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Review

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How Can We Identify Very High-Risk Heterozygous Familial Hypercholesterolemia?

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Heterozygous familial hypercholesterolemia (HeFH) is a genetic disorder that elevates low-density lipoprotein cholesterol and increases the risk of premature atherosclerotic cardiovascular disease (ASCVD). However, despite their atherogenic lipid profiles, the cardiovascular risk of HeFH varies in each individual. Their variety of phenotypic features suggests the need for better risk stratification to optimize their therapeutic management. The current review summarizes three potential approaches, including (1) definition of familial hypercholesterolemia (FH)-related risk scores, (2) genetic analysis, and (3) biomarkers. The International Atherosclerosis Society has recently proposed a definition of severe FH to identify very high-risk HeFH subjects according to their clinical characteristics. Furthermore, published studies have shown the association of FH-related genetic phenotypes with ASCVD, which indicates the genetic analysis's potential to evaluate individual cardiovascular risks. Biomarkers reflecting disease activity have been considered to predict the formation of atherosclerosis and the occurrence of ASCVD in HeFH subjects. Incorporating these risk stratifications will be expected to allocate adequate intensity of lipid-lowering therapies in HeFH subjects, which ultimately improves cardiovascular outcomes.

Key words: **Heterozygous familial hypercholesterolemia, Atherosclerotic cardiovascular disease, Risk score, Severe FH, Gene mutation, Biomarker**

Abbreviations: **ApoB=apolipoprotein B, ASCVD=atherosclerotic cardiovascular disease , CAD=coronary artery disease, CI=confidence interval, CVD=cardiovascular disease, FH=familial hypercholesterolemia, HDL= high-density lipoprotein, HDL-C=high-density lipoprotein cholesterol, HeFH=heterozygous familial hypercholesterolemia, HR=hazard ratio, IAS=international atherosclerosis society, IVUS=intravascular ultraspund, LDL-C=low-density lipoprotein cholesterol, LDLR=low-density lipoprotein receptor, Lp(a)=lipoprotein a, OR=odds ratio, PCSK9=proprotein convertase subxilisin/kexin type 9**

Introduction

Heterozygous familial hypercholesterolemia (HeFH) is a genetic disorder that elevates low-density lipoprotein cholesterol (LDL-C) significantly[1-4]. Specific gene mutations, including low-density lipoprotein receptor (*LDLR*) and proprotein convertase subxilisin/kexin type 9 (*PCSK9*), reportedly induce abnormal metabolism of low-density lipoprotein (LDL), which leads to a greater amount of LDL particles in circulation from birth[5-7]. This HeFH-related pathophysiology increases the risk of premature atherosclerotic cardiovascular disease (ASCVD), worsening their clinical outcomes[8-12]. However, despite a high cumulative burden of LDL-C, cardiovascular risk varies in each individual with

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Table 1. Clinical Risk Stratification Approaches in HeFH Patients

Authors	Subjects	Parameters for risk stratification	ASCVD risk prediction ability
Severe FH			
Pérez-Calahorra, *et al.*[19]	1732 HeFH subjects	Definition of severe FH is summarized in Fig. 1.	Univariate analysis demonstrated the association of severe FH with the presence of cardiovascular disease (OR=3.016, 95%CI=3.136-4.257, $p<0.001$). However, after adjusting traditional risk factors, this relationship did not meet statistical significance ($p=0.27$).
Humphries, *et al.*[20]	2929 HeFH subjects		A significantly higher standardized mortality ratio for coronary heart disease was observed in severe FH [220 (184-261) vs. 144 (98-203), $p=0.007$].
Funabashi, *et al.*[21]	380 HeFH subjects without ASCVD		The mean observational period was 7.4 years. Severe FH predicted a 7.76-fold greater risk for experiencing ASCVD (=cardiac death, non-fatal MI, stroke, peripheral artery disease) (HR=9.29, 95%CI=3.68-31.2, $p<0.001$). In addition, severe FH was associated with subsequent ASCVD after the occurrence of the 1st events (HR=10.6, 95%CI=3.96-28.5, $p<0.001$).
Montreal-FH-SCORE			
Paquette, *et al.*[22]	670 HeFH with *LDLR* gene variants	Sex, age, HDL-C, hypertension, smoking	Age ($\beta=0.75$), HDL-C ($\beta=-0.27$), male gender ($\beta=0.25$), hypertension ($\beta=0.19$) and smoking ($\beta=0.12$) independently predicted CVD. The AUC of Montreal-FH-SCORE incorporating these variables for CVD was 0.84 (95%CI=0.808-0.872, $p<0.0001$). In particular, its value >20 was associated with 10.3-fold higher risk of future CVD events compared to that <=20 (95%CI=6.7-15.7, $p<0.0001$).
SAFEHEART Risk Equation			
Pérez de Isla, *et al.*[23]	2404 HeFH patients	Age, male sex, history of previous ASCVD, high blood pressure, increased BMI, active smoking, and LDL-C and Lp(a) levels	The mean observational period was 5.5 years. Age, male sex, history of previous ASCVD, high blood pressure, increased BMI, active smoking, and LDL-C and Lp(a) levels are independent predictors of future occurrence of ASCVD. By using these variables, the Harrell C index was 0.85. In FH subjects without a history of ASCVD, the Harrell C index was 0.81, which was better than Framingham Risk Equation (0.78) and ACC/AHA ASCVD Pooled Cohort Risk Equations (0.8) ($p=0.045$).
FH-Risk-Score			
Paquette, *et al.*[24]	3381 HeFH patients without ASCVD	Sex, age, HDL-C, LDL-C, hypertension, smoking and Lp(a) level	A higher FH-Risk-Score was associated with worse 10-year ASCVD-free survival (5.52, 95%CI=3.94-7.73, $p<0.0001$), 10-year MACE-free survival (4.64, 95%CI=2.66-8.11, $p<0.0001$) and 30-year survival due to cardiac cause-death (10.73, 95%CI=2.51-45.79, $p=0.0014$).

