Table 13. Mutations in ATP-binding cassette transporter-1 gene in Japanese patients.

Disorder	Mutation	Nucleotide Change	Effect on Coding	Author Sequence	References
FHD	A255T	G→A at 1158	Ala→Thr at 255	Nishida Y	Biochem Biophys Res Commun 290: 713–721, 2002
TD	N935H	A→C at 3198	Asn→His at 935	Guo Z	J Hum Genet 47: 325–329, 2002
TD	N935S	A→G at 3199	Asn→Ser at 935	Guo Z	J Hum Genet 47: 325–329, 2002
FHD	3787del4bp	deletion of CGCC from 3787	premature stop at 1224	Huang W	Biochim Biophys Acta 1537: 71–78, 2001
TD	D1229N	G→A at 3805	Asp→Asn at 1229	Huang W	Biochim Biophys Acta 1537: 71–78, 2001
FHD	N1611D	A→G at 5226	Asn→Asp at 1611	Nishida Y	Biochem Biophys Res Commun 290: 713–721, 2002
TD	R1680W		Arg→Trp at 1680	Ishii J	J Hum Genet 47: 366–369, 2002
FHD	R1851X	C→T at 5946	Arg→Stop at 1851	Nishida Y	Biochem Biophys Res Commun 290: 713–721, 2002
TD	R2021W	C→T at 6181	Arg→Trp at 2021	Huang W	Biochim Biophys Acta 1537: 71–78, 2001
TD	In12–In14 del	deletion of 1221 bp	deletion of exons 13, 14	Guo Z	J Hum Genet 47: 325–329, 2002
TD	In16–In31 del	deletion of 19.9 kb	deletion of exons 17–31	Guo Z	J Hum Genet 47: 325–329, 2002

All mutations have been reported after the closing of registration to the Research Committee.
FHD: familial high-density lipoprotein deficiency, TD: Tangier disease.

Table 14. Mutations in Japanese patients with sterol strage disorders.

Disorder	Gene	Mutation	Nucleotide	Effect on Coding Change	Author Sequence	References
CTX	Cyp27	R104W[1]	C→T at codon 104	Arg→Trp at 104	Nakashima N	J Lipid Res 35: 663–668, 1994
CTX	Cyp27	E162X	G→T at codon 162	Glu→Stop at 162	Wakamatsu N	J Neurol Neurosurg Psychiatry 67: 195–198, 1999"
CTX	Cyp27	R362H	G→A at codon 362	Arg→His at 362	Chen W	Biochim Biophys Acta 1317: 119-126, 1996
CTX	Cyp27	P368R	G→C at codon 368	Pro→Arg at 368	Okuyama E	J Lipid Res 37: 631–639, 1996
CTX	Cyp27	R372Q	G→A at codon 372	Arg→Gln at 372	Chen W	J Lipid Res 38: 870–879, 1997
CTX	Cyp27	intron 7+1G→A	G→A at intron 7+1	5' splice signal	Shiga K	J Neurol Neurosurg Psychiatry 67: 675–677, 1999
CTX	Cyp27	R441W	C→T at codon 441	Arg→Trp at codon 441	Kim KS	J Lipid Res 35: 1031–1039, 1994
CTX	Cyp27	R441Q	G→A at codon 441	Arg→Gln at codon 441	Kim KS	J Lipid Res 35: 1031–1039, 1994
Sitosterolemia	ABCG5	R419H	G→A at 1396	Arg→His at 419	Lee MH	Nat Genet 27: 79–83, 2001
Sitosterolemia	ABCG5	exon 3 del	deletion of exon 3		Lu K	Am J Hum Genet 69: 278–290, 2001
Sitosterolemia	ABCG5	R408X	C→T at 1362	Arg→Stop at 408	Lu K	Am J Hum Genet 69: 278–290, 2001
Sitosterolemia	ABCG5	R389H	G→A at 1306	Arg→His at 389	Lu K	Am J Hum Genet 69:278–290, 2001
Sitosterolemia	ABCG5	R550S	A→C at 1791	Arg→Ser at 550	Lu K	Am J Hum Genet 69:278–290, 2001

ABCG5: ATP-binding cassette transporter subfamily G member 5, CTX: cerebrotendinous xanthomatosis, CYP27: sterol 27-hydroxylase.
[1]: Mutation resistered to the Research Committee

So-called "common mutations" have been described in Japanese patients with FH, CETP deficiency and LPL deficiency. It has been reported that in patients with CETP deficiency (activity) < 75% of control), 65.7% had one of the 2 common mutations (1,451 + 1G > A and D442G) or both, and that in patients with marked HALP (HDL-cholesterol > 100 mg/dl), 57.5% had at least one of these common mutations (25), suggesting that genetic diagnosis could be feasible in CETP deficiency. On the other hand, prevalence of common mutations in the LDLR gene is relatively low (3, 4), indicating that genetic diagnosis of patients with FH may not be feasible.

