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ORIGINAL RESEARCH

# Circulating Furin-Cleaved Proprotein Convertase Subtilisin/Kexin Type 9 Concentration Predicts Future Coronary Events in Japanese Subjects

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## ABSTRACT

**BACKGROUND** Proprotein convertase subtilisin/kexin type 9 (PCSK9) circulates as mature and furin-cleaved forms, which differ in their properties to degrade low-density lipoprotein (LDL) receptors.

**OBJECTIVES** In this study, we sought to investigate whether PCSK9 subtypes associate with atherosclerotic cardiovascular events.

**METHODS** We investigated 1,436 statin-naive Japanese subjects without any cardiovascular disease in the Suita Study, an epidemiologic Japanese cohort study. Total, mature, and furin-cleaved PCSK9 levels were measured by means of enzyme-linked immunosorbent assay. The occurrence of coronary and stroke events were compared in subjects stratified by PCSK9 level tertile.

**RESULTS** Total, mature, and furin-cleaved PCSK9 levels were associated with non-high-density lipoprotein cholesterol (all $P < 0.001$) and systolic blood pressure ($P = 0.001$, $P = 0.004$, and $P < 0.001$, respectively). Furthermore, only furin-cleaved PCSK9 level was correlated to high-sensitivity C-reactive protein (hs-CRP) ($P < 0.001$). During the 13.6-year observational period, furin-cleaved PCSK9 level predicted a greater likelihood of experiencing coronary events (tertile 2: hazard ratio [HR]: 2.84 [95% confidence interval [CI]: 1.21-6.65; $P = 0.01$]; tertile 3: HR: 2.81 [95% CI: 1.17-6.74; $P = 0.02$]), but not stroke (tertile 2: HR: 1.31 [95% CI: 0.72-2.40; $P = 0.36$]; tertile 3: HR: 1.27 [95% CI: 0.68-2.38; $P = 0.44$]). Total and mature PCSK9 levels were not associated with coronary events (total PCSK9: tertile 2: HR: 1.35 [95% CI: 0.68-2.68; $P = 0.39$]; tertile 3: HR: 1.13 [95% CI: 0.54-2.34; $P = 0.73$]; mature PCSK9: tertile 2: HR: 1.02 [95% CI: 0.52-2.02; $P = 0.93$]; tertile 3: HR: 0.96 [95% CI: 0.47-1.95; $P = 0.92$]) and stroke events (total PCSK9: tertile 2: HR: 0.90 [95% CI: 0.50-1.61; $P = 0.72$]; tertile 3: HR: 0.99 [95% CI:0.54-1.80; $P = 0.97$]; mature PCSK9: tertile 2: HR: 0.86 [95% CI: 0.47-1.57; $P = 0.63$]; tertile 3: HR: 1.11 [95% CI: 0.61-1.99; $P = 0.72$]), respectively.

**CONCLUSIONS** Furin-cleaved but not total and mature PCSK9 was associated with both LDL cholesterol and hs-CRP and predicted future coronary events in the primary prevention settings. Our findings provide pathophysiological insights into the properties of PCSK9 subtypes in association with coronary events. (JACC: Asia 2021;1:360–368) © 2021 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease associated with low-density lipoprotein (LDL) metabolism (1-3). It presents as mature and furin-cleaved PCSK9 in circulation (4-7). In vitro studies reported that the activity to degrade LDL receptor differs in each subtype and that mature PCSK9 exhibits a greater ability of this property compared with furin-cleaved (4-7). Because low-density lipoprotein-cholesterol (LDL-C) is an independent and established risk factor associated with atherosclerotic cardiovascular diseases, mature and furin-cleaved PCSK9 concentrations may help to stratify future risks of atherosclerotic cardiovascular diseases.

The commercially available enzyme-linked immunosorbent assay (ELISA) method enables measuring circulating total PCSK9 but not each subtype level. The association of total PCSK9 level with cardiovascular events has been recently investigated by means of cohort studies in the primary and secondary prevention settings, and the findings are not consistent (8-10). This may suggest that measuring total PCSK9 concentration does not necessarily and properly reflect its biological activity in circulation, leading to inconsistent results. Therefore, in the present study we used our recently developed ELISA, that enables quantitative measurements of mature and furin-cleaved PCSK9 levels (11), and then investigated the ability of these subtypes to predict the occurrence of coronary artery disease and stroke in Japanese subjects.

## METHODS

**SUITA STUDY.** The Suita Study is a prospective population-based cohort study of cardiovascular diseases in the urban city of Suita, Osaka, Japan. The details of the Suita Study have been described elsewhere (14). Briefly, in 1989, 6,485 Suita city residents (30-79 years of age) were enrolled as study participants. Of these, 2,315 participants underwent medical examinations from April 1994 to February 1995, and fasting serum samples in 1,676 subjects were collected and stored at −80 °C. Subjects with the following were excluded from the present analysis: a history of cardiovascular diseases (n = 68), loss to follow-up (n = 30), incident subarachnoid hemorrhage during follow-up (n = 11), use of any lipid-lowering agents at baseline (n = 72), and missing data (n = 59). The remaining 1,436 statin-naive subjects without any history of cardiovascular diseases were included into the present analysis. This study was approved by the Institutional Review Board Committee of the National Cerebral and Cardiovascular Center, and all patients gave written informed consent (M27-035-4).

