Arch. Biol. Sci., Belgrade, 59 (4), 303-309, 2007 DOI:10.2298/ABS0704303S

APOLIPOPROTEIN B GENE POLYMORPHISMS IN PATIENTS FROM SERBIA WITH ISCHEMIC CEREBROVASCULAR DISEASE

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Abstract — The plasma concentration of apoB has recently been reported to be the best lipid predictor of coronary heart disease. The possible associations of genetic markers in the apolipoprotein B gene (XbaI, EcoRI, MspI, Ins/Del, and 4311 A/G polymorphisms) were evaluated in patients with ischemic cerebrovascular disease (ICVD) and controls of equivalent BMI. The odds ratio for ICVD in the X+X+ genotype was 2.22, 95% CI 1.24-3.96 (P<0.05), while that for ICVD in the Ins/Ins genotype was 2.82, 95% CI 1.57-5.06 (P<0.05). The patients had significantly higher frequency of the 4311A allele compared to the controls (P<0.01). Our results support the assumption that apoB gene polymorphisms may contribute to the extent of cerebrovascular disease risk.

Key words: ApoB, ischemic cerebrovascular disease, gene, polymorphism, human population, Serbia

UDC 577.25:577.34
616.831-005.4(497.11)

INTRODUCTION

Most strokes are ischemic in origin; of these 80% are caused by arterial occlusion secondary to atherosclerosis (B a m f o r d et al., 1990). Evidence indicates that modifiable risk factors (such as lipids and lipoproteins) interact with genetic factors to cause stroke (E l b a z et al., 1999). Studies in twins, families, and animal models provide substantial evidence for a genetic contribution to ischemic stroke (J e f f s et al., 1997; S c h u l z et al., 2004). The genetic factors seem to be more important in large-vessel stroke and small vessel stroke than in cryptogenic stroke, and there is no epidemiological evidence for a genetic component in cardioembolic stroke (S c h u l z et al., 2004; J e r r a r d-D u n n e et al., 2003). This finding emphasizes the importance of stroke subtypes and lends support to the view that large-vessel stroke and myocardial infarction share similar pathological mechanisms and genetic susceptibility (D i c h g a n s, 2007).

Apolipoprotein B (apoB) plays a central role in lipoprotein metabolism. It is a component of chylomicrons, low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), and intermediate-density lipoproteins (IDL), as well as the ligand for the LDL receptor (M a h l e y et al., 1984). The given lipoprotein has numerous polymorphic sites. Among them are polymorphisms assigned according to the presence/absence of the cutting site of the restriction enzymes *Xba*I, *Eco*RI, and *Msp*I; the 4311 Asn→Ser substitution; and an insertion/deletion of nine base pairs (I/D) in the signal peptide (R e n g e e s et al., 1992). ApoB gene polymorphisms *Xba*I, *Eco*RI, and *Msp*I has been previously linked with variability of serum lipid levels and the risk of coronary atherosclerosis in several populations (H u m p h r i e s, 1988; K a m e r e e r et al., 1996; H a n s e n et al., 1994; D e l g h a n d i et al.; 1999, S t e p a n o v et al., 1998). I/D polymorphism has also been linked with variations in plasma cholesterol and CAD risk (H u m p h r i e s, 1988; K a m e r e e r et al., 1996; P e a c o c k et al., 1992, P e a c o c k et al., 1994). The X-, R-, and I alleles of the above-mentioned loci have been reported as risk factors for CAD (B o h n et

al.,1993; H u m p h r i e s, 1988; H e g e l e and B r e s l o w, 1987; P e a c o c k et al., 1992, P e a c o c k et al., 1994). It was also suggested that apoB gene polymorphisms may modulate plasma lipid/lipoprotein and glucose levels in patients with type 2 diabetes (D u m a n et al., 2006). The apoB gene has so far been mainly investigated in familiar hypercholesterolemia and coronary artery diseases. Accordingly, the aim of our study was to investigate the possible association of five polymorphisms in the apoB gene (*Xba*I, *Eco*RI, *Msp*I, Ins/Del, and 4311A/G) with ischemic cerebrovascular disease in Serbia.