FCHL is speculated to be the most prevalent disorder in genetic hyperlipidemia, however, the molecular mechanism has not been clarified. Similarly, the cause of FH-like syndrome, characterized by hypercholesterolemia, premature atherosclerosis and tendon xanthoma without reduction in LDLR activity, is also unknown. Further investigation should be performed to elucidate the molecular mechanisms of such disorders.

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Journal of Atherosclerosis and Thrombosis Vol. 11, No. 3
Original Articles

Clinical Features of Familial Hypercholesterolemia in Japan in a Database from 1996–1998 by the Research Committee of the Ministry of Health, Labour and Welfare of Japan

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Familial hypercholesterolemia (FH) is one of the most common primary hyperlipidemias, characterized by a heterozygous or homozygous phenotype for a severe serum low-density lipoprotein (LDL)-cholesterol level and advanced atherosclerosis, leading to coronary artery diseases (CAD). Various kinds of mutations in the LDL receptor gene responsible for the genetic disease have been identified since the human LDL receptor gene has been identified. In this study, the clinical features of FH were investigated using a database based on nationwide surveillance for primary hyperlipidemia and related disorders by the Research Committee on Primary Hyperlipidemia. The clinical features and the frequencies of accompanying vascular diseases in 660 cases of FH homozygotes and heterozygotes showed that the incidence of CAD was negatively associated with plasma HDL-cholesterol levels, but not with plasma LDL-cholesterol levels, in 641 FH heterozygotes. Risk factor analyses revealed that hypertension, male, smoking, low HDL-cholesterol levels, age > 50 y, diabetes mellitus, and hypertriglyceridemia were positive risk factors for CAD. The summarized gene analysis in FH heterozygotes showed at least 4 mutations in the LDL receptor gene as common mutations in Japan. The average serum lipids and frequency of CAD based on each common mutation suggested that their clinical features are in part determined by responsive mutations in the LDL receptor gene. ***J Atheroscler Thromb, 2004; 11: 146–151.***

Key words: Database, LDL receptor, Mutation, Heterozygote

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Introduction

Various gene abnormalities causing primary hyperlipidemia have been identified in our country. Surveillance for the current gene analysis has been started by the Research Committee on Primary Hyperlipidemia (Chairperson: Professor Toru Kita, Kyoto University), organized

by the Ministry of Health, Labour and Welfare in 1996 (1).

Familial hypercholesterolemia (FH) is one of most frequent primary hyperlipidemias. The underlying gene abnormalities have been identified on the low-density lipoprotein (LDL) receptor gene locus. A previous investigation based on the database created by the Research Committee on Primary Hyperlipidemia reported that approximately 80 mutations have been identified in various regions in the LDL receptor gene in Japan, and some of them may be more prevalent than others, comprising the so-called "common mutations" (2–4). Furthermore, the possibility of different responses of mutations against cholesterol-lowering therapy was suggested in FH (5). In this study, the clinical features of FH in Japan were investigated using the above database for mutations in Japanese patients with primary hyperlipidemia and related disorders. Additionally, the clinical phenotypes in FH with the common mutations were studied using three databases based on different areas in Japan.

Methods

The database for mutations in Japanese patients with primary hyperlipidemia and related disorders by the Research Committee on Primary Hyperlipidemia organized by the Ministry of Health, Labour and Welfare was used for the analysis in this study (2–4). The analyses of phenotypes for the common mutations were performed based on the databases provided by Drs. Maruyama and Yamashita (Osaka University), Drs. Kajinami and Mabuchi (Kanazawa University) and Drs. Bujo and Saito (Chiba University). The results are shown as mean ± SD for each index. Comparison of data was performed using the Student's *t*-test and/or ANOVA, and a value of $p < 0.05$ was considered significant. Logistic analyses were performed to obtain odds ratios for coronary artery disease (CAD).

Results

Clinical profile of familial hypercholesterolemia in Japan

The clinical features of 660 registered cases of FH (19 cases of homozygotes including two compound heterozygotes, and 641 cases of heterozygotes) were analyzed (Table 1). Both in homozygotes and heterozygotes, more women were registered than men (63% and 54%, respectively). The average age of cases for homozygotes and heterozygotes was 26 y (4–49 y) and 51 y (1–85 y), respectively. The average serum total cholesterol (TC) and LDL-cholesterol (LDL-C) of homozygous FH was 686 mg/dl and 582 mg/dl, respectively. The average serum TC and LDL-C of heterozygous FH was 324 mg/dl and 248 mg/dl, respectively. The proportion of type IIb hyperlipidemia in the WHO classification, which indicates hypertriglyceridemia as well as hypercholesterolemia, was 22% for homozygotes and 23% for heterozygotes. The serum high density lipoprotein-cholesterol (HDL-C) level was 35 mg/dl in homozygotes.

The occurrence of arcus cornea was 85% in homozygotes and 38% in heterozygotes. Tendon xanthoma was observed all in homozygotes and in 82% of heterozygotes. The occurrences of skin xanthoma were not as frequent as those of tendon xanthoma: 13% in homozygotes and 8% in heterozygotes. The occurrences of xanthelasma were 31% in homozygotes and 9% in heterozygotes. There was a history of CAD in 73% of homozygotes and 24% of heterozygotes. Other atherosclerotic diseases, cerebrovascular diseases (CVD) and arteriosclerosis obliterans (ASO), were not observed in homozygotes, and observed at 3% and 2% in heterozygotes, respectively.

