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FAMILIAL TYPE I HYPERLIPOPROTEINEMIA CAUSED BY APOLIPOPROTEIN C-II DEFICIENCY

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Summary

A study was made on the clinical and biochemical features of siblings of patients with hyperchylomicronemia and its inherited relationship. It was not a case of the classical type of familial LPL deficiency, but of familial apolipoprotein C-II deficiency. The first patient with apolipoprotein C-II deficiency was reported by Breckenridge et al. and our patients provide the basis for the second report of this new disease. Our observations in this study strongly suggest that familial apolipoprotein C-II deficiency is transmitted by an autosomal recessive mode of inheritance and heterozygotes of this disorder have no abnormalities of plasma lipid and lipoproteins in spite of the reduced plasma apolipoprotein C-II.

Key words: *Apolipoproteins — Chylomicrons — Familial hyperlipoproteinemia — Genetic disease — Lipoprotein lipase — Lipoproteins*

Introduction

Familial type I hyperlipoproteinemia is a rare disease characterized by an extreme increase of chylomicrons in the serum [1]. The biochemical defect of this disease has been thought to be an impaired clearance of chylomicrons because of deficiency of lipoprotein lipase (LPL) which catalyzes the hydrolysis of triglyceride-rich lipoproteins [1,2]. LPL localizes on the surface of

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capillary endothelium and requires a cofactor for its activity, apolipoprotein C-II [3—6], which is present in serum. The enzyme is released into plasma by intravenous injection of heparin. Postheparin plasma also contains another lipolytic enzyme, hepatic triglyceride lipase (H-TGL) [7,8], but only LPL activity is usually deficient in postheparin plasma from patients with familial type I hyperlipoproteinemia [9].

Recently, Breckenridge et al. [10] reported a patient with hypertriglyceridemia associated with deficiency of apolipoprotein C-II. The patient, a 59-year-old male, had clinical and biochemical features similar to type I hyperlipoproteinemia and apparently had LPL deficiency. In his own postheparin plasma, however, LPL was actually present, and only an activator for it — apo C-II — was absent.

In this paper, we describe two cases (a brother and a sister) of familial hyperchylomicronemia caused by apo C-II deficiency; the second case of Breckenridge-type hyperlipoproteinemia, with special reference to the genetic aspect of this new inherited disorder.

Case Report

The patients, siblings, are a 13-year-old Japanese girl (H.T.) and a 15-year-old boy (K.T.) who were referred to our lipid clinic of Osaka University Hospital because of serum turbidity. They were born to a consanguineous marriage and their parents were first cousins (Fig. 1). The patients had never experienced abdominal pain or diarrhea. They both were usually in good health and asymptomatic. Physical examination revealed well developed children. Neither hepatosplenomegaly nor xanthomas were observed. The tonsils were slightly enlarged but not pathological. The results of the laboratory examination are summarized in Table 1. They were unremarkable with the exception of very high serum triglyceride levels and a slightly elevated ASLO. Liver function tests and serum protein electrophoresis were normal. Fasting blood sugar was in the normal range and oral GTT revealed normal insulin and glucose responses. There was no sign of thyroid dysfunction. Serum triglyceride concentrations in H.T. and K.T. were 1094 mg/dl and 1090 mg/dl, and cholesterol 161 mg/dl and 204 mg/dl, respectively, on a mild fat-restricted diet. Family screening was carried
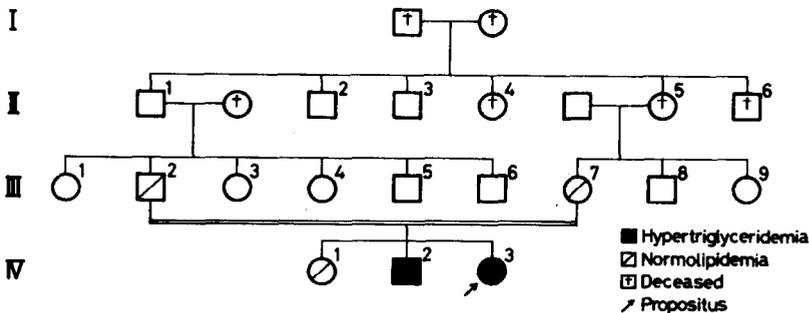

Fig. 1. T kindred, family tree.