**TOTAL, MATURE, AND FURIN-CLEAVED PCSK9 MEASUREMENT.** Total, mature, and furin-cleaved PCSK9 concentrations were measured in the stored fasting serum samples with the use of an ELISA (BML) (11-13). This sandwich ELISA enables quantitatively measurement of PCSK9 subtypes by using monoclonal antibodies. The ELISA is characterized by the use of purified recombinant human (rh) PCSK9 or cell lysate of rhΔ218PCSK9 as well as plasma samples (11). Calibration curves in the ELISA for total and mature PCSK9, rhPCSK9 protein as a primary calibrator, and rhPCSK9 culture medium as a secondary calibrator are obtained (11). The inter- and intra-assay coefficients of variance to measure each PCSK9 value were, respectively, as follows; total PCSK9 level: 7.5% and 2.3%; mature PCSK9: 7.7% and 2.2%; furin-cleaved PCSK9: 5.6% and 2.1%. The lower and upper detection limits of total, mature, and furin-cleaved PCSK9 were 4.5 and 25,000 ng/mL, 3.9 and 20,000 ng/mL, and 0.7 and 300 ng/mL, respectively (11).

**MEASUREMENT OF LIPIDS AND HIGH-SENSITIVITY C-REACTIVE PROTEIN.** LDL-C was estimated by means of the Friedewald formula in 1,344 subjects. Non-high-density lipoprotein-cholesterol (HDL-C) was calculated by subtracting HDL-C from the total cholesterol level. High-sensitivity C-reactive protein (hs-CRP) was measured by the latex turbidimetric immunoassay (LSI Medience Corp).

**CLINICAL FOLLOW-UP.** Study subjects were followed until December 31, 2013 (average follow-up period: 13.6 years). All participants received biennial medical checks at the National Cerebral and Cardiovascular Center to evaluate their condition and the occurrence of cardiovascular diseases. In addition, an annual survey with questionnaires by mail or telephone was conducted in all of the subjects. In those who had any cardiovascular diseases during the

### ABBREVIATIONS AND ACRONYMS

**CAD** = coronary artery disease

**ELISA** = enzyme-linked immunosorbent assay

**HDL-C** = high-density lipoprotein-cholesterol

**hs-CRP** = high-sensitivity C-reactive protein

**LDL** = low-density lipoprotein

**LDL-C** = low-density lipoprotein-cholesterol

**PCSK9** = proprotein convertase subtilisin/kexin type 9

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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**TABLE 1 Clinical Demographics (N = 1,436)**

| | |
|---|---|
| Age, y | 58.6 ± 12.3 |
| Male | 563 (39.2) |
| BMI, kg/m$^2$ | 22.1 ± 2.8 |
| Hypertension | 429 (29.9) |
| Type 2 diabetes mellitus | 59 (4.1) |
| Dyslipidemia | 376 (26.2) |
| Current smoking | 316 (22.0) |
| Current drinking | 651 (45.3) |
| HDL-C, mg/dL | 58.3 ± 14.1 |
| NonHDL-C, mg/dL | 141.3 ± 33.9 |
| hs-CRP, ng/mL | 0.03 (0.01-0.08) |
| Systolic BP, mm Hg | 124.7 ± 20.6 |
| Diastolic BP, mm Hg | 75.9 ± 11.2 |
| Total PCSK9, ng/mL | 224 (182-275) |
| Mature PCSK9, ng/mL | 195 (159-242) |
| Furin-cleaved PCSK9, ng/mL | 27 (22-35) |

Values are mean ± SD, n (%), or median (interquartile range).

BMI = body mass index; BP = blood pressure; HDL-C = high-density lipoprotein cholesterol; hs-CRP = high-sensitivity C-reactive protein; PCSK9 = proprotein convertase subxilisin/kexin type 9.

follow-up period, in-hospital medical records were reviewed by registered hospital physicians or research physicians who were blinded to baseline clinical demographics. Death certificates were also systematically evaluated to further identify cardiovascular diseases.

**OUTCOMES.** The primary outcome was defined as the occurrence of coronary artery disease (CAD). CAD included acute myocardial infarction, sudden cardiac death within 24 hours from the onset of symptom, and stable CAD requiring revascularization therapies. Myocardial infarction was defined according to the criteria of the Monitoring Trends and Determinants of Cardiovascular Disease (MONICA) Project (15). The secondary outcome was defined as the occurrence of: 1) stroke; and 2) the composite of coronary and stroke events. The diagnosis of stroke was based on the U.S. National Survey of Stroke criteria (16). Stroke subtypes, including ischemic, intracerebral hemorrhage, and unclassified, were diagnosed on the basis of computed tomography, magnetic resonance imaging, or autopsy results. The present study collected any coronary and stroke events during the observational period.

