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ORIGINAL ARTICLE

Association between plasma levels of PCSK9 and the presence of coronary artery disease in Japanese

Daisuke Nose[1] · Yuhei Shiga[1] · Yoko Ueda[1] · Yoshiaki Idemoto[1] · Kohei Tashiro[1] · Yasusnori Suematsu[1] · Takashi Kuwano[1] · Ken Kitajima[1] · Keijiro Saku[1,2] · Shin-ichiro Miura[1,2]

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Abstract
The ability of pro-protein convertase subtilisin/kexin type 9 (PCSK9) levels to predict the presence or severity of coronary artery disease (CAD) remains controversial. The purpose of this study was to investigate these associations. We enrolled 393 patients who were clinically suspected to have CAD or who had at least one cardiac risk factor and underwent multidetector-row computed tomography coronary angiography. The presence of CAD (≥50% coronary stenosis), the number of significantly stenosed coronary vessels, and plasma levels of PCSK9 by ELISA were analyzed. Plasma PCSK9 levels (log-transformed data) were significantly associated with the presence of CAD. Next, we divided the patients into two groups (non-statin and statin groups) according to statin treatment. PCSK9 levels in the non-statin group were significantly lower than those in the statin group. There were no significant differences in PCSK9 levels between the absence and presence of CAD in the statin group. However, in the non-statin group, PCSK9 levels in patients with CAD were significantly higher than those in patients without CAD. PCSK9 levels, in addition to age, gender, BMI, DM and HDL-C, were independently associated with the presence of CAD by a multivariable analysis. In conclusion, our results demonstrated that plasma PCSK9 levels may be a marker for evaluating the presence of CAD.

Keywords Pro-protein convertase subtilisin/kexin type 9 · Coronary artery disease · Multidetector-row computed tomography · Statin

Introduction

Pro-protein convertase subtilisin kexin-9 (PCSK9) is a protein that regulates the expression of low-density lipoprotein (LDL) receptors [1]. PCSK9 levels also seem to play a role in regulating the concentrations of apo B and TG, in addition to LDL cholesterol (LDL-C) [2–4]. However, this latter association with LDL-C is abolished by treatment with statin [5]. Recent meta-analyses have shown that PCSK9 levels might be affected by the dose and type of statin [6, 7]. Genetic studies on gain-of-function or loss-of-function mutations in PCSK9 showed that PCSK9 is associated with the risk of coronary artery disease (CAD) [8, 9]. Several studies have suggested that PCSK9 levels were associated with the degree of coronary stenosis, and might be a biomarker of CAD [10–12]. On the other hand, Zhu et al. argued that PCSK9 was unlikely to be a biomarker of atherosclerotic risk or vascular health [13]. Some equivocal reports on these relations have been reported, and this issue remains controversial [13–15].

Previous reports that supported the hypothesis of a positive relation included many subjects with severe CAD assessed by Gensini scores, and showed significant differences between the group with high scores and other groups [10, 12]. To date, several studies have indicated that the risk factors and background for CAD were significantly different among different ethnic groups, and it is unlikely that biomarkers can be generalized to other ethnicities [16, 17]. In particular, Japanese show a unique association between the lipid profile and the development of CAD [18].

The present study aimed to examine whether PCSK9 levels are useful for predicting the presence and severity

✉ Shin-ichiro Miura
miuras@cis.fukuoka-u.ac.jp

[1] Department of Cardiology, Fukuoka University School of Medicine, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan

[2] Department of Molecular Cardiovascular Therapeutics, Fukuoka University School of Medicine, Fukuoka, Japan

of CAD in Japanese, by using multidetector-row computed tomography coronary angiography (CCTA).

Methods

Study subjects

Patients were identified and recruited through a review of the CCTA database of Fukuoka University between April 2012 and July 2014. From among 424 consecutive patients, 31 lacked some data, and thus 393 were enrolled. Inclusion criteria were as follows: clinically suspected of having CAD based on abnormal findings by electrocardiography and/or chest symptoms, or with at least one cardiovascular risk factor, and age > 20. Patients with acute coronary syndrome, a history of known CAD (prior myocardial infarction, prior coronary intervention, or coronary artery bypass grafting), severe renal insufficiency [estimated glomerular filtration rate (eGFR) ≤ 30 ml/min/1.73 m^2], and missing data were excluded. The study was conducted as a single-center, cross-sectional analysis. The study complied with the Declaration of Helsinki and was approved by the Independent Review Board (IRB) of Fukuoka University Hospital (Fukuoka University Hospital EC/IRB: #09-10-02). All patients provided their written informed consent to participate before blood sampling and CCTA. The study is registered with the University Hospital Medical Information Network (UMIN000016641).

