Received: 13 December 2016 | Revised: 27 February 2017 | Accepted: 28 February 2017

DOI: 10.1002/clc.22708

PREVENTION WILEY CLINICAL CARDIOLOGY

CLINICAL INVESTIGATIONS

Correlation between genetic polymorphisms within the MAPK1/HIF-1/HO-1 signaling pathway and risk or prognosis of perimenopausal coronary artery disease

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Background: Mitogen-activated protein kinase-1 (MAPK1), as well as its downstream factors of hypoxia-inducible factor-1 (HIF-1) and heme oxygenase-1 (HO-1), have been documented to be involved in modulating development of coronary artery disease (CAD).

Hypothesis: Genetic mutations within the MAPK1/HIF-1/HO-1 signaling pathway could alter the risk of perimenopausal CAD in Chinese patients.

Methods: Peripheral blood samples were gathered from 589 CAD patients and 860 healthy controls, and 12 potential single-nucleotide polymorphisms (SNPs) were obtained from HapMap database and previously published studies. Genotyping of SNPs was implemented with the TaqMan SNP Genotyping Assays. Odds ratios (OR) and 95% confidence intervals (CI) were utilized to evaluate the correlations between SNPs and CAD risk.

Results: Regarding *MAPK1*, rs6928 (OR: 1.71, 95% CI: 1.47-1.98, $P < 0.05$), rs9340 (OR: 0.85, 95% CI: 0.73-0.99, $P < 0.05$), and rs11913721 (OR: 0.70, 95% CI: 0.52-0.95, $P < 0.05$) were remarkably associated with susceptibility to perimenopausal CAD. Of these, rs9340 and rs11913721 were also regarded as protective factors for perimenopausal CAD patients. Moreover, results of *HIF-1* indicated noticeable correlations between combined SNPs of rs1087314 and rs2057482 and risk of perimenopausal CAD (OR: 1.24, 95% CI: 1.01-1.53, $P < 0.05$; and OR: 0.71, 95% CI: 0.55-0.91, $P < 0.05$, respectively). Nonetheless, rs2071746 in *HO-1* was found to be only associated with perimenopausal CAD risk (OR: 0.67, 95% CI: 0.58-0.78, $P < 0.05$).

Conclusions: The genetic mutations within *MAPK1* (rs6928, rs9340, rs11913721), *HIF-1* (rs1087314, rs2057482), and *HO-1* (rs2071746) could alter susceptibility to perimenopausal CAD in this Chinese population.

KEYWORDS

Perimenopausal CAD, MAPK1/HIF-1/HO-1 Signaling, Single-Nucleotide Polymorphism, Susceptibility, Gene-Gene Interaction

1 | INTRODUCTION

The human perimenopausal period starts when ovarian function begins to decline and continues up to 1 year after the last menstruation.[1] Multiple epidemiologic studies have indicated that both morbidity and mortality from coronary artery disease (CAD) among postmenopausal women rose to ≥5-fold that of premenopausal women, suggesting that decreased estrogen (E2) might facilitate development of CAD.[2] Estrogen protects the cardiovascular system partly through evidently suspending oxidation actions of certain enzymes and reducing generation of oxidized low-density lipoprotein cholesterol (ox-LDL-C), further relieving injury to vascular endothelium.[3,4] Regarding hemodynamics, E2 is capable of reducing adhesion and aggregation of thrombocytes, degrading plasminogen activator inhibitor-1, as well as reducing formation of thrombus.[5] All in all, adequate E2 levels seem to prevent development of CAD through a variety of mechanisms.

One explorative study in a European population indicated that CAD was genetically relevant to genetic polymorphisms within inflammatory signaling pathways (eg, rs6928, rs9340, and rs11913721 of mitogen-activated protein kinase-1 [MAPK1]), yet an independent replication study believed that this relevance did not exist.[6] Considering hereditary variation among diverse ethnicities and tight linkage of MAPK1 with heart defects,[7] genetic mutations of *MAPK1* were still speculated to be potential risk factors for CAD. In addition, downstream factors of MAPK1, such as hypoxia-inducible factor-1 (HIF-1) and heme oxygenase-1 (HO-1), were documented to participate in modulating CAD development and they appeared as protective elements for CAD.[8,9] To be specific, rs2057482 of *HIF-1α* could be independently associated with susceptibility to CAD after removing effects of various parameters, including hypertension, high-density lipoprotein cholesterol levels, ratio of visceral to subcutaneous adipose tissue, and so on.[10] Additional polymorphisms of *HIF1A* (rs11549465, rs1087314, and rs41508050) were commonly observed among CAD patients at the initial stage, which was clinically presented as stable exertional angina.[11] As for *HO-1*, one single-nucleotide polymorphism (SNP) of T(−413)A (rs2071746) and another (GT)n dinucleotide repeat length polymorphism were reported to be markedly correlated with CAD risk, though one meta-analysis only confirmed the close correlation between (GT)n short allele (S, <25 repeats) and risk of CAD.[12,13]

