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Familial Defective Apolipoprotein B-100: a Lesson from Homozygous and Heterozygous Patients

R. ČEŠKA, M. VRABLÍK, A. HOŘÍNEK

Third Medical Clinic, First Faculty of Medicine and Prague General Hospital, Prague, Czech Republic

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Summary

Familial defective apolipoprotein B-100 (FDB) is a genetic disorder caused by a substitution of glutamine for arginine at residue 3500 of the apolipoprotein B-100 molecule. We have identified 23 heterozygotes and one homozygote for FDB (frequency 1:20) in a group of 510 patients with hypercholesterolemia. Mean age of the patients (18 females and 6 males) was 46 years. The diagnosis of FDB was based on point mutation PCR analysis of exon 26 of the apo B gene. Plasma lipids in heterozygous patients were: total cholesterol 8.76±1.2 mmol/l, triglycerides 1.42±0.5 mmol/l, HDL-cholesterol 1.43±0.3 mmol/l, LDL-cholesterol 6.69±1.2 mmol/l, apoB 1.69±0.4 g/l, Lp(a) 0.26±0.2 g/l. The most frequent apoE genotype was 3/3 (19 patients), apoE 3/4 genotype was found in 3 patients and one person had apoE 2/3. Xanthelasma palpebrarum was present in 4 patients and tendon xanthomas in 3 patients including the homozygote. Premature manifestation of coronary heart disease was revealed in 3 patients. Sixteen patients were treated with statins, a combination of statin and resin was used in 2 patients (including the homozygote), whereas six patients were treated with the diet only. We conclude that although the plasma lipid levels of total and LDL cholesterol in FDB patients are lower than in patients with familial hypercholesterolemia, the patients with FDB suffer from premature atherosclerosis. The therapeutic approach to FDB individuals and patients with familial hypercholesterolemia is very similar.

Key words

Familial hypercholesterolemia • Familial defective apo B-100 • Xanthomatosis • Coronary artery disease • Hypolipidemic drugs

Introduction

The lipoprotein receptor pathway is the primary mechanism for maintaining the homeostasis of plasma lipoproteins. The initial step in the metabolic transformation of lipoproteins is their binding to the receptors. Apolipoprotein B-100 (apoB-100) as well as apolipoprotein E (apoE) play crucial role in the interaction of lipoproteins with LDL receptors (Brown and Goldstein 1986). Apolipoprotein B is a huge protein with a molecular mass of 550 000 daltons. It is composed of 4 536 amino acids. The complete sequence and structural analysis of human apoB-100 has already been performed (Cladaras *et al.* 1986). Familial defective apoB-100 (FDB) is a genetic disorder causing hypercholesterolemia due to the presence of abnormal low density lipoproteins that bind poorly to LDL

Fax +420 2 249 20 590
E-mail: physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

receptors (Innerarity *et al.* 1987, Weisgraber *et al.* 1988, Soria *et al.* 1989).

FDB is a mirror image of familial hypercholesterolemia, a disease caused by a mutation in the LDL receptor gene, which causes a substantial decrease in the clearance of LDL particles from the plasma. In contrast to familial hypercholesterolemia (FH), plasma levels of cholesterol in FDB patients tend to be lower which could be explained by several mechanisms. (Miserez and Keller 1995).

The present results concern the screening for FDB in a cohort of hypercholesterolemic patients attending a lipid clinic. The phenotypic picture of identified FDB patients was compared to that of familial hypercholesterolemic patients.

Methods

Plasma levels of total cholesterol, triglycerides and HDL-cholesterol were measured enzymatically using sets produced by Boehringer (Manheim), LDL-cholesterol was estimated by the Friedewald formula. Apolipoprotein B and Lp(a) levels were measured according to Laurell's rocket method using the antiserum produced by Immuno (Wien). Electrophoresis of lipoproteins was performed according to Rapp and Kahlke in Šobra´s modification (Šobra 1970).

