Association of genetic variants in lncRNA GAS5/miR-21/mTOR axis with risk and prognosis of coronary artery disease among a Chinese population

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Abstract
Background: Allowing for the significance of single nucleotide polymorphisms (SNPs) in reflecting disease risk, this investigation attempted to uncover whether SNPs situated in lncRNA GAS5/miR-21/mTOR axis were associated with risk and prognosis of coronary heart disease (CHD) among a Chinese Han population.

Methods: Altogether 436 patients with CHD were recruited as cases, and meanwhile, 471 healthy volunteers were included into the control group. Besides, SNPs of GAS5/MIR-21/mTOR axis were genotyped utilizing mass spectrometry. Chi-square test was applied to figure out SNPs that were strongly associated with CHD risk and prognosis, and combined effects of SNPs and environmental parameters on CHD risk were evaluated through multifactor dimensionality reduction (MDR) model.

Results: Single nucleotide polymorphisms of GAS5 (ie, rs2067079 and rs6790), MIR-21 (ie, rs1292037), and mTOR (rs2295080, rs2536, and rs1034528) were associated with susceptibility to CHD, and also Gensini score change of patients with CHD (P < .05). MDR results further demonstrated that rs2067079 and rs2536 were strongly interactive in elevating CHD risk (P < .05), while smoking, rs6790 and rs2295080 showed powerful reciprocity in predicting Gensini score change of patients with CHD (P < .05).

Conclusion: Single nucleotide polymorphisms of lncRNA GAS5/miR-21/mTOR axis might interact with smoking to regulate CHD risk, which was conducive to diagnosis and prognostic anticipation of CHD.

KEYWORDS
coronary heart disease, lncRNA GAS5, miR-21, mTOR, prognosis, single nucleotide polymorphism

1 | INTRODUCTION

Coronary heart disease (CHD), an intricate disorder induced by mutation of single nucleotide polymorphisms (SNPs), environmental hazards, and so on, 1 is clinically manifested as insufficient blood supply for heart muscle caused by stenosis and blockage of coronary artery. 2,3 Annually, there were over 10 million people dying of cardiovascular disorders (CVD) around the globe, 4 and acute myocardial infarction (MI) was responsible for one half of the deaths. 5 Despite progresses in imaging
examination, interventional operation, and medication, numerous patients with CHD still missed the opportunity of surgery at diagnosis, owing to hidden onset and rapid progression of the disease. Therefore, exploring biomarkers for prompt diagnosis and effective treatment of CHD were crucial to reduce CHD mortality.5,7

Vast numbers of biomarkers, including C-reactive protein (CRP), interleukin-6 (IL-6) and matrix metalloproteinase-9 (MMP-9), have been documented to involve with cardiovascular dysfunction and plaque instability,8,9 and they were mostly involved in the pathogenesis of inflammation, endothelial injury, and hemostasis.10 Long non-coding RNAs (lncRNAs), identified through high-throughput sequencing,11 were also pivotal regulators of CHD etiology.10 For instance, expression of IncRNA GAS5 was higher in patients with atherosclerosis than in healthy people,12 and GAS5 knockdown could deteriorate artery remodeling and microvascular function of hypertension rat models.13 Besides, GAS5 was also able to induce cardiac abnormality by interacting with MIR-21,14,15 deletion of which could trigger thoracic aorta remodeling in mice models.16 Moreover, miR-21 expression was capable of distinguishing patients with non-ST elevation myocardial infarction (NSTEMI) from those with acute heart failure (CHF),17 which emphasized the involvement of MIR-21 in reflecting CHD severity. Furthermore, mTOR signaling, which modified T-cell differentiation and atherosclerosis formation,18 was also subjected to regulation of MIR-21.19 In summary, GASS/MIR-21/mTOR axis could matter in regulating CHD development, yet whether significant SNPs in this axis were associated with CHD risk was unclear.

