ORIGINAL ARTICLE

Thalamohippocampal atrophy in focal epilepsy of unknown cause at the time of diagnosis

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Background and purpose: Patients with chronic focal epilepsy may have atrophy of brain structures important for the generation and maintenance of seizures. However, little research has been conducted in patients with newly diagnosed focal epilepsy (NDfE), despite it being a crucial point in time for understanding the underlying biology of the disorder. We aimed to determine whether patients with NDfE show evidence of volumetric abnormalities of subcortical structures.

Methods: Eighty-two patients with NDfE and 40 healthy controls underwent magnetic resonance imaging scanning using a standard clinical protocol. Volume estimation of the left and right hippocampus, thalamus, caudate nucleus, putamen and cerebral hemisphere was performed for all participants and normalised to whole brain volume. Volumes lower than two standard deviations below the control mean were considered abnormal. Volumes were analysed with respect to patient clinical characteristics, including treatment outcome 12 months after diagnosis.

Results: Volume of the left hippocampus ($p_{(FDR\text{-}corr)} = 0.04$) and left ($p_{(FDR\text{-}corr)} = 0.002$) and right ($p_{(FDR\text{-}corr)} = 0.04$) thalamus was significantly smaller in patients relative to controls. Relative to the normal volume limits in controls, 11% patients had left hippocampal atrophy, 17% had left thalamic atrophy and 9% had right thalamic atrophy. We did not find evidence of a relationship between volumes and future seizure control or with other clinical characteristics of epilepsy.

Conclusions: Volumetric abnormalities of structures known to be important for the generation and maintenance of focal seizures are established at the time of epilepsy diagnosis and are not necessarily a result of the chronicity of the disorder.

Introduction

There is a wealth of evidence indicating that people with refractory focal epilepsy have quantitative structural brain abnormalities on magnetic resonance imaging (MRI). Atrophy of temporal lobe structures is frequently identified in patients with temporal lobe epilepsy (TLE), including the hippocampus, entorhinal cortex, perirhinal cortex and amygdala, preferentially ipsilateral to the side of seizure onset [1]. Extrahippocampal subcortical atrophy is also commonly reported, including the thalamus and striatum in both cerebral hemispheres [1,2]. However, it remains unclear whether subcortical atrophy in focal epilepsy is pre-existing, present at the time of diagnosis as a consequence of epileptogenic processes or the result of the chronicity of longstanding epilepsy and antiepileptic drug (AED) treatment. It is therefore important to determine whether brain abnormalities are already established in the early stages of epilepsy.

Despite that epileptogenesis begins prior to the onset of a first seizure [3], the earliest reliable time

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EUROPEAN JOURNAL OF NEUROLOGY

point of investigation of human epilepsy in prospective studies is at the point of diagnosis. Neuroimaging studies of patients with newly diagnosed epilepsy (NDE) have the potential to provide important information about the nature of brain abnormalities by separating pre-existing abnormalities and longstanding changes originating from recurrent seizures and chronic use of AEDs [4]. The identification of quantitative imaging abnormalities at diagnosis may provide new insights into biomarkers of pharmacoresistance and cognitive comorbidities [5,6]. Approximately 60% of patients with NDE will achieve seizure control, ~25% will develop pharmacoresistant epilepsy and the remainder will fluctuate between remission and relapse [7]. To date, markers of pharmacoresistance in patients with NDE have been limited to reports in epidemiological studies and clinical trials, and suggest, for example, that gender, treatment history, age, and time between first seizure and diagnosis may be related to pharmacoresistance [8,9]. Determining the relationship between quantitative brain imaging at diagnosis and AED treatment outcome is an important research endeavour [5]. Additionally, over 50% of patients with NDE have been found to show impairment in at least one cognitive domain [10]. Quantitative imaging studies may further contribute to the understanding of cognitive problems that may be present at point of diagnosis.

There are few quantitative neuroimaging studies in patients with newly diagnosed focal epilepsy (NDfE). Although a small number of studies have identified localised brain atrophy in patients with NDfE, findings are inconsistent. One study revealed hippocampal atrophy in patients with NDfE relative to controls [11], whereas others have found no difference in hippocampal volume between these groups [12,13]. Inconsistent findings have been reported with respect to structural changes of the cerebellum in patients with NDfE [13,14]. To our knowledge, no studies have identified thalamic atrophy in adult NDfE, although a recent study reported thalamic atrophy in drug-naïve patients with new-onset genetic generalised epilepsy (GGE) [15].

There were two primary objectives of the present study. First, we sought to determine whether atrophy of the hippocampus, thalamus, caudate nucleus, putamen and cerebral hemisphere was present at the time of diagnosis of focal epilepsy with unknown cause relative to healthy controls. Second, we aimed to explore whether volumetric changes of these structures were related to various clinical characteristics of the disorder including treatment outcome at 6 and 12 months after diagnosis.

Methods

Participants

We identified patients with archived MRI, acquired according to a clinical epilepsy protocol, within 12 months of diagnosis of focal epilepsy of unknown cause and scanned on a 3T GE Discovery (GE Healthcare) MRI system at the Walton Centre NHS Foundation Trust, Liverpool, United Kingdom, since 2015. At initial screening, 140 patients with likely NDfE and corresponding MRI for analysis were retrieved. More detailed assessment of patient clinical histories and MRI resulted in the exclusion of 58 patients due to one of the following factors: (i) first seizure with no diagnosis of epilepsy, (ii) probable idiopathic generalised epilepsy, (iii) symptomatic seizures or probable historical causes (e.g. tumour, infection, head injury), (iv) presence of epileptogenic lesion (e.g. focal cortical dysplasia, hippocampal sclerosis), or (v) unusable or unavailable MRI data for analysis. This resulted in 82 patients with NDfE of unknown cause (formerly cryptogenic focal epilepsy) with corresponding MRI data for image analysis. All patients were diagnosed by consultant neurologists at the Walton Centre NHS Foundation Trust. All images were reported nonlesional by a neuroradiologist with expertise in the assessment of MRI for epileptogenic lesions. All patients had no history of learning disability.

