

RESEARCH ARTICLE

Relation of circulating lncRNA GAS5 and miR-21 with biochemical indexes, stenosis severity, and inflammatory cytokines in coronary heart disease patients

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Abstract

Background: Long noncoding RNA GAS5 (lnc-GAS5) and its target microRNA-21 (miR-21) regulate blood lipid, macrophages, Th cells, vascular smooth muscle cells to participate in atherosclerosis, and related coronary heart disease (CHD). The study aimed to further explore the linkage of their circulating expressions with common biochemical indexes, stenosis severity and inflammatory cytokines in CHD patients.

Methods: Ninety-eight CHD patients and 100 controls confirmed by coronary angiography were enrolled. Plasma samples were collected for lnc-GAS5 and miR-21 detection by reverse transcription-quantitative polymerase chain reaction and inflammatory cytokines determination by enzyme-linked immunosorbent assay.

Results: lnc-GAS5 was increased in CHD patients compared with controls (2.270 [interquartile range [IQR]: 1.676–3.389] vs. 0.999 [IQR: 0.602–1.409], $p < 0.001$), whereas miR-21 showed opposite trend (0.442 [IQR: 0.318–0.698] vs. 0.997 [IQR: 0.774–1.368], $p < 0.001$). In aspect of their intercorrelation, lnc-GAS5 negatively linked with miR-21 in CHD patients ($p < 0.001$) instead of controls ($p = 0.211$). Interestingly, among the common biochemical indexes, lnc-GAS5 related to decreased high-density lipoprotein cholesterol ($p = 0.008$) and increased C-reactive protein (CRP) ($p < 0.001$), while miR-21 correlated with lower total cholesterol ($p = 0.024$) and CRP ($p < 0.001$) in CHD patients. As stenosis degree, lnc-GAS5 positively correlated with Gensini score ($p < 0.001$), but miR-21 exhibited negative association ($p = 0.003$) in CHD patients. In terms of inflammatory cytokines, lnc-GAS5 positively related to tumor necrosis factor α (TNF- α) and interleukin (IL)-17A, while miR-21 negatively linked with TNF- α , IL-1 β , IL-6, and IL-17 in CHD patients (all $p < 0.05$).

Conclusion: Circulating lnc-GAS5 and its target miR-21 exhibit potency to serve as biomarkers for CHD management.

KEYWORDS

coronary heart disease, inflammation, lncrna GAS5, microRNA-21, stenosis severity

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1 | INTRODUCTION

Coronary heart disease (CHD), identified as stenosis and blockage of the coronary artery lumen due to chronic lipid-induced inflammation of the vessel wall, is a common disease with an annual mortality exceeding 17 million, making it a major cause of death globally.¹⁻⁴ Meanwhile, it is estimated that there are over 3.9 million annual deaths owing to CHD in China.⁵ Early identification and treatment (*i.e.* medicinal, interventional, or surgical therapy) of CHD are associated with subsequent CHD outcomes.⁶ Thus, numerous investigations in recent years have reported multiple types of biomarkers, such as cardiac troponin-I, lipoprotein a, fibrinogen, and cystatin C, for the prediction of CHD risk.^{7,8} Despite these discovered biomarkers, the early identification and management of CHD remain undesirable. Therefore, it is necessary to continuously identify CHD biomarkers.

