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Short Communication

Hippocampal Volume Differences Between Healthy Young Apolipoprotein E ε2 and ε4 Carriers

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Abstract. The apolipoprotein E (APOE) ε4 allele is the major genetic risk factor for the development of late-onset Alzheimer's disease (AD), whereas the presence of the APOE ε2 allele seems to confer protection. Here, we report that healthy young APOE ε4 carriers have statistically significantly smaller hippocampal volumes than APOE ε2 carriers, while no differences were detected between the two groups in memory performance. The difference in hippocampal morphology is cognitively/clinically silent in young adulthood, but could render APOE ε4 carriers more prone to the later development of AD possibly due to lower reserve cognitive capacity.

Keywords: apolipoprotein E, hippocampal volume, memory function, young healthy individuals

INTRODUCTION

The apolipoprotein E (APOE) ε4 allele has emerged as the major genetic predisposition factor for the development of late-onset Alzheimer's disease (AD), while carrying the APOE ε2 allele, the frequency of which in the general population is only 0.08, may deter AD, as shown in both autopsy- and population-based studies [1–3]. Moreover, the APOE ε4 allele has been implicated in worse neuropsychological performance in the elderly and to a lesser extent in middle-aged non-demented individuals, as well as in hippocampal atrophy in cognitively healthy individuals [1, 4]. Nevertheless, it has been speculated that the findings of studies based on elderly non-demented populations were biased by the sampling of individuals with incipient AD and by the relatively limited numbers of APOE ε2 carriers due to the low prevalence of the ε2 allele in the general population [4]. We have recently reported that differences were elucidated neither in hippocampal volumes nor in memory function between 18

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healthy young adults possessing the APOE ε4 allele and 117 APOE ε4 non-carriers [5]. Upon further analysis of our research data we specifically extended our focus on the impact of the APOE ε2 and ε4 alleles on hippocampal morphology and memory function since the pathological hallmarks of AD emerge in the hippocampus, which is crucially involved in memory function [6].

MATERIALS AND METHODS

The study protocol was approved by the local Ethics Committee and was previously described [5]. In brief, healthy young adults without history of somatic diseases potentially affecting brain function, current or past psychiatric disorders, medication (except hormonal contraceptives), pregnancy and magnetic resonance imaging (MRI) contraindications were enrolled in the study after giving their written informed consent.

MRI scans were acquired on a 1.5 Tesla scanner (Siemens Magnetom Sonata, Siemens Healthcare, Erlangen, Germany). A T1-weighted 3D-sequence MPRAGE (voxel size $1 \times 1 \times 1$ mm^3, 176 slices, matrix 256×256, TR = 2030 ms, TE = 3.93 ms, SL = 1 mm) was obtained from each subject. Data were visualized using AMIRA Software 3.1®. Hippocampal volumes were traced manually on a slice-by-slice basis by two independent operators, who were blind to the cognitive performance and genetic status of the participants according the method of Pantel et al. [7]. As previously described, the intra-rater reliability for the two operators reached values of $r^2 = 0.91$ and $r^2 = 0.88$ respectively [5]. The inter-rater reliabilities were $r^2 = 0.84$ for the right and $r^2 = 0.81$ for the left hippocampus.

The memory function evaluation comprised the module A of the Inventory for Memory Diagnostics (Inventar zur Gedächtnisdiagnostik, IGD) [8], which was developed to assess middle to high memory performances. Thus it is suitable for the cognitive examination of healthy individuals. Memory function assessment took place in a standardized setting and was carried out by an experienced psychologist (T. R.-S.). Participants were tested in groups of at most 10 persons in the afternoon between 16:00–17:30.

Peripheral blood was collected from the participants. Leukocyte DNA was isolated with the Qiagen blood isolation kit according to the manufacturer's instructions (Qiagen, Hilden, Germany). The APOE genotype was analyzed as described by Hixson and Vernier [9, 10].

Statistical analyses were performed using PASW Statistics 17.0 for Windows (SPSS, Chicago IL). P values of less than 0.05 were considered to indicate statistical significance. Normal distribution was checked using the Kolmogorov-Smirnov test. Differences with regard to demographic variables, hippocampal volumes and IGD subtest scores between the APOE ε2 and ε4 carriers were tested using T-test for continuous variables and Chi-square tests for nominal (categorical) data.

RESULTS

Thirty-Three healthy young students, carrying either the APOE ε2 or the ε4 allele, were identified in the study electronic database, containing information on 135 healthy young individuals [5] (Table 1). Two heterozygous APOE ε2ε4 [5] were excluded from the current analyses. Data were normally distributed. The groups of the ε2 and ε4 carriers did not differ with regard to demographic variables. APOE ε2 carriers had larger hippocampal volumes than the ε4 carriers (right hippocampus: $p = 0.032$, left hippocampus: $p = 0.034$, total hippocampal volume: $p = 0.028$), whereas no differences were detected between the groups in memory performance ($p > 0.05$) (Table 1).

DISCUSSION

This is the first study to investigate differences in memory function and hippocampal morphology between healthy young APOE ε2 and ε4 carriers. Our results suggest that young adults in the third decade of their life possessing the APOE ε4 allele have smaller hippocampal volumes than APOE ε2 carriers. In line with our findings, previous studies have shown that healthy children and adolescents carrying the APOE ε4 allele have a thinner entorhinal cortex than APOE ε2 carriers [11]. Furthermore, spine density in cortical neurons was found to be significantly lower in somatosensory cortical layers II/III of young APOE ε4 targeted replacement (TR) mice lacking AD pathological changes compared to APOE ε2 TR mice [12]. These differences could be attributed to an elevation of oxidative insults, arising from the reported perturbations of the pro-oxidant/antioxidant balance in APOE ε4 carriers [13], as well as to alterations in synaptic connections and changes in myelination of the peripheral cortical neuropil [11].

