The official journal of the Japan Atherosclerosis Society and
the Asian Pacific Society of Atherosclerosis and Vascular Diseases

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Original Article

J Atheroscler Thromb, 2023; 30: 767-777. http://doi.org/10.5551/jat.63549

Relationship between Serum Proprotein Convertase Subtilisin/Kexin Type 9 Concentration and Prevalence of Coronary Artery Calcium in a Community-Based Sample of Japanese Men

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Aims: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a promising new target for reducing low-density lipoprotein cholesterol (LDL-C) and cardiovascular events in high-risk patients. However, the influence of circulating PCSK9 concentration on atherosclerotic plaque formation in the general population remains unknown. We assessed the relationship between serum PCSK9 concentration and coronary artery calcium (CAC) prevalence in the general population.

Methods: Community-dwelling Japanese men (n=622) aged 46–82 years without a history of cardiovascular disease and lipid-lowering medications were included. Serum PCSK9 concentration and CAC score were measured using the Agatston method, and the multivariable analysis was used to assess their association. CAC was defined as an Agatston score of >10. We conducted further analysis stratified by age (<60, 60–69, and ≥ 70 years).

Results: The average age, LDL-C, and median serum PCSK9 concentration were 68 years, 122 mg/dL, and 240 ng/mL, respectively. After multivariable adjustment for traditional cardiovascular risk factors, no significant association was observed between serum PCSK9 concentration and CAC prevalence (adjusted relative risk [aRR] 1.05, 95% confidence interval [CI] 0.97–1.13). With age stratification, serum PCSK9 concentration was significantly associated with CAC prevalence in men aged <60 years (aRR 1.38, 95% CI 1.01–1.88) but not in men aged 60–69 years (aRR 0.96, 95% CI 0.85–1.10) or ≥ 70 years (aRR 1.08, 95% CI 0.99–1.19).

Conclusions: A higher serum PCSK9 concentration was associated with a higher CAC prevalence in men aged <60 years, which was independent of traditional cardiovascular risk factors.

Key words: Atherosclerosis, Coronary artery calcium, Proprotein convertase subtilisin/kexin type 9

Introduction

Proprotein convertase subtilisin/kexin type 9 (PCSK9) plays an essential role in low-density lipoprotein cholesterol (LDL-C) regulation. PCSK9 binds the LDL receptor on the surface of hepatocytes

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Received: February 18, 2022 Accepted for publication: August 21, 2022

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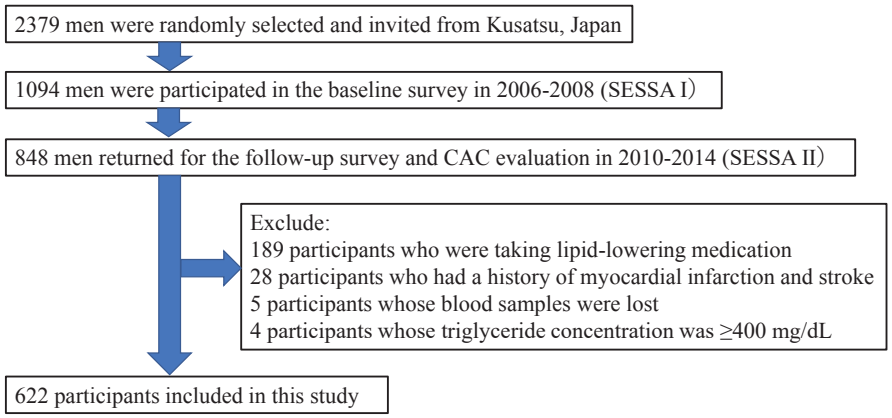

Fig. 1. Flowchart of participant selection
CAC, coronary artery calcium

and guides it for degradation[1-3]. Recently, PCSK9 has received increasing attention as a promising therapeutic target for reducing cardiovascular risk. Since 2015, PCSK9 inhibitors have been approved for clinical use. This new drug class has been reported to lower LDL-C by approximately 60% and to reduce coronary plaque volume and the risk of cardiovascular events in high-risk patients with familial hypercholesterolemia and coronary artery disease[4, 5]. However, it is unknown if PCSK9 is associated with cardiovascular outcomes in the general population.