Long noncoding RNA (lncRNA) growth arrest-specific 5 (lnc-GAS5) is a 5'-terminal oligopyrimidine class of gene that is related to inflammation, multiple cell activities, and atherosclerosis progression.^{3,9} For example, it promotes inflammation by targeting toll-like receptor 4 (TLR4).¹⁰ It regulates the apoptosis of macrophages and vascular endothelial cells *via* THP-1 cell-released exosomes in atherosclerosis.³ Moreover, it modifies cardiomyocyte apoptosis *via* semaphorin 3a in myocardial infarction.¹¹ Moreover, lnc-GAS5 downregulation defers atherosclerosis progression by facilitating reverse transportation of cholesterol and reducing intracellular lipid accumulation.⁹ microRNA-21 (miR-21), targeted by lnc-GAS5,^{12,13} has been reported to be related to inflammation and coronary stenosis.^{14,15} For instance, a study showed that miR-21 modulates T cell immune responses by enhancing extracellular signal-regulated kinase and c-Jun N-terminal kinase (JNK) signaling.¹⁵ Another study showed that miR-21 reduces in-stent restenosis by promoting vascular inflammation and remodeling by regulating anti-inflammatory M2 macrophages levels.¹⁴ Furthermore, a clinical study exhibits that single nucleotide polymorphisms of lnc-GAS5 and miR-21 are associated with the Gensini score in CHD patients.¹ However, further investigation on the potential role of lnc-GAS5 and miR-21 expressions as biomarkers in CHD patients is still required.

The present study aimed to evaluate the correlation between lnc-GAS5 and miR-21, and their association with coronary artery stenosis degree, common biochemical indexes, and inflammatory cytokines in CHD patients.

2 | METHODS

2.1 | Patients and controls

Between March, 2018 and May, 2020, 98 patients newly diagnosed with CHD after coronary angiography¹⁶ due to unexplained chest pain or suspected CHD symptoms were prospectively enrolled in this study. All enrolled CHD patients were over 18 years and presented with at least one major epicardial vessel with >50% stenosis,

as revealed by coronary angiography. Patients were ineligible for enrollment if they (a) documented other cardiac diseases apart from CHD, (b) complicated with autoimmune diseases, severe infections, severe liver or renal disease, or hematologic/solid malignancies, and (c) pregnant or breastfeeding women. In addition to 98 CHD patients, this study also recruited 100 subjects who underwent coronary angiography due to unexplained chest pain or suspected CHD symptoms but were finally identified as having diseases other than CHD. These 100 subjects served as controls for the study analysis. On the enrollment of controls, the age was limited within 50–80 years, and the sex ratio was limited in 4:1 (male vs. female), aimed at matching the age and sex of CHD patients and controls. The controls were excluded from the study if they were (a) concomitant with autoimmune diseases, severe infections, severe liver or renal disease, or hematologic/solid malignancies and (b) pregnant or breastfeeding women. All subjects provided written informed consents, and the Institutional Review Board of Huangshi Central Hospital, Affiliated Hospital of Hubei Polytechnic University, Edong Healthcare Group approved the study.

2.2 | Data recording

Demographic data, medical histories, comorbidities, and biochemical indexes of all subjects were recorded after enrollment. The Gensini score was evaluated by coronary angiography, which is a quantitative analysis for the severity of coronary artery stenosis.

2.3 | Sample collection

The peripheral blood (PB) was sampled from all enrolled subjects prior to coronary angiography. After blood sampling, the plasma samples were separated from the PB samples by centrifugalizing at 2500g for 15 min at 4°C. Afterward, plasma samples were used for the detection of lnc-GAS5 and miR-21 expression. Particularly for CHD patients, the levels of tumor necrosis factor α (TNF- α) and interleukin (IL)-1 β , IL-6, and IL-17 in the plasma samples were measured.

2.4 | lnc-GAS5 and miR-21 expressions detection

The reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay was performed to determine lnc-GAS5 and miR-21 expressions in plasma samples. Briefly, total RNA was extracted from plasma samples using the RNeasy Protect Mini Kit (Qiagen, Duesseldorf, Nordrhein-Westfalen, German) and was then reverse transcribed into cDNA using the iScript™ cDNA Synthesis Kit with random primers (Bio-Rad, CA, USA) (25°C for 5 min, 46°C for 20 min, 95°C for 1 min). Subsequently, qPCR was conducted using the THUNDERBIRD® SYBR® qPCR Mix (Toyobo, Osaka, Japan) to quantify lnc-GAS5 and miR-21 expressions (95°C for 60 s, followed

by 40 cycles of 95°C for 15 s and 60°C for 60 s), which were both calculated using the $2^{-\Delta\Delta C_t}$ method with GAPDH and U6¹⁷ as an internal reference, respectively. Primers used for PCR are displayed in Table S1.

