

The Neuroanatomical Substrate of Lexical-Semantic Decline in MCI APOE ϵ 4 Carriers and Noncarriers

Annalena Venneri, PhD,*†‡ William J. McGeown, PhD,* Roberta Biundo, PhD,*
Marco Mion, PhD,† Paolo Nichelli, MD,† and Michael F. Shanks, DPhil, FRCPsych*

Abstract: Lexical-semantic competency in mild cognitive impairment (MCI) ϵ 4 carriers was used as an endophenotype, and gray matter volume in MCI ϵ 4 carriers/noncarriers and in noncarrier controls was compared. Residual gray matter volumes were correlated with age of acquisition values for words from a category fluency task, an index of semantic competency. MCI patients had significantly impoverished lexical-semantic output compared with controls, more marked in MCI ϵ 4 carriers. Smaller volumes in the left hippocampus, bilateral regions of the uncus, and posterior cingulate cortex were associated with a tendency to retrieve earlier acquired words in the category fluency task in MCI ϵ 4 carriers, whereas poor semantic performance in MCI noncarriers was associated with smaller volumes in the left uncus, bilateral regions of the parahippocampal gyrus, and hippocampus, and also in a large number of neocortical regions. There was a significant semantic competency by genotype interaction in the left perirhinal cortex, in a number of left frontal and temporal areas and in the right inferior parietal lobule and precuneus. MCI ϵ 4 carriers, when compared with noncarriers, had lower gray matter volume values confined to the right precuneus and the cerebellum bilaterally, but the converse comparison showed that MCI noncarriers had lower values in extensive frontal, temporal, and parietal regions of the neocortex. Similar brain volumetric variations linked to genotype were found in minimal-to-mild AD. The results suggest a relatively specific impact of apolipoprotein E (APOE) ϵ 4 burden and underline the value of linguistic assessment in preclinical diagnosis.

Key Words: APOE, Alzheimer disease, magnetic resonance imaging, age of acquisition, lexical, endophenotype

(*Alzheimer Dis Assoc Disord* 2011;25:230–241)

The identification of reliable signs of Alzheimer disease (AD) at the preclinical stage is still a challenge for both clinicians and researchers, despite extensive research. Provisional clinical and research criteria have been proposed.^{1–3} These criteria are operationally useful, and represent significant improvements. It is also true, however,

that the lack of normative data for some of the measures and the difficulty in implementing them in some clinical settings, means that their actual impact on diagnostic specificity at the mild cognitive impairment (MCI) preclinical stage remains limited and validation is still ongoing. The diagnostic difficulty in AD is complicated by the lack of reliable biomarkers, which might consistently detect (or rule out) the disease, as overlapping alterations in such indices can be present in asymptomatic older individuals. One group of neuropsychological/neuroimaging studies have shown that a more accurate distinction between normal and abnormal cognitive decline can be achieved either by relying on more sophisticated methods of cognitive assessment^{4–6} or by examining brain activation patterns.⁷ This earlier work has established a link between deterioration of lexical-semantic skills and the presence of atrophy in perirhinal cortex and other temporal brain regions in clinical AD. These studies used several methods to establish semantic competency, but the age of acquisition (AoA) of items retrieved in a category fluency task seemed the most sensitive and discriminatory measure to differentiate normal and abnormal cognitive decline in established AD and might be a suitable cognitive endophenotype of the disease. The strength of the AoA parameter as a valid measure of lexical-semantic competency has been tested in patients who have an established loss of lexical-semantic abilities.⁸ The validity of this type of cognitive marker of AD at the preclinical stage is difficult to assess because only a proportion of those individuals who meet criteria for amnesic MCI progress to AD. A more recent study tested lexical-semantic competency, using the AoA parameter, in 18 MCI individuals who were carriers of the apolipoprotein E (APOE) ϵ 4 mutation and therefore at greater risk of AD. This group was compared with MCI noncarriers and controls. As in the studies of established clinical AD, MCI ϵ 4 carriers had significantly poorer performance than controls, whereas this was not the case for noncarriers.⁹ In addition, the carriers of the APOE ϵ 4 mutation were also significantly different from noncarriers. It seems, therefore, that individuals with MCI at increased risk for incident AD, even if their cognitive profile appears superficially comparable to MCI noncarriers, can be shown to have cognitive signs similar to clinical AD if a more sophisticated assessment is used. APOE ϵ 4 carriers, therefore, might be a suitable group to test the validity of the AoA endophenotype at the preclinical stage. There is further evidence of latent abnormality in carriers of genetic mutations for familial AD or carriers of susceptibility genes such as the APOE ϵ 4 mutation. A range of studies examined cognitive performance, brain structure/function, and their interaction in these at-risk healthy asymptomatic

Received for publication April 28, 2010; accepted October 14, 2010.
From the *Clinical Neuroscience Centre, University of Hull, UK;
†Department of Neuroscience, University of Modena and Reggio Emilia, Modena; and ‡S. Camillo Hospital (I.R.C.C.S.), Venice, Italy.

Supported by a grant from Ministero, Istruzione, Università, Ricerca to Annalena Venneri. Annalena Venneri and Roberta Biundo were also supported by the Marie Curie Research Training Network on Language and Brain funded by the European Commission under Framework 6 of which they were members.

The authors declare no conflicts of interest.

Reprints: Annalena Venneri, PhD, Clinical Neuroscience Centre, University of Hull, Cottingham Road, Hull HU6 7RX, England, UK (e-mail: a.venneri@hull.ac.uk).

Copyright © 2011 by Lippincott Williams & Wilkins

individuals. Asymptomatic carriers of the genetic mutation for familial AD showed poorer semantic skills than matched noncarriers.¹⁰ Healthy young carriers of the APOE ϵ 4 mutation had lower levels of cerebral metabolism in the areas characteristically affected in older patients with AD.¹¹ Lower levels of activation in bilateral and posterior inferotemporal regions, but higher level of activation in parietal structures during naming and fluency tasks were detected using functional magnetic resonance imaging (fMRI) in asymptomatic APOE ϵ 4 carriers, despite identical behavioral performance to noncarriers.^{12,13} Similarly, a recent fMRI study used a simple semantic memory activation task and found an abnormal pattern of activation in cognitively intact older adults at greater risk of AD either because they carried the APOE ϵ 4 mutation or had a family history of AD.¹⁴ Resting metabolism and brain blood flow abnormalities have been seen in APOE ϵ 4 carriers several decades before the onset of the dementia syndrome, and the presence of this allele seems to exert its influence on proficiency in some cognitive abilities, especially those strongly associated with brain regions more susceptible to AD type pathology, starting in childhood.¹⁵ MRI-based neuroanatomic studies have yielded less clear cut findings. A number of studies have reported volumetric differences in the hippocampus, which did not reach significance, between cognitively intact APOE ϵ 4 carriers and noncarriers,^{16,17} whereas 1 study found significant reductions in hippocampal volume in ϵ 4 carriers.¹⁸ Significant differences in cortical thickness of hippocampal subregions (in entorhinal cortex and subiculum but not in the main body of the hippocampus or perirhinal cortex) have been reported in cognitively normal ϵ 4 carriers.¹⁹ Similarly, selective regional effects of the APOE ϵ 4 genotype on the subfield CA3 and the dentate gyrus of the hippocampus in normal aging and AD were reported by another study.²⁰

Given this research background, it is feasible that, as observed in established AD,⁶ the substrate for the ϵ 4 carrier cognitive endophenotype identified by our earlier study of MCI individuals⁹ might be a subtle encroachment of neuropathology into perirhinal and/or neocortical areas or other regions within the limbic circuit at a time when extensive cognitive symptoms cannot be detected by conventional screening instruments [eg, Mini-Mental State Examination (MMSE)] or standard psychometric assessments.

The aim of this study was to test whether poorer lexical-semantic competency in MCI ϵ 4 carriers reflected more extensive gray matter volume loss in perirhinal cortex—a brain region, which is strongly involved in supporting semantic retrieval from long-term memory—than that observed in MCI noncarriers and in noncarrier controls. Residual gray matter volumes were correlated with a lexical-semantic competency measure, the AoA values for words produced in a category fluency task. This measure was chosen because of its established sensitivity and its discriminatory power between AD and healthy aging,⁴ and because of its suggested potential as a cognitive endophenotype of the disease. The specific impact of APOE ϵ 4 burden on regional gray matter volume loss was also assessed by direct volumetric comparisons in MCI ϵ 4 carrier/noncarrier patients and in minimal-to-mild AD ϵ 4 carrier/noncarrier patients to clarify whether differences in the patterns of atrophy aligned with any subtle cognitive difference in the lexical-semantic parameter of interest.

