CHAPTER 3

Impact of APOE ε4 and family history of dementia on grey matter atrophy in cognitively healthy middle-aged adults


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ABSTRACT

The apolipoprotein E ε4 allele (APOE4) and family history of dementia (FH) are well-known risk factors for the development of sporadic Alzheimer’s disease (AD). We assessed the effects of these risk factors on grey matter (GM) volume in 295 cognitively healthy middle-aged community-dwelling subjects. Voxel-based morphometry was used to study GM volume differences between high- and low-risk subjects, based on APOE4 carriership (n = 74), first-degree FH (n = 228) or both (n = 62). No significant results were found using a corrected p value. Using a more lenient threshold (p < 0.001 and minimum cluster size of 100 voxels), APOE4 carriers had reduced GM in the striatum compared to non-carriers. Subjects with FH had reduced GM in right precuneus compared to subjects without FH. Maternal and paternal FH provided similar atrophy patterns. APOE4 carriers with FH had GM reductions in bilateral insula compared to subjects with neither APOE4 nor FH. We conclude that a family history of dementia and APOE4 carriership are both associated with regional GM decreases in cognitively healthy middle-aged subjects, with differential effects on brain regions typically affected in AD.
INTRODUCTION

In Alzheimer’s disease (AD), the onset of symptoms is preceded by a pre-clinical phase with neurochemical, neuropathological, functional and structural brain changes [1]. Identifying pre-symptomatic brain atrophy patterns divergent from those associated with normal ageing is important as they may serve as a biomarker for early diagnosis. Subjects with an increased genetic risk for AD due to a first degree family history of dementia (FH) [2,3] or carriership of an apolipoprotein E ε4 allele (APOE4) [4] have higher chances of developing the disease. In the present study we explored whether these risk factors are associated with structural brain changes in non-demented middle-aged subjects.

Previous studies examining the effects of APOE4 on grey matter (GM) in healthy individuals have yielded inconsistent results. Some have reported GM atrophy in the medial temporal and frontotemporal regions in APOE4 carriers [5–8]; whereas others failed to find APOE4-related effects [9–12]. Few studies have examined the effects of FH on regional GM in non-demented individuals. Two relatively small voxel-based morphometry (VBM) studies found decreased GM in precuneus, parietal and frontal areas in subjects with parental family history of AD [13,14]. In both studies, FH risk was modulated by the gender of the affected parent, with more atrophy associated with maternal FH. A VBM study on subjects with both parents affected with dementia, showed reduced GM in temporal cortex and striatum [15]. One study reported decreased cortical thickness in the hippocampal region associated with FH, independent of APOE4 genotype [16]. Another did not find significant cortical thickness decreases associated with parental history of AD, but did find increased age-associated cortical atrophy in maternal AD offspring [11]. One study could only identify longitudinal effects of FH on GM without any parent-of-origin effect [10].

In this study, we used VBM to study the effects of APOE4, FH and their interaction on GM atrophy in a large cohort of cognitively healthy middle-aged subjects. Additionally, we explored differences between maternal and paternal FH.

METHODS

Subjects

We studied 295 cognitively healthy middle-aged adults enrolled in the Gipuzkoa Alzheimer Project (GAP) study, a longitudinal study on preclinical AD recruiting subjects from the general population. Inclusion criteria for the GAP study were community-dwelling middle-aged subjects without dementia and a global Clinical Dementia Rating (CDR) score ≤ 0.5. Exclusion criteria were any significant neurologic, systemic or psychiatric disorder that could cause cognitive impairment. Subjects were recruited between June 2011 and January
2013 via advertisements in the local media and presentations at the local Alzheimer's Family Association. The local Ethics Committee approved the study protocol and all subjects gave written informed consent. All subjects underwent thorough neurological and neuropsychological examination and high-resolution structural and functional MRI scans. Information on FH was obtained by structured interviews with a neurologist. During these interviews, disease history of all relatives with dementia was reconstructed including symptoms, age of onset, time course and medical diagnosis. Accordingly, FH was classified as either probably due to AD or other dementia (including frontotemporal dementia, vascular or ‘mixed’ dementia, senile dementia and unclear cases). APOE4 genotype was determined using one-stage PCR as previously described [17] and dichotomized as no APOE4 allele (APOE4-) or at least one APOE4 allele (APOE4+). In the present study we examined baseline data from the GAP study and included all subjects with a good quality 3D T1 structural MRI, known APOE4 genotype and either a first degree family history of dementia (FH+) or no family history of dementia (FH-). In the FH+ group we included all subjects with a first degree family member with any type of dementia, regardless of age of onset. In the FH- group we included all subjects who did not report a first degree or second degree family member with dementia, without considering the age at death of the family members. We excluded subjects with only second degree family history of dementia (N = 44).

