HDL Cholesterol Levels in Patients with Molecularly Defined Familial Hypercholesterolemia

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Abstract. Familial hypercholesterolemia (FH) is the most common genetic disorder leading to premature atherosclerosis. Typically, it is due to mutations in the LDL receptor gene resulting in elevated total and LDL cholesterol levels. The type of the LDL receptor gene mutations may affect the severity of hypercholesterolemia and consequently the incidence of coronary atherosclerosis. Furthermore, high-density lipoprotein (HDL) cholesterol levels have been recently shown to be an independent risk factor for coronary heart disease in this population. We examined the effect of the type of the LDL receptor gene mutations and of common gene polymorphisms possibly affecting HDL metabolism [cholesterol ester transfer protein (CETP), apolipoprotein A-IV (ApoA-IV), angiotensin converting enzyme (ACE), and apolipoprotein E (ApoE)] on HDL cholesterol levels in patients with molecularly defined heterozygous FH who were attending our lipid clinic (n=84). The nature of the LDL receptor gene mutation (81T>G, n=12; 858C>A, n=13; 1285G>A, n=12; 1646G>A, n=22; and 1775G>A, n=25) did not significantly influence HDL cholesterol levels. Unlike other gene polymorphisms, the apolipoprotein (apo) E gene polymorphism did significantly affect these levels. In fact, the presence of the E4 allele was associated with lower HDL cholesterol levels compared to patients not carrying this allele. We conclude that HDL cholesterol levels in heterozygous FH patients may be affected by the apoE gene polymorphism. *(received 11 July 2001, accepted 24 September 2001)*

Keywords: HDL cholesterol, familial hypercholesterolemia, apoE genotype, CETP gene polymorphism, apoA-IV gene polymorphism, ACE gene polymorphism

Introduction

Patients with familial hypercholesterolemia (FH) have elevated low-density lipoprotein (LDL) cholesterol levels leading to coronary heart disease (CHD) early in life. The syndrome results from mutations in the LDL receptor (LDLR) gene. The type of mutation in the LDLR gene may affect the levels of total and LDL cholesterol and, consequently, the incidence of CHD as well as the patients' responsiveness to statin treatment [1,2]. It has recently been reported that high-density lipoprotein (HDL) cholesterol levels is an independent risk factor for CHD in molecularly defined heterozygous FH [3]. It is well known that polymorphisms of some genes can affect the HDL cholesterol levels [4]. We recently detected the genetic mutations responsible for FH in our area (northwestern Greece)[5]. In this study we examine the effect of the LDLR gene mutations and gene polymorphisms possibly affecting HDL metabolism on HDL levels in a group of FH heterozygotes.

Materials and Methods

A total of 84 unrelated patients (age 8-70 yr) attending our lipid clinic with a clinical diagnosis of heterozygous FH participated in the study after providing informed consent. The diagnosis of FH was based on recently proposed clinical criteria [6]. Eighty-four healthy individuals matched for age, sex, body mass index (BMI), and smoking habits were

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Table 1. Clinical features and plasma lipid parameters in patients with familial hypercholesterolemia (FH) and controls.

Variable	FH heterozygotes	Controls	p-value
male/female	52/32	51/31	–
mean age (yr)	41	40	–
body mass index (BMI; kg/m^2)	23±4	22±4.5	–
cigarette smokers (%)	49	47.5	–
total cholesterol (TC; mg/dl)	363±83	180±45	0.00001
triglycerides (TG; mg/dl)	130±102	110±70	–
LDL-cholesterol (mg/dl)	278±83	118±46	0.0001
HDL-cholesterol (mg/dl)	48±12	55±13	0.01
Apo A-I (mg/dl)	133±34	149±28	0.05
Apo B (mg/dl)	247±70	126±38	0.0001
Lp(a) (mg/dl)	14.5 (1.2-104)	7 (0.8-34.5)	0.001

used as controls. In all heterozygous FH cases, DNA analysis for the LDL receptor gene was performed. DNA was extracted from the whole blood specimens as previously described [7]. PCR was carried out using 50 ng DNA isolated from each individual. All LDLR gene exons and the promoter were amplified essentially as previously described [8]. PCR was carried out using an MJR PTC-100 thermocycler. Restriction isotyping was used for detecting previously described LDLR gene mutations. Automated sequencing was performed using the Perkin Elmer "Big Dye Terminator Cycle Sequencing Ready Reaction Kit" as instructed by the manufacturer (Perkin-Elmer Corp.). The sequenced products were separated by capillary electrophoresis using an ABI PRISM 310 Genetic Analyzer. Sequencing Analysis and Sequence Navigator software were used to analyze the data.

The Taq1B polymorphism of the CETP gene was detected using a reported protocol [9]. Detection of the insertion/deletion polymorphism of the ACE gene was based on a reported protocol [10]. ApoE genotyping was performed as described by Hixson and Vernier [11]. Finally, apoA-IV 347 and 360 polymorphism sites were detected using the protocol described by Carrejo et al [12].

