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Effectiveness and Safety of Lipid-Lowering Drug Treatments in Japanese Patients with Familial Hypercholesterolemia: Familial Hypercholesterolemia Expert Forum (FAME) Study

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Aims: Familial hypercholesterolemia (FH) is a genetic disorder characterized by high serum levels of low-density lipoprotein (LDL)-cholesterol (LDL-C), tendon and skin xanthomas, and premature coronary artery disease (CAD). In Japan, detailed information on the current status of drug therapies for patients with FH has not been reported so far, and their efficacy and safety have not been clarified. After the introduction of ezetimibe, which can further reduce serum LDL-C levels on top of statins, the changes of management for FH patients with these drugs are of particular interest. The current study aimed to evaluate the clinical status of FH heterozygotes and homozygotes, especially focusing on the real-world lipid-lowering drug therapy, attained serum LDL-C levels, and cardiovascular events at registration and during the follow-up.

Methods: The FAME Study enrolled 762 heterozygous (including 17 newly diagnosed cases) and 7 homozygous FH patients from hospitals and clinics nationwide. Diagnosis of FH was based upon the criteria defined in the Study Report in 2008 of the Research Committee on Primary Hyperlipidemia supported by Grants-in-Aid for Scientific Research from the Japanese Ministry of Health, Labor and Welfare. Data analysis was primarily carried on heterozygous FH patients.

Results: Xanthoma or thickening of the Achilles tendon was observed in more than 80% of the patients. CAD was recorded in 23% of patients. Patients with parental and sibling CAD accounted for 47% and 24%, respectively. At baseline, patients without CAD who had LDL-C <100 mg/dL accounted for 12.3% and those with CAD who had attained the target (LDL-C <70 mg/dL) in the secondary prevention accounted for only 1.8%. In the multiple logistic analysis, male sex, age >40, heterozygous FH score >20, hypertension, and sibling CAD were significantly and positively associated with prevalent CAD, whereas serum HDL-cholesterol levels showed a significant inverse association with CAD. Patients treated with statin alone, statin + ezetimibe, statin + resin, or statin + probucol accounted for 31.1%, 26.3%, 4.0%, and 3.7%, respectively. Patients treated with three-drug combination (statin + ezetimibe + resin or statin + ezetimibe + probucol) accounted for 7.5%. Statins and ezetimibe were used in 88.0% and 48.0% at the baseline, respectively. Although high-intensity statins were mainly prescribed, statin doses were much lower than those reported in Western countries. The addition of ezetimibe resulted in ~20% reduction in serum LDL-C. CAD was diagnosed in 17 patients with 21 episodes during follow-up. The Cox hazard model analysis demonstrated that male sex, CAD at the baseline, and parental CAD were related to the development of atherosclerotic cardiovascular disease (ASCVD) events. Furthermore, an increase in serum HDL-C was associated with a significant reduction of ASCVD events, while serum LDL-C and triglyceride levels were not related to ASCVD events.

Conclusion: **The prevalence of CAD in Japanese patients with heterozygous FH is still very high. In most of the cases, the target level of serum LDL-C was not achieved for primary and secondary prevention of CAD, suggesting that a more aggressive LDL-C lowering and appropriate management of residual risks are necessary.**

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Key words: **Familial hypercholesterolemia, LDL-cholesterol, Lipid-lowering drug treatment, Ezetimibe, Coronary artery disease**

Introduction

Familial hypercholesterolemia (FH) is a genetic disorder characterized by high serum levels of low-density lipoprotein (LDL)-cholesterol (LDL-C), tendon and skin xanthomas, and premature coronary artery disease (CAD)[1]. FH is caused by pathogenic mutations in genes of the LDL receptor, apolipoprotein (apo) B-100, and proprotein convertase subtilisin/kexin type 9 (PCSK9) involved in LDL receptor pathway[2]. FH is mostly an autosomal dominantly inherited disorder, although there is a very rare form of autosomal recessive hypercholesterolemia (ARH), caused by mutations of LDL receptor adaptor protein 1 (LDLRAP1)[3].

Patients with heterozygous FH are found in 1 out of 200–500 individuals of the general population in Japan, which is similar to those in other countries[1, 4]. Meanwhile, patients with homozygous FH are observed in 1 out of 160,000–1,000,000 individuals of the general population[1, 4]. FH is the most frequent genetic disease in daily clinical practice[5]. Patients with FH are accompanied by very high levels of serum LDL-C during the fetal stage and after birth; thus, the progression of atherosclerosis begins at a young age due to the long exposure to high levels of serum LDL-C. They develop premature CAD such as angina pectoris and myocardial infarction. Mabuchi *et al.*[6] reported that coronary artery stenosis detectable by angiography occurred after 17 and 25 years of age in male and female heterozygotes, respectively. Therefore, early diagnosis and appropriate treatment are important to prevent atherosclerotic cardiovascular diseases (ASCVD)[7]. Especially, patients with homozygous FH, who are completely deficient in LDL receptor, show extremely high serum LDL-C levels and are very resistant to dietary and drug treatments.

A number of randomized large clinical trials have demonstrated the effectiveness and long-term safety of HMG-CoA reductase inhibitors (statins) on the primary and secondary prevention of ASCVD in patients with high serum LDL-C levels by lowering LDL-C[8-9]. Meta-analyses of statins have clearly demonstrated that the reduction of serum LDL-C by statins prevents coronary events[10] as well as cerebrovascular events such as ischemic stroke[11]. Therefore, stains are the first-line drugs for treatment of patients with high serum LDL-C levels. For patients with FH, high doses of high-intensity statins are generally prescribed. However, they have much higher levels of pretreatment serum LDL-C than non-FH patients, and it is very difficult to lower their serum LDL-C levels to the target levels[12]. Therefore, anion-exchange resins (cholestyramine, colestipol, colestimide, etc.) and probucol have long been used in combination on top of statins[13].

About a decade ago, ezetimibe was released in the market with a new pharmacological agent in reducing serum LDL-C. Ezetimibe was reported to inhibit the absorption of dietary cholesterol as well as biliary cholesterol, which was excreted from the liver into the small intestines via bile[14]. Later, ezetimibe was shown to bind to the cholesterol transporter Niemann-Pick C1-like 1 (NPC1L1) in the small intestines (especially jejunum)[15] and to inhibit the function of NPC1L1, leading to the 54% reduction of cholesterol absorption from the small intestines[16]. Thus, the inhibition of cholesterol synthesis in the liver by statins and that of cholesterol absorption in the small intestines by ezetimibe have made it possible to more efficiently reduce serum LDL-C levels in combination. Ezetimibe alone can decrease serum LDL-C levels by 20.4%[16]; however, it can reduce serum LDL-C levels by ~25% on top of statins[17-18].

In Japan, the detailed real-world drug therapies for patients with FH have not been reported so far, and their efficiency and safety have not been clarified yet. Especially, after the introduction of ezetimibe,

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Table 1. Criteria for Clinical Diagnosis of Heterozygous FH

Criterion item	Scoring
1. Untreated LDL-C level	160–179 mg/dL (1 point), 180–199 mg/dL (2 points), and ≥ 200 mg/dL (4 points).
2. Family history (within second-degree relatives)	Either 4 or 6 points are given dependent on conditions: 4 points for a patient with family history of premature coronary artery disease (CAD)* or LDL-C ≥ 180 mg/dL and 6 points for a patient whose second-degree relative has been diagnosed with FH.
3. Xanthoma	6 points are given if a patient has tendon xanthoma, xanthoma tuberosum, or Achilles tendon hypertrophy diagnosed with X-ray imaging or xeroradiography (≥ 9 mm on either side)
4. Juvenile corneal arcus (<50 years of age) or premature CAD	4 points are given if either of the conditions is present.
5. Genetic mutations of LDL receptor	8 points are given if present.

Definite FH is diagnosed if the heterozygous FH score is ≥ 8 points, and suspected FH is diagnosed if the score ranges 6 to 7. The heterozygous FH score is the sum of points assigned to the above criterion items 1 to 5.
*Premature CAD is defined as CAD occurring at age <55 years in men and <65 years in women.

which can further reduce serum LDL-C levels of FH patients on top of statins, the management of FH patients with these drugs in the real world is of particular interest. The current study investigated the details of long-term lipid-lowering drug therapies in Japanese patients with heterozygous FH and evaluated their effectiveness and safety. The current study also aimed to investigate the associations of clinical parameters with cardiovascular disease morbidity.

1. Subjects and Methods

The FAME Study is a multicenter observational study to investigate the current real-world therapies for patients with FH and address the effectiveness and safety of current lipid-lowering drugs. This study was registered with UMIN (UMIN000003211).

1.1 Study Subjects

It was planned to enroll 1,000 patients with heterozygous and/or homozygous FH in the current study. Patients were registered in the FAME study if they met all of the following four criteria: (1) they were diagnosed as probable or definite FH assessed by the clinical diagnostic criteria for heterozygous FH defined by the Research Committee of the Japanese Ministry of Health, Labor and Welfare (**Table 1**)[19]; (2) Patients had serum LDL-C level ≥ 100 mg/dL (if patients had been taking ezetimibe, the pretreatment level of LDL-C should be ≥ 100 mg/dL); 3) they were outpatients of participating hospitals or clinics; and (4) they gave a written informed consent.

Patients were excluded if they had serum triglycerides (TG) of ≥ 400 mg/dL; if they were suffering from severe liver dysfunction (acute phase and decompensated cirrhosis); if they had dyslipidemia secondary to hypothyroidism or pancreatitis; if they had uncontrolled diabetes mellitus (HbA1c >9%); if they were pregnant, potentially pregnant, or lactating; or if they were inappropriate for enrollment as judged by study physicians.

1.2 Background Information

Background information was obtained regarding age, sex, body height, body weight, waist circumference at the umbilical level, smoking, CAD in parents and siblings with age of onset, date of first visit, date of diagnosis of FH, type of FH (homozygote or heterozygote), diagnostic score of heterozygous FH, morbidity status (new case or treated case), presence of individual items for the diagnostic score of heterozygous FH, and complications and past diseases. The complications and past diseases included hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or use of antihypertensives), diabetes mellitus (fasting plasma glucose ≥ 110 mg/dL and/or 2-h plasma glucose ≥ 140 mg/dL), hypertriglyceridemia (≥ 150 mg/dL), renal dysfunction (serum Cr >1.1 mg/dL in men and >0.9 mg/dL in women), myocardial infarction, exertion and resting angina pectoris, percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), aortic valve stenosis, thoracic and abdominal aneurysm, peripheral artery disease, cerebral hemorrhage, cerebral infarction, and others (open-ended question).

The diagnosis of CAD was based upon the presence of diseases (I 20.0–I 25.9) of ICD10, PCI, or

CABG. Family history of CAD was designated as positive if either of the father, mother, or siblings of the patient had CAD (within the patient's second-degree relatives). The presence of xanthoma was defined when the patient had tendon xanthoma (thickening of tendons on the dorsal side of the hands, elbows, knees, or Achilles tendon hypertrophy) or xanthoma tuberosum and when the patient had a thickening of the Achilles tendons. Achilles tendon hypertrophy was diagnosed if the Achilles tendon thickness in either side was ≥ 9 mm on X-ray imaging.

1.3 Follow-Up Observation

The duration of evaluation in the current study was originally planned 4 years for patients enrolled from June 2006, to December 31, 2011. The recruitment period was extended until the end of 2012, and the duration of evaluation was 3 years for patients enrolled from January 1, 2012, to December 31, 2012.

1.4 Discontinuation Criteria of Follow-up

The follow-up was terminated when a registered patient wanted to cancel the participation in the study for some reasons.

1.5 Lipid-lowering Treatment and Use of Other Drugs

As for lipid-lowering drugs, information was obtained for each prescription with respect to class, generic or product name, daily dose, date of commencement, cessation or continuation, date of cessation if so, and date of confirmation for continued drug. The same sorts of information were obtained as to cardiovascular drugs and antidiabetic drugs. Dates of commencement and cease were also reported for use of steroids, thyroid hormones, immunosuppressive drugs, female hormone products, and others (open-ended question). Regarding LDL apheresis, information was obtained on frequency in addition to dates of start and cessation and date of confirmation when continued.

1.6 Effectiveness Parameters of Lipid-lowering Drugs

The following laboratory parameters were measured at each follow-up point in time as well as at baseline to evaluate the effectiveness of lipid-lowering treatment: total cholesterol, LDL-C (measured and estimated by the Friedewald equation), HDL-C and TG, Rf-value and presence of mid-band on polyacrylamide gel electrophoresis, remnant lipoprotein cholesterol (RLP-C) by direct or immunoabsorption method, lipoprotein (a), apolipoprotein (apo) A-1, apo B, apo E, HbA1c, fasting plasma insulin, fasting plasma glucose, and high sensitivity C-reactive protein (hs-CRP). Serum hs-CRP was to be measured at a single laboratory in the protocol, but the reported values were indicative of measurements at different laboratories. Furthermore, reported values of plasma insulin showed frequent extraordinary outliers. Thus, insulin and hs-CRP were not used in the present analysis.

