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ABSTRACT

Mild cognitive impairment (MCI) is the transition stage between the normal aging process and dementia itself. The most common clinical phenotype is amnestic MCI (aMCI) [subtypes: single domain (sMCI) and multiple domains (mMCI)], which is considered prodromal to Alzheimer's disease (AD). The APOE (apolipoprotein E) e4 allele is the most important genetic risk factor for AD, but its association with MCI onset and conversion to AD is controversial. In this follow-up study of 88 aMCI patients (68% sMCI and 32% mMCI at baseline), we examined APOE genotypes and plasma levels in relation to MCI development and progression based on their clinical/cognitive data obtained at baseline and follow-up assessment (mean follow-up time = 6.6±3.4 years). A control sample (n=164) was collected in previous investigations. The overall conversion rate to mMCI or AD was 52.2%. The APOE e4 allele was associated with a higher risk of developing MCI (OR: 2.23; 95% CI: 1.22-4.08). The conversion rate in the e4 allele carriers (32% of the sample) was 71%, and the e4 allele was associated with a higher risk of conversion to mMCI/AD (OR: 4.1; 95% CI: 1.2-13.6). APOE e2 allele carriers were 7% (all sMCI) and none progressed to mMCI/AD. Among MCI subjects, e4 carriers had the lowest plasma apoE levels (37.8±12.5mg/l), and e2 carriers had the highest (78.6±38.1mg/l). APOE *e*4 is a risk allele for the development and progression of aMCI, the APOE e2 allele seems to be protective, and apoE levels associated to them are an integral part of their action.

INTRODUCTION

With rapid aging of their populations, high-income countries face an increase in the proportion of the elderly with various forms of age-related dementia, among which Alzheimer's disease (AD) is, by far, the most frequent. A particular area of scientific and clinical research on the early detection of dementia has been the transition stage between the normal aging process and dementia itself. Mild cognitive impairment (MCI), a clinical entity that may represent this transition stage, is defined as a cognitive decline greater than that expected for an individual's age and education level but that does not interfere notably with activities of daily life. Among subjects aged 60 years and over, the estimated MCI prevalence ranges from 16 to 20% [Roberts and Knopman, 2013]. Subjects with MCI have been noted to develop dementia at a higher rate than cognitively normal subjects and the reported rates of progression from MCI to dementia range from 20% to 40% (10–15% per year). However some may remain at the MCI stage and a minority may revert to normal cognition [Roberts and Knopman, 2013]. Two clinical phenotypes of MCI are distinguished: amnestic MCI (aMCI) and non amnestic MCI (naMCI), each of which comprises subtypes of single and multiple domain classifications [Petersen et al., 2001; Petersen et al., 2009 Albert et al., 2011]. aMCI (single and multiple domain), the most common of the two, is considered a prodromal state of AD [Petersen et al., 2001; Petersen et al., 2009; Albert et al., 2011] while naMCI may progress to non-AD dementias.

Apolipoprotein E (APOE=gene, apoE=protein), a recognized genetic factor involved in AD development, plays a central role in plasma lipoprotein metabolism and lipid transport within tissues and in neuronal repair, remodeling or protection in the brain [Mahley and Rall, 2000]. APOE shows a genetic polymorphism with three common alleles APOE e2, APOE e3, and APOE e4 determined by two single nucleotide polymorphisms that cause amino acid substitutions, resulting in functional

differences in the corresponding three isoforms [Mahley and Rall, 2000]. The e2 allele has been found associated with lower levels of plasma total and low-density lipoprotein (LDL) cholesterol than those associated with the most common e3 allele, whereas the e4 allele displays an opposite pattern of higher levels of total and LDL cholesterol. Conversely, as compared with APOE e3 homozygotes, apoE levels are higher in APOE e2 carriers and lower in APOE e4 carriers [Mahley and Rall, 2000]. The e4 allele, the most important genetic risk factor for late-onset AD, not only increases the risk but also lowers the age at onset of AD. It has been hypothesized, furthermore, that the apoE isoforms differentially affect amyloid plaque deposition and clearance: the risk allele e4 has been shown to increase amyloid β (Aβ) aggregation and to impair clearance relative to the other apoE isoforms [Verghese et al., 2011]. Differently, the e2 allele seems to exert a protective effect against AD development by preventing Aβ deposition and enhancing its clearance [Suri et al., 2013].

