Circulating long non-coding RNA Coromarker expression correlated with inflammation, coronary artery stenosis, and plaque vulnerability in patients with coronary artery disease

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Abstract

Background: The aim of the study was to assess the correlation between circulating long non-coding RNA (lncRNA) OTTHUMT00000387022 (named Coromarker) expression and disease severity, inflammatory cytokine levels, and plaque vulnerability in patients with coronary artery disease (CAD).

Methods: A total of 134 participants who received coronary angiography were enrolled and classified them as CAD patients (N = 89) and controls (N = 45). Blood samples were obtained from all subjects. Quantitative polymerase chain reaction was used to evaluate Coromarker expression. The enzyme-linked immunosorbent test was used to measure inflammatory cytokines including high sensitivity C reactive protein (hsCRP), interleukin (IL)-1β (IL-1β), IL-6, NOD-like receptor protein 3 (NLRP3), and markers of coronary plaque stability including matrix metalloproteinase 9 (MMP-9) and soluble CD40 ligand (sCD40L). The severity of coronary stenosis was determined from the Gensini Score.

Results: LncRNA Coromarker expression was elevated to a greater extent in CAD patients than in control subjects before and after adjustments for age/gender (both p < 0.001); it was an independent predictor of CAD risk (area under curve: 0.824, 95% CI: 0.732–0.915). Additionally, Coromarker expression was significantly associated with Gensini Score (r = 0.574, p < 0.001), hsCRP (r = 0.221, p = 0.015), IL-1β (r = 0.351, p < 0.001), IL-6 (r = 0.286, p < 0.01), and NLRP3 levels (r = 0.312, p < 0.001). Coromarker expression was found to be linked with MMP-9 (r = 0.260, p < 0.01) and sCD40L (r = 0.441, p < 0.001).

Conclusion: Circulating IncRNA Coromarker expression correlates with increased disease severity and inflammation as well as plaque vulnerability in patients with CAD.

Keywords
coronary artery disease, coronary stenosis, inflammatory cytokines, lncRNA OTTHUMT00000387022, plaque vulnerability
1 | INTRODUCTION

Atherosclerosis (AS) is a persistent pathological process that is closely associated with inflammation. It serves as the pathological hallmark of coronary artery disease (CAD). Although risk factors (such as smoking, hypertension, hyperlipidemia, and diabetes) for CAD and strategies for their management (including with pharmacologic therapeutic drugs such as statins and antiplatelet 

2 | METHODS

2.1 | Participants

A total of 134 patients who presenting with chest pain and receiving diagnostic coronary angiography in The First Affiliated Hospital, Sun Yat-Sen University, from October 2010 to January 2021, were consecutively enrolled in this case–control study. Before angiography, all participants were received guideline-recommended drugs for CAD (antiplatelet, β-blocker, angiotensin-converting enzyme inhibitor, or angiotensin receptor blocker). The inclusion criteria were as follows: (1) over the age of 18; (2) did not have a serious infection, systematic hematological, or inflammatory disease; (3) did not receive coronary revascularization; and (4) did not have malignant tumors. The exclusion criteria included (1): had hemodynamically significant valvular heart disease, myocardiopathies, pulmonary embolism, New York Heart Association class III or IV, failure of other vital organs; (2) contraindications to coronary angiography; and (3) pregnant or lactating woman patients. Based on the results of angiography, patients were divided into CAD group (N = 89) and control group (N = 45). CAD was defined as a condition in which the patient had at least one coronary artery with 50% stenosis. Among CAD patients, 45 were diagnosed with chronic coronary syndrome (CCS) and 44 diagnosed with acute coronary syndrome (ACS) based on current guideline. This research was complied with the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-Sen University.

2.2 | Data and sample collection

Before conducting coronary angiography, we obtained detailed clinical data for all subjects, including demographic information, CAD risk factors, and biochemical parameters. We collected 10 ml peripheral blood sample from each patient within 24 h after admission. These blood samples were centrifuged at 3000g for 15 min (4°C) after collection, and plasma was isolated and stored at −80°C until measurement.

