Apolipoprotein E Polymorphism and Brain Morphology in Mild Cognitive Impairment

Philipp A. Thomann\textsuperscript{a} Ann-Sophie Roth\textsuperscript{a} Vasco Dos Santos\textsuperscript{a} Pablo Toro\textsuperscript{a} Marco Essig\textsuperscript{b} Johannes Schröder\textsuperscript{a}

\textsuperscript{a}Section of Geriatric Psychiatry, University of Heidelberg, and \textsuperscript{b}German Cancer Research Center, Heidelberg, Germany

Key Words
Apolipoprotein E \textbullet{} Alzheimer’s disease \textbullet{} Mild cognitive impairment \textbullet{} Magnetic resonance imaging \textbullet{} Voxel-based morphometry \textbullet{} Medial temporal lobe \textbullet{} Hippocampus

Abstract
Background: The apolipoprotein E (ApoE) genotype has been confirmed as the major genetic risk factor for late-onset Alzheimer’s disease (AD). How the ApoE genotype and brain morphology relate to each other is only partly understood, particularly in mild cognitive impairment, the assumed prestage of AD. Methods: A total of 83 subjects with mild cognitive impairment (aging-associated cognitive decline criteria) were investigated with optimized voxel-based morphometry (VBM). We tested for differences in gray and white matter densities between groups according to their ApoE status, i.e. e4 allele noncarriers (n = 42), subjects with one e4 allele (n = 27) and subjects with two e4 alleles (n = 14). Results: In individuals carrying two e4 alleles, VBM revealed a decline in gray matter density predominantly in the medial temporal lobe region. Subjects with a single copy of the e4 allele exhibited gray matter atrophy in the right inferior frontal gyrus. With respect to white matter changes, atrophy was only found in subjects homozygous for e4 and confined to the right superior and middle temporal gyrus. Conclusion: Our findings support the hypothesis that the ApoE genotype in mild cognitive impairment might be associated with structural changes typically found in the early stages of AD.

Introduction
Sporadic Alzheimer’s disease (AD) is the most common cause of dementia among the elderly, and neuro-pathologically characterized by the presence of large numbers of neurofibrillary tangles and neuritic plaques within the cerebral cortex. After advanced age itself, the apolipoprotein E (ApoE) allele e4 has been confirmed as the strongest risk factor for developing AD [1]. ApoE is a lipid-transport glycoprotein and the primary apolipoprotein observed in the central nervous system, where it is synthesized in a variety of cell types [2]. ApoE is encoded by a polymorphic gene localized on chromosome 19, and exists as 3 alleles designated as e2, e3 and e4, which are responsible for the 3 major isoforms ApoE1, ApoE2 and ApoE4, respectively [3]. Although the precise role of ApoE in the pathogenesis of AD is still poorly un-
understood, emerging data strongly suggest that ApoE4 in particular contributes to this disease by interacting with different factors through various pathways [4].

Structural neuroimaging provides us with increasing evidence that ApoE status contributes to brain morphometric changes in that, when compared to subjects negative for e4, both heterozygous and homozygous e4 carriers show atrophy predominantly in AD-typical cortical regions, i.e. the substructures of the medial temporal lobe [5]. However, while the effect of ApoE polymorphism on brain structure has been addressed in numerous studies of both healthy controls and AD patients, it still remains underinvestigated in mild cognitive impairment, the assumed prestage of AD.

The objective of the present study was therefore to examine the impact of the ApoE genotype on cerebral morphology in subjects with mild cognitive impairment. In order to account for potential associations throughout the entire brain, we used optimized voxel-based morphometry (VBM) for the analysis of structural data. In the first VBM study, Pennanen et al. [6] revealed MCI subjects homozygous for the e4 allele to be characterized by atrophy pronounced in the right amygdala and the right parahippocampal gyrus. In their study, the right parahippocampal gyrus was, although to a lower extent, also atrophic in cases heterozygous for e4 when compared to e4 noncarriers. However, putative white matter alterations were not addressed. Since previous studies on myelination and fiber integrity indicated white matter changes to be at least partly related to presence of the e4 allele [7, 8], we conducted an additional VBM analysis of white matter segments.