APOE seems to exert an effect in cognitively normal subjects as well. The APOE e4 allele has been implicated in the normal cognitive decline with advancing age, affecting episodic memory and executive functioning in particular [Reitz and Mayuex, 2010; Wisdom et al., 2011; Davies et al., 2014]. Research on whether APOE genotypes may differentially influence the risk for developing MCI has linked the e4 allele with an increased risk of MCI or progression from MCI to dementia AD [Petersen, et al., 2009; Reitz and Mayeux, 2010] , but this link has proved uncertain [Brainerd et al., 2013; Barabash et al., 2009; Tyas et al., 2008]. Inconsistent findings stem from differences in sample size, short follow-up duration or the clinical classification of MCI status. As mentioned above, only aMCI (single or multiple domain aMCI) is thought to progress to AD.

Unlike the recognized role of APOE polymorphism in AD , the clinical relevance of plasma apoE concentration in AD pathophysiology remains unclear.

APOE genotypes influence plasma apoE level and between 6 and 30% of its total variability can be explained by common APOE polymorphisms [Siest et al., 2000a]. Investigations to identify a useful biomarker that assesses the risk of developing AD or its presence have found that plasma apoE levels are much lower in AD patients than in healthy controls, indicating that apoE concentration may serve as a potential clinical marker for AD [Siest et al., 2000b; Corbo et al., 2006; Wang et al., 2014]. Although there is little data on plasma apoE concentrations in MCI subjects, and in relation to conversion from MCI to AD, it has been observed that apoE levels are lower in such subjects than in controls [Gupta et al., 2011; Hye et al., 2014]

In this follow-up study we investigated the possible role of the APOE gene as a whole in MCI development and progression to AD by examining the distribution of APOE genotypes and plasma apoE concentrations in a sample of MCI subjects. To reduce the effect of heterogeneity, all the subjects included in the study met the criteria for amnestic MCI, which manifests with prominent memory impairment and is likely progresses to AD [Petersen et al., 2009; Albert et al., 2011]. In addition, because the number of affected domains is a relevant parameter for defining the extent of disease severity and the likelihood of progression to AD, all subjects were assessed for cognitive impairment in one or more domains.

MATERIALS AND METHODS

Subjects

The study sample was 88 patients (61.4% women) consecutively admitted to the Alzheimer's Disease Center of Neurology Division of Verona Hospital. Patients were living in the community and had been referred by their general practitioner. They were Caucasians, born in a limited geographical area in northern Italy (district of Verona and Veneto region). Clinical and cognitive data were obtained at baseline and follow-up

assessment. Blood samples were collected at follow-up. The mean age at baseline was 70.7 ± 6.5 years and the mean duration of follow-up was 6.6± 3.4 years

MCI diagnosis was established according to internationally accepted criteria [Petersen et al., 2009; Albert et al., 2011]: (1) subjective complaint of a memory (or other cognitive) deficit confirmed by a relative or caregiver; (2) impairment in a single or multiple cognitive domains; (3) normal performance of daily living activities as measured with ad hoc scales; and (4) no dementia. Cognitive function impairment was assessed using the Mental Deterioration Battery (MDB) (Carlesimo et al. 1996) which assesses attention, verbal memory, verbal fluency, and constructive praxis The impact of dementia on everyday activities was rated and excluded according to Clinical Dementia Rating (CDR) scale scores and two clinical interviews with both the patient and the informant (Instrumental Activities of Daily Living and Basic Activities of Daily Living) [Katz et al., 1970; Lawton and Brody, 1969]. The absence of global deterioration and dementia was assessed using the Mini-Mental State Examination (MMSE) [Folstein et al., 1975] and the Diagnostic and Statistical Manual of Mental Disorders criteria [DSM-IV-AmericanPsychiatric Association, 2000]. Amnestic MCI was categorized as single domain MCI (sMCI) if only one domain was impaired, and multiple domain MCI (mMCI) if two or more domains were impaired. Patients were excluded if they had a history of head injury, psychiatric disorders, neurological diseases or severe sensorial deficits. AD diagnosis was established independently according to DSM-IV-TR and National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorder Association (NINCDS-ADRDA) criteria [McKhann et al., 1984]. Follow-up evaluation comprised standardized neuropsychological assessment comparable to that performed at baseline.

