ICAM-1 and MKL-1 polymorphisms impose considerable impacts on coronary heart disease occurrence

Cungang Wu | Chao Huang

1Department of Ultrasonography, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou, China
2Department of Stomatology, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou, China

Correspondence
Chao Huang, Department of Stomatology, The First Affiliated Hospital of Jinzhou Medical University, Jinzhou, Liaoning 121001, China.
Email: boxcnw@163.com

Abstract
This study was aimed to explore the correlation of intercellular adhesion molecule-1 (ICAM-1) K469E and megakaryoblastic leukaemia factor-1 (MKL-1) −184C/T polymorphisms with the susceptibility to coronary heart disease (CHD) in the Chinese Han population. 100 CHD patients and 91 healthy people that had no blood connection with each other were enrolled in this case-control study. ICAM-1 and MKL-1 polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach. Multiple logistic regression was used to analyse the correlation between polymorphisms of ICAM-1 and MKL-1 and CHD susceptibility. Differences of genotype and allele frequencies of the two SNPs between case and control groups were analysed by chi-square test. Odds ratios (ORs) and 95% confidence intervals (CIs) were indicated relative susceptibility of CHD. The distributions of ICAM-1 and MKL-1 polymorphisms in each group conformed to Hardy-Weinberg equilibrium (HWE). After adjusting for traditional risk factors, the TT genotype frequency of MKL-1 −184C/T polymorphism was found significantly higher in case group than in control group (P < .05). Meanwhile, T allele frequency increased in case group compared with control group, and the differences had statistical significance (P = .04, OR = 2.34, 95% CI = 1.34-5.26). Logistic regression analysis in this study proved that smoking, hypertension, diabetes and triglyceride (TG) were all risk factors for CHD. ICAM-1 K469E polymorphism has no association with the onset of CHD. But MKL-1 −184C/T polymorphism is associated with the risk of CHD and T allele might be a susceptibility factor for CHD.

KEYWORDS
coronary heart disease, ICAM-1, MKL-1, polymorphism

1 | INTRODUCTION

Coronary heart disease (CHD) is due to myocardial ischaemia, hypoxia or necrosis, which is caused by coronary morphological changes of stenosis or obstruction, or coronary functional change of vascular spasm based on atherosclerosis.1-3 CHD is the most common and most frequent cardiovascular disease (CVD).4 At present, it has become one of the main diseases threatening the health of the people around the world, and its morbidity and mortality are increasing in China in recent decades.5,6

Coronary heart disease is a complex disease. Its specific pathogenesis is not yet fully understood. But most scholars believe that
the onset of the disease is closely related to environmental and genetic factors. Studies have shown that intercellular adhesion molecule-1 (ICAM-1), as an important inflammatory mediator and credible inflammatory marker, is a momentous factor for inducing local inflammation and thrombosis and plays a vital role in the pathological process of ischaemia cardio-cerebrovascular diseases. The increased ICAM-1 levels are proved to be relevant to the risk of CHD. In addition, megakaryoblastic leukaemia factor-1 (MKL-1), also known as myocardin-related transcription factor-A (MRTF-A), is a member of the family of myocardin-related transcription factors (MRTFs). The literature has reported that MKL-1 might influence the formation and maintaining process of the cardiovascular system by regulating the abnormal proliferation of smooth muscle cells and take part in the occurrence and development process of CHD via RhoA and transforming growth factor-beta1 (TGF-β)-dependent channels. However, the domestic and foreign research results about the relationship between polymorphisms of ICAM-1 and MKL-1 and CHD were inconclusive and contradictory.

Therefore, in the present study, we discussed the correlation of ICAM-1 K469E and MKL-1 -184C/T polymorphisms with CHD susceptibility, and explored the effect of other environmental exposures on the onset of CHD.

2 | MATERIALS AND METHODS

2.1 | Cases and controls

The case group of the study enrolled 100 CHD patients including 58 males and 42 females. The mean age of the patients, who were diagnosed in Cardiology department of The First Affiliated Hospital of Jinzhou Medical University, was 59.4 ± 7.33 years old. Coronary angiography examinations of every patient showed that more than one main coronary artery of the cases had 50% or higher level of stenosis. Patients suffered from cardiomypathy, haemorrhagic diseases, renal failure and malignant tumours were eliminated. The control group recruited 91 homochronous healthy persons (53 males and 42 females) from the physical examination centre of the same hospital, with a mean age of 60.3 ± 6.79 years old. The controls had no malignant tumours and immune inflammatory diseases, and they were proved without CHD history by electrocardiograph (ECG) examinations. All of the subjects were Chinese Han population, and they had no blood relationship with each other. Informed consents were obtained from each participant. And the study was approved by the Ethics Committee of The First Affiliated Hospital of Jinzhou Medical University.

