Apolipoprotein E (APOE) polymorphism influences serum APOE levels in Alzheimer's disease patients and centenarians

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Vascular factors may play a role in the etiology of Alzheimer's disease (AD) and increased serum apolipoprotein E (APOE) levels in AD could be of interest, as APOE concentration is associated with vascular disease. Aims of this study were to evaluate the influence of APOE genotype on serum APOE levels, and, secondly, to study serum APOE concentrations in relation to age and AD. APOE genotypes, serum total cholesterol, LDL cholesterol, HDL cholesterol, total cholesterol/HDL cholesterol ratio, triglycerides, and serum APOE were performed on 52 healthy centenarians, 49 AD patients, 45 age-matched controls, and 72 young healthy adults. In all study population a significant trend in reduction of serum APOE levels from APOE ϵ2- to ϵ4 carriers was observed. The difference in serum APOE levels among age groups significantly decreased in ϵ4 carriers only, including HDL cholesterol; no significant differences between AD patients and age-matched controls were found. In these highly selected populations, APOE genotype distribution strongly influences serum APOE concentration, not suggesting, at present, a possible role as a biochemical marker for AD, but only as a putative longevity factor. *NeuroReport* 14:605–608 © 2003 Lippincott Williams & Wilkins.

Key words: Alzheimer's disease; Apolipoprotein E genotype; Centenarians; Longevity; Serum apolipoprotein E

INTRODUCTION

Apolipoprotein E (APOE) is a polymorphic protein involved in transport and redistribution of lipids in various tissues. Three major APOE isoforms, E2, E3, and E4, which are coded by the alleles ϵ2, ϵ3, and ϵ4 at a single locus on chromosome 19, have been identified. The APOE ϵ4 allele is associated with high serum total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and apolipoprotein B levels in many populations [1], and has been found to increase risk for coronary artery disease (CAD) and myocardial infarction [2]. Several studies have also shown that APOE ϵ4 allele is the strongest risk factor for sporadic and familial late-onset Alzheimer's disease (AD) [3]. Further, a significantly diminished prevalence of the APOE ϵ4 allele in centenarians has been observed [4,5]. Finally, physiological serum APOE concentrations, which vary from 30 to 250 mg/l [6], have been shown to modulate lipid metabolism [7], suggesting that APOE concentration, in addition to APOE polymorphism, might be a risk factor for CAD and cerebrovascular disease (CVD) [6,8]. Interestingly, increased serum APOE levels have been observed in early- and late-onset AD patients compared with controls [8], supporting the growing evidence that vascular factors play a role in the etiology of AD. In this study, we analyzed the relationship between serum APOE concentrations and APOE polymorphism in AD patients, and age-matched healthy controls, centenarians, and young healthy adults. In these populations, differences in serum APOE levels have been evaluated in relation to age and AD.

SUBJECTS AND METHODS

Fifty-two centenarians, 49 AD patients, and 117 healthy subjects (45 age-matched controls and 72 young adults), unrelated caregivers (spouses, friends or neighbors) or volunteers, were evaluated in the Centre for Aging Brain, Memory Unit, Department of Geriatrics, University Hospital, Bari, Italy, between November 1997 and March 1999. Centenarians were recruited from all over Apulia region (Southern Italy), at their home, by Central Population Register. The AD group included 49 patients with sporadic disease. Clinical diagnosis of probable AD was performed according to the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association criteria [10]. The ascertainment, diagnosis and collection of cases, centenarians and controls has been described in detail elsewhere [5,11]. The four groups of the present study had

the same geographic origin and were sex-matched, reflecting the sex differential in longevity and AD in Italy [2]. In these selected populations, APOE polymorphism, gender, body mass index (BMI) calculated as weight/height^2 (kg/m^2), TC, triglycerides (TG), LDL-C, HDL cholesterol (HDL-C), and TC/HDL-C ratio were studied, as known or plausible factors that can affect serum APOE concentration [12,13]. There was no lipid-lowering treatment in the whole sample. The study protocol received the approval from the Ethical Committee of the University of Bari. Informed written consent was obtained from all subjects and/or their relatives prior to collecting of blood sample.