Data on the control sample were collected during previous investigations; the sample was composed of 164 unrelated subjects (61.9% women; mean age 70.7±8.9 years) residing in the same geographic area as the patients. None of the controls presented signs of degenerative neurological disease. They were either the healthy spouses of the patients or subjects whose scores were below the threshold values on the same tests used for establishing MCI/LOAD diagnosis.

The protocol for the collection of biological material for the scientific studies was approved by the institutional ethics committees. Informed consent was obtained from all subjects.

Laboratory Methods

Venous blood was drawn in EDTA from all subjects after overnight fasting. Genomic DNA was extracted from whole blood using the salting out method described by Miller et al. [7]. APOE common genotypes were detected by restriction fragment length polymorphism (RFLP) analysis according to Wenham et al. [16].

Plasma apoE levels were measured using an immunonephelometric assay (Siemens Healthcare Diagnostics, Marburg Germany) and expressed in mg/L.

Statistical analysis

Allelic frequencies were calculated by the gene-counting method. Agreement of the observed genotype frequencies with those expected according to the Hardy-Weinberg equilibrium was verified with the chi-square test. Differences in frequencies between patients and controls were compared using a chi-square test or Fisher's exact test when small values were present. The risk of developing MCI and the risk of conversion to mMCI or AD associated with APOE genotypes was estimated according to the adjusted odds ratio (OR) obtained from logistic regression analysis after entering sex, age, and APOE genotypes as covariates. Analysis of variance (ANOVA) was

performed to compare the mean apoE levels associated with APOE genotype. When the data did not meet the assumptions of ANOVA, the Kruskall-Wallis test was used.

RESULTS

Table 1 presents the baseline clinical characteristics of the aMCI subjects by diagnosis. There were no significant differences in the demographic and clinical characteristics between the sMCI (68%) and mMCI (32%) subjects, except for age at first diagnosis (p=0.02) and MMSE scores (p=0.0004). The clinical characteristics at follow-up are reported in Table 2. The clinical evaluation at the follow-up identified 38.6% sMCI, 25.0% mMCI, and 36.4% AD subjects. The three groups differed significantly for mean MMSE scores and age, with the lowest age noted in the sMCI group and the highest in the AD group. The change in cognitive impairment, measured as the difference between MMSE scores at follow-up and baseline, was -2.8 ± 4.5 (mean ± s.d.). Multiple regression analysis showed that the magnitude of cognitive impairment increased with age (b=-0.22, 95% CI: -0.38/-0.07, p=0.005) and for male sex (b=-3.2, 95% CI: -5.3/-1.1, p=0.004) but did not depend on follow-up duration or years of education. At follow-up, conversion to mMCI or AD was noted in 52.2% of MCI subjects. Among the subjects diagnosed with sMCI at baseline, conversion to mMCI or to AD was observed in 43.3% (23.3% and 20%, respectively), and 71.4% of those diagnosed with mMCI at baseline converted to AD. The difference in the conversion rate between the two baseline MCI groups was significant (p=0.01). Also there was a significant difference in mean MMSE scores between the two MCI groups at follow-up: the baseline MMSE scores of the sMCI subjects who converted were significantly lower than those of the non-converted sMCI subjects (27.6±2.1 and 28.9±1.2, respectively, p=0.009). Similarly, the baseline MMSE scores of the converted mMCI subjects were significantly lower than those of the non-converted mMCI subjects

(25.4±3.2 and 28.1±1.2, respectively, p=0.05). Nearly significant differences in the conversion rate according to sex (a greater propensity of males to conversion) and age (higher age of converted subjects at baseline and follow-up, p=0.06 and 0.08 respectively) were observed (Table 2). Years of education had no effect on the conversion rate.

