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A Multi-Cohort Study of ApoE ε4 and Amyloid-β Effects on the Hippocampus in Alzheimer’s Disease

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\textsuperscript{1}Data used in preparation of this article were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at: http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf

\textsuperscript{2}http://aibl.csiro.au/about/aibl-research-team/

\textsuperscript{3}http://www.imagen-europe.com

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INTRODUCTION

The apolipoprotein E (APOE) gene is a well-established genetic risk factor for the development of late-onset Alzheimer’s disease (AD) and since its discovery, a large body of research has been conducted to explain its role in AD pathophysiology [1–3]. The APOE ε4 allele, a genetic risk factor known to substantially increase the risk of AD in a dose-dependent fashion, is associated with higher amyloid-β (Aβ) deposition [4, 5]. By contrast, the APOE ε2 allele has been suggested to confer a protective effect against AD [6].

Hippocampal volumetry has been shown to be a sensitive, albeit non-specific marker of neurodegeneration in AD. Previously, it has been used to demonstrate accelerated rates of hippocampal atrophy in ε4 carriers with amnestic mild cognitive impairment (MCI) [7, 8]. Although the exact temporospatial relationship between Aβ and tau in the pathological cascade of AD is unclear, it has been suggested that both proteinopathies may have a synergistic effect on neuronal toxicity [9]. Emerging data also suggests that these pathological processes that influence cognitive decline in AD are moderated by APOE ε4 through both Aβ-dependent and Aβ-independent mechanisms [10]. However, evidence for the direct mechanistic actions of APOE ε4 is mixed.

Despite the numerous studies, our limited understanding of APOE ε4 risk in asymptomatic individuals and individuals with varying stages of AD pathophysiology warrants further study. Evidence...
has suggested that equivocal findings in neuroimaging studies of APOE ε4 may be attributed to the lack of specificity between changes that are due to normal aging and those that manifest as a result of pathological neurodegeneration [11]. Furthermore, some APOE neuroimaging findings originate from studies with limitations in sample size and hence study power [12]. This is further exacerbated by the varying distribution of APOE ε4 carrier status across different samples. However, recent neuroimaging APOE studies with well-characterized samples (between 400–700 subjects) are beginning to address this methodological issue [13, 14]. Also, recent neuroimaging evidence from young APOE ε4 carriers has also shown that structural and functional alterations in the brain may precede Aβ pathology [15, 16]. This had led researchers to postulate that APOE ε4 may exert neurodevelopmental changes that provide a foothold for the pathological cascade of AD later in life.

In order to capture the heterogeneity of varying AD risk, we combined several well-characterized cohort studies to evaluate neuroanatomic the effect of APOE genotype on hippocampal volumes. The first dataset in our study, known as the AD and normal aging dataset ($n = 1,781$) consisted of imaging data from three large multi-center AD consortiums, (i) The Alzheimer’s Disease Neuroimaging Initiative (ADNI), (ii) the AddNeuroMed study (ANM), and (iii) the Australian Imaging, Biomarkers and Lifestyles (AIBL) study, as well as a sample of non-demented individuals from the Swedish National Study on Aging and Care in Kungsholmen (SNAC-K) and a dementia study from King’s College London, UK (BRC-AD). The sample of non-demented individuals from the SNAC-K study were considered separate from healthy controls as the recruitment criteria for these subjects was epidemiological in nature and we could not exclude the possibility of memory impairment in some subjects. In our second dataset, we used a large sample of healthy 14-year-old adolescents ($n = 1,387$) in order to determine the neurodevelopmental effect of APOE ε4 genotype on hippocampal volume. This is particularly important because adolescence is a time of substantial dynamic neurobiological and behavioral changes. These changes are often beneficial and can optimize the brain for adult maturation, but can also confer neural vulnerabilities for certain types of psychiatric or neurological illness.

The aim of this study was to firstly evaluate the neuroanatomic effect of APOE genotype on the hippocampus in the AD and normal aging dataset. Previous studies have demonstrated a linear effect of APOE genotype on disease risk, with ε2 carriers possessing a low risk of developing AD, and ε4 carriers possessing the greatest risk [17]. We aimed to test if this linear stepwise effect of APOE genotype also imparted a similar neuroanatomic effect on the hippocampus across the different stages of AD pathophysiology. We then tested the neuroanatomic effect of APOE genotype on hippocampal volumes of healthy 14-year-old adolescents to determine the role of APOE in adolescent brain development.

On the other hand, converging evidence suggests that APOE ε4 modifies Aβ accumulation and may have downstream effects on tau neurodegeneration [18]. To further elucidate the mechanisms of this proposed synergistic relationship, we aimed to examine whether high levels of Aβ deposition would lead to greater hippocampal loss compared to low levels of Aβ deposition in APOE ε4 carriers.

**MATERIALS AND METHOD**

**Datasets**

Cohort specific inclusion criteria and details of the study design can be found in previous publications [19–23]. Table 1 provides details of the number of subjects from each cohort included in the AD and normal aging dataset.

