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Heritability of alpha and sensorimotor network changes in temporal lobe epilepsy

Running head: Alpha-related imaging endophenotypes for mTLE

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35 **Abstract**

36 **Objective:** Electroencephalography features in the alpha band have been shown to differ between
37 people with epilepsy and healthy controls. Here, in a group of patients with mesial temporal lobe
38 epilepsy, we seek to confirm these electroencephalography features, and using simultaneous
39 functional magnetic resonance imaging, we investigate whether brain networks related to the alpha
40 rhythm differ between patients and healthy controls. Additionally, we investigate whether alpha
41 abnormalities are found as an inherited endophenotype in asymptomatic relatives.

42

43 **Methods:** We acquired scalp electroencephalography and simultaneous electroencephalography
44 and functional magnetic resonance imaging in 24 unrelated patients with unilateral mesial temporal
45 lobe epilepsy, 23 asymptomatic first-degree relatives of patients with mesial temporal lobe epilepsy,
46 and 32 healthy controls. We compared peak alpha power and frequency from
47 electroencephalographic data in patients and relatives to healthy controls. We identified brain
48 networks associated with alpha oscillations and compared these networks in patients and relatives to
49 healthy controls.

50

51 **Results:** Patients had significantly reduced peak alpha frequency across all electrodes.
52 Asymptomatic relatives also had significantly reduced peak alpha frequency primarily over central
53 electrodes. Both patients and asymptomatic relatives showed a combination of increased activation
54 and a failure of deactivation in relation to alpha oscillations compared to healthy controls in the
55 sensorimotor network.

56

57 **Interpretation:** Genetic factors may contribute to the shift in peak alpha frequency and alterations in
58 brain networks related to alpha oscillations. These may not entirely be a consequence of anti-epileptic
59 drugs, seizures or hippocampal sclerosis and deserve further investigation as mechanistic
60 contributors to mesial temporal lobe epilepsy.

61

62 Introduction

63 Mesial temporal lobe epilepsy (mTLE) is the most common type of medically refractory focal epilepsy
64 in adults. Most adults with mTLE present with hippocampal atrophy or hippocampal sclerosis, which
65 may be amenable to surgical treatment¹. Abnormalities in patients with hippocampal sclerosis extend
66 beyond the hippocampus and are thought to involve a network of regions including temporal, thalamic
67 and limbic regions²; evidence for whether abnormalities precede seizure onset or are a result of long-
68 term seizures is scarce and mixed^{3,4}. While mTLE is generally thought of as an acquired disorder,
69 there is emerging evidence for a genetic link in sporadic mTLE^{5,6}, and studies have found alterations
70 in structural brain morphology in asymptomatic relatives of patients with HS^{7,8}. This structural
71 alteration in asymptomatic relatives suggests an inherited trait that precedes seizure onset⁹.

72

73 Alpha is one of the main background electroencephalography (EEG) physiological rhythms observed
74 primarily over the bilateral posterior (occipital) areas when subjects are awake and relaxed, and their
75 eyes are closed. Its frequency, from late childhood through adulthood is within the 8 to 13 Hz range.
76 Alpha activity is typically attenuated (or blocked) by both visual and non-visual stimuli, and mental
77 tasks¹⁰. Recent studies have shown evidence for active involvement of alpha activity in cognitive
78 processes^{11–13}, and an emerging theory is that the alpha-rhythm governs neural excitability through
79 top-down modulated cognitive control networks¹⁴. The neural substrates underpinning alpha activity
80 are still not well understood but are thought to be thalamo-cortical in origin¹⁵, based on animal
81 models^{16–19} and neuroimaging studies in humans^{20–24}.

82

83 It is known that there are alterations in alpha activity in patients with epilepsy, but these alterations
84 are rarely reported or described²⁵. While it is known that alpha activity is influenced by, among other
85 things, anti-epileptic drugs^{26,27}, reductions in peak alpha frequency (PAF) have been shown to be
86 epilepsy-specific²⁵ and to distinguish between focal and generalised epilepsy²⁸ and changes in both
87 frequency and power of alpha activity are related to the severity and type of seizures after accounting
88 for anti-epileptic drug load^{28,29}.

89

90 Alpha power and peak frequency have been shown to be highly reproducible within individuals^{30,31},
91 and highly heritable^{32,33}. There exists a large amount of inter-individual variation related to age,
92 memory and cognition, and neurological conditions^{34–38}, which suggest that alpha activity measures
93 may be too unspecific to be considered biomarkers or endophenotypes¹⁴. However, there is some
94 evidence for a brain network endophenotype for idiopathic generalised epilepsy derived from the “low
95 alpha” frequency band³⁹. It is so far unknown whether there may be functional or structural network
96 alterations related to alpha activity in relatives of patients with mTLE.

97

98 The present study seeks to characterise EEG alpha band power and peak frequency in patients with
99 mTLE and asymptomatic relatives compared to healthy controls and investigate whether the
100 functional brain networks related to alpha oscillations differ in patients with mTLE and asymptomatic
101 relatives compared to healthy controls.

102

103 **Materials and methods**

104 **Participants**

105 The study was performed at the National Institute for Health Research/Wellcome Trust King’s Clinical
106 Research Facility at King’s College Hospital, London, United Kingdom. All experimental procedures
107 were reviewed and approved by the London – Bromley National Research Ethics Service. Written
108 informed consent was obtained from each participant after all procedures were fully explained.

109

110 Twenty-four unrelated patients with mTLE were recruited from outpatient epilepsy and neurology
111 clinics in hospitals in south London. The diagnosis of mTLE was made on the basis of clinical
112 evaluation including history, seizure semiology, scalp EEG, and conventional clinical MRI reported by
113 experienced neuroradiologists. Patients who had other pathologies, for example malformations of
114 cortical development or tumours, who had undergone surgical resection of the affected temporal lobe,

115 or who had recent invasive brain investigations (including depth electrode recordings) were excluded
116 from the study.

117

118 Twenty-three asymptomatic first-degree relatives were recruited either through patients included in
119 the study or through patients who had a diagnosis of mTLE but were themselves excluded from the
120 study due to a history of surgical resection or recent invasive brain investigations. (Note that recruiting
121 in this way means that while we refer to them as “relatives”, some members of this group have no
122 patient to whom they are related in the patient group). Thorough clinical interview of these relatives
123 revealed no current or previous diagnosis of neurological disorders, and no history of symptoms or
124 clinical events suggestive of epileptic seizures. Scalp EEG, carried out as part of the study, showed
125 no epileptiform discharges in any relative.