Single nucleotide polymorphisms in GASS/MIR-21/mTOR have been widely indicated to associate with disease progression. For instance, rs55829688 and rs2067679 of GAS5 were associated with severity of acute myelocytic leukemia (AML), and rs6790 was reported to lower risk of anemia.20 Despite unclear implication in disease etiology so far, rs17359906 of GAS5 was also worthy of attention for its enhancer-like function.20 Besides, rs1292037 (A>G) and rs13137 (A>G) of MIR-21 could affect cisplatin/paclitaxel resistance of patients with cervical cancer (CC).21 In addition, rs2295080 (C>A) of mTOR, which influenced mTOR expression, was associated with enhancive risk of cancers, including renal cell cancer, prostate cancer, gastric cancer, and esophageal squamous cell carcinoma.22 What’s more, patients with small-cell lung cancer (SCLC) carrying rs2536 (TT) of mTOR were more likely to benefit from chemoradiotherapy than patients with homozygote CC,23 and carriage of rs11121704 (TT), rs1034528 (CG/CC), and rs3806317 (GA/GG) could enlarge cancer risk or worsen prognosis of patients with cancer.22,24 Within spite of these findings, a finite number of researches were available to explain the association of these significant SNPs with CHD risk.25

Hence, this investigation was aimed at elucidating the potential association of SNPs in GASS/MIR-21/mTOR axis with CHD risk, which might be conducive to CHD diagnosis and treatment.26

2 | MATERIALS AND METHODS

2.1 | Collection of CHD patients

From April 2017 to February 2019, 436 patients with CHD, diagnosed by coronary angiography (CAG) according to Judkins method,27,28 were recruited from the First Naval Hospital of Southern Theater Command. They were incorporated under following conditions: (a) over 50 years old; (b) in accordance with CHD diagnostic criteria which was formulated by American College of Cardiology/American Heart Association in 2007; and (c) coronary angiography revealed that stenosis was present in one of three major vessels, or main branches of coronary was ≥50%. The patients would be excluded if (a) they were complicated by acute/chronic infection, valvular heart disease, hematological diseases, peripheral vascular disease, severe liver/kidney insufficiency, arrhythmia, systemic immune disease, tumor, or chronic obstructive pulmonary disease; (b) they underwent CHD-relevant treatments before, such as intervention, bypass, and intravenous thrombolysis; and (c) their cognition was impaired.

Simultaneously, healthy volunteers (n = 471) satisfying below conditions were recruited 29:(a) they hardly suffered from chest distress, chest pain, hypertension, hyperlipidemia, diabetes, CHD, cardiac failure, chronic renal insufficiency, peripheral vascular disease, or cerebral stroke; (b) they had no symptoms of myocardial ischemia, according to result of electrocardiograph (ECG); (c) they were not obese, with waist circumference of <90 cm among males and waist circumference of <80 cm among females; and (d) stenosis of their coronary vessels and related main branches were <10%. This study was approved by the First Naval Hospital of Southern Theater Command and Ethics Association of the First Naval Hospital of Southern Theater Command, and patients have signed informed consents.

2.2 | Genotyping of SNPs

Around 2 ml venous blood was taken from each subject after their admission, and the blood samples were reserved at −20°C for later usage. Genomic DNAs, extracted from peripheral blood samples with TIANamp Genomic DNA kit (TIANGEN Biotech, Beijing, China), were treated by 1% agarose gel electrophoresis. The DNA samples were qualified, when their A260/A280 ratio was within the scope of 1.7 ~ 1.9, after examination by ultraviolet (UV) spectrophotometer (Thermo). Integrity of the DNA samples was confirmed adopting 0.8% agarose gel electrophoresis, concentration of DNA in each sample was adjusted to >20 ng/μL. With primers detailed in Table S1, SNPs of GASS (ie, rs2067079, rs6790, rs17359906, and rs55829688), MIR-21 (ie, rs1292037 and rs13137), and mTOR (ie, rs2295080, rs2536, rs11121704, and rs1034528) were genotyped with mass spectrometry analysis platform (model: MassARRAY, Sequenom corporation). The SNPs were genotyped by two operators through double-blind manner, and >10% of the samples were randomly screened to re-identify their genotypes. The genotyping results were acceptable only when results of two examinations were consistent.
2.3 | Statistical analyses

All the statistical analyses were completed with SPSS 19.0 software. Genotype frequencies of SNPs between case group and control group were compared by chi-square test, and genetic distribution of the SNPs conformed to Hardy-Weinberg equilibrium (HWE) (Table S2). Odds ratio (OR) and 95% confidence interval (CI) were employed to evaluate association of SNPs with CHD risk and prognosis. MDR 0.5.1 software\textsuperscript{30} was applied to assess the interaction of SNPs and environmental exposures on CHD risk and prognosis.