**AD and Normal Aging Dataset ($n = 1,781$)**

**Alzheimer’s Disease Neuroimaging Initiative ($n = 779$)**

A detailed description of the study design can be found on the ADNI webpage (http://www.adni-info.org). Data was obtained for subjects from the ADNI online database (http://adni.loni.usc.edu). Subjects were between 55 and 90 years of age. ADNI was approved by the institutional review board and ethics committees of participating institutions, and written informed consent was obtained from all participants or their next of kin.

i. **AD ($n = 177$):** General inclusion/exclusion criteria: 1) MMSE scores between 20 and 26; 2) CDR of 0.5 or 1.0; 3) met NINCDS/ADRDA criteria for probable AD.

ii. **MCI ($n = 383$):** General inclusion/exclusion criteria: 1) subjects had MMSE scores between 24 and 30 (inclusive); 2) memory complaint
Table 1
Number of subjects obtained from each cohort study in the AD and normal aging dataset (n = 1,781)

<table>
<thead>
<tr>
<th>Cohort Study</th>
<th>Clinical Diagnosis</th>
<th>Number of subjects (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s Disease Neuroimaging Initiative (n = 779)</td>
<td>Alzheimer’s disease</td>
<td>177 (22.7%)</td>
</tr>
<tr>
<td></td>
<td>Mild Cognitive Impairment</td>
<td>383 (49.2%)</td>
</tr>
<tr>
<td></td>
<td>Healthy Controls</td>
<td>219 (28.1%)</td>
</tr>
<tr>
<td>AddNeuroMed study (n = 303)</td>
<td>Alzheimer’s disease</td>
<td>109 (36.0%)</td>
</tr>
<tr>
<td></td>
<td>Mild Cognitive Impairment</td>
<td>97 (32.0%)</td>
</tr>
<tr>
<td></td>
<td>Healthy Controls</td>
<td>97 (32.0%)</td>
</tr>
<tr>
<td>Australian, Imaging, Biomarkers and Lifestyles (AIBL) study (n = 228)</td>
<td>Alzheimer’s disease</td>
<td>46 (20.2%)</td>
</tr>
<tr>
<td></td>
<td>Mild Cognitive Impairment</td>
<td>42 (18.4%)</td>
</tr>
<tr>
<td></td>
<td>Healthy Controls</td>
<td>140 (61.4%)</td>
</tr>
<tr>
<td>Biomedical Research Centre for Dementia, King’s College London (BRC-AD) study (n = 89)</td>
<td>Alzheimer’s disease</td>
<td>33 (37.1%)</td>
</tr>
<tr>
<td>Swedish National study on aging and care in Kungscolmen (SN-ACK) (n = 382)</td>
<td>Healthy Controls</td>
<td>56 (62.9%)</td>
</tr>
<tr>
<td></td>
<td>Healthy Controls</td>
<td>382 (100%)</td>
</tr>
</tbody>
</table>

had objective memory loss measured by education adjusted scores on Wechsler Memory Scale Logical Memory II; 3) CDR of 0.5; 4) absence of significant levels of impairment in other cognitive domains, essentially preserved activities of daily living, and an absence of dementia.

iii. Cognitively normal (CN) (n = 219): General inclusion/exclusion criteria: 1) MMSE scores between 24 and 30 (inclusive); 2) CDR of zero; 3) they were non-depressed, non MCI, and non-demented.

AddNeuroMed (ANM) study (n = 303)
Information regarding the study design and enrolment criteria has been previously described elsewhere [21, 24]. All AD and MCI subjects were recruited from the local memory clinics of one of the six participating sites while the control subjects were recruited from non-related members of the patient’s families, caregiver’s relatives, and social centers for the elderly or GP surgeries. ANM was approved by the South London and Maudsley NHS Foundation Trust ethics committee and other ethical review boards of each participating country. Ethics committee approval was obtained at each of the participating centers in accordance with the Alzheimer’s Associations published recommendations.

i. AD (n = 109): Inclusion criteria: 1) ADRDA/NINCDS and DSM-IV criteria for probable AD. 2) Mini-Mental State Examination (MMSE) score ranged from 12 to 28; 3) age 65 years or above. Exclusion criteria: 1) Significant neurological or psychiatric illness other than AD; 2) Significant unstable systematic illness or organ failure.

ii. MCI (n = 97) and CN (n = 97): Inclusion criteria: 1) MMSE score range between 24 and 30; 2) Geriatric Depression Scale score less than or equal to 5; 3) age 65 years or above; 4) medication stable; 5) good general health. Exclusion criteria: 1) met the DSM-IV criteria for dementia; 2) significant neurological or psychiatric illness other than AD; 3) significant unstable systematic illness or organ failure.

iii. The distinction between MCI and CN individuals was based on two criteria: 1) subject scores 0 on Clinical Dementia Rating Scale (CDR) = CN; 2) Subject scores 0.5 on CDR = MCI. For the MCI subjects, it was preferable that the subject and informant reported occurrence of memory problems. All AD subjects had a CDR score of 0.5 or above.

