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Variable Expression of Hypercholesterolemia in Apolipoprotein E2* (Arg136 → Cys) Heterozygotes

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Summary

In the process of population screening for apo E gene polymorphism with the PCR and subsequent restriction analysis, we identified a female who demonstrated heterozygosity for an unusual restriction fragment caused by the loss of a CfoI restriction site. Sequence analysis of the apo E gene was performed and a carrier of the mutant allele with C → T substitution at cDNA position 3817 was identified, which caused an Arg136 → Cys change. The first-line relatives have been screened for this rare mutation with PCR and restriction analysis of PCR products. The complete lipoprotein parameters have been determined in the probands family. In the family, only one child had the same mutant allele as his mother had. The proband (7.49 mmol/l) with her siblings had hypercholesterolemia and a high body mass index (BMI 31.6 kg/m^2). By contrast, her son had a normal lipid spectrum with normal BMI. We described the mutation apo E2* (Arg136 → Cys) in a family with elevated lipid levels, but there was no confirmation of the connection between this mutation and type III hyperlipoproteinemia or hyperlipoproteinemia at all. In the case of this mutation, other factors (mainly genetic) are important for the development of lipid metabolism disorders.

Key words

Apolipoprotein E • PCR • DNA sequencing • Rare mutation • Lipid metabolism

Introduction

The gene for apolipoprotein (apo) E (localized in the apo E-CI-CII gene cluster on chromosome 19) (Scott *et al.* 1985) determines three common apo E variants (e2, e3 and e4) and subsequently six genotypes – three homozygous (apo E 2/2, 3/3 and 4/4) and three heterozygous (apo E 3/2, 4/3 and 4/2) (Davignon *et al.* 1988). The frequency of alleles and genotypes of apo E varies among different populations (mainly across races); however, the e3 allele and the E 3/3 genotype are invariably dominant (Davignon *et al.* 1988, Gerdes *et al.* 1992).

Apo E consists of 299 amino acids (Rall *et al.* 1982) and is a constituent of very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL) and high density lipoproteins (HDL). It serves as a ligand for the low-density lipoproteins (LDL) receptor (Mahley *et al.* 1981) and putative chylomicron-remnant receptor (probably identical with the LDL receptor-related protein) (Kowal *et al.* 1989, Hussain *et al.* 1991). It is responsible for VLDL catabolism and, partly, also for cholesterol redistribution through HDL.

Apo E4 (Cys 112 → Arg) and apo E2 (Arg 158 → Cys) differ from the most common apo E3 by single amino acid substitution. The substantial number

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Table 1. Known rare mutations of human apo E gene.

Apo E allele	Compared to the normal apo E3 allele	
E1	Gly127 → Asp, Arg158 → Cys	Feusnerr *et al.* 1992
E1	Arg158 → Cys, Leu252 → Glu	van den Maagdenberg *et al.* 1993
$E1_{Hammersmith}$	Lys146 → Asn, Arg147 → Trp	Hoffer *et al.* 1996
$E1_{Harisburg}$	Lys146 → Glu	Mann *et al.* 1989
$E1_{Bethesda}$	details unknown	Gregg *et al.* 1983
$E2_{Christchurch}$	Arg136 → Ser	Wardell *et al.* 1987
E2′	Arg145 → Cys	Rall *et al.* 1982b)
E2″	Lys146 → Gln	de Knijf *et al.* 1994a)
E2‴	Arg142 →Leu, Arg158 → Cys	Richard *et al.* 1995
E2⁗	Arg25 → Cys	Matsunaga *et al.* 1999
E2⁗′	Arg158 → Cys, Val236 → Glu	van den Maagdenberg *et al.* 1993
E2⁗″	Arg134 → Gln	de Knijf *et al.* 1994b)
$E2_{Dunedin}$	Arg228 → Cys	Wardell *et al.* 1991b)
$E2_{Fukuoka}$	Arg224 → Gln	Moriyama *et al.* 1996
E3′	Arg136 → His	Minnich *et al.* 1995
E3″	Cys112 → Arg, Arg142 → Cys	Horie *et al.* 1992
E3‴	Ala99 → Thr, Ala152 → Pro	Mc Lean *et al.* 1984
E3⁗	Cys112 → Arg, Arg251 → Gly	van den Maagdenberg *et al.* 1993
$E3_{Leiden}$	Cys112 → Arg, duplication of AA 121 - 127	Havekes *et al.* 1986
$E3_{Freiburg}$	Thr42 → Ala	Wieland *et al.* 1991
$E4_{Freiburg}$	Leu28 → Pro, Cys112 → Arg	Wieland *et al.* 1991
$E4_{Pittsburg}$	Leu28 → Pro	Kamboh *et al.* 1999
$E4_{Philadelphia}$	Glu13 → Lys, Arg145 → Cys	Lohse *et al.* 1992a)
E4′	Cys112 → Arg, Arg274 → His	van den Maagdenberg *et al.* 1993
E4″	Ser296 →Arg	van den Maagdenberg *et al.* 1993
$E5_{Frankfurt}$	Gln81 → Lys, Cys112 → Arg	Ruzicka *et al.* 1993
E5′	Pro84 → Arg, Cys112 → Arg	Wardel *et al.* 1995
E5″	Glu212 → Lys	Feussner *et al.* 1996c)
E5‴	Glu3 → Lys	Matsunaga *et al.* 1995
E7	Glu244 → Lys, Glu245 → Lys	Matsunaga *et al.* 1995
E_{Sendai}	Arg145 → Pro	Oikawa *et al.* 1997
E_{Tokyo}	deletion of aminoacids 141-143	Konishi *et al.* 1999
E_{Kochi}	Arg145 → His	Suehiro *et al.* 1990
E	Glu3 → Lys, Glu13 → Lys	Mailly *et al.* 1991
E	A → G substitution in 3′splice site of the third intron	Cladaras *et al.* 1987
E	deletion of aminoacids 156-173	Ando *et al.* 1999
E	deletion of bp 4037-4046	Feusnerr *et al.* 1996b)
"0" allele (premature stop codon)	1) G deletion in codon 31 2) G → A in codon for Trp209	Feusnerr *et al.* 1992 Lohse *et al.* 1992b)