STUDY 1: RELATIONSHIP BETWEEN APOE ϵ 4 GENOTYPE, SEMANTIC LOSS, AND BRAIN VOLUME IN PATIENTS WITH MCI

Methods

Sample

Twenty-eight patients with amnesic MCI took part in this study. There were 16 males and 12 females in the group. Eleven matched controls (3 males and 8 females) were also tested. The patients in the MCI group were recruited from a large pool of referrals to the Neuropsychology Service of the Neurology Clinic at the University of Modena and Reggio Emilia, Italy. A diagnosis of amnesic MCI was reached based on published criteria.^{21,22} No patient with other MCI subtypes was included in the sample and none of the MCI patients had a family history for AD. To exclude the presence of dementia, all individuals had comprehensive clinical and neuropsychological examinations [including assessment of activities of daily living (ADL)], and did not meet the international published guidelines for the diagnosis of different types of dementia.^{23–26} Individuals were included only if there was no neuroimaging evidence of cortical or subcortical vascular lesions on the MRI scan (as seen on a T2-weighted axial scan) and if there was no history of hypertension, diabetes mellitus, transient ischemic attacks, or cardiovascular problems. Additional exclusion criteria included the presence of significant symptoms of depression, claustrophobia, a history of psychiatric disorders, and treatment with antipsychotic or psychoactive medication at the time of investigation. All MCI individuals and controls were assessed using an extensive neuropsychological battery (Table 1). All of the tests included in the neuropsychology battery have norms and cut-offs available for the Italian population. ADL and instrumental ADL were also assessed with formal scales.²⁷ All MCI participants and controls were community-dwelling individuals who had no difficulties in carrying out ADL and achieved normal scores on the ADL and instrumental ADL scales. Additional informal semistructured clinical questioning highlighted no difficulties in planning and initiating complex functional activities in the MCI group. A blood sample was also collected to determine the APOE status of both MCI and control participants. On the basis of their genetic profile, the MCI group was divided into a carrier subgroup including 14 individuals heterozygous for the APOE ϵ 4 allele (ϵ 3 ϵ 4) and a noncarrier subgroup including 14 people homozygous and heterozygous for the APOE ϵ 3 allele (ϵ 3 ϵ 3/ ϵ 3 ϵ 2). There were no APOE ϵ 4 carriers among those individuals in the control group. MCI ϵ 4 carriers (7 male and 7 female) had a mean MMSE score of 26.57 (SD 2.59), a mean age of 66.78 (SD 7.33), and a mean education of 12.29 (SD 4.75). The MCI noncarriers (9 male and 5 female) had a mean MMSE score of 27.07 (SD 2.16), a mean age of 67.78 (SD 7.36), and a mean education of 7.57 (SD 3.43). Control older adults were selected using the exclusion selection criteria used for the standardization of the Wechsler Memory Scale III and Wechsler Adult Intelligence Scale III.^{28,29} This group had a mean MMSE score of 29.09 (SD 1.04), a mean age of 61.55 (SD 5.55), and a mean education of 10.82 (SD 4.46). The control group and the 2 MCI subgroups differed for education [$F_{(2,36)} = 4.51$, $P < 0.05$] and MMSE scores [$F_{(2,36)} = 4.79$, $P < 0.05$], but did not differ for age [$F_{(2,36)} = 2.81$, ns]. An additional analysis showed that the 2 MCI subgroups did

TABLE 1. Mean (SD) Age, Education, and Scores of MCI Carriers and Noncarriers of the APOE ε4 Allele and of the Noncarrier Controls Obtained on Each Test in the Neuropsychological Battery

	MCI APOE ε4 Carriers	MCI APOE ε4 Noncarriers	Controls	P
Age	66.77 (7.33)	67.78 (7.36)	61.55 (5.55)	0.073
Education	12.29 (4.75)*	7.57 (3.43)	10.82 (4.46)	0.040
Tests				
Mini-Mental State Examination	26.57 (2.59)	27.07 (2.16)	29.09 (1.04)	0.014
Verbal paired associates	4.65 (1.82)†	4.71 (1.64)†	10.73 (3.94)	0.000
Letter fluency (no words)	21.64 (11.50)	23.14 (9.35)	30.90 (10.03)	0.079
Category fluency (no words)	28.07 (11.00)†	26.93 (7.86)†	39.90 (5.01)	0.001
Category fluency (AoA)	4.64 (0.83)†	4.84 (0.56)†	6.53 (0.96)	0.001
Category fluency (typicality)	4.54 (0.77)	4.35 (0.34)	4.27 (0.20)	0.414
Category fluency (length)	5.81 (1.65)	5.91 (0.49)	6.00 (0.19)	0.657
Digit span forward	5.86 (0.86)	5.26 (0.77)	5.45 (1.37)	0.387
Digit span backward	3.29 (1.71)	3.20 (0.82)	4.45 (1.37)	0.079
Visual-spatial span	4.00 (0.96)	3.84 (0.84)	4.54 (0.69)	0.078
Visual-spatial supraspan learning	17.74 (6.82)	17.44 (8.47)	24.76 (4.23)	0.064
Raven colored progressive matrices PM47	24.96 (5.04)†	25.67 (6.21)	31.00 (3.90)	0.007
Token test	33.43 (1.49)	32.06 (4.18)	34.14 (1.58)	0.611
Digit cancellation	44.00 (11.25)	41.69 (13.30)	52.45 (6.42)	0.074
Rey complex figure direct copy	31.28 (4.21)	27.58 (10.58)	33.27 (2.69)	0.301
Rey complex figure delayed copy	10.78 (5.79)	11.28 (5.96)	17.05 (7.01)	0.074
Stroop test error interference effect	1.07 (1.10)	2.35 (2.27)	0.18 (0.60)	0.124
Stroop test time interference effect	23.78 (10.25)	34.28 (15.66)	23.09 (11.67)	0.310

*Significantly different from noncarriers.

†Significantly different from controls.

AoA indicates age of acquisition; MCI, mild cognitive impairment.

not differ for sex ($\chi^2 = 0.58$, $P = 0.445$). Informed consent was obtained from all participants, as specified by the Local Ethics Committee at the institution where data collection took place.

Neuropsychological Assessment

All participants completed a comprehensive neuropsychological test battery. The battery included the Italian version of the MMSE,³⁰ tests of language comprehension,³¹ tests of category and letter fluency,³² tests of short-term and long-term memory (verbal and nonverbal),^{33–35} a test of abstract reasoning,³⁶ and tests of attention.^{34,37} Table 1 summarizes the neuropsychological profile of the 2 genetically defined MCI subgroups and of the control group.

Lexical-Semantic Assessment

Individual lexical-semantic competency was established for each person, by determining the individual lexical-semantic attributes of items produced in the category fluency test. This test required participants to orally generate as many items as possible within 60 seconds. Two trials were administered: 1 for the animal category and 1 for fruit. In addition to taking into account the total number of items produced in the 2 trials, the AoA of each acceptable generated word was determined. A mean AoA was then computed for each participant. AoA values for words were obtained by asking a sample of 46 healthy older adults [25 females, 21 males, mean age 68.87 (7.68), mean education 9.76 (SD 5.09), mean MMSE 28.69 (1.03)] to rate the AoA of 289 words (66 fruit and 223 animal words) following the procedure reported in the study by Forbes-McKay et al.⁴ These raters were recruited in the same geographical area and from a similar socio-cultural background as the MCI and control participants enrolled in this

study and were also comparable for age [$F_{(3,81)} = 2.47$, ns] and education [$F_{(3,81)} = 2.00$, ns]. Post-hoc tests showed that there were no differences in age and education between the rater group and either the control group or the MCI subgroups. Each participant was presented with a random list of all 289 items and asked to estimate the age (in years) at which they had learned a given word and its meaning in spoken or written form. Harmonic mean AoA ratings for each item were calculated and used in the analyses. Ratings acquired in this way have been shown to correlate highly with objective measures of AoA and therefore have good validity.³⁸ In addition, word typicality and length for each of the words produced in the category fluency task were also determined. For typicality, the procedure was similar to that used for determining the AoA parameter. Raters (the same as above) were given a list of all items split into 2 categories (animal and fruit). They were requested to rate the typicality of each item by using a 7-point Likert type rating scale, from 7 (most typical) to 1 (least typical). On the basis of the instructions given by Larochelle et al.,³⁹ they were asked to rate how well each exemplar (eg, apple) represented its specific category (eg, fruit). To control for order effects, the exemplars were shown in random order to raters.⁴

Item length was also assessed by counting the number of letters for each of the words produced in the category fluency task. Item length was determined by counting the number of letters for each acceptable item produced.