2.5 | Inflammatory cytokine determination

Inflammatory cytokines, including TNF- α , IL-1 β , IL-6, and IL-17, were determined by enzyme-linked immunosorbent assay (ELISA) using the following ELISA kits: TNF alpha Human ELISA Kit, IL-1 beta Human ELISA Kit, IL-6 Human ELISA Kit, and IL-17A Human ELISA Kit. All ELISA Kits were purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). The ELISA procedures were implemented in terms of the ELISA Technical Guideline provided by the manufacturer on the official website.

2.6 | Statistical analysis

Descriptive analysis was performed using mean value, standard deviation, median value, interquartile range (IQR), frequency, and percentage, according to characteristics of variable distribution and normality. Statistical inference for difference comparison was completed using the Chi-square test, Student's *t*-test, or Wilcoxon rank-sum test. The association between two continuous variables was estimated using Spearman's rank correlation test. Receiver-operating characteristic (ROC) analysis was used to estimate the performance of variables in distinguishing CHD patients from controls. SPSS 26.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis, and GraphPad Prism 7.02 (GraphPad Software Inc., San Diego, CA, USA) was used for figure plotting. A *p* value < 0.05 indicated statistical significance.

3 | RESULTS

3.1 | Comparison of clinical characteristics between CHD patients and controls

As shown in Table 1, the mean age of CHD patients and controls is 63.5 ± 9.2 and 61.9 ± 6.9 years, respectively; meanwhile, the proportion of male CHD patients and controls was 78% and 80%, respectively, presenting no difference in age or gender (both *p* > 0.05). Notably, diabetes mellitus rate (25.5% vs. 14.0%), median C-reactive protein (CRP) level (9.0 [6.8–12.1] mg/L vs. 6.5 [2.6–10.3] mg/L), and median Gensini score (43.9 ± 28.1 vs. 1.3 ± 2.0) of CHD patients were higher than those of controls (all *p* < 0.05). However, the median high-density lipoprotein cholesterol (HDL-C) level was lower in CHD patients (0.9 [0.8–1.1] mmol/L) than in controls (1.0 [0.8–1.2] mmol/L; *p* = 0.049). No differences in other clinical characteristics were observed between CHD patients and controls (all *p* > 0.05; Table 1).

3.2 | Expression of lnc-GAS5 and miR-21 in CHD patients and controls

lnc-GAS5 expression was remarkably higher in CHD patients than in controls with respective median (IQR) values of 2.270 (1.676–3.389) and 0.999 (0.602–1.409) (*p* < 0.001; Figure 1A). It was of excellent value in discriminating CHD patients from controls with AUC of 0.915 (95%CI: 0.879–0.952; Figure 1B). miR-21 expression was lower in CHD patients than in controls, with respective median (IQR) values of 0.442 (0.318–0.698) and 0.997 (0.774–1.368) (*p* < 0.001; Figure 1C), indicating a good discriminating value for CHD with AUC of 0.852 (95%CI: 0.799–0.905; Figure 1D).

In addition, a higher lnc-GAS5 level is associated with a lower miR-21 level in CHD patients (*r* = -0.440, *p* < 0.001); however, no association between lnc-GAS5 with miR-21 was observed in controls (*r* = -0.126, *p* = 0.211) (Figure 2A,B).

3.3 | Correlation of lnc-GAS5 and miR-21 with Gensini score and biochemical indexes in CHD patients

As shown in Figure 3, elevated lnc-GAS5 expression was correlated with increased Gensini scores in CHD patients (*r* = 0.427, *p* < 0.001; Figure 3A). Nevertheless, higher miR-21 expression was correlated with lower Gensini score in CHD patients (*r* = -0.300, *p* = 0.003; Figure 3B).