TABLE 1

LABORATORY MEASUREMENTS OF H.T. AND K.T.

	H.T.		K.T.	
Urinalysis				
Protein	(—)		(—)	
Sugar	(—)		(—)	
Blood analysis				
RBC ($\times 10^4$/mm^3)	504		476	
Hb (g/dl)	14.6		13.6	
Ht (%)	40.2		37.9	
WBC (/mm^3)	5200		4100	
Serum chemistry				
Na (μEq/l)	137		137	
K (μEq/l)	4.6		4.7	
Cl (μEq/l)	96		98	
Urea nitrogen (mg/dl)	16.2		16.2	
Uric acid (mg/dl)	4.2		3.7	
Amylase (Somogyi U)	75		84	
Total protein (g/dl)	7.6		7.5	
Protein electrophoresis				
Albumin (%)	67.2		69.2	
α_1-Globulin (%)	3.1		2.7	
α_2-Globulin (%)	8.9		8.8	
β-Globulin (%)	6.4		6.8	
γ-Globulin (%)	14.0		12.2	
Cholesterol (mg/dl)	161		204	
Triglycerides (mg/dl)	1094		1090	
ZTT (U)	12		9	
GOT (U)	20		18	
GPT (U)	8		6	
Alkaline phosphatase (K.A.U.)	34.9		24.1	
γ-GTP (mU/ml)	2		2	
T$_4$ (μg/dl)	7.0		6.3	
Serological test				
CRP	(—)		(—)	
ASLO (T.U.)	240		160	
RA	(—)		(—)	
0-GTT (100 g glucose)	BS [a]	IRI [b]	BS [a]	IRI [b]
0 (min)	82	16	80	11
30	119	41	119	24
60	93	33	103	27
90	93	20	98	15
120	93	25	102	17
180	62	11	89	6

[a] Blood sugar (mg/dl); [b] immunoreactive insulin (μU/ml).

out for the parents and another sibling. The clinical and biochemical characteristics of the members of this family are listed in Table 2.

Materials and Methods

For preparative ultracentrifugation, blood samples were collected into EDTA-2Na (1 mg/ml) following an overnight fast. Plasma was separated promptly after low-speed centrifugation at room temperature and kept at 4°C

TABLE 2

CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF MEMBERS OF THE T FAMILY

Kindred position [a]	Age (yr)	Sex	Height (cm)	Weight (kg)	Serum lipids (mg/dl)			Lipoprotein phenotype	O-GTT [e]	Liver
					Ch [b]	TG [c]	PL [d]			
III-2 (father)	47	M	165.2	71.5	174	58	186	normal	borderline	not palp. [f]
III-7 (mother)	40	F	153.2	64.5	176	128	215	normal	borderline	not palp.
IV-1 (sister)	19	F	147.5	56.5	179	105	190	normal	normal	not palp.
IV-2 (patient K.T.)	15	M	171.2	67.0	204	1090	199	Type I	normal	not palp.
IV-3 (patient H.T.)	13	F	144.2	35.0	161	1094	190	Type I	normal	not palp.

[a] As shown in Fig. 1; [b] cholesterol; [c] triglycerides; [d] phospholipid; [e] oral glucose tolerance test; [f] not palpable.

until analysis. Other studies were performed on blood serum unless otherwise indicated.

Lipids were extracted with chloroform-methanol according to the method of Folch et al. [11]. Cholesterol was determined by the method of Zurkowski [12] and phospholipid was obtained by a modification of the method of Cahn et al. [13]. Triglycerides were determined by periodate oxidation and reaction with acetylacetone following treatment of the serum with Zeolite [14]. The amount of total apoproteins in the lipoprotein fractions was determined by the method of Lowry et al. [15], using crystalline bovine serum albumin as a standard. Reaction mixtures were extracted twice with chloroform after color development.

The plasma lipoproteins were analyzed using a combination system of polyacrylamide gel electrophoresis (PAGE), preparative ultracentrifugation and heparin-manganase precipitation. PAGE was performed on Sudan Black B-prestained serum samples using a 3.0% running gel for lipoprotein separation by the method of Narayan et al. [16]. Lipoprotein patterns were obtained by densitometric scanning with a densitometer (Fuji FD-A-IV).

