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Apolipoprotein E and Apolipoprotein CI Polymorphisms in the Czech Population: Almost Complete Linkage Disequilibrium of the Less Frequent Alleles of Both Polymorphisms

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Summary

Apolipoproteins E and CI are the predominant components of triglyceride-rich lipoproteins. The genes are located in one gene cluster and both are polymorphic. Three allelic (*ε2*, *ε3* and *ε4*) polymorphisms of the *APOE* gene influence plasma cholesterol levels. The distribution of these alleles differ between ethnic groups. PCR genotyping was used to determine the *APOE* and *APOCI* allele incidence in a representative group of 653 probands (302 men and 351 women) of Czech origin. The observed relative frequencies for the *ε2*, *ε3* and *ε4* alleles were 7.1 %, 82.0 % and 10.9 %, respectively, and are similar to other middle European populations. *APO ε4* carriers have the highest and *APO ε2* carriers the lowest levels of plasma total cholesterol ($p<0.0001$) and LDL cholesterol ($p<0.0001$). The frequency of the insertion (I) allele (HpaI restriction site present) of the *APOCI* polymorphism was 18.5 %. *APOCI* I/I homozygotes have the highest level of triglycerides ($p<0.003$). An almost complete linkage disequilibrium of the insertion allele of *APOCI* with the *APOE* alleles *ε2* and *ε4* has been detected and suggests that the deletion in the *APOCI* gene probably follows the deriving of all three *APOE* alleles on the *APO ε3* allele background.

Key words

Apolipoprotein E • Apolipoprotein CI • Genetic polymorphism • Czech population • Cholesterol • Triglycerides

Introduction

The genes for apolipoprotein E *(APOE)* and apolipoprotein CI *(APOCI)* are localized in the *APOE-CI-CII* gene cluster on chromosome 19 (Scott *et al.* 1985). The frequencies of common alleles (*ε2* [arg 158 → cys], *ε3* and *ε4* [cys 112 → arg]) of *APOE* gene vary among different populations; however, the *ε3* allele is the most common (Gerdes *et al.* 1992). Numerous rare mutations in the *APOE* gene have been described (for review see Hubáček *et al.* 2000, 2002). Apo E is the surface protein of chylomicrons, very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL). Apo E serves as a ligand for binding of lipoproteins to the lipoprotein

receptors (LDL receptor, VLDL receptor and LDL receptor-related protein), and is thus important in triglyceride-rich particle catabolism. A number of studies (reviewed by Davignon *et al.* 1988, Dallongeville 1994) demonstrated that the *ε4* allele is associated with increased levels and the *ε2* allele with decreased levels of plasma cholesterol. The *ε4* allele has also been shown to be a risk factor for myocardial infarction (reviewed by Davignon *et al.* 1988, Dallongeville 1994, Wilson *et al.* 1996).

Apo CI occurs in triglyceride-rich lipoproteins and inhibits the binding of beta VLDL and intermediate density lipoproteins (IDL) to the lipoprotein receptors (Sehayek and Eisenberg 1991, Swaney and Weisgraber 1994). The Hpa I polymorphism of *APOCI* gene has been previously described (Klasen *et al.* 1987, Smit *et al.* 1988). The role of this polymorphism in hyperlipidemia type III (Klasen *et al.* 1987) and in determination of plasma levels of remnant particles (Waterworth *et al.* 2000) has been suggested. The presence of the Hpa I restriction site in the *APOCI* promoter is due to a CGTT insertion at position -317, which has a negative effect on *APOCI* transcription (Xu *et al.* 1999).

The *APO ε4* allele and the *APOCI* insertion allele have been described as risk factors for Alzheimer´s disease (Duara *et al.* 1996, Poduslo *et al.* 1998). Since both polymorphisms have also been described as risk factors for type III hyperlipidemia (manifest predominantly in *APO ε2/ε2* homozygotes; but just about 5 % of the *APO ε2/ε2* homozygotes develop hyperlipidemia of type III) (Klasen *et al.* 1987), it is of special interest whether these two polymorphisms have an allelic association in the general population.