The diagnosis of the FDB mutation was based on an analysis of genomic DNA, prepared either by a rapid method using the DNA isolation kit – ReadyAmp ™Genomic DNA Purification System (Promega, USA) or by a standard salting out method (Miller *et al.* 1988). Five µl of DNA was used for the PCR reaction. The FDB mutation was determined using the PCR method and restriction enzyme isoform genotyping by ScaI (Promega, USA) restriction endonuclease (Geisel *et al.* 1991). A single nucleotide G to A transition at position 10708 in exon 26 of the apoB gene creates a ScaI restriction site. In case of the familial defective apolipoprotein B-100, the amplified DNA fragment is cut by ScaI, whereas the wild type DNA is resistant to ScaI digestion. Considering the difficulties with correct setting of the restriction enzyme cleavage (ScaI sensitivity to the salt concentration), we used the modified protocol with negative control (Geisel *et al.* 1993). A second ScaI restriction site was introduced in the upstream primer. This additional ScaI site is present in both the normal and the mutated alleles so that the restriction enzyme cleavage process can be simply controlled. DNA fragments were then separated by electrophoresis in 2 % agarose gel. Determination of apoE genotype was done by PCR and HhaI (CfoI) restriction enzyme isoform genotyping method (Hixson and Vernier 1990) using primers published by Crook *et al.* (1994). Fragments (91, 81, 72 and 48 base pairs) were separated by vertical electrophoresis in 10 % polyacrylamide gel.

Table 1. Phenotypic characteristics of the patients heterozygous for familial defective apolipoprotein B-100.

	N	Mean age (years)	Total cholesterol (mmol/l)	Triglycerides (mmol/l)	HDL cholesterol (mmol/l)	LDL cholesterol (mmol/l)	ApoB (g/l)	Lp(a) (g/l)
Men	5	45	8.8±0.9	1.5±0.2	1.3±0.3	6.9±0.9	1.7±0.4	0.3±0.0
Women	18	46	8.7±1.3	1.4±0.6	1.6±0.3	6.5±1.3	1.7±0.4	0.2±0.2
Together	23	46	8.9±1.2	1.4±0.5	1.4±0.3	6.7±1.2	1.7±0.4	0.3±0.2

Results

Five hundred and ten patients with hypercholesterolemia attending a lipid clinic were screened for the mutation causing FDB. In this group, 23 heterozygotes and one homozygote for FDB were identified. They were members of 4 families (15 patients) and 9 unrelated affected persons (frequency 1:20,

corrected 1:39). The mean age of the heterozygous patients (5 male and 18 female) was 46 years. Plasma lipid and lipoprotein levels are shown in Table 1. The following apo E genotypes were found in heterozygous FDB patients: E 3/3 (19 patients), E 3/4 (3 patients) and E 2/3 (1 patient). The homozygote had the apoE 3/3 genotype, his plasma lipid levels are shown in Table 2.

Table 2. Phenotypic characteristics of the homozygous male patient for familial defective apolipoprotein B-100.

Age (first examination)	Total cholesterol (mmol/l)	Triglyceride (mmol/l)	HDL cholesterol (mmol/l)	LDL cholesterol (mmol/l)	apoB (g/l)	Lp(a) (g/l)
34	10.2	1.07	1.17	8.50	2.27	0.17

Table 3. Phenotypic characteristics of the patients with familial hypercholesterolemia (Šobra 1970).

n	MI (%)	AP (%)	HT (%)	DM (%)	BMI>30 (%)	TC (mmol/l)	TG (mmol/l)	AC (%)	XL (%)	XO (%)
142	25.4	9.2	17.5	0	31.9	9.21±2.3	1.1±0.48	25.4	19.0	8.4

n – number of patients, MI – myocardial infarction, AP – angina pectoris, HT – arterial hypertension, DM – diabetes mellitus, BMI – body mass index (kg/m^2), TC – total cholesterol, TG – triglycerides, AC – arcus corneae senilis, XL – xanthelasma palpebrarum, XO – tendon xanthomas

The homozygous patient suffered from two myocardial infarctions, the first at the age of 34, the second at 42. Because of diffuse changes revealed by coronary angiography no revascularization was apparent. This patient was described previously in a case report (Hořínek *et al.* 1999). Coronary artery disease was also proved in two FDB heterozygous women. One woman had angina pectoris since the age of 57 and at the age of 63 four-fold coronary artery bypass grafting (CABG) was performed. The second woman suffered from myocardial infarction at 55. In addition, a proband in N. family, where six FDB patients were identified, had the first myocardial infarction at 36 and died from a second infarction at 38. The diagnosis of FDB in this woman was not done because she died in 1986. When discussing the prevalence of coronary artery disease in FDB patients, it is noteworthy that nine of the FDB patients were less than 35 years old. Xanthelasma palpebrarum was found in four heterozygotes and tendon xanthomas were present in two patients. Sixteen FDB heterozygotes were treated with statins, one patient received statin and resin, six patients were on a diet only. The homozygous patient was treated with high doses of simvastatin (40 mg daily).