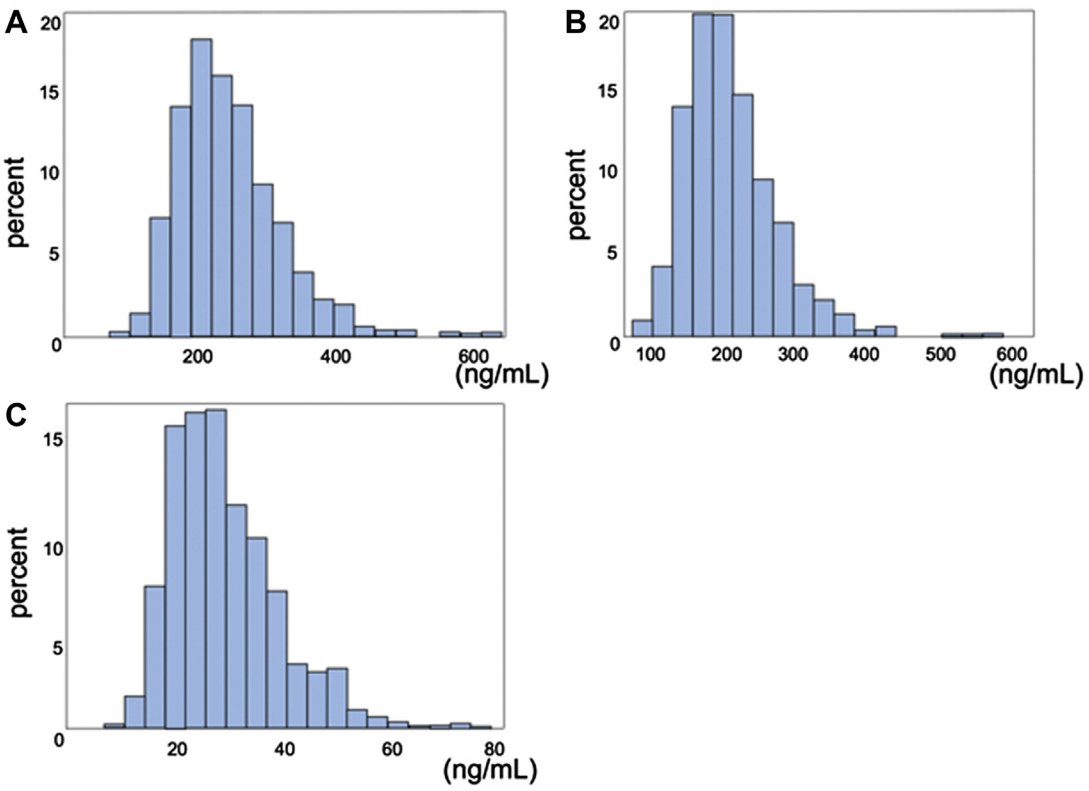
**STATISTICAL ANALYSIS.** Continuous variables are expressed as mean ± SD or median (interquartile range) when not normally distributed. Categoric variables are expressed as percentage. The relationships of clinical and biochemical parameters with each PCSK9 measure (continuous variable) were characterized by Spearman's rank correlation coefficients. A Cox proportional hazard model was used to evaluate the relationship between tertiles of each PCSK9 level and clinical outcomes (coronary and stroke events) after adjusting for age and sex (model 1). The relationship between furin-cleaved PCSK9 level and coronary events was further analyzed by using 2 models: model 2: age, sex, hypertension, diabetes mellitus, current smoking, and current drinking; model 3: age, sex, hypertension, diabetes mellitus, current smoking, current drinking, and HDL-C and hs-CRP levels. A value of $P < 0.05$ was considered to be significant. All statistical analyses were performed with the use of SAS software, version 9.1.3 (SAS Institute).

## RESULTS

**CLINICAL DEMOGRAPHICS OF STUDY PARTICIPANTS.** Clinical characteristics are summarized in Table 1. Study subjects had a low prevalence of risk factors (hypertension 29.9%, type 2 diabetes mellitus 4.1%, dyslipidemia 26.2%) and a mean age of 58.6 years, and 39.2% were male. In addition, normal levels of lipids parameters, hs-CRP, and blood pressure were observed (Table 1). Median levels of total, mature, and furin-cleaved PCSK9 were 224, 195 and 27 ng/mL, respectively (Table 1). The distributions of these PCSK9 concentrations were skewed to the right, as shown in Figure 1.

