Journal of the American Heart Association

ORIGINAL RESEARCH

Circulating Mature PCSK9 Level Predicts Diminished Response to Statin Therapy

Naoto Kuyama, MD; Yu Kataoka, MD, PhD; Misa Takegami, PhD, MPH; Kunihiro Nishimura, MD, PhD, MPH; Mariko Harada-Shiba, MD, PhD; Mika Hori, PhD; Masatsune Ogura, MD, PhD; Fumiyuki Otsuka, MD, PhD; Yasuhide Asaumi, MD, PhD; Teruo Noguchi, MD, PhD; Kenichi Tsujita, MD, PhD; Satoshi Yasuda, MD, PhD

BACKGROUND: Statin-mediated efficacy of lowering low-density lipoprotein (LDL) cholesterol varies in each individual, and its diminished response is associated with worse outcomes. However, there is no established approach to predict hyporesponse to statins. PCSK9 (proprotein convertase subxilisin/kexin type 9) is a serine-protease associated with LDL metabolism, which circulates as mature and furin-cleaved PCSK9. Since mature PCSK9 more potently degrades the LDL receptor, its evaluation may enable the identification of statin hyporesponders.

METHODS AND RESULTS: We analyzed 101 statin-naive patients with coronary artery disease who commenced a statin. PCSK9 subtypes at baseline and 1 month after statin use were measured by ELISA. Hyporesponse to statins was defined as a percent reduction in LDL cholesterol <15%. The relationship between each PCSK9 subtype level and hyporesponse to statins was investigated. Statins significantly lowered LDL cholesterol level (percent reduction, 40%±21%), whereas 11% of study participants exhibited a hyporeseponse to statins. Multivariable logistic regression analysis demonstrated that baseline mature PCSK9 level was an independent predictor for hyporesponse to statins even after adjusting clinical characteristics (mature PCSK9 per 10-ng/mL increase: odds ratio [OR], 1.12; 95% CI, 1.01–1.24 [*P*=0.03]), whereas furin-cleaved level was not (per 10-ng/mL increase: OR, 1.37; 95% CI, 0.73–2.58 [*P*=0.33]). Receiver operating characteristic curve analysis identified mature PCSK9 level of 228 ng/mL as an optimal cutoff to predict hyporesponse to statins (area under the curve, 0.73 [sensitivity, 0.91; specificity, 0.56]).

CONCLUSIONS: Baseline mature PCSK9 level >228 ng/mL is associated with hyporesponse to statins. This finding suggests that mature PCSK9 might be a potential determinant of hyporesponse to statins.

Key Words: LDL-C ■ proprotein convertase subxilisin/kexin type 9 ■ statin

Achieving a lower level of low-density lipoprotein (LDL) cholesterol (LDL-C) with a lipid-lowering drug has become a cornerstone for the prevention of atherosclerotic cardiovascular disease.[1–3] Statins are a major therapeutic agent used to lower LDL-C levels by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A reductase, which induces reduced secretion of apolipoprotein B–containing lipoproteins from the liver and the upregulation of LDL receptor (LDLR) activity.[4] This agent has been shown to lower LDL-C level by approximately 30% to 50%.[5,6] However, the extent of statin-mediated LDL-C lowering is not consistent among each individual. Recent studies reported that 11% to 20% of patients receiving a statin exhibited its diminished response and an elevated cardiovascular risk.[5,7] In addition, hyporesponse to a statin defined as a percent reduction of LDL-C <15% was associated with substantial atheroma progression.[7] These observations suggest the importance to elucidate factors causing this poor response to a statin, which may

Correspondence to: Yu Kataoka, MD, PhD, Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, 6-1 Kishibe-Shimmachi, Suita, Osaka, Japan. E-mail: yu.kataoka@ncvc.go.jp

Supplementary Material for this article is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.120.019525.