126

127 Thirty-two healthy control participants with no current or previously diagnosed personal or family
128 history of neurological disorders were recruited for comparison (Table 1A).

129

130 Full clinical information for patients is given in Supplementary Table 1 and details of relatives and
131 clinical information of their associated probands given in Supplementary Table 2.

132

133 **Data acquisition**

134 **Study design**

135 Participants had a 20-minute EEG recording outside the MRI scanner, a high-resolution structural T1-
136 weighted MRI scan and a 10-minute simultaneous EEG and functional MRI (fMRI) recording in the
137 scanner. During EEG and fMRI recordings, participants were instructed to stay awake and relax with
138 their eyes closed.

139

140 To increase power to detect pathological differences, imaging and EEG data from patients with right-
141 sided mTLE (n = 7; 38.9%) and relatives of patients with right-sided mTLE (n = 5; 41.7%) were left-

142 to-right flipped so that the ipsilateral side is on the left. All changes were considered as ipsilateral or
143 contralateral to the pathological hippocampus in patients. For consistency, a similar proportion of
144 healthy control data (n = 12; 38.7%) was randomly chosen to be side flipped.

145

146 Not all participants completed the simultaneous EEG and fMRI investigations due to reasons including
147 claustrophobia and equipment problems. The subset of participants with complete data is described
148 in Table 1B.

149

150 **EEG data**

151 EEG data were recorded using a 64-channel MRI-compatible EEG system (Brain Products GmbH,
152 Munich, Germany). All participants were fitted with a BrainCap MR EEG cap with 63 Ag/AgCl
153 electrodes arranged according to the extended international 10-20 system with the reference
154 electrode placed between Fz and Cz and the ground between Fz and Fpz. The electrocardiogram
155 (ECG) was recorded at a sampling frequency of 5kHz using the BrainVision Recorder software (Brain
156 Products). EEG recordings outside the scanner were performed in an electrically shielded Faraday
157 cage room.

158

159 **MRI data**

160 MRI was performed on a General Electric 3T MR750 scanner (GE Healthcare Systems, Chicago,
161 USA) using the body coil for radiofrequency transmission and a 12-channel head coil for signal
162 reception. Resting-state fMRI data were acquired using a gradient echo echo-planar imaging
163 sequence in a plane parallel to the AC-PC line, 2160ms repetition time (TR), 25ms echo time, 75° flip
164 angle, 36 interleaved slices of 64×64 matrix size, giving a 211×211mm field of view with a voxel size
165 of 3.3×3.3×3.3mm. Simultaneous EEG was recorded during the fMRI scan at a sampling frequency
166 of 5kHz with the SyncBox device (BrainProducts) used to synchronise EEG and fMRI acquisition. A
167 three-dimensional inversion recovery-prepared spoiled gradient-echo image was acquired in the
168 sagittal plane with 270mm field of view, 256×256 matrix (resulting in an in-plane voxel size of

169 1.05×1.05mm), 196 sagittal slices, 1.2mm slice thickness, 7.312ms repetition time, 400ms inversion
170 time, 3.016ms echo time and 11° excitation flip angle.

171

172 **Data analysis**

173 **EEG power spectral analysis**

174 EEG data acquired outside the scanner was used for the power spectral analysis conducted in
175 MATLAB (R2015b, The MathWorks Inc., Natick, MA, 2015) using tools from the FieldTrip EEG
176 software toolbox⁴⁰. In previous work, we have shown that the choice of segment does not affect the
177 analysis²⁸. We conducted the analysis on the first 5 minutes of EEG recording to exclude any possible
178 confounding effect of the choice and length of segments. Data were re-referenced to the average of
179 all channels except the Fp1, Fp2 and ECG electrodes, and de-trended. Segments were visually
180 inspected for artefacts and the presence of interictal discharges in patients.

181

182 Data were bandpass filtered between 0.5 and 70Hz and the power spectral density for each
183 participant's segment was estimated using Welch's method with a window length of 4s and 50%
184 overlap, giving a frequency resolution of 0.25Hz. The relative power at each frequency resolution
185 point was computed as a fraction of the total power between 0.5 and 70Hz. Data were band-passed
186 within the alpha frequency band (6-13Hz) with the lower boundary of the alpha band modified to
187 include "low alpha" frequencies⁴¹, since previous work has shown alterations in the low alpha band in
188 epilepsy^{28,39}. The peak power (maximum power) and peak frequency (frequency at which the
189 maximum power occurs) were computed for each subject. Statistical group comparisons of peak
190 power and peak frequency were restricted to parietal and occipital channels, where the alpha rhythm
191 is most prominently expressed. We used one-tailed two-sample t-tests (with the hypothesis that
192 patients and relatives would show reduced alpha power and frequency compared to healthy controls)
193 and controlled the false discovery rate (FDR; over 17 parietal and occipital channels) using the
194 Benjamini-Yekutieli procedure with $\alpha = 0.05$.

195

196 We performed three sub-group analyses to investigate the effects of medication, seizure control and
197 relatedness. Since carbamazepine is known to cause slowing of the alpha frequency, we split the
198 patient group into patients taking carbamazepine ($n = 8$) and patients who were not ($n = 16$). To
199 examine the effect of seizure control, we split the patient group into patients with good ($n = 4$) and
200 poor ($n = 20$) seizure control (with the threshold for poor seizure control at ≥ 4 seizures/year, as
201 defined in ²⁸). We had seven pairs of related patients and relatives in this analysis. In the final sub-
202 group analysis, to exclude any confounding effects of relatedness, we excluded four patients and
203 three relatives so that all patients and relatives remaining in the analysis were unrelated. In each of
204 these sub-group analyses, peak power and frequency in the alpha band were compared to healthy
205 controls as described above.

206

207 **Simultaneous EEG-fMRI**

208 EEG data recorded in the scanner were pre-processed using BrainVision Analyzer (version 2.0, Brain
209 Products) to remove MR gradient and ballistocardiogram artefacts from the simultaneous EEG-fMRI
210 data. A sliding average template of MRI scanner artefacts using the average of 21 TR intervals
211 identified by the gradient onset markers was subtracted from the EEG signal to correct for MR gradient
212 artefacts. Data were downsampled to 250Hz. The peaks of the R-waves were identified from the ECG
213 signal in a semi-automated manner using a template pulse wave, and subsequently visually checked
214 and adjusted. Ballistocardiogram artefacts were corrected by subtracting a sliding average template
215 for the R-waves from the EEG.

