Clinical and Biochemical Characteristics of Familial Hyperalphalipoproteinemia Associated with Complete Deficiency of Cholesteryl Ester Transfer Activity*

Shizuya YAMASHITA**1, Naohiko SAKAI**1, Yuhya UHEYAMA**1, Tadashi NAKAMURA**1, Masaharu KUBO**1, Yuji MATSUZAWA**1, Hisashi AKIOKA**2, Kazuko HIRANO**3, Tadashi HOSHINO**4, Hiroyuki KAKO**5, Tadao YASUGI**5 and Seiichiro TARUI**1

Introduction

Many epidemiological studies have demonstrated that elevated levels of high density lipoprotein (HDL)-cholesterol are negatively correlated with the incidence of coronary heart disease¹⁻³⁾, suggesting a role of HDL in preventing the development of atherosclerotic lesions⁴⁾. Familial hyperalphalipoproteinemia has been considered to be a genetic disorder characterized by elevated levels of serum HDL-cholesterol⁵⁾. We found two families of familial hyperalphalipoproteinemia with corneal opacification and coronary heart disease^{6,7)}. These probands showed very low activity of hepatic triglyceride lipase and abnormal cholesteryl ester net transfer. In addition, we reported three probands with familial hyperalphalipoproteinemia due to complete deficiency of cholesteryl ester transfer activity (CETA)⁸⁾. These data suggest that familial hyperalphalipoproteinemia may include several disease entities which are different in their pathogenesis.

Recently, we also found two other unrelated probands of cholesteryl ester transfer protein activity deficiency. In the present study, we summarized the clinical and biochemical characteristics of 5 probands with familial hyperalphalipoproteinemia due to complete deficiency of cholesteryl ester transfer activity and also show a preliminary studies on the abnormality in the CETP gene of the patients.

Materials and Methods

The clinical characteristics of the 5 probands are shown in Table 1. Blood samples were collected after overnight fasting. Serum lipids were determined by enzymatic methods using commercial kits. The serum HDL-cholesterol was measured by heparin-Ca⁺⁺ precipitation method. Concentrations of serum apolipoproteins A-I, A-II, B, C-II, C-III and E were determined by single radial immunodiffusion method. Serum lipoproteins were separated by both preparative sequential ultracentrifugation⁹⁾ and analytical ultracentrifugation¹⁰⁾. Plasma lipoprotein profiles were also examined by gel permeation chromatography using combined G5000PW and G3000SW columns obtained from TOSOH (Tokyo, Japan) and the concentration of cholesterol in the eluents was monitored¹¹⁾. Non-denaturing polyacrylamide gradient gel electrophoresis was performed by the method of Blanche et al.¹²⁾ for HDL and McNamara et al.¹³⁾ for LDL using commercial gels from Pharmacia-LKB (PAA 2/16 and 4/30). After the electrophoresis, the gels were stained

* This work was presented at the 20th Annual Meeting of Japan Atherosclerosis Society in June, 1988 (Sendai)

**1 Second Department of Internal Medicine, Osaka University Medical School, Osaka 553, Japan

**2 Nissei Hospital, Osaka

**3 Sakai City Health Research Institute, Sakai

**4 Byoutai-seiri Research Institute, Tokyo

**5 Second Department of Internal Medicine, Nihon University School of Medicine, Tokyo

Received: December 8, 1989

Accepted: March 19, 1990

Table 1 Clinical profiles and serum lipid and apo-protein levels in the probands with complete deficiency of cholesteryl ester transfer activity

	Case 1	Case 2	Case 3	Case 4	Case 5
Age (Y)	45	36	50	54	50
Sex	M	M	M	F	M
CHD	—	—	—	—	—
Corneal Opacity	—	—	—	—	—
T. Chol*	300	340	223	275	341
HDL-Chol*	236	231	157	175	281
TG*	252	329	77	71	74
Apo A-I*	233	258	210	175	232
Apo A-II*	51	55	44	34	37
Apo B*	67	51	80	67	63
Apo C-II*	8.7	9.6	5.4	4.2	10.6
Apo C-III*	34.4	41.2	13.6	15.9	35.3
Apo E*	11.4	19.2	6.2	9.3	21.6

* mg/dl CHD : coronary heart disease

with either Sudan Black B (for lipids) or Coomassie Brilliant Blue G-250 (for protein). Postheparin lipolytic activities (PHLA) were measured as described previously¹⁴. CETA was measured as described previously⁸) using [³H]-cholesteryl ester-labeled HDL₃ as a donor and unlabeled VLDL+LDL (d<1.060 g/ml) as an acceptor, respectively. Genomic DNA of the patients and normolipidemic controls was isolated from white blood cells and digested with several restriction enzymes including Taq I. Southern blotting analysis¹⁵) was performed using cholesteryl ester transfer protein (CETP) cDNA (pCETP. 11) provided by Dennis T. Drayna (Genentec, Inc., San Francisco, CA)¹⁶.

