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ORIGINAL RESEARCH

Patients With *LDLR* and *PCSK9* Gene Variants Experienced Higher Incidence of Cardiovascular Outcomes in Heterozygous Familial Hypercholesterolemia

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BACKGROUND: Patients with familial hypercholesterolemia who harbored both low-density lipoprotein receptor (*LDLR*) and *PCSK9* (proprotein convertase subtilisin/kexin type 9) gene variants exhibit severe phenotype associated with substantially high levels of low-density lipoprotein cholesterol. In this study, we investigated the cardiovascular outcomes in patients with both *LDLR* and *PCSK9* gene variants.

METHODS AND RESULTS: A total of 232 unrelated patients with *LDLR* and/or *PCSK9* gene variants were stratified as follows: patients with *LDLR* and *PCSK9* (*LDLR/PCSK9*) gene variants, patients with *LDLR* gene variant, and patients with *PCSK9* gene variant. Clinical demographics and the occurrence of primary outcome (nonfatal myocardial infarction) were compared. The observation period of primary outcome started at the time of birth and ended at the time of the first cardiac event or the last visit. Patients with *LDLR/PCSK9* gene variants were identified in 6% of study patients. They had higher levels of low-density lipoprotein cholesterol (P=0.04) than those with *LDLR* gene variants. On multivariate Cox regression model, they experienced a higher incidence of nonfatal myocardial infarction (hazard ratio, 4.62; 95% CI, 1.66–11.0; P=0.003 versus patients with *LDLR* gene variant). Of note, risk for nonfatal myocardial infarction was greatest in male patients with *LDLR/PCSK9* gene variants compared with those with *LDLR* gene variant (86% versus 24%; P<0.001).

CONCLUSIONS: Patients with *LDLR/PCSK9* gene variants were high-risk genotype associated with atherogenic lipid profiles and worse cardiovascular outcomes. These findings underscore the importance of genetic testing to identify patients with *LDLR/PCSK9* gene variants, who require more stringent antiatherosclerotic management.

Key Words: cardiovascular outcome ■ familial hypercholesterolemia ■ LDLR gene ■ PCSK9 gene

Familial hypercholesterolemia (FH) is a genetic disorder caused by variants in low-density lipoprotein receptor (*LDLR*) gene, *PCSK9* (proprotein convertase subtilisin/kexin type 9) gene, and apolipoprotein B (*APOB*) gene. These genetic variants can cause elevation of low-density lipoprotein cholesterol (LDL-C) levels and tendon or skin xanthomas,[1–3] which lead to the higher risk of atherosclerotic cardiovascular disease (ASCVD) in FH.[4] These observations emphasize the clinical importance of the genotype that causes FH because of its association with metabolic and cardiovascular risks.

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CLINICAL PERSPECTIVE

What Is New?

- Patients with low-density lipoprotein receptor (*LDLR*) and *PCSK9* (proprotein convertase subtilisin/kexin type 9) gene variants were familial hypercholesterolemia genotype, which causes an elevated risk of nonfatal myocardial infarction.

What Are the Clinical Implications?

- Risk stratification according to sex and genotype may be a potential risk stratification tool to identify high-risk patients with familial hypercholesterolemia who need more intense antiatherosclerotic therapies.

Nonstandard Abbreviations and Acronyms

FH	familial hypercholesterolemia
HeFH	heterozygous familial hypercholesterolemia
LDLR	low-density lipoprotein receptor
PCSK9	proprotein convertase subtilisin/kexin type 9
TAUSSIG	Trial Assessing Long Term Use of PCSK9 Inhibition in Subjects With Genetic LDL Disorders

Recent genetic analyses of FH have identified patients with variants in 2 different causative genes. In recent published analyses from Japan,[5,6] patients with both *LDLR* and *PCSK9* gene variants (*LDLR*/*PCSK9* gene variants) were detected in 1.2% to 4.0% of Japanese patients with FH. Although such patients represent a small fraction of patients with FH overall, the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel has proposed that this genotype represents a severe form of FH.[3] The presence of both causative gene variants has the potential to substantially elevate circulating LDL-C level. Given that LDL-C is a major driver of atherosclerosis in FH, this feature may be an important atherogenic substrate responsible for ASCVD in patients with *LDLR*/*PCSK9* gene variants.[6,7] However, the clinical demographics and outcomes in patients with *LDLR*/*PCSK9* gene variants remain to be fully elucidated. In this study, we investigated the prevalence, clinical characteristics, and cardiovascular outcomes of patients with *LDLR* and *PCSK9* gene variants.

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Population

This study retrospectively analyzed 377 Japanese unrelated patients with clinically diagnosed heterozygous FH (HeFH) who underwent genetic testing to identify variants in *LDLR* gene and/or *PCSK9* gene at the National Cerebral and Cardiovascular Center, Osaka, Japan, between 2005 and 2016. HeFH was diagnosed on the basis of Japanese Atherosclerosis Society Criteria 2017: having ≥2 of the following factors: LDL-C ≥180 mg/dL, tendon/skin xanthoma, and a family history of FH or premature coronary artery disease within second-degree relatives.[8] We excluded 124 patients without *LDLR* or *PCSK9* gene variants. Furthermore, in 253 patients who had gene variant in *LDLR* gene and/or *PCSK9* gene, the following patients were excluded: those with 2 gene variants in *LDLR* gene (n=1) and *PCSK9* gene (n=1), those with both *LDLR* gene variants and *PCSK9* gene loss-of-function variants (n=5), and those who were aged <20 years (n=14). The remaining 232 unrelated patients with FH were included into the current analysis (Figure 1). The protocol of this study was approved by the institutional review board of the National Cerebral and Cardiovascular Center (approval No. M17-56). Each patient gave written informed consent to participate in the study. All clinical investigations were conducted in accordance with the principles of the Declaration of Helsinki.

DNA Analysis

DNA analyses were conducted on genomic DNA extracted from patients' whole blood using an automated DNA extraction machine (QIAsymphony; QIAGEN, Valencia, CA). All coding regions and the exon-intron boundary sequence of the *LDLR* and *PCSK9* genes were examined by direct sequencing, as described previously.[6] Samples without variants in both the *LDLR* gene and the *PCSK9* genes were analyzed for large deletions or insertions in the *LDLR* gene by the multiplex ligation-dependent probe amplification method using the P062B *LDLR* multiplex ligation-dependent probe amplification kit (MRC Holland, Amsterdam, the Netherlands). The pathogenicity of *LDLR* and *PCSK9* gene variants was assessed according to the American College of Medical Genetics criteria.[9] With regard to *PCSK9* gene variant, the current analysis included 3 variants (p.Val4Ile, p.Glu32Lys, and p.Arg496Trp)[6,10,11] that are rare (allele frequency <1%) among East Asian population.[12] *PCSK9* p.Val4Ile gene variant is rated as benign using American College of Medical Genetics

