Coll. Antropol. **30** (2006) 2: 387–394
Original scientific paper

Apolipoprotein E Polymorphism in Relation to Plasma Lipid Levels and Other Risk Factors of Atherosclerosis in Two Ethnic Groups from Slovakia

Daniela Siváková[1], Mária Zacharová[1], Juraj Gašparovič[2], Katarína Rašlová[2], Ladislava Wsólová[2], Zuzana Bašistová[1] and Pavel Blažíček[3]

[1] Department of Anthropology, Faculty of Sciences, Comenius University, Bratislava, Slovak Republic
[2] Institute of Preventive and Clinical Medicine, Slovak Medical University, Bratislava, Slovak Republic
[3] Metabolic Clinic, Military Hospital, Bratislava, Slovak Republic

A B S T R A C T

The influence of apolipoprotein E (ApoE) genotypes on plasma lipid levels and interaction with other environmental factors was determined in two Slovakian population samples; 146 Romany and 351 Slovak individuals. The two samples differ significantly in the distribution of E3/3 genotypes ($p<0.014$) and E3/2 ($p<0.035$). Analysis of variance did not reveal any significant effect of the ApoE genotypes on any of the plasma lipid levels in the Romany individuals. In the Slovak sample the variation in plasma low-density lipoprotein cholesterol (LDL-C) levels was significantly associated with the ApoE genotypes ($p=0.012$). We detected decreased LDL-C concentrations in males with E2 genotype when compared with E3 and E4 carriers ($p=0.008$). Further, the E2 genotype was found to be associated with high triglycerides levels ($p=0.009$). The ethnic samples differ significantly in the prevalence of metabolic syndrome and in the case of males of diabetes. Both the Romany and the Slovak males can be considered as having a more atherogenic profile compared with the females.

***Key words**: ApoE, lipids, risk factors, atherosclerosis, Slovakia*

Introduction

Apolipoprotein E (ApoE) plays a central role in lipid metabolism as a ligand for two cell-surface lipoprotein receptors, which mediate the cellular uptake of specific lipoproteins such as intermediate-density lipoprotein and chylomicron remnants[1]. The ApoE gene has three common alleles[2], ApoE*2, ApoE*3 and ApoE*4[2], the frequencies of which differ significantly among diverse ethnic groups[3–6]. Despite the wide variation observed in allele frequency distribution, ApoE*3 was found to be the most common allele. Intensive investigations have established that allele variation in the ApoE gene has a significant effect on interindividual variation in total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels and on risk of cardiovascular disease in the general population[7–9].

The Slovak population occupies the upper part of the cardiovascular mortality scale[10]. Although in the last few years there seems to be a slight decrease[11] it does not substantially change the Slovak position as compared to other countries with active atherosclerosis prevention programs. Thus, the study of factors that are involved in the aetiology of cardiovascular disease is very topical. Some studies dealing with genetic and environmental risk factor contributions to the development of coronary atherosclerosis in families with and without history of early myocardial infarction have already been conducted[12–15]. However, in the general population and the Romany, the second most numerous Slovak minority, data regarding genetic polymorphisms and their relationships with plasma lipid levels are scarce[16,17]. Although no offi-

Received for publication February 28, 2005

cial statistics exist, available data suggest that poor health status, morbidity and total premature mortality in the Romany are probably three times as prevalent as in the general Slovak population[18]. The majority of studies on genetic markers in different Romany samples from Slovakia showed that their gene pool differs significantly from the Slovak population and that gene frequencies of several genetic markers are close to some Indian population groups[19]. However, conclusive heterogeneity exists among different Romany groups in Slovakia as well as diversification on the European scale[20]. Keeping this in view, the aim of this article is to compare these two population samples with respect to distribution of ApoE genotypes and frequencies, to estimate if different ApoE genotypes could influence the plasma lipid and lipoprotein levels and to analyse the interaction with other variables associated with coronary risk factors.

Materials and Methods

Study subjects

Romany subjects were inhabitants of the village Zlaté Klasy with a large Roma community, situated close to the capital city Bratislava. At the time of data collection, of the total of 3,322 inhabitants, nearly 50 percent were Romany subjects. A sample of 150 adult volunteers (68 males and 82 females) was recruited and interviewed during medical examination. Trained anthropologists collected data. The sample is representative of the general Romany population with the inbreeding coefficient from genealogy equal to 0.00016.

Three hundred and fifty one Slovak subjects (174 men and 177 women), were participants of the CINDI (Countrywide Integrated Noncommunicable Diseases Intervention) survey from Banská Bystrica (Middle Slovakia)[21]. The collected data were provided for further processing and the ApoE polymorphism was detected chronologically at the same laboratory, by one of the co-authors. The data collection had similar design in both studies.

Anthropometric measurements (body weight and height, circumference of waist and hips) were taken along with medical examination. Body mass index (BMI) was calculated as body weight divided by height squared and waist to hip ratio (WHR) was calculated as the circumference of the waist divided by the circumference of hips.

