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RESEARCH ARTICLE

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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.[6,7]

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 lncRNA 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, *GAS5*/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 *GAS5*/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 *GAS5*/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 *GAS5* (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.

TABLE 1 Comparison of clinical features between CHD patients and healthy controls

Clinical features	CHD group	Control group	t/χ^2	*P* value
Number	436	471		
Age (y)	62.31 ± 12.15	61.26 ± 11.93	1.313	.190
Sex				
Female	146 (33.49%)	185 (39.28%)	3.277	.070
Male	290 (66.51%)	286 (60.72%)		
Clinical types				
SAP	139 (31.81%)			
UAP	150 (34.55%)			
AMI	147 (33.64%)			
Type 2 diabetes mellitus				
Positive	167 (38.30%)	152 (32.27%)	3.612	.057
Negative	269 (61.70%)	319 (67.73%)		
Hypertension				
Positive	194 (44.50%)	175 (37.15%)	5.055	.025
Negative	242 (55.50%)	296 (62.85%)		
Lipid abnormality				
Positive	189 (43.35%)	176 (37.37%)	3.368	.067
Negative	247 (56.65%)	295 (62.63%)		
Smoking				
Positive	234 (53.67%)	212 (45.01%)	6.792	.009
Negative	202 (46.33%)	259 (54.99%)		
Alcohol				
Positive	213 (48.85%)	203 (43.10%)	3.019	.082
Negative	223 (51.15%)	268 (56.90%)		
BMI (kg/m^2)	25.73 ± 10.66	24.93 ± 9.02	1.223	.222
Ccr (mL/min)	74.62 ± 18.02	84.77 ± 23.16	7.326	<.001
hs-CRP (mg/L)	2.23 ± 0.81	1.86 ± 0.37	8.956	<.001
TC (mmol/L)	4.51 ± 1.23	4.42 ± 0.85	1.290	.198
TG (mmol/L)	1.72 ± 1.06	1.39 ± 0.84	5.215	<.001
HDL-C (mmol/L)	1.23 ± 0.39	1.45 ± 0.44	7.944	<.001
LDL-C (mmol/L)	2.57 ± 1.04	2.39 ± 0.88	2.821	.005

Abbreviations: AMI, acute myocardial infarction; BMI, body mass index; Ccr, creatinine clearance rate; CHD, coronary heart disease; HDL-C, high-density lipoprotein cholesterol; hs-CRP, hs-C reactive protein; LDL-C, low-density lipoprotein cholesterol; SAP, stable angina pectoris; TC, total cholesterol; TG, triacylglycerol; UAP, unstable angina pectoris.

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[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

TABLE 2 Association of single nucleotide polymorphisms in lncRNA GAS5/miR-21/mTOR axis with CHD risk

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
				303	569	461	481		
			Dominant model	WW	WM + MM	WW	WM + MM	1.30 (0.94, 1.80)	.107
				81	355	108	363		
			Recessive model	WW + WM	MM	WW + WM	MM	2.88 (2.18, 3.81)	<.001
				222	214	353	118		
	rs6790	G>A	Allelic model	W	M	W	M	0.59 (0.49, 0.72)	<.001
				613	259	549	393		
			Dominant model	WW	WM + MM	WW	WM + MM	0.59 (0.45, 0.77)	<.001
				211	225	168	303		
			Recessive model	WW + WM	MM	WW + WM	MM	0.36 (0.24, 0.55)	<.001
				402	34	381	90		
	rs17359906	G>A	Allelic model	W	M	W	M	1.09 (0.90, 1.32)	.377
				523	349	63.428	358		
			Dominant model	WW	WM + MM	WW	WM + MM	1.04 (0.80, 1.36)	.806
				164	272	181	290		
			Recessive model	WW + WM	MM	WW + WM	MM	1.27 (0.89, 1.81)	.186
				359	77	403	68		
	rs55829688	T>C	Allelic model	W	M	W	M	0.84 (0.70, 1.01)	.071
				447	425	443	499		
			Dominant model	WW	WM + MM	WW	WM + MM	0.80 (0.59, 1.09)	.152
				114	322	104	367		
			Recessive model	WW + WM	MM	WW + WM	MM	0.79 (0.59, 1.06)	.130
				333	103	339	132		
miR-21	rs1292037	T>C	Allelic model	W	M	W	M	1.76 (1.42, 2.18)	<.001
				178	694	293	649		
			Dominant model	WW	WM + MM	WW	WM + MM	1.48 (0.95, 2.31)	.082
				35	401	54	417		
			Recessive model	WW + WM	MM	WW + WM	MM	2.11 (1.61, 2.76)	<.001
				143	293	239	232		

