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MRI and genetic correlates of cognitive function in elders with memory impairment

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Abstract

The present study investigated the relationship between genetic variation, MRI measurements and neuropsychological function in a sample of 58 elders exhibiting memory decline. In agreement with previous reports, we found that the ϵ4 allele of the apolipoprotein E (APOE) and the D allele of the angiotensin converting enzyme (ACE) polymorphisms negatively modulated the cognitive performance. Further, we found an association between the A allele of the apolipoprotein C1 (APOC1) polymorphism and poorer memory and frontal lobe function. No clear associations emerged between MRI measures of white matter lesions (WML) or hippocampal sulcal cavities (HSC) and the cognitive performance after controlling for age effects. Further, the degree of WML or HSC lesions was in general not predisposed genetically except for the presence of the A allele of the APOC1 polymorphism that was related to a higher severity of HSC scores. Our results suggest that WML or HSC do not represent important brain correlates of genetic influences on cognitive performance in memory impaired subjects.

1. Introduction

Magnetic resonance imaging (MRI) T2-weighted white matter hyperintensities or computed tomography (CT) leukoaraiosis [32] are commonly seen with advancing age [54]. Several studies have reported associations between the amount of white matter lesions (WML) and neuropsychological performance in nondemented subjects, especially in the cognitive domains of speed processing and frontal lobe executive functions [11–14,24,31,39,64,65,72,75,80]. There is increased evidence that WML are genetically modulated. In a study of 74 monozygotic and 71 dizygotic elders it was found substantial contribution of genetic factors to WML regardless of differences in brain size and age effects [20]. Specific genes have also been associated to the presence of WML, such as the ACE D/D genotype of the angiotensin converting enzyme genetic polymorphism and the apolipoprotein E ϵ2 and ϵ4 alleles [2,17,67].

The angiotensin I converting enzyme (ACE) is a component of the renin angiotensin system (RAS) associated to vascular physiology and pathology. The ACE also promotes the conversion of angiotensin I to angiotensin II a potent vasopressor and an inhibitor of achetilcoline release [7]. ACE concentrations have been found to be increased in several cortical and subcortical regions of patients presenting Alzheimer disease (AD) [4,8]. There is also growing evidence that the ACE genetic polymorphism could modulate the cognitive function in humans. Some recent studies have related the D allele (or genotypes including this allele) with dementia or cognitive decline in the elderly [3,28,53,59]. However, other possible roles of this polymorphism in further conditions involving cognitive impairment such as the presence of silent cerebrovascular disease remains a question of debate [2,21,40,46,51,76].

The apolipoprotein E (APOE) polymorphism includes three alleles (ϵ2/ϵ3/ϵ4). The presence of the APOE ϵ4 rep-

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resents the major genetic risk factor for late-onset AD in a dose-dependent manner [70,81]. Human APOE ε4 expressed in transgenic mice is related to an increased number neurodegenerative changes associated with aging when compared to the effects of the APOE ε3 human allele [18]. In elderly humans and in AD, the APOE ε4 allele is related to hippocampal and enthorrinal cortex volume reduction visible with MRI (for a review see [43]). The APOE ε4 allele is also associated with cognitive decline in normal elders [52], and has been also described as a risk factor for ischemic cerebrovascular disease [48]. The APOE gene locates in a region expanding 45 kilobases (19q13.2) which includes other related genes (such as those of the apolipoproteins C1 and C2) and presents strong linkage disequilibrium with the APOC1 locus. The APOC1 gene contains a polymorphism that consists of two alleles (A and B) which differ in a dinucleotid change in its promoter region. It has been suggested that some of the effects of APOE in relation to AD could be modulated by a nearby gene, most probably the APOC1 locus [26]. Further, direct evidence for the association between APOC1 polymorphism and dementia has been reported: an increase in the presence of the APOC1 A allele has been found among AD patients when compared to age matched controls [27,56].

Alongside WML, small areas of signal abnormality localized in the hippocampus have been reported in subjects without specific clinical signs or symptoms [16]. These round or curvilinear areas, called "hippocampal sulcal cavities (HSC)" appear along the course of the vestigial hippocampus and are isointense in respect to the CSF in both T1-weighted and T2-weighted images. Recently, the APOE polymorphism was associated to the presence of HSC in normal elders, those bearing the APOE ε2 or ε4 alleles exhibiting higher HSC scores (HSCS) than those APOE ε3/ε3 subjects [6]. To our knowledge, the possible relationship between HSC and neuropsychological performance has not been studied.

Age-associated memory impairment, as described by a workgroup of the National Institute of Mental Health (NIMH) [22] defines nondemented elder presenting subjective complaints of progressive memory loss and memory scores 1 SD below the mean in standardized neuropsychological tests. Although the original NIMH criteria were established to define a population of the normal aging, there is now evidence suggesting that this condition could represent an intermediate status between usual aging and dementia [55]. Thus, determining which variables are associated to a greater decline of cognitive function in these memory impaired subjects is a matter of interest. Follow-up studies in this condition demonstrated that cognitive decline in AAMI was associated with poor memory and frontal lobe performance in base-line measures [33]. We have recently reported evidence that the cognitive profile of AAMI is genetically influenced. In our previous studies, we found that among AAMI, those subjects bearing the APOE ε4 or the ACE D alleles exhibited poorer performance in neuropsychological tests of memory and frontal lobe respectively [9,10]. Moreover, recent unpublished observations from our group suggested that the prevalence of the A allele of the APOC1 polymorphism is overepresented in AAMI samples compared to controls.