For Sources of Funding and Disclosures, see page 7.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- In statin-naive patients, an elevated mature PCSK9 (proprotein convertase subtilisin/kexin type 9) level, but not a furin-cleaved level, is significantly associated with hyporesponse to statins.

What Are the Clinical Implications?

- Evaluation of mature PCSK9 level might be a useful approach to predict response to a statin.

Nonstandard Abbreviations and Acronyms

LDLR	low-density lipoprotein receptor
PCSK9	proprotein convertase subtilisin/kexin type 9
rhPCSK9	recombinant human PCSK9

enable us to identify hyporesponders to statin therapy and then allocate adequate lipid-lowering therapy.

LDL metabolism is regulated by PCSK9 (proprotein convertase subtilisin/kexin type 9). PCSK9 is a protease that regulates LDL metabolism by degrading LDLR,[8,9] suggesting that the increased level of PCSK9 may attenuate the response to a statin. However, recent studies did not show any association of PCSK9 level with a change in on-treatment LDL-C following statin therapy.[10,11] Pathophysiologically, PCSK9 exists as mature and furin-cleaved forms in circulation. Several basic studies have demonstrated that mature PCSK9 has the ability to degrade LDLR, whereas furin-cleaved level has little activity modulating LDLR.[12–14] This observation leads to the hypothesis that the evaluation of these two subtypes may help to predict poor response to a statin. Therefore, the current study aimed to investigate the association of two PCSK9 subtypes with the degree of LDL-C lowering after treatment with a statin.

METHODS

Because of the sensitive nature of the data collected for this study, requests to access the data set from other researchers will require the approval from the corresponding author and the research ethics committee.

Study Design and Protocol

The current investigation is a single-center prospective observational study designed to evaluate whether two PCSK9 subtypes are associated with the degree of lowering LDL-C under statin therapy. The inclusion criteria included: (1) patients aged ≥18 years; (2) statin-naive patients; (3) patients with acute coronary syndrome or stable coronary artery disease (CAD); and (4) patients with a clinical indication to commence statin treatment according to Japanese guidelines.[15,16] This study excluded patients who were previously treated with any lipid-lowering therapy. During the study period (from February 2018 to November 2019), we screened a total of 127 statin-naive patients with CAD at the National Cerebral and Cardiovascular Center, Suita, Japan (Figure 1). Of these, the following numbers of patients were excluded from analysis: 10 patients did not agree to enroll in the study; and 4 patients discontinued a statin before the 1-month visit because of a statin-related side effect. Ezetimibe was added to the statin therapy before the 1-month visit in 7 patients. There were no data about lipid parameters and PCSK9 level in 5 patients. As a consequence, the remaining 101 statin-naive patients with CAD were included into the current analysis (Figure 1).

With regard to the study protocol, after informed consent was obtained, a blood sample was collected before the commencement of a statin. The blood sample was drawn from a peripheral vein in the morning after overnight fasting. It was stored at a temperature of −80°C within 2 hours after blood collection. A fixed dose of a statin was commenced and then continued for 1 month. The selection of the statin and its dose was left to each physician's discretion. High-intensity statin use was defined as rosuvastatin ≥10 mg, pitavastatin ≥4 mg, or atorvastatin ≥20 mg.[17] Other medical therapies except nonstatin lipid-lowering agents were allowed to be