Words could not be rated for frequency as spoken frequency values in Italian are not available for most of the items produced by patients and controls.

Structural MRI Scanning: Acquisition and Analysis

Three-dimensional T1-weighted MRI images were acquired on a 3.0T Philips system with a Turbo Field

Echo sequence. Voxel dimensions were $1.00 \times 1.00 \times 1.00$ mm. Field of view was 256 mm with a matrix size of $256 \times 256 \times 124$, TR 9.9 ms, TE 4.6 ms, flip angle 8 degrees, and total duration 4 minutes 41 seconds. A number of preprocessing steps were followed to isolate the gray matter from the 3-dimensional T1-weighted structural scans before performing the statistical analysis using SPM8 (Wellcome Centre for Neuroimaging, London, UK). Initially the T1 images were manually aligned to approximate Montreal Neurological Institute (MNI) space, before segmenting into gray matter, white matter, and cerebrospinal fluid using the default segmentation procedure available in SPM8.⁴⁰ The Diffeomorphic Anatomical Registration Through Exponentiated Lie (DARTEL) algebra toolbox⁴¹ was then used in compliance with the guidelines supplied within the SPM8 manual (<http://www.fil.ion.ucl.ac.uk/spm/doc/manual.pdf>). The first step was to import the parameters from the initial SPM segmentation into DARTEL, a process that generates rigidly aligned gray matter and white matter segments. The DARTEL toolbox was then used to diffeomorphically align all the participants' brain images to their common average shape by simultaneously maximizing the overlap of gray and white matter.⁴² The population average template created by DARTEL registration, was affine registered with the MNI space,⁴³ and the resulting affine transform composed with the nonlinear deformations estimated by DARTEL. These composed mappings were used to normalize spatially the gray matter tissue maps of the individual participants to MNI space, in a way that preserves the tissue volumes.⁴⁴ Smoothing was also carried out with a 6 mm full width at half maximum Gaussian kernel to reduce possible error from between-subject variability in local anatomy and render the data more normally distributed. The smoothed gray matter segments were entered into the statistical analyses. These segments were entered into a voxel-based multiple regression analysis to investigate linear correlations between gray matter volume and mean AoA values for retrieved words. Age, number of years of education, MMSE, sex, total intracranial volume, genotype, and group membership (patient or control) were also included in this analysis as covariates. Group was included as covariate in the model because a potential interaction was expected. Independent t tests were also used for group comparisons (controls vs MCI ϵ 4 carriers and MCI noncarriers, MCI ϵ 4 carriers vs MCI noncarriers, AD ϵ 4 carriers vs AD noncarriers) in which age, number of years of education, MMSE, sex, and total intracranial volume were included as covariates. Analysis of variance (ANOVA) was used to test the interaction between endophenotype (high/low semantic competency based on the median AoA score split) and genotype (ϵ 4 carriers vs noncarriers). The x , y , z coordinates of significant areas obtained from the analyses were first converted into Talairach coordinates using the Matlab function `mnit2tal` (<http://imaging.mrc-cbu.cam.ac.uk/downloads/MNI2tal/mnit2tal.m>) and then identified using the Talairach Daemon Client (<http://www.talairach.org/>). The nonstationary cluster extent correction toolbox (<http://fmri.wfubmc.edu/cms/NS-General>) was used. Unless otherwise specified, height threshold was set at $P < 0.05$ corrected. A standard Bonferroni correction was used for most clusters. In those cases when a cluster did not survive correction but which were located in areas that were expected a priori, a minimum uncorrected significance level of $P < 0.01$ was chosen with only areas with a

T value greater than 2.5 considered as significant. Height threshold for the ANOVA was set at $P < 0.05$ uncorrected. A T2-weighted axial scan was also acquired before the 3-dimensional scan acquisition to better highlight any vascular load and to ensure that all participants included in the 3-dimensional structural imaging study had no significant vascular burden.

Results

Analyses of covariance were carried out to compare scores on the various neuropsychological tests. The controls and MCI group and subgroups showed no difference in age or sex, but there was a significant difference in education, which was later included as covariate in all behavioral analyses. Post-hoc comparisons showed that there was no significant between-group difference in most tests either between MCI and controls or between the 2 genetically determined MCI subgroups. Significant differences were seen for scores on the paired associates learning task ($P < 0.00001$) and category fluency task ($P < 0.01$) on which scores of MCI ϵ 4 carriers and noncarriers differed significantly from those of controls. Scores of MCI ϵ 4 carriers and noncarriers did not differ, however. A significant between-group difference was also found on the Raven PM47 test on which scores of the MCI ϵ 4 carriers were significantly lower than those of controls ($P < 0.05$). There was no significant difference between MCI noncarriers and controls, or between MCI ϵ 4 carriers and MCI noncarriers. Except for the ones on the paired associates test, all scores on the other tests were in most cases well above the relevant inferential population based cut-off score established in the norms for each test. Although for some tests statistically significant between-group differences were present, individual patient scores were within the normal limits for the appropriate age-education-sex range.

A further analysis of covariance was carried out using AoA values from category fluency word production as the dependent variable (see mean AoA values for controls and MCI subgroups in Table 1). There was a significant difference among the 3 groups in lexical performance [$F_{(2,35)} = 20.03$, $P < 0.001$]. Post-hoc Scheffe analysis showed that the AoA values of both MCI subgroups were significantly different from controls ($P < 0.001$). The 2 MCI subgroups did not differ from one another, but AoA values were lower in the MCI ϵ 4 carrier subgroup. The same analysis was also carried out for typicality values and word length. No significant differences between the MCI and control groups or the MCI subgroups were found [$F_{(2,35)} = 1.37$, ns and $F_{(2,35)} = 0.36$, ns for typicality and length, respectively].

Voxel-based Correlation

AoA: Gray matter segments and AoA values from MCI patients and controls were all entered in a regression model. AoA values showed significant positive correlations in a number of brain areas which included the parahippocampal gyrus (extending into the most anterior portion, the uncus) bilaterally, lateral regions of the temporal lobe bilaterally, bilateral frontal cortex, and the left cerebellum. Lower gray matter volume values in these regions were associated with the retrieval of earlier acquired words during the category fluency task. Table 2 and Figure 1 show a detailed summary of these findings.

TABLE 2. Positive Correlation Between Gray Matter Volume Values and the Age of Acquisition Values of the Words Produced by Patients and Controls in the Category Fluency Task

Brain Area	Left/Right	Brodmann Area	Cluster Size	Z Value at Local Maximum	Talairach Coordinates		
					x	y	z
Age of acquisition							
Precentral gyrus	L	4	4442	4.31	−45	−10	42
Postcentral gyrus	L	3		4.30	−42	−21	43
Middle frontal gyrus	L	10	15380*	3.72	−12	53	6
Inferior frontal gyrus	L	47		3.47	−46	32	−7
Hippocampus	L			3.26	−33	−12	−14
Uncus	L	28		2.83	−28	4	−23
Parahippocampus	L	34		1.81	−22	1	−11
Precentral gyrus	R	6	2636	3.66	38	−9	47
Middle frontal gyrus	R	9		3.12	42	21	36
Superior temporal gyrus	R	38	10403*	3.31	50	13	−11
Inferior parietal gyrus	R	40		3.29	56	−33	33
Parahippocampal gyrus	R	28		3.01	24	−14	−19
Uncus	R	28		2.99	24	5	−23
Parahippocampal gyrus	R	34		2.88	14	−11	−17
	R	28		2.45	20	−19	−14
Superior temporal gyrus	L	41	1961	3.08	−49	−16	−1
Insula	L	13		2.59	−40	−14	13
Cerebellum	L		5823	2.81	−2	−63	−29

*Corrected.

L indicates left; R, right.