Serum lipids and CAD in heterozygous FH

In heterozygous FH, the clinical profiles of the 296 male cases were compared with those of the 345 female cases (Table 2). The average age of the male and female cases was 49 y and 54y, respectively. The average body mass index (BMI) of the male and female cases was 23.5 kg/m^2 and 22.6 kg/m^2, respectively. The average serum cholesterol levels of heterozygous FH were not significantly different between the males and females. The TG and

Table 1. Clinical features of FH in Japan.

	Homozygotes	Heterozygotes
n	19 (2:comp.hetero)	641
Sex (M/F)	7/12	296/345
Age (y)	26 ± 14 (19)	51 ± 15 (548)
BMI (kg/m^2)	17.2 ± 3.3 (8)	23.0 ± 3.3 (566)
TC (mg/dl)	686 ± 250 (19)	324 ± 71 (568)
LDL-C (mg/dl)	582 ± 132 (15)	248 ± 67 (512)
TG (mg/dl)	157 ± 117 (17)	132 ± 85 (551)
HDL-C (mg/dl)	35 ± 21 (16)	47 ± 14 (517)
IIa/IIb	14/4	430/130
Arcus cornea (%)	85% (13)	38% (498)
Xanthoma (%)	100% (16)	87% (556)
Xanthelasma (%)	31% (16)	9% (556)
Skin (%)	13% (16)	8% (556)
Tendon (%)	100% (16)	82% (556)
CAD (%)	73% (15)	24% (538)
CVA (%)	0% (3)	3% (477)
ASO (%)	0% (3)	2% (475)

Mean ± SD, Numbers in parentheses show the cases for analysis.

HDL-C levels were significantly higher in the males than in the females; the proportion of type IIb hyperlipidemia was higher in the males than in the females. There were no significant differences between the males and females in the occurrence of arcus cornea, skin xanthoma, or tendon xanthoma.

Figure 1 shows the relationships between serum lipids and occurrence of CAD in heterozygous FH. There was no obvious relationship between TC, LDL-C or triglyceride (TG), and CAD occurrence. However, the occurrence was most frequent in cases with LDL-C of more than 320 mg/dl or TG of more than 250 mg/dl. Notably, there was a clear negative tendency in the relationship between CAD occurrence and HDL-C; an increased HDL-C level was associated with a decreased CAD occurrence, and the occurrence at an HDL-C level of more than 60 mg/dl was reduced to about one-fifth of that at less than 35 mg/dl.

Males with FH showed a significantly higher occurrence of CAD than females (Fig. 2). There was no significant difference in CVD occurrence. Males with FH showed a higher tendency of occurrence of ASO than females, although not significant by. Further analysis of the heterozygotes was performed separately for type IIa and IIb hyperlipidemia, in order to determine the significance of accompanying hypertriglyceridemia for the occurrence of CAD (Fig. 3). There was no significant difference in CAD occurrence between the two types in the males. In the females, the cases with type IIb showed an obviously increased CAD occurrence compared to the cases with

Table 2. Clinical features of FH heterozygotes in Japan.

	Males	Females
n	296	345
Age (y)	49 ± 13 (244)	54 ± 16 (304)
BMI (kg/m²)	23.5 ± 3.3 (260)	22.6 ± 3.2 (306)
TC (mg/dl)	324 ± 70 (261)	325 ± 72 (307)
LDL-C (mg/dl)	249 ± 132 (261)	248 ± 69 (279)
TG (mg/dl)	153 ± 99 (256)	114 ± 65 (295)**
HDL-C (mg/dl)	42 ± 12 (237)	50 ± 15 (280)**
IIa/IIb	179/81	230/49*
Arcus cornea (%)	40% (229)	36% (269)
Xanthoma (%)	88% (254)	88% (302)
Xanthelasma (%)	6% (254)	10% (302)
Skin (%)	11% (254)	10% (302)
Tendon (%)	82% (254)	83% (302)

Mean ± SD, Numbers in parentheseis show the cases for analysis.
**: $p < 0.001$, *: $p < 0.0001$, vs male

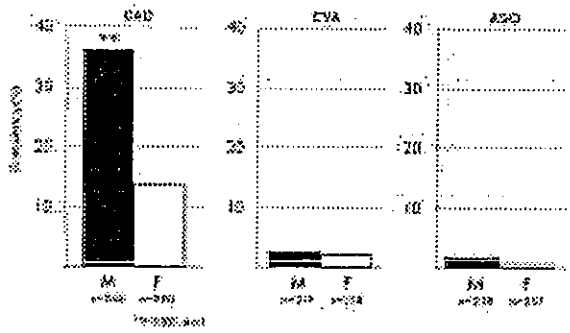

Fig. 2. Occurrence of vascular complications in FH heterozygotes.