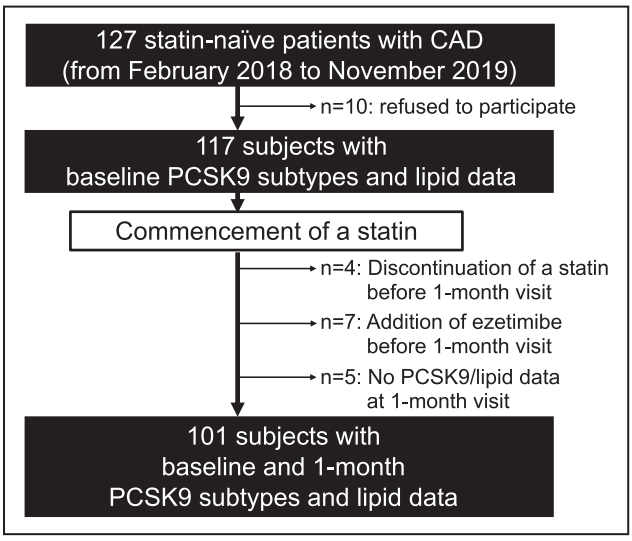

Figure 1. Study design.
CAD indicates coronary artery disease; and PCSK9, proprotein convertase subtilisin/kexin type 9.

concomitantly used during the study period. Clinical follow-up was performed at 1 month after the therapy, and fasting blood samples were collected again. This study was approved by the institutional review board of the National Cerebral and Cardiovascular Center, Suita, Japan (M28-119-4). Each patient gave written informed consent to participate in the study. All clinical investigations were conducted in accordance with the principles of the Declaration of Helsinki.

PCSK9 Subtypes' Measurements and Evaluation of Lipid Parameters

Mature and furin-cleaved PCSK9 concentrations were measured at baseline and 1 month after the commencement of a statin in the stored serum samples using an ELISA (BML, Inc).[18–21] This novel sandwich ELISA has been developed by our coauthors (M.H-S. and M.H.).[18] It enables us to quantitatively measure PCSK9 subtypes by using monoclonal antibodies. The ELISA is characterized by using purified rhPCSK9 (recombinant human PCSK9) or cell lysate of rh⊿218PCSK9 as well as plasma samples.[18] 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.[18] The interassay and intra-assay coefficients of variance to measure each PCSK9 value are as follows: mature PCSK9, 7.7% and 2.2%, and furin-cleaved PCSK9, 5.6% and 2.1%, respectively. The lower and upper detection limits of mature and furin-cleaved PCSK9 were 3.9 and 20 000 ng/mL, and 0.7 and 300 ng/mL, respectively.[19]

LDL-C level was calculated by its direct measurement as previously reported.[22–24] Hyporesponse to a statin was defined as percent reduction in LDL-C <15% according to the published article.[6] Fasting serum levels of total cholesterol and high-density lipoprotein cholesterol were measured by enzymatic methods (Sekisui Medical) using an automated analyzer (Hitachi Labospect 008; Hitachi-Hitec).

Statistical Analysis

Continuous variables were expressed as mean±SD and compared using *t* test if data were normally distributed. Non-normally distributed continuous data were summarized as median (interquartile range) and compared using Wilcoxon signed rank test. Spearman correlation coefficient test was used to examine the relationship between the percent reduction of LDL-C and the percent change in each PCSK9 subtypes' concentration. Logistic regression analysis was conducted to elucidate the association of each PCSK9 subtypes' level with a hyporsponse to a statin. A multivariable logistic regression analysis was performed to evaluate the relationship between each PCSK9 subtypes' level and a hyporeponse to a statin using the following 2 models: (1) adjusted by age and sex; and (2) adjusted by age, sex, hypertension, diabetes mellitus, estimated glomerular filtration rate, types of statin, and high-intensity statin use. Receiver operating characteristic curve analysis was performed to assess the ability of mature PCSK9 level for the prediction of a hyporesponse to a statin after statin therapy. All *P* values <0.05 were considered statistically significant. All analyses were performed using SAS version 9.4 (SAS Institute).

RESULTS

Clinical Demographics of Study Participants

Clinical characteristics of the study participants are shown in Table 1. Patients had a median age of 72 years, 79% were men, and they had a high prevalence of coronary risk factors (hypertension 68%, type 2 diabetes mellitus 49%, dyslipidemia 50%, and smoking 27%). Furthermore, 70% of the study participants presented with acute coronary syndrome, and multivessel disease was observed in 45% of the study population. Percutaneous coronary intervention was performed in 81% of the study population, and the remaining patients were medically managed.

