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MIF Gene Polymorphism rs755622 Is Associated With Coronary Artery Disease and Severity of Coronary Lesions in a Chinese Kazakh Population

A Case–Control Study

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Abstract: Inflammation plays an important role in the pathogenesis of atherosclerosis. Recent studies indicate that macrophage migration inhibitory factor (MIF) is a potent proinflammatory cytokine which mediates the inflammatory process during atherosclerosis.

The polymorphism of *MIF* gene (rs755622 [−173G/C], rs1007888, and rs2096525) were genotyped by TaqMan single nucleotide polymorphism (SNP) genotyping assay in 320 patients with coronary artery disease (CAD) and 603 controls in a Chinese Kazakh population. Coronary angiography was performed on all CAD patients and Gensini score was used to assess the severity of coronary artery lesions.

The frequency of the CC genotype and C allele of rs755622 were significantly higher in CAD patients than that in control subjects (8.4% vs. 5.1%, $P < 0.001$, 30.3% vs. 22.1%, $P < 0.001$, respectively). Multivariate logistic regression analysis showed that individuals with CC genotype or C allele had a higher risk for CAD (CC genotype vs. GG genotype, OR = 2.224, 95% CI, 1.239–3.992, $P = 0.007$, and C allele vs. G allele, OR = 1.473, 95% CI, 1.156–1.876, $P = 0.002$, respectively). Moreover, CAD patients with rs755622 C allele (CC + CG genotype) have higher levels of Gensini score when compared to C allele noncarriers (32.74 ± 26.66 vs. 21.44 ± 19.40, $P < 0.001$, adjusted).

Our results suggested that the CC genotype and C allele of *MIF* rs755622 SNP may be a genetic marker for the risk of CAD and potentially predict the severity of CAD in Chinese Kazakh population.

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Abbreviations: ACS = acute coronary syndrome, AMI = acute myocardium infarction, ApoE$^{-/-}$ = apolipoprotein E-deficient, BMI = body mass index, CAD = coronary artery disease, CI = confidence interval, CK-MB = creatinine kinase-MB, CRS = cardiovascular risk survey, DBP = diastolic blood pressure, HDL-C = high density lipoprotein-cholesterol, HWE = Hardy–Weinberg equilibrium, IL-6 = interleukin 6, LAD = left anterior descending, LDL-C = low density lipoprotein-cholesterol, MAF = minor allele frequency, MIF = migration inhibitory factor, MMP = matrix metalloproteinase, OR = odds ratio, PCR = polymerase chain reaction, SBP = systolic blood pressure, SD = standard deviation, SNP = single nucleotide polymorphism, STEMI = ST-segment elevation myocardial infarction, TC = total cholesterol, TG = triglyceride, TNF-α = tumor necrosis factor-α.

INTRODUCTION

Formation and development of atherosclerosis in coronary arteries are critical processes in the pathogenesis of coronary artery disease (CAD).[1] It has been known that inflammation plays an important role in the pathogenesis of atherosclerosis.[2] Recruitment of inflammatory cells including monocytes and T-lymphocyte into the vascular injury site, that mediated by cytokines and chemokines is an initial phase of atherosclerosis.[3] Macrophage migration inhibitory factor (MIF), a potent proinflammatory cytokine, is involved in this process.[4] Available evidence shows that MIF has been implicated in early atheromatous plaque formation and progression of atherosclerotic lesions.[5] Pharmacological inhibition of MIF or anti-MIF antibody treatment in apolipoprotein E-deficient (ApoE$^{-/-}$) mice markedly reduces intimal Mac-1-positive macrophages accumulation,[6,7] and MIF blockage in ApoE$^{-/-}$ mice leads to a shift in the cellular composition of neointimal plaques toward a stabilized phenotype with reduced macrophage/foam cell content and increased smooth muscle cells content.[6] Thus, elevation of MIF levels may contribute to CAD development.[4] Muller et al[5] reported that higher plasma levels of MIF were found in patients with acute coronary syndrome (ACS) compared to stable angina and control subjects. An association between the higher plasma levels of MIF and worse long-term outcome in patients with stable CAD and type 2 diabetes