ASCVD=atherosclerotic cardiovascular disease, BMI=body mass index, CI=confidence interval, CVD=cardiovascular disease, FH=familial hypercholesterolemia, HeFH=heterozygous familial hypercholesterolemia, HDL-C=high-density lipoprotein cholesterol, LDL-C=low-density lipoprotein cholesterol, LDLR=low-density lipoprotein receptor, Lp(a)=lipoprotein a, MACE=major cardiovascular event, OR=odds ratio

HeFH[13, 14]. This suggests a variety of phenotypic features in HeFH and the need to establish better risk stratification for optimization of therapeutic management according to their future ASCVD risks. The current review summarizes several potential approaches to identify patients with HeFH with an increased cardiovascular risk.

Risk Stratification Approach Using Clinical Characteristics of HeFH (Table 1)

According to a large body of clinical evidence about atherogenic risk factors, several ASCVD risk calculations have been already established, which included the Framingham Risk Score[15], the Pooled Cohort Equation[16], and the European Systematic Coronary Risk Evaluation (SCORE)[17]. However,

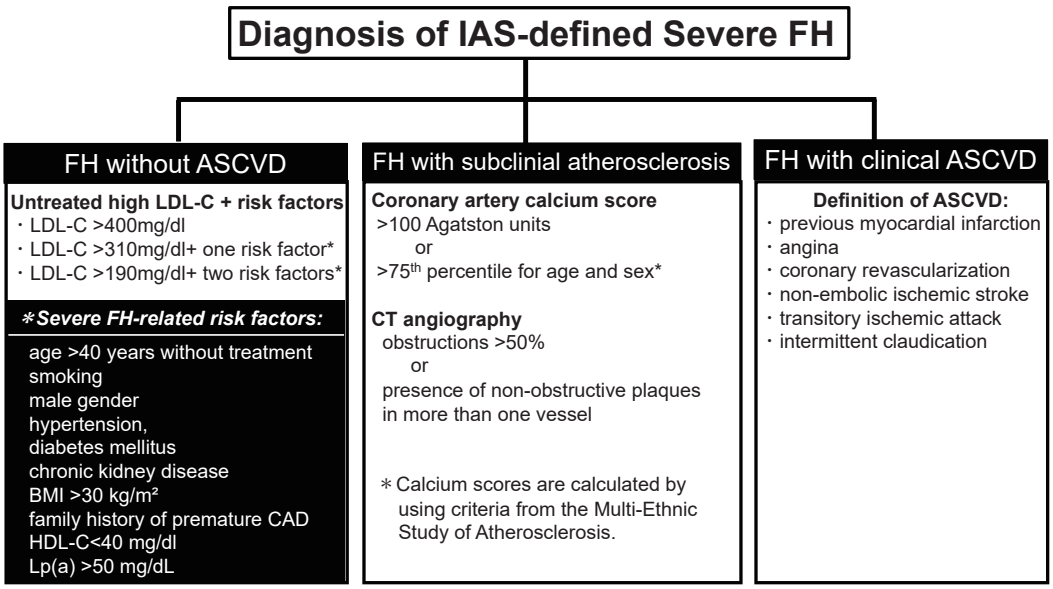

Fig. 1. Three Definitions of Severe FH Defined by IAS Statement

ASCVD=atherosclerotic cardiovascular disease, BMI=body mass index, CAD=coronary artery disease, CT=computed tomography, FH=familial hypercholesterolemia, HDL-C=high-density lipoprotein cholesterol, IAS=international atherosclerosis society, LDL-C=low-density lipoprotein cholesterol, Lp(a)=lipoprotein a

these risk calculators were not developed in patients with FH, and therefore lead to the underestimation of the familial hypercholesterolemia (FH)-related ASCVD risk. A better risk stratification approach which adequately validated in patients with FH is warranted in the clinical settings. The followings are clinically applicable tools for cardiovascular risk estimation in FH.

(a) International Atherosclerosis Society (IAS)-Proposed Severe FH

Published studies of HeFH have reported clinical risk factors associated with their ASCVD. By incorporating this evidence, the IAS has recently proposed a definition of severe FH[18]. This definition includes three different approaches based on the clinical status of patients with HeFH (Fig. 1). As shown in Fig. 1, in subjects with HeFH without any history of ASCVD, severe FH is defined according to untreated LDL-C level and the number of severe FH-related risk factors (Fig. 1). If patients with HeFH have a subclinical coronary atherosclerotic feature on computed tomography imaging, its degree of coronary artery calcification or coronary artery stenosis characteristics guide to diagnosing severe FH. Patients with HeFH who already have a history of ASCVD are also defined as severe FH. ASCVD includes a history of myocardial infarction, angina pectoris, coronary revascularization, nonembolic ischemic stroke, transitory ischemic attack, and intermittent claudication (Fig. 1).

Pérez-Calahorra *et al.* conducted a cross-sectional analysis investigating the association of severe FH with cardiovascular disease. Univariate analysis identified that severe FH was associated with the presence of cardiovascular disease [odds ratio (OR)=3.016, 95% confidence interval (CI)=3.136–4.257, $p<0.001$][19]. One recent study investigated whether IAS-defined severe FH could predict cardiac mortality. This study analyzed 2929 definite or possible subjects with HeFH diagnosed by Simon Broome criteria[20]. The frequency of severe FH was 67.7% (=1982/2929). Severe FH subjects more likely had coronary heart disease, accompanied by atherosclerotic risk factors, including obesity and smoking. Additionally, they exhibited 64% higher mortality of coronary heart disease compared to nonsevere ones ($p=0.007$). While this study suggests the potential usefulness of severe FH definition to predict future cardiac mortality, this analysis includes patients with HeFH with ASCVD and without ASCVD.