MATERIAL AND METHODS

Sample

Blood samples were obtained from 60 patients who had suffered a completed stroke or a transient ischemic attack. These were proven by computer tomography or magnetic resonance of the brain. Atherosclerosis of the eye bottom as well as both carotids and vertebral arteries was assessed by ultrasound examination. The control group consisted of 245 unrelated healthy Serbian subjects whose annual health examination showed them to be free of cerebrovascular, cardiovascular, or chronic inflammatory disease. The control group was body mass index matched (BMI) with patients. Informed consent was obtained from each participant in the study. Personal data (age, sex, weight, height, and blood pressure) were obtained from all participants. All subjects with a personal or family history of diabetes and/or thyroid dysfunction were excluded, as well as individuals taking any lipid-lowering drugs.

Blood samples were collected from participants after 12 hours of fasting. The total plasma cholesterol (TC) and triglyceride (TG) levels were determined on a Monarch Plus apparatus (Instrumentation Laboratory, Lexington, USA) using enzymatic colorimetric methods. The HDL cholesterol (HDLC) was determined after dextran sulfate – Mg^{2+} precipitation of VLDL and LDL, using the CHOD-PAP method. The LDL cholesterol (LDLC) was calculated using the Friedewald formula (F r i e d e w a l d et al., 1972) for participants with triglyceride levels <4.5 mmol/l. All reagent kits were from Instrumentation Laboratory (Lexington, USA). Serum apoA-I and serum apoB were quantified by immunonephelometry with reagents from Beckman Instruments (Fullerton, CA).

DNA analysis

Genomic DNA was isolated from whole blood cells by proteinase K digestion and phenol/chloroform extraction (K u n k e l et al., 1977). Genomic fragments containing apoB gene polymorphisms *Xba*I (codon 2488, exon 26), *Eco*RI (codon 4154, exon 29), *Msp*I (codon 3611), point mutation A/G at nucleotide 12932 (codon 4311, exon 29), and Ins/Del (signal peptide) were amplified by the polymerase chain reaction (PCR) on a Touch Down™ thermal cycler (Hybaid, Teddington, UK). Genotypes were determined by RFLP and/or gel electrophoresis as previously described (G l i š i ć et al., 1995; G l i š i ć et al., 1997; G l i š i ć and A l a v a n t i ć, 1996; R a j p u t-W i l l i a m s et al., 1988) and visualized by the GDS8000 gel documentation system (Ultra Violet Products Inc., Upland, CA).

Statistical analysis

Conformance of the allele frequencies to Hardy-Weinberg equilibrium proportions was tested by the χ^2 test. Genotype and allele frequencies in different groups were compared by the gene counting method and chi-squared analysis. The unadjusted odds ratios and their 95% confidence intervals (CI) were also calculated. The Student t-test was used to compare differences between two means. If the distribution of quantitative variables was skewed, log-transformed values were used for the analysis. In all tests, differences with two-tailed alpha–probability (P) ≤0.05 were considered statistically significant. The correction for multiple testing was performed by multiplying the *p* value by the number of polymorphisms analyzed in the study. For the analysis, we used the Statistica software package (Version 5, Stat Soft Inc., 1997).

RESULTS

Description of the population

Descriptive statistics of concomitants and their

Table 1. Study subject characteristics

Parameter	ICVD Patients	Controls	P value*
n	60	245	
Age (years)	50.73 ± 13.09	40.48 ± 15.01	<0.05
Smokers-n (%)*	38 (63.3)	154 (62.9)	NS
Body mass index (kg/m^2)	25.42 ± 2.74	26.32 ± 3.69	NS
Total cholesterol (mmol/l)	6.07 ±1.51	5.75 ± 0.68	NS
HDL-cholesterol (mmol/l)	1.20 ± 0.49	1.55 ± 0.39	<0.05
LDL-cholesterol (mmol/l)	3.65 ± 1.48	3.72 ± 1.06	NS
Triglycerides (mmol/l)#	2.05± 0.86	1.38 ± 0.80	<0.05
Systolic blood pressure (mm Hg)#	161.10±29.52	129.55±22.16	<0.05
Diastolic blood pressure (mm Hg)#	97.13±14.41	82.26±12.06	<0.05
apoA (g/L)	1.36±0.33	1.31±0.38	NS
apoB (g/L)	1.46±0.53	0.97±0.36	<0.05

Values are expressed as means ± SD; # analyses were performed with log transformed values; P value from t-test; *χ^2 –test; NS non-significant.

lipid and lipoprotein variables are presented in Table 1. The patient group was generally older and had significantly lower HDL, but also higher triglyceride and apoB levels. Blood pressure values were significantly higher in the patients than in the controls.