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mellitus was reported in a prospective case–cohort study in Japanese.[8] More recently, results from animal and human studies also suggested that MIF may be a biomarker in acute myocardium infarction (AMI).[9] Chan et al[10] find that plasma MIF levels were elevated in a high proportion of ST-segment elevation myocardial infarction (STEMI) patients at the first obtainable sample and these levels were predictive of final infarct size and the extent of cardiac remodeling. Therefore, MIF may be a novel biomarker for the risk of CAD/ACS. Except inflammatory status, genetic factors also influence circulating MIF level.[11–13] It was reported that the *MIF* gene polymorphism rs755622 (−173G/C) located in the promoter was related to plasma MIF levels in Adult-onset Still disease and the variant may contribute to the disease susceptibility.[11] In vitro and in vivo studies also suggested that rs755622 was associated with increased gene expression and protein levels of MIF.[12] MONICA/KORA Augsburg study showed that GG genotype of rs1007888 in *MIF* gene was associated with the high level of MIF and could be thought of as susceptibility factor for type 2 diabetes Mellitus in German women.[13] The MOCICA/KORA study and Shan et al reported that the polymorphism of *MIF* gene was associated with CAD in German and Chinese Han population.[14,15] Studies have showed that mutation of *MIF* gene were also associated with rheumatoid arthritis,[16] Behcet disease,[17] Vogt-Koyanagi-Harada,[18] and inflammatory bowel disease.[19]

There is more than 1.35 million Kazakh population, ranking third, less than Uygur (8.8 million) and Han (8.75 million) in Xinjiang, northwestern part of China.[20] Previous studies have reported that there was a paradoxical relationship in the Kazakh population with a lower prevalence of CAD despite having more risk factors of CAD such as obesity, high-salty and high-fat diet and high prevalence of hypertension compared to other ethnicities.[21,22] Considering the different genetic background and the role of MIF regulating atherosclerotic inflammation, we hypothesize that *MIF* gene is an important factor for CAD. In this study, we investigate whether the variants in *MIF* gene are associated with susceptibility of CAD in a Chinese Kazakh population. We also explore the association between the polymorphisms of *MIF* gene and the severity of coronary artery lesions in CAD patients.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University. It was conducted according to the standards of the Declaration of Helsinki. Written informed consent was obtained from all of the participants.

Study Population

All of the participations were selected from Kazakh population who live in Xinjiang, northwestern part of China, including 320 CAD patients and 603 controls. All CAD patients were recruited at the First Affiliated Hospital of Xinjiang Medical University from 2008 to 2013. The patients with typically chest pain, electrocardiographic changes (new pathologic Q waves, at least 1 mm ST elevation in any 2 or more contiguous limb leads or a new left bundle branch block, or new persistent ST-T wave changes diagnostic of a non-Q wave MI) and serum creatinine kinase-MB isoenzyme (CK-MB) elevations (more than 3-fold higher than the upper reference limit) were examined by coronary angiography according to the guidelines.[10,23,24] CAD was defined as the presence of at least one significant coronary artery stenosis with more than 50% reduction luminal diameter based on coronary angiography. Exclusion criteria were those with concomitant valvular heart disease, congenital heart disease, and/or nonischemic cardiomyopathy. We randomly selected 603 age and sex matched participants as the control group. All control subjects were from the cardiovascular risk survey (CRS) study. The design of CRS study has been previously reported.[25,26] In brief, it was a cross-sectional study to investigate risk factors for cardiovascular diseases in the multiethnic population in Xinjiang, northwestern part of China, conducted during October 2007 and March 2010. This study consisted of interviews, physical examinations, and data from blood sample analyses. Individuals were excluded from this study if they had: a history of CAD; electrocardiographic signs of CAD; regional wall motion abnormalities; relevant valvular abnormalities in echocardiograms and/or carotid atherogenesis.

Definition of Cardiovascular Risk Factors

Hypertension was defined as history of hypertension and/or an average systolic blood pressure (SBP) $\geq$140 mm Hg and/or an average diastolic blood pressure (DBP) $\geq$90 mm Hg on at least 2 separate occasions according to the medical examination and history. Diabetes was defined as history or presence of diabetes and/or a fasting plasma glucose level $>$7.0 mmol/L (126 mg/dl) on 2 separate occasions, or a random glucose value of $>$11.1 mmol/L (200 mg/dl) on $\geq$1 occasion. Body mass index (BMI) was calculated from standardized measurements of height and weight. Individuals reporting regular tobacco use in the previous 6 months were considered as current smokers.