With regard to statin therapy, rosuvastatin was used in >70% of study patients, whereas the frequency of pitavastatin and atorvastatin use was 16% and 8%, respectively. Fifty-four percent of patients received a high-intensity statin. In addition to statin therapy, established medical therapies were concomitantly used at 1 month after baseline as follows: aspirin, 88%; angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker, 65%; and β-blocker, 54%. Since a drug-eluting stent was implanted in 81% of patients, a P2Y12 inhibitor was used in addition to aspirin (clopidogrel 27%, prasugrel 54%).

LDL-C Control Following Commencement of a Statin

The serial change in lipid parameters is summarized in Table 2. As expected, a significant reduction of LDL-C level was observed at 1 month after statin therapy (*P*<0.001), which corresponded to a percent reduction of 40%±21%. Furthermore, 52% of study participants achieved LDL-C <1.8 mmol/L at 1 month. By contrast, despite the commencement of a statin, 11% of study patients exhibited a percent reduction of LDL-C <15%, suggesting a hyporesponse to the therapy. In addition to lowering LDL-C, statins significantly reduced participants' total cholesterol level and increased their high-density

Table 1. Baseline Clinical Demographics (N=101)

Age, y	72 (61–77)
Men, n (%)	80 (79)
BMI, kg/m^2	23±3
Hypertension, n (%)	69 (68)
Diabetes mellitus, n (%)	49 (49)
Dyslipidemia, n (%)	51 (50)
Chronic kidney disease, n (%)	32 (32)
Smoking, n (%)	27 (27)
History of myocardial infarction, n (%)	6 (6)
History of stroke, n (%)	5 (5)
History of PAD, n (%)	4 (4)
CAD type, n (%)	
Acute coronary syndrome	71 (70)
Stable CAD	30 (30)
Multivessel disease	44 (45)
Biochemistry data	
Glycated hemoglobin, %	5.9 (5.6–6.3)
eGFR, mL/min per 1.73m^2	68±21
Systolic BP, mm Hg	124±18
Diastolic BP, mm Hg	71±14
Baseline medication use, n (%)	
Aspirin	7 (7)
ACEI/ARB	18 (18)
β-Blocker	10 (9)
Concomitant medications use	
Lipid-lowering therapy, n (%)	
Statin	101 (100)
Rosuvastatin	77 (76)
Pitavastatin	16 (16)
Atorvastatin	8 (8)
High-intensity statin use*	55 (54)
Other established medication, n (%)	
Aspirin	89 (88)
Clopidogrel	27 (27)
Prasugrel	55 (54)
ACEI/ARB	66 (65)
β-Blocker	55 (54)

Continuous variables are expressed as mean±SD, and non-normally distributed continuous data are summarized as median (interquartile range).

ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blockers; BMI, body mass index; BP, blood pressure; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; and PAD, peripheral arterial disease.

*Rosuvastatin ≥10 mg, pitavastatin ≥4 mg, and atorvastatin ≥20 mg.

lipoprotein cholesterol level (P<0.001 and P=0.003, respectively).

Mature and Furin-Cleaved Levels Under Statin Therapy

In the current study population, median mature and furin-cleaved PCSK9 levels at baseline were 225 ng/mL and 22 ng/mL, respectively. The distribution of each PCSK9 subtype concentration is shown in Figure 2. Following the commencement of a statin, a significant increase of mature and furin-cleaved PCSK9 levels was observed at 1 month (Table 2).