2.3 | Disease severity assessment

We used the Gensini scoring method to determine coronary artery narrow degree. This method took into account both the multiplication factor for the major vascular segment and the degree of luminal narrowing. The following scores were assigned after assessing the extent of luminal narrowing: 1 (25% lumen diameter reduction), 2 (50% lumen diameter reduction), 4 (75% lumen diameter reduction), 8 (90% lumen diameter reduction), and 32 (total occlusion). Each main coronary segment multiplying factor (such as 0.5 or 5) was determined based on the importance of the position of the lesion in coronary circulation. We determined the final Gensini Score by multiplying the luminal stenosis score with the main coronary segment factor.

2.4 | Quantitative reverse transcription PCR (qRT-PCR)

We used qRT-PCR to detect the level of IncRNA Coromarker in the plasma of all the participants. The process of RNA extraction was conducted using the RNApure total RNA fast isolation kit (BioTeke). Purity and concentration of the extracted RNA were assessed using an Eppendorf Biophotometer D30 (Eppendorf). A260/A280 ratios of 1.9 to 2.1 and A260/A230 ratios >2 were required for sample RNAs. By electrophoresis on 1% agarose gel, RNA integrity was assessed. According to the manufacturer’s recommendations, 1 μg of total RNA was reverse transcribed into cDNA using the HiScript II Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme). Subsequently, qRT-PCR was performed using Hieff TMqPCR SYBR® Green Master Mix (Low Rox Plus) (Yeasen) with Line Gene 9600 (Bioneer). The 20 μl reaction mixture was incubated at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 15 s, annealing at 60°C for 20 seconds and extention at 72°C for 20 s. The formula 2^△△Ct was used to calculate the relative expression levels of the IncRNA Coromarker, with β-actin serving as the internal reference. For the qRT-PCR
analysis, the primers designed by Primer Premier 5.0 were as follows: Coromarker forward primer: AGGCTGGTGAAGCTATGG, reverse primer: ACCACAATGCCTTTGAGCCTAATCT; β-actin forward primer: AGATCAAGATCATTGCTCCTC; reverse primer: ACGCAGCTCAGTACAGTCC; The cDNA was amplified in triplicate.

2.5 | Enzyme-linked immunosorbent assay (ELISA)

The plasma levels of high-sensitivity C reactive protein (hsCRP), interleukin (IL)-1β (IL-1β), IL-6, NOD-like receptor protein 3 (NLRP3), matrix metalloproteinase 9 (MMP-9), and soluble CD40 ligand (sCD40L) in control subjects and CAD patients was measured by commercial ELISA kits (R&D), in accordance with the manufacturer’s instructions. The quality control of ELISAs was presented in Table S1.

2.6 | Statistics

We used the SPSS 22.0 (IBM) and GraphPad Prism 6.0 (GraphPad Software) software for statistical analysis. We performed the Kolmogorov–Smirnov test to determine the normality of continuous data; values were expressed in terms of mean± standard deviation, median and interquartile range values. Categorical variables were presented as count and percentage values. Student’s t test, Wilcoxon’s rank sum test, or chi-square test were used for comparing two groups. The Kruskal–Wallis rank sum test was used for comparing three groups. The lncRNA Coromarker level that could be used for the prediction of CAD risk was evaluated using a receiver operating characteristic (ROC) curve. Then, propensity score matching was used to select the age- and gender-matched cohort. The Spearman 2-way test was used to investigate the relationships between the Coromarker expression and Gensini Score, hsCRP, IL-1β, IL-6, NLRP-3, troponin T, MMP-9, and sCD40L levels.

3 | RESULTS

3.1 | Baseline characteristics

In terms of demographics, the ages of the CAD participants and control individuals were 65.2±9.5 years and 62.7±8.5 years, respectively (p = 0.132). There were 67 males and 22 females in the CAD patient group, while the control group included 24 males and 21 females (p = 0.010). No differences were observed in the body mass index between the two groups (p = 0.108). In terms of CAD risk factors, higher rates of smoking (p = 0.024), diabetes mellitus (p = 0.015), and hyperlipidemia (p < 0.001) were observed in patients with CAD, whereas no difference was observed in the prevalence of hypertension (p = 0.420) or hyperuricemia (p = 0.112) between the CAD group and control group. The CAD subjects had a higher Gensini Score (52.3 [38.3–78]) compared with that observed for control subjects (3.5 [2.5–5.8]; p < 0.001). In terms of biochemical indices, no difference was observed in the fasting blood glucose (p = 0.056), triglyceride (p = 0.356), total cholesterol (p = 0.481), or low-density lipoprotein cholesterol (p = 0.939) levels between the