3 | RESULTS

3.1 | Comparison of clinical features between CHD patients and healthy controls

Patients with CHD and healthy controls were matched in terms of mean age, gender distribution, BMI, history of alcoholic consumption, type 2 diabetes onset, and presence of dyslipidemia (\( P > .05 \)). However, patients with CHD were associated with higher prevalence of hypertension (44.50%) and smoking history (53.67%) than healthy volunteers (\( P < .05 \)) (Table 1). Besides, hs-C-reactive protein
| Gene    | rs number | Allele change | Model       | Case genotype | Control genotype | OR (95% CI)     | P value |
|---------|-----------|---------------|-------------|---------------|------------------|-----------------|---------|
| GAS5    | rs2067079 | C>T           | Allelic model | W M           | W M              | 1.80 (1.49, 2.17) | <.001   |
|         |           |               | Dominant model | WW WM + MM   | WW WM + MM      | 1.30 (0.94, 1.80) | .107    |
|         |           |               | Recessive model | WW + WM MM    | WW + WM MM      | 2.88 (2.18, 3.81) | <.001   |
|         | rs6790    | G>A           | Allelic model | W M           | W M              | 0.59 (0.49, 0.72) | <.001   |
|         |           |               | Dominant model | WW WM + MM   | WW WM + MM      | 0.59 (0.45, 0.77) | <.001   |
|         |           |               | Recessive model | WW + WM MM    | WW + WM MM      | 0.36 (0.24, 0.55) | <.001   |
|         | rs17359906| G>A           | Allelic model | W M           | W M              | 1.09 (0.90, 1.32) | .377    |
|         |           |               | Dominant model | WW WM + MM   | WW WM + MM      | 1.04 (0.80, 1.36) | .806    |
|         |           |               | Recessive model | WW + WM MM    | WW + WM MM      | 1.27 (0.89, 1.81) | .186    |
|         | rs55829688| T>C           | Allelic model | W M           | W M              | 0.84 (0.70, 1.01) | .071    |
|         |           |               | Dominant model | WW WM + MM   | WW WM + MM      | 0.80 (0.59, 1.09) | .152    |
|         |           |               | Recessive model | WW + WM MM    | WW + WM MM      | 0.79 (0.59, 1.06) | .130    |
| miR-21  | rs1292037 | T>C           | Allelic model | W M           | W M              | 1.76 (1.42, 2.18) | <.001   |
|         |           |               | Dominant model | WW WM + MM   | WW WM + MM      | 1.48 (0.95, 2.31) | .082    |
|         |           |               | Recessive model | WW + WM MM    | WW + WM MM      | 2.11 (1.61, 2.76) | <.001   |