216

217 The alpha power time-series was extracted from the pre-processed in-scanner EEG data using
218 MATLAB. EEG data were averaged over the three occipital electrodes (O1, O2 and Oz) and a short-
219 time Fourier transform was applied to compute the spectrogram using windows equal to the fMRI data
220 sampling TR of 2.16s with no overlap. To account for intra-subject variation of peak alpha frequency,
221 the alpha power time series was computed as the mean alpha power over a narrow band of ± 2 Hz
222 around the peak alpha frequency for each subject. Outliers in the mean alpha power time series were

223 identified as data points where the amplitude exceeded the standard boxplot outlier definition, $Q3 +$
224 $1.5 \times IQR$, and replaced by linearly interpolated values. Each alpha power time series was then
225 truncated to exclude the first 5 and last 5 TR of data to exclude end effects from average template
226 correction and scaled between 0 and 1.

227

228 fMRI data were pre-processed using FSL's FEAT software (v6.0)⁴². fMRI data pre-processing
229 involved motion correction, spatial smoothing with a 6mm full width at half maximum Gaussian filter,
230 and bandpass filtering between 0.01Hz and 0.12Hz. Each subject's native space data were co-
231 registered to the Montreal Neurological Institute (MNI) standard space image by way of a linear
232 transform to the subject's high-resolution T1-weighted image and then a non-linear registration to
233 standard space. To model and exclude effects of noise within the data, several nuisance regressors
234 were used as variables of non-interest in the subsequent general linear model analysis. These were
235 the average signal each from the white matter and cerebrospinal fluid, and the 6 motion parameters.

236

237 For each subject, a voxel-wise whole-brain general linear model analysis was implemented with FEAT
238 with the alpha power time series as the variable of interest and the nuisance regressors as variables
239 of non-interest. The alpha power time series was convolved with the canonical double-gamma
240 hemodynamic response function to take into account the delay associated with the blood-oxygen-
241 level-dependent fMRI (BOLD-fMRI) response. Group analyses were performed in FEAT with a mixed-
242 effects analysis of covariance model with age and sex as covariates. Contrasts for the main effect of
243 group and group differences between healthy controls and each of the patient and relative groups
244 were set up and significant effects were identified with a cluster threshold of $p < 0.05$, FWE-corrected.

245

246 We used the normalised alpha power estimated from the O1, O2 and Oz electrodes as a measure of
247 vigilance during the simultaneous EEG-fMRI recording⁴³. For each subject, we estimated the
248 normalised alpha power averaged across the three occipital electrodes using 10 second sliding
249 windows across the in-scanner EEG data (after discarding the first and last 5 TR as above). We then

250 estimated the slope of the normalised alpha power for each subject and compared this between
251 groups using a one-way analysis of variance.

252

253 **Results**

254 The participant groups were not significantly different in age or gender for any of the datasets when
255 assessed using one-way analyses of variance and χ^2 tests.

256

257 **Alterations in the EEG power spectrum**

258 Peak power and peak frequency within the alpha frequency band were averaged across group, and
259 patients with mTLE and asymptomatic relatives were compared to healthy controls. No significant
260 differences in peak power in either patients or relatives from controls were present (Fig 1A). PAF was
261 significantly reduced in patients with mTLE compared to healthy controls across all parietal and
262 occipital channels ($p < 0.05$, FDR-corrected; Fig 1B). Asymptomatic relatives also showed a reduction
263 in PAF compared to healthy controls in 14 of the 17 parietal and occipital channels ($p < 0.05$, FDR-
264 corrected; Fig 1B).

265

266 Peak alpha frequency remained significantly reduced compared to healthy controls in the sub-group
267 who were not on carbamazepine (Supplementary Figure 1), in the sub-group of patients with poor
268 seizure control (Supplementary Figure 2) and in both sub-groups of unrelated patients and relatives
269 (Supplementary Figure 3).

270

271 **EEG alpha correlates of BOLD-fMRI network**

272 Regions showing positive and negative correlations of BOLD-fMRI activity and EEG alpha oscillations
273 across all subjects are shown in Fig 2A ($p < 0.05$, FWE-corrected). Positive correlations were primarily
274 found in the bilateral thalamus and parahippocampal gyrus, brainstem and subcallosal cortex.
275 Negative correlations were primarily seen in bilateral cortical regions in the dorsal attention network,

276 including the middle and inferior frontal gyri, superior parietal lobes, lateral occipital cortices and
277 inferior frontal gyri.

278

279 Compared to healthy controls, patients showed significantly higher BOLD signal correlations with
280 alpha activity in regions of the sensorimotor network, including the bilateral pre- and post-central gyri
281 extending into the supplementary motor area as well as in regions of the cingulo-opercular/insular
282 network, including the anterior cingulate, bilateral insulae and frontal and parietal opercula ($p < 0.05$,
283 FWE-corrected; Fig 2B). Relatives also showed significantly higher BOLD signal correlations with
284 alpha activity compared to healthy controls in regions of the sensorimotor network, including the
285 bilateral pre- and post-central gyri and anterior cingulate, in addition to the occipital cortex ($p < 0.05$,
286 FWE-corrected; Fig 2C).

287

288 Both patients and relatives appeared to show a combination of higher activation (or higher correlation
289 with alpha oscillations) and a failure of deactivation (i.e. a failure to decouple with alpha oscillations)
290 compared to healthy controls who mainly showed reduced correlation with alpha in these regions (Fig
291 3). There were no significantly lower BOLD correlations with alpha in either the patients or relatives
292 compared to healthy controls.

293

294 The slope of the peak alpha power was not significantly different between groups: $F(2,62) = 1.361$, p
295 $= 0.264$, indicating that the level of vigilance between groups was not significantly different across the
296 EEG-fMRI recording.