We recently analyzed 380 Japanese patients with HeFH defined by the Japan Atherosclerosis Society to elucidate their cardiovascular outcomes[21]. This analysis included only patients with HeFH without any history of ASCVD, which indicated that this analysis focused on whether the definition of severe FH could identify patients with high risk in the

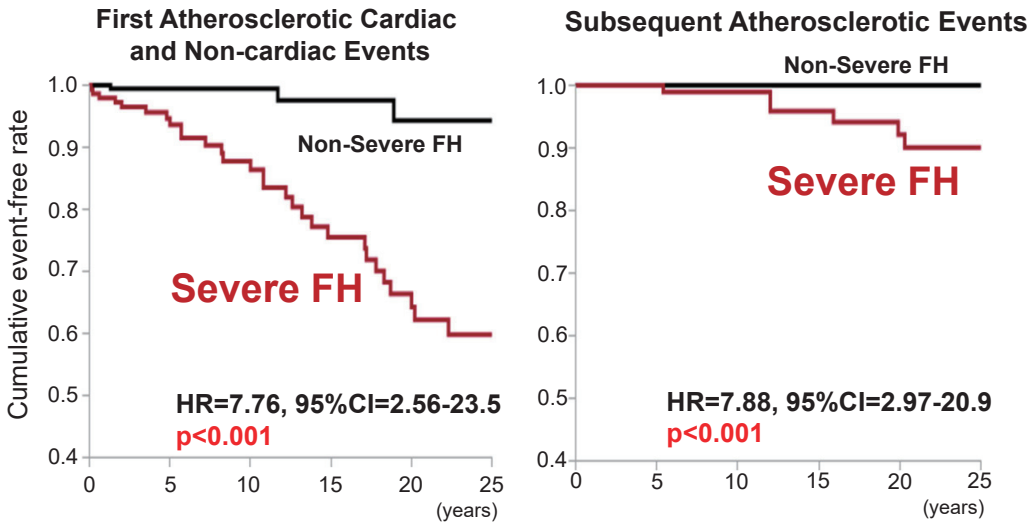

Fig. 2. First and Subsequent ASCVD Risks in Severe FH Subjects

CI=confidence interval, FH=familial hypercholesterolemia, HR=hazard ratio

primary prevention settings[21]. In this study, 40.3% (=153/380) of Japanese HeFH exhibited severe FH. Japanese severe FH is characterized as a higher untreated LDL-C (283 +/– 64 vs. 212 +/– 57 mg/dL, $p<0.001$), triglyceride (median 118 vs. 91 mg/dL, $p<0.001$) and Lp(a) levels (median: 22.7 vs. 16.7 mg/dL, $p=0.03$). Furthermore, HeFH-related physical characteristics was more frequently observed (tendon xanthomas: 73.2 vs. 47.1%, $p<0.001$, skin xanthomas: 15.7 vs. 5.7%, $p=0.002$, corneal arcus: 36.0 vs. 18.0%, $p<0.001$). During the 7.4-year observational period, severe FH was associated with a 7.76-fold greater likelihood of experiencing ASCVD (=cardiac death, nonfatal MI, stroke, peripheral artery disease) [hazard ratio (HR)=9.29, 95% CI=3.68–31.2, $p<0.001$] **(Fig. 2)**. Of note, this cardiovascular risk continued to exist even after the occurrence of the 1st ASCVD events in severe FH (HR=10.6, 95% CI=3.96–28.5, $p<0.001$) **(Fig. 2)**. These observations suggest severe FH criteria as a clinically applicable tool to find patients with HeFH with a substantially elevated future risk of ASCVD in the primary prevention settings.

(b) Montreal-FH-SCORE

The Montreal-FH-SCORE, established in 2017, is an ASCVD risk calculator specifically focused on FH subjects[22]. It uses five clinical risk factors: age, HDL-C, male gender, hypertension, and smoking. Age ($\beta=0.75$), high-density lipoprotein cholesterol (HDL-C) ($\beta=-0.27$), male gender ($\beta=0.25$), hypertension ($\beta=0.19$), and smoking ($\beta=0.12$) independently predicted cardiovascular disease (CVD). The area under the Montreal-FH-SCORE curve incorporating these CVD variables was 0.84 (95% CI=0.808–0.872, $p<0.0001$). Its value, >20, was particularly associated with a 10.3-fold higher risk of future cardiovascular disease (CVD) events compared to that <=20 (95% CI=6.7–15.7, $p<0.0001$).

(c) SAFEHEART Risk Equation

The aforementioned Montreal-FH-SCORE was validated in a retrospective cohort only. It is not fully validated in a prospective cohort. Pérez de Isla *et al.* established the SAFEHEART Risk Equation using a prospective FH registry[23]. This study included 2404 patients with HeFH, and the mean observational period was 5.5 years. Age, male sex, history of previous ASCVD, high blood pressure, increased body mass index, active smoking, and LDL-C and lipoprotein a [Lp(a)] levels are independent predictors of future occurrence of ASCVD. By using these variables, the Harrell C index was 0.85. In subjects with FH without a history of ASCVD, the Harrell C index was 0.81, which was better than Framingham Risk Equation (0.78) and American College of Cardiology/American Heart Association ASCVD Pooled Cohort Risk Equations (0.8) ($p=0.045$).

(d) FH-Risk-SCORE

One limitation in the SAFEHEART Risk Equation is that this cohort included FH patients in primary and secondary prevention settings. Another limitation is that the SAFEHEART included only Spanish individuals with FH and no other ethnicities. The FH-Risk SCORE was developed using a large

multinational prospective cohort of patients with FH without any history of ASCVD[24]. This risk score includes sex, age, HDL-C, LDL-C, hypertension, smoking, and Lp(a) level. A higher FH-Risk-SCORE was associated with worse 10-year ASCVD-free survival (5.52, 95% CI=3.94–7.73, $p<0.0001$), 10-year event-free survival (4.64, 95% CI=2.66–8.11, $p<0.0001$), and 30-year survival due to cardiac cause-death (10.73, 95% CI=2.51–45.79, $p=0.0014$).

While these risk stratifications could be integrated into our clinical practice of HeFH, future investigation is needed to determine whether clinical management according to these risk stratifications could improve HeFH outcomes.

Predictive Ability of FH Causative Gene Mutation Analysis for ASCVD risks (Table 2)

Evaluation of causative gene mutations in subjects with HeFH is an important approach to understanding the mechanism causing their abnormal lipid metabolism[7-9]. Given that an elevated LDL-C level caused by genetic mutation has been considered as a driver to promote atherosclerosis of HeFH, genetic risk evaluation is expected as another important approach to evaluate FH-related ASCVD risks.

