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S.A, M.A.K, C.L.Y: study concept and design, acquisition of data, analysis and interpretation of data, manuscript writing.
M.K.S, M.K, M.Z, T.Z., M.H.N, M.K.M.A, V.R.F, M.H.T: acquisition of data, critical revision of manuscript for intellectual context.
M.F., I.L.C, C.P.D., C.L.C, F.C., G.D.J., N. D: study supervision, study concept and design, critical revision of manuscript for intellectual context.

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ABSTRACT:

Objective: To examine cerebral cortex thickness in asymptomatic first-degree relatives of patients with mesial temporal lobe epilepsy (MTLE).

Methods: We investigated 127 asymptomatic first-degree relatives of patients with MTLE due to hippocampal sclerosis (HS) [mean age ± SD = 39.4 ± 13 years] and 203 healthy control individuals [mean age ± SD = 36.0 ± 11 years]. Participants underwent a comprehensive clinical evaluation and structural brain magnetic resonance imaging (MRI) at three study sites. Images were processed simultaneously at each site using a surface-based morphometry (SBM) method to quantify global brain measures, hippocampal volumes, and cerebral cortical thickness. Differences in brain measures between relatives of patients and controls were examined using generalized models, while controlling for relevant covariates, including age and sex.

Results:

None of the asymptomatic first-degree relatives of MTLE+HS patients showed evidence of HS on qualitative image assessments. Compared to the healthy controls, the asymptomatic relatives of patients displayed no significant differences in intracranial volume, average hemispheric surface area, or hippocampal volume. Similarly, no significant cerebral cortical thinning was identified in the relatives of patients. This was consistent across the three cohorts.

Conclusion:

Lack of cortical thickness changes in the asymptomatic relatives of patients indicates that the previously characterized MTLE+HS-related cortical thinning is not heritable, and is likely driven by disease-related factors. This finding therefore argues for early and aggressive intervention in patients with medically intractable epilepsy.

INTRODUCTION:

Neocortical thinning is a recognized morphometric trait associated with mesial temporal lobe epilepsy (MTLE).[1-5] Widespread cerebral cortical thinning has been identified in MTLE patients relative to healthy controls within several cortical regions, including the mesiotemporal, limbic, and central sensorimotor cortices.[1-4] This pattern of cortical thinning appeared progressive in patients with medically intractable epilepsy,[2, 6] and thus it has mostly been attributed to the effect of disease chronicity, including poorly controlled seizures.[2] While this explanation is plausible, a relatively similar pattern of cortical thinning has been described in patients with well-controlled epilepsy,[3] suggesting that poorly controlled seizure activity alone is not the sole driver of such cortical thickness alterations.

Using population-based twin cohort studies, the heritability of cerebral cortical thickness was previously established.[7, 8] However, it is not clear if genetic factors play a role in determining MTLE-related cortical thinning. Previously, subtle MRI-based morphologic anomalies were identified in the hippocampi of asymptomatic first-degree relatives of MTLE patients with hippocampal sclerosis (HS), suggesting a genetic predisposition to hippocampal structural alterations.[9, 10] It remains unknown, however, if thinning of relevant cortical regions is also present in family members of MTLE patients.

In an effort to explore this question, the present study examined cerebral cortical thickness in asymptomatic first-degree relatives of patients with MTLE+HS.

METHODS:

Participants:

A total of 127 asymptomatic first-degree relatives of MTLE+HS patients [mean age ± standard deviation (SD) = 39.4 ± 13 years] and 203 healthy controls [mean age ± (SD) = 36.0 ± 11 years] were included in this study. These participants were from three independent cohorts, each had its own healthy control group (see Table 1). Diagnosis of MTLE+HS in probands was established according to the International League Against Epilepsy definitions and based on comprehensive assessments that included clinical, electroencephalographic, and MRI findings.[11] HS was diagnosed based on qualitative radiological assessments of T1 and T2-weighted MR images by qualified neuroradiologists and confirmation of unilateral hippocampal atrophy by standard manual hippocampal volumetry using T1-weighted images.

At each site, first-degree relatives of MTLE+HS patients were invited and evaluated locally. All participants, including the first-degree relatives of patients and healthy controls, underwent a comprehensive clinical evaluation and were screened for a history of seizures, including childhood febrile seizures (FS), other initial precipitating insults, such as head trauma or central nervous system (CNS) infection, and neurologic or psychiatric morbidities. A description of the study participants is detailed in Table 1.