The extent of atherosclerosis was evaluated by intima–media thickness (IMT) of carotid arteries measured in accordance with the Guidelines for Evaluation of Ultrasound Analysis of Carotid Arteries[20] and Achilles tendon thickness on X-ray. The IMT was measured annually, and Achilles tendon thickness was evaluated biennially. As for the patients of delayed recruitment (January to December in 2012), Achilles tendon thickness was evaluated at baseline and 3 years of follow-up.

1.7 Safety Parameters of Lipid-lowering Drugs

Laboratory parameters for safety assessment included serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (γ-GT), creatine kinase (CK), and creatinine. Systolic and diastolic blood pressure, pulse, smoking status, body weight, and waist circumference were also monitored. Adverse events during the follow-up were reported together with the date of onset, severity, outcomes, and causality in relation to drug use. The causal relationship with a specified drug was classified into "unrelated", "probably unrelated", "undeniable", and "causally related" and a responsible drug was specified. Abnormalities in the laboratory measurements were also documented as adverse events. Selected cardiovascular events of the reported adverse events were used for the analysis in relation to serum lipid levels during the follow-up period. More details are described in Appendix, regarding (1) the investigation schedules and parameters, (2) data management strategy for patients treated with ezetimibe, (3) evaluation of effectiveness and safety, and (4) criteria for cerebrovascular or cardiovascular event occurrence.

1.8 Ethical Issues

The study protocol was initially reviewed and approved by the Institutional Review Board (IRB) of Osaka University Hospital and thereafter by the IRBs of the participating institutions. Before conducting the study, investigators obtained IRB approval and permission from the head of each institution. Informed consent was obtained from each FH patient who participated in the current study. If patients were

under 16 years old, a written informed consent was obtained from their legally authorized representatives. If patients were 16–19 years old, a written informed consent was obtained from both patients and their legally authorized representatives.

1.9 Statistical Analyses

Descriptive statistics were used to describe the demographic, clinical, and laboratory parameters of the registered patients. Proportions and means with standard deviation (SD) were calculated for categorical variables and for continuous variables, respectively. The between-group difference was assessed by unpaired t-test for means and by Fisher's exact test for proportions. The within-group change in continuous parameters was assessed by paired t-test.

The associations of serum lipids and clinical covariates with prevalent CAD were assessed by univariate and multivariate logistic regression analyses. Prevalent CAD included the history of angina pectoris, myocardial infarction, PCI, and CABG. In the follow-up analysis of ASCVD events, the Cox proportional hazard model was used to evaluate the associations with serum lipids and other clinical covariates. The ASCVD events included new or recurrent episodes of CAD, aortic valvular stenosis surgical procedure, aortic aneurysm surgical procedure, and cerebral infarction which occurred during the observation period. Few patients reported multiple ASCVD events during the observation period, and the first events were used in the analysis. Statistical significance was declared if two-sided P value was less than 0.05.

2. Results

2.1 Background Characteristics of Heterozygous FH Patients

A total of 803 patients were enrolled from 52 hospitals and clinics across the nation. After exclusion of 15 patients with unknown sex ($n=4$), unknown age ($n=5$), and age less than 15 years ($n=6$), 788 patients remained. Of these, patients with heterozygous FH score unrecorded ($n=9$) or less than 6 points ($n=10$) were further excluded. Patients with homozygous FH ($n=7$) were analyzed separately from those with heterozygous FH. The final heterozygous FH subjects used for the analysis were 762.

Patients were categorized into 4 groups with respect to treatment and diagnosis (new or known case): 713 patients (93.6%) under cholesterol-lowering treatment, 17 newly diagnosed cases (2.2%), 5 cases (0.7%) previously diagnosed without treatment, and 27 cases (3.5%) with no information regarding treatment or diagnosis.

Lipid profile at the baseline and follow-up points in time were analyzed for patients under cholesterol-lowering treatment and those with newly diagnosed patients separately. **Table 2** summarizes the background characteristics of heterozygous FH patients in each sex as well as in both sexes combined. The mean age at registration was 55.5 years in total, 53.3 years in 325 males and 57.0 years in females. Overall, the heterozygous FH score was approximately 13 on average, and the mean period from FH diagnosis was nearly 9 years. Body mass index (BMI) was slightly higher in males than in females. The mean waist circumference was 86 cm and 81 cm in males and females, respectively. The percentage of patients with genetic mutation of LDL receptor was 12.7% in both sexes (14.2% in males and 11.7% in females). Current smokers accounted for 12.7% in males and 5.3% in females. Xanthoma or thickening of the Achilles tendon was observed in approximately 80% of patients. CAD of parents was observed in 46.7%, whereas that of their siblings was reported in 24.5%. Hypertension and diabetes mellitus were observed in 31% and 17.5%, respectively. CAD was twice more frequent in men (32.9%) than in women (15.3%). It is noteworthy that the prevalence of cerebrovascular diseases, including cerebral infarction, was only 3.7% in total, which was much less than that of CAD. Lipid-lowering treatments were given in 96% of patients. Cardiovascular drugs such as antihypertensive and antiplatelet drugs were used for 48.6% of male patients and 28.3% of female patients.

2.2 Factors Associated with Prevalent CAD in Heterozygous FH Patients at Registration

Table 3 presents the results of logistic regression analysis on prevalent CAD at baseline in relation to clinical and laboratory parameters ($n=762$). Factors under study were sex, age, BMI, hypertension, diabetes mellitus, smoking status, parental and sibling CAD, and serum levels of LDL-C, HDL-C, and TG.

In the univariate analysis, male sex, age ≥ 40, heterozygous FH score ≥ 15, BMI ≥ 22.5, hypertension, diabetes mellitus, past smoking, and parental and sibling CAD showed a significantly increased prevalence odds of CAD. Regarding the serum lipid levels, a 10 mg/dL increase in serum HDL-C was associated with an odds ratio (OR) of 0.60 (95% CI 0.52–0.68), while serum LDL-C or TG levels were not related to the prevalence of CAD.

Genetic mutations of LDL receptor[21, 22] and xanthoma are important components of the FH score, and it may be of interest to examine the associations of these factors with CAD. Xanthoma and genetic

Table 2. Baseline Characteristics of the Registered Patients with Heterozygous FH

Variable	Both sexes		Male		Female	
	*N**	Value	*N**	Value	*N**	Value
Mean (SD)						
Age (year)	762	55.5 (15.4)	325	53.3 (15.0)	437	57.0 (15.6)
Heterozygous FH score	762	13.1 (4.8)	325	13.6 (5.2)	437	12.7 (4.6)
Years from FH diagnosis	742	9.4 (8.7)	316	9.5 (9.0)	426	9.4 (8.5)
Height (cm)	741	161 (9)	319	168 (6)	422	155 (6)
Body weight (kg)	745	61 (12)	320	69 (11)	425	54 (9)
Body mass index (kg/m^2)	729	23.3 (3.5)	316	24.3 (3.4)	413	22.5 (3.4)
Waist circumference (cm)	388	83 (10)	173	86 (9)	215	81 (10)
Systolic blood pressure	720	124 (16)	308	125 (15)	412	123 (17)
Diastolic blood pressure	720	73 (10)	308	74 (10)	412	72 (10)
HbA1c JDS (%)	576	5.6 (0.8)	254	5.6 (0.7)	322	5.6 (0.8)
Number (%)						
Genetic mutation of LDL receptor	762	97 (12.7)	325	46 (14.2)	437	51 (11.7)
Current smoking	747	63 (8.4)	315	40 (12.7)	432	23 (5.3)
Xanthoma/ATT	762	633 (83.1)	325	284 (87.4)	437	349 (79.9)
Parental CAD	597	279 (46.7)	242	109 (45.0)	355	170 (47.9)
Sibling CAD	507	124 (24.5)	217	54 (24.9)	290	70 (24.1)
Hypertension	762	236 (31.0)	325	118 (36.3)	437	118 (27.0)
Diabetes mellitus	762	133 (17.5)	325	68 (20.9)	437	65 (14.9)
CAD	762	174 (22.8)	325	107 (32.9)	437	67 (15.3)
Cerebrovascular diseases	762	28 (3.7)	325	14 (4.3)	437	14 (3.2)
Cerebral infarction	762	23 (3.0)	325	12 (3.7)	437	11 (2.5)
Lipid-lowering treatment	758	727 (95.9)	323	311 (96.3)	435	416 (95.6)
Use of cardiovascular drugs	758	280 (36.9)	323	157 (48.6)	435	123 (28.3)
β-Blockers	758	62 (8.2)	323	42 (13.0)	435	20 (4.6)
ACE inhibitors	758	20 (2.6)	323	13 (4.0)	435	7 (1.6)
ARB	758	105 (13.9)	323	56 (17.3)	435	49 (11.3)
CCB	758	110 (14.5)	323	46 (14.2)	435	54 (12.4)
Antiplatelet drugs	758	169 (22.3)	323	106 (32.8)	435	63 (14.5)
Use of antidiabetic drugs	758	64 (8.4)	323	35 (10.8)	435	29 (6.7)

ACE: angiotensin-converting enzyme; ARB: angiotensin II receptor blockers; ATT: Achilles tendon thickening; CAD: coronary artery disease; CCB: calcium channel blockers; FH: familial hypercholesterolemia; JDS: Japan Diabetes Society.
*Denominators were not uniform because of missing information.

mutations of LDL receptor were each significantly associated with increased prevalence odds of CAD in the univariate logistic analysis, but the associations were not significant in the multivariate analysis. Univariate OR for xanthoma was 2.78 (95% CI 1.55–4.98), and multivariate OR was 1.59 (95% CI 0.83–3.05) when the FH score was replaced with xanthoma in the multivariate model. The corresponding OR for genetic mutation of LDL receptor was 2.02 (95% CI 1.28–3.20) and 1.47 (95% CI 0.84–2.58), respectively.

In the multivariate analysis, male sex, age ≥ 40, heterozygous FH score ≥ 20, hypertension, and sibling CAD demonstrated significantly higher ORs of the prevalence of CAD. A significant inverse association with serum HDL-C levels remained in the multivariate analysis.

2.3 Baseline and Follow-Up Laboratory Data

2.3.1 Lipid Profile at the Baseline

Lipid profile, intima–media thickness (IMT), and Achilles tendon thickness at baseline are shown for patients under lipid-lowering treatment and newly diagnosed untreated patients separately **(Table 4)**. Serum LDL-C levels of newly diagnosed untreated patients were very high at 258 ± 92 (mean ± SD) mg/dL, whereas those of patients under treatment were reduced to 141 ± 41 mg/dL. Serum Lp(a) levels showed a tendency to be increased in both groups, although they were less than 40 mg/dL. Serum apoB

Table 3. Logistic Regression Analysis on Prevalent Coronary Artery Disease (CAD) at Baseline in Relation to Clinical and Laboratory Parameters (N=762)

Parameter	Total, *n*	CAD, *n* (%)	Univariate OR (95% CI)	Multivariate OR (95% CI)*
Sex				
Female	437	67 (15.3)	1.00 (referent)	1.00 (referent)
Male	325	107 (32.9)	2.71 (1.91–3.84)	2.37 (1.48–3.77)
Age (year)				
<40	125	8 (6.4)	1.00 (referent)	1.00 (ref)
40–54	189	33 (17.5)	3.09 (1.38–6.95)	2.60 (1.09–6.19)
55–69	303	78 (25.7)	5.07 (2.37–10.9)	2.68 (1.16–6.23)
70+	145	55 (37.9)	8.94 (4.05–19.7)	3.90 (1.54–9.88)
Heterozygous FH score				
<10	146	22 (15.1)	1.00 (referent)	1.00 (referent)
10–14	390	78 (20.0)	1.41 (0.84–2.36)	1.48 (0.83–2.63)
15–19	149	40 (26.8)	2.07 (1.16–3.70)	1.95 (0.97–3.92)
20+	77	34 (44.2)	4.46 (2.35–8.44)	3.49 (1.58–7.69)
Body mass index (kg/m^2)				
<22.5	331	63 (19.0)	1.00 (referent)	1.00 (referent)
22.5–24.9	184	51 (27.7)	1.63 (1.07–2.49)	1.06 (0.63–1.78)
25.0+	214	56 (26.2)	1.51 (1.00–2.27)	0.80 (0.47–1.36)
Unknown	33	4 (12.1)	0.59 (0.20–1.73)	0.64 (0.16–2.52)
Hypertension				
(–)	526	79 (15.0)	1.00 (referent)	1.00 (referent)
(+)	236	95 (40.3)	3.81 (2.68–5.43)	2.22 (1.43–3.47)
Diabetes mellitus				
(–)	629	119 (18.9)	1.00 (referent)	1.00 (referent)
(+)	133	55 (41.4)	3.02 (2.03–4.50)	1.56 (0.94–2.59)
Smoking				
Never	544	100 (18.4)	1.00 (referent)	1.00 (referent)
Past	140	57 (40.7)	3.05 (2.04–4.55)	1.12 (0.66–1.87)
Current	63	12 (19.0)	1.04 (0.54–2.03)	0.60 (0.27–1.36)
Unknown	15	5 (33.3)	2.22 (0.74–6.64)	1.20 (0.32–4.49)
Parental CAD				
(–)	318	50 (15.7)	1.00 (referent)	1.00 (referent)
(+)	279	69 (24.7)	1.76 (1.17–2.64)	1.26 (0.77–2.05)
Unknown	165	55 (33.3)	2.68 (1.72–4.17)	1.85 (1.06–3.22)
Sibling CAD				
(–)	383	70 (18.3)	1.00 (referent)	1.00 (referent)
(+)	124	52 (41.9)	3.23 (2.08–5.02)	1.94 (1.12–3.36)
Unknown	255	52 (20.4)	1.15 (0.77–1.71)	1.17 (0.71–1.93)
LDL cholesterol (mg/dL)				
per 10 mg/dL increase	740†	169 (22.8)	0.91 (0.87–0.95)	0.95 (0.91–1.00)
HDL cholesterol (mg/dL)				
per 10 mg/dL increase	759†	173 (22.8)	0.60 (0.52–0.68)	0.71 (0.62–0.82)
Triglycerides (mg/dL)				
per 10 mg/dL increase	760†	173 (22.8)	1.01 (0.98–1.03)	0.99 (0.96–1.03)