The Australian, Imaging, Biomarkers, and Lifestyles (AIBL) study (n = 228)
The AIBL study is a prospective longitudinal study of aging, integrating data from neuroimaging, biomarkers, lifestyle, clinical, and neuropsychological analysis. Detailed information about the study design has been described in previous publications [20, 25]. CN individuals were recruited by advertisement in the community while MCI and AD patients were recruited from tertiary memory disorders clinics or private geriatricians, psychiatrists, and neurologists that subspecialize in dementia. All participants were at least 60 years of age, in good general health with no history of stroke or other neurological disease. The institutional ethics committees of Austin Health, St Vincent’s Health, Hollywood Private Hospital and Edith Cowan University approved the AIBL study, and
all volunteers gave written informed consent before participating.

i. AD (n = 46): Inclusion criteria: 1) all met NINCDS-ADRDA criteria for probable AD; 2) had a CDR of 1 or more.

ii. MCI (n = 42): Inclusion criteria: 1) met criteria of subjective and objective cognitive difficulties in the absence of significant functional loss; 2) had a CDR of less than 1. 52 MCI participants fulfilled criteria for “amnestic” MCI, and 5 were non-amnestic cases (4 were non-amnestic multi-domain and 1 was non-amnestic single domain).

iii. CN (n = 140): Inclusion criteria: participants were separated in those who reported subjective memory complaints (n = 95) and those who did not (n = 82), according to their response to the question: “Do you have any difficulty with your memory?”

**Biomedical Research Centre for Mental Health and Dementia Cohort, King’s College London (BRC-AD) (n = 89)**

BRC-AD is a neuroimaging study which was designed to establish imaging markers for the earlier detection and diagnosis of AD. Data was collected at the Institute of Psychiatry, Psychology, and Neuroscience, King’s College London, UK. A total of 89 subjects (AD: 33, CN:56) were obtained with APOE data for this study. Diagnostic inclusion and exclusion criteria for this study were exactly the same as for the ANM study.

**Swedish National study on Aging and Care in Kungsholmen (SNAC-K) study (n = 382)**

Participants were recruited from a larger population-based epidemiological study, the SNAC-K. In this study, participants were randomly selected to take part from the island of Kungsholmen in central Stockholm to examine aging in late adulthood [26]. During the first data collection, a subsample of non-institutionalized and non-disabled participants were randomly selected to undergo MRI. Participants with dementia diagnoses, schizophrenia diagnosis, bipolar disorder diagnosis, self-reported stroke, stroke observed on MRI, self-reported Parkinson’s disease, or epilepsy, were excluded. The study design has been described in detail elsewhere [22]. We used a sample of 459 individuals from SNAC-K, who underwent MRI imaging and APOE genotyping. 77 subjects with suboptimal MRI images and neurological and/or psychiatric conditions were excluded. The SNAC-K study was population-based, therefore subjects in this sample were considered a heterogeneous sample of elderly participants and treated separate from our sample CN individuals.

The SNAC-K study complies with the declaration of Helsinki, and has been approved by the ethical committee at Karolinska Institutet. All subjects gave informed consent, and in the case of severe cognitive impairment consent was collected from next-of-kin.

**Neuroimaging-Genetics IMAGEN Study (n = 1,387)**

This is the first European multi-center study combining genetics with behavioral and neuropsychological measures, functional and structural neuroimaging, and genome-wide association analyses in 2,000 healthy 14-year-old adolescents. A description of the study design are provided in Schumann et al. [23]. We selected 1,387 healthy adolescents with available MRI data and ApoE status information.

Genome-wide genotyping was performed using Illumina Quad 610 and 660 arrays (San Diego, CA, USA). Quality control of the genome-wide data was performed and samples with the following criteria were excluded: genotype call rate < 95%, and those with discordance between clinical and genotypic gender. Single nucleotide polymorphisms (SNP) quality control filters were used as described in the ENIGMA consortium imputation protocol (http://enigma.ini.usc.edu/protocols/genetics-protocols/). Further details on the imputation of unobserved SNP’s to determine APOE status are described in detail elsewhere [27].

**Image acquisition**

High resolution 3D T1-weighted MRI were acquired for each subject and a comprehensive quality control procedure was applied to all MR images according to the AddNeuroMed study quality control framework [24, 28].

**ADNI:** The protocol included a high resolution T1 weighted sagittal 3D MP-RAGE volume (voxel size 1.1 × 1.1 × 1.2 mm³), and axial proton density with T2 weighted fast spin echo images. MRI scanner protocols from models of General Electric (GE) Healthcare, Philips Medical Systems, and Siemens Medical Solutions were supported.
ANM: Data acquisition took place using six different 1.5T MR systems (4 General Electric, 1 Siemens, and 1 Picker). At each site a quadrature birdcage coil was used for RF transmission and reception. Data acquisition was designed to be compatible with the ADNI. The imaging protocol included a high-resolution sagittal 3D T1-weighted MPRAGE volume (voxel size $1.1 \times 1.1 \times 1.2$ mm$^3$) and axial proton density/T2-weighted fast spin echo images. The BRC-AD study protocol was designed to be the same as the ANM protocol.

AIBL: T1-weighted MRI was obtained using the ADNI 3D Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence, with $1 \times 1$ mm in-plane resolution and $1.2$ mm slice thickness, TR/TE/T1 = 2300/2.98/900, flip angle 9° and field of view $240 \times 256$ and 160 slices. T2 FSE and FLAIR sequences were also obtained. The AIBL protocol has been described in extensive detail previously [20].

SNAC-K: MRI scanning was undertaken on a 1.5T scanner (Philips Intera, Netherlands) on which 3D FFE (fast field echo) T1, Axial SE (spin echo) Proton Density/T2, DTI (Diffusion Tensor Imaging), and Axial FLAIR (fluid-attenuated inversion recovery) were acquired. In this study, the 3D FFE T1 images (TR = 15 ms, TE = 7 ms, Flip angle = 15°, number of slices = 128, thickness = 1.5 mm, in-plane resolution = $0.94 \times 0.94$ mm, no gap, Field of view = 240, matrix = $256 \times 256$) were used.