Standard Protocol Approvals, Registrations, and Patient Consents:

The research ethics committee of each participating study centre independently approved this study and informed consent was obtained from each participant.

MR Imaging Acquisition:

High-resolution T1-weighted brain magnetic resonance images (MRI) were acquired locally from participants at each site. For cohort 1, MRI scans were acquired using a 1.5 Tesla MRI scanner (Signa, GE, Milwaukee, WI, U.S.A.) at Beaumont Hospital, Dublin, Ireland. A three-dimensional T_1-weighted spoiled gradient recalled (SPGR) sequence [TR/TE = 10.1/4.2 millisecond (msec), TI = 450 msec, flip angle = 20 degrees] with 124 sagittal slices was used to acquire the images.

For cohort 2, participants were scanned using a 3.0 Tesla TIM Trio Siemens scanner (Erlangen, Germany) at the Florey Institute of Neuroscience and Mental Health, Austin campus, Melbourne, Australia. A three-dimensional T1-weighted magnetization prepared rapid gradient echo (MPRAGE) sequence [TR/TE = 1900/2.6 msec, TI = 900 msec, flip angle = 9 degrees) with 192 sagittal slices was acquired.

For cohort 3, MRI scans were acquired using a 3.0 Tesla MRI scanner (Philips Achieva) at the University of Campinas, Campinas, São Paulo, Brazil. A three-dimensional T1- weighted turbo field echo (TFE) sequence [TR/TE = 7.0/3.2 msec, flip angle = 8 degrees] with 180 sagittal slices was obtained.

MRI Processing:

Image processing was conducted simultaneously at each site. Images were processed using FreeSurfer 5.3.0 (http://surfer.nmr.mgh.harvard.edu/), a well validated fully automated surface-based morphometry (SBM) tool. A detailed description of the FreeSurfer image-processing pipeline is provided elsewhere.[12-14]

Briefly, image processing starts with removal of nonbrain tissue, intensity normalization, transformation to Talairach space, and segmentation of the subcortical white matter and deep gray matter structures. The gray-white matter boundary is then determined and topological defects are corrected. This is followed by deformation of gray-white matter boundary outward to generate the pial surface as indicated by intensity gradient.[12] Once generated, convoluted reconstruction of the cortical surfaces is inflated to a smooth surface, allowing sulcal and gyral folds to be viewed. Subsequently, each vertex on the inflated surface is registered to a sphere, which is then mapped to an average surface template for optimal alignment of the cortical fold patterns in a way that minimizes metric distortion.[13]

Quality control of image segmentation and cerebral cortex surface reconstructions were conducted according to ENIGMA quality control procedures (http://enigma.ini.usc.edu/protocols/imaging-protocols). Participants with poor image segmentation and surface reconstructions were excluded. No manual edits were performed. In this study, FreeSurfer was applied to reconstruct cerebral cortex surfaces and estimate intracranial volume (ICV), global cerebral hemispheric surface area, and hippocampal volumes as described previously.[1] In each participant, cerebral cortex thickness was quantified at each vertex across the entire cortex as the average of the shortest distance between the gray-white matter surface and the gray matter-cerebrospinal fluid (CSF) surface.[14]

Data and Statistical Analyses:

Each site tested for differences in age between first-degree relatives of patients and controls using an unpaired, two-tailed *t*-test. Additionally, sex differences between

relatives of patients and controls were tested using a chi-squared test in SPSS Statistics package (IBM Corp., Version 24.0).

Firstly, a generalized linear model (GML) was employed to assess differences in global brain measures and hippocampal volumes between first-degree relatives of MTLE+HS patients and healthy controls in each cohort. Age and sex were included as covariates for ICV assessment, whereas age, sex, and ICV were included as covariates for hemispheric cerebral surface area and hippocampal volume assessments. Adjusted group mean differences and standard errors (calculated for each center) were pooled across study sites using random-effects, restricted maximum likelihood method of meta-analysis via the *R* package, *metafor*.[15]

Next, we examined for group differences in cortical thickness at each vertex across the entire cerebral cortex within sites by computing a GLM of the effect of case-control status, while controlling for age and sex effect. Data were smoothed using a 10-mm full width half maximum (FWHM) Gaussian kernel, and a false discovery rate (FDR) of $p < 0.05$ was applied to correct for multiple comparisons. To illustrate the across sites effect, mean cortical thickness maps for the first-degree relatives and controls within sites were pooled together and presented (see Figure 2).

