Familial Combined Hyperlipidemia (FCHL) in Children: the Significance of Early Development of HyperapoB Lipoproteinemia, Obesity and Aging

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It is not easy to make diagnose FCHL in children, since a clear expression of lipoprotein abnormality is unlikely and standard criteria have not yet been established. We investigated eight cases of childhood FCHL and their families with respect to familial history, anthropometric parameters and serum lipoprotein levels, to explore the characteristics of childhood FCHL. To diagnose childhood FCHL it is necessary to clarify both the family history and lipid profiles of the parents. In this study, two prominent features were suggested; that serum TG level is affected by both obesity and age, and also in particular, that a significantly elevated level of serum apoB is a predominant feature of FCHL in childhood. It was found that hyperapoB may be revealed antecedently without other lipid abnormalities at an early age. Regardless of other lipoprotein abnormality, it was suggested that hyperapoB might be added to the early diagnostic criteria for FCHL. *J Atheroscler Thromb*, 2002; 9: 314-320.

Key words: Familial combined hyperlipidemia (FCHL), HyperapoB, Apo E polymorphism, IIb phenotype

Introduction

Familial combined hyperlipidemia (FCHL) was first reported by Goldstein *et al.* in 1973 (1). It is thought to demonstrate various phenotypes of hyperlipidemia (Type IIa, IIb, IV, V) within first degree relatives, is dominantly inherited and frequently causes coronary artery disease (CAD), so it might be the consequence of a single gene abnormality. However, it is a heterogeneous disorder influenced by several genes, and by interactions between these genes and the environment (2). It occurs in about 1% of the general population, and is responsible for 34% of hyperlipidemic coronary artery disease in Japan (3).

It had been considered that lipid abnormalities in FCHL usually do not appear in childhood, but recently, with westernization of the Japanese lifestyle, there has been a

gradual increase in childhood obesity and hyperlipidemia. A Japanese nationwide study also revealed in children increased serum levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG)(4). Showing that lipid abnormalities are now expressed in childhood FCHL. Cortner *et al.* (5) reported that FCHL was the leading cause of familial hyperlipidemia in pediatric referred lipid clinics. However, the degree of FCHL expression in childhood is controversial (1, 3, 6).

In adult FCHL, the association of several lipoprotein abnormalities has been reported, consisting of elevated serum TG and LDL-C levels, a low high-density lipoprotein cholesterol (HDL-C) level and an increased level of apolipoprotein B (apoB) (1,7). FCHL appears to be a heterogeneous condition associated with obesity as well as several different genetic and metabolic alterations (8,9). These lipoprotein abnormalities indicate an increased risk of developing CAD. Thus, FCHL should be identified in children as an early risk for future premature CAD.

However, there has been no formal report of childhood FCHL in our country, so the association of FCHL with obesity and the influence of age on lipid phenotype ex-

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pression remains obscure. Therefore, we studied the features of affected families and the probands in eight cases of childhood FCHL that were identified in our pediatric lipid and obesity clinics.

Subjects and Methods

Eight children (six male, two female) with FCHL were examined. Their ages ranged from 7 months to 19 years, with an average of 9.7 years. The study observation period ranged from two to eight years (Table 1).

The diagnosis of FCHL was made according to the criteria recommended by the Primary Hyperlipidemia Research Group formed by the Japanese Ministry of Health, Labour and Welfare (10). The criteria comprise both major and minor items as follows: Major items: 1) proband reveals phenotypic expression of IIa, IIb or IV, 2) affected first degree relatives show one of the above phenotypes, and at least one of them (including the proband) shows IIb. 3) Familial hypercholesterolemia should be excluded. Minor items: 1) serum TC level less than 300mg/dl in most affected families and proband, 2) observed phenotypic change (e.g., from IIa to IIb), 3) worsening of hyperlipidemia at postpubertal age, 4) not associated with achilles tendon xanthomatosis.