297

298 **Discussion**

299 The present study identified EEG peak alpha frequency reductions and functional brain network
300 alterations associated with alpha oscillations in both patients with mTLE and asymptomatic relatives
301 compared to healthy controls. Analysis of the EEG power spectrum revealed a shift of the alpha
302 rhythm towards lower frequencies in both patients with mTLE and asymptomatic relatives. The PAF

303 shift was observed across all EEG channels in patients, while in relatives it was seen primarily over
304 fronto-central channels. With simultaneous EEG-fMRI, we showed that cortical regions in the
305 sensorimotor network failed to deactivate in relation to alpha oscillations in both patients and
306 asymptomatic relatives compared to healthy controls. In addition, patients also showed this pattern of
307 increased fMRI activation and a failure to deactivate with alpha oscillations in the cingulo-
308 opercular/insular network. Reduced PAF and altered topographical distribution of EEG alpha power
309 in patients with epilepsy has been reported previously, and we replicate this finding in independent
310 data here. We show here, for the first time, that brain activity related to the alpha rhythm differs
311 between patients with epilepsy and healthy controls. We also show, for the first time, that reduced
312 PAF and altered brain activity related to the alpha rhythm, differ between asymptomatic relatives and
313 healthy controls. These findings in relatives suggest that alterations in alpha activity in patients with
314 epilepsy are not necessarily related to AEDs or seizures, and are an inherited endophenotypic
315 predisposition to epilepsy, which is currently mechanistically unexplained.

316

317 **Peak alpha frequency shift**

318 The shift in PAF toward lower frequencies in patients has been demonstrated previously in both
319 patients with focal and generalized epilepsy, and is thought to be linked to poor seizure control²⁸. We
320 also found evidence for a fronto-central PAF decrease in asymptomatic relatives. Importantly, the
321 asymptomatic relatives in this study were unmedicated, hence the reduced PAF cannot be attributed
322 to effects of antiepileptic drugs. The slowing of the alpha rhythm has also been reported in several
323 other neurological and psychiatric disorders including depression and Alzheimer's disease^{19,44}. This
324 suggests that the PAF on its own may not be a specific enough measure to serve as an
325 endophenotype for mTLE and may instead point to a more general indicator of susceptibility to
326 abnormal brain function.

327

328 **Brain network alterations related to alpha oscillations**

329 Across the whole group of participants, we show positive correlations in the thalamus and negative
330 correlations in the dorsal attention network. The pattern of positive and negative correlations of brain
331 fMRI activity with alpha oscillations is largely in line with the literature in this field¹⁴.

332

333 Group comparisons showed higher correlation of alpha oscillations with regions of the sensorimotor
334 network in both patients with mTLE and asymptomatic relatives compared to healthy controls. In
335 healthy controls, cortical regions mainly showed decreased activation with alpha oscillations. In
336 patients, there was a combination of increased activation and a failure to deactivate in regions of the
337 sensorimotor network and the cingulo-opercular/insular network. Interestingly, in the sensorimotor
338 network, relatives also showed the same pattern of increased brain activation and a failure to
339 deactivate in relation to alpha oscillations.

340

341 Alpha oscillations are thought to govern cortical excitability, or a rhythmic inhibition, where increases
342 in alpha power generally result in an increase in inhibition and hence a decrease in cortical
343 excitability^{14,45,46}. The increased correlation between alpha oscillations and sensorimotor network
344 activity observed in patients and relatives could suggest that alpha oscillations have a reduced effect
345 of inhibition over sensorimotor network activity.

346

347 Compared to healthy controls, patients with mTLE also had higher correlations with alpha in the
348 cingulo-opercular/insular network. It is unclear what the link is between this network and alpha
349 oscillations in mTLE. The cingulo-opercular/insular network is thought to underpin “tonic alertness”
350 through the modulation of alpha oscillations⁴⁷. The insular cortex has also been implicated in
351 ictogenicity in mTLE^{48,49}. As one of the few regions with direct connections to the cholinergic basal
352 forebrain, it has been suggested that the link between the alpha rhythm and cholinergic basal
353 forebrain activity is modulated by the insula⁵⁰.

354

355 This study represents the first investigation of functional brain changes related to the altered alpha
356 rhythm in patients with mTLE and asymptomatic relatives. The findings suggest that the shift in PAF

357 and alterations in brain function related to the alpha rhythm may deserve further investigation as an
358 endophenotype for mTLE and may not entirely be a consequence of anti-epileptic drugs, seizures or
359 hippocampal sclerosis.

360

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368

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373 the Department of Health.

374

375 **Author Contributions**

376 MPR, GJB and SNY conceived and designed the study. MK and RDCE contributed primary patient
377 referrals and clinical data. SNY, CT and EA acquired the study data and conducted the analyses.
378 SNY drafted the manuscript. All authors reviewed the paper.

379

380 **Conflicts of Interest**

381 The authors report no conflicts of interest relevant to this work.

382

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502

503 **Figure Legends**

504 **Figure 1. EEG topographical plots for the alpha band.** Group-averaged EEG topographical plots
505 of (A) peak power and (B) peak frequency in the alpha frequency band. In the patients' and relatives'
506 plots, channels that show a significant group difference from healthy controls are indicated by pink
507 dots ($p < 0.05$, FDR-corrected across parietal and occipital channels only). "L" indicates the left or
508 ipsilateral side. Pat: patients with mTLE, Rel: asymptomatic relatives, Con: healthy controls.

509

510 **Figure 2. BOLD fMRI correlates of EEG alpha oscillations.** (A) Regions showing positive and
511 negative fMRI correlations with EEG alpha oscillations across all subjects. (B) Regions showing
512 higher correlations with alpha oscillations in (B) patients and (C) asymptomatic relatives compared to
513 healthy controls. Voxels in the sensorimotor region that were significantly different from controls in
514 the relatives group had high overlap with voxels found significantly different from controls in the patient
515 group. Images show Z-statistics at a cluster threshold of $p < 0.05$ (FWE-corrected). Positive values
516 are shown in red/yellow and negative values in blue. MNI coordinates are shown above each slice.
517 "L" represents the left or ipsilateral side.

518

519 **Figure 3. Mean group difference in correlation with alpha oscillation.** (A) Clusters showing
520 significantly higher correlation with alpha oscillation in patients compared to healthy controls. (B)
521 Clusters showing significantly higher correlation with alpha oscillation in relatives compared to healthy
522 controls. The mean parameter estimates are shown on the upper axes and the mean group
523 differences shown on the lower axes as bootstrap sampling distributions. Mean differences are
524 depicted as dots; 95% confidence intervals are indicated by the ends of the vertical error bars. Pat:
525 patients with mTLE, Rel: asymptomatic relatives, Con: healthy controls. Figures created on
526 www.estimationstats.com.