### Table 1. Demographic and clinical characteristics of the three ApoE groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>e4−/− (1) (n = 42)</th>
<th>e4+/− (2) (n = 27)</th>
<th>e4+/+ (3) (n = 14)</th>
<th>F value</th>
<th>d.f.</th>
<th>p value</th>
<th>Duncan test (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (female/male)</td>
<td>27/15</td>
<td>15/12</td>
<td>9/5</td>
<td>n. sig.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>71.48 ± 5.19</td>
<td>72.19 ± 4.90</td>
<td>69.63 ± 3.48</td>
<td>1.508</td>
<td>2, 80</td>
<td>0.23</td>
<td>1 = 2 = 3</td>
</tr>
<tr>
<td>Education, years</td>
<td>10.02 ± 2.23</td>
<td>10.04 ± 2.53</td>
<td>10.00 ± 1.6</td>
<td>0.001</td>
<td>2, 80</td>
<td>0.99</td>
<td>1 = 2 = 3</td>
</tr>
<tr>
<td>MMSEb</td>
<td>26.76 ± 1.41</td>
<td>24.44 ± 1.63</td>
<td>26.29 ± 1.44</td>
<td>0.700</td>
<td>2, 80</td>
<td>0.50</td>
<td>1 = 2 = 3</td>
</tr>
<tr>
<td>Verbal fluencyb</td>
<td>−0.61 ± 1.02</td>
<td>−0.46 ± 1.18</td>
<td>−0.71 ± 1.07</td>
<td>0.296</td>
<td>2, 80</td>
<td>0.74</td>
<td>1 = 2 = 3</td>
</tr>
<tr>
<td>Boston Naming Testb</td>
<td>−0.58 ± 1.18</td>
<td>−0.97 ± 1.66</td>
<td>−1.02 ± 1.72</td>
<td>0.849</td>
<td>2, 80</td>
<td>0.43</td>
<td>1 = 2 = 3</td>
</tr>
<tr>
<td>Word list (immediate recall)b</td>
<td>−1.74 ± 1.33</td>
<td>−1.53 ± 1.10</td>
<td>−1.77 ± 1.80</td>
<td>0.233</td>
<td>2, 80</td>
<td>0.79</td>
<td>1 = 2 = 3</td>
</tr>
<tr>
<td>Word list (delayed recall)b</td>
<td>−1.58 ± 1.35</td>
<td>−1.47 ± 1.21</td>
<td>−2.37 ± 1.07</td>
<td>2.587</td>
<td>2, 80</td>
<td>0.08</td>
<td>1, 2 &gt; 3</td>
</tr>
<tr>
<td>Word list (recognition)b</td>
<td>−1.66 ± 2.83</td>
<td>−1.84 ± 2.91</td>
<td>−1.73 ± 2.51</td>
<td>0.032</td>
<td>2, 80</td>
<td>0.97</td>
<td>1 = 2 = 3</td>
</tr>
<tr>
<td>Constructional praxisb</td>
<td>−0.27 ± 1.44</td>
<td>−0.64 ± 1.59</td>
<td>−0.02 ± 1.34</td>
<td>0.934</td>
<td>2, 80</td>
<td>0.40</td>
<td>1 = 2 = 3</td>
</tr>
<tr>
<td>Constructional praxis (recall)b</td>
<td>−1.00 ± 1.62</td>
<td>−0.98 ± 1.74</td>
<td>−1.57 ± 1.21</td>
<td>0.766</td>
<td>2, 80</td>
<td>0.47</td>
<td>1 = 2 = 3</td>
</tr>
</tbody>
</table>

Values presented as means ± SD; d.f. = degrees of freedom; n. sig. = not significant.

