The official journal of the Japan Atherosclerosis Society and
the Asian Pacific Society of Atherosclerosis and Vascular Diseases

Original Article

J Atheroscler Thromb, 2022; 29: 1201-1212. http://doi.org/10.5551/jat.62989

Familial Hypercholesterolemia in Patients with Acute Coronary Syndrome: Genetic Insights from EXPLORE-J

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Aim: Genetic testing can provide a definitive diagnosis of familial hypercholesterolemia (FH). However, accessibility of genetic testing may be limited in certain countries where it is not considered "standard of care," including Japan. In addition, mutations responsible for FH cannot be identified in approximately 30% of patients.

Methods: EXPLORE-J is a multicenter, prospective, observational study of patients presenting with acute coronary syndrome (ACS). The genetic data were analyzed and adjudicated as pathogenic, indeterminate, or nondetectable pathogenic variant.

Results: Of 1,944 patients, 431 underwent genetic screening. Overall, most patients had nonpathogenic variants of *LDLR*, *LDLRAP1*, or *PCSK9* (n=396, 91.9%). Of the 25 (5.8%) patients with pathogenic variants, variants of the *LDLR* gene and the *PCSK9* gene were seen in 10 and 15 patients, respectively. Indeterminate variants were observed in 10 (2.3%) patients. Of the 431 patients, eight (1.9%) met the criteria for a diagnosis of FH using the Japanese Atherosclerosis Society (JAS) 2017 guidelines. When genetic data were incorporated, 33 (7.7%) patients met the JAS guidelines. No patients with FH pathogenic variants satisfied the JAS clinical criteria for a diagnosis of FH.

Conclusions: The results revealed a higher prevalence of genetic mutations of FH among Japanese patients with ACS and a low sensitivity of the FH diagnostic criteria of the JAS 2017 guidelines. These findings highlight the difficulties of FH diagnosis in patients with ACS in the acute phase and suggest the importance of genetic testing and family history.

Key words: Familial hypercholesterolemia, Genetic testing, Japan, Acute coronary syndrome

Introduction

Familial hypercholesterolemia (FH) is an inherited autosomal dominant disease that is characterized by severely elevated low-density lipoprotein cholesterol (LDL-C), early-onset coronary artery disease (CAD), and tendon or cutaneous xanthomas[1-3]. The prevalence of FH worldwide is approximately 1 in 200–500, and the estimated number of cases of FH in Japan is 300,000 [4]. However,

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Received: May 10, 2021 Accepted for publication: August 1, 2021

in Japan, less than 1% of those with FH are estimated to have been diagnosed using the Dutch Lipid Clinical Network guidelines[5].

The diagnosis of FH may include a combination of family history, clinical signs (e.g., tendon xanthomas), and LDL-C levels. Although there are several guidelines for the clinical diagnosis of FH, including the Japan Atherosclerosis Society (JAS) 2017 guidelines[6], the Dutch Lipid Clinical Network guidelines[5], and the Simon Broome Register guidelines[7], there are differences between these guidelines in the criteria used to diagnose FH. The Dutch Lipid Clinical Network and Simon Broome Register guidelines include genetic testing, whereas the JAS 2017 guidelines currently do not.

Genetic testing can provide a definite diagnosis of FH by detection of pathogenic mutations in the genes coding for the low-density lipoprotein receptor (LDLR), apolipoprotein B (apoB), and proprotein convertase subtilisin/kexin type 9 (PCSK9)[8-10], or a rare recessive form of FH, autosomal recessive hypercholesterolemia, which is caused by loss-of-function mutations in the LDLR adaptor protein-1 (LDLRAP1)[8, 11]. However, accessibility of genetic testing may be limited in countries where it is not considered "standard of care," including Japan[12], and in up to 30% of cases a mutation cannot be identified[13].

EXPLORE-J is a prospective, large-scale, observational study using data from an acute coronary syndrome (ACS) registry, conducted at 59 centers in Japan[14]. ACS encompasses a range of conditions compatible with acute myocardial ischemia and/or myocardial infarction (MI), including ST-segment elevation MI, non–ST-segment elevation MI, and unstable angina[15]. Large observational studies in Europe have investigated the prevalence of FH in patients with ACS, including genetically confirmed cases[16, 17]. However, the evidence base for Japan is lacking, and the JAS 2017 guidelines have higher cut-off values for LDL-C and Achilles tendon thickness (ATT) when identifying possible FH[3, 14]. To date, results from the EXPLORE-J study have highlighted the prevalence of FH in patients with ACS in Japan[18], as well as described lipid management at the time of ACS[19]. Using the JAS 2017 guidelines, FH was approximately five times more prevalent in patients with ACS than in the general population[18].

Aim

The overall aim of EXPLORE-J was to evaluate lipid management and persistent cardiovascular risk in patients hospitalized for ACS, with a focus on underlying hypercholesterolemia and FH[14]. The current analysis of the EXPLORE-J registry aimed to identify the characteristics of patients with confirmed pathogenic variants associated with FH. In addition, this analysis aimed to evaluate FH prevalence in patients with ACS, both with and without genetic testing, according to the JAS 2017 guidelines, the Dutch Lipid Clinical Network guidelines, and the Simon Broome Register guidelines.

