



# Aortic Root Calcification Score as an Independent Factor for Predicting Major Adverse Cardiac Events in Familial Hypercholesterolemia

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**Aim:** The aims of this study were: 1) to determine whether the accumulation of aortic root calcification (ARC) assessed using coronary computed tomography angiography (CCTA) can predict future cardiovascular events, and 2) to estimate the onset and progression of ARC in patients with familial hypercholesterolemia (FH).

**Methods:** One hundred thirteen consecutive Japanese patients with heterozygous FH (male=54, mean age=52.1±15.6 years, mean LDL-C=299.0±94.6 mg/dL), without known coronary artery disease, who underwent 64-detector row CCTA were retrospectively evaluated. ARC was defined as the presence of calcium at the aortic root. The extent of ARC was expressed in Agatston units as the ARC-score. Major adverse cardiac events (MACE) were defined as either cardiac death, ST elevated myocardial infarction (STEMI), non-ST elevated myocardial infarction (NSTEMI), unstable angina pectoris (UAP), planned percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), or stroke. The periods to MACE were estimated using multivariate logistic regression analysis.

**Results:** During the follow-up period (median 1635 days), 19 instances of MACE occurred. Multivariate logistic regression analysis revealed that ARC was a significant independent predictor of MACE (OR=1.48; 95% CI 1.11–1.87,  $p<0.001$ , respectively). The regression equations were  $Y=0.09X-1.59$  ( $R^2=0.34$ ,  $p<0.001$ ) in males and  $Y=0.08X-1.60$  ( $R^2=0.13$ ,  $p<0.05$ ) in females.

**Conclusions:** ARC was significantly associated with future MACE in Japanese patients with heterozygous FH. ARC may start to develop, on average, at 17.4 and 19.7 years of age in males and females, respectively, with heterozygous FH.

**Key words:** Familial hypercholesterolemia, Coronary computed tomography angiography, Aortic valve calcification

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## Introduction

Familial hypercholesterolemia (FH; OMIM #143890) is characterized by the triad of (1) primary hyper-LDL-cholesterolemia, (2) tendon xanthomas, and

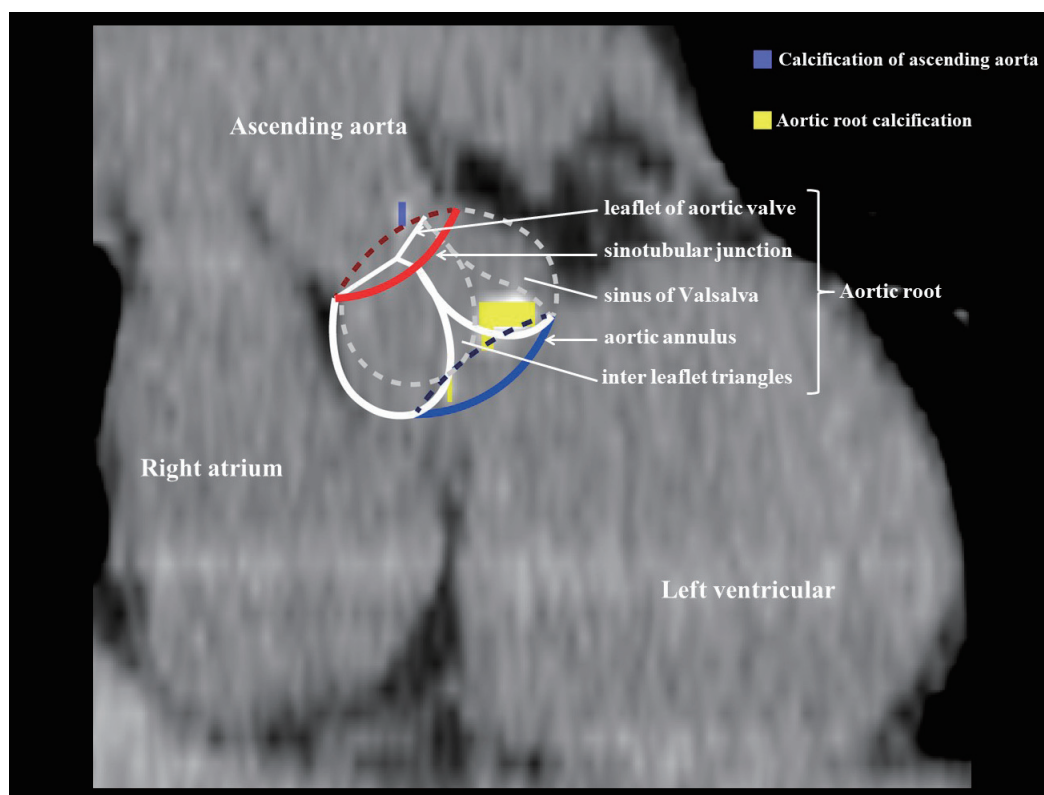
(3) premature coronary artery disease (CAD)<sup>1,2</sup>. Coronary computed tomography angiography (CCTA), a noninvasive imaging modality, is quite useful for the accurate detection and exclusion of CAD in the general population<sup>3-5</sup>. We demonstrated the prognostic utility of coronary plaque burden assessed by CCTA in patients with FH<sup>6</sup>. On the other hand, it has been shown that the calcium burden of the aortic valve is larger in patients with FH compared to the general population<sup>7</sup>. CCTA can assess the degree of the calcium burden in the aortic valve, which could be associated with cardiovascular events<sup>8,9</sup>. In addition, pati-