| Parameters           | CAD patients (n = 89) | Controls (n = 45) | p value |
|----------------------|-----------------------|------------------|---------|
| Age (years)          | 65.2 ± 9.5            | 62.7 ± 8.5       | 0.132   |
| Gender (Male/Female) | 67/22                 | 24/21            | 0.01    |
| BMI (kg/m²)          | 23.2 ± 7.5            | 25.1 ± 3.6       | 0.108   |
| Smoke (n/%)          | 32 (35.9)             | 7 (15.6)         | 0.024   |
| Hypertension (n/%)   | 63 (70.8)             | 28 (62.2)        | 0.42    |
| Diabetes mellitus (n/%)| 36 (40.4)             | 8 (17.8)        | 0.015   |
| Hyperlipidemia (n/%) | 68 (76.4)             | 14 (31.1)        | <0.001  |
| Hyperuricemia (n/%)  | 40 (44.9)             | 17 (37.8)        | 0.112   |
| Gensini score        | 52.3 (38.3, 78)       | 3.5 (2.5, 5.8)   | <0.001  |
| FBG (mmol/L)         | 6.69 ± 2.43           | 5.87 ± 1.90      | 0.056   |
| TG (mmol/L)          | 1.53 ± 0.70           | 1.41 ± 0.65      | 0.356   |
| TC (mmol/L)          | 4.40 ± 1.33           | 4.56 ± 1.03      | 0.481   |
| HDL-C (mmol/L)       | 0.98 ± 0.20           | 1.18 ± 0.23      | <0.001  |
| LDL-C (mmol/L)       | 2.83 ± 0.99           | 2.84 ± 0.77      | 0.939   |
| Serum creatinine (mmol/L) | 91.81 ± 29.76   | 72.75 ± 15.46     | <0.001  |

Note: Data were presented as mean value ± standard deviation, median and 25th–75th value or count (percentage). Significance of the comparison was determined by t test, Wilcoxon rank sum test or chi-square test. p value <0.05 was considered significant. Bold values indicate the statistical significance.

Abbreviations: BMI, body mass index; CAD, coronary artery disease; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.
CAD group and control group. The clinical features of patients are shown in Table 1.

3.2 | The expression and predictive value of lncRNA Coromarker

Coromarker expression was higher in CAD group (median value, 2.525 [1.826–3.242]) than in controls (median value, 1.298 [1.070–1.752]; p<0.001; Figure 1A) prior to the adjustment of values. In addition, the Coromarker was a predictor of elevated risk for CAD (AUC: 0.866, 95% CI: 0.800–0.931; Figure 1B). After adjusting for age and gender, Coromarker expression (median value, 2.280 [1.758–2.724]) in CAD patients was still greater than those in the control individuals (median value, 1.298 [1.034–1.897]; p<0.001; Figure 1C) and linked with increased CAD risk (AUC: 0.824, 95% CI: 0.732–0.915; Figure 1D).

3.3 | Correlation between lncRNA Coromarker and degree of coronary stenosis

As shown in Figure 2, the expression of Coromarker was associated with a higher Gensini Score in CAD patients (r = 0.574, p<0.001). This may imply that CAD patients with higher Coromarker expression had more severe coronary stenosis.

**FIGURE 1** Expression of the lncRNA Coromarker and its predictive value for CAD risk before and after age/gender adjustment.

Before adjustment, lncRNA Coromarker expression (A) and its predictive value for CAD risk (B). After adjustment by age/gender, lncRNA Coromarker expression (C) and its predictive value for CAD risk (D). AUC, area under curve; CHD, coronary heart disease; CI, confidence interval; lncRNA, long non-coding RNA.
3.4 | Correlation between IncRNA Coromarker and inflammatory cytokines

As demonstrated in Figure 3, Coromarker expression was significantly linked with inflammatory cytokines levels, including hsCRP ($r = 0.221, p = 0.015$), IL-1β ($r = 0.351, p < 0.001$), IL-6 ($r = 0.286, p < 0.01$), and NLRP3 ($r = 0.312, p < 0.001$). These findings suggest that Coromarker was linked to higher levels of inflammation in CAD patients.