Additional clinical data were obtained by searching through hospital electronic records. We obtained age at diagnosis and seizure type (focal aware seizures [FAS], focal impaired awareness seizures [FIAS] and focal to bilateral tonic-clonic seizures [FBTCS]) for patients. Fifty-one (62.2%) had undergone electroencephalography (EEG), and we recorded whether interictal abnormalities were captured. Seizure status at 6 and 12 months after diagnosis, hereon referred to as seizure outcome, was obtained for 58 patients at 6 months and 48 patients at 12 months. For comparison with patients, we used imaging data from a cohort of 40 healthy adult controls who were scanned as part of a different study [16] but who had the equivalent MRI scans acquired on the same MRI scanner for comparative analysis. The North West – Liverpool research ethics committee approved this study (14/NW/0332).

MRI acquisition

The standard 3T MRI protocol for patients with a new presentation of seizures at our centre included a high in-plane resolution T1-weighted fluid attenuated

inversion recovery (FLAIR) MRI acquisition of the whole brain (echo time 1.5 ms, repetition time 2500 ms, flip angle 111°, voxel size 0.4 mm × 0.4 mm, slice thickness 3.0 mm, field of view 220 mm, matrix size 320 × 384). This sequence was used for analysis in the present study. Other sequences acquired for diagnostic purposes but not used for analysis included axial T2-weighted and coronal T2-FLAIR scans.

MRI analysis

Given the slice thickness of the T1-weighted FLAIR images, we were unable to reliably apply automated image analysis tools to extract subcortical and hemispheric volume. We therefore used rigorous manual techniques to estimate the volume of subcortical and hemispheric structures. The volume of the left and right hippocampus, thalamus, caudate nucleus, putamen and cerebral hemispheres was quantified for all participants using the Cavalieri method of design-based stereology [17]. This approach has been frequently applied to MRI data in epilepsy studies [12,18–20], has been considered the benchmark measurement approach to which automated MRI techniques have been compared [19], and provides a mathematically unbiased and validated approach to estimate brain compartment volume [17,21].

Using Easymeasure software [19], each volume of interest (VOI) was estimated using a series of parallel two-dimensional (2D) magnetic resonance (MR) sections set at a constant distance apart. A randomly orientated grid of pixels was overlaid on each section, and points intersecting each region of interest (ROI) were counted separately for the left and right structures of each patient and control. The pixel size used for point counting was altered depending on the size of the ROI (pixel sizes: hippocampus, 4; thalamus, 6; caudate nucleus, 5; putamen, 6; and cerebral hemisphere, 30) to optimise the sampling density [17]. The number of points transecting the ROI was multiplied by distance between each consecutive section to produce volume estimates. Given that nuclei (e.g., thalamic nuclei) and subregions (e.g., hippocampal cornu ammonis) of structures measured are almost indistinguishable on clinical MRI, we measured the structures as an entire complex.

Stereological point counting on MR images for volume estimation of the hippocampus, thalamus, caudate nucleus and putamen is shown in Fig. 1. Detailed information on the hippocampal VOI is provided elsewhere [20]. Moving along its longitudinal axis, the hippocampus is bound superiorly by the white, myelinated fibres of the alveus and often by an additional region of cerebrospinal fluid superior to the alveus. The hippocampus was differentiated anteriorly from the amygdala through visualisation of the alveus. The posterior boundary of the hippocampus was reached when the lateral ventricles divide into the frontal and temporal horns. The hippocampal VOI comprised the hippocampus proper, dentate gyrus, alveus, subiculum, presubiculum and parasubiculum; the amygdala, uncus, choroid plexus and grey matter above the alveus were not included in the measurements.

The anterior border of the thalamus began immediately posterior to the anterior commissure and maintained a close relationship with the internal capsule laterally and the central canal of the ventricles medially; the posterior border of the thalamus was the pulvinar. Measurements ended with the formation of the atrium of the ventricles. The zona incerta formed the inferior border of the thalamus. We excluded the subthalamic nuclei, substantia nigra and red nuclei from thalamic measurements. The lateral and medial geniculate bodies and the habenular nucleus were also excluded [19]. The posterior border of the caudate nucleus was considered the last slide in which the caudate tail was still superior to the lateral ventricle. Caudate nucleus and putamen measurements ended with the formation of the atrium from the temporal and frontal horns of the lateral ventricle. Neither caudate nucleus nor putamen measurements included the striatal cell bridges connecting the two nuclei or the nucleus accumbens. The medial and lateral borders of the putamen were the internal capsule and external capsule, respectively [18]. The posterior border of the putamen often coincides with the appearance of the medial and lateral geniculate bodies. Measurement of the entire cerebral hemispheres was also obtained that included all supratentorial grey and white matter, excluding the brainstem and cerebellum. All subcortical and hemispheric volumes were normalised using whole brain volumes (summation of left and right cerebral hemispheric volumes); the proportion of each subcortical and hemispheric volume relative to whole brain volume was calculated.