Routine Blood Test

Fasting peripheral blood samples were obtained from all participants for the assessment of routine biochemical variables. Total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), and triglyceride (TG) were measured by standard enzymatic methods using Dimension AR/AVL Clinical Chemistry System (DADE Bchring, Newark, NJ) in the Central Laboratory of the First Affiliated Hospital of Xinjiang Medical University.

Coronary Angiography

Coronary angiography was performed in all CAD patients. Gensini score: angiographic stenosis of a culprit artery in the range of 0% to 25% was scored as 1 point, stenosis in the range of 25% to 50% was scored as 2 points, 50% to 75% was scored as 4 points, 75% to 90% was scored as 8 points, 90% to 99% was scored as 16 points, and total occlusion was scored as 32 points. A multiplier was assigned to each main vascular segment based on the functional significance of the myocardial area supplied by that segment: 5 for the left main coronary artery, 2.5 for the proximal segment of the left anterior descending (LAD) coronary artery and the proximal segment of the circumflex artery, 1.5 for the mid-segment of the LAD, 1.0 for the right coronary artery, the distal segment of the LAD, mid-distal region of the circumflex artery, the posterolateral artery, and the obtuse marginal artery, and 0.5 for other segments.

Angiographic evaluations were reviewed by 2 independent interventional cardiologists blinded to the study information. In case of disagreement, the decision was based on the judgment of the third, more experienced cardiologist.

Polymorphism Selection

We used the minor allele frequency (MAF) $\geq$0.1 and linkage disequilibrium patterns with $r^2 \geq 0.5$ as a cut off by

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the Haploview 4.2 software to select the Tag single nucleotide polymorphism (TagSNPs) based on the Hapmap human SNP database (www.hapmap.org). Finally, we selected three SNPs (rs7556222, rs1007888, rs2096525) located in different areas of *MIF* gene. The rs755622 was located in the promoter region, rs1007888 was located in the translation termination codon and rs2096525 was located in the first intron of *MIF* gene.

Genotyping of MIF Gene

Blood samples were collected from all participants. Genomic DNA was extracted from the peripheral leukocytes using standard phenol-chloroform method and stored at −80°C for future analysis. We used the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA) to genotype the polymorphism of *MIF* gene. The primers and probes used in assay were chosen according to the information at the ABI website (http://myscience.appliedbiosystems.com). Applied Biosystems 7900HT Standard Real-Time Polymerase Chain Reaction (PCR) System was used for the DNA amplification. The results of each polymorphism of *MIF* gene were read by the Sequence Detection Systems automation controller software v2.3 (ABI). The reaction system of PCR amplification was as follows: 3 μL of TaqMan Universal Master Mix, 0.12 μL probes and 1.88 ddH_2O in a 6 μL final reaction volume containing 1 μL DNA (50 ng). Amplification cycling conditions were as follows: 95°C for 5 minutes; 40 cycles of 95°C for 15 seconds; and 60°C for 1 minute. Samples with ambiguous genotypes that were not separated by discrete clusters were regenotyped.

Statistical Analysis

Analyses were carried out using SPSS version 17.0 (SPSS, Inc., Chicago, IL). Continuous variables were expressed as mean ± standard deviation (SD) and the difference between CAD and control groups was detected by Student *t* test. Categorical variables were presented as proportions and the difference between two groups was detected by Chi-square test. Chi-square test was also used to calculate Hardy–Weinberg equilibrium (HWE) of the frequencies of genotype between CAD and control subjects. The association between *MIF* gene polymorphisms and CAD was evaluated by multiple logistic regression analysis. All odds ratios (ORs) were adjusted for age, gender, diabetes, hypertension, blood glucose, BMI, smoking and plasma lipid (HDL-C, LDL-C, TG, and TC). For each OR, the 95% confidence interval (CI) was calculated. Multiple linear regression analysis was performed to assess whether the *MIF* genetic variants were the independent explanatory factors for angiographic severity of coronary artery lesions which was assessed by Gensini scores in CAD patients. The power of this study was calculated by Power and Sample Size Program (Version 3.0.43).[27] Statistical significance was set at $P < 0.05$ (2-tailed).

