UDC 575
DOI: 0.2298/GENSR1303865M
Original scientific paper

DISTRIBUTION OF APOLIPOPROTEIN E GENE POLYMORPHISM IN STUDENTS AND IN HIGH-EDUCATED ELDERLY FROM SERBIA

Nela MAKSIMOVIĆ, Ivana NOVAKOVIĆ, Vesna RALIĆ and Elka STEFANOVA

Faculty of Medicine, University of Belgrade, Belgrade, Serbia

Maksimović N, I. Novaković, V. Ralić, and E.Stefanova (2013): *Distribution of apolipoprotein e gene polymorphism in students and in high-educated elderly from Serbia.* Genetika, Vol 45, No. 3, 865-872.

Apolipoprotein E (ApoE) play important role in lipid metabolism and in processes of remodeling and reparation in central nervous system. Three common ApoE isoforms, ApoE2, ApoE3 and ApoE4, show strong genetic determination by ε2, ε3, and ε4 allele. In human genome gene encoding Apolipoprotein E (*APOE*) is located on cromosome 19, and ε2/ε3/ε4 haplotype system is defined by 2 non-synonymous single nucleotide polymorphisms (SNPs) in the *APOE* exon 4. The frequency of the three *APOE* alleles and corresponding genotypes varies across human populations, with possible clinical implications. At least, variable distribution of ε4 allele may contribute to the regional risk of cardiovascular and Alzheimer's diseases. Allele-frequency comparisons between younger and older populations suggest an effect of *APOE* on mortality, but these data are not consistently confirmed. In the present study we have analyzed the distribution of *APOE* gene polymorphism in a group of University students and retained University professors living in Serbia. After DNA extraction from peripheral blood samples, the *APOE* genotype was determined by polymerase chain reaction (PCR) followed with HhaI restriction digestion. We found no statistically significant difference in alleles and genotypes distribution between younger and elder group of participants. Also, there was no significant difference compared to *APOE* data previously obtained in YUSAD cohort of healthy school children (15 y of age) from different regions of Serbia. In both of our groups, as well as in YUSAD cohort, frequency of *APOE* ε4 allele was <10%. The observed frequencies are lower than in neighboring countries, but similar with Spanish data and some Asian populations. Our results do not support important role of *APOE* ε4 in the morbidity and mortality in Serbian population, but gene-environmental-social interactions should be considered.

Key words: apolipoprotein E, gene polymorphism, population study, Serbia, ε4 allele

Corresponding author: Ivana Novaković, Faculty of Medicine, University of Belgrade,dr Subotića 8, 11000 Belgrade, Serbia, phone: +381 11 3607040. fax: +381 11 3607042, email: novivana@eunet.rs

INTRODUCTION

Apolipoprotein E (ApoE) E has a crucial role in the metabolism of plasma lipoproteins, as well as in lipid synthesis and in reparative and remodelling processes in central nervous system. As one of the major protein constituents of several lipoprotein classes, ApoE serves as a ligand for the low density lipoprotein (LDL) receptor and LDL receptor-related protein (LRP) and enables lipids removal from plasma. In brain, ApoE is involved in endogenous cholesterol and amyloid protein metabolism as well as in synaptic plasticity during reparation and remodelling. Three common ApoE isoforms, ApoE2, ApoE3 and ApoE4, show strong genetic determination by ε2, ε3, and ε4 allele forms, respectively (SVOBODOVA *et al*., 2007; YU *et al.,* 2007).

The gene encoding ApoE (*APOE* gene) in human genome maps on the long arm of chromosome 19. The ε2/ε3/ε4 haplotype system is defined by 2 non-synonymous single nucleotide polymorphisms (SNPs) in *APOE* exon 4. Both SNPs are C/T substitutions changing arginine to cysteine in ApoE at amino acid position 112 and 158, respectively. The allelic compositions of the haplotypes are TT for ε2, TC for ε3, and CC for ε4, with corresponding protein isoforms Cys,Cys for ApoE2, Cys,Arg for ApoE3 and Arg,Arg for ApoE4 (YU *et al.,* 2007). The most frequent allele in human populations is ε3, with overall prevalence of 70–80%.