527

528 **Supplementary Figure 1. EEG topographical plots for the alpha band in the sub-group analysis**
529 **investigating effect of carbamazepine therapy.** Group-averaged EEG topographical plots of (A)

530 peak power and (B) peak frequency in the alpha frequency band. In the patients' plots, channels that
531 show a significant group difference from healthy controls are indicated by pink dots ($p < 0.05$, FDR-
532 corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat (no
533 CAR): patients with mTLE who are not taking carbamazepine, Pat (CAR): patients with mTLE who
534 are taking carbamazepine, Con: healthy controls.

535

536 **Supplementary Figure 2. EEG topographical plots for the alpha band in the sub-group analysis**
537 **investigating effect of seizure control.** Group-averaged EEG topographical plots of (A) peak power
538 and (B) peak frequency in the alpha frequency band. In the patients' plots, channels that show a
539 significant group difference from healthy controls are indicated by pink dots ($p < 0.05$, FDR-corrected
540 across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat (poor control):
541 patients with mTLE who have ≥ 4 seizure per year, Pat (good control): patients with mTLE who have
542 < 4 seizures per year, Con: healthy controls.

543

544 **Supplementary Figure 3. EEG topographical plots for the alpha band in the sub-group analysis**
545 **excluding the effect of relatedness.** Group-averaged EEG topographical plots of (A) peak power
546 and (B) peak frequency in the alpha frequency band. In the patients' plots and relatives' plots,
547 channels that show a significant group difference from healthy controls are indicated by pink dots (p
548 < 0.05 , FDR-corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral
549 side. Pat: patients with mTLE (unrelated to relatives included in this analysis), Rel: asymptomatic
550 relatives of patients with mTLE (unrelated to patients included in this analysis), Con: healthy controls.

551

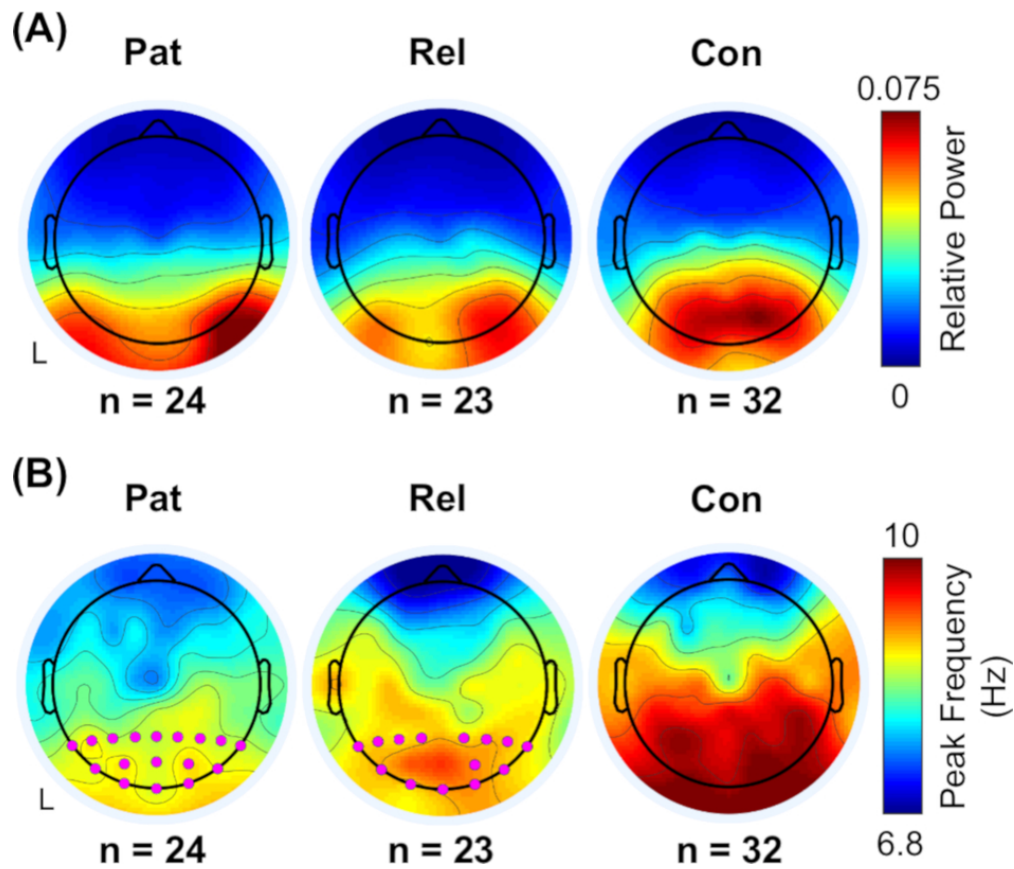
552 **Tables**553 **Table 1. Demographic information for all participants and clinical information for patients.**

	Patients	Relatives	Controls
(A) Participants with EEG data only			
Number	24	23	32
Age (years)	40.2 ± 11.9	36.7 ± 13.0	36.9 ± 10.8
Sex (male/female)	13/11	10/13	16/16
mTLE side^a (right/left)	10/14	9/14	-
Epilepsy onset age (years)	21.8 ± 9.9	-	-
Duration of epilepsy (years)	18.0 ± 14.0	-	-
Seizure frequency (/month)^b	5.6 ± 6.3	-	-
(B) Participants with simultaneous EEG-fMRI data			
Number	22	18	25
Age (years)	39.3 ± 12.7	35.9 ± 13.3	34.8 ± 7.9
Sex (male/female)	11/11	8/10	13/12
mTLE side^a (right/left)	9/13	8/10	-
Epilepsy onset age (years)	23.1 ± 9.1	-	-
Duration of epilepsy (years)	15.6 ± 13.7	-	-
Seizure frequency (/month)^b	5.8 ± 6.6	-	-