Statistical analysis

The data did not meet the assumptions of parametric tests; therefore, nonparametric tests were used to analyse the data. For patient-control analysis, all volumes were analysed using nonparametric Mann-Whitney *U* tests in SPSS (version 25, https://www.ibm.com/products/spss-statistics; IBM). Given the significant sex difference between patients and controls, and the higher average age of patients compared to controls, we

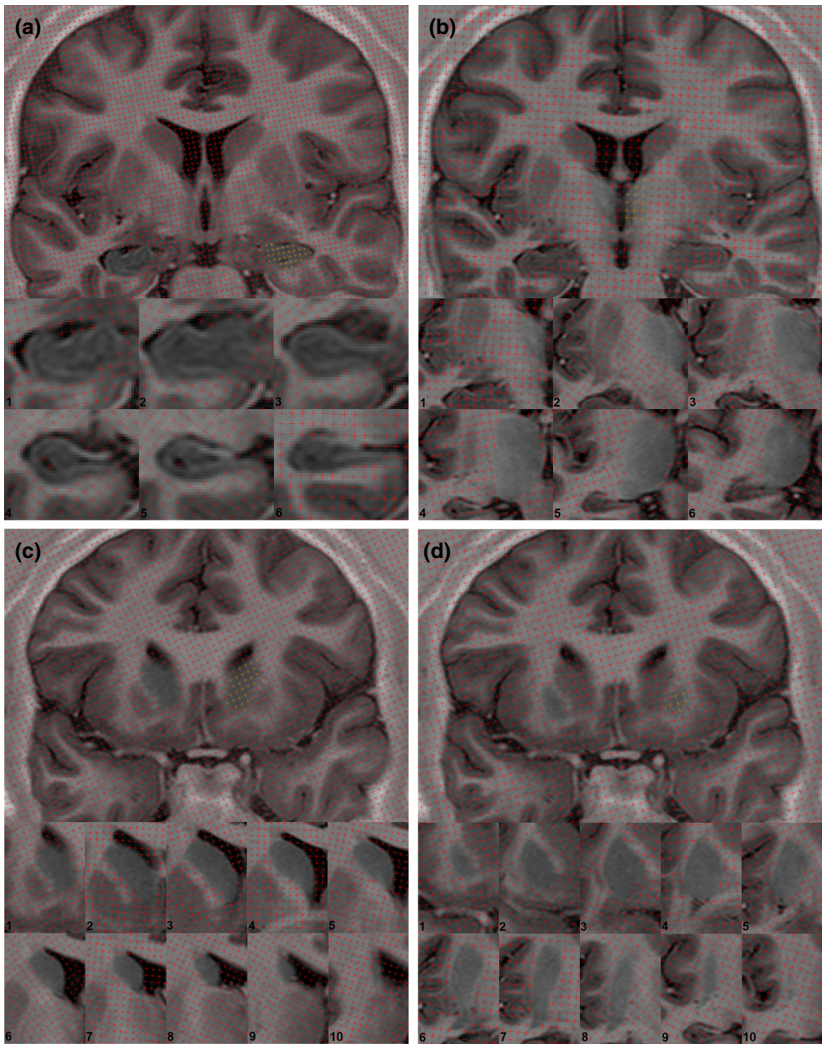

Figure 1 T1-weighted fluid attenuated inversion recovery coronal sections through an exemplar patient showing point counting for stereology through the subcortical volumes of interest. For each structure, point counts are removed in the left hemisphere and coloured orange in the right hemisphere. (A) hippocampus. (B) thalamus. (C) Caudate nucleus. (D) Putamen. Zoomed sections at the bottom of each panel show point counting in a rostral (top left) to caudal (bottom right) direction.

performed all statistical analyses on normalised volumes, with their residuals corrected for age and sex in a confound-only regression model. Volumes lower than two standard deviations of the control mean were considered abnormal and suggestive of structural atrophy in individual patients. Spearman's correlations were used to investigate relationships between volumes and age at first seizure, years between first seizure and diagnosis, and age at diagnosis. Categorical analysis of clinical and neuroimaging data was performed using χ^2 tests. Multiple comparisons were corrected using the false discovery rate (FDR), and results were considered statistically significant at $p < 0.05$.

Results

Clinical data

Table 1 presents a summary of the demographic information for patients and controls, and clinical data for patients. There was no significant difference between the age of patients (when diagnosed) and healthy controls (at time of MRI) ($t = 2.8$, $p = 0.13$). However, there was a significant sex difference between patients and controls, a reflection of more males in the patient group and more females in the control group ($\chi^2 = 4.72$, $p = 0.03$). More patients had FIAS compared to FAS (57.3% vs. 42.7%) and most patients

experienced FBTCS (88.9%). The majority (76.5%) of patients had a normal inter-ictal EEG. Two patients did not commence AED treatment. These patients did not have outcome data. For the remainder of the patient cohort, the first AED used was lamotrigine (n = 47, 59%), levetiracetam (n = 14, 18%), zonisamide (n = 8, 10%), carbamazepine (n = 6, 8%), sodium valproate (n = 3, 4%), oxcarbazepine (n = 1, 1%) and phenytoin (n = 1, 1%). We were able to obtain seizure status at 6 and 12 months postdiagnosis for 58 (40% seizure free) and 48 (50% seizure free) patients, respectively. There were no significant differences in demographic data, incidence of FAS, FIAS or FBTCS, or normal/abnormal EEG between patients who were seizure free and those who continued to experience seizures at 6 or 12 months.

