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Gene polymorphisms of CETP and apolipoprotein E in elderly subjects with cognitive impairment

Research Article

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Abstract: The association of cholesteryl ester transfer protein (*CETP*) and apolipoprotein E (*APOE*) gene polymorphisms with mild cognitive impairment (MCI) is under debate. Our aim was to evaluate the relationship between *APOE* and *CETP* genotypes with healthy ageing. We analysed 267 elderly subjects (55 to 80+ years), 163 with MCI and 104 healthy, and 50 healthy control subjects (35 to 55 years) from a Romanian population. Biochemical parameters and thyroid hormones were assayed in plasma. *APOE* and *CETP TaqIB* gene polymorphisms were determined. Elderly subjects had higher frequency of ε3/ε2 genotype (14.6% *vs*. 4%, P<0.001) than controls. Elderly subjects with MCI had lower high density lipoproteins (HDL) cholesterol (P=0.031), apoA-I (P=0.018), T3 (P=0.002), T4 (P=0.028) and TSH (P=0.001) hormone levels, higher systolic blood pressure (P=0.005), lower frequency of *CETP B2* allele than the age-matched subjects. Healthy elderly subjects had *CETP B2* allele associated with higher plasma apoA-I (P=0.021), lower circulating collagen (P=0.001) levels, and an increased frequency of the combined *APOE ε2- CETP B2* genotype (18.3%) relative to MCI elderly subjects (7.6%, P=0.011). Healthy elderly subjects are characterized by higher HDL cholesterol, apoA-I levels and higher frequency of the combined *APOE ε2* and *CETP B2* alleles, indicating this pattern as representative for healthy ageing.

Keywords: *Ageing • Apolipoprotein E • Cholesteryl ester transfer protein • Cognitive impairment • Gene polymorphism.*

1. Introduction

Cholesteryl ester transfer protein (CETP) and apolipoprotein E (apoE) are two important proteins involved in lipid metabolism and known to affect the plasma lipid concentration [1]. ApoE is the major apolipoprotein constituent of triglyceride-rich very low density lipoproteins (VLDL), low density lipoproteins (LDL) and chylomicron remnant particles and serves as a high affinity ligand of the hepatic LDL receptor and LDL receptor-related protein-1 (LRP-1), important for lipoprotein catabolism in the liver. ApoE is encoded by a polymorphic gene (*APOE*) located on chromosome 19 and which has three common alleles: *ε2, ε3, ε4*. These three alleles are translated into 3 isoforms of apoE (E2, E3, E4) by the substitution of Arginine and Cysteine at positions 112 (rs429358) and/or 158 (rs7412) in the protein, determining the apparition of six different *APOE* genotypes [2]. Data from literature identify *APOE* genotype as a strong genetic risk factor for various ageing-related diseases, including dementia. Carriers of *ε4* allele have an increased risk of developing cognitive disabilities [3,4], whereas *ε2* allele carriers are relatively protected [5,6].

CETP is a hydrophobic glycoprotein which transfers cholesterol esters from the high density lipoproteins (HDL) to triglycerides-rich particles (such as VLDL) in exchange for the triglycerides from the latter [7]. The gene coding for CETP encompasses 16 exons and is localized on chromosome 16q21. It has been demonstrated that the *CETP* gene is polymorphic, one of the most widely studied polymorphisms being *TaqIB* (rs708272), which results from a silent mutation in nucleotide 277, in intron 1 of the gene [8]. The mutation determines the presence or absence of a *TaqIB* restriction site, which leads to three possible genotypes associated with B1 and B2 alleles. Published data demonstrate that the *B2* allele is associated with lower

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CETP levels as compared to the *B1* allele, and that the *CETP TaqIB* genotype is strongly associated with the risk of cardiovascular diseases (CVD), carriers of *B2B2* genotypes having higher levels of HDL cholesterol and a lower risk of CVD [9]. Recently, Murphy *et al.* showed that CETP polymorphisms associate with brain structure, atrophy rate, and Alzheimer's disease risk in an APOE-dependent manner [10], raising the importance to study the variations of the two genes in a interaction approach.

The aim of our study was to determine the association between *APOE* and *CETP TaqIB* gene polymorphisms with healthy ageing in a Romanian population. We considered healthy ageing the process in which subjects do not have CVD and cognitive impairment.

2. Experimental Procedures

2.1 Subjects

The study included 317 subjects of both genders, divided by age into two groups: (1) 267 elderly subjects aged 55-80+ years and (2) 50 control subjects between 35-55 years, selected from a Romanian population. The elderly subjects were also divided by mini mental state examination score (MMSE) into (i) 163 elderly subjects with mild cognitive impairment (MMSE<28), and (ii) 104 elderly healthy subjects without cognitive impairment (MMSE≥28). The MMSE (or Folstein) test is a brief 30-point questionnaire test that was used to screen for cognitive impairment [11]. From these subjects, 211 were women (72.3%) and 81 men (27.7%), with the same gender distribution in each group. From each subject, fasting blood samples were taken on ethylene-diamine-tetra-acetic acid for biochemical and hormonal analysis and DNA isolation from circulating leukocytes was done. The body mass index (BMI), waist circumference, systolic and diastolic blood pressure (BP) were recorded for each subject. All participants gave written informed consent.

2.2 Plasma assays

Plasma cholesterol, HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), total apoB, apoA-I, glucose, triglycerides, collagen, C-reactive protein (CRP), thyroid hormones (free T3, T3, T4 and thyroid stimulating hormone (TSH)) levels were assayed using commercial kits, within the current analysis with automated biochemical or immuno-turbidimetric analyzers in the Hospital laboratory.