Several previous epidemiological studies have assessed the relationship between serum PCSK9 concentration and cardiovascular outcomes in the general population with inconsistent results[6, 7]. A previous meta-analysis of nine longitudinal cohort studies demonstrated that baseline PCSK9 concentration predicted total cardiovascular events in apparently healthy subjects but not in populations with established cardiovascular or renal disease[8]. Thus, heterogeneity in the characteristics of the target population of each epidemiological study might have caused these inconsistent results.

Few studies have investigated the direct association between PCSK9 concentration and coronary atherosclerotic plaque formation, which is a preliminary stage of cardiovascular disease, in the general population. Additionally, we hypothesized that the association between PCSK9 concentration and coronary atherosclerotic plaque formation is stronger in younger people than in older people, as age itself is a main driver of cardiovascular disease, and younger populations generally have fewer cardiovascular risk factors and healthier than older populations[9, 10].

Coronary artery calcium (CAC) is a well-established marker of both coronary and systemic atherosclerosis, as well as cardiovascular outcomes[11, 12]. In the present study, we assessed the relationship between serum PCSK9 concentration and CAC prevalence in a community-based sample of Japanese men. Additionally, we further assessed these associations after age stratification.

Methods

Study Participants

The Shiga Epidemiological Study of Subclinical Atherosclerosis (SESSA) is a cross-sectional and prospective cohort study designed to assess the risk factors associated with the prevalence and progression of subclinical atherosclerosis in the general population. The details of the SESSA are described elsewhere[13, 14]. In brief, 1094 community-dwelling Japanese men aged 40–79 years were randomly selected from Kusatsu, Shiga, Japan. The participation rate was 46%. The baseline survey was performed from 2006 to 2008 (SESSA I). The follow-up survey was performed from 2010 to 2014 (SESSA II). Among the SESSA I participants, 848 agreed to participate in SESSA II. In the present study, we excluded 189 participants who were taking lipid-lowering medication[15], 28 who had a history of myocardial infarction and stroke, 5 whose blood samples were lost, and 4 whose triglycerides concentration was ≥ 400 mg/dL because we used the Friedewald formula[16] to estimate LDL-C concentration. Consequently, 622 participants were analyzed in this study **(Fig. 1)**. We obtained written informed consent from all participants. This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the institutional review board of Shiga University of Medical Science.

Measurements of Risk Factors

Trained nurses measured the height and weight of the participants following the standardized protocol. Body mass index (BMI) was calculated as weight (in kg) divided by height squared (in m^2). Blood pressure was measured twice in the sitting position after 5 min of rest using an automated sphygmomanometer (BP-8800; Omron Colin, Tokyo, Japan). The average of two blood pressure measurements was used in the analysis. Hypertension was defined as a blood pressure of ≥ 140/90 mmHg and/or current use of antihypertensive medications. Blood samples were collected after fasting for 12 h. Serum was separated by centrifugation (1000 g for 15 min at 4℃) less than 90 min after blood collection. Routine laboratory testing, including diabetic and lipid profiles, was performed, and other samples were immediately frozen at −80℃ until further analysis. Total cholesterol and triglycerides concentrations were measured using an enzymatic assay. High-density lipoprotein cholesterol (HDL-C) was measured using a direct method. LDL-C was estimated using the Friedewald formula[16]. Diabetes mellitus was defined as a glycated hemoglobin (National Glycohemoglobin Standardization Program) of ≥ 6.5%, a fasting blood glucose concentration of ≥ 126 mg/dL, or current use of antidiabetic medications. Data on smoking and drinking habits and medical history were obtained using questionnaires. Smoking habits were defined as either "current," "past," or "never." Past smokers were those who quit and who did not smoke in the 30 days preceding the study. Pack-year smoking was calculated by multiplying the average number of packs smoked per day by the number of smoking years.

Measurement of PCSK9 Concentration

Two forms of PCSK9 have been reported to have existed in circulation, namely, mature and furin-cleaved PCSK9 with different properties to degrade LDL receptors[17, 18]. In 2018, stored blood samples were thawed, and total, mature, and furin-cleaved PCSK9 concentrations were measured at the clinical chemistry laboratory of BML Inc. using a sandwich enzyme-linked immunosorbent assay kit (BML Inc., Tokyo, Japan), as previously reported[19]. The intra-assay and interassay coefficients of variance of total, mature, and furin-cleaved PCSK9 were 2.3% and 7.5%, 2.2% and 7.7%, and 2.1% and 5.6%, respectively[20].