Blood samples were collected in EDTA vacutainers. Plasma total cholesterol and triglycerides levels were determined using enzymatic methods, high-density lipoprotein cholesterol (HDL-C) was measured by dextran sulphate precipitation, and low density lipoprotein cholesterol (LDL-C) was calculated using the formula of Friedewald et al.[22]. The atherogenic indices considered were the ratio of TC/HDL-C and logarithm of ratio (TG/HDL-C)[23].

DNA was isolated from peripheral blood leukocytes by the standard phenol extraction technique. The ApoE genotypes were determined by polymerase chain reaction (PCR) using the following primers:

5´ – GGC CCA GGC CCG GCT GGG CGC GGA – 3´

5´ – CGG ATG GCG CTG AGG CCG CGC TCG – 3´

The amplified DNA was cleaved with *Hha*I followed by electrophoresis on a polyacrylamide gel[24].

Blood pressure was measured twice in the sitting position after 10 minutes of rest using a mercury sphygmomanometer. Subjects with hypertension were selected according to blood pressure (systolic >155 mmHg and/or diastolic >95 mmHg). Subjects with diabetes mellitus (DM) were selected according to level of glucose>7 mmol/L.

Metabolic syndrome (MS) defined by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III)[25], represents a clustering of risk factors and is diagnosed when three or more indicators are present. Inclusion criteria were the following: abdominal obesity: waist: >102 cm males, >88 cm females, hypertriglyceridemia: >1.7 mmol/L, HDL-C: males <1.0 mmol/L, females <1.3 mmol/L, blood pressure: ≥130/85 mmHg, fasting glycaemia: >6.1 mmol/L.

Education was classified into three categories, namely elementary, apprentice and higher education.

The normal distribution of examined parameters was tested by the Kolmogorov-Smirnov test and Shapiro-Wilk test. Differences between the quantitative parameters were compared by Student's *t* test or by Mann-Whitney test for parameters that showed non-normal distribution. Allele frequencies were tested for the Hardy-Weinberg equilibrium. Mean adjusted lipid levels were compared among ApoE genotype groups using analysis of variance (ANOVA) or Kruskal-Wallis tests. For this purpose subjects were grouped as ApoE2 carriers (ApoE 2/2 and ApoE 2/3 genotypes), ApoE3 carriers (ApoE 3/3 genotype) and ApoE4 carriers (ApoE 3/4 and ApoE 4/4 genotypes). The regression analysis was performed to estimate the effect of apoE alleles and other risk factors like age, sex, ethnicity, BMI, WHR and education on plasma lipid levels.

These analyses were performed using SPSS (version 13.0) for Windows.

The Ethics Committee of the Institute of Preventive and Clinical Medicine (Bratislava) approved the study.

Results and Discussion

Characteristics of the two population samples by sex are given in Table 1. Both males and females of these samples differ significantly in mean age. It is interesting to note that the Roma subjects, being younger than the Slovaks, are significantly shorter in their body height. The variables related to obesity (BMI and WHR indices) show very high average values in both groups and significant differences between the groups. A majority of the Slovak subjects, both males and females, were characterized by BMI as overweight, while most Roma subjects can be characterized as obese. In the Romany sample

TABLE 1
CHARACTERISTICS OF STUDY SUBJECTS BY SEX IN TWO SAMPLES FROM SLOVAKIA (EXPRESSED AS X AND 95% CONFIDENCE INTERVALS, N AND %)