Association Between PCSK9 Subtype Levels and Hyporesponse to a Statin

Logistic regression analyses were conducted to evaluate the association of each PCSK9 subtypes' level with a hyporesponse to a statin under the 1-month statin use (Table 3). In the unadjusted model, the baseline mature PCSK9 level was significantly associated with a hyporesponse to a statin (odds ratio [OR], 1.08; 95% CI, 1.01–1.15 [P=0.02]; per 10-ng/mL increase), whereas the baseline furin-cleaved PCSK9 level at baseline was not (OR, 1.37; 95% CI, 0.88–2.11 [P=0.16]; per 10-ng/mL increase). In the multivariable models, the baseline mature PCSK9 level was still an independent determinant predicting a hyporesponse to a statin, even after adjusting by age and sex (OR, 1.11; 95% CI, 1.03–1.20 [P=0.009]), and adjusted by age, sex, hypertension, diabetes mellitus, estimated glomerular filtration rate, high-intensity statin use, and its types (OR, 1.12; 95% CI, 1.01–1.24 [P=0.03]).

Receiver operating characteristics curve analysis elucidated the optimal cutoff value of the baseline mature PCSK9 to predict a hyporesponse to a statin as 228 ng/mL (area under the curve, 0.73 [sensitivity, 91%; specificity, 56%]) (Figure 3). With regard to the association between percent change in each PCSK9 subtype and the degree of statin-mediated LDL-C reduction, both the percent change in mature and furin-cleaved PCSK9 did not exhibit any significant relationships with the percent reduction of LDL-C (Figure S1).

DISCUSSION

While numerous large-scale clinical trials have consistently demonstrated the clinical benefit of a statin to reduce the frequency of atherosclerotic cardiovascular disease,[1,5,6] its efficacy to lower LDL-C differs in each individual, and some patients do not fully respond to the therapy.[5,25] In the present study, each of two PCSK9 subtypes exhibited different properties in the degree of lowering LDL-C under statin use. In particular, an elevated mature PCSK9 level was associated with an attenuated efficacy for the statin to lower LDL-C level, whereas an elevated furin-cleaved level was not. These findings indicate the association of mature PCSK9 with LDL metabolism under statin therapy.

In the current analysis, the degree of LDL-C lowering with a statin was associated with an elevation of mature PCSK9 level but not furin-cleaved level. This

Table 2. Serial Change in Lipid Parameters

	Baseline (n=101)	1 mo (n=101)	*P* Value
LDL-C, mmol/L	3.1±0.9	1.8±0.5	<0.001
Achieved LDL-C <1.8 mmol/L, n (%)	…	53 (52)	…
Percent reduction in LDL-C (%)	…	40±21	…
Percent reduction in LDL-C<15%, n (%)	…	11 (11)	…
Total cholesterol, mmol/L	5.0±1.0	3.7±0.6	<0.001
HDL-C, mmol/L	1.2 (1.0–1.5)	1.2 (1.1–1.6)	0.003
Mature PCSK9, ng/mL	225 (187–284)	312 (265–376)	<0.001
Furin-cleaved PCSK9, ng/mL	22 (17–28)	26 (19–32)	<0.001

Continuous variables are expressed as mean±SD, and non-normally distributed continuous data are summarized as median (interquartile range).
HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and PCSK9, proprotein convertase subtilisin/kexin type 9.

finding further supports the different properties of each PCSK9 subtype in vivo. PCSK9 is initially produced as an ≈75 kDa proprotein. Mature PCSK9 is formed by removal of the signal peptide and secreted consisting of a prodomain, catalytic domain, and C-terminal domain.[25] The prodomain is associated with the C-terminal domain after autocatalytic cleavage, and PCSK9 has degradation activity of LDLR not by its proteolytic active site but by binding of its catalytic domain and prosegment with the epidermal growth factor A of LDLR.[26] Given that statins lower LDL-C level through the overexpression of LDLR at the surface of a hepatocyte,[27] an increased level of mature PCSK9 could attenuate the efficacy of a statin, resulting in hyporesponse. With regard to furin-cleaved PCSK9, furin cleaves some of the mature PCSK9 at the 218 RFHR position of the catalytic domain, resulting in prosegment detachment and loss of binding ability to LDLR.[12] This property could account for the absence of an association between furin-cleaved PCSK9 and the extent of LDL-C lowering.

