.67	-0.65	-0.96 to -0.36	<0.001	0.003
Siblings	2.65	2.32-2.80	-0.43	-0.61 to -0.21	<0.001	0.001
Controls	2.94	2.77-3.24	--	--	--	--
Left hippocampal volume						
Patients	2.43	2.07-2.67	-0.30	-0.48 to -0.06	0.02	0.02
Siblings	2.39	2.18-2.53	-0.37	-0.53 to -0.20	<0.001	0.002
Controls	2.71	2.37-2.89	--	--	--	--
Left TLE						
Left hippocampal volume						
Patients	2.05	1.93-2.33	-0.42	-0.72 to -0.10	0.01	0.03
Siblings	2.44	2.31-2.66	-0.34	-0.44 to -0.19	0.007	0.009
Controls	2.71	2.37-2.89	--	--	--	--
Right hippocampal volume						
Patients	2.68	2.49-2.71	-0.39	-0.67 to -0.10	0.06	0.06
Siblings	2.71	2.50-2.89	-0.41	-0.58 to -0.14	0.02	0.02
Controls	2.94	2.77-3.24	--	--	--	--
Right TLE						
Right hippocampal volume						
Patients	1.92	1.39-2.62	-0.75	-1.1 to -0.36	<0.001	0.007
Siblings	2.57	2.28-2.78	-0.46	-0.70 to -0.17	0.003	0.009
Controls	2.94	2.77-3.24	--	--	--	--
Left hippocampal volume						
Patients	2.55	2.29-2.91	-0.16	-0.36 to 0.08	0.17	0.26
Siblings	2.31	2.09-2.51	-0.40	-0.66 to -0.12	0.002	0.009
Controls	2.71	2.37-2.89	--	--	--	--

Table 2: Statistical results of hippocampal volumes among three groups. Volumetric group-differences (patients vs. healthy controls; siblings vs. healthy controls) were analysed using a full-factorial general linear model with age, sex, educational level, Mini Mental State Examination (MMSE) scores, and total intracranial volume (TIV) as covariates of no interest. TLE, temporal lobe epilepsy; IQR, interquartile range; ß, effect sizes; CI, confidence intervals; p_{uncorr}, uncorrected p-values; p_{BS}, bias-corrected p-values.

Significant clusters	Points	Resels	P value
All subjects			
Patients			
Right lateral rim	224	8.7	<0.00001
Right inferior medial rim	75	4.2	0.0003
Right superior medial head	36	2.5	0.006
Left medial body	44	2.1	0.01
Siblings			
Right lateral tail	48	1.9	0.02
Right head	37	1.6	0.04
Left TLE			
Patients			
Left inferior head	39	2.2	0.01
Left tail	30	1.7	0.03
Right TLE			
Patients			
Right lateral side, inferior surface, and superomedial head	404	18.5	<0.00001
Siblings			
Right lateral body/tail	83	3.4	0.001
Right inferior head	35	1.6	0.04

Table 3: Statistical results of hippocampal surface morphology among three groups.
We statistically compared point-wise displacement values on hippocampal surfaces using fixed-effect linear models implemented in SurfStat. We used age, sex, level of education, and MMSE scores as covariates of no interest. TIV was used as an additional covariate for hippocampal shape analysis. TLE, temporal lobe epilepsy.