Variable	Females					Males				
	Slovak N=177		Romany N=82		p	Slovak N=174		Romany N=68		p
Age (years)	46.2	(45.0–47.4)	39.9	(36.8-42.9)	***	47.7	(46.4–49.0)	41.3	(37.9–44.7)	**
Weight (kg)	70.6	(68.6–72.6)	75.3	(70.8–79.7)	ns	83.4	(81.6–85.2)	89.1	(84.8–93.5)	*
Height (cm)	164.0	(163.1–164.0)	159.5	(158.3–160.8)	***	175.1	(174.1–176.1)	171.9	(170.4–173.4)	**
BMI (kg/m^2)	26.3	(25.5–27.0)	29.6	(27.9–31.3)	**	27.2	(26.6–27.8)	30.1	(28.8–31.5)	***
WHR	80.2	(79.3–81.2)	86.2	(84.3–88.1)	***	89.8	(89.0–90.7)	96.5	(94.6–98.3)	***
SBP (mmHg)	129.3	(125.8–132.7)	124.3	(119.2–129.4)	ns	137.3	(134.0–140.7)	130.1	(124.6–135.6)	ns
DBP (mmHg)	82.7	(80.8–84.6)	76.7	(73.9–79.6)	**	87.4	(85.6–89.2)	81.4	(78.6–84.2)	**
Glucose (mmol/L)	5.3	(5.9–0.6)	5.3	(4.9–6.7)	ns	5.4	(5.2–5.5)	6.3	(5.6–7.0)	ns
TC (mmol/L)	5.9	(5.7–6.2)	4.8	(4.5–5.0)	***	5.8	(5.6–6.0)	5.1	(4.7–5.4)	***
LDL-C (mmol/L)	3.7	(3.5–3.8)	3.0	(2.8–3.1)	***	3.5	(3.4–3.7)	3.0	(2.8–3.2)	***
HDL-C (mmol/L)	1.5	(1.5–1.6)	1.1	(1.1–1.2)	***	1.3	(1.3–1.4)	1.0	(1.0–1.1)	***
TG (mmol/L)	1.6	(1.3–1.9)	1.5	(1.3–1.7)	ns	2.2	(1.9–2.5)	2.6	(1.8–3.4)	ns
log(TG/HDL-C)	–0.05	(–0.09–(–0.003))	0.08	(0.03–0.14)	**	0.11	(0.05–0.16)	0.26	(0.16–0.35)	**
TC/HDL	4.4	(3.7–5.0)	4.3	(4.1–4.6)	ns	4.7	(4.5–4.9)	5.2	(4.7–5.7)	*
MS	41	(23.3%)	28	(36.8%)	*	39	(23.1%)	30	(44.8%)	**
Hypertention	34	(19.2%)	14	(17.9%)	ns	49	(28.2%)	12	(17.6%)	ns
Diabetes Mellitus	10	(5.6%)	6	(7.3%)	ns	7	(4.0%)	15	(22.1%)	***
Education										
– elementary	17	(9.6%)	61	(78.2%)	***	10	(5.8%)	40	(58.8%)	***
– apprentice	129	(72.9%)	17	(21.8%)	***	122	(70.5%)	28	(41.2%)	***
– higher education	31	(17.5%)	0	0	***	41	(23.7%)	0	0	***

BMI – body mass index, WHR – waist to hip ratio, SBP – systolic blood pressure, DBP – diastolic blood pressure, TC – total cholesterol, LDL-C – low-density lipoprotein cholesterol, HDL-C – high-density lipoprotein cholesterol, TG – triglycerides, TC/HDL-C and log(TG/HDL-C) – atherogenic indices, MS – metabolic syndrome, *p<0.05, **p<0.01, ***p<0.001

52.7% of individuals were obese (BMI>30.0) vs. 22.1% of all Slovak persons. Both the Romany females and males differed significantly from the Slovaks in the average values of central obesity index (WHR). In 32% of Roma people vs. 5.5% of Slovaks the WHR index borders on a need for health care and suggests an androgynous type of fat distribution, which is more dangerous from the atherogenic point of view.

The mean values of diastolic blood pressure, TC, LDL-C and HDL-C were significantly higher in the Slovak females and males, respectively, than in the Romany individuals. The mean plasma atherogenic index (expressed as log TG/HDL-C) is significantly lower among the Slovak females and males than in the Roma individuals.

The mean value of another index of plasma lipids (TC/HDL-C) differs significantly only between males of the two groups, and is lower in Slovaks. Further, we have found that the prevalence of MS is significantly different between these ethnic samples and higher in the Slovak females and males than in the Romany subjects. The male series also differ in prevalence of DM (Romany males 22.05% vs. Slovak males 4.0%). No significant differences have been observed between the ethnic samples with respect to prevalence of hypertension.

The ethnic samples differ significantly in the level of education. The Romany had only elementary and apprentice education and differ significantly from the Slovaks, among whom 17.5% of females and 23.7% of males had higher education, in addition.

The observed values of BMI and WHR indices in the Romany subjects are much higher than those found in the Slovak patients who survived premature myocardial infarction[14]. The observed high mean values of both the obesity indices are not symptomatic only for the present study. Similar results were published in the study of adult population from Bratislava[26]. Among adult participants of the MONICA study from Middle Slovakia overweight by BMI was reported in 52.4% of females and 69.1% of males[27]. The only study from Slovakia[28], which examined lipid and non-lipid cardiovascular risk parameters in a young Gypsy population, showed significant prevalence of obesity among them (20% of Romany subjects had BMI>30.0 vs 8% of Slovak subjects). These young individuals had a significantly decreased HDL-C level and significantly higher values of TG and atherogenic index expressed as ratio TC/HDL-C than the young Slovaks. These findings are not in conformity with our results except for the decreased HDL-C level found in the Romany subjects. Particularly the atherogenic index (TC/HDL-C) in our study gives contradictory result. However, our Roma subjects had significantly higher values of another plasma parameter calculated as log(TG/HDL-C). This index was recently suggested, among the other new indicators of atherogenic risk[23], as a more precise measure of atherogenic profile of plasma examined in a large sample of Czech and Slovak subjects and compared with the French PRIME study and the Canadian HATS study.

