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ORIGINAL ARTICLE

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Apolipoproteins E and C1 and brain morphology in memory impaired elders

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Abstract Previous research has shown that polymorphisms of the apolipoproteins E (*APOE*) and *APOC1* represent genetic risk factors for dementia and for cognitive impairment in the elderly. The brain mechanisms by which these genetic variations affect behavior or clinical severity are poorly understood. We studied the effect of *APOE* and *APOC1* genes on magnetic resonance imaging measures in a sample of 50 subjects with age-associated memory impairment. The *APOE E4* allele was associated with reduced left hippocampal volumes and *APOE*E3* status was associated with greater frontal lobe white matter volumes. However, no *APOE* effects were observed when analyses accounted for other potential confounding variables. The effects of *APOC1* on hippocampal volumes appeared to be more robust than those of the *APOE* polymorphism. However, no modulatory effects on brain morphology outside the medial temporal lobe region were observed when demographic variables, clinical status, and other anatomical brain measurements were taken into consideration. Our results suggest that the role of the *APOC1* polymorphism in brain morphology of the cognitively impaired elderly should be examined in further studies.

Keywords Apolipoproteins · Frontal lobe · Hippocampus · Magnetic resonance imaging · Volumetry

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Introduction

Age-associated memory impairment (AAMI) is an operational term first described by the US National Institute of Mental Health (NIMH) to define memory problems in otherwise healthy subjects above 50 years of age [1]. Some reports indicated that subjects fulfilling the criteria might represent a heterogeneous aged population, most of whom were unlikely to develop dementia [2, 3]. However, the concept of AAMI has been criticized because of the high prevalence of cases among the elderly in some studies. This indicates that the term is too broad or general to define a condition presenting with cognitive impairment [4, 5, 6, 7, 8]. Furthermore, certain cross-sectional [9, 10, 11] and follow-up studies [12] of the outcome of these subjects and their biological and behavioral characteristics suggest that AAMI is not a variant of normal aging.

The apolipoprotein E (*APOE*) E4 variant (*APOE*4*) is a major genetic risk factor for Alzheimer's disease (AD) [13, 14, 15] and forms a cluster with the genes encoding the apolipoproteins C1 (*APOC1*) and C2. Previous studies reported an association of the *APOC1* allele (the insertion allele) or the haplotypes including this allele and E3 or E4 from APOE polymorphism and AD [16, 17, 18, 19, 20, 21]. The association of the *APOC1* gene with cognitive impairment, however, remains to be clarified, since these findings have not been replicated in all populations studied [22, 23].

We recently showed that AAMI was associated with an increased presence of the *APOC1*A* allele compared

with control subjects. Relative risks, assessed by odd ratios for the presence of this allele (2.66) and the A/A homozygous state (11.47), were consistent with a dosage effect of the allele associated with AAMI. Additionally, memory impaired subjects showed *APOE*2*-high and *APOE*3*-low prevalence, with an odds ratio of 2.18 for the presence of the *E2* allele and 7.85 for the absence of the *E3* allele [24]. In a subsequent study, we aimed to define some brain mechanisms that could explain the effects of the genetic variants. We hypothesized that they would probably be associated with the presence of silent cerebrovascular pathology, such as white matter abnormalities or lacunar infarcts on magnetic resonance imaging (MRI). However, we failed to confirm this hypothesis and suggested that other mechanisms, such as increased susceptibility to brain atrophy, may underlie the basis of the genetic associations [25].