Lipoprotein determinations and APOE genotyping: Blood samples were taken after a 13 h overnight fast; serumAPOE concentrations were measured by nephelometry (Nephelometer 100 Analyzer, Behring, Germany). APOE genotypes, TC, TG, LDL-C, and HDL-C were determined as reported in detail elsewhere [5,14].

Statistical analysis: The statistical analysis was performed by the Pearson χ^2 test to determine whether the observed APOE genotype frequencies were in agreement with those determined by the Hardy-Weinberg law. Allele frequencies were determined by allele counting. To express variances of the allele frequencies, we used 95% confidence intervals, the upper and lower values of which were calculated according to Nielsen's formulas. The Cochran–Armitage trend test was carried out to evaluate the trend across APOE allele frequencies in young and middle-aged subjects, and centenarians. Then, to evaluate differences between APOE allele frequencies, a Pearson χ^2 test was performed. The results of statistical inference were adjusted according to Bonferroni inequality. In this analysis we used the χ^2 value corresponding to $0.05/6 = 0.83\%$ for each of the individual comparisons. Finally, all subjects were divided according to the APOE carrier in three subgroups: $\epsilon 2/2$, $\epsilon 2/3$, and $\epsilon 2/4$ ($\epsilon 2$ carrier), $\epsilon 3/3$ ($\epsilon 3$ homozygous), and $\epsilon 4/3$ and $\epsilon 4/4$ ($\epsilon 4$ carrier). To study the association between serum APOE levels and AD and between serum APOE levels and age, independently of the APOE carriers, we used an analysis of co-variance on fixed effects factorial design adding the APOE carriers to the model. The trend in serum APOE levels among the APOE carriers was tested by polynomial orthogonal contrasts. To examine whether possible or known determinants of serum APOE (TC, LDL-C, HDL-C, TG, and TC/HDL-C ratio, and BMI) could explain association between serum APOE levels and AD and between serum APOE levels and age we included these variables by stepwise procedure in the statistical model. Statistical differences in APOE serum levels in $\epsilon 4$-carriers among age groups and between AD patients and age-matched controls were performed by contrasts. The threshold of significance was set at $p < 0.05$. Statistical analyses were performed with BMDP software, version 7.0 (Los Angeles, CA, USA).

RESULTS

The APOE genotypes and the allele frequencies in young healthy subjects, age-matched controls, centenarians, and AD patients are shown in Table 1. The frequency of the different APOE genotypes in our population was in Hardy–Weinberg equilibrium (young healthy subjects: Pearson $\chi^2 = 2.32$, df $= 3$, $p = 0.51$; age-matched controls: Pearson $\chi^2 = 4.88$, df $= 3$, $p = 0.18$; centenarians: Pearson $\chi^2 = 1.48$, df $= 3$, $p = 0.69$; AD patients: Pearson $\chi^2 = 3.66$, df $= 3$, $p = 0.30$). No significant trend in age was observed for $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ (Cochran–Armitage trend test: $\chi^2 = 0.09$, $p < 0.93$; $\chi^2 = 0.64$, $p < 0.52$, and $\chi^2 = 1.23$, $p < 0.22$, respectively). Statistically significant differences in $\epsilon 4$ allele frequencies were found between centenarians and AD patients (Pearson $\chi^2 = 11.8$, Bonferroni $p < 0.05$) and between young healthy subjects and AD patients (Pearson $\chi^2 = 30.4$, Bonferroni

Table 1. Socio-demographic and clinical features in young healthy adults, age-matched controls, centenarians, and Alzheimer's disease (AD) patients. Values are mean ($\pm$ s.d.), or ratio (%), or frequency (95% CI).