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**Fig. 1.** Measurement of aortic root calcification (ARC) score. The purple block indicates calcification of the ascending aorta. The yellow block indicates ARC. The red line indicates the sinotubular junction. The blue line indicates the aortic annulus.

ents with FH displayed an increased burden of ascending aorta atherosclerosis in comparison with patients with hypercholesterolemia without a gene mutation<sup>10</sup>. Moreover, CCTA could help us to estimate when and how rapidly aortic root calcification (ARC) develops in patients with FH. Here we aimed to test 2 hypotheses in our FH cohort without known CAD: (1) ARC assessed by CCTA is associated with future coronary events beyond established risk factors in patients with FH; and (2) we can estimate the onset and progression of ARC in patients with FH assuming a linear model of plaque progression.

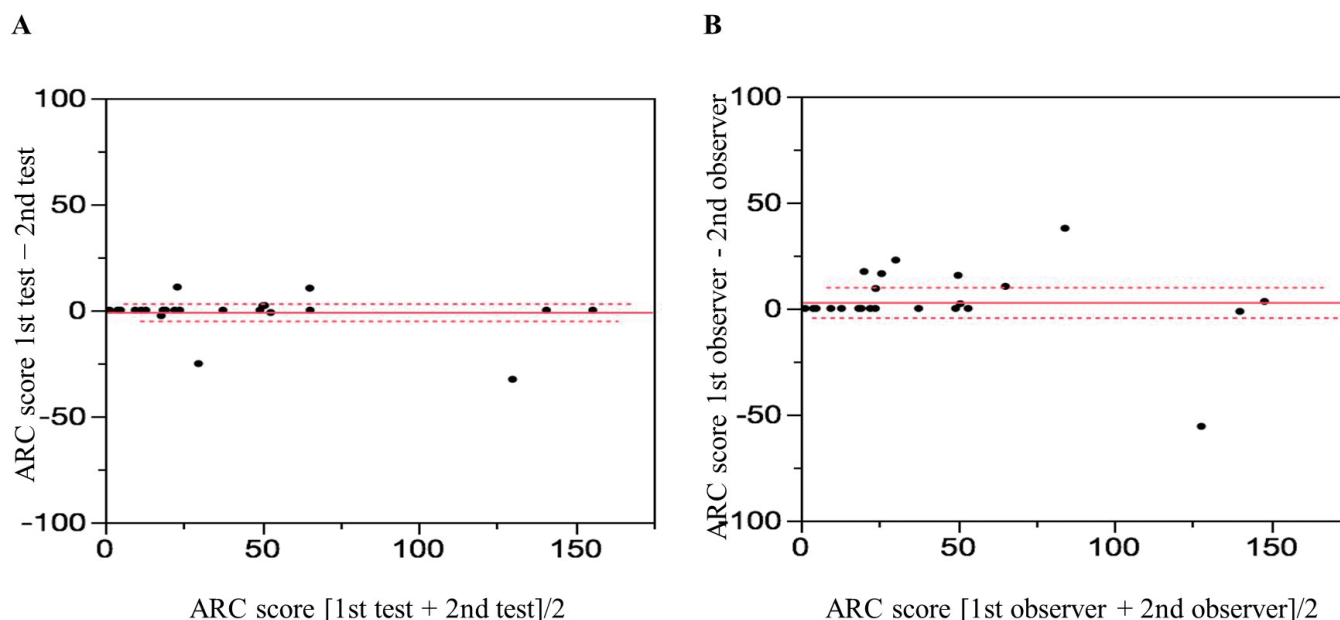
## Methods

The institutional review board approved the study protocol. All patients gave written informed consent.