of rare apo E mutations (often associated with type III hyperlipoproteinemia) has been described (Table 1). Unlike apo E4 and apo E3, apo E2 has a significantly lower affinity for the LDL receptor. The higher total and LDL cholesterol levels are associated with the e4 allele while lower total cholesterol (TC) and LDL-cholesterol (LDL-C) levels are associated with the e2 allele (Davignon *et al.* 1988).

The effect of apo E is not restricted only to the metabolism of triacylglycerol-transporting particles, but it also influences the intestinal absorption and catabolism of cholesterol. Probands with the e2 allele absorb less cholesterol and probands with e4 allele more cholesterol compared to homozygotes E 3/3 (Kesäniemi *et al.* 1987, Miettinen *et al.* 1992, Gytling and Myettinen 1992). The catabolism of the sterol core is the highest in probands with the e2 allele (Miettinen *et al.* 1992, Gytling and Myettinen 1992). The studies addressing the effect of apo E on endogenous cholesterol synthesis did not give any definitive results (Roe *et al.* 1991, Miettinen *et al.* 1992, Gytling and Myettinen 1992).

Here, we describe a rare mutation of apo E2* (Arg136 → Cys) which was identified in apo E polymorphism screening in the Czech population. One proband demonstrated heterozygosity for an unusual restriction fragment, originating from the loss of CfoI restriction site in the apo E gene.

Patients and Methods

Uncoagulated blood for isolation of the genetic material was diluted with sterile water at a ratio of 1:1 and stored at –20° C. DNA was isolated using the standard method (Miller *et al.* 1988).

Analysis of the polymorphism in the apo E gene was performed using polymerase chain reaction (PCR) with subsequent restriction of the product by the enzyme CfoI as described elsewhere (Hixson and Vernier 1990). Lipoprotein fractions (LDL and HDL) were isolated by ultracentrifugation (39,000 rpm, 18 h, 12° C) in a Beckman 50.4 Ti rotor on an L7 Ultracentrifuge (Beckman Instruments, Inc., California, USA).

Lipid parameters and apo B were measured enzymatically by the WHO Lipid Reference Centre at the Institute for Clinical and Experimental Medicine on a Roche COBAS MIRA autoanalyzer (Hoffmann-La Roche, Switzerland) using reagents from Boehringer Mannheim Diagnostics (Indianapolis, IN).

The PCR product of 5′end of exon 4 apo E gene was cloned with a SureClone Ligation Kit (Pharmacia Biotech, Sweden) in the pUC18 plasmide and fluorescent sequenced with a Cy5 AutoRead Sequencing Kit (A.L.F. System, Pharmacia Biotech, Sweden).

Results

The proband was 54-year-old caucasian woman, menopausal and a non-smoker, who suffered an attack of renal colic in 1995.