IMAGEN: MRI images were acquired using 3T MRI systems from major manufacturers (Siemens, Philips, Bruker, and General Electric). The protocol included a high-resolution 3D T1-weighted ultra-fast gradient echo volume (voxel size $1.1 \times 1.1 \times 1.1$ mm$^3$) and axial proton density T2-weighted fast spin echo images based on the ADNI study protocol.

Aβ PET methods

Data on Aβ imaging with positron emission tomography (PET) was accessed for a total of 95 ADNI and 57 AIBL ε4 carriers. Aβ imaging for these subjects was conducted using either $^{11}$C-Pittsburgh Compound B (PiB), or $^{18}$F-florbetapir. PET scans that were acquired as close as possible to the structural MRI scans in the ADNI and AIBL study were chosen. The PET imaging methodology of the ADNI and AIBL studies has been extensively described elsewhere [29, 30]. For PiB PET, the measure of amyloid burden was calculated by averaging the ratio of cortical to cerebellar signal (SUVR) measurements from frontal, parietal, anterior cingulate, and parietal regions of interest [29]. For $^{18}$F-florbetapir, the SUVR was calculated for 6 pre-defined regions of interest (frontal, temporal, parietal, anterior cingulate, posterior cingulate, and precuneus). The whole cerebellum was used as a reference region for both PiB and $^{18}$F-florbetapir PET.

Of the 95 subjects that underwent PET neuroimaging in the ADNI study, 66 were scanned using $^{18}$F-florbetapir and 29 using PiB. For the AIBL study, all subjects underwent PET neuroimaging using PiB. The global measure of amyloid burden was used to define participants as Aβ positive (Aβ+) and Aβ negative (Aβ–). PiB participants were classified as Aβ+ when the measure of amyloid burden was ≥ 1.5 [30] and $^{18}$F-florbetapir participants were classified as Aβ+ if the measure of amyloid burden was ≥ 1.11 [31].

Image analysis

Volumetric segmentation of the hippocampus was performed using FreeSurfer (5.1.0). FreeSurfer utilizes an affine rigid linear transformation and combines spatial information about voxel intensity relative to a probability distribution for tissue classes [32]. The Freesurfer segmentation process includes motion correction of volumetric T1-weighted images, removal of non-brain tissue using a hybrid watershed/surface deformation procedure [33], automated Talairach transformation, segmentation of the subcortical white matter and deep grey matter volumetric structures (including hippocampus, amygdala, caudate, putamen, ventricles) [34], intensity normalization [33], tessellation of the grey matter white matter boundary, automated topology correction [35, 36], and surface deformation following intensity gradients to optimally place the grey/white and grey/cerebrospinal fluid borders at the location where the greatest shift in intensity defines the transition to the other tissue class [37–39]. Further details of the segmentation approach have been described previously [34]. Quality control of hippocampal images were performed by visualizing the subcortical segmentation borders of the hippocampus for every subject. Images reflecting a poor segmentation of the hippocampal structures were excluded from the study. Hippocampal volumes were normalized by subject intracranial volume (volume$\text{normalized} = \text{volume}_{\text{raw}} / 1,000/\text{intracranial volume}$) to correct for individual differences in head size.
**Statistical analysis**

To compare demographic statistics, Fisher’s exact tests and ANOVA with Tukey-Kramer HSD post-hoc tests were used. A linear mixed model regression was used to compare hippocampal volumes by APOE genotype ($\varepsilon2/\varepsilon3/\varepsilon4$). In accordance with previous work, 44 $\varepsilon2/\varepsilon4$ individuals were excluded from the analysis due to the opposing protective effect of the $\varepsilon2$ allele and detrimental effect of $\varepsilon4$ allele [40]. The model treated subject age, gender, and baseline diagnosis as fixed terms in the final model. Interaction terms for APOE genotype and diagnosis were also included and image acquisition site was included as a random effect term.

Hippocampal comparisons were also performed in a sample of subjects from the ADNI and AIBL cohorts who were divided into $\alpha\beta+$ and $\alpha\beta–$ participants. Pairwise multiple comparisons were corrected using a stringent Bonferroni correction method. The R statistical software environment, version 3.1.1, was used to perform all statistical analyses in RStudio. The nlme v3.1–117 [41] package was used to create linear-mixed effects models and multcomp v1.3–6 [42] package for post-hoc comparisons.

**RESULTS**

**Demographic characteristics**

In the AD group, a difference in age between APOE groups was significant ($p = 0.016$) with $\varepsilon2$ carriers being significantly older than $\varepsilon3$ carriers and $\varepsilon4$ carriers. For MCI subjects, $\varepsilon2$ and $\varepsilon3$ carriers had significantly higher MMSE scores than $\varepsilon4$ carriers ($p = 0.016$). No other demographic characteristics differed between the groups (Table 2).