Methods

Study Design and Patients

EXPLORE-J was a multicenter, prospective, observational study that was conducted between April 2015 and August 2018. Full details of the methods have been published previously[14]. In brief, patients with ACS who required hospitalization were recruited and registered at 59 sites between April 2015 and August 2016. Patients were then followed up for 2 years. Baseline data, including LDL-C levels, presence of xanthoma, and family history, were reported at Visit 1, within 14 days after hospitalization due to ACS.

Genetic Examinations

Separate informed consent was obtained for genetic examinations. Single nucleotide variations or insertion/deletion in genes encoding key proteins were evaluated. Mutations in genes encoding key proteins involved in the LDLR endocytic and recycling pathways were analyzed (*LDLR*; *LDLRAP1*; *PCSK9*). The *APOB* gene was not analyzed because no cases of *APOB* mutations had been previously reported in the Japanese population when the analysis was conducted[6]. Genetic mutations were categorized as nonpathogenic variant (–), indeterminate pathogenic variant (±, not reported previously, but potentially pathogenic), and pathogenic variant (+, reported pathogenicity of FH). The variants detected in the *LDLR* or *PCSK9* genes were classified as pathogenic using ClinVar, the Leiden Open Variation database, and population data from the Exome Aggregation Consortium, the Japanese Human Genetic Variation Database, and the Tohoku Medical Megabank Organization, as well as *in silico* tools/software, and functional data based on guidelines edited by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.

Diagnosis of FH

In this study, three sets of diagnostic guidelines for FH were used for each patient: the JAS 2017 guidelines[6], the Dutch Lipid Clinical Network

Table 1. Diagnostic guidelines for FH

Diagnostic guidelines	Criteria
The Japanese Atherosclerosis Society guidelines [6]	**At least two of the following:** · Hyper LDL-C (≥ 180 mg/dL before treatment) - Secondary hyperlipidemia is excluded - Lipid levels before treatment are considered if patients were on medications - FH is strongly suspected if LDL-C is ≥ 250 mg/dL · Tendon xanthoma (tendon xanthoma in the back of hand, elbow, knee, etc.; or Achilles tendon thickening ≥ 9 mm) or tuberous xanthoma (excluding xanthelasma of eyelid) · Familial history of FH (blood relatives within the second degree of kinship) or early-onset CAD (male subjects aged <55 years; female subjects aged <65 years) Diagnosis by genetic testing is advised if FH is suspected
The Dutch Lipid Clinical Network guidelines for FH[a] [5]	**Family history:** First-degree relative with known premature coronary and vascular disease (1 point); first-degree relative with known LDL-C level above the 95th percentile[b] (1 point); first-degree relative with tendon xanthoma and/or corneal arcus[b] (2 points); children aged <18 years with LDL-C level above the 95th percentile[b] (2 points) **Clinical history:** Patient with premature cerebral or peripheral vascular disease (1 point); or patient with premature CAD (2 points) **Physical examination:** Corneal arcus prior to age 45 years[b] (4 points); or tendinous xanthoma (6 points) **Genetic testing:** Functional mutation in the *LDLR*, *APOB*[b], or *PCSK9* genes (8 points) **LDL-C levels:** 155–189 mg/dL (1 point); 190–249 mg/dL (3 points); 250–329 mg/dL (5 points); ≥ 330 mg/dL (8 points)
The Simon Broome guidelines[c] [7]	**Criterion 1:** Total cholesterol levels >290 mg/dL or LDL-C >190 mg/dL in adults; or total cholesterol levels >260 mg/dL or LDL-C >155 mg/dL in children[b] **Criterion 2:** Tendon xanthomas in the patient, or tendon xanthomas in a first- or second-degree relative[b] **Criterion 3:** DNA-based evidence of an *LDLR* mutation, familial defective apoB-100[b], or a *PCSK9* mutation **Criterion 4:** Family history of myocardial infarction in a second-degree relative <50 years of age or first-degree relative <60 years of age[b] **Criterion 5:** Family history of elevated total cholesterol >290 mg/dL in an adult first- or second-degree relative; or family history of elevated total cholesterol >260 mg/dL in a child, brother, or sister aged ≤ 16 years[b]

ApoB, apolipoprotein B; CAD, coronary artery disease; FH, familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/kexin type 9.
[a] 3–5 points indicate possible FH, 6–8 points indicate probable FH, and >8 points indicate definite FH.
[b] Criteria not collected as part of the EXPLORE-J study.
[c] Criterion 1+ 2 or 3 indicated definitive FH; criterion 1 +4 or 5 indicated possible FH.

guidelines[5], and the Simon Broome guidelines[7]. Details of each are shown in Table 1. Diagnoses of FH by the JAS 2017 guidelines were conducted both with and without genetic testing as a definitive criterion (identification of a pathogenic variant led to a positive FH diagnosis; functional variants in *LDLR* and *PCSK9* genes were screened in EXPLORE-J).

