Increased amyloid burden in patients with childhood-onset epilepsy five decades later

Juho Joutsa, MD, PhD[1,2,3]; Juha O. Rinne, MD, PhD[1,2]; Bruce Hermann, PhD[4]; Mira Karrasch, PhD[5]; Anu Anttinen, MD[2]; Shlomo Shinnar, MD, PhD[6]; Matti Sillanpää, MD, PhD[7,8];

[1]Turku PET Centre, [2]Division of Clinical Neurosciences, [3]Department of Clinical Neurophysiology, Turku University Hospital, 20500 Turku, Finland; [4]Department of Neurology, University of Wisconsin School of Medicine and Public Health, 1685 Highland Avenue, Madison WI 53792; [5]Department of Psychology, Abo Akademi University, 20500 Turku, Finland; [6]Departments of Neurology, Pediatrics and Epidemiology and Population Health, Montefiore Medical Center, Albert Einstein College of Medicine, 111 E. 210th Street Bronx, NY 10467; [7]Departments of Public Health and [8]Child Neurology, 20014 University of Turku, Finland

Correspondence:
Juho Joutsa, M.D., Ph.D.
MGH/MIT/HMS Athinoula A.
Martinos Center for Biomedical Imaging
149 13th street, room 2301
Charlestown, MA 02129
email: jjoutsa@mgh.harvard.edu
tel: +1.617.726.3197
fax: +1.617.726.7422

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Abstract

Importance: The impact of childhood epilepsy on later life cognitive and brain health is an unclear and little explored issue.

Objective: To determine if adult persons with a history of childhood-onset epilepsy exhibit increased brain amyloid accumulation, possibly predisposing to accelerated cognitive impairment or even frank cognitive disorders in later life.

Design: The participants with childhood-onset epilepsy were originally enrolled at the mean age of 5.1 (SD 4.5, range 0–14) years. After a mean 52.5 (SD 4.0) years follow-up, subjects, together with matched population based controls, were scanned with amyloid ligand 11C-labeled Pittsburgh compound B (PIB) positron emission tomography.

Setting: The study was conducted in a unique population-based cohort of subjects with childhood-onset epilepsy in Southwestern Finland prospectively followed for more than 50 years along with population based controls.

Participants: Forty-one subjects with childhood-onset epilepsy and 46 matched controls.

Main outcome measures: The specific PIB uptake was quantified as region-to-cerebellar cortex ratio. Tracer uptake was evaluated visually, and analyzed voxel-by-voxel over the entire brain to investigate the spatial distribution of amyloid deposition. We hypothesized that childhood-onset epilepsy, particularly in subjects with APOE ε4 allele, is associated with increased brain amyloid accumulation.

Results: 22% of the subjects with childhood-onset epilepsy compared to 6.5% of controls had a visually abnormal amyloid scan (P=0.037). In semiquantitative analyses, there was a significant interaction effect indicating higher frontal PIB

uptake in APOE ε4 allele carriers compared to non-carriers in subjects over controls. In addition, there was a significant group effect showing higher tracer uptake in subjects compared to controls.

Conclusions and relevance: Subjects with childhood-onset epilepsy, and particularly APOE ε4 carriers, have increased brain amyloid load at late middle age. Thus, epilepsy is linked with a biomarker, which might be related to an accelerated brain ageing and can be considered a neurobiological predisposition to later life cognitive disorders.

Introduction

Approximately one out of 100 suffers from epilepsy prior to age 18.[1] Somatic comorbidities and cognitive problems are common even among patients with uncomplicated syndromes,[2] and particularly chronic medication-resistant epilepsy is associated with abnormal prospective cognitive trajectories in middle aged patients.[3]

Population-based studies have shown that prevalence rates of epilepsy and dementia overlap in the elderly.[4] Seizures in Alzheimer's disease become more common after longer disease duration and more advanced neurodegeneration, but also occur more frequently in patients with young-onset disease or genetic mutations associated with excessive brain amyloid beta (Aβ) accumulation.[5,6] Translational evidence provides further support to the link between amyloid pathology and epilepsy.[6] For instance, in transgenic mouse models of Alzheimer's disease that overproduce Aβ, the mice develop seizures along with the increase in Aβ levels and age. On the other hand, epilepsy can be considered as a model of chronic neuronal over activity and stress, which might predispose the patients for increased amyloid accumulation and neuronal damage.[7]

Despite the fact that neurological disorders with amyloid pathology are associated with epileptic seizures, human studies investigating amyloid deposition in patients with established epilepsy are scarce.[4] Some neuropathological examinations in the 1990's of resected brain tissue specimens from patients with treatment-resistant temporal lobe epilepsy suggested a link between epilepsy and abnormalities in local brain Aβ function.[8,9] Patients with long-standing treatment-resistant temporal lobe

epilepsy showed increased amyloid production[8] and abnormally high number of amyloid plaques compared to age-matched non-epileptic controls, in an age-accelerated fashion.[9] However, it is not known if amyloid accumulation is linked only to local pathological brain processes, or whether there is more general amyloid pathology in epilepsy, as the brain functional abnormalities in epilepsy are not limited to the area of epileptic discharges.[2]