Allele ε4 is associated with increased plasma total and LDL cholesterol and ApoB levels, and it is designed as risk factor for cardiovascular diseases (CVD). Usually, allele ε2 is associated with lower total and LDL cholesterol levels, but its relation to the risk of atherosclerosis is controversial (NOVAKOVIĆ *et al*., 2010; SVOBODOVA *et al.,* 2007). Regarding ApoE role in central nervous system, ε4 allele is well known genetic risk factor for Alzheimer's disease, confirmed in genome wide association studies (GWAS) (GHARESOURAN *et al.,* 2013; BELBIN *et al.,* 2011; YU *et al*., 2007). Also, there are number of reports of *APOE* ε4 association with affective and cognitive disorders, subarachnoid hemorrhage etc. (KAUSHAL *et al.,* 2007).

The frequency of the three *APOE* alleles and corresponding genotypes varies across human populations, with possible clinical implications. At least, variable distribution of ε4 allele may contribute to the regional risk of cardiovascular and Alzheimer's diseases. Allele-frequency comparisons between younger and older populations suggest an effect of *APOE* on mortality, but these data are not consistently confirmed (SCHUPF *et al.,* 2013; ROSVALL *et al.,* 2009). Serbian population shows high prevalence of CVD, which are still major cause of death in the country (DAMNJANOVIĆ *et al.,* 2011). On the other hand, population age-structure is not convenient, with high prevalence of aging-related neurodegenerative disorders, such as Alzheimer's disease. Under these circumstances, the evaluation of *APOE* allele distribution in different age-related groups from Serbia could be interesting from genetic, as well as from epidemiological and clinical viewpoints. We therefore studied the *APOE* gene polymorphism in a group of University students and retained University professors living in Serbia.

MATERIALS AND METHODS

Apo E genotype was examined in two groups of subjects from Serbian population: a group of University students (N=548, 188 males, 366 females, age 21+/-2y) and a group of retained University professors (N=94, all men, age 83+/-3y). The young participants were recruited from University of Belgrade, and they arrived from different parts of Serbia. The senior participants were part of Serbian cohort in Seven Countries Study (ALONSO *et al.,* 2009). For

molecular genetic analysis, 5ml peripheral blood samples were collected and DNA was extracted using salting-out method.

The *APOE* genotype was determined by polymerase chain reaction (PCR) followed with *HhaI* restriction digestion of PCR products and electrophoresis of restriction fragments on polyacrylamide gels (HIXON and VERNIER, 1990). Each of the genotype forms was distinguished by a unique combination of *HhaI* fragment sizes that enabled unambiguous typing of all homozygotic and heterozygotic combinations. *HhaI* cleaves at GCGC encoding 112arg (ε4) and 158arg (ε3, ε4), but does not cut at GTGC encoding 112cys (ε2, ε3) and 158cys (ε2). PCR was conducted in a thermal cycler ABI 2700 (Applied Biosystems, USA). The oligonucleotide primers used for PCR amplification were P1 (5´-TCCAAGGAGCTGCAGGCGGCGCA-3´) and P2 (5´-ACAGAATTCGCCCCGGCCTGGTACACTGCCA-3´). The amplification mixture contained 2 mM MgCl2, 1U of DreamTaq DNA Polymerase (Fermentas, Germany), 0.2 mM of each dNTP (Promega, Madison, WI), 0.8 μM of each primer and 300ng DNA in a final volume of 25 μl. The PCR conditions were: initial denaturation at 95°C for 4 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing and elongation at 67.5°C for 1 min 30 s. A final extension step at 72°C for 10 min was done. The amplification generated a DNA fragment of 227 bp. After amplification, 20 μl of the PCR product were directly digested with 12 units of the restriction endonuclease *HhaI* (Promega) at 37°C overnight. Gene fragments were separated using 10 % vertical polyacrylamide gel electrophoresis and detected by ethidium bromide staining under ultraviolet illumination, using an appropriate DNA size marker.

The allelic and genotypic frequencies of *APOE* were estimated by counting alleles and genotypes and calculating sample proportions; the statistical significance of differences of frequencies between groups was compared by $\chi 2$ test.