ApoE allele and genotype frequencies observed in both samples are presented in Table 2. Hardy-Weinberg equilibrium can be assumed only for the Slovak sample (Slovak χ^2=6.48, df 3, Romany χ^2=39.88, df 3). The two samples do not differ significantly in the gene frequencies (p=0.181). Neither female nor male series are not significantly different in the gene frequencies (females p=0.720, males p=0.161)). However, these samples differ significantly in the distribution of particular genotypes (p=0.04). The same results were obtained when only the three most frequent genotypes in both samples (E3/2, E3/3, E4/3), were compared (χ^2=8.9, df 2, p=0.012). In the Romany sample significantly more homozygous E3/3 (p=0.035) and fewer E3/2 heterozygous genotypes (p=0.014) were observed than in the Slovak sample. In addition, we have tested the ratio of »protective« (E2/2, E2/3) and »atherogenic« (E4/4, E3/4) genotypes in each sample, respectively, but this did not show any significant difference.

TABLE 2
APOE ALLELE AND GENOTYPE FREQUENCIES IN TWO SAMPLES FROM SLOVAKIA

	Slovak sample N=351						Romany sample N=146					
Allele	Males		Females		All		Males		Females		All	
ApoE*2	0.0718		0.0678		0.0698		0.0303		0.0563		0.0445	
ApoE*3	0.8477		0.8616		0.8547		0.9091		0.8875		0.8973	
ApoE*4	0.0805		0.0706		0.0755		0.0606		0.0500		0.0582	
Genotype	N	%	N	%	N	%	N	%	N	%	N	%
E2/2	0	0	0	0	0	0	1	1.5	1	1.25	2	1.4
E3/2	23	13.2	18	10.2	41	11.7	1	1.5	7	8.75	8	5.5*
E3/3	125	71.8	135	76.3	260	74.1	57	86.4	66	82.5	123	84.2*
E4/2	2	0.6	6	3.4	8	2.3	1	1.5	0	0	1	0.68
E4/3	22	12.6	17	9.6	39	11.1	6	7.6	3	3.75	8	5.5
E4/4	2	0.6	1	0.5	3	0.85	1	1.5	3	3.75	4	2.7

*p<0.05

Deviation from the Hardy-Weinberg equilibrium in the Romany sample is difficult to explain. Genotyping error is unlikely, because the same method was used for detection and the same person detected the genotypes. In an inbred population, the low proportion of heterozygous may be due to inbreeding. However, the inbreeding coefficient estimated in this sample (0.00016) is one and two orders of magnitude lower than the values estimated in other highly endogamous and inbred Romany groups from Slovakia (e.g. in Podhorany, East Slovakia, F= 0.0065, and in the Walachian group, West Slovakia, F= 0.017)[29,30]. The value of F obtained in this sample falls within the range reported for large populations in Europe (range from 0.00001 to 0.00077 in the second half of the 20th century)[31]. Thus, it seems that it is sample size rather than consanguinity that is responsible for the observed deviation, for in our previous paper the same Romany sample was in genetic equilibrium with respect to another polymorphic system[17].

The Apo E allele frequencies in the Slovak sample are similar to those found in southern rather than central Europe. The allele E*4 in southern Europe ranges from 0.069 in Turkey to 0.091 in Spain, the allele E*3 from 0.856 in Spain to 0.876 in Greece. The allele E*2 is similar to the frequency in Turkey (0.067) or in Spain (0.065)[4,6,32]. On the other hand, in central Europe the frequency of the allele E*4 is generally over 10% and ranges from 0.107 in Switzerland to 0.163 in Belgium, while the allele E*3 ranges from 0.752 in Netherlands to 0.807 in Hungary. The allele E*2 attains the highest frequency in France (0.106) and the lowest in Finland (0.043)[6,33].

TABLE 3
LIPID AND LIPOPROTEIN LEVELS ACCORDING TO APOE GENOTYPE GROUPS IN THE SLOVAK MALES, FEMALES, TOTAL SLOVAK AND ROMANY SAMPLES (EXPRESSED AS X AND 95% CI)