The subjects were screened for obesity using standard procedures for weight and length measurement. Obesity was judged as a value of more than 20% overweight ((actual weight - ideal weight) / ideal weight $\times$ 100). Ideal weights according to age, sex and height were obtained from the Japanese Ministry of Education and Science (11).

Blood samples were obtained by venipuncture in the morning before breakfast. TC and TG levels were measured by enzymatic methods; HDL-C was determined by a direct enzymatic method; LDL-C was calculated by means of the Friedewald equation; Serum levels of apolipoprotein AI, B, CII were measured by immunoelectrophoretic techniques (Sanwa Chemical Co. Ltd., Tokyo, Japan); LDL receptor activity was estimated by the mitogen-stimulated proliferation of lymphocytes using a flow cytometric assay (12, 13). (When the measurement value was set at 100 in healthy adult controls, its value in normal children (41 children, aged from 4 to 16 years) was $80.2 \pm 13.8\%$ (mean $\pm$ SD), unpublished data.)

The apolipoprotein E (apoE) gene was analyzed by the polymerase chain reaction restriction (PCR) fragment length polymorphism (RFLP) method(14). Hyperlipidemia was judged as an LDL-C and/or TG level above the 95th percentile in the proband, parents or affected families.

To establish the pedigree and the presence of dominantly inherited hyperlipidemia, blood samples were obtained from the proband, both parents and available siblings. In all cases in this study, the families showed hyperlipidemia in two successive generations (proband and parents) and occasionally hyperlipidemia or prema-

ture CAD in the third generation (grandparents).

Welch's *t*-test was performed to assess whether differences in the mean values between subjects and healthy schoolchildren controls were significant or not (Table 1). A *p* value less than 0.05 was considered statistically significant.

Results

1 Association with Obesity (Table 2)

Of eight children, five were more than 20% overweight, but none were more than 50% overweight (severe obesity).

2 Lipoprotein and Apolipoprotein Analysis (Tables 1, 2 and 3)

Overall, the mean values of TC and LDL-C were 267 mg/dl and 172 mg/dl, respectively. Both values were significantly higher than those of the controls ($p < 0.05$). Case 1 revealed a TC value over 300 mg/dl at the first visit, which then decreased during the course of investigation to 271 and 231 mg/dl (Table 4). All families were examined for LDL receptor activity in lymphocytes, and all revealed that the estimated LDL receptor function was within the normal limits (the normal range was considered to be above the value of mean -2SD) (Fig. 1; cases 1, 2 and 3). The mean HDL-C value was 54.1 mg/dl; none of the subjects showed a value of less than 40 mg/dl. The mean TG value was 202.8 mg/dl; five cases showed a value of more than 150 mg/dl, and they were also more than 8 years

Table 1. Characteristics of Childhood FCHL and Healthy School Children (ref. 22, 26)

	FCHL Cases (mean $\pm$ SD)	School children ^{22,26)} (mean $\pm$ SD)
No	n = 8	n = 289
Sex (M:F)	6:2	146:143
Age (years)	9.7 $\pm$ 5.7	12.5 $\pm$ 0.5
% ideal weight (%)	19.9 $\pm$ 22.3	M:5.0 $\pm$ 15.4, F:2.2 $\pm$ 16.4
BMI (kg/m ²)	21.6 $\pm$ 5.6	18.9 $\pm$ 3.3
TC (mg/dl)	267 $\pm$ 77.5	169 $\pm$ 26.2
LDL-C (mg/dl)	172.4 $\pm$ 89.7	93.1 $\pm$ 23.7
HDL-C (mg/dl)	54.1 $\pm$ 15.3	60.5 $\pm$ 11.9
TG (mg/dl)	202.8 $\pm$ 142.7	78.7 $\pm$ 34.0
ApoAI (mg/dl)	129.8 $\pm$ 15.3	135 $\pm$ 18.9
ApoB (mg/dl)	126.6 $\pm$ 39.3	63 $\pm$ 15
LDL-C/ApoB	1.42 $\pm$ 0.29	1.44 $\pm$ 0.14
%LDLR (%)	73 $\pm$ 14	—