CAC Assessments

CAC was measured by a 16-channel multidetector-row computed tomography using an Aquilion scanner (Toshiba, Tokyo, Japan). Images were obtained of every 3-mm slice from the aortic root to the heart with a scan time of 320 ms. Images were obtained at 70% of the cardiac cycle with electrocardiogram triggering during a single breath hold. Calculation of CAC was performed using AccuImage software (AccuImage Diagnostics, San Francisco, CA, USA). CAC was defined as a minimum of three contiguous pixels (area = 1 mm^2) with a density of ≥ 130 HU. Peak density (HU) and area (mm^2) were measured for each high-density lesion in the epicardial coronary arteries, and CAC was calculated following the widely accepted Agatston method[21]. All images were assessed by one trained physician who was blinded to the participants' clinical information. The standardized protocol for CAC assessment was developed from a separate cohort study by our research group[22], in which the scan reproducibility showed an intraclass correlation of 0.98 [23, 24]. In the current study, the presence of CAC was defined as an Agatston score of > 10, which is predictive of an increased cardiovascular risk[25]. We also performed the same analyses using an Agatston score of > 0 to define the presence of CAC, and similar results were obtained (data not shown). There were eight and two participants with an Agatston score of > 100 and > 300, respectively, in the < 60-year age group; thus, we could not appropriately analyze using these thresholds.

Statistical Analysis

Calculations were performed using SPSS, version 28.0 (IBM Corp., Armonk, NY, USA). Normally distributed continuous variables are expressed as mean ± standard deviation (SD). Nonnormally distributed continuous variables are expressed as median (interquartile range). Categorical variables are expressed as number (percentage). We compared normally distributed continuous variables using analysis of variance and nonnormally distributed variables using the Kruskal–Wallis test, whereas categorical variables were compared using Fisher's exact test or chi-square test. Correlations between total, mature, and furin-cleaved PCSK9 concentrations and other continuous variables of interest were analyzed using Spearman's correlation analysis. Univariable and multivariable Poisson regression with robust error variance[26] were used to estimate the relative risk (RR) and 95% confidence interval (CI) per 1 SD of the serum PCSK9 concentration for the presence of CAC. This is because the prevalence of CAC was > 10% in this study, and odds ratios could not be interpreted as RRs. Model 1 was adjusted for age, BMI, smoking habit (current, past, never), pack-year smoking, alcohol

Table 1. Baseline characteristics of the study participants stratified by PCSK9 concentration

The cohort included 622 men aged 46–82 years from 2010 to 2014, who were recruited from Kusatsu City, Shiga, Japan

	Overall n=622	Lower PCSK9 n=311	Higher PCSK9 n=311	p-value
Age, years	68 ± 9	70 ± 8	66 ± 9	<0.001
Body mass index, kg/m^2	23.0 ± 2.9	22.6 ± 2.7	23.4 ± 3.0	0.001
Smoking, *n* (%)				0.583
Current	134 (21.5)	65 (20.9)	69 (22.2)	
Past	372 (59.8)	183 (58.8)	189 (60.8)	
Never	116 (18.6)	63 (20.3)	53 (17.0)	
Pack-year smoking	22.0 (5.1 – 41.0)	22.0 (4.5 – 40.0)	22.5 (6.5 – 41.4)	0.516
Alcohol intake (g/week)	98.0 (2.0 – 231.8)	69.1 (0.7 – 196.0)	134.6 (10.4 – 277.4)	<0.001
Diabetes mellitus, *n* (%)	109 (17.6)	59 (19.0)	50 (16.1)	0.352
Fasting glucose, mg/dL	100 ± 20	100 ± 21	101 ± 19	0.582
Glycated hemoglobin, %	5.9 ± 0.8	5.9 ± 0.8	5.9 ± 0.7	0.764
Antidiabetic medication use, *n* (%)	69 (11.1)	35 (11.3)	34 (10.9)	0.898
Hypertension, *n* (%)	329 (52.9)	165 (53.1)	164 (52.7)	0.936
Systolic blood pressure, mmHg	132 ± 17	131 ± 17	133 ± 17	0.149
Diastolic blood pressure, mmHg	77 ± 11	76 ± 11	79 ± 10	0.002
Antihypertensive medication use, *n* (%)	200 (32.3)	99 (31.9)	101 (32.6)	0.864
Total cholesterol, mg/dL	205 ± 35	198 ± 32	212 ± 36	<0.001
HDL-cholesterol, mg/dL	60 ± 13	59 ± 16	61 ± 17	0.097
Triglycerides, mg/dL	99 (70 – 138)	92 (65 – 121)	111 (76 – 152)	<0.001
LDL-cholesterol, mg/dL	122 ± 32	118 ± 30	125 ± 32	0.002
Total PCSK9, ng/mL	240 (205 – 291)	205 (179 – 221)	291 (256 – 329)	<0.001
Mature PCSK9, ng/mL	219 (186 – 265)	186 (166 – 202)	265 (235 – 306)	<0.001
Furin-cleaved PCSK9, ng/mL	20 (17 – 25)	17 (15 – 20)	25 (21 – 28)	<0.001
Presence of CAC, *n* (%)	346 (55.6)	173 (55.6)	173 (55.6)	1.000
CAC score, Agatston	20.3 (0.0 – 151.3)	22.8 (0.0 – 145.4)	19.7 (0.0 – 159.2)	0.837

