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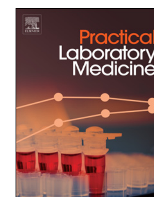

Lipoprotein metabolism in familial hypercholesterolemia: Serial assessment using a one-step ultracentrifugation method

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ABSTRACT

Objectives: It is well known that familial hypercholesterolemia (FH) is a common inherited disorder that can markedly elevate the level of plasma LDL cholesterol. However, little data exists regarding the clinical impact of the plasma triglyceride (TG)-rich lipoprotein fraction, including VLDL and IDL, in FH. Thus, we assessed the hypothesis that the mutations in the LDL receptor modulate lipoprotein metabolism other than the LDL fraction.

Design and methods: We investigated plasma lipoprotein with a one-step ultracentrifugation method for 146 controls (mean age $=61.4\pm17.1$ yr, mean LDL cholesterol $=92.7\pm61.2$ mg/dl), 213 heterozygous mutation-determined FH subjects (mean age $=46.0\pm18.0$ yr, mean LDL cholesterol $=225.1\pm61.2$ mg/dl), and 16 homozygous/compound heterozygous mutation-determined FH subjects (mean age $=26.9\pm17.1$ yr, mean LDL cholesterol $=428.6\pm86.1$ mg/dl). In addition, we evaluated cholesterol/TG ratio in each lipoprotein fraction separated by ultracentrifugation.

Results: In addition to total cholesterol and LDL cholesterol levels, VLDL cholesterol (19.5 ± 10.4, 25.2 ± 19.3, 29.5 ± 21.4 mg/dl, respectively) and IDL cholesterol (8.3 ± 3.7, 16.8 ± 11.5, 40.0 ± 37.3 mg/dl, respectively) exhibited a tri-model distribution according to their status in LDL receptor mutation(s). Moreover, the ratios of cholesterol/TG of each lipoprotein fraction increased significantly in heterozygous FH and homozygous/compound heterozygous FH groups, compared with that of controls, suggesting that the abnormality in LDL receptor modulates the quality as well as the quantity of each lipoprotein fraction.

Conclusions: Our results indicate that cholesterol in TG-rich lipoproteins, including VLDL and IDL, are significantly higher in FH subjects, revealing a tri-modal distribution according to the number of LDL receptor mutations.

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1. Introduction

Familial hypercholesterolemia (FH) is the most common and most severe monogenic hypercholesterolemia characterized by excess deposition of cholesterol in tissues leading to tendon xanthomas and premature coronary artery disease [1–3]. Patients with FH have mutant allele(s) of either of three FH-associated genes (FH genes), namely LDL receptor, apolipoprotein B-100 and proprotein convertase subtilisin/kexin 9 (PCSK9) genes [2], among which, LDL receptor is the leading cause of this disorder. Individuals with two mutations in the LDL receptor gene (homozygous FH) display extremely severe hyper-LDL-cholesterolemia, usually over 400 mg/dl, and develop cutaneous xanthomas, coronary artery disease, and aortic valve stenosis in childhood [1,2]. If the LDL-cholesterol level is not effectively treated, homozygous FH die prematurely from an acute coronary event or heart failure [4]. The frequency of heterozygous FH in the general population has been estimated at about 1 in 500 almost all over the world. Recent advances in genetic analysis has enabled the accuracy of

Abbreviations: FH, familial hypercholesterolemia; PCSK9, proprotein convertase subtilisin/kexin 9; LDL-C, LDL cholesterol; TC, total cholesterol; TG, triglyceride; HDL-C, HDL cholesterol; VLDL-C, VLDL cholesterol; IDL-C, IDL cholesterol

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diagnosis of heterozygous FH and revealed a much higher frequency of FH in certain populations, such as the Afrikaners, Christian Lebanese, Finns, and French–Canadians due to a founder gene effect [1].

Triglyceride (TG)-rich lipoprotein, such as VLDL and IDL, are associated with coronary artery disease [5,6]. However, little data exists regarding the impact of LDL receptor mutations on the metabolism of TG-rich lipoprotein in FH, which could be an additive risk factor for the development of coronary atherosclerosis. Here, we investigated whether the mutations in the LDL receptor modulate lipoprotein metabolism other than the LDL fraction using a one-step ultracentrifugation method.

2. Materials and methods

2.1. Study subjects

We investigated plasma lipoprotein by an ultracentrifugation method for 146 controls, 213 heterozygous mutation-determined FH subjects, and 16 homozygous/compound heterozygous mutation-determined FH subjects. All of the FH subjects have (a) mutant allele (s) in LDL receptor gene. The characteristics of the study subjects are listed in Table 1.