A total of 134 consecutive patients with FH without known CAD who underwent 64-detector row CCTA between May 2007 and May 2017 due to any clinical indications, including chest symptoms, signs of cardiac diseases, peripheral artery disease, cerebrovascular disease, or multiple coronary risk factors were retrospectively analyzed. We used the diagnostic criteria

for FH by the Japan Atherosclerosis Society<sup>11</sup>. We excluded patients with a known history of coronary disease. We defined major adverse cardiac events (MACE) as either cardiac death, ST elevated myocardial infarction (STEMI), non-ST elevated myocardial infarction (NSTEMI), unstable angina pectoris (UAP), planned percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), or stroke.

Hypertension was defined as systolic blood pressure of at least 140 mmHg, diastolic blood pressure of at least 90 mmHg, or use of antihypertensive medication. The presence of diabetes was defined as previously described by the Japan Diabetes Society<sup>12</sup> or the use of diabetes medication. Body mass index (BMI) was defined as body weight in kilograms divided by the square of height measured in meters. Serum concentrations of total cholesterol, triglyceride, and HDL-C were determined enzymatically while the patients were not given any lipid lowering drugs as their baseline level. The patients who received some type of a lipid lowering drug had the same examination after administration of statin therapy. LDL-C concentrations were calculated using the Friedewald formula. Most of the patients were assessed simultaneously with CCTA,



**Fig. 2.** Bland-Altman analysis for the measurement of ARC score

Bland-Altman analysis demonstrated good agreement between intra-observer (a) and inter-observer (b) measurements.

while some of the subjects were assessed a few months before the assessment with CCTA.

CCTA was performed with a dual source 64-slice system (Somatom Definition Flash; Siemens Medical System, Erlangen, Germany). The detailed scan protocol has been described previously<sup>6</sup>.

Two experienced radiologists, blinded with regards to the clinical status, evaluated all CCTA scans separately. Lesions were classified as ARC if they were located within the aortic root, inclusive of the aortic annulus or coronary artery ostia, and contained  $\geq 3$  contiguous pixels with an attenuation value  $> 130$  HU (**Fig. 1**)<sup>7,13</sup>. The ARC score was defined as the quantity of ARC expressed in Agatston units referring to the same definition as for coronary artery calcification, using dedicated software (SYNAPSE VINCENT; Fuji Film Medical, Tokyo, Japan). We referred to contrast-enhanced scans when the exact location of calcified lesions was unclear.

Categorical variables are expressed as percentages. The Fisher exact test or chi-square test was used as appropriate. Continuous variables with a normal distribution are reported as mean ( $\pm$  SD), and were compared using unpaired Student's *t*-tests. Kaplan-Meier curves were compared between the MACE of patients with ARC and those without ARC with the log-rank test. We initially analyzed all available risk factors using a univariate model, then multivariate logistic regression analysis was performed using only the covariates that were significantly associated with MACE in the

univariate analysis. Intraobserver/interobserver variability between readers was assessed using the Bland-Altman method and the coefficient of variation (CV) for 30 randomly selected patients. Most statistical analyses were conducted using JMP® 13 (SAS Institute Inc., Cary, NC, USA) except for receiver-operating characteristic (ROC) curve analysis, which was performed using R version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria, <http://www.R-project.org/>); *p* values  $< 0.05$  were considered statistically significant.

## Results

Intra- and interobserver reproducibility for measurements of the ARC score are shown in **Fig. 2**. Bland-Altman analysis demonstrated good agreement, with an intra-observer CV of 13.2% (**Fig. 2a**) and inter-observer CV of 19.9% (**Fig. 2b**).

One hundred and thirteen patients with heterozygous FH, whose ages ranged from 13 to 84 years, were included in this analysis (male=54, mean age=  $52.1 \pm 15.6$  years, mean LDL-C=  $299.0 \pm 94.6$  mg/dL). The median follow-up period was 1635 days. The clinical characteristics of patients with or without subsequent MACE are shown in **Table 1**. The frequencies of the traditional coronary risk factors, such as age, hypertension, and diabetes mellitus were significantly higher, while HDL-C and post-treatment LDL-C were significantly lower, in patients with FH that developed