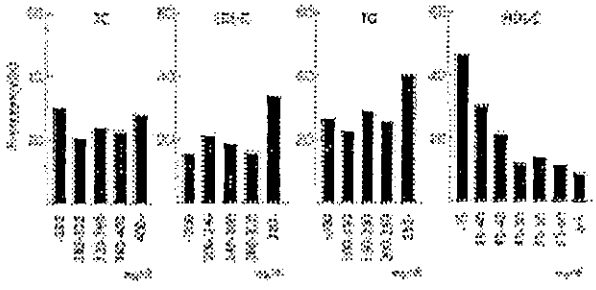

Fig. 1. Serum lipids and occurrence of CAD in FH homozygotes and heterozygotes.

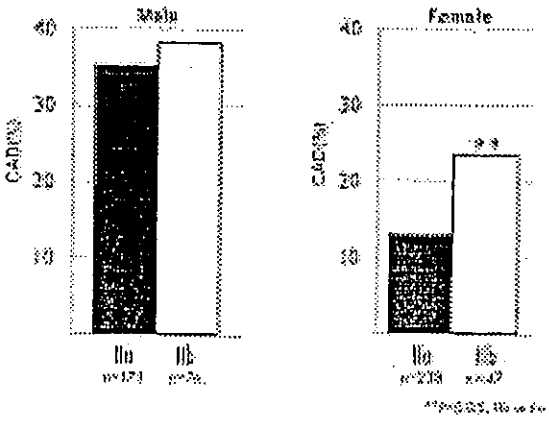

Fig. 3. Occurrence of CAD in the presence or absence of TG > 150 mg/dl in FH heterozygotes.

type IIa. Next, the significance of hypoalphalipoproteinemia (HDL-C of less than 40 mg/dl) was analyzed (Fig. 4). The cases with hypoalphalipoproteinemia showed increased CAD occurrence in males. Together with the results in Figure 1, serum HDL-C level seems to be rather well or related with CAD occurrence in FH heterozygotes.

Logistic regression analyses for CAD revealed that male, age > 50 y, smoking, hypertension, diabetes mellitus, TG > 150 mg/dl and HDL < 40 mg/dl were associated with an increased risk of CAD (Fig. 5). The cumulative incidence of CAD is shown in Fig. 6. Males with FH developed CAD 10-20 years earlier than females with FH.

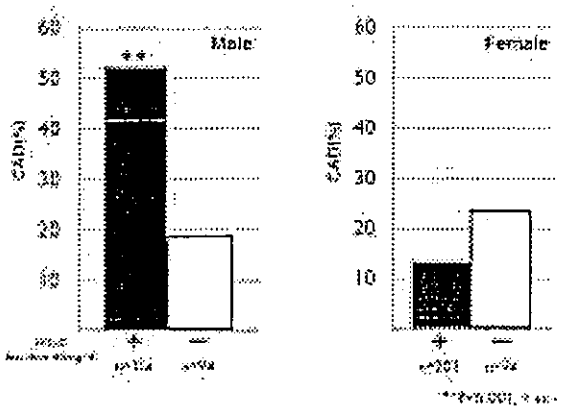

Fig. 4. Occurrence of CAD in the presence or absence of HDL-C < 40mg/dl in FH heterozygotes.

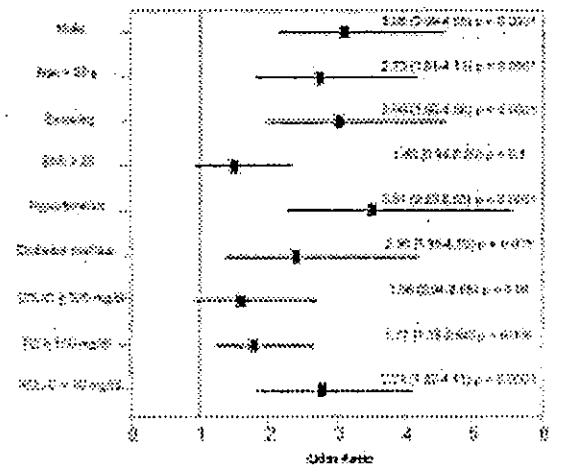

Fig. 5. Association between occurrence of CAD and conventional coronary risk factors in FH heterozygotes.

Phenotype of heterozygous cases with the "common mutations" in the LDL receptor gene

Common mutations in the LDL receptor gene have been suggested to exist in FH heterozygotes in Japan (6-8). The common mutations consist of four mutations: K790X in exon 17, C317S in exon 7 (FH Wakayama), P664L in exon 14 (FH Kanazawa-2) and 1845 + 2 T to C (FH Niigata). Previous studies have suggested that the total number of cases with the four mutations accounts for about 30% of heterozygous FH in Japan (6, 7). In order to determine the frequencies and clinical features of cases with the common mutations in various areas in Japan, the four mutations were intensively analyzed in the cases with FH in the Chiba area, and the frequencies and phenotypes were analyzed in comparison with previous data from other areas. The occurrences of the four mutations in the 154 cases of heterozygous FH in Chiba were observed as 5.8%, 4.5%, 5.2% and 1.9% for 1847TC, K790X, P664L and C317S, respectively (Table 3). The frequencies in Chiba were about one-half and one-third to-fourth for 1847TC and C317S, respectively, compared to those in the Osaka area. The frequencies of K790X and P664L were almost the same between both areas. The frequencies of P664L in both areas were also similar to that in the Kanazawa area. The frequency of cases with the four mutations was up to 17.5% of all FH cases analyzed in the Chiba area.