Abnormal ECG (sinus rhythm, heart rate 71/min, deep Q, I, aVL, negative T wave in III and aVF) was found accidentally while hospitalized because of a head injury, which she sustained in 1996. She had no history of ischemic heart disease, hypertension (systolic pressure 139 mm Hg, diastolic pressure 90 mm Hg), or diabetes mellitus. She was overweight (height 157 cm, weight 77.8 kg, waist hip ratio 0.74). Physical examination did not reveal any abnormalities. Serum total, LDL and HDL cholesterol values were elevated, triacylglycerols, fasting glucose, and insulin were slightly above normal limits (Table 2).

All living first-degree relatives of the proband (Fig. 1) were examined (brother, sister, son). Father died aged 82, the cause of death was a fifth myocardial infarction; he suffered his first attack in his 60's. Her mother died because of stroke at the age of 63. Proband's brother has established ischemic heart disease and suffered myocardial infarction at the age of 52; in addition he is a type-II diabetic treated by antidiabetics. Her siblings (brother and sister) have elevated lipid values, approximately to the same level as the proband. The lipid profile of her son was normal (Table 2).

CfoI restriction analysis of the PCR product by mother and her son provided an unusual restriction fragment of approx. 110 bp (Fig. 1). Sequence analysis illustrates that they are carriers of the already described (Walden *et al.* 1994, Feussner *et al.* 1996) C → T substitution at position 3817 of apo E cDNA (data not shown).

Discussion

Rare mutations in the apo E gene (Table 1) have very often been described in patients with different types of severe hyperlipoproteinemia.

During population screening, we identified C3817 → T (Arg136 → Cys) mutation of apo E in a 54-year-old caucasian woman with a positive family history of cardiovascular diseases, abnormal ECG, and impaired lipoprotein metabolism.