Intriguingly, MAPK1, HIF-1, and HO-1 seemed to be interrelated with the functional role of E2 within the human body. For instance, phosphorylated MAPK1 might accelerate estrogen receptor-α (ERα) turnover, and sometimes inhibited ERα expression, which greatly affected estrogen action.[14,15] Moreover, E2 also served to induce activation of HIF-1 on the precondition that the phosphatidylinositol 3-kinase/Akt pathway was activated,[16] and HIF-1 activation might also be initiated via ER-related PI3K/Akt and MAPK1 pathways.[17] Ultimately, HO-1 was involved in the attenuation of lung injury mediated by E2.[18]

In view of the complicated relationships among E2, the MAPK1/HIF-1/HO-1 signaling pathway, and potential risk of CAD, we intended to discover whether SNPs within *MAPK1*, *HIF-1*, and *HO-1* could explain the high risk of perimenopausal CAD.

2 | METHODS

2.1 | Study populations

This retrospective study collected 1449 female patients who underwent coronary arteriography (CAG) at Cangzhou Central Hospital of Heibei Medical University from January 2007 to June 2010. The patients were divided into a CAD group (n = 589) and a control group (n = 860), according to CAG results. Then, based on menopausal status, the patients in the CAD group were divided into a premenopausal CAD group (n = 167) and a postmenopausal CAD group (n = 422). The statistical power of sample size in this study was calculated to be 0.949 via the software of Power and Sample Size Program, which was very robust. The patients were excluded if (1) they did not have complete medical records; (2) they were diagnosed simultaneously with other myocardial diseases or severe disorders of the liver, kidney, blood, and so on; (3) they suffered from amenorrhoea and psychosis; (4) they received estrogen-replacement therapy; or (5) they had undergone surgery or trauma within the past 1 month. The informed consents were signed by all participants, and the ethics committee of Cangzhou Central Hospital (Heibei province) approved this study.

2.2 | Assessment of aspirin resistance

After extracting 4 mL of fasting blood from each subject, the blood was anticoagulated with 1:9 sodium citrate, and the platelet aggregation rates were accordingly detected. The aspirin resistance was confirmed when the platelet aggregation rate induced by 10 μmol/L ADP was ≥70% and the platelet aggregation rate induced by 0.5 mmol/L arachidonic acid was ≥20%.[19]

2.3 | Coronary arteriography

Participants were included in the CAD group if ≥1 of their arteries had >50%-diameter stenosis.[20] Lesions of CAD patients were classified as single-, double-, and triple-vessel lesions based on the amount of stenosed coronary vessel. The classification (type A, type B, and type C) regarding complexity of the CAD lesions was made in accordance with the principles of the American College of Cardiography/American Heart Association.[21] The SYNTAX score was calculated (http://www.syntaxscore.com), and the 3 grades were classified as low risk (score ≤23), moderate risk (score 23–32), and high risk (score ≥33).

2.4 | Gensini scoring

The Gensini score was used to evaluate the degree of coronary artery stenosis.[20] Specifically, the scoring was applied as follows: (1) score of 1 when the proportion of stenosis was ≤25%; (2) score of 2 when the proportion of stenosis was 25% to 50%; (3) score of 4 when the proportion of stenosis was 50% to 75%; (4) score of 8 when the proportion of stenosis was 75% to 90%; (5) score of 16 when the proportion of stenosis was 90% to 99%; and (6) score of 32 when the proportion of stenosis was 100%. The coefficients for different segments of the coronary arteries were as follows: left main coronary artery (×5), the proximal left anterior descending branch (×2.5), left anterior descending branch in the middle (×1.5), far-left anterior descending branch (×1), the first diagonal branch (×1), the second diagonal branch (×0.5), and the proximal left circumflex branch (×2.5). The final scoring was set as the sum of all the scores. Finally, we divided participants into 2 groups according to Gensini score: group 1 (total score ≤34) and group 2 (total score >34).