(Continues)

TABLE 2 (Continued)

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

TABLE 3 Association of haploid of significant SNPs in the lncRNA GAS5/miR-21/mTOR axis with CHD risk

SNP	Haplotype	CHD group		Control group		OR (95% CI)	*P* value
		Freq	Num	Freq	Num		
rs2067079_rs6790 _rs1292037_rs2295080 _rs2536_rs1034528	TACTCG	0.05	22	0.032	15	1.62 (0.83-3.16)	.157
	TGCTCG	0.118	51	0.044	21	2.84 (1.68-4.80)	<.001
	TGCTTG	0.046	20	0.041	19	1.14 (0.60-2.17)	.682
	TGCGCG	0.053	23	0.031	15	1.69 (0.87-3.29)	.116
	CGCTCG	0.063	28	0.043	20	1.55 (0.86-2.79)	.144

Abbreviations: CHD, coronary heart disease; CI, confidence interval; Freq, frequency; Num, number; OR, odds ratio.

(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 lncRNA 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% = 1.42-2.18, $P < .001$; Recessive model: OR = 2.11, 95CI% = 1.61-2.76, $P < .001$). Concerning *mTOR*, mutant alleles of rs2295080 (G>T), rs2536 (T>C), and rs1034528 (G>C) were all hazard factors for CHD onset under the allelic model (OR = 1.53, 95CI% = 1.26-1.86, $P < .001$; OR = 2.35, 95CI% = 1.93-2.85, $P < .001$; OR = 1.32, 95CI% = 1.08-1.61, $P = .006$). In addition, haploid TGCTCG raised CHD risk significantly in comparison with other haploids (OR = 2.84, 95CI% = 1.68-4.80, $P < .001$) (Table 3).

3.3 | Correlation between SNPs in lncRNA 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, haploid TGCTC 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 lncRNA 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

TABLE 4 Association of single nucleotide polymorphisms in lncRNA GAS5/miR-21/mTOR axis with Gensini score of CHD patients

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	W	M	1.51 (1.14, 2.00)	.004
				119	281	184	288		
			Dominant model	WW	WM + MM	WW	WM + MM	1.29 (0.79, 2.11)	.306
				33	167	48	188		
			Recessive model	WW + WM	MM	WW + WM	MM	1.80 (1.23, 2.63)	.002
				86	114	136	100		
	rs6790	G>A	Allelic model	W	M	W	M	0.76 (0.60, 0.96)	.027
				613	259	304	168		
			Dominant model	WW	WM + MM	WW	WM + MM	0.69 (0.50, 0.95)	.025
				211	225	93	143		
			Recessive model	WW + WM	MM	WW + WM	MM	0.71 (0.41, 1.22)	.222
				402	34	211	25		
	rs17359906	G>A	Allelic model	W	M	W	M	0.89 (0.68, 1.17)	.399
				246	154	277	195		
			Dominant model	WW	WM + MM	WW	WM + MM	0.77 (0.52, 1.14)	.180
				82	118	82	154		
			Recessive model	WW + WM	MM	WW + WM	MM	1.04 (0.63, 1.70)	.862
				164	36	195	41		
	rs55829688	T>C	Allelic model	W	M	W	M	1.25 (0.96, 1.63)	.102
				193	207	254	218		
			Dominant model	WW	WM + MM	WW	WM + MM	1.50 (0.97, 2.32)	.070
				44	156	70	166		
			Recessive model	WW + WM	MM	WW + WM	MM	1.21 (0.78, 1.88)	.396
				149	51	184	52		
miR-21	rs1292037	T>C	Allelic model	W	M	W	M	1.28 (0.92, 1.79)	.144
				73	327	105	367		
			Dominant model	WW	WM + MM	WW	WM + MM	2.25 (1.05, 4.80)	.032
				10	190	25	211		
			Recessive model	WW + WM	MM	WW + WM	MM	1.12 (0.75, 1.67)	.597
				63	137	80	156		
	rs13137	A>T	Allelic model	W	M	W	M	0.94 (0.69, 1.28)	.699
				302	98	351	121		
			Dominant model	WW	WM + MM	WW	WM + MM	0.91 (0.62, 1.33)	.610
				115	85	130	106		
			Recessive model	WW + WM	MM	WW + WM	MM	1.02 (0.47, 2.20)	1.000
				187	13	221	15		