Furthermore, lnc-GAS5 was negatively correlated with HDL-C level (*r* = -0.268, *p* = 0.008) and positively correlated with CRP level (*r* = 0.459, *p* < 0.001) in CHD patients. miR-21 was negatively correlated with total cholesterol (TC) (*r* = -0.228, *p* = 0.024) and CRP levels (*r* = -0.424, *p* < 0.001) in CHD patients. No correlation of lnc-GAS5 and miR-21 with other biochemical indexes was found in CHD patients (Table 2).

3.4 | Correlation of lnc-GAS5 and miR-21 with inflammatory cytokines in CHD patients

lnc-GAS5 expression was positively correlated with both TNF- α (*r* = 0.272, *p* = 0.007) and IL-17 (*r* = 0.308, *p* = 0.002), but not with IL-1 β (*r* = 0.178, *p* = 0.079) or IL-6 (*r* = 0.165, *p* = 0.104) in CHD patients (Figure 4A–D). miR-21 expression was negatively correlated with all tested inflammatory cytokines including TNF- α (*r* = -0.266, *p* = 0.008), IL-1 β (*r* = -0.220, *p* = 0.029), IL-6 (*r* = -0.294, *p* = 0.003), and IL-17 (*r* = -0.355, *p* < 0.001) in CHD patients (Figure 4E–H).

4 | DISCUSSION

Previous studies have disclosed that several noncoding RNAs possess the potential as CHD biomarkers, such as lncRNA-FA2H-2, lncRNA metastasis-associated lung adenocarcinoma transcript 1

TABLE 1 Clinical characteristics

Items	Controls (N = 100)	CHD patients (N = 98)	p value
Age (years), mean \pm SD	61.9 \pm 6.9	63.5 \pm 9.2	0.185
Gender, n (%)			0.943
Male	80 (80.0)	78 (79.6)	
Female	20 (20.0)	20 (20.4)	
BMI (kg/m ²), mean \pm SD	23.6 \pm 3.1	23.9 \pm 2.8	0.479
Smoke, n (%)	34 (34.0)	44 (44.9)	0.117
Family history of CHD, n (%)	19 (19.0)	24 (24.5)	0.349
Complications			
Hypertension, n (%)	64 (64.0)	73 (74.5)	0.110
Hyperlipidemia, n (%)	42 (42.0)	50 (51.0)	0.203
Hyperuricemia, n (%)	31 (31.0)	35 (35.7)	0.482
DM, n (%)	14 (14.0)	25 (25.5)	0.042
Biochemical indexes			
FBG (mmol/L), mean \pm SD	5.5 \pm 1.0	5.7 \pm 1.1	0.236
Scr (μ mol/L), mean \pm SD	75.0 \pm 14.0	77.7 \pm 15.6	0.204
SUA (μ mol/L), median (IQR)	360.7 (310.7–402.2)	345.2 (311.2–391.7)	0.352
TG (mmol/L), median (IQR)	1.5 (0.9–2.4)	1.7 (0.9–2.3)	0.744
TC (mmol/L), mean \pm SD	4.5 \pm 1.0	4.8 \pm 1.0	0.129
LDL-C (mmol/L), mean \pm SD	2.8 \pm 0.7	3.0 \pm 0.7	0.130
HDL-C (mmol/L), median (IQR)	1.0 (0.8–1.2)	0.9 (0.8–1.1)	0.049
CRP (mg/L), median (IQR)	6.5 (2.6–10.3)	9.0 (6.8–12.1)	<0.001
Gensini score, mean \pm SD	1.3 \pm 2.0	43.9 \pm 28.1	<0.001

Abbreviations: BMI, body mass index; CHD, coronary heart disease; CRP, C-reactive protein; DM, diabetes mellitus; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; Scr, serum creatinine; SD, standard deviation; SUA, serum uric acid; TC, total cholesterol; TG, triglyceride.

