Supplemental materials

1. Cohort recruitment and follow-up:

The Connecticut cohort was recruited from the offices of 16 of 17 pediatric neurologists practicing in the state of Connecticut. Eligible patients were diagnosed for the first time between 1993 and 1997 with epilepsy and had their initial unprovoked seizure before their 16th birthday. Following an informed consent and assent process, information about the initial presentation was collected from parent interview and from all relevant neurological records. The type of epilepsy and seizure types were classified according to the contemporary classifications available at the time by three pediatric neurologists who specialized in epilepsy (e1). The cohort was followed prospectively by phone contact 3 to 4 times a year and, with permission, review of interim neurological records every six months. Epilepsy diagnosis and seizure types as well as characterization of the underlying cause were revised based on accumulated information two (e2), five, and nine years after initial diagnosis for each child. During the follow-up period, study participants who attained the age of majority were invited to continue participating as adults. A new consent process was followed and form signed. At approximately nine years after each study participant's entry into the cohort, a research assessment was offered which include, among other procedures, an epilepsy protocol research MRI (2002-2006).

2. MRI protocols:

Yale: Subjects were imaged on either a 1.5T Siemens Sonata MR scanner (Siemens, Erlangen, Germany) or a Genesis Signa scanner. Structural MRI was obtained using a whole-brain T1-weighted coronal 3D MPRAGE sequence acquisition (TE = 4.38ms, TR = 1730ms, TI = 1100ms, flip angle = 15°, matrix size = 256 × 256, FOV = 27cm, body transmit/head receive coil) with contiguous coronal slices of 1.6mm thickness. Coronal 3mm thick high resolution FSE T2W sections through temporal lobe were performed to evaluate amygdala and hippocampal atrophy and signal,

Hartford: Subjects were imaged on either a 1.5T GE Signa MR scanner (General Electric, Milwaukee, WI, USA) or a Signa Excite scanner. A whole-brain T1- weighted coronal 3D spoiled gradient recovery (SPGR) sequence was used (TE = 2.64ms, TR = 13.76ms, TI = 450ms, flip angle = 20°, matrix size = 256 × 192, FOV = 27 × 18cm, head transmit/receive coil) with contiguous coronal slices of 1.6mm thickness. Coronal 3mm thick high resolution FSE T2W sections through temporal lobe were performed to evaluate amygdala and hippocampal atrophy and signal,

4. Hippocampal Volumetry: Hippocampi were manually delineated in a posterior to anterior direction. The most posterior slice was selected as one slice anterior to the slice in which the fornix was visible in full profile. This slice was selected to reduce measurement error due to partial voluming of the hippocampus as the alignment of the hippocampal tail moved from orthogonal to the coronal acquisition plane to an inferior-superior direction. The hippocampus was outlined on each slice and the area of each slice was summed to give an estimate of the hippocampal volume in voxels. For each subject the right hippocampus was measured first, then the image flipped around the vertical axis and the left hippocampus was measured. The volume of the hippocampus was converted to cubic millimeters (mm3) by multiplying by the voxel size.

Brain Volume: Brain tissue was segmented from non-brain structures using the software program BET, provided as part of the FSL software package (http://www.fmrib.ox.ac.uk/fsl). The segmentation results were visually inspected to ensure correct segmentation. In some cases some non-brain tissue was included. In these cases a correct segmentation was obtained by providing a centre of mass estimate for the brain as input to the BET program to improve the starting estimates for brain segmentation.

Hippocampal Volumes adjusted for Brain Volume: Hippocampal volumes were adjusted for brain volume using a covariance method that is commonly used for this purpose (e.g. e3, e4).
For ease of interpretation, corrected left and right hippocampal volumes were then transformed to z-scores based upon the control group's left and right hippocampal volume means and standard deviations.

5.. Correction of hippocampal volume for brain volume:

The equation used for adjusting the hippocampal volume for brain volume based on the control sample is as follows:

$$HippVol_{corrected} = HippVol_{measured} - gradient \times (BrainVol_{measured} - BrainVol_{mean})$$

where *gradient* is the slope of a linear regression fit of the left and right control hippocampal volumes to the control brain volumes, pooled across the two sites; and $BrainVol_{mean}$ is the average brain volume of

the controls. In this study the average control brain volume was 1545523 mm^3 and the gradient was 1.303×10^{-3}. The gradient we obtained in this series of controls is highly comparable to that which was obtained using the same methods in an entirely independent series but using the same approach, 0.0017 (e5).

4. Automated Hippocampal Volumetry: Software-based automated hippocampal volumes were estimated using the same T1-weighted whole brain MRI scans, in addition to manual hippocampal estimates. Automated hippocampal volume estimates were obtained using the subcortical gray matter segmentations obtained by Freesurfer (version 5.0 (e6)). Default Freesurfer processing settings were used. Previous research has indicated that manual hippocampal volume estimates are more sensitive to pathology-based volume change than Freesurfer-based automated estimate (e7, e8) therefore the automated estimates were used as a secondary analysis to confirm findings from the hippocampal volume estimates obtained from the manual segmentations.

References:

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e4. Jack CR Jr, Twomey CK, Zinsmeister AR, Sharbrough FW, Petersen RC, Cascino GD. Anterior temporal lobes and hippocampal formations: normative volumetric measurements from MR images in young adults. Radiology 1989;172:549-554.

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e7. Pardoe HR, Pell GS, Abbott DF, Jackson GD. Hippocampal volume assessment in temporal lobe epilepsy: How good is automated segmentation? Epilepsia 2009;50:2586-2592.

e8. Tae WS, Kim SS, Lee KU, Nam EC, Kim KW. Validation of hippocampal volumes measured using a manual method and two automated methods (FreeSurfer and IBASPM) in chronic major depressive disorder. Neuroradiology 2008;50:569-581.