(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
				99	301	173	299		
			Dominant model	WW	WM + MM	WW	WM + MM	1.84 (1.04, 3.27)	.036
				20	180	40	196		
			Recessive model	WW + WM	MM	WW + WM	MM	1.98 (1.35, 2.90)	<.001
				79	121	133	103		
	rs2536	T>C	Allelic model	W	M	W	M	1.38 (1.02, 1.86)	.037
				99	301	147	325		
			Dominant model	WW	WM + MM	WW	WM + MM	2.06 (1.14, 3.72)	.015
				18	182	40	196		
			Recessive model	WW + WM	MM	WW + WM	MM	1.22 (0.83, 1.79)	.310
				81	119	107	129		
	rs11121704	C>T	Allelic model	W	M	W	M	1.04 (0.78, 1.38)	.806
				263	137	314	158		
			Dominant model	WW	WM + MM	WW	WM + MM	1.13 (0.77, 1.65)	.527
				88	112	111	125		
			Recessive model	WW + WM	MM	WW + WM	MM	0.88 (0.5, 1.54)	.647
				175	25	203	33		
	rs1034528	G>C	Allelic model	W	M	W	M	1.22 (0.92, 1.61)	.170
				250	150	316	156		
			Dominant model	WW	WM + MM	WW	WM + MM	1.14 (0.78, 1.67)	.507
				81	119	103	133		
			Recessive model	WW + WM	MM	WW + WM	MM	1.70 (0.96, 3.02)	.069
				169	31	213	23		

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 lncRNA GAS5/miR-21/mTOR axis with Gensini score of CHD patients

		Gensini ≥ 30 group		Gensini < 30 group			
SNP	Haplotype	Freq	Num	Freq	Num	OR (95% CI)	*P* value
rs2067079_ rs6790_ rs1292037_ rs2295080_ rs2536	TACTC	0.097	19	0.074	18	1.27 (0.65, 2.49)	.484
	TACTT	0.032	6	0.033	8	0.88 (0.30, 2.58)	.818
	TACGC	0.032	6	0.044	10	0.70 (0.25, 1.96)	.494
	TGCTC	0.226	45	0.132	31	1.92 (1.16, 3.17)	.010
	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
	TGTTC	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.

TABLE 6 The MDR model concerning SNP-SNP and SNP-environmental exposure interactions

Indicator	Interaction	Best model	Training accuracy (%)	Testing accuracy (%)	CVC	χ^2	*P* value	OR	95% CI
CHD	SNP-SNP	rs2536	63.90%	63.90%	10/10	64.24	<.001	3.21	2.40-4.28
		rs2067079, rs2536	74.26%	73.94%	10/10	198.54	<.001	9.73	6.93-13.65
		rs2067079, rs6790, rs2536	79.37%	77.97%	9/10	287.95	<.001	15.82	11.19-22.37
	SNP-En	rs2536	63.90%	63.90%	10/10	64.24	<.001	3.21	2.40-4.28
		rs2067079, rs2536	74.26%	73.94%	10/10	198.54	<.001	9.73	6.93-13.65
		Smoking, alcohol, rs1292037	81.31%	79.81%	8/10	334.56	<.001	23.69	16.14-34.77
Change of Gensini score	SNP-SNP	rs2295080	56.35%	56.35%	10/10	6.27	.012	1.67	1.12-2.48
		rs6790, rs2295080	58.27%	49.97%	7/10	14.01	<.001	2.29	1.48-3.54
		rs6790, rs2295080, rs2536	62.91%	54.92%	7/10	31.3	<.001	3.27	2.14-4.99
	SNP-En	Smoking	60.47%	60.47%	10/10	15.61	<.001	2.26	1.50-3.40
		Smoking, rs6790	61.76%	58.97%	9/10	20.08	<.001	2.53	1.68-3.80
		Smoking, rs6790, rs2295080	65.16%	60.82%	10/10	35.35	<.001	3.49	2.29-5.30

Abbreviations: CHD, coronary heart disease; MDR, multifactor dimensionality reduction; SNP, single nucleotide polymorphism; SNP-En, SNP-Environment.

