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**Assessment of Coronary Atherosclerosis in Patients with Familial Hypercholesterolemia by  
Coronary Computed Tomography Angiography**

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## Abstract

The aims of this study were 1) to determine whether the accumulation of coronary plaque burden assessed with coronary computed tomography angiography (CCTA) can predict future events, and 2) to estimate the onset and progression of coronary atherosclerosis in patients with familial hypercholesterolemia (FH). Consecutive 101 Japanese heterozygous FH patients (male=52, mean age=56±16 years, mean LDL-C=264±58 mg/dL) who underwent 64-detector row CCTA without known coronary artery disease were retrospectively evaluated by assigning a score (0-5) to each of 17 coronary artery segments according to the Society of Cardiovascular Computed Tomography guidelines. Those scores were summed and subsequently natural log-transformed. The periods to major adverse cardiac events (MACE) were estimated using multivariable Cox proportional hazards models. During the follow-up period (median 941 days), 21 MACE had occurred. Receiver-operating characteristic curve analyses identified a plaque burden score of 3.35 (raw score 28.5) as the optimal cutoff for predicting a worse prognosis. Multivariate Cox regression analysis identified the presence of a plaque score  $\geq 3.35$  as a significant independent predictor of MACE (HR=3.65; 95% CI 1.32 to 25.84,  $p<0.05$ ). The regression equations were  $Y=0.68X-15.6$  ( $r=0.54$ ,  $p<0.05$ ) in male and  $Y=0.74X-24.8$  ( $r=0.69$ ,  $p<0.05$ ) in female heterozygous FH patients. In conclusion, coronary plaque burden identified in a noninvasive, quantitative manner was significantly associated with future coronary events in Japanese patients with heterozygous FH and that coronary atherosclerosis may start to develop, on average, at 23 and 34 years of age in male and female heterozygous FH patients, respectively.

**Keywords:** Familial hypercholesterolemia, Coronary computed tomography angiography, plaque burden score

## **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 that progressed during the last decade, permits accurate detection and exclusion of CAD.<sup>3-5</sup> In addition, the prognostic utility of CCTA for the general population has been clearly demonstrated by a number of prior studies.<sup>6,7</sup> However, few data exists regarding the clinical prognostic performance of CCTA in FH. Moreover, such modality could help us to estimate when and how rapidly coronary atherosclerosis in FH patients develop.<sup>8</sup> Here, we build on these observations to test 2 hypotheses: (1) plaque burden assessed by CCTA are associated with future coronary events beyond established risk factors in FH patients; and (2) we can estimate the onset and progression of coronary atherosclerosis in FH patients assuming linear model of plaque progression. We tested these hypotheses in our mutation determined heterozygous FH cohort without known CAD.

## **Methods**

A total of 104 consecutive FH patients without known CAD exhibiting a single mutation in the LDL receptor or proprotein convertase subtilisin/kexin type 9 (PCSK9) gene who underwent 64-detector row CCTA between January 2008 and December 2012 due to any clinical indications, including chest symptom, signs of cardiac diseases, peripheral artery disease, cerebrovascular disease, or multiple coronary risk factors were retrospectively analyzed. Among a total of 104 heterozygous FH patients, 3 subjects with poor images (2.9%) were excluded, thus, 101 subject whose ages range from 22 to 84 years with heterozygous FH patients remained in this analysis

(male = 52, mean age = 56±16 years, mean LDL-C = 264±58 mg/dL). The characteristics of the study subjects are listed in **Table 1** and **Supplementary Table 1**. Median follow-up period was 941 days. 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), staged percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG).

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. Presence of diabetes was defined as previously described by Japan Diabetes Society,<sup>9</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. LDL-C concentrations were calculated using the Friedewald formula.<sup>10</sup> Genomic DNA was isolated from peripheral blood white blood cells according to standard procedures and was used for PCR. Genotypes of all the participants in this study were determined as previously described.<sup>11-13</sup> The institutional review board approved the study protocol. All patients gave written informed consent.

CCTA was performed with a dual source 64-slice (Somatom Definition Flash; Siemens Medical System, Erlangen, Germany). A noncontrast-enhanced scan was performed to assess coronary calcium and defined the anatomical range for subsequent CCTA. This scan was automatically triggered and performed using the following scan parameters: collimation, 0.6 mm; gantry rotation time, 280 ms; tube voltage, 120 kV; and tube current, 100 mA. For the contrast-enhanced scan, collimation was 0.6 mm and gantry rotation time was 280 ms. The tube voltage and current were 120 kV and 340 mA, respectively. Fifty to 80 mL of nonionic iodinated contrast (370 mg iodine/mL,

Iopamidol-370, Bayer Healthcare Pharmaceuticals, Osaka, Japan) was injected using a dual-flow injector (Dual Shot GX, Nemoto Kyorindo, Tokyo, Japan) via an antecubital vein. The iodine load was based on body weight. Image acquisition was manually triggered upon arrival of contrast in the left main coronary trunk. Patients with a heart rate greater than 100 beats per minute and with no contraindications to beta-blockers received intravenous beta-blocker therapy (Landiolol Hydrochloride 0.125 mg/kg) just before the computed tomographic scan. Multiple phases were employed to assess the images of different arteries. In addition, we constructed three-dimensional rotation images to assess the diagonal and other small branches.

