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Distribution of Apolipoprotein E (APOE) Genotypes in a Siberian Female Population Sample

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ABSTRACT Variations at the apolipoprotein E (APOE) locus influence lipid and lipoprotein levels in the normal population, and are associated with premature coronary artery disease. In the present study, the APOE genotypes and allele frequencies in 299 women from Novosibirsk (West Siberia) were determined. APOE genotypes were detected by restriction isotyping after amplification of the genomic DNA by PCR. APOE*3 was found to be the predominant allele (allele frequency = 0.769). The frequency of APOE*2 and APOE*4 alleles were found to be 0.048 and 0.182, respectively. The results are compared with those reported for major human populations of the world. Moreover, genotypic relationship with quantitative lipid levels (total cholesterol, high-density lipoprotein cholesterol, and triglycerides) were examined. Subjects with APOE*2 allele showed much lower total choletsterol and HDL values as compared to APOE*4 allele carriers supporting the notion that E*4 allele is a susceptibility factor for cardiovascular diseases.

INTRODUCTION

Apolipoprotein E (APOE) is a constituent of the plasma-circulating chylomicrons, chylomicron remnants, liver-derived very-low-density lipoprotein (VLDL), intermediate-density lipoproteins (IDL) and high-density lipoprotein (HDL). APOE plays an important regulatory role in lipid metabolism through its ability to bind to LDL receptors. Our appreciation of its role continues to expand as additional aspects of its function are discovered. APOE affects the levels of all lipoproteins, either directly or indirectly by modulating their receptor-mediated clearance or lipolytic processing and the production of hepatic very low density lipoproteins (Mahley and Huang 1999). The APOE gene is 3.6 kb long, contains four exons and three introns and maps on chromosome 19q13.2 (Zannis et al. 1993). APOE gene is polymorphic and exists in six different isoprotein forms, designated E2/E2, E2/E3, E3/E3, E2/E4, E3/E4 and E4/E4 which are the gene products of three APOE alleles, E*2, E*3 and E*4, respectively (Utermann et al. 1977; Bouthillier et al. 1983). E3/E3 is the most common of these isoforms, and is distinguished by cysteine at position 112 (112 cys) and arginine at position 158 (158 arg) in the receptor-binding region of APOE (Weisgraber et al. 1981). The E4/E4 isoform (112 arg and 158 arg) is associated with increased levels of total cholesterol and betalipoprotein (Boerwinkle et al. 1987; Boerwinkle and Utermann, 1988), and increased susceptibility to heart disease (Davignon et al. 1988; Yamamura et al. 1992; Eicher et al. 1993; Lehtinen et al. 1995; Sheehan et al. 2000). Many recent studies have shown that APOE gene locus influences not only the levels of certain lipoprotein variables during young adulthood, but also modulates the association between obesity and dyslipidemias (Srinivasan et al. 2001). Population screening studies around the world indicate a wide range in the frequency distribution of APOE alleles (Table 1).

In the present study we report on the distribution of APOE genotypes in a female Siberian

Table 1: Distribution of various APOE alleles (frequency range) among major human populations*

Population Group	*E*2*	*E*3*	*E*4*
Europeans	0.044-0.119	0.64-0.898	0.052-0.310
Africans	0.031-0.116	0.536-0.850	0.085-0.407
Asians	0.02-0.140	0.620-0.870	0.070-0.240
Native Americans	0.0-0.014	0.720-0.911	0.089-0.280
Oceanians	0.0-0.110	0.486-0.740	0.260-0.360

*Modified from Carbo and Scacchi (1999).

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Table 2: Restriction fragments for the identification of different APOE genotypes

Genotype	*E2/E2*	*E2/E3*	*E3/E3*	*E2/E4*	*E3/E4*	*E4/E4*	*Undigested*
Fragment size							223-227
	91	91	91	91	91	-	
	83	83	-	83	-	-	
	-	-	-	72	72	72	
	-	48	48	48	48	48	
	-	35	35	35	35	35	
	38	38	38	38	38	38	
	-	-	-	19**	19**	19**	

** = too small to be detected usually

population sample from Novosibirsk.Frequency distribution of the three major alleles is compared with that reported for the major world populations. Moreover, the mean average plasma lipid values were correlated with various APOE genotypes.

