
SHORT COMMUNICATION

Familial Dysbetalipoproteinemia in Three Patients with apoE 2*(Arg136→Cys) Gene Variant

M. VRABLÍK, A. HOŘÍNEK, R. ČEŠKA, T. ŠTULC, T. KVASNIČKA

Third Internal Department, First Faculty of Medicine, Charles University, Prague, Czech Republic

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Summary

Apolipoprotein E (apoE) is a polymorphic protein which occurs in three common isoforms and more than 25 rare variants. Some of the rare apoE variants have been implicated in a dominant mode of inheritance of familial dysbetalipoproteinemia (FD). We have identified three unrelated apoE 2*(Arg136→Cys) carriers with FD. This finding supports the notion that although apoE 2*(Arg136→Cys) mutation is perhaps not sufficient to cause FD itself, the presence of other genetic and/or environmental factors can lead to the phenotypic expression of the disease in the carriers.

Key words

Apolipoprotein E • Rare variants • Familial dysbetalipoproteinemia

Apolipoprotein E (apoE) is a polymorphic protein associated with plasma lipoproteins which usually occurs in three isoforms designated E 2, E 3 and E 4 (Hořejší and Češka 2000). Except for these more than 25 rare apoE variants have been identified to date. Some of them have been reported to cause familial dysbetalipoproteinemia (FD) with a dominant mode of inheritance (De Knijff *et al.* 1994).

FD is an inherited disorder of the metabolism of plasma cholesterol and triglyceride rich chylomicron and VLDL remnants. Affected individuals present grossly elevated both cholesterol and triglyceride levels in the plasma and develop accelerated and premature atherosclerosis (Rall and Mahley 1992).

Ninety percent of the patients with FD are homozygous for apoE 2/2 genotype. The remainder is

represented by apoE 2 heterozygous patients or carriers of apoE variants or compounds (Utterman 1987).

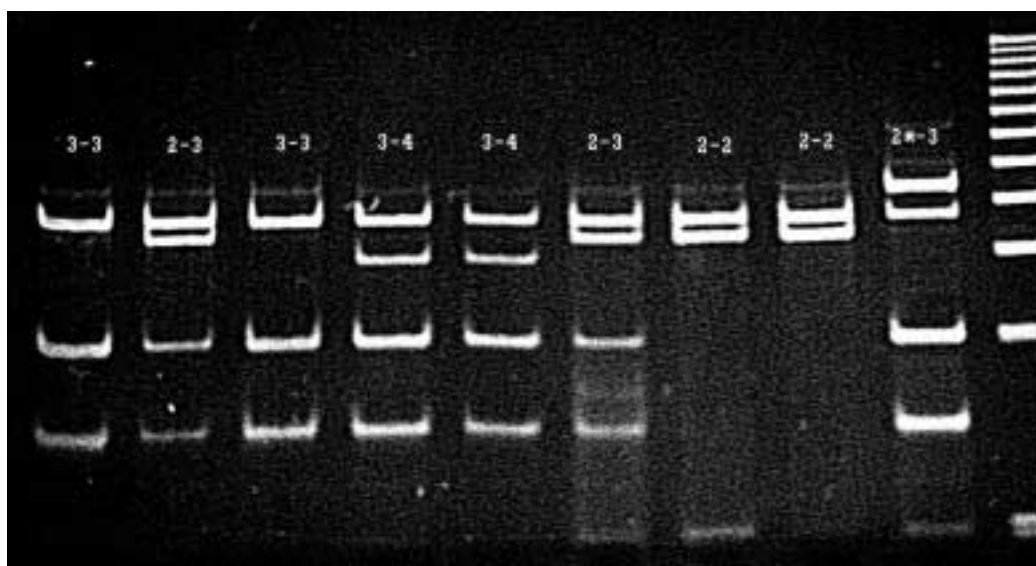
Some of the rare apoE variants have been implicated in dominant mode of inheritance of FD. Hubáček *et al.* (2002) reported on a heterozygous apolipoprotein E 2*(Arg136→Cys) carrier without dyslipidemia. Here we present findings of three unrelated apoE 2*(Arg136→Cys) individuals with familial dysbetalipoproteinemia (FD) who have been detected during routine examination of the apoE genotype in FD patients.

Three probands (2 females, 1 male) were identified among thirteen FD individuals followed at the lipid clinic of the Third Department of Medicine of the General Faculty Hospital in Prague. They were unrelated. Their phenotypic characteristics and basic data from their medical history are shown in Table 1.

Table 1. Phenotypic characteristics of the apoE 3/2*(Arg136→Cys) probands. Pretreatment lipid values are shown.

Sex	age	BMI	apoE	TC	TG	HDL-c	VLDL-c/TG	ApoB	Lp(a)	X	CAD	FaH
	years				mmol/l				g/l			
f	84	27.7	2*/3	7.24	4.78	1.04	0.44	1.33	0.05	–	+	+
f	77	23.4	2*/3	11.1	8.58	1.00	0.42	1.54	0.33	–	+	+
m	58	29.0	2*/2	7.38	3.11	1.33	0.47	1.30	0.06	–	–	–

Sex – female (f), male (m); age – years, BMI – body mass index (kg/m^2); apoE – apoE genotype; TC – total cholesterol; TG – triglycerides; HDL-c – HDL cholesterol; VLDL-c/TG – VLDL to total triglycerides ratio; apoB – apolipoprotein B; Lp(a) – lipoprotein (a); X – xanthomatosis; CAD – coronary artery disease; FaH – family history of hyperlipoproteinemia/coronary artery disease.

