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 by a marked elevation of low-density lipoprotein cholesterol (LDL-C) levels.1 This atherogenic substrate more likely causes atherosclerotic disease of the coronary artery (CAD). In addition, recent studies reported that polyvascular disease is a common complication in patients with 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.
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.

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-Hitc, 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).

**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
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) $\geq 50$ 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).

### 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     |

*Tested using analysis of variance. Other comparisons were conducted by Kruskal-Wallis test.

### Table 2. Lipid-Lowering Therapies and Lipid Control

| Lipid-lowering therapy                        | 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    |

*Tested using analysis of variance. Other comparisons were conducted by Kruskal-Wallis test.
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, 1.39–9.00], \( P = 0.008 \)), 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

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**Table 3. Multivariable Analysis of Predictors for PolyVD**

| Predictors                     | 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.
Funabashi et al. Lp(a) and Polyvascular Disease in HeFH

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. 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, 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, and the International Atherosclerosis Society has considered all of these features as important factors to define severe FH. Furthermore, these risk stratification approaches have predicted an elevated

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.
risk of ASCVD including not only CAD but stroke and PAD. 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. 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. 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.). 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.

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Supplemental Material
Tables S1–S3
Figure S1

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| 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  |
| Chrom | Reference | Variation | Description | Genotype | Pathogenicity | rs_ID | Comments |
|-------|-----------|-----------|-------------|----------|--------------|-------|----------|
| 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 | 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 | Pathogenic | 1       |
| 4     | 11105576  | c.670_682dup | p.(Glu228Glyfs*4) | N/A | Pathogenic | 2       |
| 4     | 11105579  | c.673_681dup | p.(Lys225_Asp227dup) | Likely pathogenic | rs155580342 | 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 | 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 | 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 | Uncertain significance | 4       |
| 9     | 11113298  | c.1207T>C  | p.(Phe403Leu) | Likely pathogenic | rs879254831 | Likely pathogenic | 4       |
| Chromosome | Gene Location | Variant Type | Phenotype | Variant ID | Interpretation |
|------------|---------------|--------------|-----------|------------|----------------|
| 9          | 11113307      | c.1216C>T    | p.(Arg406Trp) | Pathogenic/Likely pathogenic | rs121908043 | Likely pathogenic |
| 9          | 11113343      | c.1252G>A    | p.(Glu418Lys) | Likely pathogenic | rs869320651 | Uncertain significance |
| 9          | 11113356      | c.1265T>G    | p.(Leu422Arg) | N/A | N/A | Uncertain significance |
| 9          | 11113388      | c.1297G>C    | p.(Asp433His) | Pathogenic/Likely pathogenic | rs121908036 | Pathogenic |
| 9          | 11113571      | c.1395T>G    | p.(Tyr465*) | N/A | N/A | Pathogenic |
| 10         | 11113364      | c.1469G>A    | p.(Trp490*) | Pathogenic | rs875989922 | Pathogenic |
| 10         | 11113365-11113664 | c.1477_1488del | p.(Ser493_Gly496 del) | N/A | N/A | Pathogenic |
| 10         | 11113678      | c.1502C>T    | p.(Ala501Val) | Conflicting interpretations of pathogenicity | rs755667663 | Uncertain significance |
| 10         | 11113743      | c.1567G>A    | p.(Val523Met) | Pathogenic/Likely pathogenic | rs28942080 | Likely pathogenic |
| 10         | 11113763      | c.1586+1G>A  | Splicing error | Pathogenic/Likely pathogenic | rs755389753 | Pathogenic |
| 11         | 11116125      | c.1618G>A    | p.(Ala540Thr) | Pathogenic/Likely pathogenic | rs769370816 | Uncertain significance |
| 11         | 11116209      | c.1702C>G    | p.(Leu568Val) | Pathogenic/Likely pathogenic | rs746959386 | Pathogenic |
| 12         | 11116859      | c.1706A>G    | p.(Asp569Gly) | N/A | N/A | Pathogenic |
| 12         | 11116900      | c.1747C>T    | p.(His583Tyr) | Conflicting interpretations of pathogenicity | rs730882109 | Uncertain significance |
| 12         | 11116936      | c.1783C>T    | p.(Arg595Trp) | Conflicting interpretations of pathogenicity | rs373371572 | Likely pathogenic |
| 12         | 11117000      | c.1845+2T>C  | Splicing error | Pathogenic/Likely pathogenic | rs778408161 | Pathogenic |
| 13         | 11120117      | c.1871_1873del | p.(Ile624del) | Pathogenic/Likely pathogenic | rs879255062 | Likely pathogenic |
| 14         | 11120408      | c.2026G>A    | p.(Gly676Ser) | Conflicting interpretations of pathogenicity | rs745753810 | Uncertain significance |
| Chrom | Gene   | Variant | Mutation Description | Pathogenicity Description | ClinVar ID | PhENO | Pathogenicity |
|------|--------|---------|----------------------|---------------------------|------------|-------|--------------|
| 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_2418delAGAAG | 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
| 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 Nucleotide change | LDLR Effect of protein | PCSK9 Nucleotide change | PCSK9 Effect of protein | N |
|-----------------------|-----------------------|------------------------|------------------------|---|
| ex 2-6 dup            |                       | c.94G > A              | p.(Glu32Lys)           | 1 |
| c.68-1G>C             | Splicing error        | c.94G > A              | p.(Glu32Lys)           | 1 |
| c.418G>A              | p.(Glu140Lys)         | c.10G > A              | p.(Val4Ile)            | 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)            | 1 |
| c.1147T>G             | p.(Phe383Val)         | c.10G > A              | p.(Val4Ile)            | 2 |
| 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*
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).