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ORIGINAL RESEARCH

Characterization of Polyvascular Disease in Heterozygous Familial Hypercholesterolemia: Its Association With Circulating Lipoprotein(a) Levels

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BACKGROUND: Heterozygous familial hypercholesterolemia (HeFH) more likely exhibits extensive atherosclerotic disease at multiple vascular beds. Lipoprotein(a) (Lp(a)) is an atherogenic lipoprotein that elevates HeFH-related atherosclerotic cardiovascular disease risks. Whether circulating Lp(a) level associates with polyvascular propagation of atherosclerosis in subjects with HeFH remains uncertain.

METHODS AND RESULTS: The current study analyzed 370 subjects with clinically diagnosed HeFH who received evaluation of systemic arteries. Polyvascular disease (polyVD) was defined as more than 2 coexisting atherosclerosis conditions including coronary artery disease, carotid stenosis, or peripheral artery disease. Clinical characteristics and lipid features were analyzed in subjects with HeFH and polyVD; 5.7% of patients with HeFH (21/370) had polyVD. They were more likely to have a clustering of risk factors, tendon (P<0.001) and skin xanthomas (P=0.004), and corneal arcus (P=0.026). Furthermore, an elevated Lp(a) level (P=0.006) and a greater frequency of Lp(a) level ≥50 mg/dL (P<0.001) were observed in subjects with HeFH and polyVD. On multivariable analysis adjusting risk factors and lipid-lowering agents, Lp(a) ≥50 mg/dL (odds ratio [OR], 5.66 [95% CI, 1.68–19.0], P=0.005), age, and family history of premature coronary artery disease independently predicted polyVD in subjects with HeFH. Of note, the prevalence of polyVD rose to 33.3% in patients with HeFH and age >58 years old, family history of premature coronary artery disease, and Lp(a) ≥50 mg/dL (OR, 10.3 [95% CI, 3.12–33.4], P<0.001).

CONCLUSIONS: An increased level of circulating Lp(a) levels predicted concomitance of polyVD in patients with HeFH. The current findings suggest subjects with HeFH and Lp(a) ≥50 mg/dL as a high-risk category who require meticulous screening of systemic vascular beds.

Key Words: atherosclerosis ■ familial hypercholesterolemia ■ lipoprotein(a) ■ polyvascular disease

Heterozygous familial hypercholesterolemia (HeFH) is a genetic disorder that is characterized as a marked elevation of low-density lipoprotein cholesterol (LDL-C) levels.[1] This atherogenic substrate more likely causes atherosclerotic coronary artery disease (CAD). In addition, recent studies reported extensive disease formation including not only coronary artery but peripheral arteries in HeFH.[2] Because this polyvascular atherosclerotic propagation has been shown to associate with more frequent occurrence of cardiovascular events,[3] the concomitance of polyvascular disease (polyVD) may be an important disease substrate that worsens cardiovascular outcomes in subjects with HeFH. However, the frequency, clinical characteristics, and predictors of polyVD in the setting of HeFH remain to be fully elucidated.

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CLINICAL PERSPECTIVE

What Is New?

- An increased level of circulating lipoprotein(a) levels (≥50 mg/dL) was associated with concomitant polyvascular disease in patients with heterozygous familial hypercholesterolemia.

What Are the Clinical Implications?

- Patients with heterozygous familial hypercholesterolemia and lipoprotein(a) ≥50 mg/dL are a high-risk category for atherosclerotic cardiovascular disease and screening of systemic vascular beds is advisable.

Nonstandard Abbreviations and Acronyms

HeFH	heterozygous familial hypercholesterolemia
LDLR	low-density lipoprotein receptor
Lp(a)	lipoprotein(a)
polyVD	polyvascular disease

Recent study has shown an elevated circulating Lp(a) level in patients with CAD receiving coronary artery bypass grafting who had polyVD.[4] Given that Lp(a) is an atherogenic lipoprotein that promotes foam cell and necrotic core formations and inflammatory and prothrombotic activities,[5] these proatherogenic properties of Lp(a) may be an important driver causing polyVD in subjects with HeFH. Therefore, the current study sought to investigate clinical characteristics of HeFH with polyVD and its association with circulating Lp(a) levels.

METHODS

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

Study Population

The present study retrospectively analyzed 481 patients who were clinically diagnosed with HeFH at the National Cerebral and Cardiovascular Center between January 1, 1978 and December 31, 2016. All of these subjects received genetic analysis of low-density lipoprotein receptor (*LDLR*) and *PCSK9* genes at our institute from January 1, 2005 to December 31, 2016.[6,7] CAD, carotid stenosis, and/or peripheral artery disease (PAD) were assessed in relation to clinical care. Evaluation of peripheral arteries was received in clinically indicated cases. Of these, 111 subjects were excluded because they did not receive any evaluation of their peripheral arteries. The remaining 370 patients with evaluation of peripheral arteries were included in the current analysis. HeFH was diagnosed according to the Japan Atherosclerosis Society guidelines as follows: subjects who fulfilled at least 2 of the clinical characteristics criteria including (1) untreated LDL-C level ≥180 mg/dL, (2) tendon xanthoma (tendon xanthoma on the dorsal hands, elbows, and knees, or Achilles tendon thickening) or nodular xanthoma on the skin, and (3) a history of familial hypercholesterolemia or premature CAD within second degree of relatives.[8] Patients with Achilles tendon thickening (≥9mm) on radiography are considered as having xanthoma.[8] Premature CAD is defined as CAD in men younger than 55 years old and women younger than 65 years old.[8] A cutoff value of LDL-C was selected according to 1 published study analyzing 1356 Japanese patients with dyslipidemia.[8] This paper showed that the percentage of patients with and without FH with their LDL-C ≥180 mg/dL were 24.5% and 94.5%, respectively. In addition, better sensitivity with similar specificity was observed by using 180 mg/dL as its cutoff value compared with 190 mg/dL.[8] The research protocol was approved by the ethics committee of our institution (M17-056). 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.