Methods

We determined the *APOE* and *APOCI* polymorphisms in 653 Czechs (302 males and 351 females, a 1 % representative selected population sample according to the protocol of the "MONICA project" [1983]). The sample of 287 probands (136 men and 151 women, respondence 70 %) represented an 8-year cohort selected in 1988 from the Central Region of Bohemia (Benešov) (Hubáček *et al.* 2001a). The Northern Region of Bohemia (Litoměřice) was represented by 366 probands (166 men and 200 women, respondence 65.2 %) (Hubáček *et al.* 1999). Written informed consent was obtained from the study participants and the local ethic committee approved the design of the study.

DNA was isolated by a standard method from EDTA blood (Miller *et al.* 1988). Analysis of genetic polymorphisms was performed using PCR and restriction analysis (Hixson and Vernier 1990, Nillesen *et al.* 1990).

The lipoprotein parameters were measured enzymatically by the WHO Regional Lipid Reference Center in Prague, on a Roche COBAS MIRA autoanalyzer, using enzymatic methods with reagents from Boehringer-Mannheim Diagnostics and Hoffmann-La Roche as described elsewhere (Škodová *et al.* 1993). LDL was calculated by the Friedewald's formula.

Table 1. Frequencies of the *APOE* genotypes in two Czech districts

	Central Bohemia						**Northern Bohemia**					
APOE	**Population**		**Males**		**Females**		**Population**		**Males**		**Females**	
	N	%	N	%	N	%	N	%	N	%	N	%
2/2	0	0	0	0	0	0	3	0.8	1	0.6	2	1.0
3/2	38	13.3	13	9.6	25	16.6	37	10.1	15	9.0	22	11.0
3/3	197	68.6	91	66.9	106	70.2	247	67.5	111	66.9	136	68.0
4/3	42	14.6	26	19.1	16	10.6	66	18.0	30	18.1	36	18.0
4/4	4	1.4	2	1,5	2	1.3	7	1.9	7	4.2	0	0
4/2	6	2.1	4	2.9	2	1.3	6	1.7	2	1.2	4	2

The observed numbers of genotypes were compared to the expected frequencies (Hardy-Weinberg equilibrium) by the chi-square test. Triglycerides were logarithmically transformed before the analysis. For the *APOE* polymorphism analysis, the subjects were divided into three groups, carriers of the *ε2* allele (*ε2/ε2*, *ε3/ε2*),

ε3/ε3 homozygotes, and carriers of the *ε4* allele (*ε4/ε4* and *ε4/ε3*). Subjects with the *ε4/ε2* genotype (n = 12) were excluded from statistical analysis. Statistical analysis was performed using the ANOVA and a test for linear association was selected for analysis of lipid parameters.

Table 2. Lipid parameters and *APOE* polymorphism in the Czech population. *APO ε4* carriers have the highest and *APO ε2* carriers the lowest levels of plasma total cholesterol and LDL cholesterol.

	Population			
APOE	**N**	**Total cholesterol***	**LDL cholesterol***	**Triglyceride**
ε2/ε2 + ε3/ε2	78	5.17 ± 1.27	2.88 ± 0.88	2.01 ± 2.01
ε3/ε3	444	5.72 ± 1.17	3.55 ± 1.02	1.90 ± 1.49
ε4/ε3 + ε4/ε4	119	5.92 ± 1.03	3.78 ± 1.01	1.90 ± 1.27
	Males			
APOE	**N**	**Total cholesterol***	**LDL cholesterol***	**Triglyceride**
ε2/ε2 + ε3/ε2	29	5.21 ± 1.45	2.83 ± 0.72	2.59 ± 2.98
ε3/ε3	202	5.65 ± 1.11	3.52 ± 0.96	2.21 ± 1.84
ε4/ε3 + ε4/ε4	65	6.01 ± 0.96	3.87 ± 0.92	2.06 ± 1.50
	Females			
APOE	**N**	**Total cholesterol***	**LDL cholesterol***	**Triglyceride**
ε2/ε2 + ε3/ε2	49	5.15 ± 1.17	2.91 ± 0.96	1.67 ± 1.00
ε3/ε3	242	5.77 ± 1.21	3.57 ± 1.07	1.64 ± 1.06
ε4/ε3 + ε4/ε4	54	5.81 ± 1.11	3.69 ± 1.11	1.72 ± 1.01