**MRI acquisition**

Whole-brain scans were obtained using a 3 T scanner (Siemens Magnetom Tim Trio) using a 32-channel head coil. Isotropic structural 3D T1-weighted images were acquired using a sagittal magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence (1.25 mm x 1.25 mm x 1.25 mm voxels, repetition time (TR) = 2300 ms, echo time (TE) = 2.86 ms, inversion time (TI) = 900 ms, flip angle = 9 degrees), which were used for VBM analysis. A 2D fat-saturated FLAIR sequence (0.9 mm x 0.9 mm x 3 mm voxels, TR = 9000 ms, TE = 79 ms, TI = 2500 ms) was acquired for the visual rating of white matter hyperintensities (WMH).

**Voxel-based morphometry**

The structural 3D T1 images were segmented using the VBM8 toolbox (Gaser, Department of Psychiatry, University of Jena, Germany) as implemented in Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology, London, UK) running in MATLAB 2011a (MathWorks Inc., Natick, MA, USA). Default parameters of VBM8 were used unless specified otherwise. The VBM8 toolbox provides an integrated pre-processing pipeline for image segmentation including nonlinear DARTEL warping to Montreal Neurological Institute (MNI) space [18]. Resulting GM images (1.5 mm³ voxels)
were modulated for non-linear effects only, compensating for volume changes during normalization while correcting for brain size. Images were smoothed using an isotropic Gaussian filter of 8 mm full-width at half-maximum (FWHM). To limit the analysis to areas of GM, absolute threshold masking with a threshold of 0.2 was used. After processing, the quality of the segmentations was visually checked and none had to be excluded. Total GM, white matter (WM) and total intracranial volumes (TIV, i.e. GM + WM + CSF) were derived from segmented images in native space.

WMH were rated on FLAIR images using the three-point Fazekas scale ranging from 0 (no WMH) to 3 (large confluent areas of WMH) [19].

**Statistical Analysis**

GM differences between high and low risk groups were assessed by voxel-wise statistical comparisons using the general linear model (GLM) implemented within SPM8. APOE4 genotype (dichotomized by the presence of an APOE4 allele) and FH were entered in a 2 x 2 full factorial design adjusting for age and gender. Additionally, interactions of FH and APOE4 with age were modelled. For FH, parent of offspring effects were examined in a second 3 x 2 full factorial design with FH (FH, maternal FH (FHm), paternal FH (FHp)) and APOE4, adjusting for age and gender. We compared FH- to FHm (N = 138) and FHp (N = 58) separately and FHm to FHp directly.

Since we were able to examine a large sample of healthy subjects, we incorporated the entire brain in our analysis, without making use of an inclusive mask. Statistical maps were first examined with a cluster level threshold corrected for multiple comparisons (p < 0.05, derived from uncorrected voxel threshold of p < 0.001 with minimum cluster size of 501 voxels), derived from Monte Carlo simulations (3dClutSim, AFNI, http://afni.nimh.nih.gov). Since we expected to find only subtle GM volume changes to be related to AD risk, we also performed an exploratory analysis with a more lenient thresholding approach: uncorrected voxel level threshold of p < 0.001 with a minimum cluster size of 100 voxels. We only considered supratentorial clusters.

One-way analysis of variance, Kruskall-Wallis or Chi-square tests were used when appropriate to compare the four groups (FH-APOE4-; FH-APOE4+; FH+APOE4-; FH+APOE4+) on demographic and clinical information using SPSS (version 20; IBM) with p < 0.05 considered statistically significant.
RESULTS

Characteristics of subjects are summarized in Table 1. 25% of participants were APOE4 carriers and 77% had a first degree FH of dementia. There were small but statistically significant differences in age and MMSE between groups. The FH+APOE4+ group was younger than the FH-APOE4- and FH-APOE4+ groups. The MMSE was lower in FH-APOE4- than FH+APOE4+. There were no statistically significant group differences in gender, years of education, total GM and WM, TIV and WMH.