MRI volumetric changes in NDfE

Volumetric descriptive statistics are provided in Table 2. Volume of the left hippocampus (U = 1232, $p_{(FDR\text{-}corr)}$ = 0.04), and left (U = 1002, $p_{(FDR\text{-}corr)}$ = 0.002) and right (U = 1228, $p_{(FDR\text{-}corr)}$ = 0.04) thalamus was significantly smaller in patients compared to controls (Fig. 2). There was a trend for the right hippocampus to be smaller in patients relative to controls (U = 1311, $p_{(FDR\text{-}corr)}$ = 0.09). There were no significant differences ($p_{(FDR\text{-}corr)} < 0.05$) or trends for differences in volume of the left or right caudate nucleus, putamen or whole cerebral hemisphere between patients and controls. In patients, average volume of the left hippocampus was decreased by 6.9%, right hippocampus by 6.5%, left thalamus by 6.4%, and right thalamus by 4.1%, relative to controls. Individual volumetric analysis revealed abnormal volume of the left hippocampus in nine (11%) patients, the right hippocampus in four (4.9%) patients, the left thalamus in 14 (17.1%) patients, and the right thalamus in seven (8.5%) patients.

Table 1 Demographic and clinical data

Clinical variable	Patients	Controls
n	82	40
Age at diagnosis/MRI	38.04 (10.8)	32.50 (8.9)
Sex	50 M/32 F*	16 M/24 F*
FAS	35/ 82 (42.7%)	—
FIAS	47/ 82 (57.3%)	—
FBTCS	72/ 81 (88.9%)	—
Normal EEG	39/ 51 (76.5%)	—
Abnormal EEG	12/ 51 (23.5%)	—
Seizure free, 6 months	23/ 58 (39.7%)	—
Seizure free, 12 months	24/ 48 (50%)	—

Note: Mean age at diagnosis/MRI is presented with standard deviation. Abbreviations: EEG, electroencephalography; F, female; FAS, focal aware seizures; FBTCS, focal to bilateral tonic-clonic seizures; FIAS, focal impaired awareness seizures; M, male; MRI, magnetic resonance imaging. *Significantly different (χ^2 = 4.72, p = 0.03).

MRI correlations with clinical variables

There were no associations between volumes and seizure outcome at 6 or 12 months, EEG finding, loss of awareness during seizures, history of FBTCS, age at first seizure, years between first seizure and diagnosis or age at diagnosis. Table 3 presents the descriptive and statistical comparisons between outcome groups at 12 months. There were also no clinically significant differences between the individual patients who had significant loss of hippocampal and thalamic volume and those who did not.

Discussion

In the present study, we sought to establish whether structures that are known to show atrophy in focal epilepsy also show evidence of volume loss at the time of diagnosis of epilepsy. We report significant volume loss of the thalamus and hippocampus in adults with NDfE. Additionally, we aimed to explore whether clinical variables were associated with volume changes. Atrophy of subcortical structures was not related to seizure outcome at either 6 or 12 months or any other clinical characteristic of epilepsy.

Biological and clinical implications

Of the limited number of quantitative MRI studies that exist in NDfE, the focus has been on the hippocampus. Our results are in support of those studies that reported hippocampal atrophy at diagnosis [11,12,22] and are in contrast to those that did not report atrophy [13]. Patients with chronic epilepsy have shown a 13% to 16% reduction in hippocampal volume compared to controls [22]; we identified a 6.5% to 6.9% decrease in hippocampal volume, which may indicate hippocampal atrophy is present at time of diagnosis and may worsen with the progression of epilepsy. Although only left hippocampal volume reduction was significant after FDR correction, mean right hippocampal volume was similarly reduced in patients. This may suggest that both hippocampi are equally impacted at diagnosis, and clearer insights would be achieved if the epileptogenic zone could be lateralised in newly diagnosed patients. The present study also sought to determine whether extrahippocampal subcortical atrophy was present at diagnosis. To our knowledge, the present study is the first to identify thalamic atrophy in adults with a new

Table 2 Results of volumetric comparisons between patients and controls

	Patients			Controls			
Structure	Mean	SD	Abnormal, *n*, %	Mean	SD	Abnormal, *n*, %	*U*, $p_{(FDRcorr)}$
Left hippocampus							
%CorrAgeSex	0.190	0.033	9, 11	0.204	0.025	1, 2.5	$U = 1232$, $p = 0.04$
cm^3	2.075	0.427	19, 23.2	2.270	0.233	0	—
Right hippocampus							
%CorrAgeSex	0.188	0.034	4, 4.9	0.201	0.031	0	$U = 1311$, $p = 0.09$
cm^3	2.058	0.450	10, 12.2	2.221	0.292	1, 2.5	—
Left thalamus							
%CorrAgeSex	0.642	0.068	14, 17.1	0.686	0.055	1, 2.5	$U = 1002$, $p = 0.002$
cm^3	7.021	0.915	6, 7.3	7.609	0.934	0	—
Right thalamus							
%CorrAgeSex	0.638	0.069	7, 8.5	0.665	0.062	1, 2.5	$U = 1228$, $p = 0.04$
cm^3	6.986	0.938	6, 7.3	7.362	0.934	0	—
Left caudate							
%CorrAgeSex	0.371	0.054	5, 6.1	0.368	0.040	1, 2.5	$U = 1621$, $p = 0.46$
cm^3	4.054	0.634	3, 3.7	4.063	0.487	0	—
Right caudate							
%CorrAgeSex	0.363	0.050	0	0.355	0.044	1, 2.5	$U = 1513$, $p = 0.39$
cm^3	3.963	0.579	2, 2.4	3.932	0.509	0	—
Left putamen							
%CorrAgeSex	0.467	0.051	3, 3.7	0.464	0.043	0	$U = 1623$, $p = 0.46$
cm^3	5.091	0.493	1, 1.2	5.111	0.474	0	—
Right putamen							
%CorrAgeSex	0.473	0.053	2, 2.4	0.469	0.044	0	$U = 1545$, $p = 0.39$
cm^3	5.164	0.539	0	5.176	0.551	1, 2.5	—
Left hemisphere							
%CorrAgeSex	49.98	0.66	5, 6.1	49.95	0.50	0	$U = 1550$, $p = 0.39$
cm^3	550.3	56.7	1, 1.2	549.5	52.5	0	—
Right hemisphere							
%CorrAgeSex	50.02	0.66	4, 4.9	50.05	0.50	0	$U = 1550$, $p = 0.39$
cm^3	550.2	56.5	1, 1.2	551.4	51.3	0	—