**Comparing the effect of APOE genotype on hippocampal volume in the AD, and normal aging dataset (n = 1,781) and the IMAGEN study of healthy 14-year-old adolescents (n = 1,387)**

In AD patients, $\varepsilon4$ carriers had significantly smaller hippocampal volumes than non-carriers. A significant linear stepwise reduction in hippocampal volume was observed with $\varepsilon2$ carriers possessing the largest volumes, $\varepsilon3$ carriers possessing intermediate volumes and $\varepsilon4$ carriers possessing the smallest volumes. This pattern was also observed in the MCI group (Table 3). The effect of the $\varepsilon4$ allele on hippocampal volume was found to be moderately dose-dependent in AD patients [left region: Cohen’s $d = 0.10$, $p \leq 0.001$; right region: Cohen’s $d = 0.22$, $p \leq 0.0001$] and MCI subjects [left region: Cohen’s $d = 0.15$, $p \leq 0.001$; right region: Cohen’s $d = 0.19,$ $p \leq 1$].

The effect of APOE genotype on hippocampal volumes in CN individuals was not significant (left region: $p = 0.052$; right region: $p = 0.053$) and the magnitude of this non-significant difference in both regions was small (Cohen’s $d = 0.16$; effect size $r = 0.08$). This non-significant pattern was also observed in elderly individuals from the SNAC-K study, as well as in healthy adolescents from the IMAGEN study (Table 3). Figure 1 displays hippocampal volumes by APOE genotype in the AD and normal aging dataset and IMAGEN study of adolescents.

Hippocampal volumes of AD $\varepsilon4$ carriers were found to be significantly smaller than MCI, CN, and non-demented $\varepsilon4$ carriers. This pattern was also observed in MCI $\varepsilon4$ carriers who had significantly smaller volumes than CN, and non-demented $\varepsilon4$ carriers (Table 4). In carriers of the $\varepsilon2$ allele, the MCI and CN groups possessed significantly larger hippocampal volumes than $\varepsilon2$ carriers of the AD group (Table 5).

**Comparison of hippocampal volumes by ApoE $\varepsilon4$ and $\alpha\beta$ deposition**

In this analysis, $\varepsilon4$ carriers from the ADNI and AIBL cohort studies were selected and divided into $\alpha\beta+$ and $\alpha\beta–$ participants to assess if the effect of APOE $\varepsilon4$ on the hippocampus is modified by levels of $\alpha\beta$ deposition. Descriptive information for this sample is shown in Table 6. Among $\varepsilon4+$ CN individuals, hippocampal volumes between $\alpha\beta+$ and $\alpha\beta–$ participants did not significantly differ (left region: $p = 0.692$; right region: $p = 0.946$). MCI $\alpha\beta+$ participants were found to have significantly smaller hippocampal volumes than MCI $\alpha\beta–$ participants for the right hippocampus (left hippocampus: $p = 0.295$; right hippocampus: $p = 0.054$). Hippocampal volumes of MCI $\alpha\beta+$ $\varepsilon4+$ participants did not differ when compared to AD $\alpha\beta+$ $\varepsilon4+$ participants. AD $\alpha\beta+$ $\varepsilon4+$ participants possessed significantly smaller hippocampal volumes than (i) the CN $\alpha\beta–$ $\varepsilon4+$ group (left region: Cohen’s $d = -1.05$; $p \leq 0.001$; right region: Cohen’s $d = -1.03$; $p \leq 0.001$) and the (ii) CN $\alpha\beta+$ $\varepsilon4+$ group (left region: Cohen’s $d = -1.09$; $p \leq 0.001$; right region: Cohen’s $d = -1.02$; $p \leq 0.001$). The complete results for these comparisons are shown in Table 7. There was no interaction
observed between gender and ε4 status in hippocampal volumes between groups. Hippocampal differences between the different Aβ+ and Aβ− participants are shown in Fig. 2.

**DISCUSSION**

Prior neuroimaging studies of APOE ε4 have helped define our current observation of structural changes in the brain, but the mechanisms associated with the detrimental effect of APOE ε4, particularly across the different stages of AD, still remain poorly understood. With recent studies proposing a neurodevelopmental foothold of APOE ε4 on the brain [43, 44], understanding whether atrophy in AD-susceptible areas, such as the hippocampus, are attributed to pre-clinical manifestations of the disease, or whether these constitute a part of non-specific normal aging is of great importance for earlier diagnosis.

Here we present the largest cross-sectional multi-cohort study of APOE and hippocampal volume to date (n = 3,168) and discuss a number of key findings. Firstly, a linear neuroanatomic effect of the APOE genotype was observed for hippocampal volumes of AD and MCI subjects, whereby ε4 carriers presented with the lowest volumes, ε3 homozygotes possessed intermediate volumes, and ε2 carriers possessed the largest volumes. As expected, ε4 carriers in the AD and the MCI group had significantly lower hippocampal volumes when compared to CN

