Table 1. Characteristics of patients with CAD and controls

Characteristics	CAD group	Controls	P
Number	166	130	n.s.
Male sex	(90)[b]	(82)	n.s.
Age (years)	51 ± 7[a]	52 ± 7	0.13
BMI (kg/m^2)	26.9 ± 2.9	27.1 ± 3.4	n.s.
Arterial hypertension	66 (40)	46 (35)	n.s.
Diabetes mellitus	16 (10)	5 (4)	<0.05
Smoking habit	108 (65)	45 (35)	<0.001
Total cholesterol (mmol/l)	6.5 ± 1.6	6.4 ± 1.7	n.s.
HDL cholesterol (mmol/l)	1.1 ± 0.4	1.2 ± 0.5	<0.001
LDL cholesterol (mmol/l)	4.4 ± 1.5	4.1 ± 1.6	n.s.
Triglycerides (mmol/l)	2.3 ± 1.6	2.1 ± 1.5	n.s.

[a]Mean ± SD
[b]The percent values are given in parentheses.

Table 2. ApoE allele frequencies in patients with CAD and in the control group

Phenotype	CAD		Control group	
	number	%	number	%
e2e2	0	0	0	0
e2e3	9.6	16.0	15.4	20.0
e2e4	3.0	5.0	0	0
e3e3	71.7	119.0	70	91.0
e3e4	15.7	26.0	13.8	18.0
e4e4	0	0.0	0.8	1.0
Total	100.0	166.0	100	130.0
Allele				
e2	6.3	7.7		
e3	84.3	84.6		
e4	9.4	7.7		

genotypes was found in lipid parameters (total cholesterol 6.19 ± 1.39 mmol/l vs. 6.29 ± 1.49 mmol/l, P = 0.65; LDL cholesterol 4.04 ± 1.31 mmol/l vs. 4.14 ± 1.62 mmol/l, P = 0.67; HDL cholesterol 1.13 ± 0.57 mmol/l vs. 1.10 ± 0.44 mmol/l, P = 0.73; triglyceride levels 2.27 ± 1.19 mmol/l vs. 2.36 ± 1.24 mmol/l, P = 0.67). When we wanted to assess the effect of smoking in subjects with the GG genotype only, there was a difference in the LDL cholesterol level, but not in other lipid parameters (total cholesterol 6.21 ± 1.38 mmol/l vs. 6.63 ± 1.68 mmol/l, P = 0.08; LDL cholesterol 4.06 ± 1.31 mmol/l vs. 4.53 ± 1.47 mmol/l, P = 0.03; HDL cholesterol 1.15 ± 0.58 mmol/l vs. 1.19 ± 0.45 mmol/l, P = 0.57; triglyceride levels 2.24 ± 1.19 mmol/l vs. 2.14 ± 1.81 mmol/l, P = 0.68).

Univariate analysis revealed no association between e3e4 plus e4e4 genotypes of apoE gene polymorphism and CAD risk (OR = 1.1; 95% CI = 0.6–2.1, P = 0.8) (Table 4) and no association between the apoA1 gene promoter polymorphism (GG genotype) and CAD risk (OR = 0.7; 95% CI = 0.5–1.2, P = 0.19) (Table 4). No evidence for a synergistic interaction between e3e4 plus e4e4 genotypes and apoA1-GG genotype on a risk of CAD was found (OR = 1.3, 95% CI = 0.6–2.9; P = 0.5) (Table 4).

One individual with familial defective apoB100 (Arg3500Gln) was found in each group. No homozygote was found in either group. Univariate analysis revealed no association between the familial defective apoB100 and CAD risk (OR = 0.8, 95% CI = 0.5–12.8; P = 0.87).

Discussion

In this study a low frequency of the e4 allele was noted (9.4% in the study group and 7.4% in the control group). This is the first report on apoE allele frequencies in Slovenia. It is interesting that a similar prevalence of the e4 allele was found in some Middle European countries, such as Italy, Austria and Slovakia (Gabelli et al., 1990;

Table 3. Plasma lipids (mean ± SD, mmol/l) of subjects (patients and controls) with apoE risk genotypes (e3e4+e4e4) compared with other genotypes

	e3e4+e4e4 genotypes	e2e2+e2e3+e3e3+e2e4	P
Total cholesterol	6.96 ± 1.50[a]	6.38 ± 1.58	0.023
LDL cholesterol	4.70 ± 1.14	4.20 ± 1.49	0.040
HDL cholesterol	1.24 ± 0.70	1.15 ± 0.45	0.300
Triglycerides	2.50 ± 2.20	2.20 ± 1.34	0.200

[a]Mean ± SD, mmol/l

Table 4. Distribution of risk genotypes in each group

Variable	CAD (%)	Controls (%)	P	RR(95% CI)[a]
e3e4+e4e4 genotype[b]	26 (15.7)[c]	19 (14.6)	0.8	1.1 (0.6 – 2.1)
GG genotype[d]	89 (53.6)	76 (61.3)	0.19	0.7 (0.5 – 1.2)
e3e4+e4e4 genotype[b] + GG genotype[d]	18 (10.8)	11 (8.5)	0.5	1.3 (0.6 – 2.9)