Materials and Methods

Subjects

A total of 83 patients with mild cognitive impairment as defined by the concept of aging-associated cognitive decline (AACD) [9] participated in the study. The AACD concept considers declines over a broad potential range of cognitive domains, namely memory and learning, attention and concentration, thinking, language and visuospatial functioning. Longitudinal studies confirmed the AACD criteria as a stable and broad concept for mild cognitive impairment that was instrumental in the prediction of the future development of dementia [10, 11]. Study subjects were recruited consecutively through the Section of Geriatric Psychiatry at the University of Heidelberg, Germany. Investigations were approved by the local ethics committee and written informed consent was obtained from all participants after the procedures of the study had been fully explained.

Clinical Evaluation and ApoE Genotyping

Clinical evaluation included ascertainment of personal and family history and thorough physical, neurological and neuropsychological examination. None of the participants had a lifetime history of neurological or severe medical illness, head injury or substance abuse. Global cognitive deficits were assessed using the Mini-Mental State Examination (MMSE) [12]. Cognitive performance was investigated with a standardized extensive neuropsychological test battery (Consortium to Establish a Registry for Alzheimer’s Disease – CERAD) [13].

In all study subjects, genomic DNA was extracted from whole blood using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) following the manufacturer’s instructions. ApoE genotyping using the LightCycler technology was performed as previously described [14].

MRI Acquisition

MR images were obtained on a 1.5-T clinical MR scanner (Magnetom Vision, Siemens Medical Solutions, Erlangen, Ger-
A T<sub>1</sub>-weighted coronal high-resolution volume acquisition was performed (repetition time = 10 ms, echo time = 4 ms, flip = 13°, field of view = 250 mm, matrix = 256 × 256, 128 contiguous slices, 1.8 mm) and supplemented with axial T<sub>2</sub>-weighted and FLAIR images in order to exclude secondary causes of cognitive impairment and ischemic changes, respectively. Vascular changes were quantified in the T<sub>2</sub>-weighted sequences according to the European Task Force on Age-Related White Matter Changes (ARWMC) rating scale, and interpreted in relation to the amnestic risk factors. Amyloid angiopathic changes were evaluated on the gradient echo sequences. Patients with pronounced microvascular changes (ARWMC grade 1–2) were excluded from further evaluation. The acquired images were anonymized by the removal of the Digital Imaging and Communication in Medicine header information.

Voxel-Based Morphometry

SPM2 software (http://www.fil.ion.ucl.ac.uk/spm) was used for voxel-based analysis. Initially, all structural images were visually checked for artifacts, and the origin was placed on the anterior commissure. A previously described optimized VBM protocol [15] was applied in order to minimize the probability of misclassifications within the tissue segmentation. Briefly, this method comprises the following steps: (1) creation of a customized group and tissue specific template; (2) segmentation of native MRI images into tissue classes; (3) normalization of gray and white matter images to the group specific template; (4) reapplication of calculated normalization parameters to the structural images and reslicing them to a voxel size of 1 × 1 × 1 mm<sup>3</sup>; (5) resegmentation of normalized structural images; (6) smoothing of tissue segments with a 10-mm full-width at half-maximum Gaussian kernel.

Statistical Analysis

SPSS for Windows (version 14) was used for statistical analysis of demographical and clinical data; p values less than 0.05 were considered significant.

According to their ApoE status, subjects were assigned to the following 3 groups: ε4 allele noncarriers (ε4–/–, n = 42), subjects heterozygous for the ε4 allele (ε4+/–, n = 27) and subjects homozygous for the ε4 allele (ε4++/+, n = 14). We tested for brain morphological group differences including potential confounding factors, i.e. age, gender, and education as nuisance covariates. In order to control the rate of false-positive results, t maps were thresholded at p < 0.05, corrected for multiple comparisons (family-wise error correction over the whole brain as defined in the SPM software), with an extend threshold of 50 voxels.

Results

Demographic and clinical characteristics of the study sample are reported in table 1. According to statistical analysis, the 3 groups that were either negative, heterozy-

Fig. 1. Brain regions associated with ApoE genotype in AACD/mild cognitive impairment. a Less gray matter in ε4+/– versus ε4–/–. b Less gray matter in ε4+/+ versus ε4–/–. c Less gray matter ε4+/+ versus AACD ε4+/–. d Less white matter in ε4+/+ versus ε4–/–. Differences significant at p < 0.05, corrected for multiple comparisons; extent threshold = 50 voxels.
gous or homozygous for the e4 allele did not differ significantly with respect to age, gender, education or global cognitive functioning.