Table 1: Population characteristics by family history and APOE4 groups.

<table>
<thead>
<tr>
<th></th>
<th>FH- APOE4-</th>
<th></th>
<th>FH+ APOE4-</th>
<th></th>
<th>FH+ APOE4+</th>
<th></th>
<th>p overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>N, % in FH subgroup</td>
<td>55 (82)</td>
<td>12 (18)</td>
<td>166 (73)</td>
<td>62 (27)</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>58.4 ± 7.0</td>
<td>61.1 ± 9.9</td>
<td>56.5 ± 6.6</td>
<td>54.9 ± 6.0</td>
<td>&gt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex, % male</td>
<td>31 (56)</td>
<td>6 (50)</td>
<td>64 (39)</td>
<td>27 (43)</td>
<td>0.10</td>
<td></td>
<td></td>
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<tr>
<td>Education, y</td>
<td>14.4 ± 3.6</td>
<td>12.8 ± 3.8</td>
<td>13.7 ± 3.6</td>
<td>14.3 ± 3.6</td>
<td>0.35</td>
<td></td>
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<td>MMSE</td>
<td>28.4 ± 1.1</td>
<td>28.9 ± 1.1</td>
<td>28.7 ± 1.2</td>
<td>28.9 ± 1.1</td>
<td>0.03</td>
<td></td>
<td></td>
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<tr>
<td>TAVEC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Free short delay</td>
<td>10.3 ± 3.0</td>
<td>11.4 ± 2.8</td>
<td>10.8 ± 2.9</td>
<td>10.9 ± 3.3</td>
<td>0.68</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free long delay</td>
<td>10.5 ± 3.0</td>
<td>11.2 ± 3.0</td>
<td>11.0 ± 3.1</td>
<td>11.4 ± 3.4</td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMT-A</td>
<td>34.0 ± 13.2</td>
<td>34.4 ± 5.6</td>
<td>34.9 ± 13.7</td>
<td>32.4 ± 9.8</td>
<td>0.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMT-B</td>
<td>70.8 ± 30.8</td>
<td>72.9 ± 39.5</td>
<td>77.0 ± 45.3</td>
<td>76.7 ± 36.8</td>
<td>0.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boston Naming Test</td>
<td>55.0 ± 3.9</td>
<td>55.4 ± 3.0</td>
<td>54.7 ± 4.4</td>
<td>55.8 ± 3.6</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDR</td>
<td>0.02 ± 0.1</td>
<td>0.04 ± 0.1</td>
<td>0.04 ± 0.1</td>
<td>0.04 ± 0.1</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TIV, ml</td>
<td>1428 ± 131</td>
<td>1374 ± 88</td>
<td>1387 ± 136</td>
<td>1398 ± 129</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMV, ml</td>
<td>642 ± 52</td>
<td>617 ± 52</td>
<td>628 ± 57</td>
<td>633 ± 54</td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WMV, ml</td>
<td>541 ± 65</td>
<td>512 ± 30</td>
<td>526 ± 61</td>
<td>537 ± 65</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fazekas score</td>
<td>0.56 ± 0.7</td>
<td>0.42 ± 0.5</td>
<td>0.54 ± 0.6</td>
<td>0.52 ± 0.7</td>
<td>0.95</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD or count (%). FH- = no family history of dementia; FH+ = first degree relative with dementia; MMSE = mini mental state examination; TAVEC = spanish version of California Verbal Learning Test; TMT; trail making test; CDR = clinical dementia rating; TIV = total intracranial volume; GMV = grey matter volume; WMV = white matter volume.

Effects of FH and APOE4 on grey matter atrophy

No significant group differences were found for either APOE4 or FH using the threshold corrected for multiple comparisons. Using a more lenient threshold ($p < 0.001$ uncorrected with minimal cluster size of 100 voxels), both APOE4+ and FH+ were associated with
GM decreases in several regions (Figure 1; Table 2). Subjects with APOE4+ had less GM in striatum bilaterally but more GM in the right superior frontal gyrus when compared to APOE4- subjects. Compared to FH-, subjects with FH+ had reduced GM in the right precuneus. There were no regions of decreased GM in FH- compared to FH+. Percent difference maps of the main effect comparisons are displayed in Supplementary Figure 1.