Evaluation of coronary stenosis using CCTA

All patients were scanned by CCTA as previously described [19]. The use and dose of beta-blocker was left to the physician's discretion, according to the baseline heart rate and body weight. All segments were assessed according to the 15-segment American Heart Association coronary artery model [20]. Significant coronary artery stenosis was defined as narrowing of the coronary lumen ≥ 50% using multiplanar reconstructions or cross-sectional images. Subjects were classified into 3 groups [0, single (1), and multi (2 + 3) vessel disease (VD) groups] according to the number of coronary stenosed vessels. CCTA was assessed by trained cardiologists using Ziostation (Ziosoft Inc., Tokyo, Japan), a computed tomography workstation. These cardiologists were blinded to the clinical characteristics and the blood tests. Overall, the severity of CAD was assessed according to the number of VD and the Gensini score. This scoring system was established to define the severity of coronary stenosis. The details have been reported elsewhere [21]. Coronary artery calcium score was assessed by using the Agatston score.

Evaluation of CAD risk factors

In all subjects, age, gender, body mass index (BMI), systolic blood pressure, diastolic blood pressure, serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), LDL-C, uric acid (UA), blood glucose, hemoglobin A1c (HbA1c), smoking (current and past smokers), family history [myocardial infarction, angina pectoris or sudden death] and medication use were evaluated as cardiovascular risk factors by cardiologists. These cardiologists were blinded to the CCTA results and all clinical information. Concentrations of TC, TG, HDL-C, LDL-C and UA were measured by enzymatic methods. Plasma levels of PCSK9 were measured using a commercial sandwich enzyme immunoassay (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. BMI was calculated as weight (kg)/height $(m)^2$. Patients who had a current SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or who were receiving anti-hypertensive therapy were considered to have hypertension (HTN). Patients with LDL-C ≥ 140 mg/dl, TG ≥ 150 mg/dl, and/or HDL-C < 40 mg/dl, or who were being treated with lipid-lowering therapy were defined as DL. The HbA1c value was evaluated according to the National Glycohemoglobin Standardization Program. Patients were considered to have diabetes mellitus (DM) if they satisfied any of the diagnostic criteria defined by the Japan Diabetes Society [fasting blood sugar level ≥ 126 mg/dL, 2-h 75 g OGTT glucose level ≥ 200 mg/dL or random blood sugar level ≥ 200 mg/dL (venous plasma levels), or if they were taking glucose-lowering drugs with a clear diagnosis]. The National Kidney Foundation has defined chronic kidney disease (CKD) as a GFR of less than 60 ml/min/1.73 m^2 [22]. Estimated glomerular filtration rate (eGFR) was determined using the abbreviated equation as modified for Japanese by the Japanese Society of Nephrology; 194 × [age (years)] − 0.287 × [serum Cr (mg/dl)] − 1.094 × [0.739 if female] [23]. CKD was defined as an eGFR level < 60 ml/min/1.73 m^2.

Statistical analysis

All calculations were performed with the statistical software JMP® 13 (SAS Institute Inc., Cary, NC, USA). Continuous data are reported as mean ± standard deviation (SD) or as median values (interquartile range). Categorical and continuous variables were compared between the groups by the *t* test or Mann–Whitney *U* test, respectively. When continuous variables did not show a normal distribution, expressed as a median value and interquartile range, we used the Wilcoxon rank-sum test. The Spearman

rank correlation coefficient was used to evaluate associations between the groups. Log-normalization was used for PCSK9 levels, which were not normally distributed. Changes in quantitative results of PCSK9 between no- or 1 VD and 2 + 3 VD were determined by one-way ANOVA with a multiple comparison Tukey–Kramer HSD test, or Kruskal–Wallis test with the Steel–Dwass test where appropriate. A multivariable analysis was performed using a logistic regression analysis for independent variables (age, gender, family history, BMI, smoking, HTN, DM, DL, CKD, statin treatment, HDL-C, LDL-C in addition to PCSK9 levels) that were related to the presence or absence of CAD. A value of $p < 0.05$ was considered significant.