2.5 | DNA extraction and genotyping

About 5 mL of fasting venous blood was drawn by venipuncture from each participant. The blood sample was conserved in EDTA tubes and centrifuged at 4000 *g* for 5 minutes to collect supernatant. Then the DNA extraction from blood samples was conducted with a kit (TaKaRa Biotechnology [Dalian] Co., Ltd., Shiga, Japan). The TaqMan

TABLE 1 Association of SNPs within *MAPK1*, *HIF-1α*, and *HO-1* with development of CAD among groups of pre- and postmenopausal women

			A Genotype			B Genotype			Allelic Model		Dominant Model		Recessive Model	
Gene	rs Number (W > M)	A vs B	WW	WM	MM	WW	WM	MM	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
MAPK1	rs6928 (C > G)	CAD vs control	133	297	159	328	393	139	1.71 (1.47-1.98)	<0.01	2.11 (1.67-2.68)	<0.01	1.92 (1.48-2.48)	<0.01
		Pre-CAD vs post-CAD	46	84	37	87	213	122	0.76 (0.59-0.98)	0.03	0.68 (0.45-1.03)	0.07	0.70 (0.46-1.07)	0.10
	rs9340 (G > A)	CAD vs control	231	282	76	302	408	150	0.85 (0.73-0.99)	0.03	0.85 (0.69-1.06)	0.15	0.72 (0.53-0.97)	0.03
		Pre-CAD vs post-CAD	64	74	29	167	208	47	1.17 (0.90-1.52)	0.23	1.05 (0.73-1.52)	0.78	1.68 (1.01-2.77)	0.04
	rs2298432 (A > C)	CAD vs control	106	289	194	132	407	321	0.86 (0.74-1.01)	0.06	0.83 (0.62-1.09)	0.18	0.82 (0.66-1.03)	0.09
		Pre-CAD vs post-CAD	32	82	53	74	207	141	0.93 (0.72-1.21)	0.61	0.90 (0.57-1.42)	0.64	0.93 (0.63-1.36)	0.70
	rs9610470 (T > C)	CAD vs control	348	207	34	482	322	56	0.90 (0.76-1.07)	0.25	0.88 (0.71-1.09)	0.25	0.88 (0.57-1.37)	0.57
		Pre-CAD vs post-CAD	98	59	10	250	148	24	1.02 (0.76-1.38)	0.88	1.02 (0.71-1.47)	0.90	1.06 (0.49-2.26)	0.89
	rs11913721 (A > C)	CAD vs control	232	285	72	314	404	142	0.86 (0.74-1.00)	0.05	0.88 (0.71-1.10)	0.27	0.70 (0.52-0.96)	0.02
		Pre-CAD vs post-CAD	61	74	32	171	211	40	1.34 (1.03-1.74)	0.03	1.18 (0.82-1.71)	0.37	2.26 (1.37-3.75)	<0.01
HIF-1	rs11549465 (C > T)	CAD vs control	324	10	255	449	11	400	0.89 (0.76-1.03)	0.11	0.89 (0.72-1.10)	0.29	0.88 (0.71-1.08)	0.23
		Pre-CAD vs post-CAD	91	3	73	233	7	182	1.03 (0.80-1.33)	0.84	1.03 (0.72-1.48)	0.87	1.02 (0.71-1.47)	0.90
	rs11549467 (G > A)	CAD vs control	326	17	246	454	19	387	0.89 (0.77-1.03)	0.12	0.90 (0.73-1.11)	0.34	0.88 (0.71-1.08)	0.22
		Pre-CAD vs post-CAD	92	8	67	234	9	179	0.96 (0.74-1.24)	0.76	1.01 (0.71-1.45)	0.94	0.91 (0.63-1.31)	0.61
	rs10873142 (C > T)	CAD vs control	45	209	335	71	347	442	1.16 (0.98-1.38)	0.08	1.09 (0.74-1.60)	0.68	1.24 (1.01-1.53)	0.04
		Pre-CAD vs post-CAD	23	66	78	22	143	257	0.56 (0.43-0.75)	<0.01	0.34 (0.19-0.64)	<0.01	0.56 (0.39-0.81)	<0.01
	rs2057482 (T > C)	CAD vs control	20	93	476	9	125	726	0.71 (0.55-0.91)	0.01	0.30 (0.14-0.66)	<0.01	0.78 (0.59-1.02)	0.07
		Pre-CAD vs post-CAD	1	27	139	19	66	337	1.48 (0.96-2.28)	0.08	7.83 (1.04-58.94)	0.02	1.25 (0.78-2.00)	0.35
	rs41508050 (C > T)	CAD vs control	581	8	0	843	17	0	0.68 (0.29-1.59)	0.38	0.68 (0.29-1.59)	0.37	—	—
		Pre-CAD vs post-CAD	165	2	0	416	6	0	0.84 (0.17-4.19)	0.83	0.84 (0.17-4.21)	0.83	—	—
HO-1	rs2071746 (A > T)	CAD vs control	183	288	118	184	417	259	0.67 (0.58-0.78)	<0.01	0.60 (0.48-0.77)	<0.01	0.58 (0.45-0.75)	<0.01
		Pre-CAD vs post-CAD	52	82	33	131	206	85	0.99 (0.77-1.28)	0.94	1.00 (0.68-1.47)	0.98	0.98 (0.62-1.53)	0.92
	(GT)n repeat (S > L)	CAD vs control	116	320	153	243	436	181	1.34 (1.15-1.55)	<0.01	1.65 (1.28-2.11)	<0.01	1.36 (1.06-1.73)	0.02
		Pre-CAD vs post-CAD	40	93	34	76	227	119	0.76 (0.59-0.98)	0.03	0.70 (0.45-1.08)	0.10	0.65 (0.42-1.00)	0.05