**RELATIONSHIPS OF TOTAL, MATURE, AND FURIN-CLEAVED PCSK9s WITH CLINICAL RISK MARKERS.** Table 2 presents the associations of each PCSK9 level with atherogenic parameters in the present subjects. All 3 PCSK9 levels were significantly and positively correlated with age (total PCSK9: $r = 0.09$ [95% CI: 0.047-0.150; $P < 0.001$, mature PCSK9: $r = 0.09$ [95% CI: 0.039-0.142; $P < 0.001$]; furin-cleaved PCSK9: $r = 0.11$ [95% CI: 0.067-0.169; $P < 0.001$]), nonHDL-C (total PCSK9: $r = 0.19$ [95% CI: 0.141-0.241; $P < 0.001$]; mature PCSK9: $r = 0.16$ [95% CI: 0.115-0.217; $P < 0.001$]; furin-cleaved PCSK9: $r = 0.28$ [95% CI: 0.236-0.332; $P < 0.001$]), and systolic blood pressure levels (total PCSK9: $r = 0.08$ [95% CI: 0.034-0.137; $P = 0.001$]; mature PCSK9: $r = 0.07$ [95% CI: 0.023-0.127; $P = 0.004$]; furin-cleaved PCSK9: $r = 0.12$ [95% CI: 0.073-0.175; $P < 0.001$]) (Table 2). Similar relationships of total and furin-cleaved PCSK9 levels with body mass index were observed (total PCSK9: $r = 0.06$ [95% CI: 0.016-0.120; $P = 0.009$]; furin-cleaved PCSK9: $r = 0.18$ [95% CI: 0.131-0.232; $P < 0.001$]). In addition, furin-cleaved PCSK9 level was associated with hs-CRP ($r = 0.18$ [95% CI: 0.136-0.237; $P < 0.001$]) and diastolic blood pressure levels ($r = 0.08$ [95% CI: 0.035-0.138; $P = 0.001$]), whereas others were not (hs-CRP: total

**FIGURE 1** **Distributions of Total, Mature, and Furin-Cleaved PCSK9 Levels**




Total, mature, and furin-cleaved PCSK9 levels were measured with the use of an enzyme-linked immunosorbent assay (BML). All of these measures showed nonnormal distribution. Median values (interquartile ranges) of **(A)** total, **(B)** mature, and **(C)** furin-cleaved PCSK9 levels were 224 (182-275), 195 (159-242), and 27 (22-35) ng/mL, respectively. PCSK9 = proprotein convertase subtilisin/kexin type 9.

PCSK9: $r = 0.04$ [95% CI: -0.007-0.095; $P = 0.096$]; mature PCSK9 $r = 0.01$ [95% CI: -0.033-0.070; $P = 0.482$]; diastolic blood pressure level: total PCSK9: $r = 0.03$ [95% CI: −0.012 to 0.091; $P = 0.136$]; mature PCSK9: $r = 0.02$ [95% CI: -0.022-0.081; $P = 0.263$]) (Table 2). Regarding each PCSK9 measure, there were positive relationships among total, mature, and furin-cleaved PCSK9 levels (total-mature PCSK9: $r = 0.99$ [95% CI: 0.992-0.994; $P < 0.001$]; total-furin-cleaved PCSK9: $r = 0.71$ [95% CI: 0.686-0.737; $P < 0.001$]; mature-furin-cleaved PCSK9: $r = 0.62$ [95% CI: 0.596-0.659; $P < 0.001$]) (Table 2).

**CORONARY AND STROKE EVENTS IN THE SUITA STUDY.** In the present study, during the 13.6-year observational period (range 0.002-19.7 years), there were 50 coronary and 51 stroke events. Details of each coronary and cerebrovascular event are summarized in Table 3. Coronary events include 29 acute myocardial infarction, 1 sudden cardiac-death, and 20 stable CAD requiring revascularization (Table 3).

**THE 3 PCSK9 MEASURES AND OUTCOMES.** The relationships of each tertile of the total, mature, and furin-cleaved PCSK9 levels with clinical outcomes are summarized in Table 4. The second and third tertiles of total PCSK9 level did not elevate future risks of coronary events (second tertile: hazard ratio [HR]: 1.35 [95% confidence interval (CI): 0.68-2.68; $P = 0.39$]; third tertile: HR: 1.13 [95% CI: 0.54-2.34; $P = 0.73$]), stroke (second tertile: HR: 0.90 [95% CI: 0.50-1.61; $P = 0.72$]; third tertile: HR: 0.99 [95% CI: 0.54-1.80; $P = 0.97$]), and composite events (second tertile: HR: 0.98 [95% CI: 0.60-1.59; $P = 0.93$]; third tertile: HR: 1.09 [95% CI: 0.67-1.78; $P = 0.71$]) (Table 4). Similar relationships were observed between mature PCSK9 levels and these clinical outcomes. An elevated level of mature PCSK9 level was not associated with the occurrence of coronary events