In the present study we sought to further investigate the effects of both *APOE* and *APOC1* genes on brain characteristics in AAMI subjects. The presence of the *APOE*4* allele has been associated with increased atrophy of the hippocampus in AD patients and probably in non-demented elders as well [26]. Its possible role in other brain regions has not been so widely studied. Its influence on brain measures other than medial temporal lobe structure has only been assessed in a few studies of AD patients, which have suggested that *APOE* may have a region-specific effect on brain morphology [27, 28, 29]. To our knowledge, there are no data on the effects of *APOC1* on brain characteristics. Due to the importance of the hippocampus in memory functioning [30], and given that this is the cognitive domain that is most impaired in our subjects, we hypothesized that, if present, the brain effects of both genetic polymorphisms would be mainly seen in this region. Finally, and following on from our previous results on association studies, we hypothesized that the genetic effects on brain atrophy would probably be stronger for *APOC1* polymorphism than for the *APOE* gene.

Materials and methods

Subjects

Fifty subjects fulfilling the NIMH criteria for AAMI [1] were recruited. The subjects were selected from a larger sample of AAMI subjects evaluated in our previous association study [24]. For the present investigation, all subjects carrying the *APOE*2* or *APOE*4* alleles underwent MRI, as did a randomly selected sample of subjects with *APOE E3/E3* genotype. This procedure was established to ensure that the representation of the *E2* and *E4* alleles would be sufficient for statistical analysis. The final sample was divided into three *APOE* groups (*APOE*4* carriers=13, *APOE E2/E3*=14, and *APOE E3/E3*=23); no *APOE*2/E2* subjects were found. For the *APOC1* genotype, subjects were divided into two groups according to the presence (*APOC1*A*/- cases=27) or absence (*APOC1*B/B* homozygous=23) of the *APOC1*A* allele.

All subjects underwent extensive neuropsychological examinations to rule out dementia or other neuropsychiatric disorders. Possible depression was assessed by means of Hamilton's depression scale, anxiety disorder by the STAI questionnaire, and delusions and/or hallucinations using the CAMDEX battery. Language was evaluated with the Boston naming test and the Token test, praxis with the Kohs cubes from Wechsler Intelligence Scale-Revised scale, Luria's ideomotor, and ideational praxis, and gnosis with the Poppelreuter test and Luria's watches test [31]. Following the original AAMI criteria [1], the main inclusion variable was the reporting of progressive memory loss to the physician and scores of 1 SD below the mean of standardized norms for young adults on neuropsychological tests of secondary memory such as the Rey Auditory Verbal Learning Test or subtests of the Wechsler Memory Scale Revised [31]. Mean age of the entire sample was 65.5 years (SD=9.27 years); 70% were women, and mean MMSE score was 27.3 (SD=1.40).

Genetic analyses and DNA typing

APOE and *APOC1* polymorphisms were determined in all cases from frozen blood samples as described previously [24]. Briefly, at the *APOE* locus, the polymorphism of the three common variants *E2*, *E3*, and *E4* was analyzed. The *APOC1* locus was investigated for nucleotide changes affecting the *Hin*cII restriction sequence. Polymerase chain reaction (Thermal Cycler, Perking Elmer Cetus) was used to amplify the common alleles of the *APOE* and *APOC1* genes [32, 33].

MRI acquisition and volumetric measures

We studied the following cerebral measures: whole brain volumes [grey matter, white matter and cerebrospinal fluid (CSF)], frontal lobe volumes (white and grey matter), ventricular system, and hippocampal volumes. These brain regions were selected because previous reports had suggested that they may be influenced by *APOE* polymorphism in AD patients [27, 28, 29] or because they are believed to be predictors of dementia in elders with mild cognitive impairment [34].

All MRI scans were acquired on a 1.5T Signa (General Electric, Milwaukee, Wis., USA). The protocol included axial T2Wdual FSE (4000TR/20–100TE/1nex/3-mm slice thick) and coronal three-dimensional (SPGR 300TR/min full TE/20 flipα/1nex/1-mmslice.thick. recon.), field of view 24×24 and matrix 256×256. Whole brain volume measurements were determined using the following procedures. A T2 volume was reconstructed for each subject from the DICOM raw data by means of the 'Vool Tool' option of the ANALYZE software (Biomedical Imaging Resource, Mayo Foundation, Rochester, Minn., USA). Volumes were further saved in ANALYZE 7.5 format with an adequate ScaleFactor (i.e., 0.001685) to allow processing with Statistical Parametric Mapping software (SPM99, Wellcome Department of Cognitive Neurology, London). All automated image processing was performed using SPM99 running in Matlab 6.0 (MathWorks, Natick, Mass., USA).

