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Featured Article

Effects of *APOE*-ε4 allele load on brain morphology in a cohort of middle-aged healthy individuals with enriched genetic risk for Alzheimer's disease

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Abstract

Introduction: Apolipoprotein E (*APOE*)-ε4 is the major genetic risk factor for Alzheimer's disease. However, the dose-dependent impact of this allele on brain morphology of healthy individuals remains unclear.

Methods: We analyzed gray matter volumes (GMvs) in a sample of 533 healthy middle-aged individuals with a substantial representation of ε4-carriers (207 heterozygotes and 65 homozygotes).

Results: We found *APOE*-ε4 additive GMv reductions in the right hippocampus, caudate, precentral gyrus, and cerebellar crus. In these regions, the *APOE* genotype interacted with age, with homozygotes displaying lower GMv after the fifth decade of life. *APOE*-ε4 was also associated to greater GMv in the right thalamus, left occipital gyrus, and right frontal cortex.

Discussion: Our data indicate that *APOE*-ε4 exerts additive effects on GMv in regions relevant for Alzheimer's disease pathophysiology already in healthy individuals. These findings elucidate the mechanisms underlying the increased Alzheimer's disease risk in ε4-carriers, suggesting a dose-dependent disease vulnerability on the brain structure level.

Keywords: Alzheimer's disease; *APOE*-ε4; Healthy individuals; Voxel-based morphometry; Gray matter volumes; Aging

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1. Background

Alzheimer's disease (AD) is an irreversible neurodegenerative disorder characterized by progressive cognitive impairment and a characteristic pattern of regional brain atrophy that starts in the hippocampus and medial temporal regions and then spreads to other cortical areas [1]. The two major neuropathologic features of AD are extracellular fibrillary amyloid β (Aβ) plaques and intracellular neurofibrillary tau tangles. AD pathology develops slowly with a protracted preclinical phase characterized by abnormal cerebral Aβ deposition in cognitively intact individuals [2]. The apolipoprotein E (*APOE*)-ε4 represents the major genetic risk factor for late-onset AD, with increasing copies of the ε4 allele being associated to greater AD risk and younger age of disease onset [3,4]. Three common polymorphic alleles referred as ε2, ε3, and ε4 code, respectively, three distinct apoeE isoforms, which differ among each other by a single amino acid, and depending on the allele pairs, they result in six possible diploid haplotypes. In the brain, apoE mediates neuronal delivery of cholesterol, which is an essential component for axonal growth, synaptic formation, and remodeling, and has an important role in Aβ metabolism. The apoE4 isoform (coded by the *APOE*-ε4 allele) has been shown to be less efficient in Aβ clearance and cholesterol transport than the other isoforms (apoE2 > apoE3 > apoE4) [4]. *APOE*-ε4-homozygous (HO) individuals are therefore an interesting population for studying the long-term effects of the lack of expression of the more efficient apoE isoform. Regarding the pathogenesis of AD, the mechanisms through which *APOE*-ε4 increases risk can be regarded as conferring a loss of neuroprotective function, gain of neurotoxic function, or both [4]. *APOE*-ε4 allele dose effects on memory decline have been reported in cognitively healthy individuals [5], and a dose-dependent hippocampal gray matter (GM) degeneration has been observed in AD patients [6,7]. However, only a few reports have assessed the impact of *APOE*-ε4 homozygosity on the brain morphology of healthy middle-aged subjects. One cross-sectional study found significantly reduced hippocampal volume in healthy *APOE*-ε4 homozygotes, as compared to ε4-heterozygotes and noncarriers (NC), without however reporting any ε4 dose-dependent effects [8]. In the follow-up of this study, an age-related hippocampal volume loss was found only in homozygotes, again not supporting for *APOE*-ε4 additive effects in this region [9]. On the other hand, Chen et al. [10] reported a significant correlation between *APOE*-ε4 gene dose and higher annualized rates of whole-brain atrophy in healthy individuals.

Aside from these reports, most of the studies in the literature pooled together *APOE*-ε4 homozygotes and heterozygotes into a single *APOE*-ε4 carrier category. Although longitudinal studies reported significantly faster decays of regional gray matter volume (GMv) in healthy carriers compared to NC [11,12], most cross-sectional studies which determined volumes in *a priori*–defined AD-sensitive cerebral regions did not find significant group differences [13–15]. Similarly, null findings were reported in studies using voxelwise techniques, such as voxel-based morphometry (VBM) [16–18]. By contrast, two studies using VBM reported GMv differences in brain regions including the hippocampus, lingual gyrus, and precuneus, when comparing carriers to NC [19,20]. More recently, Ten Kate et al. [21] reported decreased volume in the precuneus and insula in healthy middle-aged carriers of the risk allele as well as an interaction between *APOE* status and age in determining GMv in temporal and occipital regions. Discrepancies among studies might be in part due to the volumetric technique adopted and the difference in the samples' age, where studies including relatively older individuals tend to report significant differences compared to those including younger samples.

In the present study, we sought to determine the effects of *APOE*-ε4 allele load on brain morphology to better characterize the mechanisms through which *APOE*-ε4 confers an increased risk to develop AD in the healthy population. To this end, we used VBM in a cohort of healthy middle-aged individuals enriched for this genetic risk for AD (261 NC, 207 *APOE*-ε4 heterozygotes, and 65 *APOE*-ε4 homozygotes). We also sought to determine potential interactions between *APOE* status and age in determining GMv variability.

2. Methods

2.1. Study participants

The recruitment for the study consisted of two steps. First, 2743 cognitively healthy volunteers aged between 45 and 76 years were enrolled in the ALFA (ALzheimer and FAmilies) study, a large cohort program pointing to the identification of neuroimaging biomarkers of preclinical AD in the general population [22]. Exclusion criteria included performance exceeding established cutoff for a number of cognitive tests and presence of a psychiatric diagnosis [22]. Second, after *APOE* genotyping, all participants homozygous for the ε4 allele as well as carriers of the ε2 allele were invited to undergo magnetic resonance imaging (MRI) scanning along with ε4-heterozygous (HE) and NC matched for age and sex. This sampling strategy resulted in 576 study participants, of which 43 had to be discarded due to either MRI incidental findings or poor image quality, resulting in the final sample included in our study of 533 individuals. Demographic characteristics of the participants are summarized in Table 1 and Supplementary Table 1. For the statistical analyses, participants were pooled according to the cumulative presence of the ε4 allele, that is, NC, HE, and HO. Total intracranial volume as well as gender and education years did not differ among the three groups. However, HO individuals were significantly younger than NC and HE