Definition of PolyVD

PolyVD was defined as the presence of more than 2 coexisting atherosclerosis conditions, including CAD, carotid stenosis, or PAD. CAD was defined as the presence of at least 1 segment with >50% diameter stenosis at left main coronary artery and/or >75% diameter stenosis at right and/or left coronary arteries by coronary angiography.[9] Carotid stenosis was defined as the presence of >50% stenosis by the NASCET (North American Symptomatic Carotid Endarterectomy Trial) on duplex ultrasonography.[10] PAD was defined as the presence of intermittent claudication, an ankle/arm index <0.9 or stenosis of peripheral arteries with diameter stenosis >50% on angiography or ultrasonography. The concomitance of atherosclerosis in the current study subjects was evaluated at the most recent visit.

Measurement of Lipid Parameters

The current study collected lipids data at recent visit of study subjects. Fasting serum levels of total cholesterol, triglycerides, high-density lipoprotein cholesterol, and Lp(a) were measured by enzymatic methods

(Sekisui Medical, Tokyo, Japan) using an automated analyzer (Hitachi Labospect 008; Hitachi-Hitec, Tokyo, Japan). LDL-C levels were calculated by the Friedewald formula, except for triglyceride levels >400 mg/dL.[11] High-intensity statin was defined as either atorvastatin ≥20 mg, rosuvastatin ≥10 mg, or pitavastatin ≥4 mg.[12]

Statistical Analysis

Results are shown as percentages for categorical variables and mean±SD for continuous variables. When variables were not normally distributed, their results are expressed as median (interquartile range). Clinical characteristics, lipid-lowering therapies, and on-treatment lipid parameters were compared by ANOVA for continuous variables as appropriate. Categorical variables were compared using the Kruskal-Wallis test as appropriate. Multivariable logistic regression was used to calculate odds ratios (ORs) and 95% CIs after controlling simultaneously for potential confounders. The model included risk factors that demonstrated an association with stenotic atherosclerosis in univariate analysis. A value of $P<0.05$ was considered statistically significant. All statistical analyses were performed using the SAS software, version 13.0.0 (SAS Institute Inc, Cary, NC) or STATA 15 (Stata Corp, College Station, TX).

RESULTS

Frequency of PolyVD in HeFH

In the current study, 72.4% (=268/370) of patients with HeFH did not have any atherosclerotic cardiovascular disease (=nonatherosclerosis), whereas 21.9% (=81/370) and 5.7% (=21/370) of them exhibited 1 atherosclerosis condition (=1 atherosclerosis) and polyVD, respectively (Figure 1). Figure 2 summarizes the frequency of each atherosclerotic cardiovascular disease, and the overlapped area indicates the presence of polyVD. As expected, CAD was the most frequent concomitant disease (26.8%=99/370), followed by carotid stenosis (4.6%=17/370) and PAD (3.0%=11/370). With regard to polyVD, the concomitance of CAD with carotid stenosis or PAD was observed in 3.5% (=13/370) and 1.1% (=4/370), respectively. In addition, 1.1% (=4/370) of study subjects had all atherosclerosis conditions (Figure 2).

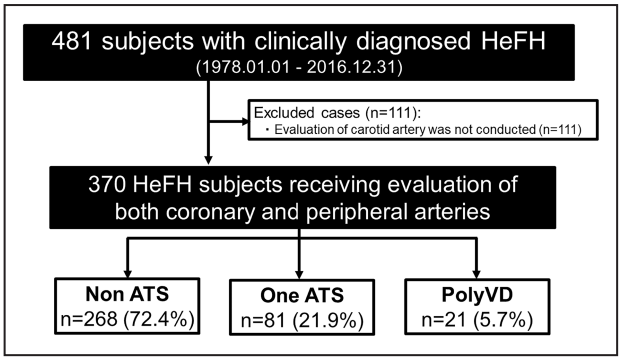

Figure 1. Patients' disposition.
ATS indicates atherosclerosis; HeFH, heterozygous familial hypercholesterolemia; and polyVD, polyvascular disease.

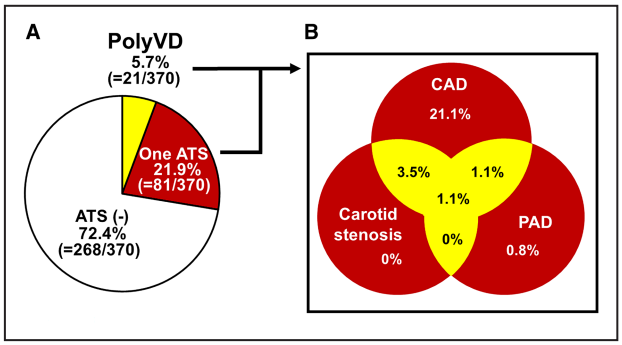

Figure 2. Characteristics of polyVD in HeFH.
A, Frequency of polyVD. **B,** Characteristics of concomitant ATS in HeFH with polyVD. ATS indicates atherosclerosis; CAD, coronary artery disease; HeFH, heterozygous familial hypercholesterolemia; PAD, peripheral artery disease; and polyVD, polyvascular disease.