Both subjects with maternal (FHm) and paternal (FHp) dementia had the same cluster of decreased GM in right precuneus when compared to FH-. Additionally, F Hp showed a small cluster of decreased GM in the right insula when compared to FHm (p < 0.001 uncorrected, details not shown). We performed an additional analysis with more strict family history encoding, including only subjects whose parents both reached the age of 70 in the FH- group (N = 34) and subjects who had dementia likely to be due to AD with an age of onset below 75 and other parent reaching at least age of 70 in the FHm (N = 34) and F Hp (N = 25) groups. This resulted in the same cluster of decreased GM in the right precuneus for the whole group, as well as for maternal and paternal FH separately, provided the threshold for significance was lowered even further in the VBM analysis (Supplementary Table 1). At this lower threshold, additional decreases in GM associated with FH+ were found.

**Figure 1: Effects of APOE4 and family history (FH) on GM atrophy, assessed by voxel-based morphometry.**

In green decreased GM in striatum of APOE4 carriers compared to non-carriers. In red decreased GM in right precuneus for FH+. In blue decreased GM in bilateral insular regions for highest risk subjects (FH+ APOE4+) versus lowest risk subjects (FH- APOE4-). Background image is the population average. Colour bar range is based on Z scores.
Table 2: Brain regions with grey matter atrophy.

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Brain region</th>
<th>Coordinates x,y,z (mm)</th>
<th>Cluster extent (voxels)</th>
<th>Z score</th>
<th>p value uncorrected</th>
</tr>
</thead>
<tbody>
<tr>
<td>More atrophy in FH+ compared to FH-</td>
<td>Precuneus R</td>
<td>24, -72, 37</td>
<td>261</td>
<td>3.73</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>More atrophy in APOE4+ compared to APOE4-</td>
<td>Caudate R</td>
<td>21, 6, 16</td>
<td>157</td>
<td>3.94</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Caudate L</td>
<td>-16, 8, 19</td>
<td>231</td>
<td>3.73</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>More atrophy in APOE4- compared to APOE4+</td>
<td>Superior frontal</td>
<td>14, -3, 72</td>
<td>112</td>
<td>4.04</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>gyrus L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction FH*APOE4</td>
<td>Parahippocampal</td>
<td>-28, -61, -9</td>
<td>207</td>
<td>4.02</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>gyrus L</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More atrophy in FH+APOE4+ compared to FH-APOE4-</td>
<td>Insula L</td>
<td>-38, -7, 3</td>
<td>1184</td>
<td>4.49</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Insula R</td>
<td>40, -10, 7</td>
<td>273</td>
<td>3.86</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

GM = grey matter; FH = family history of dementia; FH+ = first degree relative with dementia; L = left hemisphere; R = right hemisphere.

There was an interaction between FH and APOE4 on GM volume in the left parahippocampal gyrus, which was driven by decreased GM volume for APOE4+ in FH- only. Although we did not find other FH by APOE4 interactions at our statistical threshold, when we directly compared FH status within APOE4+ by choosing the appropriate contrasts in the existing model, FH+ had decreased GM volume in the right precuneus compared to FH-. In APOE4 non-carriers, subjects with FH+ had decreased GM volume in the left insula and bilaterally in the temporal region compared to FH- (p < 0.001 uncorrected, details not shown). Finally, we compared subjects with the highest risk (FH+APOE4+) with the lowest risk subjects (FH-APOE4-) and found clusters of decreased GM volume bilaterally in the insula for the high-risk group (Figure 1; Table 2).

Interaction with age

We observed an interaction effect between age and APOE4 carrihership on GM volume in the left temporal and occipital lobe (Figure 2A). Subsequent analysis showed that only APOE4- subjects had GM loss with advancing age in these regions, compared to APOE4+ subjects (Figure 2B-C). There was no interaction effect between age and FH on GM volume.
DISCUSSION

This study contributes to the evidence that increased risk for AD according to APOE4 genotype or FH is already associated with GM reductions in various brain regions in cognitively healthy middle-aged subjects, although the effect is moderate and only discovered at an uncorrected threshold. We found effects of these AD-risk factors on the precuneus, striatum and insular regions, which are typically affected in AD.