Apolipoprotein E (APOE) is an important factor in neuronal repair and thus the APOE genotype might be a crucial factor in determining individual capacity to endure neuronal stress related to epileptic seizures.[10] Of the APOE genotypes, ε4 allele leads to the least effective of isoforms and ε4 allele has been recognized as a major risk factor for increased Aβ accumulation and Alzheimer's disease in the general population.[10] Interestingly, the APOE ε4 allele has also been linked with poorer memory performance and neurodegeneration in patients with treatment-resistant temporal lobe epilepsy.[11,12] Thus, APOE genotype might be an important determinant of brain ageing and vulnerability to excess amyloid accumulation also in patients with epilepsy.

In the present study, we investigated brain Aβ accumulation in subjects with childhood-onset epilepsy and matched controls. The study was carried out with a well-known population-based cohort of epilepsy patients that has been prospectively followed from their childhood in the early 1960's through their lives until late middle age.[13,14] Brain amyloid accumulation was investigated using positron emission tomography (PET) with amyloid ligand [^{11}C]Pittsburgh Compound B (PIB). We hypothesized that subjects with childhood-onset epilepsy, and particularly subjects

with APOE ε4 allele, would show an increase in brain PIB uptake predisposing them to development of progressive neurodegenerative disorders, such as Alzheimer's disease.

Materials and Methods

The study protocol was approved by the Institutional Review Board (Diary No. 120/2008/26.1.2009 §454) and the study was conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all study participants.

Participants

Subjects with childhood-onset epilepsy were originally recruited from Turku University Hospital district area (total population approx. 700,000) during years 1961–1964 and followed prospectively over the decades.[13] The original cohort included 100 subjects with uncomplicated epilepsy (i.e. no major neurological comorbidity, as described in more detail earlier[14]). Using stratified random sampling, controls for subjects with uncomplicated epilepsy, matched for age, gender and place of domicile, were randomly chosen from the general population of the study area in 1992.[14] Although not all subject-control pairs were anymore available at present, the remaining controls continued to serve as a control group on group-level comparisons.

The subjects were all Caucasian, on average five years old at baseline and 56 years at present. The mean (SD) follow-up of the subjects was 52.5 (4.0) years. The subject recruitment, epilepsy classification, follow-up design and the 50-year clinical outcome of the Turku Adult Childhood Onset Epilepsy (TACOE) study has been described in detail previously.[13-16] Overall 51 (70% of 73 eligible) subjects and 52 (of 78 available) matched controls participated to the TACOE study. Of the participants,

86% (41 subjects and 46 controls) underwent PET scanning with PIB and were included in the present study. Thirteen participants (7 subjects, 6 controls) declined, two subjects were excluded because of structural brain lesions in MRI and one subject discontinued the PET imaging due to a panic attack in the scanner.

All participants were clinically examined by a consultant neurologist. Individual epilepsy syndromes were classified to idiopathic and cryptogenic, as described earlier.[17] The neuropsychological examination included ten validated tests examining episodic memory, semantic memory, language function, executive function, and visuo-motor function [Digit span, Similarities and Digit Symbol from the Finnish WAIS-R; The Trail Making Test A and B; Finnish WMS-R Logical memory (Story A, immediate and delayed recall); Controlled Word Association Test; Finnish Boston Naming Test; Wordlist learning and Clock drawing from the CERAD]. Neuropsychological test scores were z-transformed based on the distribution in the 48 neurologically healthy participants in the control group. The procedure and test outcomes have been described in detail in a separate paper.[18] Cognitive impairment was defined as having a z-score of at least 1.5 SD below the mean in three or more tests.[19] The APOE genotyping was performed as described previously.[20]

Imaging

Participants were scanned with 3T Siemens Verio scanner (Siemens AG, München, Germany) to evaluate the presence of structural brain abnormalities and to provide anatomical reference (3D T1-weighted MRI with 1 mm isotropic voxels) for PET image processing. In addition, axial T2PD, DTI, T2*, and FLAIR sequences were

obtained for clinical evaluation. One subject and one control had a contraindication for MRI and were therefore only scanned with PET.

[^{11}C]PIB (N-methyl-[^{11}C]-2-(4'-methylaminophenyl)-6-hydroxybezothiazole) was synthesized as described previously.[21] The scanning was performed using Siemens High Resolution Research Tomograph (HRRT; Siemens AG, München, Germany). Antecubital vein was cannulated for tracer administration, and head motion was controlled using an individually fitted thermoplastic mask during the scanning. A mean (SD, range) 444 (100, 206–561) MBq rapid bolus of PIB was administered in the antecubital vein prior the scanning. Three 10-minute frames were obtained beginning at 60 minutes from the injection. Head motion tracking was applied during the scanning using a plastic cap with infrared light detectors (Polaris, Northern Digital Inc., Canada) attached on top of the thermoplastic mask. Motion tracking data was available from 85% of participants included to the SPM analyses (33 subjects and 40 controls).