Clinical Demographics of HeFH With PolyVD

Table 1 describes clinical characteristics in subjects with HeFH stratified according to the number of concomitant atherosclerosis conditions. Subjects with HeFH and polyVD were more likely to be older ($P<0.001$), male ($P<0.001$), and have a history of hypertension ($P<0.001$), type 2 diabetes ($P<0.001$), and smoking habit ($P<0.001$) with family history of premature CAD ($P<0.001$). Furthermore, tendon ($P<0.001$) and skin xanthomas ($P=0.004$) and corneal arcus ($P=0.026$) were more frequently observed in subjects with HeFH and polyVD (Table 1). *LDLR* pathogenic variants are dominant characteristics of genetic variants in the current study subjects, and there were no significant differences in the proportion of each HeFH genotype across the groups (Table 1). Evaluated gene variants in this study was summarized in Tables S1–S3.

Lipid-Lowering Therapies

The use of lipid-lowering agents and on-treatment lipid profiles are shown in Table 2. Patients with polyVD more frequently received intensive lipid-lowering management including high-intensity statin ($P<0.001$), ezetimibe ($P<0.001$), and PCSK9 (proprotein convertase subtilisin/kexin type 9) inhibitor; $P<0.001$ (Table 2). As a consequence, patients with HeFH and polyVD were more likely to exhibit a lower LDL-C ($P<0.001$) with a greater frequency of achieving LDL-C <70 mg/dL ($P<0.001$), whereas their

Table 1. Baseline Clinical Characteristics

	Non ATS (n=268)	One ATS (n=81)	PolyVD (n=21)	*P* value
Age, y	52.0±19.5	65.9±14.5	76.6±10.1	<0.001*
Male sex, n (%)	91 (34.0)	52 (64.2)	16 (76.2)	<0.001
Hypertension, n (%)	40 (14.9)	39 (48.2)	15 (71.4)	<0.001
Diabetes, n (%)	1 (0.4)	5 (6.2)	3 (14.3)	<0.001
Smoker, n (%)	55 (20.5)	43 (53.1)	17 (81.0)	<0.001
Family history of premature coronary artery disease, n (%)	32 (11.9)	40 (49.4)	13 (61.9)	<0.001
Tendon xanthomas, n (%)	152 (56.7)	64 (79.0)	17 (81.0)	<0.001
Skin xanthomas, n (%)	25 (9.3)	11 (13.6)	7 (33.3)	0.004
Corneal arcus, n (%)	67 (25.0)	32 (39.5)	8 (38.1)	0.026
Genotype of familial hypercholesterolemia				
LDLR pathogenic variants, n (%)	157 (58.6)	46 (56.8)	15 (71.4)	0.467
PCSK9 pathogenic variants, n (%)	20 (7.5)	4 (4.9)	1 (4.8)	0.681
LDLR and *PCSK9* pathogenic variants, n (%)	11 (4.1)	5 (6.2)	1 (4.8)	0.738

ATS indicates atherosclerosis; *LDLR*, low-density lipoprotein cholesterol receptor; *PCSK9*, proprotein convertase subtilisin/kexin type 9; and polyVD, polyvascular disease.
*Tested using analysis of variance. Other comparisons were conducted by Kruskal-Wallis test.

on-treatment Lp(a) was significantly higher compared with those without non atherosclerosis and with 1 atherosclerosis condition (*P*=0.002) (Table 2). Furthermore, patients with polyVD were more likely to have a greater proportion of on-treatment Lp(a) ≥50 mg/dL (*P*<0.001) with lower high-density lipoprotein cholesterol (*P*<0.001) and higher triglyceride (*P*<0.001) levels (Table 2).

Association of Lp(a) With PolyVD in Subjects With HeFH

Figure 3 illustrates the distribution of atherosclerosis in association with Lp(a) levels. In subjects with HeFH and Lp(a) <30 mg/dL, over 75% of them did not have any atherosclerosis and the frequency of polyVD was only 3.2%. However, in association with an increased level of Lp(a), subjects with HeFH more likely exhibited concomitantly 1 atherosclerosis condition and polyVD (*P*<0.001 for trend). In particular, the proportion of subjects with HeFH and 1 atherosclerosis condition and polyVD was 27.6% and 17.2%, respectively (Figure 3). The overall prevalence of polyVD is likely to be positively associated with greater age, and analysis was added. Although no significant differences were observed, vascular prevalence tended to increase with greater age (*P*=0.092) (Figure S1).

Uni- and multivariable logistic regression analyses were performed to elucidate clinical parameters that

Table 2. Lipid-Lowering Therapies and Lipid Control

	Non ATS (n=268)	One ATS (n=81)	PolyVD (n=21)	*P* value
Lipid-lowering therapy				
Statin, n (%)	228 (85.1)	76 (93.8)	19 (90.5)	0.105
High-intensity statin, n (%)	122 (45.5)	58 (71.6)	13 (61.8)	<0.001
Ezetimibe, n (%)	136 (50.8)	59 (72.8)	19 (90.5)	<0.001
Proprotein convertase subtilisin/kexin type 9 inhibitor, n (%)	28 (10.5)	24 (29.6)	8 (38.1)	<0.001
On-treatment lipid parameters				
Low-density lipoprotein cholesterol, (mg/dl)	124±50.2	90±37.8	91±32.8	<0.001*
High-density lipoprotein cholesterol (mg/dl)	60±14.4	51±14.4	44±10.7	<0.001*
Triglyceride (mg/dl)	78 [58–113]	93 [63–134]	106 [87–156]	<0.001*
Lp(a) (mg/dl)	14.9 [6.9–30.9]	18.4 [9.0–42.7]	49.0 [17.6–70.7]	0.002*
Lp(a) ≥50 mg/dlL, n (%)	32 (12.4)	16 (20.5)	10 (47.6)	<0.001

ATS indicates atherosclerosis; Lp(a), lipoprotein(a); and polyVD, polyvascular disease.
*Tested using analysis of variance. Other comparisons were conducted by Kruskal-Wallis test.