The clinical features of FH with the common mutations were analyzed next. The serum levels of both TC and LDL-C in the cases with the common mutations were increased compared to the average levels of the 641 cases with heterozygous FH (Table 4). The summarized data of the Chiba and Osaka areas (provided by Drs. Maruyama and Yamashita) showed that cases with any

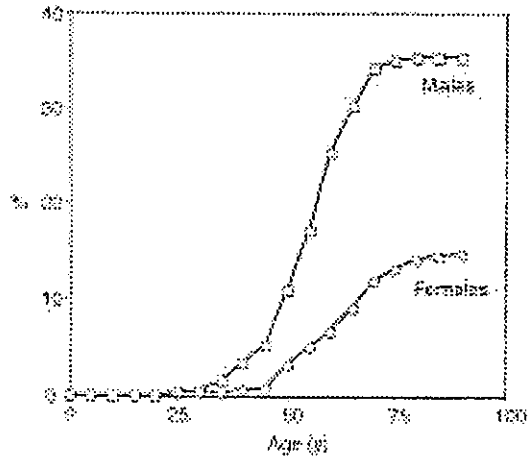

Fig. 6. Cumulative incidence of CAD in FH heterozygotes.

of the common mutations showed increased average TC and LDL-C levels compared to those in the data of heterozygous FH (Table 5). The frequencies of CAD occurrence were 52%, 54%, 36% and 54% for 1847TC, K790X, P664L and C317S, respectively. These occurrences in the cases with the common mutations were increased compared to those in the data of heterozygous FH, although the average age was younger in the cases with the common mutations. However, the average levels of TC and LDL-C of the cases with P664L (FH Kanazawa 2) in the Kanazawa area (provided by Drs. Kajinami and Mabuchi) were obviously decreased compared to the summarized levels (Table 6).

Discussion

The clinical features of FH in Japan were investigated using a database mutations in Japanese patients with primary hyperlipidemia and related disorders. Additionally, clinical phenotypes in FH with the common mutations were studied using three databases based on different areas in Japan. The clinical features and the frequencies of accompanying vascular diseases in 660 FH homozygotes and heterozygotes suggested that the occurrence of CAD has increased in males, and has not changed much in females, compared to that in the database from 1986, respectively (9). However, it is impossible to compare the occurrence exactly as the criteria for the definition of FH is not the same between the previous and current studies. There was no clear relationship between CAD occurrence and TC level in the annual report of the research group for primary hyperlipidemia in 1986, which is not clearly different from the results of this study. However, the annual report in 1986 showed a relationship between TG and CAD, which was not obviously observed in this study. The relationship

Table 3. Frequencies of common mutations in 3 areas in Japan.

Mutation	Chiba (n = 154)	Osaka (n = 120)	Kanazawa (n = 201)
1847TC	5.8% (9)	13.3% (16)	
K790X	4.5% (7)	6.7% (8)	
P664L (Kanazawa 2)	5.2% (8)	3.3% (4)	3.0% (6)
C317S	1.9% (3)	6.7% (8)	
Tonami 1			5.0% (10)
Tonami 2			5.5% (11)
Total	17.5% (27)	30.0% (36)	13.4% (27)

Table 4. Clinical features of FH heterozygotes with common mutations in the Chiba area.

Mutation	K790X	P664L	1847TC	C317S
n	7	8	9	3
Age (y)	50 ± 12	50 ± 17	50 ± 5	33 ± 20
Sex (M/F)	5/2	2/6	4/5	2/1
BMI (kg/m²)	23.2 ± 2.7	22.8 ± 2.7	21.3 ± 4.1	20.8 ± 1.6
TC (mg/dl)	406 ± 25	398 ± 73	384 ± 35	349 ± 70
LDL-C (mg/dl)	335 ± 40	323 ± 77	329 ± 43	292 ± 65
TG (mg/dl)	164 ± 86	146 ± 54	118 ± 57	133 ± 39
HDL-C (mg/dl)	38 ± 11	46 ± 13	44 ± 14	31 ± 4
ATT (max, mm)	14 ± 8	11 ± 5	19 ± 7	8 ± 1
Xanthoma (%)	20	17	25	0
CAD (%)	40	43	50	33

Mean ± SD, Xanthoma does not include Achilles tendon thickness.

Table 5. Clinical features of FH heterozygotes with common mutations in 3 areas (National Cardiovascular Center, Osaka University, Chiba University).

