Review > Int J Angiol. 2021 Mar;30(1):22-28. doi: 10.1055/s-0041-1722875. Epub 2021 Feb 18.

Effect of *PCSK9* E670G and R46L Polymorphisms on Major Adverse Cardio-Cerebrovascular Events in Patients with ST-Segment Elevation Myocardial Infarction Undergoing Primary Percutaneous Coronary Intervention

Anwar Santoso [1], Yulianto Yulianto [1], Hendra Simarmata [1], Abhirama Nofandra Putra [2], Erlin Listiyaningsih [3]

Affiliations + expand

PMID: 34025093 PMCID: PMC8128487 DOI: 10.1055/s-0041-1722875

Free PMC article

The Scientific Advisory Board

The EBI Literature Services Advisory Board meets annually and advises the EMBL-EBI on all aspects of the Literature Databases and Services, putting the development of these resources in the context of the scientific community within Europe and beyond. More specifically for Europe PMC, they review progress on the development of the service over the past year, and the plans for development for the forthcoming year. They advise the Europe PMC Funder Committee as to the overall effective use of funds from the Europe PMC grant. The Scientific Advisory Board members are:

- Jürg Bähler (Chair), University College London, UK
- Mohlopheni Marakalala, Africa Health Research Institute, South Africa
- Veronique Kiermer, PLOS, USA
- Jonny Coates, William Harvey research institute, QMUL, UK
- Alfonso Valencia, Barcelona Supercomputing Centre, Spain
- Bianca Kramer, Utrecht University, the Netherlands
- Gemma Villanueva, The Cochrane Collaboration, Oxford, UK

The Funder Committee

The Funder Committee meets twice a year to review completed development, comment on future development and approve the release of funds on behalf of the Funders' Group.

- Geraldine Clement-Stoneham, MRC (Chair)
- Andrew Knowles, CRUK
- Tobias Philipp, SNSF
- Hannah Hope, Wellcome
- Ian Coltart, WHO
- Inesa Thomsen, DHSC
- Matthew Lam, Worldwide Cancer Research
- Bela Desai, Wellcome Trust/DBT India Alliance
- Chris Macdonald, Pancreatic Cancer UK
- Dagmar Meyer, ERC (non-voting)

Funder Committee Terms of Reference

The Funders' Group

The Funders' Group consists of representation from all Europe PMC Funders and meets annually.

Effect of *PCSK9* E670G and R46L Polymorphisms on Major Adverse Cardio-Cerebrovascular Events in Patients with ST-Segment Elevation Myocardial Infarction Undergoing Primary Percutaneous Coronary Intervention

Anwar Santoso, FIHA, PhD[1] Yulianto Yulianto, FIHA[1] Hendra Simarmata, FIHA[1]
Abhirama Nofandra Putra, FIHA[2] Erlin Listiyaningsih, PhD[3]

[1] Department of Cardiology, Vascular Medicine, Harapan Kita Hospital, National Cardiovascular Centre, Universitas Indonesia, Jakarta, Indonesia
[2] Department of Cardiology, Vascular Medicine, Gunung Jati Government Hospital, Kesambi - Cirebon, West Java, Indonesia
[3] Faculty of Medicine, University of Hamka, Ciledug, Banten, Indonesia

Address for correspondence Anwar Santoso, FIHA, PhD, Department of Cardiology, Vascular Medicine, Universitas Indonesia, Harapan Kita Hospital, National Cardiovascular Centre, Jalan Letjen S. Parman kav 87, Jakarta 11420, Indonesia (e-mail: anwarsantoso@inaheart.org).

Int J Angiol

Abstract

Major adverse cardio-cerebrovascular events (MACCE) in ST-segment elevation myocardial infarction (STEMI) are still high, although there have been advances in pharmacology and interventional procedures. Proprotein convertase subtilisin/Kexin type 9 (*PCSK9*) is a serine protease regulating lipid metabolism associated with inflammation in acute coronary syndrome. The MACCE is possibly related to polymorphisms in *PCSK9*. A prospective cohort observational study was designed to confirm the association between polymorphism of E670G and R46L in *the PCSK9* gene with MACCE in STEMI. The Cox proportional hazards model and Spearman correlation were utilized in the study. The Genotyping of *PCSK9* and ELISA was assayed. Sixty-five of 423 STEMI patients experienced MACCE in 6 months. The E670G polymorphism in *PCSK9* was associated with MACCE (hazard ratio = 45.40; 95% confidence interval: 5.30–390.30; $p = 0.00$). There was a significant difference of PCSK9 plasma levels in patients with previous statin consumption (310 [220–1,220] pg/mL) versus those free of any statins (280 [190–1,520] pg/mL) ($p = 0.001$). E670G polymorphism of *PCSK9* was associated with MACCE in STEMI within a 6-month follow-up. The plasma PCSK9 level was higher in statin users.