Abbreviations: CAD, coronary artery disease; CI, confidence interval; HIF-1α, hypoxia inducible factor-1α; HO-1, heme oxygenase-1; M, mutation allele; MAPK1, mitogen activated protein kinase-1; OR, odds ratio; SNP, single-nucleotide polymorphism; W, wild allele.

SNP Genotyping Assays (Applied Biosystems, Foster City, CA) was used for genotyping. The primers were designed according to the information provided by Applied Biosystems (see Supporting Information, Table 1, in the online version of this article). The operation was conducted strictly according to the kit protocol. The thermal cycling conditions were as follows: 50°C for 2 minutes; 95°C for 10 minutes; 50 cycles of 95°C for 15 seconds; and 60°C for 1 minute.

2.6 | Statistical analysis

Statistical analyses were performed using SPSS software version 13.0 (SPSS Inc., Chicago, IL). The *t* test was used for comparisons of 2 independent groups. The χ^2 test was performed to find the difference in groups. For all tests, $P < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Association of genetic polymorphisms within MAPK1/HIF-1/HO-1 pathway and CAD risk

The baseline clinical characteristics among non-CAD, premenopausal CAD, and postmenopausal CAD groups are compared in Supporting Information, Table 2, in the online version of this article. Regarding the genetic polymorphism of *MAPK1* (Table 1), it was noted that the CAD risk of G allele carriers in rs6928 was significantly higher than C allele carriers (odds ratio [OR]: 1.71, 95% confidence interval [CI]: 1.47-1.98, $P < 0.05$). The subjects carrying genotypes GG + CG of rs6928 showed greater risk of CAD than subjects carrying homozygote CC (OR: 2.11, 95% CI: 1.67-2.68, $P < 0.05$). Moreover, the allelic model indicated that the frequency of the rs6928 (G) allele in postmenopausal CAD group was statistically higher than that in the premenopausal CAD group (OR: 0.76, 95% CI: 0.59-1.98, $P < 0.05$). The rs9340 was also found to be significantly correlated with CAD development when compared with healthy controls (OR: 0.85, 95% CI: 0.73-0.99, $P < 0.05$). The frequency of rs9340 (G > A) also displayed significant distinctions between pre- and postmenopausal CAD groups in the recessive model (OR: 1.68, 95% CI: 1.01-2.77, $P < 0.05$). Interestingly, the results of recessive model suggested the strong association of rs11913721 (A > C) with higher CAD risk among postmenopausal women than premenopausal ones (OR: 2.26, 95% CI: 1.37-3.75, $P < 0.05$).

Scarcely any significant associations were found between mutations of *HIF-1α* rs11549465, rs11549467, and rs41508050 with CAD risk among each pair of groups. However, rs10873142(C > T) was associated with less risk of CAD among premenopausal women when compared with postmenopausal ones in the allelic model (OR: 0.56, 95% CI: 0.43-0.75, $P < 0.05$), and rs2057482 also seemed to regulate susceptibility to CAD with significance in the allelic model (OR: 0.71, 95% CI: 0.55-0.91, $P < 0.05$).

For the investigation on *HO-1*, the rs2071746(A > T) was found to be associated with altered susceptibility to CAD group in the allelic

TABLE 2 Association of SNPs within *MAPK1*, *HIF-1α*, and *HO-1* with Gensini score