Table 3 presents the distribution of APOE genotypes and allele frequency in the different groups. There were significant differences in APOE genotype and allele frequencies among the MCI subjects and the controls (p= 0.03 and p=0.005, respectively) mainly due to the higher number of APOE e4 carrying genotypes among the MCI patients. To better evaluate the risk of MCI development associated with carrying or not the APOE e4 allele, we performed a logistic regression analysis and included in the model, together with APOE e4 allele, potential confounding factors such as age and sex. The analysis showed that carrying the APOE e4 allele is associated with a significantly increased risk of developing MCI (O.R.= 2.23 95% CI 1.22-4.08, p=0.009), while age and sex are not. Comparison of the distribution of APOE genotypes in the MCI subgroups at baseline and follow-up showed that, at baseline, e4/e4 homozygotes were present only in the mMCI group and APOE e2 carrying genotypes were present only in the sMCI group. Significant differences in allele frequencies were found only between the controls and the mMCI subjects (p<0.001). The difference was particularly evident on comparison of allele frequencies: APOEe4 allele frequency in the mMCI subjects was more than twice that of the sMCI subjects and three times that of the controls, while the APOE e2 allele was completely absent (Table 3). Subgroup analysis of APOE genotype and allele distributions at follow-up (sMCI, mMCI, AD) showed a clear difference between sMCI on one side and mMCI and AD on the other, which was more marked for the allele frequencies. The APOE allele frequencies among the sMCI subjects were very similar to those of the control group (p= 0.71), whereas

the frequencies among the mMCI and the AD were similar (p=0.79), with the e4 allele frequency about three times higher than that of the sMCI subjects and the e2 allele completely absent.

APOE genotype appeared to influence conversion of MCI subjects to AD (from sMCI to mMCI or AD, and from mMCI to AD) as well. APOE genotype and allele frequencies in the converted subjects differed significantly from the not-converted (p=0.014) and the controls (p<0.0001), while the APOE allele frequencies in the not-converted subjects were similar to those in the controls (p=0.80). Within the total MCI sample, 48% of the e3/e3 subjects converted to AD (or from sMCI to mMCI), none of the e3/e2 subjects converted to AD or mMCI, and 71% of the e4 carriers converted to AD or mMCI. (Fig. 1). Logistic regression analysis was then applied to correctly estimate the specific role of the APOE e4 allele on the risk of conversion. In this analysis, the dependent variable was conversion to mMCI/AD or not and the explanatory variables were APOE e4 carrying genotype, together with sex, age at baseline, and MMSE at baseline, which are recognized factors influencing conversion to mMCI or to AD. The analysis showed that carrying the APOE e4 allele significantly increases the risk of conversion as compared with the APOE e3/e3 and e3/e2 genotypes (O.R.= 4.1, 95%C.I. 1.2- 13.6, p=0.02) and is independent of the presence of the other risk factors [age at baseline: O.R=1.1, 95%C.I. 1.0-1.2 p=0.02; MMSE scores: O.R.= 0.62, 95%C.I. 0.46-0.83, p=0.001; sex (males): O.R. 2.71, 95%C.I. 0.9-8.1, p=0.07].

ApoE plasma levels were determined in the MCI sample at the follow-up. Plasma apoE levels were lower among the males (38.8±13.5 mg/l) than the females (47.2±19.0 mg/dl, p=0.01). Though there was no significant difference in apoE levels between the three patient groups (sMCI, mMCI, AD) (Table 2), a downward trend with increasing disease severity was observed (sMCI>mMCI> AD). Similarly, plasma apoE levels were slightly higher in the not-converted than in the converted subjects (Table

2). The apoE levels in the whole patient sample differed significantly for APOE genotype (Fig. 2), with the APOE e3e2 genotypes showing the highest values (78.6±38.1 mg/l), and the APOE e 4/e3 (37.9±9.9 mg/l) and APOE e 4/e4 (37.5±23.9 mg/l) genotypes the lowest. The APOE e3/e3 showed intermediate values (42.4±10.1 mg/l).

DISCUSSION

Early recognition of MCI has opened new possibilities for early treatment. But it has also raised complex questions about the role known risk factors for dementia play in the progression of MCI to dementia and to AD in particular. In this study we analysed APOE genotypes and plasma levels to determine the possible involvement of the APOE gene in the progression of MCI to AD .