CAD: coronary artery disease; FH: familial hypercholesterolemia; HDL: high-density lipoprotein; LDL: low-density lipoprotein
*Patients with missing values for serum lipids were excluded (n=740).
†Patients with a missing value were deleted.

Table 4. Lipid Profile, Intima-media Thickness and Achilles Tendon Thickness at Baseline by Treatment Status

Lipid and related parameters	Under treatment		Newly diagnosed		*P*-value
	N	Mean (SD)	*N*	Mean (SD)	
Total cholesterol (mg/dL)	700	217 (45)	17	333 (96)	$<10^{-21}$
Triglycerides (mg/dL)	712	106 (65)	17	111 (64)	0.77
HDL cholesterol (mg/dL)	711	55 (17)	17	53 (10)	0.58
LDL cholesterol (mg/dL)	693	141 (41)	17	258 (92)	$<10^{-26}$
Rf	160	0.34 (0.04)	3	0.32 (0.02)	0.55
Remnant lipoprotein cholesterol (mg/dL)	325	5.3 (4.3)	10	7.1 (3.6)	0.18
Lp (a) (mg/dL)	399	31.6 (34.5)	10	28.5 (20.7)	0.77
Apolipoprotein A-I (mg/dL)	412	139 (31)	15	134 (19)	0.55
Apolipoprotein B (mg/dL)	413	112 (28)	15	160 (31)	$<10^{-9}$
Apolipoprotein E (mg/dL)	396	4.4 (1.4)	13	5.4 (1.8)	0.02
Maximal IMT (mm)*	511	1.57 (0.85)	12	1.40 (0.89)	0.49
Mean IMT (mm)†	459	0.89 (0.33)	11	0.92 (0.61)	0.75
Achilles tendon thickness (mm)†	486	11.4 (4.1)	14	10.9 (3.3)	0.64

IMT: intima media thickness of the carotid arteries; Rf: Relative to front.
*The highest of the right and left values.
†Average of the right and left values.

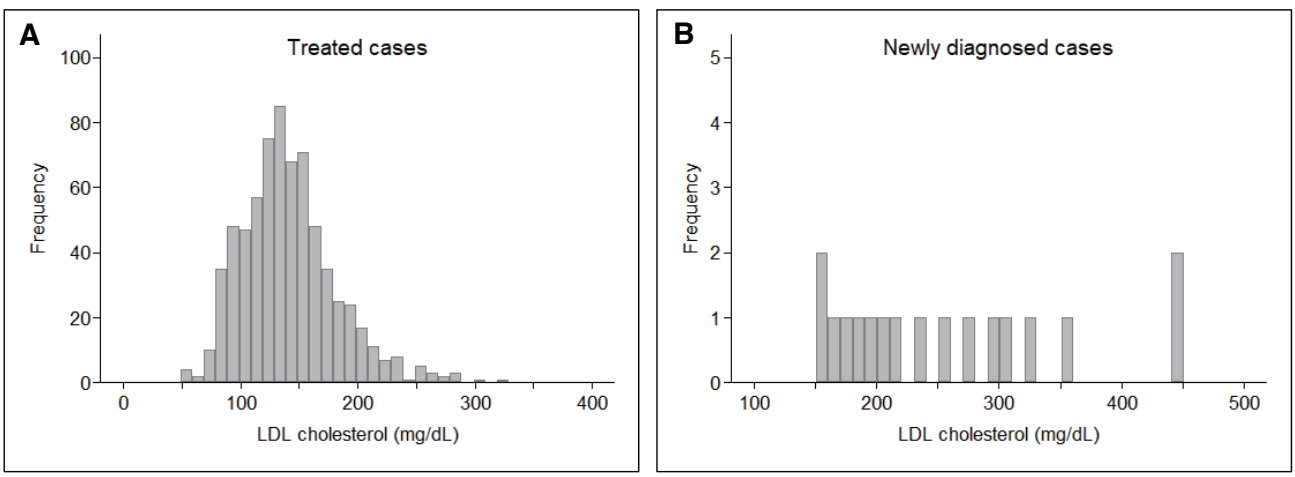

Fig. 1. Distribution of serum LDL-cholesterol levels at the baseline in the treated cases (A) and newly diagnosed cases (B) of heterozygous FH

levels of newly diagnosed untreated patients were high at 160±31 mg/dL, whereas those of patients under treatment were reduced to 112±28 mg/dL. Maximal IMT was very thick in both patients under treatment and newly diagnosed untreated patients. The mean Achilles tendon thickness was 10.9 mm and 11.5 mm in newly diagnosed untreated patients and in patients under drug treatment, respectively.

Fig. 1 shows the distribution of serum LDL-C levels in treated patients (panel A) and those who were newly diagnosed as heterozygous FH (panel B). In the drug-treated heterozygous FH patients, the LDL-C level varied markedly from 49 to 323 mg/dL. Despite a variety of drug treatments, only 15.0% (104/693) of treated patients with heterozygous FH showed serum LDL-C levels <100 mg/dL, i.e., the target level for primary prevention defined by the Japanese Society of Atherosclerosis Guidelines 2017 [23]. Most of the patients still showed markedly high serum LDL-C levels. Patients without CAD who had LDL-C <100 mg/dL accounted for 12.3% (65/528), and those with CAD who had attained the target (LDL-C <70 mg/dL) in the secondary prevention accounted for only 1.8% (3/165). In the newly diagnosed heterozygous FH patients, the LDL-C level varied markedly from 151 to 443 mg/dL.

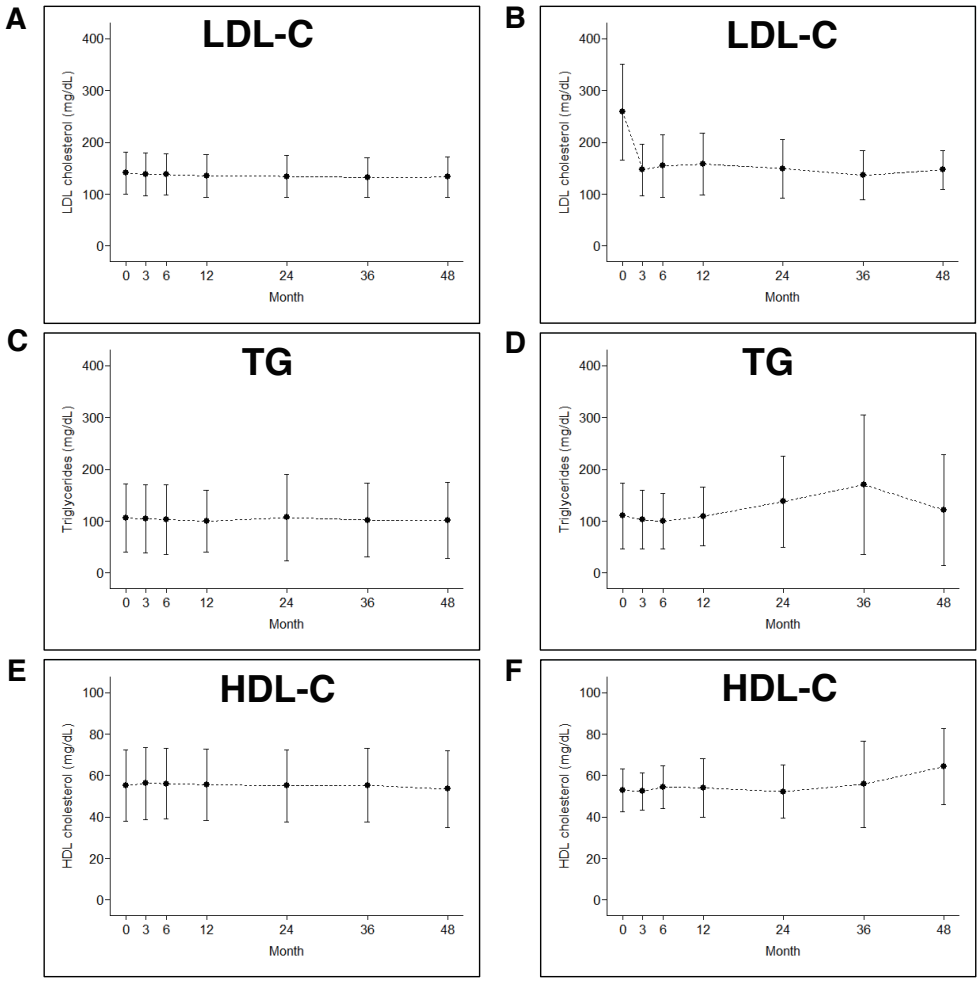

Fig. 2. Changes in the serum levels of LDL-C, TG, and HDL-C in heterozygous FH patients under lipid-lowering treatment at registration (left panels A, C, and E) and newly diagnosed heterozygous FH patients (right panel B, D, and F)

In all panels, points and bars indicate means and standard deviations, respectively.

2.3.2 Lipid Profile during the Follow-Up Period

Fig. 2 (left panel) illustrates the serum LDL-C, TG, and HDL-C levels during the 4 years of follow-up of heterozygous FH patients under lipid-lowering treatment. The serum LDL-C levels were around 132–138 mg/dL at the follow-up, which were much higher than those recommended by the Japan Atherosclerosis Society (JAS) Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2017 [23].

The mean serum TG levels were 106 mg/dL at the baseline and remained stable around 100–107 mg/dL at the follow-up. The mean serum HDL-C levels were 55 mg/dL at the baseline and remained stable around 54–56 mg/dL at the follow-up. These data suggested that only serum LDL-C levels remained still high in heterozygous FH patients in Japan before the launch of PCSK9 inhibitors in 2016.

Heterozygous FH patients under lipid-lowering treatment at registration showed nearly constant levels of LDL-C during the follow-up (141 mg/dL at baseline and around 132–138 mg/dL at follow-up). Similarly, serum TG, HDL-C, Rf, RemL-C, Lp(a) and apoAI, apoB and apoE, fasting plasma glucose, and HbA1c levels remained stable **(Supplementary Table 1)**.

Fig. 2 (right panel) illustrates the serum LDL-C, TG, and HDL-C levels during the 4 years of follow-up of heterozygous FH patients who were newly diagnosed as heterozygous FH. Mean of LDL-C averaged over the follow-up measurements was 149 ± 52 (mean ± SD) mg/dL and was markedly and significantly lower than the mean at baseline (P=0.0001). The serum LDL-C levels were reduced but still high despite drug treatments. Serum TG and HDL-C levels seemed to vary with follow-up months,

Table 5. Lipid-lowering Drug Regimens for Heterozygous FH Patients

Regimen	Baseline (N=758*)	End of follow-up (N=743†)
	n (%)	n (%)
No treatment	31 (4.1)	8 (1.1)
Statin alone	236 (31.1)	197 (26.5)
Ezetimibe alone	26 (3.4)	21 (2.8)
Statin + ezetimibe	199 (26.3)	246 (33.1)
Statin + resin	30 (4.0)	26 (3.5)
Statin + probucol	28 (3.7)	18 (2.4)
Ezetimibe + resin	3 (0.4)	4 (0.5)
Ezetimibe + probucol	2 (0.3)	4 (0.5)
Statin + ezetimibe + resin	19 (2.5)	26 (3.5)
Statin + ezetimibe + probucol	38 (5.0)	47 (6.3)
Drug + apheresis	21 (2.8)	16 (2.2)
Apheresis alone	2 (0.3)	3 (0.4)
Others	123 (16.2)	127 (17.1)

*Excluding 4 patients with no information on treatment.
†Excluding 19 patients without either information on treatment (n=4) or follow-up information (n=18).