[a]risk ratio (95 % confidence interval)
[b]apolipoprotein E gene polymorphism
[c]percent value in parentheses
[d]apolipoprotein A-1 gene promoter polymorphism

Hallman et al., 1991; Raslova et al., 1998). In this study we found no statistically significant differences in apoE allele frequencies between the cases and controls. The frequency of the e4 allele varies among nations; it is 4.9% in the Chinese population, 39% in Aboriginal Australians, whereas in Europe it is distributed along a decreasing north/south gradient (Van Bockxmeer, 1994; Kao et al. 1995; Siest et al., 1995). Some have suggested that the differences in the e4 allele frequency among populations could explain the different prevalence rates of cardiovascular diseases (Siest et al., 1995). It is interesting that Orientals have a low incidence of CAD and a low prevalence of the e4 allele, whereas Aboriginal Australians have a high incidence of CAD and high prevalence of the e4 allele (Van Bockxmeer, 1994). According to the national statistics, cardiovascular diseases are the main cause of mortality in Slovenia (Moravec-Berger and Turk, 1993), which is not consistent with the low prevalence of the e4 allele in Slovenia. Very likely, the discrepancy is due to the multifactorial nature of CAD.

The patients and control subjects with e3e4 and e4e4 genotypes had higher total and LDL cholesterol levels than the subjects with other apoE genotypes, which is consistent with some other studies (Lehtinen et al., 1995; Gylling et al., 1997). The patients and control subjects with the GG genotype of the apoA1 gene promoter polymorphism did not have lower serum HDL cholesterol levels than those with AG or AA genotypes, and the polymorphism did not affect the total cholesterol, LDL cholesterol, and triglyceride levels. Our results are in accordance with the findings of Smith et al. (1992) and Civeira et al. (1993), who noted no effect of this polymorphism on the HDL cholesterol level. The lower serum HDL cholesterol level in patients than in the control group was most probably not due to the apoA1 gene promoter polymorphism, but might be due to other factors, such as smoking. When we wanted to assess the effect of genotypes in smokers only, no difference between the GG genotype and AG or AA genotypes was found in the HDL cholesterol level as well as in other lipid parameters. When we wanted to assess the effect of smoking in subjects with the GG genotype only, there was no difference in the HDL cholesterol level.

In spite of the effect of the apoE phenotype on lipid parameters (total and LDL cholesterol levels), univariate analysis did not show e3e4 and e4e4 genotypes to be risk factors for CAD in this study. Similarly, the apoA1-GG genotype was not associated with CAD. No evidence for a synergistic interaction between the apoE gene polymorphism (e3e4 plus e4e4 genotypes) and GG genotype of the apoA1 gene promoter polymorphism on the CAD risk was found (OR = 1.3, 95% CI = 0.6–2.9; P = 0.5).

One individual with familial defective apoB100 (Arg3500Gln) was found in each group. In a larger study on the Danish population, this mutation was associated with a sevenfold increased risk for CAD (Tybjaerg-Hansen et al., 1998).

We may conclude that the apoE gene polymorphism affects the total and LDL cholesterol levels, whereas neither the apoE gene polymorphism nor apoA1 gene promoter polymorphism have been shown to be independent risk factors for CAD in Slovenia.

Acknowledgements

The authors thank Ms. Mojca Pirc for revising the English text.

References

Boerwinkle, E., Ellsworth, D. L., Hallman, D. M., Biddinger, A. (1996) Genetic analysis of atherosclerosis: a research paradigm for common chronic diseases. *Hum. Mol. Genet.* **5,** 1405-1410.

Brown, M. S., Goldstein, J. L. (1986) A receptor-mediated pathway for cholesterol homeostasis. *Science* **232,** 34-47.

Civeira, F., Pocovi, M., Cenarro, A., Garces, C., Ordovas, J. M. (1993) Adenine for guanine substitution – 78 base pairs 5' to the apolipoprotein A-I gene: relation with high density lipoprotein cholesterol and apolipoprotein A-I concentration. *Clin. Genet.* **44,** 307-312.

Castelli, W. P. (1983) Cardiovascular disease and multifactorial risk: challenge of the 1980s. *Am. Heart. J.* **106,** 1191-1200.

Chetlin, M. D., Sokolow, M., McIlroy, M. B. (1993) Coronary heart disease. In: *Clinical Cardiology,* eds. Chetlin, M. D., Sokolow, M., McIlroy, M. B., pp. 147-246, Appleton&Lange, East Norwalk.

Eto, M., Watanabe, K., Makino, I. (1992) Increased frequency of apoplipoprotein e2 and e4 alleles in patients with ischemic heart disease. *Clin. Genet.* **36,** 183-188.

Gabelli, C., Martini, S., Barbato, G. M. et al. (1990) Allelic distribution of apolipoprotein E isoforms in Northern Italy (Abstract). International Symposium on Lipoprotein Metabolism and Lipid-lowering Agents, The Hague.