Image preprocessing

The images were preprocessed using Statistical Parametric Mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) running in Matlab 2011a (Mathworks Inc., Natick, MA, USA). First, the between-frame motion was corrected and the images were coregistered to the individual T1-weighted MR images using mutual information algorithm. Then, the motion-corrected and coregistered images were normalized to MNI space using SPM unified segmentation algorithm applying the structural information of the T1-weighted image.[22] For the normalization of the

two subjects without brain MRI, a PET template was created using the calculated average of the normalized images of all other participants. Reference region standardized uptake values (SUVs) were calculated, and specific PIB uptake was quantified as the region-to-cerebellar cortex ratio from 60 to 90 minutes from the injection of the tracer.[23] The images were smoothed using 10 mm full-width at half maximum (FWHM) Gaussian kernel to improve the signal-to-noise ratio.

As quality control, within-frame head motion was estimated by calculating the amplitude of the transposition within each frame. In addition, displacement from the transmission scan position was estimated by calculating the mean transposition of each frame. Head motion parameters were averaged across the analyzed frames for statistical analyses. There were no significant difference in the average within-frame motion (Mann-Whitney U=637.5 P=0.98), frame disposition from the transmission position (U=649.5, P=0.91), injected PIB dose per weight (Student's t-test t_{85}=0.70, P=0.49), or reference region standardized uptake values between the subjects and controls (t_{85}=0.62, P=0.54).

Visual analysis

Visual analysis was carried out for all 87 participants with anonymized MNI space region-to-cerebellum ratio images by consensus statement of two experienced readers blinded to the clinical data. As epilepsy-related amyloid accumulation may not necessarily follow similar spatial distribution across all epilepsy syndromes, PIB scan was deemed abnormal if the tracer uptake in gray matter was higher than or equal to that in the white matter in any of the following regions: frontal lobe, posterior

cingulate/precuneus, parietal lobe, or temporal lobe. Thus, in addition to Alzheimer type pathology, also more focally restricted increase in PIB uptake was evaluated as abnormal.

Statistical analysis

The demographics were compared between the subjects and controls using independent t-test or Mann-Whitney U-test (continuous variables) and Fisher's Exact Test (categorical variables), as appropriate. For t-tests the normality of the distribution of continuous variables was inspected visually and equality of variances using Levene's test. Differences in visual analysis were investigated using Fisher's Exact Test, where one-sided test was chosen based on the *a priori* hypothesis of increased, and not decreased, amyloid accumulation in subjects with epilepsy compared to non-epileptic controls. In addition, differences in visual analysis between subjects with and without APOE ε4 allele, active vs. remitted epilepsy, presence/absence of cognitive impairment or presence/absence of cerebrovascular disease were compared using Fisher's Exact Test.

One subject was excluded from the whole-brain SPM analyses because of inappropriate field-of-view (FOV) excluding part of the cerebellum from the PET image. Thus, the final sample in the SPM analyses included 40 subjects and 46 controls. An analysis mask was created to limit the search volume to the cerebral gray matter. PIB uptake was examined voxel-by-voxel over the search volume using the general linear model (GLM) with group and APOE genotype (presence of ε4 allele) as explanatory variables, and age as a covariate of no interest. The effects were

investigated on cluster level using family-wise error (FWE) correction. The findings were confirmed and magnitudes of the group differences estimated in the PFC and whole cerebral cortex by extracting average ratio values using automated anatomical labelling (AAL) library regions (excluding non-brain and white matter from the regions) and using GLM corresponding to the SPM analyses. The associations between other explanatory variables and cerebral PIB uptake were investigated using the ROI data using GLM. Model fit in the linear models was inspected visually from the standardized residuals. The statistical analyses, except for voxel-by-voxel SPM analyses, were run in SPSS Statistics 21 (IBM Corp., Armonk, NY). P values less than 0.05 were considered significant.

Results

Demographics

While cognitive impairment was more common in the subject group, there were no significant differences between the subjects and controls in age, body mass index (BMI), family history of dementia or APOE genotype (Table 1). When comparing the TACOE study participants included vs not included in the PIB PET study, there were no significant differences in subjects vs controls (P=0.41), age (t_{101}=-0.26, P=0.80), gender (P=0.57), BMI (t_{101}=0.17, P=0.87), duration of AED medication (U=181.5, P=0.58) or cumulative years with seizures (U=192, P=0.76). Participants included in the study were more likely to be cognitively normal than participants not included in the study [presence of cognitive impairment 28% versus 56% (P=0.039), respectively]. The epilepsy characteristics are presented in Table 2.