**Table 1.** Baseline characteristics

	All Subjects ( <i>n</i> = 113)	MACE (+) ( <i>n</i> = 19)	MACE (-) ( <i>n</i> = 94)	<i>p</i> value
Male	54 (47.8%)	7 (36.8%)	47 (50.0%)	n.s.
Age, yrs	52.1 ± 15.6	61.2 ± 14.2	50.3 ± 15.2	< 0.05
Body mass index, kg/m <sup>2</sup>	23.6 ± 3.2	24.1 ± 3.8	23.5 ± 3.1	n.s.
Smoking (Current/former)	32 (30.5%)	8 (42.1%)	24 (27.9%)	n.s.
Hypertension	49 (43.4%)	13 (68.4%)	36 (38.3%)	< 0.05
Diabetes mellitus	26 (23.2%)	9 (47.4%)	17 (18.3%)	< 0.05
HbA1c, %	5.9 ± 1.0	6.7 ± 1.8	5.8 ± 0.7	< 0.001
Defined mutation	78 (69.0%)	15 (78.9%)	63 (67.0%)	n.s.
Lipids				
Total cholesterol, mg/dl	385.2 ± 99.1	389.9 ± 104.2	384.2 ± 98.7	n.s.
LDL-C, mg/dl	299.0 ± 94.6	309.7 ± 113.4	296.7 ± 90.9	n.s.
HDL-C, mg/dl	54.2 ± 13.6	49.0 ± 12.8	55.3 ± 13.6	n.s.
Triglyceride, mg/dl	147.5 ± 93.0	169.3 ± 85.9	142.9 ± 94.4	n.s.
Lp(a), mg/dl	34.9 ± 36.0	34.0 ± 20.2	35.1 ± 38.5	n.s.
Post-treatment LDL-C, mg/dl	140.7 ± 46.4	109.2 ± 28.5	148.2 ± 46.8	< 0.05
Percent reduction of LDL-C, %	52.3 ± 14.6	61.5 ± 16.3	50.1 ± 13.3	< 0.05
Statin use	107 (94.7%)	18 (94.7%)	89 (94.7%)	n.s.
Ezetimibe use	51 (45.1%)	13 (68.4%)	38 (40.4%)	n.s.
Cholestimide use	19 (16.8%)	5 (26.3%)	14 (14.9%)	n.s.
ARC positive	67 (59.3%)	16 (84.2%)	51 (54.3%)	< 0.05
ARC score log	2.9 ± 2.7	5.0 ± 2.4	2.4 ± 2.6	< 0.001

MACE: major adverse cardiac events, ARC: aortic root calcification

**Table 2.** Factors associated with major adverse cardiac events

	patient (s)
Cardiac death	0
ST elevated myocardial infarction	0
Non-ST elevated myocardial infarction/unstable angina pectoris	4
Planned percutaneous coronary intervention/coronary artery bypass grafting	11
Congestive heart failure	3
Stroke	1

MACE compared to those without MACE. Interestingly, the extent of ARC was significantly greater in patients with FH with MACE than those without MACE. **Table 2** shows the MACE that occurred during the follow-up period. **Supplementary Table 1** shows the clinical characteristics of patients with or without ARC. We found that the patients who were ARC positive were older, had a higher BMI, and more likely to have hypertension and diabetes than expected. In addition, when we divided the patients based on the ARC score higher or lower than the threshold value for MACE, the patients with high ARC scores were older and much more likely to have hypertension (**Supplementary Table 2**).

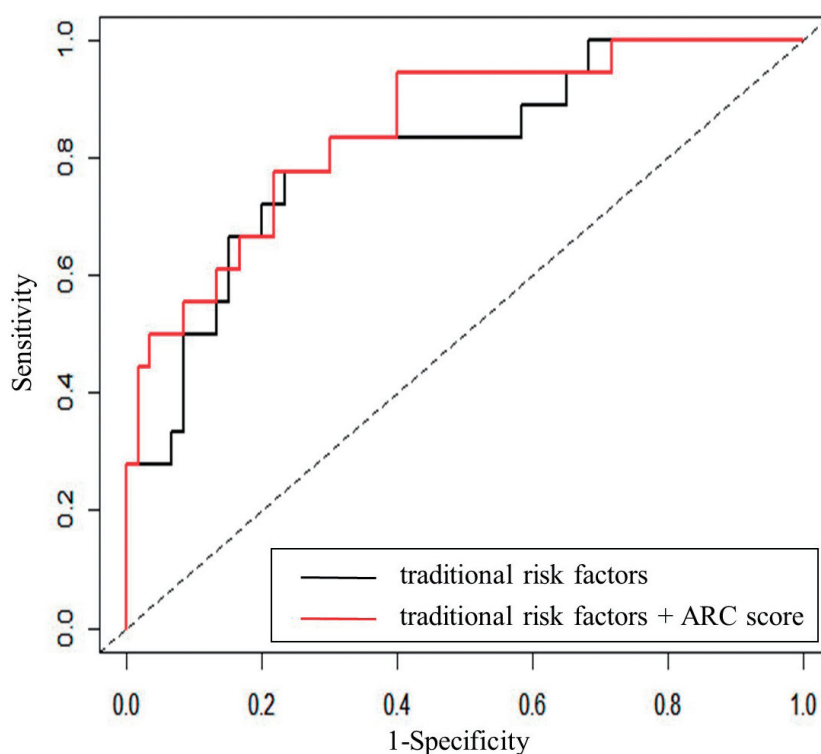
Univariable logistic regression analysis showed that age, hypertension, diabetes mellitus, post-treatment LDL-C, and ARC score were significantly associated with MACE (**Table 3**). In the multivariable logistic regression analysis, the ARC score remained significantly associated with MACE.