Lower and higher PCSK9 was defined by lower and higher than 240mg/ml (median) in PCSK9, respectively. Normally distributed continuous variables are expressed as mean ± standard deviation. Non–normally distributed continuous variables are expressed as median (interquartile range). Categorical values are expressed as number (percentage). CAC, coronary artery calcium; HDL, high–density lipoprotein; LDL, low–density lipoprotein; PCSK9, proprotein convertase subtilisin/ kexin type 9.

intake (g/week), diabetes mellitus, and hypertension. Model 2 was adjusted for the variables included in Model 1 and LDL-C. Model 3 was adjusted for the variables included in Model 2, HDL-C, and triglycerides. A subgroup analysis was performed after stratification by age (<60, 60–69, and ≥ 70 years). A p value of <0.05 was considered as statistically significant.

Results

Baseline Characteristics

Table 1 shows the baseline characteristics of the participants. The average age, LDL-C, and median total PCSK9 concentration were 68 years, 122 mg/dL, and 240 ng/mL, respectively. The distribution of serum PCSK9 concentration among the overall participants was positively skewed (Fig. 2). The prevalence of CAC was 55.6%. After dividing participants into lower or higher than 240 ng/mL (median) of total PCSK9 concentration, a higher total PCSK9 concentration was significantly associated with younger age, higher BMI, higher alcohol intake, and higher triglycerides and LDL-C concentrations. Table 2 shows the baseline characteristics of each age group. BMI, the rate of never smokers, triglycerides concentration, LDL-C concentration, and total and mature PSCK9 concentrations were significantly higher in men aged <60 years than in men aged 60–69 or ≥ 70 years. Pack-year smoking; the prevalence of diabetes mellitus, hypertension, and CAC; and Agatston score were significantly higher in men aged 60–69 and ≥ 70 years than in men aged < 60 years. In contrast, no significant difference was observed in furin-cleaved PCSK9 concentration among the age groups.

Correlations between Serum PCSK9 Concentration and other Variables

Table 3 shows the correlations of total, mature,

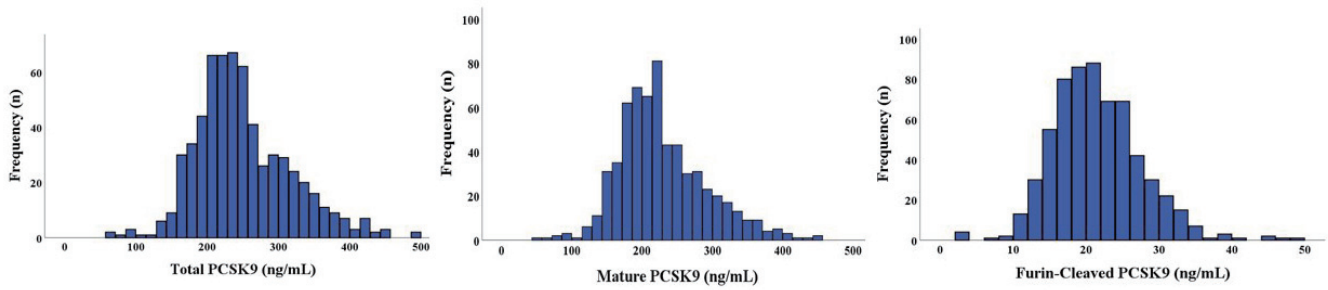

Fig. 2. Distribution of serum PCSK9 concentration among the overall cohort

PCSK9, proprotein convertase subtilisin/kexin type 9

Table 2. Baseline characteristics of the study participants stratified by age group