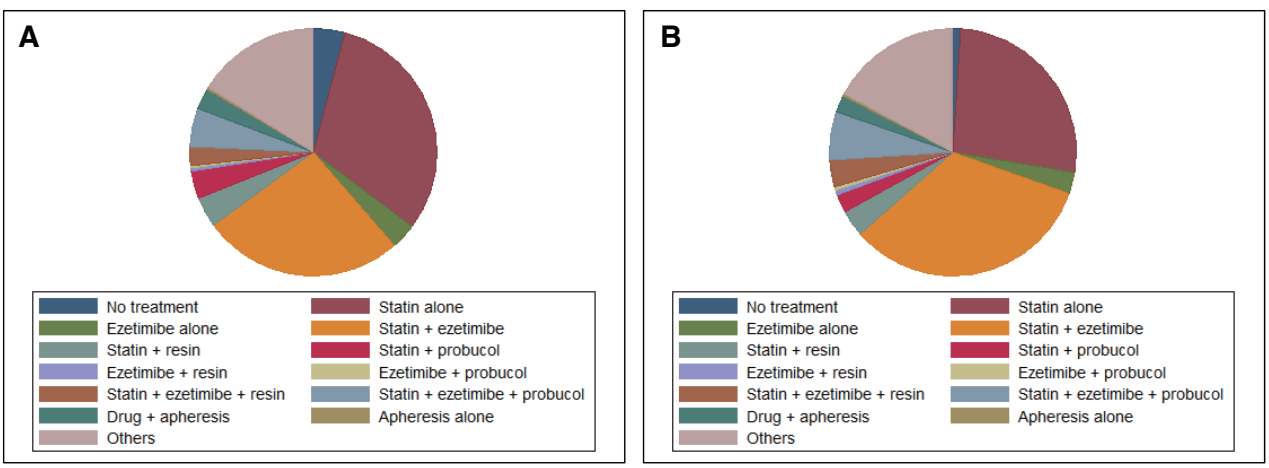

Fig. 3. Lipid-lowering regimens at baseline (A) and at the end of follow-up (B) for all heterozygous FH patients

but the variations were within random fluctuation. Two-way ANOVA with repeated follow-up measurements resulted in P=0.13 and P=0.59 for TG and for HDL-C, respectively, regarding variation over time. Other values of Rf, RemL-C, Lp(a) and apoA-I, apoB, and apoE remained stable **(Supplementary Table 2)**.

In the treated cases and newly diagnosed cases combined, 16.4% (88/537) attained the target of primary intervention (<100 mg/dL), and 4.8% (8/166) attained the target of secondary prevention (<70 mg/dL) during the follow-up treatment. The most recent available LDL-C was used for the follow-up measurement.

2.3.3 Other Laboratory and Clinical Data at Baseline and during Follow-up

Heterozygous FH patients under lipid-lowering treatment at baseline showed an increase in maximal IMT, mean IMT, and Achilles tendon thickness, whereas no remarkable findings were noted in other laboratory and clinical parameters. During the 4 years of follow-up, maximal IMT, mean IMT, and Achilles tendon thickness also showed no significant increase. Other parameters on laboratory tests, blood pressure, pulse, body weight, and waist circumference also remained stable during the follow-up **(Supplementary Table 1)**.

Heterozygous FH patients who were newly diagnosed at registration showed an increase in maximal IMT, mean IMT, and Achilles tendon

thickness, whereas no remarkable findings were noted in other laboratory and clinical parameters. During the follow-up, fasting plasma glucose, HbA1c levels, blood pressure, pulse, body weight, and waist circumference remained stable. The changes in maximal IMT, mean IMT, and Achilles tendon thickness could not be significant due to the small number of patients **(Supplementary Table 2)**.

5. Lipid-Lowering Therapies

Of the 762 patients with heterozygous FH, 4 were excluded from the analysis due to the lack of information on the treatment. Thus, 758 heterozygous FH patients were analyzed at baseline **(Table 5 and Fig. 3)**. Additional 15 patients were excluded in the analysis on the treatment at the end of follow-up. At the baseline, 4.1% of the patients were not under lipid-lowering treatment, and 3.4% of the patients were treated with ezetimibe alone. Patients treated with statin alone, statin + ezetimibe, statin + resin, or statin + probucol accounted for 31.1%, 26.3%, 4.0%, and 3.7%, respectively. Patients treated with three-drug combination (statin + ezetimibe + resin or statin + ezetimibe + probucol) accounted for 7.5%. Overall, statins were used in 88.0% (667/758) of patients, and ezetimibe was used in 48.0% (364/758) of patients at the baseline **(Supplementary Table 3)**. Patients treated with LDL apheresis and drug accounted for 2.8%, and those with apheresis alone accounted for only 0.3%.

At the end of follow-up, only 1.1% of patients were those without treatment, and 2.8% of patients were those treated with ezetimibe alone. Patients treated with statin alone, statin + ezetimibe, statin + resin, or statin + probucol accounted for 26.5%, 33.1%, 3.5%, and 2.4%, respectively. Patients treated with three-drug combination (statin + ezetimibe + resin or statin + ezetimibe + probucol) accounted for 9.8%. Overall, statin use and ezetimibe use accounted for 91.1% (677/743) and 59.8% (444/743), respectively **(Supplementary Table 3)**.

Furthermore, the monotherapy with resin was used for 2.0% (15/758) of patients at baseline and 1.1% (8/743) of patients at the end of follow-up. All of these patients were women, and the sex difference was statistically significant at baseline ($P=0.0003$) and at the end of follow-up ($P=0.01$). Women with resin monotherapy were much younger than those with other regimens, mean age being 34.5 years (SD 15.3 years) versus 58.6 years (SD 14.4 years) at the baseline ($P=5.4\times10^{-10}$). No information was available regarding pregnancy and breastfeeding.

Regarding the intensity of statins, high-intensity statins such as atorvastatin ($n=303$), rosuvastatin ($n=267$), and pitavastatin ($n=180$) were most frequently prescribed **(Supplementary Table 3)**. Mild or moderate-intensity statins such as pravastatin, simvastatin, and fluvastatin were not often administered. Ezetimibe was administered in 482 patients (64.3%). Resins were almost exclusively colestimide. Probucol monotherapy was prescribed for three patients at baseline and six patients at the end of follow-up. Probucol was prescribed for 148 patients (19.7% of those treated with lipid-lowering drugs) during the whole period. N-3 polyunsaturated fatty acids (n-3 PUFA) were prescribed for 71 patients (9.5%) during the whole period. LDL apheresis was prescribed for 25 patients (3.3% of total FH heterozygotes) during the whole period.

Regarding the dose of statins, the medians (ranges) of the maximum doses of individual statins prescribed during the observation period were atorvastatin 20 (2.5–40) mg, fluvastatin 30 (20–60) mg, pitavastatin 2 (1–4) mg, pravastatin 10 (5–30) mg, rosuvastatin 10 (2.5–20) mg, and simvastatin 10 (5–20) mg.

6. Effects of Intestinal Cholesterol Transporter Inhibitor, Ezetimibe, on Serum Lipid Levels in Patients with Heterozygous FH

Just before the start of this FAME study, ezetimibe became available in Japan. There were 42 patients who added ezetimibe on the date of registration or within 7 days thereafter. **Fig. 4** shows the add-on effect of ezetimibe on serum LDL-C levels in patients with heterozygous FH. The addition of ezetimibe caused a reduction of serum LDL-C (panel A) of approximately 30–35 mg/dL and a percent change of serum LDL-C (panel B) of approximately 20%.

7. Adverse Events and Factors Associated with Cardiovascular Events

7.1 Adverse Events

Supplementary Table 4 presents a list of all reported adverse events (AEs). The number of patients and episodes are shown. Cardiovascular diseases occurred in 36 patients with 44 episodes. CAD was diagnosed in 17 patients with 21 episodes. Eleven cases with PCI with 15 episodes are included. AEs which might be related to lipid-lowering drug therapies were rhabdomyolysis in one case (suspected case with no observed elevation of CK) and myalgia in three cases (four episodes), respectively. The elevation of CK occurred in three cases (four episodes) and that

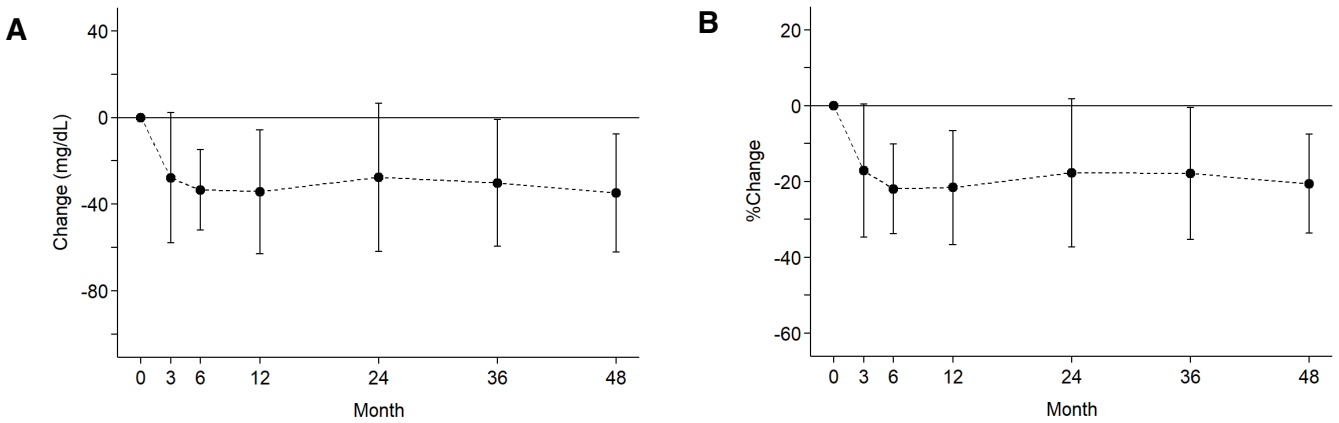

Fig. 4. Effects of intestinal cholesterol transporter inhibitor, ezetimibe, on serum LDL-cholesterol levels in patients with heterozygous FH

Panel A: Changes of serum LDL-cholesterol levels (mg/dL)
Panel B: Percent changes of serum LDL-cholesterol levels (%)
In all panels, points and bars indicate means and standard deviations, respectively.

Table 6. Cardiovascular Events in the 4-Year Follow-up Period

Adverse events	No. of patients	No. of episodes	Remark
Cardiovascular diseases	36	44	
Coronary artery disease	17	21	
Aortic stenosis surgery	3	3	
Arrhythmia	4	4	
Heart failure	2	4	3 episodes of one patient
Subarachnoid hemorrhage	1	1	
Subdural hemorrhage	2	2	
Cerebral infarction	2	2	
Aortic aneurysm surgery	4	4	
Others*	3	3	

*Including subclavian artery stenosis, vertebrobasilar insufficiency, and aortic aneurysm diagnosed accidentally at abdominal CT screening.

of liver enzyme in four cases (four episodes).

Eight deaths were recorded. The underlying causes were leukemia (n=2), acute myocardial infarction (n=1), heart failure due to drug-induced bradycardia (n=1), subarachnoid hemorrhage (n=1), rupture of aortic aneurysm (n=1), respiratory failure (n=1), and unknown cause (n=1).

7.2 Factors Associated with Cardiovascular Events

Table 6 summarizes the details of cardiovascular events that occurred during the 4-year follow-up period. A total 44 episodes of cardiovascular events were reported in 36 patients. Several patients had multiple episodes in the same and different categories of cardiovascular events. CAD events occurred in 17 patients: angina pectoris (n=2), myocardial infarction (n=2), PCI (n=12), and CABG (n=1). ASCVD events included CAD, aortic valvular stenosis surgery, cerebral infarction, and aortic aneurysm with rupture or surgical procedure. ASCVD events were used as outcomes in the Cox proportional hazard model analysis.