			Pre-CAD Group (n = 167)				Post-CAD Group (n = 422)			
Gene	SNP	Genotype	≤34	>34	OR (95% CI)	*P* Value	≤34	>34	OR (95% CI)	*P* Value
MAPK1	rs6928	CC	38	8	Ref	Ref	52	35	Ref	Ref
		CG	66	18	0.77 (0.31-1.94)	0.58	115	98	0.79 (0.48-1.31)	0.36
		GG	23	14	0.35 (0.13-0.95)	0.04	56	66	0.57 (0.33-1.00)	0.05
	rs9340	GG	47	17	Ref	Ref	81	86	Ref	Ref
		GA	58	16	1.31 (0.60-2.87)	0.50	119	89	1.42 (0.94-2.14)	0.09
		AA	22	7	1.14 (0.41-3.14)	0.80	23	24	1.02 (0.53-1.94)	0.96
	rs11913721	AA	43	18	Ref	Ref	80	91	Ref	Ref
		AC	60	14	1.79 (0.81-4.00)	0.15	115	96	1.36 (0.91-2.04)	0.13
		CC	24	8	1.26 (0.48-3.32)	0.65	28	12	2.65 (1.27-5.56)	0.01
HIF-1α	rs10873142	CC	17	6	Ref	Ref	12	10	Ref	Ref
		CT	53	13	1.44 (0.47-4.37)	0.52	89	54	1.37 (0.56-3.39)	0.49
		TT	57	21	0.96 (0.33-2.76)	0.94	122	135	0.75 (0.31-1.81)	0.52
	rs2057482	TT	0	1	Ref	Ref	5	14	Ref	Ref
		TC	21	6	—	0.08	22	44	1.40 (0.45-4.39)	0.56
		CC	106	33	—	0.08	196	141	3.89 (1.37-11.05)	0.01
HO-1	rs2071746	AA	35	17	Ref	Ref	62	69	Ref	Ref
		AT	63	19	1.61 (0.74-3.49)	0.23	114	92	1.38 (0.89-2.14)	0.15
		TT	29	4	3.52 (1.07-11.64)	0.03	47	38	1.38 (0.80-2.38)	0.25
	(GT)n repeat	SS	29	11	Ref	Ref	33	43	Ref	Ref
		SL	71	22	1.22 (0.53-2.84)	0.64	120	107	1.46 (0.87-2.47)	0.15
		LL	27	7	1.46 (0.50-4.32)	0.49	70	49	1.86 (1.04-3.33)	0.04

Abbreviations: CAD, coronary artery disease; CI, confidence interval; HIF-1α, hypoxia inducible factor-1α; HO-1, heme oxygenase-1; M, mutation allele; MAPK1, mitogen activated protein kinase-1; OR, odds ratio; post-CAD, postmenopausal CAD; pre-CAD, premenopausal CAD; Ref, reference; SNP, single-nucleotide polymorphism.

TABLE 3 Association of haplotypes within *MAPK1*, *HIF-1α*, and *HO-1* with CAD risk in pre- and postmenopausal groups

Haplotype	Frequency		χ^2 Test	OR (95% CI)	*P* Value
	Case	Control			
C-G-A-T-C-A-S	20 (0.034)	21 (0.035)	0.03	0.95 (0.51-1.77)	0.87
C-G-A-T-C-A-L	23 (0.038)	18 (0.030)	0.63	1.29 (0.69-2.41)	0.43
C-G-A-T-C-T-L	18 (0.030)	21 (0.035)	0.24	0.85 (0.45-1.62)	0.63
	Pre-CAD	Post-CAD			
C-G-A-T-C-A-S	6 (0.033)	14 (0.033)	0.03	1.09 (0.41-2.88)	0.87
C-G-A-T-C-A-L	5 (0.030)	17 (0.040)	0.36	0.74 (0.27-2.03)	0.55

Abbreviations: CAD, coronary artery disease; CI, confidence interval; HIF-1α, hypoxia inducible factor-1α; HO-1, heme oxygenase-1; MAPK1, mitogen activated protein kinase-1; OR, odds ratio; post-CAD, postmenopausal CAD; pre-CAD, premenopausal CAD.

model (OR: 0.67, 95% CI: 0.58-0.78, $P < 0.05$). According to (GT)n repeats, another *HO-1* variant was divided as class S less repeats (≤25), M,[22–27] and class L more repeats (≥32). In our study, as the M allele was not found, the participants were grouped into SS, SL, and LL genotypes. It was shown that the L allele was correlated with greater risk of CAD (OR: 1.34, 95% CI: 1.15-1.55) and displayed close association with higher CAD risk among the postmenopausal patients than premenopausal ones (OR: 0.76, 95% CI: 0.59-0.98, $P < 0.05$).

3.2 | Association of SNPs within *MAPK1*, *HIF-1α*, and *HO-1* with CAG characteristics and Gensini score of CAD patients

After that, we further investigated whether the above significant variants of *MAPK1* (rs6928, rs9340, rs11913721), *HIF-1α* (rs10873142, rs2057482), and *HO-1* (rs2071746, (GT)n repeat) in pre- and postmenopausal CAD groups were significantly correlated with the amount of artery lesions and coronary artery positions (see Supporting Information, Table 3, in the online version of this article). Moreover, genotypes of *MAPK1* rs6928 (GG vs CG; $\chi^2 = 62.02$; $P < 0.01$) and *HIF-1α* rs2057482 (CC vs TC; $\chi^2 = 8.26$; $P = 0.04$) GG and CG presented distinct frequencies among subgroups of coronary artery positions when postmenopausal CAD patients were considered.