**TABLE 2 Associations of PCSK9 and Its Subtypes With Atherogenic Parameters**

| | Total PCSK9 | | Mature PCSK9 | | Furin-cleaved PCSK9 | |
|---|---|---|---|---|---|---|
| | *r* | *P* Value | *r* | *P* Value | *r* | *P* Value |
| Age | 0.09 | <0.001 | 0.09 | <0.001 | 0.11 | <0.001 |
| *r*, 95% CI | 0.047-0.150 | | 0.067-0.169 | | 0.039-0.142 | |
| BMI | 0.06 | 0.009 | 0.04 | 0.079 | 0.18 | <0.001 |
| *r*, 95% CI | 0.016-0.120 | | 0.131-0.232 | | −0.005 to 0.098 | |
| HDL-C | 0.01 | 0.651 | 0.02 | 0.42 | −0.05 | 0.059 |
| *r*, 95% CI | −0.040-0.064 | | −0.101-0.022 | | −0.030-0.073 | |
| Non-HDL-C | 0.19 | <0.001 | 0.16 | <0.0001 | 0.28 | <0.001 |
| *r*, 95% CI | 0.141-0.241 | | 0.236-0.332 | | 0.115-0.217 | |
| hs-CRP | 0.04 | 0.096 | 0.01 | 0.482 | 0.18 | <0.001 |
| *r*, 95% CI | −0.007-0.095 | | 0.136-0.237 | | −0.033-0.070 | |
| Systolic BP | 0.08 | 0.001 | 0.07 | 0.004 | 0.12 | <0.001 |
| *r*, 95% CI | 0.034-0.137 | | 0.073-0.175 | | 0.023-0.127 | |
| Diastolic BP | 0.03 | 0.136 | 0.02 | 0.263 | 0.08 | 0.001 |
| *r*, 95% CI | −0.012 to 0.091 | | 0.035-0.138 | | −0.022 to 0.081 | |
| Each PCSK9 measure | | | | | | |
| Total PCSK9 | - | - | 0.99 | <0.001 | 0.71 | <0.001 |
| *r*, 95% CI | - | | 0.992-0.994 | | 0.686-0.737 | |
| Furin-cleaved PCSK9 | 0.71 | <0.001 | 0.62 | <0.001 | - | - |
| *r*, 95% CI | 0.686-0.737 | | 0.596-0.659 | | - | |
| Mature PCSK9 | 0.99 | <0.001 | - | - | 0.62 | <0.001 |
| *r*, 95% CI | 0.992-0.994 | | - | | 0.596-0.659 | |

BMI = body mass index; BP = blood pressure; CI = confidence interval, HDL-C = high-density lipoprotein cholesterol; hs-CRP = high-sensitivity C-reactive protein; PCSK9 = proprotein convertase subxilisin/kexin type 9.

(second tertile: HR: 1.02 [95% CI: 0.52-2.02; $P = 0.93$]; third tertile: HR: 0.96 [95% CI: 0.47-1.95; $P = 0.92$]), stroke (second tertile: HR: 0.86 [95% CI: 0.47-1.57; $P = 0.63$]; third tertile: HR: 1.11 [95% CI: 0.61-1.99; $P = 0.72$]), and composite events (second tertile: HR: 0.91 [95% CI: 0.56-1.46; $P = 0.70$]; third tertile: HR: 0.91 [95% CI: 0.56-1.49; $P = 0.73$]) (Table 4). In contrast, the present analysis showed an increased risk of coronary and composite events in association with higher furin-cleaved PCSK9 levels (coronary events: second tertile: HR: 3.08 [95% CI: 1.32-7.22; $P = 0.009$]; third tertile, HR: 2.93 [95% CI: 1.23-6.98; $P = 0.01$]; composite events: second tertile, HR: 1.99 [95% CI: 1.17-3.39; $P = 0.01$]; third tertile: HR: 1.94 [95% CI: 1.12-3.36; $P = 0.01$]) (Table 4). On multivariable analysis adjusting for age, sex, hypertension, diabetes mellitus, current smoking, current drinking, and HDL-C and hs-CRP levels, the association of higher furin-cleaved levels with a greater frequency of coronary and composite events remained (coronary events: second tertile: HR: 2.84 [95% CI: 1.21-6.65; $P = 0.01$]; third tertile: HR: 2.81 [95% CI: 1.17-6.74; $P = 0.02$]; composite events: HR: 1.74 [95% CI: 1.01-3.01; $P = 0.04$]; third tertile: HR: 1.76 [95% CI: 0.99-3.13; $P = 0.05$]) (Table 4). In 1,344 subjects with LDL-C level at baseline, the association of each PCSK9 measure with outcomes is summarized in Supplemental Table 3. Even after adjusting for LDL-C level, an increased risk of coronary and composite events was still observed in association with furin-clevaed PCSK9 level (coronary events: second tertile: HR: 2.29 [95% CI: 0.94-5.53; $P = 0.06$]; third tertile, HR: 2.52 [95% CI: 1.01-6.27; $P = 0.04$]; composite events: HR: 1.77 [95% CI: 1.01-3.11; $P = 0.04$]; third tertile: HR: 1.93 [95% CI: 1.07-3.51; $P = 0.02$]) (Supplemental Table 3). In contrast to coronary and composite events, furin-cleaved PCSK9 level did not predict the occurrence of stroke events (second tertile: HR: 1.31 [95% CI: 0.72-2.40; $P = 0.36$]; third tertile: HR: 1.27 [95% CI: 0.68-2.38; $P = 0.44$]) (Table 4). The relationship of each PCSK9 subtype with

**TABLE 3 The Occurrence of Coronary and Cerebrovascular Events in Entire Subjects (N = 1,436)**

| | |
|---|---|
| CAD events | 50 (3.5) |
| Acute myocardial infarction | 29 (2.0) |
| Sudden cardiac death | 1 (0.1) |
| Stable CAD requiring revascularization | 20 (1.4) |
| Stroke events | 51 (3.5) |
| Ischemic stroke | 44 (3.1) |
| Unclassified strokes | 7 (0.4) |

Values in n (%).
CAD = coronary artery disease.

ischemic stroke and intracerebral hemorrhage is summarized in Supplemental Table 4. Sex differences in the relationship between PCSK9 measures and outcomes are presented in Supplemental Table 5.