Genotype Group	E2		E3		E4		p
Slovak Males	N=23		N=125		N=24		
TC (mmol/L)	5.4	(4.9–5.8)	5.9	(5.7–6.1)	5.8	(5.3–6.3)	0.230
HDL-C (mmol/L)	1.4	(1.2–1.6)	1.3	(1.2–1.4)	1.3	(1.2–1.5)	0.399
TG (mmol/L)	2.7	(1.2–3.6)	2.2	(1.9–2.5)	1.3	(1.0–1.6)	0.009
LDL-C (mmol/L)	2.9	(2.5–3.4)	3.6	(3.4–3.8)	3.9	(3.5–4.3)	0.008
TC/HDL-C	4.1	(3.5–4.6)	4.9	(4.5–5.1)	4.5	(4.0–5.0)	0.056
log(TG/HDL-C)	0.09	(–0.06–0.23)	0.14	(0.08–0.20)	–0.06	(–0.17–0.04)	0.024
Slovak Females	N=18		N=135		N=18		
TC (mmol/L)	6.4	(4.5–8.3)	5.9	(5.7–6.0)	6.1	(5.5–6.6)	0.451
HDL-C (mmol/L)	1.6	(1.4–1.8)	1.5	(1.5–1.6)	1.5	(1.3–1.7)	0.571
TG (mmol/L)	2.6	(–0.2–5.4)	1.5	(1.3–1.6)	1.8	(1.4–2.2)	0.130
LDL-C (mmol/L)	3.7	(2.8–4.6)	3.7	(3.5–3.8)	3.7	(3.3–4.2)	0.567
TC/HDL-C	3.6	(3.1–4.1)	4.5	(3.6–5.4)	4.3	(3.5–5.0)	0.322
log(TG/HDL-C)	–0.14	(–0.27–(–0.01))	–0.05	(–0.10–(–0.00))	0.02	(–0.13–0.18)	0.252
All Slovaks	N=41		N=260		N=42		
TC (mmol/L)	5.9	(5.0–6.7)	5.9	(5.7–6.0)	5.9	(5.6–6.3)	0.165
HDL-C (mmol/L)	1.5	(1.4–1.7)	1.4	(1.4–1.5)	1.4	(1.3–1.5)	0.551
TG (mmol/L)	2.5	(1.2–3.8)	1.8	(1.7–2.0)	1.5	(1.2–1.7)	0.405
LDL-C (mmol/L)	3.3	(2.8–3.7)	3.6	(3.5–3.8)	3.8	(3.5–4.1)	0.012
TC/HDL-C	3.9	(3.5–4.2)	4.7	(4.2–5.1)	4.4	(4.0–4.8)	0.077
log(TG/HDL-C)	–0.01	(–0.11–0.09)	0.04	(–0.00–0.08)	–0.03	(–0.11–0.06)	0.391
All Romany	N=10		N=123		N=12		
TC (mmol/L)	4.9	(3.5–6.3)	4.9	(4.7–5.1)	5.0	(4.6–5.5)	0.627
HDL-C (mmol/L)	1.0	(0.9–1.2)	1.0	(1.0–1.1)	1.04	(1.0–1.1)	0.401
TG (mmol/L)	3.6	(–1.1–8.4)	1.9	(1.6–2.2)	2.2	(1.3–3.0)	0.419
LDL-C (mmol/L)	2.6	(1.8–3.3)	3.0	(2.8–3.1)	3.2	(2.8–3.6)	0.258
TC/HDL-C	5.6	(2.2–8.9)	4.6	(4.4–4.9)	4.9	(4.2–5.6)	0.507
log(TG/HDL-C)	0.27	(–0.11–0.65)	0.14	(0.09–0.20)	0.26	(0.08–0.43)	0.390

TC – total cholesterol, LDL-C – low-density lipoprotein cholesterol, HDL-C – high-density lipoprotein cholesterol, TG – triglycerides, TC/HDL-C and log(TG/HDL-C) – atherogenic indices

TABLE 4
REGRESSION ANALYSES OF THE SELECTED CONFOUNDER EFFECTS ON LIPID LEVELS IN ROMANY AND SLOVAK STUDY SUBJECTS FROM SLOVAKIA

Dependent Variable	Independent Variable	Coefficient	95% CI	p
TC	Age	0.029	(0.017–0.042)	<0.001
	Sex	0.209	(–0.018–0.435)	0.071
	Ethnicity	1.419	(0.921–1.916)	<0.001
	ApoE	0.017	(–0.166–0.199)	0.859
	BMI	0.014	(–0.011–0.040)	0.270
	WHR	0.027	(0.019–0.034)	<0.001
	Education	0.054	(–0.183–0.292)	0.655
LDL-C	Age	0.019	(0.008–0.029)	<0.001
	Sex	0.186	(0.009–0.362)	0.039
	Ethnicity	0.670	(0.286–1.054)	0.001
	ApoE	0.020	(–0.122–0.162)	0.784
	BMI	0.017	(–0.003–0.37)	0.094
	WHR	0.015	(0.009–0.021)	<0.001
	Education	0.028	(–0.157–0.212)	0.768
HDL-C	Age	0.001	(–0.003–0.004)	0.768
	Sex	0.093	(0.014–0.172)	0.021
	Ethnicity	–1.080	(–1.626–(–0.534))	<0.001
	ApoE	–0.001	(–0.050–0.049)	0.983
	BMI	–0.013	(–0.020–(–0.006))	<0.001
	WHR	–0.009	(–0.016–(–0.003))	0.005
	Education	–0.006	(–0.072–0.060)	0.858
TG	Age	0.024	(0.004–0.043)	0.016
	Sex	–0.635	(–0.982–(–0.288))	<0.001
	Ethnicity	0.508	(–0.254–1.269)	0.191
	ApoE	0.054	(–0.225–0.334)	0.703
	BMI	0.032	(–0.008–0.071)	0.113
	WHR	0.004	(–0.007–0.015)	0.464
	Education	–0.025	(–0.389–0.338)	0.891