The T2-weighted images were transferred into a standardized space by means of an automated spatial normalization (12-parameter affine transformation followed by non-linear iterations using 7×8x7 basis functions), using the SPM99-T2 Template image and the sinc interpolation (9×9x9) option. The normalized whole-brain images were automatically segmented into separate images representing probability maps for grey matter, white matter, and CSF using the combined pixel intensity and a priori knowledge approach integrated in SPM99, and supplemented by the "lots of inhomogeneity corrections" option. We used an automatic Matlab routine to obtain volumetric measurements of the three tissue compartments.

Medial temporal lobe boundaries were manually traced on T1-weighted contiguous coronal slices (1-mm thickness) by means of ANALYZE software (Mayo Foundation). The hippocampus was measured starting from its appearance below the amygdala. Its posterior boundary was identified as the point where both the crura of fornix and the superior colliculi were visible. Frontal lobe volumes were delimited from T2-weighted MRI axial slices by using the region of interest tool of the MRICRO software (Nottingham, UK), creating an individual frontal mask that was applied to

the images. Inferior-posterior boundaries of the frontal lobes were delimited by the basal portion of the lateral cissure. Medial surface was defined by the presence of the basal ganglia or the frontal horn of the lateral ventricles. Ventricular volume was measured in T2 axial images, by using a semiautomated method implemented in the Region of Interest option of ANALYZE software. This tool selects an intensity boundary in the brain image, which then has to be corrected manually. Since images were T2 weighted, ventricular regions yielded a much higher intensity value than adjacent structures. Since the fourth ventricle was difficult to identify in most cases, the lowest slice in which we measured ventricular system was defined arbitrarily as the slice in which ventricular temporal horn was visible. The uppermost slice was the last one where the lateral ventricle was visible. With this method, ventricular measure includes mainly lateral and third ventricle volumes. The volume of the region across successive slices was finally estimated automatically by the ANALYZE program. Regional brain volumes were corrected for brain size following the covariance estimate method proposed by Jack et al. [35] and validated by Free et al. [36]. The use of this procedure allowed the correction of hippocampal, grey and white matter frontal lobe volumes and ventricular system via the following equation:

$$\mathrm{CRV} = \mathrm{ORV} - \mathrm{Grad}(\mathrm{CM_i} - \mathrm{CMmean})$$

where CRV represents the corrected regional volume, ORV is the original cerebral volume, and Grad refers to the gradient of the regression line between the regional measure and the cerebral volume. $\mathrm{CM_i}$ is the value of the cerebral volume for each subject and CM mean represents the mean cerebral volume for the sample of subjects.

Results

Compared with women, men fulfilling the AAMI criteria presented lower volumes of grey matter (t=3, P<0.004) and a trend towards increased CSF measures (t=1.87, P<0.07), although the ages of males and females were comparable (t=1.49, P<0.14). Age correlated significantly and negatively with hippocampi (right hippocampus r=–0.44, P<0.001, left hippocampus r=–0.47, P<0.0001), grey frontal (r=–59, P<0.0001), white matter (r=–0.55, P<0.001), and grey matter volumes (r=–0.53, P<0.001) and positively with the global CSF compartments (r=0.78, P<0.0001) and ventricular system (r=0.56, P<0.0001). In the light of these results, age and/or gender were used as covariates when appropriate in further analyses.