We evaluated the lifestyle and clinical parameters, which may be related to the development of cardiovascular events over a 4-year follow-up (n=749). The average of serum lipid levels on treatment before the events was used. Four patients with missing values for serum lipids were excluded, and finally 745 patients were analyzed. Table 7 summarizes the results of Cox hazard model analysis on the occurrence of ASCVD. From multivariate analysis, male gender (HR 4.30; 95% CI: 1.41–13.1), CAD at the baseline (HR 4.42; 95% CI: 1.68–11.6), and parental CAD (HR 3.24; 95% CI: 1.12–9.39) were related to the

Table 7. Cox Hazard Model Analysis on Cardiovascular Events Over a 4-Year Follow-up in Relation to Lifestyle and Clinical Parameters (N=749)

Parameter	Total, *n*	CVD, *n* (%)	Univariate HR (95% CI)	Multivariate HR (95% CI)*
Sex				
Female	430	6 (1.4)	1.00 (referent)	1.00 (referent)
Male	319	19 (6.0)	4.22 (1.68–10.6)	4.30 (1.41–13.1)
Age (year)				
<40	122	1 (0.8)	1.00 (referent)	1.00 (ref)
40–54	186	5 (2.7)	3.07 (0.36–26.3)	2.31 (0.26–20.8)
55–69	299	11 (3.7)	4.11 (0.53–31.9)	3.22 (0.38–27.5)
70+	142	8 (5.6)	6.19 (0.77–49.5)	5.88 (0.59–58.5)
Heterozygous FH score				
<10	142	2 (1.4)	1.00 (referent)	1.00 (referent)
10–14	384	18 (4.7)	3.09 (0.72–13.3)	2.96 (0.64–13.7)
15–19	147	2 (1.4)	0.89 (0.12–6.30)	0.49 (0.06–4.10)
20+	76	3 (3.9)	2.32 (0.39–13.9)	0.80 (0.11–5.71)
Body mass index (kg/m^2)				
<22.5	325	8 (2.5)	1.00 (referent)	1.00 (referent)
22.5–24.9	180	5 (2.8)	1.11 (0.36–3.40)	0.79 (0.23–2.65)
25.0+	211	11 (5.2)	2.23 (0.90–5.56)	1.66 (0.55–5.03)
Unknown	33	1 (3.0)	1.32 (0.16–10.5)	2.55 (0.27–24.1)
CAD at baseline				
(–)	578	8 (1.4)	1.00 (referent)	1.00 (referent)
(+)	171	17 (9.9)	6.81 (2.94–15.8)	4.42 (1.68–11.6)
Hypertension				
(–)	516	10 (1.9)	1.00 (referent)	1.00 (referent)
(+)	233	15 (6.4)	3.23 (1.45–7.18)	1.42 (0.53–3.84)
Diabetes mellitus				
(–)	618	18 (2.9)	1.00 (referent)	1.00 (referent)
(+)	131	7 (5.3)	1.71 (0.71–4.09)	0.88 (0.31–2.50)
Smoking				
Never	534	15 (2.8)	1.00 (referent)	1.00 (referent)
Past	139	6 (4.3)	1.41 (0.55–3.64)	0.31 (0.10–0.98)
Current	62	3 (4.8)	1.64 (0.48–5.68)	0.78 (0.19–3.25)
Unknown	14	1 (7.1)	3.35 (0.44–25.4)	0.98 (0.09–10.7)
Parental CAD				
(–)	312	6 (1.9)	1.00 (referent)	1.00 (referent)
(+)	275	14 (5.1)	2.55 (0.98–6.64)	3.24 (1.12–9.39)
Unknown	162	5 (3.1)	1.61 (0.49–5.28)	1.25 (0.34–4.54)
Sibling CAD				
(–)	377	13 (3.4)	1.00 (referent)	1.00 (referent)
(+)	123	5 (4.1)	1.20 (0.43–3.36)	0.76 (0.25–2.30)
Unknown	249	7 (2.8)	0.88 (0.35–2.20)	1.04 (0.36–3.00)
LDL cholesterol (mg/dL)				
per 10 mg/dL increase	745	25 (3.4)	0.93 (0.83–1.05)	1.02 (0.90–1.16)
HDL cholesterol (mg/dL)				
per 10 mg/dL increase	748	25 (3.3)	0.58 (0.44–0.77)	0.70 (0.50–0.98)
Triglycerides (mg/dL)				
per 10 mg/dL increase	748	25 (3.3)	1.00 (0.93–1.07)	0.96 (0.87–1.06)

CAD: coronary artery disease; FH: familial hypercholesterolemia; HDL: high-density lipoprotein; LDL: low-density lipoprotein
*Patients with missing values for serum lipids were excluded (n=745).

development of ASCVD events. Furthermore, serum HDL-C (per 10 mg/dL increase) showed a reduction of ASCVD events (HR 0.70; 95% CI: 0.50–0.98). In contrast, age, heterozygous FH score, BMI, hypertension, diabetes mellitus, smoking, sibling CAD, serum LDL-C, and TG levels were not related to the development of ASCVD events.

8. Clinical Data of Patients with Homozygous FH

Supplementary Tables 5 and 6 summarize the baseline characteristics and laboratory data of registered FH homozygotes. Five out of seven patients (71.4%) had CAD and had been treated with PCI or CABG. However, the patients never suffered from cerebrovascular diseases. Although the number of homozygotes is small, their mean LDL-C was 185 mg/dL probably due to the effect of LDL apheresis. All of the patients were on treatment with LDL apheresis[24].

Discussion

1. Clinical Characteristics of Patients with Heterozygous FH in Japan

The current FAME trial aimed to evaluate the clinical phenotypes, real-world therapies, and lipid management levels of patients with heterozygous FH in Japan. The prevalence of CAD was as high as 23% at baseline. It is well known that CAD is highly frequent in patients with heterozygous FH[1, 6], but the magnitude of increased risk of CAD in these patients compared with the general population or subjects without FH has not been documented in Japan. The expected number of prevalent CAD cases was estimated to be 5.51 based on the sex- and 5-year age-specific prevalence rates of CAD in the Patient Survey in 2011[25]. The ratio of observed versus expected cases of prevalent CAD was 31.6 (174/5.51). The increase was substantial, but the ratio was probably overestimated because of different ascertainments in the two surveys and a selection bias of FH patients. In the current study, patients with heterozygous FH were enrolled mainly from core hospitals rather than private clinics; therefore, patients may have suffered from CAD and were hospitalized. Only a single disease of primary concern is enumerated in patients with multiple diseases in the Patient Survey, whereas diagnosis and care for CAD may be more intensive in patients with heterozygous FH. With respect to cerebral infarction, the ratio of observed versus expected prevalent cases was 3.85 (23/5.97). Although it is difficult to infer a role of heterozygous FH in the occurrence of CAD and cerebral infarction based on prevalence data, the substantial difference in prevalence between CAD and cerebral infarction deserves discussion. The onset of cerebral infarction is generally much later than CAD onset; thus, more intense therapy after CAD may deter the progression toward cerebral infarction. Alternatively, high-risk patients may have failed to survive until ages at which cerebral infarction becomes notably more frequent.

In the annual report of the Research Group for Primary Hyperlipidemia in 1986 (Group Leader: Seiichiro Tarui)[26], the occurrence of CAD was 22.2% and 14.7% in males and in females, respectively, although the mean age was not reported. Male FH heterozygotes showed a significantly higher occurrence of CAD than females. In the later study of the Research Group for Primary Hyperlipidemia from a database from 1996 to 1998 (Group Leader: Toru Kita)[27], the occurrence of CAD has slightly increased in males (~36%) and was not changed in females (~14%). These two reports of the Research Group for Primary Hyperlipidemia were based upon the data before initiation of drug treatment. In the current study, most of the heterozygous FH patients had been treated with lipid-lowering drugs. The mean age of heterozygous FH patients was similar to that reported by Bujo *et al.*[27], in which the occurrence of CAD was slightly decreased in males (32.9%) and mildly increased in females (15.3%), respectively.

FH is characterized by premature onset of CAD. The EXPLORE-J study[28] reported the prevalence of FH was 2.7% of patients with acute coronary syndrome (ACS) in Japan, using Achilles tendon thickness measurement by X-ray. The rate of FH among patients with ACS was at least five times higher than in the general population (0.2%–0.5%)[29-30]. Another study in Japan reported that the prevalence of FH was 5.7% in patients with ACS[31], while a Swiss study reported that it was 1.6% using the Dutch Lipid Clinic Network algorithm[32]. In the EXPLORE-J study[28], the prevalence of FH was higher in patients under 40 years of age than in those over 40 years (8.3% vs. 2.6%). Thus, FH appears to be more common among patients with early onset of ACS.

We analyzed the factors associated with prevalent CAD in heterozygous FH patients at registration, most of whom were under treatment and some of whom were newly diagnosed. In logistic regression analysis on prevalent CAD at baseline **(Table 3)**, male gender, age ≥ 40, heterozygous FH score ≥ 15, BMI ≥ 22.5, hypertension, diabetes mellitus, past smoking, and parental and sibling CAD showed significantly increased prevalence odds of CAD in the univariate analysis. A 10 mg/dL increase in serum HDL-C was

associated with an OR of 0.60 (95%CI: 0.52–0.68), while serum LDL-C and TG levels were not related to the prevalence of CAD. In the multivariate analysis, male gender, age ≥ 40, heterozygous FH score ≥ 20, hypertension, and sibling CAD demonstrated significantly higher ORs of the prevalence of CAD. A significant inverse association with serum HDL-C remained in the multivariate analysis. In the annual report of the Research Group for Primary Hyperlipidemia in 1986 [26], hypertriglyceridemia but not LDL-C level was positively associated with prevalent CAD in FH heterozygotes. Another report of Bujo *et al.* [27] based upon the database from 1996 to 1998 showed that hypertension, male gender, smoking, low HDL-C levels, age >50 years, diabetes mellitus, and hypertriglyceridemia were positive risk factors for CAD. However, in the current study, a positive association between serum TG level and CAD could not be demonstrated. Moreover, serum LDL-C level at registration was not related to CAD in patients with heterozygous FH in the current study who were mostly under treatment. In contrast, heterozygous FH patients who had either LDL-C ≥ 260 mg/dL or Achilles tendon thickness (ATT) ≥ 14.5 mm before treatment showed a 23.94-fold higher risk of CAD than those with LDL-C <260 mg/dL and ATT < 14.5 mm[33]. Lifelong cumulative LDL-C levels are reportedly important for the development of CAD in FH patients, and there may be a threshold for CAD onset[6]. The LDL-C levels before drug treatment may positively correlate with lifelong cumulative LDL-C levels. However, the LDL-C levels at registration may not reflect cumulative LDL-C levels. Moreover, the LDL-C levels at registration were still much higher than the target levels. A longer follow-up of FH patients may provide more information regarding the contribution of attained LDL-C levels to the possible reduction of CAD events.

2. Real-World Drug Therapies for FH Heterozygotes in Japan

We evaluated what kind of lipid-lowering drugs was used for treating heterozygous FH patients **(Table 5)**. At the baseline, 4.1% of the patients were not under lipid-lowering treatment, and 3.4% of the patients were treated with ezetimibe alone. Patients treated with statin alone, statin + ezetimibe, statin + resin, or statin + probucol accounted for 31.1%, 26.3%, 4.0%, and 3.7%, respectively. Patients treated with three-drug combination (statin + ezetimibe + resin or statin + ezetimibe + probucol) accounted for 7.5%. In total, statins were used in 88.0%, and ezetimibe was used in 48.0% of patients at the baseline.

The type and intensity of statins and their dose **(Supplementary Table 3)** were then evaluated. Medians of the maximum doses of individual statins prescribed during the observation period were atorvastatin 20 mg, fluvastatin 30 mg, pitavastatin 2 mg, pravastatin 10 mg, rosuvastatin 10 mg, and simvastatin 10 mg, respectively. These doses were much lower compared with those prescribed in Western countries[34-35], suggesting that statins at a low dose may be effective for Asian populations, especially Japanese. Harada-Shiba *et al.* have shown that statin use was independently associated with the difference in age at the onset of CAD in Japanese patients with heterozygous FH[36].

In the current study, probucol was prescribed for 19.7% of patients treated with lipid-lowering drugs during the whole period. Probucol is a potent antioxidative drug which lowers serum HDL-C as well as LDL-C. Probucol has been prescribed especially for FH patients with a marked tendon xanthoma and xanthelasma[37]. Although probucol reduces serum HDL-C[38], it was significantly effective for the prevention of secondary cardiovascular events in the retrospective cohort of heterozygous FH patients (POSITIVE trial)[39]. Probucol reduces serum HDL-C, but it enhances reverse cholesterol transport through the activation of plasma cholesteryl ester transfer protein and hepatic scavenger receptor class B type I (SR-BI), thereby leading to the regression of xanthomas and possibly cardiovascular events[40-41]. In another trial (PROSPECTIVE)[42], probucol was given to patients with prior CAD, and cardiovascular events rate tended to be lower in probucol-treated group than in probucol-non-treated group, although no statistical significance was observed.