2.3 DNA genotyping

APOE (rs429358 and rs7412) and *CETP TaqIB* (rs708272) gene polymorphisms were determined by PCR-restriction fragment length polymorphism (RFLP) method, by using genomic DNA isolated from peripheral leukocytes.

APOE genotyping. A fragment of 244 bp in exon 4 of the *APOE* gene was amplified by PCR using specific primers, as previously described [12]. After PCR amplification, 10 U of *CfoI* (*Sigma-Aldrich Co., St. Louis, MO, USA*) or *HhaI* (*Promega GmbH, Mannheim, Germany*) was added for enzymatic digestion of 8 µl of the amplification product (37ºC, overnight). The digested product was run in 12% polyacrylamide gel. Each of the six possible genotypes was recognized by the unique combination of bands. The presence of 35, 38, 48, and 91 bp bands, corresponded to the *APOE ε3* allele, 35, 38, 48, and 72 bp bands to the *ε4* allele and 38, 83, and 91 bp to the *ε2* allele. The six *APOE* genotypes identified by this procedure in the selected subjects were: *3/3, 4/3, 3/2, 2/2, 4/2, 4/4*.

CETP genotyping. A fragment of 1420-bp was amplified by PCR using specific primers, as previously described [13]. The PCR products were digested with 10 U/µl reaction *TaqIB* restriction enzyme (*Promega GmbH, Mannheim, Germany*) and the digestion product was run on a 2% agarose gel. The *CETP B1B1* genotype was identified as two bands of 750 bp and 670 bp, the *B1B2* genotype as 750 bp, 670 bp and 1420 bp, and the *B2B2* genotype as the starting undigested 1420 bp on the agarose gels.

All the PCR reactions were performed in a *Thermo PX2* thermal cycler (*Thermo Fisher Scientific, Waltham, MA, USA*). The resulting enzyme-digested fragments of the PCR products fractionated on 16% polyacrylamide or 2% agarose gels were stained with ethidium bromide and visualized under UV light with an *ImageMaster* imaging system (*Amersham-Pharmacia Biosciences, Piscataway, NJ, USA*).

2.4 Statistical analysis

Statistical analysis was performed using the SPSS 15.0 software for Windows (*SPSS Inc., Chicago, IL, USA*). Allele and genotype frequencies were determined by direct counting. The normal quantitative variables (anthropometric, biochemical and hormonal data) were expressed as means ± standard deviation (SD) and were analysed by using two-sample *T*-test or *ANOVA* test. For some statistical analysis, heterozygous and homozygous genotype carriers were pooled to obtain a better significance (*i.e.* biochemical parameters in aged subjects with cognitive impairment B1B1 as compared to B1B2+B2B2). Significant differences of continuous variables were assessed by using two tests, the Student's T-test, analysis of variance (One-way ANOVA) (biochemical, hormonal and

anthropological parameters), and for categorical ones we used χ-square test, and Mantel-Haenszel common odds ratios (OR) estimates (alleles and genotypes frequencies, combined genotypes analysis). The threshold for statistical significance was set to 5% (P-values lower than 0.05).

3. Results

3.1 Plasma parameters of the subjects

Biochemical, endocrine and anthropometric mean values of the parameters of elderly and control subjects are presented in Table 1. The elderly subjects had statistically significant increased plasma HDL-C and circulating collagen, and decreased waist circumference and thyroid hormone T3 levels, as compared to control subjects.

The elderly subjects with cognitive impairment presented statistically significant increased systolic BP and plasma collagen levels compared to the elderly healthy subjects. The levels of HDL-C, apolipoprotein A-I main HDL protein, TSH, T3 and T4 hormones in the plasma of elderly subjects with cognitive impairment were decreased as compared to the elderly healthy subjects (Table 2).

3.2 Distribution of *APOE* alleles and genotypes in elderly and control subjects

The *APOE* genotypes did not deviate from Hardy-Weinberg equilibrium in the entire group, in control, as well as in elderly subjects group. Analysis of the *APOE* gene polymorphisms in all the studied subjects revealed the following general distribution: *ε3*- 82.5%, *ε2*- 8.8%, *ε4*- 8.8%. The corresponding frequencies of the *APOE* genotypes were: *3/3* - 66.8%, *4/3* - 17.5%, *3/2* - 13.9%, *2/2* - 1.8%, *4/2* and *4/4* - 0%. The distribution of *APOE* alleles did not differ between the elderly and control subjects, only an increase of the *APOE* 3/2 genotype frequency observed in the elderly subjects (15.7% *vs* 4.0%, χ^2-test P<0.001) (Figure 1).

