

# 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 ( $\epsilon 2$ ,  $\epsilon 3$  and  $\epsilon 4$ ) 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  $\epsilon 2$ ,  $\epsilon 3$  and  $\epsilon 4$  alleles were 7.1 %, 82.0 % and 10.9 %, respectively, and are similar to other middle European populations. *APOE*  $\epsilon 4$  carriers have the highest and *APOE*  $\epsilon 2$  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  $\epsilon 2$  and  $\epsilon 4$  has been detected and suggests that the deletion in the *APOCI* gene probably follows the deriving of all three *APOE* alleles on the *APOE*  $\epsilon 3$  allele background.

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## 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 ( $\epsilon 2$  [arg 158  $\rightarrow$  cys],  $\epsilon 3$  and  $\epsilon 4$  [cys 112  $\rightarrow$  arg]) of *APOE* gene vary among

different populations; however, the  $\epsilon 3$  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  $\epsilon 4$  allele is associated with increased levels and the  $\epsilon 2$  allele with decreased levels of plasma cholesterol. The  $\epsilon 4$  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*  $\epsilon 4$  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*  $\epsilon 2/\epsilon 2$  homozygotes; but just about 5 % of the *APO*  $\epsilon 2/\epsilon 2$  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, response 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, response 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

<i>APOE</i>	Central Bohemia						Northern Bohemia					
	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  $\epsilon 2$  allele ( $\epsilon 2/\epsilon 2$ ,  $\epsilon 3/\epsilon 2$ ),

$\epsilon 3/\epsilon 3$  homozygotes, and carriers of the  $\epsilon 4$  allele ( $\epsilon 4/\epsilon 4$  and  $\epsilon 4/\epsilon 3$ ). Subjects with the  $\epsilon 4/\epsilon 2$  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*  $\epsilon 4$  carriers have the highest and *APO*  $\epsilon 2$  carriers the lowest levels of plasma total cholesterol and LDL cholesterol.

Population				
<i>APOE</i>	N	Total cholesterol*	LDL cholesterol*	Triglyceride
$\epsilon 2/\epsilon 2 + \epsilon 3/\epsilon 2$	78	$5.17 \pm 1.27$	$2.88 \pm 0.88$	$2.01 \pm 2.01$
$\epsilon 3/\epsilon 3$	444	$5.72 \pm 1.17$	$3.55 \pm 1.02$	$1.90 \pm 1.49$
$\epsilon 4/\epsilon 3 + \epsilon 4/\epsilon 4$	119	$5.92 \pm 1.03$	$3.78 \pm 1.01$	$1.90 \pm 1.27$
Males				
<i>APOE</i>	N	Total cholesterol*	LDL cholesterol*	Triglyceride
$\epsilon 2/\epsilon 2 + \epsilon 3/\epsilon 2$	29	$5.21 \pm 1.45$	$2.83 \pm 0.72$	$2.59 \pm 2.98$
$\epsilon 3/\epsilon 3$	202	$5.65 \pm 1.11$	$3.52 \pm 0.96$	$2.21 \pm 1.84$
$\epsilon 4/\epsilon 3 + \epsilon 4/\epsilon 4$	65	$6.01 \pm 0.96$	$3.87 \pm 0.92$	$2.06 \pm 1.50$
Females				
<i>APOE</i>	N	Total cholesterol*	LDL cholesterol*	Triglyceride
$\epsilon 2/\epsilon 2 + \epsilon 3/\epsilon 2$	49	$5.15 \pm 1.17$	$2.91 \pm 0.96$	$1.67 \pm 1.00$
$\epsilon 3/\epsilon 3$	242	$5.77 \pm 1.21$	$3.57 \pm 1.07$	$1.64 \pm 1.06$
$\epsilon 4/\epsilon 3 + \epsilon 4/\epsilon 4$	54	$5.81 \pm 1.11$	$3.69 \pm 1.11$	$1.72 \pm 1.01$

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

## Results and Discussion

The frequencies of *APOE* and *APOC1* genotypes are in the Hardy-Weinberg equilibrium. The *APOE* and *APOC1* 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*  $\epsilon 4$  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 *APOC1* 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*  $\epsilon 4$  carriers have the highest and *APO*  $\epsilon 2$  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 *APOC1* 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 *APOC1* insertion allele and both *APO*  $\epsilon 4$  (98 %) and *APO*  $\epsilon 2$  alleles (95 %) in the Czech European population (Table 4). It was

previously reported that the association between the *APO*  $\epsilon 4$  allele and the *APOC1* 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 *APOC1* 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 *APOC1* 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 *APOC1* gene and lipid parameters in the Czech population: *APOC1* I/I homozygotes have the highest level of triglycerides.