Subjects carrying the *APOE*4* allele had lower left hippocampal volumes than those homozygous *APOE*3/E3* (F=12.32, P<0.001), although they did not differ when compared with *APOE*2* individuals (F=1.55, P<0.23). No differences were found for *APOE*3/E3* compared with *APOE*E2* carriers (F=1.78, P<0.19) in left hippocampal volumes. Right hippocampus was not related to *APOE* status after adjusting for age effects. *APOE*3/E3* carriers showed larger white matter volumes than *APOE*2* carriers (F=4.82, P<0.04) but did not differ from *APOE*4* bearers (F=2.52, P<0.12). *APOE*2* and *APOE*4* carriers had comparable frontal lobe white matter values (F=0.38, P<0.54) (Table 1).

For the *APOC1* genotype, individuals carrying the *APOC1*A* allele had lower corrected volumes of both right and left hippocampi than *APOC1 B/B* subjects (Fig. 1 and Table 2). *APOC1*A* carriers exhibited greater atrophy in white matter measurements of the frontal lobe. Finally, neither *APOE* nor *APOC1* polymorphisms were related to ventricular system volume estimations or asymmetrical measures of brain volumes.

To elucidate whether the contribution of *APOE* and *APOC1* genes improved the prediction of frontal and hippocampal measures after taking into account demographic, clinical, and cognitive characteristics, we performed stepwise multiple regression analyses including predictor variables in the following order: age, years of education, gender, depression score (Hamilton's scale for depression), MMSE score, the remaining brain variables,

Table 1 Brain volumetric measures in age-associated memory impaired (AAMI) subjects according to *APOE* polymorphism (*CSF* cerebrospinal fluid)

APOE	*E2/E3*	*E3/E3*	*E4/-*	*P<*
Brain	1158.91 (8.46)	1159.50 (10.92)	1161.83 (7.65)	0.74
White matter	504.86 (18.15)	510.24 (17.49)	504.54 (20.79)	0.27
Grey matter	460.26 (19.67)	448.96 (19.91)	444.86 (20.32)	0.25
CSF	193.79 (21.77)	200.30 (26.50)	212.76 (38.13)	0.38
Ventricular system	28029.96 (18479.76), *28186.17 (18439.62)*	25107.00 (12503.19), *25227.00 (12202.61)*	27972.72 (13609.04), *27930.20 (13607.85)*	0.30
Left hippocampus	2545.96 (355.259), *2541.50 (353.41)*	2623.68 (260.87), *2622.98 (260.46)*	2289.51 (307.84), *2310.77 (289.51)*	0.01
Right hippocampus	2464.10 (371.74), *2441.789 (339.58)*	2560.48 (281.44), *2560.46 (281.44)*	2340.34 (287.92), *2353.10 (284.539)*	0.08
Grey frontal	144390.43 (10521.34), *144502.00 (10485.88)*	138876.65 (8842.19), *138846.65 (8411.63)*	133531.58 (11709.88), *135100.90 (9597.87)*	0.17
White frontal	164468.21 (5557.93), *164356.64 (5477.44)*	170701.57 (10074.69), *170571.56 (9651.47)*	164754.92 (7150.22), *165728.98 (5816.51)*	0.05

Values for whole brain, grey and white matter, and CSF are given in cm^3 whereas volumes for regional areas studied are expressed in mm^3. Hippocampal, ventricular, and frontal lobe corrected volumes are given in *italics* after correction for whole brain measurements. *P* Statistical significance derived from analyses of variance (ANOVA) from corrected value analyses using age and gender as covariates