In the amnestic MCI sample as a whole, conversion rate was 52.3% from diagnosis at baseline, but the conversion rates among the sMCI subjects was significantly lower than among the mMCI subjects (43.3% vs. 71.4%). Greater cognitive impairment (lower MMSE scores) was a significant predictor of progression among both the sMCI (to mMCI or to AD) and the mMCI subjects. This reflects the typical pathway of conversion from aMCI to AD which proceeds with a gradual increase in cognitive disabilities as the condition evolves from sMCI to mMCI and eventually to AD. [Petersen et al., 2009; Roberts and Knopman, 2013]. Age also seemed to be an important predictor of conversion from sMCI to AD, with higher mean age associated with greater disease severity (sMCI <mMCI <AD). This pattern was found both for age at baseline and age at follow-up and is consistent with previous studies [Roberts and Knopman, 2013] suggesting that age may act as a prevalent risk factor [Tyas et al.,2007], in the progression to dementia. Finally, gender appeared to influence progression of disease; although the proportion of females was higher in the

sample (61.4%), more males converted to mMCI or AD and male sex correlated significantly with changes in cognitive impairment. Published data on the sex ratio among MCI subjects are derived largely from prevalence data and are mixed, but a higher prevalence of males is consistently reported [Petersen et al., 2010; Roberts and Knopman, 2013] .

Within the total sample of MCI subjects, analysis of the distribution of APOE genotypes showed that carrying the APOE e4 allele is associated with a significantly higher risk for aMCI development (O.R.=2.2). However, when the sMCI /mMCI subtype classification at baseline was taken into account, only the mMCI subgroup differed significantly from the controls, with an e4 allele frequency about three times that of the controls (0.286 vs. 0.088). Subgroup analysis at follow-up highlighted a clear distinction between the two MCI subtypes in the distribution of APOE genotypes and alleles: the sMCI subjects and the controls had similarly low e4 allele frequencies while the mMCI subjects and the AD patients had similarly high frequencies. At the genotype level, it is noteworthy that e4/e4 homozygotes were found at baseline only among the mMCI subjects and at follow-up among the mMCI and the AD subjects. Logistic regression analysis showed that carrying the e4 allele confers a significantly increased risk of conversion to mMCI or AD independent of age or cognitive impairment severity, two other important predictors of disease progression. But the difference between the two MCI subtypes also concerns the APOE e2 allele, which was present only in the sMCI subjects at both baseline and follow-up, since none of the e3/e2 carriers converted to mMCI or AD, whereas 48% of the e3/e3homozygotes and 71% of the e4 carriers did. Due to the low e2 allele frequency, the result was not statistically significant, but it did seem to confer the APOE e2 allele a protective role against progression of cognitive deterioration.

Numerous studies have investigated the relationships between APOE genotypes, MCI development, and conversion to dementia/AD, but the overall picture is controversial. Some studies observed that carrying the APOE e4 was associated with an increased risks of developing MCI in healthy subjects, but not with progression from MCI to dementia [Brainerd et al., 2013; Barabash et al., 2009; Tyas et al., 2008]. Other investigations provided evidence that the presence of APOE e4 was associated with a more rapid progression from MCI to AD (Petersen et al. 1995; Aggarwal et al., 2005; Blom et al., 2009]. Underlying the discrepancies might be the heterogeneity of MCI samples: while naMCI can evolve into various forms of dementia, such as frontotemporal dementia or dementia with Lewy bodies [Roberts and Knopman, 2013], aMCI (single or multiple domains) is assumed to progress to AD, for which the e4 allele is a major risk factor. In the present study we have tried to reduce as much as possible the heterogeneity of the sample, including only the subjects who met the criteria for aMCI, [Petersen et al., 2009; Albert et al., 2011], and identifying the two subtypes (sMCI and mMCI) associated with increasing disease severity. The results of the study provides evidence for involvement of the APOE e4 allele in both the development and progression of MCI (aMCI). However, the analysis of the distribution of APOE allele frequencies at follow-up showed that the allele frequencies in the sMCI subjects and the controls were similar, whereas the allele frequencies in the mMCI subjects were very similar to those in the AD subjects. This suggests that the risk associated with the APOE e4 allele is stronger in the conversion from sMCI to mMCI than in the subsequent progression to AD, where it could act in conjunction with other risk factors. The same line of reasoning could be applied to the APOE e2 allele, which was completely absent among the converted subjects. This finding, although limited by the small sample number in the present study, suggests a protective action of APOE e2 against severe cognitive impairment, in agreement with previous studies showing that

e2 allele leads to a decreased AD-related neuropathology, and may also act delaying the clinical manifestations of AD [Suri et al., 2013; Iacono et al., 2015].