Fig. 1 Pedigree of families with familial hyperalphalipoproteinemia.

Fig. 2 Cholesteryl ester transfer activity in the patients with hyperalphalipoproteinemia.

Table 2 Lipid concentrations of lipoprotein fractions in the probands

	Case 1	Case 2	Case 3	Case 4	Case 5	Control (M, n=14)	
CH*	VLDL	21.7	14.5	15.0	4.5	5.1	10.2± 4.2**
	IDL	3.4	1.9	0.8	2.1	2.1	4.7± 2.2
	LDL	72.0	95.2	65.7	104.1	176.0	110.4± 26.6
	HDL ₂	171.2	219.6	111.8	126.0	190.7	24.6± 8.6
	HDL ₃	21.2	26.5	44.3	19.0	24.9	25.8± 5.2
TG*	VLDL	218.8	125.7	90.8	21.6	36.1	39.4± 19.1
	IDL	6.9	8.1	6.9	4.4	5.7	8.5± 2.1
	LDL	17.9	22.2	25.0	31.3	34.1	27.9± 4.6
	HDL ₂	6.0	8.3	28.8	1.6	4.9	10.8± 3.0
	HDL ₃	2.9	3.4	18.7	1.1	1.1	12.8± 2.6

* mg/dl, ** Mean ± S.D.

Results and Discussion

Table 1 shows the clinical profiles of the 5 probands. None of the patients had any signs of coronary heart disease, corneal opacification and tendon xanthoma. The results of exercise electrocardiogram showed no remarkable findings. The serum concentration of total cholesterol ranged from 223 to 341 mg/dl, while that of HDL-cholesterol was extremely elevated at 157–281 mg/dl. Two patients were accompanied by hypertriglyceridemia. Serum concentrations of apolipoprotein A-I, A-II, C-II, C-III and E were also highly elevated, whereas that of apolipoprotein B was low. Inheritance of hyperalphalipoproteinemia was demonstrated in all families (Fig. 1).

Figure 2 shows the CETA of the probands as well as that of two patients accompanied by low hepatic triglyceride lipase activity, corneal opacifi-

cation and coronary heart disease^{6,7}. Cases 1–5 were totally deficient in CETA, while the latter two patients were not deficient in CETA.

As shown in Table 2, sequential ultracentrifugation disclosed that LDL-cholesterol was slightly decreased and that the increase of HDL-cholesterol was due to the marked increment of HDL₂-cholesterol.

Serum of patients and normolipidemic controls was subjected to high performance liquid chromatography using gel permeation columns and the concentration of cholesterol in the eluents was monitored. Figure 3 shows the elution patterns of serum from patients and a representative normolipidemic control. In the control, the first high peak corresponded to LDL and the latter low peak to HDL. In the patients with complete deficiency of CETA, the peak of LDL was low and

Fig. 3 Elution patterns of sera from patients with complete deficiency of cholesteryl ester transfer activity by HPLC.

the elution position shifted significantly to the right, suggesting that the mean particle size of LDL of the patients is smaller than that of normal LDL. In these patients, on the other hand, the peak of HDL was very high and significantly shifted to the left, suggesting that the mean particle size of HDL of the patients is markedly larger than that of control HDL. Especially in Case 5 (Fig. 3, lower panel), the difference of the lipoprotein profile was so striking. The boundary of LDL and

Fig. 4 Schlieren patterns of sera from patients with complete deficiency of cholesteryl ester transfer activity.

Fig. 5 Gradient gel electrophoresis of sera from the patients with complete deficiency of cholesteryl ester transfer activity.

HDL was unclear and LDL appeared as only a shoulder of the HDL peak.

Figure 4 shows the Schlieren patterns of patients' sera in analytical ultracentrifugation. In a normolipidemic control, one symmetrical peak of LDL was detected, while in the patients LDL was heterogenous and composed of several subspecies. The most predominant subpopulation of LDL in

the patients was heavier or smaller than that in normolipidemic controls.