Just before the FAME study was started, an intestinal cholesterol transporter inhibitor, ezetimibe, was released on the Japanese market. The add-on effect of ezetimibe on serum LDL-C levels was investigated in 42 patients with heterozygous FH **(Fig. 4)**. Serum LDL-cholesterol levels were reduced by 30~40 mg/dL on top of lipid-lowering drugs such as statins, with a mean reduction of LDL-C of approximately 20%. Since PCSK9 inhibitors, evolocumab and alirocumab, were launched into the Japanese market in 2016, these drugs were not prescribed for FH patients in the current study.

3. Management of Serum LDL-C Levels

In heterozygous FH patients for primary prevention, it was desirable to set a management target for the LDL-C level at <100 mg/dL, and it was also acceptable to aim for <50% of the pretreatment level if the management target for LDL-C is not achieved

according to the Japanese Guidelines for Diagnosis and Treatment of Familial Hypercholesterolemia 2017[43]. In heterozygous FH patients for secondary prevention, the target LDL-C level for management was set at <70 mg/dL. However, in the current study, despite the combination of drugs including statins and ezetimibe, the mean serum LDL-C levels were 141±41 mg/dL at the baseline and 133±39 mg/dL at 4 years of follow-up in patients under lipid-lowering treatment at registration **(Supplementary Table 1)**. Furthermore, the mean serum LDL-C levels were 258±92 mg/dL at the baseline and 147±38 mg/dL at 4 years of follow-up in patients who were newly diagnosed with heterozygous FH **(Supplementary Table 2)**. Patients without CAD who had LDL-C < 100 mg/dL accounted for only 12.3%, and those with CAD who had attained the target LDL-C <70 mg/dL in the secondary prevention accounted for only 1.8%. Therefore, the management of LDL-C in heterozygous FH patients for both primary and secondary prevention was quite inadequate.

4. Factors Associated with Cardiovascular Events during Follow-Up

A total of 44 episodes of cardiovascular events occurred in 36 patients during the 4-year follow-up period **(Table 6)**. ASCVD events included CAD, aortic valvular stenosis surgery, cerebral infarction, and aortic aneurysm with rupture or surgical procedure. CAD events occurred in 17 patients, including angina pectoris (n=2), myocardial infarction (n=2), PCI (n=12), and CABG (n=1). ASCVD events included new onset as well as recurrent events and were used as outcomes in the Cox proportional hazard model analysis. Multivariate analysis demonstrated that male gender, CAD at the baseline, and parental CAD were related to the development of cardiovascular events. Furthermore, an increase in serum HDL-C (per 10 mg/dL increase) was associated with a significant reduction of ASCVD events, while serum LDL-C and TG levels were not related to the development of ASCVD events. Therefore, we could not demonstrate the threshold of LDL-C level for primary and secondary prevention of cardiovascular events. However, since LDL-C levels are the most important risk factor for ASCVD in FH when we compare the development of ASCVD between FH homozygotes and heterozygotes, LDL-C levels should be managed appropriately. The reasons why LDL-C levels were not related to the development of ASCVD events at registration and during follow-up may be as follows: (1) the target LDL-C levels were not attained in most of FH heterozygotes, (2) young FH heterozygotes without other risks are usually treated with higher LDL-C levels than older FH heterozygotes, and (3) FH heterozygotes with prior CAD or with high risks are usually treated more intensively to lower LDL-C levels by a combination of statins, ezetimibe, resins, and probucol. In fact, young patients (<55 years old) had much higher means of serum LDL-C than older patients (≥ 55 years old) at baseline (159 mg/dL vs. 138 mg/dL, $P<10^{-8}$) and during the follow-up (149 mg/dL vs. 130 mg/dL, $P<10^{-9}$). When a high-risk group was arbitrarily defined as those with hypertension, diabetes mellitus, parental CAD, sibling CAD, or ever smoking, mean LDL-C levels were lower in high-risk patients compared with those with no such conditions at baseline (143 mg/dL vs. 154 mg/dL, P=0.01) and during the follow-up (134 mg/dL vs. 147 mg/dL, $P<10^{-4}$). Therefore, LDL-C levels after drug treatment may not simply reflect the risk for ASCVD in a short period of time.

A notable finding was that serum HDL-C level was inversely associated with ASCVD. Baseline serum HDL-C level was associated with a decreased prevalence of CAD in the cross-sectional analysis, and follow-up HDL-C was inversely related to atherosclerotic disease events in the longitudinal analysis. Serum HDL-C level seems to be more predictive of CAD and ASCVD than LDL-C in heterozygous FH patients under lipid-lowering treatment. This protective effect of HDL may reflect the pretreatment level of serum HDL-C in each patient. Ogura *et al.*[44] evaluated the HDL-C level and cholesterol efflux capacity in 227 heterozygous FH patients under drug treatment. The mean level of HDL-C was significantly lower in patients with ASCVD, and increased efflux capacity was correlated with the reduction of ASCVD risk even after the addition of HDL-C level as a covariate. Reduction of cholesterol efflux capacity was correlated with the presence of corneal arcus. Inverse relationships between cholesterol efflux capacity and ATT as well as carotid IMT were also demonstrated, confirming the role of HDL in the prevention of ASCVD.

5. Long-Term Safety of Lipid-Lowering Drugs for Patients with Heterozygous FH

The current study has also assessed the AEs associated with lipid-lowering therapy in patients with heterozygous FH. Rhabdomyolysis was reported in one patient, but this case did not show an elevation of CK. Therefore, this case may have complained of muscle-related symptoms. Myalgia occurred in three patients. Five patients were associated with other signs of musculoskeletal diseases. The details of AEs **(Supplementary Table 4)** including cardiovascular events **(Table 6)** during the 4-year follow-up period

suggested that severe AEs, such as rhabdomyolysis liver dysfunction, are very rare. Only a small number of patients developed signs of musculoskeletal diseases.

Statin use was reportedly associated with the elevation of fasting plasma glucose and HbA1c[45-46] as well as the development of newly diagnosed diabetes mellitus[47-48]. While fasting plasma glucose levels were almost constant throughout the observation period, HbA1c levels seemed to have increased at 4 years of follow-up compared with the baseline value **(Supplementary Table 1)**. However, in the analysis of 246 patients under treatment at baseline who had HbA1c measurements at baseline and 4 years, the mean increase of HbA1c at 4 years from baseline was less in patients who had ever used statin than those who have not used statin (0.21% vs. 0.47%), but no statistical significance was observed ($P=0.21$).

Limitation of the Study

There are several limitations in the current study. Firstly, the untreated lipid levels were unavailable in most of the subjects in the current study.

Secondly, the diagnosis of heterozygous FH was based upon the scoring system of the Annual Report of the Research Committee on Primary Hyperlipidemia of the Ministry of Health and Welfare of Japan reported by Harada-Shiba *et al.*[19]. The JAS has proposed the diagnostic criteria for definite FH, which are much simpler than the criteria used in the FAME study[49] in the Guidelines for the Management of Familial Hypercholesterolemia 2012[29] and the revised version of 2017[23]. In the JAS criteria[23, 49], definite FH is diagnosed if there are at least any two of the three conditions (untreated LDL-C ≥ 180 mg/dL, presence of tendon or tuberous xanthoma, and familial history of FH or family history of premature CAD). These three conditions are the same as items 1 to 3 in the FAME scoring system, except for the absence of LDL-C ≥ 180 mg/dL in the definition of family history of FH. The definite cases of heterozygous FH numbered 718 in the FAME study, and 19 (2.7%) of them did not meet the criteria of definite FH in the JAS guideline. The failure in defining FH was due to the lack of juvenile corneal arcus or premature CAD (item 4) and genetic mutation of LDL receptor (item 5) in the JAS criteria. On the other hand, 2 (4.5%) of the 44 suspected FH cases in the FAME study were classified as definite cases in the JAS criteria. Both of the patients had untreated LDL-C of 189–199 mg/dL (2 points) and family history of FH (4 points). Therefore, the current JAS criteria may need some modification to augment the ability of detecting FH.

Thirdly, the data were collected from facilities all over Japan, and the laboratory tests and the analysis of ATT and ultrasound examination of carotid arteries were not uniform, suggesting some variations in the data collection among participating facilities.

Fourthly, 4 years may not be enough to analyze the factors associated with cardiovascular events during follow-up.

Conclusions

The FAME Study was performed after the launch of cholesterol absorption inhibitor, ezetimibe, but PCSK9 inhibitors were not used during the follow-up period. Therefore, the levels of serum LDL-C and cardiovascular event rate after starting the use of PCSK9 inhibitors may be another interesting issue to be addressed in future studies. Currently, the Research Committee on Primary Hyperlipidemia of the Ministry of Health and Welfare of Japan is performing a registration study (PROLIPID[50]) of patients with primary hyperlipidemia, including FH patients.

In conclusion, the current study has demonstrated the clinical status of FH heterozygotes and homozygotes including the levels of serum LDL-C and cardiovascular event rate. A variety of lipid-lowering drugs were prescribed in combination. The combination of statins and ezetimibe was most frequently prescribed. Although high-intensity statins were mainly prescribed, statin doses were much smaller than those reported in the Western countries. The target level of serum LDL-C was not achieved in most of the cases for primary and secondary prevention of CAD. There was no concern regarding the long-term safety of lipid-lowering drugs. The current study also investigated the associations of clinical parameters with cardiovascular disease morbidity. Logistic regression analysis demonstrated that male sex, CAD at the baseline, and parental CAD were related to the development of cardiovascular events. Furthermore, higher serum HDL-C was associated with a significant reduction of cardiovascular events, while serum LDL-C and TG levels were not related to the development of cardiovascular events.

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Conflict of Interest

Hidenori Arai has received honoraria from Sanofi, Daiichi-Sankyo Co., Ltd., MSD K.K., Kowa Pharmaceutical Co., Ltd., and Pfizer Co., Ltd. Hideaki Bujo has nothing to disclose. Hiroyuki Daida has received honoraria from Amgen Inc., Daiichi-Sankyo Co., Ltd., Kowa Pharmaceutical Co., Ltd., and MSD K.K., and received clinical research funding from Canon Medical Systems Corporation, Philips Japan, Ltd., Toho Holdings Co., Ltd., Asahi Kasei Corporation, and Inter Reha Co., Ltd. HD has also received scholarship grants from Nippon Boehringer Ingelheim Co., Ltd., Otsuka Pharmaceutical Company, Ltd., Sanofi K.K., MSD K.K., Daiichi-Sankyo Co., Ltd., Pfizer Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Astellas Pharma Inc., Takeda Pharmaceutical Co., Ltd., Teijin Pharma, Ltd., Shionogi & Co., Ltd., Actelion Pharmaceuticals, Ltd., Actelion Ltd., Kowa Pharmaceutical Co., Ltd., Bayer Yakuhin, Ltd. HD has also courses endowed by companies, including Philips Japan, Ltd., ResMed, Fukuda Denshi Co., Ltd., and Paramount Bed Co., Ltd. Mariko Harada-Shiba has received stock holdings or options from Liid Pharma, honoraria from Amgen Inc., Astellas Pharma Inc., Sanofi K.K., and scholarship grants from Aegerion Pharmaceuticals, Inc., Recordati Rare Diseases Japan, and Kaneka Cooperation. Shun Ishibashi has received honoraria from Kowa Pharmaceutical Co., Ltd., and a scholarship grant from Ono Pharmaceutical Co., Ltd. Nobuhiko Koga has nothing to disclose. Daisaku Masuda has received clinical research funding from MSD K.K., Takeda Pharmaceutical Co., Ltd., Daiichi-Sankyo Co., Ltd., Kowa Company, Ltd., Otsuka Pharmaceutical Co., Ltd., and scholarship grants from Skylight Biotec, Inc., Pfizer Japan Inc., Amgen Astellas Biopharma K.K., and Sanofi K.K. Shinichi Oikawa has nothing to disclose. Shizuya Yamashita has received honoraria from Amgen Astellas BioPharma K.K., Kowa Pharmaceutical Co. Ltd., Sanofi K.K., MSD K.K., Bayer Yakuhin, Ltd., clinical research fundings from Ono Pharmaceutical Co., Ltd., Hitachi Chemical Diagnostics Systems Co., Ltd., Takeda Pharmaceutical Company Ltd, Mitsubishi Tanabe Pharma Corporation, Rohto Pharmaceutical Co., Ltd., Astellas Pharma Inc., Nippon Boehringer Ingelheim Co., Ltd., MSD K.K., Bayer Yakuhin, Ltd., scholarship grants from Astellas Pharma Inc., Nippon Boehringer Ingelheim Co., Ltd., MSD K.K., Bayer Yakuhin, Ltd., and Courses endowed by Izumisano City.