Two experienced radiologists, blinded with regard to the clinical status, evaluated all CCTA scans separately. In spite of our efforts, the segments that were uninterpretable were scored as the same as most proximal segment which was interpretable (72 segments among total of 1717 segments: 4.2%). Discrepancies in evaluation were resolved during a consensus reading. Angiographic analysis by coronary computed tomography was performed according to a 17-segment American Heart Association classification.<sup>14</sup> Coronary plaque burden was assessed by assigning a score (0 to 5) to each of 17 coronary artery segments according to the Society of Cardiovascular Computed Tomography (SCCT) guidelines (0 Normal: absence of plaque and no luminal stenosis; 1 Minimal: plaque with < 25% stenosis; 2 Mild: 25%–49% stenosis; 3 Moderate: 50%–69% stenosis; 4 Severe: 70%–99% stenosis; 5 Occluded).<sup>15</sup> Those scores were summed and natural log-transformed, due to its skewed distribution.

Categorical variables were expressed as percentages. The Fisher exact test or chi-square test was used as appropriate. Continuous variables with a normal distribution were shown as mean ( $\pm$ SD), and were compared using unpaired Student's *t*-tests. The plaque burden score cut-off value was determined on the basis of receiver-operating characteristic (ROC) curve analysis. The cumulative

fraction of events was estimated as 1 minus the Kaplan-Meier estimate of survival free of MACE. Differences in the cumulative fraction of events between subgroups were assessed by the log-rank test according to the cut-off. We initially analyzed all available risk factors using a univariate model, then multivariate Cox 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 coefficient of variation (CV) with 20 randomly selected subjects. All statistical analyses were conducted using R.<sup>16</sup> All p values < 0.05 were considered statistically significant.

## Results

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, diabetes mellitus, smoking habits, and BMI were significantly higher while HDL-C was significantly lower in FH patients with MACE compared to those without MACE. Interestingly, plaque burden was significantly greater in FH patients with MACE than those without MACE. The genetic backgrounds of the study participants are shown in **Supplementary Table 1**. A nonsense mutation (c.2431A>T) in the LDL receptor gene was common (40%), and the remaining participants carried 26 other different gene mutations, including PCSK9.

Intra- and interobserver reproducibility for measurements of plaque burden scores are shown in **Figure 1**. Bland-Altman analysis demonstrated good agreements between both within intraobserver with a CV of 9.1% (**Figure 1A**) and within interobserver with a CV of 9.9% (**Figure 1B**) measurements.

To evaluate if coronary plaque burden and traditional risk factors were determinants of the

occurrence of MACE, univariate analysis was performed (**Table 2**). The median of the coronary plaque burden score was 2.78 (raw score 16.1). As a result, age, hypertension, diabetes mellitus, smoking, BMI, and plaque burden score  $\geq$  median were significant predictors for MACE (**Table 2**). In addition, multivariate analysis showed that the presence of hypertension and a plaque burden score  $\geq$  median were significant independent prognostic factors (**Table 2**).

Based on the ROC curve analysis, the optimal plaque burden score cutoff value for developing MACE was 3.35 (raw score 28.5), the sensitivity and specificity of which were 85.7% and 82.5%, respectively with an AUC of 0.90 (**Figure 2A**). **Table 3** compares the clinical profiles of patients with a plaque burden score  $\geq$  3.35 and those with  $<$  3.35. The frequencies of the traditional coronary risk factors, such as age, hypertension, diabetes mellitus, and smoking habit were higher in patients with a plaque burden score  $\geq$  3.35 than those  $<$  3.35. Moreover, BMI and the duration under statin therapy were greater in patients with a plaque burden score  $\geq$  3.35 than those with a score  $<$  3.35.

The cumulative event rate curve revealed that those patients with a plaque burden score  $\geq$  3.35 had a significantly higher event rate than those with a score  $<$  3.35 (**Figure 2B**). **Table 4** shows the MACE during the follow-up period. As many as 18 among 30 patients with a plaque burden score  $\geq$  3.35 had MACE during the follow-up period.

There was no one who exhibited coronary ostial stenosis associated with supralvalvular stenosis which is specific to FH. However, as much as 26 among 101 (26%) heterozygous FH patients exhibited aortic valve calcification detected by CCTA.

We also investigated if the particular mutation status (c.2431A>T) could affect any risk factors including lipids, as well as the outcomes; however, there is no difference observed in the group with this mutation and that with other mutations (**Supplementary Table 2, 3**).

Finally, we evaluated the correlation coefficient between age and raw plaque burden score in



each gender (**Figure 3**). The regression equations were  $Y=0.68X - 15.6$  ( $r = 0.54, p < 0.05$ ) in male and  $Y= 0.74X - 24.8$  ( $r = 0.69, p < 0.05$ ) in female heterozygous FH patients even after accounting for other risk factors, such as LDL-C, HDL-C, hypertension, diabetes, smoking. These results suggest that coronary atherosclerosis may start to develop, on average, at 23 and 34 years of age in male and female heterozygous FH patients, respectively.

## **Discussion**

In this study, we evaluated plaque burden score assessed with CCTA among mutation determined heterozygous FH patients and found that such score was associated with future coronary events beyond established risk factors, and that we can estimate the onset and progression of coronary atherosclerosis in FH patients assuming a linear model of plaque progression.