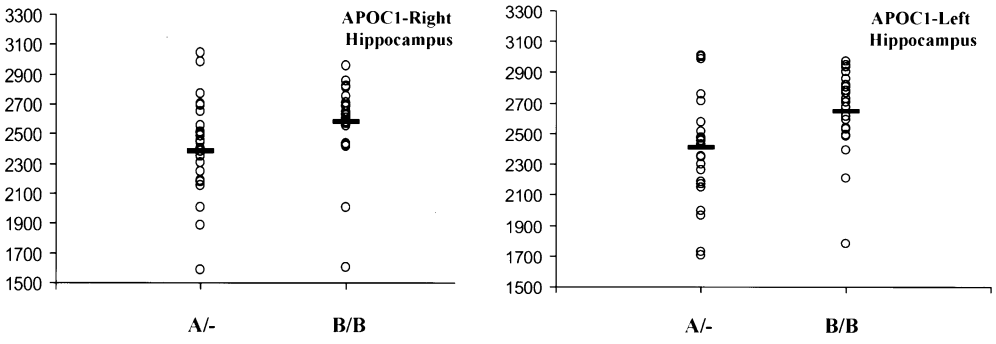

Fig. 1 Volumetric measures (in mm^3) of right and left hippocampi in subjects according to *APOC1* genotype. *Horizontal black bars* indicate the mean for each group. The significant *P* values of the resulting comparison between the two groups are reported in Table 2

Table 2 Brain volumetric measures in AAMI subjects according to *APOC1* polymorphism

APOC1	*A/-*	*B/B*	*P<*
Brain	1161.01 (7.91)	1158.87 (10.90)	0.40
White matter	504.63 (19.07)	510.33 (17.48)	0.16
Grey matter	451.65 (21.43)	450.37 (19.69)	0.97
CSF	204.73 (31.71)	198.18 (26.02)	*0.18*
Ventricular system	29113.87 (15885.99), *29064.83 (15880.15)*	23802.23 (12235.38), *24061.87 (11986.01)*	0.13
Left hippocampus	2407.29 (337.06), *2413.62 (329.97)*	2641.32 (268.49), *2639.06 (267.64)*	0.004
Right hippocampus	2386.47 (321.34), *2391.80 (306.93)*	2581.66 (284.55), *2581.09 (284.48)*	0.02
Grey frontal	138841.74 (12223.27), *139172.72 (11749.93)*	139483.69 (8366.91), *139377.77 (8347.00)*	0.97
White frontal	164818.15 (6124.78), *165062.45 (5617.18)*	170455.57 (10282.50), *170207.21 (9996.97)*	0.03

Values for whole brain, grey and white matter, and CSF are given in cm^3 whereas volumes for regional areas studied are expressed in mm^3. Hippocampal, ventricular, and frontal lobe corrected volumes are given in *italics* after correction for whole-brain measurements. *P* Statistical significance derived from analyses of variance (ANOVA) from corrected values analyses using age and gender as covariates

and both genetic polymorphisms. Frontal lobe grey volume was associated with total brain grey volume (t=13.37, P<0.00001), years of formal education (t=2.51, P<0.02), and total brain volume (t=2.49, P<0.02). Frontal lobe white matter was only predicted by total brain white matter (t=3.54, P<0.0009). The variables that were entered in the regression equation to predict right hippocampus size were age (t=3.78, P<0.0005) and *APOC1* polymorphism (t=2.65, P<0.011). Finally, significant predictors for left hippocampus were age (t=5.85, P<0.0001), *APOC1* genotype (t=3.89, P<0.0003), and gender (t=2.34, P<0.02).

Discussion

The results of our single comparison analyses, showing reduced hippocampal volumes in presence of *APOE*4*, are in accordance with some but not all previous reports in normal aging, memory impaired subjects, and AD patients [26]. The fact that the presence of the *E4* allele mainly compromised the left hippocampus may be related to the observation that the most-sensitive test for detecting memory impairment among our subjects was a verbal learning test. Only a few cases were diagnosed on the basis of their visual memory performance. It should be emphasized, however, that when results derived from multiple regression analyses were considered, the *APOE* effects on hippocampal structures were no longer seen. This observation suggests that if real the effect of *APOE* polymorphism on brain morphology among the non-demented elderly is confounded by or is strongly dependent on other variables such as age, gender, or the *APOC1* status.