Visual analysis

Visual analysis revealed nine (22%) subjects and three (6.5%) controls to have abnormal PIB images showing high cortical uptake in at least in one of the evaluated brain regions (P=0.037). The proportion of subjects with abnormal PIB scans did not reach significance between subjects with (n=12) versus without (n=29) APOE ε4 allele (42% vs 14%, P=0.064). The etiology of epilepsy (idiopathic/cryptogenic), presence/absence of cognitive impairment or cerebrovascular disease were not associated with abnormal PIB scan status (P>0.1). Visually, there was no clear correspondence between the epileptic focus and PIB uptake in subjects with

localizable epilepsy syndromes (i.e. in subjects with temporal, occipital or frontal epilepsy syndromes; n=12).

Semiquantitative analyses

In the whole brain SPM analysis, there was a significant group x APOE genotype effect showing higher increase in frontal and insular PIB uptake in APOE ε4 carriers in subjects compared to controls (blue clusters in Figure 1). In addition, subjects showed higher frontal PIB uptake compared to controls (red-yellow clusters in Figure 1). The findings were confirmed with PFC and cerebral cortex ROIs (Figure 2). The group x APOE genotype interaction and main effect of group remained significant when excluding outlier values from GLM analyses. However, the main effect of APOE genotype in the PFC was no longer significant.

When examining subjects by etiology, the APOE genotype effect on PIB uptake was related specifically to the idiopathic epilepsy syndromes (Figure 3). Note however that the number of ε4-carriers in each subject group were small. Subjects with/without active epilepsy, cognitive impairment, family history of dementia or cerebrovascular disease did not differ significantly in PFC or whole brain PIB uptake. Neither were there any significant associations between PFC or whole brain PIB uptake and age of epilepsy onset, duration of active epilepsy or duration of AED use. In addition, PFC or whole brain PIB uptake did not correlate with average z-score ($r=0.20$, $p=0.24$ and $r=0.15$, $p=0.38$, respectively) or individual scores (all $p>0.05$) in cognitive tests.

Discussion

Here we show, for the first time, that childhood-onset epilepsy is associated with increased brain amyloid deposition at late middle age. We show that increased amyloid deposition can be observed in a sample of unselected subjects with a wide variety of different epilepsy syndromes mostly in remission and many off-medication already for decades. Thus, the results suggest that increased brain amyloid deposition may be linked to the pathophysiology of epilepsy rather than to the seizure control and duration of active epilepsy. Furthermore, individuals with APOE ε4 allele and idiopathic epilepsy syndromes might be particularly vulnerable for the development of amyloid pathology.

Earlier work with parallel neuroimaging and tissue specimen showed that brain amyloid accumulation can be reliably measured using PIB PET.[24] Brain amyloid accumulation occurs in normal brain ageing, and the prevalence of amyloid plaques (and abnormal PIB imaging) in asymptomatic individuals increases in parallel with age.[25,26] The frequency of abnormal PIB imaging finding has been estimated to be approximately 10% in the age group of 50-60 years,[25] which corresponds well to the proportion of abnormal PIB scans in controls (6.5%) in the present study. However, our epilepsy group exhibited substantially higher prevalence (22%) of abnormal PIB imaging corresponding roughly to the prevalence estimates of a decade older population.[25] Thus, the results suggest that subjects with epilepsy show accelerated brain ageing in terms of amyloid accumulation compared to non-epileptic general population controls.

According to the suggested Alzheimer's disease biomarker model, amyloid deposition can be observed as an early presymptomatic phenomenon in Alzheimer's disease, and abnormally high brain amyloid load in asymptomatic individuals predicts future cognitive impairment.[27,28] However, the amyloid cascade hypothesis assumes that brain amyloid accumulation is only the initial trigger of series of molecular events in the brain leading to Alzheimer's disease pathology.[29] Thus, the increased amyloid accumulation in epilepsy could be interpreted as a predisposing factor or even an early sign of a neurodegenerative process such as Alzheimer's disease. It should however be noted that also other factors, such as concomitant tau-pathology, might be needed to trigger neurobiological processes leading to clinical Alzheimer's disease. For instance, patients with chronic medication-resistant epilepsy with traumatic brain injury have been shown to have increased tau protein accumulation, which was associated with higher Braak staging of Alzheimer's disease at autopsy.[30]

The increase in brain PIB uptake in subjects with childhood-onset epilepsy was strongly associated with APOE genotype (ε4 allele). Furthermore, although idiopathic epilepsy syndromes tended to have more favorable clinical outcome compared to cryptogenic syndromes, subjects with idiopathic epilepsy seemed to be particularly vulnerable for amyloid accumulation associated with APOE ε4 allele.[14,16] It is possible that subjects with idiopathic syndromes also have other genetic defects that predispose them to the adverse effects of neuronal stress that is exaggerated by low functioning neuronal repair mechanisms associated with APOE ε4. It is also of interest that, although the present sample included a substantial proportion of subjects (18 out of 41) with cognitive impairment, cognitive impairment was not associated with brain amyloid deposition [in the cross-sectional analyses]. However, epilepsy is

known to be associated with marked cognitive impairment even in the absence of comorbid Alzheimer's disease,[2] and human *post mortem* and *in vivo* studies have shown that amyloid pathology can be observed over a decade before the manifestation of Alzheimer's disease.[25,31] Therefore, the findings indicate that the increased amyloid accumulation here likely mostly represents presymptomatic increase in brain amyloid burden when amyloid accumulation is not associated with cognitive performance.[27]