Heterozygous FH have a mutant allele of either of three FH-associated genes (FH genes), namely LDL receptor, apolipoprotein B-100 or PCSK9 genes,<sup>17</sup> and the frequency of which is estimated to be at least 1 in 500 general populations worldwide.<sup>18</sup> Those patients exhibit premature coronary atherosclerosis due to extremely high LDL-C levels, thus their risk of future coronary events needs to be assessed. In the current study, plaque burden score assessed with CCTA successfully estimated future MACE. In addition to those prognostic values of plaque burden score, calculating those scores quantitatively based on SCCT guideline instead of adopting any other published dichotomized scoring system,<sup>5,19,20</sup> we could estimate the onset and progression of CAD in FH patients assuming linear model of plaque progression. The regression lines from age and plaque score suggested that coronary atherosclerosis might start to develop at 23 and 34 years of age in male and female FH patients, respectively, even under statin therapy. In contrast, our

previous study, conducted before the approval of statins in 1989 showed that coronary artery stenosis detectable by conventional angiography occurred around 17 and 25 years of age in male and female FH patients, respectively.<sup>8</sup> These differences in age (6 years in male, and 9 years in female) might reflect a delay in the development of coronary atherosclerosis using statin in patients with FH. We may consider the examination by CCTA around such ages in FH patients.

There are only a few papers from Brazil, Spain and The Netherlands investigating the clinical application of CCTA for FH.<sup>21-24</sup> Although those papers demonstrated that CCTA could detect substantial coronary plaque burden in FH, especially in mutation-positive FH, few of them addressed the simple issue regarding the predictive prognostic value of this modality.

This study has several limitations. (1) This study was a retrospective analysis of data from a single center with a relatively small sample size, thus our results need to be validated through prospective multi-center studies. (2) Because some patients with FH were not examined by CCTA due to physicians' and patients' selection, there is likely to be some bias. (3) Although we have validated the reproducibility of this measurement, the assessments of stenotic severity of calcified plaque by CCTA were still difficult, leaving the possibility that plaque burden was overestimated. (4) Measurement of the Agatston score, which is widely used to assess the burden of coronary artery calcium in a quantitative manner,<sup>25</sup> was not routinely available in this cohort. (5) Our assumption of the development of coronary atherosclerosis in FH is based on linear model (and on the findings from the age 22 to 84 years), which may not be applicable to the younger FH patients. (6) Majority of events occurred close after the CT scan, which reflected the fact that the findings of CCTA could lead to the coronary angiogram as well as to the coronary revascularization procedure.

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## Figure Legends

### Figure 1. Bland-Altman analysis for the measurement of plaque burden score

Bland-Altman analysis demonstrated good agreements between the measurements (A) within intraobserver and (B) within interobserver.

### Figure 2. Receiver-operating characteristic (ROC) curve analysis and Survival analysis

(A) Receiver-operating characteristic (ROC) curve analysis revealed a plaque burden score of 3.35 as the optimal cutoff for predicting MACE, the sensitivity and specificity of which were 85.7% and 82.5%, respectively with an AUC of 0.90.

(B) Cumulative event rates according to the cut-off. Blue dotted line indicates subjects with a plaque burden score  $\geq 3.35$ . Red solid line indicates subjects with a plaque burden score  $< 3.35$ .

### Figure 3. Plots of correlation between age (X) and plaque burden score (Y) in male (A) and female (B) heterozygous FH patients.

The regression equations are  $Y=0.68X - 15.6$  ( $r = 0.54$ ,  $p < 0.05$ ) in male heterozygotes, and  $Y=0.74X - 24.8$  ( $r = 0.69$ ,  $p < 0.05$ ) in female heterozygotes, respectively. The solid lines indicate the regression line. The dotted lines indicate 95% confidence interval. Plaque burden score was obtained by assigning scores (0 to 5) to each of 17 coronary artery segments; the highest possible plaque burden score is 60.



**Table 1. Baseline characteristics divided by the presence of major adverse cardiac event**

Variable	Major adverse cardiac event		p value
	YES (n=21)	NO (n=80)	
Age (years)	59.4±14.8	50.1±13.7	< 0.05
Men	12 (57%)	40 (50%)	n.s.
Hypertension	19 (79%)	15 (19%)	< 0.05
Diabetes mellitus	11 (52%)	11 (14%)	< 0.05
Smoker	17 (81%)	19 (24%)	< 0.05
BMI (kg/m <sup>2</sup> )	25.8±3.8	23.4±2.9	< 0.05
Total cholesterol (mg/dL)	339±54	347±62	n.s.
Low-density lipoprotein cholesterol (mg/dL)	265±65	260±55	n.s.
High-density lipoprotein cholesterol (mg/dL)	43±10	57±14	< 0.05
Triglyceride (mg/dL)	147±60	132±80	n.s.
Plaque burden score	3.64±0.33	2.13±1.25	< 0.05
Statins	15 (71%)	52 (65%)	n.s.
Statins duration (years)	9±9.3	7.2±8.6	n.s.

**Table 2. Univariate and multivariate Cox regression analysis of risk factors for major adverse cardiac event**

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	p value	Hazard ratio	95% CI	p value
Age	1.038	1.006-1.072	< 0.05			
Men	0.986	0.413-2.354	n.s.			
Hypertension	20.82	4.842-89.55	< 0.05	7.521	1.581-37.76	< 0.05
Diabetes mellitus	3.833	1.620-9.066	< 0.05			
Smoker	6.336	2.214-18.9	< 0.05			
Body mass index	1.171	1.037-1.324	< 0.05			
Total cholesterol	1.004	0.9972-1.011	n.s.			
Low-density lipoprotein cholesterol	1.005	0.9978-1.012	n.s.			
High-density lipoprotein cholesterol	0.9773	0.9449-1.011	n.s.			
Triglyceride	1.002	0.9966-1.007	n.s.			
plaque burden score $\geq$ median (2.78)	13.93	4.082-47.51	< 0.05	5.424	1.411-20.85	< 0.05
Statins	1.006	0.9594-1.055	n.s.			