Discussion

The frequency of heterozygotes for the familial defective apolipoprotein B-100 (FDB) in the general European and North American population ranges between 1:500 to 1:700 (Humphries and Talmud 1995). In a Swiss study, a much higher incidence of FDB heterozygotes was found. The prevalence of carriers of this metabolic defect corresponding to about 1:209 in Switzerland suggests that this mutation possibly originates in this European region (Miserez *et al.* 1994). On the contrary, a Finnish study proved the absence of FDB in Finnish patients with hypercholesterolemia (Hämäläinen *et al.* 1990). In most lipid clinics, 2-5 % of patients diagnosed familial

hypercholesterolemia have FDB (Myant 1993). However, more recent studies have indicated that the clinical picture of patients with FDB is usually less severe than in FH patients (Brousseau *et al.* 1995, Schuster *et al.* 1990) who tend to have higher plasma levels of total and LDL cholesterol as well as an earlier onset of premature atherosclerosis with its complications. In a pilot study, a group of 142 subjects with FH treated at the lipid clinic of the Third Department of Medicine in Prague were followed by Šobra (1970). The phenotypic characteristics of this group are shown in Table 3. The data obtained most recently in a large study concerning FH patients are summarized in Table 4 (Simon Broome Register Group 1999). In both above studies the plasma lipid levels of total and LDL cholesterol in FH patients were higher than in FDB patients identified in our present study.

Table 4. Phenotypic characteristics of patients with familial hypercholesterolemia followed by the Simon Broome Register Group (1999).

	Men (n=605)	Women (n=580)
Age	40.3	43.9
MI	83 (13.7%)	38 (6.6%)
AP	118 (19.5%)	93 (16.2%)
HT	40 (6.6%)	56 (9.7%)
DM	7 (1.2%)	3 (0.5%)
BMI>30	22 (3.6%)	20 (3.4%)
TC (mmol/l)	8.5±2.1	9.0±2.4
TG (mmol/l)	1.4±1.2-1.6	1.2±1.2-1.3
HDL-C (mmol/l)	1.1±0.3	1.3±0.3
LDL-C (mmol/l)	6.6±2.1	7.0±2.4

n – number of patients, MI – myocardial infarction, AP — angina pectoris, BMI – body mass index(kg/m^2), HT – arterial hypertension, DM – diabetes mellitus, TC – total cholesterol, TG – triglycerides, HDL-C – HDL cholesterol, LDL-C – LDL cholesterol.

The incidence of obesity in FH is lower or the same as in the general population. Serum leptin levels do not correlate with plasma lipid levels (Haluzík *et al.* 1999). In accordance with the results of other authors, it was concluded in the latter study that in FH patients the most effective therapy of FH is the treatment with HMG-CoA reductase inhibitors which substantially improved the prognosis of patients heterozygous for familial hypercholesterolemia.

Only four unrelated FDB homozygotes have been reported to date (Funke *et al.* 1992, Gallagher and Myant 1992, März *et al.* 1992, 1993). The prevalence of coronary artery diseases varies in different studies but our findings are comparable with the data of most authors. For the treatment of the patients, we mainly use statins which have been shown, despite former skepticism concerning the use of these drugs in FDB, as effective and safe in the FDB patients. The treatment is carried out in accordance with the guidelines of the European Atherosclerosis Society and the National Cholesterol Education Program, USA. In the treatment of the homozygote, we used different treatment regimes including a combination of three hypolipidemic drugs. Acceptable lipid values were attained in this man using higher doses of simvastatin.

Despite the fact that our group of patients with familial defective apolipoprotein B-100 is not large, we could draw some conclusions. In comparison with patients heterozygous for familial hypercholesterolemia, the plasma lipid levels of FDB heterozygotes are lower. These findings are even more pronounced when homozygotes for both diseases are compared. FDB represents a significant risk factor for coronary artery disease. Xanthomatosis is an important clinical sign not only for familial hypercholesterolemia but also for FDB patients. Recently, it has become evident that the therapeutic approach for patients with familial hypercholesterolemia and those with FDB is very similar or even the same.

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Reprint requests

Dr. M. Vrablík, Third Faculty of Medicine, Charles University, U nemocnice 1, 128 21 Prague 2, Czech Republic.
e-mail: michal.vrablik@post.cz