Genotypes and susceptibility to ICVD

The genotype and allele frequencies of apoB gene polymorphisms *Xba*I, *Eco*RI, *Msp*I, Ins/Del, and 4311 A/G in the patients with ICVD and controls are shown in Table 2. The observed genotype frequencies did not significantly differ from expected values according to the Hardy-Weinberg equilibrium, except for the Ins/Del polymorphism ones in the controls (G l i š i ć et al., 1997). The frequency of the apoB X+ allele was significantly higher in the ICVD patients than in the controls (P<0.05). The odds ratio (OR) for ICVD in the X+X+ genotype was 2.22, 95% CI 1.24-3.96. Frequency of the apoB Ins allele was also significantly higher in the ICVD patients compared to the controls (P<0.05). The OR for ICVD in the Ins/Ins genotype was 2.82, 95% CI 1.57-5.06. The patients with ICVD had significantly higher frequency of the apoB 4311A allele compared to the controls (P<0.01). We did not calculate the OR for carriership of the A allele, since all investigated patients had carrier status. No significant differences of genotype and allele frequency distribution for either the *Eco*RI or the *Msp*I polymorphism at the apoB gene were observed between the patients and the controls.

DISCUSSION

The plasma concentration of apoB has recently been reported to be the best lipid predictor of coronary heart disease (S n i d e r m a n and M a r c o v i n a , 2006; P i s c h o n et al., 2005). There is growing evidence indicating that a number of risk factors are shared between coronary heart disease and cerebrovascular disease (P e a r s o n et al., 2002). Although cerebrovascular insufficiency may be caused by a variety of pathophysiological mechanisms, it is known that many possible risk factors (such as disturbance of lipid profile and sequence variations in genes coding for apolipoproteins) can accelerate the development of atherosclerosis and result in stroke. Information about the effects of apoB gene polymorphisms in ICVD still remains scanty. Previously, Ins/Del polymorphism of the apoB gene has been linked with CAD risk (H a n s e n et al., 1994). Our results are compatible with those linking the Ins allele or the Ins/Ins genotype with CAD or with severity of coronary atherosclerosis at the first angiography (R e g i s-B a i l e y et al.,

Table 2. Genotype and allele frequencies of apolipoprotein B-100 gene polymorphisms XbaI, EcoRI, MspI, Ins/Del, and 4311 A/G in ICVD patients and controls

apoB	Genotypes and alleles	ICVD patients		Controls		P (χ^2)
		n=60	%	n =245	%	
*Xba*I	X+X+	37	61.67	103	42.04	
	X+X-	20	33.33	124	50.61	<0.05
	X-X-	3	5.00	18	7.35	
Allele	X+	94	78.33	330	67	<0.05
	X-	26	21.67	160	33	
*Eco*RI	R+R+	31	51.67	146	68.60	
	R+R-	26	43.33	91	29.70	NS
	R-R-	3	5.00	8	1.70	
Allele	R+	88	73.33	383	78.16	
	R-	32	26.67	107	21.84	NS
*Msp*I	M+M+	23	38.33	118	48.16	
	M+M-	34	56.67	115	46.94	NS
	M-M-	3	5.00	12	4.90	
Allele	M+	80	66.67	351	71.63	
	M-	40	33.33	139	28.37	NS
Ins/Del	InsIns	29	48.33	61	24.90	
	InsDel	21	35.00	155	63.26	<0.001
	DelDel	10	16.67	29	11.84	
Allele	Ins	79	65.83	276	56.32	<0.05
	Del	41	34.16	214	43.67	
4311	AA	31	51.66	97	39.59	
	AG	29	48.33	115	46.94	<0.05
	GG	0	0	33	13.47	
Allele	A	91	75.83	309	63.06	<0.01
	G	29	24.17	181	36.94	