(a) Gene Mutations and Cardiovascular Outcomes

Evidence suggests the association of FH gene mutations with ASCVD. Khera *et al.* investigated three causative gene mutations [*LDLR*, apolipoprotein B (*APOB*), and proprotein convertase subxilisin/kexin type 9 (*PCSK9*)] in 26025 subjects from 7 case-control studies and 5 prospective cohort studies[25]. In this analysis, 6.7% of subjects exhibited LDL-C level ≥ 190mg/dL. Of these, FH mutation appeared in 1.7% of them. Additionally, the presence of FH mutation was associated with an increased risk of coronary artery disease (CAD). In detail, compared to those with LDL-C <130 mg/dl but not FH mutation, subjects with LDL-C ≥ 190mg/dL alone confer a 6.0-fold greater likelihood of experiencing CAD (95% CI=5.2–6.9, $p<0.001$). This CAD risk was substantially elevated in those with both LDL-C ≥ 190mg/dL and FH mutation (OR=22.3, 95% CI=10.7–53.2, $p<0.001$). These findings highlight that FH genetic mutations could evaluate the risk of ASCVD.

Our recent analysis has focused on cardiovascular outcomes in subjects with HeFH with both *LDLR* and *PCSK9* gene variants (*LDLR*/*PCSK9* gene variants)[26]. In this study, including 232 Japanese subjects with HeFH, 6.0% (=13/232) exhibited *LDLR*/*PCSK9* gene variants, followed by *LDLR* and *PCSK9* in 78.9% and 15.1%, respectively. During the observational period (53 +/– 17 years), *LDLR*/*PCSK9* gene variants were associated with an increased risk of nonfatal myocardial infarction (HR=4.26, 95% CI=1.66–11.0, $p=0.003$) **(Fig. 3)**. Of particular interest, the occurrence of myocardial infarction rose to 86% in male patients with *LDLR*/*PCSK9* gene variants. Another study also reported an elevated LDL-C level in double heterozygous subjects with HeFH (*LDLR*/*APOB* or *LDLR*/*PCSK9*)[27] **(Fig. 4)**.

Detailed characteristics of *PCSK9* gene variants were evaluated by another study in 269 clinically diagnosed Japanese patients with HeFH[28]. This study detected 11 *PCSK9* gene variants. In those without *LDLR* gene variant, LDL-C level and the frequency of CAD were comparable in those with any *PCSK9* gene variant (*PCSK9* V4I, L21_22insL/A53V, and E32K). By contrast, in subjects with HeFH with *LDLR* gene variant, the concomitance of *PCSK9* V4I gene variant significantly increased LDL-C level ($p=0.0036$) and risk of CAD ($p=0.048$). These observations indicate that elevation of cardiovascular risk accelerates according to the concomitance of both *LDLR* and *PCSK9* gene variants, specifically *PCSK9* V4I, in subjects with HeFH.

Detailed and complete genetic variations are evaluable by whole-genome sequencing. Recent studies reported the association of monogenic and polygenic mutations with ASCVD risks[29, 30]. This study investigated 2081 patients with early-onset myocardial infarction from 4 racial subgroups hospitalized in the United States[29]. Whole-genome sequencing was conducted to evaluate monogenic and polygenic mutations. The average age of early-onset myocardial infarction was 48 years old. The prevalence of Caucasian, African-American, Hispanic, and Asian subjects was 75%, 16%, 8%, and 2%, respectively. The genomes of 2081 subjects were compared with those of 3761 control subjects. FH mutation (*LDLR*, *APOB*, or *PCSK9*) was identified in 1.7% of subjects (0.6% in control subjects). All of these are *LDLR*, and there were no patients with *APOB* or *PCSK9* gene variants. This FH mutation was associated with a 3.76-fold elevated risk of early-onset myocardial infarction (95% CI=2.12–6.82, $p<0.0001$) on logistic regression model analysis. The polygenic score was calculated, and then the top 5% high polygenic score was analyzed as a carrier group. In this analysis, 17.3% of subjects exhibited a high polygenic score (5.0% in the control group), and it predicted an increased risk of early-onset myocardial infarction (3.73, 95% CI=3.06–4.56, $p<0.0001$). A high polygenic score was particularly associated with a 5.1-