Keywords
- *PCSK9* gene polymorphism
- ST-elevation myocardial infarction
- major adverse cardio-cerebrovascular events
- primary PCI
- cardiovascular diseases
- high-sensitivity C-reactive protein

Incidence of acute ST-segment elevation myocardial infarction (STEMI) has decreased over the last decades, mostly in developed, higher-income countries. Otherwise, acute MI (either STEMI or non-STEMI) has increased in developing, lower-income countries.[1] International guidelines have advocated invasive procedures in all except low-risk patients with acute coronary syndrome (ACS); the Major adverse cardio-cerebrovascular events (MACCE) in STEMI is still high. However, there have been advances in pharmacology and interventional procedures.

PCSK9 is a serine protease that promotes the catabolism of low-density lipoprotein (LDL) receptors (LDLR), through

Thieme Medical Publishers, Inc., 333 Seventh Avenue, 18th Floor, New York, NY 10001, USA

DOI https://doi.org/10.1055/s-0041-1722875.
ISSN 1061-1711.

which it substantially affects lipid metabolism. Accordingly, the abolition of *PCSK9* physiological activity limits LDLR degradation, promoting the clearance of LDL cholesterol (LDL-C) and other atherogenic lipoproteins from the circulation.[2] The immense potential of *PCSK9* activity might extend beyond post-ACS. A recent study has proposed that *PCSK9* inhibition could be beneficial during the early phase of ACS treatment due to some of its pleiotropic actions.[3] Indeed, studies in animals and humans have indicated that *PCSK9* inhibition might affect not only LDL-C reduction but also other aspects of lipoprotein metabolism, thrombosis, immune function, and inflammation.[4]

Genetic variants that increase LDL-C have also been associated with increased susceptibility to coronary heart disease (CHD). Accordingly, several polymorphisms of the *PCSK9* gene, located on chromosome 1p32.3 and encompasses 12 exons, may positively or negatively modulate plasma cholesterol levels, consequently influencing the risk for CHD. The gain-of-function (GOF) single nucleotide polymorphism (SNP) E670G in the *PCSK9* gene reduces LDLR expression on the cell surface, which inhibits cellular uptake of serum LDL-C and ultimately elevates LDL-C, leading to autosomal dominant hypercholesterolemia and premature atherosclerosis.[5,6] Instead, loss-of-function (LOF) R46L mutations promote LDL-C uptake and lower serum cholesterol, thereby conferring protection against CHD.[7,8]

Though several studies have investigated the association between the two variants above and lipid levels and CHD susceptibility in different populations, the results have been inconsistent. Moreover, no study has yet determined the association and interaction between the two variants and the MACCE among patients with STEMI. Consequently, determining the association between *PCSK9* gene variants and MACCE among patients with STEMI undergoing primary percutaneous coronary intervention (PCI) may be clinically justified and relevant, given that it allows for secondary prevention wherein patients at high risk of an CHD event can be identified.

Methods

Study Population and Study Design

This was a single-center prospective cohort observational study designed to determine the association between *PCSK9* E670G and R46L polymorphisms and MACCE among patients with STEMI undergoing primary PCI.

Adult patients (18 years or older) admitted because of STEMI with an onset of $<$12 hour and undergoing primary PCI were included. Acute STEMI was diagnosed based on the increase in cardiac biomarker levels (hs-Troponin-T) with at least one value above the 99th percentile of the upper reference limit and clinical evidence of acute myocardial ischemia (i.e., symptoms of myocardial ischemia and electrocardiogram changes indicating new-onset ischemia),[9] then they were enrolled consecutively. Accordingly, patients with STEMI receiving fibrinolytic therapy; those with non-STEMI or unstable angina and those with a history of cancer, severe renal insufficiency (estimated glomerular filtration rate $<$ 30 mL/min), hepatic insufficiency, acute severe infection, and the inability to provide consent were excluded.

The study was approved by the Institutional Review Board and Local Ethics Committee of National Cardiovascular Centre—Harapan Kita Hospital in Jakarta, Indonesia (No: LB.02.01/VII/307/KEP.078/2–18) and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. All patients admitted to the emergency unit with life-threatening conditions agreed to participate and might have provided written informed consent the morning after admission, given the need for prioritizing emergency medical procedures.

Biomarker Measurements

Anthropometric data, medical history, and comorbidities were recorded and documented, and serum samples were obtained on the first day of admission and before statin administration. Peripheral venous blood samples were drawn upon entrance (day 0) and then centrifuged to obtain plasma samples. Samples were centrifuged at 2,100 g for 10 minutes and then kept as aliquots at −80°C in the Biomolecular Laboratory of Harapan Kita Hospital—NCC, Jakarta.