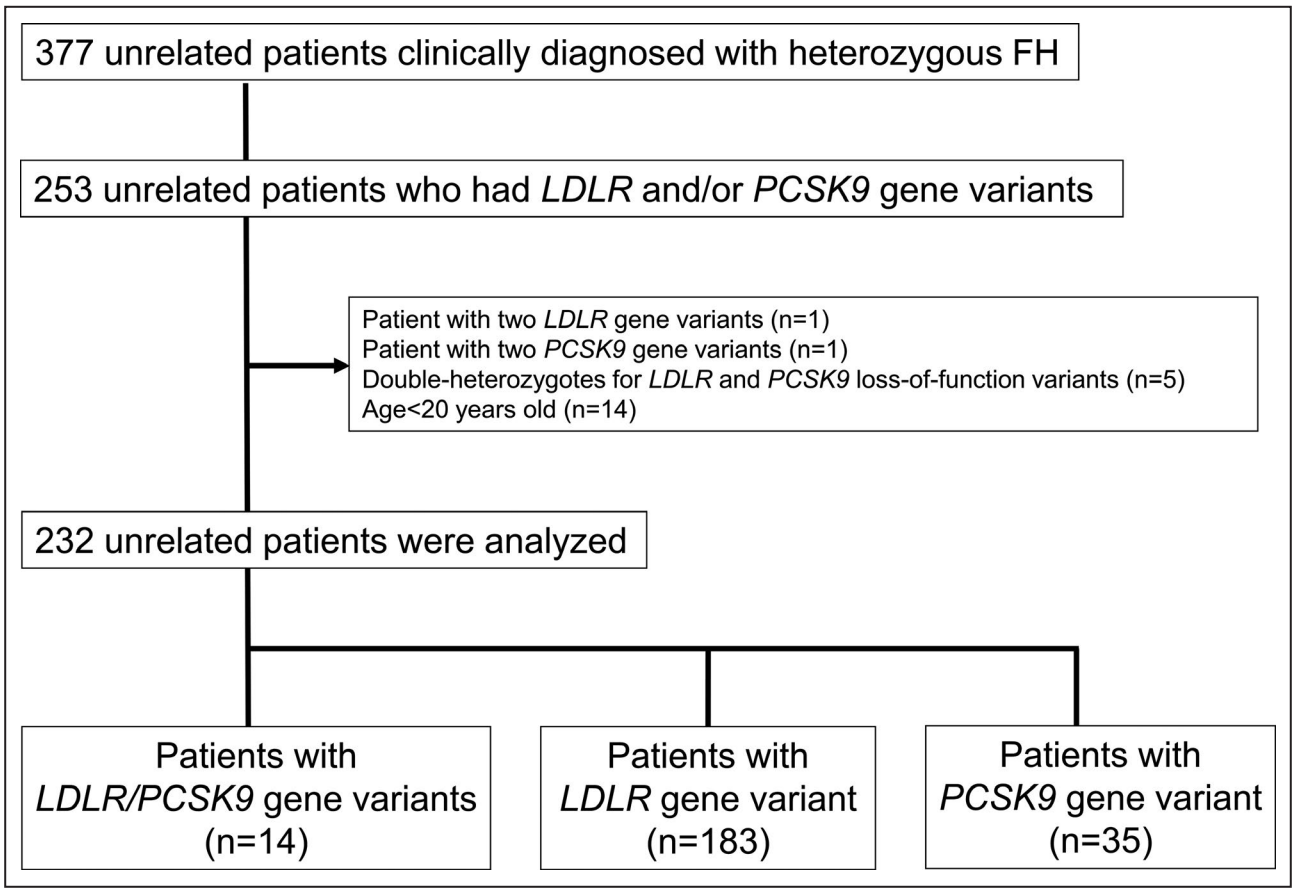

Figure 1. Flowchart of the study patients.
FH indicates familial hypercholesterolemia; *LDLR*, low-density lipoprotein receptor; and *PCSK9*, proprotein convertase subtilisin/kexin type 9.

criteria,[13] whereas its potential association for serum LDL-C level has been shown by our published study.[6] Therefore, in the current study, we included (1) all of these 3 *PCSK9* gene variants and (2) those except *PCSK9* p.Val4Ile gene variant into the analysis. All variants were denoted using known and accepted nomenclature based on the full lengths of the splice variants with 860 and 692 amino acids (National Center for Biotechnology Information reference sequence NM_000527.4 for *LDLR* gene and NM_174936.3 for *PCSK9* gene).[14,15]

Cardiovascular Outcomes

The primary outcome was defined as the occurrence of nonfatal myocardial infarction (MI). The secondary outcome included the occurrence of nonfatal MI and coronary revascularization. Because any cardiac cause of death was not observed in the current study subjects, we did not include this event in either primary or secondary outcomes. MI was defined as the presence of cardiac ischemic symptoms with biomarker evidence of myocardial injury and electrocardiographic changes suggestive of new ischemia (new ST-T changes or new left bundle-branch block) or the development of pathological Q waves on electrocardiography.[16] Coronary revascularization was defined as percutaneous coronary intervention or coronary artery bypass grafting for any reason. These outcomes were determined through medical record review and, when necessary, through a mailed questionnaire or telephone follow-up.

Measurement of Lipid Parameters

LDL-C levels at baseline were calculated by the Friedewald formula, except for triglyceride levels >400 mg/dL.[17] In patients who had already received lipid-lowering agents at their first visit, we estimated baseline LDL-C levels according to the type and dose of their lipid-lowering medication, applying a correcting factor for LDL-C based on the reported efficacy of each drug, as performed previously in similar analyses.[18–21] Fasting serum levels of total cholesterol, triglycerides, high-density lipoprotein cholesterol, and lipoprotein (a) were measured by enzymatic methods (Sekisui Medical, Tokyo, Japan) using an automated analyzer (Hitachi Labospect 008; Hitachi-Hitec, Tokyo, Japan). Apolipoproteins were measured by turbidimetric

Table 1. Clinical Demographics

Demographics	Patients With *LDLR* Gene Variant (n=183)	Patients With *PCSK9* Gene Variant (n=35)	*P* Value*	Patients With *LDLR/PCSK9* Gene Variants (n=14)	*P* Value†
Age, y	43±16	53±13	<0.001	53±13	0.66
Male sex, n (%)	92 (51)	17 (49)	0.85	7 (50)	0.98
BMI ≥30 kg/m^2, n (%)	0 (0)	1 (3)	0.05	1 (7)	0.02
Hypertension, n (%)	42 (23)	7 (20)	0.68	5 (36)	0.34
Diabetes mellitus, n (%)	4 (2)	1 (3)	0.82	0 (0)	0.43
Smoking history, n (%)	75 (41)	15 (43)	0.36	8 (57)	0.29
Family history of coronary artery disease, n (%)	53 (29)	2 (6)	0.16	4 (29)	0.92
Any xanthomas, n (%)	138 (76)	18 (51)	0.04	12 (86)	0.61
Corneal arcus, n (%)	68 (37)	8 (23)	0.33	5 (36)	0.92

Categorical variables (sex, BMI ≥30 kg/m^2, history of hypertension, diabetes mellitus, and smoking, family history of coronary artery disease, and presence of any xanthomas and corneal arcus) were expressed as number (percentage) and compared using the Fisher's exact test. Normally distributed continuous variables (age) were expressed as mean±SD and compared using permutation test. BMI indicates body mass index; *LDLR,* low-density lipoprotein receptor; and *PCSK9,* proprotein convertase subtilisin/kexin type 9.

*Patients with *LDLR* gene variant vs those with *PCSK9* gene variant.

†Patients with *LDLR* gene variant vs those with *LDLR/PCSK9* gene variants.

immunoassay (Nittobo Medical, Tokyo, Japan) using an automated analyzer (JCA-BM8060; JEOL, Tokyo, Japan) by LSI Medience Corporation (Tokyo, Japan).

Statistical Analysis

Two-group comparison was conducted using the Fisher's exact test for the categorical variables. Normally distributed continuous variables were expressed as mean±SD, and nonnormally distributed continuous data were summarized as the median (interquartile range). Both continuous variables were compared using permutation test with the perm package of R. The Kaplan-Meier method was used to estimate survival curves for primary and secondary outcomes, and the exact log-rank test was used to assess differences between patients with *LDLR* gene variant and those with *PCSK9* gene variant and between patients with *LDLR* gene variant and those with *LDLR* and *PCSK9* gene variants.[22] The current study collected lifetime cardiac events after the birth, but not those after the first visit to our clinic. This is because 82% of primary outcome in study subjects occurred before their first visit (Table S1). To evaluate true cardiac event risks in the current study population, the observation period started after the time of birth, and it ended at the time of the first cardiac event or the last visit. Patients who were free from primary outcome at the last visit were considered as censored observation. Cox proportional hazards model was used to identify high-risk cause of primary outcome using following covariates determined before the analysis (model 1: sex and genotype; and model 2: sex, history of hypertension, diabetes mellitus, smoking, and genotype). In addition, this analysis further adjusted the following 2 variables: duration until achieving LDL-C goal (defined as >50% decrease of LDL-C from baseline or on-treatment LDL-C <100 mg/dL) from the time of birth and that during optimal LDL-C control under the therapy.[8,23] Because history of hypertension, diabetes mellitus, smoking, duration until achieving LDL-C goal from the time of birth, and that during optimal LDL-C control under the therapy were time varying, we analyzed these covariates as time-varying covariates.[24] $P<0.05$ was considered statistically significant. All analyses were performed with SPSS (SPSS Japan, Tokyo, Japan), STATA 13 (StataCorp, College Station, TX), and R 3.6.3 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Genetic Features in Patients With FH

In this study, the prevalence rates of patients with *LDLR/PCSK9* gene variants, those with *LDLR* gene variants, and those with *PCSK9* gene variants were 6%, 80%, and 14%, respectively. In patients with *LDLR/PCSK9* gene variants, 13 *LDLR* gene variants and 2 *PCSK9* gene variants were identified (Table S2). All variants identified in this cohort were listed in Tables S3 and S4.[13]

Clinical Demographics in Patients With Different Gene Variants

We summarize baseline clinical characteristics of the patients with *LDLR* and/or *PCSK9* gene variants (Table 1). There were no significant differences in the percentages of men and the prevalence of coronary risk factors in patients with *LDLR* gene variant compared with those with *PCSK9* gene variant and in

patients with *LDLR* gene variant compared with those with *LDLR/PCSK9* gene variants (Table 1). For lipid profiles at baseline, LDL-C level was highest in patients with *LDLR/PCSK9* gene variants (316±75 mg/dL) compared with those with *LDLR* gene variant (273±72 mg/dL; *P*=0.04) and those with *PCSK9* gene variant (219±58 mg/dL) (Table 2). Baseline triglycerides, apolipoprotein A-I, apolipoprotein A-II, and apolipoprotein C-II were higher in patients with *PCSK9* gene variant than patients with *LDLR* gene variants (*P*<0.001, *P*=0.001, *P*<0.001, and *P*<0.001, respectively), whereas the levels of lipoprotein (a), apolipoprotein C-III, and apolipoprotein E were similar.