Table 2. Genetic Risk Stratification Approaches in HeFH Patients

Authors	Subjects	Evaluated genetic variants	Findings
Khera, *et al.*[25]	26025 FH subjects	three causative gene mutations (*LDLR*, *APOB* and *PCSK9*)	6.7% of subjects exhibited LDL-C level ＞＝190mg/dl. Of these, FH mutation was observed in 1.7% of them. In addition, the presence of FH mutation was associated with an increased risk of CAD. In detail, compared to those with LDL-C ＜130mg/dl but not FH mutation, subjects with LDL-C ＞190mg/dl alone confer 6.0-fold greater likelihood experiencing CAD (95%CI＝5.2-6.9, p ＜0.001). In those with both LDL-C ＞190mg/dl and FH mutation, this CAD risk substantially elevated (OR＝22.3, 95%CI＝10.7-53.2, p＜0.001).
Doi, *et al.*[26]	232 HeFH subjects	causative gene mutations (*LDLR*, *PCSK9* and *LDLR*/*PCSK9*)	*LDLR*/*PCSK9* gene variants were observed in 6% of study subjects. HeFH subjects with this gene variant more likely exhibited a higher LDL-C level compared to that in subjects with *LDLR* (316+/-75 mg/dL vs. 273+/-72 mg/dL, p＝0.04). During the observational period (53+/-17 years), a greater frequency of non-fatal MI was observed in HeFH subjects with *LDLR*/*PCSK9* compared to those with *LDLR* (p＝0.02). Even after adjusting clinical characteristics, the presence of *LDLR*/*PCSK9* gene variants still predicted an increased risk of non-fatal MI (HR＝6.08, 95%CI＝2.29-16.1, p＜0.001 vs. *LDLR* alone).
Sjouke, *et al.*[27]	56 HeFH subjects and 18 unaffected relatives	*LDLR*/*APOB* and *LDLR*/*PCSK9* gene variants	This study included 28 double heterozygotes (23 *LDLR*/*APOB* and 5 *LDLR*/*PCSK9* mutation carriers). A higher LDL-C level was observed in double heterozygotes compared to heterozygous HeFH subjects and unaffected relatives (324 +/- 108 vs. 216 +/- 85, 96 +/- 42 mg/dl, p＜0.01), whereas homozygous/compound heterozygous *LDLR* mutation carriers (502+/-197 mg/dL, p＜0.001) had a higher LDL-C level compared to that in double heterozygotes.
Khera, *et al.*[29]	2081 patients with early-onset MI and 3761 control subjects	•FH mutation (*LDLR*, *APOB* or *PCSK9*) •Polygenic score was calculated in each individual, and then top 5% high polygenic score was analyzed as carrier group. Authors recently conducted polygenic score derivation by using recent GWAS studies including subjects of European ancestry for five diseases (CAD, atrial fibrillation, diabetes mellitus, inflammatory bowel disease and breast cancer). It was validated by the UK Biobank (Khera, *et al.* Nat Genet, 2018; 50: 1219-1224).	The averaged age of early-onset myocardial infarction was 48 years old. The prevalence of white, black, Hispanic and Asian subjects was 75, 16, 8 and 2%, respectively. The genomes of 2081 subjects were compared with those of 3761 control subjects. FH mutation (*LDLR*, *APOB* or *PCSK9*) was identified in 1.7% of subjects (0.6% in control subjects). All of these are *LDLR*, and there was no patients with *APOB* or *PCSK9* gene variants. On logistic regression model analysis, this FH mutation was associated with a 3.76-fold elevated risks of early-onset myocardial infarction (95%CI＝2.12-6.82, p＜0.0001). Polygenic score was calculated and then top 5% high polygenic score was analyzed as carrier group. In this analysis, 17.3% of subjects exhibited high polygenic score (5.0% in control group), and it predicted an increased risk of early-onset MI (3.73, 95%CI＝3.06-4.56, p＜0.0001). In particular, a high polygenic score was associated with a 5.1-fold increased risk in white subjects compared to a 2.0-, 3.4-, and 3.3-fold risks in black, Hispanic and Asian subjects, respectively. 0.2% of subjects had both FH mutation and a high polygenic score. Their mean LDL-C level was 235 mg/dL, compared to 202 mg/dL in FH mutation alone and 130 mg/dL in high polygenic score alone and 122 mg/dL in those without any genetic features.
D'Erasmo, *et al.*[30]	370 clinically-diagnosed FH subjects	*LDLR*, *APOB*, *APOE*, *PCSK9*, *LDLRAP1*, *STAP1* and *LIPA* genes were analyzed. Pathogenecity was evaluated according to the American College of Medical Genetics classification. A weighted LDL-C-raising polygenic risk score was calculated by 6SNPs (rs4299376, rs1367117, rs6511720, rs629301, rs7412, rs429358). Polygenic risk score ＞0.69 was defined as polygenic hypercholesterolemia.	56.5% of study subjects (n＝209) were classified as monogenic FH. In the remaining subjects (n＝161), polygenic hypercholesterolemia was observed in 89 patients (55.3%＝89/161). Monogenic FH more likely had a higher untreated LDL-C level compared to polygenic ones (258.5 vs. 213.8 mg/dl, p＜0.001). There was a trend toward a greater degree of coronary artery calcification in monogenic FH [14.5 (0-161.6) vs. 0 (0-31.8), p＝0.05).

APOB=apolipoprotein B, CAD=coronary artery disease, CI=confidence interval, FH=familial hypercholesterolemia, GWAS=genome-wide association study, HeFH=heterozygous familial hypercholesterolemia, LDL-C=low-density lipoprotein cholesterol, LDLR=low-density lipoprotein receptor, LDLRAP1= low-density lipoprotein receptor adapter protein 1, LIPA=lysosomal acid lipase A, MI=myocardial infarction, OR=odds ratio, PCSK9=proprotein convertase subxilisin/kexin type 9, STAP1=signal transducing adaptor family member 1

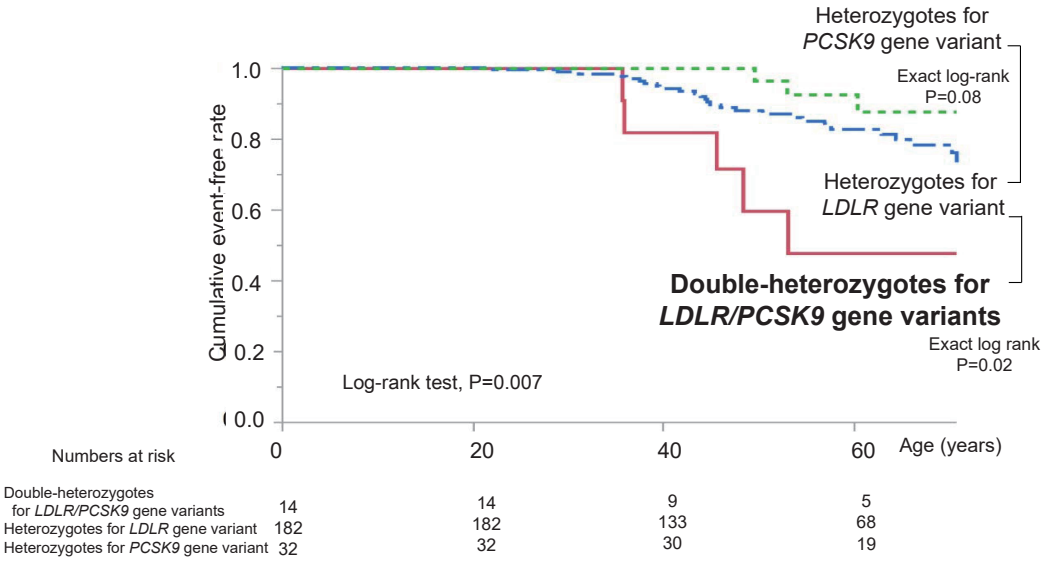

Fig. 3. Cardiovascular Outcomes in HeFH Subjects with Both LDLR and PCSK9 Gene Variants