3.3 Association of *APOE* alleles and genotypes with biochemical parameters in elderly and control subjects

Elderly carriers of the *ε2* allele had increased plasma levels of triglycerides (P=0.041) as compared with

Parameter	Elderly (N=267)	Control (N=50)	T-test *P value*
Age, years	67.2 ± 7.5	48.0 ± 4.9	<0.001*
Body mass index	26.5 ± 4.5	27.2 ± 2.9	0.304
Waist circumference, cm	87.7 ± 14.5	99.6 ± 13.5	0.001*
Systolic blood pressure, mmHg	136.2 ± 22.3	130.6 ± 16.3	0.239
Diastolic blood pressure, mmHg	79.0 ± 12.7	81.0 ± 11.3	0.409
Total cholesterol, mM	5.94 ± 1.11	5.98 ± 0.94	0.867
Triglycerides, mM	1.46 ± 0.75	1.74 ± 1.13	0.089
HDL-Cholesterol, mM	1.57 ± 0.51	1.34 ± 0.42	0.031*
LDL-Cholesterol, mM	4.02 ± 1.19	4.66 ± 1.40	0.063
Total apoB, mg/dl	122.2 ± 55.7	134.1 ± 40.1	0.241
ApoA-I, mg/dl	190.8 ± 57.8	184.5 ± 46.6	0.587
Fasting glucose, mM	5.68 ± 1.47	5.44 ± 1.08	0.321
Insulin, mU/l	9.14 ± 5.47	11.27 ± 8.66	0.237
HOMA Index	2.36 ± 1.94	2.74 ± 2.35	0.573
Collagen, ng/ml	0.72 ± 0.33	0.61 ± 0.30	0.015*
CRP, mg/L	0.44 ± 0.70	0.38 ± 0.51	0.644
T3 hormone, ng/ml	0.94 ± 0.26	1.14 ± 0.33	0.015*
Free T3 hormone, pg/ml	1.43 ± 0.60	1.61 ± 0.53	0.644
T4 hormone, μg/dl	7.38 ± 1.52	7.06 ± 1.68	0.196
TSH, μU/ml	1.87 ± 1.23	2.27 ± 1.55	0.478

Table 1. Plasma biochemical, endocrine and anthropometric parameters of elderly and control subjects.

*The values are given as mean ± standard deviation; *P value <0.05 was considered statistically significant.*

Parameter	Elderly subjects with cognitive impairment (N=163)	Healthy elderly subjects (N=104)	T-Test *P value*
Age, years	68.3 ± 7.6	65.4 ± 6.9	0.282
Body mass index	26.8 ± 4.38	26.1 ± 4.5	0.859
Waist circumference, cm	84.8 ± 13.2	88.5 ± 14.9	0.417
Systolic blood pressure, mmHg	139.6 ± 22.4	131.7 ± 21.7	0.010*
Diastolic blood pressure, mmHg	79.2 ± 12.3	78.7 ± 13.2	0.772
Total cholesterol, mM	5.96 ± 1.11	5.91 ± 1.13	0.882
Triglycerides, mM	1.52 ± 0.75	1.38 ± 0.63	0.226
HDL-Cholesterol, mM	1.51 ± 0.46	1.67 ± 0.56	0.043*
LDL-Cholesterol, mM	3.89 ± 1.05	4.21 ± 1.37	0.060
Total apoB, mg/dl	119.8 ± 38.4	124.8 ± 70.6	0.432
ApoA-I, mg/dl	181.1 ± 47.9	201.7 ± 65.9	0.026*
Fasting glucose, mM	5.66± 1.56	5.71 ± 1.32	0.717
Insulin, mU/l	8.95 ± 4.79	9.42 ± 6.37	0.523
HOMA Index	2.20 ± 1.98	2.48 ± 1.91	0.320
Collagen, ng/ml	0.81 ± 0.36	0.65 ± 0.29	0.007*
CRP, mg/L	0.53 ± 0.88	0.34 ± 0.39	0.078
T3 hormone, ng/ml	0.85 ± 0.23	1.01 ± 0.27	<0.001*
Free T3 hormone, pg/ml	1.38 ± 0.52	1.48 ± 0.66	0.301
T4 hormone, μg/dl	7.05 ± 1.38	7.65 ± 1.59	0.024*
TSH, μU/ml	1.49 ± 0.79	2.17 ± 1.42	0.001*

Table 2. Plasma biochemical, endocrine and anthropometric parameters of the elderly subjects studied.

*The values are given as means ± standard deviation; *P value <0.05 was considered statistically significant.*

control subjects, while no association of *APOE* alleles with other biochemical parameters was observed (data not shown). No association between *APOE* alleles or genotypes with pathological values of biochemical parameters, CRP and thyroid hormones levels was observed in the elderly group.

3.4 Distribution of *APOE* alleles and genotypes in elderly subjects with cognitive impairment

The distribution of *APOE* alleles and genotypes did not differ between the elderly subjects with cognitive impairment and the elderly healthy subjects (Figure 1 and Table 5 – Supplementary Material). However, *APOE 3/2* genotype frequency was higher in the elderly healthy subjects (20.4%), while in the group of elderly subjects with cognitive impairment it was only 12.8%; the difference was not statistically significant (χ^2-test P-value NS (not significant), OR=1.651, 95% CI 0.834-3.265, P-value NS). No association between *APOE* alleles or genotypes with pathological values of biochemical parameters, CRP and thyroid hormones levels was observed in the elderly subjects with cognitive impairment.

3.5 Distribution of *CETP* alleles and genotypes in elderly and control subjects

The *CETP* genotypes did not deviate from Hardy-Weinberg equilibrium in the entire group, in controls, as well as in elderly subjects group. The overall distribution of *CETP TaqIB* alleles in the studied group was: 60.6% for *B1* and 39.4% for *B2*, while the genotype distribution was: 36.3% for *B1B1*, 48.5% for *B1B2*, and 15.2% for *B2B2*. No statistical differences in the distribution of *CETP* alleles and genotypes were observed for the elderly subjects as compared to controls (Figure 2 and Table 3).