The following criteria were assessed as they are included in all three guidelines for the diagnosis of FH: LDL-C, ATT, and family history of CAD. The highest available measurement of LDL-C obtained at or before Visit 1 was used for the diagnosis of FH. LDL-C was also measured (calculated or direct) prior to hospitalization without treatment (for patients who had available LDL-C values off statin treatment any time before hospitalization) and after hospitalization. In this study, both calculated (e.g., by the Friedewald equation) and/or direct (e.g., by homogeneous direct methods) LDL-C values were collected. For these analyses, calculated LDL-C values were used if available; if calculated LDL-C values were not available, direct LDL-C values were used. ATT was measured at the central reading laboratory by blinded investigators. In addition, family history of CAD was collected at enrollment to help facilitate FH diagnosis.

Statistical Analyses

Baseline characteristics and LDL-C values between genetic variants were described by mean, median, standard deviation, and range for continuous data, and by proportion in each category for categorical data. For comparisons of baseline

Table 2. Baseline characteristics by genetic variants[18]

	Nonpathogenic variant (–) (*n*=396)	Indeterminate variant (±) (*n*=10)	Pathogenic variant (+) (*n*=25)	Total (*n*=431)	*P*-value
Age, years, mean (SD)	65.7 (11.7)	69.7 (12.2)	63.6 (10.8)	65.7 (11.7)	0.378
Male, *n* (%)	317 (80.1)	7 (70.0)	19 (76.0)	343 (79.6)	0.528
Female, *n* (%)	79 (19.9)	3 (30.0)	6 (24.0)	88 (20.4)	
BMI, kg/m^2, mean (SD)	24.3 (3.8)[a]	24.1 (3.3)	23.0 (3.3)	24.3 (3.8)[b]	0.221
Weight, kg, mean (SD)	65.5 (13.4)[a]	64.0 (15.9)	62.9 (12.7)	65.3 (13.4)[b]	0.633
STEMI, *n* (%)	270 (68.2)	7 (70.0)	18 (72.0)	295 (68.4)	0.732
NSTEMI, *n* (%)	52 (13.1)	1 (10.0)	1 (4.0)	54 (12.5)	
Unstable angina, *n* (%)	74 (18.7)	2 (20.0)	6 (24.0)	82 (19.0)	
Diabetes mellitus, *n* (%)	144 (36.4)	3 (30.0)	6 (24.0)	153 (35.5)	0.446
Hypertension, *n* (%)	305 (77.0)	10 (100.0)	17 (68.0)	332 (77.0)	0.123
Dyslipidemia, *n* (%)	306 (77.3)	9 (90.0)	23 (92.0)	338 (78.4)	0.152
Therapy before hospitalization, *n* (%)					
Statin	97 (24.5)	1 (10.0)	10 (40.0)	108 (25.1)	0.133
Intensive statin[c]	16 (4.0)	0 (0.0)	0 (0.0)	16 (3.7)	0.449
Ezetimibe	4 (1.0)	0 (0.0)	1 (4.0)	5 (1.2)	0.347
Statin + ezetimibe	0 (0.0)	0 (0.0)	1 (4.0)	1 (0.2)	-

BMI, body mass index; NSTEMI, non–ST-elevation myocardial infarction; SD, standard deviation; STEMI, ST-elevation myocardial infarction. Baseline characteristics were collected at Visit 1.
[a] *n*=395. [b] *n*=430. [c] Atorvastatin ≥ 20 mg, rosuvastatin ≥ 10 mg, and pitavastatin ≥ 4 mg.

characteristics and LDL-C values, *P*-values are provided for baseline characteristics based on Fisher's exact test for categorical variables and analysis of variance tests for continuous variables. A two-sided *P*-value of <0.05 was considered significant. Comparisons between diagnostic guidelines for genetic variants are descriptive only. Receiver operating characteristic (ROC) curves were plotted for LDL-C and ATT to FH diagnosis, and cut-off values were obtained using the Youden index[20].

Ethics Approval and Consent to Participate

This study was conducted in compliance with the Declaration of Helsinki (amended in October 2013) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects (enacted on December 22, 2014). Prior to the study initiation, the investigator or sub-investigators submitted the protocol and informed consent form to the ethical review committee of each study center and obtained their approval. Patient anonymity was protected by the use of subject identification codes. A co-operation fee of 5000 Japanese yen (approximately US$42 or €37 in October 2015) for study participation was provided for each patient on request from the study center. All patients were required to provide written informed consent.

Results

In total, 1,944 patients were included in the EXPLORE-J study. Of these, 431 underwent genetic testing and were analyzed. Pathogenic variants were observed in 25 (5.8%) patients. Of these 25 patients, 10 (40.0%) had a pathogenic variant of *LDLR*, and 15 (60.0%) had a pathogenic variant of *PCSK9* **(Supplementary Table 1)**. No patients had pathogenic variants of *LDLRAP1*. Indeterminate variants were observed in 10 (2.3%) patients **(Supplementary Table 2)**. Of these, five (50.0%) patients had indeterminate variants of *LDLR* and another five (50.0%) had indeterminate variants of *PCSK9*. Overall, most patients had nonpathogenic variants of *LDLR*, *LDLRAP1*, or *PCSK9* (*n*=396, 91.9%).