*Values are given in mmol/l as means ± S.D. * $p<0.0001$*

Results and Discussion

The frequencies of *APOE* and *APOCI* genotypes are in the Hardy-Weinberg equilibrium. The *APOE* and *APOCI* genotype incidence did not differ between both districts (see Table 1 for *APOE* polymorphism). In the pooled sample, males had a slightly higher frequency of the *APO ε4* allele compared to the females (13.2 % vers. 8.8 %, $p<0.05$). The difference was observed predominantly in the Central Region of Bohemia, and is probably caused by chance. The genotype frequencies of the *APOE* and *APOCI* polymorphisms in the Czech population (Tables 1 and 3) are similar to the frequencies of those that have been reported for other middle European populations (Gerdes *et al.* 1992, Duara *et al.* 1996, Xu *et al.* 1999). We have previously suggested the gender specific influence of the *APOE* polymorphism on plasma lipids (Hubáček *et al.* 2001b). In the overall Czech population, no sex specific effect of the *APOE* polymorphism on plasma lipids has been detected. The usual association between lipid parameters and *APOE* polymorphism had been described. *APO ε4* carriers have the highest and *APO ε2* carriers the lowest levels of plasma total cholesterol ($p<0.0001$) and LDL cholesterol ($p<0.0001$), but they do not differ in triglycerides levels (Table 2). Similar associations have been observed, if males and females were analyzed separately.

No associations have been detected between the total or LDL cholesterol levels and the *APOCI* polymorphism (Table 3), but in a pooled population sample I/I homozygotes have the highest level of triglycerides ($p<0.003$).

We have found an almost absolute association (linkage disequilibrium) between the *APOCI* insertion allele and both *APO ε4* (98 %) and *APO ε2* alleles (95 %) in the Czech European population (Table 4). It was

previously reported that the association between the *APO ε4* allele and the *APOCI* I allele was lower both in Caucasian (about 85 %) and African (about 60 %) populations (Xu *et al.* 1999, Seixas *et al.* 1999). The almost absolute association between the *APOE* and *APOCI* alleles must be taken into account in any study on the effects of these two polymorphisms on lipid metabolism. This very strong linkage disequilibrium almost excludes the insertion (H2) allele of the *APOCI* gene as a separate or additional risk factor for familial dyslipidemia of type III or for Alzheimer's disease, but the real (if any) role of this polymorphism in type III dyslipidemia and Alzheimer's disease needs to be further evaluated.

Table 3. HpaI (I/D) polymorphism of the *APOCI* gene and lipid parameters in the Czech population: *APOCI* I/I homozygotes have the highest level of triglycerides.

APOCI	N	%	Total cholesterol	LDL cholesterol	Triglyceride*
D/D	436	66.8	5.74 ± 1.19	3.56 ± 1.02	1.89 ± 1.45
D/I	193	29.6	5.58 ± 1.17	3.42 ± 1.06	1.92 ± 1.67
I/I	24	3.6	5.75 ± 1.30	3.33 ± 1.26	2.69 ± 1.62

*Values are given in mmol/l as means ± S.D. * p<0.003*

Table 4. Linkage disequilibrium between the *APOE* and *APOCI* genotypes in the Czech population.

APOE			ε2/ε2	ε3/ε2	ε3/ε3	ε4/ε3	ε4/ε4	ε4/ε2
		N	3	75	444	108	11	12
	D/D	436	0	0	434	2	0	0
APOCI	D/I	193	0	74	10	105	0	4
	I/I	24	3	1	0	1	11	8

It has been reported (Hanlon and Rubinsztein 1995, Seixas *et al.* 1999) that *ε4* allele in the *APO* gene, is the ancestral one and *ε3* and *ε2* were derived by two following mutations at amino acid positions 112 and 158. Using the results from complete haplotype analysis of the *APOE* gene (Fullerton *et al.* 2000), we suggest that the deletion in the *APOCI* gene probably follows after splitting of all three *APOE* alleles on *APO* ε*3* background. Thus the deletion in the *APOCI* gene occurred at least 200 000 years ago. Additional detailed haplotype analysis of the *APOE-APOCI-APOCII* gene cluster is necessary for explaining the exact origin and age of the *APOCI* deletion allele.

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Reprint requests

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