We investigated whether the addition of the ARC score increased the accuracy of risk discrimination beyond established traditional risk factors, including age, sex, BMI, hypertension, diabetes, smoking, and post-treatment LDL-C. The C-statistic increased by adding the ARC score to the traditional risk factors from 0.811 to 0.852, although it did not reach statistical significance (**Fig. 3**, *p* = n.s.). Kaplan-Meier curves

**Table 3.** Major adverse cardiac events during follow-up period

	univariable			multivariable		
	odds ratio	95% CI	<i>p</i> value	odds ratio	95% CI	<i>p</i> value
Male	1.714	0.633-4.964	0.292			
Age	1.053	1.016-1.096	0.004	1.018	0.958-1.082	0.557
BMI	1.054	0.902-1.227	0.502			
Smoking	1.879	0.656-5.225	0.234			
Hypertension	3.491	1.261-10.702	0.016	1.325	0.288-6.433	0.717
Diabetes mellitus	4.024	1.405-11.559	0.010	1.727	0.450-6.369	0.418
Total cholesterol	1.001	0.995-1.006	0.836			
LDL-C	1.001	0.997-1.008	0.389			
Post-treatment LDL-C	0.965	0.942-0.984	<0.001	0.969	0.943-0.990	0.003
ARC score	1.500	1.210-1.944	<0.001	1.408	1.110-1.869	0.029

ARC: aortic root calcification



**Fig. 3.** Receiver-operating characteristic (ROC) curves of established risk factors and ARC score in predicting MACE.

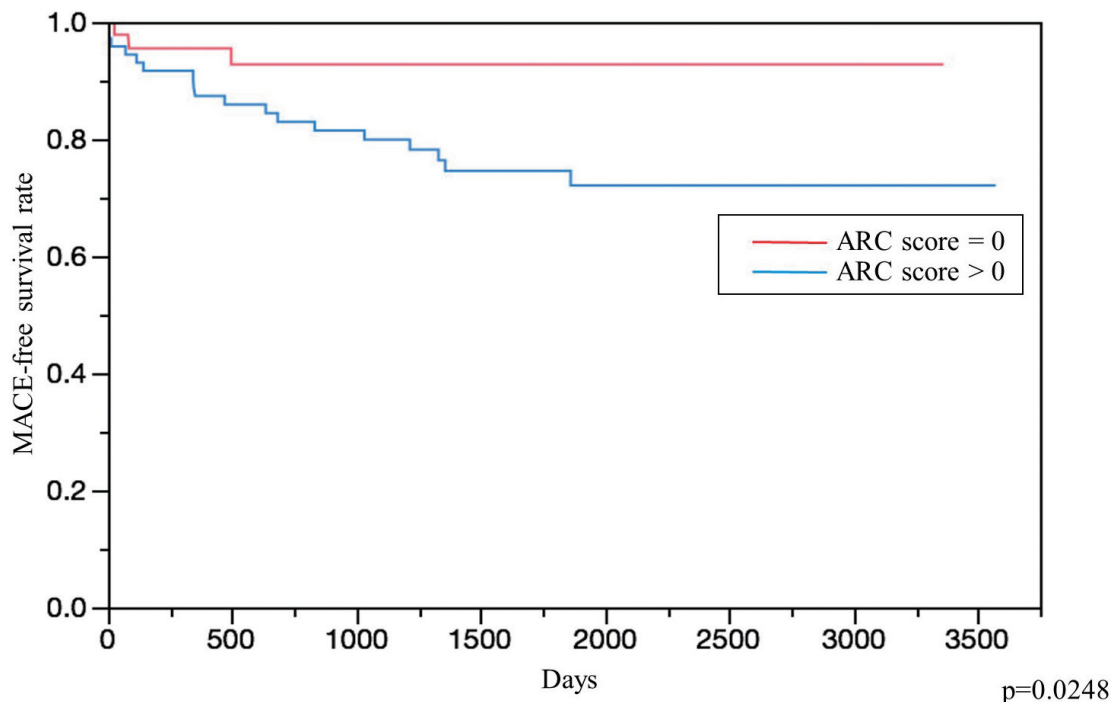
The black line indicates traditional risk factors, including age, sex, BMI, hypertension, diabetes, posttreatment levels of LDL-C, and smoking.