1996; Peacock et al., 1992). In the present study, individuals carrying the Ins/Ins genotype presented 2.82-fold increased risk for development of ICVD. Although our study was limited in the number of ICVD patients, this risk remained significant even after correction for multiple testing. It was also recently reported that the Ins/Ins genotype confers a 2.2 times higher risk for an unfavorable course of ischemic heart disease in the population of Russia (Zateishchikov et al., 2004).

*Xba*I polymorphism of the apoB gene has also been linked with atherosclerosis in a number of studies from different populations. Even frequency of the rare X+ allele was significantly lower in the Chinese Han population than that reported in

Caucasians (0.027 vs. 0.418) (X- was the most frequent one in this population), and higher frequency of the X+ allele was found in the Chinese Han atherosclerotic cerebral infarction group compared to controls (0.053 vs. 0.027, $P < 0.05$) (W a n g et al., 1999). Alto-Setala and co-workers discerned no statistically significant association between *Xba*I alleles and cerebrovascular atherosclerosis (A l t o-S e t a l a et al., 1998), while two other studies showed a higher prevalence of the X+X+ genotype or X+ allele in patients with arterial disease (M a n s u r et al., 2000; M o n s a l v e et al., 1988), which is in agreement with our results. Individuals carrying the X+X+ genotype had a 2.22-fold increased risk of developing ICVD. This association was no longer significant after correction for multiple testing.

The point mutation (A/G) at codon 4311 of the apoB gene was less thoroughly examined in recent reports. In the control group, frequency of the allele G (0.37) is among the highest in Caucasian populations. Rare homozygotes (GG) were not present in the Serbian ICVD group. A similar trend of lower GG frequency was observed in patients with myocardial infarction compared to controls (M o r e e l et al., 1992). The 4311 polymorphism had a significant effect on high density lipoprotein (HDL) cholesterol levels in a study of young myocardial infarction survivors and healthy population-based individuals (P e a c o c k et al., 1992). In our study, patients with ICVD had a significantly higher frequency of the A allele, which remained significant after correction for multiple testing.

We did not observe significant differences of genotype distribution and relative allele frequencies for the *Eco*RI and *Msp*I polymorphisms in the apoB gene. These data are in accordance with some of previous reports (D e l g h a n d i et al., 1999; S a l a z a r et al., 2000). Others found the common M+ allele of the *Msp*I RFLP polymorphism more frequently present in CAD patients than in controls, but no significant differences of allele frequencies were observed for the *Xba*I and *Eco*RI polymorphisms (S t e p a n o v et al., 1998).

It is very important that the control group in the present study was matched with patients according to BMI values, since recent findings showed that increased BMI is a risk factor for both total and ischemic stroke (H u et al., 2007). In addition to the different design of the previous studies, inconsistencies of the results might be attributable to different apoB haplotype distributions in the different populations studied. Also, most previous studies were focused on the effect of apoB gene polymorphisms on changes in lipid levels. There is a possibility that some of the polymorphisms may act through mechanisms not directly related to influence on measured lipid traits. Also, the significant effect of certain polymorphisms that we found could be due to linkage disequilibrium with other functional genetic markers.

There are no previous data on genotype distribution, allele frequencies or correlation of the apoB Ins/Del, *Eco*RI, *Msp*I, *Xba*I, and 4311 A/G polymorphisms with ICVD in Serbian patients. Our study suggests association between polymorphisms in the apoB gene and ICVD in subjects of Serbian origin and supports the assumption that apoB polymorphisms may contribute to the extent of cerebrovascular risk. Insight into the genetic profile of affected subjects before the onset of ICVD clinical symptoms could have immediate clinical and public health benefits in predicting ICVD risk. Future studies on larger and independent samples and in different populations could confirm our results and elucidate these relations with a higher power of clarification.

Acknowledgment — This work was funded by the Serbian Ministry of Science (Grant No. M145023).