554 Data are means ± standard deviations.

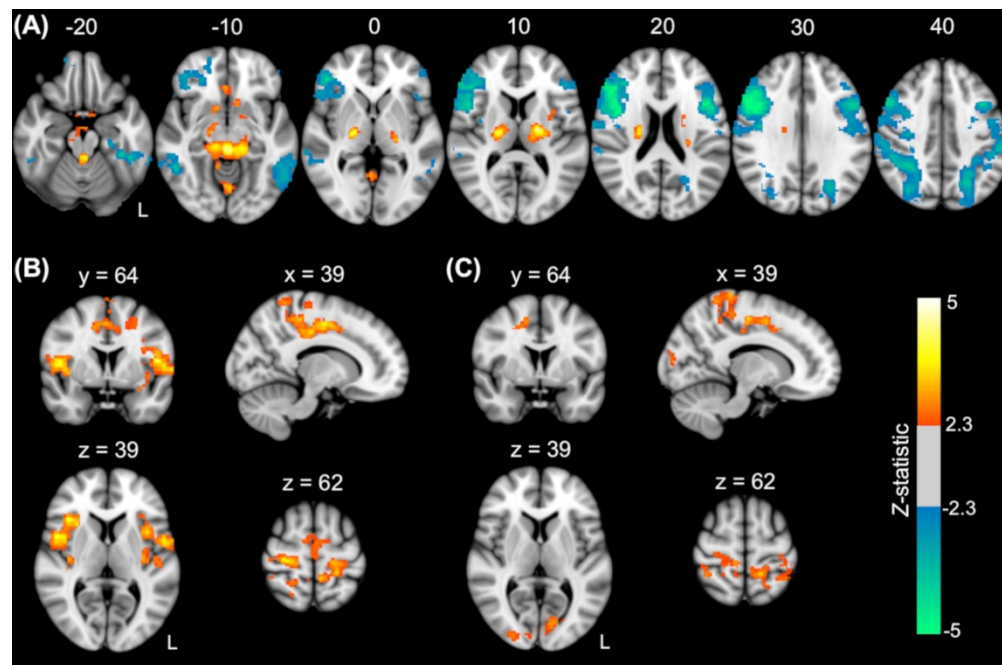
555 ^a Refers to side of seizure onset in Patients group, and of the proband in Relatives group.556 ^b Data unavailable for one patient.

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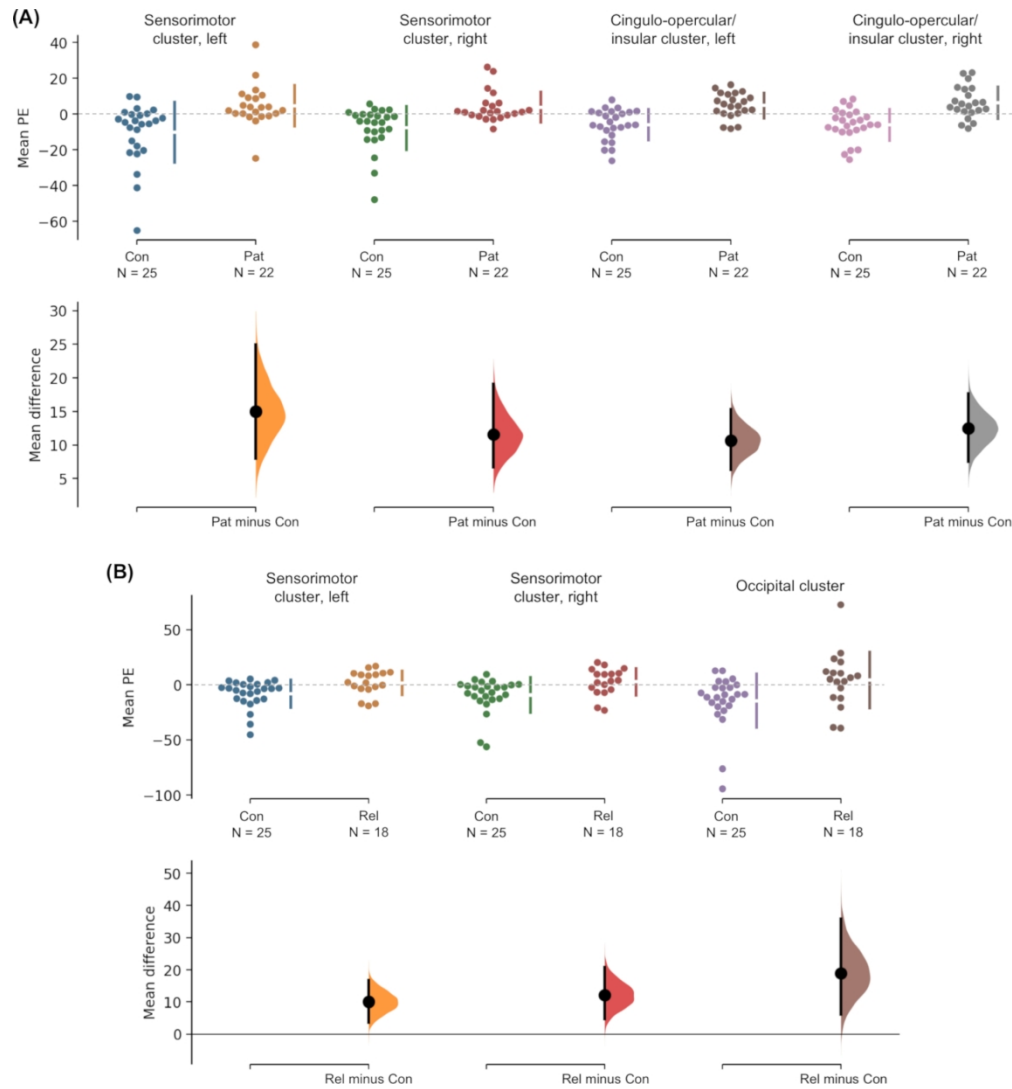
EEG topographical plots for the alpha band. Group-averaged EEG topographical plots of (A) peak power and (B) peak frequency in the alpha frequency band. In the patients' and relatives' plots, channels that show a significant group difference from healthy controls are indicated by pink dots ($p < 0.05$, FDR-corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat: patients with mTLE, Rel: asymptomatic relatives, Con: healthy controls.

80x68mm (300 x 300 DPI)



BOLD fMRI correlates of EEG alpha oscillations. (A) Regions showing positive and negative fMRI correlations with EEG alpha oscillations across all subjects. (B) Regions showing higher correlations with alpha oscillations in (B) patients and (C) asymptomatic relatives compared to healthy controls. Voxels in the sensorimotor region that were significantly different from controls in the relatives group had high overlap with voxels found significantly different from controls in the patient group. Images show Z-statistics at a cluster threshold of $p < 0.05$ (FWE-corrected). Positive values are shown in red/yellow and negative values in blue. MNI coordinates are shown above each slice. "L" represents the left or ipsilateral side.