Data Availability:

Any data not published within the article will be shared, in an anonymized form, by request from any qualified investigator.

RESULTS:

In each cohort, no significant sex or age differences were noted between the relatives of MTLE+HS patients and the healthy controls. Cohort 1 only included siblings of patients. Meanwhile, cohort 2 and 3 were comprised of siblings, parents, and offspring of probands (see Table 1 for further details). All family members in cohort 1 and cohort 2 were first-degree relatives of patients with medically refractory MTLE+HS. Fourteen (27.5%) individuals in cohort 3 were relatives of patients with drug-responsive MTLE+HS. None of asymptomatic relatives of patients reported prior history of head injury or CNS infection. No history of childhood FS was reported in the Irish or Australian samples (cohort 1 and 2). In the Brazilian cohort (cohort 3), four (7.8%) family members reported a history of childhood FS, two were parents and the other two were offspring of probands. Fourteen (53.8%) family members of the Australian probands (cohort 2) and one (2%) family member of the Brazilian probands (cohort 3) reported a family history of epilepsy in more than one first-degree relative.

Qualitative assessments of brain MRI by qualified experts revealed no evidence of HS in any of the asymptomatic first-degree relatives of patients. On quantitative assessment, the asymptomatic relatives of MTLE+HS patients displayed no significant differences in ICV, hippocampal volume, or hemispheric cerebral surface area when compared to the healthy controls (see Figure 1 and 2). This was consistent within and across sites (see Table 2 and 3).

Similarly, using a vertex-wide approach, no significant local cerebral cortical thickness differences were noted between the asymptomatic first-degree relatives of patients and the healthy controls after expected corrections for multiple comparisons

(see Figure 3 and 4). Supplementary Figure 1 illustrates the cerebral cortical thinning patterns identified in MTLE+HS patients (not included in the current study) when compared to the same healthy controls at each study site.

DISCUSSION:

Examining three independent cohorts of asymptomatic first-degree relatives of MTLE+HS patients, we identified no significant regional thinning of the cerebral cortex in the asymptomatic relative of patients when compared to healthy controls. In addition, after controlling for age, sex, and ICV, no significant differences were noted in hippocampal volume or hemispheric cortical surface area. Lack of cortical thickness changes in the asymptomatic relatives of patients indicates that the previously identified MTLE+HS-related cortical thinning (see Supplementary Figure 1) is unlikely to be heritable.[1-4]

Widespread structural atrophy often accompanies hippocampal atrophy in MTLE+HS and extends beyond the epileptogenic zones to involve many interconnected brain regions.[16, 17] Structural atrophy has been noted in several subcortical gray matter structures, including the amygdala and thalamus,[18, 19] the ipsilateral temporal cortex,[20, 21] and fronto-limbic cortical regions. [2-4,22] This brain atrophy appeared progressive over the course of the disease, especially in patients with medically intractable epilepsy.[2, 6] In order to disentangle disease-related from genetically-mediated effects, the role of genetic factors in determining MTLE+HS-related brain alteration has been explored in a number of family-based quantitative MRI studies.[9,10, 23] Subtle hippocampal morphologic anomalies were identified in asymptomatic relatives of

patients with strong family history of epilepsy,[9, 10] supporting a genetic role and possible familial predisposition to hippocampal alterations in MTLE+HS.[9] In the current study, using a validated automated segmentation method (FreeSurfer), we found no significant reduction in total hippocampal volume in the asymptomatic family members of MTLE+HS patients. It is worth noting, however, that FreeSurfer's hippocampal volume estimation is less sensitive in detecting atrophy related to HS when compared to manual volumetry, despite the high correlations between the two hippocampal segmentation methods.[24] Therefore, the current findings does not rule out the possibility of subtle hippocampal abnormalities in the relatives of patients. To potentially disentangle subtle hippocampal anomalies in the asymptomatic relatives of patients, future studies are encouraged to examine hippocampal shape or subfield surface changes. These advanced morphologic measures could be superior to hippocampal volumetry in detecting subtle morphologic changes not often captured by total hippocampal volume estimation.[25, 26]