There are some limitations in the present study. First, our study was cross-sectional and did not allow for assessing the temporal course of the amyloid accumulation. Second, as the mean age of the participants was 56 years, the prevalence of Alzheimer's disease is still very low at this age. The sample was also heterogeneous in terms of epilepsy syndromes (and used medications), which limits the power to detect associations between individual epilepsy syndromes and/or their associated genetic mutations and amyloid accumulation.[32] We also lacked detailed data about individual seizure types and number of seizures. Note, however, that all subjects had uncomplicated epilepsy, which reduces the variety of etiologies. Finally, the sample included in the present imaging study showed some selection bias, as participants with cognitive impairment were less likely to be included in the study, which might lead to underestimation of the group differences and explain why APOE ε4 effect on cerebral PIB uptake was not seen in the control group.

In conclusion, childhood-onset epilepsy appears to be associated with increased amyloid accumulation in late middle age even among cases in remission without medication for decades. The findings suggest a link between epilepsy, APOE genotype and amyloid pathology, encouraging to further research on brain ageing in

epilepsy. Further follow-up with repeated amyloid assessments are needed to confirm the findings and test this hypothesis.

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TACOE study group: Anu Anttinen, MD (Turku University Hospital); Matti Erkinjuntti, MD, PhD (Turku University Hospital); Bruce Hermann, PhD (University of Wisconsin); Juho Joutsa, MD, PhD (University of Turku); Mira Karrasch, PhD (Åbo Akademi University); Juha O. Rinne, MD, PhD (University of Turku); Maiju Saarinen, MSSc (Biostat) (University of Turku); Shlomo Shinnar, MD, PhD (Albert Einstein College of Medicine); Matti Sillanpää, MD, PhD (University of Turku); Pirkko Sonninen, MD (Turku University Hospital); Petri Tiitta, MA (Åbo Akademi University).

References

1. Silverberg JI, Joks R, Durkin HG. Allergic disease is associated with epilepsy in childhood: a US population-based study. *Allergy.* 2014;69(1):95-103.
2. Lin JJ, Mula M, Hermann BP. Uncovering the neurobehavioural comorbidities of epilepsy over the lifespan. *Lancet.* 2012;380(9848):1180-1192.
3. Hermann BP, Seidenberg M, Dow C, et al. Cognitive prognosis in chronic temporal lobe epilepsy. *Ann Neurol.* 2006;60(1):80-87.
4. Hermann B, Seidenberg M, Sager M, et al. Growing old with epilepsy: the neglected issue of cognitive and brain health in aging and elder persons with chronic epilepsy. *Epilepsia.* 2008;49(5):731-740.
5. Larner AJ. Epileptic seizures in AD patients. *Neuromolecular Med.* 2010;12(1):71-77.
6. Chin J, Scharfman HE. Shared cognitive and behavioral impairments in epilepsy and Alzheimer's disease and potential underlying mechanisms. *Epilepsy Behav.* 2013;26(3):343-351.
7. Cirrito JR, Yamada KA, Finn MB, et al. Synaptic activity regulates interstitial fluid amyloid-beta levels in vivo. *Neuron.* 2005;48(6):913-922.
8. Sheng JG, Boop FA, Mrak RE, Griffin WS. Increased neuronal beta-amyloid precursor protein expression in human temporal lobe epilepsy: association with interleukin-1 alpha immunoreactivity. *J Neurochem.* 1994;63(5):1872-1879.
9. Mackenzie IR, Miller LA. Senile plaques in temporal lobe epilepsy. *Acta Neuropathol.* 1994;87(5):504-510.
10. Hirsch LJ. ApoE, MemorE, and EpilepsE. *Epilepsy Curr.* 2007;7(6):149-150.
11. Busch RM, Lineweaver TT, Naugle RI, et al. ApoE-epsilon4 is associated with reduced memory in long-standing intractable temporal lobe epilepsy. *Neurology.* 2007;68(6):409-414.
12. Aboud O, Mrak RE, Boop FA, Griffin WS. Epilepsy: neuroinflammation, neurodegeneration, and APOE genotype. *Acta Neuropathol Commun.* 2013;1:41.
13. Sillanpää M. Medico-social prognosis of children with epilepsy. Epidemiological study and analysis of 245 patients. *Acta Paediatr Scand Suppl.* 1973;237:3-104.
14. Sillanpää M, Jalava M, Kaleva O, Shinnar S. Long-term prognosis of seizures with onset in childhood. *N Engl J Med.* 1998;338(24):1715-1722.
15. Sillanpää M, Jalava M, Shinnar S. Epilepsy syndromes in patients with childhood-onset seizures in Finland. *Pediatr Neurol.* 1999;21(2):533-537.
16. Sillanpää M, Anttinen A, Rinne JO, et al. Childhood-onset epilepsy five decades later. A prospective population-based cohort study. *Epilepsia.* 2015;56(11):1774-1783.
17. Sillanpää M, Shinnar S. Long-term mortality in childhood-onset epilepsy. *N Engl J Med.* 2010;363(26):2522-2529.