RESULTS AND DISCUSSION

The frequencies of *APOE* genotypes are presented in Table 1. The observed distributions were compared by $\chi 2$ test and no statistical significance was found (p=0.829). (Table 1)

Table 1. The frequencies of APOE genotypes in Serbian groups of University students and retained University professors

APOE genotypes	students						professors	
	M&F	%	M	%	F	%	M	%
ε 2/ ε 2	0	0	0	0	0	0	1	1
ε 2/ ε 3	55	10.1	13	7.1	42	11.5	10	11
ε3/ ε 3	426	77.7	148	81.3	278	76.0	69	73
ε 2/ ε 4	6	1.1	1	0.5	5	1.4	3	3
ε 3/ ε 4	57	10.4	20	11.1	37	10.0	11	12
ε 4/ ε 4	4	0.7	0	0.0	4	1.1	0	0
total	548	100.0	182	100.0	366	100.0	94	100

(M = males, F = females) χ^2 =0.374 , p =0.829

Table 2 shows the allelic frequencies of *APOE* in present study and in several European countries; the frequency of the risk *APOE* ε4 allele in Serbian population is the lowest among the listed countries. Data for YUSAD study (Yugoslav study of precursors of atherosclerosis in school children) are from cohort of more than 500 school children (15y) living in different regions of Serbia (DAMNJANOVIĆ *et al.,* 2011). (Table 2)

Table . The frequencies of APOE ε2, ε3, and ε4 alleles in Serbia, some other European countries and USA (in alphabetic order)

Population	ε2	ε3	ε4	χ^2	p
Serbia - students	**0.056**	**0.879**	**0.065**	**0**	**1**
Serbia - professors	**0.0797**	**0.8457**	**0.0744**	**5.449**	**0.076**
Serbia – YUSAD	0.067	0.863	0.070	1.316	0.518
Croatia	0.069	0.787	0.144	36.294	<0.0001
Finland	0.064	0.807	0.129	24.721	<0.0001
France	0.087	0.782	0.131	34.609	<0.0001
Germany	0.082	0.782	0.136	35.643	<0.0001
Hungary	0.065	0.808	0.127	23.678	<0.0001
Spain	0.075	0.849	0.075	3.99	0.136
Turkey	0.061	0.860	0.079	1.782	0.410
USA	0.072	0.786	0.140	34.632	<0.0001

(students, professors = our results; other = from DAMNJANOVIĆ et al., 2011)

We determined *APOE* genotypes in total number of 642 subjects from Serbia, belonging to two age-related groups: University students and retained University professors. We found no statistically significant difference in alleles and genotypes distribution between younger and elder group of participants (p=0.076 and 0.829 for alleles and genotypes, respectively). Also, there was no significant difference (p=0.518) compared to *APOE* data previously obtained in cohort of YUSAD study, which comprises more than 500 healthy school children (15 y of age) from different regions of Serbia (DAMNJANOVIĆ *et al.,* 2011).

In both of our groups, as well as in YUSAD cohort, frequency of *APOE* ε4 allele was <10% (6.5%, 7.44% and 7.0%, in students, professors and school children, respectively). The observed frequencies are significantly lower than in neighboring countries (Croatia, Hungary p<0.0001), but similar with Spanish data (p=0.136) and some Asian populations (Turkey p=0.410).

It is well documented that the distribution of *APOE* alleles varies across populations (DAMNJANOVIĆ *et al.,* 2011; MAKSIMOVIĆ *et al.,* 2009; SVOBODOVA *et al.,* 2007). In general, the

European populations traditionally have higher *APOE* ε4 frequency than Asians. The cause for this regional variability is still not clear. Allele ε4 is assumed as an ancestral form, but due to mechanisms of negative selection its frequency has been decreased, and ε3 allele has become most frequent in contemporary populations. Notably, the frequency of ε4 appears to be higher in northern regions of Europe than in southern regions, thus following the incidence of CVD. In Asia, a similar trend has not been described (SVOBODOVA *et al.,* 2007).

Low *APOE* ε4 allele frequency in Serbia is not consistent with high prevalence of CVD in the country. However, analyses of lipid profiles in YUSAD study showed significantly higher mean level of total cholesterol and in ε4 carriers, even in childhood (DAMNJANOVIĆ *et al.,* 2011).