Results

Patient characteristics in all patients and the non-CAD and CAD groups

Table 1 shows the clinical characteristics in all patients, and the non-CAD and CAD groups. Among all patients, the median age was 66 years, 51% of patients were male and 34% were treated with statins. The patients with CAD were older and had a higher prevalence of DM, HTN, and CKD. The use of angiotensin II receptor blocker, calcium

Table 1 Patient characteristics in all patients, non-CAD and CAD groups

	All patients ($n = 393$)	Non-CAD group ($n = 182$)	CAD group ($n = 211$)	p value non-CAD vs. CAD
Age, years	66 (59–73)	63 (56–70)	69 (61–75)	< 0.0001
Sex male, % (*n*)	51 (202)	45 (82)	57 (120)	< 0.019
BMI, kg/m^2	24 (22–26)	24 (22–26)	23 (21–26)	< 0.21
Family history, % (*n*)	26 (102)	24 (44)	27 (58)	< 0.46
Smoking, % (*n*)	38 (149)	33 (60)	42 (89)	< 0.061
HTN, % (*n*)	72 (283)	63 (115)	80 (168)	< 0.0003
DM, % (*n*)	23 (89)	16 (29)	28 (60)	< 0.003
DL, % (*n*)	67 (262)	62 (113)	71 (149)	< 0.074
CKD, % (*n*)	31 (124)	25 (46)	37 (78)	< 0.013
TG, mg/dl	117 (87–158)	109 (79–154)	124 (94–163)	< 0.017
LDL-C, mg/dl	115 ± 30	115 ± 30	114 ± 29	< 0.78
HDL-C, mg/dl	51 (44–62)	54 (45–66)	50 (43–59)	< 0.002
HbA1c, % (*n*)	5.7 (5.4–6.2)	5.7 (5.3–6.1)	5.9 (5.5–6.4)	< 0.0003
Fasting glucose, mg/dl	100 (93–113)	99 (93–107)	102 (93–121)	< 0.041
UA, mg/dl	5.4 (4.5–6.3)	5.4 (4.5–6.3)	5.4 (4.5–6.3)	< 0.93
eGFR, ml/min/1.73 m^2	67 (57–77)	70 (60–80)	64 (55–75)	< 0.002
PCSK9, ng/ml	213 (169–268)	203 (156–257)	223 (172–278)	< 0.006
Log PCSK9	5.35 ± 0.36	5.29 ± 0.39	5.40 ± 0.33	< 0.004
Medication				
ARB, % (*n*)	38 (149)	31 (56)	44 (93)	< 0.007
ACE-I, % (*n*)	3 (12)	2 (3)	4 (9)	< 0.12
CCB, % (*n*)	36 (141)	27 (50)	43 (91)	< 0.001
BB, % (*n*)	11 (43)	7 (12)	15 (31)	< 0.009
Diuretic, % (*n*)	10 (41)	9 (17)	11 (24)	< 0.51
Statin, % (*n*)	34 (134)	27 (50)	40 (84)	< 0.01
SU, % (*n*)	9 (39)	7 (12)	13 (27)	< 0.037
BG, % (*n*)	6 (24)	6 (11)	6 (13)	< 0.96
DPP4-I, % (*n*)	11 (43)	8 (14)	14 (29)	< 0.052

Continuous variables are expressed as mean ± SD. When continuous variables did not show a normal distribution, the variables are expressed as a median value and interquartile range

CAD coronary artery disease, *BMI* body mass index, *HTN* hypertension, *DM* diabetes mellitus, *DL* dyslipidemia, *CKD* chronic kidney disease, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *HbA1c* glycosylated hemoglobin A1c, *UA* uric acid, *eGFR* estimated glomerular filtration rate, *PCSK9* pro-protein convertase subtilisin/kexin type 9, *ARB* angiotensin II receptor blocker, *ACE-I* angiotensin-converting enzyme inhibitor, *CCB* calcium channel blocker, *BB* beta-blocker, *DU* diuretic, *SU* sulfonylurea, *BG* biguanide, *DPP4-I* dipeptidyl-peptidase 4 inhibitor

channel blocker (CCB), beta-blocker (BB), sulfonylurea, and statin were significantly higher in the CAD group.

Patient characteristics in all patients, and in patients with non-CAD and CAD in the non-statin and statin groups

Table 2 shows the clinical characteristics of all patients and patients with non-CAD and CAD in the non-statin and statin groups. The patients treated with statin had significantly lower levels of LDL-C, and their morbidity percentages of CAD, DM, and HTN, but not DL, were also higher than those in patients without statin. Second, patients with statin and non-statin treatment were further divided into two groups: CAD and non-CAD groups. In the non-statin group, patients with CAD were significantly older, included more smokers and CKD, and had higher levels of serum HbA1c and lower levels of HDL-C than those without CAD. In the non-statin and statin groups, patients with CAD had a higher percentage of HTN than those without CAD. With regard to medications, anti-hypertensive drugs in the statin group were significantly higher than those in the non-statin group. In the non-statin group, patients with CAD had a higher percentage of CCB, while in the statin group, they had a higher percentage of BB.