Participants included 622 men aged 46–82 years from 2010 to 2014, who were recruited from Kusatsu City, Shiga, Japan.

	age <60 *n*=89	age 60–69 *n*=236	70 ≤ age *n*=297	*p*-value
Age, years	51 ± 4	66 ± 3	75 ± 3	<0.001
Body mass index, kg/m^2	23.8 ± 3.0	23.2 ± 2.9	22.7 ± 2.7	0.003
Smoking, *n* (%)				<0.001
Current	31 (34.8)	59 (25.0)	44 (14.8)	
Past	38 (42.7)	140 (59.3)	194 (65.3)	
Never	20 (22.5)	37 (15.7)	59 (19.9)	
Pack-year smoking	15.0 (0.9 – 28.4)	23.8 (7.5 – 42.0)	23.0 (5.6 – 44.8)	0.006
Alcohol intake (g/week)	84.0 (10.5 – 227.9)	110.0 (10.4 – 255.1)	84.7 (0.7 – 206.9)	0.042
Diabetes mellitus, *n* (%)	9 (10.1)	39 (16.5)	61 (20.6)	0.062
Fasting glucose, mg/dL	98 ± 14	99 ± 20	102 ± 21	0.135
Glycated hemoglobin, %	5.8 ± 0.5	5.9 ± 0.7	5.9 ± 0.9	0.688
Antidiabetic medication, *n* (%)	2 (2.2)	24 (10.2)	43 (14.5)	0.005
Hypertension, *n* (%)	28 (31.5)	123 (52.1)	178 (59.9)	<0.001
Systolic blood pressure, mmHg	128 ± 16	131 ± 16	134 ± 18	0.021
Diastolic blood pressure, mmHg	82 ± 12	79 ± 10	75 ± 10	<0.001
Antihypertensive medication, *n* (%)	11 (12.4)	78 (33.1)	111 (37.6)	<0.001
Total cholesterol, mg/dL	216 ± 38	207 ± 32	200 ± 35	<0.001
HDL-cholesterol, mg/dL	60 ± 13	61 ± 17	59 ± 16	0.367
Triglycerides, mg/dL	107 (78 – 157)	104 (70 – 145)	96 (68 -127)	0.024
LDL-cholesterol, mg/dL	130 ± 37	121 ± 32	119 ± 29	0.015
Total PCSK9, ng/mL	267 (219 – 326)	243 (207 – 296)	230 (200 – 267)	<0.001
Mature PCSK9, ng/mL	247 (198 -308)	221 (188 – 272)	209 (182 – 244)	<0.001
Furin-cleaved PCSK9, ng/mL	21 (17 – 27)	21 (17 – 25)	20 (17 – 24)	0.131
Presence of CAC, *n* (%)	23 (25.8)	129 (54.7)	194 (65.3)	<0.001
CAC score, Agatston	0.0 (0.0 – 11.6)	22.6 (0.0 – 153.3)	48.2 (0.0 – 213.9)	<0.001

Normally distributed continuous variables are expressed as mean±standard deviation. Non-normally distributed continuous variables are expressed as median (interquartile range). Categorical values are expressed as number (percentage). CAC, coronary artery calcium; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9.

and furin-cleaved PCSK9 concentrations with other variables according to Spearman's correlation analysis. Total and mature PCSK9 concentrations were more strongly correlated with age (ρ=–0.22, p<0.001; ρ=–0.23, p<0.001) and triglycerides (ρ=0.25, p<0.001; ρ=–0.26, p<0.001) than furin-cleaved PCSK9 concentration. Furin-cleaved PCSK9 concentration was more strongly correlated with LDL-C (ρ=–0.29, p<0.001) than total and mature PCSK9 concentrations. Mature and furin-cleaved PCSK9 concentrations were both highly correlated with total PCSK9 concentration (ρ=–0.99, p<0.001;