18. Karrasch M, Tiitta P, Hermann B, et al. Cognitive outcome in childhood-onset epilepsy: A five decade prospective cohort study. *Int J Neuropsychol Soc.* In press.
19. LJ I, CB A. An empirical approach to determining criteria for abnormality in test batteries
with multiple measures. *Neuropsychology.* 1996;10:120-124.
20. Jänis MT, Siggins S, Tahvanainen E, et al. Active and low-active forms of serum phospholipid transfer protein in a normal Finnish population sample. *J Lipid Res.* 2004;45(12):2303-2309.
21. Kemppainen NM, Aalto S, Wilson IA, et al. Voxel-based analysis of PET amyloid ligand [11C]PIB uptake in Alzheimer disease. *Neurology.* 2006;67(9):1575-1580.
22. Ashburner J. A fast diffeomorphic image registration algorithm. *Neuroimage.* 2007;38(1):95-113.
23. Lopresti BJ, Klunk WE, Mathis CA, et al. Simplified quantification of Pittsburgh Compound B amyloid imaging PET studies: a comparative analysis. *J Nucl Med.* 2005;46(12):1959-1972.
24. Leinonen V, Alafuzoff I, Aalto S, et al. Assessment of beta-amyloid in a frontal cortical brain biopsy specimen and by positron emission tomography with carbon 11-labeled Pittsburgh Compound B. *Arch Neurol.* 2008;65(10):1304-1309.
25. Rowe CC, Ellis KA, Rimajova M, et al. Amyloid imaging results from the Australian Imaging, Biomarkers and Lifestyle (AIBL) study of aging. *Neurobiol Aging.* 2010;31(8):1275-1283.
26. Morris JC, Roe CM, Grant EA, et al. Pittsburgh compound B imaging and prediction of progression from cognitive normality to symptomatic Alzheimer disease. *Arch Neurol.* 2009;66(12):1469-1475.
27. Jack CR, Knopman DS, Jagust WJ, et al. Tracking pathophysiological processes in Alzheimer's disease: an updated hypothetical model of dynamic biomarkers. *Lancet Neurol.* 2013;12(2):207-216.
28. Doraiswamy PM, Sperling RA, Johnson K, et al. Florbetapir F 18 amyloid PET and 36-month cognitive decline: a prospective multicenter study. *Mol Psychiatry.* 2014;19(9):1044-1051.
29. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science.* 2002;297(5580):353-356.
30. Thom M, Liu JY, Thompson P, et al. Neurofibrillary tangle pathology and Braak staging in chronic epilepsy in relation to traumatic brain injury and hippocampal sclerosis: a post-mortem study. *Brain.* 2011;134(Pt 10):2969-2981.
31. Braak H, Braak E, Bohl J, Reintjes R. Age, neurofibrillary changes, A beta-amyloid and the onset of Alzheimer's disease. *Neurosci Lett.* 1996;210(2):87-90.
32. Kawas C, Gray S, Brookmeyer R, Fozard J, Zonderman A. Age-specific incidence rates of Alzheimer's disease: the Baltimore Longitudinal Study of Aging. *Neurology.* 2000;54(11):2072-2077.

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Figure legends

Figure 1. Increased PIB uptake in subjects with childhood-onset epilepsy

BLUE SCALE: Group x APOE genotype effect showing higher increases in PIB uptake in APOE ε4 allele carriers in subjects compared to controls. RED-YELLOW SCALE: Group effect showing higher PIB uptake in subjects compared to controls. The images are thresholded to show only clusters with cluster-level family-wise error (FWE) corrected $P<0.05$. Color scales represent the voxel t-values.

Figure 2. PIB uptake in subjects and controls according to the APOE genotype

A. Prefrontal cortex and B. cerebral cortex uptakes. Age-adjusted GLM analysis P values are presented. Number of participants without/with APOE ε4 allele were 33/13 and 29/12 in controls and subjects, respectively.

Figure 3. PIB uptake according to the epilepsy type and APOE genotype

A. Prefrontal cortex and B. cerebral cortex uptakes. Age-adjusted GLM analysis P values are presented. Number of participants without/with APOE ε4 allele were 33/13, 11/5 and 17/7 in controls, subjects with cryptogenic epilepsy and subjects with idiopathic epilepsy, respectively.