(Continues)
| Gene | rs number | Allele change | Model               | Case genotype | Control genotype | OR (95% CI) | P value |
|------|-----------|---------------|---------------------|----------------|------------------|-------------|---------|
| rs13137 | A>T       | Alleric model  | W                   | M              | W                | 1.21 (0.97, 1.50) | .082    |
|       |           |               | 653                 | 219            | 738              | 204         |         |
|       |           | Dominant model | WW                  | WM + MM        | WW               | 1.26 (0.97, 1.64) | .087    |
|       |           |               | 245                 | 191            | 291              | 180         |         |
|       |           | Recessive model| WW + WM             | MM             | WW + WM          | 1.28 (0.73, 2.24) | .390    |
|       |           |               | 408                 | 28             | 447              | 24          |         |
| mTOR  | rs2295080 | G>T           | Alleric model       | W              | M                | 1.53 (1.26, 1.86) | <.001   |
|       |           |               | 272                 | 600            | 386              | 556         |         |
|       |           | Dominant model | WW                  | WM + MM        | WW               | 1.15 (0.79, 1.67) | .458    |
|       |           |               | 60                  | 376            | 73               | 398         |         |
|       |           | Recessive model| WW + WM             | MM             | WW + WM          | 2.09 (1.60, 2.73) | <.001   |
|       |           |               | 212                 | 224            | 313              | 158         |         |
| rs2536 | T>C       | Alleric model  | W                   | M              | W                | 2.35 (1.93, 2.85) | <.001   |
|       |           |               | 246                 | 626            | 452              | 490         |         |
|       |           | Dominant model | WW                  | WM + MM        | WW               | 2.18 (1.54, 3.08) | <.001   |
|       |           |               | 58                  | 378            | 118              | 353         |         |
|       |           | Recessive model| WW + WM             | MM             | WW + WM          | 3.22 (2.44, 4.23) | <.001   |
|       |           |               | 188                 | 248            | 334              | 137         |         |
| rs11121704 | C>T    | Alleric model  | W                   | M              | W                | 0.86 (0.71, 1.04) | .116    |
|       |           |               | 577                 | 295            | 590              | 352         |         |
|       |           | Dominant model | WW                  | WM + MM        | WW               | 0.79 (0.61, 1.03) | .081    |
|       |           |               | 199                 | 237            | 188              | 283         |         |
|       |           | Recessive model| WW + WM             | MM             | WW + WM          | 0.89 (0.61, 1.30) | .560    |
|       |           |               | 378                 | 58             | 402              | 69          |         |
| rs1034528 | G>C      | Alleric model  | W                   | M              | W                | 1.32 (1.08, 1.61) | .006    |
|       |           |               | 566                 | 306            | 668              | 274         |         |
|       |           | Dominant model | WW                  | WM + MM        | WW               | 1.39 (1.07, 1.81) | .014    |
|       |           |               | 184                 | 252            | 237              | 234         |         |
|       |           | Recessive model| WW + WM             | MM             | WW + WM          | 1.52 (0.99, 2.34) | .055    |
|       |           |               | 382                 | 54             | 431              | 40          |         |

Abbreviations: CHD, coronary heart disease; CI, confidence interval; M, mutant allele; OR, odds ratio; W, wild allele.
(hs-CRP), triacylglycerol (TG), and low-density lipoprotein cholesterol (LDL-C) levels were significantly increased, yet creatinine clearance rate (Ccr) and high-density lipoprotein cholesterol (HDL-C) levels revealed a dramatic drop in CHD population, when compared with healthy controls (P < .05).

### 3.2 Associations of SNPs in IncRNA GAS5/miR-21/mTOR axis with CHD risk

Allele T of rs2067079 (C>T) could increase the likelihood of CHD onset as relative to allele C (Allelic model: OR = 1.80, 95CI% = 1.49-2.17, P < .001; Recessive model: OR = 2.88, 95CI% = 2.18-3.81, P < .001) (Table 2). By contrast, allele A of rs6790 (G>A) was prone to reduce CHD risk in comparison with allele G (Allelic model: OR = 0.59, 95CI% = 0.49-0.72, P < .001; Dominant model: OR = 0.59, 95CI% = 0.45-0.77, P < .001; Recessive model: OR = 0.36, 95CI% = 0.24-0.54, P < .001). With respect to SNPs of MIR-21, both allele C and homozygote CC of rs1292037 (T>C) were strongly associated with elevated susceptibility to CHD (Allelic model: OR = 1.76, 95CI% = 1.31-2.36, P < .001; Dominant model: OR = 1.84, 95CI% = 1.04-3.27, P = .036; rs2536: Allelic model: OR = 1.38, 95CI% = 1.02-1.86, P = .037, Dominant model: OR = 2.06, 95CI% = 1.14-3.72, P = .015). Furthermore, haplotype TGCTG composed by rs2067079 (C>T), rs6790 (G>A), rs1292037 (T>C), rs2295080 (G>T), and rs2536 (T>C) could be a high-risk factor for coronary stenosis, due to its high prevalence in high Gensini score group than those with low Gensini score (OR = 1.92, 95%CI = 1.16-3.17, P = .010) (Table 5).