Voxel-based Group Comparisons

MCI versus Controls: Significant gray matter volume differences from noncarrier healthy controls were detected in the MCI APOE $\epsilon 4$ carriers. Lower gray matter volume values in the MCI $\epsilon 4$ carrier subgroup were found in several limbic regions including posterior cingulate, hippocampus, and adjacent regions in the medial temporal cortex. Significant clusters were also found in the right precuneus, temporal neocortex, and cerebellum bilaterally. Detailed areas where lower gray matter volume values were found in MCI $\epsilon 4$ carriers are shown in Table 3A.

MCI noncarriers also had significantly lower volume values in a range of brain regions when compared with healthy controls. Significant clusters were found in the hippocampus and adjacent structures in the medial temporal cortex bilaterally. Extensive additional significant clusters were present in frontal cortex bilaterally, in the left temporal cortex and on the right in the posterior cingulate and other structures in the parietal and occipital cortex (Table 3B).

MCI $\epsilon 4$ Carriers versus MCI Noncarriers: Significantly less gray matter volume in MCI $\epsilon 4$ carriers when compared with MCI noncarriers was found in the right precuneus and in the cerebellum bilaterally (Table 4A, Fig. 2A).

The direct comparison of MCI noncarriers/ $\epsilon 4$ carriers showed that MCI noncarriers had more extensive regions of lower gray matter volume values (Fig. 2B). Multiple areas of the neocortex showed lower volume values in MCI noncarriers than in MCI $\epsilon 4$ carriers and these included the left inferior frontal gyrus, in addition to the right superior temporal gyrus, inferior temporal gyrus, and fusiform gyrus. Significantly lower volume values were also found in the precentral gyrus, postcentral gyrus, and medial frontal gyrus bilaterally (Table 4B, Fig. 2B).

Relationship Between the AoA Endophenotype and APOE $\epsilon 4$ Status: To test the relationship between semantic competency as an AD endophenotype (high/low semantic competency expressed by AoA values) and genotype for the APOE gene ($\epsilon 4$ carrier/noncarrier status), the gray matter

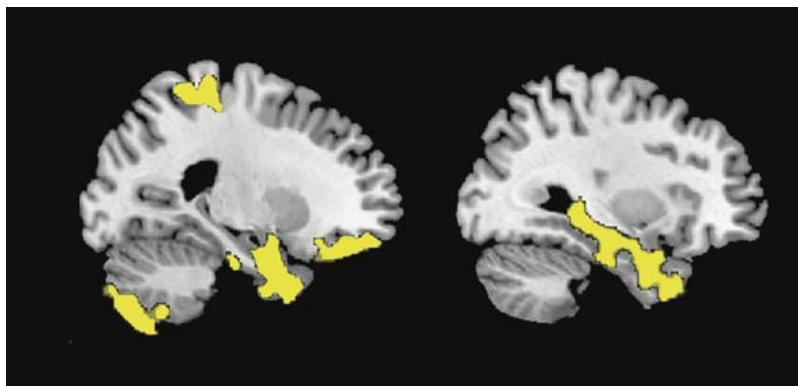
**FIGURE 1.** Areas of significant correlation between age of acquisition values and gray matter volume values.

TABLE 3. Areas of Significantly Lower Gray Matter Volume Values in MCI ϵ 4 Carriers and MCI Noncarriers When Compared With Noncarrier Controls

Brain Area	Left/Right	Brodmann Area	Cluster Size	Z Value at Local	Talairach Coordinates		
				Maximum	x	y	z
A, Controls versus MCI ϵ 4 carriers							
Precuneus	R	7	2720	3.76	6	−50	49
Posterior Cingulate gyrus	R	31		2.79	8	−37	24
	L	23		2.69	−4	−37	22
Cerebellum	L		421	3.61	−12	−58	−35
	L			2.74	−8	−63	−26
	R		4968	3.46	27	−46	−27
	R			3.34	14	−60	−34
Inferior frontal gyrus	R	47	771	2.95	27	23	−6
Hippocampus	L		477	2.81	−26	−15	−9
Uncus	L	28		2.67	−24	5	−20
	R	28	240	2.63	32	1	−28
Cerebellum	L		542	2.55	−34	−77	−28
Middle temporal gyrus	R	20	477	2.46	48	5	−23
	R	21		2.39	50	−13	−17
B, Controls versus MCI noncarriers							
Inferior temporal gyrus	L	20	8756*	3.68	−51	−39	−15
Middle frontal gyrus	L	47		3.39	−38	42	−10
Superior temporal gyrus	L	38		3.28	−38	19	30
Uncus	L	28		3.04	−27	7	−22
Fusiform gyrus	L	36		2.84	−36	−27	−19
Parahippocampal gyrus	L	28		2.73	−22	4	−18
Hippocampus	L			2.56	−26	−12	−9
Superior temporal gyrus	L	38		2.36	−38	−11	−27
Middle frontal gyrus	R	8	9633*	3.49	34	19	41
Middle occipital gyrus	R	18		3.46	39	−80	−10
Fusiform gyrus	R	20		3.28	50	−34	−18
Superior temporal gyrus	R	38		2.95	32	1	−28
Parahippocampal gyrus	R	36		2.69	32	−14	−35
Hippocampus	R			2.58	34	−32	−5
Posterior cingulate gyrus	R	31		2.41	10	−37	33
Precuneus	R	7	2079	3.43	10	−59	47
Cuneus	R	18	4001	3.43	10	−88	13
Lingual gyrus	R	17		3.39	22	−90	2

*Corrected.

MCI indicates mild cognitive impairment L, left; R, right.

images of the MCI patients were entered in a 2×2 ANOVA. A significant interaction between semantic competency and genotype was found in a number of areas. Significant clusters included the left medial frontal gyrus; parahippocampal gyrus; fusiform gyrus; inferior, middle, and superior temporal gyri; the putamen; the caudate nucleus; and the anterior cingulate cortex. A significant cluster was also found in the right inferior parietal lobule and precuneus (Table 5, Fig. 3). To illustrate the interaction in perirhinal cortex in more detail, data from the area in the parahippocampal gyrus where a significant interaction was present in the MCI group were extracted from the AoA regression analysis and plotted by group/subgroup in a scatter plot (Fig. 4).

Discussion

The findings support the hypothesis that the AoA measure is an appropriate endophenotype for AD. A significant interaction between semantic competency (expressed by AoA values) and genotype (APOE ϵ 4 carriers/noncarriers) was found in the perirhinal cortex suggesting that the AoA measure is highly sensitive to early damage in this region of the hippocampal complex. When directly compared, the pattern of atrophy in the 2 MCI subgroups

showed distinct differences. Atrophy was confined to mediotemporal structures in carriers of the ϵ 4 mutation, whereas in noncarriers atrophy was more widespread and involved several neocortical regions in frontal and parietal cortex. The relatively small sample size, however, could have exaggerated the differences between the 2 genetic profiles. To test the robustness of this finding, the pattern of atrophy was studied in a sample of established mild AD patients in which carriers of the APOE ϵ 4 mutation were better represented.

STUDY 2: THE RELATIONSHIP BETWEEN APOE ϵ 4 GENOTYPE AND BRAIN VOLUME IN PATIENTS WITH MINIMAL-TO-MILD AD

Methods

Sample

Thirty patients with probable AD of minimal-to-mild severity participated in this study. They were recruited through referrals to our outpatient memory clinic in United Kingdom. Clinical and neuropsychological assessments

TABLE 4. Areas of Significantly Lower Gray Matter Volume Values in MCI $\epsilon 4$ Carriers Compared With MCI Noncarriers and MCI Noncarriers Compared With MCI $\epsilon 4$ Carriers

Brain Area	Left/Right	Brodmann Area	Cluster Size	Z Value at Local	Talairach Coordinates		
				Maximum	x	y	z
A, MCI ε4 carriers versus MCI noncarriers*							
Cerebellum	R		188	4.18	39	−42	−30
	R			2.83	27	−43	−33
	L		689	3.77	−28	−36	−25
Precuneus	R	7	41	2.96	4	−48	49
B, MCI noncarriers versus MCI ε4 carriers							
Precentral gyrus	L	6	3037	3.83	−51	−7	36
Postcentral gyrus	L	2		3.37	−62	−20	23
Medial frontal gyrus	L	6	7990	3.79	−2	7	52
Inferior frontal gyrus	L	11		3.54	−26	27	−14
Medial frontal gyrus	L	10		3.35	−4	56	8
Fusiform gyrus	R	19	2301	3.73	46	−69	−11
Inferior Temporal gyrus	R	37		3.41	53	−63	−11
Superior temporal gyrus	R	22	2949	3.47	59	−27	7
	R	42		3.08	59	−24	15
Inferior parietal lobule	R	40		2.98	51	−26	26
Postcentral gyrus	R	5	2045	3.43	3	−32	61
Medial frontal gyrus	R	6		3.16	2	−24	66
Middle frontal gyrus	R	6	2653	3.10	28	5	53
Precentral gyrus	R	6		3.05	60	1	31

*Height threshold $T = 2.5$.