Mutation	K790X	P664L	1847TC	C317S
n	13	16	29	13
Age (y)	44 ± 14	43 ± 18	44 ± 14	41 ± 14
Sex (M/F)	6/7	4/12	12/17	7/6
BMI (kg/m²)	23.2 ± 1.7	22.7 ± 2.4	21.1 ± 2.9	22.2 ± 2.2
TC (mg/dl)	414 ± 90	377 ± 63	355 ± 74	381 ± 67
LDL-C (mg/dl)	346 ± 99	300 ± 64	282 ± 74	320 ± 63
TG (mg/dl)	149 ± 68	131 ± 55	140 ± 78	134 ± 55
HDL-C (mg/dl)	40 ± 12	51 ± 12	45 ± 14	34 ± 7
CAD (%)	54	36	52	54

Mean ± SD.

Table 6. Clinical features of FH heterozygotes with common mutations in the Kanazawa area.

Mutation	Kanazawa 2	Tonami 1	Tonami 2
n	15	22	34
Age (y)	40 ± 16	48 ± 19	52 ± 21
TC (mg/dl)	309 ± 45	338 ± 42	310 ± 69
LDL-C (mg/dl)	251 ± 43	272 ± 43	222 ± 61
TG (mg/dl)	126 ± 51	97 ± 41	158 ± 126
HDL-C (mg/dl)	35 ± 14	46 ± 12	40 ± 11

Mean ± SD.

observed between HDL-C and CAD was almost the same in both reports. The importance of accompanying hypertriglyceridemia in females and hypoalphalipoproteinemia in males for an increased occurrence of CAD was clarified in FH. Risk factor analyses revealed that hypertension, male, smoking, low HDL-cholesterol levels (< 40 mg/dl), age > 50 y, diabetes mellitus, and hypertriglyceridemia (> 150 mg/dl) were positive risk factors for coronary heart disease. The cumulative incidence of CAD showed that males with FH developed CAD 10–20 years earlier than females with FH.

A summarized gene analysis for hyperlipidemia showed at least four mutations in the LDL receptor gene as common mutations in Japan (6,7). The increased average serum lipids and frequencies of CAD suggested the differing phenotypic severity among FH cases based on various mutations. Further analysis of the clinical features of cases with the common mutations should be performed to determine the clinical severity in these cases.

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Survey of gene polymorphisms on five genes related to triglyceride and HDL-cholesterol in the general Japanese population in 2000

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Running title: Survey of gene polymorphism in Japanese

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Abstract

We studied the association of six common gene polymorphisms of four genes related to lipid metabolism with serum lipid levels. We selected single-nucleotide polymorphisms (SNPs) in the cholesteryl ester transfer protein (*CETP*), lipoprotein lipase (*LPL*), hepatic triglyceride lipase (*LIPC*), and apolipoprotein CIII (*APOC3*), and studied 2267 individuals in the general Japanese populations. There was a significant interaction in *CETP* polymorphism (D442G, Int14 +1 G_A, and TaqIB), *LPL* polymorphism (S447X), and *LIPC* polymorphism (-514_CT) with HDL-cholesterol levels. We also found a significant interaction in *LPL* polymorphism (S447X) and *APOC3* polymorphism (SstI) with triglyceride levels. This is the largest database showing the association of common genetic variants in lipid metabolism with serum lipid levels in the general Japanese population. Further study is necessary to elucidate the role of these gene polymorphisms on cardiovascular events.

Introduction

Hyperlipidemia is a major risk factor for coronary artery disease (CAD) [1]. In contrast to the sharp decline in both serum cholesterol and the mortality from CAD in the United States and Western Europe, remarkable increases in serum cholesterol levels as well as CAD mortality have been anticipated in the Asian-Pacific area due to industrialization and modernization of the lifestyle [2]. The importance of lifestyle is also proved by the fact that Japanese who migrated to Hawaii and California, for example, showed higher levels of serum cholesterol and a higher incidence of CAD than people in Japan [3]. Thus, dietary habits and other environmental factors affect serum cholesterol levels and CAD mortality in the population. However, the genetic trait is also an important determinant of serum lipid levels.

Major mutations have been described coding for the low-density lipoprotein (LDL) receptor, apolipoprotein B, and so forth, affecting mainly the serum LDL-cholesterol levels [4, 5]. However, plasma triglyceride (TG) and high-density lipoprotein (HDL) cholesterol levels are also considered established risk factors for CAD [6]. Therefore, association of common gene variants at candidate genes with changes in TG and HDL-cholesterol levels would be important determinants for CAD risk. Considering the recent prevalence of the metabolic syndrome, it would be also intriguing to examine the effect of these gene polymorphisms on the development of the metabolic syndrome. So far in Japan, however, a large-scale analysis has not been performed on common gene variants related to lipid metabolism.