<table>
<thead>
<tr>
<th>ε2 Carriers (n = 344)</th>
<th>ε3 Carriers (n = 1754)</th>
<th>ε4 Carriers (n = 1070)</th>
<th>p-value</th>
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<tbody>
<tr>
<td>ε2/ε2 (n = 326)</td>
<td>ε3</td>
<td>ε4/ε3 (n = 893)</td>
<td>ε4/ε4 (n = 177)</td>
</tr>
<tr>
<td>n</td>
<td>n</td>
<td>n</td>
<td>n</td>
</tr>
<tr>
<td>Number of subjects (n (%))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>78.1 ± 6.6</td>
<td>76.9 ± 7.4</td>
<td>74.9 ± 6.7</td>
</tr>
<tr>
<td>Male (n (%))</td>
<td>4 (23.5)</td>
<td>56 (44.8)</td>
<td>112 (50.2)</td>
</tr>
<tr>
<td>Education (years)</td>
<td>12.0 ± 3.6</td>
<td>11.8 ± 5.1</td>
<td>12.2 ± 4.5</td>
</tr>
<tr>
<td>MMSE</td>
<td>21.3 ± 5.8</td>
<td>22.1 ± 4.1</td>
<td>22.1 ± 3.7</td>
</tr>
<tr>
<td>ICV (mL)</td>
<td>1437 ± 165</td>
<td>1520 ± 166</td>
<td>1532 ± 188</td>
</tr>
<tr>
<td>Number of subjects (n (%))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.1 ± 7.4</td>
<td>75.3 ± 7.5</td>
<td>74.2 ± 6.9</td>
</tr>
<tr>
<td>Male (n (%))</td>
<td>15 (2.9)</td>
<td>144 (26.7)</td>
<td>152 (29.1)</td>
</tr>
<tr>
<td>MMSE</td>
<td>27.6 ± 1.4</td>
<td>27.1 ± 1.8</td>
<td>26.8 ± 1.8</td>
</tr>
<tr>
<td>ICV (mL)</td>
<td>1491 ± 173</td>
<td>1558 ± 173</td>
<td>1559 ± 169</td>
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<tr>
<td>Number of subjects (n (%))</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>75.8 ± 6.5</td>
<td>75.9 ± 6.3</td>
<td>75.6 ± 6.2</td>
</tr>
<tr>
<td>Male (n (%))</td>
<td>28 (5.5)</td>
<td>139 (27.1)</td>
<td>84 (16.4)</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.9 ± 1.1</td>
<td>29.0 ± 1.1</td>
<td>29.1 ± 1.1</td>
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<tr>
<td>ICV (mL)</td>
<td>1506 ± 174</td>
<td>1521 ± 162</td>
<td>1518 ± 160</td>
</tr>
<tr>
<td>Number of subjects (n (%))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.9 ± 7.8</td>
<td>70.1 ± 8.6</td>
<td>69.5 ± 8.6</td>
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<tr>
<td>Male (n (%))</td>
<td>15 (3.9)</td>
<td>102 (26.7)</td>
<td>37 (9.7)</td>
</tr>
<tr>
<td>MMSE</td>
<td>29.2 ± 0.9</td>
<td>29.2 ± 1.0</td>
<td>29.1 ± 1.1</td>
</tr>
<tr>
<td>ICV (mL)</td>
<td>1478 ± 265</td>
<td>1509 ± 248</td>
<td>1522 ± 275</td>
</tr>
</tbody>
</table>

MMSE, Mini-Mental State Examination; ICV, intracranial volume; CANTAB SWM, Cambridge Neuropsychological Test Automated Battery [CANTAB] Spatial Working Memory; IQ, intelligence quotient. Data are Mean ± SD. Percentages are displayed in parentheses.
individuals and a population-based sample of elderly non-demented individuals from the SNAC-K study. These findings are in agreement with previous studies demonstrating a distinct neuroanatomic effect of APOE genotype on brain structure, as well as studies reporting smaller hippocampal volumes in MCI subjects with prodromal stages of AD. Furthermore, our finding of larger hippocampal volumes in e2 carriers in older healthy groups supports the e2 allele’s suggested effect of protection against neurodegeneration. Previous cellular models have advocated its role in a disease staving protective effect, in particular its ability to modify the neuropathological effects of Aβ accumulation [45].
Fig. 1. Hippocampal volumes and ApoE genotype in the AD and normal aging dataset and IMAGEN study. Shown are hippocampal volumes from (a) the left and (b) the right region in the AD and normal aging dataset by baseline diagnosis ApoE genotype (ε2 carriers, ε3 homozygotes, and ε4 carriers). Hippocampal volumes from (c) the left and (d) right region of healthy 14-year-old adolescents in the IMAGEN study. SNAC-K indicates Swedish National study on Aging and Care in Kungsholmen.

### Table 4

Hippocampal volume comparisons of ApoE ε4 carriers (ε++) from different subject groups

<table>
<thead>
<tr>
<th></th>
<th>AD ε4+ group (n = 223)</th>
<th>MCI ε4+ group (n = 260)</th>
<th>healthy controls ε4+ group (n = 171)</th>
<th>SNAC-K elderly ε4+ group (n = 98)</th>
<th>p-value</th>
<th>Pairwise Difference (t-value; p-value)</th>
</tr>
</thead>
</table>
| Left Hippocampus | 1.79 (0.02; 1.75–1.84) | 1.97 (0.02; 1.93–2.02) | 2.30 (0.03; 2.25–2.36) | 2.56 (0.06; 2.45–2.67) | <0.0001 | AD versus MCI = –5.7; <0.001
|             |                        |                         |                                     |                                  |         | AD versus CTL = –14.0; <0.001
|             |                        |                         |                                     |                                  |         | AD versus SNAC-K = –8.7; <0.001
|             |                        |                         |                                     |                                  |         | MCI versus CTL = –9.0; <0.001
|             |                        |                         |                                     |                                  |         | MCI versus SNAC-K = –6.3; <0.001
|             |                        |                         |                                     |                                  |         | SNACK versus CTL = 1.9; 0.208
| Right Hippocampus | 1.81 (0.02; 1.77–1.86) | 2.01 (0.02; 1.97–2.05) | 2.33 (0.03; 2.27–2.39) | 2.53 (0.06; 2.42–2.64) | <0.0001 | AD versus MCI = –6.0; <0.001
|             |                        |                         |                                     |                                  |         | AD versus CTL = –13.4; <0.001
|             |                        |                         |                                     |                                  |         | AD versus SNAC-K = –7.9; <0.001
|             |                        |                         |                                     |                                  |         | MCI versus CTL = –8.1; <0.001
|             |                        |                         |                                     |                                  |         | MCI versus SNAC-K = –5.3; <0.001
|             |                        |                         |                                     |                                  |         | SNACK versus CTL = 1.2; 0.579