Table 3. Correlations between serum PCSK9 concentration and other variables

Variables	Total PCSK9		Mature PCSK9		Furin-cleaved PCSK9	
	ρ	p–value	ρ	p–value	ρ	p–value
Age, years	–0.22	<0.001	–0.23	<0.001	–0.10	0.017
Body mass index, kg/m^2	0.11	0.008	0.10	<0.001	0.12	0.003
Pack-year smoking	0.06	0.137	0.07	0.100	–0.02	0.715
Alcohol intake, g/week	0.16	<0.001	0.17	<0.001	0.01	0.814
Fasting glucose, mg/dL	0.05	0.226	0.04	0.284	0.10	0.016
Glycated hemoglobin, %	0.04	0.384	0.02	0.565	0.13	0.001
Systolic blood pressure, mmHg	0.08	0.038	0.09	0.033	0.05	0.246
Diastolic blood pressure, mmHg	0.14	<0.001	0.15	<0.001	0.05	0.219
LDL-cholesterol, mg/dL	0.16	<0.001	0.14	<0.001	0.29	<0.001
HDL-cholesterol, mg/dL	0.04	0.275	0.03	0.471	0.14	<0.001
Triglycerides, mg/	0.25	<0.001	0.26	<0.001	0.12	0.004
Total PCSK9, ng/mL	–	–	0.99	<0.001	0.78	<0.001
Mature PCSK9, ng/mL	0.99	<0.001	–	–	0.74	<0.001
Furin-cleaved PCSK9, ng/mL	0.78	<0.001	0.74	<0.001	–	–

Correlations were analyzed using Spearman's correlation analysis. HDL, high-density lipoprotein; LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9.

$\rho = -0.78$, $p < 0.001$).

Association of PCSK9 with CAC Prevalence

No significant association was observed between total PCSK9 concentration (per 1 SD increase) and CAC prevalence among the overall cohort (RR 1.04, 95% CI 0.97–1.11; **Table 4**). However, total PCSK9 concentration was significantly associated with CAC prevalence among men aged <60 years in the univariable analysis (RR 1.35, 95% CI 1.04–1.77) and the multivariable analysis (RR 1.38, 95% CI 1.01–1.88), even after adjusting for traditional coronary risk factors and lipid parameters, including LDL-C concentration. Total PCSK9 concentration was not associated with CAC prevalence in men aged 60–69 years (RR 0.96, 95% CI 0.85–1.10) and men aged ≥ 70 years (RR 1.08, 95% CI 0.99–1.19).

Mature and furin-cleaved PCSK9 concentrations (per 1 SD increase) were both significantly associated with CAC prevalence among men aged <60 years (RR 1.36, 95% CI 1.01–1.83; RR 1.66, 95% CI 1.01–2.71). Unexpectedly, furin-cleaved PCSK9 was significantly associated with CAC prevalence among men aged ≥ 70 years (RR 1.11, 95% CI 1.02–1.20).

Discussion

In this study, we assessed the relationship between serum PCSK9 concentration and CAC prevalence in apparently healthy Japanese men without lipid-lowering medications. Serum PCSK9 concentration was significantly associated with CAC prevalence among men aged <60 years, independent of other traditional coronary risk factors and lipid parameters, including LDL-C. A recent study demonstrated that serum PCSK9 concentration was independently associated with the presence and severity of CAC in asymptomatic patients with familial hypercholesterolemia[27]. However, to the best of our knowledge, few studies have reported the relationship between serum PCSK9 concentration and CAC prevalence in the general population. The results of our study suggest that serum PCSK9 concentration is associated with earlier-stage atherosclerosis. However, further studies are warranted to confirm our results.

In the current study, a higher total PCSK9 concentration was associated with younger age, increased alcohol intake, and higher triglycerides and LDL-C concentrations. Previous studies reported that circulating PCSK9 concentration is associated with age, LDL-C, triglycerides, HDL-C, insulin resistance, smoking, and BMI[6, 28-30]. However, these associations were inconsistent and not well established, apart from the associations with LDL-C and triglycerides. The associations between total PCSK9 and LDL-C and triglycerides were often modest but consistent, and we found similar associations in this study.

In this study, total PCSK9 concentration was associated with CAC prevalence among men aged < 60 years, independent of other traditional coronary risk factors. However, this relationship was not observed among men aged ≥ 60 years. Dyslipidemia is one of the established cardiovascular risk factors.