Baseline characteristics are shown in **Table 2**. Overall, the baseline characteristics of the 431 patients who underwent genetic testing were similar to those previously reported for the entire EXPLORE-J population (mean [standard deviation (SD)] age, 66.0 [12.2] years; men, 80.3%; mean [SD] body mass index, 24.2 [3.6] kg/m^2)[18]. Patients with pathogenic variants had generally higher LDL-C levels at baseline compared with patients with nonpathogenic or indeterminate variants before hospitalization (without medication) and after hospitalization **(Table 3)**. Other baseline characteristics were similar between the

Table 3. LDL-C levels by genetic variants

LDL-C levels, mg/dL, mean (SD)	Nonpathogenic variant (–) (*n*=396)	Indeterminate variant (±) (*n*=10)	Pathogenic variant (+) (*n*=25)	Total (*n*=431)	*P*-value
n	392	10	25	427	
Maximum value at baseline	125.1 (41.0)	135.5 (33.2)	157.2 (57.3)	127.2 (42.5)	<0.001
n	88	5	3	96	
Measurement without medication before hospitalization	131.5 (37.4)	137.4 (41.3)	179.7 (99.2)	133.3 (40.4)	0.123
n	374	10	24	408	
First measurement after hospitalization	119.4 (40.4)	126.9 (38.0)	148.1 (51.9)	121.2 (41.6)	0.004
n	346	10	24	380	
Direct method	93.3 (31.1)	107.7 (27.7)	130.9 (51.1)	96.1 (33.8)	<0.001
n	343	10	24	377	
Calculated	91.9 (31.3)	106.6 (27.4)	124.3 (38.9)	94.4 (32.7)	<0.001

LDL-C, low-density lipoprotein cholesterol; SD, standard deviation.

Table 4. The percentage of patients meeting the JAS guideline criteria by genetic variants

JAS guideline criteria		All patients (*n*=431)	Nonpathogenic variants (–) and indeterminate variants (±) (*n*=406)	Pathogenic variants (+) (*n*=25)
Family history of early-onset CAD[a]	No	325 (75.4)	308 (75.9)	17 (68.0)
	Yes	29 (6.7)	28 (6.9)	1 (4.0)
	Unknown	77 (17.9)	70 (17.2)	7 (28.0)
ATT	<9 mm	358 (83.1)	337 (83.0)	21 (84.0)
	≥ 9 mm	19 (4.4)	17 (4.2)	2 (8.0)
	Unknown	54 (12.5)	52 (12.8)	2 (8.0)
Baseline LDL-C levels (maximum value)	<180 mg/dL	384 (89.1)	385 (89.9)	19 (76.0)
	≥ 180 mg/dL	43 (10.0)	37 (9.1)	6 (24.0)
	Unknown	4 (0.9)	4 (1.0)	0 (0.0)

[a]Male <55 years, female <65 years.
ATT, Achilles tendon thickness; CAD, coronary artery disease; JAS, Japan Atherosclerosis Society; LDL-C, low-density lipoprotein cholesterol.

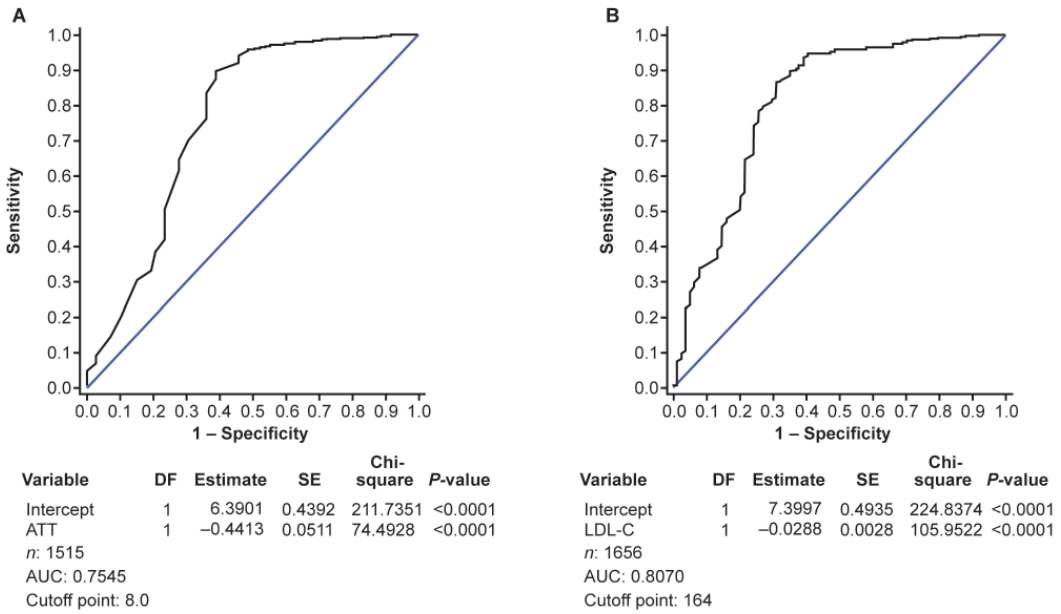
genetic variants, with no difference observed for any baseline characteristic **(Table 2; Table 3)**.

The percentage of patients meeting each of the three sections (family history of early-onset CAD, ATT, maximum baseline LDL-C levels) of the JAS guidelines by genetic variant is shown in **Table 4**. Family history was available for most patients across all genetic variants (overall, 77 [17.9%] patients had unknown [information unavailable] family history of early-onset CAD).