## DISCUSSION

Two major subtypes of PCSK9 exist in the circulation, with different properties to degrade LDL receptors. Whether these PCSK9 forms associate with atherosclerotic cardiovascular events remains uncertain. In the present study, the furin-cleaved form of PCSK9 predicted future coronary events in Japanese participants without any history of cardiovascular events. In contrast, the mature subtype did not exhibit any relationships with cardiovascular outcomes (**Central Illustration**). These findings suggest the importance of considering PCSK9 subtypes in circulation for risk stratification of coronary events in the primary prevention setting.

Published studies used ELISAs that could measure total PCSK9 concentration alone, and thus do not consider its circulating active forms (8-10). Given differences in structural and biological characteristics between furin-cleaved and mature PCSK9s (4-7), the aforementioned ELISAs were limited in accurately evaluating activity of circulating PCSK9 and its relationship with cardiovascular outcomes. The present study used a novel sandwich ELISA enabling quantitatively measurement of PCSK9 subtypes with the use of monoclonal antibodies (11-13). We observed that future risks of coronary events significantly increased in association with furin-cleaved PCSK9 concentration but not total and mature ones. This observation underscores the biological activities of PCSK9 for predicting future cardiovascular risks.

In vitro analyses reported a shorter half-life furin-cleaved PCSK9 with reduced efficiency to degrade LDL receptor compared with the mature form (4,5,17). However, how much these distinct properties of PCSK9 subtypes clinically affect lipid and other risk factors have not yet been characterized. In our healthy Japanese subjects who did not take any lipid-lowering therapies, both furin-cleaved and mature PCSK9s were significantly associated with LDL-C, nonHDL-C, and systolic blood pressure levels. Of note, furin-cleaved PCSK9 positively correlated with hs-CRP level and body mass index, whereas mature PCSK9 did not. These findings indicate that furin-cleaved PCSK9 may still be an active form that affects lipid metabolism, inflammatory activity, and metabolic parameters.

Recent mechanistic studies showed that PCSK9 induces secretion of proinflammatory cytokines in macrophages, liver cells, and a variety of tissues. Ding et al reported that PCSK9 promoted Toll-like receptor 4 expression and nuclear factor κB activation in the rabbit thoracic aorta (18-20). In addition, PCSK9 interacts with lectin-type oxidized LDL receptor 1, which activates the renin-angiotensin system (20). To date, there are no dedicated in vitro studies to provide mechanistic insights into the proinflammatory effect of furin-cleaved PCSK9. However, because there was a positive relationship of hs-CRP with furin-cleaved but not mature PCSK9 levels, this subtype may harbor more proatherogenic properties that could be a driver causing coronary events.

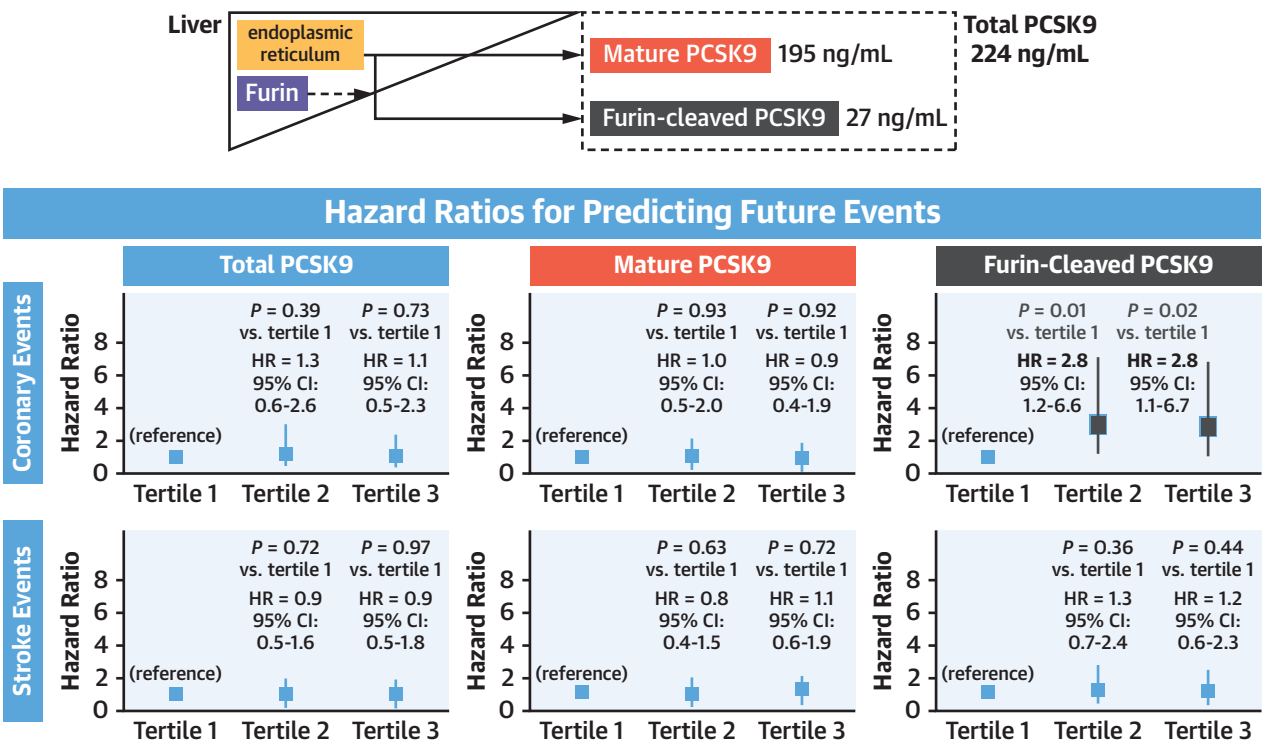
Although the current analysis showed the association of circulating furin-cleaved PCSK9 concentration with coronary events, its pathophysiology in vivo is not yet fully determined. We observed that furin-