	Young healthy adults	Age-matched controls	Centenarians	AD patients
Age (years)	31.8 $\pm$ 8.5	65.8 $\pm$ 11.6	100 $\pm$ 2	71.6 $\pm$ 9.3
Gender (women)	49 (68.1%)	32 (71.1%)	36 (69.2%)	34 (69.4%)
Body mass index (kg/m^2)	25.3 $\pm$ 4.4	28.4 $\pm$ 5.0	23.9 $\pm$ 3.0	23.4 $\pm$ 4.2
Serum APOE (mg/l)	40.0 $\pm$ 10.0	44.0 $\pm$ 7.0	42.0 $\pm$ 13.0	39.0 $\pm$ 10.0
TC (mmol/l)	4.5 $\pm$ 0.8	5.6 $\pm$ 1.2	4.3 $\pm$ 1.2	4.9 $\pm$ 1.0
LDL-C (mmol/l)	2.7 $\pm$ 0.7	3.7 $\pm$ 1.0	2.6 $\pm$ 0.9	3.1 $\pm$ 0.9
HDL-C (mmol/l)	1.4 $\pm$ 0.3	1.2 $\pm$ 0.4	1.2 $\pm$ 0.4	1.3 $\pm$ 0.4
TG (mmol/l)	0.8 $\pm$ 0.4	1.6 $\pm$ 1.0	1.3 $\pm$ 0.6	1.3 $\pm$ 0.5
TC/HDL ratio	3.3 $\pm$ 1.0	5.0 $\pm$ 1.8	3.9 $\pm$ 1.1	4.1 $\pm$ 1.2
APOE genotypes				
$\epsilon 2/2$	0/72 (0%)	1/45 (2.2%)	0/52 (0%)	0/49 (0%)
$\epsilon 2/3$	14/72 (19.4%)	20/45 (44.4%)	12/52 (23.1%)	2/49 (4.1%)
$\epsilon 2/4$	0/72 (0%)	1/45 (2.2%)	0/52 (0%)	2/49 (4.1%)
$\epsilon 3/3$	50/72 (69.4%)	17/45 (37.8%)	37/52 (71.2%)	32/49 (65.3%)
$\epsilon 3/4$	7/72 (9.7%)	5/45 (11.1%)	3/52 (5.8%)	11/49 (22.4%)
$\epsilon 4/4$	1/72 (1.4%)	1/45 (2.2%)	0/52 (0%)	2/49 (4.1%)
APOE alleles				
$\epsilon 2$	0.10 (0.06–0.16 95% CI)	0.26 (0.17–0.36 95% CI)	0.12 (0.06–0.20 95% CI)	0.04 (0.01–0.11 95% CI)
$\epsilon 3$	0.84 (0.77–0.89 95% CI)	0.66 (0.55–0.75 95% CI)	0.86 (0.77–0.92 95% CI)	0.79 (0.69–0.86 95% CI)
$\epsilon 4$	0.06 (0.03–0.12 95% CI)	0.09 (0.04–0.17 95% CI)	0.03 (0.01–0.09 95% CI)	0.17 (0.11–0.27 95% CI)

CI, confidence intervals; apolipoprotein E, APOE; total cholesterol, TC; LDL cholesterol, LDL-C; HDL cholesterol, HDL-C; triglycerides, TG.

Table 2. Mean serum apolipoprotein E (APOE) levels (mg/l) in each subgroup, stratified according to the APOE carriers.

	Young healthy adults		Age-matched controls		Centenarians		AD patients	
	Mean ± s.d.	Adjusted mean ± s.e.[a]	Mean ± s.d.	Adjusted mean ± s.e.[a]	Mean ± s.d.	Adjusted mean ± s.e.[a]	Mean ± s.d.	Adjusted mean ± s.e.[a]
ϵ2 carrier[b]	48.5 ± 7.9	47.4 ± 2.3	46.9 ± 4.9	49.0 ± 1.8	46.2 ± 10.4	46.5 ± 2.4	42.5 ± 12.4	44.4 ± 4.2
ϵ3 homozygous	39.0 ± 8.9	37.2 ± 1.2	43.0 ± 6.8	43.2 ± 2.1	40.8 ± 12.8	42.1 ± 1.4	40.6 ± 10.0	40.5 ± 1.5
ϵ2 carrier[c]	29.8 ± 4.7	28.0 ± 3.0	34.8 ± 8.0	35.5 ± 3.5**	35.05 ± 6.1	38.1 ± 4.9*	32.8 ± 5.6	32.4 ± 2.4

[a]Adjusted for total cholesterol (TC), LDL cholesterol, HDL cholesterol (HDL-C), triglycerides, TC/HDL-C ratio, and body mass index. Standard error of the adjusted cell means for serum APOE levels. s.e.m. is the s.d. of the sampling distribution. Essentially, this is a measure of the variability of a measure over repeated sampling.
[b]APOE η2/η2, η2/η3, and η2/η4 genotypes.
[c]APOE η3/η4 and η4/η4 genotypes.
*$p < 0.05$;
**$p < 0.01$.