Use of Lipid-Lowering Therapy and On-Treatment Lipid Parameters

Table 3 shows a comparison of lipid-lowering therapy and on-treatment lipid profiles. Over 85% of study patients received statin. Of note, high-intensity statin and PCSK9 inhibitor, including evolocumab and alirocumab, were more frequently used in patients with *LDLR/PCSK9* gene variants (high-intensity statin: *P*=0.03; PCSK9 inhibitor: *P*<0.001; evolocumab: *P*=0.002; and alirocumab: *P*=0.05 versus patients with *LDLR* gene variant). As a consequence, these therapeutic differences were associated with a greater absolute (*P*<0.001 versus patients with *LDLR* gene variant) and percentage reduction of LDL-C (*P*=0.008 versus patients with *LDLR* gene variant) in patients with *LDLR/PCSK9* gene variants. Furthermore, a higher proportion of these patients achieved LDL-C <100 mg/dL (*P*=0.002) and percentage reduction of LDL-C <50% (*P*=0.04) in patients with *LDLR/PCSK9* gene variants compared with those with *LDLR* gene variant (Table 4).

Occurrence of Primary and Secondary Outcomes in Patients With *LDLR/PCSK9* Gene Variants

The frequencies of primary and secondary outcomes are summarized in Table 5, Table S1, and Table S5. During the observational period (mean, 53±17 years), 39 patients experienced primary outcome (mean time to event occurrence, 50±14 years). Moreover, higher occurrences of primary (nonfatal MI: exact log-rank: *P*=0.02) outcomes were observed in patients with *LDLR/PCSK9* gene variants compared with those with *LDLR* gene variant (Figure 2A). Univariate Cox proportional hazards model identified significantly higher incidence of nonfatal MI in male patients (*P*<0.001), patients with smoking history (*P*=0.002), and patients with *LDLR/PCSK9* gene variants (*P*=0.03 versus patients with *LDLR* gene variants). On multivariate Cox proportional hazards model analysis, which included sex and genotype as covariates (model 1), patients with *LDLR/PCSK9* gene variants had significantly higher likelihood of experiencing nonfatal MI than those with *LDLR* gene variant (hazard ratio [HR], 3.21; 95% CI, 1.20–7.20; *P*=0.02). We further adjusted the history of hypertension, diabetes mellitus, and smoking as covariates. Patients with *LDLR* and *PCSK9* gene variants still had a significantly higher incidence of primary outcome compared with those with *LDLR* gene variant (HR, 4.26; 95% CI, 1.66–11.0; *P*=0.003) (model 2) (Table 6). Furthermore, additional analysis adjusted (1) the duration from birth to achieving LDL-C goal and (2) the duration during optimal LDL-C control under the therapy as well. The mean values of these variables were 52.0 and 1.8 years, respectively. Even after adjusting these variables, patients with *LDLR*

Table 2. Baseline Lipid Profiles

Variable	Patients With *LDLR* Gene Variant (n=183)	Patients With *PCSK9* Gene Variant (n=35)	*P* Value*	Patients With *LDLR/PCSK9* Gene Variants (n=14)	*P* Value†
LDL-C, mg/dL	273±72	219±58	<0.001	316±75	0.04
HDL-C, mg/dL	52±16	58±13	0.03	50±21	0.64
Triglycerides, mg/dL	91 (70–138)	139 (88–195)	<0.001	133 (68–167)	0.32
Lp(a), mg/dL	29±26	28±22	0.85	27±17	0.82
Apolipoprotein A-I, mg/dL	127±33	147±25	0.001	117±38	0.31
Apolipoprotein A-II, mg/dL	27±6	33±6	<0.001	27±6	0.91
Apolipoprotein B, mg/dL	127±39	108±37	0.01	135±29	0.43
Apolipoprotein C-II, mg/dL	3.8±1.7	5.8±1.9	<0.001	4.1±1.5	0.61
Apolipoprotein C-III, mg/dL	9.5±8.6	11.0±3.2	0.25	9.0±2.4	0.75
Apolipoprotein E, mg/dL	4.3±1.3	4.2±0.8	0.68	4.2±1.0	0.76

Normally distributed continuous variables (LDL-C, HDL-C, Lp[a], apolipoprotein A-I, apolipoprotein A-II, apolipoprotein B, apolipoprotein C-II, apolipoprotein C-III, and apolipoprotein E) were expressed as mean±SD, and nonnormally distributed continuous variables (triglycerides) were expressed as median (interquartile range). Both continuous variables were compared using permutation test. HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; *LDLR,* low-density lipoprotein receptor; Lp(a), lipoprotein (a); and *PCSK9*, proprotein convertase subtilisin/kexin type 9.

*Patients with *LDLR* gene variant vs those with *PCSK9* gene variant.

†Patients with *LDLR* gene variant vs those with *LDLR/PCSK9* gene variants.

Table 3. On-Treatment Lipid-Lowering Therapies

Variable	Patients With *LDLR* Gene Variant (n=183)	Patients With *PCSK9* Gene Variant (n=35)	*P* Value*	Patients With *LDLR/PCSK9* Gene Variants (n=14)	*P* Value†
Statin, n (%)	160 (88)	32 (91)	0.93	12 (86)	0.54
High-intensity statin, n (%)	6 (16)	2 (6)	0.07	6 (43)	0.03
Ezetimibe, n (%)	105 (58)	11 (31)	0.002	6 (43)	0.21
Cholestyramine, n (%)	32 (18)	0 (0)	<0.001	2 (14)	0.71
PCSK9 inhibitor, n (%)	10 (5)	0 (0)	0.06	6 (43)	<0.001
Evolocumab, n (%)	6 (3)	0 (0)	0.14	4 (29)	0.002
Alirocumab, n (%)	4 (2)	0 (0)	0.23	2 (14)	0.05
Lipoprotein apheresis, n (%)	1 (0)	0 (0)	0.26	0 (0)	0.70

Categorical variables (use of statin, high-intensity statin, ezetimibe, cholestyramine, PCSK9 inhibitor, evolocumab, alirocumab, and lipoprotein apheresis; patients with LDL-C <100 mg/dL; and patients with percentage reduction of LDL-C <50%) were expressed as number (percentage) and compared using the Fisher's exact test. *LDLR* indicates low-density lipoprotein receptor; and *PCSK9*, proprotein convertase subtilisin/kexin type 9.

*Patients with *LDLR* gene variant vs those with *PCSK9* gene variant.

†Patients with *LDLR* gene variant vs those with *LDLR/PCSK9* gene variants.

and *PCSK9* gene variants still had higher incidence of nonfatal MI in subjects with HeFH (HR, 6.08; 95% CI, 2.29–16.1; *P*<0.001 versus patients with *LDLR* gene variant).

We evaluated the study population after excluding the patients with *PCSK9* p.Val4Ile gene variant. Similar to the aforementioned results, patients with *LDLR/PCSK9* gene variants had higher LDL-C level (Figure S1) and worse cardiovascular outcomes than those with *LDLR* or *PCSK9* gene variants (Figure S2A and S2B).

Risk Stratification of Primary Outcome, According to Sex and Genotype

The incidence of primary outcome was further investigated in subgroups stratified according to sex and genotype (Figure 3). Men and patients with *LDLR/PCSK9* gene variants were associated with more frequent occurrence of primary outcome (male patients versus female patients, *P*=0.006; patients with *LDLR/PCSK9* gene variants versus patients with *LDLR* gene variant, *P*=0.03). Of note, in men, patients with *LDLR/PCSK9* gene variants had a higher risk of primary outcome than patients with *LDLR* gene variant (*P*<0.001), whereas the incidence of primary outcome was not statistically significant in female subjects (Figure 3).

DISCUSSION

Our analyses provide clinical evidence of cardiovascular risk in patients with *LDLR/PCSK9* gene variants. Although the prevalence of this genotype was 6% in our Japanese cohort, the lipid profiles and cardiovascular outcomes of the patients were distinct, characterized by higher levels of LDL-C as well as more frequent occurrence of nonfatal MI. The current findings support a consensus statement from the International Atherosclerosis Society Severe Familial

Table 4. On-Treatment Lipid Levels

Variable	Patients With *LDLR* Gene Variant (n=183)	Patients With *PCSK9* Gene Variant (n=35)	*P* Value*	Patients With *LDLR/PCSK9* Gene Variants (n=14)	*P* Value†
On-treatment LDL-C, mg/dL	136±49	116±46	0.03	110±52	0.05
Absolute reduction of LDL-C, mg/dL	121±71	96±54	0.01	206±114	0.008
Percentage reduction of LDL-C, %	44±21	45±25	0.66	61±26	0.03
Patients with LDL-C <100 mg/dL, n (%)	33 (18)	13 (37)	0.01	8 (57)	0.002
Patients with percentage reduction of LDL-C <50%, n (%)	87 (48)	14 (40)	0.78	10 (71)	0.04

Categorical variables (patients with LDL-C <100 mg/dL and patients with percentage reduction of LDL-C <50%) were expressed as number (percentage) and compared using the Fisher's exact test. Normally distributed continuous variables (on-treatment LDL-C, absolute reduction of LDL-C, and percentage reduction of LDL-C) were expressed as mean±SD and compared using permutation test. LDL-C indicates low-density lipoprotein cholesterol; *LDLR*, low-density lipoprotein receptor; and *PCSK9*, proprotein convertase subtilisin/kexin type 9.