In a previous investigation, comparable, but subtle, morphologic changes were identified within the anteromedial temporal cortex in MTLE+HS patients and their asymptomatic siblings (included in this study sample).[27] These morphologic findings were related to changes in cerebral cortex surface area and not cortical thickness, providing additional evidence to a possible genetic contribution to localized structural changes within the temporal lobe.[27] In contrast, the current findings of no significant cortical thinning in the first-degree relatives of patients are in line with our previous findings of lack of widespread brain tissue alterations, including absence of white matter tract atrophy.[23, 28] These findings, thus, implicate disease-related factors, including recurrent seizures, underlying pathologic process, or other global exposure,

such as the effect of anti-epileptic medications, and argue for early and more aggressive intervention in patients with medically intractable epilepsy.[2, 29]

This study had few limitations. Firstly, we analyzed each data set separately. This was addressed by performing a meta-analysis to allow pooling of data across the three sites. Secondly, the control group lacked first-degree relatives of participants. Paired first-degree relatives of healthy individuals can offer an ideal control group to disentangle disease-related from genetically mediated brain structural changes. Thirdly, in this study, we focused on investigating cortical thickness. Other cerebral cortex traits, including surface area, morphology and gyrification, were not examined. Investigating such morphologic traits could be an alternative approach to explore the underpinning mechanisms of MTLE-related brain structural alterations.

REFERENCES:

1. Alhusaini S, Doherty CP, Palaniyappan L, Scanlon C, Maguire S, Brennan P, Delanty N, Fitzsimons M, Cavalleri GL. Asymmetric cortical surface area and morphology changes in mesial temporal lobe epilepsy with hippocampal sclerosis. *Epilepsia* 2012; 53:995-1003.
2. Bernhardt BC, Worsley KJ, Kim H, Evans AC, Bernasconi A, Bernasconi N. Longitudinal and cross-sectional analysis of atrophy in pharmacoresistant temporal lobe epilepsy. *Neurology* 2009; 72:1747–54.
3. Labate A, Cerasa A, Aguglia U, Mumoli L, Quattrone A, Gambardella A. Neocortical thinning in "benign" mesial temporal lobe epilepsy. *Epilepsia* 2011; 54:712–7.
4. Mueller SG, Laxer KD, Barakos J, Cheong I, Garcia P, Weiner MW. Widespread neocortical abnormalities in temporal lobe epilepsy with and without mesial sclerosis. *Neuroimage* 2009; 46:353-9.
5. 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.
6. Caciagli L, Bernasconi N, Weibe S, Koepp MJ, Bernasconi N, Bernhardt BC. A meta-analysis on progressive atrophy in intractable temporal lobe epilepsy: Time is brain? *Neurology* 2017; 89:506-15.
7. Panizzon MS, Fennema-Notestine C, Eyler LT, Jernigan TL, Prom-Wormley E, Neale M, Jacobson K, Lyons MJ, Grant MD, Franz CE, Xian H, Tsuang M, Fischl B, Seidman L, Dale AM, Kremen WS. Distinct genetic influences on cortical surface area and thickness. *Cereb Cortex* 2009; **19**:2728–35.

8. Strike LT, Hansell NK, Couvy-Duchesne B, Thompson PM, de Zubicaray GI, McMahon KL, Wright MJ. Genetic complexity of cortical structure: Differences in genetic and environmental factors influencing cortical surface are and thickness. *Cereb Cortex* 2018 (in press).
9. Tsai MH, Pardoe HR, Perchyonok Y, Fitt GJ, Scheffer IE, Jackson GD, Berkovic SF. Etiology of hippocampal sclerosis: evidence for a predisposing familial morphologic anomaly. *Neurology* 2013; 81:144-9
10. Kobayashi E, Li LM, Lopes-Cendes I, Cendes F. Magnetic resonance imaging evidence of hippocampal sclerosis in asymptomatic, first-degree relatives of patients with familial mesial temporal lobe epilepsy. *Arch Neurol* 2002; 59: 1891–4.
11. Wieser HG, ILAE Commission on Neurosurgery of epilepsy. ILAE Commission report: mesial temporal lobe epilepsy with hippocampal sclerosis. *Epilepsia* 2004; 45:695-714.
12. Fischl B, Salat D, Busa E, Albert M, Dieterich M, Haselgrove C, van der Kouwe A, Killiany R, Kennedy D, Klaveness S, et al. Whole brain segmentation: automated labelling of neuroanatomical structures in the human brain. *Neuron* 2002; 33:341–55.
13. Fischl B, Sereno M, Dale AM. Cortical surface-based analysis. II: inflation, flattening, and a surface-based coordinate system. *Neuroimage* 1999 9:195–207.
14. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci USA* 2000; 97:11050–5.
15. Viechtbauer W. Conducting meta-analyses in R with metaphor package. *J Stat Soft* 2010; 36:1-48.