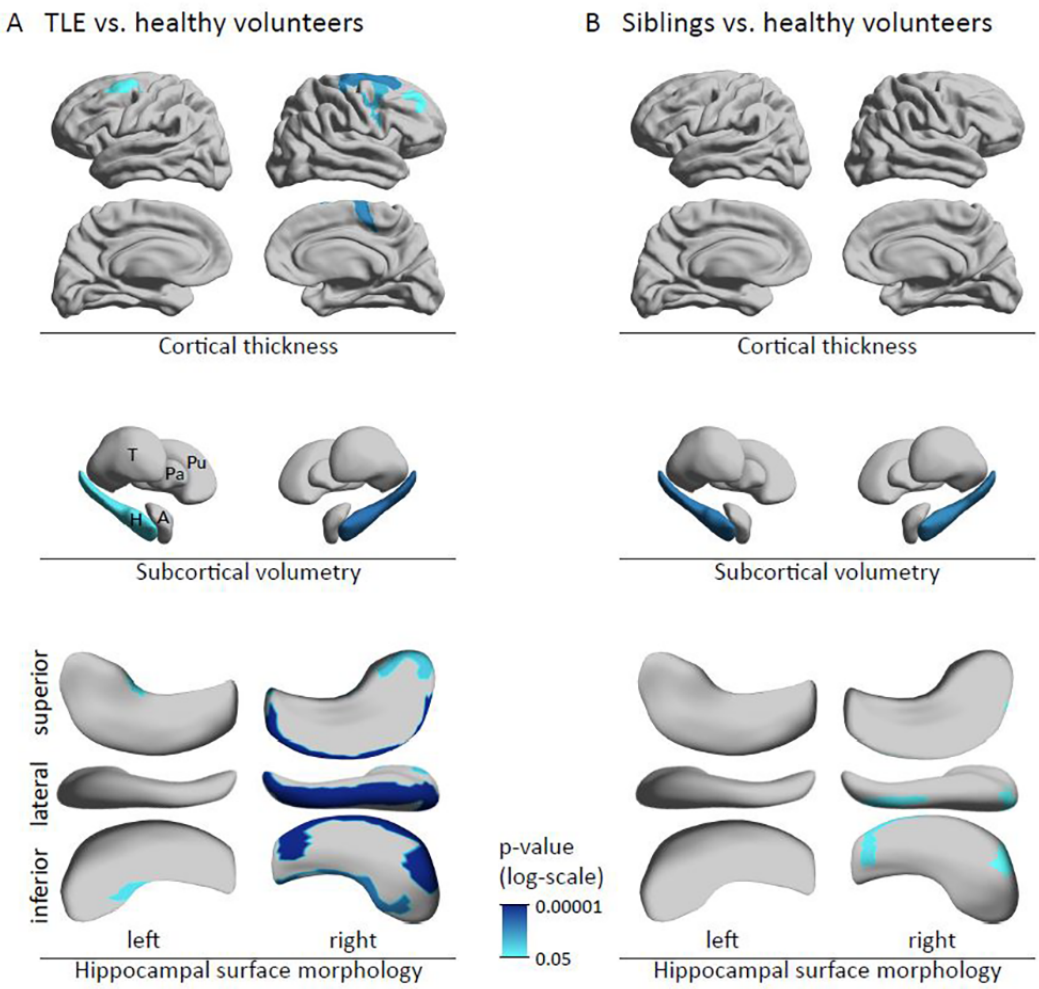

Figure 1: *Overall group findings.*

Displayed are comparisons of patients with TLE (**Panel A**, n=18) and their unaffected siblings (**Panel B**, n=18) with unrelated healthy volunteers (n=18). The top panels show significant clusters of cortical thinning on medial and lateral views of the cerebral hemispheres. The middle panels show volumetric findings for the hippocampus (H), amygdala (A), thalamus (T), putamen (Pu), and pallidum (Pa) in the left and right hemispheres. The bottom panels show significant focal inward deformations (i.e. atrophy) on superior, lateral, and inferior views of the left and right hippocampal surface reconstructions.