### 3.3 Correlation between SNPs in IncRNA GAS5/miR-21/mTOR axis and CHD prognosis

Coronary heart disease patients with smaller Gensini score (<30) were designated into ones with favorable prognosis, while CHD patients with larger Gensini score (≥30) were considered to be with poor prognosis (Table 4). We observed that patients with CHD carrying allele T of rs2067079 were associated with higher Gensini score than those carrying allele C (Allelic model: OR = 1.51, 95CI% = 1.14-2.00, P = .004; Recessive model: OR = 1.80, 95CI% = 1.23-2.63, P = .002), while allele A of rs6790 (G>A) served as a protector against coronary stenosis, with higher frequency in small Gensini score group than allele G (Allelic model: OR = 0.76, 95CI% = 0.60-0.96, P = .027; Dominant model: OR = 0.69, 95CI% = 0.50-0.96, P = .025). In addition, CHD patients with rs1292037 (CC/TC) were more likely to show higher Gensini score than those with homozygote TT (Dominant model: OR = 2.25, 95CI% = 1.05-4.80, P = .032). As for mTOR, rs2295080 (G>T) and rs2536 (T>C) were associated with severe coronary stenosis (ie high Gensini score) under allelic and dominant models (rs2295080: Allelic model: OR = 1.76, 95CI% = 1.31-2.36, P < .001, Dominant model: OR = 1.84, 95CI% = 1.04-3.27, P = .036; rs2536: Allelic model: OR = 1.38, 95CI% = 1.02-1.86, P = .037, Dominant model: OR = 2.06, 95CI% = 1.14-3.72, P = .015). Furthermore, haplotype TGCTG composed by rs2067079 (C>T), rs6790 (G>A), rs1292037 (T>C), rs2295080 (G>T), and rs2536 (T>C) could be a high-risk factor for coronary stenosis, due to its high prevalence in high Gensini score group than those with low Gensini score (OR = 1.92, 95%CI = 1.16-3.17, P = .010) (Table 5).

### 3.4 Interactive effect of SNPs in IncRNA GAS5/miR-21/mTOR axis and environmental exposures on CHD risk and prognosis

Among SNPs that significantly affected CHD risk, rs2067079 (C>T) and rs2536 (T>C) were strongly interactive in boosting CHD risk, with testing accuracy of 73.94% and cross-consistency of 10/10 (Table 6, Figure 1). Rs2067079 (C>T), rs6790 (G>A), and rs2536 (T>C) also showed strong interaction in triggering CHD susceptibility (testing accuracy: 77.97%; cross-consistency: 9/10). After taking environmental parameters into consideration, the 2-order model (ie, rs2067079 [C>T] and rs2536 [T>C]) still demonstrated powerful interaction in inducing CHD risk (testing accuracy: 73.94%; cross-consistency: 10/10). Besides, smoking, rs6790 (G>A) and rs2295080 (G>T) constituted the optimal 3-order interaction in predicting Gensini score of patients with CHD, with testing accuracy of 60.82% and cross-consistency of 10/10 (Table 6, Figure 2).