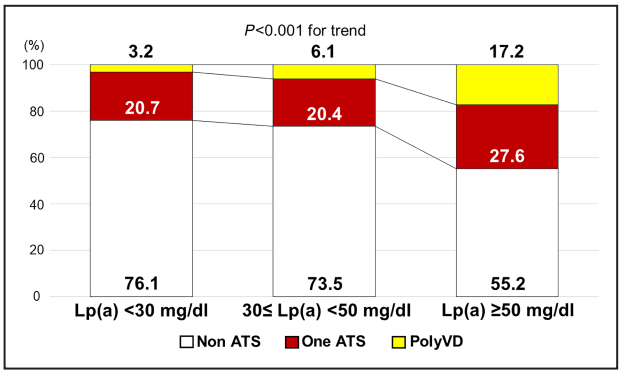

Figure 3. Frequency of polyVD in association with Lp(a) levels.
ATS indicates atherosclerosis; Lp(a), lipoprotein(a); and polyVD, polyvascular disease.

predicted the concomitance of polyVD in subjects with HeFH. Univariate analysis showed age (OR, 1.08 [95% CI, 1.05–1.12], *P*<0.001), male sex (OR, 4.61 [95% CI, 1.65–12.9], *P*=0.004), hypertension (OR, 8.54 [95% CI, 3.21–22.8], *P*<0.001), diabetes (OR, 9.53 [95% CI, 2.20–41.2], *P*=0.003), smoking (OR, 10.9 [95% CI, 3.57–33.2], *P*<0.001), family history of premature CAD (OR, 6.25 [95% CI, 2.50–15.7], *P*<0.001), PCSK9 inhibitor (OR, 3.51 [95% CI, 1.39–8.90], *P*=0.008), LDL-C (OR, 0.99 [95% CI, 0.97–1.00], *P*=0.01), high-density lipoprotein cholesterol (OR, 0.93 [95% CI, 0.89–0.96], *P*<0.001) and triglyceride (OR, 1.01 [95% CI, 1.00–1.02], *P*=0.006), and Lp(a) ≥50 mg/dL (OR, 5.47 [95% CI, 2.20–13.6], *P*<0.001) were significant predictors of polyVD in subjects with HeFH. On multivariable analysis, Lp(a) ≥50 mg/dL still continued to associate with the concomitance of polyVD (OR, 5.66 [95% CI, 1.68–19.0], *P*=0.005) (Table 3).

The frequency of polyVD was further investigated in subgroups stratified according to the presence of independent predictors for polyVD (age >58 years old [=median value], family history of premature atherosclerotic cardiovascular disease [ASCVD]), and (Lp(a) ≥50 mg/dL) (Figure 4). In patients with HeFH and Lp(a) ≥50 mg/dL alone, the prevalence of polyVD was 17.2%, which was significant higher compared with those with Lp(a) <50 mg/dL (OR, 5.47 [95% CI, 2.20–13.6], *P*<0.001). Moreover, polyVD was observed in over 20% of subjects with HeFH with both their age >58 years old and Lp(a) ≥50 mg/dL (OR, 9.30 [95% CI, 3.65–23.7], *P*<0.001) or HeFH with both Lp(a) ≥50 mg/dL and family history of premature ASCVD (OR, 7.21 [95% CI, 2.31–22.5], *P*<0.001) (Figure 4). Of note, the prevalence of polyVD rose to 33.3% in patients with HeFH and all of these clinical characteristics (OR, 10.3 [95% CI, 3.12–33.4], *P*<0.001) (Figure 4).

DISCUSSION

The concomitance of ASCVD substantially affects cardiovascular outcomes in subjects with HeFH, which indicates a clinical need to identify factors associated with its atherosclerotic severity. In the current study, throughout evaluation of systemic arteries, the concomitance of polyVD was identified in 5.7% of Japanese

Table 3. Multivariable Analysis of Predictors for PolyVD

	Unadjusted			Adjusted		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Age (per a year)	1.08	(1.05–1.12)	<0.001	1.07	(1.02–1.13)	0.012
Male sex	4.61	(1.65–12.9)	0.004	1.52	(0.29–7.91)	0.620
Hypertension	8.54	(3.21–22.8)	<0.001	2.11	(0.62–7.22)	0.234
Diabetes	9.53	(2.20–41.2)	0.003	4.59	(0.51–41.1)	0.173
Smoker	10.9	(3.57–33.2)	<0.001	5.42	(1.16–25.4)	0.032
Family history of premature CAD	6.25	(2.50–15.7)	<0.001	3.21	(1.00–10.3)	0.049
Tendon xanthomas	2.62	(0.86–7.94)	0.065			
LDLR pathogenic variants	1.80	(0.68–4.74)	0.221			
High-intensity statin	1.53	(0.62–3.77)	0.354			
PCSK9 inhibitor	3.51	(1.39–8.90)	0.008	1.76	(0.45–6.84)	0.415
On-treatment LDL-C (per mg/dl)	0.99	(0.97–1.00)	0.012	0.99	(0.98–1.02)	0.817
On-treatment HDL-C (per mg/dl)	0.93	(0.89–0.96)	<0.001	0.98	(0.93–1.02)	0.307
On-treatment triglyceride (per mg/dl)	1.01	(1.00–1.02)	0.006	1.01	(0.99–1.02)	0.309
Lipoprotein(a) ≥50 mg/dL	5.47	(2.20–13.6)	<0.001	5.66	(1.68–19.0)	0.005

Adjusted odds ratios were calculated by a multivariable logistic regression. This model included the following variables: age, sex, hypertension, diabetes, smoker, family history of premature CAD, tendon xanthomas, *LDLR* pathogenic variants, high-intensity statin, PCSK9 inhibitor, on-treatment LDL-C, on-treatment HDL-C, On-treatment triglyceride, lipoprotein(a) ≥50 mg/dL. CAD indicates coronary artery disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; *LDLR*, low-density lipoprotein receptor; OR, odds ratio; and polyVD, polyvascular disease.