Biomarker measurement was performed by individuals blinded to the patient's clinical outcome data. *PCSK9* analysis was performed on plasma samples using the OmniKine Human *PCSK9* colorimetric ELISA kit (OmniKine kit MBS9502070, MyBiosource, Inc, San Diego, CA 92195–3308) according to the manufacturer's instructions. Briefly, plasma samples were first spun for 15 minutes at 1,000 rpm (rotation per minute) and 10-fold diluted in 10% fetal bovine serum–phosphate buffer saline. It was subsequently reacted in a "sandwich" format to capture antibodies for specific particular epitopes on human *PCSK9* and secondary detection antibodies. Finally, colorimetric reactions were obtained by peroxidase enzymes and TMB (tetramethylbenzidine) substrate reaction. An excellent standard curve formed was then utilized to measure the PCSK9 levels. The latex agglutination method was used to measure plasma high-sensitivity C-reactive protein (hs-CRP) using CRPHS Cobas from Roche Diagnostics (Roche Diagnostics, Risch-Rotkreuz; Mannheim, Germany). Different laboratory technicians performed plasma PCSK9 and hs-CRP assays. All procedures strictly followed the manufacturer's instructions.

Genotyping

The *PCSK9* genetic variability of R46L and E670G were determined using genomic DNA obtained from peripheral blood mononuclear cells with the QIAmp DNA Mini Kit (Qiagen, Hilden, Germany), then analyzed using TaqMan SNP Genotyping Assays (Thermo Fisher Scientific Inc, Applied Biosystem, NYSE; TMO). The TaqMan SNP genotyping assay ID number: c_2018188_10 (rs11591147) and ID number: c_998744_10 (rs505151) were respectively used to identify R46L and E670G variants. Briefly, the PCR (polymerase chain reactions) were conducted in 96-well plates, in which each well contained 10 ng genomic DNA in a final volume of 10 µL. This final volume consisted of 5 µL of each TaqMan SNP assay, and 5 µL of each TaqMan GTXpress master mix (Thermo Fischer Scientific Inc, Applied Biosystem, NYSE; TMO). To validate the amplification

of entire samples, assessment of reagent qualities, and detection of contamination and possible technical errors, negative controls, namely nontemplate control, was utilized. The amplification process was conducted by real-time PCR—Fast Advance type 7500 (Thermo Fisher Scientific Inc, Applied Biosystem, NYSE; TMO).

Clinical Outcomes

The primary outcome of the present study was the association between *PCSK9* polymorphisms and the occurrence of MACCE within 6 months of onset, whereas the secondary outcome was the correlation between the plasma PCSK9 and inflammation indicated by hs-CRP concentrations. The primary outcome was defined as the first occurrence of any composite outcome of all-cause mortality, recurrent MI, re-hospitalization, urgent revascularization procedure, and ischemic stroke within the 6-month follow-up period. Exposures were defined as polymorphisms of E670G and R46L in *the PCSK9* gene, namely: homozygote/heterozygote of both variants versus wild type.

Post-discharge follow-ups were conducted to collect information regarding medication adherence, risk factor control, and any clinical outcome during the follow-up period.

Statistical Analysis

Continuous variables were presented as mean ± standard deviation or median (interquartile range or between upper and lower quartiles), depending on the normality of their distribution, whereas categorical variables were expressed as proportions. Chi-square or Fisher's exact tests were utilized to compare categorical variables. The correlation between plasma PCSK9 and hs-CRP concentration was determined using the Spearman correlation analysis. Differences in plasma PCSK9 concentrations between patients who did and did not receive statins were presented using a boxplot and measured using Fischer's exact test.

The association between *PCSK9* gene variants and the MACCE was evaluated using the Cox proportional-hazards model and expressed as hazard ratios (HR) and 95% confidence intervals (CIs). Potential determinants showing a univariate association with MACCE ($p < 0.25$) were identified and included in the multivariate analysis model. This measure was intended to control for confounding variables to avoid the spurious association. All hypothesis tests were two-sided, with the significance level set at 5%, whereas sufficient statistical power was confirmed (≥0.80).

Results

Patients' Characteristics

This study was conducted between September 1, 2018, and April 30, 2019, at the National Cardiovascular Centre Harapan Kita Hospital in Jakarta, Indonesia. This was a tertiary referral teaching hospital for cardiovascular diseases (CVDs) accredited by JCI (Joint International Commission) no: CN 3599–1 and was effective from June 20, 2019, through June 19, 2022. The first and last patients were enrolled on September 2, 2018, and April 30, 2019.

The study subjects consisted of 423 patients admitted for STEMI undergoing primary PCI, among whom 31 (7.3%) had previously taken statins, and 392 were free of any lipid-lowering medications. Subjects had a mean age of 59.2 (± 10) years, and a vast majority (85.8%) identified as male. Among the included patients, 2.1% had the *PCSK9* E670G polymorphism and carried the 670G allele. Unfortunately, no *PCSK9* R46L polymorphisms were encountered despite extending the recruited sample to 610 subjects. Patients' characteristics are presented in ►**Table 1**. Interestingly, 2.1% of subjects with a heterozygote AG allele were male, and no homozygote GG allele was found (►**Table 1** and ►**Fig. 1**).