The red line indicates traditional risk factors and ARC score.

revealed that patients with any ARC had a significantly higher event rate than those without ARC (**Fig. 4**).

Finally, we evaluated the correlation coefficient between age and ARC score for each sex (**Fig. 5**), because a previous study demonstrated that the onset of coro-

nary disease was significantly earlier in males with FH than in females<sup>6</sup>. The regression equations were  $Y = 0.09X - 1.59$  ( $R^2 = 0.34$ ,  $p < 0.001$ ) in males and  $Y = 0.08X - 1.60$  ( $R^2 = 0.13$ ,  $p < 0.05$ ) in females with heterozygous FH. These results suggest that ARC may



**Fig. 4.** Kaplan-Meier event curves for MACE.

The red line indicates patients whose ARC score=0.  
The blue line indicates patients whose ARC score > 0.

start to develop, on average, at 17.4 and 19.7 years of age in male and female patients with heterozygous FH.

## Discussion

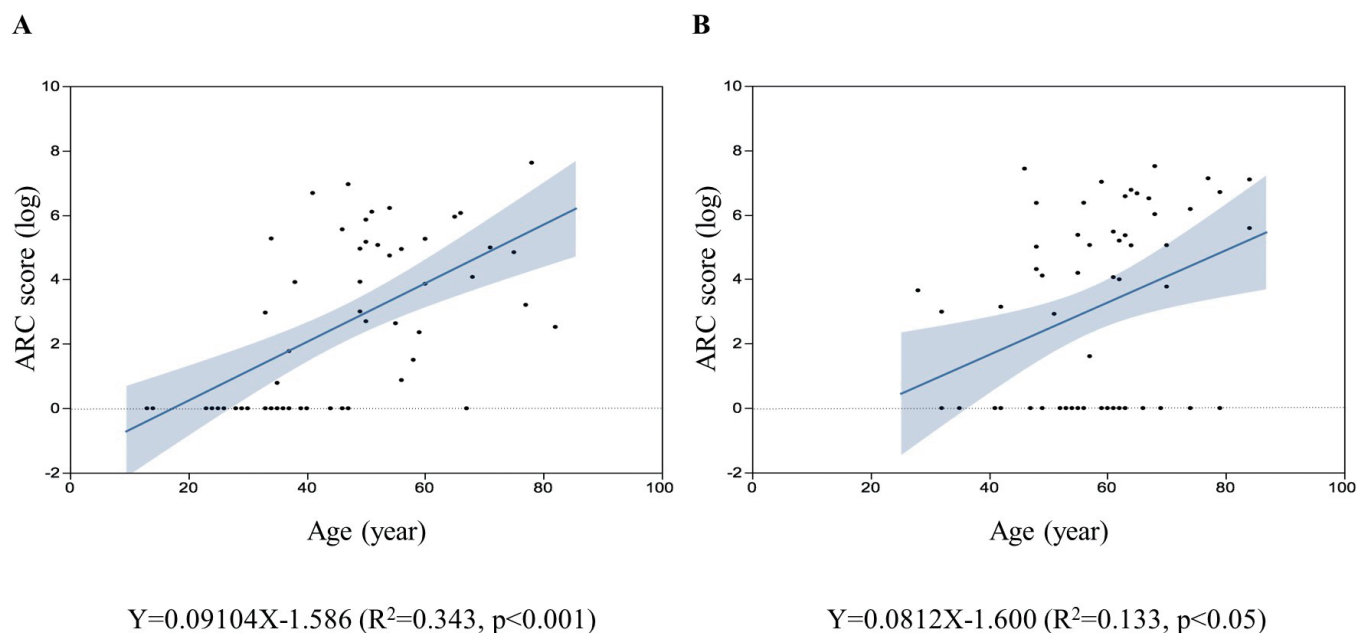
In this study, we evaluated ARC assessed with CCTA among patients with FH and found that the extent of ARC was associated with future coronary or cardiovascular events beyond established risk factors, and that we can estimate the onset and progression of ARC in patients with FH, assuming a linear model of progression.