ATT was available for most patients across all genetic variants (overall, 54 [12.5%] patients had unknown ATT [ATT unavailable]). Baseline LDL-C was available for most patients (overall, four [0.9%] patients had unknown baseline LDL-C [baseline LDL-C unavailable]).

Of the patients with known pathogenic variants (*n*=25; **Supplementary Table 1**), none of the patients would have been diagnosed with FH according to the JAS 2017 guidelines. Similarly, of the patients with indeterminate variants (*n*=10; **Supplementary Table 2**), no patients would have been diagnosed with FH according to the JAS 2017 guidelines. In addition, two (8.0%) patients with pathogenic variants had ATT ≥ 9 mm; however, their highest LDL-C values were 153 and 108 mg/dL, respectively, which did not meet the LDL-C criterion for FH as per the JAS guidelines.

The percentage of patients meeting criteria for each of the three guidelines for the diagnosis of FH (the JAS guidelines, the Dutch Lipid Clinical Network guidelines, or the Simon Broome guidelines) is shown



Variable	DF	Estimate	SE	Chi-square	*P*-value
Intercept	1	6.3901	0.4392	211.7351	<0.0001
ATT	1	–0.4413	0.0511	74.4928	<0.0001
n: 1515					
AUC: 0.7545					
Cutoff point: 8.0					

Variable	DF	Estimate	SE	Chi-square	*P*-value
Intercept	1	7.3997	0.4935	224.8374	<0.0001
LDL-C	1	–0.0288	0.0028	105.9522	<0.0001
n: 1656					
AUC: 0.8070					
Cutoff point: 164					

Fig. 1. ROC curve of FH-related parameters on FH judgment via JAS guidelines and FH genetic test for (A) ATT (mm) and (B) LDL-C (max value on/before Visit 1; mg/dL)

ATT, Achilles tendon thickness; AUC, area under the curve; DF, degree of freedom; FH, familial hypercholesterolemia; JAS, Japanese Atherosclerosis Society; LDL-C, low-density lipoprotein cholesterol; ROC, receiver operating characteristic; SE, standard error.

in **Supplementary Fig. 1**. In total, 398 (92.3%) patients did not have FH according to any of the three guidelines. Thirty-three (7.7%) patients had definitive FH according to at least one of the guidelines, and 17 (3.9%) patients had FH according to both the Dutch Lipid Clinical Network and Simon Broome guidelines. Overall, six (1.4%) patients had definitive FH according to all three guidelines.

Without genetic testing, only eight (1.9%) of the 431 patients met the JAS 2017 guidelines for FH, compared with definite FH according to the Dutch Lipid Clinical Network guidelines (n=6, 1.4%) or the Simon Broome guidelines (n=6, 1.4%). When the JAS 2017 guidelines were supplemented with genetic data (data not shown), the proportion of patients considered to have FH (n=33, 7.7%) was similar to that of definite FH diagnosed using Dutch Lipid Clinical Network guidelines (n=17, 3.9%) and the Simon Broome guidelines (n=31, 7.2%).

ROC curves of parameters of FH diagnosis via the JAS 2017 guidelines and FH genetic mutations are shown in **Fig. 1**. Cut-offs of LDL-C 164 mg/dL and ATT 8.0 mm have the highest sensitivity and specificity to identify patients with FH when used as part of the JAS guidelines.

Discussion

This analysis of a subgroup of patients with ACS from the EXPLORE-J study who underwent FH genetic testing (n=431) provides several major findings: i) overall, 25 (5.8%) patients had pathogenic variants for FH, mostly in *PCSK9*; ii) no patients with pathogenic variants met the threshold for FH according to the JAS guidelines; iii) when the JAS guidelines were supplemented with genetic data, the proportion of patients considered to have FH was similar to that of the Dutch Lipid Clinical Network and Simon Broome guidelines; and iv) when using the JAS guidelines for FH, according to the ROC parameter of values analysis, cut-off values of LDL-C 164 mg/dL and ATT 8.0 mm have the highest sensitivity and specificity to identify patients with FH.

Of the 25 patients who had pathogenic variants for FH, many (n=15, 60%) had a pathogenic variant of PCSK9. There were more mutations in the *PCSK9* gene (associated with higher risk of CAD) than previously reported in Japanese patients with pathogenic variants associated with FH[10, 21]. This may be due to differences in the population, region, and family history of the patient samples in the previous studies. Additionally, the prevalence of the E32K variant of PCSK9 in Japanese patients with pathogenic variants for FH may be higher than that in other countries[22-24]. Patients who have this mutation show milder phenotypes compared with those with LDLR mutations[25]. Therefore, it may be possible that some Japanese FH patients with this mutation do not meet

the criteria of FH in the JAS 2017 guidelines for this reason and are therefore not diagnosed as having FH.

It is surprising that none of the patients with pathogenic variants would be diagnosed with FH using the JAS 2017 guidelines. Their LDL-C levels were much lower than those of statin-naïve FH patients without ACS. One reason for the lack of patients with pathogenic variants meeting the JAS guidelines' definition of FH could be that 40% of these patients were receiving a statin. Another reason is that LDL-C levels decrease during the acute phase of ACS. It has been reported that LDL-C can decrease by up to 48% after acute MI[26]. These may have masked their untreated LDL-C levels by lowering LDL-C below the 180 mg/dL threshold required to identify FH with the JAS 2017 guidelines. Cut-off levels of LDL-C for the diagnosis of FH in the JAS guidelines were determined by using the pre-treatment LDL-C levels in patients with an FH diagnosis and those without[27]. Furthermore, the JAS guidelines criteria are not designed for patients in the acute phase of ACS.