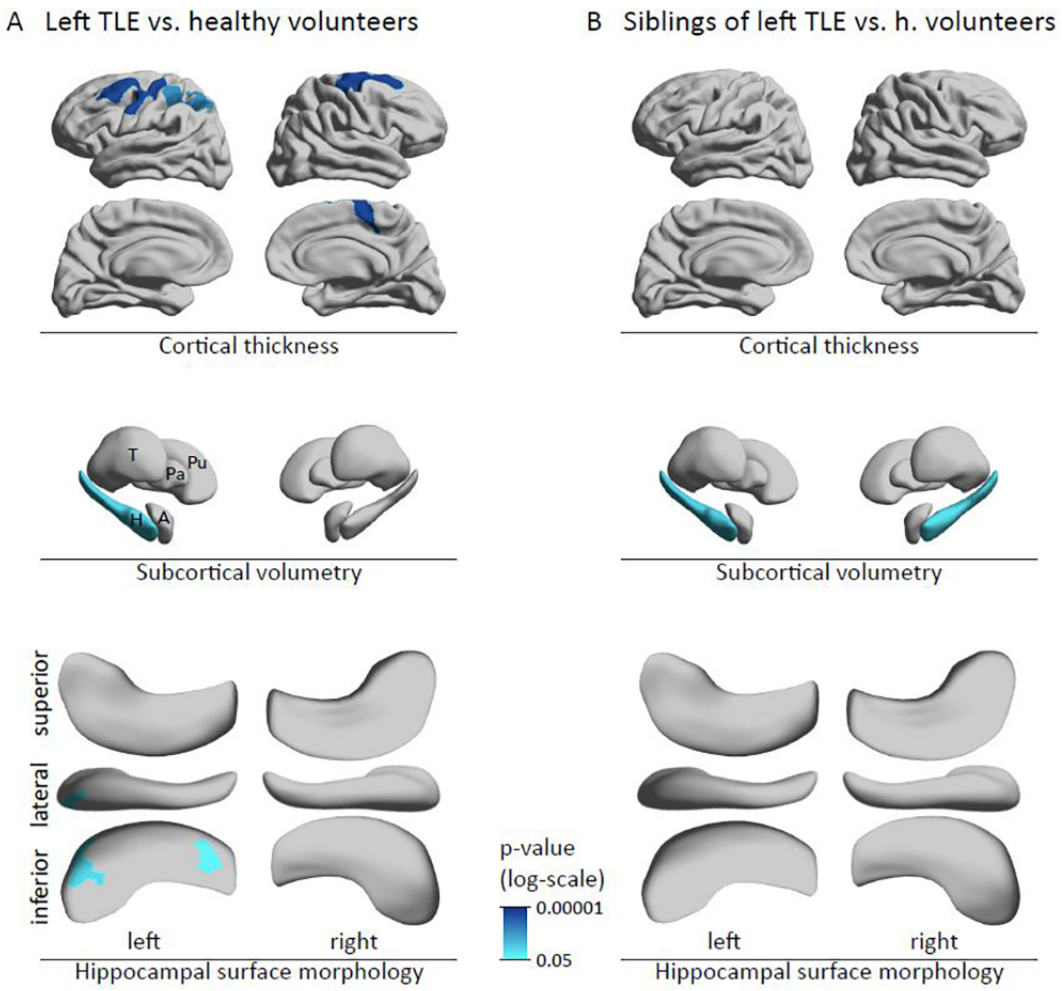

Figure 2: *Patients with left TLE and their siblings.*

Displayed are comparisons of patients with left TLE (**Panel A**, n=6) and their unaffected siblings (**Panel B**, n=6) with unrelated healthy volunteers (n=18). The top panels show significant clusters of cortical thinning on medial and lateral views of the cerebral hemispheres. The middle panels show volumetric findings for the hippocampus (H), amygdala (A), thalamus (T), putamen (Pu), and pallidum (Pa) in the left and right hemispheres. The bottom panels show significant focal inward deformations (i.e. atrophy) on superior, lateral, and inferior views of the left and right hippocampal surface reconstructions.