*Patients with *LDLR* gene variant vs those with *PCSK9* gene variant.

†Patients with *LDLR* gene variant vs those with *LDLR/PCSK9* gene variants.

Table 5. Summary of Primary and Secondary Outcomes

Variable	All Patients (n=232)	Patients With *LDLR/PCSK9* Gene Variants (n=14)	Patients With *LDLR* Gene Variant (n=183)	Patients With *PCSK9* Gene Variant (n=35)
Primary outcome: nonfatal MI, n (%)	39 (17)	6 (43)	30 (16)	3 (9)
Secondary outcome: a composite of nonfatal MI and coronary revascularization, n (%)	69 (30)	7 (50)	55 (30)	7 (20)

Categorical variables were expressed as number (percentage). *LDLR* indicates low-density lipoprotein receptor; MI, myocardial infarction; and *PCSK9*, proprotein convertase subtilisin/kexin type 9.

Hypercholesterolemia Panel that proposed patients with 2 different causative gene variants as a severe genotype of FH.[3]

The poor cardiovascular outcomes in patients with *LDLR/PCSK9* gene variants could be explained by their more atherogenic lipid profiles based on genetic characteristics. LDLR is a major contributor to low-density lipoprotein (LDL) metabolism. *LDLR* gene variant elevates LDL particles via the diminished quality and/or quantity of LDLR[25] and promotes production of apolipoprotein B-100 from hepatocytes.[26] PCSK9 itself induces an elevation of LDL particles in circulation because of the degradation of LDLR.[27] In addition to *LDLR* gene variant, the concomitance of *PCSK9* gain-of-function gene variant could further promote atherogenicity. These basic mechanisms suggest the concomitance of both *LDLR* and *PCSK9* gene variants as a considerably atherogenic genetic phenotype that exhibits substantially high LDL-C level and a greater frequency of ASCVD.

Differences in baseline triglycerides and apolipoprotein C-II across the groups are another interesting observation in the current analysis. We observed higher triglyceride levels in patients with *PCSK9* gene variant alone and those with *LDLR/PCSK9* gene variants. Patients with *PCSK9* gain-of-function gene variant are characterized as having degradation of LDLR because of higher PCSK9 affinity for LDLR. Given that PCSK9 has been shown to degrade LDLR as well as LDL receptor-related protein-1 (LRP1) and very-LDLR,[28] this property of PCSK9 may result in an elevated level of triglyceride-rich lipoprotein. In response to this PCSK9-mediated elevation of triglyceride-rich lipoprotein, which apolipoprotein C-II is a component of (eg, very-LDL), apolipoprotein C-II might be elevated.

The clinical significance of *PCSK9* gene variants, especially *PCSK9* p.Val4Ile gene variant, has not been fully annotated. Although this variant has been reported to elevate LDL-C level in patients with *LDLR* gene variant,[6] the American College of Medical Genetics guidelines classified it as benign one. In Korean subjects, those with *PCSK9* p.Val4Ile gene variant did not necessarily exhibit an elevated LDL-C level,[29] suggesting that the pathogenicity of *PCSK9* p.Val4Ile gene variant is still inconsistent. However, in our analysis, a substantially heightened cardiac risk of *LDLR/PCSK9* gene variants existed regardless of including or excluding *PCSK9* p.Val4Ile gene variant. This finding underscores the concomitance of *LDLR* and *PCSK9* gene variants to considerably modify atherogenic properties of HeFH.

The current observation highlights the importance of sex difference in cardiac outcomes of HeFH. Similar to our findings, previous reports showed a higher cardiovascular risk in male subjects with HeFH than female subjects with HeFH.[30,31] One of possible mechanisms behind these observations could be atheroprotective properties of sex hormones, such as estrogen. Because estrogen has been shown to modulate inflammation and oxidative stress, these estrogen-mediated effects may account for different cardiovascular outcomes between male and female patients with HeFH. Our findings as well as data from previous reports indicate sex as an important clinical characteristic to stratify future cardiovascular risks in subjects with HeFH.

The current observation provides additional evidence that supports genetic testing to refine risk stratification of cardiovascular events in subjects with HeFH. Accumulating findings through numerous genetic studies have shown that pathogenic variants and their severity, causing HeFH, were associated with the degree of hypercholesterolemia and the risk for the development of coronary artery diseases. However, possibly because of its costing issue as well as ethical reasons, genetic testing is underused in the clinical settings.[32] Our analysis demonstrated patients with *LDLR/PCSK9* gene variants as another prognostic genotype exhibiting high LDL-C and worse cardiovascular risk as well. In particular, cardiac event rate markedly increased in “male” with HeFH with this genetic variant. This approach could help to identify patients with high-risk HeFH who require intensified lipid-lowering therapies.

The high cardiovascular risk in patients with *LDLR/PCSK9* gene variants emphasizes the need to adopt more stringent lipid management in these patients. In the TAUSSIG (Trial Assessing Long Term Use of PCSK9 Inhibition in Subjects With Genetic LDL Disorders), patients with *LDLR/PCSK9* gene variants

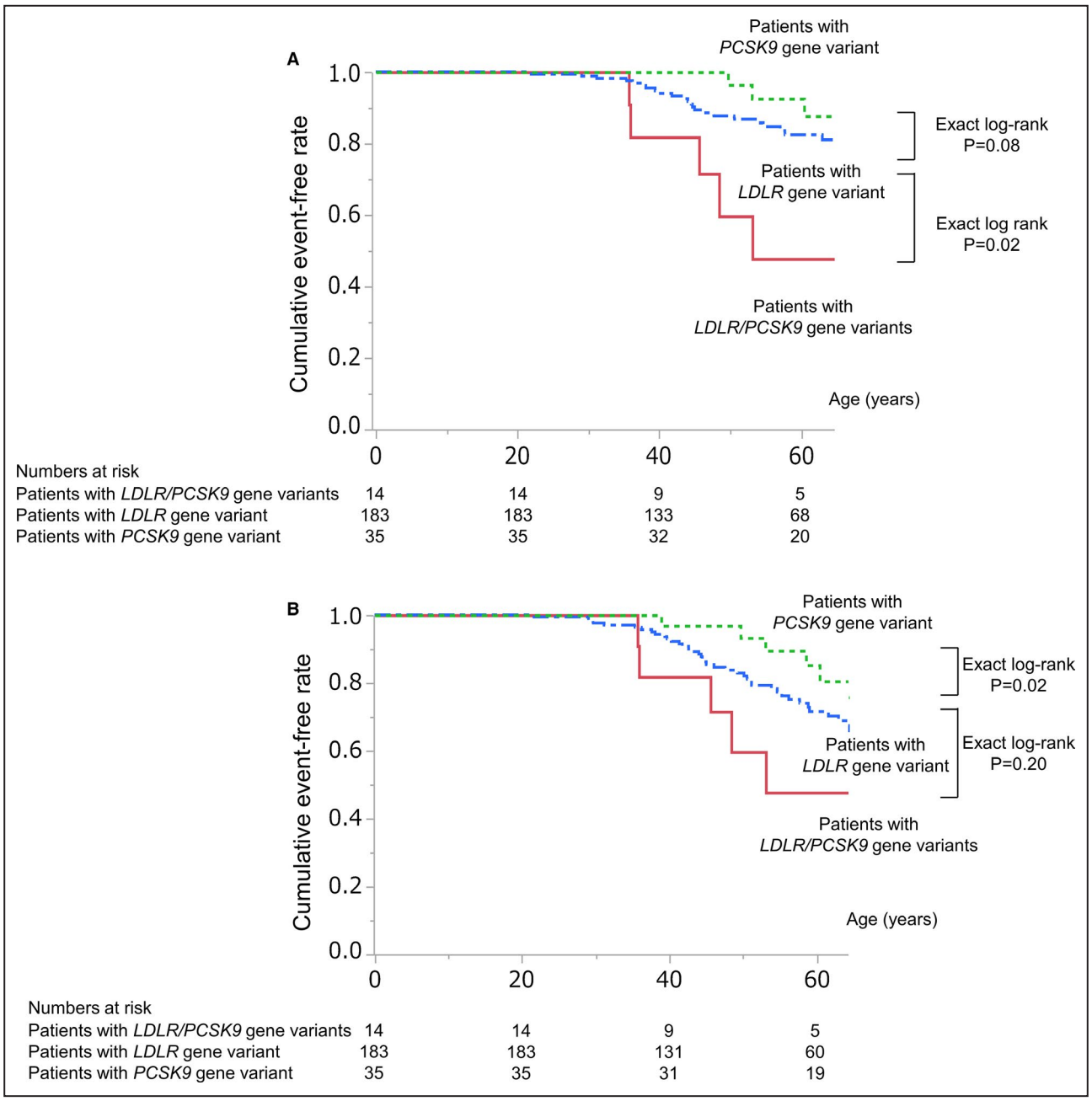

Figure 2. Comparison of prognostic influence of genotype.
Prognostic influence of genotype in patients with familial hypercholesterolemia on primary outcome (nonfatal myocardial infarction [MI]) (**A**) and secondary outcome (nonfatal MI and coronary revascularization) (**B**). Solid red, blue dash-dotted, and green dotted lines indicate event-free survival curves for patients with low-density lipoprotein receptor (*LDLR*)/*PCSK9* (proprotein convertase subtilisin/kexin type 9) gene variants, patients with *LDLR* gene variant, and patients with *PCSK9* gene variant, respectively.

responded well to PCSK9 inhibitor, evolocumab.[33] This observation indicates the importance of commencing PCSK9 inhibitors earlier to prevent future ASCVD in patients with *LDLR/PCSK9* gene variants.