Because of no significant difference in *APOE* alleles and genotypes distribution in a group age ≤25y and in seniors, we could not confirm the role of *APOE* in mortality as well as in longevity in Serbian population. However, this conclusion should be accepted cautiously, because of demographic profile of our advanced-age participants. Our senior group comprised male participants with highest education (PhD degree at least). Several recent studies from different parts of world: Italy (MARENGONI *et al.,* 2011), Sweden (WANG *et al.,* 2012; ROSVALL *et al.,* 2009), Canada (MENG and D'ARCY, 2013), USA (PETERSEN *et al*., 2010), showed that education level and gender, as well as leisure activities, diet, could modify ε4 allele related risks. Generally, lower education level is associated with higher dementia risk in ε4 allele carriers, but some investigators did not confirm this influence (CHEN *et al*., 2010). The ε4 allele in *APOE* gene accompanied with the linked G allele in rs2075650 of *TOMM40* gene have been associated with increased mortality and the ε2 allele with decreased mortality in advanced age, although inconsistently (SCHUPF *et al.,* 2013). Schupf's group findings support the hypothesis that both reduction in the frequency of the ε4 allele and increase in the frequency of the ε2 allele contribute to longevity (SCHUPF *et al*., 2013).

Similarly, Rosvall's group reported increased mortality-risk of 22% among elderly with the ε4 allele, whereas a 28% decreased mortality-risk was detected in those with the ε2 allele compared to those with the ε3ε3 genotype (ROSVALL *et al.,* 2009). Adjustment for severe vascular events did not change the observed risks. In this investigation dementia accounted for the majority of the increased mortality-risk associated with the ε4 allele, but the protective effect of the ε2 allele remained. Also, both effects of the ε 4 allele and the ε2 allele were strongly modified by gender. A 49% elevated risk for death in men was related to the ε4 allele, and a 36% decreased mortality-risk was found in women with the ε2 allele. These findings suggest different roles for the APOE alleles in survival by gender in old age.

Again, some studies showed only weak or even lack of these associations (VAN GERVEN *et al.,* 2012; CHEN *et al*., 2010; WELSH-BOHMER *et al.,* 2009) , and our investigation supports these observations. In order to solve such controversies, very recent studies suggest that single SNP approaches may be inadequate to identify genetic risks. An alternative approach is the use of multilocus genotype patterns (MLGPs) that combine SNPs at different susceptibility genes (BARRAL *et al*., 2012). Genome wide association studies (GWAS) also confirmed modest effect of single SNPs in complex traits, such as CVD or longevity. On the other hand, huge amount of data obtained by GWASs encourages MLGPs strategy. In addition, gene-environmental-social interactions during human life are very important and may be another part of "missing heritability" explanation (NOVAKOVIĆ *et al.,* 2013, GRÜNBLATT *et al.,* 2009).

ACKNOWLEDGEMENT

This work is supported by Serbian Ministry of education, science and technology development, Grant No. ON175091

Received June 20th, 2013
Accepted October 05th, 2013

REFERENCES

ALONSO, A., JR. D.R.JACOBS, A.MENOTTI, A.NISSINEN, A.DONTAS, D. KROMHOUT (2009): Cardiovascular risk factors and dementia mortality: 40 years of follow-up in the Seven Countries Study. J. Neurol. Sci. *280*: 79-83

BARRAL, S., T.BIRD, A.GOATE, M.R.FARLOW, R.DIAZ-ARRASTIA, D.A.BENNETT, GRAFF- N.RADFORD, B.F.BOEVE, R.A. SWEET, Y.STERN, R.S.WILSON, T.FOROUD, J.OTT, R.MAYEUX (2012): Genotype patterns at PICALM, CR1, BIN1, CLU, and APOE genes are associated with episodic memory. Neurology *78*: 1464-1471.

GRÜNBLATT, E., S.ZEHETMAYER, J.BARTL, C.LÖFFLER, I.WICHART, M.K.RAINER, S.JUNGWIRTH, P.BAUER, W.DANIELCZYK, K.H.TRAGL, P.RIEDERER, P.FISCHER (2009): Genetic risk factors and markers for Alzheimer's disease and/or depression in the VITA study. J. Psychiatr. Res. *43*: 298-308.

BELBIN, O., M.M.CARRASQUILLO, M.CRUMP, O.J.CULLEY, T.A.HUNTER, L.MA, G.BISCEGLIO, F.ZOU, M.ALLEN, D.W.DICKSON, N.R.GRAFF-RADFORD, R.C.PETERSEN, K.MORGAN, S.G. YOUNKIN (2011): Investigation of 15 of the top candidate genes for late-onset Alzheimer's disease. Hum. Genet. *129*: 273–282.