LDLR=low-density lipoprotein receptor, PCSK9=proprotein convertase subxilisin/kexin type 9

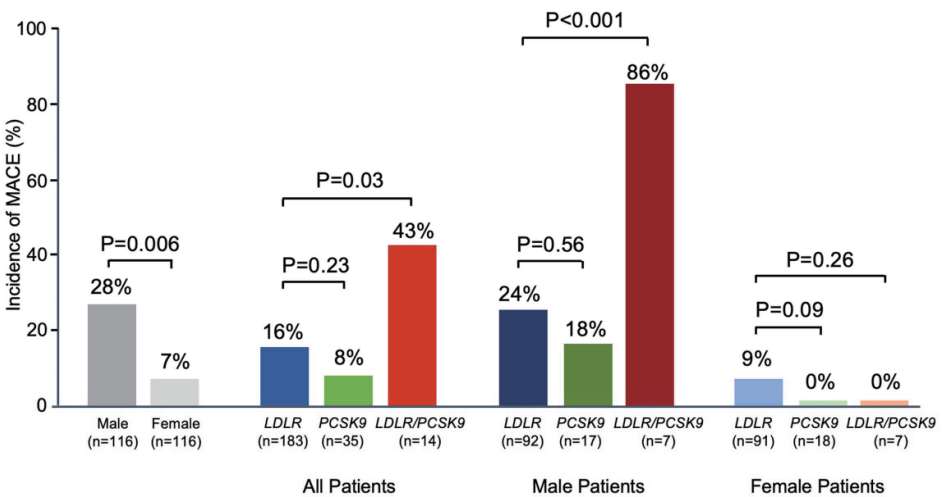

Fig. 4. The Incidence of MACE in Association with Gender and LDLR/PCSK9 Gene Variants

LDLR=low-density lipoprotein receptor, MACE=major cardiovascular events, PCSK9=proprotein convertase subxilisin/kexin type 9

fold increased risk in white subjects compared to a 2.0-, 3.4-, and 3.3-fold risk in African-American, Hispanic, and Asian subjects, respectively.

Biomarkers and ASCVD in HeFH Subjects (Table 3)

(a) Lp(a)

Lp(a) is consisted of apolipoprotein B100 covalently bound to the glycoprotein apolipoprotein(a)[31, 32]. A growing body of evidence suggests the association of Lp(a) with ASCVD in primary and secondary prevention settings of HeFH[33-41]. In the SAFEHEART study analyzing 1960 FH and 957 non-FH subjects, an increased Lp(a) level appeared in HeFH subjects with ASCVD[33]. Furthermore, Lp(a) level was an independent predictor for ASCVD in HeFH subjects. Of note, Lp(a) >50 mg/dL with *LDLR* negative mutation was associated with the greatest cardiovascular risk. The SAFEHEART study also reported another analysis of 2927 family members from 755 subjects with HeFH[35]. During over 5 years' follow-up, FH subjects experienced a 2.47-fold greater risk for ASCVD or death. Furthermore, in subjects