TC – total cholesterol, LDL-C – low-density lipoprotein cholesterol, HDL-C – high-density lipoprotein cholesterol, TG – triglycerides, BMI – body mass index, WHR – waist to hip ratio

The allele frequencies in the Romany sample are similar to those reported e.g. from Sardinia (E*2=0.050, E*3=0.898, E*4=0.052)[4] and from different Indian groups for which relatively low frequency of the alleles ApoE*4 and ApoE*2, respectively (E*2=0.030–0.070, E*4=0.055–0.080), and high frequency of the allele ApoE*3 are characteristic (E*3=0.875–0.889)[6,33]. However, our Romany sample differs remarkably from the frequencies recorded in Hungarian Gypsy children (ApoE*2=0.089, ApoE*3=0.738 and ApoE*4=0.173)[34].

The impact of ApoE genotype groups on quantitative levels of lipids in the Slovak males, females and the total Slovak and Romany samples is shown in Table 3. Since no significant differences were observed in any plasma lipids between genotype groups in the Romany males and females, only results for the total sample are presented. However, even in the total sample no significant association has been revealed, though the Romany E2 carriers showed lower LDL-C and TC levels than the E3 and E4 subjects. In the total Slovak sample only the value related to LDL-C appeared significant among the three-genotype groups (p=0.012). Particularly the Slovak males contributed to this result since the E2 carriers presented the lowest values and the E4 carriers the greatest values of LDL-C (p=0.008). In this case also TG levels showed association with apoE genotypes (p=0.009); the E4 carriers have the lowest values and the E2 genotype group the highest values of plasma TG. In males the three-genotype groups differ significantly (p=0.024) in the value of log(TG/HDL-C).

The associations between the allele E*2 and decreased LDL-C and TC levels and increased HDL-C level and

between the allele*4 and increased total-C and LDL-C levels have been determined in many studies independently of ethnic group[3,35].

In our study we did not confirm such association among the Roma subjects though the tendency to lower LDL-C in the E2 carrier was suggested. A remarkably different effect of apoE genotypes on serum lipid parameters was revealed in the Hungarian Gypsy children[34]. They had significantly lower TC, LDL-C and TG levels in the E3 genotype group than in the E2 and E4 groups. Only the HDL-C levels did not differ significantly among the genotype groups, as in our Romany sample. In the Slovak sample a significant effect of apoE genotypes on lipid levels was revealed only in males and the total sample. An interaction of sex with the effects of apoE polymorphism was recorded also in Spain – in women it influenced plasma cholesterol, LDL and HDL levels, whereas in men polymorphism was associated with variations in TG and VLDL lipid levels[36].

Differences between the sexes, as in our sample, have also been found in the Czech population where the ApoE*4 allele was associated with higher TC and LDL-C levels only in males[37]. On the other hand, in a Mexican population this effect was observed only in female carriers of the E3/4 genotype[38].

No association between the ApoE genotypes and indices of obesity (BMI and WHR), were revealed either in the Slovak or in the Romany subjects (data not shown).

The results on regression analysis of selected confounders with lipid and lipoprotein levels in the Romany and Slovak study subjects are in Table 4. Significant association was recorded with total cholesterol and age ($p<0.001$), ethnicity ($p<0.001$) and WHR ($p<0.001$). Similarly the level of LDL-C interacts with age ($p<0.001$), ethnicity ($p=0.001$), WHR ($p<0.001$) and in addition with sex ($p=0.039$). Significant positive association was recorded between HDL-C and sex ($p=0.021$), and negative with ethnicity ($p<0.001$), BMI ($p<0.001$) and WHR ($p=0.005$). The level of TG is associated significantly with age ($p=0.016$) and sex ($p<0.001$).

Because in the analysis the variable sex indicates male as (1) and female as (2) the result suggests that females had higher LDL-C and HDL-C than males and TG are higher by males (–0.635). Interaction with ethnicity (Slovak=1, Roma=2) shows that Roma subjects had higher levels of TC and LDL-C and lower HDL-C. High level of TC is influenced by high value of WHR index, while the high level of HDL-C is influenced by lower values of WHR and BMI indices.

The regression analysis showed no significant association of ApoE genotypes and level of education with lipids and lipoproteins.