CHEN, C.H., T.MIZUNO, R.ELSTON, M.M.KARIUKI, K.HALL, F.UNVERZAGT, H.HENDRIE, S.ATERE, P.KIOY, N.B.PATEL, R.P.FRIEDLAND, R.N. KALARIA (2010): A comparative study to screen dementia and APOE genotypes in an ageing East African population. Neurobiol. Aging. *31*: 732-740.

DAMNJANOVIĆ, T., I.NOVAKOVIĆ, V.DIKLIĆ, N.MAJKIĆ, S.SIMEUNOVIĆ, M.VUKOTIĆ, S.NEDELJKOVIĆ: Genetic markers of atherosclerosis in YUSAD study -2. in: Nedeljković S., Simeunović S., Novaković I., Marisavljević D., Vukotić M. (ed.) (2011): Yugoslav study of precursors of atherosclerosis in school children – 20 years of the follow up. Faculty of Medicine, CIBID, Belgrade, 453-470.

GHARESOURAN, J., M.REZAZADEH, S. MOJTABA MOHADDES ARDEBELI (2013): Investigation of five polymorphic DNA markers associated with late onset Alzheimer disease. Genetika *45*: 503-514.

HIXON, J.E., D.T. VERNIER (1990): Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J. Lipid Res. *31*: 545-548.

KAUSHAL, R., D.WOO, P.PAL, M.HAVERBUSCH, X H.I, C.MOOMAW, P.SEKAR, B.KISSELA, D.KLEINDORFER, M.FLAHERTY, L.SAUERBECK, R.CHAKRABORTY, J.BRODERICK, R. DEKA (2007): Subarachnoid hemorrhage: tests of association with apolipoprotein E and elastin genes. BMC Medical Genetics *8*: 49-55.

MAKSIMOVIĆ, N., E.STEFANOVA, I.NOVAKOVIĆ, T.STOJKOVIĆ, M.BAJČETIĆ, T.DAMNJANOVIĆ, B JEKIĆ, L.LUKOVIĆ, B.POPOVIĆ, V.KOSTIĆ (2009): Study of association between *APOE* genotype and cognitive performance in young adults. European Human Genetics Conference, Barcelona, Spain, European J. Hum. Genet.*17*:233.

MARENGONI, A., L.FRATIGLIONI, S.BANDINELLI, L.FERRUCCI (2011): Socioeconomic status during lifetime and cognitive impairment no-dementia in late life: the population-based aging in the Chianti Area (INCHIANTI) Study. J. Alzheimers Dis. *24*: 559-568.

MENG, X., C. D'ARCY (2013): Apolipoprotein E gene, environmental risk factors, and their interactions in dementia among seniors. Int. J. Geriatr. Psychiatry *28*:1005-1014.

NOVAKOVIĆ, I., N.MAKSIMOVIĆ, S.CVETKOVIĆ, D.CVETKOVIĆ (2010): Gene polymorphisms as markers of disease susceptibility. J. Med. Biochem. *29*: 1-5.

NOVAKOVIĆ, I., N.MAKSIMOVIĆ, A.PAVLOVIĆ, M.ŽARKOVIĆ, B. ROVČANIN, D. MIRKOVIĆ, T.PEKMEZOVIĆ, D.CVETKOVIĆ (2013): Introduction to molecular genetic diagnosis. J. Med. Biochem. DOI: 10.2478/jomb-2013-0039

PETERSEN, R.C., R.O.ROBERTS, D.S.KNOPMAN, Y.E.GEDA, R.H.CHA, PANKRATZ V.S., B.F.BOEVE, E.G.TANGALOS, R.J.IVNIK, W.A. ROCCA (2010): Prevalence of mild cognitive impairment is higher in men. The Mayo Clinic Study of Aging. Neurology. *75*: 889-897.

ROSVALL, L., D.RIZZUTO, H.X.WANG, B.WINBLAD, C.GRAFF, L.FRATIGLIONI (2009): APOE-related mortality: effect of dementia, cardiovascular disease and gender. Neurobiol. Aging. *30*: 1545-1551.

SCHUPF, N., S.BARRAL, T.PERLS, A.NEWMAN, K.CHRISTENSEN, B.THYAGARAJAN, M.PROVINCE, W.R K.OSSI, R. MAYEUX (2013): Apolipoprotein E and familial longevity. Neurobiol. Aging. *34*: 1287-1291.