Levels of PCSK9 in all patients and the non-CAD and CAD groups

In all patients, PCSK9 levels (log-transformed data) in the CAD group were significantly higher than those in the non-CAD group (5.40 ± 0.33 vs. 5.29 ± 0.39, $p = 0.004$, Table 1 and Fig. 1a). In addition, in all patients, PCSK9 levels in the statin group were significantly higher than those in the non-statin group (5.49 ± 0.37 vs. 5.28 ± 0.34, $p < 0.01$, Table 2 and Fig. 1a). In the non-statin group, patients with CAD showed significantly higher PCSK9 levels than those without CAD (5.35 ± 0.31 vs. 5.21 ± 0.36, $p = 0.0008$) (Table 2 and Fig. 1b), although there was no significant difference between the CAD and non-CAD groups in the statin group (Table 2 and Fig. 1c).

To examine the association between PCSK9 levels and the severity of CAD, we analyzed VD in all patients, and the non-statin and statin groups (Fig. 2). In the non-statin group, PCSK9 levels in patients with 1 or 2 + 3 VD were significantly higher than those in patients with 0 VD (5.20 ± 0.03 vs. 5.37 ± 0.04 vs. 5.33 ± 0.04, $p = 0.003$), whereas there was no significant difference between patients with 1 VD and 2 + 3 VD (Fig. 2b). In addition, there were no significant differences among patients with 0 VD, 1 VD and 2 + 3 VD in the statin group (Fig. 2c).

Furthermore, to determine the correlation between the Gensini score and PCSK9 levels, we performed correlation analyses. PCSK9 levels were not associated with the Gensini score in all patients with CAD ($|\rho| = 0.072$, $p = 0.30$), or in either the statin ($|\rho| = 0.079$, $p = 0.48$) or non-statin group ($|\rho| = 0.12$, $p = 0.17$). There was no correlation between PCSK9 levels and Agatston score in all patients ($|\rho| = 0.059$, $p = 0.24$), or in either the statin ($|\rho| = 0.007$, $p = 0.94$) or non-statin group ($|\rho| = 0.007$, $p = 0.91$).

Univariable and multivariable logistic regression analysis regarding the association with CAD in all patients and the statin and non-statin groups

Finally, a logistic regression analysis that corrected for independent variables was performed in all patients, and the statin and non-statin groups (Table 3). PCSK9 levels, in addition to age, gender, BMI, DM and HDL-C were independently associated with the presence of CAD in all patients. PCSK9 levels, in addition to age, gender, BMI, HDL-C and LDL-C were also independently associated with the presence of CAD in the non-statin group, whereas BMI and DM, but not PCSK9 levels, were associated with the presence of CAD in the statin group.

Discussion

This study investigated the associations between plasma PCSK9 levels and the presence of CAD and statin treatment, and further evaluated the associations with the severity of CAD. The results indicated that PCSK9 levels in patients with CAD were significantly higher than those in patients without CAD in the non-statin group, but were not associated with the presence of CAD in the statin group. Finally, PCSK9 levels, in addition to age, gender, BMI, DM and HDL-C, were independently associated with the presence of CAD by a multivariable analysis.

Although various trials have evaluated PCSK9 as a biomarker for CAD, the results remain controversial [12–14, 24, 25]. The discrepant results are partly due to differences in patient characteristics and treatment. Various atherosclerosis imaging studies have been performed to investigate the association between PCSK9 levels and cardiovascular disease, such as carotid intima-media wall thickness, coronary artery calcium, and the severity of coronary artery disease [11, 25, 26]. The use of statins leads to an increase in the PCSK9 level due to an increase in its expression in hepatocytes [27, 28]. It has been reported that statin increased the activity of sterol regulatory element-binding protein-2 [29]. Therefore, we examined the association between PCSK9 levels and CAD depending on the presence or absence of statin treatment. A previous retrospective cohort study reported that the PCSK9 level was associated with the incidence of CVD [15]. Some reports

Table 2 Patient characteristics of all patients, the patients with non-CAD and CAD in the non-statin and statin groups