180x119mm (300 x 300 DPI)



Mean group difference in correlation with alpha oscillation. (A) Clusters showing significantly higher correlation with alpha oscillation in patients compared to healthy controls. (B) Clusters showing significantly higher correlation with alpha oscillation in relatives compared to healthy controls. The mean parameter estimates are shown on the upper axes and the mean group differences shown on the lower axes as bootstrap sampling distributions. Mean differences are depicted as dots; 95% confidence intervals are indicated by the ends of the vertical error bars. Pat: patients with mTLE, Rel: asymptomatic relatives, Con: healthy controls. Figures created on www.estimationstats.com.

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Supplementary Table 1. Further clinical details of patients in the study.

ID	Age	Sex	Onset age	Localization features	Seizure frequency	Medication (daily dose)	Febrile seizures?	EEG?	EEG-fMRI?
PAT01	41	F	24	Right sided HS on MRI.	9-10	LMT (550); LEV (1500); PER (4); CLB (60)	*	Yes	Yes
PAT02	35	M	15	Left sided HS on MRI.	2	LMT (400); VPA (900); CLB (10)	*	Yes	No
PAT03	43	F	20	Left sided HS on MRI. Bitemporal seizure onset on intracranial EEG.	3-7	CAR (1400); LMT (200)	No	Yes	Yes
PAT04	57	F	5	Left sided HS on MRI. Left temporal discharges on EEG.	6-7	LEV (875); CIT (30)	No	Yes	Yes
PAT05	22	M	16	Left sided HS on MRI.	20-24	CAR (1200)	No	Yes	Yes
PAT06	34	M	11	Right sided HS on MRI. Right anterior temporal spike and wave epileptiform discharges on EEG.	2-3	PHB (60); VPA (1200); OLA (7.5); CIT(*)	No	Yes	Yes
PAT07	52	F	15	Loss of digitation in the left hippocampal head. Left temporal hypometabolism on FDG PET. Left temporal discharges on EEG.	4	LAB (150); CIT (50); LOR (2)	No	Yes	Yes
PAT08	51	F	31	Left sided HS on MRI.	<1	LAC (400)	*	Yes	Yes
PAT09	31	M	25	Right sided HS on MRI.	16-20	CAR (800); LEV (200); CLB (10)	*	Yes	Yes
PAT10	48	M	33	Right sided HS on MRI.	<1	LEV (3000); TOP (100)	Yes	Yes	Yes
PAT11	31	M	21	Right sided HS on MRI.	3-4	LEV (3000); ZON (200); CLN (2)	No	Yes	Yes

PAT12	58	F	47	Left sided HS on MRI.	3-4	TOP (200); CLB (10)	*	Yes	Yes
PAT13	47	M	40	Right sided HS on MRI	<1	LEV (400)	*	Yes	No
PAT14	24	M	22	Ectopic grey matter lateral to body of right hippocampus. Focal temporal lobe seizures and right temporal discharges on EEG.	3-4	CAR (400)	Uncertain	Yes	Yes
PAT15	25	M	23	MRI normal. Left temporal focal seizures on EEG. Seizure semiology suggestive of left TLE onset.	3-4	VPA (800); TOP (300)	No	Yes	Yes
PAT16	43	F	40	Left sided HS on MRI	4	CAR (800)	No	Yes	Yes
PAT17	23	M	22	Right sided HS on MRI	<1	ZON (200)	Yes	Yes	Yes
PAT18	47	M	15	Normal MRI. Left temporal focal seizures on EEG.	1-2	CAR (600)	No	Yes	Yes
PAT19	24	F	22	Left sided HS on MRI	*	*	No	No	Yes
PAT20	57	M	25	Right sided HS on MRI	<1	LMT (300)	*	Yes	Yes
PAT21	37	F	27	Left sided HS on MRI	12	CAR (400)	Yes	Yes	Yes
PAT22	31	F	22	Left sided HS on MRI	3	LEV (3000)	No	Yes	Yes
PAT23	44	F	1	Left sided HS on MRI.	4	CAR (1200); CLB (10)	Uncertain	Yes	No
PAT24	22	F	18	Left sided HS on MRI. Seizure semiology: posturing of right hand.	1-2	None at time of scan	No	Yes	Yes
PAT25	52	M	25	Right sided HS on MRI.	15	LMT (750); PER (8)	*	Yes	Yes

* indicates missing information; HS = Hippocampal Sclerosis; FS = Febrile Seizures. Seizure frequency is the approximate number of seizures per month.

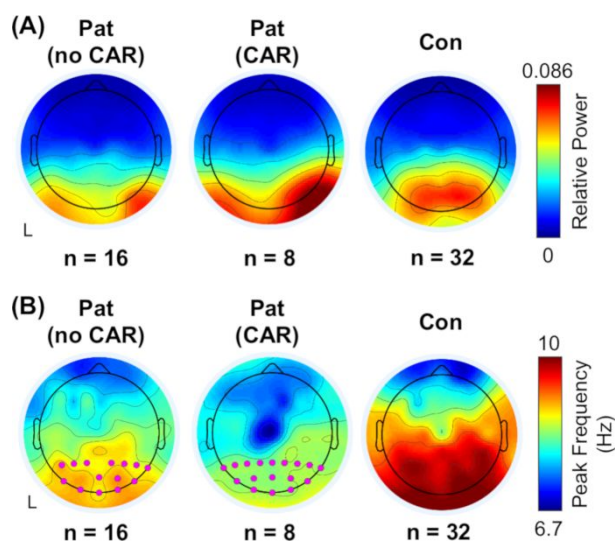
Drug abbreviations: CAR = Carbamazepine, CIT = Citalopram, CLB = Clobazam, CLN = Clonazepam, LAC = Lacosamide, LEV = Levetiracetam, LMT = Lamotrigine, LOR = Lorazepam, OLA = Olanzapine, PER = Perampanel, PHB = Phenobarbitone, PHE = Phenytoin, TOP = Topiramate, VPA = Valproate, ZON = Zonisamide.

Supplementary Table 2. Further details of relatives and clinical details of associated probands.