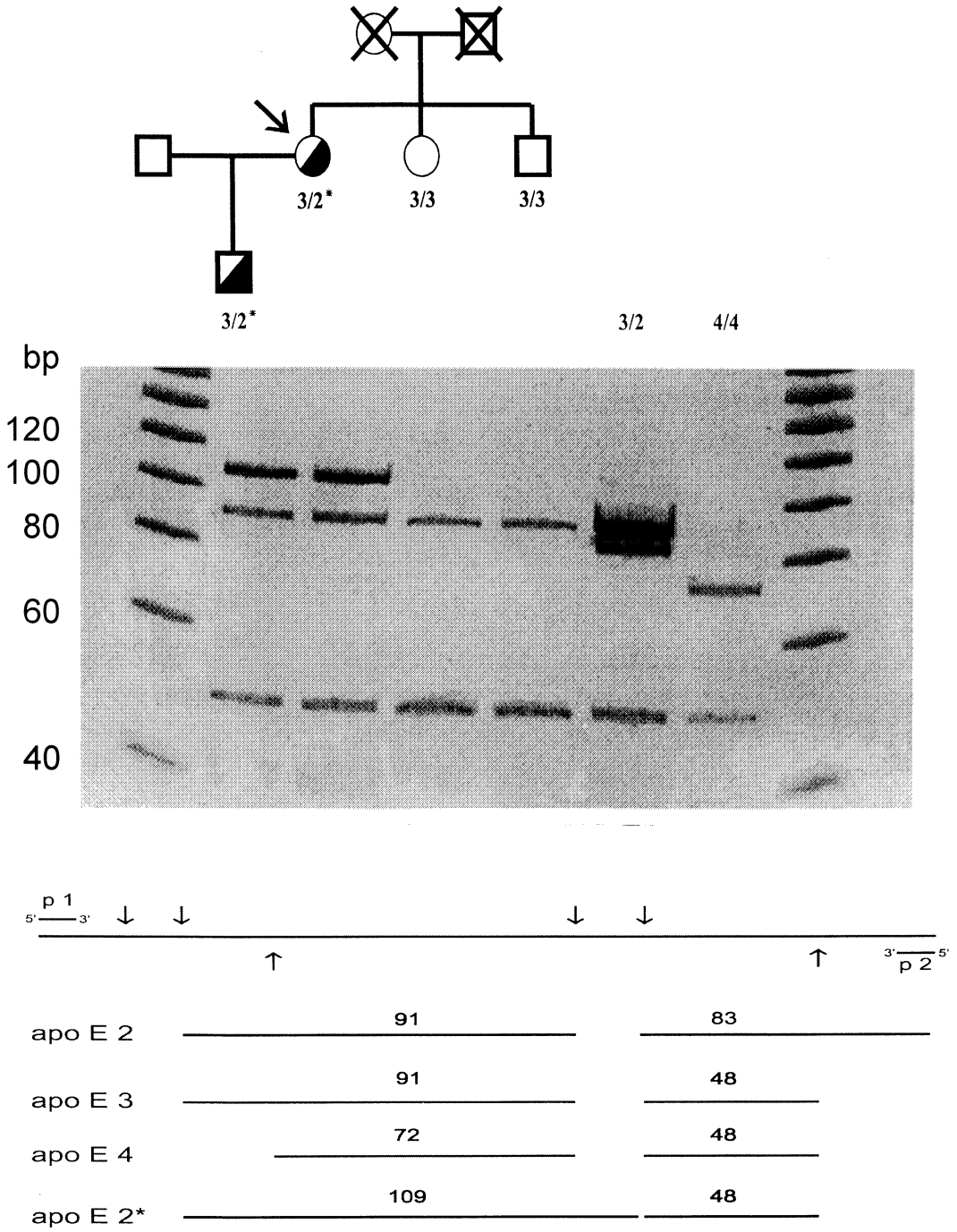

Fig 1. *Pedigree of the family with apo E2* (Arg136 → Cys) (upper panel). Proband is identificated in pedigree by arrow. Apo E genotyping by PCR and restriction analysis in the four consanguinic family members and using standard 3/2 and 4/4 genotype (middle panel). PCR product is cleaved constant at four (↓) and polymorphic at two (↑) positions. Mutation caused the loss of one of the constant cleavage positions, resulting in the production of an unusual restriction fragment of approx. 110 bp. This fragment showed a mutation that caused the loss of a nonpolymorphic restriction site in codons for AA 136 or 137 (lower panel).*

Table 2. Characterization of the family with apo E2* (Arg136 → Cys) mutation.

	Proband	Brother	Sister	Son
ApoE	3/2*	3/3	3/3	3/2*
TC (mmol/l)	7.49	7.34	7.06	3.74
TG (mmol/l)	1.75	1.61	1.23	1.33
HDL-C (mmol/l)	2.16	1.28	1.76	1.17
LDL-C (mmol/l)	4.53	5.33	4.75	1.97
Glucose (mmol/l)	5.2	13.6	6.3	4.8
Age	54	57	62	24
CAD	? *	+	–	–
BMI (kg/m^2)	31.6	33.69	36.03	19.6
Waist/hip ratio	0.74	1.09	0.84	0.87
SBP/DBP (mm Hg)	139/90	139/81	125/75	101/63

*(BMI - body mass index, BP – blood pressure, CAD – coronary artery disease, HDL-C – HDL cholesterol, LDL-C – LDL cholesterol, TC – total cholesterol, TG – triacylglycerols, * abnormal ECG without history of CAD)*

Among all her living first-line relatives, this mutation was present only in her 24-year-old son with apparently normal lipid values.

The C → T substitution at cDNA position 3817 which caused the change of cysteine for arginine at position 136 is localized on the border within the putative apo E binding domain for the LDL receptor (Wilson *et al.* 1991).

This mutation of apo E has been described (Walden *et al.* 1994) to decrease VLDL uptake by macrophages. However, this alteration is not as pronounced as that in macrophages isolated from the patient with type III hyperlipoproteinemia with the apo E genotype 2/2.

The apo E2* allele (Arg136 → Cys) was formerly detected in normal or late-onset type III hyperlipoproteinemia and in heterozygosity for allele apo e2 (Walden *et al.* 1994) or alleles e3 and e4 (Feussner *et al.* 1996). No relationship has been identified between investigated family and those previously reported (Walden *et al.* 1994, Feussner *et al.* 1996). Two of the probands described here were heterozygous for mutant apo E2* (Arg136 → Cys) and a normal apo e3 allele. They had very different lipid values, probably due to differences in the age, sex, and BMI. The mother was overweight with hypercholesterolemia in contrast to her child whose BMI and lipid values were within normal limits. The high HDL cholesterol in mother and her sister (in spite of the fact that both women were postmenopausal), was not found in male family members. Female sex could be the cause of this difference.

It should be noted that although both of the proband's siblings had evidence of hypercholesterolemia neither of them was a carrier of the rare apo E Arg136 → Cys allele. Thus an additional (and yet unidentified) defect in lipoprotein metabolism might be prevalent in the family.

We may thus conclude that single C → T mutation at position 3817 in the apo E gene is not mandatory for the manifestation of type III hyperlipoproteinemia in all cases. In the case reported in this paper, the presence of the e3 allele could modify the final lipoprotein phenotype in the apo E2* (Arg136 → Cys) carrier. Apparently in the case of this mutation, other factors, probably mainly genetic (e2 allele) and to a less extent environmental (age, sex, weight), are necessary for the manifestation of a particular hyperlipidemic pattern. If carriers of this abnormal apo E2* allele are in certain circumstances at increased risk of cardiovascular diseases needs further evaluation.

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Reprint requests

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