**Fig. 1.** DNA band patterns of apoE genotypes. 3-3 – genotype apoE 3/3; 2-3 – genotype apoE 2/3; 2-2 – genotype apoE 2/2; 3-4 – genotype apoE 3/4; 2*-3 – genotype apoE 2*(Arg136→Cys)/3

Plasma lipid levels were assayed using standard automated enzymatic methods (Cobas Mira, Roche). The electrophoresis of plasma lipoproteins in 0.8 % agarose gel in Rapp and Kahlke's modification revealed a broad β band corresponding to "floating" β VLDL. The diagnosis of FD was confirmed by preparative ultracentrifugation confirming the presence of β VLDL with VLDL-cholesterol to total serum triglycerides ratio over 0.3.

The apoE genotype was determined using the restriction isoform genotyping of PCR product with CfoI restriction endonuclease (Hixson and Vernier 1990). Presence of apoE 2*(Arg136→Cys) gene variant causes

creation of an atypical 109 bp long DNA fragment which originates from the loss of CfoI restriction site in the apo E gene (Fig. 1). The mutation was verified by direct sequencing using an automated sequence analyzer (ABI PRISM 310).

Identification of three unrelated probands with apoE 2*(Arg136→Cys) gene variant among patients with FD supports the notion that this variant can, under certain circumstances, be associated with dominant forms of FD. The frequency of the variant in studied FD patients is much higher (1:4) than in the general population where the frequency is estimated to be lower than 1:1000 (Hubáček *et al.* 2000). The two female probands were

heterozygotes apoE 2*(Arg136→Cys)/3 and the male proband was an apoE 2*(Arg136→Cys)/2 individual. Both female probands had a strongly positive familial as well as a personal history of coronary artery disease and their lipid levels in the plasma were markedly elevated. They both were older than the male proband. It is possible that age, gender and estrogen deficit could involve some of the factors necessary for manifestation of the disease. However, other genetic factors (e.g. LPL gene variants) leading to deterioration of VLDL metabolism can be implicated. In the male proband the phenotypic picture was milder. It can be presumed that the second apoE 2 allele which the proband carried was the factor leading to manifested FD. An effort has been made to identify all carriers of apoE 2*(Arg136Cys) variant in the probands' pedigrees. However, only a daughter of the male proband was available for testing. She was not hyperlipidemic and her apoE genotype was apoE 2/3. Parents of the two female probands were reported to be also hyperlipidemic, but this could not be confirmed by control examination as the parents were not alive. Both female probands did not have any children.

The apoE 2*(Arg136→Cys) mutation was first described by Walden *et al.* (1994) who identified an apoE 2/2*(Arg136→Cys) heterozygote with FD and extreme obesity. The mutation was also present in the father of the proband who was a heterozygote carrying apoE 3/2*(Arg136→Cys) genotype as well as in two brothers of the proband (both apoE 2/2*(Arg136→Cys) carriers). One of the brothers was slightly hypercholesterolemic. None had signs of FD and the authors therefore concluded that apoE 2*(Arg136→Cys) mutation was associated with a recessive rather than a dominant mode of inheritance (Walden *et al.* 1994).

Later, Feussner *et al.* (1996) found a heterozygous apoE 4/2*(Arg136→Cys) individual with a typical clinical picture of FD and suggested that the apoE

2* (Arg136→Cys) might be considered a cause of dominance of FD. In the following study of the same authors, other four carriers of the variant were identified. A proband and her father were apoE 3/2*(Arg136→Cys) heterozygotes with FD. Other two young relatives of the proband carrying apoE 4/2*(Arg136→Cys) and apoE 3/2*(Arg136→Cys) genotypes displayed dyslipoproteinemia. The authors concluded that apoE 2*(Arg136→Cys) predisposes to late-onset dominance of FD while another environmental or genetic factors are necessary for its expression (Feussner *et al.* 1998).

In contrast to Feussner and collaborators, März *et al.* 1998) have identified four apoE 3/2*(Arg136→Cys) probands none of them having FD. Therefore these authors came to conclusion that apoE 2*(Arg136→Cys) is unlikely to cause dominance of FD. Similarly, Hubáček *et al.* (2002) reported no signs of FD in a heterozygous individual with apoE 2*(Arg136→Cys)/3 genotype. The same authors previously described some putative reasons for variable expression of hyperlipidaemia in apoE 2*(Arg136→Cys) carriers (Hubáček *et al.* 2000).

In the present study, we have identified three unrelated carriers of apoE 2*(Arg136→Cys) gene variant. ApoE 2*(Arg136→Cys) mutation is almost certainly not sufficient to cause familial dysbetalipoproteinemia itself, but the presence of other genetic and/or environmental factors can lead to the phenotypic expression of the disease in the carriers. If this complementary underlying factor is the same for apoE 2*(Arg136→Cys) variant and for „classical“ FD associated with apoE 2 homozygosity remains to be clarified by further research.

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Reprint requests

Michal Vrablik, MD, PhD., U nemocnice 1, 128 08 Prague 2, Czech Republic, e-mail: michal.vrablik@seznam.cz