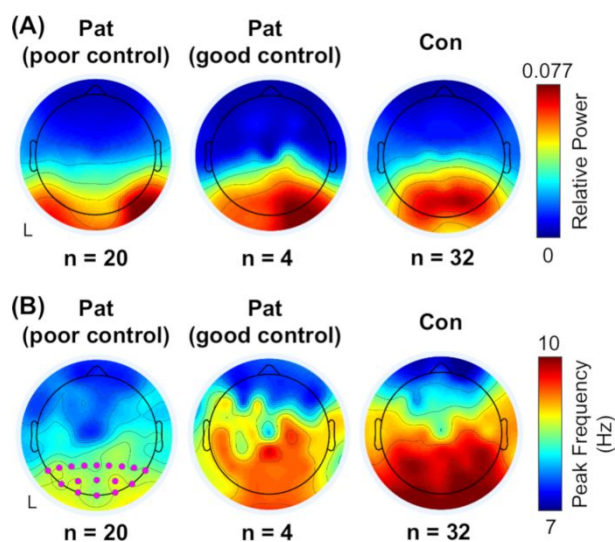
ID	Age	Sex	Relationship to proband	Proband mTLE details	Proband in study	EEG?	EEG-fMRI?
REL01	49	M	Son of female patient	Age of onset 48. Left HS on MRI. Patient has well controlled seizures on medication and has not undergone surgery.	No	Yes	Yes
REL02	24	F	Daughter of female patient	See Supplementary Table 1.	PAT07	Yes	Yes
REL03	34	M	Twin brother of male patient	See Supplementary Table 1.	PAT06	Yes	Yes
REL04	37	F	Daughter of female patient	See Supplementary Table 1.	PAT04	Yes	No
REL05	60	F	Mother of male patient	See Supplementary Table 1.	PAT11	Yes	Yes
REL06	31	F	Daughter of male patient	Age of onset 40. Right HS on MRI. No history of febrile convulsions. Patient has had right temporal lobectomy and amygdalohippocampectomy and is currently seizure free on medication.	No	Yes	Yes
REL07	17	M	Son of female patient	Age of onset 10-15 years. Left HS on MRI. No history of febrile seizures. Patient has had amygdalohippocampectomy and was seizure free for 7 years, but seizures have recently recurred. Updated telemetry showed left sided abnormalities and independent right sided abnormalities, with some evidence for right sided hypometabolism on PET.	No	Yes	Yes
REL08	25	M	Brother of female patient	Age of onset 19. Right HS on MRI. No febrile seizures. Patient has had right temporal hippocampectomy and was seizure free for a period before a recurrence of nocturnal seizures. Pathology confirmed hippocampal sclerosis.	No	Yes	Yes
REL09	30	M	Son of female patient	Age of onset 10. Left HS on MRI. Patient has had left temporal hippocampectomy. Pathology confirmed hippocampal sclerosis plus FCD type 2b. Seizures remain post-surgery.	No	Yes	Yes
REL10	44	M	Brother of male patient	Early age of onset. Right HS on MRI. Patient has not undergone surgery.	No	Yes	Yes
REL11	25	M	Brother of male patient	See Supplementary Table 1.	PAT13	Yes	Yes

REL12	54	F	Mother of female patient	See Supplementary Table 1.	PAT19	Yes	Yes
REL13	24	F	Daughter of female patient	Early age of onset. Right HS on MRI. Patient has not undergone surgery.	No	Yes	Yes
REL14	18	F	Sister of male patient	See Supplementary Table 1.	PAT15	Yes	No
REL15	40	F	Sister of female patient	Early age of onset. Bilateral HS on MRI. Patient has not undergone surgery.	No	Yes	Yes
REL16	40	F	Sister of female patient	Early age of onset. Bilateral HS on MRI. Patient has not undergone surgery.	No	Yes	Yes
REL17	20	F	Daughter of female patient	Age of onset 33. Left HS on MRI. Patient has had left temporal lobectomy. Pathology confirmed hippocampal sclerosis. Seizures remain post-surgery.	No	Yes	Yes
REL18	60	F	Mother of female patient	Age of onset 5. Left HS on MRI showing volume loss and hyperintensity on T2-weighted MRI. Prolonged febrile seizures at 8 months. Patient has had left temporal lobectomy and is now seizure free.	No	Yes	Yes
REL19	28	M	Brother of female patient	Age of onset 5. Left HS on MRI showing volume loss and hyperintensity on T2-weighted MRI. Prolonged febrile seizures at 8 months. Patient has had left temporal lobectomy and is now seizure free.	No	Yes	Yes
REL20	31	F	Sister of female patient	Early age of onset. Right HS on MRI. Patient has not undergone surgery.	No	Yes	Yes
REL21	49	M	Brother of female patient	Early age of onset. Left HS on MRI. Patient has not undergone surgery.	No	Yes	No
REL22	47	M	Son of female patient	Early age of onset. Left HS on MRI. Patient has not undergone surgery.	No	Yes	No
REL23	44	F	Daughter of male patient	Early age of onset. Right HS on MRI. Patient has not undergone surgery.	No	Yes	No

Supplementary Figure 1. EEG topographical plots for the alpha band in the sub-group analysis investigating effect of carbamazepine therapy. Group-averaged EEG topographical plots of (A) peak power and (B) peak frequency in the alpha frequency band. In the patients' plots, channels that show a significant group difference from healthy controls are indicated by pink dots ($p < 0.05$, FDR-corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat (no CAR): patients with mTLE who are not taking carbamazepine, Pat (CAR): patients with mTLE who are taking carbamazepine, Con: healthy controls.



Supplementary Figure 2. EEG topographical plots for the alpha band in the sub-group analysis investigating effect of seizure control. Group-averaged EEG topographical plots of (A) peak power and (B) peak frequency in the alpha frequency band. In the patients' plots, channels that show a significant group difference from healthy controls are indicated by pink dots ($p < 0.05$, FDR-corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat (poor control): patients with mTLE who have ≥ 4 seizure per year, Pat (good control): patients with mTLE who have < 4 seizures per year, Con: healthy controls.



Supplementary Figure 3. EEG topographical plots for the alpha band in the sub-group analysis excluding the effect of relatedness. Group-averaged EEG topographical plots of (A) peak power and (B) peak frequency in the alpha frequency band. In the patients' plots and relatives' plots, channels that show a significant group difference from healthy controls are indicated by pink dots ($p < 0.05$, FDR-corrected across parietal and occipital channels only). "L" indicates the left or ipsilateral side. Pat: patients with mTLE (unrelated to relatives included in this analysis), Rel: asymptomatic relatives of patients with mTLE (unrelated to patients included in this analysis), Con: healthy controls.