MATERIALS AND METHODS

The study was carried out on blood samples collected under the WHO multinational program MONICA („Monitoring of Trend and Determinants in Cardiovascular Diseases„). lt is a larger project that includes an investigation of cardiovascular disorders, diabetes mellitus, anemias, alcohol consumption and other medical anomalies. A total of 875 women (age = 25-65 yrs). were investigated, using confidential interviews with the help of questionnaires and medical examinations. Venous blood was obtained for biochemical and genetic analysis from 299 females. DNA samples were prepared from clotted blood using conventional methods (treatment with proteinase K, phenol-chloroform extraction and ethanol precipitation). For DNA amplification the following primers (5'-CTG GGC GCG GAC ATG GAG GAC GT -3') and (5'-GAT GGC GCT GAG GCC GCG CTC G-3') were used as forward and reverse, respectively. Twenty five microliters of the PCR reaction mixture contained 0.5 µl of 10 mM solution of dNTP, 2.5 µl of PCR buffer, 0.5 µl of 100 pM solution of each primer, 0.8 µl of 50 mM $MgC1_2$, 13 µl H_20, 1 µl DMSO, 5 µml solution Q, 1 µl DNA and 1 unit of Taq polymerase. Amplification was performed in the following mode: 1 cycle: 4 min at 95^0C, 2 min at 65^0C; 35 cycles: 30 sec at 95^0C, 50 sec at 65^0C, 10 sec at 72^0C; 1 cycle: 10 min at 72^0C.

Genotyping was carried out according to the method of Hixon and Vernier (1990) as described before (Benkmann et al. 1996). Eight microliters of PCR product was digested with the restriction enzyme HhaI (CfoI) for ovenight. Restriction sites: E2: position 112 (Cys) and position 158 (Cys): no HhaI restriction site; E3: position 112 (Cys) and position 158 (Cys→ Arg): one HhaI restriction site; E4: position 112 (Cys→ Arg) and position 158 (Cys→ Arg): two HhaI restriction sites. The DNA fragments were separated in 8% polyacrylamide gel and the DNA bands were visualized by silver staining. The size and number of the restriction fragments obtained for various APOE genotypes are shown in table 2.

RESULTS AND DISCUSSION

The distribution of APOE genotypes and allele frequency in the Novosibirsk sample are presented in table 3.

Table 3: Distribution of APOE genotypes and allele frequency in Siberia (n=299)

Genotype	*Genotype frequency (%)*	*Allele frequency*		
E2/E2	0.33	**E2**	**E3**	**E4**
E2/E3	7.69	0.048	0.769	0.182
E3/E3	56.19			
E2/E4	1.34			
E3/E4	33.78			
E4/E4	0.67			

Only scarce data are available for the distribution of APOE allele frequencies in Russian populations (Kamboh et al. 1996; Skobeleva et al. 1997). To our knowledge the present study is the first of its kind reporting the distribution of APOE alleles in a white female population of West Siberia. Population genetic studies have revealed that APOE*3 is the most frequent allele among all human populations (Hallman et al. 1991; Sandholzer et al. 1995; Benkmann et al. 1996;

Beranek et al. 1999; Corbo and Scacchi, 1999; Panza et al. 1999; de Andrade et al. 2000; Gamboa et al. 2000; Schiele et al. 2000). In our present study we found the prevalence of E*2 allele as 0.048, E*3 allele as 0.769 and E*4 allele as 0.182. These allele frequencies are very similar to those reported for other North European populations (see Table 1).

The WHO multinational MONICA project has been established to measure trends in cardiovascular mortality and coronary heart disease and cerebrovascular disease morbidity and to assess the extent to which these trends are related to changes in the known risk factors at the same time in defined communities in different countries. A relatively high rate of mortality, morbidity and lethality due to cardiovascular diseases has been reported for Russia (Stegmayr et al. 2000), and in particular for the inhabitants of Novosibirsk (Feigin et al. 1996; Gafarov 2000; Gafarov and Gagulin 2000). Thus, it was of great interest to look into the distribution of APOE genotypes in a representative Siberian population sample and correlate the effect of APOE polymorphism on serum levels of cholesterol, HDL-cholesterol and triglycerides. The lipid values in different genotypic groups of APOE gene are presented in table 4.

Table 4: Total cholesterol, HDL and triglyceride values in different APOE genotypes (n=299)

Genotype	*Total cholesterol*	*HDL-cholesterol*	*Triglyceride*
E2/E2	121.0 ±	49.0 ±	239 ±
E2/E3	169.2 ± 51.8	61.4 ± 12.4	79.2 ± 30.1
E3/E3	197.7 ± 44.6	57.9 ± 12.5	105.4 ± 53.9
E2/E4	194.3 ± 55.1	45.8 ± 7.6	156.0 ± 77.8
E3/E4	205.4 ± 46.4	55.4 ± 13.7	109.9 ± 57.3
E4/E4	190.5 ± 17.7	63.5 ± 4.9	79.0 ± 7.1

The values are given in mg/dl

Indeed, subjects with APOE*2 allele showed lower mean average total cholesterol and HDL values as compared to homozygous and heterozygous APOE*4 allele carriers supporting the notion that APOE*4 reflects a genetic susceptibility factor for cardiovascular diseases. Thus, both environmental and genetic factors are associated with cardiovascular disease risk.

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