MCI indicates mild cognitive impairment; L, left; R, right.

were as in Study 1 except that the English version of the tests was used. All selected patients met the National Institute of Neurological and Communication Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria for a diagnosis of probable AD of mild severity,²³ and none had radiological evidence of ischemic brain disease. Other causes of dementia were excluded according to published clinical criteria.^{24–26} Blood samples were also collected to determine their APOE status. There were 20 APOE $\epsilon 4$ carrier patients (12 male and 8 female), 9 homozygous ($\epsilon 4/\epsilon 4$) and 11 heterozygous ($\epsilon 4/\epsilon 3$), and 10 noncarrier patients (7 male and 3 female). AD $\epsilon 4$ carriers had a mean MMSE score of 22.58 (SD 2.85), a mean age of 76.60 (SD 9.86), and a mean education of 9.58 (SD 1.12).

AD noncarriers had a mean MMSE score of 23.50 (SD 3.17), a mean age of 76.60 (SD 6.70), and a mean education of 13.20 (SD 3.42). The AD $\epsilon 4$ carrier and AD noncarrier subgroups did not differ for age [$F_{(1,28)} = 0.01$, ns] and mean MMSE score [$F_{(1,28)} = 0.47$, ns], but a significant difference was present for education [$F_{(1,28)} = 18.07$, $P < 0.001$]. Informed consent was obtained from all participants, as specified by the Regional Ethics Committee.

Structural MRI Scanning: Acquisition and Analysis

The same scanning protocol and methods of analysis were used as for Study 1 except that scanning was carried out on a 1.5 T scanner.

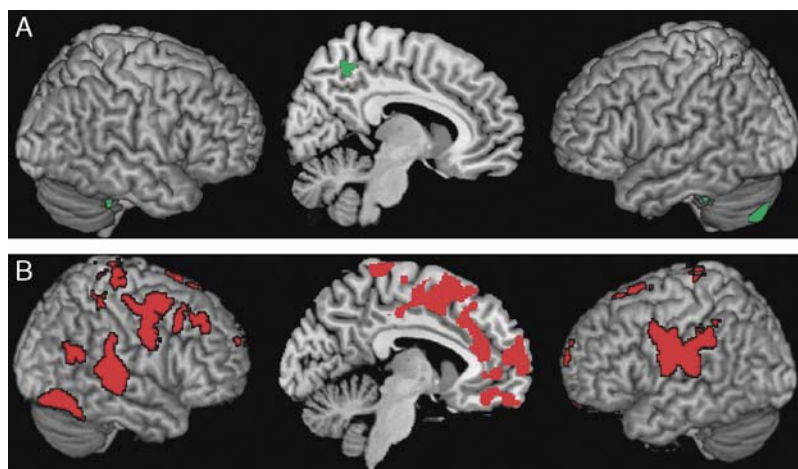
**FIGURE 2.** A, Areas of volumetric difference in MCI $\epsilon 4$ carriers when compared with MCI noncarriers. B, Areas of significantly lower gray matter volume values in MCI noncarriers when compared with MCI $\epsilon 4$ carriers. MCI indicates mild cognitive impairment.

TABLE 5. Interaction Between APOE ϵ 4 Status (Carrier, Noncarrier) and AoA Ability Level (High Competence, Low Competence)

Brain Area	Left/Right	Brodmann Area	Cluster Size	F Value at Local Maximum	Talairach Coordinates		
					x	y	z
Medial frontal gyrus	L	10	2956*	51.00	−3	51	7
Anterior cingulate gyrus	L	32		23.97	−4	26	28
Anterior cingulate gyrus	L	25	5242	37.44	−4	6	−4
Putamen	L			29.76	−15	11	−12
Caudate nucleus (head)	L			25.80	−13	25	−5
Parahippocampal gyrus	L	36	2626	27.20	−24	−40	−12
Cerebellum	L			18.23	−22	−35	−23
Parahippocampal gyrus	L	34		4.78	−22	1	−11
Fusiform gyrus	L	19	585	24.34	−48	−66	−11
	L	37		21.79	−46	−52	−18
Postcentral gyrus	R	40	374	22.84	42	−32	51
Inferior parietal lobule	R	40		9.09	43	−40	49
Postcentral gyrus	R	3		6.94	40	−19	51
Superior temporal gyrus	L	41	1963	20.34	−55	−18	9
Inferior temporal gyrus	L	21		15.76	−64	−4	−19
Superior temporal gyrus	L	22	589	11.89	−59	−56	8
Middle temporal gyrus	L	39		12.41	−46	−61	24
Precuneus	R	7	462	11.99	16	−68	48

*Corrected.

AoA indicates age of acquisition; L, left; R, right.

Results

Voxel-based Group Comparisons

AD ϵ 4 Carriers versus AD Noncarriers: Significant clusters of lower volumetric values in AD ϵ 4 carriers were found in right precuneus, cuneus, posterior cingulate cortex and superior frontal gyrus, and in the medial frontal gyrus bilaterally when compared with AD noncarriers (Fig. 5A).

AD noncarriers had significantly lower gray matter volume values in extensive regions of the temporal, frontal, and parietal neocortex bilaterally (Fig. 5B). Additional significant clusters of lower volumetric values were also found in the thalamus bilaterally, in the right putamen and in bilateral cerebellum.

Discussion

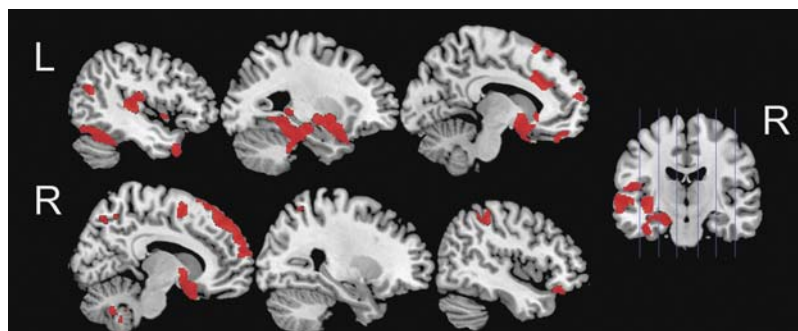
The findings of this study fully replicated those of the MCI sample. Atrophy was confined to limbic and midline structures in carriers of the ϵ 4 mutation, whereas in noncarriers atrophy involved several structures in frontal, parietal, and temporal cortex.

GENERAL DISCUSSION

This study identified distinct structural differences between MCI ϵ 4 carriers/noncarriers of the APOE ϵ 4

isoform when compared with healthy noncarriers. In MCI ϵ 4 carriers, lower gray matter volume values were mainly identified in medial temporal structures, posterior cingulate, and parietal cortex. MCI noncarriers also had lower gray matter volume values in medial temporal structures, but large significant clusters were also present in a number of neocortical regions bilaterally. Direct MCI ϵ 4 carrier/noncarrier comparisons showed that MCI ϵ 4 carriers had substantially smaller gray matter volumes in parietal regions and in the cerebellum, whereas MCI noncarriers seemed to have smaller gray matter volume values in other extensive parts of the neocortex bilaterally. At the cognitive level, the MCI group showed impoverished lexical-semantic output in the category fluency task compared with controls, with the MCI ϵ 4 carrier subgroup producing the most impoverished output of all. A voxel-based correlation analysis showed that higher gray matter volume values in the parahippocampal gyrus (including the most anterior portion, the uncus) bilaterally and in bilateral frontotemporal-parietal areas were associated with retrieval of later acquired words in the category fluency task.