**Table 3. Baseline characteristics divided by the plaque score cut-off value**

Variable	Plaque burden score		p value
	≥ 3.35 (n=32)	< 3.35 (n=69)	
Age (years)	61.0±13.7	48.0±12.8	< 0.05
Men	15 (47%)	37 (54%)	n.s.
Hypertension	23 (72%)	11 (16%)	< 0.05
Diabetes mellitus	15 (47%)	7 (10%)	< 0.05
Smoker	19 (59%)	17 (25%)	< 0.05
Body mass index (kg/m <sup>2</sup> )	25.7±3.3	23.1±2.9	< 0.05
Total cholesterol (mg/dL)	341±49	346±59	n.s.
Low-density lipoprotein cholesterol (mg/dL)	262±70	264±53	n.s.
High-density lipoprotein cholesterol (mg/dL)	44±12	56±13	< 0.05
Triglyceride (mg/dL)	129±84	138±99	n.s.
Plaque burden score	3.68±0.22	1.86±1.15	< 0.05
Statins	25 (78%)	42 (61%)	n.s.
Statins duration (years)	10.5±9.2	6.2±8.2	< 0.05

**Table 4. Major adverse cardiac event during the follow-up period**

Variable	Plaque burden score		p value
	≥ 3.35 (n=32)	< 3.35 (n=69)	
Composite endpoint			
All major adverse cardiac event	18 (56%)	3 (4%)	< 0.05
Acute coronary syndrome-related event	5 (16%)	0 (0%)	n.s.
Coronary events			
Cardiac death	1 (3%)	0 (0%)	n.s.
ST elevated myocardial infarction	2 (6%)	0 (0%)	n.s.
Unstable angina pectoris/non-ST elevated myocardial infarction	2 (6%)	0 (0%)	n.s.
Staged-PCI/CABG	13 (41%)	3 (4%)	< 0.05