In 2000 we have conducted the lipid survey in the general Japanese population of 12,839 people all over the country (J Atheroscler Thromb, in press). In this survey we also tried to examine the frequency of common gene polymorphisms of four genes related to lipid metabolism and show the association with serum lipid levels. Among the proteins involved in lipid metabolism, we chose the following 4 genes because of the association with TG or HDL-cholesterol. Cholesteryl ester transfer protein (CETP) facilitates the transfer of cholesteryl ester from HDL to apolipoprotein B-containing lipoproteins [7]. CETP is a key protein in reverse cholesterol transport and its deficiency is associated with hyperalphalipoproteinemia [8-10]. Among several polymorphisms of the *CETP* gene, G to A substitution at the 5' splice donor site of intron 14 (Int14 +1 G_A) and a missense mutation of exon 15 (D442G) in the *CETP* gene are common mutations of hyperalphalipoproteinemia in Japanese [11, 12]. The Int14 +1 G_A mutation results in a null allele: homozygotes with the mutation have no CETP in plasma and have marked elevation of HDL-cholesterol [9]. TaqIB polymorphism of the *CETP* gene is one of the most studied polymorphisms worldwide. The D442G mutant is near the carboxy terminal region of CETP shown to be essential for the function [13, 14]. The B2 allele of TaqIB polymorphism in intron 1 is associated with decreased CETP levels and high HDL-cholesterol levels [15] and with coronary heart disease risk in Framingham Study [16]. Therefore, we selected these three polymorphisms for our analysis.

Lipoprotein lipase (LPL) is one of the key enzymes in the metabolism of the TG-rich lipoproteins. Among the several polymorphisms of the *LPL* gene we chose S447X polymorphism,

which is common with the allele frequency being approximately 20% in healthy individuals [17-19]. Hepatic lipase (LIPC) is also a member of the lipase superfamily and plays an important role in the metabolism and modeling of both pro- and anti-atherogenic lipoproteins [20]. Among the several polymorphisms we selected -514C_T SNP. This polymorphism, located in the promoter region of the *LIPC* gene, has been demonstrated to influence LIPC activity levels [21]. Apolipoprotein CIII (apoCIII) can inhibit LPL and reduces the uptake of TG-rich remnant particles and the SstI polymorphism of the *APOC3* gene has been shown to associate with hypertriglyceridemia and CAD in various human populations [22-26]. Therefore, we also examined these polymorphisms in the general Japanese population.

The aim of this study was, therefore, to examine the incidence of these gene polymorphisms and their contribution to lipid concentrations in the general Japanese populations.

Methods

Designs and Data Collection

This work is part of the Serum Lipid Level Survey 2000 from various areas around Japan. The Ethics committee, graduate school and faculty of Medicine, Kyoto University approved the study protocol and all subjects provided written informed consent for participation of gene analysis. The handling of DNA samples was followed by the guideline from the Ministry of Health, Labor, and Welfare. In the Serum Lipid Survey 2000, a total of 12,839 subjects were recruited at 36 hospitals across the country. These subjects in the present study were participants in the survey at 9 hospitals

with whom informed content for genotyping was sought for. Of 12,839 subjects, 2267 (17.7%) with no lipid-altering medication were randomly selected for the present study. In some institutes the information on sex was not disclosed.

Laboratory Methods

All serum and blood samples were obtained in the fasting state. All lipid and other analyses were conducted on venous blood samples within one week of collection at BML (Saitama, Japan). Serum cholesterol and TG levels were measured by enzymatic assay. HDL-cholesterol and LDL-cholesterol were measured enzymatically by a kit from Daiichi Kagaku Co. Ltd. (Tokyo, Japan). The results of lipid analyses in the four surveys were indirectly standardized according to the criteria of the CDC Lipid Standardization Program [24]. DNA was extracted by QIAamp DNA blood kit (Qiagen, Hilden, Germany).

Detection of gene mutations by Invader® assay

We applied the Invader® assay for screening three known mutations of the *CETP* gene, one of the *LIPC* genes, one of the *LPL* genes, and one of the *APOC3* genes, as previously described [25]. In brief, the probe/Invader®/MgCl2 mixture was prepared by combining 3 μl of primary probe/Invader® mix and 5 μl of 22.5 mM MgCl2 per reaction. The primary probes/Invader® mixture contained 3.5 μmol/l wild primary probe, 3.5 μmol/l mutant primary probe, 0.35 μmol/l Invader® oligonucleotide, and 10 mmol/l MOPS. Eight microliter of primary probe/Invader®/MgCl2 mixture was added into 96 well plate. Seven microliters of 5 fmol/l

synthetic target oligonucleotides, 10 μg/ml yeast tRNA (no target blank), and genomic DNA samples (15 ng/μl) were added, which were denatured by incubation at 95°C for 10 min. After 15 μl of mineral oil (Sigma, St. Louis, MO) were overlayed into all reaction wells, the plate was incubated isothermally at 63°C for 4 h in the DNA thermalcycler (PTC-200; MJ Research, Watertown, MA) and then kept at 4°C until fluorescence measurements. The fluorescent intensities were measured by the fluorescence microtiter plate reader (Cytoflour 4000; Applied Biosystems) with excitation, 485 nm/20 nm (Wavelength/Bandwidth) and emission, 530 nm/25 nm for FAM; excitation, 560 nm/20 nm and emission, 620 nm/40 nm for RED. The genotyping was analyzed by calculation with the ratios of net counts with wild primary probe to net counts with mutant primary probe. The probes used in this study were designed and synthesized by Third Wave Technologies, Inc (Madison, WI).