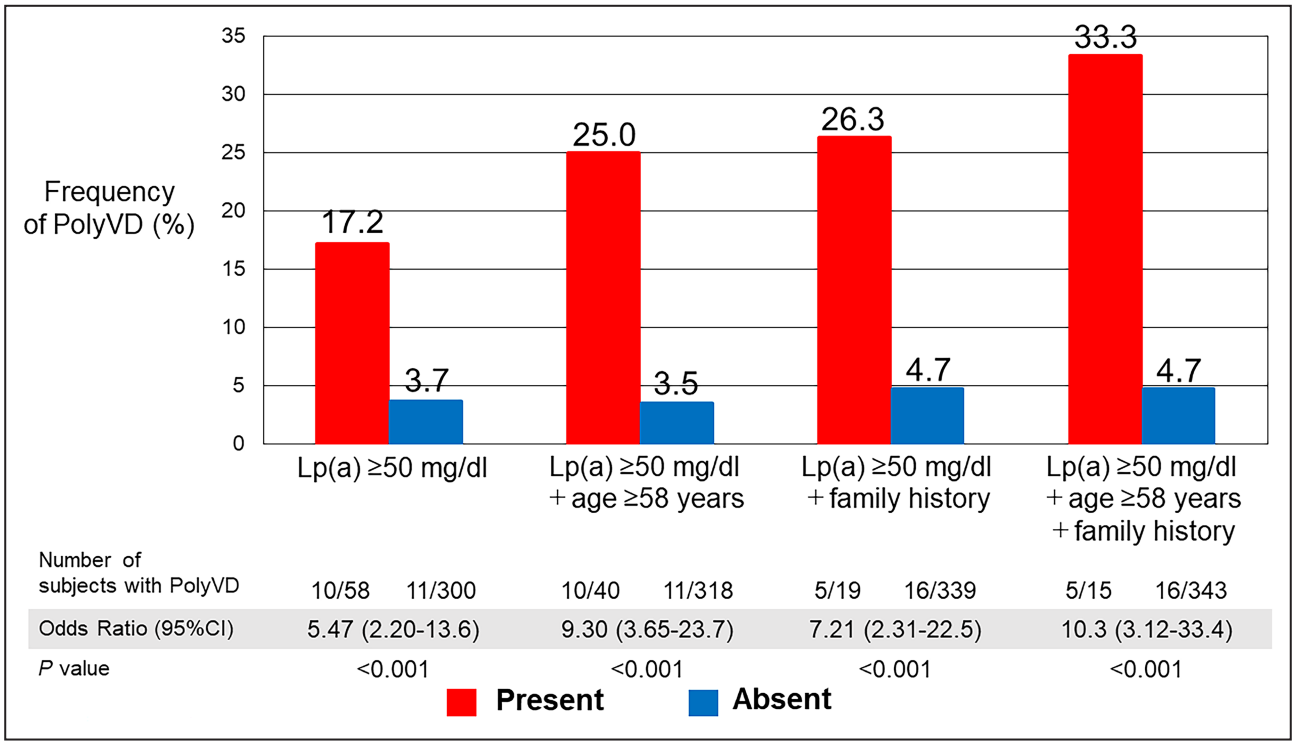

Figure 4. A risk of concomitant polyVD in subgroups of HeFH subjects.
HeFH indicates heterozygous familial hypercholesterolemia; Lp(a), lipoprotein(a); and polyVD, polyvascular disease.

subjects with HeFH. In addition to a clustering of atherogenic risk factors, Lp(a) ≥50 mg/dL was associated with the presence of polyVD in subjects with HeFH. Of note, the frequency of polyVD substantially rose to 33.3% in subjects with HeFH and Lp(a) ≥50 mg/dL, in addition to their older age and family history of premature CAD. Our findings support circulating Lp(a) level as an important clinical tool to identify very high-risk subjects with HeFH concomitantly exhibiting polyVD.

The current study provides additional insights into Lp(a) as an important contributor to atherosclerosis in subjects with HeFH. The SAFEHEART (Spanish Familial Hypercholesterolemia Cohort Study) study reported that Lp(a) level, especially its value ≥50 mg/dL, independently predicted the presence of cardiovascular disease including CAD or PAD in Spanish subjects with HeFH.[13] In addition, a greater frequency of severe aortic valve stenosis requiring surgical procedure has been observed in subjects with HeFH and a higher circulating Lp(a) level in the SAFEHEART study. These observations highlight that circulating Lp(a) could induce propagation of atherosclerosis in multiple vascular beds. In our analysis, Lp(a) ≥50 mg/dL in Japanese subjects with HeFH reflected polyvascular involvement of atherosclerosis under lipid-lowering therapies. Given that Lp(a) has been considered to accelerate atherogenesis via its intimal deposition, proinflammatory oxidized phospholipids and impaired fibrinolysis, these Lp(a)-related properties may cause systemic atherosclerotic formation and progression in subjects with HeFH.

Although polyVD has been reported to worsen cardiovascular outcomes, its diagnosis requires evaluation of multiple arteries by using various modalities and therefore it is always challenging to conduct these screening in appropriate subjects. In the current study, we observed that the prevalence of polyVD increased pertinent to Lp(a) levels. In particular, in subjects with HeFH and Lp(a) <30 mg/dL, 3.2% concomitantly had polyVD. By contrast, its frequency was almost 6 times greater in those with Lp(a) ≥50 mg/dL. Given that recent studies consistently reported the predictive ability of Lp(a) ≥50 mg/dL in CAD, stroke, and PAD,[13,14] measurement of Lp(a) levels may guide physicians to identify subjects with HeFH who require polyvascular beds' evaluation in the clinical settings.