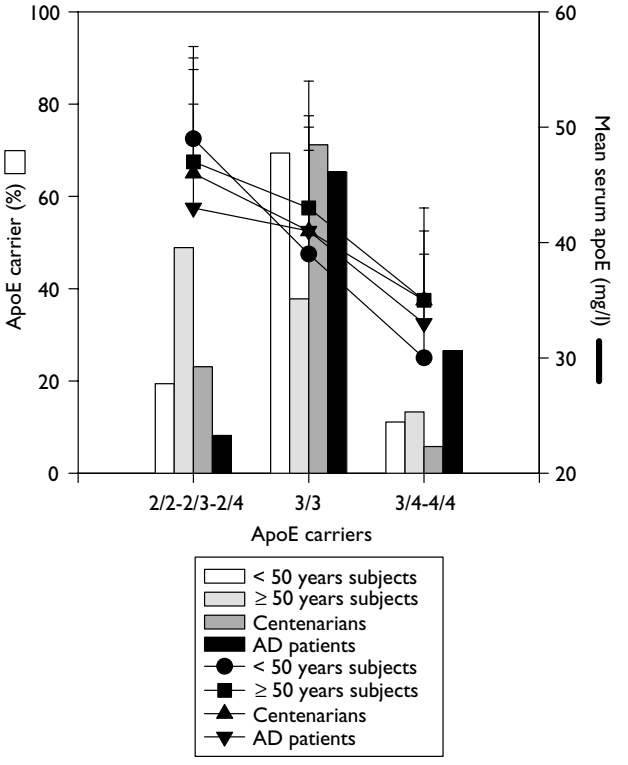

Fig. 1. (**1**) Bar plot for counts of young healthy adults, age-matched controls, centenarians, and Alzheimer's disease (AD) patients stratified according to the apolipoprotein E (APOE) carriers. (**2**) Line plot for mean values of serum APOE levels in young healthy adults, age-matched controls, centenarians, and AD patients within the three categories of APOE carriers.

$p < 0.01$) as well as between AD patients and age-matched controls (Pearson $\chi^2 = 17.6$ Bonferroni $p < 0.01$).

Serum APOE levels in each sub-group, and stratified according to the APOE carriers, are shown in Table 2. A significant trend in reduction of serum APOE levels from APOE ϵ2- to ϵ4 carrier was observed (orthogonal polynomial contrasts: $F = 27.0$, $p < 0.01$; Fig. 1). No significant differences in serum APOE levels with respect to age were found (centenarians *vs* age-matched controls, $F = 0.12$, $p = 0.74$; centenarians *vs* young healthy adults, $F = 0.42$, $p = 0.52$; age-matched controls *vs* young healthy adults, $F = 1.35$, $p = 0.25$) and between AD patients and age-matched controls ($F = 1.47$, $p = 0.23$). When we adjusted for biological factors and lipoprotein traits, the difference in serum APOE levels among age groups in ϵ4 carriers significantly decreased only including HDL cholesterol (centenarians *vs* young healthy adults, $F = 6.38$, $p < 0.05$; age-matched controls *vs* young healthy adults, $F = 8.7$, $p < 0.01$), and no significant differences between AD patients and age-matched controls were found (Table 2). A significant trend in reduction of serum APOE levels from APOE ϵ2- to ϵ4 carrier was confirmed after adjustments.