Family history is associated with precuneus atrophy

Having a first degree relative with dementia was associated with atrophy of the right precuneus. The precuneus is notoriously involved in AD, especially in patients with early onset AD [20]. Previous VBM studies examining cognitively healthy subjects have also reported decreased GM volume in AD-related areas [13–15], albeit some only longitudinally [10]. In contrast to two studies who reported relatively more GM atrophy for FHm than FHp in frontal, precuneal and temporal regions [13,14] but in agreement with another [10], we did not find pronounced parent of origin effects on GM atrophy. Compared to FH-, both FHp and FHm had similarly decreased GM volume in the right precuneus and when directly comparing FHm to FHp, we only observed a small cluster of decreased GM volume
for FHp in the right insula. Differences between studies examining FH could partially be explained by differences in age of the populations studied. Those studies that found a pronounced parent-of-origin effect [13,14] examined older populations than in our present sample, suggesting that differences between maternal and paternal FH may only become apparent at an older age.

**APOE4 is associated with striatal atrophy**

In APOE4 carriers, we found decreased GM volume in bilateral caudate nucleus. Atrophy of the striatum has been reported in AD [21,22], is associated with progression from MCI to AD [23] and was also described in healthy subjects at increased risk for AD due to APOE4 carriership [24]. The caudate nucleus is involved in several cognitive processes including planning of goal-directed behaviour and learning from action outcomes [25]. PiB-PET studies have shown accumulation of amyloid-beta in the striatum of relatively young (mean age: 56) non-demented subjects with both parents affected by AD [15] and in asymptomatic mutation carriers (presenilin-1) for early-onset AD [26]. Combined with results from the current study, these findings suggest that the striatum is affected early in subjects at increased genetic risk for AD.

In contrast to some previous studies [5,6], we did not find any effect of APOE4 on the hippocampus. A possible explanation is the relatively young age of the present cohort. Those previous studies have examined older subjects [5,6], whereas other studies examining younger populations also did not find an effect of APOE4 on hippocampal atrophy [9,10,12]. Older age cohorts may have a higher prevalence of subjects with preclinical AD (evidence of amyloid pathology) or hippocampal atrophy may only become apparent at a later age [27].

Compared to APOE4-, we found preserved GM volume in APOE4+ in the right superior frontal gyrus. This is consistent with results from a previous VBM study in cognitively healthy elderly, who also found less reduced GM in the frontal lobe in APOE4+ [6]. In AD, APOE4 carriers have relatively spared frontal lobes on MRI and preserved executive functioning on neuropsychology compared to subjects without APOE4 [28–30]. Together these findings support the idea of region-specific effects of APOE4 on grey matter atrophy associated with AD, which may already be detected in cognitively healthy subjects with this risk factor.

**Double risk of family history and APOE4**

When comparing the extreme phenotypes of ‘double risk’ (FH+ and APOE4+) versus lowest risk (FH- and APOE4-), we found bilateral insular atrophy. Insular atrophy has previously been reported in subjects with AD [21,22] and in APOE4 carriers with mild cognitive
impairment (MCI) [31] but has to our knowledge not yet been described in cognitively healthy subjects at increased risk for AD. The insula is located centrally in the cerebral hemisphere and is highly connected to multiple brain areas; such as limbic structures, ventral striatum and sensory cortical areas [32]. Functionally, the insula is involved in a large variety of processes, including speech production, limbic integration and somatosensory functions [32]. In AD, insular atrophy may be responsible for behavioural abnormalities and altered self-awareness [33].

The prevalence of APOE4+ is higher in subjects with FH [34]. Part of the increased risk for AD due to a positive family history may be explained by APOE4 genotype inheritance but family history also comprises additional genetic factors, as well as other risk factors related to socioeconomic status and environment. Our results suggest that APOE4+ and FH+ have separate effects on GM atrophy. In APOE4 non-carriers FH+ was associated with more atrophy in temporal and insular regions while in APOE4 carriers FH+ was associated with atrophy of the precuneus. Both APOE4 genotype and FH of dementia are associated with an earlier age of onset of AD [35,36], which is typically associated with atrophy of precuneus [20].