In conclusion, our data suggested differences between ethnic samples in genetic variation at the ApoE locus. We did not determine an effect of ApoE polymorphism on lipid levels in the Romany subjects. In view of conventional risk factors for cardiovascular disease the studied subjects possess a different risk factor profile. The Roma subjects have risky HDL-C and obesity markers (BMI and WHR), and the Slovaks LDL-C and blood pressure. The studied samples differ in predisposition to the insulin resistance status, too. However, to detect the effective role of any single genetic factor in determining the variable or disease prevalence in a population, will depend partly on the gene's frequency and partly on the gene's interactions with other genetic and environmental factors. Because this is the first epidemiological study among Roma people in Slovakia dealing with relation of lipid profile and ApoE genotypes, further studies on risk factors could be clarified on a larger sample and through the analysis of different apolipoprotein loci[39,40] that are thought to be involved in susceptibility to cardiovascular disease as suggested by other authors.

REFERENCES

1. MAHLEY, R. W., Science, 240 (1988) 622. — 2. UTERMANN, G., M. HEES, A. STEINMETZ, Nature, 269 (1977) 604. — 3. HALLMAN, D. M., E. BOERWINKLE, N. SAHA, C. SANDHOLZER, H. J. MENZEL, A. CZARAR, G. UTERMANN, Am. J. Hum. Genet., 49 (1991) 338. — 4. CORBO, R. M., R. SCACCHI, L. MUREDDU, G. MULAS, S. CASTRECHINI, P. RIVASI, Hum. Biol., 71 (1999) 933. — 5. GARCÉS, C., M. CANTOS, M. BENAVENTE, J. J. GRANIZO, B. CANO, E. VITURRO, M. DE OYA, Hum. Biol., 76 (2004) 615. — 6. SINGH, P., M. SINGH, U. GERDES, S. S. MASTANA, Anthrop. Anz., 59 (2001) 27. — 7. DAVIGNON, J., R. E. GREGG, C. F. SING, Atherosclerosis, 8 (1988) 1. — 8. RALL, S. C. JR., R. W. MAHLEY, J. Intern. Med., 231 (1992) 633. — 9. ORDOVAS, J. M., L. A. CUPPLES, P. W. WILSON, C. LAHOZ, D. LEVY, J. D. OTVOS, J. R. MCNAMARA, E. GAGNE, M. HAYDEN, E. SHAEFER, Atherosclerosis, 11 (1998) 425. — 10. WHO: World Health Statistic Annual. (Geneva, 1995). — 11. EGNEROVÁ, A., A. BEZEČNÁ, Lek Obz, 46 (1997) 1. — 12. RAŠLOVÁ, K., B. SMOLKOVÁ, J. FROHLICH, Atherosclerosis, 134 (1997) 67. — 13. RAŠLOVÁ, K., A. BEDEROVÁ, J. GAŠPAROVIČ, P. BLAŽÍČEK, B. SMOLKOVÁ, Physiol. Res., 49 (2000) 651. — 14. RAŠLOVÁ, K., B. SMOLKOVÁ, B. VOHNOUT, J. GAŠPAROVIČ, J. FROHLICH, Metabolism, 50 (2001) 24. — 15. DOBIÁŠOVÁ, M., K. RAŠLOVÁ, K. RAUCHOVÁ, B. VOHNOUT, K. PTÁČKOVÁ, J. FROLICH, Physiol. Res., 50 (2001) 1. — 16. ZACHAROVÁ, M., K. RAŠLOVÁ, J. KRAJČOVIČ, B. VOHNOUT, M. AVDIČOVÁ, Bull. Slov. antropol. Spoloč., 3 (2000) 80. — 17. GAŠPAROVIČ, J., K. RAŠLOVÁ, Z. BAŠISTOVÁ, M. ZACHAROVÁ, L. WSÓLOVÁ, M. AVDIČOVÁ, P. BLAŽÍČEK, J. LIETAVA, D. SIVÁKOVÁ, Physiol. Res., 53 (2004) 218. — 18. GINTER, E., M. KRAJČOVIČOVÁ-KUDLÁČKOVÁ, O. KAČALA, V. KOVAČIC, M. VALACHOVIČOVÁ, Bratisl. Lek Listy, 102 (2001) 479. — 19. BERNASOVSKÝ, I., J. BERNASOVSKÁ: Anthropology of Romanies (Gypsies). (NAUMA and Universitas Masarykiana, Brno, 1999). — 20. MASTANA, S. S., S. S., PAPIHA, Z. Morph. Anthrop., 79 (1992) 43. — 21. AVDIČOVÁ, M., A. EGNEROVÁ, F. HRUBÁ: Evaluation of the CINDI program in Slovakia. (State Institute of Public Health, Banská Bystrica, 2000). — 22. FRIEDEWALD, W. T., R. L. LEVY, D. S. FREDRICKSON, Clin. Chem., 18 (1972) 499. — 23. DOBIÁŠOVÁ, M., J. FROHLICH, G. LUC, A. MYDLILOVÁ, J. PITHA, R. POLEDNE, K. RAŠLOVÁ, M. ŠAMÁNEK, Z. URBANOVÁ, Cardiol., 11 (2002) K/C 3A. — 24. HIXSON, J. E., D. T. VERNIER, J. Lipid. Res., 31 (1990) 545. — 25. RAŠLOVÁ, K., S. FILIPOVÁ, Z. MIKEŠ, I. TKÁČ, J. TURAY, Interná Medicína, 3 (2003) 10. — 26. GEROVÁ, Z., I. PANÁKOVÁ, M. MATUŠKOVÁ, Bratisl Lek Listy, 100 (1999) 231. — 27. ZACHAROVÁ, M., K. RAŠLOVÁ, M. AVDIČOVÁ, D. Siváková, Bull. Slov. Antropol. Spoloč., 5 (2002) 136. — 28. KRAJČOVIČOVÁ-KUDLÁČKOVÁ, M., P. BLAŽÍČEK, V. SPUSTOVÁ, M. VALACHOVIČOVÁ, E. GINTER, Bratisl Lek Listy, 105 (2004) 256. — 29. CHLEBOVSKÁ, K., I. BERNASOV-