and its potential target miR-125b, and miR-92a.^{18–20} As described above, lnc-GAS5 and miR-21, as inflammatory regulators, play vital roles in diversified pathophysiological processes of the cardiovascular system.^{3,9,14} The current study exhibited that lnc-GAS5 was highly expressed, while miR-21 was insufficiently expressed in CHD patients. ROC curves displayed that both lnc-GAS5 and miR-21 were able to discriminate CHD patients from controls with AUC values over 0.850. At the same time, lnc-GAS5 was negatively correlated with miR-21 in CHD patients. The possible reasons might be: (1) lnc-GAS5 regulated the systematic inflammation (via the miR-23a-3p/TLR4¹⁰ or the miR-223-3p/NLRP3 pathway²¹) and the growth of endothelial cells (via miR-194-3p-targeted thioredoxin-interacting protein axis²²), while miR-21 regulated inflammatory cytokines (via JNK signaling pathway²³ or the phosphatidylinositol 3-hydroxy kinase [PI3K]/protein kinase B [AKT] pathway²⁴); thus, the dysregulation of lnc-GAS5 and miR-21 increased the risk of CHD. (2) The negative correlation between lnc-GAS5 and miR-21 might result from that lnc-GAS5 acted as a negative regulator of miR-21.^{12,13} In the current study, all subjects in the CHD cohort were newly diagnosed with CHD. Thus, they did not receive any CHD treatment prior to the current study. Blood samples were collected before coronary angiography, while the patients received treatment after coronary

angiography. Therefore, treatment should have no effect on lnc-GAS5 and miR-21 expression.

The Gensini score is a meaningful scoring system for measuring the general severity of coronary artery stenosis in CHD.²⁵ A clinical study found that single nucleotide polymorphisms of lnc-GAS5 (rs2067079 and rs6790) and miR-21 (rs1292037) are linked with Gensini score alteration in CHD patients.¹ However, another clinical study showed a lack of association between serum exosomal miR-21 level and Gensini score in patients with acute myocardial infarction or unstable angina.²⁶ Our study revealed that lnc-GAS5 was positively correlated with the Gensini score, while miR-21 was negatively correlated with the Gensini score in CHD patients. This could be due to the following: (1) lnc-GAS5 aggravated CHD by regulating endothelial cell growth via the miR-194-3p/thioredoxin-interacting protein axis²²; (2) lnc-GAS5 enhanced inflammation via the facilitation of miR-21-5p-mediated TLR4/nuclear factor- κ B signaling,²⁷ resulting in more severe vessel stenosis, thus worsening CHD; (3) miR-21 mediated the severity of CHD by regulating the proliferation and migration of vascular smooth muscle cells via the protein kinases B/extracellular signal-regulated kinase signaling pathway.²⁸ Furthermore, our study also showed that lnc-GAS5 was negatively correlated with HDL-C; meanwhile, miR-21 was negatively

FIGURE 1 Lnc-GAS5 and miR-21 expression. Comparison of Lnc-GAS5 expression in CHD patients compared to controls (A); discrimination of CHD patients from controls by Lnc-GAS5 (B); comparison of miR-21 expression in CHD patients compared to controls (C); discrimination of CHD patients from controls by miR-21 (D). AUC, area under curves; CHD, coronary heart disease; CI, confidential interval; Lnc-GAS5, long noncoding RNA growth arrest-specific 5; miR-21, microRNA-21