To further characterize the biobehavioral determinants of age-associated memory impairment, in the present study we sought to investigate the separate effects and the interaction of genetic and MRI measures on neuropsychological decline in these memory impaired elders.

2. Method

2.1. Subjects

AAMI subjects were recruited from different health centers and geriatric homes from the Northeastern Iberian Peninsula, in the area of Barcelona. During the years 1997–1999, one-hundred subjects fulfilling NIMH-AAMI criteria were selected. Briefly, these individuals are above 50 and experience subjective progressive memory loss which is confirmed by their physicians or relatives. Despite their memory problems, these persons are not demented according to general measures (MMSE $\geq$ 24; cut-off for AAMI criteria [22]) and a comprehensive neuropsychological battery including assessments of language, praxis, gnosis and abstract reasoning as described elsewhere [10]. Additionally, these subjects present no medical condition according to routine laboratory tests and medical examinations, no history of neuropsychiatric disorder, current depression (Hamilton's scale cut-off 12) or mental retardation (Vocabulary score of Wechsler Adult Intelligence Scale cutoff 9 scaled score). Of all 100 AAMI, those presenting the APOE ε2 or ε4 alleles (n = 36) and a random sample of APOE ε3/ε3 subjects (n = 29) were selected for possible MRI examinations. Five subjects carrying APOE ε2 or ε4 genotypes declined to participate in the MRI study and in two cases the MRI examination could not be finished because the subjects complained of discomfort inside the scanner. Thus 58 AAMI subjects had complete MRI, neuropsychological and genetic testing. All subjects gave informed consent to participate in the study which was approved by the local ethics committee.

2.2. MRI examinations

All MR scans were performed on a 1.5T Signa (General Electric, Milwaukee, WI). The protocol included axial T2Wdual FSE (4000TR/20–100TE/1nex/3 mm slice thick.) and coronal 3D SPGR 300TR/min full TE/20 flipα/1nex/1 mmslice.thick. recon.), field of view (FOV) 24 $\times$ 24 and matrix 256 $\times$ 256. HSC were identified on T2-weighted images using a method previously described [6]. Briefly, each HSC of more than 3 mm in maximal diameter received a score of 3 and each HSC of less than 3 mm received a