Quantitative ultracentrifugation was carried out in a Hitachi model 55P-7 ultracentrifuge using a RP-40 rotor head according to the method of Hatch and Lee [17]. Plasma samples were centrifuged at 25,000 rpm for 25 min in order to separate chylomicrons. Very low density lipoprotein (VLDL, $d < 1.006$) was isolated in the top fraction from chylomicron-free plasma after 18 h of ultracentrifugation at 40,000 rpm in a solvent with a density of 1.006. The heparin-manganase precipitation method [18] was used to precipitate low density lipoprotein (LDL) from the bottom fraction ($d > 1.006$) of the last ultracentrifugation and high density lipoprotein (HDL) was obtained as the supernatant. The concentrations of cholesterol and triglyceride were measured in each of these lipoprotein fractions.

Tetramethylurea (TMU)-soluble apolipoproteins in chylomicrons and VLDL were analyzed by PAGE according to the method of Kane [19] with a minor modification. Washed chylomicrons or VLDL preparations containing approximately 60—100 μg of total protein were mixed with TMU. After delipidation, an aqueous solution of Bromophenol Blue (BPB) was added as an indicator to each preparation. Electrophoresis was carried out using a 7.5% running gel containing 6 M urea. Gels were stained by Amido Black 10B for 30 min and destained in 7% acetic acid solution followed by densitometer scanning. The relative concentration of apo C-II in VLDL was determined as the ratio of the scanned area of apo C-II peak to that of apo C-III peaks (C-III-1 plus C-III-2) in densitometric scanning of electrophoretograms of TMU-soluble apo-VLDL.

Apo C-II was isolated by the following procedures. TMU-soluble apo-VLDLs from patients with hypertriglyceridemia were separated by electrophoresis on large preparative polyacrylamide gels and visualized under ultraviolet light after staining with the magnesium salt of 8-anilino-1-naphthalene sulfonic acid in saturated $(NH_4)_2SO_4$ [20]. The fluorescent bands of apo C-II were cut out and extracted with water. The extract containing apo C-II was dialyzed and lyophilized.

Postheparin plasma was obtained at 10 min after intravenous injection of sodium heparin (0.1 mg/kg body weight) after an overnight fast. Samples were

collected into tubes containing 0.1 ml of 0.1 M sodium citrate per ml. The plasma was separated by low-speed centrifugation at 4°C. Postheparin lipolytic activity (PHLA) was determined by the modified method of Okuda et al. [21]. The assay system consisted of 0.05 ml of 1 : 4 diluted Ediol, 0.45 ml of 0.1 M Tris-HCl buffer pH 9.0 containing 5% (w/v) bovine serum albumin (Fraction V) with or without 2 M NaCl (final concentration 0.9 M) and 0.5 ml of diluted postheparin plasma. When serum or apo C-II was added to the assay mixture as an activator, 0.25 ml of postheparin plasma and 0.25 ml of serum or apo C-II in saline were used instead of diluted postheparin plasma. After incubation for 1 h at 37°C, released free fatty acids (FFA) were extracted and titrated by the method of Dole [22]. The results were expressed as μEq of released FFA/ml of postheparin plasma/h. The activity obtained in high-salt buffer corresponded to the activity of H-TGL [7,8]. The activity of LPL was calculated by subtracting the value of H-TGL from the total activity obtained in low-salt conditions.

The ability of human serum to activate guinea pig lipoprotein lipase was measured according to the method of Chu et al. [23]. The guinea pig blood samples were withdrawn after 4 min following the intracardiac injection of sodium heparin (20 U/kg body weight). Guinea pig postheparin plasma and human sera obtained from members of the T family and from healthy normo-lipidemic volunteers were stored at −10°C until use. Lipolytic activity of guinea pig postheparin plasma showed a linear increase in proportion to the amount of standard human serum added to the assay mixture at least up to 0.6 ml. Accordingly, the assay was carried out after addition of 0.4 ml of test serum. The activity was expressed arbitrarily as units percent of a standard serum after subtraction of basal lipolytic activity of guinea pig postheparin plasma.