Table 4. Association between PCSK9 concentration (per 1 SD increase) and CAC prevalence

Group	Univariable	Multivariable (Model 1)	Multivariable (Model 2)	Multivariable (Model 3)
	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)
Total PCSK9				
Overall	1.04	1.08*	1.06	1.05
n=622	(0.97 – 1.11)	(1.00 – 1.15)	(0.99 – 1.14)	(0.97 – 1.13)
age <60	1.35*	1.45**	1.38*	1.38*
n=89	(1.04 – 1.77)	(1.09 – 1.91)	(1.02 – 1.88)	(1.01 – 1.88)
age 60–69	1.00	1.01	0.99	0.96
n=236	(0.89 – 1.13)	(0.89 – 1.14)	(0.88 – 1.12)	(0.85 – 1.10)
70 ≤ age	1.11*	1.09*	1.09	1.08
n=297	(1.02 – 1.20)	(1.00 – 1.19)	(0.99 – 1.19)	(0.99 – 1.19)
Mature PCSK9				
Overall	1.03	1.08*	1.06	1.05
n=622	(0.96 – 1.11)	(1.00 – 1.15)	(0.99 – 1.14)	(0.97 – 1.13)
age <60	1.34*	1.42*	1.36*	1.36*
n=89	(1.03 – 1.73)	(1.08 – 1.87)	(1.01 – 1.83)	(1.01 – 1.83)
age 60–69	1.01	1.01	1.00	0.97
n=236	(0.89 – 1.13)	(0.90 – 1.14)	(0.89 – 1.13)	(0.85 – 1.11)
70≤ age	1.11*	1.09	1.08	1.08
n=297	(1.02 – 1.20)	(1.00 – 1.19)	(0.99 – 1.18)	(0.99 – 1.18)
Furin-cleaved PCSK9				
Overall	1.06	1.07*	1.05	1.04
n=622	(0.99 – 1.13)	(1.00 – 1.14)	(0.98 – 1.13)	(0.97 – 1.12)
age <60	1.37*	1.61**	1.62*	1.66*
n=89	(1.04 – 1.81)	(1.14 – 2.28)	(1.04 – 2.52)	(1.01 – 2.71)
age 60–69	0.95	0.94	0.91	0.89
n=236	(0.83 – 1.08)	(0.83 – 1.06)	(0.80 – 1.04)	(0.78 – 1.02)
70 ≤ age	1.12**	1.11*	1.10*	1.11*
n=297	(1.05 – 1.20)	(1.03 – 1.19)	(1.02 – 1.19)	(1.02 – 1.20)

Model 1 was adjusted for age, body mass index, smoking habit (current, past, never), pack-year smoking, alcohol intake (g/week), diabetes mellitus, hypertension. Model 2 was adjusted for the variables included in Model 1 and LDL-C. Model 3 was adjusted for the variables included in Model 2, HDL-C, and triglycerides. CAC, coronary artery calcium; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RR, relative risk; PCSK9, proprotein convertase subtilisin/kexin type 9; SD, standard deviation. *$p<0.05$. **$p<0.01$.

However, the relationship between dyslipidemia and cardiovascular events often attenuated or diminished in the elderly populations as previously reported[31, 32], although underlying mechanisms are not fully elucidated. Age itself is a main driver of atherosclerosis, and elderly people are often affected with multiple cardiovascular risk factors than younger people[33]. The influence of dyslipidemia on atherosclerosis might be suppressed by that of age itself and the accumulation of through life exposure to other risk factors. In this study, pack-year smoking and the prevalence of diabetes mellitus and hypertension were significantly higher in men aged ≥ 60 years than in men aged <60 years. The influence of these established risk factors, along with older age itself, might have contributed to the development of CAC to a greater degree than PCSK9 concentration in men aged ≥ 60 years. However, this was not elucidated in this study.

Previous studies have reported that gain-of-function variants of PCSK9 are associated with increased LDL-C and more severe coronary artery disease[34, 35]. Furthermore, the significant correlation between PCSK9 concentration and carotid artery intimal–medial thickness independent of other cardiovascular risk factors was revealed in the general population[36, 37]. The prospective cohort study of community-dwelling subjects aged 60 years at enrollment found a significant relationship between serum PCSK9 concentration and cardiovascular

events[6]. A meta-analysis of nine longitudinal studies found that baseline PCSK9 concentration predicted total cardiovascular events only in apparently healthy subjects and not in populations with established cardiovascular or renal disease[8]. All the results of the abovementioned studies appear to support our results.