3.6 Association of *CETP* genotypes with biochemical parameters in elderly and control subjects

The *CETP* B1B2 and B2B2 genotypes were correlated with lower waist circumference (P=0.002), lower levels of LDL-C (P=0.028) and apoB (P=0.050) in elderly subjects compared to controls. There was no association between the other biochemical parameters, CRP and thyroid hormones levels with *CETP* genotype distribution in elderly and control groups (data not shown).

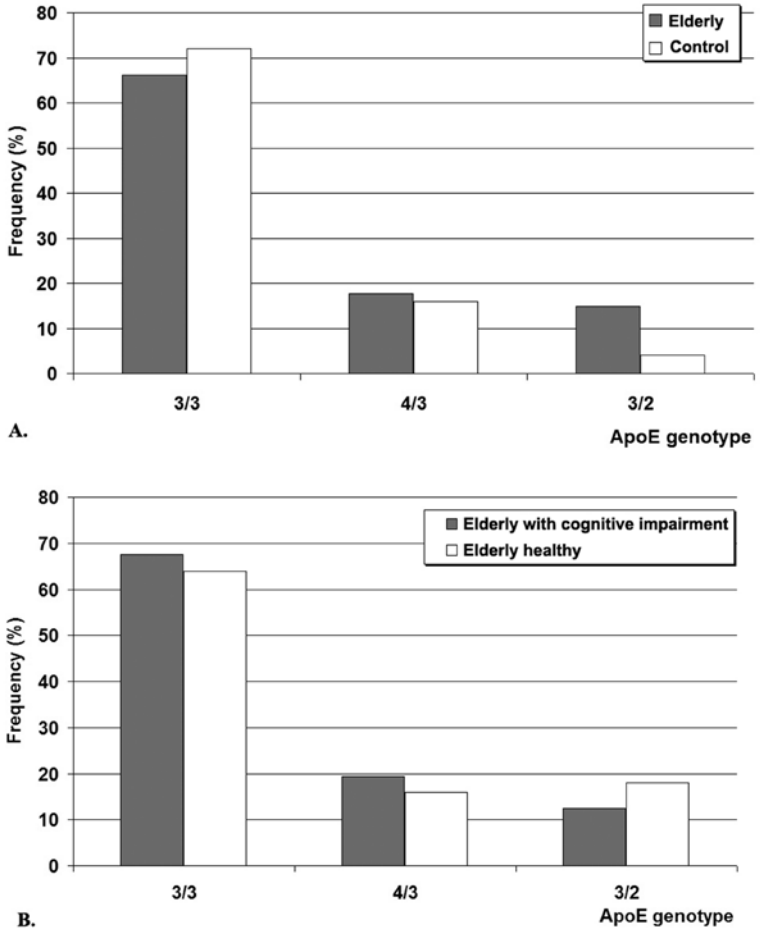

Figure 1. Distribution of *APOE* genotypes in elderly and control subjects (A), in elderly subjects with and without cognitive impairment (B). Note the increase of *APOE 3/2* genotype frequency (15.7% *vs* 4.0%, χ^2-test $P<0.001$) in elderly *vs*. control subjects. The other *APOE* genotypes are not presented (*4/2, 2/2* and *4/4*) due to their very low frequencies (0-1%).

CETP TaqIB	Groups	Frequency (%)	χ^2-test P-value	OR /P-value (95% CI)
B1 allele	Elderly	84.5	0.744	1.346 /0.616
	Control	88.0		(0.384-4.722)
	Elderly with cognitive impairment	85.7	0.319	0.781 /0.303
	Age-matched subjects	82.4		(0.387-1.579)
B2 allele	Elderly	64.1	0.663	0.841 /0.656
	Control	60.0		(0.362-1.951)
	Elderly with cognitive impairment	58.4	0.017*	1.985 /0.018*
	Age-matched subjects	73.6		(0.1.127-3.496)

Table 3. Distribution of *CETP* alleles in the studied subjects.

*OR=odds ratio; CI=confidence interval; *P value <0.05 was considered statistically significant.*