Lexical-semantic competency is normally supported by an extensive neuronal network, which includes limbic, neocortical, and cerebellar structures. Limbic structures,

**FIGURE 3.** Areas of significant semantic competency by genotype interaction.

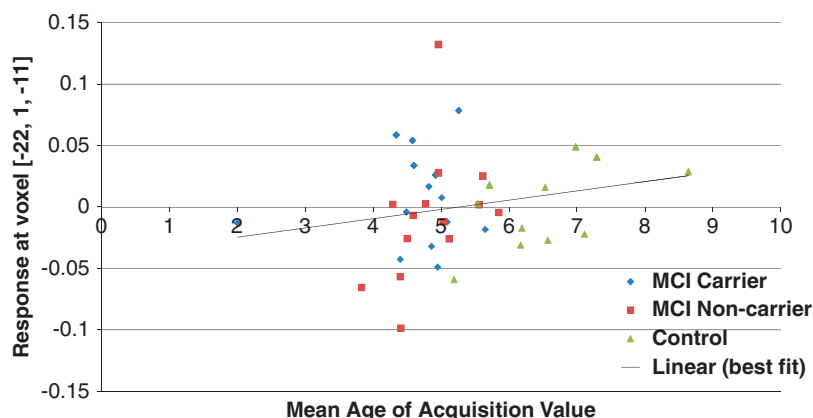


FIGURE 4. Scatter plot showing the correlation between age of acquisition values and gray matter volume values in MCI $\epsilon 4$ carriers, MCI noncarriers, and controls in the region of significant semantic competency by genotype interaction in the left parahippocampal gyrus. MCI indicates mild cognitive impairment.

especially perirhinal cortex, facilitate retrieval from long-term memory, whereas frontal, temporal, and parietal structures bilaterally are associated with semantic and lexical aspects of stored items. These extensive correlations in neocortical regions lend further support to the view that semantic representations are widespread in the neocortex, especially in frontal and temporal regions and are in line with earlier findings in established AD.⁶ A significant correlation was also found in the cerebellum. Cerebellar involvement in verbal fluency and in category fluency especially, has been found by other studies. Earlier functional and lesion studies have shown that the cerebellum plays an important part in verbal fluency, and have suggested a supportive function for higher cognitive processes associated with crossed frontal areas.^{45–49} Some authors have suggested that the cerebellum may be the repository of internal models that make a replica of essential elements of mental representations stored in the cerebral cortex.⁵⁰ In this framework, the involvement of the cerebellum in mental search and retrieval from long-term memory as required by the category fluency task is plausible.

Earlier MRI morphometric studies of APOE $\epsilon 4$ effects on brain structure have focused either on examining cognitively intact APOE $\epsilon 4$ young or older adult carriers or on morphometric measures of patients with clinically diagnosed AD.^{19,51,52} A few studies are available in which the effect of APOE $\epsilon 4$ on atrophy or progression of atrophy in the brain of MCI patients has been investigated. Significant differences especially in the hippocampus have been found and those which have assessed progression have reported accelerated atrophy in APOE $\epsilon 4$ carriers, taking place primarily in the hippocampus.^{53–57} One study more specifically reported greater atrophy in amygdala, parahippocampal gyrus, and in the medial dorsal nucleus of the thalamus but only in homozygous MCI APOE $\epsilon 4$ carriers.⁵⁸ The present morphometric study is the first to focus on the preclinical MCI stage of AD, to include healthy noncarriers and to examine the link between the added neuropathological burden of APOE $\epsilon 4$ and degraded lexical-semantic skills. Despite the small sample size, this design has clarified the morphometric differences between the genetically defined MCI subgroups and controls. The different patterns of atrophy in the MCI $\epsilon 4$ carriers and

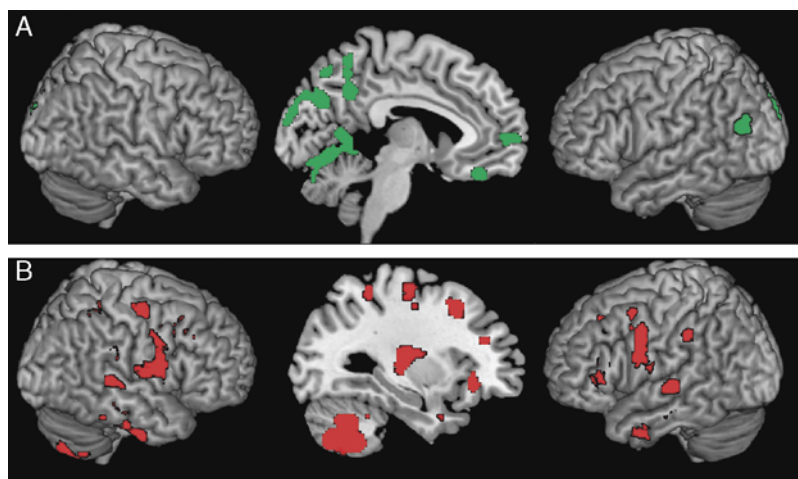


FIGURE 5. A, Areas of significant volumetric difference in AD $\epsilon 4$ carriers when compared with AD noncarriers. B, Areas of significantly lower gray matter volume values in AD noncarriers when compared with AD $\epsilon 4$ carriers. AD indicates Alzheimer disease.

MCI noncarriers are of particular interest and confirm earlier indications of a differential regional impact of AD pathology in mediotemporal and limbic areas in APOE ϵ 4 carriers.⁵¹ The difference in the pattern of atrophy of the 2 MCI subgroups suggests that in MCI noncarriers the level of cognitive decline might be due to the greater volume differences found in neocortical areas. In contrast in MCI ϵ 4 carriers, morphometry suggests a greater impact of AD pathology on the most anterior parts of the parahippocampal cortex in the medial temporal region, and on relevant structures in the limbic system. Volumetric differences are, therefore, more confined in the ϵ 4 carrier MCI subgroup but of greater impact on cognitive performance as ϵ 4 carriers showed the most pronounced lexical-semantic degradation in their linguistic output. A significant interaction between semantic competency values and genotype was found in the left perirhinal cortex supporting the idea that atrophy in this region has the greatest impact on performance on the category fluency task. Degraded functioning of limbic structures such as cingulate cortex were also reported in an fMRI study of a large sample of asymptomatic individuals with family history of AD (and therefore at greater genetic risk for AD), although no specific differences owing to APOE ϵ 4 were found.⁵⁹ It may be, therefore, that limbic structures are especially sensitive to the pathological effects of APOE ϵ 4.

This finding also adds value to the validity of a cognitive measure of lexical competence such as AoA in MCI as a useful early marker of abnormal cognitive decline. Other studies in clinically established AD have supported the discriminant power of detailed linguistic assessment as a way of detecting very early semantic degradation.^{4,6,7,14} The greater impoverishment of these skills in MCI ϵ 4 carriers seems to reflect the greater disruption caused by selective and delimited pathological involvement of structures within the limbic system, but without the hippocampus. The structures involved are part of the Papez circuit and have an important role in retrieval from long-term memory.

In MCI noncarriers, a significant but less marked impoverishment of lexical-semantic output might reflect the overall more extensive gray matter volume differences in limbic and neocortical frontal, temporal, and parietal areas in both hemispheres. Gray matter volume differences in a number of neocortical temporal regions, such as the temporal poles and the fusiform gyri, were significantly greater in MCI noncarriers compared with MCI ϵ 4 carriers. It is plausible that in MCI noncarriers, minimal lexical-semantic impairments might be related to an overall loss of cognitive efficiency owing to widespread atrophy, rather than to AD neuropathology specifically involving the perirhinal cortex. In the greatest proportion of MCI noncarriers, a number of causal factors are likely to contribute to cognitive decline. It follows that indices (such as AoA) which are more specifically affected from the outset in instances of typical AD may be better preserved in the noncarrier population at an early stage. Stated in another way, it is well known that there is a lot of heterogeneity in the MCI stage and that the available clinical criteria may not achieve high diagnostic specificity. This is not to say that only MCI APOE ϵ 4 carriers are converting to AD and the MCI APOE noncarriers are not, but it is well known from earlier large conversion studies that the APOE ϵ 4 mutation is a predictor of conversion⁶⁰

and therefore it is reasonable to assume that the decline in ϵ 4 carriers is more likely to be caused by AD neurodegeneration. In noncarriers this probability is lower and so it is more likely that pathological processes other than AD might be important contributors to cognitive decline in a good proportion of cases.