Note: Mean and standard deviation (SD) of subcortical and hemispheric volumes, expressed as percentage of whole brain volume and their residuals corrected for age and sex (%CorrAgeSex), and raw volume (cm^3). For each structure, the number (and percentage) of patients with volumes lower than the normal limits is indicated as abnormal (*n*, %).

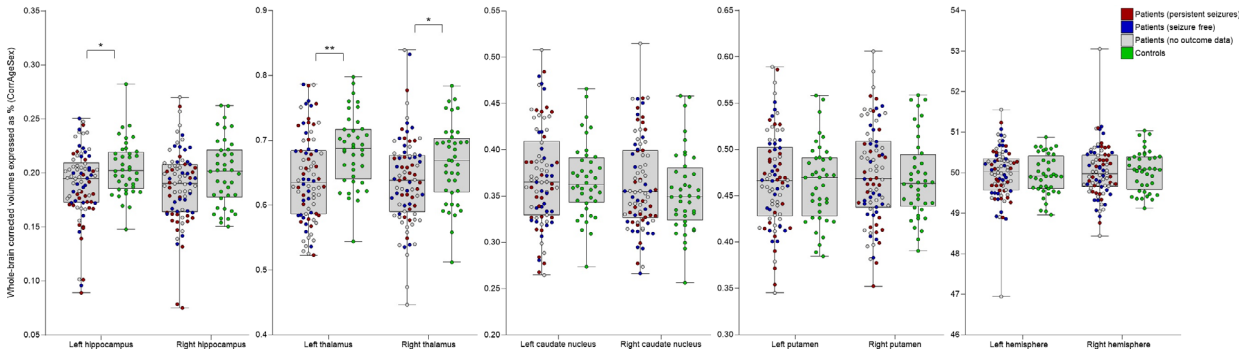

Figure 2 Scatterplots with minimum, 25th percentile, median, 75th percentile, and maximum normalised and corrected volume measurements of brain structures in seizure-free patients, patients with persistent seizures, and healthy controls. Statistically significant between groups: *($p_{(FDRcorr)} < 0.05$), **($p_{(FDRcorr)} < 0.01$).

diagnosis of focal epilepsy. A previous study in a small sample of patients with NDfE did not report thalamic atrophy [13]. Thalamic atrophy has been reported to be almost as common as hippocampal atrophy in a meta-analysis of voxel-based morphometry studies of refractory TLE [23], and is observed in a range of longstanding focal and generalised epilepsy disorders [2,24]. Thalamic volume loss has also been

Table 3 Results of clinical and volumetric comparisons between seizure-free patients and patients with persistent seizures at 12 months

Structure	Seizure free		Persistent seizures		χ^2, U, $p_{(FDRcorr)}$
n (%)	24 (50)		24 (50)		—
Male/female	14 (58.3)	10 (41.7)	14 (58.3)	10 (41.7)	$\chi^2 = 0$, $p = 1.0$
FAS/FIAS	11 (45.8)	13 (54.2)	7 (29.2)	17 (70.8)	$\chi^2 = 1.42$, $p = 0.49$
FBTCS/no FBTCS[a]	22 (91.7)	2 (8.3)	18 (75)	5 (20.8)	$\chi^2 = 1.67$, $p = 0.49$
Normal/ abnormal EEG[b]	11 (45.8)	2 (8.3)	7 (29.2)	7 (29.2)	$\chi^2 = 3.64$, $p = 0.41$
Mean/ SD					
Age	39.71	12.22	37.00	10.25	$U = 251$, $p = 0.45$
Age of onset	32.21	12.33	29.33	11.01	$U = 250$, $p = 0.45$
Left hippocampus					
%CorrAgeSex	0.193	0.032	0.175	0.035	$U = 185$, $p = 0.34$
cm^3	2.101	0.369	1.926	0.507	—
Right hippocampus					
%CorrAgeSex	0.189	0.028	0.172	0.041	$U = 201$, $p = 0.37$
cm^3	2.070	0.336	1.892	0.520	—
Left thalamus					
%CorrAgeSex	0.651	0.071	0.653	0.068	$U = 280$, $p = 0.98$
cm^3	7.156	0.906	7.157	0.917	—
Right thalamus					
%CorrAgeSex	0.654	0.064	0.646	0.051	$U = 257$, $p = 0.98$
cm^3	7.209	0.923	7.084	0.792	—
Left caudate					
%CorrAgeSex	0.367	0.053	0.365	0.058	$U = 288$, $p = 1.0$
cm^3	4.038	0.647	3.982	0.577	—
Right caudate					
%CorrAgeSex	0.355	0.052	0.363	0.051	$U = 255$, $p = 0.98$
cm^3	3.894	0.614	3.963	0.503	—
Left putamen					
%CorrAgeSex	0.468	0.045	0.462	0.055	$U = 276$, $p = 0.98$
cm^3	5.134	0.367	5.059	0.618	—
Right putamen					
%CorrAgeSex	0.469	0.047	0.461	0.054	$U = 271$, $p = 0.98$
cm^3	5.150	0.528	5.042	0.571	—
Left hemisphere					
%CorrAgeSex	50.00	0.63	49.94	0.54	$U = 264$, $p = 0.98$
cm^3	553.7	56.2	549.7	56.7	—
Right hemisphere					
%CorrAgeSex	50.00	0.63	50.06	0.54	$U = 264$, $p = 0.98$
cm^3	553.1	55.4	550.8	56.6	—