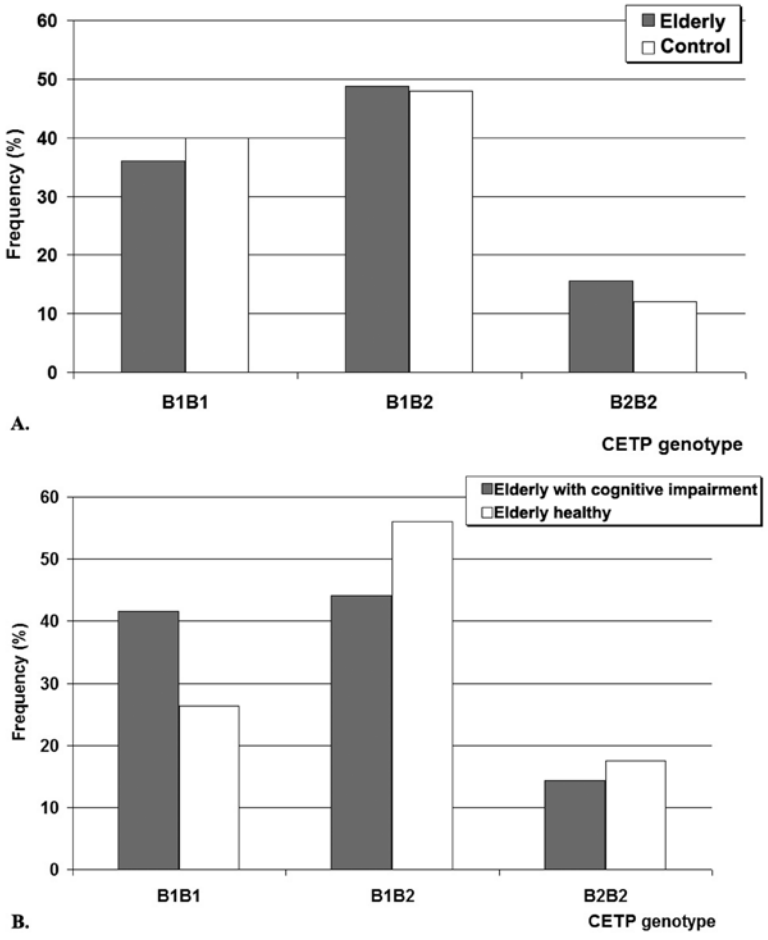

Figure 2. Distribution of *CETP* genotypes in elderly and control subjects (A), and in elderly subjects with and without cognitive impairment (B).

3.7 Distribution of *CETP* alleles and genotypes in elderly subjects with cognitive impairment

The distribution of *CETP* genotypes differed between the elderly subjects with and without cognitive impairment (P=0.026) (Figure 2). *CETP B2* allele was present in 58.4% of elderly subjects with cognitive impairment, while in elderly healthy subjects *B2* allele frequency was 73.6%, a statistically significant difference (P=0.017), which suggests an association of *B2* allele with healthy ageing (Table 3). Interestingly, an increased frequency of the *B1B1* genotype in elderly subjects with cognitive impairment (41.6%) was also observed, as compared to age-matched subjects without cognitive impairment (26.4%, P=0.019) (Figure 2).

3.8 Association of *CETP* genotypes with biochemical parameters in elderly subjects with cognitive impairment

The *CETP B1B2* genotype in elderly subjects with cognitive impairment was associated with lower plasma apoA-I (P=0.021) and higher collagen type I (P=0.001) levels, as compared to the elderly healthy subjects (Figures 3A, B). Moreover, the *B1B1* genotype was associated with higher systolic BP in elderly subjects with cognitive impairment, as compared to the elderly healthy subjects (Figure 3C). There was no difference between *CETP* genotypes distribution and hormonal status in elderly subjects with and without cognitive impairment. CETP activity measured in plasma isolated from all subjects did not differ between the elderly and control groups, as well as between elderly subjects with and without cognitive impairment (data not shown).

3.9 Combined genotypes of *APOE* and *CETP* in cognitive impairment

In order to evaluate the association between the *APOE* and *CETP* gene polymorphisms, we analysed the distribution of the six possible combined genotypes defined by the *APOE ε3/ε4/ε2* and *CETP B1/B2*