The patients with FH developed premature coronary atherosclerosis due to extremely high LDL-C levels, thus their risk of future coronary events needs to be assessed, since their lifetime risk is still diverse<sup>14</sup>. In the current study, ARC assessed with CCTA successfully estimated future MACE in this high-risk population. There are several reports investigating the significance of aortic calcification in patients with FH<sup>15-17</sup>; however, few studies exist concerning the association between aortic calcification and cardiovascular events in patients with FH. Our study adds evidence that the extent of ARC is significantly associated with cardiovascular events in patients with FH. We speculate the cause of the strong association between ARC and MACE is the fact that ARC can reflect different risk factors,

including unknown ones.

In our previous report, we found that the coronary plaque burden might start to develop at 23 and 34 years of age in male and female patients with heterozygous FH, respectively<sup>6</sup>. In this study, we could estimate the onset and progression of ARC in patients with FH assuming a linear model of plaque progression. The regression lines from age and ARC suggested that ARC might start to develop in the teenage years in both genders, even under statin therapy. In addition, we showed that ARC might start to develop much earlier than coronary plaques in this high-risk population. ARC can be assessed less invasively without using any contrast agents, compared to coronary plaque burden. Accordingly, we suggest the assessment of ARC prior to coronary plaque burden at this younger age.

The pathologic mechanism of aortic calcification is not understood completely in heterozygous FH. In addition, mechanistic insight into the development of aortic calcification, which is earlier than that of coronary plaque formation is still unclear in this study. Aortic calcifications are thought to be due to a complex interplay between inflammation, vascular injury, and osteogenesis<sup>18</sup>. On the other hand, there are some reports that statins might lead to an increase in coronary calcium score and contribute to vascular calcifications, although this remains controversial<sup>19-21</sup>. In this



**Fig. 5.** Plots of the correlation between age (X) and aortic root calcification score (Y) in male (a) and female (b) patients with FH. The regression equations are  $Y=0.09X-1.59$  ( $R^2=0.34$ ,  $p<0.05$ ) in males and  $Y=0.08X-1.60$  ( $R^2=0.13$ ,  $p<0.05$ ) in females with heterozygote FH. The solid lines indicate the regression lines. The dotted lines indicate the 95% confidence interval.

To quantify the ARC, as well as the coronary calcium score, CCTA without contrast was performed. Calcification was attributed to the aortic root if it was clearly part of the sinuses of the valsalva, valve cusps, aortic annulus, and the sinotubular junction. Calcifications above the sinotubular junction and calcifications of coronary arteries other than the ostia were removed by manual segmentation.

study, multivariate analysis suggested that the post-treatment levels of LDL-C were inversely correlated with MACE. Accordingly, lowering LDL-C aggressively could be an effective way to manage such high-risk patients. Further studies, investigating the effects of lipid-lowering agents on the development of calcification as well as plaque formation in patients with/without FH will give us insights into those points.

This study has several limitations. First, this study was conducted retrospectively from a single center using a relatively small sample size. Second, selection bias exists regarding the indication of CCTA, leading to an attenuation of the estimated age of the development of atherosclerosis. Third, our assumption concerning the development of ARC in FH is based on a linear model that may not be applicable to younger patients with FH. The significance of ARC assessed with CCTA may be high, although future prospective, multi-center studies are necessary to confirm the present results.

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### Conflicts of Interest

Hayato Tada has received research grants from Takeda Science Foundation, Mochida Memorial Foundation, Japan Research Promotion Society for Cardiovascular Diseases, Sanofi K.K., and Astellas Foundation for Research on Metabolic Disorders. Kenshi Hayashi has received research grants from Takeda Science Foundation and Mitsubishi Tanabe Pharma. Atsushi Nohara and Hiroshi Mabuchi have received research grants from MSD K.K., Sanofi K.K., Shionogi & Co., Ltd., Kowa Co., Ltd., Astellas Pharma Inc., AstraZeneca K.K., Keiai-Kai Medical Corp., and Biopharm of Japan Co. Masakazu Yamagishi has received lecture fees from Astellas Pharma Inc., Daiichi-Sankyo Co., Ltd., Shionogi & Co., Ltd., and Kowa Co., Ltd. Masaki Kawashiri has received lecture fees from Amgen Astellas Biopharma K.K., Astellas Pharma Inc., and Sanofi K.K.