The current study provides insights into the association of a statin-mediated PCSK9 increase with its response. Statins increase the activity and nuclear translocation of sterol regulatory element-binding protein 2, which activates PCSK9 genes.[26,27] In our analysis, following commencement of a statin, a significant elevation of both mature and furin-cleaved PCSK9 levels was observed. In particular, mature PCSK9 was still a dominant subtype in circulation

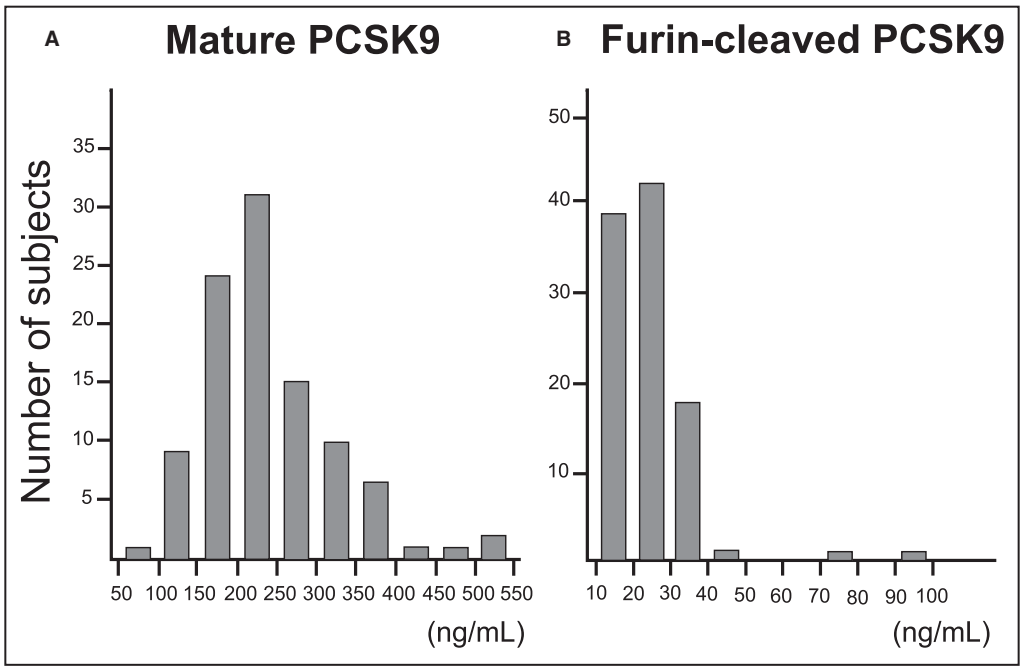

Figure 2. The distribution of two PCSK9 (proprotein convertase subtilisin/kexin type 9) subtypes' concentrations. The histograms of each PCSK9 subtypes' concentration.

Table 3. Logistic Regression Analysis for Predicting Hyporesponse to Statins (Percent Reduction in LDL-C <15%) by Each PCSK9 Subtypes' Concentration

	Unadjusted Model		Age- and Sex-Adjusted Model		Multivariate-Adjusted Model*	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
Mature PCSK9 (per 10, ng/mL)	1.08 (1.01–1.15)	0.02	1.11 (1.03–1.20)	0.009	1.12 (1.01–1.24)	0.03
Furin-cleaved PCSK9 (per 10, ng/mL)	1.37 (0.88–2.11)	0.16	1.38 (0.89–2.15)	0.15	1.37 (0.73–2.58)	0.33

LDL-C indicates low-density lipoprotein cholesterol; OR, odds ratio; and PCSK9, proprotein convertase subtilisin/kexin type 9.
*Adjusted by sex, age, hypertension, diabetes mellitus, estimated glomerular filtration rate, type of statin, and high-intensity statin use.