Lower apolipoprotein A-I level in patients with *LDLR/PCSK9* gene variants indicates this apolipoprotein as a potential therapeutic target to mitigate their cardiovascular risks. Apolipoprotein A-I harbors a variety of atheroprotective properties, including cholesterol efflux capacity.[34] Because of these attractive antiatherosclerotic effects, a variety of apolipoprotein A-I mimetic peptides have been developed, and their clinical benefit has been investigated. However, recent clinical trials did not find any favorable benefit of these agents to halt coronary atherosclerosis in patients with ASCVD.[35] In addition, Ditiatkovski et al reported that in vivo functional properties of apolipoprotein A-I mimetic peptide were not necessarily the same as their

Table 6. Cox Proportional Hazards Model for Primary Outcome (Nonfatal MI)

Variable	Unadjusted			Adjusted					
				Model 1			Model 2		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Men	4.30	(2.07–10.1)	<0.001	4.73	(2.28–11.1)	<0.001	32.3	(0.22–4700)	0.17
Hypertension	0.92	(0.46–1.84)	0.81	…	…	…	0.33	(0.01–7.57)	0.49
Diabetes mellitus	1.93	(0.46–8.04)	0.37	…	…	…	0.12	(0.0001–120)	0.55
Smoking history	3.42	(1.55–7.54)	0.002	…	…	…	6.74	(0.09–510)	0.68
LDLR gene variants	Reference	…	…	Reference	…	…	Reference		
vs *PCSK9* gene variant	0.41	(0.10–1.15)	0.10	0.39	(0.09–1.10)	0.09	0.45	(0.13–1.53)	0.21
vs *LDLR/PCSK9* gene variants	2.97	(1.11–6.68)	0.03	3.21	(1.20–7.20)	0.02	4.26	(1.66–11.0)	0.003

Unadjusted HRs for nonfatal MI were calculated by a univariate Cox proportional hazards model. Adjusted HRs were calculated by a multivariate Cox proportional hazards model using (model 1: sex and genotype; and model 2: sex, history of hypertension, diabetes mellitus, and smoking, and genotype) as covariates listed before analysis. HR indicates hazard ratio; *LDLR*, low-density lipoprotein receptor; MI, myocardial infarction; and *PCSK9*, proprotein convertase subtilisin/kexin type 9.

in vitro ones.[36] These findings suggest the complexity of apolipoprotein A-I functionality, which requires a better approach to evaluate its in vivo efficacy on atherosclerotic plaques. Difficulties still exist to translate antiatherosclerotic effects of apolipoprotein A-I into the clinical settings.

Several caveats should be noted. First, this was an observational study conducted in a single center, and the numbers of unrelated patients with *LDLR/PCSK9* gene variants and those who experienced cardiovascular events were relatively small. In addition, the use and selection of lipid-lowering therapy were conducted according to individual physicians' discretion. The current study analyzed lifetime cardiac events after the birth, but not those after the first visit to our clinic. This is because 82% of primary outcomes occurred before their first visit. These analyses may induce immortal time bias. The CIs of HRs in some variables are wide. This is possibly because of small numbers of study subjects in patients with *PCSK9* gene variants alone (n=35) and with *LDLR/PCSK9* gene variants (n=14). We stratified subjects according to types of gene variants but not their putative functions. The patients with *LDLR/PCSK9* gene variants and those with *LDLR* gene variant may include a wide range of biologic properties, which may also cause their wide CI (model 1: 95% CI, 1.20–7.20; model 2: 95% CI, 1.66–11.0). Because *APOB*

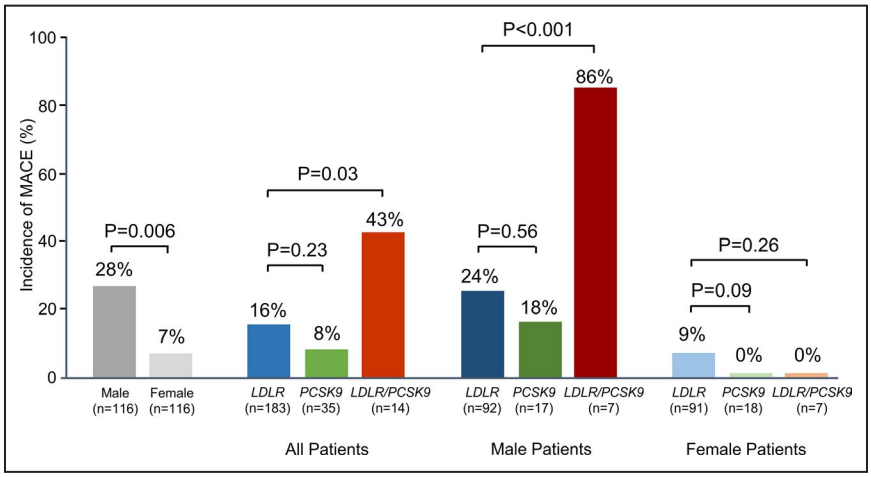

Figure 3. Risk for primary outcome (nonfatal myocardial infarction [MI]), stratified according to sex and genotype.
Genotype alone or in combination with sex difference and rate of nonfatal MI is shown. Low-density lipoprotein receptor (*LDLR*) indicates patients with *LDLR* gene variant; *LDLR/PCSK9* (proprotein convertase subtilisin/kexin type 9), patients with *LDLR* and *PCSK9* gene variants; and *PCSK9*, patients with *PCSK9* gene variant; MACE indicates major adverse cardiovascular event.

gene variant has not been identified in the Japanese population,[5] clinical demographics and outcomes in patients with *LDLR* and *APOB* or *APOB* and *PCSK9* gene variants remain unknown.

In conclusion, patients with *LDLR/PCSK9* gene variants were associated with more atherogenic lipid profiles and a greater likelihood of experiencing ASCVD. Our findings suggest that patients with *LDLR/PCSK9* gene variants are a high-risk FH category who warrant intensive and personalized management.

ARTICLE INFORMATION

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Supplementary Material

Tables S1–S5
Figures S1–S2

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SUPPLEMENTAL MATERIAL

Table S1. Summary of Primary and Secondary Outcomes and the Percentage of Events Before the Diagnosis of FH.

	All patients (n=232)	Patients with *LDLR* gene variant (n=183)	Patients with *PCSK9* gene variant (n=35)	Patients with *LDLR/PCSK9* gene variants (n=14)
Primary outcome: Non-fatal MI				
Subjects who experienced events in lifetime, n (%)	39 (17)	30 (16)	3 (9)	6 (43)
Subjects who experienced events after FH diagnosis, n (%)	7 (3)	5 (3)	0 (0)	2 (14)
Subjects who experienced events before FH diagnosis, n (%)	32 (14)	25 (13)	3 (9)	4 (29)
Subjects who occurred events both before and after FH diagnosis, n (%)	0 (0)	0 (0)	0 (0)	0 (0)
% of events before FH diagnosis in their lifetime, %	82	83	100	67
Secondary outcome: A composite of non-fatal MI and coronary revascularization				
Subjects who experienced events in lifetime, n (%)	69 (30)	55 (30)	7 (20)	7 (50)
Subjects who experienced events after FH diagnosis, n (%)	53 (23)	42 (23)	5 (14)	6 (43)
Subjects who experienced events before FH diagnosis, n (%)	27 (12)	21 (11)	3 (9)	3 (21)
Subjects who occurred events both before and after FH diagnosis, n (%)	11 (5)	8 (4)	1 (3)	2 (4)
% of events before FH diagnosis, %	39	38	43	43

Categorical variables were expressed as n (%).