Table 3. Biomarkers Associated with ASCVD Risks in HeFH Subjects

Authors	Subjects	Outcomes	Findings
Lp(a)			
Alonso, *et al.*[33]	1960 FH and 957 non-FH from SAFEHEART	CVD (=MI, angina pectoris, revascularization, ischemic stroke or TIA, PAD, abdominal aortic aneurism)	FH with CVD more likely had a higher Lp(a) level compared those without CVD (43.4 (18.2-84.3) vs. 21.3 (8.9-53.9), $p<0.0001$). The Cox proportional hazards model demonstrated independent predictors of CVD which included Lp(a) (OR=1.008, $p<0.0001$), male (OR=2.738, $p<0.0001$), smoking (OR=1.906, $p<0.0001$), xanthomas (OR=1.488, $p<0.01$), HDL-C<40 mg/dL (OR=1.419, $p<0.03$) and BMI (OR=1.035, $p<0.02$).
Cao, *et al.*[34]	393 HeFH subjects	CVE (=fatal and non-fatal myocardial infarction, fatal and non-fatal stroke, post-discharge coronary revascularization and cardiac death)	During the observational period (36.5 months), HeFH subjects with a higher Lp(a) was associated with a significantly lower event-free survival ($p=0.004$) (HR=2.03 (1.28-3.21), $p=0.002$). Adding Lp(a) to the Cox model improved C-statistic to 0.796 (95%CI=0.751-0.841, $p=0.001$), net reclassification (24.2%, 95%CI=1.4%-43.6%, $p=0.041$) and integrated discrimination (5.4%, 95%CI=2.2%-12.8%, $p=0.037$).
Ellis, *et al.*[35]	Family members ($n=2927$) from 755 HeFH cases in SAFEHEART	CVD (=MI, angina pectoris, revascularization, ischemic stroke or TIA, PAD, abdominal aortic aneurism)	Through the cascade screening ($n=2927$), 18.5, and 6.6% of them exhibited Lp(a) 50-99 mg/dl and >=100 mg/dl, respectively. Adjusting for clinical characteristics, patients with elevated Lp(a) alone (HR=3.17, $p=0.02$) and FH alone (HR=2.47, $p=0.03$) conferred an elevated risk for CVD compared to those without any these features.
Pérez de Isla, *et al.*[36]	5022 subjects from SAFEHEART (FH: $n=3712$, non-affected relatives: $n=1310$)	Severe aortic valve stenosis requiring surgical aortic valve replacement	The frequency of AVR due to severe AS was 1.48 and 0.27% in HeFH and non-affected relatives, respectively (OR=5.71, 95%CI=1.78-18.4, $p=0.003$) during 7.48-year follow-up period. Cox regression analysis identified Lp(a) level as an independent predictor for AVR (HR=1.013, 95%CI=1.009-1.018, $p<0.001$), in addition to age (HR=1.089, 95%CI=1.063-1.12, $p<0.001$), a history of ASCVD (HR=16.89, 95%CI=6.93-41.23, $p<0.001$), hypertension (HR=7.48, 95%CI=3.95-14.2, $p<0.001$) and LDL-C-years (HR=1.013, 95%CI=1.009-1.016, $p<0.001$).
Naito, *et al.*[37]	399 HeFH subjects from FAME study	CAD, cerebral infarction and PAD	Japanese HeFH patients with an elevated Lp(a) level more likely harboured a greater risk of future cardiovascular events ($p=0.02$ for trend).
Zawacki, *et al.*[38]	129 HeFH pediatric patients	ASCVD (=myocardial infarction, percutaneous coronary intervention, coronary artery bypass surgery, cerebrovascular accident, peripheral vascular disease)	HeFH pediatric patients with a family history of early-onset ASCVD had an elevated Lp(a) level compared to those with late-onset ASCVD (OR=3.77, 95% CI=1.16-12.25, $p=0.027$), whereas they did not necessarily had an elevated LDL-C (OR=0.45, 95%CI=0.11-1.80, $p=0.26$).
Alonso, *et al.*[39]	161 molecularly defined FH	CAC score	After adjusting age, sex, BMI, glycemia, statin intensity, smoking and high blood pressure, Lp(a) ($\beta=0.158$, $p=0.03$) and PCSK9 ($\beta=0.179$, $p=0.02$) were independent predictors for positive CAC scores. these markers predicted a positive CAC score in HeFH subjects.
HDL			
Ogura, *et al.*[46]	227 HeFH subjects	ASCVD (=CAD, stroke and revascularization)	The mean values for cholesterol efflux capacity 0.88±0.14. A lower cholesterol efflux capacity was observed in HeFH subjects with ASCVD. Logistic regression analysis demonstrated cholesterol efflux capacity as an independent predictor for ASCVD ($p=0.02$).
CRP			
Mohrschladt, *et al.*[49]	337 FH patients	CVD	The presence of CVD was associated with a higher CRP level in HeFH patients (2.26mg/l vs. 1.55 mg/l, $p<0.001$).
Wissen, *et al.*[50]	325 FH patients	Intima media thickness of carotid artery	Atorvastatin 80mg and simvastatin 40mg decreased CRP level. The degree of CRP reduction with these statins was associated with slowing progression of intima media thickness.
PCSK9			
Kataoka, *et al.*[56]	138 HeFH subjects	Atheroma volume on intravascular ultrasound	Mature PCSK9 level was associated with percent atheroma volume (r=0.78, $p=0.003$), whereas vessel volume did not change across any mature PCSK9 levels (r=0.05, $p=0.78$). As a consequence, smaller lumen volume was observed in association with mature PCSK9 level (r=0.65, $p=0.009$). By contrast, furin-cleaved (r=0.12, $p=0.45$) and total PCSK9 (r=0.37, $p=0.25$) levels did not associate with percent atheroma volume. Multivariate analysis revealed that mature PCSK9 level independently contributed to percent atheroma volume (odds ratio: 1.45, 95% confidence interval: 1.11-1.67, $p=0.01$).

ASCVD=atherosclerotic cardiovascular disease, BMI=body mass index, CAC=coronary artery calcification, CAD=coronary artery disease, CRP=c-reactive protein, CVD=cardiovascular disease, FH=familial hypercholesterolemia, HDL-C=high-density lipoprotein cholesterol, HeFH=heterozygous familial hypercholesterolemia, Lp(a)=lipoprotein a, PAD=peripheral artery disease, PCSK9=proprotein convertase subxilisin/kexin type 9

with HeFH with an elevated Lp(a) level, their cardiovascular risk was greatest (HR=4.40, 95% CI=1.92–10.07, $p<0.001$), independent of conventional risk factors. A recent study provided additional evidence about Lp(a) association with aortic valve stenosis in subjects with HeFH[36]. The frequency of aortic valve stenosis requiring surgical procedure was 1.48% in subjects with HeFH compared to 0.27% in non-FH ones (OR=5.71, 95% CI=1.78–18.4, $p=0.003$) during a 7.4-year follow-up period. Moreover, an increased Lp(a) level independently predicted the need for a surgical procedure of aortic valve stenosis in patients with HeFH. The FAME study reported whether Lp(a) predicts cardiovascular events' risks in Japanese subjects with HeFH[37]. In this study, 399 Japanese patients with HeFH were analyzed, and their Lp(a) level was 20.8 (11.3–38.0) mg/dL. During the 3-year observational period, Japanese patients with HeFH with an elevated Lp(a) level more likely harbored a greater risk of future ASCVD events ($p=0.02$ for trend). Mechanistic studies using computed tomography imaging and coronary angiography revealed a greater amount of coronary artery calcification and more complex features of coronary artery stenosis in patients with HeFH associated with a higher Lp(a) level[39, 40].

(b) HDL

The functionality of high-density lipoprotein (HDL) confers the degree of its atheroprotective property[42, 43], and therefore it has been considered to affect future cardiovascular risks. Several studies reported diminished functions of HDL in patients with FH. In one previous study analyzing 259 subjects with FH and 208 subjects without FH, the size of HDL was significantly smaller in FH subjects[44]. This characteristic of HDL particularly was more prevalent in men than women. Another study analyzed major components of reverse cholesterol transport in 12 subjects with FH and 12 healthy subjects[45]. Large HDL2 particles in patients with FH exhibited a diminished cholesterol efflux capacity via scavenger-BI and ATP Binding Cassette Subfamily G Member 1-dependent pathways. Additionally, cholesteryl ester transfer protein-mediated cholesterol ester transfer more likely occurred from HDL2 and HDL3 particles to LDL, whereas the degree of delivering cholesterol ester within HDL to the liver was reduced.

Regarding the association of HDL functionality with atherosclerosis in subjects with HeFH, Ogura *et al.* reported that a decreased cholesterol efflux capacity was associated with the concomitance of corneal arcus (OR per 1-SD increase=0.98, 95% CI=0.95–1.00, $p=0.03$), Achilles tendon thickness ($p<0.0001$) and carotid intima-media thickness ($p<0.0001$)[46]. Moreover, a greater degree of HDL-medicated cholesterol efflux capacity predicted a lower risk of ASCVD (OR per 1-SD increase=0.95, 95% CI=0.90–0.99, $p=0.02$)[46]. These findings suggest the evaluation of HDL functionalities as a potential measure to predict atherosclerotic cardiovascular risks in patients with HeFH.