3.5 | Association of IncRNA Coromarker with plaque vulnerability

Forty-five and 44 patients were diagnosed with CCS and ACS, respectively. LncRNA Coromarker expression was remarkably elevated in ACS participants, compared with CCS patients ($p = 0.017$, Figure 4A) and control subjects ($p < 0.001$, Figure 4A). In addition, Coromarker expression was positively associated with troponin T ($r = 0.546, p < 0.001$, Figure 4B), MMP-9 ($r = 0.260, p < 0.01$, Figure 4C), and sCD40L ($r = 0.441, p < 0.001$, Figure 4D) levels. These results suggest that Coromarker was associated with coronary plaque stability.

4 | DISCUSSION

AS is the most common pathological mechanism of CAD. It is characterized by the lipid-rich plaque formation on the inner walls of arteries.$^{1,2}$ Inflammation was considered to plays a pivotal role in the development and progression of AS.$^{19–20}$ LncRNAs are epigenetic, transcriptional, and translational regulators that coordinate and integrate numerous signaling pathways involved in processes such as cellular proliferation, differentiation, and organ development.$^{6–8}$ Increasing evidence suggests that IncRNAs play an important role in the cardiovascular system and are associated with susceptibility to CAD. For example, in patients with serious CAD, the expression of IncRNA nuclear-enriched abundant transcript 1 (NEAT1) and IncRNA-NEAT1/miR-125a axis increased, and these changes were strongly correlated with the accumulation of significant adverse cardiac and cerebrovascular events.$^{21}$ In a recent study, RNA-seq was used to examine the IncRNA and mRNA expression profiles in peripheral blood mononuclear cells of patients with unstable angina and healthy coronary artery controls. The findings showed that many IncRNAs were differentially expressed and were considered as potential biomarkers for the diagnosis of unstable angina.$^{22}$ These findings suggest that IncRNAs could play a role in the development of CAD.

Coromarker is a novel IncRNA that was discovered by Cai et al.$^{13}$ Cai et al. performed microarray-based differential expression
profiling of IncRNAs and found that the expression of 86 IncRNAs was remarkably elevated in circulating peripheral blood monocytes and plasma in CAD patients. After the confirmation of these results by qRT-PCR, only three IncRNAs (OTTHUMT00000387022, BAT5, and IL21R-AS1) could be considered as candidate markers of CAD. Upon performing ROC analysis in a larger cohort, IncRNA OTTHUMT00000387022 was identified as the best candidate biomarker for CAD and was renamed as Coromarker. The study by Cai et al. also facilitated the identification of 35 and 33 genes that were positively and negatively correlated with the Coromarker expression levels, respectively. The results of functional enrichment analysis revealed that genes that were positively correlated with Coromarker were implicated in signal transduction, transmembrane transportation, and synaptic transmission, while the genes that were negatively correlated with Coromarker were involved in innate immune response generation, cytokine signaling, and apoptotic signaling pathways, all of which may affect AS development.13,14

In this study, it is found that the circulating Coromarker expression was elevated in CAD patients, and the ROC curve demonstrated a strong predictive value for CAD risk. These results could be attributable to the positive regulation of systemic inflammation by the IncRNA Coromarker, which results in CAD development, as indicated by the positive correlation with inflammatory cytokines, including hsCRP, IL-1β, and IL-6. The Coromarker expression level was also linked to the Gensini Score, indicating that it was associated with coronary artery stenosis. It was hypothesized that Coromarker might regulate the transcription of pro-inflammatory factors, increase the cytokine expression, and accelerate AS development and aggravate the degree of coronary stenosis. It was thought that IL-1 and IL-6 were crucial in the development of AS. IL-1β upregulates the expression of adhesion molecules on the endothelial cell surface, promotes vascular smooth muscle cell proliferation, and stimulates the production of cytokines implicated in AS development.23 IL-6 activates endothelial cells, accelerates coagulation, and promotes lymphocyte proliferation and differentiation.24