Data are presented as the mean of normalized hippocampal volumes (Volume/ICV × 1000). SE with min-max is shown in parentheses.
studies have since shown that APOE e4 is linked to Aβ deposition and may exert a synergistic effect to promote cognitive decline [49, 50].

To test whether an Aβ-dependent effect of APOE e4 would be associated with greater hippocampal volume loss, we examined the combined effect of APOE e4 and Aβ on the hippocampus. We found no significant differences in hippocampal volume between CN Aβ+ e4+ individuals and CN Aβ− e4+ individuals, suggesting that in healthy

| Table 5 |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | AD e2+ group     | MCI e2+ group    | healthy controls | SNAC-K elderly   |                  |                  |
|                  | (n=17)           | (n=29)           | (n=66)           | (n=45)           |                  |                  |
|                  | e3e2 (n=17)      | e3e2 (n=29)      | e3e2 (n=64)      | e3e2 (n=42)      |                  |                  |
|                  | e2e2 (n=0)       | e2e2 (n=0)       | e2e2 (n=2)       | e2e2 (n=3)       |                  |                  |
| Left Hippocampus | 2.00 (0.11; 1.77–2.22) | 2.22 (0.06; 2.09–2.35) | 2.40 (0.04; 2.32–2.47) | 2.72 (0.08; 2.56–2.87) | 0.0003 | AD versus MCI = –2.1; 0.141 |
|                  | Right Hippocampus | 2.04 (0.12; 1.80–2.29) | 2.27 (0.06; 2.14–2.39) | 2.43 (0.04; 2.35–2.51) | 2.66 (0.08; 2.50–2.82) | 0.0035 | AD versus MCI = –3.2; 0.144 |

| Table 6 |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | AD Aβ+           | MCI Aβ+          | MCI Aβ−          | CN Aβ+           | CN Aβ−           |                  |
|                  |                  |                  |                  |                  |                  |                  |
| Age (years)      | 70.2 ± 7.3       | 72.5 ± 7.3       | 77.3 ± 4.9       | 77.1 ± 3.4       | 75.1 ± 4.0       | 0.041 |
| Male (%)         | 4 (7.1)          | 37 (66.1)        | 5 (8.9)          | 4 (7.1)          | 6 (10.7)         | 0.113 |
| MMSE             | 23 ± 1.7         | 27.2 ± 1.7       | 27.1 ± 1.9       | 29.4 ± 0.8       | 29.4 ± 0.9       | <0.001 |
| ICV (mL)         | 1556 ± 210       | 1583 ± 198       | 1494 ± 176       | 1429 ± 98        | 1591 ± 209       | 0.08 |
|                  |                  |                  |                  |                  |                  |                  |
| AIBL study (n=57)|                  |                  |                  |                  |                  |                  |
| Number of subjects (n) | 7 (12.2) | 1 (1.8) | 3 (5.3) | 20 (35.0) | 26 (45.6) | — |
| Age (years)      | 77.7 ± 7.8       | 58 ± —           | 79.7 ± 12.0      | 78.1 ± 6.5       | 71.5 ± 5.8       | 0.001 |
| Male (%)         | 1 (3.7)          | 1 (3.7)          | 1 (3.7)          | 10 (37.0)        | 14 (51.9)        | 0.296 |
| MMSE             | 19.4 ± 3.8       | 27.0 ± —         | 27.7 ± 2.5       | 27.4 ± 3.1       | 27.2 ± 4.3       | <0.001 |
| ICV (mL)         | 1466 ± 87        | 1515 ± —         | 1592 ± 301       | 1535 ± 159       | 1545 ± 165       | 0.781 |

MMSE, Mini-Mental State Examination; ICV, intracranial volume. Bolded values are significant at p < 0.05 or the p < 0.001 level. In the ADNI study, of the 95 subjects who underwent PET neuroimaging, 66 were scanned using 11F-florbetapir and 29 using 11C-Pittsburgh Compound B (PiB). In the AIBL study, all subjects underwent PET neuroimaging using PiB.

| Table 7 |
|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | CN Aβ− (n=34)    | CN Aβ+ (n=34)    | MCI Aβ− (n=10)   | MCI Aβ+ (n=27)   | AD Aβ+ (n=15)    | p-value | Pairwise Difference (Cohens d; p-value) |
|                  |                  |                  |                  |                  |                  |                  |                  |
| Left Hippocampus | 2.42 (0.052)     | 2.39 (0.053)     | 2.18 (0.095)     | 1.97 (0.04)      | 1.88 (0.078)     | <0.0001 | CN Aβ+ = –0.08; 0.692 |
|                  | 2.45 (0.056)     | 2.44 (0.058)     | 2.27 (0.01)      | 1.94 (0.043)     | 1.90 (0.084)     | <0.0001 | MCI Aβ+ = –0.42; 0.0001 |