A number of studies of frontotemporal dementia have reported that decreased gray matter and perfusion in the temporal poles is associated with poorer semantic abilities.^{61–63} A previous morphometric study on patients with early AD has also reported a relationship between both mediotemporal and temporal neocortical regions and semantic fluency.⁶ In addition, functional imaging studies of semantic fluency have also highlighted the potential involvement of neocortical temporal regions, reporting activation during the task in the middle temporal gyrus, the superior temporal gyrus, and in the fusiform gyrus.^{64,65} Again, therefore, lower gray matter volume values across a greater number of limbic and neocortical structures within the lexical-semantic network identified by the voxel-based correlation analysis might contribute to the impaired lexical-semantic abilities of the MCI noncarriers in the current study, whereas in ϵ 4 carriers more limited damage to crucial regions, such as those in perirhinal cortex, is sufficient and of greater impact on their performance.

MCI ϵ 4 carriers and MCI noncarriers had, overall, comparable levels of cognitive performance despite evident volumetric differences in limbic and neocortical structures. This observation suggests that, despite the extensive areas of lower gray matter volume values in MCI noncarriers, the impact of this widespread atrophy on cognitive performance was limited compared with that expected. In contrast, in MCI ϵ 4 carriers atrophy was more circumscribed but already sufficient to produce a significant level of cognitive impairment. In MCI noncarriers there might be a more efficient neuronal connectivity in the premorbid state, which in this instance increases cerebral reserve and advantages the compensatory processes which sustain competence, even in the presence of extensive neocortical damage. In contrast, at this preclinical stage, MCI ϵ 4 carriers, despite much more limited volumetric differences in neocortex, seem to have less efficient neuronal connectivity and cognitive impairments are detectable even with limited neuronal loss. It might be objected that the 2 genetically determined subgroups are small and therefore conclusions about their relative volumetric differences remain questionable. Supporting evidence is available, however, from the additional disclosure in this study of a similar pattern of contrasting volumetric differences in the sample of minimal-to-mild AD ϵ 4 carrier patients compared with matched noncarrier AD patients of comparable clinical severity. All this evidence seems consistent with the suggestion that genetic mutations accelerate burden on neurons, leading to plasticity failure and poor neuronal repair.^{66,67} This, in turn, manifests phenotypically as more severe cognitive impairment than one might expect at a stage when neuropathological spread is still relatively limited.

More generally, the finding of significantly lower gray matter volume values in frontal areas in some of the MCI patients is of course also consistent with the early emergence of neurobehavioral symptoms including dysthymia and apathy in population-based studies of MCI.^{68,69} An earlier voxel-based morphometry paper on neuropsychiatric symptoms in early AD has investigated this issue

specifically, finding an association between atrophy in subcortical nuclei and frontal cortex and apathy.⁷⁰ The finding of significant atrophy in the cerebellum is also interesting and supports earlier findings of frequent falls, gait deficits, and amyloid accumulation in the cerebellum of AD and MCI patients.^{71,72}

In conclusion, the combination of behavioral and morphometric measurement has provided an opportunity to better test the sensitivity of lexical-semantic parameters to the earliest signs of AD pathology. This approach has also contributed to a better understanding of the effects of increased genetic risk on regional pathology and on specific forms of cognitive decline. This is the first study which has tested the association between a specific cognitive endophenotype and regional atrophy in genetically defined MCI and early AD subgroups. Additional larger studies will be important to clarify and validate the relationships between structural differences, isoform burden, and lexical-semantic loss in early and preclinical AD.

ACKNOWLEDGMENTS

The authors thank Patrizia Panzetti, Maria Grazia Venneri, Tommaso Trenti, and Nicoletta Lelli for their assistance at various stages of this study.

REFERENCES

- Petersen RC, Smith GE, Waring SC, et al. Mild cognitive impairment: clinical characterization and outcome. *Arch Neurol*. 1999;56:303–308.
- Petersen RC, Ivnik RJ, Boeve BF, et al. Outcome of clinical subtypes of mild cognitive impairment. *Neurology*. 2004;62:A295.
- Dubois B, Feldman HH, Jacova C, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. *Lancet Neurol*. 2007;6:734–746.
- Forbes-McKay KE, Ellis AW, Shanks MF, et al. The age of acquisition of words produced in a semantic fluency task can reliably differentiate normal from pathological age related cognitive decline. *Neuropsychologia*. 2005;43:1625–1632.
- Venneri A, Forbes-McKay KE, Shanks MF. Impoverishment of spontaneous language and the prediction of Alzheimer's disease. *Brain*. 2005;128:E27.
- Venneri A, McGeown WJ, Hietanen HM, et al. The anatomical bases of semantic retrieval deficits in early Alzheimer's disease. *Neuropsychologia*. 2008;46:497–510.
- McGeown WJ, Shanks MF, Forbes-McKay KE, et al. Patterns of brain activity during a semantic task differentiate normal aging from early Alzheimer's disease. *Psychiatry Res Neuroimaging*. 2009;173:218–227.
- Lambon Ralph MA, Graham KS, Ellis AW, et al. Naming in semantic dementia—what matters? *Neuropsychologia*. 1998;36:775–784.
- Biundo R, Gardini S, Concarl L, et al. Genetic influences on the decline of semantic skills in Mild Cognitive Impairment. *J Int Neuropsychol Society*. 2010. In press.
- Arango-Lasprilla JC, Cueto F, Valencia C, et al. Cognitive changes in the preclinical phase of familial Alzheimer's disease. *J Clin Exp Neuropsychol*. 2007;29:892–900.
- Reiman EM, Chen KW, Alexander GE, et al. Functional brain abnormalities in young adults at genetic risk for late-onset Alzheimer's dementia. *Proc Natl Acad Sci U S A*. 2004;101:284–289.
- Smith CD, Andersen AH, Kryscio RJ, et al. Altered brain activation in cognitively intact individuals at high risk for Alzheimer's disease. *Neurology*. 1999;53:1391–1396.
- Smith CD, Andersen AH, Kryscio RJ, et al. Women at risk for AD show increased parietal activation during a fluency task. *Neurology*. 2002;58:1197–1202.
- Seidenberg M, Guidotti L, Nielson KA, et al. Semantic memory activation in individuals at risk for developing Alzheimer disease. *Neurology*. 2009;73:612–620.
- Raber J. The impact of APOE on cognition and behavior. *Alzheimer Dement: J Alzheimer Assoc*. 2009;5:168.
- Jack CR Jr, Petersen RC, Xu YC, et al. Hippocampal atrophy and apolipoprotein E genotype are independently associated with Alzheimer's disease. *Ann Neurol*. 1998;43:303–310.
- Reiman EM, Uecker A, Caselli RJ, et al. Hippocampal volumes in cognitively normal persons at genetic risk for Alzheimer's disease. *Ann Neurol*. 1998;44:288–291.
- Plassman BL, Welsh-Bohmer KA, Bigler ED, et al. Apolipoprotein E epsilon 4 allele and hippocampal volume in twins with normal cognition. *Neurology*. 1997;48:985–989.
- Burggren AC, Zeineh MM, Ekstrom AD, et al. Reduced cortical thickness in hippocampal subregions among cognitively normal apolipoprotein E ε4 carriers. *Neuroimage*. 2008;41:1177–1183.
- Mueller SG, Schuff N, Raptentsetsang S, et al. Selective effect of Apo ε4 on CA3 and dentate in normal aging and Alzheimer's disease using high resolution MRI at 4 T. *Neuroimage*. 2008;42:42–48.
- Petersen RC, Doody R, Kurz A, et al. Current concepts in mild cognitive impairment. *Arch Neurol*. 2001;58:1985–1992.
- Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med*. 2004;256:183–194.
- McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34:939–944.
- Brun A, Englund B, Gustafson L, et al. Clinical and Neuropathological criteria for frontotemporal dementia. *J Neurol Neurosurg Psychiatry*. 1994;57:416–418.
- Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. *Neurology*. 1993;43:250–260.
- McKeith IG, Galasko D, Kosaka K, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. *Neurology*. 1996;47:1113–1124.
- Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist*. 1969;9:179–186.
- Wechsler D. *Wechsler Adult Intelligence Scale*. 3rd ed. San Antonio: The Psychological Corporation; 1997.
- Wechsler D. *Wechsler Memory Scale III*. San Antonio: The Psychological Corporation, Harcourt Brace & Co.; 1997.
- Measso G, Cavarzeran F, Zappalà G, et al. The mini-mental state examination: normative study of an Italian random sample. *Develop Neuropsychol*. 1993;9:77–85.
- De Renzi E, Faglioni P. Normative data and screening power of a shortened version of the Token Test. *Cortex*. 1978;14:41–49.
- Novelli G, Papagno C, Capitani E, et al. Tre test clinici di produzione lessicale. Taratura su soggetti normali. *Arch Psicol Neurol Psichiatr*. 1986;47:477–506.
- Novelli G, Papagno C, Capitani E, et al. Tre test clinici di memoria verbale a lungo termine. Taratura su soggetti normali. *Arch Psicol Neurol Psichiatr*. 1986;47:278–296.
- Spinnler H, Tognoni G. Standardizzazione e taratura italiana di test neuropsicologici. *Ital J Neurol Sci*. 1987;6:1–120.
- Caffarra P, Vezzadini G, Dieci F, et al. Rey-Osterrieth complex figure: normative values in an Italian population sample. *Neurol Sci*. 2002;22:443–447.
- Basso A, Capitani E, Laiacona M. Raven's coloured progressive matrices and brain damage: normative values on 305 adult normal controls. *Funct Neurol*. 1987;1:189–194.