To gain more insight into the mechanism that underpins the role of the Coromarker in inflammation, the association between the expression of Coromarker and NLRP3 plasma level was evaluated in
this study. The Coromarker expression was significantly correlated with the level of NLRP3. The NLRP3 signaling pathway was the central mediator and a key participant in inflammation-associated processes. The activation of NLRP3 resulted in the activation of caspase-1 protease. This induced an increase in the production and release of powerful inflammatory cytokines including IL-1β and IL-6, which are important for vascular pathogenesis.25,26 The NLRP3 inflammasome has been shown to participate in AS development and progression in atherosclerotic mouse models.27,28 AS severity was linked to the levels of downstream cytokines of the NLRP3 inflammasome in individuals with established CAD. This phenomenon has inspired clinical trials for the investigation of anti-inflammatory therapies.29 The specific molecular mechanism by which the lncRNA Coromarker affects the regulation of inflammation, however, has to be investigated further in studies involving animals or cell lines.

The majority of cases of ACS are caused by thrombotic occlusions in coronary arteries that are caused by an atherosclerotic plaque rupture, which can result in a life-threatening thromboembolic event. It is well known that MMP-9 and sCD40L are biomarkers of plaque rupture or vulnerability. CD40L can activate plaque macrophages; the activated macrophages then produce MMPs that facilitate the disintegration of the extracellular plaque matrix, thus causing plaque instability and resulting in a fatal ACS event. The predictive value of plasma MMP-9 and sCD40L levels in ACS patients has been previously described.30-32 Elevated serum MMP-9 and sCD40L levels were associated with plaque rupture, as compared to the levels observed in stable angina pectoris patients.33,34 In this study, Coromarker expression were higher in ACS patients compared with non-ACS subjects and were positively correlated with troponin T, MMP-9, and sCD40L levels. These findings indicate that lncRNA Coromarker was associated with the coronary plaque stability. It is hypothesized that the Coromarker’s pro-inflammatory activities have a significant impact on the development of coronary plaque.

This study had some limitations: (1) the sample size was small, which could have resulted in reduced statistical efficiency, when compared to studies involving larger sample sizes; thus, it would be beneficial to further confirm the results using larger sample sizes; (2) all the patients enrolled in this study were from a single center, which could have resulted in selection bias; and (3) the impact of lncRNA Coromarker on prognosis was not assessed, and future cohort studies need to be conducted to investigate the role of lncRNA Coromarker in CAD.

In conclusion, the circulating lncRNA Coromarker expression was linked to coronary stenosis, inflammation, and plaque vulnerability.

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**CONFLICT OF INTEREST**

None.

**DATA AVAILABILITY STATEMENT**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**REFERENCES**

1. Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med*. 1999;340(2):115-126.
2. Libby P, Theroux P. Pathophysiology of coronary artery disease. *Circulation*. 2005;111(25):3481-3488.
3. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*. 2005;352(16):1685-1695.
4. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet*. 2016;388(10053):1545-1602.
5. GBD 2017 Causes of Death Collaborators. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet*. 2018;392(10159):1736-1788.
6. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. *Annu Rev Biochem*. 2012;81:145-166.
7. Wang KC, Chang HY. Molecular mechanisms of long noncoding RNAs. *Mol Cell*. 2011;43(6):904-914.
8. Ng SY, Lin L, Soh BS, Stanton LW. Long noncoding RNAs in development and disease of the central nervous system. *Trends Genet*. 2013;29(8):461-468.
9. Klattenhoff CA, Scheuermann JC, Surface LE, et al. Braveheart, a long noncoding RNA required for cardiovascular lineage commitment. *Cell*. 2013;152(3):570-583.
10. Grote P, Wittler L, Hendrix D, et al. The tissue-specific lncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. *Dev Cell*. 2013;24(2):206-214.
11. Holdt LM, Beutner F, Scholz M, et al. ANRIL expression is associated with atherosclerosis risk at chromosome 9p21. *Arterioscler Thromb Vasc Biol*. 2010;30(3):620-627.
12. Burd CE, Jeck WR, Liu Y, Sanoff HK, Wang Z, Sharpless NE. Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk. *PLoS Genet*. 2010;6(12):e1001233.
13. Cai Y, Yang Y, Chen X, et al. Circulating lncRNA OTTHUMP00000387022 from monocytes as a novel biomarker for coronary artery disease. *Cardiovasc Res*. 2016;112(3):714-724.
14. Yang Y, Cai Y, Wu G, et al. Plasma long non-coding RNA, coromarker, a novel biomarker for diagnosis of coronary artery disease. *Clin Sci*. 2015;129(8):675-685.
15. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. 2016;37(27):2129-2200.
16. Knuuti J, Wijns W, Saraste A, et al. 2019 ESC guidelines for the diagnosis and management of chronic coronary syndromes. *Eur Heart J*. 2020;41(3):407-477.
17. Collet JP, Thiele H, Barbato E, et al. 2020 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J*. 2021;42(14):1289-1367.
18. Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol*. 1983;51(3):606.
19. Ott SJ, El Mokhtari NE, Musfeldt M, et al. Detection of diverse bacterial signatures in atherosclerotic lesions of patients with coronary heart disease. *Circulation*. 2006;113(7):929-937.
20. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. Science. 2015;349(6245):316-320.