The absence of differences between healthy APOE ε4 and ε2 carriers in memory performance points

Table 1
Summary of demographic, volumetric and neuropsychological data and genotypes of the study sample

	APOE ε2 allele carriers ε2ε3 ($n=15$), ε2ε2 ($n=2$) Mean (SD)	APOE ε4 allele carriers ε3ε4 ($n=12$), ε4ε4 ($n=4$) Mean (SD)	Inferential statistics
Demographic characteristics			
Age (in years)	24.7 (3.2)	24.2 (4.1)	$t=-0.41$, $P=0.688$
Education (in years)	17.35 (2.69)	16.56 (2.07)	$t=-0.94$, $P=0.353$
Gender (male/ female)	5/12	7/9	χ^2Fischer's exact test $p=0.481$
Handedness (right/left)	17/0	15/1	χ^2 Fischer's exact test $p=0.485$
Hippocampal data			
Hippocampus (right) (cm^3)	2.38 (0.26)	2.16 (0.30)	$t=-2.25$, $p=0.032$
Hippocampus (left) (cm^3)	2.22 (0.25)	2.01 (0.28)	$t=-2.22$, $p=0.034$
Total Hippocampus Volume (cm^3)	4.59 (0.50)	4.17 (0.55)	$t=-2.34$, $p=0.026$
Neurocognitive assessment			
IGD Working memory score	54.00 (10.15)	54.13 (6.04)	$t=-0.04$, $p=0.966$
IGD Learning ability score	54.51 (3.22)	53.50 (3.97)	$t=-0.73$, $p=0.473$
IGD Delayed recall score	48.59 (6.52)	46.69 (4.33)	$t=-0.98$, $p=0.335$
IGD Verbal memory score	63.47 (7.45)	60.81 (5.50)	$t=-1.16$, $p=0.255$
IGD Visual memory score	50.41 (7.29)	51.00 (5.09)	$t=0.28$, $p=0.791$

out that the deleterious cognitive effects associated with APOE ε4 are more apparent in later life, even though mild functional neural perturbations have been detected in healthy young ε4 carriers. Resting glucose metabolism reduction in brain regions affected by AD pathology, such as the parietal, temporal, and prefrontal cortices [14], decreased fractional anisotropy values and increase in mean duffusivity values [15], as well as alterations in activation patterns [16] and lower mitochondrial cytochrome oxidase activity [17] were reported in healthy adults possessing the ε4 allele. The absence of differences in memory function between ε4 and ε2 carriers in our study and between ε4 carriers and non-carriers in previous studies [5, 16] implies that the brain preserves enough redundancy and efficiency at young age to avoid decline in clinical cognitive performance despite the presence of neural perturbations in healthy young individuals with the ε4 allele. Taking into account the difference in hippocampal volumes, it is likely that APOE ε4 carriers are more vulnerable to the cortical thinning observed in aging [18] and AD [6], since less cortical thinning is necessary in key brain regions in ε4 carriers before a critical anatomical threshold is passed, and the neural dysfunctions become clinically evident as very mild cognitive deficits. Interestingly, aging is associated with a progressive decrease in spine density in APOE ε4 TR mice without AD pathology, whereas no changes in spine density over time were observed in APOE ε2 TR mice [12]. Thus, it can be argued that the observed differences in hippocampal volumes are related to a lower reserve cognitive capacity in APOE ε4 carriers and to a lower resistance to AD pathology in comparison with individuals with the APOE ε2 allele [19]. It is of note that neither APOE ε4 nor APOE ε2 carriers differed significantly in hippocampal volume and memory function from the healthy young ε3 homozygotes, who were enrolled in the study [5], though the ε3 homozygotes had higher hippocampal volume compared to ε4 carriers and lower compared to participants possessing the ε2 allele (data not shown). Interestingly, it has recently been reported that elderly healthy APOE ε2 carriers have slower rates of hippocampal atrophy compared to ε3ε3 homozygotes [20]. Differences in rates of atrophy in hippocampus between young individuals, possessing the APOE ε2 allele, and ε3 and ε4 carriers warrant investigation especially in the light of the observation that brain mass begins to decline at the age of 20 years [5].

Attempting to link a change in genotype with variations in cognitive function or brain morphology is a daunting task. It should be underscored, that it is often unclear how gene variants result in the phenotypic variations. It is possible that APOE ε2 and ε4 alleles exert a direct influence on hippocampal morphology, but it is also plausible that the genetic variation mediates an effect through some other downstream functional change, or through the regulation of some other genes.

The current study should be viewed in the light of some limitations. Despite its high reliability and reproducibility, the method employed for volumetric measurement of hippocampi depends on the varying capacity of the tracers to identify the borders of a brain region of particular morphological complexity. Moreover the study sample was relatively small and was restricted to university students of a narrow age range. As a result the generalization of the yielded results warrants further investigation.

In conclusion, our data indicate a genetically-determined difference in hippocampus morphology between APOE ε2 and ε4 carriers which is cognitively/clinically silent in young adulthood, but which could render individuals possessing the ε4 allele more prone to the later development of AD.

DISCLOSURE STATEMENT

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=849).

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