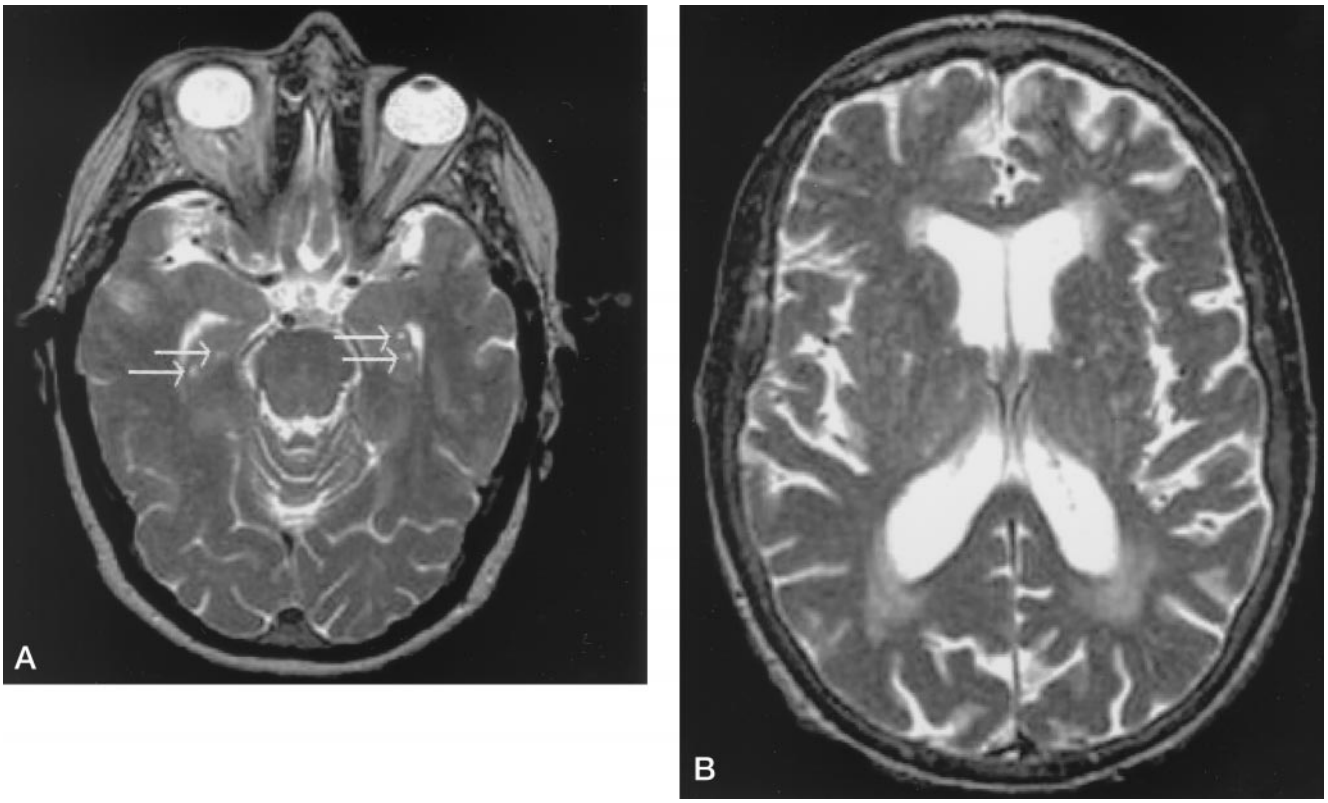

Fig. 1. A) Hippocampal sulcal cavities (arrows) on a T2-weighted MRI axial image of a 66-year-old women presenting memory impairment. B) T2-weighted axial slice of a 80-year old man fulfilling the AAMI criteria presenting mild ventricular enlargement and cortical atropy and moderate to severe periventricular hyperintensities in the anterior and posterior horns of the lateral ventricles. No HSC or WML scores are given for these two subjects because the total score for each subject was based on all slices.

score of 1. Maximal diameter length was automatically calculated by tracing a line along the HSCs. HSC were rated in three to four axial slices in both right and left hippocampi (Fig. 1, a). We scored all HSCs present in each slice. Thus, we did not consider the possibility that the same particular WML was in fact present in more than one image. Scoring was undertaken in this way because we decided that the thickness of the slices (3 mm) prevented us from reliably concluding whether there was a single large HSC or several independent HSCs. WML were evaluated according to a previous published scale [62] although we did not grade infratentorial lesions. Periventricular intensities (PVH), white matter hyperintensities (WMH) and subcortical hyperintensities (SH) were evaluated in all axial slices for each subject (Fig. 1, b). The scale for WMH and SH ranges from 0 (absence) to 6 (confluent) with intermediate values depending on the number and size of the findings. The scale for PVH goes from 0 (absent) to 2 ($>$5 mm). For WMH the maximal score for a subject is 24 and the minimum is 0 since there are four regions (4×6) suitable for scoring (the four lobules). For the SH the maximal score is 30 and the minimum again 0 since here there are five regions (5×6) to score (caudate, putamen, globus pallidus, thalamus and internal capsule). Finally the maximum score for a subject for PVH is 6 and the minimum 0 since there are three regions (3×2) suitable to score (occipital and frontal caps and bands of lateral ventricles). All slices including MRI measures were examined simultaneously by two experienced raters blind to neuropsychological and genetic analyses and the scores were given by consensus. All MRI examinations were performed within 90 days of neuropsychological testing.

2.3. Genetic polymorphisms

We selected three genes previously associated with cognitive impairment or the presence of WML. The ACE gene (17q23), presents a 287-bp *Alu* insertion (I)/deletion (D) polymorphism resulting in three genotypes: heterozygous (I/D), D allele homozygous (D/day) and I allele homozygous (I/I). APOE and APOC1 loci, encoding for important apolipoproteins, are located at the 19q13.2 region of chromosome 19. At the APOE locus, the polymorphism of the three common variants, ϵ2, ϵ3 and ϵ4, due to Cys-Arg substitutions at the 112 and 158 amino acid positions was analyzed using the restriction endonuclease *HhaI*. The APOC1 locus was tested for nucleotide changes affecting the *HincII* restriction sequence (the A allele) in the promoter region of the gene. This polymorphism results in three genotypes: APOC1 A homozygous (A/A), heterozygous (A/B) and APOC1 B homozygous (B/B). The full procedures used in the present study to determine these genetic

polymorphisms are described elsewhere [30,50,77]. For statistical comparisons groups were divided as follows according to the distinct genotypes: APOE: APOE $\epsilon 2/\epsilon 3$ ($n = 15$), APOE $\epsilon 3/\epsilon 3$ ($n = 29$) and APOE $\epsilon 4$ carriers ($n = 14$, no APOE $\epsilon 2/\epsilon 2$ cases were observed); ACE: ACE I/I ($n = 9$), ACE D carriers (ACE D/D and ACE I/D, $n = 49$); APOC1: APOC1 B/B ($n = 29$) and APOC1 A carriers (APOC1 A/A and APOC1 A/B, $n = 29$).

2.4. Neuropsychological assessment

We focused our cognitive assessment on memory and frontal lobe functions because both cognitive domains are believed to be affected in AAMI [34] and have been considered to be reliable predictors of dementia in these individuals [33]. Memory test included the following: Logical Memory, Visual Reproduction and Visual Paired Associates (Wechsler Memory Scale Revised, WMS-R) and the Rey Auditory Verbal Learning Test (RAVLT). Immediate measures (first trial in the case of RAVLT) and delayed free recall (after 30 min) were obtained from all memory tests. Frontal lobe tests were Verbal Fluency (sum of letters F, A and S, 1 min, and category fluency: animals 1 min), Tower of Hanoi test and Trail Making Test (forms A and B) [44].