The most widely reported functional role of PCSK9 is the regulation of LDL-C metabolism. However, recent studies have suggested that PCSK9 has multiple functions in the progression of atherosclerosis aside from LDL-C regulation. For instance, very-LDL-C (VLDL-C), which is known as remnant cholesterol or triglyceride-rich lipoprotein, is also regulated by PCSK9 [37, 38]. PCSK9 inhibitors were also reported to lower triglycerides and raise HDL-C and apolipoprotein A-1 [39, 40]. Lipoprotein(a) is an LDL particle, and previous epidemiological studies have consistently demonstrated it to be an independent risk factor for atherosclerotic cardiovascular disease[41]. PCSK9 inhibitors have also been found to reduce lipoprotein(a) by approximately 25%–30%, although the underlying mechanism of this effect remains unknown[42]. Mechanisms other than LDL-C regulation might have led to our observations. However, we did not measure VLDL-C or lipoprotein(a) in this study. Thus, further studies are warranted to confirm this hypothesis.

Previous studies have reported that mature PCSK9 has greater properties to degrade LDL receptors, whereas furin-cleaved PCSK9 was reported to have none or much less activity to regulate LDL receptors[17, 18]. A previous study reported that mature PCSK9, but not furin-cleaved PCSK9, was associated with coronary plaque volume in patients with heterozygous familial hypercholesterolemia and coronary artery disease[20]. In contrast, it is also reported that furin-cleaved PCSK9, but not mature PCSK9, was associated with future coronary events in Japanese subjects[43]. There are still limited in vitro and in vivo studies regarding the proatherogenic properties of these two PCSK9 subtypes, and further studies are warranted. In the present study, both mature and furin-cleaved PCSK9 concentrations were positively correlated with triglycerides or LDL-C, and both subtypes were associated with CAC prevalence in men aged <60 years. It might be observed as long as total PCSK9 concentration was associated with CAC prevalence, because both mature and furin-cleaved PCSK9 concentrations are highly correlated with total PCSK9 concentration in this study. Unexpectedly, furin-cleaved PCSK9 was associated with CAC prevalence in men aged ≥ 70 years in this study. We could not estimate if this result was caused by a chance or by unmeasured specific properties of furin-cleaved PCSK9 from the results of this study, and further studies are warranted.

Study Limitations

This study has several limitations. First, the relatively small number of participants might have caused insufficient statistical power, and the cross-sectional nature of this study did not allow us to make causative conclusions. Second, the participants were solely Japanese men in this study. Third, serum PCSK9 concentrations were reported to be higher in women than in men and in postmenopausal women than in premenopausal women[28]. Thus, our findings cannot be generalized to other populations. Fourth, we could only measure circulating PCSK9 concentration and not intracellular or intraplaque PCSK9 concentration in this study. PCSK9 is also reported to be expressed in the small intestinal cells, endothelial cells, vascular smooth muscle cells, and atherosclerotic plaque, which might have affected our results. Finally, unmeasured confounding factors or drugs other than lipid-lowering medication might have affected our results[44].

Conclusion

We found a significant association between serum PCSK9 concentration and CAC prevalence independent of other established risk factors, including LDL-C, in apparently healthy middle-aged men without lipid-lowering medications.

Acknowledgements

The authors would like to thank staff members of the Shiga Epidemiological Study of Subclinical Atherosclerosis study (SESSA). The SESSA Research Group is composed of the following investigators:

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Financial Support

The SESSA (Shiga Epidemiological Study of Atherosclerosis) has been supported by JSPS KAKENHI Grant Number JP13307016, JP17209023, JP21249043, JP23249036, JP25253046, JP15H02528, JP18H04074, and JP18K17378 from the Ministry of Education, Culture, Sports, Science and Technology Japan, by grant R01HL068200 from NIH, from Glaxo-Smith Kline GB. The present study was initiated and analyzed by the authors. The funding sources listed above have no role in the study design, collection.

Conflict of Interest

The authors do not have any conflict of interest to declare.

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