Notably, four patients with pathogenic variants were reported by their attending physician to have been diagnosed with FH despite not meeting the JAS guidelines for FH during the EXPLORE-J study using ATT. Of these patients, three had LDL-C $<$180 mg/dL and had statin use prior to the index ACS. This further supports the view that signs of FH might be masked in some patients through prior statin use, especially in this population for whom early initiation of high-intensity statin is recommended or who are already on statins at presentation of ACS[6].

Statin use can also mitigate the development of xanthoma and reduce ATT[28], and some patients may therefore not have had ATT ≥ 9 mm due to prior statin use. A previous study in Japanese patients with ACS (n=296) demonstrated a prevalence of statin use similar to that in the present study (23.3% vs. 25.1%, respectively)[29]. However, the detection rate of ATT ≥ 9 mm here (19/377; 5.0%) was lower than that reported by Ohmura *et al.* (53/296; 17.9%)[29]. Because not all patients with FH have ATT ≥ 9 mm, this may account for differences between these two studies. Patients in the Ohmura *et al.* study were from residential areas of Tokyo. In this study, patients were enrolled from all over Japan, which may account for differences[29].

In addition, the percentage of patients with FH according to the JAS guidelines reported by Ohmura *et al.* was 5.7%[29], whereas in this study 1.9% of patients would be diagnosed with FH using the JAS 2017 guidelines (without additional genetic testing) and 7.7% would be diagnosed with FH when the JAS guidelines were supplemented with genetic testing[29]. FH status was determined using the JAS 2017 guidelines with and without additional genetic testing. When genetic testing was incorporated with the JAS 2017 guidelines, the proportion of patients identified as having FH was similar to those diagnosed with definite FH by the Dutch Lipid Clinical Network and Simon Broome guidelines (3.9% and 7.2%, respectively). This suggests that the JAS guidelines without genetic testing might be insufficient for diagnosis of FH in patients with ACS in Japan. In addition, the cut-off point of LDL-C and ATT was analyzed using ROC curves of parameters. According to these ROC curves, we propose that cut-off values of LDL-C 165 mg/dL and ATT 8.0 mm could lead to more accurate diagnosis of FH if incorporated into the current JAS guidelines.

Because of variability in LDL-C after ACS, availability of genetic testing in patients with suspected FH is important in this population. Currently, there are a limited number of hospitals in Japan that can conduct genetic testing for FH, and genetic testing for FH is not reimbursed by health insurance providers[6]. Stigma of genetic disease (e.g., social perceptions and fears of insurance denial) and lack of resources (such as accessibility to geneticists and genetic counseling) may also hinder diagnosis of FH[30, 31]. These barriers need to be removed to improve the diagnostic rate of FH and allow cascade screening, which will lead to appropriate lipid-lowering therapy with high-dose statins, ezetimibe, and PCSK9 inhibitors for these patients, lowering their risk of premature cardiovascular events. However, although genetic testing will improve diagnosis in this population, many patients with FH will not have an identifiable mutation. Therefore, clinical evaluation of those presenting with ACS remains crucial.

Interestingly, although a direct comparison may not be meaningful because of the small sample size, a lower proportion of patients with pathogenic variants had a family history of CAD than had those without pathogenic variants (4.0% vs. 6.9%). This may be due to underreporting or the fact that patients with pathogenic variants (and their families) have severe enough hypercholesterolemia that they are already treated with statins, thereby reducing CAD.

This study is limited by the nature of the observational study design. In this analysis, untreated LDL-C levels were only available for three of the patients with pathogenic variants. Ohmura *et al.* also highlighted the difficulties of collecting LDL-C values for use in the diagnosis of FH and that collection of blood samples varied among the facilities included in their study[29]. Furthermore, information on family

history was unknown for 77 (17.9%) patients overall and seven (28.0%) patients with pathogenic variants. In addition, not all patients received genetic testing, and available data for each criterion in the JAS, Dutch Lipid Clinical Network, and Simon Broome guidelines were not complete for all patients. Furthermore, although *APOB* mutations are generally not found in the Japanese population, and so were not included in the genetic testing used here, sporadic mutations may occur. In fact, subsequent to this analysis, the first case of FH due to a known pathogenic *APOB* gene variant was reported in a Japanese family[32]. Although this study has only examined variants of the *LDLR* and *PCSK9* genes, it might be more informative for FH diagnosis if polygenic variants are examined, such as *APOE4* and the sporadic mutations of *APOB*. Finally, the generalizability of these findings is limited to the Japanese population.

Conclusion

These results revealed genetic mutations of FH found among Japanese patients with ACS. However, to increase the diagnostic accuracy of patients presenting with ACS, the diagnostic thresholds used in the JAS guidelines may need to be re-evaluated for these patients. These findings highlight the importance of genetic testing and family history in the diagnosis of FH in patients with ACS, as well as screening and diagnosis of FH before the initiation of statins in patients that may have FH. A substantial number of high-risk patients could benefit from genetic testing. In addition, we propose that cut-off values of LDL-C 165 mg/dL and ATT 8.0 mm could improve diagnosis of FH if incorporated into the current JAS guidelines.