RESULTS

Clinical Characteristics of Participants

A total of 320 CAD patients (mean age 57.48 ± 8.46 and 54.4% men) and 603 controls (mean age 56.62 ± 9.51 and 48.8% men) were recruited in the present study. Clinical characteristics of all participants at baseline are summarized in Table 1. We found that the CAD patients had lower HDL-C and higher glucose, LDL-C, TG, and higher prevalence of hypertension and diabetes compared with controls (all $P < 0.05$). There were no differences between the groups with respect to age, gender, BMI, smoking, and TC.

TABLE 1. General Characteristics of Study Population Including Controls and CAD Patients

	Control (n = 603)	CAD (n = 320)	*P*-Value
Age (years)	56.62 ± 9.51	57.48 ± 8.46	0.158
Male gender, n (%)	294 (48.8)	174 (54.4)	0.112
BMI (kg/m^2)	24.34 ± 3.30	24.74 ± 3.44	0.088
Smoking, n (%)	152 (25.2)	89 (27.8)	0.388
Hypertension, n (%)	235 (39.0)	154 (48.1)	0.008
Diabetes, n (%)	76 (12.6)	68 (21.3)	0.001
Glucose (mmol/L)	5.20 ± 1.72	5.64 ± 1.94	0.001
TG (mmol/L)	1.40 ± 0.95	1.70 ± 0.91-	<0.001
TC (mmol/L)	4.25 ± 1.67	4.35 ± 1.23	0.207
HDL-C (mmol/L)	1.23 ± 0.40	1.00 ± 0.35	<0.001
LDL-C (mmol/L)	2.14 ± 0.79	2.26 ± 0.90	0.031

Continuous variables are expressed as mean ± SD. Categorical variables are expressed as percentages.

The *P*-value of the continuous variables was calculated by the unpaired *t* test. The *P*-value of the categorical variables was calculated by χ^2 test.

BMI = body mass index, CAD = coronary artery disease, HDL-C = high-density lipoprotein-cholesterol, LDL-C = low density lipoprotein-cholesterol, TC = total cholesterol, TG = triglyceride.

Polymorphism of *MIF* Genes

Three SNPs of *MIF* [rs755622 (−173G/C), rs1007888, and rs2096525] were genotyped in 320 CAD patients and 603 controls. The genotypes and alleles frequencies of the 3 SNPs examined in CAD patients and controls are summarized in Table 2. The MAF of rs755622, rs1007888, and rs2096525 were 30.3%, 50.8%, and 19.8%, respectively. All the genotypes frequencies were in HWE ($P > 0.05$). There was no significant difference in the distribution of genotypes or alleles rates in rs1007888 and rs2096525 between CAD and control groups ($P > 0.05$). However, significant differences of genotypic and allelic distribution were found in rs755622. The frequency of the CC genotype was higher in CAD patients than that in control subjects (8.4% vs. 5.1%, $P < 0.001$). Moreover, the frequency of the C allele was higher in the CAD than that in control group (30.3% vs. 22.1%, $P < 0.001$, Table 2).

Association Between *MIF* Gene Polymorphism and Risk and Severity of CAD

Single factor logistic regression analysis showed that the CC genotype and C allele in rs755622 were the risk factors for CAD (CC genotype vs. GG genotype, OR = 2.089, 95% CI, 1.206–3.619, $P = 0.008$, and C allele vs. G allele, OR = 1.53, 95% CI, 1.232–1.900, $P < 0.001$, respectively). Corresponding to these findings, multivariate logistic regression analysis was used to detect the association between rs755622 polymorphisms and susceptibility of CAD. After adjusting the confounding factors such as hypertension, diabetes, and HDL-C, TG, the CC genotype and C allele were remained the risk factors for CAD (CC genotype vs. GG genotype, OR = 2.224, 95% CI, 1.239–3.992, $P = 0.007$, and C allele vs. G allele, OR = 1.473, 95% CI, 1.156–1.876, $P = 0.002$, respectively, Table 3). Furthermore, the association between the 3 polymorphisms and the severity of coronary artery lesions was analyzed among CAD patients. Patients with rs755622 GG genotype had significantly lower

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TABLE 2. Association Analyses Between Genotypes and Allele of *MIF* Gene Polymorphisms in Control Subjects and Patients With Coronary Artery Disease