Similarly, the rs6928 (GG vs CC) displayed significantly different frequencies among CAD groups with single-, double- and triple-vessel lesions regardless of menopausal status (all $P < 0.05$; see Supporting Information, Table 4, in the online version of this article). Furthermore, rs9340 (GA vs GG), rs11913721 (CC vs AA), rs2057482 (TC vs TT), and (GT)n repeat (LL vs SS, SL vs SS) were also remarkably associated with different amounts of vessel lesions (all $P < 0.05$).

The results of χ^2 tests on *MAPK1* (rs6928, rs9340, rs11913721), *HIF-1α* (rs10873142, rs2057482), and *HO-1* (rs2071746) among type A, B, and C lesions revealed that mutations of rs9340 and rs2071746 could cause discrepant types of CAD lesions in the premenopausal CAD group, whereas mutations of rs6928, rs2071746, and (GT)n repeat exhibited a similar tendency within postmenopausal CAD women (see Supporting Information, Table 5, in the online version of this article).

Concerning the SYNTAX score (see Supporting Information, Table 6, in the online version of this article), the results of premenopausal CAD group indicated that mutations of rs10873142 (CT vs CC), rs2071746 (AT vs AA), and (GT)n repeats (LL vs SS, SL vs SS) were significantly correlated with the risk degree (all $P < 0.05$). Slightly different from the premenopausal women, the distributive frequencies of genotypes within rs6928 (CG vs CC, GG vs CC), rs9340 (AA vs GG), rs11913721 (AC vs AA, CC vs AA), and rs2071746 (AT vs AA, TT vs AA) were shown to be significantly distinct among the postmenopausal CAD group (all $P < 0.05$).

Additionally, the GG genotype of rs6928 was strongly associated with higher Gensini score (>34) in premenopausal CAD (OR: 0.35, 95% CI: 0.13-0.95, $P = 0.04$) and postmenopausal CAD patients (OR: 0.57, 95% CI: 0.33-1.00, $P = 0.05$) than the homozygote CC (Table 2). Also, the CC genotype of rs11913721 and rs2057482, as well as LL genotype of (GT)n repeats, were found to cause elevated risk of CAD among postmenopausal CAD patients (CC vs AA, OR: 2.65, 95% CI: 1.27-5.56, $P = 0.01$; CC vs TT, OR: 3.89, 95% CI: 1.37-11.05, $P = 0.01$; and LL vs SS, OR: 1.86, 95% CI: 1.04-3.33, $P = 0.04$).

3.3 | Association of haplotypes within *MAPK1*, *HIF-1α*, and *HO-1* with risk and prognosis of CAD

Then we investigated the role of haplotypes within *MAPK1* (rs6928, rs9340, rs11913721), *HIF-1α* (rs10873142, rs2057482), and *HO-1* (rs2071746, [GT]n repeat) in formation of coronary lesions and prognosis of CAD among pre- and postmenopausal women; however, no significant difference was found (Tables 3 and 4).

3.4 | Polymorphisms affecting aspirin resistance in CAD patients

Furthermore, we investigated the association of *MAPK1*, *HIF-1α*, and *HO-1* polymorphisms with aspirin resistance in CAD patients

TABLE 4 Association of haplotypes within *MAPK1*, *HIF-1α*, and *HO-1* with Gensini scores

Group	Haplotype	Frequency		χ^2 Test	OR (95% CI)	*P* Value
		≤34	>34			
Pre-CAD	C-G-A-T-C-A-S	4 (0.03)	2 (0.04)	0.30	0.62 (0.11-3.51)	0.58
	C-G-A-T-C-A-L	4 (0.03)	1 (0.03)	0.04	1.27 (0.14-11.69)	0.83
Post-CAD	C-G-A-T-C-A-L	9 (0.04)	9 (8.67)	0.00	1.00 (0.39-2.57)	0.87
	G-G-A-T-C-A-S	7 (0.03)	11 (11.05)	0.93	0.62 (0.24-1.64)	0.34
	G-G-A-T-C-A-L	10 (0.04)	12 (0.05)	0.19	0.83 (0.35-1.95)	0.66
	G-G-A-T-C-T-L	8 (0.04)	9 (0.04)	0.06	0.88 (0.34-2.34)	0.81

Abbreviations: CAD, coronary artery disease; CI, confidence interval; HIF-1α, hypoxia inducible factor-1α; HO-1, heme oxygenase-1; MAPK1, mitogen activated protein kinase-1; OR, odds ratio; post-CAD, postmenopausal CAD; pre-CAD, premenopausal CAD.