Tables

Table 1. Demographics of subjects with childhood-onset epilepsy and controls on follow-up of five decades

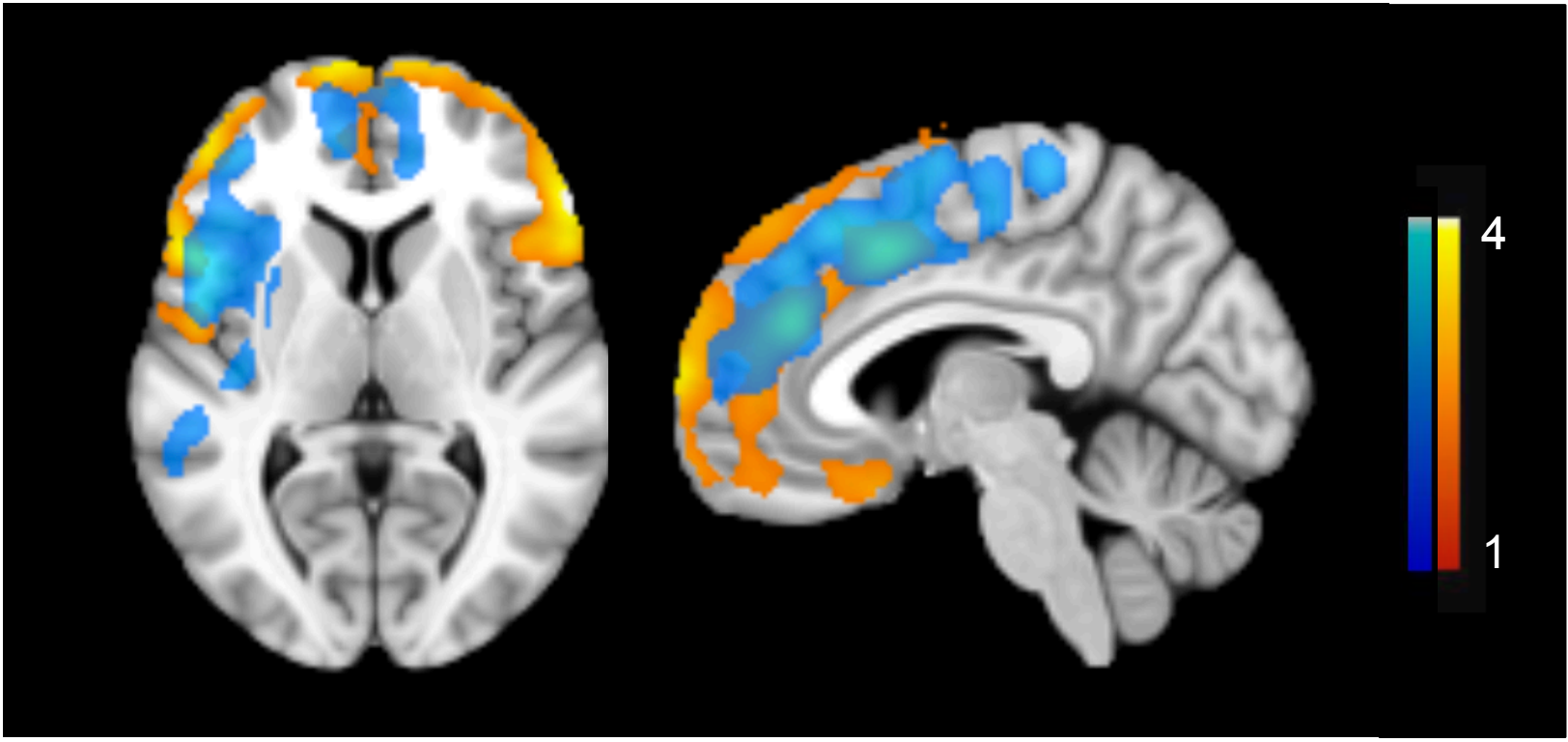
	Subjects (n=41)	Controls (n=46)	t_{85}	P
Age (y)	56 (4.3, 48–63)	56 (4.3, 49–64)	0.02	0.99
Sex (m/f)	15/26	17/29	-	1.0
BMI (kg/m^2)	28.7 (4.8, 18-42)	27.4 (5.8, 17-42)	-1.1	0.27
APOE ε4 allele	12 (29%)	13 (28%)	-	1.0
Family history of dementia	18 (44%)	22 (48%)	-	0.83
Cognitively impaired[1]	18 (44%)	6 (13%)	-	0.002