PCI: percutaneous coronary intervention, CABG: coronary artery bypass grafting

Figure 1

Intraobserver

Interobserver

A

B

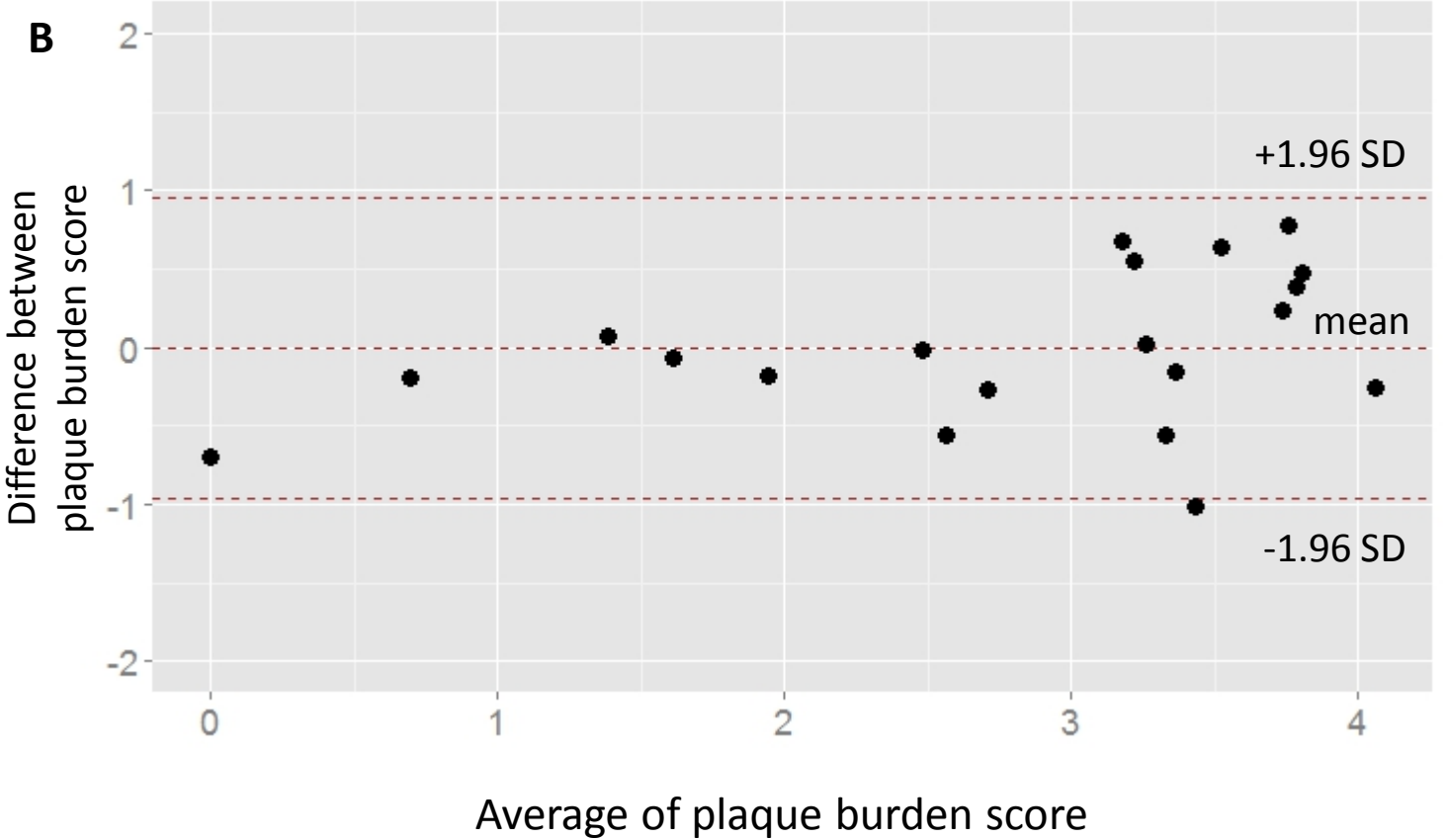
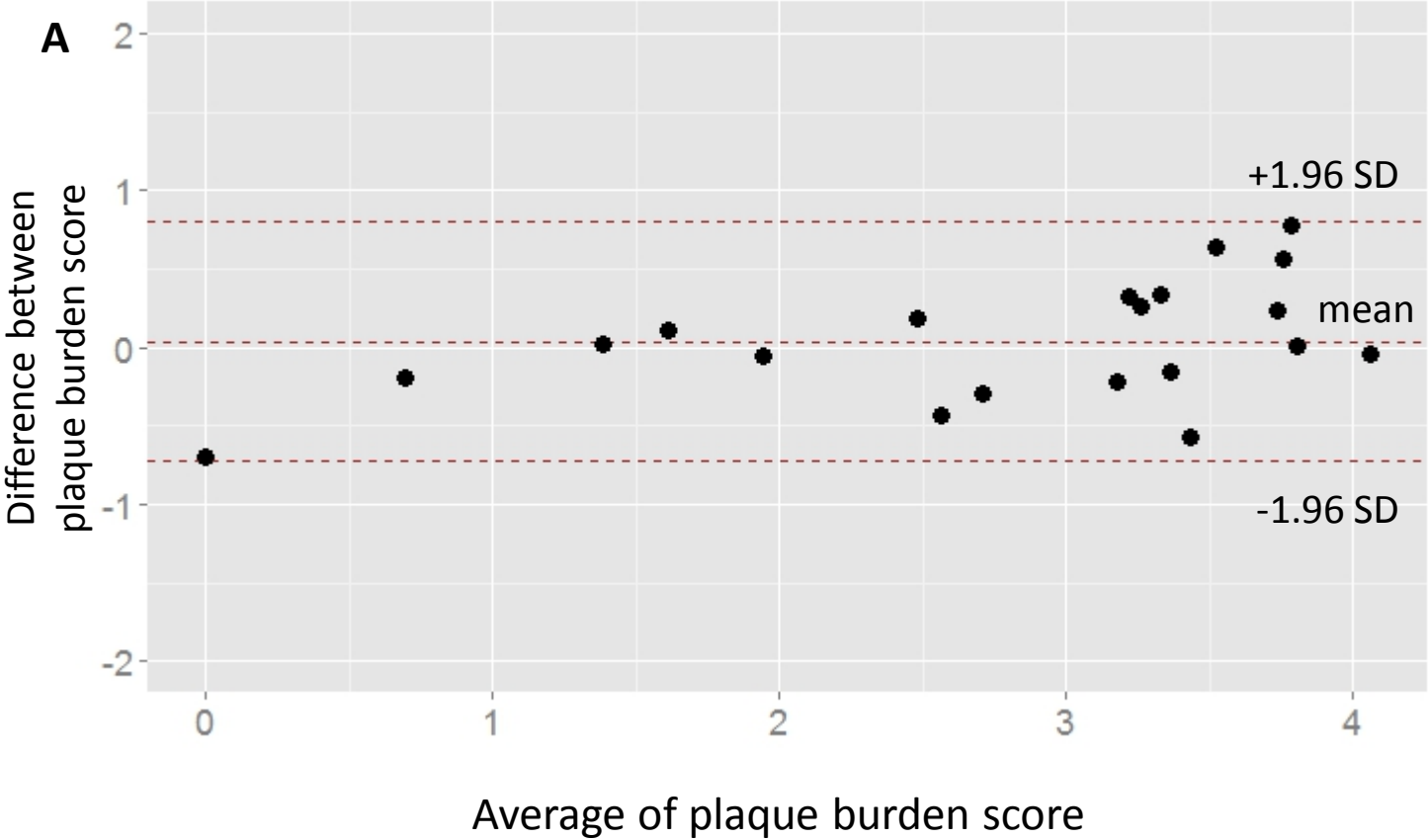
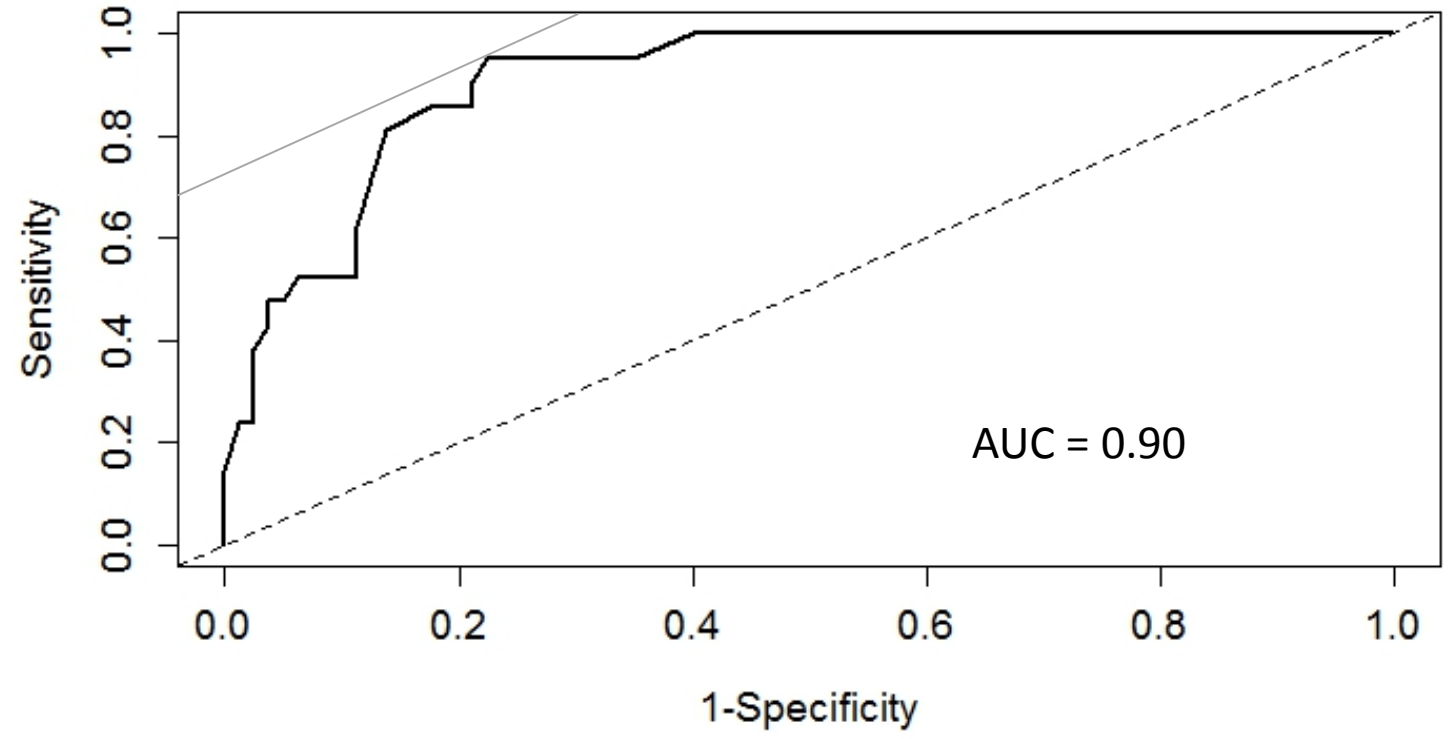
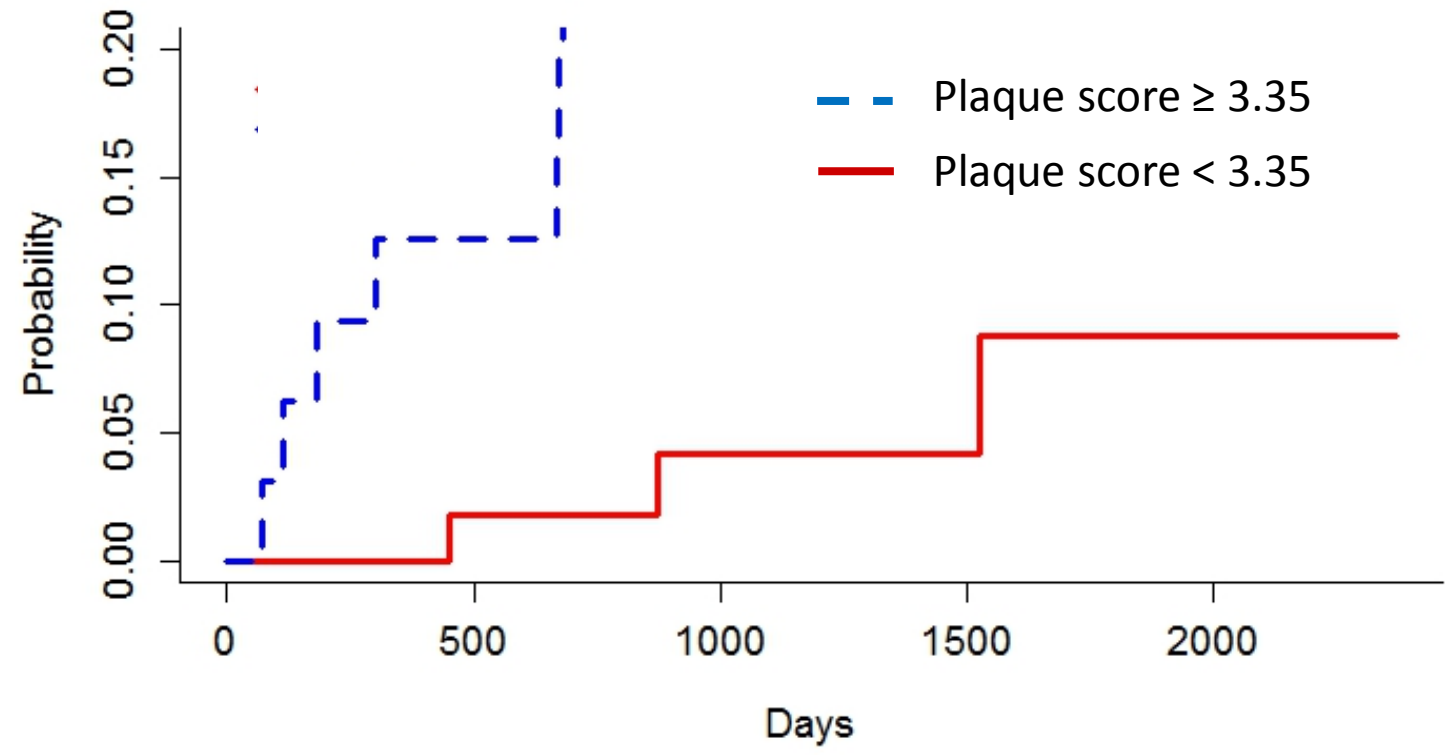