These findings were also confirmed by the non-denaturing polyacrylamide gradient gel electrophoresis of lipoproteins. As shown in Fig. 5 (left panel), the LDL of the patients was stained very faintly with Sudan Black B, appeared as extremely heterogenous bands and contained smaller LDL as the major subpopulation. On the other hand, the HDL of the patients were markedly large compared with normal HDL (Fig. 5, right panel). Especially in Case 5, a dense band of markedly large HDL was identified between the position of LDL and HDL₂. These lipoproteins may correspond to HDLc which has been detected in hypercholesterolemic animals after loading cholesterol¹⁷.

Lipoprotein lipase (LPL) and hepatic triglyceride lipase (H-TGL) are considered to participate in the interconversion of HDL subspecies (HDL₂ and HDL₃)¹⁸. Postheparin lipolytic activities

Table 3 Postheparin lipolytic activities of the probands

	LPL*	H-TGL*
Case 1	17.2	22.7
Case 2	19.4	25.7
Case 3	6.5	32.6
Case 4	15.3	16.9
Case 5	18.2	19.4
Controls	9.4±2.3**	20.1±10.4

* μ moles/ml/hr, ** Mean $\pm$ S.D.

Fig. 6 Southern blotting of patients' DNA digested with Taq I using CETP cDNA probe. H: Hyper-HDL, N: Normolipidemia.

were measured after injection of heparin (50 U/kg body weight). As shown in Table 3, LPL activity was very high in the patients except for Case 3. However, in contrast to two hyperalphalipoproteinemic cases with corneal opacification as reported^{6,7)}, the H-TGL activity of the patients was within normal limits.

In order to further examine the possible abnormalities in the CETP gene, genomic DNA of the patients was digested with a restriction enzyme Taq I and Southern blotting analysis was then performed using [α -³²P]-labeled CETP cDNA. In normal subjects, two RFLP's with Taq I have been reported (polymorphism A: 9.0 and 7.5 kb, polymorphism B: 5.3 and 4.4 kb)¹⁶⁾. As shown in Fig. 6, the patients' DNA (Cases 1 and 4) showed usual RFLP's with fragments similar in size suggesting that there might be no large-scale alterations in the CETP gene in these CETA-deficient subjects. Similar results were obtained using different restriction enzymes including Pst I, Sac I, Ban I, and Msp I. This was also true in other probands (data not shown). The abnormality in the CETP gene of these patients could, therefore, be a point mutation or a very small deletion or insertion (several base pairs). Further studies are in progress in order to identify the biochemical and molecular basis of the plasma CETA deficiency.

Conclusion

The clinical and biochemical characteristics of cholesteryl ester transfer protein activity deficiency were analyzed in 5 probands with familial hyperalphalipoproteinemia. The present data show that it is accompanied by a unique lipoprotein profile characterized by small polydisperse LDL and large HDL rich in cholesteryl ester. From the Southern blotting analysis of patients' DNA, there might be no gross alterations in the CETP gene

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Summary

We report the clinical and biochemical characteristics of familial hyperalphalipoproteinemia associated with complete deficiency of plasma cholesteryl ester transfer protein (CETP) activity. The study subjects are 5 probands whose serum high density lipoprotein (HDL)-cholesterol levels ranged from 157 to 281 mg/dl. Serum total cholesterol and apolipoproteins A-I, A-II, C-III and E were also elevated, while apolipoprotein B was decreased. Cholesteryl ester had accumulated in the HDL₂ fraction, which was large and rich in apolipoprotein E. The CETP activity was totally deficient in the probands, which was considered to be the cause of hyperalphalipoproteinemia. Non-denaturing polyacrylamide gradient gel electrophoresis, high performance liquid chromatography with gel permeation column and analytical ultracentrifugation disclosed small polydisperse low

density lipoproteins (LDL) along with the markedly large HDL. In a patient whose HDL-cholesterol was 281 mg/dl, lipoproteins with size range between LDL and HDL₂ were also detected. Lipoprotein lipase activity was very high in 4 of these patients, while hepatic triglyceride lipase activity was within normal limits. Restriction fragment length polymorphism (RFLP) of patients' DNA was examined in Southern blotting analysis using CETP-cDNA as a probe. The hybridization patterns in the probands were not significantly different from those of normal controls suggesting that there may be no gross alterations in the CETP gene.

Key words: cholesteryl ester transfer protein, familial hyperalphalipoproteinemia, restriction fragment length polymorphism (RFLP), gene.