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50) Prospective Registry Study of Primary Dyslipidemia (PROLIPID) (UMIN000042782)

Appendix

1. Investigation Schedules and Parameters

Investigation Schedules

	Registration period	Observational period					
	At registration	3 months after ± 1 month	6 months after ± 1 month	1 year after ± 2 months	2 years after ± 2 months	3 years after ± 2 months	4 years after ± 2 months
Background of patients	●						
Adherence	←	—	—	—	—	—	→
Combination therapy, Combination drugs	←	—	—	—	—	—	→
Blood pressure	●	●	●	●	●	●	●
Body weight, Waist circumference	●			●	●	●	●
Smoking habbit	●			●	●	●	●
Special tests (hs-CRP)	●	●	●	●	●	●	●
Serum lipids, lipoprotein lipids, lipoprotein subfractions, remnant lipoproteins, Lp(a), apolipoproteins	●	●	●	●	●	●	●
Blood biochemical tests	●	●	●	●	●	●	●
Other laboratory tests	●	●	●	●	●	●	●
IMT	●			●	●	●	●
Achilles tendon thickness	●				●	(●)	●
Cardiovascular events	←	—	—	—	—	—	→
Adverse effects	←	—	—	—	—	—	→

2. Data Management Strategy for Patients Treated with Ezetimibe

2.1. Cases Who Have Already Been Treated with Ezetimibe

For cases who have already been treated with ezetimibe, the following laboratory and clinical data at the visit prior to ezetimibe administration should be recorded in addition to the data at registration: serum lipids (total cholesterol, LDL-C (desirable to be calculated by Friedewald's formula), HDL-C, TG), polyacrylamide gel electrophoresis, remnant lipoprotein cholesterol (RemL-C), Lp(a), apolipoprotein (apo) A-1, apo B, apo E, HbA1c, insulin, fasting plasma glucose, IMT, and Achilles tendon thickness (mm).

2.2. Cases for Whom Ezetimibe Has Been Added at the Registration

For cases for whom ezetimibe has been added at the registration, laboratory and clinical data for registration should be evaluated and described as those at registration.

2.3. Cases for Whom Ezetimibe Has Been Added after the Registration

For cases for whom ezetimibe has been added after the registration, the following laboratory and clinical data both at the registration and at the time point of starting ezetimibe should be recorded: serum lipids (total cholesterol, LDL-C (desirable to be calculated by Friedewald's formula), HDL-C, TG), polyacrylamide gel electrophoresis, remnant lipoprotein cholesterol (RemL-C), Lp(a), apolipoprotein (apo) A-1, apo B, apo E, HbA1c, insulin, fasting plasma glucose, IMT, and Achilles tendon thickness (mm).

3. Evaluation of Effectiveness and Safety

The following information of parameters on the effectiveness and safety of lipid-lowering drugs was

evaluated by subgroup analyses in terms of the following factors.

1. Age
2. Gender
3. Presence or absence of diabetes mellitus
4. Presence or absence of hypertension
5. Number of risk factors: 1) Diabetes mellitus, 2) Hypertension, 3) Low HDL-cholesterolemia, 4) Hypertriglyceridemia
6. Presence or absence of smoking habit
7. Presence or absence of metabolic syndrome (MetS)
8. Presence or absence of chronic kidney disease (CKD) or proteinuria: Stratified analysis was performed.
9. Drugs for treatment of dyslipidemia: Presence or absence of drug treatment before registration and combined drug treatment during the study period
10. Stratified analysis by each serum lipid value at registration and during follow-up
11. Cases treated with ezetimibe

4. Criteria for Cerebrovascular or Cardiovascular Event Occurrence

Events were judged if either of the following occurred.

4.1. Cardiac Death/Sudden Death

Cardiac sudden death or fatal myocardial infarction, fatal cerebral infarction, fatal cerebral hemorrhage.

4.1.1. Cardiac Sudden Death

Cardiac sudden death excludes death due to stroke and includes the cases as follows:

1) Cases who died within one hour after serious chest symptoms or those who died almost at the same time of onset

2) Cases without acute or chronic changes or events (including in-hospital events) other than atherosclerotic coronary artery disease that may have a fatal clinical course

3) Cases due to unexpected intrinsic death who were discovered at home or at places other than hospitals

4) Cases who were identified to have cardiovascular diseases at autopsy, leading to death (excluding asymptomatic myocardial infarction)

4.1.2. Fatal Myocardial Infarction

Death related to myocardial infarction (cases with persistent angina for more than 30 minutes, and/or with signs suspicious of myocardial infarction or definite diagnosis of myocardial infarction in more than 2 leads of electrocardiogram, and/or with an elevation of myocardial enzymes).

4.1.3. Fatal Cerebral Infarction

Deaths due to cerebral infarction (cases diagnosed to have infarction lesions by CT, MRI or autopsy corresponding to clinical focal signs).

4.1.4. Fatal Cerebral Hemorrhage

Deaths due to cerebral hemorrhage (cases diagnosed to have hemorrhagic lesions by CT, MRI or autopsy), excluding hemorrhagic cerebral infarction.

4.2. Nonfatal Myocardial Infarction

Cases who survived from myocardial infarction (cases with persistent angina for more than 30 minutes, and/or with signs suspicious of myocardial infarction or definite diagnosis of myocardial infarction in more than 2 leads of electrocardiogram, and/or with an elevation of myocardial enzymes).

4.3. Coronary Revascularization (PCI or CABG)

4.4. Nonfatal Stroke

Nonfatal cerebral infarction, or nonfatal cerebral hemorrhage (excluding transient ischemic attack (TIA)), or TIA.

(1) Nonfatal cerebral infarction: cases who survived from cerebral infarction and whose infarction lesion(s) corresponding to clinical signs and symptoms were confirmed by CT or MRI.

(2) TIA: cases whose focal neurological symptoms occurred suddenly, but disappeared within 24 hours, and whose infarction lesion(s) corresponding to clinical signs and symptoms were not confirmed by CT or MRI.

(3) Nonfatal cerebral hemorrhage: cases who survived from cerebral hemorrhage and whose hemorrhagic lesion(s) corresponding to clinical signs and symptoms were confirmed by CT or MRI (excluding hemorrhagic cerebral infarction).

Supplementary Table 1. Laboratory and Clinical Data at Baseline and during Follow-up among Heterozygous FH Patients under Lipid-lowering Treatment at Registration (*N*=713)

Parameter		Baseline	3 months	6 months	1 year	2 years	3 years	4 years
TC (mg/dL)	*N*	700	586	586	626	584	525	376
	Mean (SD)	217 (45)	215 (46)	214 (44)	211 (47)	211 (46)	208 (44)	207 (48)
TG (mg/dL)	*N*	712	598	601	636	594	537	383
	Mean (SD)	106 (65)	105 (65)	103 (67)	100 (59)	107 (84)	102 (71)	102 (74)
HDL-C (mg/dL)	*N*	711	598	601	636	594	536	382
	Mean (SD)	55 (17)	56 (17)	56 (17)	56 (17)	55 (17)	55 (18)	54 (19)
LDL-C (mg/dL)	*N*	693	581	576	621	574	521	370
	Mean (SD)	141 (41)	138 (41)	138 (40)	135 (42)	134 (41)	132 (38)	133 (39)
Rf	*N*	160	120	124	147	119	106	71
	Mean (SD)	0.34 (0.04)	0.33 (0.05)	0.34 (0.05)	0.34 (0.04)	0.33 (0.04)	0.32 (0.03)	0.33 (0.04)
Midband, *n* (%)	*N*	242	171	180	212	190	158	97
	n (%)	86 (35.5)	68 (39.8)	62 (34.4)	77 (36.3)	70 (36.8)	55 (34.8)	43 (44.3)
RemL-C (mg/dL)	*N*	325	291	284	332	310	241	138
	Mean (SD)	5.3 (4.3)	5.5 (4.6)	5.8 (5.8)	5.1 (4.0)	5.7 (6.0)	6.0 (8.3)	5.6 (7.4)
Lp (a) (mg/dL)	*N*	399	317	307	348	331	289	200
	Mean (SD)	31.6 (34.5)	31.8 (34.2)	30.4 (31.4)	30.4 (33.0)	33.0 (40.3)	33.3 (39.8)	38.4 (47.4)
ApoA-I (mg/dL)	*N*	412	340	338	407	362	291	182
	Mean (SD)	139 (31)	140 (31)	139 (31)	139 (31)	140 (31)	139 (32)	139 (35)
ApoB (mg/dL)	*N*	413	340	338	407	362	292	183
	Mean (SD)	112 (28)	108 (28)	108 (29)	105 (28)	105 (25)	103 (25)	103 (24)
ApoE (mg/dL)	*N*	396	334	330	396	348	282	177
	Mean (SD)	4.4 (1.4)	4.3 (1.3)	4.3 (1.3)	4.3 (1.3)	4.3 (1.4)	4.3 (1.4)	4.4 (1.7)
FPG (mg/dL)	*N*	604	501	505	561	538	486	338
	Mean (SD)	104 (24)	105 (41)	103 (25)	103 (23)	102 (20)	102 (21)	102 (26)
HbA1c JDS (%)	*N*	546	430	432	480	455	406	282
	Mean (SD)	5.6 (0.8)	5.7 (0.9)	5.6 (0.8)	5.6 (0.8)	5.6 (0.8)	5.7 (0.7)	5.9 (0.8)
Max IMT (mm)*	*N*	511	–	–	383	368	311	217
	Mean (SD)	1.57 (0.85)	–	–	1.56 (0.81)	1.56 (0.82)	1.53 (0.85)	1.61 (0.90)
Mean IMT (mm)†	*N*	459	–	–	359	349	290	204
	Mean (SD)	0.89 (0.33)	–	–	0.90 (0.34)	0.92 (0.38)	0.91 (0.37)	1.00 (0.46)
Achilles tendon thickness (mm)†	*N*	486	–	–	–	227	–	95
	Mean (SD)	11.4 (4.1)	–	–	–	11.7 (4.3)	–	11.4 (4.1)
ALT (IU/L)	*N*	696	570	580	627	583	526	372
	Mean (SD)	27 (15)	28 (16)	26 (16)	26 (15)	25 (15)	26 (25)	24 (13)
AST (IU/L)	*N*	695	569	573	626	582	524	375
	Mean (SD)	25 (13)	27 (11)	25 (11)	26 (10)	26 (11)	27 (43)	25 (10)
γ-GT (IU/L)	*N*	650	538	539	588	550	497	352
	Mean (SD)	37 (44)	37 (40)	36 (39)	34 (37)	37 (46)	36 (44)	32 (31)
CPK (IU/L)	*N*	655	548	554	597	559	510	362
	Mean (SD)	129 (99)	128 (74)	129 (74)	136 (142)	128 (74)	135 (105)	134 (94)
Creatinine (mg/dL)	*N*	670	546	551	603	566	512	360
	Mean (SD)	0.72 (0.20)	0.72 (0.21)	0.71 (0.22)	0.71 (0.18)	0.73 (0.29)	0.74 (0.33)	0.75 (0.33)
SBP (mmHg)	*N*	675	438	438	505	566	507	355
	Mean (SD)	124 (16)	125 (17)	124 (16)	124 (16)	125 (16)	125 (18)	124 (17)
DBP (mmHg)	*N*	675	438	437	505	566	507	353
	Mean (SD)	73 (10)	73 (11)	73 (10)	73 (10)	73 (10)	73 (11)	71 (11)
Pulse	*N*	469	311	312	369	393	393	256
	Mean (SD)	71 (11)	72 (12)	71 (12)	71 (11)	72 (12)	73 (12)	73 (11)
Body weight (kg)	*N*	650	334	341	459	502	453	331
	Mean (SD)	61 (12)	62 (12)	62 (12)	61 (12)	60 (12)	60 (12)	60 (12)
Waist circumference (cm)	*N*	306	84	78	187	161	149	87
	Mean (SD)	83 (10)	84 (9)	85 (10)	84 (9)	84 (10)	83 (10)	82 (11)

Rf: Relative to front. RemL-C: remnant lipoprotein cholesterol. FPG: fasting plasma glucose. IMT: intima media thickness of the carotid arteries.
*The highest of the right and left values.
†Average of the right and left values.