### 4 DISCUSSION

With advances in human genome project and haplotype HapMap program, considerable findings have been documented to account...
| Gene  | rs number | Allele change | Model          | Gensini ≥ 30 group | Gensini < 30 group | OR (95% CI) | P value |
|-------|-----------|---------------|----------------|-------------------|-------------------|-------------|---------|
| GAS5  | rs2067079 | C>T           | Allelic model  | W                 | M                 | 1.51 (1.14, 2.00) | .004    |
|       |           |               |                | 119               | 281               |             |         |
|       |           |               | Dominant model | WW + MM           | WW + MM           | 1.29 (0.79, 2.11) | .306    |
|       |           |               |                | 33                | 167               |             |         |
|       |           |               | Recessive model| WW + WM           | MM                 | 1.80 (1.23, 2.63) | .002    |
|       |           |               |                | 86                | 114               |             |         |
|       | rs6790    | G>A           | Allelic model  | W                 | M                 | 0.76 (0.60, 0.96) | .027    |
|       |           |               |                | 613               | 259               |             |         |
|       |           |               | Dominant model | WW + MM           | WW + MM           | 0.69 (0.50, 0.95) | .25    |
|       |           |               |                | 211               | 225               |             |         |
|       |           |               | Recessive model| WW + WM           | MM                 | 0.71 (0.41, 1.22) | .222    |
|       |           |               |                | 402               | 34                |             |         |
|       | rs17359906| G>A           | Allelic model  | W                 | M                 | 0.89 (0.68, 1.17) | .399    |
|       |           |               |                | 246               | 154               |             |         |
|       |           |               | Dominant model | WW + MM           | WW + MM           | 0.77 (0.52, 1.14) | .180    |
|       |           |               |                | 82                | 118               |             |         |
|       |           |               | Recessive model| WW + WM           | MM                 | 1.04 (0.63, 1.70) | .862    |
|       |           |               |                | 164               | 36                |             |         |
|       | rs55829688| T>C           | Allelic model  | W                 | M                 | 1.25 (0.96, 1.63) | .102    |
|       |           |               |                | 193               | 207               |             |         |
|       |           |               | Dominant model | WW + MM           | WW + MM           | 1.50 (0.97, 2.32) | .070    |
|       |           |               |                | 44                | 156               |             |         |
|       |           |               | Recessive model| WW + WM           | MM                 | 1.21 (0.78, 1.88) | .396    |
|       |           |               |                | 149               | 51                |             |         |
| miR-21| rs1292037 | T>C           | Allelic model  | W                 | M                 | 1.28 (0.92, 1.79) | .144    |
|       |           |               |                | 73                | 327               |             |         |
|       |           |               | Dominant model | WW + MM           | WW + MM           | 2.25 (1.05, 4.80) | .032    |
|       |           |               |                | 10                | 190               |             |         |
|       |           |               | Recessive model| WW + WM           | MM                 | 1.12 (0.75, 1.67) | .597    |
|       |           |               |                | 63                | 137               |             |         |
|       | rs13137   | A>T           | Allelic model  | W                 | M                 | 0.94 (0.69, 1.28) | .699    |
|       |           |               |                | 302               | 98                |             |         |
|       |           |               | Dominant model | WW + MM           | WW + MM           | 0.91 (0.62, 1.33) | .610    |
|       |           |               |                | 115               | 85                |             |         |
|       |           |               | Recessive model| WW + WM           | MM                 | 1.02 (0.47, 2.20) | 1.000   |
|       |           |               |                | 187               | 13                |             |         |

(Continues)
### TABLE 4 (Continued)

| Gene | rs number | Allele change | Model | Gensini ≥ 30 group | Gensini < 30 group | OR (95% CI) | P value |
|------|------------|---------------|-------|-------------------|-------------------|-------------|---------|
| mTOR | rs2295080  | G>T           | Allelic model | W | M | W | M | 1.76 (1.31, 2.36) | <.001 |
|      |            |               | Dominant model | WW | WM + MM | WW | WM + MM | 1.84 (1.04, 3.27) | .036 |
|      |            |               | Recessive model | WW + WM | MM | WW + WM | MM | 1.98 (1.35, 2.90) | <.001 |
| rs2536 | T>C       | Allelic model | W | M | W | M | 1.38 (1.02, 1.86) | .037 |
|      |            |               | Dominant model | WW | WM + MM | WW | WM + MM | 2.06 (1.14, 3.72) | .015 |
|      |            |               | Recessive model | WW + WM | MM | WW + WM | MM | 1.22 (0.83, 1.79) | .310 |
| rs11121704 | C>T     | Allelic model | W | M | W | M | 1.04 (0.78, 1.38) | .806 |
|      |            |               | Dominant model | WW | WM + MM | WW | WM + MM | 1.13 (0.77, 1.65) | .527 |
|      |            |               | Recessive model | WW + WM | MM | WW + WM | MM | 0.88 (0.5, 1.54) | .647 |
| rs1034528 | G>C       | Allelic model | W | M | W | M | 1.22 (0.92, 1.61) | .170 |
|      |            |               | Dominant model | WW | WM + MM | WW | WM + MM | 1.14 (0.78, 1.67) | .507 |
|      |            |               | Recessive model | WW + WM | MM | WW + WM | MM | 1.70 (0.96, 3.02) | .069 |

Abbreviations: CHD, coronary heart disease; CI, confidence interval; M, mutant allele; OR, odds ratio; W, wild allele.