	Non-statin group ($n=259$)				Statin group ($n=134$)			
	All patients ($n=259$)	Non-CAD group ($n=132$)	CAD group ($n=127$)	p value non-CAD vs. CAD	All patients ($n=134$)	Non-CAD group ($n=50$)	CAD group ($n=84$)	p value non-CAD vs. CAD
Age, years	65 (58–73)	63 (55–70)	69 (60–75)	< 0.0001	67 (60–74)	64 (58–71)	69 (62–75)	< 0.059
Male, % (n)	52 (134)	45 (59)	59 (75)	< 0.021	51 (68)	46 (23)	54 (45)	< 0.40
BMI, kg/m^2	23 (21–26)	24 (22–26)	23 (21–26)	< 0.22	24 (22–26)	24 (22–27)	24 (22–26)	< 0.39
Family history, smoking, % (n)	24 (63)	25 (33)	24 (30)	< 0.80	29 (39)	22 (11)	33 (28)	< 0.16
Smoking, % (n)	39 (102)	33 (44)	46 (58)	< 0.042	35 (47)	32 (16)	37 (31)	< 0.57
HTN, % (n)	67 (173)	60 (79)	74 (94)	< 0.016	82 (110)**	72 (36)	88 (74)	< 0.019
DM, % (n)	18 (47)	14 (18)	23 (29)	< 0.055	31 (42)**	22 (11)	37 (31)	< 0.072
DL, % (n)	49 (128)	48 (63)	51 (65)	< 0.58	100 (134)**	100	100	
CKD, % (n)	31 (79)	24 (32)	37 (47)	< 0.026	34 (45)	26 (13)	37 (31)	< 0.19
TG, mg/dl	117 (87–158)	108 (79–154)	125 (96–162)	< 0.042	117 (90–163)	112 (78–156)	124 (93–166)	< 0.16
LDL-C, mg/dl	119 ± 30	117 ± 27	120 ± 32	< 0.362	107 ± 27**	110 ± 35	104 ± 23	< 0.27
HDL-C, mg/dl	51 (44–63)	53 (45–68)	49 (42–58)	< 0.005	52 (45–62)	54 (47–62)	51 (44–61)	< 0.31
HbA1c, %	5.7 (5.3–6.2)	5.6 (5.3–6.0)	5.8 (5.5–6.3)	< 0.002	5.9 (5.5–6.5)**	5.8 (5.6–6.3)	5.9 (5.5–6.8)	< 0.19
Fasting glucose, mg/dl	100 (93–112)	100 (94–108)	102 (93–118)	< 0.19	99 (93–116)	98 (92–105)	102 (93–127)	< 0.089
UA, mg/dl	5.4 (4.5–6.2)	5.3 (4.5–6.2)	5.4 (4.5–6.3)	< 0.61	5.6 (4.5–6.5)	5.7 (4.5–7.8)	5.5 (4.5–6.3)	< 0.22
eGFR, ml/min/1.73 m^2	68 (57–78)	70 (61–82)	65 (55–76)	< 0.019	64 (57–75)	68 (59–77)	64 (55–73)	< 0.064
PCSK9, ng/ml	196 (159–247)	182 (150–235)	213 (169–261)	< 0.003	252 (201–303)**	252 (210–294)	253 (197–304)	< 0.67
Log PCSK9	5.28 ± 0.34	5.21 ± 0.36	5.35 ± 0.31	< 0.0008	5.49 ± 0.37**	5.51 ± 0.40	5.47 ± 0.34	< 0.51
CAD, % (n)	49 (127)				63 (84)*			
VD 0		100 (132)	0			100	0	
VD 1		0	45 (57)			0	32 (27)	
VD 2 + 3		0	55 (70)			0	68 (57)	
Gensini score		2.5 (0–6)	15 (10–23)	< 0.0001		2.5 (0–6)	18 (13–27)	< 0.0001
Medication								
ARB, % (n)	29 (76)	26 (33)	34 (43)	< 0.12	54 (73)**	46 (23)	60 (50)	< 0.13
ACE-I, % (n)	2 (3)	2 (2)	2 (3)	< 0.62	5 (7)	2 (1)	7 (6)	< 0.17
CCB, % (n)	31 (81)	23 (30)	40 (51)	< 0.002	45 (60)**	40 (20)	48 (40)	< 0.39
BB, % (n)	8 (22)	7 (9)	10 (13)	< 0.32	16 (21)*	6 (3)	21 (18)	< 0.012
Diuretic, % (n)	10 (27)	11 (14)	10 (13)	< 0.92	10 (14)	6 (3)	13 (11)	< 0.18
SU, % (n)	7 (19)	6 (8)	9 (11)	< 0.42	15 (20)	8 (4)	19 (16)	< 0.071
BG, % (n)	4 (11)	5 (6)	4 (5)	< 0.81	10 (13)	10 (5)	10 (8)	< 0.93
DPP4-I, % (n)	9 (23)	8 (10)	10 (13)	< 0.45	15 (20)	8 (4)	19 (16)	< 0.071

Continuous variables are expressed as mean ± SD. When continuous variables did not show a normal distribution, the variables are expressed as a median value and interquartile range