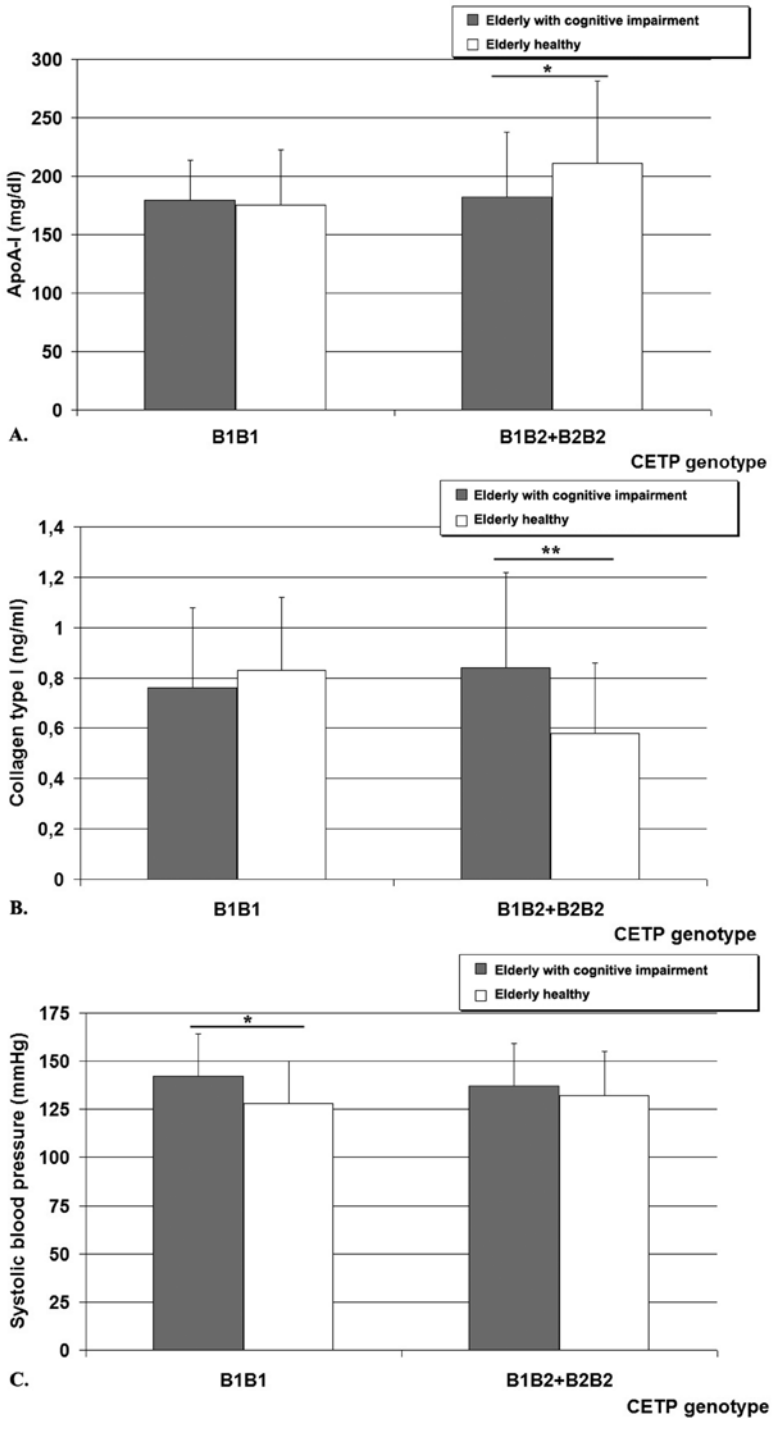

Figure 3. Distribution of plasma levels of apoA-I (A) and collagen type I (B), and systolic blood pressure (C) in elderly subjects with and without cognitive impairment as a function of CETP genotypes (*P<0.05; **P<0.01).

alleles for the studied subjects. We found that there was no association between *APOE ε4* distribution and *CETP B1* distribution within any of the studied groups (Table 4). Moreover, we observed an interesting association of the *APOE ε2* allele distribution with the *CETP B2* allele in elderly subjects: healthy elderly subjects had an increased frequency of the *APOE ε2*/*CETP B2* combined genotype (18.3%) as compared to the mentally affected elderly subjects (7.6%, P=0.011) (Table 4). There was no statistically significant

Combined genotype	Groups	Frequency (%)	χ^2-test P-value	OR /P-value (95% CI)
APOE ε3 /CETP B1 (common)	Elderly	83.1	0.693	0.812 /0.493
	Control	80.0		(0.288-2.288)
	Elderly with cognitive impairment	84.9	0.349	0.723 /0.250
	Elderly healthy	80.2		(0.366-1.428)
APOE ε3 /CETP B2	Elderly	62.7	0.294	0.644 /0.237
	Control	52.0		(0.282-1.472)
	Elderly with cognitive impairment	57.5	0.030*	1.847/0.031*
	Elderly healthy	71.4		(1.059-3.221)
APOE ε4 /CETP B1	Elderly	14.5	0.842	1.122 /0.822
	Control	16.0		(0.364-3.459)
	Elderly with cognitive impairment	16.0	0.380	0.712 /0.281
	Elderly healthy	12.0		(0.332-1.524)
APOE ε2 /CETP B2	Elderly	11.6	0.582	0.663 /0.585
	Control	8.0		(0.148-2.958)
	Elderly with cognitive impairment	7.6	0.011*	2.703 /0.014*
	Elderly healthy	18.3		(1.227-5.952)

Table 4. Frequencies of some combined genotypes defined by *APOE ε3/ε4/ε2* and *CETP B1/B2* alleles in the studied subjects.

*OR= odds ratio; CI = confidence interval; *P value <0.05 was considered statistically significant.*

difference for the distribution of the other two combined genotypes (*APOE ε3/CETP B2* and *APOE ε4/CETP B2*) (data not shown). However, no association was found for any of these combined genotypes with biochemical and metabolic parameters, CRP and thyroid hormones levels in the studied subjects.

4. Discussion

In the present study we evaluated the association of *APOE* and *CETP TaqIB* gene polymorphisms with successful ageing in a selected group of Romanian subjects by analysing their cognitive status and biochemical and hormonal parameters.

We observed that both distributions of *APOE* and CETP *TaqIB* alleles and genotypes in the studied groups were similar to the one reported for other European-Caucasian populations [14,15]. Polymorphism of the *APOE* gene was proposed as an important risk factor for Alzheimer's disease and other neurological disabilities associated with ageing [16-18].

Data from the present study show no association between the frequency of the *ε4* allele and biochemical or hormonal parameters in elderly carriers relative to control subjects. Our results are in good agreement with other reports showing that the *APOE ε4* allele is not associated with cognitive deficit [19-21]. Other investigators, however, reported that the presence of at least one *ε4* allele was associated with faster cognitive decline in Alzheimer's disease patients [22]. The presence of the *ε4* allele was also recently associated with impaired memory function in both middle-aged and older subjects with mild cognitive impairment [23]. A recent genome-wide association study confirms APOE as one of the major gene influencing survival in long-lived individuals [24]. This study independently confirmed the APOE-longevity association, thus strengthening the conclusion that this locus is a very, if not the most, important genetic factor influencing longevity.

We observed no association of the *ε2* allele or *ε2*-containing genotypes with ageing or with cognitive impairment, either in men or women. However, we found that the elderly carriers of the *ε2* allele had increased levels of triglycerides relative to control subjects. In contrast, Martins *et al*. demonstrated that *APOE* genotype strongly predicts the rate of cognitive decline in Alzheimer's disease, a dose-response relation existing with the *APOE ε4* allele, while the *APOE ε2* allele was protective [25].

We investigated a possible association between *APOE* alleles, thyroid hormone levels and cognitive

impairment. Our results show no statistical association between *APOE* polymorphisms and any of the above parameters in the elderly subjects. However, we demonstrate that elderly subjects with cognitive impairment have lower T3, T4 and TSH hormone levels, and higher systolic BP, compared to elderly healthy subjects. This result was in agreement with van Osch *et al.*, who reported that Alzheimer's disease patients had significantly lower levels of TSH [26]. Lower levels of TSH were associated with a more than two-fold increased risk of Alzheimer's disease, independently of other risk factors.