Abbreviations: EEG, electroencephalography; FAS, focal aware seizures; FBTCS, focal to bilateral tonic-clonic seizures; FIAS, focal impaired awareness seizures. [a]FBTCS data not available for one patient with persistent seizures. [b]EEG data not available for 11 (45.8%) seizure-free patients and 10 (41.7%) patients with persistent seizures.

reported in children with NDfE [25] and patients with new-onset GGE [15]. Consistent with our findings, volume loss of the thalamus in both hemispheres is frequently reported in patients with chronic focal epilepsy [2,24,26–28]. Taken together, our results suggest that thalamo-hippocampal atrophy is likely established prior to the onset of habitual epilepsy; further hippocampal and thalamic damage may occur as the disorder becomes longstanding, particularly in refractory cases [29–31].

There are very few existing studies that have attempted to predict pharmacoresistance from the point of diagnosis of focal epilepsy using advanced imaging in a way that resembles work predicting surgical outcome in focal epilepsy [27,32]. This is an unmet need in the early stages of the disorder [4,5,33]. Having a reliable imaging biomarker of the health issues patients will experience (e.g., uncontrolled seizures, memory impairment) from diagnosis will provide clinicians and patients with realistic expectations and could serve to assist the patient management pathway (e.g., earlier use of adjunctive/ alternative therapies in patients likely to be pharmacoresistant) [33]. In the present study, we have reported that gross neuroanatomical volume of subcortical structures is not related to seizure control at 6 or 12 months after diagnosis. It is likely that imaging markers of pharmacoresistance, if found, will be microstructural, functional, or metabolic. Interestingly, in patients with longstanding focal epilepsy, MR spectroscopy

hippocampal *N*-acetylaspartate/creatine measurements have been related to seizure control [34].

Approximately 50% of patients with NDfE exhibit impairment in at least one cognitive domain [10]. Drug-naïve patients with NDfE show significant impairment in memory, sustained attention, executive functioning, mental flexibility and psychomotor speed relative to healthy volunteers [35–40]. Cognitive deficits are therefore not necessarily a result of the chronicity of the disorder and may be at least partly driven by the pathological processes that lead to the generation of spontaneous seizures. Although we did not assess cognition in the current study, atrophy of subcortical structures may be related to cognitive impairment in patients with NDfE.

Methodological issues

Following diagnostic MRI, most patients with NDfE will not undergo further investigation, which is usually undertaken in those with a refractory course, for who more precise localisation of the seizure focus is more likely as more seizures are witnessed and following increasingly detailed imaging, EEG and neuropsychological evaluation. Our sample of patients with NDfE is therefore likely to be clinically heterogenous in terms of seizure foci, despite commonalities in a new diagnosis of focal epilepsy of unknown cause and nonlesional MRI. It is difficult, most often impossible, to discern the epileptogenic focus in patients with a new diagnosis of focal epilepsy; diagnosis here is based on an epileptologist's expertise in ascribing a likely diagnosis based on very few—sometimes singular—past events described by the patient and/or their family soon after the seizure(s). Inter-ictal EEGs are most frequently (76.5% of our sample) unrevealing and provide little diagnostic or localising information; the majority of patients with new-onset seizures do not show inter-ictal epileptiform activity on clinical EEG [41–43]. As such, our imaging findings are 'collapsed' across patients with likely newly diagnosed nonlesional temporal and frontal lobe epilepsy, which constitutes the vast majority of focal epilepsies. This is an inherent shortcoming of our pragmatic approach, but this is necessary in imaging studies of NDfE [13,44]. The epileptogenic focus could be determined by long-term follow-up of newly diagnosed patients who later become refractory, experience many more seizures and be evaluated using a variety of investigative tools. However, most patients will not experience further seizures after starting AED treatment [7]; long-term follow-up is difficult in these patients. Furthermore, as per the exclusion criteria for this study, all patients did not have relevant histories that could be used to predict brain atrophy or pharmacoresistance. Future studies may relax exclusion criteria to identify predictors of pharmacoresistance in a more heterogeneous sample. We suggest that what is lost through the inclusion of a highly phenotyped group of patients is gained through a pragmatic approach to studying all nonlesional patients with a new diagnosis of focal epilepsy.

The standard MRI protocol for patients with a new presentation of seizures at our institution does not include three-dimensional volume scans that would be amenable to automated segmentation techniques. It was necessary for us to apply manual volumetric analysis due to the 3-mm slice thickness of the coronal 2D T1-weighted FLAIR MRI scans. Despite this being a time-inefficient way of obtaining morphometric data, there were distinct advantages to our approach. First, manual measurement of brain regions is considered the gold standard, and stereology provides a mathematically unbiased and validated approach to estimate brain compartment volume [17,21]. Second, despite the nonisotropic voxel size, the high in-plane resolution and contrast of the scans provided excellent grey–white matter differentiation, even in regions where the grey matter and white matter borders are difficult to establish (e.g., the thalamus). Ultimately, gross brain volumetry appears not to be a biomarker of pharmacoresistance in patients with NDfE; more advanced imaging methods that permit analysis of brain connectivity and microscopic brain architecture in prospectively recruited newly diagnosed cohorts may identify such prognostic markers [33].