APOC1	N	%	Total cholesterol	LDL cholesterol	Triglyceride*
D/D	436	66.8	5.74 $\pm$ 1.19	3.56 $\pm$ 1.02	1.89 $\pm$ 1.45
D/I	193	29.6	5.58 $\pm$ 1.17	3.42 $\pm$ 1.06	1.92 $\pm$ 1.67
I/I	24	3.6	5.75 $\pm$ 1.30	3.33 $\pm$ 1.26	2.69 $\pm$ 1.62

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

**Table 4.** Linkage disequilibrium between the *APOE* and *APOC1* genotypes in the Czech population.

APOE		$\epsilon 2/\epsilon 2$	$\epsilon 3/\epsilon 2$	$\epsilon 3/\epsilon 3$	$\epsilon 4/\epsilon 3$	$\epsilon 4/\epsilon 4$	$\epsilon 4/\epsilon 2$
<i>APOC1</i>	N	3	75	444	108	11	12
	D/D	436	0	0	434	2	0
	D/I	193	0	74	10	105	4
	I/I	24	3	1	0	1	8

It has been reported (Hanlon and Rubinsztein 1995, Seixas *et al.* 1999) that  $\epsilon 4$  allele in the *APO* gene, is the ancestral one and  $\epsilon 3$  and  $\epsilon 2$  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 *APOC1* gene probably follows after splitting of all three *APOE* alleles on *APO*  $\epsilon 3$  background. Thus the deletion in the *APOC1* gene

occurred at least 200 000 years ago. Additional detailed haplotype analysis of the *APOE-APOC1-APOC2* gene cluster is necessary for explaining the exact origin and age of the *APOC1* deletion allele.

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### References

- DALLONGEVILLE J: Apolipoprotein E polymorphism and atherosclerosis risk. In: *Genetic Factors in Coronary Heart Disease*. U GOLDBOURT, U DE FAIRE, K BERG (eds), Kluwer Academic Publishers, Dordrecht, Boston, London, 1994, pp 289-298.
- DAVIGNON J, GREGG RE, SING CF: Apolipoprotein E and atherosclerosis. *Atherosclerosis* **8**: 1-21, 1988.
- DUARA R, BARKER WW, LOPEZ-ALBEROLA G, LOEWENSTEIN DA, GRAU LB, GILCHRIST D, SERUSH S, ST GEORGE-HYSLOP S: Alzheimer's disease: interaction of apolipoprotein E genotype, family history of dementia, gender, education, ethnicity, and age of onset. *Neurology* **46**: 1575-1579, 1996.