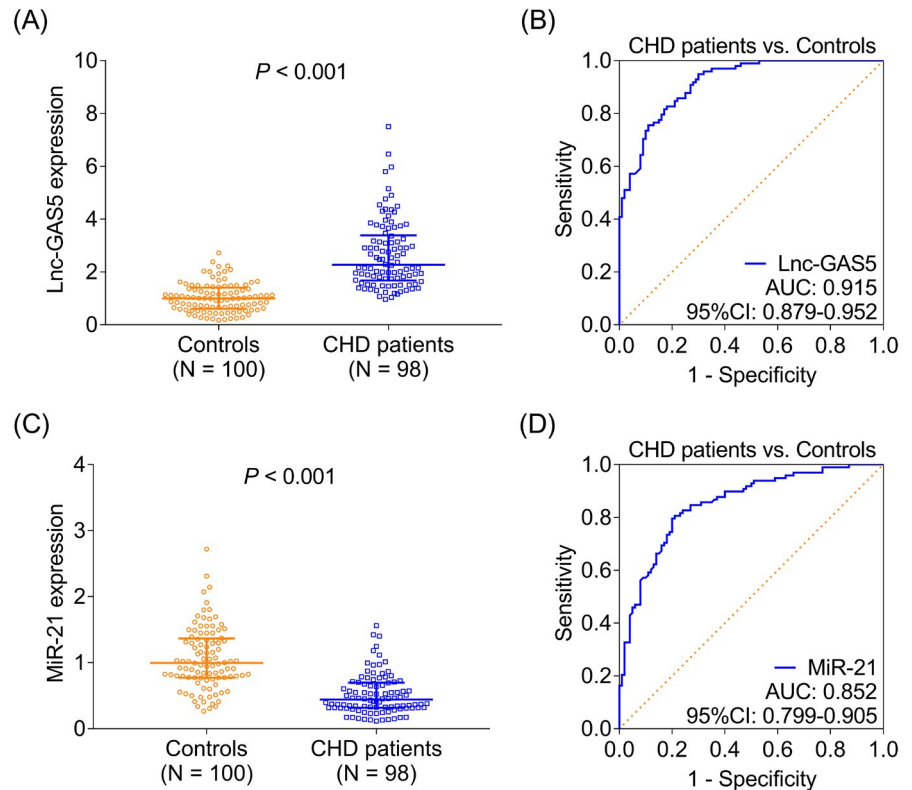


FIGURE 2 Association of Lnc-GAS5 with miR-21. The correlation of Lnc-GAS5 expression with miR-21 expression in controls (A) and CHD patients (B). CHD, coronary heart disease; Lnc-GAS5, long noncoding RNA growth arrest-specific 5; miR-21, microRNA-21

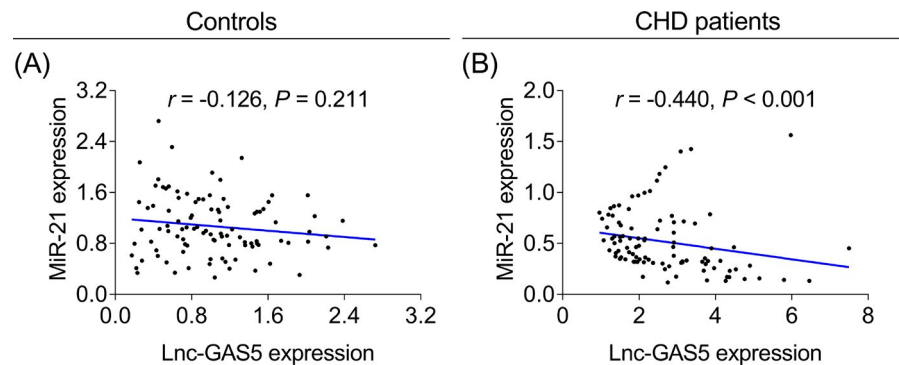
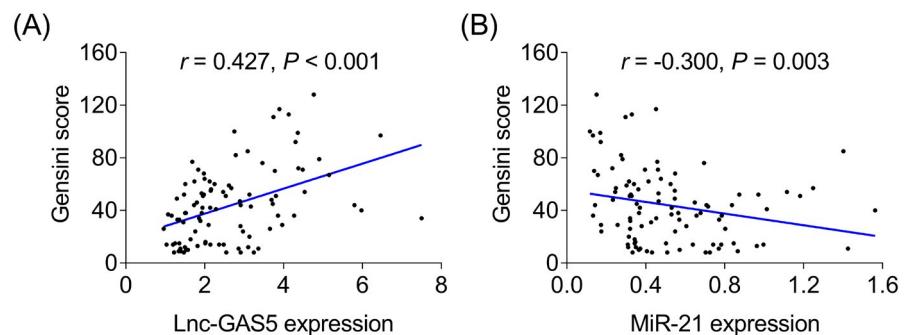