2.2. Genetic analyses

Genomic DNA was isolated from peripheral blood white blood cells using Genomic DNA Purification Kit (Gentra Systems, Minneapolis, MN) and was used for PCR. The genotypes of all the participants in this study were determined as previously described [7–10]. Genetic analyses were approved by the Ethics Committee of Kanazawa University and carried out in accordance with the Declaration of Helsinki (2008) of the World Medical Association. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Informed consents were obtained from all subjects.

2.3. Biochemical analyses

Fasting blood samples were drawn for assays either before the lipid-lowering treatment or after discontinuation of medication for at least 4 weeks. Blood samples were stored at 4 °C immediately in the pharmaceutical refrigerators (MPR-721, Panasonic healthcare, Tokyo, Japan) until the process to obtain serum and plasma for within an hour. Serum concentrations of total cholesterol (TC), TG, and HDL cholesterol (HDL-C) were determined enzymatically (Qualigent®, Sekisui Medical, Tokyo, Japan) using automated instrumentation (LABOSPECT 008, Hitachi High-Technologies, Tokyo, Japan) based on the assays previously described [11–13]. LDL-C concentrations were derived using the Friedewald formula [14]. Plasma were processed to ultracentrifugation immediately after the acquisition.

2.4. Ultracentrifugation analyses

We used a one-step ultracentrifugation method as described in Fig. 1 [15–17]. Plasma (600 μL) was divided into three different tubes (each containing 200 μL), then NaBr solutions with different specific gravity (d=1.006, 1.032, and 1.120) were added. The least dense solution (d=1.006) was overlayed with the plasma, and the other two solutions (d=1.032, and 1.120) were mixed with plasma, adjusting the gravities to d=1.019, and 1.063, respectively. Subsequently, the same three tubes were ultracentrifuged at 42,000 rpm, for 12 h at 10 °C in a Beckman 42 Ti rotor. The top 200 μL containing VLDL ($d<1.006$ g/ml), and the bottom 200 μL containing IDL, LDL, and HDL were extracted separately in the tube with the least dense solution. Similarly, the top 200 μL containing VLDL and IDL ($d<1.019$ g/ml), and the bottom 200 μL containing LDL and HDL were extracted separately in the tube with the higher density solution. Finally, the top 200 μL containing VLDL, IDL, and LDL ($d<1.063$ g/ml), and the bottom 200 μL containing HDL were extracted separately in the tube with the highest density solution. Cholesterol ester (calculated as total cholesterol minus free cholesterol), and TG were analyzed in each ultracentrifugally separated lipoprotein as described above.

Table 1
Characteristics of the study subjects.

Variable	Controls (n=146)	Heterozygous FH (n=213)	Homozygous/compound heterozygous FH (n=16)
Age (yr)	61.4 ± 17.1	46.0 ± 18.0*	26.9 ± 17.1**
Men (%)	91 (62%)	87 (41%)	11 (69%)
BMI (kg/m^2)	25.8 ± 3.8	23.4 ± 2.9	25.4 ± 4.4
TC (mg/dl)	193 ± 21	321 ± 66**	701 ± 160**
VLDL-C (mg/dl)	19.5 ± 10.4	25.2 ± 19.3*	29.5 ± 21.4**
IDL-C (mg/dl)	8.3 ± 3.7	16.8 ± 11.5**	40.0 ± 37.3**
LDL-C (mg/dl)	93 ± 61	225 ± 61**	429 ± 86**
HDL-C (mg/dl)	57 ± 17	53 ± 25	39 ± 10*
TG (mg/dl)	88 ± 12	142 ± 73**	170 ± 86**
VLDL-TG (mg/dl)	42.0 ± 24.0	56.2 ± 55.3*	43.9 ± 28.6
IDL-TG (mg/dl)	7.0 ± 3.2	11.5 ± 7.2*	13.8 ± 10.4**
LDL-TG (mg/dl)	19.0 ± 6.3	35.0 ± 16.4**	68.2 ± 29.5**
HDL-TG (mg/dl)	12.1 ± 6.3	12.8 ± 5.4	12.0 ± 7.2

FH: familial hypercholesterolemia, BMI: body mass index, TC: total cholesterol, VLDL-C: VLDL cholesterol, IDL-C: IDL cholesterol, LDL-C: LDL cholesterol, HDL-C: HDL cholesterol, TG: triglyceride,VLDL-TG: VLDL triglyceride, IDL-TG: IDL triglyceride, LDL-TG: LDL triglyceride, and HDL-TG: HDL triglyceride.

* $p<0.05$.

** $p<0.0001$ vs controls.

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