Results

Serum lipids, lipoproteins and apolipoproteins

The serum lipid levels and the lipid composition of lipoproteins in members of the T family are summarized in Tables 2 and 3. In patients H.T. and K.T. serum triglyceride concentrations were very high, 1094 mg/dl and 1090 mg/dl,

TABLE 3

LIPOPROTEIN LIPID CONCENTRATIONS IN THE PATIENTS AND THEIR PARENTS

Subject	Cholesterol (mg/dl)					Triglycerides (mg/dl)			
	Total	Chylo. [a]	VLDL	LDL [c]	HDL [c]	Total	Chylo. [a]	VLDL	LDL + HDL [b]
Patient H.T.	161	98	35	12	16	1094	813	219	62
Patient K.T.	204	119	45	23	17	1090	823	198	69
Father	174	—	2	129	43	58	—	10	48
Mother	176	—	21	105	50	128	—	67	61

[a] Chylomicrons.
[b] Bottom fractions isolated by ultracentrifugation at d = 1.006 from chylomicron-free plasma.
[c] Isolated by heparin-manganese precipitation method from ultracentrifugal bottom fraction at d = 1.006.

respectively, but serum cholesterol levels were within the normal range, 161 mg/dl and 204 mg/dl, on a mild fat-restricted diet. The lipoprotein pattern on PAGE revealed a high chylomicron peak in each patient but normal patterns were obtained from other relatives (Fig. 2). By ultracentrifugal analysis, more than 80% of plasma triglycerides were recovered in the chylomicron fraction from each patient. The amount of cholesterol in this fraction was 98 mg/dl in H.T. and 119 mg/dl in K.T., corresponding to more than half the value for total plasma cholesterol. However, HDL- and LDL-cholesterol were markedly reduced, 12 mg/dl and 16 mg/dl in H.T., and 23 mg/dl and 17 mg/dl in K.T., respectively. In contrast, the parents and an elder sister had completely normal serum lipid and lipoprotein levels.

The densitometric pattern of apolipoproteins in the chylomicron fractions is shown in Fig. 3. The C apoproteins are clearly separated into C-I, C-II, C-III-1 and C-III-2 on PAGE [19], as shown in the control. In our PAGE system, the Rf values for apo C-II, apo C-III-1 and apo C-III-2 (with BPB band as a reference) were 0.52—0.54, 0.60—0.62 and 0.66—0.68, respectively. In contrast to the control, apo C-II band was not detected in chylomicron apoproteins obtained from patient K.T. (Fig. 3). The same result, not shown in the figure, was obtained for VLDL apoprotein in this patient. The lack of apo C-II on PAGE was also observed in triglyceride-rich lipoproteins from patient H.T. On the other hand, the apo C-II band was clearly observed on PAGE of apo-VLDL in their parents. However, the relative concentration of their apo C-II was reduced, approximately 50% of the mean control value (Table 4).
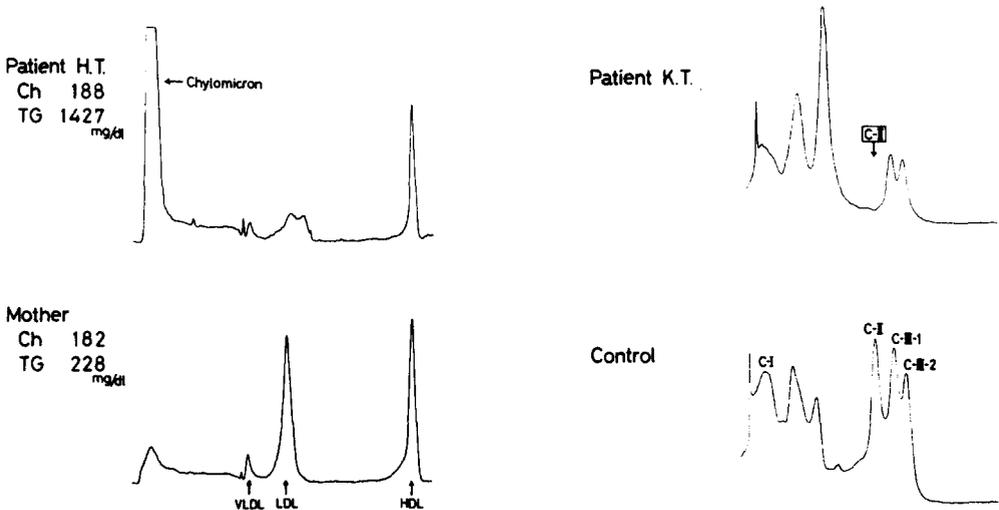