**TABLE 4 Hazard Ratios (95% Confidence Intervals) for Predicting Future Coronary and Stroke Events**

| | Tertile 1 (Reference) | Tertile 2 | Tertile 3 |
|---|---|---|---|
| Total PCSK9 | | | |
| Coronary events[a] | 1.00 | 1.35 (0.68-2.68) | 1.13 (0.54-2.34) |
| *P* value vs tertile 1 | – | 0.39 | 0.73 |
| Stroke events[a] | 1.00 | 0.90 (0.50-1.61) | 0.99 (0.54-1.80) |
| *P* value vs tertile 1 | – | 0.72 | 0.97 |
| Composite events[a] | 1.00 | 0.98 (0.60-1.59) | 1.09 (0.67-1.78) |
| *P* value vs tertile 1 | – | 0.93 | 0.71 |
| Mature PCSK9 | | | |
| Coronary events[a] | 1.00 | 1.02 (0.52-2.02) | 0.96 (0.47-1.95) |
| *P* value vs tertile 1 | – | 0.93 | 0.92 |
| Stroke events[a] | 1.00 | 0.86 (0.47-1.57) | 1.11 (0.61-1.99) |
| *P* value vs tertile 1 | – | 0.63 | 0.72 |
| Composite events[a] | 1.00 | 0.91 (0.56-1.46) | 0.91 (0.56-1.49) |
| *P* value vs tertile 1 | – | 0.70 | 0.73 |
| Furin-cleaved PCSK9 | | | |
| Coronary events | | | |
| Model 1[a] | 1.00 | 3.08 (1.32-7.22) | 2.93 (1.23-6.98) |
| *P* value vs tertile 1 | – | 0.009 | 0.01 |
| Model 2[b] | 1.00 | 2.89 (1.23-6.78) | 2.75 (1.15-6.57) |
| *P* value vs tertile 1 | – | 0.01 | 0.02 |
| Model 3[c] | 1.00 | 2.84 (1.21-6.65) | 2.81 (1.17-6.74) |
| *P* value vs tertile 1 | – | 0.01 | 0.02 |
| Stroke events[a] | 1.00 | 1.31 (0.72-2.40) | 1.27 (0.68-2.38) |
| *P* value vs tertile 1 | – | 0.36 | 0.44 |
| Composite events | | | |
| Model 1[a] | 1.00 | 1.99 (1.17-3.39) | 1.94 (1.12-3.36) |
| *P* value vs tertile 1 | – | 0.01 | 0.01 |
| Model 2[b] | 1.00 | 1.88 (1.10-3.21) | 1.87 (1.08-3.24) |
| *P* value vs tertile 1 | – | 0.02 | 0.02 |
| Model 3[c] | 1.00 | 1.74 (1.01-3.01) | 1.76 (0.99-3.13) |
| *P* value vs tertile 1 | – | 0.04 | 0.05 |

[a]Adjusted for age and sex. [b]Adjusted for age, sex, hypertension, diabetes mellitus, current smoking, and current drinking. [c]Adjusted for age, sex, hypertension, diabetes mellitus, current smoking, current drinking, high-density lipoprotein cholesterol, and high-sensitivity C-reactive protein.