Figure 2

Receiver-operating characteristic curve

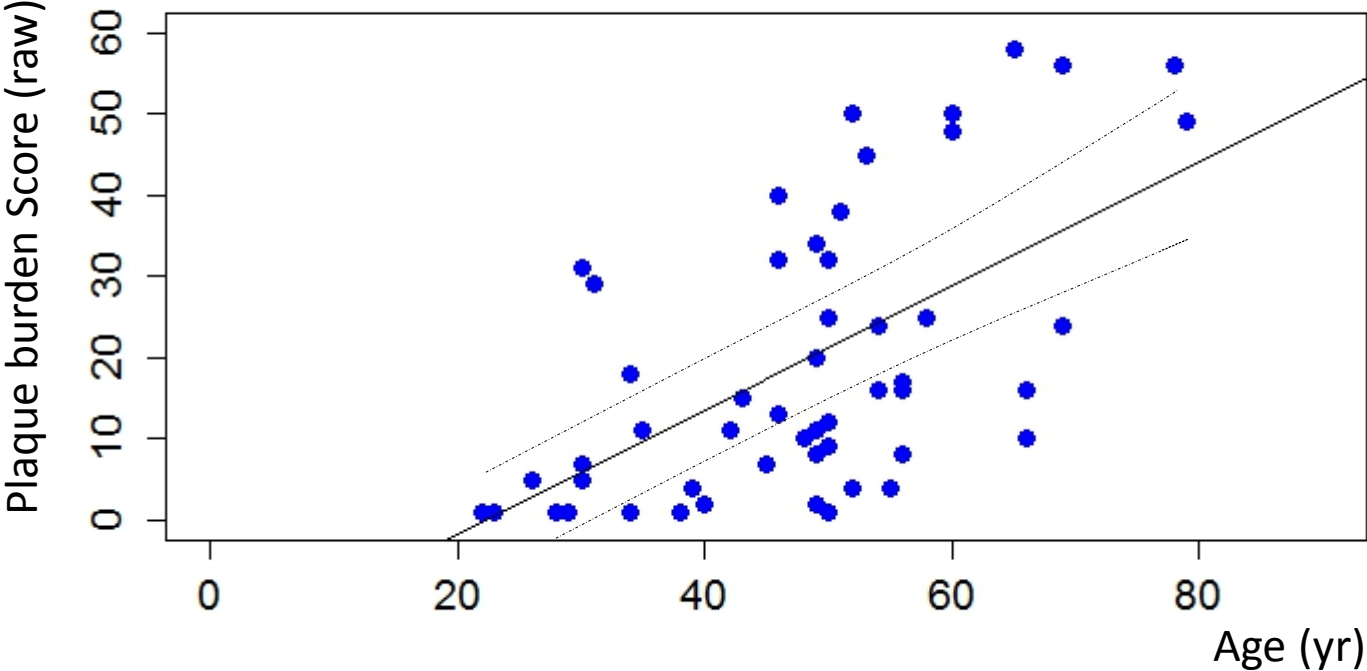


Cumulative incidents



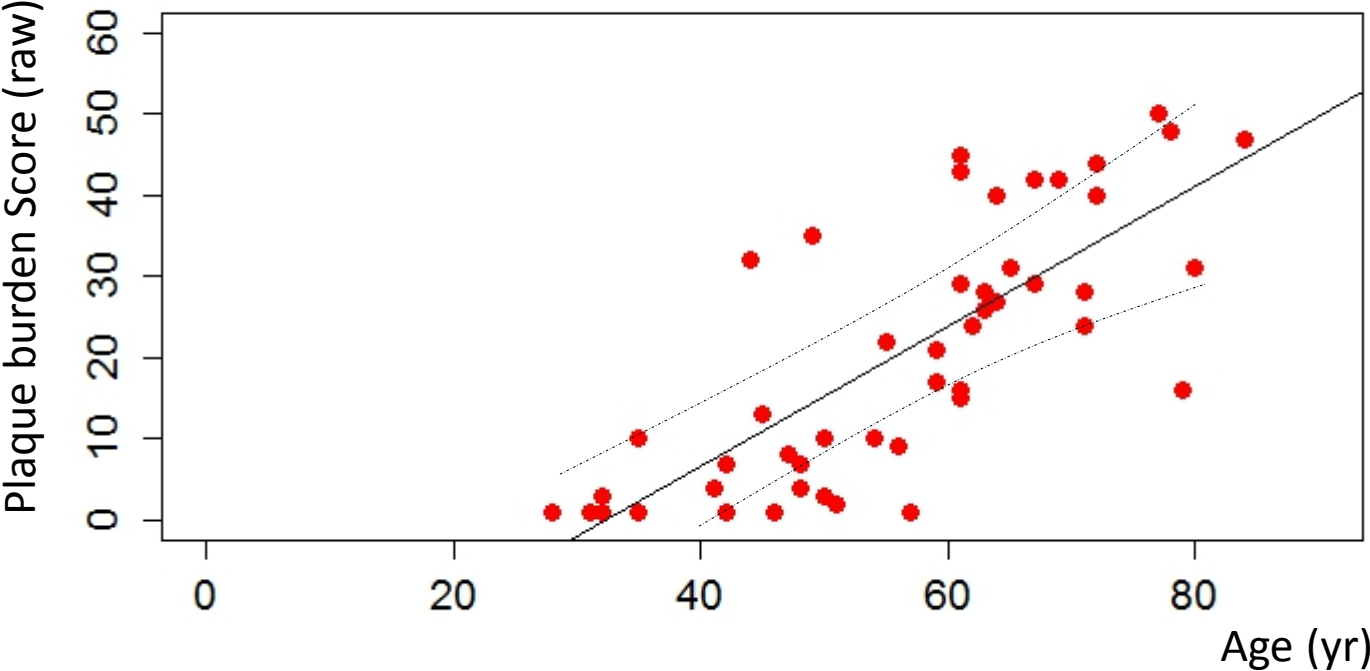
**Figure 3**

**A**



**Male**

**B**



**Female**

Supplementary Table1. Genetic backgrounds

Gene	Nucleotide Change	Mutation Type	Effect on Protein	Number of Patients
LDLR	c.68-?_313+?del	Large deletion	Truncated protein	5
LDLR	c.191-?_940+?dup	Large duplication	Truncated protein	2
LDLR	c.344G>A	Missense	Arg-His	1
LDLR	c.413C>G	Nonsense	Ser-stop	3
LDLR	c.539G>A	Nonsense	Trp-stop	1
LDLR	c.662_665dupACTG	Duplication	Frameshift/stop	1
LDLR	c.682G>A	Missense	Glu-Lys	2
LDLR	c.797A>G	Missense	Asp-Gly	1
LDLR	c.901G>T	Missense	Asp-Tyr	1
LDLR	c.1012T>A	Missense	Cys-Ser	2
LDLR	c.1285G>A	Missense	Val-Met	1
LDLR	c.1297G>C	Missense	Asp-His	2
LDLR	c.137G>A	Missense	Cys-Tyr	1
LDLR	c.1432G>A	Missense	Gly-Ala	1
LDLR	c.1474G>A	Missense	Asp-Asn	1
LDLR	c.1567G>A	Missense	Val-Met	1
LDLR	c.1689dupC	Duplication	Frameshift/stop	1
LDLR	c.1702C>G	Missense	Leu-Val	1
LDLR	c.1778dupG	Duplication	Frameshift/stop	1
LDLR	c.1845+2T>C	Splicing	exon13 skip	1
LDLR	c.1871_1873delTCA	Deletion	603 Ile deletion	3
LDLR	c.2054C>T	Missense	Pro-Leu	6
LDLR	c.2140+1G>T	Splicing	exon15 skip	2
LDLR	c.2141-?_2311+?del	Large deletion	Truncated protein	3