Fig. 2. Densitometric patterns of polyacrylamide gel electrophoresis of serum lipoproteins in patient H.T. (upper) and her mother (lower). The pattern in patient K.T. was similar to that in H.T., and patients' father and sister, to that in their mother.

Fig. 3. Densitometric patterns of polyacrylamide gel electrophoresis of TMU-soluble apoproteins in chylomicrons from patient K.T. (upper) and from a control subject with hyperchylomicronemia previously classified as LPL deficiency (lower). [C-II] (upper panel) indicates the position of apoprotein C-II peak, if present, judged by the Rf value.

TABLE 4

RELATIVE CONCENTRATION OF APOLIPOPROTEIN C-II IN VLDL FROM MEMBERS OF THE T FAMILY AND CONTROL SUBJECTS

Subject	Ratio of $\dfrac{\text{Apo C-II [a]}}{\text{Apo-C-III}}$	% of control value
Patient H.T.	0	0
Patient K.T.	0	0
Father	0.19	48
Mother	0.17	43
Controls	0.40 ± 0.10 [b]	100

[a] Calculated from areas of densitometric peaks. See text.
[b] Mean ± SD for 5 normolipidemic healthy subjects.

Postheparin lipolytic activity

As familial type I hyperlipoproteinemia was strongly suggested in these patients by the lipid and lipoprotein analyses, LPL and H-TGL activities were measured using selective NaCl inactivation in the postheparin plasma. In both patients (H.T. and K.T.) LPL activities were markedly decreased. Furthermore, H-TGL activities were also significantly lower than normal (Fig. 4). Their parents and elder sister, however, had normal LPL with subnormal or normal H-TGL activities (Fig. 4). When the serum from normolipidemic subjects was added to the assay mixture for PHLA, as shown in Methods, LPL activity markedly increased into or above the normal range (Fig. 5). H-TGL was not activated by normal serum. Activation of LPL by human serum was not observed in their parents and other hyperlipidemic subjects. When purified apo C-II (15 μg) was added to the assay mixture in place of whole serum, LPL activity increased approximately to the same extent (Fig. 5).
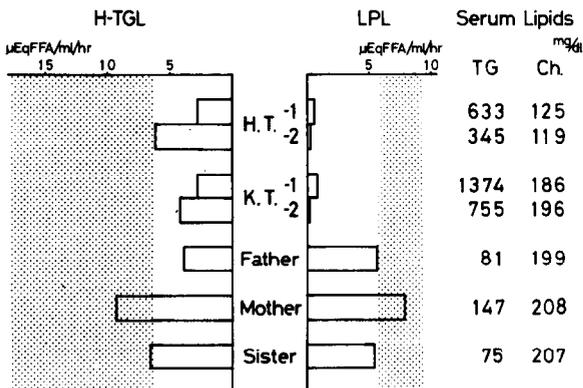