Polymorphisms	Controls, n (%)	CAD, n (%)	*P*-Value*	*P*-Value†
rs755622				
GG	367 (60.9)	153 (47.8)		
CG	205 (34.0)	140 (43.8)		
CC	31 (5.1)	27 (8.4)	0.733	<0.001
G allele	939 (77.9)	446 (69.7)		
C allele	267 (22.1)	194 (30.3)		<0.001
rs1007888				
AA	147 (24.4)	79 (24.7)		
AG	317 (52.6)	157 (49.1)		
GG	139 (23.1)	84 (26.3)	0.205	0.498
A allele	611 (50.7)	315 (49.2)		
G allele	595 (49.3)	325 (50.8)		0.555
rs2096525				
TT	418 (69.3)	204 (63.7)		
CT	169 (28.0)	105 (32.8)		
CC	16 (2.7)	11 (3.4)	0.826	0.221
T allele	1005 (83.3)	513 (80.2)		
C allele	201 (16.7)	127 (19.8)		0.089

CAD = coronary artery disease.
*P values for Hardy–Weinberg equilibrium in CAD patients and controls.
†P values for distribution frequency for genotype and allele of the 3 SNPs in *MIF* gene.

level of Gensini score (21.44 ± 19.40) than those with CG genotype (31.96 ± 26.86, $P < 0.001$, adjusted) or CC genotype (36.78 ± 25.68, $P < 0.001$, adjusted). In addition, CAD patients with rs755622 C allele carriers (CC + CG genotype) have high levels of Gensini score when compared to C allele noncarriers (32.74 ± 26.66 vs. 21.44 ± 19.40, $P < 0.001$, adjusted, Figure 1). However, these differences were not observed in the other 2 SNPs.

Power of Study

Our data indicated that the probability of exposure of CC genotype among controls was 0.051 and OR in CAD patients relative to control subjects was 2.224. The Power and Sample Size Program showed that the power of this study was 0.861.

TABLE 3. Multiple Logistic Regression Analysis for CAD Patients and Control Subjects

Risk Factors	OR	95% CI	*P*-Value
rs755622 (CC vs. GG)	2.224	1.239–3.992	0.007
C allele vs. G allele	1.473	1.156–1.876	0.002
Hypertension	1.778	1.503–2.104	<0.001
Diabetes	1.696	1.383–2.081	<0.001
TG	1.013	0.849–1.147	0.859
HDL-C	0.614	0.409–0.922	0.019

CI = confidence interval, HDL-C = high density lipoprotein-cholesterol, OR = odds ratio, TG = triglyceride.

DISCUSSION

The pathogenesis of CAD which cannot be explained solely by traditional risk factors and it has been the subject of intense investigation. Variations in several genes involved in inflammation [NFKB1, interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), and C-reactive protein] have been reported to be associated with susceptibility to CAD.[28–31] In the present study, we found a significant association between the polymorphism of rs755622 of *MIF* gene and CAD in a Chinese Kazakh population.

It is generally believed that MIF was a pleiotropic cytokine to regulate the activation of macrophage and lymphocyte in the inflammatory response. Studies have shown MIF was a key modulator of many chronic inflammatory diseases, such as rheumatoid arthritis,[32] inflammatory bowel disease,[33] and glomerulonephritis.[34] An important role of MIF on atherosclerosis has also been reported, including promoting migration and recruitment of monocytes and T-lymphocyte into atherosclerotic lesions,[35] enhancing secretion of inflammatory mediators such as TNF-α and nitric oxide (NO), and expression of matrix metalloproteinases (MMP-1, -9, and -12).[36]

In the case–control study presented herein, we firstly investigated the association between the variants of *MIF* gene (rs755622, rs1007888, and rs2096525) and CAD in a Chinese Kazakh population. The rs755622 C allele frequency of Kazakh CAD patients in our study was 30.3% which was much higher than that in reported Chinese Han CAD patients (8%). We also found that the rs755622 C allele frequency was higher in Kazakh control population (22.1%) than that in Caucasian (13.5% in