DISCUSSION

This study confirms the differences in APOE ϵ4 allele frequency between centenarians and young healthy adults, and between AD patients and age-matched controls [3–5]. In all study populations, the presence of APOE ϵ4 allele was found to be associated with lower serum APOE levels, whereas the ϵ2 allele with higher levels. It has been reported that in a normal population serum APOE levels were higher in APOE ϵ2 carriers, with ϵ2 homozygous subjects having the highest APOE levels [3]. The effect of APOE polymorphism on serum APOE level, accounting for 20% of interindividual variability of APOE concentration, suggests that APOE polymorphism influences primarily APOE-containing lipoproteins [3]. Our data are consistent with the concept that APOE2 is catabolized more slowly than APOE4. Several studies have shown significant associations between plasma APOE levels and APOE isoforms [12,16,17] and genotypes [13,18]. These findings indicate that APOE locus is the most important factor controlling serum APOE levels, together with waist-to-hip ratio, oral contraceptive intake, puberty, and BMI [12,13]. Our study provides a novel finding that APOE genotype has a clear effect on serum APOE levels in both AD patients and centenarians. The influence of APOE genotype on serum APOE level in centenarians demonstrates that this influence is present across all age categories [17,18].

To date, the results on serum APOE levels in AD are controversial. An increase in APOE concentrations has been reported in AD patients [8]. In a recent study on late-onset AD patients from Northern Italy, serum APOE levels were similar in patients and controls [15], and a post-mortem study confirmed these results [19]. Our data are in contrast to these findings, and consistent with others in which serum

APOE level differences between AD patients and controls mainly resulted from the distribution of the APOE genotypes [20]. In the present study, we found lower serum APOE levels, and higher ϵ4 allele frequency in AD cases than in age-matched controls, but only the difference in APOE ϵ4 allele frequency showed statistically significance. Our study suggests that serum APOE levels not only are unrelated to AD, but that AD patients are somewhat protected from age-associated increases in serum APOE levels (Table 2), and associated increased risk for CAD and CVD [6,8]. In fact, the higher prevalence of the ϵ4 allele in AD patients strongly influences the decrease in serum APOE levels. These results probably reflect a limit of the study due to the little sample examined and the higher number of multiple comparisons performed in the statistical analysis. In larger sample in the Rotterdam study serum APOE levels were significantly lower in AD patients than in controls, when adjusted for age and gender, and of borderline statistical significance when adjusted for BMI, protein, and albumin levels [20]. In another recent study, APOE concentrations were lower in AD patients and non-demented ϵ4 carrier controls than in those non ϵ4 carriers [21]. Finally, a study on nine European populations showed a clear decrease in APOE serum levels in AD populations, but ϵ4 allele and APOE concentration were independently associated with development of AD, though without adjustment for other lipid parameters [22]. These contrasting findings in the regulatory role of APOE polymorphism on APOE serum levels in AD subjects may be explained by different methods, lack of standardization, different ages of AD patients, different stage of the disease, and geographical differences. We suggest this last one as source of variability, due to an increasing North–South gradient in serum APOE concentration independent of age, sex and APOE genotype [23].

To our knowledge, this is the first report on serum APOE levels in centenarians. Significant differences in serum APOE levels with respect to age were found in ϵ4 carriers between young healthy adults and age-matched controls, and between young healthy adults and centenarians, but only after adjustment for serum HDL-C levels. In fact, in normolipidemic subjects, the majority of APOE ($> 60\%$) in plasma was associated with HDL [24]. In a recent study, serum APOE concentrations were determined in 4284 subjects aged 4–71 years: females exhibited higher APOE values than males until the age of 17–26 years, while after the age of 26 years serum APOE concentrations were higher in men than in women [12]. The decrease of serum APOE levels with age in children, with a synergistic effect of age and puberty, and a lack of age variation in adults were in agreement with other findings [25]. The role of serum APOE concentration in extreme longevity may be explained by the relevance of this factor in CVD [9]. Furthermore, although it has an uncertain relationship with AD, serum APOE concentration has been shown to modulate lipid metabolism [7] and APOE concentration, in addition to polymorphism, might be a risk factor for CAD [6].

CONCLUSION

In the present study, in all study populations, we demonstrate a significant trend in reduction of serum APOE levels from APOE ϵ2 to ϵ4 carriers and significant differences in serum APOE levels with respect to age in ϵ4 carriers, but only after adjustment for HDL-C. At present, serum APOE levels could not be used as a biochemical marker for AD instead of APOE genotyping in neuroepidemiological studies. Further, studies are needed to investigate in depth the role of different common APOE polymorphisms in controlling serum APOE levels in AD and the possible role of APOE concentration as a longevity factor.

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