3. Results

3.1. Genetic influences on cognitive function

Within each genetic category (APOE, ACE and APOC1) subjects did not differ in age, years of education, Hamilton's depression score or on the presentation of a recent history or current diagnosis of arterial hypertension (not shown). For neuropsychological comparisons, those AAMI subjects carrying the APOE $\epsilon 4$ performed significantly worse on long term Visual Reproduction (least-significant difference (DMS) post-hoc: $P < 0.02$) and on verbal fluency (letters fluency, $P < 0.03$) when compared to APOE $\epsilon 3/\epsilon 3$ subjects. No difference was found between APOE $\epsilon 4$ and APOE $\epsilon 2/\epsilon 3$ groups ($P < 0.29$ and $P < 0.84$ respectively). Additionally APOE $\epsilon 3/\epsilon 3$ subjects exhibited better scores in MMSE when compared to both APOE $\epsilon 2/\epsilon 3$ ($P < 0.004$) and APOE $\epsilon 4/-$ ($P < 0.001$) groups. Further, the presence of the ACE D allele was associated with reduced frontal lobe function (movements in the Tower of Hanoi test) compared to those ACE I/I homozygous. These results regarding the APOE and ACE polymorphisms in relation to cognitive performance in AAMI subjects, essentially replicate our previous studies in other samples [9,10]. Interestingly and not previously reported, for APOC1, those carriers of the APOC1 A allele obtained poorer scores in MMSE, verbal memory, and frontal lobe tests of verbal fluency and in TMT-B, compared to APOC1 B/B subjects. For the significant results in neuropsychological tests see Table 1.

Table 1
Significant differences in neuropsychological tests in AAMI according to APOE, APOC1 and ACE genotypes

	APOE			
	$\varepsilon 2/\varepsilon 3$	$\varepsilon 3/\varepsilon 3$	$\varepsilon 4/-$	F
MMSE	26.8 (1.4)	28 (1.3)	26.6 (0.9)	7.91***
Memory				
Long term visual reproduction	18.1 (11.1)	22.4 (10)	13.9 (11.1)	3.19*
Frontal lobe				
Letter fluency	26.4 (12.2)	33.9 (11.9)	25.5 (10.43)	3.41*
	APOC1			
	A/-	B/B	-	t
MMSE	26.7 (1.3)	28 (1.3)		3.71****
Memory				
Short term RAVLT	3.8 (1.2)	4.5 (1.4)		2.45**
Frontal lobe				
Letter fluency	2 (11.1)	33.9 (12.1)		2.57**
TMT-B	188.6 (80.5)	143.6 (62.2)		2.18*
	ACE			
	D/-	I/I	-	t
MMSE	27.3 (1.4)	27.7		0.74
Frontal lobe				
Tower of Hanoi (movements)	12.5 (6.4)	8 (1.6)	-	2.08*

Values are given in means (standard deviations). F: F value of one-way analysis of variance. t: t value of student's t-test for independent samples. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. RAVLT: Rey Auditory Verbal Learning Test. Tower of Hanoi (movements) refers to the movements needed to solve the task. TMT-B is given in seconds. Higher scores in these two tests indicate worse performance. Differences among APOE groups are reported in the text. APOE: Apolipoprotein E polymorphism; ACE: Angiotensin Converting Enzyme polymorphism; APOC1: Apolipoprotien C1 polymorphism.

3.2. MRI measures and relation to other variables

A minimal degree of WML in at least one of the brain areas studied was found in 56 (95.7%) subjects. However, the lesions were found to be generally mild (i.e. 48.3% subjects scored within the absent-mild degree of WMH and only 12% presented confluent WMH). Fifty four (93.1%) subjects presented scores of at least 1 in HSC measures of either hippocampi, suggesting that these are very common findings in memory impaired subjects. Age correlated strongly with HSCS, WMH and PVH, scores but did not reach statistical significance in relation with the amount of SH after excluding two outliers (see Fig. 2). No WML or HSC differences appeared between subjects with recent history or current diagnosis of arterial hypertension ($n = 17$) or those presenting normotensive values ($n = 41$), although those presenting arterial hypertension obtained higher scores on all MRI measures (Table 2). Neuropsychologically group comparison achieved statistical significance for verbal memory (Logical Memory) (Table 3). No differences on frontal lobe measures were found (not shown). Higher amounts of WML and HSCS were associated with