16. Miller JW. Teasing out the anatomy of mesial temporal lobe epilepsy. *Epilepsy Curr* 2011; 11:145-6.
17. Bernhardt BC, Hong S, Bernasconi A, Bernasconi N. Imaging structural and functional brain networks in temporal lobe epilepsy. *Front Hum Neurosci* 2013; 7:624.
18. Bernasconi N, Bernasconi A, Caramanos SB, Antel B, Andermann F, Arnold DL. Mesial temporal damage in temporal lobe epilepsy: a volumetric MRI study of the hippocampus, amygdala, and parahippocampal region. *Brain* 2003; 126:462-9.
19. Alhusaini S, Doherty CP, Scanlon C, Ronan L, Maguire S, Borgulya G, Brennan P, Delanty N, Fitzsimons M, Cavalleri GL. A cross-sectional MRI study of brain regional atrophy and clinical characteristics of temporal lobe epilepsy with hippocampal sclerosis. *Epilepsy Res* 2012; 99:156-66.
20. Doherty CP, Fitzsimons M, Meredith G, Thornton J, McMackin D, Farrell M, Phillips J, Staunton H. Rapid stereological quantitation of temporal neocortex in TLE. *Magn Reson Imaging* 2003; 21:511-8.
21. Bernhardt BC, Kim H, Bernasconi M. Patterns of subregional mesiotemporal disease progression in temporal lobe epilepsy. *Neurology* 2013; 81:1840-7.
22. Voets NL, Bernhardt BC, Kim H, Yoon U, Bernasconi N. Increased temporolimbic cortical folding complexity in temporal lobe epilepsy. *Neurology* 2011; 76:138-44.
23. Alhusaini S, Scanlon C, Ronan L, Maguire S, Meaney JP, Fagan AJ, Boyle G, Borgulya G, Iyer PM, Brennan P, Costello D, Chaila E, Fitzsimons M, Doherty CP, Delanty N, Cavalleri GL. Heritability of subcortical volumetric traits in mesial temporal lobe epilepsy. *PLoS ONE* 2013; 8:e61880.

24. Pardoe HR, Pell GS, Abbott DF, Jackson GD. Hippocampal volume assessment in temporal lobe epilepsy: How good is automated segmentation? *Epilepsia*; 2009; 50:2586-92.
25. Bernhardt BC, Kim H, Bernasconi N. Patterns of subregional mesiotemporal disease progression in temporal lobe epilepsy. *Neurology* 2013; 81:1840-7.
26. Schoene-Bake JC, Keller SS, Niehusmann P, Vomering E, Elger C, Deppe M, Weber B. In vivo mapping of hippocampal subfields in mesial temporal lobe epilepsy: relation to histopathology. *Hum Brain Mapp* 2014; 35:4718-28.
27. Alhusaini S, Whelan CD, Doherty CP, Delanty N, Fitzsimons M, Cavalleri GL. Temporal cortex morphology in mesial temporal lobe epilepsy patients and their asymptomatic siblings. *Cereb Cortex* 2016; 26:1234-41.
28. Vaughan DN, Raffelt D, Curwood E, Tsai MH, Tournier JD, Connelly A, Jackson GD. Tract-specific atrophy in focal epilepsy: Disease, genetics, or seizures? *Ann Neurol* 2017; 81:240-50.
29. Laxer KD, Trinka E, Hirsch LJ, Cendes F, Langfitt J, Delanty N, Resnick T, Benbadis SR. The consequences of refractory epilepsy and its treatment. *Epilepsy Behav* 2014; 37:59-70.

FIGUERS LEGENDS:

Figure 1: Forest plots for group mean differences of left and right hippocampal volumes between the asymptomatic relatives of MTLE+HS patients and healthy controls. Diamonds indicate pooled group mean differences. The horizontal lines indicate 95% confidence intervals.

Figure 2: Forest plot for group mean differences of the intracranial volume (reported in cm^3) and hemispheric cerebral surface area (reported in mm^2) between the asymptomatic relatives of MTLE+HS patients and healthy controls. Diamonds indicate pooled group mean differences. The horizontal lines indicate 95% confidence intervals.