Although *APOE* gene polymorphism is associated with variable risks for several diseases, no clear relationship has been demonstrated with vascular frailty. We report here that none of the *APOE* alleles or genotypes were associated with decreased levels of type I collagen, an accepted index of frailty, in elderly subjects relative to controls, in a good agreement with the results of Rockwood *et al.* [27].

Our data show that the analysed *CETP TaqIB* alleles and associated genotypes have the same distribution in both elderly and control subjects, in good agreement with Ordovas *et al.* [9]. The *CETP* B2 allele is associated with lower LDL-cholesterol and total apoB levels in elderly subjects relative to young controls. We observed an association of the *B2* allele with diminished obesity in elderly subjects, indicated by a lower waist circumference as compared to controls, while similar data were reported by Brousseau *et al.* [28].

Our observations show that CETP activity measured in plasma isolated from all subjects did not differ between the elderly and control groups, as well as between elderly subjects with and without cognitive impairment (data not shown). Arai *et al*. observed that although heterozygosity for the *CETP B2* allele was consistently associated with higher HDL-cholesterol levels both in centenarians and controls, the distribution of *CETP TaqIB* alleles did not differ between the two groups [13].

To the best of our knowledge, there are no published data on the role of *CETP TaqIB* polymorphism in ageing and ageing-related cognitive impairment. Our results show that the distribution of *CETP TaqIB* genotypes is similar in elderly subjects, either with or without cognitive impairment. However, we found an increased frequency of the *B1B1* genotype in elderly subjects with cognitive impairment as compared to healthy elderly subjects, and this result might be of particular interest. Moreover, the *CETP B2* allele has a lower frequency in elderly subjects with cognitive impairment. We also observed the association of the *B1* allele with decreased plasma apoA-I concentrations and increased collagen levels, while the *B1* allele was associated with increased systolic BP in cognitively-impaired elderly subjects. Our results suggest that the *CETP B2* allele may be protective against cerebrovascular diseases.

The data reported here support the importance of the distribution of combined *APOE* and *CETP* genotypes in elderly subjects. We demonstrate an interesting association of the *APOE* ε2 allele and the *CETP B2* allele with healthy ageing. Our results confirm and expand Muendlein *et al*. previous report on the synergistic effect of *APOE ε3/ε2/ε4*, *CETP TaqIB* and *APOC3 -482C>T* polymorphisms in Caucasian patients with CVD [29].

In summary, we propose that the *CETP B2* allele may play a protective role in successful ageing and longevity that could be enhanced by the co-existence of *APOE ε2* allele. Our results suggest that the population analysis of genetic risk factors for cognitive impairment in ageing should consider the possible contribution of these combined genotypes.

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References

[1] Humphries S.E., Morgan L., Genetic risk factors for stroke and carotid atherosclerosis: insights into pathophysiology from candidate gene approaches, Lancet Neurol., 2004, 3, 227-235

[2] Seripa D., D'Onofrio G., Panza F., Cascavilla L., Masullo C., Pilotto A., The genetics of the human APOE polymorphism, Rejuvenation Res, 2011, 14, 491-500

[3] Corder E.H., Saunders A.M., Strittmatter W.J., Schmechel D.E., Gaskell P.C., Small G.W., et al., Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families, Science, 1993, 261, 921-923

[4] Bender A.R., Raz N., Age-related differences in memory and executive functions in healthy APOE varepsilon4 carriers: The contribution of individual differences in prefrontal volumes and systolic blood pressure, Neuropsychologia, 2012