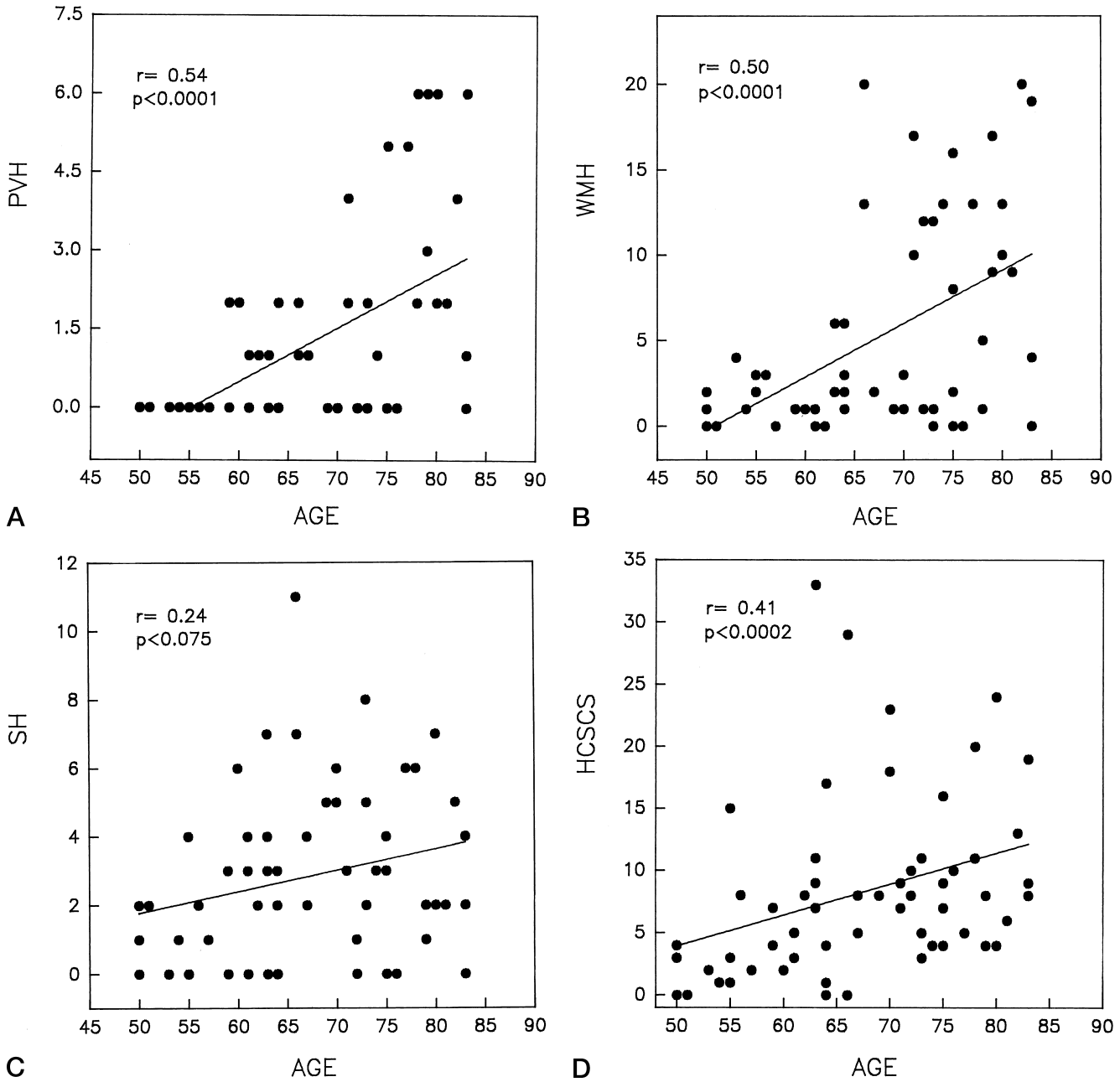

Fig. 2. Correlations between age and MRI findings. A) PVH: Periventricular hyperintensities. B) WMH: White matter hyperintensities. C) SH: Subcortical hyperintensities. D) HSCS: Hippocampal sulcal cavity scores, for both right and left hippocampi.

lower cognitive function but most of the significant associations disappeared after adjusting for age effects (Table 4). Further ANCOVA analyses controlling for age effects revealed no differences on any of the neuropsychological tests when comparing subjects presenting severe (confluent) WML to those without severe lesions (not shown).

3.3. Influence of genotypes on MRI findings

No statistically significant difference was found for APOE, ACE or APOC1 genetic polymorphisms in the degree of WML or HSC (see Table 5). To determine whether

Table 2
MRI findings according to the presence or absence of recent history or current diagnosis of hypertension

	Hypertensives	Normotensives	t
White matter hyperintensities	6.4 (6.8)	4.7 (5.6)	0.99
Periventricular hyperintensities	1.4 (1.8)	1.1 (1.9)	0.41
Subcortical hyperintensities	4.8 (5.1)	2.8 (3.2)	1.77
HSCS left	3.8 (3.8)	3.4 (2.9)	0.44
HSCS right	5.1 (4.5)	4 (2.9)	1.05

Values are given in means (standard deviations). HSCS: Hippocampal sulcal cavities; left and right refer to one or another hippocampus. No t-test was statistically significant at $p < 0.05$ level.

Table 3
Memory scores according to the presence or absence of recent history or current diagnosis of hypertension

	Hypertensives	Normotensives	t
Short term RAVLT	4.3 (1.3)	4.1 (1.4)	0.58
Long term RAVLT	6.6 (2.4)	6.6 (2.9)	0.13
Wechsler Memory Scale Revised			
Short term Logical Memory	7.8 (3.9)	10.3 (3.9)	2.20*
Long term Logical Memory	5.6 (3.9)	8 (4.2)	2.03*
Short term Visual Reproduction	25.2 (10.2)	23.6 (9.8)	0.57
Long term Visual Reproduction	22.1 (12.6)	18.1 (10.2)	1.25
Short term Visual Associates	4.1 (1.8)	3.9 (1.4)	0.32
Short term Visual Associates	2.6 (1.9)	2.9 (1.8)	0.49

Values are given in means (standard deviations). t: t value of student's t-test for independent samples. *p < 0.05. RAVLT: Rey Auditory Verbal Learning Test.

the severity of lesions was genetically modulated, the number of subjects with severe WML or HSC (defined as equal or scoring above the 75th percentile) was compared to a group without severe lesions (scores below 75th percentile). The only statistically significant difference was found for the APOC1 genotype. Subjects carrying the A allele presented more severe HSCS in the left hippocampus compared to APOC1 B/B subjects (see Fig. 3).