TABLE 5 Association of SNPs within *MAPK1*, *HIF-1α*, and *HO-1* with treatment efficacy of diverse treatments

			Pre-CAD Group (n = 167)				Post-CAD Group (n = 422)			
Gene	SNP	Genotype	Normal	AR	OR (95% CI)	*P* Value	Normal	AR	OR (95% CI)	*P* Value
MAPK1	rs6928	CC	40	6	Ref	Ref	79	8	Ref	Ref
		CG	75	9	1.25 (0.42-3.76)	0.69	186	27	0.7 (0.30-1.60)	0.39
		GG	31	6	0.78 (0.23-2.64)	0.68	103	19	0.55 (0.23-1.32)	0.18
	rs9340	GG	56	8	Ref	Ref	146	21	Ref	Ref
		GA	63	11	0.82 (0.31-2.18)	0.69	184	24	1.10 (0.59-2.06)	0.76
		AA	27	2	1.93 (0.38-9.71)	0.42	38	9	0.61 (0.26-1.43)	0.25
	rs11913721	AA	52	9	Ref	Ref	149	22	Ref	Ref
		AC	67	7	1.66 (0.58-4.74)	0.34	184	27	1.01 (0.55-1.84)	0.98
		CC	27	5	0.93 (0.28-3.07)	0.91	35	5	1.03 (0.37-2.92)	0.95
HIF-1α	rs10873142	CC	20	3	Ref	Ref	17	5	Ref	Ref
		CT	56	10	0.84 (0.21-3.36)	0.81	123	20	1.81 (0.60-5.45)	0.29
		TT	70	8	1.31 (0.32-5.41)	0.71	228	29	2.31 (0.79-6.74)	0.12
	rs2057482	TT	1	0	Ref	Ref	10	9	Ref	Ref
		TC	20	7	—	0.56	56	10	5.04 (1.64-15.51)	<0.01
		CC	125	14	—	0.74	302	35	7.77 (2.96-20.41)	<0.01
HO-1	rs2071746	AA	42	10	Ref	Ref	110	21	Ref	Ref
		AT	76	6	3.02 (1.02-8.88)	0.04	179	27	1.27 (0.68-2.35)	0.45
		TT	28	5	1.33 (0.41-4.32)	0.63	80	5	3.05 (1.10-8.45)	0.03
	(GT)n repeat	SS	33	7	Ref	Ref	66	10	Ref	Ref
		SL	81	12	1.43 (0.52-3.96)	0.49	198	29	1.03 (0.48-2.24)	0.93
		LL	27	7	0.82 (0.26-2.62)	0.74	104	15	1.05 (0.45-2.48)	0.91

Abbreviations: CAD, coronary artery disease; CI, confidence interval; HIF-1α, hypoxia inducible factor-1α; HO-1, heme oxygenase-1; MAPK1, mitogen activated protein kinase-1; OR, odds ratio; post-CAD, postmenopausal CAD; pre-CAD, premenopausal CAD; Ref, reference; SNP, single-nucleotide polymorphism.

(Table 5). The postmenopausal CAD patients who carried C allele of rs2057482 had significantly decreased aspirin-resistance risk when compared with allele T (CC vs TT, OR: 7.77, 95% CI: 2.96-20.41, $P < 0.01$; TC vs TT, OR: 5.04, 95% CI: 1.64-15.51, $P < 0.01$). The subjects with rs2071746 AT in the premenopausal CAD group and those with rs2071746 TT in the postmenopausal CAD group also tended to have lower aspirin resistance (AT vs AA, OR: 3.02, 95% CI: 1.02-8.88, $P = 0.04$; TT vs AA, OR: 3.05, 95% CI: 1.10-8.45, $P = 0.03$).

4 | DISCUSSION

It was widely investigated that the role of SNPs within MAPK1/HIF-1/HO-1 pathway in development and prognosis of CAD might be significant within diverse populations,[6,28] yet finite sample size and incomplete experimental design limited the credibility of the aforementioned study results to be applied to specific Chinese Han populations. Besides, postmenopausal women were 4-fold more likely to develop CAD than premenopausal ones, emphasizing that E2 functioned to protect the cardiovascular system.[29] And among premenopausal women age <60 years, estrogen-replacement therapy could decrease risk of CAD by 32%.[30] Also, because the MAPK1/HIF-1/HO-1 pathway was correlated with production of E2 within the human body,[22,31] we hoped to compare the associations of SNPs within *MAPK1/HIF-1/HO-1* with CAD risk among premenopausal and postmenopausal women.