Conclusions

Many specialist institutions and research centres do not see patients with epilepsy until it is well established, which may contribute to the lack of imaging studies of NDfE. In the present imaging study, we have studied a comparatively large number of patients with NDfE and report that atrophy of the hippocampi and thalami, ordinarily reported to be atrophic in longstanding and refractory focal epilepsy, is established at the time of diagnosis. It remains uncertain as to whether this atrophy is congenital, a consequence of epileptogenic processes or a combination of both.

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Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

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.

Data availability statement

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

References

1. Bonilha L, Keller SS. Quantitative MRI in refractory temporal lobe epilepsy: relationship with surgical outcomes. *Quant Imaging Med Surg* 2015; **5:** 204–224.
2. Whelan CD, Altmann A, Botia JA, *et al*. Structural brain abnormalities in the common epilepsies assessed in a worldwide ENIGMA study. *Brain* 2018; **141:** 391–408.
3. Velisek L, Moshe SL. Temporal lobe epileptogenesis and epilepsy in the developing brain: bridging the gap between the laboratory and the clinic. Progression, but in what direction? *Epilepsia* 2003; **44**(Suppl 12)**:** 51–59.
4. Pohlmann-Eden B. Conceptual relevance of new-onset epilepsy. *Epilepsia* 2011; **52**(Suppl 4)**:** 1–6.
5. Pohlmann-Eden B, Crocker CE, Schmidt MH. A conceptual framework for the use of neuroimaging to study and predict pharmacoresistance in epilepsy. *Epilepsia* 2013; **54**(Suppl 2)**:** 75–79.
6. Pohlmann-Eden B, Aldenkamp A, Baker GA, *et al*. The relevance of neuropsychiatric symptoms and cognitive problems in new-onset epilepsy - Current knowledge and understanding. *Epilepsy Behav* 2015; **51:** 199–209.
7. Brodie MJ. Road to refractory epilepsy: the Glasgow story. *Epilepsia* 2013; **54**(Suppl 2)**:** 5–8.
8. Bonnett L, Smith CT, Smith D, Williamson P, Chadwick D, Marson AG. Prognostic factors for time to treatment failure and time to 12 months of remission for patients with focal epilepsy: post-hoc, subgroup analyses of data from the SANAD trial. *Lancet Neurol* 2012; **11:** 331–340.
9. Mohanraj R, Brodie MJ. Early predictors of outcome in newly diagnosed epilepsy. *Seizure* 2013; **22:** 333–344.
10. Taylor J, Kolamunnage-Dona R, Marson AG, *et al*. Patients with epilepsy: cognitively compromised before the start of antiepileptic drug treatment? *Epilepsia* 2010; **51:** 48–56.
11. Kalviainen R, Partanen K, Aikia M, *et al*. MRI-based hippocampal volumetry and T2 relaxometry: correlation to verbal memory performance in newly diagnosed epilepsy patients with left-sided temporal lobe focus. *Neurology* 1997; **48:** 286–287.
12. Salmenpera T, Kononen M, Roberts N, Vanninen R, Pitkanen A, Kalviainen R. Hippocampal damage in newly diagnosed focal epilepsy: a prospective MRI study. *Neurology* 2005; **64:** 62–68.
13. Park KM, Han YH, Kim TH, *et al*. Cerebellar white matter changes in patients with newly diagnosed partial epilepsy of unknown etiology. *Clin Neurol Neurosurg* 2015; **138:** 25–30.
14. Hagemann G, Lemieux L, Free SL, *et al*. Cerebellar volumes in newly diagnosed and chronic epilepsy. *J Neurol* 2002; **249:** 1651–1658.
15. Perani S, Tierney TM, Centeno M, *et al*. Thalamic volume reduction in drug-naive patients with new-onset genetic generalized epilepsy. *Epilepsia* 2018; **59:** 226–234.
16. Kreilkamp BAK, Lisanti L, Glenn GR, *et al*. Comparison of manual and automated fiber quantification tractography in patients with temporal lobe epilepsy. *Neuroimage Clin* 2019; **24:** 102024.
17. Roberts N, Puddephat MJ, McNulty V. The benefit of stereology for quantitative radiology. *Br J Radiol* 2000; **73:** 679–697.
18. Keller SS, Ahrens T, Mohammadi S, *et al*. Microstructural and volumetric abnormalities of the putamen in juvenile myoclonic epilepsy. *Epilepsia* 2011; **52:** 1715–1724.
19. Keller SS, Gerdes JS, Mohammadi S, *et al*. Volume estimation of the thalamus using freesurfer and stereology: consistency between methods. *Neuroinformatics* 2012; **10:** 341–350.
20. Keller SS, Mackay CE, Barrick TR, Wieshmann UC, Howard MA, Roberts N. Voxel-based morphometric comparison of hippocampal and extrahippocampal abnormalities in patients with left and right hippocampal atrophy. *NeuroImage* 2002; **16:** 23–31.
21. Doherty CP, Fitzsimons M, Holohan T, *et al*. Accuracy and validity of stereology as a quantitative method for assessment of human temporal lobe volumes acquired by magnetic resonance imaging. *Magn Reson Imaging* 2000; **18:** 1017–1025.
22. Saukkonen A, Kalviainen R, Partanen K, Vainio P, Riekkinen P, Pitkanen A. Do seizures cause neuronal damage? A MRI study in newly diagnosed and chronic epilepsy. *NeuroReport* 1994; **6:** 219–223.
23. Barron DS, Fox PM, Laird AR, Robinson JL, Fox PT. Thalamic medial dorsal nucleus atrophy in medial temporal lobe epilepsy: a VBM meta-analysis. *Neuroimage Clin* 2012; **2:** 25–32.
24. Keller SS, Roberts N. Voxel-based morphometry of temporal lobe epilepsy: an introduction and review of the literature. *Epilepsia* 2008; **49:** 741–757.
25. Yoong M, Hunter M, Stephen J, *et al*. Cognitive impairment in early onset epilepsy is associated with reduced left thalamic volume. *Epilepsy Behav* 2018; **80:** 266–271.
26. Keller SS, Richardson MP, O'Muircheartaigh J, Schoene-Bake JC, Elger C, Weber B. Morphometric MRI alterations and postoperative seizure control in refractory temporal lobe epilepsy. *Hum Brain Mapp* 2015; **36:** 1637–1647.
27. Keller SS, Richardson MP, Schoene-Bake JC, *et al*. Thalamotemporal alteration and postoperative seizures in temporal lobe epilepsy. *Ann Neurol* 2015; **77:** 760–774.
28. Szabo CA, Lancaster JL, Lee S, *et al*. MR imaging volumetry of subcortical structures and cerebellar hemispheres in temporal lobe epilepsy. *AJNR Am J Neuroradiol* 2006; **27:** 2155–2160.