3.4. Discriminant variables of cognitive function in AAMI

Poorer scores on memory and frontal lobe functions were found to constitute neuropsychological predictors of dementia in previous follow-up studies of AAMI. To examine the manner in which genetic and MRI conditions best differentiated low from "high" memory and frontal lobe performers,

Table 4
Partial correlations (controlling for age effects) among neuropsychological measures, WML and HSC findings

	WMH	PVH	SH	LHSCS	RHSCS
Memory					
Short term RAVLT	.10	−.13	−.02	−.07	−.09
Long term RAVLT	.07	−.18	.05	.24	−.02
WMS-R					
Short term Logical Memory	−.21	−.24	−.11	−.09	−.22
Long term Logical Memory	−.11	−.16	−.06	−.17	−.26
Short term Visual Reproduction	−.06	.30*	.01	.05	−.15
Long term Visual Reproduction	.08	.16	−.08	.12	−.05
Short term Visual Associates	.04	−.03	−.05	.05	.19
Long term Visual Associates	−.20	−.09	−.13	−.01	.13
Frontal lobe					
Fonetic Fluency	.03	.08	.02	−.19	−.21
Semantic Fluency	−.13	.02	−.19	.22	−.18
TMT-A	−.05	−.17	−.07	.33*	.32*
TMT-B	−.07	.01	.08	.10	−.04
Tower of Hanoi (time)	−.30*	−.28	−.11	−.16	−.18
Tower of Hanoi (movements)	−.24	.20	−.12	−.03	−.09

WMH: White matter hyperintensities, PVH: Periventricular hyperintensities; SH: Subcortical hyperintensities, LHSCS: Left hippocampal sulcal cavity scores; RHSCS: Right hippocampal sulcal cavity scores. *p < 0.05.

Table 5
Distributions of WML and HSC measures according to the distinct genotypes

	WMH	PVH	SH	LHSCS	RHSCS
APOE					
ε2/ε3	4 (6.4)	1.2 (2.1)	2.8 (3.4)	4.7 (3.9)	5.3 (4.9)
ε3/ε3	5.2 (5.6)	0.9 (1.4)	3.5 (4)	2.8 (2.8)	3.8 (3.1)
ε4/ε-	6.6 (6.4)	2 (2.2)	3.9 (4.2)	3.8 (3)	4.4 (3.2)
F value	0.67	1.66	0.28	1.72	0.89
ACE					
D/-	5.2 (6.1)	1.2 (1.7)	3.2 (3.2)	3.6 (.32)	4.4 (3.4)
I/I	5.4 (5.7)	2 (2.3)	4.3 (4.9)	3.8 (4.9)	3.4 (3.2)
t-test value	0.13	1.33	0.79	0.10	0.51
APOC1					
A/-	5.1 (6.2)	1.4 (1.9)	3.3 (3.8)	4.3 (3.5)	5.1 (4.1)
B/B	5.3 (5.9)	1.1 (1.7)	3.4 (4)	2.8 (2.8)	3.6 (3.1)
t-test value	0.17	0.64	0.10	1.79*	1.53

Values are given in means (standard deviations). WMH: White matter hyperintensities, PVH: Periventricular hyperintensities; SH: Subcortical hyperintensities, LHSCS: Left hippocampal sulcal cavity scores; RHSCS: Right hippocampal sulcal cavity scores. APOE: Apolipoprotein E polymorphism; ACE: Angiontensin Convering Enzyme polymorphism; APOC1: Apolipoprotein C1 polymorphism. *p < 0.08.

two discriminant function analyses were used. A "memory" and a "frontal lobe" dichotomic variables were generated. Subjects performing above the 50th percentile in long term RAVLT and long term Visual Reproduction were given a

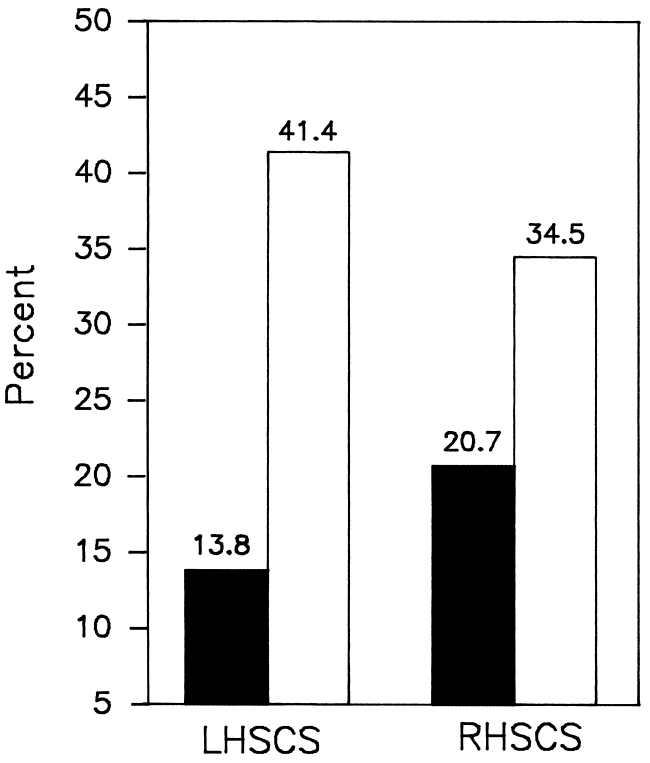

Fig. 3. Percentage of cases presenting severe hippocampal sulcal cavity scores (HSCS) for left (LHSCS; $\chi^2 = 5.52$; $P < 0.019$) and right (RHSCS; $\chi^2 = 1.38$; $P < 0.240$) hippocampi in relation to the APOC1 genetic polymorphism (black bars: APOC1 B/B; white bars: APOC1 A/-).