Furthermore, no significant differences in demographic and risk factors were observed between those with a heterozygote (AG) and wild-type (AA) allele, except for age ($p = 0.00$), thrombolysis in MI (TIMI) risk score > 4 ($p = 0.005$) and MACCE proportion ($p = 0.00$). All heterozygote patients were older, had more significant TIMI risk scores, and eventually had higher MACCE proportions than wild-type subjects (►**Table 1**).

During 6 months follow-up after index STEMI, 65 of 423 STEMI (15.4%) patients experienced MACCE. The Kaplan-Meier curve demonstrated that those with AG polymorphism had significantly more adverse events than wild type (►**Fig. 2**). Multivariate analysis showed significant positive associations between Killip class status (II-IV) (HR = 4.85; 95% CI: 2.30–10.20; $p = 0.00$) and E670G polymorphism (HR = 45.40; 95% CI: 5.30–390.30; $p = 0.00$) and MACCE among patients with STEMI (►**Table 2**).

As expected, a significant difference in PCSK9 levels was observed between patients who did (310 [220–1220] pg/mL) and did not (280 [190–1520] pg/mL) ($p = 0.001$) take any statins/lipid-lowering drugs. Moreover, no correlation was observed between PCSK9 concentration and hs-CRP levels among patients with STEMI in the scatter plot ($p = 0.88$) (►**Figs. 3** and **4**).

Discussion

The present study genotyped two *PCSK9* gene polymorphisms, namely, E670G (rs505151) and R46L (rs11591147) as exposures and determined the association between these variants and MACCE. Accordingly, our results demonstrated a significant association between E670G, a GOF polymorphism in the *PCSK9* gene, and the occurrence of MACCE within 180 days follow-up, as shown in Kaplan-Meier curve with HR: 7.47; 95% CI: 3.57–15.70; $p = 0.00$ (►**Fig. 2**). However, after adjustment in multivariate analysis, E670G polymorphism's association appeared to be the most important predictor to attenuate along with the Killip class (►**Table 2**). This has been the first study conducted in Asia for our ethnicity to the best of our knowledge.

Among the included patients, only 2.1% exhibited E670G polymorphism. Unfortunately, no R46L polymorphisms had been encountered despite increasing the number of subjects to 610. Consequently, we could not determine the interaction between such genotypic variants and phenotype in the studied ethnicity. The present study found no correlation

Table 1 Baseline characteristic of the subjects

Variable	Heterozygote (AG)	Wild type (AA)	*p*-Value
Demographic			
Age (Year), median (IQR)	64 (7)	54 (13)	0.00
Male, n (%)	9 (2.1)	414 (97.9)	0.61
Risk factor			
Hypertension, *n* (%)	5 (55.6)	248 (59.9)	1.00
Diabetes mellitus, *n* (%)	4 (44.4)	146 (35.3)	0.73
Dyslipidemia, *n* (%)	1 (11.1)	104 (25.1)	0.46
Smoker, *n* (%)	3 (33.3)	278 (67.1)	0.07
Family history, *n* (%)	1 (11.1)	58 (14)	1.00
Clinical characteristic			
Onset, *n* (%)			
<6 h	4 (44.4)	197 (47.6)	1.00
≥ 6 h	5 (55.6)	217 (52.4)	1.00
Killip Class I, *n* (%)	6 (66.7)	360 (87)	0.11
Killip Class II-IV, *n* (%)	3 (33.3)	54 (13)	0.11
TIMI score >4, *n* (%)	7 (77.8)	123 (29.7)	0.00
Anterior infarction, *n* (%)	1 (11.1)	48 (11.6)	0.60
Previous statin, *n* (%)	1 (11.1)	30 (7.3)	0.50
TIMI flow post intervention (TIMI 3)	3 (66.7)	210 (50.7)	0.50
Length of stay >5 d	3 (33.3)	129 (31.2)	0.57
Systolic ejection fraction (>40%)	2 (25)	171 (42.3)	0.48
MACCE	8 (88.9)	57 (13.8)	0.00
Laboratory parameter			
LDL-C (mg/dL), median (IQR)	126.29 (49)	127 (47)	0.73
HDL-C (mg/dL), median (IQR)	36 (15)	36 (12)	0.71
Triglyceride (mg/dL), median (IQR)	116 (129)	144 (89)	0.66
TC (mg/dL), median (IQR)	190 (58)	176 (50)	0.92
eGFR (mg/dl), median (IQR)	87 (39)	85 (38)	0.74
PCSK9 level (pg/mL), median (IQR)	260 (128)	280 (98)	0.43

Abbreviations: e-GFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein-cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein-cholesterol; MACCE, major adverse cardio-cerebrovascular events; PCSK9, proprotein convertase subtilisin/kexin type 9; TC, total cholesterol; TIMI, thrombolysis in myocardial infarction.

between plasma PCSK9 levels and inflammatory response indicated by hs-CRP concentration ($p = 0.88$) (►**Fig. 3**).