CAD coronary artery disease, *BMI* body mass index, *HTN* hypertension, *DM* diabetes mellitus, *DL* dyslipidemia, *CKD* chronic kidney disease, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *HbA1c* glycosylated hemoglobin A1c, *UA*, uric acid, *eGFR* estimated glomerular filtration rate, *PCSK9* pro-protein convertase subtilisin/kexin type 9, *VD* vessel disease (the number of significantly stenosed coronary vessels), *ARB* angiotensin II receptor blocker, *ACE-I* angiotensin-converting enzyme inhibitor, *CCB* calcium channel blocker, *BB* beta-blocker, *DU* diuretic, *SU* sulfonylurea, *BG* biguanide, *DPP4-I* dipeptidyl-peptidase 4 inhibitor

*$p < 0.05$ vs. all patients in the non-statin group. **$p < 0.01$ vs. all patients in the non-statin group

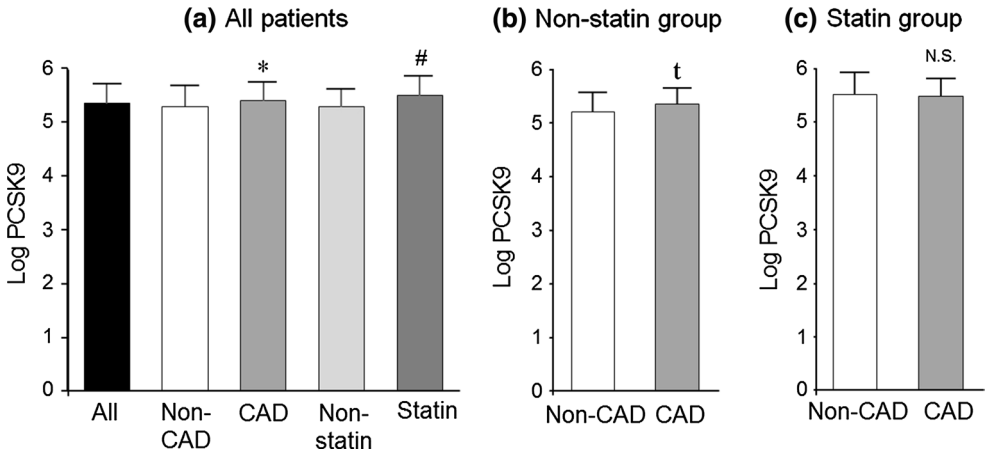

Fig. 1 Plasma levels of PCSK9 in all patients and the non-CAD, CAD, non-statin and statin groups (**a**). Plasma levels of PCSK9 in patients with and without CAD in the non-statin (**b**) and statin (**c**) groups. *$p=0.004$ vs. the non-CAD group in all patients. #$p<0.01$ vs. the non-statin group in all patients. $^{t}p=0.0008$ vs. patients without CAD (non-CAD) in the non-statin group. N.S., not significant

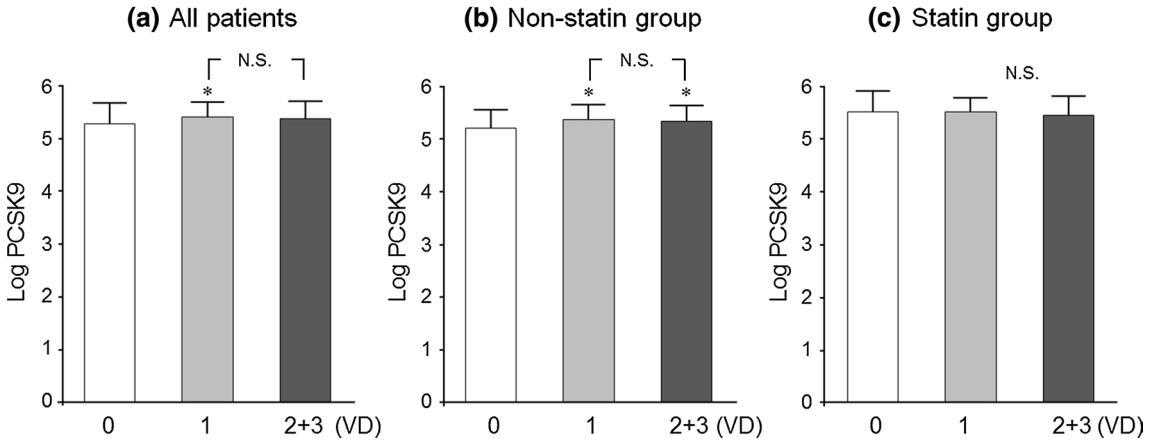

Fig. 2 Plasma levels of PCSK9 in the 0 VD, 1 VD and multi- (2 + 3) VD in all patients (**a**) and the non-statin (**b**) and statin (**c**) groups. *$p<0.05$ vs. 0 VD. N.S., not significant

have mentioned that PCSK9 levels were elevated by statin use, and the levels without statin were associated with coronary atherosclerosis [30, 31]. In the present study, although subjects were enrolled consecutively, including DL, the PCSK9 levels in CAD patients in the non-statin group were significantly higher than those in non-CAD subjects, which was consistent with the results in previous studies noted above. Finally, we could find that the PCSK9 levels were independently associated with the presence of CAD in all patients and in the non-statin group, but not in the statin group probably due to changing of PCSK9 levels by statin treatment.