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,[31] yet *IL-6* promoter-174CC decreased CHD risk among a Scottish population.[32] 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,[33] 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.[34] 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,[35-37] 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),[38] and GAS5 level in airway epithelial cells and airway smooth muscle cells could be raised by pro-inflammatory factors.[39] 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,[20,40] 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.[41,42] Here, we introduced MIR-21, whose expression was abnormally high in peripheral blood mononuclear cell (PBMC) of patients with CHD.[43] The MIR-21 not merely prohibited angiogenesis of endothelial progenitor cells (EPCs) in CHD,[44] but also promoted apoptosis of cardiomyocytes.[45] Altogether, MIR-21 was a pronounced regulator of cardiovascular diseases, and its SNPs, rs1292037 (T>C), and rs13137 (A>T), were associated with enhancive CHD risk and poor CHD prognosis (Tables 2-5). Apart from SNPs, the biological function of MIR-21 could be altered by other mechanisms,[46] such as DNA methylation,[47] so transcriptional regulation of MIR-21 required further exploration.

Furthermore, *mTOR* signaling exerted vital roles in promoting atherosclerosis development.[48] That was because blockage of *mTOR* signaling could down-regulate expression of inflammatory cytokines[49] and drive selective clearance of macrophages and vascular endothelial cells,[50,51] which altogether delayed atherosclerosis progression.

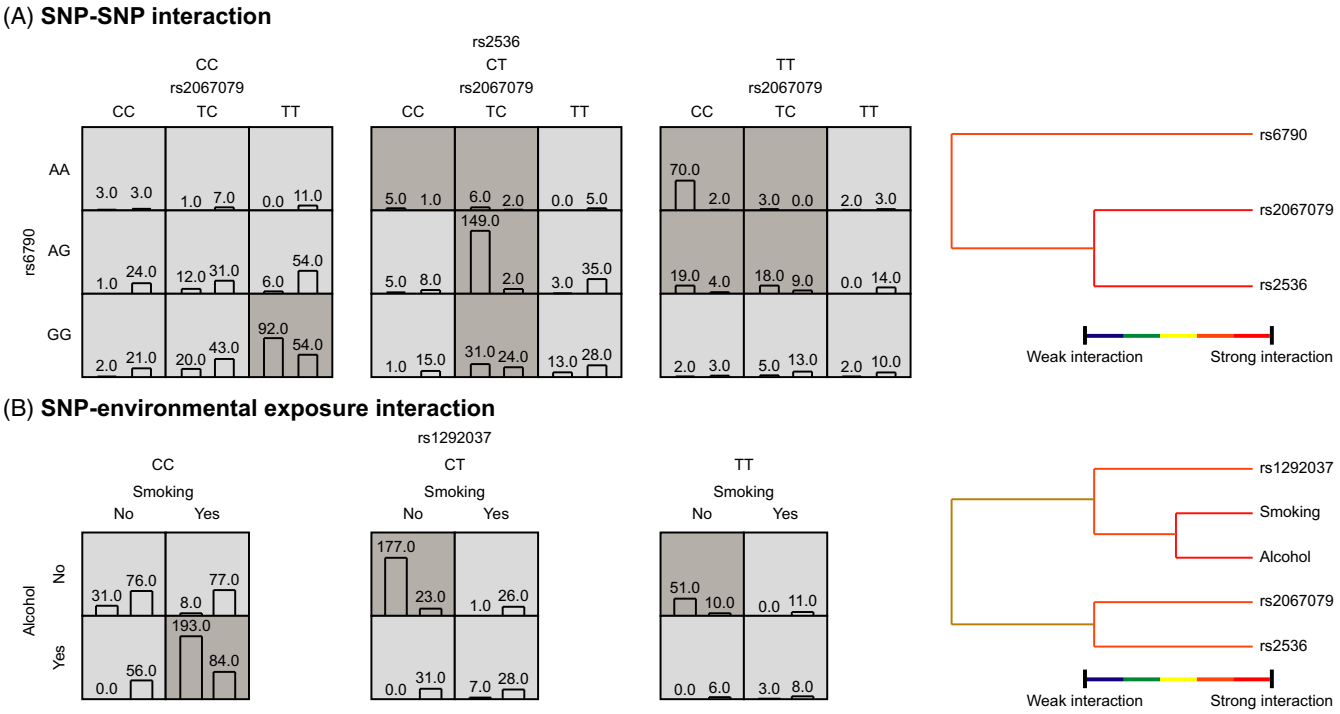

FIGURE 1 Combination of risk factors that produced interactions in association with CHD risk, as well as tree diagram for SNP-SNP (A) interaction and SNP-environmental exposure (B) interaction. CHD: coronary heart disease. Bars in each box represented the number of case group (left) and that of control group (right)