SKÝ, J. BERNASOVSKÁ, Bull. Slov. Antropol. Spoloč., 1 (1998) 49. — 30. FERÁK, V., D. SIVÁKOVÁ, Z. SIEGLOVÁ, Bratisl Lek Listy, 87 (1987) 168. — 31. VOGEL, F., A. G. MOTULSKY: Human Genetics. (Springer-Verlag, Berlin, Heidelberg, New York, 1979). — 32. AL-BUSTAN, S. A., M. A. ALNAQEEB, B. C. ANNICE, G. IBRHIM, J. AL-RUBAIAN, A. H. AHMED, T. M. REFAI, Hum. Biol., 77 (2005) 487. — 33. THELMA, B. K., R. C. JUYAL, H. H. DODGE, R. PANDAV, V. CHANDRA, M. GANGULI, Hum. Biol., 73, (2001) 135. — 34. SZALAI, C., A. CZINNER, A. CSÁSZÁR, Eur. J. Pediatrics, 159 (2000) 257. — 35. LARSON, I. A., J. M. ORDOVAS, C. DE LUCA, Atherosclerosis, 148 (2000) 327. — 36. GOMEZ-CORONADO, D., J. J. LVAREZ, A. ENTRALA, Atherosclerosis, 147 (1999) 67. — 37. HUBÁČEK, J. A., D. M. WATERWORTH, R. POLEDNE, J. PITHA, Z. ŠKODOVÁ, S. E. HUMPHRIES, P. J. TALMUD, Clin. Biochem., 34 (2001) 113. — 38. GAMBOA, R., G. VARGAS-ALARCÓN, A. MEDINA-URRUTIA, G. CARDOSO-SALDANA, G. HERNÁDEZ-PACHECO, J. ZAMORA-GONZÁLES, C. POSADAS-ROMERO, Hum. Biol., 73 (2001) 835. — 39. CALO, C. M., L. VARESI, M. MEMMI, P. MORAL, G. VONNA, Coll. Antropol., 27 (2003) 105. — 40. AGARWAL, D. P., I. J. H. G., 1 (2001) 233.

D. Siváková

Department of Anthropology, Faculty of Sciences, Comenius University, Mlynská dolina B2, 842 15 Bratislava 4, Slovak Republic
e-mail: sivakova@fns.uniba.sk

POLIMORFIZAM APOLIPOPROTEINA E U ODNOSU NA RAZINU LIPIDA U PLAZMI I DRUGE FAKTORE RIZIKA ZA ATEROSKLEROZU U DVIJE ETNIČKE SKUPINE IZ SLOVAČKE

S A Ž E T A K

Utjecaj genotipova apolipoproteina E (apoE) na lipide u plazmi te interakcija s drugim okolišnim faktorima određeni su u dvije slovačke populacije; 146 Roma i 351 Slovaka. Ove dvije populacije značajno se razlikuju u raspodjeli genotipova E3/3 ($p<0.014$) i E3/2 ($p<0.035$). Analiza varijance nije pokazala nikakav značajni učinak ApoE genotipova na razinu bilo kojeg lipida u plazmi u romskoj populaciji. U populaciji Slovaka varijacije u razini kolesterola lipoproteina niske gustoće (LDL-C) značajno su povezane s ApoE genotipovima ($p=0.012$). Uočili smo smanjenu koncentraciju LDL-C kod muškaraca s E2 genotipom u usporedbi s nositeljima E3 i E4 genotipova ($p=0.008$). Nadalje, utvrđena je povezanost E2 genotipa s povišenom razinom triglicerida u plazmi ($p=0.009$). Dvije etničke skupine značajno se razlikuju u prevalenciji metaboličkog sindroma i dijabetesa kod muškaraca. U obje skupine, i kod Roma i kod Slovaka, muškarci pokazuju veću sklonost aterosklerozi u usporedbi sa ženama.