37. Caffarra P, Vezzadini G, Dieci F, et al. Una versione abbreviata del test di Stroop. Dati normativi nella popolazione italiana. *Nuova Riv Neurol*. 2002;12:111–115.
38. Morrison CM, Chappell TD, Ellis AW. Age of acquisition norms for a large set of object names and their relation to adult estimates and other variables. *Q J Exp Psychol*. 1997;50A:528–559.
39. Larochelle S, Richard S, Soulières I. What some effects might not be: the time to verify membership in “Well defined” categories. *Q J Exp Psychol*. 2000;53:929–961.
40. Ashburner J, Friston KJ. Unified segmentation. *Neuroimage*. 2005;26:839–851.
41. Ashburner J. A fast diffeomorphic image registration algorithm. *Neuroimage*. 2007;38:95–113.
42. Ashburner J, Friston KJ. Computing average shaped tissue probability templates. *Neuroimage*. 2009;45:333–341.
43. Evans AC, Collins DL, Mills SR, et al. 3D statistical neuroanatomical models from 305 MRI volumes. Proceedings IEEE-Nuclear Science Symposium and Medical Imaging Conference. San Francisco, CA, USA: IEEE, 1993:1813–1817.
44. Davatzikos C, Genc A, Xu D et al. Voxel-based morphometry using the RAVENS maps: methods and validation using simulated longitudinal atrophy. *Neuroimage*. 2001;14:1361–1369.
45. Schlosser R, Hutchinson M, Joseph S, et al. Functional magnetic resonance imaging of human brain activity in a verbal fluency task. *J Neurol Neurosurg Psychiatry*. 1998;64:492–498.
46. Gourovitch ML, Kirkby BS, Goldberg TE, et al. A comparison of rCBF patterns during letter and semantic fluency. *Neuropsychology*. 2000;14:353–360.
47. Hubrich-Ungureanu P, Kaemmerer N, Henn FA, et al. Lateralized organization of the cerebellum in a silent verbal fluency task: a functional magnetic resonance imaging study in healthy volunteers. *Neurosci Lett*. 2002;319:91–94.
48. Ravnkilde B, Videbech P, Rosenberg R, et al. Putative tests of frontal lobe function: a PET-study of brain activation during Stroop’s Test and verbal fluency. *J Clin Exp Neuropsychol*. 2002;24:534–547.
49. Richter S, Gerwig M, Aslan B, et al. Cognitive functions in patients with MR-defined chronic focal cerebellar lesions. *J Neurol*. 2007;254:1193–1203.
50. Ito M. Control of mental activities by internal models in the cerebellum. *Nat Rev Neurosci*. 2008;9:304–313.
51. Lehtovirta M, Laakso MP, Frisoni GB, et al. How does the apolipoprotein E genotype modulate the brain in aging and in Alzheimer’s disease? A review of neuroimaging studies. *Neurobiol Aging*. 2000;21:293–300.
52. Filippini N, Rao A, Wetten S, et al. Anatomically-distinct genetic associations of APOE epsilon4 allele load with regional cortical atrophy in Alzheimer’s disease. *Neuroimage*. 2009;44:724–728.
53. Farlow MR, He Y, Tekin S, et al. Impact of APOE in mild cognitive impairment. *Neurology*. 2004;63:1898–1901.
54. Morra JH, Tu Z, Apostolova LG, et al. Automated mapping of hippocampal atrophy in 1-year repeat MRI data from 490 subjects with Alzheimer’s disease, mild cognitive impairment, and elderly controls. *Neuroimage*. 2009;45:S3–S15.
55. van de Pol LA, Van Der Flier WM, Korf ESC, et al. Baseline predictors of rates of hippocampal atrophy in mild cognitive impairment. *Neurology*. 2007;69:1491–1497.
56. Fleisher A, Grundman M, Jack CR Jr, et al. Sex, apolipoprotein E epsilon 4 status, and hippocampal volume in mild cognitive impairment. *Arch Neurol*. 2005;62:953–957.
57. Hamalainen A, Grau-Olivares M, Tervo S, et al. Apolipoprotein E epsilon 4 allele is associated with increased atrophy in progressive mild cognitive impairment: a voxel-based morphometric study. *Neurodegenerat Dis*. 2008;5:186–189.
58. Pennanen C, Testa C, Boccardi M, et al. The effect of apolipoprotein polymorphism on brain in mild cognitive impairment: a voxel-based morphometric study. *Dement Geriatr Cogn Disord*. 2006;22:60–66.
59. Bassett SS, Yousem DM, Cristinzio C, et al. Familial risk for Alzheimer’s disease alters fMRI activation patterns. *Brain*. 2006;129:1229–1239.
60. Petersen RC, Smith GE, Ivnik RJ, et al. Apolipoprotein E status as a predictor of the development of Alzheimer’s disease in memory-impaired individuals. *JAMA*. 1995;273:1274–1278.
61. Noppeney U, Patterson K, Tyler LK, et al. Temporal lobe lesions and semantic impairment: a comparison of herpes simplex virus encephalitis and semantic dementia. *Brain*. 2007;130:1138–1147.
62. Williams GB, Nestor PJ, Hodges JR. Neural correlates of semantic and behavioural deficits in frontotemporal dementia. *Neuroimage*. 2005;24:1042–1051.
63. Desgranges B, Matuszewski V, Piolino P, et al. Anatomical and functional alterations in semantic dementia: a voxel-based MRI and PET study. *Neurobiol Aging*. 2007;28:1904–1913.
64. Birn RM, Kenworthy L, Case L, et al. Neural systems supporting lexical search guided by letter and semantic category cues: a self-paced overt response fMRI study of verbal fluency. *Neuroimage*. 2010;49:1099–1107.
65. Gaillard WD, Sachs BC, Whitnah JR, et al. Developmental aspects of language processing: fMRI of verbal fluency in children and adults. *Hum Brain Mapp*. 2003;18:176–185.
66. Mesulam MM. Neuroplasticity failure in Alzheimer’s disease: bridging the gap between plaques and tangles. *Neuron*. 1999;24:521–529.
67. Mesulam MM. A plasticity-based theory of the pathogenesis of Alzheimer’s disease. *Ann N Y Acad Sci*. 2000;924:42–52.
68. Lyketsos CG, Lopez O, Jones B, et al. Prevalence of neuropsychiatric symptoms in dementia and mild cognitive impairment: results from the cardiovascular health study. *JAMA*. 2002;288:1475–1483.
69. Palmer K, Berger AK, Monastero R, et al. Predictors of progression from mild cognitive impairment to Alzheimer disease. *Neurology*. 2007;68:1596–1602.
70. Bruen PD, McGeown WJ, Shanks MF, et al. Neuroanatomical correlates of neuropsychiatric symptoms in Alzheimer’s disease. *Brain*. 2008;131:2455–2463.
71. Lerner AJ. The cerebellum in Alzheimer’s disease. *Dement Geriatr Cogn Disord*. 1997;8:203–209.
72. Verghese J, Robbins M, Holtzer R, et al. Gait dysfunction in mild cognitive impairment syndromes. *J Am Geriatr Soc*. 2008;56:1244–1251.