When compared to e4 allele noncarriers, subjects with a single e4 allele showed a significantly decreased gray matter density in the right inferior frontal gyrus (fig. 1a; table 2). Patients homozygous for the e4 allele exhibited, when contrasted to e4 allele noncarriers, gray matter reduction in the left hippocampus, parahippocampal gyrus, and amygdala as well as in the right amygdala (fig. 1b; table 2). In subjects either homozygous or heterozygous for the e4 allele, the former group was characterized by reduced gray matter density in the left entorhinal cortex (fig. 1c; table 2).

In VBM analysis of white matter segments, individuals homozygous for the e4 allele showed reduced white matter density in the right superior and middle temporal gyrus when compared to e4 allele noncarriers (fig. 1d; table 2). In contrast, no significant differences in white matter segments arose between e4 allele noncarriers and subjects heterozygous for the e4 allele, or between the latter group and homozygous e4 allele carriers.

**Discussion**

This study examined the influence of the ApoE genotype on brain morphology in subjects with mild cognitive impairment by using optimized VBM of both gray and white matter segments. Cerebral gray matter atrophy was found to be most prominent in individuals homozygous for the e4 allele and included the left hippocampus, parahippocampal gyrus and amygdala, and the right amygdala. The ApoE impact appeared to be dose dependent, with homozygous e4 allele carriers showing a lower gray matter density in the right entorhinal cortex when compared to those heterozygous for the e4 allele. Compared to e4 allele noncarriers, subjects with a single e4 allele showed a significantly decreased gray matter density in the right inferior frontal gyrus. Changes in white matter were restricted to the comparison between homozygous e4 allele carriers and subjects negative for e4, comprising a reduced density of the right superior and middle temporal gyrus in the former group.

Our finding of ApoE-related medial temporal lobe atrophy corroborates the results of recent structural neuroimaging studies, which demonstrated significantly smaller hippocampal volumes in e4 allele carriers with mild cognitive impairment [16–18]. In line with our finding of a gene dose effect, the 2 studies, which accounted for the number of e4 alleles, revealed hippocampal atrophy to be most prominent in individuals homozygous for e4 with heterozygous e4 allele carriers ranking in between [16, 17]. The mentioned studies performed region of interest measurement, which is based upon the a priori definition of specific brain regions by a neuroanatomically skilled rater. While region of interest analysis is time-consuming, observer dependent and restricted to specific cerebral structures, somewhat newer automated computer-based imaging techniques like VBM enable us to reliably and rapidly assess volume or shape changes throughout the entire brain [19]. In the present study, whole brain analysis seemed to be particularly suitable to us, since structural imaging data on healthy controls and AD patients suggest the ApoE genotype to not only influence hippocampal morphology but also to affect other cerebral sites, e.g. parahippocampal white matter [20], frontal and temporal cortical fields, and the cerebellum [21], the corpus callosum and temporal lobe white matter [8], frontal white matter [7] and whole brain volume [22].

With respect to gray matter changes, our findings are generally in line with those of Pennanen et al. [6], who used optimized VBM to determine the association between ApoE status and brain morphology in a group of 51 subjects with mild cognitive impairment. They demonstrated gray matter atrophy to be most prominent in

![Table 2. ApoE genotype and regional brain atrophy in subjects with AACD/mild cognitive impairment](image-url)