under statin therapy. However, these increases in PCSK9 subtypes' concentrations did not necessarily affect the magnitude of LDL-C lowering. Circulating LDL-C level generally depends on the balance between cholesterol synthesis and its clearance. The commencement of a statin triggers reduced cholesterol synthesis, LDLR overexpression, and the secretion of PCSK9 subtypes.[10,28,29] Since some circulating PCSK9, especially the mature form, can bind to LDLR and then both removes from the circulation, the increased amount of PCSK9 subtypes under the therapy may not invariably reflect the true degree of its statin-mediated secretion. Therefore, evaluating the PCSK9 subtypes' levels before statin use may be a better clinically applicable tool to estimate the extent of LDL-C reduction following statin use.

Predicting a poor response to a statin by evaluating the PCSK9 subtypes may help to customize a lipid-lowering therapy for each individual. Recently, an injectional agent for PCSK9 modulation has been used to substantially lower LDL-C levels. While its favorable efficacy on atherosclerotic cardiovascular disease has been demonstrated,[30,31] its high medication cost has been a barrier to patients requiring more potent agents. Our observation suggests that measuring the mature PCSK9 level might guide physicians to select potent lipid-lowering agent. In addition, this approach may have great potential for statin hyporesponders to better understand the expected effect of a statin, its related-future cardiovascular risks, and the need for additional lipid-lowering drugs.

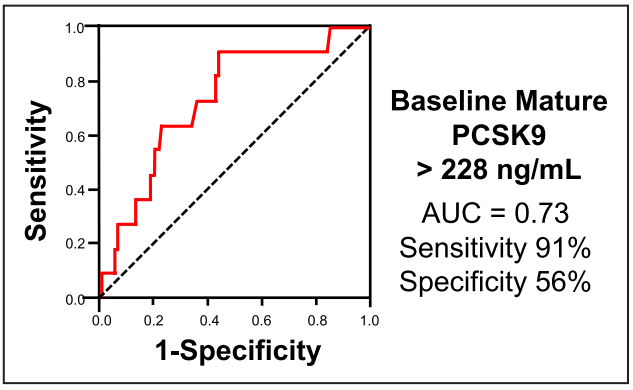

Figure 3. Receiver operating characteristics analysis for the baseline mature PCSK9 (proprotein convertase subtilisin/kexin) level to predict a hyporesponse to a statin (defined as percent reduction of low-density lipoprotein cholesterol <15%).
AUC indicates area under the curve.

Several caveats should be noted. First, this investigation was a single-center study, and the number of poor responses to a statin was relatively small. Second, the type and dose of a statin was selected by each physician, which may be a potential bias. Third, the approved maximum dose of statins (atorvastatin 20 mg, rosuvastatin 10 mg, and pitavastatin 4 mg) in Japan is lower than those in Europe and the United States. Therefore, the current study does not evaluate the association of PCSK9 with LDL-C level under the guideline-recommended high-intensity statin regimen (atorvastatin 40 mg to 80 mg and rosuvastatin 40 mg) in other countries. However, all of the study population exhibited CAD at baseline, and therefore physicians commenced an adequate statin dose and intensity following the cholesterol guideline from the Japan Atherosclerosis Society.[16] Fourth, adherence to statin therapy was assessed by patients' self-reports and not by research nurses. Hence, there is a possibility that poor adherence might have existed in some patients, which may have affected their response to a statin.

CONCLUSIONS

The baseline mature PCSK9 level predicted a hyporesponse to statin therapy in patients with CAD, whereas the furin-cleaved level did not. As expected, all of these PCSK9 levels significantly increased at 1 month after the therapy commenced. However, this statin-mediated increase in PCSK9 did not help to estimate response to a statin. Our findings reveal that mature PCSK9 level before statin treatment might be an important marker to identify statin hyporesponders who require more potent lipid-lowering therapy.

ARTICLE INFORMATION

Received September 25, 2020; accepted April 5, 2021.