REFERENCES:

Alto-Setala, K., Palomaki, H., Miettinen, H., Vuorio, A., Kuusi, T., Raininko, R., Salonen, O., Kaste, M., and *K. Kontula* (1998). Genetic risk factors and ischemic cerebrovascular disease: role of common variation of the genes encoding apolipoproteins and angiotensin-converting enzyme. *Ann. Med.* **30**, 224-233.

Bamford, J., Sandercock, P., Dennis, M., Burn, J., and *C. Warlow* (1990). A prospective study of acute cerebrovascular disease in the community: the Oxfordshire Community Stroke Project--1981-86. 2. Incidence, case fatality rates and overall outcome at one year of cerebral infarction, primary intracerebral and subarachnoid hemorrhage. *J. Neurol. Neurosurg. Psychiatry* **53**,16-22.

Bohn, M., Bakken, A., Erikssen, J., and *K. Berg* (1993). XbaI

polymorphism in DNA at the apolipoprotein B locus is associated with myocardial infarction (MI). *Clin Genet*. **44**, 241-248.

Delghandi, M, Thangarajah, R, Nilsen, M, Grimsgaard, S, Bonaa, K. H., Tonstad, S, and L. Jorgensen (1999). DNA polymorphisms of the apolipoprotein B gene (XbaI, EcoRI, and MspI RFLPs) in Norwegians at risk of atherosclerosis and healthy controls. *Acta. Cardiol.* **54**, 215-225.

Dichgans, M. (2007). Genetics of ischaemic stroke. *Lancet. Neurol.* **6**,149-161.

Duman, B.S., Ozturk, M., Yilmazer, S., Cagatay, P., and *H. Hatemi* (2006). Apolipoprotein B gene variants are involved in the determination of blood glucose and lipid levels in patients with non-insulin dependent diabetes mellitus. *Cell. Biochem. Funct.* **24**, 261-267.

Elbaz, A., and *P. Amarenco* (1999). Genetic susceptibility and ischemic stroke. *Curr. Opin. Neurol.* **12,** 47–55.

Friedewald, W. T., Levy, R. I., and *D. S. Fredrickson* (1972). Estimation of the concentration of LDL cholesterol in plasma without use of preparative ultracentrifuge. *Clin. Chem*. **18**, 499-502.

Glišić, S., and *D. Alavantić* (1996). A simple PCR method for detection of defined point mutations. *Trends Genet.* **12**, 391-392.

Glišić, S., Prljić, J., Radovanović, N., and *D. Alavantić* (1997). Study of apoB gene signal peptide insertion/deletion polymorphism in a healthy Serbian population: no association with serum lipid levels. *Clin. Chem. Acta* **263**, 57-65.

Glišić, S., Savić, I., and *D. Alavantić* (1995). Apolipoprotein B gene DNA polymoprphisms (EcoRI and MspI) and serum lipid levels in the Serbian healthy population: interaction of rare alleles and smoking and cholesterol levels. *Genet. Epidemiol.* **12**, 499-508.

Hansen, P. S., Klausen, I. C., Lemming, L., Gerdes, L. U., Gregersen, N., and *O. Faergeman* (1994). Apolipoprotein B gene polymorphisms in ischemic heart disease and hypercholesterolemia: effects of age and sex. *Clin. Genet.* **45**, 78-83.

Hegele, R.A., and *J. L. Breslow* (1987). Apolipoprotein genetic variation in the assessment of atherosclerosis susceptibility. *Genet. Epidemiol*. **4**, 163-184.

Hu, G., Tuomilehto, J., Silventoinen, K., Sarti, C., Mannisto, S., and *P. Jousilahti* (2007). Effects of body mass index, waist circumference, and waist-hip ratio on the risk of total and type-specific stroke. *Arch. Intern. Med.* **167**, 1420-1427.

Humphries, S. E. (1988). DNA polymorphisms of the apolipoprotein genes-their use in the investigation of the genetic component of hyperlipidaemia and atherosclerosis. *Atherosclerosis*. **72**, 89-108.