Welch's *t*-test

TC: Total cholesterol, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TG: Triglycerides, ApoAI: Apolipoprotein AI, ApoB: Apolipoprotein B, %LDLR: LDL-receptor activity in lymphocytes measured by flowcytometric assay

Table 2. Profile of Each Case

Case	Sex	Age (Y)	% Overweight (%)	BMI (Kg/m ²)	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	TG (mg/dl)
1.	M	0.7	-0.1	17.5	454	388	54	59
2.	M	4	-7.5	14.6	218	152	55	54
3.	M	8	27.4	21.3	257	143	60	272
4.	M	10	43.2	23.7	226	138	44	218
5.	F	10	-8.1	16.1	250	153	88	44
6.	M	12	22.9	21.4	220	102	41	386
7.	M	14	46.4	28.6	265	176	50	197
8.	F	19	35.3	29.8	246	127	41	392

% Overweight (%) = (actual weight - ideal weight) / ideal weight × 100

Table 3. Apoprotein Analysis, ApoE Polymorphism and LDL Receptor Activity

Case	Apo-AI (mg/dl)	Apo-B (mg/dl)	Apo-CII (mg/dl)	LDLC/ApoB	Apo-E polymorphism	%LDLR
1.	123	207	—	1.87	E3/3	54
2.	115	96	—	1.58	E3/3	79
3.	133	101	7.8	1.67	E3/3	70
4.	138	103	—	1.34	E3/3	—
5.	160	101	2.2	1.51	E3/2	87
6.	134	106	11.7	0.96	E3/2	—
7.	123	143	5.3	1.23	E4/3	—
8.	112	156	4.7	1.23	E4/3	—

Table 4. Changes in serum lipoprotein levels in cases 1-3

		'98.1	'98.6	'99.6	'00.7	'01.7
Case 1.	Age	7M	1Y	2Y	3Y	4Y
	TC (mg/dl)	454	264	259	271	231
	TG (mg/dl)	59	72	111	62	181
	LDLC (mg/dl)	388	196	173	209	138
	HDLC (mg/dl)	54	54	64	63	57
Case 2.	Age	4Y	5Y		6Y	7Y
	TC (mg/dl)	218	214		172	202
	TG (mg/dl)	54	56		99	72
	LDLC (mg/dl)	152	147		110	125
	HDLC (mg/dl)	55	56		50	63
Case 3.	Age	5Y			7Y	8Y
	TC (mg/dl)	172			201	257
	TG (mg/dl)	69			139	272
	LDLC (mg/dl)	96			111	143
	HDLC (mg/dl)	60			61	60

old and had associated obesity. On the other hand, three cases were type IIa in the Fredrickson/WHO classification of hyperlipidemia; all except one was less than 5 years old and they were all nonobese.

The apoAI level as well as that of HDL-C decreased, while the apoB level increased, coinciding with the LDL-C level in the subjects. Although there seemed to be an

age difference between the subjects and healthy schoolchildren controls, it was not statistically significant. The apoB level was markedly increased in the subjects, who showed twice the control level ($p < 0.005$). Serum apoCII was measured in five cases, four of which revealed an increased level compared with the data from healthy schoolchildren reported by Kim *et al.* (15).

Fig. 1. Cases 1, 2 and 3 were all type IIa, and their father was type IIb with small dense LDL. Case 4 was type IIb and his mother was IIa. His elder brother was also obese, but he did not have hyperlipidemia. Case 5 was type IIa and her father was IIb. Her paternal grandfather died of acute myocardial infarction in his young middle age. Case 8 was type IIb consistently and her mother was IIa.