So far, there have been few studies on the relationship between the PCSK9 level and the severity of CAD among populations with a lower incidence of ischemic heart disease, such as in Japanese compared to Western populations. The fact is thought to be due to differences in lifestyle and the anatomy of the coronary arteries [32–34]. In the present study, patients with CAD had similar levels of LDL-C and non-HDL-C as in previous studies [10–12]. Recent studies have suggested that LDL-C or non-HDL-C levels are associated with the severity of coronary atherosclerosis [35, 36]. However, unlike these reports, our previous report about the evaluation of coronary stenosis in Japanese showed no linear

Table 3 The univariable and multivariable logistic regression analysis regarding the association with CAD in all patients, the statin and non-statin groups

All patients						
Variables	Univariable			Multivariable		
	OR	95% CI	*p*	OR	95% CI	*p*
Age	1.05	1.03–1.07	< 0.0001	1.05	1.02–1.08	< 0.0001
Male	1.61	0.42–0.92	< 0.020	2.10	1.21–3.65	< 0.007
BMI	0.96	0.90–1.01	< 0.12	0.92	0.86–0.99	< 0.021
Family history	1.19	0.75–1.87	< 0.45	1.34	0.80–2.24	< 0.26
Smoking	1.48	0.98–2.24	< 0.06	1.12	0.65–1.94	< 0.67
HTN	2.28	1.45–3.57	< 0.0003	1.62	0.95–2.75	< 0.075
DM	2.10	1.28–3.45	< 0.003	2.20	1.24–3.91	< 0.006
CKD	1.73	1.12–2.68	< 0.013	1.11	0.67–1.85	< 0.69
Log PCSK9	2.27	1.27–4.01	< 0.004	2.65	1.36–5.14	< 0.003
Statin	1.75	1.14–2.67	< 0.010	1.45	0.87–2.40	< 0.15
HDL-C	0.98	0.96–0.99	< 0.0005	0.98	0.96–0.99	< 0.008
LDL-C	1.00	0.99–1.01	< 0.78	1.01	1.00–1.01	< 0.18
Statin group						
Variables	Univariable			Multivariable		
	OR	95% CI	*p*	OR	95% CI	*p*
Age	1.04	1.00–1.07	0.034	1.03	0.98–1.07	0.26
Male	1.35	0.67–2.73	0.40	1.44	0.52–3.98	0.48
BMI	0.37	0.057–2.43	0.30	0.88	0.77–1.00	0.047
Family history	1.77	0.79–3.98	0.16	2.24	0.89–5.62	0.078
Smoking	1.24	0.59–2.61	0.56	1.30	0.48–3.56	0.61
HTN	2.88	1.17–7.11	0.021	2.01	0.70–5.77	0.19
DM	2.07	0.93–4.63	0.068	3.31	1.24–8.84	0.013
CKD	1.66	0.78–3.69	0.20	1.46	0.57–3.75	0.43
Log PCSK9	0.72	0.27–1.91	0.51	0.66	0.22–1.98	0.45
HDL-C	1.00	0.97–1.02	0.69	0.99	0.96–1.02	0.60
LDL-C	0.99	0.98–1.02	0.27	0.99	0.98–1.01	0.37
Non-statin group						
Variables	Univariable			Multivariable		
	OR	95% CI	*p*	OR	95% CI	*p*
Age	1.05	1.03–1.08	< 0.0001	1.07	1.04–1.11	< 0.0001
Male	1.78	1.09–2.92	< 0.021	3.03	1.46–6.29	< 0.002
BMI	0.95	0.89–1.02	< 0.15	0.91	0.83–1.00	< 0.049
Family history	0.93	0.53–1.64	< 0.8	1.04	0.53–2.02	< 0.91
Smoking	1.68	1.02–2.78	< 0.042	1.16	0.57–2.37	< 0.68
HTN	1.91	1.13–3.24	< 0.015	1.51	0.80–2.85	< 0.21
DM	1.87	0.98–3.58	< 0.054	1.65	0.76–3.57	< 0.20
CKD	1.84	1.07–3.14	< 0.027	0.91	0.47–1.75	< 0.77
Log PCSK9	3.65	1.67–7.99	< 0.0007	6.23	2.44–15.9	< 0.0001
HDL-C	0.97	0.95–0.99	< 0.0006	0.97	0.95–0.99	< 0.003
LDL-C	1.00	1.00–1.01	< 0.38	1.01	1.00–1.02	< 0.022