In the current study, in addition to Lp(a) level, age and family history of premature CAD were associated with polyVD in subjects with HeFH. Of particular interest, almost one third of subjects with HeFH and all of these characteristics had polyVD. Recently proposed FH-related risk scores have included age and Lp(a) level,[15] and the International Atherosclerosis Society has considered all of these features as important factors to define severe FH.[16] Furthermore, these risk stratification approaches have predicted an elevated

risk of ASCVD including not only CAD but stroke and PAD.[2] Collectively, this evidence as well as our findings support the importance of considering comprehensive of atherogenic risks including age, family history of premature CAD, and Lp(a) for estimating systemic atherosclerotic disease substrates in subjects with HeFH.

Consistent findings about the association of Lp(a) with ASCVD have stimulated considerable interests whether elevated Lp(a) level would be a potential therapeutic target to mitigate ASCVD risks. The subanalysis of ODYSSEY Outcomes (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) reported that the presence of polyVD conferred a substantially elevated risk of future cardiovascular events in patients with acute coronary syndrome.[17] Despite their worse clinical outcome, a greater absolute risk reduction was observed following alirocumab use. In the prespecified analysis of the FOURIER (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk) trial, patients with higher Lp(a) level exhibited a greater benefit with evolocumab, reflected by a greater absolute reduction in Lp(a) and greater cardiovascular risk reduction in subjects with established ASCVD.[18] The potential antiatherosclerotic benefits of targeting Lp(a) may be derived by treating subjects with a higher Lp(a) level. The dedicated future studies are expected to elucidate whether pharmacological modulation of circulating Lp(a) may be effective in subjects with HeFH and Lp(a) ≥50 mg/dL.

Several limitations should be considered to interpret the current findings. First, this is a retrospective observational study conducted at a single center in Japan. The number of subjects with HeFH, especially those with polyVD, is relatively small. Second, the use and the selection of lipid-lowering therapy were undertaken according to each physician's discretion but not in randomized fashion. This may be potential selection bias. Third, the current study included Japanese subjects with FH according to the Japan Atherosclerosis Society guidelines of FH diagnosis. Whether the observation can be translated to non-Japanese patients with FH warrants further investigation. Finally, the definition of polyvascular disease was based on published papers (Song et al., Tmoyan et al.).[4,19] Therefore, the present definition does not include patients with stroke events, which may be a possible selection bias.

Conclusions

In conclusion, 5.7% of Japanese subjects with HeFH concomitantly exhibited polyVD. Subjects with HeFH and polyVD more likely had atherogenic risk factors and HeFH-related physical characteristics, accompanied by an elevated Lp(a) level. Even after adjusting clinical characteristics and LDL-C levels, Lp(a) ≥50 mg/dL predicted polyVD in subjects with HeFH receiving lipid-lowering therapies. The current observation underscores circulating Lp(a) level as a way to identify very high-risk subjects with HeFH concomitantly exhibiting polyVD.

ARTICLE INFORMATION

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Supplemental Material

Tables S1–S3
Figure S1

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SUPPLEMENTAL MATERIAL

Table S1. Included *LDLR* Gene Variants

Exon No.	Genomic location GRCh38 (Chr19)	Nucleotide change	Effect of protein	ClinVar	rs number	Variant rating according to ACMG guideline	N
1	11089567	c.20_21del	p.(Lys7Ilefs*44)	N/A	N/A	Pathogenic	2
1	11100222	c.68-1G>C	Splicing error	Pathogenic	rs879254397	Pathogenic	4
2	11100249	c.94_111del	p.(Phe32_Gly37del)	N/A	N/A	Likely pathogenic	1
2	11100294	c.139G>A	p.(Asp47Asn)	Conflicting interpretations of pathogenicity	rs778284147	Uncertain significance	1
3	11102756	c.283T>G	p.(Cys95Gly)	Conflicting interpretations of pathogenicity	rs879254456	Likely pathogenic	5
3	11102757	c.284G>T	p.(Cys95Phe)	Pathogenic/ Likely pathogenic	rs879254457	Uncertain significance	1
3	11102758	c.285C>A	p.(Cys95*)	Pathogenic	rs139400379	Pathogenic	2
3	11102774	c.301G>A	p.(Glu101Lys)	Pathogenic/Likely pathogenic	rs144172724	Likely pathogenic	1
3	11102783-11102785	c.310_312del	p.(Cys104del)	N/A	N/A	Pathogenic	2
4	11105250	c.344G>A	p.(Arg115His)	Conflicting interpretations of pathogenicity	rs201102461	Uncertain significance	4
4	11105295	c.389dup	p.(Asp131Argfs*49)	Pathogenic	rs879254510	Pathogenic	5
4	11105314	c.408del	p.(Asp136Glufs*70)	N/A	N/A	Pathogenic	1
4	11105324	c.418G>A	p.(Glu140Lys)	Pathogenic/Likely pathogenic	rs748944640	Pathogenic	4
4	11105384	c.478T>C	p.(Cys160Arg)	Pathogenic/Likely pathogenic	rs879254540	Likely pathogenic	5
4	11105406	c.500G>A	p.(Cys167Tyr)	Likely pathogenic	rs879254548	Uncertain significance	1

