

SHORT COMMUNICATION

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## Rare Variant of Apolipoprotein E (Arg136→Ser) in Two Normolipidemic Individuals

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### Summary

Through the analysis of the common apolipoprotein (apo) E gene polymorphism in large Caucasian population study with the PCR and subsequent restriction analysis, we have identified carriers of mutant allele Arg136→Ser. Both of them (71-years-old female and her 43-years-old son) have normal lipid parameters. We suggest that Arg136→Ser mutation in apoE is not necessarily connected with elevated lipid levels in all cases. Furthermore, so far unidentified factors (environmental and/or genetic) are important for the development of lipid metabolism disorders in apoE Arg136→Ser mutation carriers.

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### Key words

Apolipoprotein E • Rare mutation • Lipid metabolism

Apolipoprotein E (apoE) gene (OMIM database +107741) determines three common variants - apoE2 (Arg158→Cys), apoE3 and apoE4 (Cys112→Arg). Although the frequency of apoE alleles varies among different populations, the E3 allele is invariably dominant (Davignon *et al.* 1988, Gerdes *et al.* 1992). A high number of rare variants has been described (Hubáček *et al.* 2000).

ApoE plays an important role in the metabolism of triacylglycerol-rich lipoproteins and is described as an important determinant of serum cholesterol level. Carriers of the allele E4 have a higher level of plasma low density lipoproteins (LDL) cholesterol and carriers of the allele E2 have a lower level of LDL cholesterol compared to carriers of the common 3/3 genotype (Davignon *et al.*

1988). Roughly 5 % of apoE2/E2 homozygotes suffer from hyperlipoproteinemia type III.

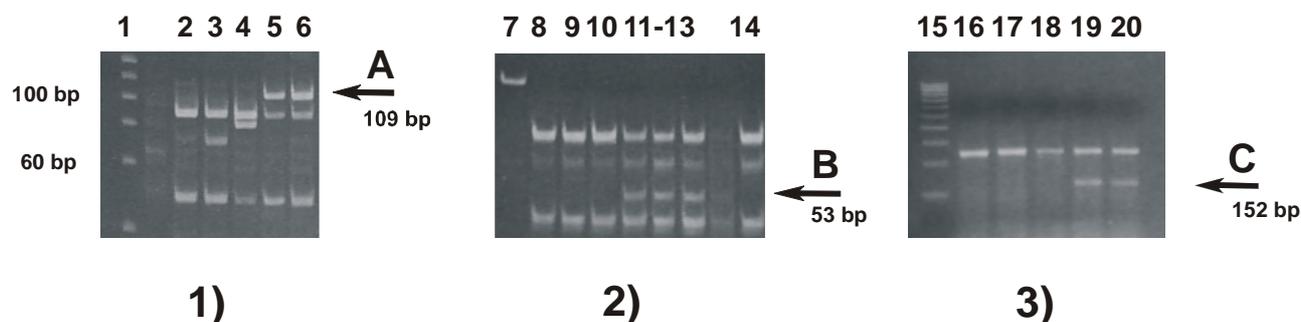
We have screened common apoE polymorphism in the Caucasian population (more than 4000 individuals analyzed) (partially published in Hubáček *et al.* 2003) with the PCR and restriction analysis with restriction enzyme CfoI (Hixson and Vernier 1990). This leads to detection of an uncommon restriction fragment of the size 109 bp, originated from the loss of CfoI restriction site in the apoE gene, in one proband.

By the PCR directed mutagenesis and restriction analysis (Hubáček *et al.* 2002), the allele Cys136 was excluded. Through the additional restriction of the same PCR product with enzyme HphI (Pocovi *et al.* 1996), a carrier of the apoE2\* allele C3817→A (Arg136→Ser)

has been detected. The second allele was the common apoE3 variant. The son of the proband has the same genotype as his mother (Fig. 1).

The proband was postmenopausal, past-smoker woman aged 71 years, with normal BMI (25.2 kg/m<sup>2</sup>), suffering coronary artery disease (but without the family history) and with normal lipid values (total-cholesterol

5.00 mmol/l, LDL-cholesterol 3.05 mmol/l, HDL-cholesterol 1.05 mmol/l, triglycerides 0.72 mmol/l). Her son, overweight (BMI 29.7 kg/m<sup>2</sup>) non-smoker, aged 43 years had lipid values in recommended range (total-cholesterol 4.92 mmol/l, LDL-cholesterol 3.17 mmol/l, HDL-cholesterol 1.10 mmol/l, triglycerides 1.43 mmol/l). They were not on lipid lowering treatment.



**Fig. 1.** Results of the APOE genotyping. **Left panel:** In comparison to common APOE genotypes (2 - APOE3/3, 3 - APOE4/3, 4 - APOE3/2) an unusual restriction fragment (A) occurs in both the proband (5) and her son (6). **Middle panel:** The carrier of the allele Arg136 → Cys (DNA samples 11-13) is excluded – the characteristic restriction fragment (B) is missing not just in common APOE genotypes (7 – PCR product, 8 - APOE3/3, 9 - APOE4/3, 10 - APOE3/2), but also in proband's sample (14). **Right panel:** In the proband (19) and her son (20) the restriction fragment C, characteristic only for Arg 136 → Ser, is present. The usual APOE genotypes are included for comparison (16 - APOE3/3, 17 - APOE4/3, 18 - APOE3/2)

Rare mutations in the apoE gene have been very often described in patients with different types of severe hyperlipoproteinemia. ApoE gene position 3817 (aminoacid 136) is the most commonly mutated. Interestingly, all four nucleotides occur at this position, coding for four different aminoacids. The common allele has Arg at position 136, relative common is substitution for Cys (Hubáček *et al.* 2000, Vráblík *et al.* 2003), whereas Ser and His (Minnich *et al.* 1996) are rare at these positions.

The apoE2\* allele (Arg136→Ser) was first described in a New Zealand patient (apoE-*Christchurch*) with hyperlipidemia type III (Wardell *et al.* 1987). In Italian patients, HLP III was obvious just with the simultaneous presence of the mutation and apoE2 allele (Rolleri *et al.* 2003). Interestingly, the same genotype apoE2/apoEArg136→Ser, was detected in siblings with type V hyperlipoproteinemia (Violettes *et al.* 2000).

Together with our results, the apoE allele (Arg136→Ser) itself in a heterozygous form with the common apoE3 allele is not sufficient for the expression of obvious dyslipidemia. A negative effect of the

Arg136→Ser mutation is expressed just in connection with less common apoE variants – apoE2 and, in less extent, apoE4. Although the apoE genotyping was performed in dozens of population studies, it is interesting to note that the authors have never mentioned the presence of this mutation in healthy normolipidemic individuals.

So far, we have genotyped more than 4000 individuals for apoE polymorphism. This is the first reported Arg136→Ser heterozygote detected in the Czech region. We can estimate that the population frequency of this apoE mutation is very low. Still, there could be theoretically hundreds or thousands carriers of this apoE mutation. It is therefore important to know, whether this also occurs in common population, not only in patients with some types of hyperlipidemias.

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