Jeffs, B., Clark, J. S., Anderson, N. H., Gratton, J., Brosnan, M. J., Gauguier, D., Reid, J. L., Macrae, I. M., and A. F. *Dominiczak* (1997). Sensitivity to cerebral ischaemic insult in a rat model of stroke is determined by a single genetic locus. *Nat. Genet.* **16,** 364–367.

Jerrard-Dunne, P., Cloud, G., Hassan, A., and *H. S. Markus* (2003). Evaluating the genetic component of ischemic stroke subtypes: a family history study. *Stroke* **34,** 1364–1369.

Kammerer, C. M., VandeBerg, J. L., Haffner, S. M., and *J. E. Hixon* (1996). Apolipoprotein B (apoB) signal peptide length polymorphisms are associated with apoB, low density lipoprotein cholesterol, and glucose levels in Mexican Americans. *Atherosclerosis* **120**:37-45.

Kunkel, L. M., Smith, K. D., Boyer, S. H., Borgaonkar, D. S., Wachtel, S. S., Miller, O. J., Breg, W. R., Jones, H. W. Jr., and *J. M. Rary* (1977). Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. *Proc. Natl. Acad. Sci. USA* **74**, 1245-1249.

Mahley, R. W., Innerarity, T. L., Rall, S. C. Jr., and *K. H. Weisgraber* (1984). Plasma lipoproteins: apolipoprotein structure and function. *J. Lipid Res*. **25**, 1277-1294.

Mansur, A. P., Annicchino-Bizzacchi, J., Favarato, D., Avakian, S. D., Cesar, L. A. M., and *J. A. F. Ramires* (2000). Angiotensin-converting enzyme and apolipoprotein B polymorphism in coronary artery disease. *Am. J. Cardiol.* **85**, 1089-1093.

Monsalve, M. V., Young, R., Jobsis, J., Wiseman, S. A., Dhamu, S., Powell, J. T., Greenhalgh, R.M., and *S. E. Humphries* (1988). DNA polymorphisms of the gene for apolipoprotein B in patients with peripheral arterial disease. *Atherosclerosis* **70,** 123-129.

Moreel, J. F. R., Roizes, G., Evans, A. E., Arveiler, D., Cambou, J. P., Souriau, C., Parra, H. J., Desmarais, E., Fruchart, J. C., Ducimetiere, P., and *F. Cambien* (1992). The polymorphism ApoB/4311 in patients with myocardial infarction and controls: the ECTIM study. *Hum Genet.* **89**, 169-175.

Peacock, R., Dunning, A., Hamsten, A., Tornvall, P., Humphries, S., and *P. Talmud* (1992). Apolipoprotein B gene polymorphisms, lipoproteins and coronary atherosclerosis: a study of young myocardial infarction survivors and healthy population-based individuals. *Atherosclerosis* **92**, 151-164.

Peacock, R., Hamsten, A., Johansson, J., Nilsson-Ehle, P., and *S. Humphries* (1994). Association of genotypes at the apolipoprotein AI-CIII-AIV, apolipoprotein B and lipoprotein lipase gene loci with coronary atherosclerosis

and high density lipoprotein subclasses. *Clin. Genet.* **46**, 273-282.

Pearson, T. A., Blair, S. N., Daniels, S. R., Eckel, R. H., Fair, J. M., Fortmann, S. P., Franklin, B. A., Goldstein, L. B., Greenland, P., Grundy, S. M., Hong, Y., Miller, N. H., Lauer, R. M., Ockene, I. S., Sacco, R. L., Sallis, J. F. Jr., Smith, S. C. Jr., Stone, N. J., and *K. A. Taubert* (2002). AHA guidelines for primary prevention of cardiovascular disease and stroke: 2002 update: consensus panel guide to comprehensive risk reduction for adult patients without coronary or other atherosclerotic vascular diseases. American Heart Association Science Advisory and Coordinating Committee. *Circulation.* **16**, 388-391.

Pischon, T., Girman, C. J., Sacks, F. M., Rifai, N., Stampfer, M. J., and *E. B. Rimm* (2005). Non-high-density lipoprotein cholesterol and apolipoprotein B in the prediction of coronary heart disease in men. *Circulation*. **112**, 3375–3383.