**CENTRAL ILLUSTRATION** **PCSK9 Subtypes and Coronary and Stroke Events**

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PCSK9 is a serine protease circulating in mature and furin-cleaved PCSK9 forms. Mature PCSK9 is the more dominant subtype in circulation (median total, mature, and furin-cleaved PCSK9 levels: 224, 195, and 27 ng/mL, respectively). During the 13.6-year observational period, total PCSK9 (tertile 2: hazard ratio [HR]: 1.35 [95% confidence interval [CI]: 0.68-2.68; $P = 0.39$]; tertile 3: HR: 1.13 [95% CI: 0.54-2.34; $P = 0.73$]) and mature PCSK9 (tertile 2: HR: 1.02 [95% CI: 0.52-2.02; $P = 0.93$]; tertile 3: HR: 0.96 [95% CI: 0.47-1.95; $P = 0.92$]) did not associate with the occurrence of future coronary events. In contrast, furin-cleaved PCSK9 level predicted a greater likelihood of experiencing coronary events even after adjusting for clinical characteristics (tertile 2: HR: 2.84, 95% CI = 1.21-6.65; $P = 0.01$]; tertile 3: HR: 2.81, 95% CI = 1.17-6.74; $P = 0.02$]). There was no association of total, mature, and furin-cleaved PCSK9 levels with future stroke events (total PCSK9: tertile 2: HR: 0.90 [95% CI: 0.50-1.61; $P = 0.72$]; tertile 3: HR: 0.99 [95% CI: 0.54-1.80; $P = 0.97$]; mature PCSK9: tertile 2: HR: 0.86 [95% CI: 0.47-1.57; $P = 0.63$]; tertile 3: HR: 1.11 [95% CI: 0.61-1.99; $P = 0.72$, furin-cleaved PCSK9: tertile 2: HR: 1.31 [95% CI: 0.72-2.40; $P = 0.36$]; tertile 3: HR: 1.27 [95% CI: 0.68-2.38; $P = 0.44$]). Each **square** and **line** indicate odds ratio and 95% confidence interval, respectively. PCSK9 = proprotein convertase subtilisin/kexin type 9.

cleaved PCSK9 level was positively associated with mature PCSK9 level in our study population (**Table 2**). This finding suggests that a synthesis of mature PCSK9 at hepatocytes might directly promote a production and secretion of furin-cleaved PCSK9. However, the proportion of furin-cleaved to mature PCSK9 varies in the published data (12,13). Other studies reported that concentration of furin-cleaved PCSK9 in patients with CAD was from around one-third to less than one-tenth of mature PCSK9, whereas this proportion was one-eighth in our healthy Japanese subjects. This observation indicates that cleavage via furin could also affect furin-cleaved PCSK9 level. How much this furin-derived property regulates circulating levels of PCSK9 subtypes requires further investigation.

Published studies investigated the predictive ability of total PCSK9 level with a composite outcome of cardiovascular events, but not cerebrovascular events alone (8-10). Our cohort had more cerebrovascular events compared with coronary events, which reflects national trends of coronary and stroke events as reported by 8 Japanese cohort studies from 1990 to 2010 (21,22). Despite a higher occurrence of cerebrovascular events in our study subjects, we did not find any association with each PCSK9 level. As mentioned above, total, furin-cleaved, and mature PCSK9 levels were positively associated with non-HDL-C levels.

Considering that non-HDL levels do not necessarily increase the risk of stroke events in Japanese subjects (23), this may suggest that PCSK9 levels have a limited ability to estimate future risk of stroke events in the primary prevention settings.

**STUDY LIMITATIONS.** A number of caveats should be noted. First, the number of coronary and stroke events was relatively small. In particular, the frequency of hard cardiac events was low, which might have limited the power to detect statistical significance. Second, the present findings were in healthy Japanese subjects in an urban area. It is unknown whether similar findings would be observed in individuals living in rural areas. Third, 60.8% of the study population was female, which is different from other cohort studies analyzing PCSK9 levels. Because the association of PCSK9 with cardiovascular events was more robust in men (8), a greater frequency of woman may affect the present findings. Finally, there were no data about the commencement of lipid-lowering therapy including a statin during the observational period.

## CONCLUSIONS

Compared with furin-cleaved PCSK9, the mature form was more dominant in circulation of Japanese healthy subjects. Total PCSK9 and the subtypes levels were associated with LDL-C, non-HDL-C and systolic blood pressure levels, whereas only furin-cleaved PCSK9 level exhibited a significant relationship with both hs-CRP and diastolic blood pressure. Furthermore, an elevated level of furin-cleaved PCSK9 predicted the occurrence of coronary events in the primary prevention settings, whereas total and mature PCSK9 levels did not. Our findings provide additional pathophysiological insights into the metabolism of PCSK9 associated with coronary events.

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## PERSPECTIVES

**COMPETENCY IN PATIENT CARE:** Measurement of furin-cleaved PCSK9 may be a clinically applicable tool for risk stratification of future coronary events in Japanese subjects without any history of cardiovascular diseases.

**TRANSLATIONAL OUTLOOK:** Future studies are warranted to elucidate whether furin-cleaved PCSK9 is a therapeutic target for the prevention of coronary events in the primary prevention settings.

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**KEY WORDS** cohort study, coronary, proprotein convertase subtilisin/kexin type 9, stroke

**APPENDIX** For supplemental tables, please see the online version of this paper.