Fig. 4. Postheparin lipolytic activity and serum lipid concentrations in patients and their relatives. Each assay was performed as described in Methods. Both patients were examined before (H.T. —1 and K.T. —1) and after (H.T. —2 and K.T. —2) a low-fat diet. Serum lipid concentrations were measured in blood samples obtained before heparin injection. Shaded areas represent the normal range (mean ± SD).
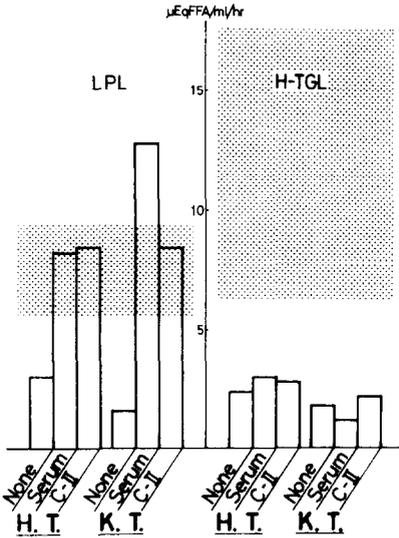
Fig. 5. Lipoprotein lipase and hepatic triglyceride lipase activities in postheparin plasma from patients estimated after the addition of normal serum or purified apo C-II to the assay mixture. 0.25 ml of serum from a normolipidemic subject or 15 μg of apo C-II were added to the assay mixture. Experiments were carried out as shown in the text. Shaded areas represent the normal range (mean ± SD).
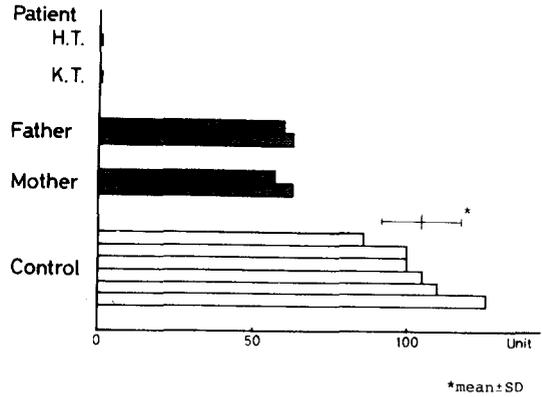
Fig. 6. The levels of lipase activator in the serum of patients and their parents. Results are expressed in arbitrary units percent of the value obtained for a standard serum after subtraction of basal lipolytic activity of guinea pig postheparin plasma. Assay was performed twice in the same serum samples against different guinea pig postheparin plasma in the parents. Control subjects are six healthy volunteers without hyperlipoproteinemia.

The LPL activator assay

Since there is no activator for LPL in guinea pig postheparin plasma [24], triglycerides cannot be hydrolyzed unless rat or human serum is added [25,26]. The activating ability of sera from patients and their parents was determined as lipolytic activity when a constant volume (0.4 ml) of test serum was added to the assay system. In contrast to the healthy control, serum from the patients (H.T. or K.T.) did not activate guinea pig LPL, as shown in Fig. 6. Furthermore, serum from the parents showed approximately only 60% of the control activation ability value, 105 ± 13 U (Fig. 6).

Discussion

Our patients (H.T. and K.T.) had once been thought to have familial type I hyperlipoproteinemia (familial LPL deficiency), because they have heavy chylomicronemia and an apparent deficiency of LPL shown by routine PHLA activity. Hyperlipoproteinemia secondary to systemic lupus erythematosus, diabetes mellitus, hypothyroidism or paraproteinemia, was ruled out by physical and laboratory examinations. Apoprotein analysis of triglyceride-rich lipoproteins demonstrated the lack of apo C-II in both our patients, whereas apo C-II is one of the major apoproteins in triglyceride-rich lipoproteins from normal subjects. Apo C-II has been detected in those other cases of type I

hyperlipoproteinemia with LPL deficiency (Fig. 3). The functional role of the various apolipoproteins has not until now been completely understood. However, it is well known that apo C-II is an important activator for LPL [3—6]. Although a marked reduction in LPL activity was observed by the standard assay procedure of PHLA, where postheparin plasma is used as an enzyme source as well as a sole source of the activator, activity completely recovered after addition of normal serum or purified apo C-II.

These observations indicate that the patients H.T. and K.T. are not really deficient in LPL and that the cause of their chylomicronemia is a deficiency in its activator. On the other hand, patients with typical type I hyperlipoproteinemia show a real deficiency of LPL in postheparin plasma [9] as well as in adipose tissue [27]. This means that LPL activity cannot further increase after addition of normal serum or apo C-II [9,28]. Chylomicron triglycerides in our patients with familial apo C-II deficiency were not hydrolyzed by LPL — inactivated by the lack of an activator — so that the chylomicronemia appeared the same as in the classical type of familial LPL deficiency.

The analysis of apoproteins in HDL was not performed in this study. However, based upon the observation that the serum from the patients failed to activate LPL in guinea pig postheparin plasma (Fig. 6), it can easily be presumed that the same deficiency of apo C-II is present in HDL as in chylomicrons and VLDL, because the C apolipoproteins are interchangeable between triglyceride-rich lipoproteins and HDL in the process of lipoprotein metabolism [29].