[5] West H.L., Rebeck G.W., Hyman B.T., Frequency of the apolipoprotein E epsilon 2 allele is diminished in sporadic Alzheimer disease, Neurosci. Lett., 1994, 175, 46-48
[6] Berlau D.J., Corrada M.M., Head E., Kawas C.H., APOE epsilon2 is associated with intact cognition but increased Alzheimer pathology in the oldest old, Neurology, 2009, 72, 829-834
[7] Dixit M., Bhattacharya S., Mittal B., Association of CETP TaqI and APOE polymorphisms with type II diabetes mellitus in North Indians: a case control study, BMC Endocr Dis, 2005, 5, 7
[8] Drayna D., Lawn R., Multiple RFLPs at the human cholesteryl ester transfer protein (CETP) locus, Nucleic Acids Res., 1987, 15, 4698
[9] Ordovas J.M., Cupples L.A., Corella D., Otvos J.D., Osgood D., Martinez A., et al., Association of cholesteryl ester transfer protein-TaqIB polymorphism with variations in lipoprotein subclasses and coronary heart disease risk: the Framingham study, Arterioscler. Thromb. Vasc. Biol., 2000, 20, 1323-1329
[10] Murphy E.A., Roddey J.C., McEvoy L.K., Holland D., Hagler D.J., Jr., Dale A.M., et al., CETP polymorphisms associate with brain structure, atrophy rate, and Alzheimer's disease risk in an APOE-dependent manner, Brain Imaging Behav, 2012, 6, 16-26
[11] Folstein M.F., Folstein S.E., McHugh P.R., "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician, J Psychiatr Res, 1975, 12, 189-198.
[12] Hixson J.E., Vernier D.T., Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI, J. Lipid Res., 1990, 31, 545-548
[13] Arai Y., Hirose N., Yamamura K., Nakazawa S., Shimizu K., Takayama M., et al., Deficiency of choresteryl ester transfer protein and gene polymorphisms of lipoprotein lipase and hepatic lipase are not associated with longevity, J Mol Med (Berl)., 2003, 81, 102-109
[14] Chiodini B.D., Franzosi M.G., Barlera S., Signorini S., Lewis C.M., D'Orazio A., et al., Apolipoprotein E polymorphisms influence effect of pravastatin on survival after myocardial infarction in a Mediterranean population: the GISSI-Prevenzione study, Eur. Heart J., 2007, 28, 1977-1983
[15] Sima A., Iordan A., Stancu C., Apolipoprotein E polymorphism-a risk factor for metabolic syndrome, Clin Chem Lab Med., 2007, 45, 1149-1153
[16] Evans R.M., Hui S., Perkins A., Lahiri D.K., Poirier J., Farlow M.R., Cholesterol and APOE genotype interact to influence Alzheimer disease progression, Neurology, 2004, 62, 1869-1871
[17] Mahley R.W., Huang Y., Weisgraber K.H., Detrimental effects of apolipoprotein E4: potential therapeutic targets in Alzheimer's disease, Curr. Alzheimer Res., 2007, 4, 537-540
[18] Kulminski A., Ukraintseva S.V., Arbeev K.G., Manton K.G., Oshima J., Martin G.M., et al., Association between APOE epsilon 2/epsilon 3/epsilon 4 polymorphism and disability severity in a national long-term care survey sample, Age Ageing., 2008, 37, 288-293
[19] Garcia A.N., da Silva H.A., Silva R.C., Leal E.M., Rodrigues L., da Silva V.C., et al., APOE-epsilon4 polymorphism and cognitive deficit among the elderly population of Fernando de Noronha, Arqu. Neuropsiquiatr., 2008, 66, 298-302
[20] Alexander D.M., Williams L.M., Gatt J.M., Dobson-Stone C., Kuan S.A., Todd E.G., et al., The contribution of apolipoprotein E alleles on cognitive performance and dynamic neural activity over six decades, Biol. Psychol., 2007, 75, 229-238
[21] Brainerd C.J., Reyna V.F., Petersen R.C., Smith G.E., Taub E.S., Is the apolipoprotein e genotype a biomarker for mild cognitive impairment? Findings from a nationally representative study, Neuropsychology, 2011, 25, 679-689
[22] Cosentino S., Scarmeas N., Helzner E., Glymour M.M., Brandt J., Albert M., et al., APOE epsilon 4 allele predicts faster cognitive decline in mild Alzheimer disease, Neurology, 2008, 70, 1842-1849
[23] Ramakers I.H., Visser P.J., Aalten P., Bekers O., Sleegers K., van Broeckhoven C.L., et al., The association between APOE genotype and memory dysfunction in subjects with mild cognitive impairment is related to age and Alzheimer pathology, Dement. Geriatr. Cogn. Disord., 2008, 26, 101-108
[24] Nebel A., Kleindorp R., Caliebe A., Nothnagel M., Blanche H., Junge O., et al., A genome-wide association study confirms APOE as the major gene influencing survival in long-lived individuals, Mech Ageing Dev, 2011, 132, 324-330
[25] Martins C.A., Oulhaj A., de Jager C.A., Williams J.H., APOE alleles predict the rate of cognitive decline in Alzheimer disease: a nonlinear model, Neurology, 2005, 65, 1888-1893.
[26] van Osch L.A., Hogervorst E., Combrinck M., Smith A.D., Low thyroid-stimulating hormone as an independent risk factor for Alzheimer disease, Neurology, 2004, 62, 1967-1971

[27] Rockwood K., Nassar B., Mitnitski A., Apolipoprotein E-polymorphism, frailty and mortality in older adults, J. Cell Mol. Med., 2008, 12, 2754-2761

[28] Brousseau M.E., O'Connor J.J., Jr., Ordovas J.M., Collins D., Otvos J.D., Massov T., et al., Cholesteryl ester transfer protein TaqI B2B2 genotype is associated with higher HDL cholesterol levels and lower risk of coronary heart disease end points in men with HDL deficiency: Veterans Affairs HDL Cholesterol Intervention Trial, Arterioscler. Thromb. Vasc. Biol., 2002, 22, 1148-1154

[29] Muendlein A., Saely C.H., Marte T., Schmid F., Koch L., Rein P., et al., Synergistic effects of the apolipoprotein E epsilon3/epsilon2/epsilon4, the cholesteryl ester transfer protein TaqIB, and the apolipoprotein C3 -482 C>T polymorphisms on their association with coronary artery disease, Atherosclerosis, 2008, 199, 179-186

Appendix – Supplementary Material

APOE allele	Groups	Frequency (%)	χ^2-test P-value	OR /P-value (95% CI)
ε3 allele	Aged Control	98.4 92.0	0.223	0.188 /0.120 (0.033-1.081)
	Aged with cognitive impairment Age-matched subjects	99.4 96.8	0.117	0.194 /0.158 (0.020-1.889)
ε4 allele	Aged Control	17.7 20.0	0.772	1.165 /0.153 (0.415-3.271)
	Aged with cognitive impairment Age-matched subjects	18.6 16.1	0.622	0.842 /0.423 (0.425-1.669)
ε2 allele	Aged Control	16.1 12.0	0.594	0.713 /0.596 (0.204-2.494)
	Aged with cognitive impairment Age-matched subjects	13.5 20.4	0.147	1.651 /0.150 (0.834-3.265)

Table 5. Distribution of *APOE* alleles in the studied subjects.

COR= odds ratio; CI = confidence interval; a P value <0.05 was considered statistically significant.