Data Analyses

Differences in means were evaluated by analysis of variance. The χ^2-square test was used to compare the incidence of each genotype. The analysis was performed by SPSS software (ver. 11.5)(Chicago, IL).

Results

We investigated the frequency and phenotypic association of the common polymorphisms of *CETP*, *LPL*, *LIPC*, and *APOC3* genes at the population level in 2,267 subjects. Table 1 summarizes the mean serum lipid levels in the participants in this study. The mean age, total

cholesterol, TG, HDL-cholesterol, and LDL-cholesterol levels in this population were similar to the level of all the participants of 12,839 people in the Serum Lipid Survey 2000. We also found that the medians of total, LDL-, and HDL-cholesterol levels did not differ appreciably from the means, thereby excluding gross right-hand tailing of the distribution (data not shown). These data indicate that the participants for the gene analysis are representative of the general Japanese population.

Table 2 summarizes the association of the gene polymorphisms with serum lipid levels in all the participants. Tables 3 and 4 show the analysis in male and female participants, respectively. We tested and found that Hardy-Weinberg equilibrium was the case for all the SNPs, supporting the assumptions of random mating in this population except *CETP* Int14 +1 G_A, in which no homozygote was found in this population.

The incidence of heterozygote mutations of D442G and Int14 +1 G→A of the *CETP* gene was 8.1 and 0.6 %, respectively. These mutations were associated with higher HDL-cholesterol. The heterozygous mutation of D442G was also associated with lower TG level only in men. Although the incidence of homozygous mutation of D442G and heterozygous mutation of Int14 +1 G→A was quite low and the difference was not significant, the TG levels tended to be higher. The incidence of B1B1, B1B2, and B2B2 genotypes the *CETP* TaqIB polymorphism was 35.8, 48.4, and 15.8%, respectively. The B2 allele of the *CETP* TaqIB polymorphism was associated with higher HDL-cholesterol levels in all the participants, men, and women. Although the difference was not statistically significant, the participants with B2 allele tended to have lower TG levels, which is

different from the results with homozygous mutation of D442G and heterozygous mutation of Int14 +1 G→A.

We then determined the polymorphism of *LPL* S447X mutations in this population. The incidence of heterozygous and homozygous mutations in the *LPL* gene was 20.7 and 1.3%, respectively. The mutation in the *LPL* S447X site was associated with higher HDL-cholesterol and lower TG levels, although the difference of HDL-cholesterol in men or that of TG in women was not statistically significant, possibly due to the small sample number.

The incidence of CC, CT, and TT genotype of *LIPC* in the Japanese was 24.9, 50.4, and 24.7%, respectively. Overall, the T allele was associated with an increase in HDL-cholesterol levels. However, the statistical significance was not found in men. The TG levels do not seem to be affected by this SNP.

The incidence of S1S1, S1S2, and S2S2 genotypes of the *APOC3* SstI polymorphism was 42.0, 45.8, and 12.2%, respectively. Although the HDL and LDL-cholesterol levels were similar in all the genotypes, the S2 allele was associated with higher TG levels in all the participants and in men, but not in women. Among the SNPs studied, no polymorphism was found to affect the LDL-cholesterol levels.

To determine the contribution of CETP and LPL gene polymorphism to hyperalphacholesterolemia (2.58 mmol/l or over) and hypoalphacholesterolemia (1 mmol/l or under), we divided all the participants into 3 groups according to HDL-cholesterol levels; 1 mmol/l

or under, 1 to 2.58 mmol/l, and 2.58 mmol/l or over. We then assessed the incidence of each genotype. The incidence of hyper- and hypoalphacholesterolemia was 8.3 and 1.8%, respectively. Among the genes studied we found 3 gene polymorphisms were associated with the incidence of high HDL-cholesterol (2.58 or over) (Table 5). Participants with B2B2 of *CETP* TaqIB had a higher incidence of high HDL-cholesterol levels than the others. Heterozygotes of *CETP* D442G polymorphism had a higher incidence of higher HDL-cholesterol levels than wild type. Homozygotes of *LPL* S447X polymorphism had a higher incidence of higher HDL-cholesterol levels than the others.

Discussion

In this study we have demonstrated the frequency of six common gene polymorphisms of the four genes related to lipid metabolism and its incidence and association with serum lipid levels in the general Japanese population. Because this is the largest analysis in the Japanese population, these data would be useful for the future analysis in the general Japanese population.

The prevalence of the D442G and Int14 +1 G_A mutations is very high in the general Japanese population, with heterozygote frequencies of 7 and 1 %, respectively [9, 10, 26, 27]. Our large scaled study showed similar frequencies of these mutations, with 8.1 and 0.6 %, respectively, indicating that our study population represents the general Japanese population and confirmed that the frequency of these mutations is quite frequent in our population. Because these mutations are associated with lower CETP activities [26], the plasma level of HDL-cholesterol is higher in