4	11105436	c.530C>T	p.(Ser177Leu)	Pathogenic/Likely pathogenic	rs121908026	Pathogenic	3
4	11105495	c.589T>C	p.(Cys197Arg)	Likely pathogenic	rs730882085	Pathogenic	1
4	11105560	c.654_682del	p.(Pro220Lysfs*10)	N/A	N/A	Pathogenic	2
4	11105567	c.661G>T	p.(Asp221Tyr)	Pathogenic/Likely pathogenic	rs875989906	Likely pathogenic	1
4	11105573	c.667_680dup	p.(Asp227Glufs*43)	N/A	N/A	Pathogenic	1
4	11105576	c.670_682dup	p.(Glu228Glyfs*4)	N/A	N/A	Pathogenic	2
4	11105579	c.673_681dup	p.(Lys225_Asp227dup)	Likely pathogenic	rs1555803425	Likely pathogenic	4
4	11105588	c.682G>A	p.(Glu228Lys)	Pathogenic/Likely pathogenic	rs121908029	Pathogenic	3
5	11106666	c.796G>A	p.(Asp266Asn)	Pathogenic/Likely pathogenic	rs875989907	Likely pathogenic	1
6	11107461	c.888G>A	p.(Cys296*)	Pathogenic	rs879254708	Pathogenic	5
6	11107439	c.865T>C	p.(Cys289Arg)	N/A	N/A	Uncertain significance	1
6	11107513	c.939C>A	p.(Cys313del)	Pathogenic	rs13306512	Pathogenic	1
7	11110685	c.974G>A	p.(Cys325Tyr)	Likely pathogenic	rs879254746	Uncertain significance	1
7	1110696	c.985T>G	p.(Cys329Glu)	Pathogenic/Likely pathogenic	N/A	Pathogenic	2
7	11110723	c.1012T>A	p.(Cys338Ser)	Pathogenic/Likely pathogenic	rs879254753	Pathogenic	18
7	11110766	c.1055G>A	p.(Cys352Tyr)	Pathogenic/Likely pathogenic	rs193922566	Likely pathogenic	1
8	11111515	c.1062dup	p.(Ile355Tyrfs*3)	Pathogenic	rs879254775	Pathogenic	1
8	11111519	c.1066G>C	p.(Asp356His)	Conflicting interpretations of pathogenicity	rs767767730	Uncertain significance	2
8	11111565-11111585	c.1112_1132del	p.(Leu371_Cys377 del)	N/A	N/A	Pathogenic	2
8	11111577	c.1124A>G	p.(Tyr375Cys)	Pathogenic/Likely pathogenic	rs879254800	Likely pathogenic	3
8	11111600	c.1147T>G	p.(Phe383Val)	N/A	N/A	Uncertain significance	4
9	11113298	c.1207T>C	p.(Phe403Leu)	Likely pathogenic	rs879254831	Likely pathogenic	4

9	11113307	c.1216C>T	p.(Arg406Trp)	Pathogenic/Likely pathogenic	rs121908043	Likely pathogenic	3
9	11113343	c.1252G>A	p.(Glu418Lys)	Likely pathogenic	rs869320651	Uncertain significance	1
9	11113356	c.1265T>G	p.(Leu422Arg)	N/A	N/A	Uncertain significance	2
9	11113388	c.1297G>C	p.(Asp433His)	Pathogenic/Likely pathogenic	rs121908036	Pathogenic	9
10	11113571	c.1395T>G	p.(Tyr465*)	N/A	N/A	Pathogenic	1
10	11113645	c.1469G>A	p.(Trp490*)	Pathogenic	rs875989922	Pathogenic	1
10	11113653-11113664	c.1477_1488del	p.(Ser493_Gly496 del)	N/A	N/A	Pathogenic	1
10	11113678	c.1502C>T	p.(Ala501Val)	Conflicting interpretations of pathogenicity	rs755667663	Uncertain significance	1
10	11113743	c.1567G>A	p.(Val523Met)	Pathogenic/Likely pathogenic	rs28942080	Likely pathogenic	1
10	11113763	c.1586+1G>A	Splicing error	Pathogenic/Likely pathogenic	rs755389753	Pathogenic	5
11	11116125	c.1618G>A	p.(Ala540Thr)	Pathogenic/Likely pathogenic	rs769370816	Uncertain significance	1
11	11116209	c.1702C>G	p.(Leu568Val)	Pathogenic/Likely pathogenic	rs746959386	Pathogenic	8
12	11116859	c.1706A>G	p.(Asp569Gly)	N/A	N/A	Pathogenic	1
12	11116883	c.1730G>A	p.(Trp577*)	Pathogenic	rs138947766	Pathogenic	1
12	11116900	c.1747C>T	p.(His583Tyr)	Conflicting interpretations of pathogenicity	rs730882109	Uncertain significance	1
12	11116936	c.1783C>T	p.(Arg595Trp)	Conflicting interpretations of pathogenicity	rs373371572	Likely pathogenic	2
12	11117000	c.1845+2T>C	Splicing error	Pathogenic/Likely pathogenic	rs778408161	Pathogenic	23
13	11120117	c.1871_1873del	p.(Ile624del)	Pathogenic/Likely pathogenic	rs879255062	Likely pathogenic	1
14	11120408	c.2026G>A	p.(Gly676Ser)	Conflicting interpretations of pathogenicity	rs745753810	Uncertain significance	1