Affiliations

Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center, Osaka, Japan (N.K., Y.K., F.O., Y.A., T.N., S.Y.); Department of Cardiovascular Medicine, Graduate School of Medical Sciences, Kumamoto University, Japan (N.K., K.T.); Department of Preventive Medicine and Epidemiology (M.T., K.N.); and Department of Molecular Innovation in Lipidology (M.H., M.H., M.O.), National Cerebral and Cardiovascular Center, Osaka, Japan; Department of Endocrinology, Research Institute of Environmental Medicine, Nagoya University, Japan (M.H.); and Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan (S.Y.).

Acknowledgments

We thank the Biobank at the National Cerebral and Cardiovascular Center for their assistance with blood sample storage.

Sources of Funding

This study was supported by the Japan Research Foundation for Clinical Pharmacology (grant number: H29).

Disclosures

Yu Kataoka has received research support from Nipro and Abbott, and honoraria from Nipro, Abbott, Kowa, Amgen, Sanofi, Astellas, Takeda, and Daiichi-Sankyo. The remaining authors have no disclosures to report.

Supplementary Material

Figure S1

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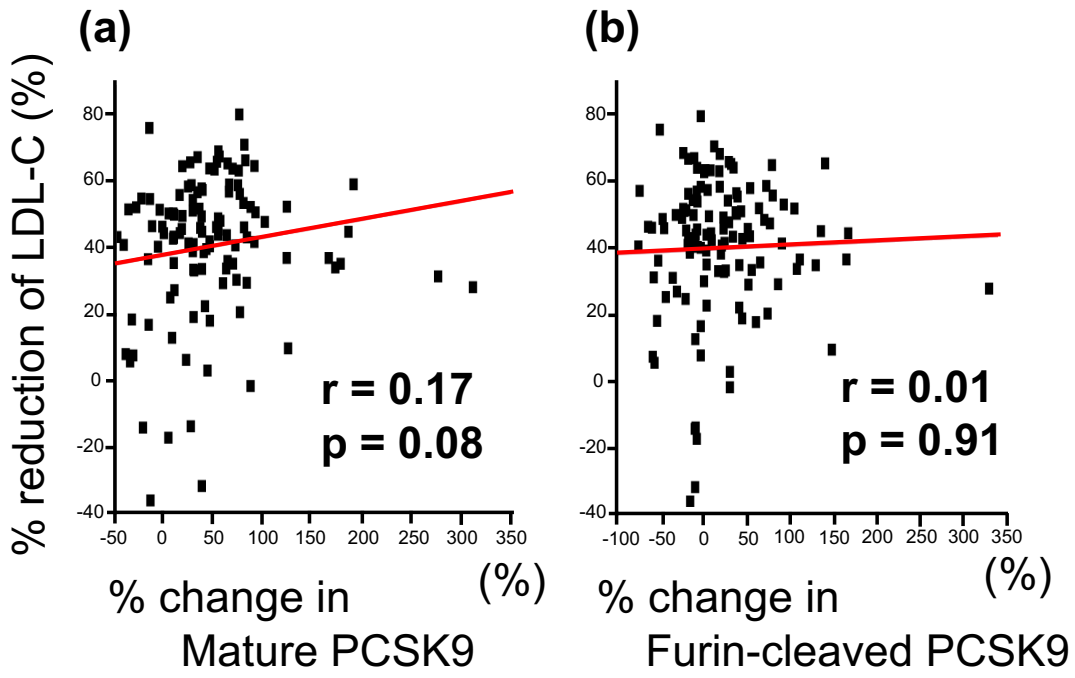
SUPPLEMENTAL MATERIAL

Figure S1. The correlation between percent changes of PCSK9 subtypes [(a): mature PCSK9, (b): furin-cleaved PCSK9] and percent reduction of LDL-C following commencement of a statin.



LDL-C, low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin/kexin 9.