FH = familial hypercholesterolemia, *LDLR* = low-density lipoprotein receptor, MI = myocardial infarction, *PCSK9* = proprotein convertase subtilisin/kexin type 9.

Table S2. Gene Variants Detected in Patients with *LDLR* and *PCSK9* Gene Variants.

LDLR		*PCSK9*		N
Nucleotide change	Effect of protein	Nucleotide change	Effect of protein	
ex 2-6 dup		c.94G > A	p.(Glu32Lys)	1
c.68-1G>C	Splicing error	c.10G > A	p.(Val4Ile)	1
c.418G>A	p.(Glu140Lys)	c.94G > A	p.(Glu32Lys)	1
c.478T>C	p.(Cys160Arg)	c.10G > A	p.(Val4Ile)	1
c.667_680dup	p.(Asp227Glufs*43)	c.10G > A	p.(Val4Ile)	1
c.888C>A	p.(Cys296*)	c.10G > A	p.(Val4Ile)	1
c.888C>A	p.(Cys296*)	c.94G > A	p.(Glu32Lys)	1
c.1124A>G	p.(Tyr375Cys)	c.10G > A	p.(Val4Ile)	1
c.1147T>G	p.(Phe383Val)	c.10G > A	p.(Val4Ile)	1
c.1297G>C	p.(Asp433His)	c.10G > A	p.(Val4Ile)	1
c.1502C>T	p.(Ala501Val)	c.94G > A	p.(Glu32Lys)	1
c.1618G>A	p.(Ala540Thr)	c.10G > A	p.(Val4Ile)	1
c.1845+2T>C	Splicing error	c.94G > A	p.(Glu32Lys)	1
c.2389G>A	p.(Val797Met)	c.10G > A	p.(Val4Ile)	1

LDLR = low-density lipoprotein receptor, N = number, *PCSK9* = proprotein convertase subtilisin/kexin type 9.

Table S3. Included *LDLR* Gene Variants.

Exon No.	Genomic location GRCh38 (Chr19)	Nucleotide change	Effect of protein	ClinVar	CADD score	rs number	Variant rating according to ACMG guideline	N
1	11089567	c.20_21del	p.(Lys7Ilefs*44)	N/A	N/A	N/A	Pathogenic	2
1	11100222	c.68-1G>C	Splicing error	Pathogenic	29.8	rs879254397	Pathogenic	4
2	11100249	c.94_111del	p.(Phe32_Gly37del)	N/A	N/A	N/A	Likely pathogenic	1
2	11100294	c.139G>A	p.(Asp47Asn)	Conflicting interpretations of pathogenicity	25.6	rs778284147	Uncertain significance	1
3	11102756	c.283T>G	p.(Cys95Gly)	Conflicting interpretations of pathogenicity	25.5	rs879254456	Likely pathogenic	2
3	11102757	c.284G>T	p.(Cys95Phe)	Pathogenic/ Likely pathogenic	25.7	rs879254457	Uncertain significance	1
3	11102758	c.285C>A	p.(Cys95*)	Pathogenic	21.3	rs139400379	Pathogenic	1
3	11102774	c.301G>A	p.(Glu101Lys)	Pathogenic/Likely pathogenic	25.2	rs144172724	Likely pathogenic	1
3	11102788	c.313+2dup	Splicing error	Pathogenic/Likely pathogenic	N/A	rs875989897	Pathogenic	1
4	11105250	c.344G>A	p.(Arg115His)	Conflicting interpretations of pathogenicity	22.5	rs201102461	Uncertain significance	4
4	11105295	c.389dup	p.(Asp131Argfs*49)	Pathogenic	N/A	rs879254510	Pathogenic	4
4	11105301	c.395G>A	p.(Arg132Gln)	N/A	0.044	rs751519676	Uncertain significance	1
4	11105314	c.408del	p.(Asp136Glufs*70)	N/A	N/A	N/A	Pathogenic	1
4	11105324	c.418G>A	p.(Glu140Lys)	Pathogenic/Likely pathogenic	26.6	rs748944640	Pathogenic	4
4	11105364	c.458T>C	p.(Phe153Ser)	N/A	25.5	N/A	Uncertain significance	1

4	11105384	c.478T>C	p.(Cys160Arg)	Pathogenic/Likely pathogenic	26.0	rs879254540	Likely pathogenic	3
4	11105406	c.500G>A	p.(Cys167Tyr)	Likely pathogenic	24.9	rs879254548	Uncertain significance	1
4	11105436	c.530C>T	p.(Ser177Leu)	Pathogenic/Likely pathogenic	24.7	rs121908026	Pathogenic	3
4	11105560	c.654_682del	p.(Pro220Lysfs*10)	N/A	N/A	N/A	Pathogenic	3
4	11105567	c.661G>T	p.(Asp221Tyr)	Pathogenic/Likely pathogenic	26.3	rs875989906	Likely pathogenic	1
4	11105573	c.667_680dup	p.(Asp227Glufs*43)	N/A	N/A	N/A	Pathogenic	1
4	11105576	c.670_682dup	p.(Glu228Glyfs*4)	N/A	N/A	N/A	Pathogenic	1
4	11105579	c.673_681dup	p.(Lys225_Asp227dup)	Likely pathogenic	N/A	rs1555803425	Likely pathogenic	2
4	11105587	c.681C>A	p.(Asp227Glu)	Pathogenic	18.28	rs121908028	Pathogenic	2
4	11105588	c.682G>A	p.(Glu228Lys)	Pathogenic/Likely pathogenic	27.0	rs121908029	Pathogenic	3
5	11106666	c.796G>A	p.(Asp266Asn)	Pathogenic/Likely pathogenic	27.5	rs875989907	Likely pathogenic	1
6	11107461	c.888G>A	p.(Cys296*)	Pathogenic	25.3	rs879254708	Pathogenic	6
7	11110685	c.974G>A	p.(Cys325Tyr)	Likely pathogenic	25.7	rs879254746	Uncertain significance	1
7	11110723	c.1012T>A	p.(Cys338Ser)	Pathogenic/Likely pathogenic	24.8	rs879254753	Pathogenic	19
7	11110766	c.1055G>A	p.(Cys352Tyr)	Pathogenic/Likely pathogenic	29.1	rs193922566	Likely pathogenic	1
8	11111515	c.1062dup	p.(Ile355Tyrfs*3)	Pathogenic	N/A	rs879254775	Pathogenic	1
8	11111519	c.1066G>C	p.(Asp356His)	Conflicting interpretations of pathogenicity	24.1	rs767767730	Uncertain significance	1
8	11111522	c.1069G>T	p.(Glu357*)	N/A	41	N/A	Pathogenic	1
8	11111577	c.1124A>G	p.(Tyr375Cys)	Pathogenic/Likely pathogenic	24.4	rs879254800	Likely pathogenic	1
8	11111600	c.1147T>G	p.(Phe383Val)	N/A	27.9	N/A	Uncertain significance	3
9	11113298	c.1207T>C	p.(Phe403Leu)	Likely pathogenic	26.7	rs879254831	Likely pathogenic	3
9	11113307	c.1216C>T	p.(Arg406Trp)	Pathogenic/Likely pathogenic	26.4	rs121908043	Likely pathogenic	2

9	11113343	c.1252G>A	p.(Glu418Lys)	Likely pathogenic	25.6	rs869320651	Uncertain significance	1
9	11113354	c.1263C>A	p.(Ser421Arg)	N/A	14.08	rs752942769	Uncertain significance	1
9	11113356	c.1265T>G	p.(Leu422Arg)	N/A	28.9	N/A	Uncertain significance	2
9	11113388	c.1297G>C	p.(Asp433His)	Pathogenic/Likely pathogenic	31	rs121908036	Pathogenic	8
10	11113571	c.1395T>G	p.(Tyr465*)	N/A	35	N/A	Pathogenic	1
10	11113645	c.1469G>A	p.(Trp490*)	Pathogenic	43	rs875989922	Pathogenic	1
10	11113653	c.1477_1488del	p.(Ser493_Gly496del)	N/A	N/A	N/A	Likely pathogenic	1
10	11113678	c.1502C>T	p.(Ala501Val)	Conflicting interpretations of pathogenicity	23.7	rs755667663	Uncertain significance	1
10	11113743	c.1567G>A	p.(Val523Met)	Pathogenic/Likely pathogenic	29.8	rs28942080	Likely pathogenic	1
10	11113763	c.1586+1G>A	Splicing error	Pathogenic/Likely pathogenic	34	rs755389753	Pathogenic	3
11	11116125	c.1618G>A	p.(Ala540Thr)	Pathogenic/Likely pathogenic	26.7	rs769370816	Uncertain significance	2
11	11116209	c.1702C>G	p.(Leu568Val)	Pathogenic/Likely pathogenic	24.3	rs746959386	Pathogenic	8
12	11116883	c.1730G>A	p.(Trp577*)	Pathogenic	49	rs138947766	Pathogenic	1
12	11116900	c.1747C>T	p.(His583Tyr)	Conflicting interpretations of pathogenicity	24.7	rs730882109	Uncertain significance	1
12	11116936	c.1783C>T	p.(Arg595Trp)	Conflicting interpretations of pathogenicity	24.6	rs373371572	Likely pathogenic	3
12	11117000	c.1845+2T>C	Splicing error	Pathogenic/Likely pathogenic	33	rs778408161	Pathogenic	20
13	11120117	c.1871_1873del	p.(Ile624del)	Pathogenic/Likely pathogenic	N/A	rs879255062	Likely pathogenic	1
14	11120408	c.2026G>A	p.(Gly676Ser)	Conflicting interpretations of pathogenicity	25.7	rs745753810	Uncertain significance	1