### TABLE 5 Association of haploid of significant single nucleotide polymorphisms in IncRNA GAS5/miR-21/mTOR axis with Gensini score of CHD patients

| SNP | Haplotype | Gensini ≥ 30 group | Gensini < 30 group | OR (95% CI) | P value |
|-----|-----------|--------------------|--------------------|-------------|---------|
| rs2067079_ | TACTC | 0.097 19 | 0.074 18 | 1.27 (0.65, 2.49) | .484 |
| rs6790_     | TACTT | 0.032 6 | 0.033 8 | 0.88 (0.30, 2.58) | .818 |
| rs1292037_  | TACGC | 0.032 6 | 0.044 10 | 0.70 (0.25, 1.96) | .494 |
| rs2295080_  | TGCTC | 0.226 45 | 0.132 31 | 1.92 (1.16, 3.17) | .010 |
| rs2536      | TGCTT | 0.075 15 | 0.059 14 | 1.29 (0.60, 2.73) | .513 |
|            | TGCGC | 0.075 15 | 0.078 18 | 0.98 (0.48, 2.00) | .960 |
|            | TGTC | 0.050 10 | 0.037 9 | 1.33 (0.53, 3.33) | .545 |
|            | CACTC | 0.042 8 | 0.048 11 | 0.85 (0.34, 2.16) | .736 |
|            | CGCTC | 0.097 19 | 0.085 20 | 1.13 (0.59, 2.19) | .709 |
|            | CGCTT | 0.032 6 | 0.038 9 | 0.78 (0.27, 2.23) | .642 |
|            | CGCGC | 0.032 6 | 0.050 12 | 0.58 (0.21, 1.57) | .276 |

Abbreviations: CHD, coronary heart disease; CI, confidence interval; Freq, frequency; Num, number; OR, odds ratio.
for etiology of single-gene diseases. Nonetheless, genetic function in distinct disorders varied greatly, making it tough to explain pathogenesis of multifactor diseases. Furthermore, environmental factors also could act interactively with specific genes, thereby facilitating or slowing down disease progression. Therefore, it was of significance to elucidate the combined role of SNPs and environmental exposures in regulating disease risk.

There were known SNPs which affected CHD development dramatically, for example, 3′-UTR-1444C>T of CRP was associated with incremental chance of CHD onset, yet IL-6 promoter-174CC decreased CHD risk among a Scottish population. We demonstrated that SNPs in GAS5/MIR-21/mTOR axis were associated with CHD risk and prognosis (Tables 2-5), which expanded knowledge of this area. Despite shortage of direct evidence, GAS5 might still be implicated in etiology of CHD, which was generally held as an inflammatory disorder, for its relevance to inflammation. To be specific, GAS5 could prevent binding of glucocorticoid receptor (GR) to GR element (GRE), thus hindering glucocorticoid-mediated signaling which played key roles in inflammation. More than that, anomalies in glucocorticoid signaling was a major contributor to CHD onset, and high glucocorticoid content could engender cardiovascular symptoms, such as visceral obesity and hypercholesterolemia, which implied the association of GAS5 with GR-mediated inflammation underlying CHD pathogenesis. In addition, high GAS5 expression was detectable in patients with autoimmune diseases (eg, systemic lupus erythematosus and scleroderma) and infectious diseases (eg, bacteria sepsis), and GAS5 level in airway epithelial cells and airway smooth muscle cells could be raised by pro-inflammatory factors. Maybe it was due to these linkages that rs2067079 (C>T) and rs6790 (G>A) of GAS5 were markedly associated with CHD risk and prognosis (Tables 2-5), yet whether these SNPs might influence GAS5 expression in CHD was unclear. However, pathogenic SNPs of GAS5 differed among diseases, such as rs145204276 in gastric cancer and rs55829688 in acute leukemia, which could be attributed to difference in pathogenesis of diseases.