We found no significant correlation between mean plasma apoE levels and MCI severity or conversion to AD. Unfortunately we were unable to compare them with a control group, though the downward trend sMCI > mMCI>AD is consistent with previous observations of significantly lower apoE levels in AD patients as compared to controls [Siest et al., 2000b; Corbo et al., 2006; Wang et al., 2014], and less significant differences between AD and MCI subjects [Gupta et al., 2011; Gupta et al., 2015]. Our finding of lower apoE levels in the males than in the females is shared by previous studies comparing AD patients and controls [Scacchi et al, 1999; Cruchaga et al.,2012; Gupta et al., 2015], and in line with population studies which reported that the mean serum apoE concentration is lower in men than in women in healthy older individuals (> 50 years of age) [Schiele F. et al, 2000]. This difference between men and women has been suggested to depend on the effect of sex hormones in interaction with age [Haddy et al, 2002; Srivastava et al, 1997]. The greater propensity to MCI progression and greater cognitive impairment we observed in the males supports the hypothesis that, to some extent, lower apoE levels might be an additional risk factor in AD irrespective of APOE genotype [Wang et al, 2014].

Previous studies have shown a significant association between mean plasma apoE levels and APOE genotypes, with a gradient of apoE levels in the order of e3/e2>e3/e3>e3/e4≥e4/e4 [Mahley and Rall, 2000; Schiele et al., 2000]. The present study corroborates these findings: the highest apoE levels were associated with the APOE e2 allele and the lowest with the APOE e4 allele. The role played by APOE alleles in the conversion from MCI to AD (protection conferred by e2 and risk by e4) underlines an important function of apoE protein level associated with the APOE genotypes. There is increasing evidence that by itself the apoE level could be another

contributing factor in MCI/AD onset and evolution. Indeed, AD patients are noted to have lower plasma apoE levels than controls, independent of APOE genotype [Gupta et al., 2011]. Moreover, low apoE levels are correlated with greater cerebral amyloid burden [Gupta et al., 2015], and with smaller hyppocampal size, another neuroimaging marker associated with AD pathology [Teng et al., 2015]. Conversely, it could be argued that high plasma apoE levels protect against the development of AD and the pathological signs accompanying it. APOE isoforms have been shown to differentially modulate amyloid-β accumulation and clearance, but they can also exert their role through their action on synaptic plasticity and membrane remodeling after neuronal injury. [Mahley and Rall, 2000]. Collectively, the data reported in the present study suggest that protein levels associated with APOE genotype are an integral part of the action of the genotype in increasing the risk of MCI and AD or protecting against cognitive decline.

In conclusion, our findings suggest that APOE genotype and associated protein levels contribute to the progression of cognitive impairment from a single domain to multiple domains. APOE genotype may be a predictor of disease progression already in the early disease stages, and as such, potentially useful in directing clinical interventions to prevent or delay AD.

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The authors declare that there is no conflict of interest.

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Table 1. Baseline demographic characteristics of the MCI patients

	Total MCI	sMCI	mMCI	p
Total	88	60 (0.68)	28 (0.32)	
Age (years)	70.7 ± 6.5	69.6± 6.1	73.1± 6.8	0.02
Sex (males,%)	38.6	40.0	35.7	0.15
MMSE score	26.9±2.7	27.9±2.1	24.9±2.8	0.004
Education (years)	7.4±3.8	7.7±3.7	6.9±4.1	0.38

Plus-minus values are means ±standard deviation.

Table 2 Characteristics of the MCI patients at follow-up

	Follow-up						
	sMCI	**mMCI**	**AD**	P value	**Not converted**	**Converted**	P value
Follow-up	34 (38.6)	22 (25.0)	32 (36.4)		42 (47.7)	46 (52.3)	
Baseline aMCI	*34 (56.7)*	*14(23.3)*	*12(20.0)*		*34 (56.7)*	*26(43.3)*	
mMCI	*/*	*8(28.6)*	*20(71.4)*	<0.0001	*8(27.6)*	*20(71.4)*	0.01
Sex (%) males	26.5	29.4	44.1	0.18	35.3	64.7	0.06
females	46.3	22.2	31.5		55.6	44.4	
Age (years)	74.9±6.4	77.4±5.6	79.5±6.4	0.02	76.1±5.9	78.5±6.5	0.08
Follow up (years)	6.5±4.1	7.3±3.4	6.2±2.6	0.48	6.5±3.9	6.6±3.0	0.85
Education (years)	7.8±3.6	7.0±3.5	7.4±4.4	0.80	7.4±3.6	7.5±4.1	0.88
MMSE score	28.3±1.4	26.0±2.6	19.3±5.9	<0.0001	28.6±1.6	22.3±6.2	<0.0001
Plasma apoE(mg/l)	47.4± 23.4	42.8±13.0	40.4±10.5	0.54	45.7±22.0	42.0±11.0	0.80