FIGURE 3 Association of Lnc-GAS5 and miR-21 with Gensini score. The correlation of Lnc-GAS5 (A) and miR-21 (B) expressions with Gensini score in CHD patients. CHD, coronary heart disease; Lnc-GAS5, long noncoding RNA growth arrest-specific 5; miR-21, microRNA 21



correlated with TC, which could be resulted from their regulation on cholesterol and intracellular lipid accumulation in atherosclerosis.⁹ Several reports have shown that HDL-C and TC are potential risk factors for CHD.^{29,30} Thus, it could be inferred that Lnc-GAS5 and miR-21 regulated lipid metabolism to indirectly promote CHD progression. A previous study reveals that Lnc-GAS5 regulates lipid

metabolism in mice,⁹ however, whether it exerts similar regulation in human should be further explored.

Lnc-GAS5 and miR-21 are correlated with inflammatory level in cardiovascular disease patients.^{31,32} For example, a study reported that Lnc-GAS5 aggravates the inflammatory response in THP-1 macrophages by sponging miR-221 during atherosclerosis progression.³¹

Another study reported that the absence of miR-21 in macrophages promotes vascular inflammation during atherogenesis.³³ Our study showed that lnc-GAS5 was positively associated with CRP, TNF- α , and IL-17 levels, while miR-21 was negatively associated with CRP, TNF- α , IL-1 β , IL-6, and IL-17 levels in CHD patients. The explanations might be as follows: (1) lnc-GAS5 intensified inflammation and inflammatory cytokines production via multiple pathways, such as the miR-23a-3p/TLR4,¹⁰ miR-223-3p/NLRP3,²¹ and AMP-activated protein kinase pathways,³⁴ triggering increased CRP, TNF- α , and IL-17 levels; (2) miR-21 downregulated TNF- α , IL-1 β , IL-6, and IL-17 via the JNK signaling²³ and PI3K/AKT pathways,²⁴ leading to decreased pro-inflammatory cytokines and CRP levels. Meanwhile, lnc-GAS5

and miR-21 presented high correlation coefficients with IL-17, which could be explained by the regulation of lnc-GAS5 and miR-21 on T helper 17 balance and differentiation.^{35,36}

The findings of our study revealed that lnc-GAS5 and miR-21 were correlated with disease severity and inflammation of CHD. Based on these findings, it might be possible that the intensive monitoring of lnc-GAS5 and miR-21 could reflect disease severity and inflammation in CHD patients, thus giving appropriate management to CHD patients with different disease severity or inflammation level. Therefore, lnc-GAS5 and miR-21 might possess the potential to improve the management of CHD patients.

This study had a few limitations. First, the sample size was small, leading to a weak statistical power. Further study with larger sample sizes should be conducted in the future. Second, the molecular mechanisms by which lnc-GAS5 and miR-21 regulate inflammation in CHD require exploration. Third, this study only investigated the correlation of lnc-GAS5 and miR-21 with inflammation and disease severity in CHD patients; nevertheless, their correlation with long-term prognosis such as MACE requires further evaluation. Fourth, the correlation of lnc-GAS5 and miR-21 from other sources with inflammation and disease severity in CHD patients could be investigated further. Fifth, the current study did not include healthy subjects as a control cohort, but enrolled subjects who underwent coronary angiography due to unexplained chest pain or suspected CHD symptoms and were finally identified as having diseases other than CHD. Sixth, further longitudinal cohort studies could be conducted to explore the causality between lnc-GAS5/miR-21 and the change of biochemical indexes, such as CRP. Seventh, the molecular mechanisms of lnc-GAS5 and miR-21 on the pathogenesis and progression of CHD could be further investigated. Eighth, the correlation of lnc-GAS5 and miR-21 with the atherosclerotic plaque formation should be explored in further studies.