Acknowledgements

The authors thank the patients, their families, and all investigators involved in the study: Tamio Teramoto of Teikyo University (Tokyo, Japan), Shun Ishibashi of Jichi Medical University (Tochigi, Japan), Kotaro Yokote of Chiba University (Chiba, Japan), Tomonori Okamura of Keio University (Tokyo, Japan), and Hiroyuki Daida of Juntendo University (Tokyo, Japan) for collaboration and advice regarding planning of the study; Mebix (Tokyo, Japan) for assistance with study implementation/operation; BML (Tokyo, Japan) for PCSK9-related and genome-related analysis; CTD (Tokyo, Japan) for consulting; and Shizuya Yamashita of Rinku General Medical Center (Osaka, Japan) and Toru Yoshizumi of Kawasaki Hospital (Hyogo, Japan). The authors would also like to thank Yoshiharu Takagi and Yukiko Morimoto of Sanofi for providing support with statistical analysis; and Yasunori Nakahigashi and Makiko Usami of Sanofi for assistance with study implementation/operation. Medical writing assistance and editorial support, under the direction of the authors, were provided by Kate Carolan, PhD, of Prime Global (Knutsford, United Kingdom) according to Good Publication Practice guidelines (Link) and funded by Sanofi. The authors were involved in the study design as well as the collection, analysis, and interpretation of data. All authors had full access to all the data in this study and take complete responsibility for the integrity of the data and accuracy of the data analysis.

Financial Support

This study was funded by Sanofi and Regeneron Pharmaceuticals, Inc.

Conflicts of Interest

Mariko Harada-Shiba has received honoraria from Amgen, Astellas, and Sanofi; and research grants from Aegerion, Kaneka and Recordati and held stock of Liid Pharma. Junya Ako has received honoraria from Amgen and Sanofi. Atsushi Hirayama has received honoraria and research grants from Sanofi. Masato Nakamura has received honoraria from Astellas, Amgen, and Sanofi; and research grants from Sanofi. Atsushi Nohara has received honoraria from Sanofi. Kayoko Sato has received research grants from Astellas, Takeda, and Aegerion. Yoshitaka Murakami does not have any conflicts of interest to declare. Ryusuke Koshida was an employee of Sanofi K.K at the time of this study. Asuka Ozaki is an employee of Sanofi K.K. Hidenori Arai has received lecture fees from Daiichi Sankyo, Kowa, MSD, Pfizer, and Sanofi.

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Supplementary Table 1. Characteristics of patients with pathogenic variants ($n=25$)

Age, years	Sex	FH history	FH diagnosis by JAS criteria	Premature CAD family history	CAD family history	Highest LDL-C level, mg/dL	Statin use	ATT, mm	Gene	Mutation[a]	Amino acid[b]
50-59	Male	No	No	No	Yes	119	Yes	7.9	*PCSK9*	c.94G>A	p.E32K
50-59	Male	No	No	N/A	N/A	136	Yes	6.2	*LDLR*	c.344G>A	p.R115H
60-69	Male	Possible	No	N/A	N/A	146	No	8.2	*PCSK9*	c.94G>A	p.E32K
50-59	Male	No	No	N/A	N/A	154	No	6.2	*LDLR*	c.344G>A	p.R115H
70-79	Male	Possible	No	No	No	78	Yes	8.8	*PCSK9*	c.94G>A	p.E32K
70-79	Male	Possible	No	No	No	109	Yes	5.8	*LDLR*	c.344G>A	p.R115H
70-79	Female	Possible	No	No	No	245	No	6.2	*PCSK9*	c.94G>A	p.E32K
60-69	Male	Yes	No	No	Yes	221	No	5.6	*PCSK9*	c.94G>A	p.E32K
40-49	Male	Yes	No	No	Yes	168	Yes	8.9	*LDLR*	c.1845+2T>C[b]	N/A
80-89	Male	No	No	No	No	136	No	6.9	*PCSK9*	c.94G>A	p.E32K
60-69	Male	No	No	No	No	204	No	6.1	*PCSK9*	c.94G>A	p.E32K
60-69	Male	No	No	No	No	151	No	N/A	*PCSK9*	c.94G>A	p.E32K
70-79	Female	Yes	No	Yes	Yes	113	Yes	7.8	*LDLR*	c.810C>A[c]	p.C270X
50-59	Female	Yes	No	No	Yes	290	No	5.3	*PCSK9*	c.1486C>T	p.R496W
60-69	Female	No	No	No	No	98	Yes	7.4	*LDLR*	c.1784G>A	p.R595Q
70-79	Female	Yes	No	No	No	153	Yes (plus ezetimibe)	17.5	*LDLR*	c.2390-2A>T[d]	N/A
60-69	Male	No	No	No	Yes	126	No (plus fibrate)	5.7	*PCSK9*	c.94G>A	p.E32K
60-69	Male	No	No	No	Yes	188	No	6.4	*LDLR*	c.1747C>T	p.H583Y
30-39	Male	Possible	No	No	Yes	300	Yes	8.2	*PCSK9*	c.94G>A	p.E32K
60-69	Male	No	No	N/A	N/A	103	No	6.7	*LDLR*	c.1783C>T	p.R595W
60-69	Male	No	No	N/A	N/A	173	No	6.3	*PCSK9*	c.94G>A	p.E32K
70-79	Male	No	No	N/A	N/A	122	No	N/A	*LDLR*	c.1702C>G	p.L568V
70-79	Male	No	No	N/A	N/A	121	No	7.2	*PCSK9*	c.94G>A	p.E32K
60-69	Female	Yes	No	No	No	108	Yes	12.8	*PCSK9*	c.94G>A	p.E32K
60-69	Male	No	No	No	No	167	No	6.8	*PCSK9*	c.94G>A	p.E32K