Table 3. Distribution of APOE genotypes and alleles in MCI patients at baseline and follow.up

	APOE genotypes				APOE alleles		
	e3/e3	**e4/e4***	**e3/e2**	**e4/e3**	**e2**	**e3**	**e4**
Total MCI sample	54 (0.61)	4(0.05)	6(0.07)	24 (0.27)	0.032	0.784	0.182
Controls	118 (0.72)	0	17 (0.10)	29[§](0.18)	0.061	0.851	0.088
Chi square p value	0.033				0.005		
Baseline sMCI	38 (0.63)	0*	6 (0.10)*	16 (0.27)	0.05[#]	0.817	0.133
mMCI	16 (0.57)	4 (0.14)	0	8 (0.29)	/	0.714	0.286
Chi square p value	0.15				0.0146		
Follow-up sMCI	22 (0.65)	0*	6 (0.18)*	6 (0.18)	0.088[#]	0.824	0.088
mMCI	13 (0.59)	1 (0.05)	0	8 (0.36)	/	0.773	0.227
AD	19 (0.59)	3 (0.09)	0	10 (0.31)	/	0.750	0.250
Chi square p value	0.08				0.037		
Not converted	28 (0.66)	1 (0.02)*	6 (0.14)*	7 (0.17)	0.071[#]	0.821	0.107
converted	26 (0.56)	3 (0.07)	0	17 (0.37)	/	0.750	0.250
Chi square p value	0.014				0.014		

§ 3 APOE e4/e2 genotypes were included

*For chi square calculation APOE e3/e2 genotypes were pooled with APOE e3/e3 genotypes and APOE e4/e4 with APOE e4/e3 genotypes

[#] For chi square calculation APOE e2 alleles were pooled with APOE e3 alleles

Fig. 1. Conversion rate of MCI patients according to APOE genotypes.

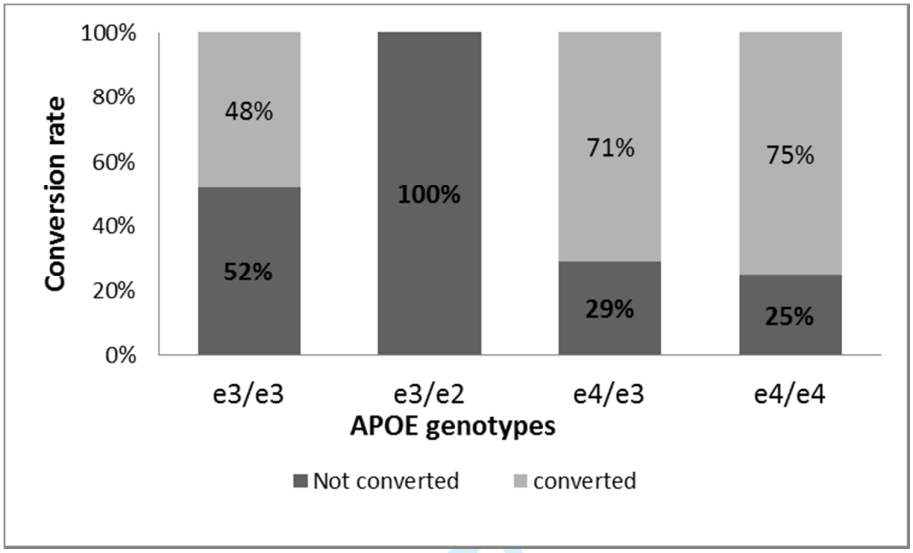

Fig. 2 Mean plasma apoE levels (mg/l) associated with APOE genotypes at follow-up.