O.I.; Obesity Index = % Overweight, OB, obesity, HT; hypertension, DM; diabetes mellitus, HL; hyperlipidemia, SIDS; sudden infant death syndrome, AMI; acute myocardial infarction

The LDL-C/ApoB ratio was not significantly lower than the control value (Table 1).

ApoE4 and E2 phenotypes were identified in four of eight children (50%). Among the four, three were obese and all showed both increased LDL-C and TG levels. The remaining subject (Case 5) was neither obese nor had an increased TG level.

3 Familial Study

Fig. 1 shows the pedigree of cases 1, 2 and 3. Two of three brothers had the type IIa lipoprotein phenotype and the other was type IIb. The phenotype of their father was type IIb, who also had small dense LDL ($\bar{D}25.2$ nm). Although their mother had hyperlipidemia, she was known to usually be normolipidemic when not pregnant. Case 1 first visited our clinic seven months after birth and had

been breast fed by his mother. Throughout the time course, the three children showed markedly varying lipid levels (Table 3).

In case 4, both brothers were obese, but the younger one had hyperlipidemia type IIb, and their mother showed IIa. In case 5, the father had type IIb, his daughter IIa, and the grandfather had died of acute myocardial infarction at 48 years of age, but it was not known whether he had hyperlipidemia or not. All families were free from obesity. Recently, in this case, the father was shown to have moderately advanced carotid arteriosclerosis by ultrasound examination. Case 8 was post-adolescent with Down syndrome. Several years previously, hyperlipidemia had been detected, and type IIb was consistently found at examination. Her family contained obese members (including herself) and diabetes mellitus.

Discussion

In the eight children with FCHL in this study, serum levels of TC, LDL-C, TG, apoB, and apoCII were increased, while the levels of HDL-C and apoAI were decreased.

Case 1 showed low LDL receptor activity in lymphocytes, 54%. Since the normal value is more than 80% in adults, more than 60% in children, and even lower in infants (unpublished data), the value of 54% was considered to be within the normal limits in individuals of this age. Beyond the age of one year in this case, the TC level decreased to 200 mg/dl, indicating marked alterations of serum lipid levels by nutritional changes and aging. There may be unknown etiological factors in the relationship between maternal hyperlipidemia and breast feeding. This case may indicate that the difficulty of FCHL diagnosis in children with one examination, because the phenotype and onset of age are variable.

Approximately 1% of the general population has FCHL. It was reported that about 30% of patients who suffer juvenile myocardial infarction before the age of 65 years had FCHL (1). In FCHL, LDL receptor activity is not lowered and the levels of TC and LDL-C are not as high as those observed in familial hyperlipidemia (FH). However, the prevalence of coronary artery disease in FCHL is high, because abnormal TG metabolism may contribute to the development of atherosclerosis. A recent study revealed that the prevalence of coronary artery disease is increased in individuals with TG levels of more than 100 mg/dl (16).

In addition, a high TG level may also be involved in increasing the levels of coagulation factor VII, fibrinogen and plasminogen activator inhibitor type I (PAI-I), acting on the coagulation-fibrinolysis system and increasing the risk of thrombosis (16).

In the FCHL children in this study, the TG level increased with the development of obesity and aging. In particular, it is reported that in individuals with high TG levels, TG level and obesity are strongly positively correlated (17). The probability of developing hyperlipidemia and hyperapoB lipoproteinemia increases with age (8). Since in FCHL, individuals possess genetic factors that make their TG metabolism susceptible to obesity and aging, they may gradually develop FCHL under their influence. High TG values can be attributed to the efflux of fatty acids from adipocytes, abnormal function of lipoprotein lipase (LPL), and the presence of abnormal apoCIII (9).