14	11120424	c.2042G>C	p.(Cys681Ser)	Likely pathogenic	26.1	rs201637900	Uncertain significance	1
14	11120436	c.2054C>T	p.(Pro685Leu)	Pathogenic/Likely pathogenic	24.9	rs28942084	Pathogenic	3
14	11120484	c.2102del	p.(Gly701Alafs*8)	N/A	N/A	N/A	Pathogenic	2
14	11123172	c.2141-2delA	Splicing error	N/A	N/A	N/A	Pathogenic	1
15	11128005	c.2312-3C>A	Splicing error	Pathogenic/Likely pathogenic	17.32	rs875989942	Pathogenic	5
16	11128085	c.2389G>A	p.(Val797Met)	Conflicting interpretations of pathogenicity	25.4	rs750518671	Likely pathogenic	4
17	11129539	c.2416dup	p.(Val806Glyfs*11)	Conflicting interpretations of pathogenicity	N/A	rs773618064	Pathogenic	3
17	11129539	c.2416_2418delinsAGAAG	p.(Val806Argfs*124)	N/A	N/A	N/A	Pathogenic	2
17	11129554	c.2431A>T	p.(Lys811*)	Pathogenic	43	rs879255211	Pathogenic	10
		ex1del		Pathogenic	N/A	N/A	Pathogenic	2
		ex2-3del		Pathogenic	N/A	N/A	Pathogenic	4
		ex2-6dup		N/A	N/A	N/A	Pathogenic	3
		ex5del		Pathogenic	N/A	N/A	Pathogenic	1
		ex7-18del		Pathogenic	N/A	N/A	Pathogenic	2
		ex13-14del		Pathogenic	N/A	N/A	Pathogenic	4
		ex13-14dup		Pathogenic	N/A	N/A	Likely pathogenic	1
		ex16-18del		N/A	N/A	N/A	Pathogenic	1
		ex17dup		N/A	N/A	N/A	Likely pathogenic	1
		ex17-18del		Pathogenic	N/A	N/A	Pathogenic	1

ACMG guideline = American College of Medical Genetics guideline, CADD score = Combined Annotation Dependent Depletion score, *LDLR* = low-density lipoprotein receptor, N = number, N/A = not applicable

Table S4. Included *PCSK9* Gene Variants.

Exon No.	Genomic location GRCh38 (Chr1)	Nucleotide change	Effect of protein	ClinVar	CADD score	rs number	Variant rating according to ACMG	N
1	55039847	c.10G > A	p.(Val4Ile)	Uncertain significance	6.659	rs186669805	Benign	16
1	55039931	c.94G > A	p.(Glu32Lys)	Conflicting interpretations of pathogenicity	22.3	rs564427867	Pathogenic	30
9	55058630	c.1486C > T	p.(Arg496Trp)	Uncertain significance	23.3	rs374603772	Likely pathogenic	3

ACMG guideline = American College of Medical Genetics guideline, CADD score = Combined Annotation Dependent Depletion score, N = number, N/A = not applicable *PCSK9* = proprotein convertase subtilisin/kexin type 9.

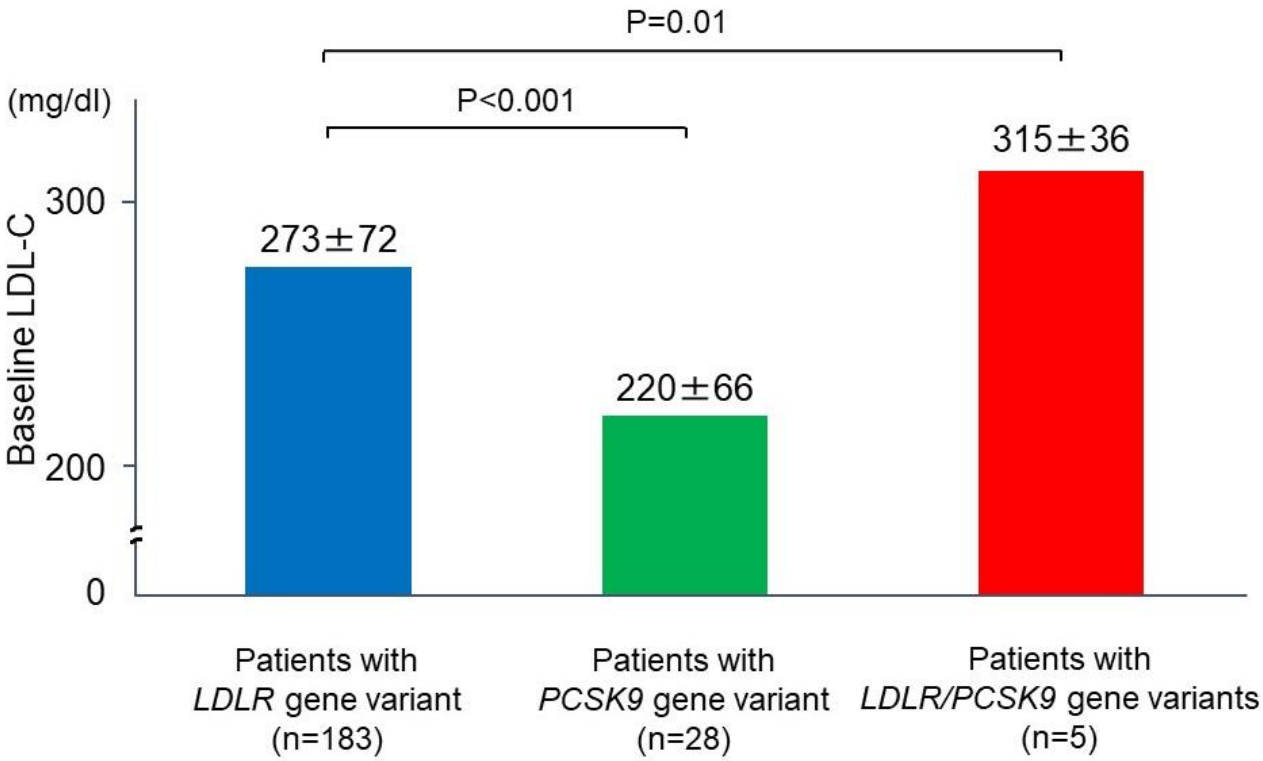
Table S5. Details of MACE.

Case No.	Age, y	Sex	Genotype	MACE	Cause of death	Culprit artery	Culprit site	Treatment Option
1	46	M	*LDLR/PCSK9*	Non-fatal MI	-	LAD	#7	PCI
2	37	M	*LDLR/PCSK9*	Non-fatal MI	-	LAD	#7	PCI
3	36	M	*LDLR/PCSK9*	Non-fatal MI	-	LCX	#14	MT
4	64	M	*LDLR/PCSK9*	Non-fatal MI	-	RCA	#2	PCI
5	53	M	*LDLR/PCSK9*	Non-fatal MI	-	RCA	#1	PCI
6	41	M	*LDLR/PCSK9*	Non-fatal MI	-	RCA	#1	PCI
7	40	M	*LDLR*	Non-fatal MI	-	RCA	#3	PCI
8	51	F	*LDLR*	Non-fatal MI	-	LAD	#6	PCI
9	71	F	*LDLR*	Non-fatal MI	-	LAD	#7	PCI
10	62	F	*LDLR*	Non-fatal MI	-	LAD	#6	PCI
11	32	F	*LDLR*	Non-fatal MI	-	RCA	#2	PCI
12	48	M	*LDLR*	Non-fatal MI	-	LAD	#7	CABG
13	42	M	*LDLR*	Non-fatal MI	-	RCA	#1	PCI
14	39	M	*LDLR*	Non-fatal MI	-	RCA	#1	MT
15	59	M	*LDLR*	Non-fatal MI	-	RCA	#2	CABG
16	39	M	*LDLR*	Non-fatal MI	-	LAD	N/A	MT
17	55	F	*LDLR*	Non-fatal MI	-	LAD	#7	MT
18	48	M	*LDLR*	Non-fatal MI	-	RCA	#2	PCI
19	45	M	*LDLR*	Non-fatal MI	-	RCA	#1, #13	CABG
20	54	M	*LDLR*	Non-fatal MI	-	LCX	#13	MT
21	45	M	*LDLR*	Non-fatal MI	-	LCX	#14	CABG