CAD coronary artery disease, *OR* odds ratio, *CI* confidence Interval, *BMI* body mass index, *HTN* hypertension, *DM* diabetes mellitus, *CKD* chronic kidney disease, *PCSK9* pro-protein convertase subtilisin/kexin type 9, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol

association between LDL-C (non-HDL-C) and the Gensini score [19]. In the present study, these cholesterol levels were also nonlinear with respect to an increase in the Gensini score (data not shown). The Seven Country Study reported that Japanese people have a very low incidence of coronary heart disease compared to Western populations, even those with the same cholesterol levels [18]. The incidence of acute myocardial infarction in Japan is approximately 25% of that in the United States [37]. With regard to these differences, Japanese tend to have a large number of stenotic vessels and a wide range of angiographically diffuse atherosclerosis [34]. On the other hand, Caucasians tend to have more severe multi-vessel disease, and more severe proximal atherosclerosis. Moreover, regarding the risk factors for CAD, HTN and coronary vasoconstriction were related to CAD in Japanese, while cholesterol was strongly related to CAD in Caucasians [34, 38]. In this study, patients who were clinically suspected of having CAD or who had at least one cardiac risk factor underwent CCTA. This might explain the relatively small number of subjects who had a very high Gensini score in this study. Over the past several years, many authors have reported that PCSK9 levels are associated with the severity of CAD as assessed by the Gensini score [10–12, 24]. However, there were no significant differences between all of the groups. Significant differences were found in comparisons with the high score groups, which had much higher Gensini scores than our patients. While the LDL-C and non-HDL-C levels were similar to those in these other reports, the Gensini score tended to be considerably lower.

Thus, our subjects likely had less severe CAD. This suggests that the difference in intermediate scores is unimportant from a clinical standpoint. From another point of view, it has been reported that PCSK9 levels are not associated with the overall plaque burden or plaque volume, but are associated with the necrotic core fraction and volume [39]. On the basis of these considerations, it seems plausible that, although PCSK9 levels can predict the presence of CAD, they are not as useful for estimating the severity of coronary artery disease. Almontashiri et al. showed that there was no association between PCSK9 levels and the number of diseased vessels, except in subjects with acute coronary syndrome [30]. An increase in PCSK9 expression leads to the degradation of LDL receptor on the liver cell surface and to an increase in LDL-C levels. Moreover, PCSK9 has been found to accumulate cholesterol, resulting in the chronic inflammatory process of atherosclerosis by enhancing oxidized LDL uptake and the expression of vascular cell adhesion protein-1 [40]. Thus, the correlation between PCSK9 levels and cardiovascular events may change with plaque progression and plaque characteristics [41]. It could be inferred that it takes a long time for PCSK9 levels to become correlated with the severity of CAD. As previously described, PCSK9 levels were significantly elevated in patients with CAD [41]. Moreover, our findings indicated that PCSK9 levels in a population with relatively low-severity CAD, such as Japanese, are less likely to predict the number and severity of diseased vessels.

This study has several limitations. First, the study was cross-sectional. Both the 1 VD and multi-VD groups were small. Compared with previous studies, fewer subjects had high-severity stenosis [10, 12]. A large number of subjects with a higher Gensini score may be needed to reveal a relationship between the PCSK9 level and the severity of CAD. Second, CCTA is not a gold standard for the evaluation of CAD, although recent studies have shown that both its sensitivity and specificity were approximately 95% of those for invasive coronary angiography for the identification of significant coronary stenosis [42]. Third, calcified lesions were omitted from the evaluation. Seventy-seven segments of 24/393 patients (6.1%) could not be evaluated due to severe calcification. Fourth, there were fewer female patients in the multi-vessel disease group. PCSK9 levels in females have been shown to be significantly higher than those in males [43]. Fifth, the patients in the statin group received various types and doses of statin (Data not shown).

Conclusion

PCSK9 levels, in addition to age, gender, BMI, DM, and HDL-C, were significantly associated with the presence of CAD by a multivariable analysis in Japanese patients. However, PCSK9 levels were not significantly associated with the severity of CAD.

Compliance with ethical standards

Conflict of interest KS and SM are Directors of NPO Clinical and Applied Science, Fukuoka, Japan. KS and SM received a grant from the Public Interest Incorporated Foundation of "Clinical Research Promotion Foundation" in Fukuoka, Japan, and part of this work was transferred to NPO Clinical and Applied Science, Fukuoka, Japan. KS has an Endowed Department of Molecular Cardiovascular Therapeutics (SM), Fukuoka University, supported by MSD Co., Ltd.

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