ATT, Achilles tendon thickness; CAD, coronary artery disease; FH, familial hypercholesterolemia; JAS, Japanese Atherosclerosis Society; LDL-C, low-density lipoprotein cholesterol; N/A, not available.

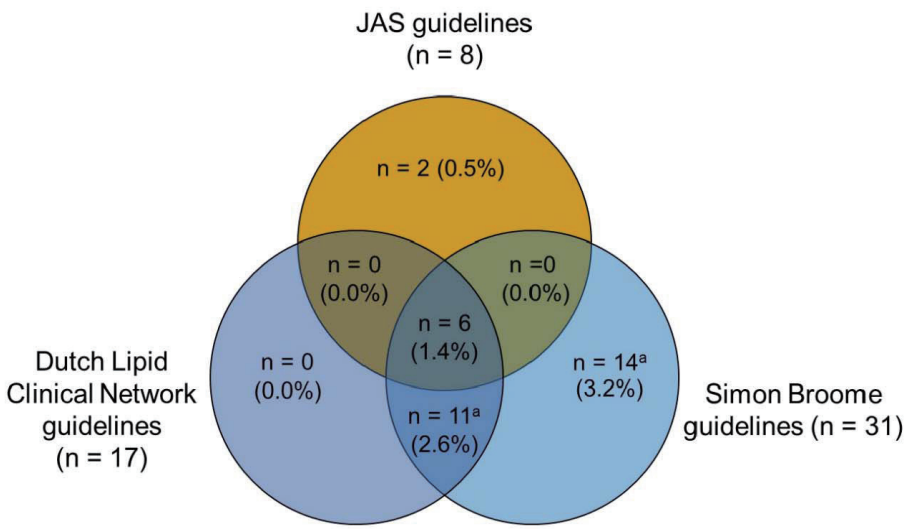
[a]Mutations were missense mutations, except for splice donor variant, stop codon mutation, and splice acceptor variant (denoted by [b], [c], and [d], respectively).

[b]Amino acid abbreviations: C, cysteine; E, glutamic acid; H, histidine; K, lysine; L, leucine; Q, glutamine; R, arginine; V, valine; W, tryptophan; X, stop codon; Y, tyrosine.

Supplementary Table 2. Characteristics of patients with indeterminate variants ($n=10$)

Age	Sex	FH history	FH diagnosis by JAS criteria	Premature CAD family history[a]	CAD family history	Highest LDL-C level	Statin use	ATT, mm	Gene	Mutation	Amino acid[b]
50-59	Male	No	No	No	No	141	No	7.7	*PCSK9*	c.1765G>A	p.V589M
60-69	Male	Possible	No	No	No	191	No	8.7	*LDLR*	c.211G>A	p.G71R
60-69	Male	No	No	N/A	N/A	142	No	6.3	*LDLR*	c.211G>A	p.G71R
70-79	Male	No	No	Yes	Yes	115	No	N/A	*PCSK9*	c.212C>T	p.P71L
80-89	Female	Yes	No	No	No	112	No	6.6	*PCSK9*	c.1954A>G	p.N652D
80-89	Male	Yes	No	No	No	91	No	5.3	*PCSK9*	c.1975C>G	p.R659G
80-89	Female	No	No	N/A	N/A	89	No	N/A	*PCSK9*	c.1727C>T	p.P576L
70-79	Female	No	No	No	No	166	No	6.2	*LDLR*	c.2257C>T	p.P753S
50-59	Male	No	No	No	Yes	158	Yes	7.4	*LDLR*	c.1546G>A	p.G516S
50-59	Male	Possible	No	No	No	150	No	10.2	*LDLR*	c.1834G>T	p.A612S

ATT, Achilles tendon thickness; CAD, coronary artery disease; FH, familial hypercholesterolemia; JAS, Japanese Atherosclerosis Society; LDL-C, low-density lipoprotein cholesterol; N/A, not available.
[a]Male <55 years, female <65 years.
[b]Amino acid abbreviations: A, alanine; D, aspartic acid; G glycine; L, leucine; M, methionine; N, asparagine; P, proline; R, arginine; S, serine; V, valine.



Supplementary Fig. 1. Diagnosis of patients with FH based on the clinical criteria of JAS, Dutch Lipid Clinical Network, and Simon Broome guidelines

FH, familial hypercholesterolemia; JAS, Japan Atherosclerosis Society.
In total, 398 patients did not meet any of the three diagnostic guidelines.
[a]All patients carry any pathogenic variants for FH (definite FH).