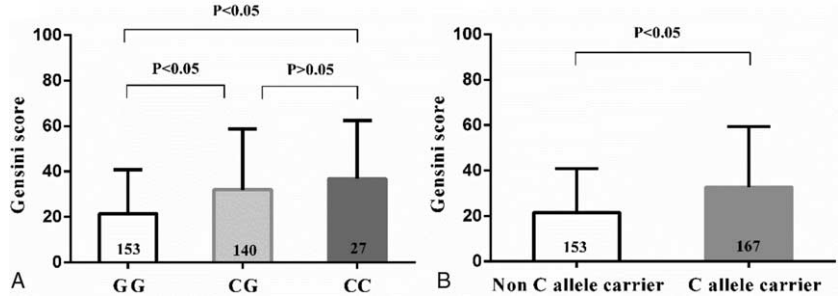

FIGURE 1. Influence of the *MIF* gene polymorphisms on Gensini score in the CAD patients. (A) Comparison among different genotypes of MIF gene. (B) Comparison between patients with C allele (carrier) and without C allele (noncarrier). The number of each group is given in the corresponding bar. *P*-values are adjusted by age, gender, diabetes, hypertension, glucose, BMI, smoking, and plasma lipid parameters.

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Czech, 5.5% in Russian, and 20% in Netherlander),[37,38] but was lower than Chinese Han control population (25.0%).[39] In our study, we found that participants with rs755622 CC genotype or C allele were more susceptible to CAD than those with GG genotype or G allele after adjustments for potential confounding factors, which was consistent with previous reports. The MONICA/KORA Augsburg study reported that *MIF* rs755622 C allele were associated with increased risk of CAD in German women after mean follow-up time of 10.3 years.[14] A number of studies also found that rs755622 C allele was a risk factor for CAD in Chinese Han population.[15,40] However, the association was not evident in Russian or Czech patients with myocardial infarction.[37] Except rs755622 G/C or C allele, Valdes-Alvarado reported that the variant in the promoter of *MIF* gene at the position of −794 (CATT) 5 to 8 was also susceptible to ACS in Mexican population.[41] In addition, the rs2070766 G allele (located in the intron region of *MIF* gene) has been found with increased risk for CAD in German women.[14] However, we did not find any difference between CAD patients and controls in variation of rs1007888 and rs2096525 which located outside of *MIF* gene promoter. This information seems to indicate that variation of *MIF* gene in the promoter more likely has a functional influence.

Gensini score is a useful angiographic scoring system which used to cumulatively quantify the extent, severity, and complexity of CAD.[42] CAD patients with high level of Gensini score are more susceptible to cardiovascular events.[43] We found that Gensini scores were significantly higher in CAD patients who with rs755622 CC genotype compared to those with GG or CG genotype. The underlying mechanisms may be related with the increased inflammatory reaction. Previous studies showed that there was a positive correlation between the Gensini score and inflammatory cytokines such as IL-6 and TNF-α[44] which both can be induced by MIF.[35] Yang et al[45] reported that MIF plasma levels may be used to predict the severity of coronary lesion. Meanwhile, *MIF* rs755622 G/C polymorphism can influence the plasma concentration of MIF and TNF-α in some inflammatory disorders.[38,46] However, precise regulatory mechanisms of this association remain unclear. Taken together, results from us and others suggest that the *MIF* rs755622 G/C polymorphism may play a critical role in the etiology of coronary artery lesions and may have a predictive value for the severity of coronary artery lesions.

Some limitations in this study should be mentioned. Firstly, this study was only focused on Chinese Kazakh patients in Xinjiang, northwestern part of China. Our findings still need to be verified in other ethnicities and in a larger population. Secondly, plasma levels of MIF and other inflammatory cytokines were not measured in this study. Therefore, we cannot exactly pinpoint the potential mechanism and functional significance of *MIF* gene polymorphism on the risk of CAD. Obtaining this information would facilitate identification and development of new therapeutic targets.

In summary, we investigated the association between *MIF* −rs755622 G/C polymorphism and CAD in a Chinese Kazakh population. Our results suggest that the CC genotype and C allele of *MIF* rs755622 SNP link to the susceptibility of CAD, and *MIF* rs755622 C allele can be potentially used as a gene marker to predict severity of coronary artery lesions. Analysis of certain *MIF* gene polymorphism may help to identify individuals with potential CAD risk, and identification of targeted *MIF* gene variation in patients with CAD may also benefit in risk stratification and management.

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