Supplementary Table 2. Laboratory and Clinical Data at Baseline and during Follow-up among Patients Who Were Newly Diagnosed with Heterozygous FH (*N*=17)

Parameter		Baseline	3 months	6 months	1 year	2 years	3 years	4 years
TC (mg/dL)	*N*	17	16	16	13	9	8	6
	Mean (SD)	333 (96)	220 (51)	229 (62)	234 (62)	230 (47)	243 (73)	236 (39)
TG (mg/dL)	*N*	17	16	16	13	10	9	6
	Mean (SD)	111 (64)	103 (56)	100 (53)	109 (56)	138 (88)	170 (134)	122 (108)
HDL-C (mg/dL)	*N*	17	16	16	13	10	9	6
	Mean (SD)	53 (10)	53 (9)	55 (10)	54 (14)	52 (13)	56 (21)	64 (18)
LDL-C (mg/dL)	*N*	17	16	16	13	9	7	6
	Mean (SD)	258 (92)	147 (49)	154 (61)	158 (60)	150 (57)	136 (47)	147 (38)
Rf	*N*	3	2	2	2	2	1	1
	Mean (SD)	0.32 (0.02)	0.32 (0.01)	0.32 (0.04)	0.32 (0.04)	0.33 (0.01)	0.31 (–)	0.31 (–)
Midband, *n* (%)	*N*	14	7	7	6	5	3	3
	n (%)	4 (28.6)	2 (28.6)	2 (28.6)	3 (50.0)	3 (60.0)	2 (66.7)	1 (33.3)
RemL-C (mg/dL)	*N*	10	7	6	6	4	3	3
	Mean (SD)	7.1 (3.6)	6.5 (5.3)	7.5 (4.5)	6.8 (3.1)	11.1 (9.0)	8.3 (5.1)	10.5 (9.4)
Lp (a) (mg/dL)	*N*	10	5	6	7	5	4	4
	Mean (SD)	28.5 (20.7)	35.2 (13.0)	31.0 (15.5)	30.2 (22.0)	43.1 (39.1)	29.8 (27.5)	16.7 (24.4)
ApoA-I (mg/dL)	*N*	15	6	8	7	5	3	4
	Mean (SD)	134 (19)	148 (13)	142 (13)	147 (29)	141 (30)	146 (41)	152 (30)
ApoB (mg/dL)	*N*	15	6	8	7	5	3	4
	Mean (SD)	160 (31)	115 (25)	116 (35)	124 (39)	109 (36)	97 (27)	121 (15)
ApoE (mg/dL)	*N*	13	6	7	7	5	3	3
	Mean (SD)	5.4 (1.8)	4.3 (0.9)	4.3 (0.9)	5.4 (1.5)	4.2 (1.1)	5.0 (1.6)	5.9 (1.1)
FPG (mg/dL)	*N*	15	13	14	12	9	7	5
	Mean (SD)	89 (9)	92 (9)	95 (12)	89 (13)	97 (10)	93 (10)	87 (12)
HbA1c JDS (%)	*N*	14	10	12	10	8	6	4
	Mean (SD)	5.3 (0.4)	5.4 (0.5)	5.3 (0.6)	5.2 (0.3)	5.4 (0.6)	5.5 (0.6)	5.1 (0.1)
Max IMT (mm)*	*N*	12	–	–	9	5	4	3
	Mean (SD)	1.40 (0.89)	–	–	1.41 (0.99)	1.78 (1.06)	1.85 (0.90)	1.73 (1.23)
Mean IMT (mm)†	*N*	11	–	–	9	6	4	3
	Mean (SD)	0.92 (0.61)	–	–	0.84 (0.37)	0.90 (0.45)	1.03 (0.57)	1.07 (0.67)
Achilles tendon thickness (mm)†	*N*	14	–	–	–	3	–	1
	Mean (SD)	10.9 (3.3)	–	–	–	9.3 (2.4)	–	7.9 (–)
ALT (IU/L)	*N*	16	15	16	13	10	8	5
	Mean (SD)	20 (9)	28 (17)	23 (9)	27 (13)	29 (16)	32 (8)	28 (9)
AST (IU/L)	*N*	16	14	16	13	10	8	5
	Mean (SD)	22 (11)	27 (16)	24 (12)	28 (22)	26 (11)	30 (12)	19 (4)
γ-GT (IU/L)	*N*	16	12	13	13	10	8	5
	Mean (SD)	21 (13)	33 (31)	35 (34)	24 (18)	30 (26)	30 (20)	27 (20)
CPK (IU/L)	*N*	16	14	14	13	9	7	5
	Mean (SD)	116 (76)	100 (47)	117 (56)	122 (94)	136 (83)	209 (321)	93 (20)
Creatinine (mg/dL)	*N*	16	12	13	12	10	8	5
	Mean (SD)	0.73 (0.19)	0.71 (0.17)	0.71 (0.16)	0.76 (0.20)	0.77 (0.20)	0.79 (0.16)	0.73 (0.11)
SBP (mmHg)	*N*	17	12	12	11	9	9	6
	Mean (SD)	119 (18)	114 (17)	117 (18)	122 (20)	124 (16)	127 (19)	119 (21)
DBP (mmHg)	*N*	17	12	12	11	9	9	6
	Mean (SD)	72 (8)	68 (6)	67 (9)	70 (12)	76 (9)	78 (13)	74 (12)
Pulse	*N*	15	10	11	9	6	6	5
	Mean (SD)	68 (9)	65 (13)	68 (13)	68 (13)	75 (18)	73 (9)	67 (10)
Body weight (kg)	*N*	17	9	8	12	8	9	5
	Mean (SD)	64 (11)	68 (9)	66 (10)	64 (14)	69 (13)	67 (13)	62 (9)
Waist circumference (cm)	*N*	7	4	5	8	4	5	4
	Mean (SD)	81 (13)	80 (10)	79 (9)	81 (12)	84 (14)	86 (11)	82 (9)

Rf: Relative to front. RemL-C: remnant lipoprotein cholesterol. FPG: fasting plasma glucose. IMT: intima media thickness of the carotid arteries.
*The highest of the right and left values.
†Average of the right and left values.

Supplementary Table 3. Lipid-lowering Treatment and Cardiovascular, Antidiabetic and Other Drugs Used during the Study Period at Baseline and at the End of Observation

Drug class/name	Whole period (n=758)*		Baseline (n=758)*		End of observation (n=743)†	
Lipid-lowering drugs	750		727		660	
(no treatment)	(8)		(31)		(83)	
Ezetimibe	482		364		406	
Statin	715		667		608	
atorvastatin		303		273		231
fluvastatin		11		8		8
pitavastatin		180		146		128
pravastatin		31		20		18
rosuvastatin		267		214		217
simvastatin		9		6		6
Resin	135		121		109	
colestimide		134		120		109
colestyramine		1		1		0
Probucol	148		124		116	
Fibrates	14		14		12	
bezafibrate		10		10		8
fenofibrate		5		4		4
Nicotinic acids	28		25		21	
niceritrol		5		5		4
tocopherol nicotinate		23		20		17
PUFA	71		53		65	
icosapentate		69		53		62
omega-3 PUFA (Lotriga)		3		0		3
Apheresis	25		23		17	
Other lipid-lowering drugs	8		0		1	
CETP inhibitor in trial		2		0		0
MK0859 in trial		5		0		1
SAR236553 in trial		1		0		0
Cardiovascular drugs	310		280		267	
$\alpha\beta$-Blockers	5		2		3	
α-Blockers	6		5		3	
β-Blockers	74		62		65	
ACE inhibitors	21		20		15	
Angiotensin II receptor blockers	131		105		106	
Calcium channel blockers	131		110		102	
Diuretics	29		23		20	
Antiplatelet agents	181		169		159	
Nitrites	7		7		6	
Other cardiovascular drugs	19		18		14	
Antidiabetic drugs	75		64		68	
α-Glucosidase inhibitors	16		14		17	
Biguanides	36		30		33	
Dipeptidyl peptidase 4 inhibitors	45		23		39	
Insulin preparations	11		9		9	
Rapid-acting insulin secretagogues	10		5		6	
Sulfonylureas	30		25		27	
Thiazolidines	16		14		14	
Other drugs	77		57		59	
Steroids	9		4		6	
Thyroid hormones	17		16		12	
Immunosuppressants	3		1		2	
Estrogen preparations	1		0		0	
Antihyperuricemics	6		6		6	
Others	51		35		35	

Values are numbers of patients.
*Excluding 4 patients without information on treatment.
†Excluding 19 patients without either information on treatment (n=4) or follow-up information (n=18). ACE: angiotensin converting enzyme; ARB: Angiotensin II receptor blocker; CCB: calcium-channel blocker.

Supplementary Table 4. List of Adverse Events

Adverse events	No. of patients	No. of episodes	Remark
Infectious diseases	3	3	
Vomiting and diarrhea	1	1	
Tuberculosis	1	1	
Herpes zoster	1	1	
Malignant neoplasms	9	10	
Gastric cancer	3	3	
Breast cancer	2	2	
Prostate cancer	1	1	
Renal pelvic cancer	1	2	Primary and recurrent episodes.
Leukemia	2	2	Two deceased.
Benign/unspecified neoplasms	2	2	
Uterine myoma	1	1	
Upper pharyngeal tumor	1	1	
Eye diseases	3	4	
Cataract	1	2	Right and left sides at two occasions.
Preretinal membrane	1	1	
Glaucoma	1	1	
Ear diseases	5	5	
Positional vertigo	4	4	
Sudden hearing loss	1	1	
Cardiovascular diseases	36	44	
(Coronary artery disease)	(17)	(21)	
Angina pectoris	1	1	
Acute myocardial infarction	3	3	One deceased.
Coronary stenosis	1	1	
PCI	11	15	
CABG	1	1	
Aortic valvular stenosis surgery	3	3	
Arrythmia	4	4	
Heart failure	2	4	Three episodes of one patient; one deceased.
Subarachnoid hemorrhage	1	1	One deceased.
Subdural hemorrhage	2	2	
Cerebral infarction	2	2	
Aortic aneurysm surgery	4	4	One deceased.
Others	3	3	
Respiratory diseases	2	3	
Pneumonia	1	2	
Respiratory failure	1	1	One deceased.
Gastrointestinal diseases	8	8	
Reflux esophagitis	1	1	
Ileus	1	1	
Others	6	6	
Dermatological diseases	4	5	
Drug eruption	1	1	
Urticaria	2	3	
Sweet's disease	1	1	
Musculoskeletal diseases	9	11	
Rhabdomyolysis	1	1	Suspected case with no observed elevation of CPK
Myalgia	3	4	
Others	5	6	
Urological diseases	1	1	
Urinary tract infection	1	1	
Laboratory findings	7	8	
CPK elevation	3	4	
Liver enzyme elevation	4	4	
Symptoms and signs only	14	16	
Injuries	5	5	
Death of unknown cause	1	1	One deceased.

Supplementary Table 5. Baseline Characteristics of the Registered Patients with Homozygous FH

Variable	*N**	Mean (SD)
Mean (SD)		
Age (year)	7	49.6 (15.9)
Years from FH diagnosis	7	21.4 (14.6)
Height (cm)	7	163 (5)
Body weight (kg)	7	62 (11)
BMI (kg/m^2)	7	23.2 (3.1)
Waist circumference (cm)	5	84 (10)
Systolic blood pressure	5	123 (17)
Diastolic blood pressure	5	63 (8)
Number (%)		
Male	7	5 (71.4)
Current smoking	7	0 (0.0)
Xanthoma/ATT	7	6 (85.7)
Parental CAD	6	6 (100.0)
Sibling's CAD	7	2 (28.6)
Hypertension	7	4 (57.1)
Diabetes mellitus	7	2 (28.6)
CAD	7	5 (71.4)
Cerebrovascular diseases	7	0 (0.0)
Cerebral infarction	7	0 (0.0)
Lipid-lowering treatment	7	7 (100.0)
Ezetimibe	7	5 (71.4)
Statin	7	7 (100.0)
Probucol	7	2 (28.6)
PUFA	7	1 (14.3)
Apheresis	7	7 (100.0)
Use of cardiovascular drugs	7	6 (85.7)
Use of antidiabetic drugs	7	1 (14.3)

ATT: Achilles tendon thickening; BMI: body mass index; CAD: coronary artery diseases, FH: familial hypercholesterolemia.
*Number of the subjects used for the calculation.

Supplementary Table 6. Baseline Laboratory Data of the Registered Patients with Homozygous FH

Laboratory tests	*n*	Mean	SD
Total cholesterol (mg/dL)	6	245	89
Triglycerides (mg/dL)	6	116	77
HDL cholesterol (mg/dL)	6	37	12
LDL cholesterol (mg/dL)	6	185	79
Rf	1	0.38	–
Remnant lipoprotein cholesterol (mg/dL)	4	6.0	4.3
Lp (a) (mg/dL)	4	46.8	57.5
Apolipoprotein A-I (mg/dL)	4	107	36
Apolipoprotein B (mg/dL)	4	133	63
Apolipoprotein E (mg/dL)	4	5.6	3.4
Maximal IMT (mm)*	5	2.18	1.63
Mean IMT (mm)†	4	1.08	0.46
Achilles tendon thickness (mm)†	5	16.3	1.3
Fasting plasma glucose (mg/dL)	4	137	78
A1c (JDS) (%)	4	5.8	1.0
ALT (IU/L)	6	48	26
AST (IU/L)	6	44	36
GTP (IU/L)	6	54	33
CPK (IU/L)	5	124	96
Cr (mg/dL)	6	0.90	0.33

IMT: intima media thickness of the carotid arteries. Rf: Relative to front.
*The highest of the right and left values.
†Average of the right and left values.