21. Liu H, Yan X, Yu J. Long noncoding RNA NEAT1/microRNA-125a axis predicts increased major adverse cardiac and cerebrovascular event risk independently in patients with unprotected left main coronary artery disease underwent coronary artery bypass grafting. J Clin Lab Anal. 2020;34(7):e23299.

22. Liu H, Yan X, Yu J. Long noncoding RNA NEAT1/microRNA-125a axis predicts increased major adverse cardiac and cerebrovascular event risk independently in patients with unprotected left main coronary artery disease underwent coronary artery bypass grafting. J Clin Lab Anal. 2020;34(7):e23299.

23. Khan R, Rheume E, Tardif JC. Examining the role of and treatment directed at IL-1β in atherosclerosis. Curr Atheroscler Rep. 2018;20(11):53.

24. Hartman J, Frishman WH. Inflammation and atherosclerosis: a review of the role of interleukin-6 in the development of atherosclerosis and the potential for targeted drug therapy. Cardiol Rev. 2014;22(3):147-151.

25. He Y, Hara H, Núñez G. Mechanism and regulation of NLRP3 inflammasome activation. Trends Biochem Sci. 2016;41(12):1012-1021.

26. Akther M, Haque ME, Park J, Kang TB, Lee KH. NLRP3 ubiquitination—a new approach to target NLRP3 inflammasome activation. Int J Mol Sci. 2021;22(16):8780.

27. Duewell P, Kono H, Rayner KJ, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. Nature. 2010;464(7293):1357-1361.

28. Westerterp M, Fotakis P, Ouimet M, et al. Cholesterol efflux pathways suppress inflammasome activation, NETosis, and atherogenesis. Circulation. 2018;138(9):898-912.

29. Satoh M, Tabuchi T, Itoh T, Nakamura M. NLRP3 inflammasome activation in coronary artery disease: results from prospective and randomized study of treatment with atorvastatin or rosuvastatin. Clin Sci. 2014;126(3):233-241.

30. Kai H, Ikeda H, Yasukawa H, et al. Peripheral blood levels of matrix metalloproteases-2 and -9 are elevated in patients with acute coronary syndromes. J Am Coll Cardiol. 1998;32(2):368-372.

31. Inokubo Y, Hanada H, Ishizaka H, Fukushima T, Kamada T, Okumura K. Plasma levels of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 are increased in the coronary circulation in patients with acute coronary syndrome. Am Heart J. 2001;141(2):211-217.

32. Målarstig A, Lindahl B, Wallentin L, Siegbahn A. Soluble CD40L levels are regulated by the −3459 A>G polymorphism and predict myocardial infarction and the efficacy of antithrombotic treatment in non-ST elevation acute coronary syndrome. Arterioscler Thromb Vasc Biol. 2006;26(7):1667-1673.

33. Fukuda D, Shimada K, Tanaka A, et al. Comparison of levels of serum matrix metalloproteinase-9 in patients with acute myocardial infarction versus unstable angina pectoris versus stable angina pectoris. Am J Cardiol. 2006;97(2):175-180.

34. Aukrust P, Müller F, Ueland T, et al. Enhanced levels of soluble and membrane-bound CD40 ligand in patients with unstable angina. Possible reflection of T lymphocyte and platelet involvement in the pathogenesis of acute coronary syndromes. Circulation. 1999;100(6):614-620.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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