29. Keller SS, Wieshmann UC, Mackay CE, Denby CE, Webb J, Roberts N. Voxel based morphometry of grey matter abnormalities in patients with medically intractable temporal lobe epilepsy: effects of side of seizure onset and epilepsy duration. *J Neurol Neurosurg Psychiatry* 2002; **73:** 648–655.
30. Kalviainen R, Salmenpera T. Do recurrent seizures cause neuronal damage? A series of studies with MRI volumetry in adults with partial epilepsy. *Prog Brain Res* 2002; **135:** 279–295.
31. Kalviainen R, Salmenpera T, Partanen K, Vainio P, Riekkinen P, Pitkanen A. Recurrent seizures may cause hippocampal damage in temporal lobe epilepsy. *Neurology* 1998; **50:** 1377–1382.
32. Bonilha L, Jensen JH, Baker N, *et al*. The brain connectome as a personalized biomarker of seizure outcomes after temporal lobectomy. *Neurology* 2015; **84:** 1846–1853.
33. de Bezenac C, Garcia-Finana M, Baker G, *et al*. Investigating imaging network markers of cognitive dysfunction and pharmacoresistance in newly diagnosed epilepsy: a protocol for an observational cohort study in the UK. *BMJ Open* 2019; **9:** e034347.
34. Campos BA, Yasuda CL, Castellano G, Bilevicius E, Li LM, Cendes F. Proton MRS may predict AED response in patients with TLE. *Epilepsia* 2010; **51:** 783–788.
35. Taylor J, Kolamunnage-Dona R, Marson AG, Smith PE, Aldenkamp AP, Baker GA. Patients with epilepsy: cognitively compromised before the start of antiepileptic drug treatment? *Epilepsia* 2010; **51:** 48–56.
36. Kalviainen R, Aikia M, Helkala EL, Mervaala E, Riekkinen PJ. Memory and attention in newly diagnosed epileptic seizure disorder. *Seizure* 1992; **1:** 255–262.
37. Aikia M, Kalviainen R, Riekkinen PJ. Verbal learning and memory in newly diagnosed partial epilepsy. *Epilepsy Res* 1995; **22:** 157–164.
38. Prevey ML, Delaney RC, Cramer JA, Mattson RH. Complex partial and secondarily generalized seizure patients: cognitive functioning prior to treatment with antiepileptic medication. VA Epilepsy Cooperative Study 264 Group. *Epilepsy Res* 1998; **30:** 1–9.
39. Aikia M, Salmenpera T, Partanen K, Kalviainen R. Verbal memory in newly diagnosed patients and patients with chronic left temporal lobe epilepsy. *Epilepsy Behav* 2001; **2:** 20–27.
40. Pulliainen V, Kuikka P, Jokelainen M. Motor and cognitive functions in newly diagnosed adult seizure patients before antiepileptic medication. *Acta Neurol Scand* 2000; **101:** 73–78.
41. Aikia M, Kalviainen R, Mervaala E, Riekkinen PJ Sr. Predictors of seizure outcome in newly diagnosed partial epilepsy: memory performance as a prognostic factor. *Epilepsy Res* 1999; **37:** 159–167.
42. Kim LG, Johnson TL, Marson AG, Chadwick DW, group MMS. Prediction of risk of seizure recurrence after a single seizure and early epilepsy: further results from the MESS trial. *Lancet Neurol* 2006; **5:** 317–322.
43. Su L, Di Q, Kwan P, *et al*. Prediction for relapse and prognosis of newly diagnosed epilepsy. *Acta Neurol Scand* 2013; **127:** 141–147.
44. Alonazi BK, Keller SS, Fallon N, *et al*. Resting-state functional brain networks in adults with a new diagnosis of focal epilepsy. *Brain Behav* 2019; **9:** e01168.

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