14	11120424	c.2042G>C	p.(Cys681Ser)	Likely pathogenic	rs201637900	Uncertain significance	1
14	11120436	c.2054C>T	p.(Pro685Leu)	Pathogenic/Likely pathogenic	rs28942084	Pathogenic	4
14	11120484	c.2102del	p.(Gly701Alafs*8)	N/A	N/A	Pathogenic	2
14	11123172	c.2141-2delA	Splicing error	N/A	N/A	Pathogenic	1
15	11128005	c.2312-3C>A	Splicing error	Pathogenic/Likely pathogenic	rs875989942	Pathogenic	6
16	11128085	c.2389G>A	p.(Val797Met)	Conflicting interpretations of pathogenicity	rs750518671	Likely pathogenic	8
17	11129539	c.2416dup	p.(Val806Glyfs*11)	Conflicting interpretations of pathogenicity	rs773618064	Pathogenic	3
17	11129539	c.2416_2418delinsAGAAG	p.(Val806Argfs*124)	N/A	N/A	Pathogenic	2
17	11129554	c.2431A>T	p.(Lys811*)	Pathogenic	rs879255211	Pathogenic	10
		ex1del		Pathogenic	N/A	Pathogenic	4
		ex2-3del		Pathogenic	N/A	Pathogenic	6
		ex2-6dup		N/A	N/A	Pathogenic	4
		ex5del		Pathogenic	N/A	Pathogenic	1
		ex7-18del		Pathogenic	N/A	Pathogenic	1
		ex9-12del		N/A	N/A	Pathogenic	4
		ex12del		N/A	N/A	Pathogenic	1
		ex13-14del		Pathogenic	N/A	Pathogenic	6
		ex13-14dup		Pathogenic	N/A	Likely pathogenic	1
		ex16-18del		N/A	N/A	Pathogenic	2
		ex17ins		N/A	N/A	Pathogenic	1
		ex17-18del		Pathogenic	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 S2. Included *PCSK9* Gene Variants

Exon No.	Genomic location GRCh38 (Chr1)	Nucleotide change	Effect of protein	ClinVar	rs number	Variant rating according to ACMG	N
1	55039847	c.10G > A	p.(Val4Ile)	Uncertain significance	rs186669805	Benign	19
1	55039931	c.94G > A	p.(Glu32Lys)	Conflicting interpretations of pathogenicity	rs564427867	Pathogenic	20
9	55058630	c.1486C > T	p.(Arg496Trp)	Uncertain significance	rs374603772	Likely pathogenic	3

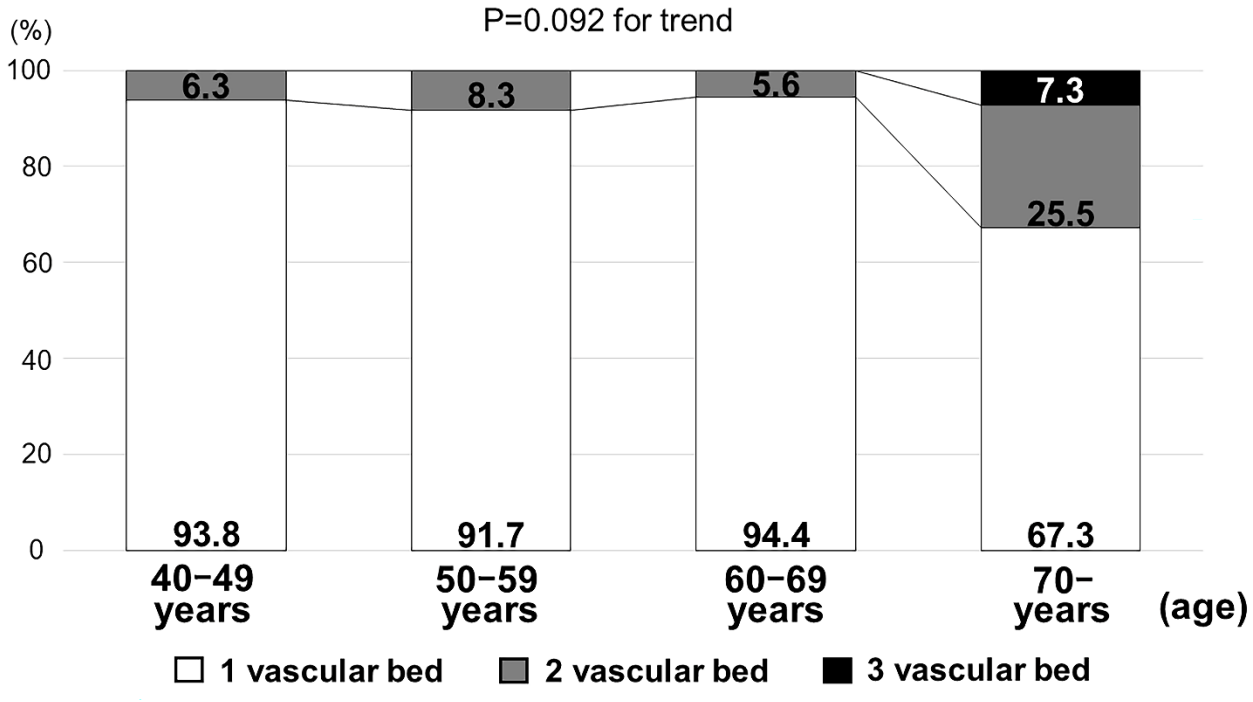
ACMG guideline = American College of Medical Genetics guideline, CADD score = Combined Annotation Dependent Depletion score, N = number, N/A = not applicable *PCSK9* = proprotein convertase subtilisin/kexin type 9.

Table S3. Gene Variants Detected in Patients with LDLR and PCSK9 Gene Variants

LDLR		*PCSK9*		
Nucleotide change	Effect of protein	Nucleotide change	Effect of protein	N
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.888C>A	p.(Cys296*)	c.10G > A	p.(Val4Ile)	1
c.985T>G	p.(Cys329Glu)	c.10G > A	p.(Val4Ile)	2
c.1066G>C	p.(Asp356His)	c.10G > A	p.(Val4Ile)	1
c.1124A>G	p.(Tyr375Cys)	c.10G > A	p.(Val4Ile)	2
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.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

Figure S1. The Prevalence of One, Two and Three ATS in Study Subjects Stratified According to Their Ages (40-49, 50-59, 60-69 and ≥ 70 years)



There was a trend toward a greater frequency of 2 and 3 ATS in those with their age ≥ 70 years (p=0.09 for trend).