Rajput-Williams, J., Wallis, S. C., Yarnell, J., Bell, G. I., Knott, T., Sweetnam, P., Cox, N., Miller, N. E, and *J. Scott* (1988). Variation of apolipoprotein-B gene is associated with obesity, high blood cholesterol levels, and increased risk of coronary heart disease. *Lancet* **2**,1442-1445.

Regis-Bailly, A., Visvikis, S., Steinmetz, J., Feldman, L., Briancon, S., Danchin, N., Zannad, F., and *G. Siest* (1996). Frequencies of five genetic polymorphisms in coronarographed patients and effect on lipid levels in a supposedly healthy population. *Clin. Genet.* **50**, 339-347.

Rengees, H. H., Peacock, R., Dunning, A. M., Talmud, P., and *S. E. Humphries* (1992). Genetic relationship between the 3'-VNTR and diallelic apolipoprotein B gene polymorphisms: haplotype analysis in individuals of European and South Asian origin. *Ann. Hum. Genet.* **56**, 11-33.

Salazar, L. A., Hirata, M. H., Giannini, S. D., Forti, N., Diament, J., Lima, T. M., and *R. D. C. Hirata* (2000). Seven DNA polymorphisms at the candidate genes of atherosclerosis in Brazilian women with angiographically documented coronary artery disease. *Clin. Chem. Acta* **300**,139-149.

Schulz, U. G., Flossmann, E., and *P. M. Rothwell* (2004). Heritability of ischemic stroke in relation to age, vascular risk factors, and subtypes of incident stroke in population-based studies. *Stroke* **35,** 819–824.

Sniderman, A. D., and *S. M. Marcovina* (2006). Apolipoprotein A1 and B. *Clin. Lab. Med.* **26**, 733-750.

Stepanov, V. A., Puzyrev, V. P., Karpov, R. S., and *A. I. Kutmin* (1998). Genetic markers in coronary artery disease in a Russian population. *Hum. Biol.* **70,** 47-57.

Wang, L., Gu, Y., and *G. Wu* (1999). The relation between polymorphisms of apolipoprotein B gene and atherosclerotic cerebral infarction. *Zhonghua. Yi. Xue. Za. Zhi.* **79**, 603-606.

Zateishchikov, D. A., Chumakova, O. S., Zateishchikova, A. A., Zotova, I. V., Minushkina, L. O., Chudakova, D. A., Nosikov, V. V., and *B. A.* (2004).. Genetic predictors of unfavorable course in high-risk patients with ischemic heart disease. Data of follow-up for two years. *Kardiologiya* **44**, 16-22.

ПОЛИМОРФИЗМИ ДНК У ГЕНУ ЗА АПОЛИПОПРОТЕИН Б КОД ПАЦИЈЕНАТА СА ИСХЕМИЈСКОМ БОЛЕШЋУ МОЗГА ИЗ СРБИЈЕ

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Концентрација аполипопротеина Б (аро В) у хуманој плазми представља најбољи липидни предиктор коронарних болести. Ген за аполипопротеин садржи велики број полиморфизама ДНК, којису до сада испитивани углавном у асоцијацији са нивоима липида и коронарним болестима. Циљ студије је био да се испита потенцијална асоцијација полиморфизама ДНК у гену за apoB (*Xba*I, *Eco*RI, *Msp*I, Ins/Del, 4311 A/G полиморфизми) са исхемијском болешћу мозга (IBM) у хуманој популацији из Србије. Узорак пацијената и контрола су одабирани по еквивалентним вредностима индекса телесне масе. Однос шанси за подложност IBM код носиоца генотипа X+X+ је био 2.22, 95% CI 1.24-3.96 (P<0.05), а код носиоца генотипа Ins/Ins 2.82, 95% CI 1.57-5.06 (P<0.05). Такође, пацијенти су имали значајно већу фреквенцију алела 4311A у односу на контроле (P<0.01). Резултати ове студије указују на значајан утицај полиморфизама ДНК у гену за аро В на повећање ризика за настанак исхемијске болести мозга.