Table e-1. Values for left and right hippocampal volumes uncorrected for brain size (raw values) and left and right Z-scores for all cases and controls with extreme Z_{HC}

Type of epilepsy and subject #	Left Volume (mm3)	Left Z_{HC}	Right Volume (mm3)	Right Z_{HC}
Controls				
1	2045	-2.11	2381	-1.19
2	2524	-1.02	2230	-2.44
Nonsyndromic-structural				
1	1713	-3.76	1967	-3.02
2	1666	-3.14	1759	-3.02
3	2525	-3	2940	-1.74
4	3284	2.03	3130	0.9
5	1190	-3.59	1340	-3.23

6	2030	-2.32	2252	-1.8
7	2998	2.09	3022	1.59
Nonsyndromic-unknown				
8	2076	-1.8	2116	-1.96
9	3123	2.13	3159	1.67
10	3010	2.26	3052	1.81
11	2018	-2.25	2415	-1.1
12	1915	-2.49	2334	-1.25
13	3022	2.07	2945	1.21
14	3396	2.44	3862	3.5
15	4229	3.1	4096	1.96
16	3295	3.22	3816	4.42
17	3147	2.41	3371	2.6
18	1689	-2.61	1903	-2.09
19	2049	-1.96	2418	-0.94
20	3059	0.89	3740	2.83
21	2749	1.36	3160	2.29
22	3352	3.06	2924	0.87
23	1878	-1.67	1640	-2.84
24	2865	1.54	3137	1.97
25	1918	-2.5	1948	-2.65
26	1990	-2.1	2129	-1.88
27	2850	2	2454	0
28	2517	-1.84	2498	-2.22
29	2338	0.8	2914	2.37
30	2881	0.24	3644	2.52
31	2693	1.77	3032	2.42
32	2512	-0.68	2201	-2.18

Table e-2: Underlying causes, MRI findings, and hippocampal results in the group with known or presumed underlying structural and related causes for their epilepsy (N=23) .

Subgroup	N	Both Z_{HC} within +/- 1.96	Either or both Z_{HC} >1.96	Either or both Z_{HC} <1.96
Normal overall MRI	10	7	1	2
Cerebral palsy and/or intellectual disability/autism spectrum disorder	9	6	1	2
Migrational disorder*	1	1	0	0
Abnormal MRI	13	9	1	3
IVH	2	1	0	1
Migrational disorder	3	3	0	0
CNS Infection	1	0	1	0
Trauma	1	0	0	1
Tumor	1	1	0	0
Neurocutaneous syndrome	2	2	0	0
Other	3	2	0	1

* The study MRI was read as normal; however a clinical MRI was read as abnormal and suggestive of a migrational defect. Pathology after surgery confirmed polymocrigyria.

Table e-3. Comparisons of means and variances using automated volume measures.

	Right HC-Z (SD)	Left HC-Z, SD	Asymmetry
Controls (N=55)	4358 (415)	4358 (415)	1.05 (0.04)
Possibly/Probably TLE (N-44)	4315 (622)	4311 (603)	1.05 (0.04)
Probably/definitely no TLE (N=64)	4194 (380)	4230 (388)	1.05 (0.04)
	p-value*	p-value*	p-value*
Control vs. TLE variance	0.002	0.01	0.78
Control vs. Not-TLE variance	0.60	0.77	0.74
TLE vs. Not-TLE variance	0.0004	0.001	1.0

*None of the differences in means approached statistical significance (all p-values >0.35). A subset of the acquired MRI scans was not suitable for automated analysis due to data format issues and imaging artifacts remote from the hippocampus that affected the software-based processing stream (8 controls , 4 non-TLE, and 5 TLE).

Table e-4: Exploratory analysis comparing cases with large and small hippocampal volumes to each other and to those with average volumes on the basis of clinical factors.

	Both Z_{HC} within 1 SD of control mean (N=51)	Either or both Z_{HC} <-1.96 or >1.96 (N=25)	Either or both Z_{HC} >1.96 (N=15)	Either or both Z_{HC} <1.96 (N=10)
Temporal lobe epilepsy*				
TLE	35 (69%)	10 (40%)	6 (40%)	4 (40%)
Not TL	16 (31%)	15 (60%)	9 (60%)	6 (60%)
Generalized tonic-clonic seizures				
Negative history	20 (39%)	11 (44%)	9 (60%)	2 (20%)
Positive history	31 (61%)	14 (56%)	6 (40%)	8 (80%)
Febrile seizures				
Negative history	45 (90%)	21 (84%)	12 (80%)	9 (90%)
Positive history	5 (10%)	4 (16%)	3 (20%)	1 (10%)
Pharmacoresistance				
Negative history	46 (90%)	19 (76%)	10 (67%)	9 (90%)
Positive history	5 (10%)	6 (24%)	5 (33%)	1 (10%)
Convulsive status epilepticus				
Negative history	46 (90%)	23 (92%)	14 (93%)	9 (90%)
Positive history	5 (10%)	2 (8%)	1 (7%)	1 (10%)

Age at onset of epilepsy*				
<1	9 (18%)	2 (8%)	0 (0)	2 (20%)
1-5	19 (37%)	8 (32%)	4 (27%)	4 (40%)
5-10	15 (29%)	10 (40%)	6 (40%)	4 (40%)
10+	8 (16%)	5 (20%)	5 (33%)	0
Cognitive status				
Within normal (IQ≥80)	45 (88%)	20 (80%)	12 (80)	8 (80%)
Below normal (IQ<80)	6 (12%)	5 (20%)	3 (25%)	2 (10%)

* See text for results of statistical tests.

Figure e-1: Original composition of the full cohort, loss to follow-up by the time of the research scan, participation in the research scan, and exclusion of scans.