<table>
<thead>
<tr>
<th>Anatomical structure</th>
<th>Cluster size, voxels</th>
<th>Peak coordinates (x, y, z)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gray matter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AADD e4+/– versus AACD e4+/–</td>
<td>84 5.11 50, 17, 15</td>
<td></td>
</tr>
<tr>
<td>Right inferior frontal gyrus (BA 44)</td>
<td>84 5.11 50, 17, 15</td>
<td></td>
</tr>
<tr>
<td>AADD e4+/– versus AACD e4+/+</td>
<td>771 5.36 19, –6, –16</td>
<td></td>
</tr>
<tr>
<td>Left hippocampus, extending to amygdala and parahippocampal gyrus (BA 36, BA 28)</td>
<td>2,958 5.91 –29, –13, –18</td>
<td></td>
</tr>
<tr>
<td>Right amygdala</td>
<td>771 5.36 19, –6, –16</td>
<td></td>
</tr>
<tr>
<td>AADD e4+/+ versus AACD e4+/+</td>
<td>68 5.52 –16, –6, –26</td>
<td></td>
</tr>
<tr>
<td>Left entorhinal cortex (BA 28)</td>
<td>68 5.52 –16, –6, –26</td>
<td></td>
</tr>
<tr>
<td><strong>White matter</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AADD e4+/– versus AACD e4+/+</td>
<td>178 5.02 39, –44, 6</td>
<td></td>
</tr>
<tr>
<td>Right superior temporal gyrus, extending to middle temporal gyrus</td>
<td>178 5.02 39, –44, 6</td>
<td></td>
</tr>
</tbody>
</table>

BA = Brodmann’s area; height threshold p < 0.05, corrected for multiple comparisons; extent threshold = 50 voxels.
homozygous $e_4$ allele carriers and to mainly occur in the substructures of the medial temporal lobe. In contrast to Pennanen et al. [6], our observed relationship between brain structural changes and ApoE genotype remained significant after correcting for multiple comparisons over the whole brain, a statistically stringent step that allows for controlling the rate of false-positive results. Hence, in our patients with mild cognitive impairment, morphological alterations related to ApoE status seem to appear to a greater extent, a finding that might be related to aspects of sample size (n = 83 in the present study vs. n = 51) and of subject selection. As indicated by a mean MMSE of 24 points, subjects enrolled by Pennanen et al. [6] most likely were in a more advanced stage of the disease than our patients (mean MMSE of ~26.5 points). This is of importance for the comparison of the results, since the ApoE genotype has been reported to be more influential in the very early, initial stages of the neurodegenerative processes, and might disappear or be masked later in the course of AD [5].

In subjects heterozygous for the $e_4$ allele, gray matter loss was confined to the right inferior frontal gyrus. Atrophic changes in the frontal cortex were also described by Wishart et al. [21], who compared whole-brain gray matter density in cognitively unaffected individuals either having a single $e_4$ copy or being $e_4$ negative.

To the best of our knowledge, this is the first report on the putative association between the ApoE genotype and both gray and white matter changes. While the present findings of gray matter alterations are consistent with previous results in subjects with mild cognitive impairment, the question whether the number of $e_4$ alleles also has an impact on cerebral white matter structure has so far not been addressed in this patient group. From a neuropathological standpoint, our result of a reduced temporal lobe white matter density in homozygous $e_4$ allele carriers seems plausible since this region is known to be affected early in the course of AD [23]. However, whether the respective white matter changes are due to ApoE effects in the central nervous system, or are secondary to destructive processes in earlier affected AD typical cortical regions, remains unsettled.

In conclusion, we showed ApoE status to significantly influence gray and white matter volume in subjects with mild cognitive impairment. The impact of the ApoE genotype was found to be pronounced in the temporal lobe and appeared to be dose dependent, i.e. atrophy increased with the number of $e_4$ alleles. One weak point of our study is that we did not include healthy comparison subjects; the interaction between genotype status and diagnosis (AACD vs. healthy controls) would have been conclusive in order to address the question of whether ApoE-related brain morphological changes remain stable or are rather altered by initial disease-related pathological processes.

The potential relevance of the ApoE genotype in the course of AD has been underlined by a recent longitudinal VBM study in mild cognitive impairment, which revealed higher baseline atrophy in $e_4$ allele carriers and, even to a greater extent, in those $e_4$-allele-positive individuals who had developed manifest AD at observed follow-up [24].

The question of whether the combination of neuroimaging and ApoE genotyping will facilitate an early diagnosis of AD needs to be addressed in larger prospective studies, including healthy controls and cognitively impaired subjects, and applying different imaging techniques.

References