- FULLERTON SM, CLARK AG, WEISS KM, NICKERSON DA, TAYLOR SL, STENGARD JH, SALOMAA V, VARTIAINEN E, PAROLA M, BOERWINKLE E, SING CF: Apolipoprotein E variation at the sequence haplotype level: implications for the origin and maintenance of a major human polymorphism. *Am J Hum Genet* **67**: 881-900, 2000.
- GERDES LU, KLAUSEN IC, SIHN I, FAERGEMAN O: Apolipoprotein E polymorphism in a Danish population compared to findings in 45 other study populations around the world. *Genet Epidemiol* **9**: 155-167, 1992.
- HANLON CS, RUBINSZTEIN DC: Arginine residues at codons 112 and 158 in the apolipoprotein E gene correspond to the ancestral state in humans. *Atherosclerosis* **112**: 85-90, 1995.
- HIXSON JE, VERNIER DT: Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res* **31**: 545-548, 1990.
- HUBÁČEK JA, PÍTHA J, ADÁMKOVÁ V, ŠKODOVÁ Z, LÁNSKÁ V, POLEDNE R: Insertion deletion polymorphism in the angiotensin converting enzyme gene in the Czech population. *Cas Lek Cesk* **138**: 596-598, 1999.
- HUBÁČEK JA, PÍTHA J, STÁVEK P, SCHMITZ G, POLEDNE R: Variable expression of hypercholesterolemia in apolipoprotein E2\* (Arg136→Cys) heterozygotes. *Physiol Res* **49**: 307-317, 2000.
- HUBÁČEK JA, WATERWORTH DM, POLEDNE R, PÍTHA J, ŠKODOVÁ Z, HUMPHRIES SE, TALMUD PJ: Genetic determination of plasma lipids and insulin in the Czech population. *Clin Biochem* **34**: 113-118, 2001a.
- HUBÁČEK JA, WATERWORTH DM, PÍTHA J, HUMPHRIES SE, TALMUD PJ, POLEDNE R: Polymorphisms in the lipoprotein lipase and hepatic lipase genes and plasma lipid values in the Czech population. *Physiol Res* **50**: 345-351, 2001b.
- HUBÁČEK JA, PÍTHA J, ŠKODOVÁ Z, POLEDNE R: Rare variant of apolipoprotein E2\* (Arg136→Cys) in a subject with normal lipid values. *Physiol Res* **51**: 107-108, 2002.
- KLASEN EC, TALMUD PJ, HAVEKES L, DE WIT E, VAN DER KOOIJ-MEIJIS E, SMIT M, HANSSON G, HUMPHRIES SE: A common restriction fragment length polymorphism of the human apolipoprotein E gene and its relationship to type III hyperlipidemia. *Hum Genet* **75**: 244-247, 1987.
- MILLER SA, DYKES DD, POLESKY HF: A simple salting out procedure for extraction DNA from human nucleated cells. *Nucleic Acid Res* **16**: 1215, 1988.
- MONICA Project: Multinational monitoring of trends and determinants in cardiovascular diseases. *Manual of operations WHO/MNC* 82.2, Nov. 1983.
- NILLESEN WM, SMEETS HJM, VAN OOST BA: Human apo CI Hpa I restriction site polymorphism revealed by the polymerase chain reaction. *Nucleic Acid Res* **11**: 3428, 1990.
- PODUSLO SE, NEAL M, HERRING K, SHELLY J: The apolipoprotein CI allele as a risk for Alzheimer's disease. *Neurochem Res* **23**: 361-367, 1998.
- SCOTT J, KNOTT TJ, SHAW DJ, BROOK JD: Localization of genes encoding apolipoproteins CI, CII, and E to the p13-cen region of human chromosome 19. *Hum Genet* **71**: 144-146, 1985.
- SEHAYEK E, EISENBERG S: Mechanism of inhibition by apolipoprotein C of apolipoprotein E-dependent cellular metabolism of human triglyceride rich lipoproteins through the low density lipoprotein receptor pathway. *J Biol Chem* **266**: 18259-18267, 1991.
- SEIXAS S, TROVOADA MJ, ROCHA J: Haplotype analysis of the apolipoprotein E and apolipoprotein CI Loci in Portugal and Sao Tome e Principe (Gulf of Guinea): Linkage disequilibrium evidence that APOE\*4 is the ancestral APOE allele. *Human Biol* **71**: 1001-1007, 1999.
- ŠKODOVÁ Z, PÍŠA Z, PIKHARTOVÁ J, CÍCHA Z, VOJTÍŠEK P, EMROVÁ R, BERKA R, HOKE M, WIESNER E, VALENTA Z, PACLT M: Development of the cardiovascular risk in the population of the Czech Republic. *Cor Vasa* **35**: 178-182, 1993.
- SMIT M, VAN DER KOOIJ-MEIJIS E, WOUTDT LP, HAVEKES LM, FRANTS RR: Exact localization of the familial dysbetalipoproteinemia associated Hpa I restriction site in the promoter region of the APOCI gene. *Biochem Biophys Res Commun* **152**: 1282-1288, 1988.
- SWANEY JB, WEISGRABER KH: Effect of apolipoprotein C-I peptides on the apolipoprotein E content and receptor-binding properties of beta-migrating very low density lipoproteins. *J Lipid Res* **35**: 134-142, 1994.

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- WATERWORTH DM, HUBÁČEK JA, PÍTHA J, KOVÁŘ J, POLEDNE R, HUMPHRIES SE, TALMUD PJ: Plasma levels of remnant particles are determined in part by variation in the APOC3 gene insulin responsive element and the APOC1-APOE cluster. *J Lipid Res* **41**: 1103-1109, 2000.
- WILSON PWF, SCHAEFER EJ, LARSON MG, ORDOVAS JM: Apolipoprotein E alleles and risk of coronary disease: A meta analysis. *Arterioscler Thromb Vasc Biol* **16**: 1250-1255, 1996.
- XU Y, BERGLUND L, RAMAKRISHNAN R, MAYEUX R, NGAI C, HOLEVAN S, TYCKO B, LEFF T, SHACHTER NS: A common Hpa I RFLP of apolipoprotein C-I increases gene transcription and exhibits an ethnically distinct pattern of linkage disequilibrium with the alleles of apolipoprotein E. *J Lipid Res* **40**: 50-58, 1999.
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**Reprint requests**

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