The father of cases 1, 2 and 3 had small dense LDL, which attracts attention as a factor accelerating the development of atherosclerosis. As reported by Nitta *et al.* (18), it was shown that neonates did not have small dense LDL, and the appearance of small dense LDL increased with age, suggesting that its appearance is greatly influenced by acquired factors. Among patients with FCHL, compared to those without coronary artery disease, those with coronary artery disease develop hypertriglyceridemia,

possess small dense LDL and show hyperapoB lipoproteinemia at a significantly higher rate (6), and furthermore, the rate seems to increase with age. The genes responsible for the development of hyperapoB lipoproteinemia and small dense LDL have not been identified (6, 19, 20), but gene clusters of AI-CIII-AIV on chromosome 11 were reported to be involved in the development of FCHL (21). These gene clusters are involved in 1) absorption of food lipids, 2) translocation of TG, 3) reverse translocation of cholesterol, 4) regulation of lecithin cholesterol acyltransferase (LCAT) activity, 5) regulation of cholesteryl ester transfer protein (CETP) activity, and 6) regulation of apoCII, which functions as a cofactor for LPL activity. They are thus considered to regulate the clearance of intrinsic TG (22). Since the gene clusters show a variety of polymorphisms, their abnormal function may be related to postprandial hypertriglyceridemia.

ApoE polymorphism was determined in the present series. It has been reported that obese adults with the apoE2 and/or E4 gene show a surprisingly high rate of lipid abnormality (23). The same results have been documented even in children, and may indicate that an early expression of lipid abnormality might occur in those with such apoE polymorphism in childhood FCHL.

The normal value of apoB in schoolchildren in Japan is 63 ± 15 mg/dl (24). The average value of apoB in the eight cases with FCHL was increased approximately two-fold. These cases showed high apoB levels of more than 90 mg/dl, including cases with normal TG levels. None of these cases showed small dense LDL. In FCHL, the synthesis of apoB-100 was increased (25), suggesting that hyperapolipoproteinemia preceded hypertriglyceridemia. Okada *et al.* (26) reported that isolated hyperapoB lipoproteinemia was observed in 28.7% of schoolchildren in Japan, who showed normolipidemia and hyperapoB lipoproteinemia, suggesting that some of them could develop FCHL in the future.

The present study revealed that individuals may develop abnormally high values of apoB even without hypertriglyceridemia and small dense LDL, providing an early diagnostic tool for FCHL, if combined with the family history and the serum values of other lipids. In adults, the progression of hyperapoB lipoproteinemia and the advent of small dense LDL can be explained by the notion that the apoB level is increased dependent upon the increment of the number of small dense LDL particles, since LDL and very low density lipoprotein (VLDL) particles each contain one molecule of apoB (27). However, these notions do not explain the factors responsible for the development of hypertriglyceridemia not accompanied by the presence of small dense LDL in children, suggesting the presence of other unknown mechanisms.

The present study suggested that patients with FCHL may be predisposed to TG metabolic abnormality and they are likely to be affected by lifestyle-related factors includ-

ing obesity, aging, diet and physical inactivity, even in children. This study also indicated a decreased risk of cardiovascular disease derived from FCHL if the lifestyle could be controlled from an early age. Although it is difficult to diagnose FCHL precisely in childhood at present, the genetic features and relation to diet in children with FCHL need to be further clarified in the future. It is expected that more effective prevention of cardiovascular disease could be achieved by the adoption of a healthy lifestyle during development.

Conclusions

To make the diagnosis of FCHL in children, blood examination of the families and parents as well as the children is needed, and the familial history related to premature CAD, obesity and diabetes mellitus without hyperlipidemia should also be determined. It has been suggested that it is worthwhile to diagnose type IIb hyperlipidemia of FCHL even in children. TG metabolic abnormalities may be associated with obesity and aging in childhood FCHL. The early expression of lipid abnormality may be attributable to the apoE2 and/or E4 gene in childhood FCHL. It was found that a higher apoB level may be revealed antecedently without other lipid abnormalities at an early age, and thus, an elevated apoB level should be added to the early diagnostic criteria of FCHL in children.

Further study is needed to clarify the relationship between the present results and genetic findings.

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