22	46	M	*LDLR*	Non-fatal MI	-	RCA	#2	PCI
23	36	M	*LDLR*	Non-fatal MI	-	LAD	#7	PCI
24	69	F	*LDLR*	Non-fatal MI	-	RCA	#2	PCI
25	63	M	*LDLR*	Non-fatal MI	-	LCX	#13	PCI
26	38	M	*LDLR*	Non-fatal MI	-	RCA	#3	PCI
27	66	M	*LDLR*	Non-fatal MI	-	RCA	#3	PCI
28	41	M	*LDLR*	Non-fatal MI	-	N/A	N/A	MT
29	64	M	*LDLR*	Non-fatal MI	-	N/A	#6, #13, #3	CABG
30	32	M	*LDLR*	Non-fatal MI	-	LAD	#6	PCI
31	30	F	*LDLR*	Non-fatal MI	-	RCA	#3	PCI
32	31	F	*LDLR*	Non-fatal MI	-	LAD	#7	PCI
33	43	M	*LDLR*	Non-fatal MI	-	LAD	#6	PCI
34	75	M	*LDLR*	Non-fatal MI	-	RCA	#2	MT
35	44	M	*LDLR*	Non-fatal MI	-	RCA	#1	PCI
36	44	M	*LDLR*	Non-fatal MI	-	LAD	#7	PCI
37	50	M	*PCSK9*	Non-fatal MI	-	LAD	#6	PCI
38	53	M	*PCSK9*	Non-fatal MI	-	RCA	#4AV	PCI
39	60	M	*PCSK9*	Non-fatal MI	-	RCA	#3	PCI

AV = atrioventricular, CABG = coronary artery bypass grafting, F = female, LAD = left anterior descending artery, LCX = left circumflex artery, *LDLR* = low-density lipoprotein receptor, M = male, MACE = major adverse cardiac events, MI = myocardial infarction, MT = medical treatment, No = number, RCA = right coronary artery, PCI = percutaneous coronary intervention, *PCSK9* = proprotein convertase subtilisin/kexin type 9

Figure S1. Comparison of LDL-C in Patients with *LDLR* Gene Variants and/or *PCSK9* (p.Glu32Lys, p.Arg496Trp) Gene Variant.

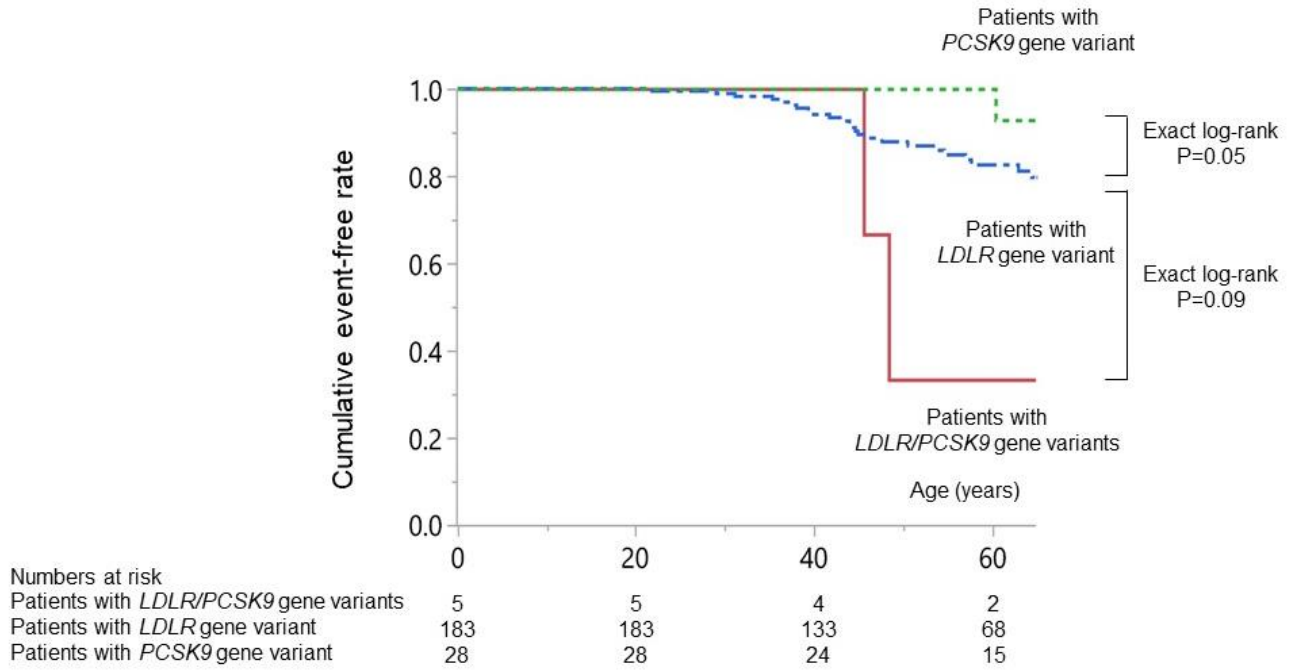


The levels of baseline LDL-C were shown. Blue, green, and red bars indicate the levels of baseline LDL-C in patients with *LDLR* gene variant, those with *PCSK9* gene variant, and those with *LDLR/PCSK9* gene variants, respectively.

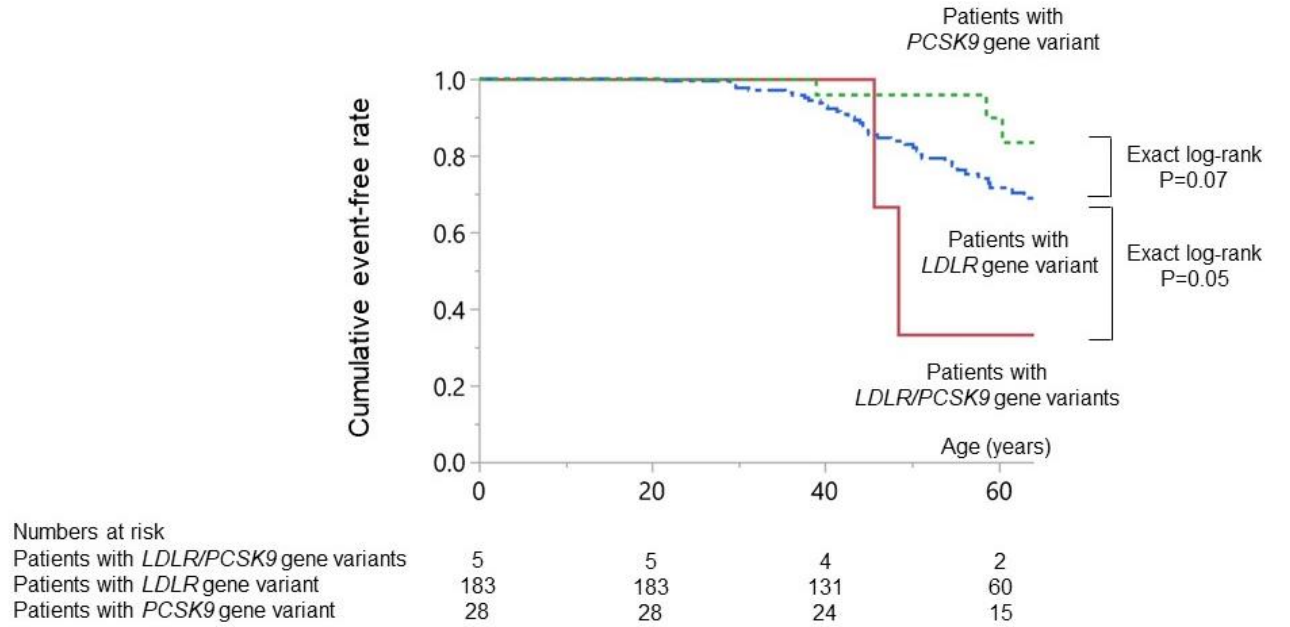
LDL-C = low-density lipoprotein cholesterol, *LDLR* = low-density lipoprotein receptor, *PCSK9* = proprotein convertase subtilisin/kexin type 9.

Figure S2. Comparison of Prognostic Influence of Genotype in Patients with *LDLR* Gene Variants and/or *PCSK9* (p.Glu32Lys, p.Arg496Trp) Gene Variant.

a.



b.



Prognostic influence of genotype in FH patients on primary outcome (non-fatal MI) (a) and

secondary outcome (non-fatal MI, and coronary revascularization) (b). Solid red, blue dash-dotted, and green dotted lines indicate event-free survival curves for patients with *LDLR/PCSK9* gene variants, patients with *LDLR* gene variant, and patients with *PCSK9* gene variant, respectively.

FH = familial hypercholesterolemia, *LDLR* = low-density lipoprotein receptor, MI = myocardial infarction, *PCSK9* = proprotein convertase subtilisin/kexin type 9