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**Supplementary Table 1.** Characteristics divided by ARCS positive or negative

	All Subjects ( <i>n</i> = 113)	ARCS positive ( <i>n</i> = 67)	ARCS negative ( <i>n</i> = 46)	<i>p</i> value
Male	54 (47.8%)	33 (49.3%)	21 (45.7%)	n.s.
Age, yrs	52.1 ± 15.6	57.3 ± 13.1	44.5 ± 15.8	< 0.001
Body mass index, kg/m <sup>2</sup>	23.6 ± 3.2	24.2 ± 3.5	22.7 ± 2.5	< 0.05
Smoking (Current/former)	32 (30.5%)	23 (34.3%)	9 (19.6%)	n.s.
Hypertension	49 (43.4%)	35 (52.2%)	14 (30.4%)	< 0.05
Diabetes mellitus	26 (23.2%)	20 (29.9%)	6 (13.0%)	< 0.05
HbA1c, %	5.9 ± 1.0	6.1 ± 1.2	5.7 ± 0.7	< 0.05
Defined mutation	78 (69.0%)	51 (76.1%)	27 (58.7%)	< 0.05
Lipids				
Total cholesterol, mg/dl	385.2 ± 99.1	405.2 ± 112.9	358.4 ± 66.3	< 0.05
LDL-C, mg/dl	299.0 ± 94.6	320.6 ± 105.2	270.9 ± 68.8	< 0.05
HDL-C, mg/dl	54.2 ± 13.6	51.6 ± 14.8	57.4 ± 11.4	< 0.05
TG, mg/dl	147.5 ± 93.0	144.4 ± 95.8	150.4 ± 90.1	n.s.
Lp(a), mg/dl	34.9 ± 36.0	36.9 ± 30.0	32.5 ± 42.6	n.s.
Post-treatment levels of LDL-C, mg/dl	140.7 ± 46.4	136.0 ± 42.9	149.1 ± 51.9	n.s.
Mean percent reduction of LDL-C, %	52.3 ± 14.6	55.6 ± 13.8	46.4 ± 14.1	< 0.05
Statin use	107 (94.7%)	66 (98.5%)	41 (89.1%)	n.s.
Ezetimibe use	51 (45.1%)	39 (58.2%)	12 (26.1%)	n.s.
Cholestimide use	19 (16.8%)	14 (20.1%)	5 (10.9%)	n.s.

ARCS: aortic root calcification score

**Supplementary Table 2.** Characteristics divided by ARCS high or low

	All Subjects ( <i>n</i> = 113)	ARCS ≥ 4.74 ( <i>n</i> = 41)	ARCS < 4.74 ( <i>n</i> = 72)	<i>p</i> value
Male	54 (47.8%)	18 (43.93%)	36 (50.0%)	n.s.
Age, yrs	52.1 ± 15.6	60.5 ± 11.8	47.3 ± 15.5	< 0.001
Body mass index, kg/m <sup>2</sup>	23.6 ± 3.2	24.1 ± 3.3	23.3 ± 3.2	n.s.
Smoking (Current/former)	32 (30.5%)	13 (31.7%)	19 (26.4%)	n.s.
Hypertension	49 (43.4%)	23 (56.1%)	26 (36.1%)	< 0.05
Diabetes mellitus	26 (23.2%)	13 (31.7%)	13 (18.3%)	n.s.
HbA1c, %	5.9 ± 1.0	6.1 ± 1.4	5.8 ± 0.8	n.s.
Defined mutation	78 (69.0%)	32 (78.0%)	46 (63.9%)	n.s.
Lipids				
Total cholesterol, mg/dl	385.2 ± 99.1	403.4 ± 113.3	374.5 ± 87.4	n.s.
LDL-C, mg/dl	299.0 ± 94.6	323.9 ± 118.1	284.3 ± 74.0	n.s.
HDL-C, mg/dl	54.2 ± 13.6	50.7 ± 16.6	56.2 ± 11.4	n.s.
TG, mg/dl	147.5 ± 93.0	143.3 ± 80.4	149.3 ± 100.1	n.s.
Lp(a), mg/dl	34.9 ± 36.0	41.4 ± 33.2	31.2 ± 37.3	n.s.
Post-treatment levels of LDL-C, mg/dl	140.7 ± 46.4	135.7 ± 45.7	143.9 ± 47.0	n.s.
Mean percent reduction of LDL-C, %	52.3 ± 14.6	55.3 ± 15.3	50.2 ± 13.8	n.s.
Statin use	107 (94.7%)	40 (97.6%)	67 (93.1%)	n.s.
Ezetimibe use	51 (45.1%)	23 (56.1%)	28 (38.9%)	< 0.05
Cholebine use	19 (16.8%)	10 (24.4%)	9 (12.5%)	n.s.

ARCS: aortic root calcification score

ARCS = 4.74 was determined by ROC analysis predicting MACE