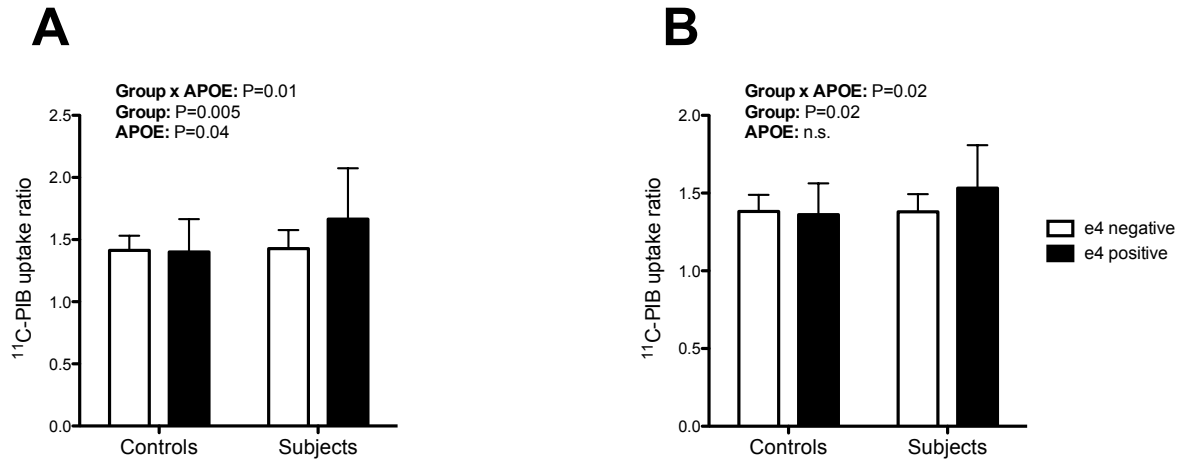
Number (percentage) of subjects or mean (SD, range) are presented together with t-test stattistics or Fisher's Exact test P-values, as appropriate. AED = antiepileptic drugs. [1]Three or more out of ten neuropsychological test scores at least 1.5 SD below mean.

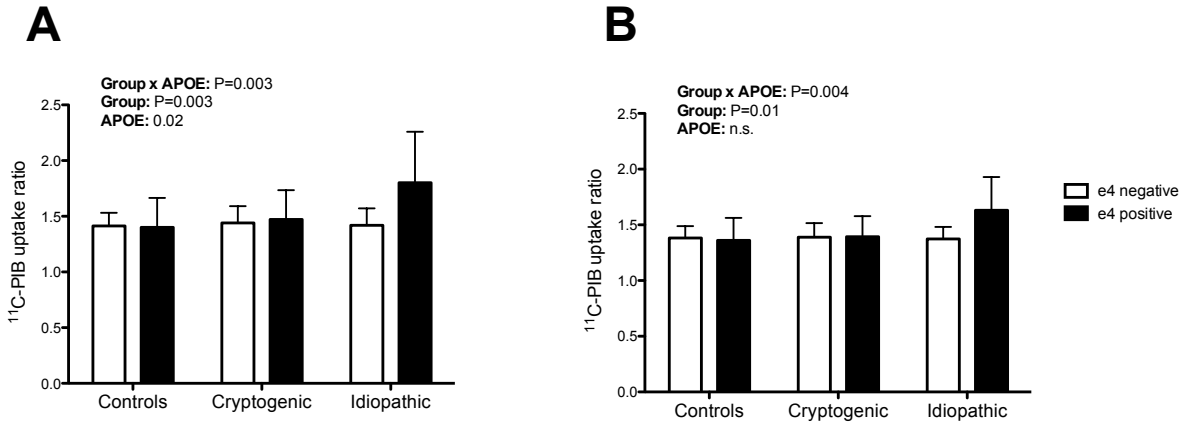
Table 2. Epilepsy characteristics

	Subjects (n=41)
Age at onset of epilepsy (y)	5.1 (4.5, 0–14)
Duration of active epilepsy (y)	17 (15, 1–49)
Duration of AED treatment (y)	21 (19, 0–55)
Current medication	9 (22%)
Epilepsy type	
Idiopathic	24 (59%)
Cryptogenic	17 (41%)
Used AEDs	
Barbiturates	33 (81%)
Hydantin	23 (56%)
Carbamazepine	9 (22%)
Succinimid	6 (15%)
Benzodiazepines	4 (9.8%)
Coxazol	5 (12%)
Other	3 (7.3%)

AED = antiepileptic medication. Mean (SD, range) or number (%) are presented, as appropriate.


4
1


A
Group x APOE: P=0.01
Group: P=0.005
APOE: P=0.04
11C-PIB uptake ratio
2.5
2.0
1.5
1.0
0.5
0.0
Controls
Subjects
B
Group x APOE: P=0.02
Group: P=0.02
APOE: n.s.
11C-PIB uptake ratio
2.0
1.5
1.0
0.5
0.0
Controls
Subjects
e4 negative
e4 positive


A
Group x APOE: P=0.003
Group: P=0.003
APOE: 0.02
11C-PIB uptake ratio
2.5
2.0
1.5
1.0
0.5
0.0
Controls
Cryptogenic
Idiopathic
B
Group x APOE: P=0.004
Group: P=0.01
APOE: n.s.
11C-PIB uptake ratio
2.5
2.0
1.5
1.0
0.5
0.0
Controls
Cryptogenic
Idiopathic
e4 negative
e4 positive