Recently Breckenridge et al. [10] have described a 59-year-old male with severe hypertriglyceridemia caused by the absence of apo C-II and suggested his disease be called apolipoprotein C-II deficiency. Our patients have a hyperlipoproteinemia with a biochemical defect similar to Breckenridge's patient. But our patients have never suffered from xanthomas, pancreatitis or diabetes mellitus. The presence of diabetes mellitus, which is not a common complication of familial LPL deficiency, must have aggravated the clinical features of the case of Breckenridge et al. Serum triglyceride concentrations in our patients were usually less than 2500 mg/dl, and relatively lower than the value of Breckenridge's patient or of subjects with typical familial LPL deficiency reported by American and European authors. The regular diet in Japan is comparatively low in fat and calories compared with that in Western countries and it is, therefore, suggested that pancreatitis and xanthomas did not develop in our patients because of the relatively low serum triglyceride levels. Youth may also have been a contributory factor.

Most cases of reported familial LPL deficiency have normal H-TGL activity in postheparin plasma [9]. However, moderately reduced H-TGL activities were obtained for both our patients, in agreement with the data for the first patient with apo C-II deficiency. Reduced H-TGL activity is frequently observed in subjects with hypertriglyceridemia [30]. The physiological role of H-TGL is at present not well known. As the recovery of H-TGL activity was obtained in patient H.T. following reduced serum triglyceride concentration on a low-fat diet (Fig. 4), the decrease in H-TGL seems to be a secondary phenomenon. Furthermore, there is a possibility that abnormal H-TGL or inhibitors of H-TGL may have been present in the patients' postheparin plasma, for

example, an inactive form of LPL in the absence of apo C-II. Ganesan et al. [31] described another LPL (LPL_{C-I}) in human postheparin plasma which requires apo C-I as an activator instead of apo C-II, and showed that LPL_{C-I} activity was absent in postheparin plasma from type I hyperlipoproteinemia. In our patients' sera the band corresponding to apo C-I was detected on PAGE (Fig. 4), although the assay of LPL_{C-I} was not performed in this study.

The parents had normal lipid concentrations and are first cousins. Two of their three children (one of each sex) have been affected with hyperchylomicronemia. Thus, it is strongly suggested that the disorder is inherited as an autosomal recessive mode. It is reasonable to presume that some members of the kindred, including the parents of the patients, must have carried a single mutant allele to become heterozygotes. The consanguineous marriage of heterozygotes could give rise to the birth of homozygotes (patients H.T. and K.T.) bearing two mutant alleles.

The LPL activator assay carried out in this study is thought to indicate only the net LPL activating power of the serum and not the exact amount of apo C-II itself, because apo C-III [5,32] and apo E [33,34] which reportedly inhibit LPL are also present in the same serum. Nevertheless, assuming that the level of LPL activating power obtained here correlates with the apo C-II level in serum, it can be postulated that apo C-II levels in the parents' sera were reduced to approximately half the mean control value (Fig. 6); this strongly suggests that the parents were heterozygotes. This consideration is also supported by the relative concentration of apo C-II in VLDL (Table 4).

The parents were normolipidemic and had almost normal LPL activity in postheparin plasma, which was not increased by the addition of normal serum. These results indicate that the heterozygote for familial apo C-II deficiency has a decreased apo C-II level in his plasma but the amount is enough to activate LPL for hydrolysis of chylomicrons, while the homozygote with no detectable apo C-II suffers from hyperchylomicronemia. Our view of the heterozygote is supported by the observation of Breckenridge et al. that the plasma triglyceride level fell dramatically in a patient with apo C-II deficiency when the apo C-II concentration in his VLDL reached only 5—10% of its normal value after infusion of normal plasma [10].

Addendum

After this manuscript was completed, Cox et al. [35] reported that apo C-II deficiency is inherited as an autosomal recessive trait. This conclusion was based on a study of the relatives of Breckenridge's patient with apo C-II deficiency. Seven homozygotes and ten heterozygotes for the disease were found in the same kindred. Our observations concerning inheritance of the disease agrees well with Cox et al.

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