SVOBODOVA, H., F.KUČERA, T.ŠTULC, M.VRABLIK, B.AMARTUSVHIN, T.S. ALTANNAVCH (2007): Apolipoprotein E Gene Polymorphism in the Mongolian Population. Folia Biologica (Praha) *53*, 138-142.

VAN GERVEN, P.W., M.P.VAN BOXTEL, E.E.AUSEMS, O.BEKERS, J. JOLLES (2012): Do apolipoprotein E genotype and educational attainment predict the rate of cognitive decline in normal aging? A 12-year follow-up of the Maastricht Aging Study. Neuropsychology *26*:459-472.

WANG, H.X., D.R.GUSTAFSON, M.KIVIPELTO, N.L.PEDERSEN, I.SKOOG, B.WINDBLAD, L.FRATIGLIONI (2012): Education halves the risk of dementia due to apolipoprotein ε4 allele: a collaborative study from the Swedish brain power initiative. Neurobiol. Aging *33*: 1007.e1-7.

WELSH-BOHMER, K.A., T.OSTBYE, L. SANDERS, C.F.PIEPER, K.M.HAYDEN, J.T.TSCHANZ, C.NORTON M., Cache Country Study Group. (2009): Neuropsychological performance in advanced age: influences of demographic factors and Apolipoprotein E: findings from the Cache County Memory Study. Clin. Neuropsychol. *23*: 77-99.

YU, C-E., H.SELTMAN, E.R.PESKIND, N.GALLOWAY, X.ZHOU P, E.ROSENTHAL, E.M.WIJSMAN, D.W.TSUANG, B. DEVLIN, G.D. SCHELLENBERG (2007): Comprehensive analysis of *APOE* and selected proximate markers for late-onset Alzheimer disease: Pattern of linkage disequilibrium and disease/marker association. Genomics *89*: 655–665.

DISTRIBUCIJA PLOMORFIZMA GENA ZA APOLIPOPROTEIN E KOD STUDENATA I PENZIONISANIH UNIVERZITETSKIH PROFESORA U SRBIJI

Nela MAKSIMOVIĆ, Ivana NOVAKOVIĆ, Vesna RALIĆ i Elka STEFANOVA

Medicinski fakultet Univerziteta u Beogradu, Beograd, Srbija

Izvod

Apolipoprotein E (ApoE) ima važnu ulogu u metabolizmu lipida, kao i u procesima remodelovanja i reparacije u centralnom nervnom sistemu. Glavne izoforme ApoE, označene kao ApoE2, ApoE3 and ApoE4, su genetički determinisane alelima ε2, ε3, and ε4 *APOE* gena. Haplotipski sitem ε2/ε3/ε4 je određen sa dva SNP lokusa u egzonu 4 *APOE* gena. Frekvenca tri *APOE* alela i odgovarajućih genotipova varira u humanim populacijama, što može da ima kliničke implikacije. Na primer, različita distribucija ε4 alela može da utiče na regionalni rizik od Alchajmerove bolesti ili kardiovaskularnih bolesti. Poređenje učestalosti *APOE* alela u populacijama mladih i populacijama starijih ukazuje na moguću ulogu ovog gena u mortalitetu, ali podaci su još uvek protivrečni. U ovoj studiji analizirali smo distribuciju polimorfizma *APOE* gena u grupi studenata i grupi penzionisanih univerzitetskih profesora iz Srbije. Nakon izolacije DNK iz prikupljenih uzoraka venske krvi, *APOE* genotipovi su određivani PCR/RFLPs metodom. U našoj studiji utvrdili smo da nema statistički značajne razlike u učestalosti genotipova i alela između analiziranih grupa, kao ni u odnosu na rezultate prethodnog ispitivanja u populaciji školske dece u Srbiji (YUSAD studija). U grupama studenata i profesora, kao i YUSAD kohorti, frekvencija *APOE* ε4 alela je bila <10%. Utvrđene frekvence su niže nego u susednim zemljama, a slične npr. španskoj i nekim azijskim populacijama. Naši rezultati nisu pokazali značajnu ulogu *APOE* ε4 alela u morbiditetu i mortalitetu u Srbiji, ali u njihovom tumačenju važne su interakcije gena i spoljne sredine.

Primljeno 20. VI 2013.
Odobreno 05. X. 2013.