Figure 3: Mean cortical thickness maps for the entire control group (n = 203) and asymptomatic first-degree relatives of patients (n = 127) are shown. For each group, maps of mean cortical thickness are also displayed across the three sites. Color bar represents cortical thickness in millimeter.

Figure 4: Statistical maps of group differences in cortical thickness between the asymptomatic first-degree relatives of patients and controls are displayed for each site prior to correction for multiple comparisons. The subtle and inconsistent effect shown across sites disappears after correcting for multiple comparisons, indicating lack of significant cortical thickness differences. Color bar represents statistical significance: controls > asymptomatic relatives of MTLE+HS patients at uncorrected $p < 0.05$.

Table 1: Demographics of the study participants across the three sites

Site:	**Dublin, Ireland**			**Melbourne, Australia**			**Campinas, Brazil**	
MRI Platform	1.5T (GE Signa)			3.0T (Siemens Trio)			3.0T (Philips Achieva)	
Group	Healthy controls	First-degree relatives of patients		Healthy controls	First-degree relatives of patients		Healthy controls	First-degree relatives of patients
Number	40	50		112	26		51	51
Age: mean (SD)	33.7 (9.9)	36.8 (10.3)		34.7 (10.6)	38.9 (12.6)		42.0 (15.4)	42.2 (15.5)
Sex: number (%)								
Male	18 (45%)	20 (40%)		63 (56.3%)	15 (57.7)		11 (21.6%)	13 (25.5%)
Female	22 (55%)	30 (50%)		49 (43.7%)	11 (42.3)		40 (78.4%)	38 (74.5%)
Relationship to proband:								
Sibling	NA	50 (100%)		NA	12 (46.2%)		NA	26 (52%)
Parent	NA	0		NA	9 (34.6%)		NA	7 (13.7%)
Offspring	NA	0		NA	5 (19.2%)		NA	18 (35.3%)
IPIs:								
FS	0	0		0	0		0	4 (7.8%)
Head trauma	0	0		0	0		0	0
CNS infection	0	0		0	0		0	0
Family history of epilepsy:								
1 relative only	0	50 (100%)		0	12 (46.2%)		0	50 (98%)
≥ 2 relative	0	0		0	14 (53.8%)		0	1 (2%)

CNS: central nervous system, FS: febrile seizures, ICV: intracranial volume, IPI: initial precipitating insult, SD: standard deviation.

Table 2: Adjusted Mean and standard error of global brain measures and hippocampal volumes across the three sites

Site:	**Dublin, Ireland**			**Melbourne, Australia**			**Campinas, Brazil**	
Group	Healthy controls (n = 40)	First-degree relatives of patients (n = 50)		Healthy controls (n = 112)	First-degree relatives of patients (n = 26)		Healthy controls (n= 51)	First-degree relatives of patients (n = 51)
Intracranial volume (ICV, cm^3):	1567.4 (21.5)	1567.6 (22.3)		1533.3 (12)	1502.4 (25)		1244.3 (30)	1235.5 (25)
Cerebral surface area (mm^2)								
Left	83563 (631)	84168 (656)		84945 (365)	850121 (767)		78537 (857)	77268 (729)
Right	83552 (619)	84546 (643)		85390 (367)	85683 (770)		78611 (864)	77232 (736)
Hippocampal volume (cm^3)								
Left	4567 (64)	4585 (67)		4498 (29)	4466 (62)		3946 (65)	4101 (55)
Right	4551 (67)	4557 (70)		4603 (30)	4594 (63)		4100 (69)	4230 (59)

ICV is reported after adjustment for age and sex. Hemispheric cerebral cortical surface area and hippocampal volumes are reported after adjustment for ICV, age, and sex. Using a generalized linear model, first-degree relatives of patients were compared to the healthy controls. No significant group differences were noted after controlling for relevant covariates including: ICV, age, and sex.

Table 3: Full meta-analysis results of group mean differences in brain measures, controlled for age, sex, and ICV

Brain measure	**Group mean difference (asymptomatic relatives of patients – healthy controls)**	**SE**	**Z value**	**P value**
Intracranial volume (ICV, cm^3):	- 15.15	18.58	- 0.82	0.414
Cerebral surface area (mm^2):				
Left hemisphere	- 55.39	547.92	- 0.10	0.92
Right hemisphere	135.72	603.21	0.22	0.82
Hippocampus volume (mm^2):				
Left hippocampus	40.31	58.19	0.69	0.48
Right hippocampus	34.42	48.77	0.71	0.49