Data are presented as the estimated marginal mean of normalized hippocampal volumes and SE in parentheses. MCI follow diagnosis was available for ADNI (0–36 months) and AIBL (0–54 months).
individuals there is no effect of APOE e4 and Aβ on the hippocampus. This is consistent with previous studies that have shown that the relationship between APOE e4, Aβ, and brain atrophy is mediated by CSF p-tau181 levels [51, 52] and, in the absence of abnormal p-tau181 levels, there is no synergistic relationship between APOE e4 and Aβ deposition. Hippocampal volumes of MCI Aβ+ e4+ individuals did not differ when compared to AD Aβ+ e4+ participants, suggesting that a similar degree of hippocampal loss that is expected in AD has already manifested in MCI subjects. Our findings are supported by empirical evidence which has shown that APOE induces intracellular degradation of Aβ peptides facilitating synaptotoxicity, neuroinflammation, and tau hyperphosphorylation [5, 53]. It is therefore possible that Aβ and the e4 in conjunction impart great levels of neuronal toxicity and injury in the presence of hippocampal neurodegeneration.

Recent studies have suggested an Aβ independent effect of the APOE e4 on neuronal integrity as another explanation for the gene’s effect on brain structure [47, 54]. A study by Dean and colleagues [44] argued that an early neurodevelopmental foothold of APOE on the brain may render individuals more susceptible to the toxic and downstream neurodegenerative effects of Aβ later in life. However, findings from our large sample of 14-year-old adolescents showed that there were no hippocampal volume differences present between the APOE groups, suggesting that e4 carriers are unlikely to be at risk in adolescence, but may perhaps develop a greater risk later in life. Similar studies, such as that of O’Dwyer and colleagues [15] reported lower hippocampal volumes in e4 carriers aged in their mid-twenties. Although differences may be attributed to the methodological approach adopted for the automated segmentation of the hippocampus, we cannot exclude the possibility of a low APOE penetrance in our young sample. Nevertheless, previous studies in younger e4 carriers have shown that APOE plays a fundamental role in modulating brain function in the absence of any differences in brain volume [47].

An important caveat when interpreting the results of this study is that the multi-cohort data was cross-sectional and a more complete understanding of how hippocampal trajectories vary with age would require longitudinal data. In particular, more neuroimaging studies need to be conducted into typical cognitive aging across the lifespan [18] in order to differentiate between brain changes that are associated with typical normal aging from those that arise from...
APOE ε4 dependent mechanisms and β-amyloidosis. Additionally, when combining data across cohort studies, it is important to consider study design differences that may complicate the interpretation of our results. For instance, the use of different AD diagnostic criteria across the different cohort studies may contribute to a level of diagnostic variability between groups. Participants from the ADNI and AIBL study were also highly educated, had few comorbidities, and were of Caucasian background. As such, future prospective studies with more representative samples should be conducted to address how these comorbidities, namely the presence of vascular disease, could potentially influence the size of the hippocampus. We demonstrated that systematic bias was not present in our dataset when comparing hippocampal volumes between APOE groups within each cohort study separately (Supplementary Material). Additional factors such as the use of two different PET radioligands meant that SUVR values of tracer uptake could not be compared as a single continuous measure. However, ongoing working groups such as the Centiloid project will further enable a more standardized approach for the direct comparability of results across different labs when different tracers and methods of analysis are employed [55].

Despite these limitations, this is first multi-cohort neuroimaging study of APOE genotype that attempts to characterize the differential risk of APOE on hippocampal volumes of subjects with varying stages of AD. Using metadata and clinical phenotyping pooled across several cohort studies, we were systematically able to demonstrate differential effects of the different APOE gene polymorphisms on hippocampal volume. An independent sample of healthy 14-year-old adolescents also provides an understanding into the role of APOE in adolescent brain neurodevelopment.

In conclusion, our findings in the largest APOE neuroimaging dataset show that hippocampal volume loss is present in patients with AD and in subjects with an increased risk of developing AD, particularly subjects with memory impairment. However, healthy older individuals did not show APOE ε4 dependent changes in the hippocampus, suggesting that the relationship between APOE ε4 and Aβ may be mediated by the presence of neurodegeneration. The same pattern was also observed in healthy young adolescents who possessed no hippocampal differences between different APOE groups. Our study thus shows hippocampal volume loss is moderated by APOE ε4 and Aβ in AD and the MCI stages of the AD pathological process. The influence of these three markers could be considered as prognostic tools in clinical trials and therapeutic interventions of AD.

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Data used in preparation of this article were obtained from the Alzheimer’s Disease Neuroimaging Initiative (ADNI) database (http://adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found on ADNI website (http://adni.loni.usc.edu/).

Data used in the preparation of this article were obtained from the Australian Imaging Biomarkers and Lifestyle Flagship Study of Ageing (AIBL) funded by the Commonwealth Scientific and Industrial Research Organisation (CSIRO), which was made available at the ADNI database (http://www.loni.usc.edu/ADNI). The AIBL researchers contributed data but did not participate in analysis or writing of this report. AIBL researchers can be found on the AIBL website (http://aibl.csiro.au).

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SUPPLEMENTARY MATERIAL

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REFERENCES