**Differential effects of age according to APOE4 genotype**

When assessing the effects of age separately in APOE4- and APOE4+, APOE4 non-carriers had more widespread age-related atrophy. There are several possible explanations for this finding. First, this may be due to a reduction in statistical power to detect age-related changes in APOE4+ subjects as a consequence of smaller group size (N = 74 APOE4+ vs N = 221 APOE4-). It can also be hypothesized that age-related GM volume decreases, similar to those observed in APOE4-, may have resulted in cognitive complaints in those with an APOE4 allele and thus non-selection of these subjects for this study. Finally, APOE4 non-carriers could be more vulnerable to age-related atrophy in this age range. Several studies have demonstrated a more aggressive disease course in AD subjects without APOE4, especially in early-onset AD (age < 65 years) [37,38]. Increased age-related atrophy in middle-aged APOE4- subjects could explain these findings.

**Strengths and limitations of the study**

A strength of our study is the relatively large sample size of well phenotyped subjects, which allowed us to incorporate the entire brain in our analysis. Most previous VBM studies examining healthy subjects at increased risk of AD have restricted their analysis using an inclusive mask to regions known to be involved in AD such as hippocampus, parahippocampal gyrus, amygdala and cingulate. Although a masking approach can increase statistical power to detect group differences, the risk is that novel areas might be
missed. Our more exploratory work revealed effects of APOE4+ and FH+ on the striatum and insular regions. In a VBM study, the amount of smoothing may influence the results, with smaller smoothing kernels more readily detecting localized effects. Therefore, statistical analysis for main effects of APOE4 and FH was repeated with data smoothed with a Gaussian kernel of 6 mm FWHM; this resulted in overlapping clusters with the ones found using the 8 mm kernel (data not shown).

This study has several limitations. Firstly, we examined cross-sectional baseline data of the GAP study cohort. Several studies have suggested that the effects of APOE4 and FH on atrophy may only be detected longitudinally [10,39–41]. The current study may thus underestimate the effects of these risk factors in cognitively healthy subjects. Longitudinal follow-up is also necessary to determine whether our observed effects are related to the development of dementia or merely a neuroanatomical feature associated with APOE4 carrieryship and family history of dementia. Secondly, FH was self-reported and this might be less reliable than information based on medical records. We chose to pool all subjects with a first degree relative with dementia, regardless of age of onset or the dementia subtype since medical records were unavailable. In the FH- group, we included all subjects who do not have a reported first or second degree family member with dementia. We chose not to exclude subjects whose parents did not reach a certain age, in order to avoid having the bias of a super-healthy control group. In our FH- group, mothers reached on average an age of 79 years old (SD 13) and fathers 73 (SD 14). An additional analysis including only subjects whose both parents reached the age of 70 in the FH- group and subjects who had dementia likely to be due to AD with an age of onset below 75 in the FH+ group resulted in the same cluster of decreased GM in the right precuneus for the whole group, as well as for maternal and paternal FH separately, provided the threshold for significance was lowered in the VBM analysis (Supplementary Table 1).

There were small but significant differences in MMSE between the four groups. Post-hoc analysis including MMSE z-scores as a covariate in the analysis yielded similar results for main effects of FH and APOE4 as the original model (data not shown). Thirdly, the percentage of our participants with a first-degree family history of dementia is relatively high. This enrichment may be caused by the recruitment strategy and that people with a positive FH are more eager to enrol in an ageing study, which might limit the generalizability of our findings. We did use a community-based sample rather than a memory clinic sample. The relatively low percentage of APOE4+ subjects in our study is consistent with reported allelic frequencies in Spain [17,42]. Unfortunately, the low number of APOE4 homozygous subjects in our study (N = 5) did not provide us enough power to perform a separate group analysis. Finally, the use of voxel-wise analysis in the present work results in a large number of statistical comparisons. Besides the primary analysis using a threshold corrected for
multiple comparisons, we examined the effects of APOE4 and FH with a more exploratory threshold, since we expected structural effects to be subtle in a cognitively healthy cohort. Given the biological plausibility of regions detected, we consider this approach valid and providing meaningful results.

**CONCLUSION**

In summary, FH and APOE4 are both associated with regional GM decreases in cognitively healthy subjects, although the effect is moderate and only detected at an uncorrected threshold. These atrophy patterns detected in subjects with an increased genetic risk for AD may reflect the earliest structural changes associated with the development of dementia. Longitudinal follow-up of the GAP study cohort is currently being collected. This data will help determine whether our findings are associated with the development of AD and thereby could serve as a biomarker for early diagnosis and trial enrichment.