Nonetheless, other studies have shown that plasma PCSK9 concentration measured during the acute phase was associated with serum hs-CRP, triglycerides, and premature CHD among those with acute MI. However, the study did not predict cardiovascular disease (CVD) recurrence during the 1-year follow-up period.[10] Our present study recruited only patients with STEMI, all of whom were managed with primary PCI as recommended by our medical care standards.

However, in another previous study,[11] only half of the recruited non-STEMI (around 17% of all subjects) and STEMI (83%) patients underwent reperfusion therapy. The present study measured PCSK9 during admission (day 0), regardless of statin consumption. Nonetheless, only around 7.3% of all subjects included herein consumed statins before admission. Another essential finding of the current study was that patients who had received statins had significantly higher plasma PCSK9 levels than those who did not ($p = 0.001$) (►**Fig. 4**). Indeed, we observed a significant increase in plasma PCSK9 levels 24 hour after statin consumption, which plateaued after 48 hours.[11,12] Moreover, plasma PCSK9 levels did not further increase with continuous statin therapy.

Previous studies have limited their analysis to patients without prior statin consumption, given that statins have been recognized to enhance plasma PCSK9 levels in human and animal studies.[13,14] Accordingly, Dubuc had been the first to encounter higher *PCSK9* mRNA expression due to statins in 2004.[13] The statin-induced upregulation of *PCSK9* expression occurs through a mechanism involving the sterol

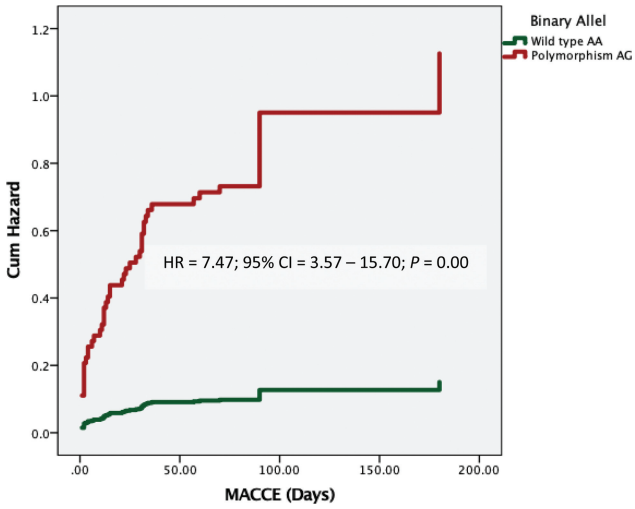

Fig. 1 Kaplan-Meier curve on E670G polymorphism of *PCSK9* and MACCE. HR, hazard ratio; MACCE, major adverse cardio-cerebrovascular events; PCSK9, proprotein convertase subtilisin/kexin type 9; 95% CI, 95% confidence interval).

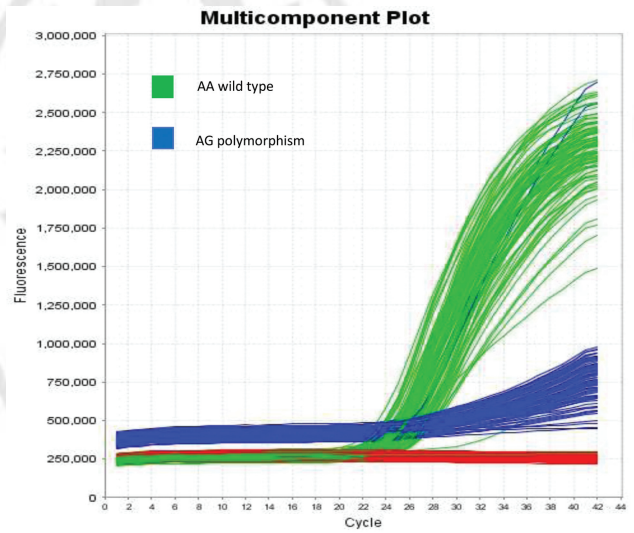

Fig. 2 Fluorescence curve of AG and GG genotype. (*Blue line representing AG polymorphism and green line representing wild type AA*).