In addition, miRNAs were also crucial in regulating CHD pathogenesis, including hypertrophy, myocardial remodeling, and angiogenesis. Here, we introduced MIR-21, whose expression was abnormally high in peripheral blood mononuclear cell (PBMC) of patients with CHD. The MIR-21 not merely prohibited angiogenesis of endothelial progenitor cells (EPCs) in CHD, but also promoted apoptosis of cardiomyocytes. Altogether, MIR-21 was a pronounced regulator of cardiovascular diseases, and its SNPs, rs1292037 (T>C), and rs6790 (G>A) of GAS5 were markedly associated with CHD risk and prognosis (Tables 2-5), yet whether these SNPs might influence GAS5 expression in CHD was unclear. However, pathogenic SNPs of GAS5 differed among diseases, such as rs145204276 in gastric cancer and rs55829688 in acute leukemia, which could be attributed to difference in pathogenesis of diseases.

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Furthermore, mTOR signaling exerted vital roles in promoting atherosclerosis development. That was because blockage of mTOR signaling could down-regulate expression of inflammatory cytokines and drive selective clearance of macrophages and vascular endothelial cells, which altogether delayed atherosclerosis progression.
FIGURE 1  Combination of risk factors that produced interactions in association with CHD risk, as well as tree diagram for SNP-SNP (A) interaction and SNP-environmental exposure (B) interaction. CHD: coronary heart disease. Bars in each box represented the number of case group (left) and that of control group (right).

FIGURE 2  Combination of risk factors that produced interactions in association with Genisini score of CHD patients, as well as tree diagram for SNP-SNP (A) interaction and SNP-environmental exposure (B) interaction. CHD: coronary heart disease. Bars in each box represented the number of case group (left) and that of control group (right).
Nevertheless, Lajoie et al\textsuperscript{12} reported that rapamycin, an inhibitor of mTOR, tended to aggravate MI severity of rat models. This contradiction was attributable to distinction in animal species, arterial disease, and treatment mode among studies. In addition, rs2295080, located in promoter of mTOR, could alter mTOR expression\textsuperscript{75} and thus deregulating mTOR signaling-induced disease onset.\textsuperscript{53,54} Besides rs2295080 (G>T), our study also revealed that rs2536 (T>C) and rs1034528 (G>C) of mTOR were hazard factors for CHD onset and prognosis (Tables 2-5), yet whether they were associated with differential expression of mTOR in CHD demanded more proof.

More deeply, MDR model clarified that rs2067079-TT of GASS synergizing with rs2536-CC of mTOR could significantly trigger CHD onset, and smoking interacting with rs6790-GG of GASS and rs2295080-TT of mTOR also displayed strong associations with CHD prognosis (Figures 1 and 2, Table 6). Actually, the non-parametric MDR was advantageous in not requiring uniform genetic model of included diseases, and it could avoid false-positive results with its cross-validation strategy, compared with traditional parametric statistics. Hence, this study offered some reliable clues about the interaction of SNPs in GASS/MIR-21/mTOR axis and smoking on CHD susceptibility and prognosis, although statistical analysis might not suffice to articulate gene-gene/environment interaction underlying disease etiology.

In conclusion, SNPs of GASS/MIR-21/mTOR axis might interact with smoking to exacerbate CHD risk and worsen CHD prognosis, although this has not been biologically confirmed. However, a series of other points reduced the persuasiveness of this study. Firstly, the patients with CHD were retrospectively included, which might lead to bias in selecting participants. Secondly, this study was based on relatively small sample size, which might blur inner relationships between SNPs/environmental exposures and CHD risk/prognosis. Thirdly, conclusion of this study, which focused on a Chinese cohort, might not be applicable to other ethnicities. Finally, in vivo and in vitro experiments were not performed to certify the biological role of GASS/MIR-21/mTOR axis underlying CHD etiology. All in all, points exemplified as above should be optimized in the future.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Li H, Liu Y, Huang J, Liu Y, Zhu Y. Association of genetic variants in IncRNA GAS5/miR-21/mTOR axis with risk and prognosis of coronary artery disease among a Chinese population. *J Clin Lab Anal*. 2020;34:e23430. [https://doi.org/10.1002/jcla.23430](https://doi.org/10.1002/jcla.23430)