In AD there is some evidence of a modulation effect of *APOE*4* in structures outside the hippocampus. However, the results are contradictory, especially when compared with those demonstrated for the medial temporal lobe region. It has recently been reported among dementia cases that patients carrying the *APOE*4* allele have larger frontal [27] and whole brain volumes [28, 29]. We found that *APOE*E3* subjects exhibited larger volumes of frontal lobe white matter than *APOE*E2* carriers, but could not find an association with increased frontal lobe or larger brain volumes in the presence of the *APOE*E4* allele. One possible explanation for the discrepancies between these results and those of studies of AD patients is that AAMI subjects appear to suffer from a relatively normal decline of memory function linked to usual aging. However, our data are consistent with a recent study including subjects with mild cognitive impairment, which represents a transitional state between normal aging and AD [37]; in that study, no significant *APOE* effects were observed using similar whole brain and medial temporal lobe measurements [38].

Although the findings derived from single comparisons show that the effect of the *APOC1*A* allele on the hippocampus may be smaller than that observed for the *APOE*4* allele (i.e., volume reductions of 8% or 9% vs. 15% for *APOE*), multiple regression analyses demonstrated a more-robust effect for *APOC1* polymorphism after adjusting for the contribution of other variables. The present findings suggesting an effect of this gene on brain morphology are also in agreement with our previous studies, indicating an effect of the *APOC1* gene on cognitive impairment and some brain characteristics, such as hippocampal sulcal cavities scores [25]. We previously hypothesized that the known interaction between APOC1 and APOE molecules could explain some of the putative effects of *APOC1* on brain morphology or functioning. In vivo evidence shows that an excess of APOC1 interferes with the APOE-mediated binding of triglyceride-rich lipoproteins to the very low-density lipoprotein receptor [39] and that the presence of the *APOC1*A* allele associates with increased transcription of the *APOC1* gene [40]. There is also evidence among AD patients that those carrying the *APOE*E4* allele have higher levels of APOC1 protein than non-E4 patients [41]. However, these assumptions must be considered merely speculative, since our methodological approach does not make it possible to establish any conclusion at the neurochemical level. Furthermore, it should be noted that most of our *APOE E3/E3* homozygotes were also *APOC1 B/B* homozygotes, whereas the proportion of *APOE E2/E3* and *APOE*4* subjects was distributed mainly among *APOC1*A* carriers. Finally, the number of subjects studied precluded an analysis of the possible effects of *APOE/APOC1* haplotypes on brain morphology. Due to these characteristics, the separate effects of *APOC1* and *APOE* might not be clearly elucidated in the present sample.

A few limitations of the present study should be considered. First, the relationship between the A variant of the *APOC1* allele and conditions involving cognitive impairment is still controversial, and has only been reported among AD cases. A second drawback is the small sample size within each genetic group, which means that the possibility of a chance finding cannot be entirely ruled out. Furthermore, it should be noted that AAMI is a debated concept. Although certain neuroimaging and neuropsychological studies support the validity of the construct as an entity that can be differentiated both from normal aging and from AD [9, 10, 11], other investigations concluded that the concept only described a heterogeneous group of normal aging [4, 7]. Finally, our study did not include a sample of age-matched controls without cognitive impairment, and so we were unable to establish whether the structural brain effects observed in relation to genetic polymorphisms were independent of the clinical deficit. In conclusion, the present findings should await further replication from other studies, including larger samples of healthy controls and patients.

In summary, our findings suggest that among subjects with cognitive impairment, the specific deleterious effects of the *APOE*E4* allele on brain morphology, if present, may be mainly restricted to the hippocampus and even in this case may be confounded by other demographic or biological variables. The effects of *APOC1* on hippocampal volumes appear to be more robust than those of the *APOE* polymorphism. However, no modulation effects on brain morphology outside the medial temporal lobe region were observed for any polymorphism when demographic variables, clinical status, and other anatomical brain measurements were taken into consideration. None of the genetic polymorphisms studied were associated with indicators of global brain atrophy such as CSF or ventricular measures.

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