Table 2 Multivariate analysis based on MACCE in STEMI patients

Variable	HR	95% CI	*p*-Value
Killip Class II-IV	4.85	2.30–10.20	0.00
Polymorphism of E670G mutant (AG)	45.40	5.30–390.30	0.00

Abbreviations: 95% CI, 95% confidence interval; HR, hazard ratio; MACCE, major adverse cardio-cerebrovascular events; STEMI, ST-elevation myocardial infarction.

regulatory binding protein-2 transcription factor.[15] Additionally, insulin concentration/resistance and tumor necrosis factor-α have affected *PCSK9* expression.[16]

The *PCSK9* gene is highly polymorphic, and functional variants affect plasma PCSK9 activity, resulting in lipid metabolism disturbances. Studies have shown that the

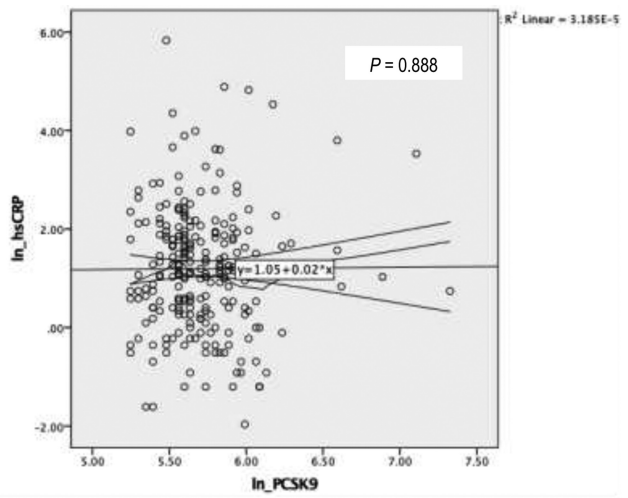

Fig. 3 Scatter plot of plasma PCSK9 and hs-CRP in STEMI patients treated with primary PCI. hs-CRP, highly sensitive C-reactive protein; PCI, percutaneous coronary intervention; PCSK9, proprotein convertase subtilisin/kexin type 9; STEMI, ST-elevation myocardial infarction.

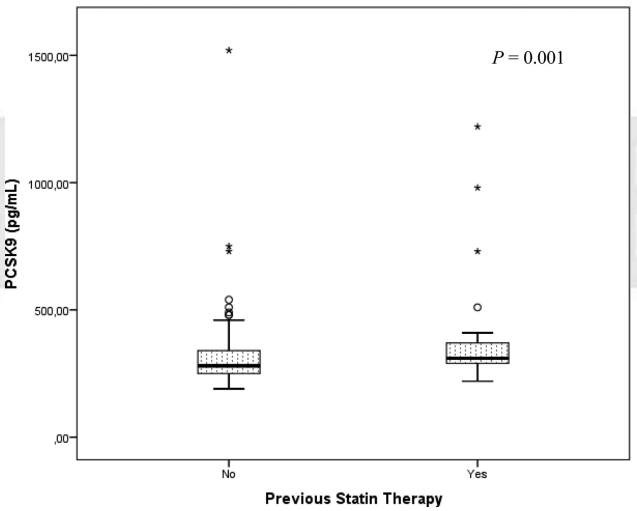

Fig. 4 Boxplot of median of PCSK9 levels between STEMI patients with previously statins versus without statins consumption. PCSK9, proprotein convertase subtilisin/kexin type 9; STEMI, ST-elevation myocardial infarction.

E670G polymorphism is an independent determinant of plasma LDL-C, conferring increased triglycerides and severity of atherosclerosis and ischemic stroke.[17–19] Meanwhile, another study found that among the Asian population, those with AG + GG genotypes had lower high-density lipoprotein cholesterol (HDL-C) levels than the wild types.[20] Moreover, among Han population G allele carriers, males had higher serum HDL-C and apoprotein A-1 levels, whereas females had lower serum Apo B levels and higher Apo A-1/Apo B ratio.[21] Among the Han people of Hainan and Northeast China who had CHD, the *PCSK9* E670G variant also showed regional differences.[6] The present study demonstrated that E670G polymorphisms could become a novel predictor of MACCE in patients with STEMI. Despite the small prevalence of the variants after multivariate analysis, these findings remain seemingly relevant.

One previous meta-analysis indicated that the *PCSK9* E670G polymorphism might be involved in coronary artery disease.[22] Despite the weak effect of a single SNP on pathophysiological processing, such genetic information may be potentially useful during lipid management and CVD risk prediction in clinical practice.

Two years after the first study reported that GOF mutations lead to hypercholesterolemia, LOF mutations emerged as described.[8] They were found to be associated with lower cholesterol levels and a reduction of CHD. Moreover, studies had observed the L46 allele showed a trend toward a protective effect (OR = 0.75, 95% CI = 0.49–1.13; $p = 0.17$), as well as a significant association between the *PCSK9* R46L variant and markers of subclinical atherosclerosis (carotid intima-media thickness), although its association with MI was not significant.[23,24] This association was plausible as the mutation decreases the phosphorylation of Ser47. Phosphorylation may play an essential regulatory role in the PCSK9 function, which is assumed to protect PCSK9 from proteolysis.[25,26] Therefore, PCSK9 secretion into plasma decreases with rs11591147.