Table 6
Standardized coefficients of the variables entered in the discriminant canonic functions classifying "low" and "high" memory and frontal lobe AAMI performers

	Memory	Frontal lobe
Age	0.845	−0.587
Education, y	−0.575	0.708
ACE genotype	0.542	-
APOC1 genotype	-	0.523
λ Wilkis/χ^2	0.561/31.51	0.632/24.99
Sig. <	0.0001	0.0001

Sig.: Significance. ACE: Angiotensin Converting Enzyme; APOC1: Apolipoprotein C1.

value ("high" memory performers) and subjects exhibiting scores below this percentile in any of these two tests were given another value (low memory performers). The same was done for the "frontal lobe" variable using the fluency for letters and the Trails. In a stepwise analyses, age, years of education and presence or absence of history or current diagnose of arterial hypertension were entered followed by the four MRI measures (PVH, WMH, SH and HSC for left and right hippocampi) and the three genetic polymorphisms (APOE, ACE and APOC1). Three variables were selected as best discriminators to classify "low" and "high" AAMI memory and frontal lobe performers. Best discriminators refers to the process by which each variable enters the algorithm in the order by which it improves the significance of the overall function. These variables were age, years of education and the ACE polymorphism in the case of the "memory" domain and age, years of education and the APOC1 polymorphism in the case of the "frontal lobe" domain. The percentage of subjects correctly classified by the canonical functions were of 81.0% in the case of memory and 79.3% in the case of frontal lobe (Table 6).

4. Discussion

In agreement with previous reports in aging [2,52,59,69, 79] we found that those AAMI subjects carrying the APOE ε4 and ACE D alleles perform poorer in measures of cognitive functioning. In former studies we speculated that these genetic variations could be associated with lower cognitive function via the presence of WML as there was some evidence of these genotype-MRI interactions from other reported studies [2,67]. Present results however, suggest that the presence of these MRI findings may not be an important underlying brain mechanism associated to neuropsychological status in AAMI, at least for the cognitive tasks used in this study. The fact that white matter abnormalities do not represent the strong brain correlates of cognitive decline in our AAMI subjects but that genetic variables are associated to the neuropsychological profile may indicate that cortical or subcortical (gray nuclei) atrophy (i.e. medial temporal lobe, basal ganglia or the frontal cortex) need to be additionally considered. In this vein, a recent study found that subjects presenting a combination of both high WMH and low total brain volumes (TBV) tended to perform less well and experience la larger 10-year decline on measures of planning, sequencing, working memory, psychomotor speed and selective attention (among others) when compared to a group with low WMH and high TBV [71].

Although there is evidence from correlative studies that the WML are associated with subtle neuropsychological deficits in nondemented subjects [11–14,23,24,31,45,64,65, 72,75,80] a number of studies failed to find significant associations with cognitive impairment [1,15,29,35,37,58, 66,74] or the progression of cognitive decline [68]. A recent study [60] found that cognitive performance correlated negatively with brain atrophy and positively with cerebral blood flow and regional brain glucose metabolism but failed to find significant associations with deep white matter lesions, suggesting that these findings could represent merely epiphenomena in that sample. We found only small correlations between neuropsychological performance and WML after correcting for age in accordance with previous reports [80]. This however, could be due to the limited number of individuals with extensive WML in our sample since previous studies suggested that a threshold for severity of lesions is needed before cognitive deficit occurs [12,24,58]. However even these subjects did not differ from others in any cognitive domain after controlling for age in agreement with previous reports [29]. Nevertheless, this conclusion needs to be taken with caution due to the small number of subjects presenting severe WML in our sample (i.e. 12% in the case of WMH).

The APOE and ACE polymorphisms represent genetic risk factors for vascular pathology such as myocardial infarction or ischemic stroke [19,48,49]. The association with silent cerebrovascular disease is however, still controversial. Despite the findings of a few studies in normal elders and demented patients that the APOE ε2 or the ε4 alleles were associated with higher prevalence of white matter lesions [17,20,67], a number of recent reports challenged such association in both types of subjects [2,5,25,36,41,60, 63]. We used the same semiquantitative scale to rate WML as in a recent study [5] where no differences could be reported for WMH or PVH among AD, vascular dementia and dementia with Lewy body cases according to the APOE genotype. Further, a large report including more than 3600 participants found no MRI differences in white matter or infarctile lesions (subclinical vascular pathology) according to the presence or absence of APOE ε4 alleles but found associations between the genetic status and lower cognitive function [42]. Our findings in AAMI subjects agree with these previous reports.

A recent published paper [2] supports evidence that the ACE D/D genotype is more prevalent among subjects presenting white matter lesions. In that study however, up to

75% of subjects received a diagnosis of dementia, and moreover, most of neuroradiological examinations were performed using CT rather than MRI. Two further MRI studies on lacunar infarct cases found positive associations with the ACE D allele [40,46] but this was not replicated in another two studies [21,76]. Finally a recent article on patients suffering from silent brain infarction (which includes small lacunar infarction, WMH and PVH) found no association with the ACE polymorphism and white matter lesions in agreement with our findings [51].