(c) C-Reactive Protein (CRP)

Accumulating evidence highlights inflammation as an important contributor to atherosclerosis, underscoring biomarkers reflecting inflammation as a potential tool for future cardiovascular risk stratification[47, 48]. Several studies have reported the association of CRP levels with ASCVD. Mohrschladt, *et al.* revealed an elevated CRP levels in subjects with HeFH with premature cardiovascular disease (2.26 vs. 1.55 mg/dL, $p<0.001$)[49]. In another analysis with carotid ultrasound evaluation and serial CRP measurement, change in CRP levels was associated with the progression rate of carotid atherosclerosis[50].

(d) PCSK9

PCSK9 is a protease that combines *LDLR*. Then, it induces to degrade LDLR, which elevates circulating LDL particles[51, 52]. Several observational studies analyzed the relationship of circulating PCSK9 levels with future cardiovascular events in healthy subjects or patients without FH[53-56]. This relationship is not fully evaluated in patients with FH. Pathophysiologically, it circulates as two subtypes—mature and furin-cleaved forms; these subtypes differ in their properties to modulate LDLR. While mature PCSK9 can degrade LDLR, furin-cleaved form has been shown to have no activity modulating LDLR[57, 58]. Our recently developed ELISA has enabled measuring these two PCSK9 concentrations quantitatively, and we found out that mature PCSK9 is a more dominant one in the circulation of FH[59]. This finding could account for an elevated LDL-C level and potential contribution to atherosclerosis in patients with FH. The association of mature and furin-cleaved PCSK9 with coronary atherosclerosis was investigated by employing intravascular ultrasound (IVUS) in 138 patients with HeFH[60]. In this analysis, average mature and furin-cleaved PCSK9 levels were 294.2±111.9 and 80.0±82.4 ng/ml, respectively. Intravascular imaging analysis indicated a greater amount of coronary atheroma associated with a higher level of mature PCSK9 (r=0.65, $p=0.009$). By contrast, there was no relationship between furin-cleaved PCSK9 level and coronary atherosclerosis (r=0.12, $p=0.45$).

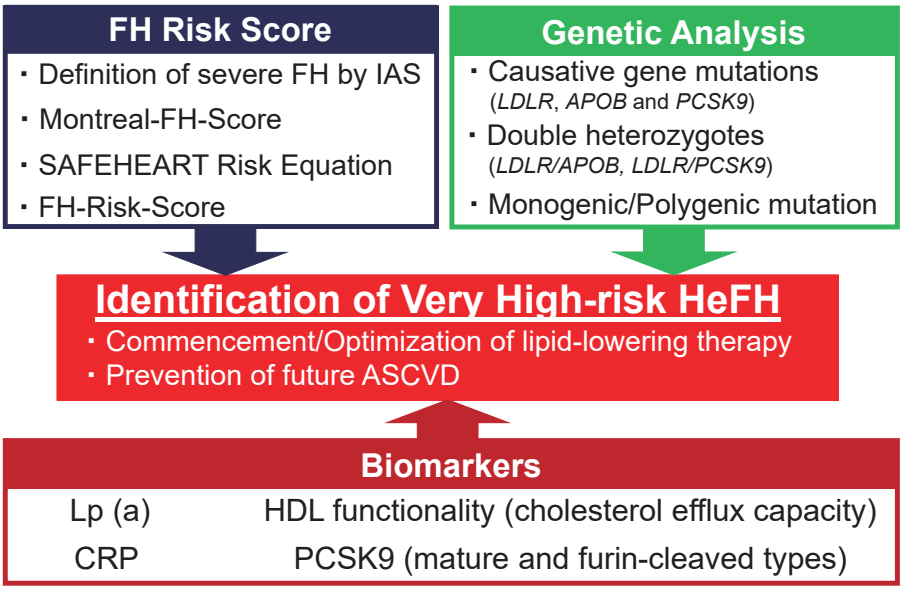

Fig. 5. Future Perspectives for Cardiovascular Risk Stratification in HeFH

APOB=apolipoprotein B, ASCVD=atherosclerotic cardiovascular disease, CRP=c-reactive protein, FH=familial hypercholesterolemia, HDL=high-density lipoprotein, HeFH=heterozygous familial hypercholesterolemia, IAS=international atherosclerosis society, LDL-C=low-density lipoprotein cholesterol, LDLR=low-density lipoprotein receptor, Lp(a)=lipoprotein a, PCSK9=proprotein convertase subxilisin/kexin type 9

Multivariate analysis demonstrated that mature but not furin-cleaved PCSK9 independently contributed to coronary atherosclerosis in patients with HeFH. Given that the degree of atheroma burden on IVUS imaging predicts future cardiovascular events[61], these findings indicate the evaluation of mature PCSK9 as a potential biomarker to stratify future cardiovascular risks.

Conclusions

A markedly high LDL-C level and its accumulation have been considered to promote cardiovascular risks in subjects with HeFH. Given their elevated premature ASCVD risks, appropriate evaluation of future cardiovascular risks in subjects with HeFH is clinically important to commence lipid-lowering therapies with their adequate intensity[62]. The current review has introduced clinical approaches to evaluate future cardiovascular events' risks in patients with HeFH. Incorporating the aforementioned tools is expected to identify very high-risk patients with HeFH who warrant more intensive lipid management (Fig. 5). This approach will lead to achieving the appropriate LDL-C goal according to the estimated cardiovascular risks of HeFH. Recently, a cross-sectional study from the European Atherosclerosis Society Familial Hypercholesterolemia Studies Collaboration has been reported by analyzing 61612 subjects with FH[63]. This large-scale study will provide additional evidence about the risk stratification of HeFH in the future. Further investigation will be needed whether clinical management according to risk stratification tools could improve cardiovascular outcomes in subjects with HeFH.

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