Regarding *MAPK1*, although rs6928 and rs9340 displayed strong associations with susceptibility to premenopausal CAD in this Chinese population, a documentary based on the European population indicated that the 2 SNPs could predict susceptibility to CAD merely in the explorative investigation, rather than the replicative one.[6] It was hypothesized that rs6928 and rs9340 were probably closely linked with onset of CAD, for that the average age of CAD patients in the explorative study was about 9 years older than the average age of patients included in the replicative study. Comparing the mean age among pre- and postmenopausal populations, it was found that the mean age of premenopausal patients was similar to that of the explorative study, which might explain the significant correlation between SNPs and susceptibility to CAD. Interestingly, even though subjects in the replicative study and those in the postmenopausal group were approximate in their mean age, their associations with CAD risk still displayed dissimilar consequences. The role of E2 might facilitate rs6928 and rs9340 mutants to be outstanding among risk factors for CAD. Moreover, the discrepant ethnicities investigated could partly contribute to differences in genetic frequency, and thereby associations with CAD risk. Furthermore, the relatively small sample size might render statistic power to be less persuasive, and the means for genetic detection differed between the 2 studies. Hence, additional case–control studies and meta-analyses are needed to confirm whether rs6928 and rs9340 were correlative polymorphisms for CAD development.

In addition, mutant alleles of *HIF-1* rs2057482 manifested lower ability to transcript than wild-type ones,[11] implying the significance of rs2057482 in maintaining transcriptional stability of *HIF-1α* mRNA. In fact, rs2057482 was situated within the 3′-untranslated region (3′UTR) of splicing variations, which might readily affect the downstream region rich in adenylate-uridylate.[23] The characteristic interaction might help to account for effects of rs2057482 on clinical manifestations of CAD patients. Furthermore, as *HIF-1α* served as a stimulus for evolution of coronary artery collateral vessels,[11,24] it was well explainable that the frequency of rs2057482 among groups of patients with single-, double-, and triple-vessel lesions displayed significant difference, irrespective of patients' menopausal condition. Consistent with previous results, rs10873142 that was located 143 bp distant from 5′ end, was also closely linked with cardiac disorders,[11] whereas rs11549465 and rs11549467 showed scarcely any sign in their remarkable correlation with CAD risk.[10,25]

Because HO-1 functioned as a catalyzer in converting heme to elements featured by antagonism of proliferation, oxidation, and inflammation (eg, ferrous iron, carbon monoxide, and biliverdin),[26] induction of *HO-1* has been deemed as a tenable therapy for cardiovascular disorders.[27] A mouse model also suggested that elevated *HO-1* expressions could, to a great extent, decrease the prevalence of atherosclerotic lesions.[32] The length polymorphism of (GT)n repeats, which was mapped in the promoter of *HO-1*, were verified to modulate HO-1 expressions during exposure to oxidative stress.[33] With failure to find possible transcription factors that bound to (GT)n repeats, the regulatory role of (GT)n repeats under exposure of high oxidative stress was principally postulated as modulation of *HO-1* promoter structures, thereby activating such critically responsive elements as TATA boxes and activator protein-1 binding sites.[34] Owing to the above pathology, (GT)n repeats have received wide recognition in either severity and prognosis of either premenopausal or postmenopausal CAD patients, though inconsistent results were also drawn.[22,35]

4.1 | Study limitations

This investigation was limited by studying only the apparent association of SNPs within the MAPK1/HIF-1/HO-1 pathway with development or prognosis of CAD, yet molecular experiments and establishment of animal models are necessary to explore the intrinsic mechanisms. Moreover, only 1 pathway that regulated occurrence of CAD was studied here. Virtually, multiple pathways relevant to lipid metabolism,[36] stress response,[37] or inflammation[38] were probable parameters affecting CAD risk. For instance, it was documented that the aberrant running of the interleukin-6/Janus kinase/signal transducers and activators of the transcription pathway might induce ischemia and even cardiomyopathy.[39,40] The sample size and ethnicity investigated also limited generalization of study results to a wider range of the population. Hence, more studies with large-scale sample sizes are needed to remedy the above shortcomings.

5 | CONCLUSION

Certain key SNPs located within *MAPK1* (eg, rs6928 and rs9340), *HIF-1* (eg, rs2057482 and rs10873142), and *HO-1* (eg, [GT]n repeats) could more or less predict the occurrence, prognosis, and even treatment efficacy of premenopausal and postmenopausal CAD, providing evidence that novel target therapies might be developed particularly for CAD in premenopausal and postmenopausal women.

Conflicts of interest

The authors declare no potential conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Guo N, Zhang N, Yan L, Cao X, Wang J, Wang Y. Correlation between genetic polymorphisms within the MAPK1/HIF-1/HO-1 signaling pathway and risk or prognosis of perimenopausal coronary artery disease. *Clin Cardiol*. 2017;40:597–604. https://doi.org/10.1002/clc.22708