The absence of such a variant in the present study may be reasonable considering its minimal frequency in some populations. Indeed, one study showed that the R46L variant was present in only 3% of the familial hypercholesterolemia population.[27] Furthermore, the *PCSK9* 46L allele is more frequent among healthy men from the United Kingdom than patients with familial hypercholesterolemia. It is strongly associated with a protective plasma lipid profile risk against CHD. However, considering its low frequency (approximately 2% of carriers), it does not significantly determine populations at risk for CHD in the United Kingdom.[28] Pharmacogenetic studies have shown that GOF and LOF variants of the *PCSK9* gene are associated with worse and better responses to statin treatment, respectively.[29,30]

The current study's strength was that the recruited subjects were better identified as a high-risk population, which is entirely different compared with other studies.[10,11,13,31] Moreover, this has been the first study to determine the association between *PCSK9* E670G and R46L polymorphisms and MACCE among patients with STEMI undergoing primary PCI. Given that the current study was a single-center prospective cohort observational study and not a nationwide study, its selected population attending the tertiary hospital can be considered a limitation.

Conclusion

The present study found that the GOF E670G polymorphism of the *PCSK9* gene was associated with MACCE among patients with STEMI undergoing primary PCI within a follow-up duration of 6 months. Additionally, prior statin consumption was significantly associated with higher plasma PCSK9 levels among patients with STEMI.

Ethical Approval

All procedures performed in the study involving human participants were following the ethical standards and had been reviewed by the Institutional Review Board and Local Ethics Committee of National Cardiovascular Centre—Harapan Kita Hospital in Jakarta, Indonesia (No: LB.02.01/VII/307/KEP.078/2–18) and conforms to the ethical guidelines of the 1964 Helsinki declaration and its amendments, and the 2016 International Ethical Guidelines for Health-related Research Involving Humans (CIOMS and WHO).

Note

Informed consent was obtained from all individuals included in the study.

Funding

This study was funded by the National Cardiovascular Centre—Harapan Kita Hospital, Jakarta—Indonesia, with the grant number: LB. 02.02/XX.8/0224/2019

Conflict of Interest

A.S., Y.Y., H.S., A.N.P., and E.L. declare that they have no conflict of interest related to this work.

References

1 Dharma S, Andriantoro H, Purnawan I, et al. Characteristics, treatment and in-hospital outcomes of patients with STEMI in a metropolitan area of a developing country: an initial report of the extended Jakarta Acute Coronary Syndrome registry. BMJ Open 2016;6(08):e012193
2 Navarese EP, Kołodziejczak M, Dimitroulis D, et al. From proprotein convertase subtilisin/kexin type 9 to its inhibition: state-of-the-art and clinical implications. Eur Heart J Cardiovasc Pharmacother 2016;2(01):44–53
3 Robinson JG, Nedergaard BS, Rogers WJ, et al;LAPLACE-2 Investigators. Effect of evolocumab or ezetimibe added to moderate- or high-intensity statin therapy on LDL-C lowering in patients with hypercholesterolemia: the LAPLACE-2 randomized clinical trial. JAMA 2014;311(18):1870–1882
4 Bittner V. Pleiotropic effects of PCSK9 (proprotein convertase subtilisin/Kexin type 9) inhibitors? Circulation 2016;134(22):1695–1696
5 Chan DC, Watts GF. Postprandial lipoprotein metabolism in familial hypercholesterolemia: thinking outside the box. Metabolism 2012;61(01):3–11
6 Aung LH, Yin RX, Miao L, et al. The proprotein convertase subtilisin/kexin type 9 gene E670G polymorphism and serum lipid levels in the Guangxi Bai Ku Yao and Han populations. Lipids Health Dis 2011;10:5
7 Abifadel M, Varret M, Rabès JP, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet 2003;34(02):154–156
8 Cohen J, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. Nat Genet 2005;37(02):161–165
9 Ibanez B, James S, Agewall S, et al;ESC Scientific Document Group. 2017 ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J 2018;39(02):119–177
10 Gao Y, Qiu Y, Wu J, et al. Acute-phase plasma PCSK9 levels and recurrent cardiovascular events in a Chinese acute myocardial infarction cohort. Cardiology 2018;141(02):88–97
11 Welder G, Zineh I, Pacanowski MA, Troutt JS, Cao G, Konrad RJ. High-dose atorvastatin causes a rapid sustained increase in