In patients and controls, blood samples were obtained after a 14 hr overnight fast for gene genotype detection as well as determination of lipid parameters. Blood samples were centrifuged for 30 min (3,600 g) and the serum was separated and stored at 4°C for analysis of lipid parameters. Serum for the assay of Lp(a) was frozen and stored at -70°C. Cholesterol and triglycerides were determined by enzymatic colorimetric assay using an RA-1000 analyzer (Technicon, Ltd), while HDL cholesterol was determined enzymatically in the supernatant after precipitation of other lipoproteins with dextran sulfate-magnesium. LDL cholesterol was calculated using the Friedewald formula. The CV values derived from a control material were 2.32% for total cholesterol, 3.24% for triglycerides, and 3.89% for HDL cholesterol.

Serum Apo A-I and Apo B were measured by immunonephelometry with the aid of an Array analyzer (Beckman Instruments, Inc). The CV values were 2.16% and 1.97%, respectively. Lp(a) was measured using a monoclonal anti-Lp(a) antibody technique by the enzyme immunoassay Macra Lp(a) (Temuro Medical Corp., Elkton, MD). The lower limit of delection was 0.8 mg/dl. In cases of Lp(a) levels <0.8 mg/dl, the value of 0.8 mg/dl was used for statistical analysis. The intra-assay and inter-assay CVs were <6% and 10.3%, respectively. Statistical analyses were performed by one-way analysis of variance (ANOVA), followed by the LSD test for paired comparisons and Student's t-test for unpaired data.

Results

The clinical and laboratory characteristics of the study population are summarized in Table 1. HDL cholesterol levels in patients with heterozygous FH attending our lipid clinic were significantly lower than in the normal controls (48.3±12.2 vs 55±13 mg/dl, p=0.01).

In the FH group, the nature of the LDL receptor gene mutation (81T>G, n=12; 858C>A, n=13; 1285G>A, n=12; 1646G>A, n=22; and 1775G>A, n=25) did not significantly affect the HDL cholesterol levels (49.2±12.3, 48.3±9.5, 52.7±13.1, 48.2±14.9, 45±10.1 mg/dl, respectively, p=0.08). However, homozygous FH patients for the 1775G>A mutation (n=7) showed significantly lower HDL cholesterol levels than the heterozygous FH patients with the same mutation (n=25) (37.5±10.2 versus 45±9.7 mg/dl, p=0.05).

Since the type of LDLR gene mutations did not significantly affect the HDL cholesterol levels in our FH patients, we examined whether gene polymorphisms that possibly affect HDL metabolism might influence the HDL cholesterol levels in the whole group of patients. As shown in Table 2, only the apolipoprotein (apo) E gene polymorphism significantly affected the HDL cholesterol levels. In fact, the presence of the allele E4 (although in a small number of patients, n=13) was associated with lower HDL cholesterol levels, compared to patients who did not carry this allele.

Discussion

Low HDL cholesterol levels are a strong independent predictor of CHD in the general population [13,14]. The risk of CHD is very high in patients with FH mainly because of high total and LDL cholesterol

Table 2. HDL-cholesterol levels in heterozygotes for familial hypercholesterolemia in relation to gene polymorphisms.

Gene	Polymorphism	N of patients	HDL-cholesterol (mg/dl)	p-value
CETP	B1B1	31	48.0±15.2	0.98
	B1B2	39	48.5±12.4	
	B2B2	14	48.4±8.8	
APOA-IV 347	AA	51	48.7±12.9	0.89
	AT +TT	33	48.3±13.1	
APO-IV 360	1/1	76	47.7±12.8	0.2
	1/2	8	53.7±13.4	
ACE	DD	30	48.9±12.8	0.56
	DI	40	49.2±11.9	
	II	14	53.9±15.6	
APOE	nonE4	71	49.3±13.0	0.03
	E4	13	39.9±8.6	

CETP, cholesterol ester transfer protein.
ApoA-IV 347, apolipoprotein A4 polymorphism at position 347.
ApoA-IV 360, apolipoprotein A4 polymorphism at position 360.
ACE, angiotensin-converting enzyme.
ApoE, apolipoprotein E.

plasma levels. Furthermore, FH is often associated with decreased HDL cholesterol levels, as confirmed in our study [16].

The effect of FH on HDL metabolism remains poorly documented. Kinetic studies suggested both increased fractional catabolic rate and decreased absolute production rate of HDL-apoA-I [15]. Our study suggests that homozygote FH patients have even lower HDL cholesterol levels than FH heterozygotes with the same mutation. Furthermore, our data point out that HDL cholesterol levels in FH heterozygotes can be affected by the apoE gene polymorphism. In fact, the presence of apoE4 is associated with lower HDL cholesterol levels. Even though in most studies the common polymorphisms of apoE do not appreciably affect HDL cholesterol concentration in heterozygous FH, low HDL cholesterol levels in apoE4 carriers have been reported in an Italian population as well as in women with heterozygous FH [16-18].

Interestingly, an expanded pool size of apoE in FH has been previously suggested [19]. ApoE-enriched HDL could be catabolised either via a specific receptor-dependent pathway, mediated by apoE, or via the hepatic apoB/E receptor, present in almost half the normal amount in heterozygous FH. Thus, higher HDL-apoE level in heterozygous FH could lead to an increased clearance of HDL in this group [15,19]. Since the allele E4 shows greater affinity with cellular receptors, HDL carrying apoE might exhibit higher catabolic rates in its presence.

We conclude that HDL cholesterol levels in heterozygous FH patients may be affected by the apoE gene polymorphism. Studies with a large number of patients are needed to confirm our results, since they were derived from a relatively small number of patients.

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