**Acknowledgements**

This work has received support from the EU/EFPIA Innovative Medicines Initiative Joint Undertaking (EMIF grant: 115372) and ZonMW (project 733050204). Funding for data acquisition has been provided by SAIOTEK Programme of the Basque Government (grant: S-PR13ZH001) and the Carlos III Institute of Health (grant: PI112-02262). The VUmc Alzheimer centre is supported by Alzheimer Nederland and Stichting VUmc fonds.
REFERENCES


## SUPPLEMENTARY DATA

**Supplementary Table 1: Brain regions with grey matter atrophy using strict family encoding.**

<table>
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<tr>
<th>Contrast</th>
<th>Functional area</th>
<th>Coordinates x,y,z (mm)</th>
<th>Cluster extent (voxels)</th>
<th>Z score</th>
</tr>
</thead>
<tbody>
<tr>
<td>More atrophy in FH+ compared to FH-</td>
<td>Precuneus R</td>
<td>21,-73,37</td>
<td>310</td>
<td>3.87</td>
</tr>
<tr>
<td></td>
<td>Precuneus L</td>
<td>-12,-63,43</td>
<td>120</td>
<td>3.21</td>
</tr>
<tr>
<td></td>
<td>Fusiform gyrus L</td>
<td>-33,-49,-21</td>
<td>323</td>
<td>3.49</td>
</tr>
<tr>
<td></td>
<td>Insula R</td>
<td>46,-39,19</td>
<td>137</td>
<td>3.14</td>
</tr>
<tr>
<td>More atrophy in FHm compared to FH-</td>
<td>Precuneus R</td>
<td>20,-72,37</td>
<td>127</td>
<td>3.41</td>
</tr>
<tr>
<td></td>
<td>Cingulate gyrus R</td>
<td>3,21,46</td>
<td>660</td>
<td>3.60</td>
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<tr>
<td></td>
<td>Insula R</td>
<td>45,-39,18</td>
<td>110</td>
<td>3.27</td>
</tr>
<tr>
<td>More atrophy in FHp compared to FH-</td>
<td>Precuneus R</td>
<td>22,-73,37</td>
<td>249</td>
<td>3.78</td>
</tr>
<tr>
<td></td>
<td>Fusiform gyrus L</td>
<td>-33,-49,-23</td>
<td>619</td>
<td>3.77</td>
</tr>
<tr>
<td>More atrophy in FHp compared to FHm</td>
<td>Inferior temporal gyrus R</td>
<td>56,-27,-20</td>
<td>223</td>
<td>3.26</td>
</tr>
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<td>Middle Occipital gyrus R</td>
<td>-28,-81,13</td>
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<td>3.11</td>
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<tr>
<td>More atrophy in FHm compared to FHp</td>
<td>AnTERIOR Cingulate R</td>
<td>15,33,24</td>
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<td>3.68</td>
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<td>Middle Occipital gyrus R</td>
<td>33,-78,-9</td>
<td>129</td>
<td>3.66</td>
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<tr>
<td></td>
<td>Cuneus L</td>
<td>2,-81,19</td>
<td>220</td>
<td>3.05</td>
</tr>
</tbody>
</table>

Abbreviations: GM = grey matter; FH = family history of Alzheimer’s disease; FH+ = first degree relative with Alzheimer’s disease; FHm = maternal family history of Alzheimer’s disease; FHp = paternal family history of Alzheimer’s disease; L = left hemisphere; R = right hemisphere. A cluster-forming threshold of $p < 0.005$ was used; only clusters with more than 100 voxels are presented. Analysis were corrected for age, gender and APOE4 status. There were no regions in which FH- had more atrophy compared to FH+. 
C. Highest risk (APOE4+ FH+) and lowest risk (APOE4- FH-) subjects

Supplementary Figure 1: Percent difference maps.
(A) Percent difference maps of group of subjects with a first degree family history of dementia and subjects with no family history of dementia. Darker regions illustrate areas of atrophy in subjects with a first degree family history of dementia. (B) Percent difference maps of APOE4 carriers and non-carriers. Darker regions illustrate areas of atrophy in APOE4 carriers. (C) Percent difference maps of highest risk subjects (FH+ APOE4+) and lowest risk subjects (FH- APOE4-). Darker regions illustrate areas of atrophy in highest risk subjects.