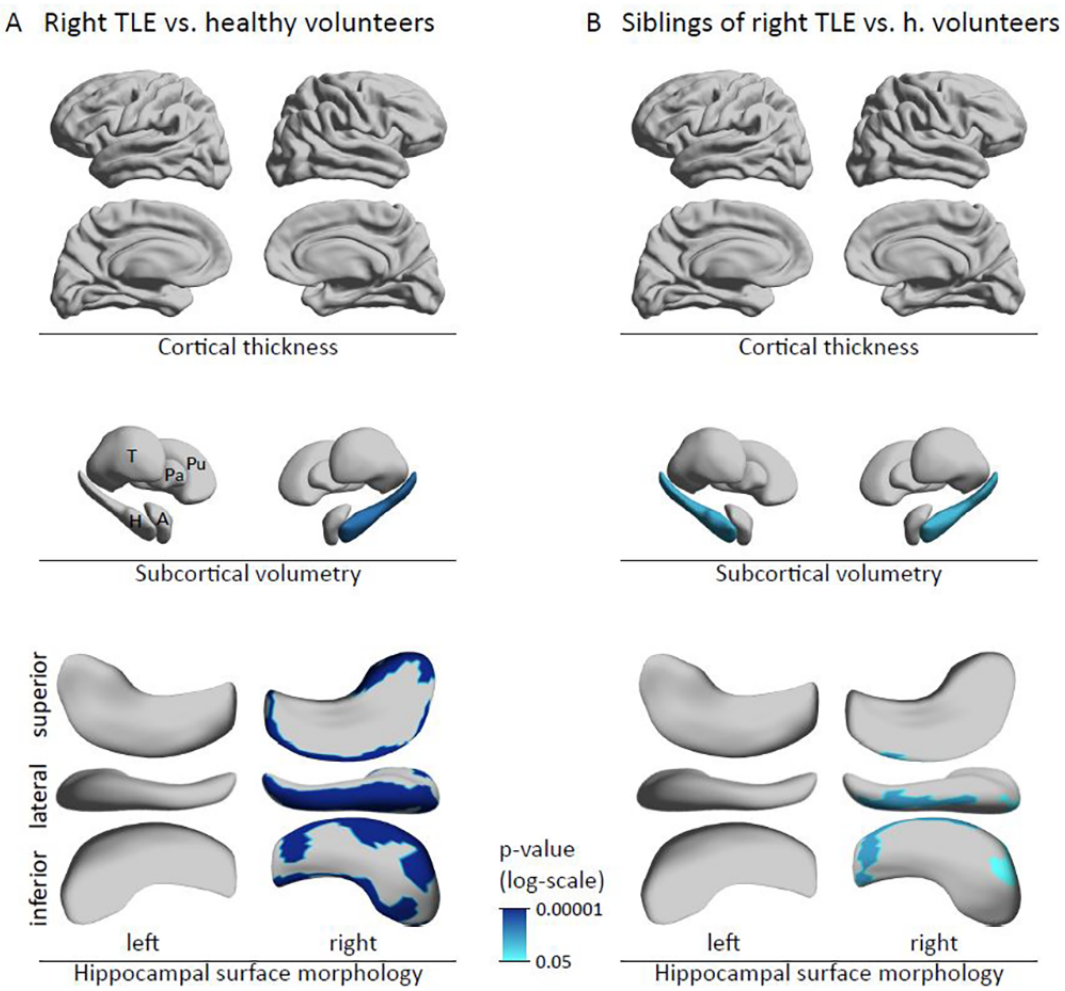

Figure 3: *Patients with right TLE and their siblings.*

Displayed are comparisons of patients with right TLE (**Panel A**, n=12) and their unaffected siblings (**Panel B**, n=12) with unrelated healthy volunteers (n=18). The top panels show significant clusters of cortical thinning on medial and lateral views of the cerebral hemispheres. The middle panels show volumetric findings for the hippocampus (H), amygdala (A), thalamus (T), putamen (Pu), and pallidum (Pa) in the left and right hemispheres. The bottom panels show significant focal inward deformations (i.e. atrophy) on superior, lateral, and inferior views of the left and right hippocampal surface reconstructions.