TABLE 2 Correlation of lnc-GAS5 and miR-21 with biochemical indexes in CHD patients

Items	lnc-GAS5 expression		MiR-21 expression	
	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
FBG	0.090	0.378	0.027	0.789
Scr	0.069	0.502	-0.109	0.285
SUA	0.186	0.066	-0.093	0.361
TG	-0.031	0.764	-0.165	0.104
TC	0.036	0.728	-0.228	0.024
LDL-C	0.090	0.377	-0.186	0.066
HDL-C	-0.268	0.008	0.024	0.818
CRP	0.459	<0.001	-0.424	<0.001

Abbreviations: CHD, coronary heart disease; CRP, C-reactive protein; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; lnc-GAS5, long noncoding RNA growth arrest-specific 5; miR, microRNA; Scr, serum creatinine; SUA, serum uric acid; TC, total cholesterol; TG, triglyceride.

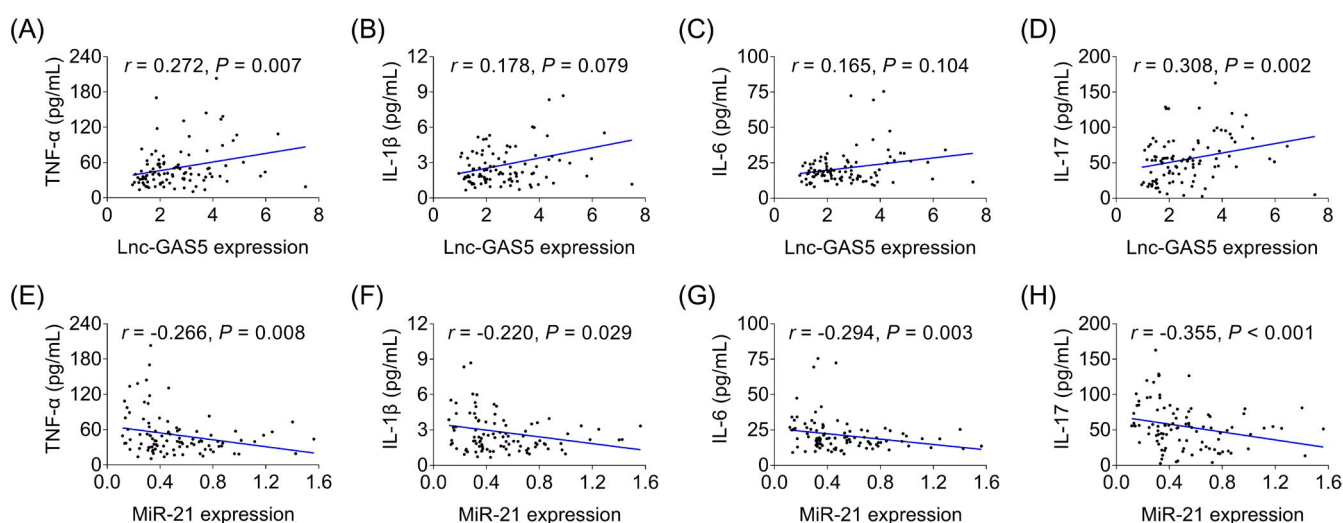


FIGURE 4 Association of lnc-GAS5 and miR-21 with inflammation. The correlation of lnc-GAS5 expression with inflammatory cytokines such as TNF- α (A), IL-1 β (B), IL-6 (C), and IL-17 (D) levels in CHD patients; the correlation of miR-21 expression with inflammatory cytokines such as TNF- α (E), IL-1 β (F), IL-6 (G), and IL-17 (H) in CHD patients. CHD, coronary heart disease; lnc-GAS5, long noncoding RNA growth arrest-specific 5; miR-21, microRNA-21; IL-1 β , interleukin 1 β ; IL-6, interleukin 6; IL-17, interleukin 17; TNF- α , tumor necrosis factor α

In conclusion, Inc-GAS5 negatively correlates with miR-21, and both are associated with the Gensini score, cholesterol level, and inflammation in CHD patients. Our study indicates that Inc-GAS5 and miR-21 may act as biomarkers to improve CHD management, which requires further validation.

CONFLICT OF INTEREST

The authors of this work have nothing to disclose.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

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