human serum PCSK9 and disrupts its correlation with LDL cholesterol. J Lipid Res 2010;51(09):2714–2721
12 Cariou B, Guérin P, Le May C, et al. Circulating PCSK9 levels in acute coronary syndrome: Results from the PC-SCA-9 prospective study. Diabetes Metab 2017;43(06):529–535
13 Dubuc G, Chamberland A, Wassef H, et al. Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. Arterioscler Thromb Vasc Biol 2004;24(08): 1454–1459
14 Mayne J, Dewpura T, Raymond A, et al. Plasma PCSK9 levels are significantly modified by statins and fibrates in humans. Lipids Health Dis 2008;7:22
15 Jeong HJ, Lee HS, Kim KS, Kim YK, Yoon D, Park SW. Sterol-dependent regulation of proprotein convertase subtilisin/kexin type 9 expression by sterol-regulatory element binding protein-2. J Lipid Res 2008;49(02):399–409
16 Glerup S, Schulz R, Laufs U, Schlüter KD. Physiological and therapeutic regulation of PCSK9 activity in cardiovascular disease. Basic Res Cardiol 2017;112(03):32
17 Chen SN, Ballantyne CM, Gotto AM Jr, Tan Y, Willerson JT, Marian AJ. A common PCSK9 haplotype, encompassing the E670G coding single nucleotide polymorphism, is a novel genetic marker for plasma low-density lipoprotein cholesterol levels and severity of coronary atherosclerosis. J Am Coll Cardiol 2005;45(10):1611–1619
18 Qiu C, Zeng P, Li X, et al. What is the impact of PCSK9 rs505151 and rs11591147 polymorphisms on serum lipids level and cardiovascular risk: a meta-analysis. Lipids Health Dis 2017;16(01):111
19 Slimani A, Harira Y, Trabelsi I, et al. Effect of E670G polymorphism in PCSK9 gene on the risk and severity of coronary heart disease and ischemic stroke in a Tunisian cohort. J Mol Neurosci 2014;53 (02):150–157
20 Cai G, Zhang B, Shi G, et al. The associations between proprotein convertase subtilisin/kexin type 9 E670G polymorphism and the risk of coronary artery disease and serum lipid levels: a meta-analysis. Lipids Health Dis 2015;14:149
21 He XM, Chen L, Wang TS, Zhang YB, Luo JB, Feng XX. E670G polymorphism of PCSK9 gene of patients with coronary heart disease among Han population in Hainan and three provinces in the northeast of China. Asian Pac J Trop Med 2016;9(02):172–176
22 Adi D, Xie X, Liu F, et al. Relationships between genetic polymorphisms of E670G in PCSK9 gene and coronary artery disease: a meta-analysis. Int J Clin Exp Med 2015;8(08):13251–13258
23 Guella I, Asselta R, Ardissino D, et al. Effects of PCSK9 genetic variants on plasma LDL cholesterol levels and risk of premature myocardial infarction in the Italian population. J Lipid Res 2010; 51(11):3342–3349
24 Dron JS, Hegele RA. Complexity of mechanisms among human proprotein convertase subtilisin-kexin type 9 variants. Curr Opin Lipidol 2017;28(02):161–169
25 Dewpura T, Raymond A, Hamelin J, et al. PCSK9 is phosphorylated by a Golgi casein kinase-like kinase ex vivo and circulates as a phosphoprotein in humans. FEBS J 2008;275(13):3480–3493
26 Mostaza JM, Lahoz C, Salinero-Fort MA, et al;investigators of the SPREDIA-2 Group. R46L polymorphism in the PCSK9 gene: relationship to lipid levels, subclinical vascular disease, and erectile dysfunction. J Clin Lipidol 2018;12(04):1039–1046.e3
27 Saavedra YG, Dufour R, Davignon J, Baass A. PCSK9 R46L, lower LDL, and cardiovascular disease risk in familial hypercholesterolemia: a cross-sectional cohort study. Arterioscler Thromb Vasc Biol 2014;34(12):2700–2705
28 Scartezini M, Hubbart C, Whittall RA, Cooper JA, Neil AH, Humphries SE. The PCSK9 gene R46L variant is associated with lower plasma lipid levels and cardiovascular risk in healthy U.K. men. Clin Sci (Lond) 2007;113(11):435–441
29 Feng Q, Wei WQ, Chung CP, et al. The effect of genetic variation in PCSK9 on the LDL-cholesterol response to statin therapy. Pharmacogenomics J 2017;17(02):204–208
30 Postmus I, Trompet S, Deshmukh HA, et al;Welcome Trust Case Control Consortium. Pharmacogenetic meta-analysis of genome-wide association studies of LDL cholesterol response to statins. Nat Commun 2014;5:5068
31 Navarese EP, Kolodziejczak M, Winter MP, et al. Association of PCSK9 with platelet reactivity in patients with acute coronary syndrome treated with prasugrel or ticagrelor: The PCSK9-REACT study. Int J Cardiol 2017;227:644–649