LDLR	c.2312-3C>A	Splicing	exon16 skip	9
LDLR	c.2431A>T	Nonsense	Lys-stop	41
PCSK9	c.94G>A	Missense	Glu-Lys	7

LDLR: LDL receptor



Supplemental Table 2. Baseline characteristics d

Variable	Genotype	
	c.2431A>T (n=41)	Others (n=60)
Age (years)	55.9±14.4	56.2±13.9
Men	23 (56%)	29 (48%)
Hypertension	16 (39%)	18 (30%)
Diabetes mellitus	11 (27%)	11 (18%)
Smoker	17 (41%)	19 (32%)
Body mass index (kg/m <sup>2</sup> )	23.8±3.5	24.1±4.0
Total cholesterol (mg/dL)	344±47	346±56
Low-density lipoprotein cholesterol (mg/dL)	264±66	265±49
High-density lipoprotein cholesterol (mg/dL)	52±11	55±10
Triglyceride (mg/dL)	142±76	139±104
Plaque burden score	2.38±0.20	2.46±0.34
Statins	30 (73%)	37 (62%)
Statins duration (years)	7.5±8.4	7.7±8.6



Supplemental Table 3. Major adverse cardiac event divid

Variable	Gen c.2431A>T (n=41)
Composite endpoint	
All major adverse cardiac event	10 (24%)
Acute coronary syndrome-related event	2 (5%)
Coronary events	
Cardiac death	1 (2%)
ST elevated myocardial infarction	1 (2%)
Unstable angina pectoris/non-ST elevated myocardial infarct	0 (0%)
Staged-PCI/CABG	8 (20%)
PCI: percutaneous coronary intervention, CABG: coronary artery bypass grafting	

ed by the genotype

otype	p value
Others (n=60)	
11 (18%)	n.s.
3 (5%)	n.s.
0 (0%)	n.s.
1 (2%)	n.s.
2 (3%)	n.s.
8 (13%)	n.s.