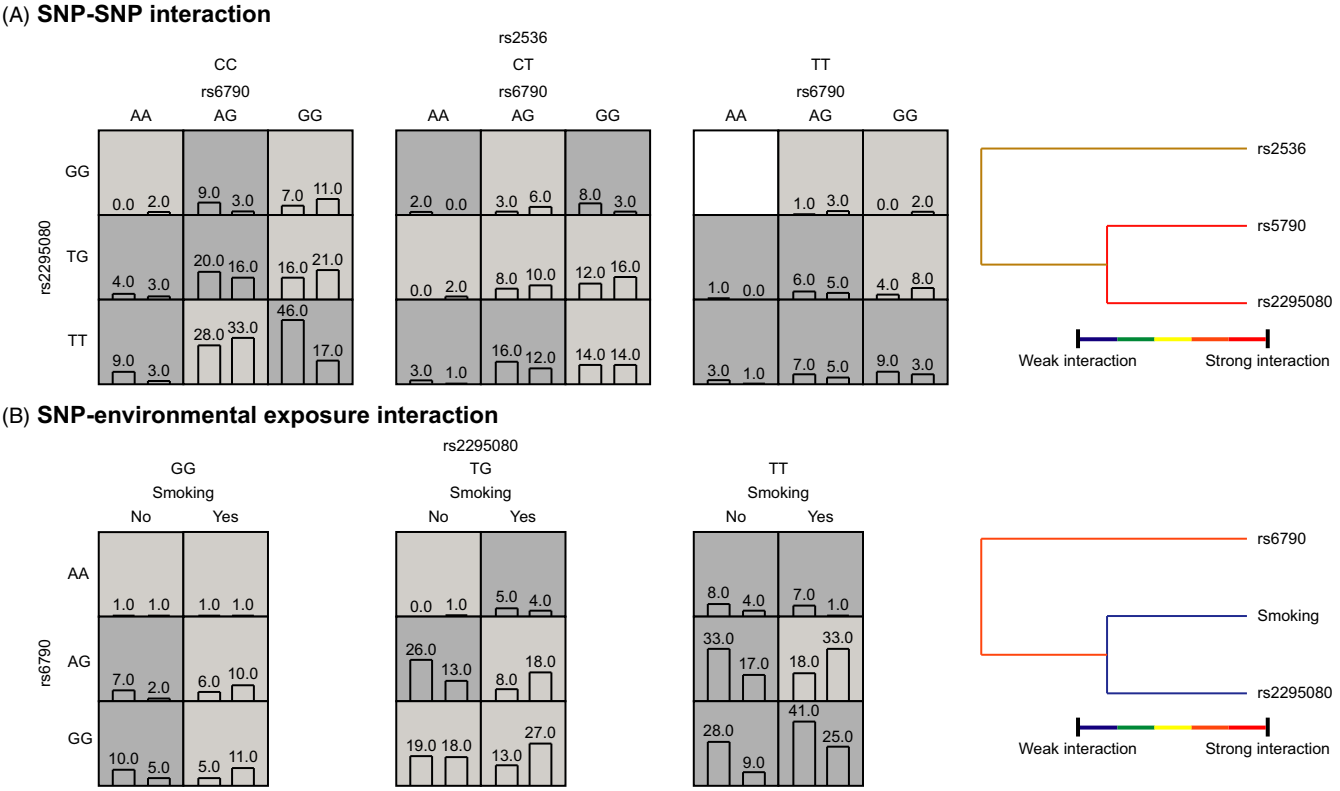

FIGURE 2 Combination of risk factors that produced interactions in association with Genisini score of CHD patients, as well as tree diagram for SNP-SNP (A) interaction and SNP-environmental exposure (B) interaction. CHD: coronary heart disease. Bars in each box represented the number of case group (left) and that of control group (right)

Nevertheless, Lajoie et al[52] 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 promotor of *mTOR*, could alter mTOR expression[25] and thus deregulating mTOR signaling-induced disease onset.[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 *GAS5* synergizing with rs2536-CC of *mTOR* could significantly trigger CHD onset, and smoking interacting with rs6790-GG of *GAS5* 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 *GAS5*/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 *GAS5*/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 *GAS5*/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.

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