To our knowledge this is the first study to describe an association between the APOC1 polymorphism and neuropsychological status. The APOE and APOC1 represent part of a genetic cluster (APOE/APOC1/APOC2) and exhibit strong linkage disequilibrium. Both the APOE ϵ4-APOC1 A and APO ϵ3-APOC A haplotypes as well as the APOC1 A allele by itself were considered to represent genetic risk factors for AD in former reports [27,56,57,61,73]. We did not analyze enough subjects to have reliable MRI and neuropsychological data on haplotypes, however our results suggest that the APOC1 polymorphism modulates the cognitive profile in memory impaired subjects. This is further supported by our unpublished data in which statistically significant increases in the frequency of the A allele were observed in AAMI when compared aged controls. The study of the mechanism by which the APOC1 A allele influences the cognitive function is beyond the scope of this report, although recent evidence suggest that the effect could be via the alteration of the biochemical proprieties of the APOE protein, as there is recent in vivo evidence that excess of APOC1 interferes with the APOE-mediated binding of triglyceride-rich lipoproteins to the very low density lipoprotein receptor (VLDLR) [38] and that the presence of the APOC1 A allele associates with increased expression of APOC1 [78]. Additionally, we found that the presence of the APOC1 A allele (rather than APOE alleles) is more prevalent among subjects suffering from severe HSCS. At present we do not have an explanation for this effect other than the possible modulative effect on APOE function.

This represents first attempt to study the neuropsychological correlates of HSC. Our findings suggest that HSC are common findings in memory impaired individuals and are not associated to a global degree of neuropsychological impairment. However, we found that the degree of HSCS was significantly associated with the time needed to execute a psychomotor speed task (TMT-A) even after the removal of age effects. Further cognitive studies are needed to explore other possible neuropsychological correlates of HSC. A recent study [6] found that the APOE ϵ2 and ϵ4 carriers exhibited higher rates of HSCS when compared to APOE ϵ3/ϵ3 subjects. Methodological approaches or sample characteristics could account for differences between two studies. First, we studied memory impaired subjects whereas previous findings were reported on normal elders. Additionally, our scores for HSC were notably higher than those previously reported (e.g. for the APOE ϵ3/ϵ3 group previous mean score was 2.9 whereas in our study it is 6.6), and this could reflect differences in the number of slices measured (we considered all T2-axial slices where the head, body or tail of the hippocampus were present) or judgment differences among raters due some of the subjective nature of the rating process. A possible explanation regarding our higher rates of score however, is that AAMI status is really associated with higher amounts of HSC since the former study [6] was performed in normal elders. Future research comparing directly the HSCS between memory impaired subjects and normal controls are needed to clarify this issue.

Some methodological caveats may help to explain discrepancies between ours and other studies, as well as some particular characteristics of our report. First, although to our best knowledge this represents the largest sample of subjects fulfilling the AAMI-NIMH criteria studied simultaneously using MRI, neuropsychological assessment and genetic analyses, future studies on larger samples would make present findings more representative and reduce the probability of not detecting a positive result due to a low statistical power. Thus, despite the use of a large sample compared to previous studies undertaken with memory-impaired subjects, that employed here is smaller than other studies performed on normal elders that found positive correlations between WML and cognitive performance (see references [23] and [45] for the largest samples reporting positive results). Second, we did not include a control group in our study since we intended to further characterize the cognitive profile in memory impaired subjects in relation to their particular genetic and MRI characteristics. Third, although frontal lobe functions were assessed, we did not use some of the frontal lobe or speed of processing neuropsychological tests (i.e. Stroop test or Wisconsin Card Sorting Test) generally described in positive studies of WML and cognitive performance. This could explain some of the differences with previous cognitive reports. Fourth, since both WMH and HSC are usually age-associated findings, in the present study we first evaluated MRI scans of normal young subjects to establish a base-line of what is not to be associated with advancing age. Our observations indicated that WMH or HSC are very rarely observed in young individuals, thus we decided to score all minimal changes in the elder sample. This could explain the high prevalence of such changes in our sample (although similar or higher prevalences in aged subjects have been reported by others, see ref. [54]) and the fact that some of our mean values are higher than others published [6] despite using the same rating methods. Notwithstanding, we believe that our methodological approach is theoretically appropriate since we aimed to evaluate MRI changes caused by advancing age.

In conclusion, our findings suggest that WML or HSCS could only explain some of the cognitive decline in memory impaired subjects. Although some neuropsychological tests such as Logical Memory strongly correlated with MRI findings, partial correlations showed that these results could be confounded by age effects. Further, these MRI findings do

not seem to be genetically determined by APOE or ACE polymorphisms although the cognitive profile of AAMI subjects is influenced by these two genes, suggesting that other brain mechanisms may be modulated genetically. Moreover, the A allele of the APOC1 genetic polymorphism previously associated to AD was related to low cognitive function in memory and frontal lobe neuropsychological tests and to the presence of severe HSC in AAMI subjects. Finally, although the results obtained using a discriminant analysis need to be validated with another samples, they suggest that the polymorphisms of ACE and APOC1 represent the best discriminators of memory and frontal lobe function respectively.

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