2.2 | Blood collection and DNA extraction

We collected 2 mL fasting venous blood of each subject and put the blood into PCR tubes, anticoagulated by EDTA. Genome DNA was extracted from 300 µL whole blood by Biospin Whole Blood Genomic DNA Extraction Kit (Sangon Company) and then stored at -70°C.

2.3 | PCR amplifications and genetic typing assay

The genotypes of ICAM-1 K469E and MKL-1 -184C/T polymorphisms were examined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach. Primer sequences for ICAM-1 K469E and MKL-1 -184C/T polymorphisms were designed by Primer Premier 5.0 and synthesized by Sang Biotech (Table 1). The PCR amplifications were performed in a total volume of 10 µL of mixture, containing 0.5 µL forward primer, 0.5 µL reverse primer, 3 µL 10 × Buffer, 1 µL dNTP, 2.5 µL MgCl₂, 0.5 µL Taq DNA polymerase and 2 µL deionized sterile water. The PCR cycle was as the following: 95°C initial denaturation for 5 minutes; followed by 32 cycles of denaturing at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension for 30 seconds at 72°C; finally extension at 72°C for 5 minutes.

The amplification products were digested by restriction enzyme BstUI at 60°C for 4 hours. Then, digested DNA products were analysed by 3% agarose gel electrophoresis. In ICAM-1 K469E polymorphism, only a 223-bp stripe was observed in KK genotype because it had no restriction enzyme cutting site; three stripes of 223, 136 and 87 bp were observed in KE genotype while two stripes of 136 and 87 bp in EE genotype. As for MKL-1 -184C/T polymorphism, the genotyping results were as follows: a stripe of 223 bp for CC genotype, three stripes of 223, 136, 87 bp for CT genotype and two stripes of 136, 87 bp for TT genotype.

2.4 | Statistical analysis

Data analysis was performed by SPSS.18 statistical software. The genotype and allele frequencies in two groups were compared by χ² test. The correlation of CAM-1 and MKL-1 gene polymorphisms with CHD was calculated by odds ratios (ORs) and 95% confidence intervals (95% CIs). Multiple logistic regression was adopted for testing the effects of other CHD risk factors. The differences had statistical significance when P < .05.

| Locus     | Primer sequence                  |
|-----------|----------------------------------|
| ICAM-1 K469E |  |
3 | RESULTS

3.1 | Characteristics of study subjects

As shown in Table 2, the differences in age, sex and body mass index (BMI) of the two groups had no statistical significance (P > .05). But the case group had more smokers and patients with hypertension and diabetes, as well as higher levels of glucose (Glu), serum total cholesterol (TC) and triglyceride (TG) than the control group (P < .05). The differences of high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels between two groups were not statistically significant (P > .05).

3.2 | Logistic regression analysis on CHD risk factors

The genotype and allele distributions of ICAM-1 K469E and MKL-1 -184C/T polymorphisms in case and control groups are shown in Table 3. As shown, the frequencies of these two tested variants did not deviate from the Hardy-Weinberg equilibrium (HWE), which indicated the representativeness of participants. In the building of Logistic regression, sex, age, smoking status, bodyweight, hypertension, diabetes, levels of Glu, TC, TG, HDL-C, LDL-C and polymorphisms of K469E and -184C/T as independent variable, and the existence of CHD as dependent variable. The results of logistic regression analysis are given in Table 4. After adjusting for confounding factors, ICAM-1 K469E polymorphism was proved to have no relation with the onset of CHD. While the distribution differences of -184C/T polymorphism TT genotype between case and control groups were of statistical significance (P < .05), logistic regression analysis offered a same result. Compared with CC genotype, TT genotype increased 4.92 times the onset risk of CHD (95% CI = 1.32-26.6). Meanwhile, T allele was closely related to CHD as well (OR = 2.34, 95% CI = 1.34-5.62). Thus, we confirmed that TT genotype and T allele were susceptible factors for CHD. Other related risk factors like smoking, hypertension, diabetes and TG level were consistent with the recognized CHD risk factors that had been studied before.

| TABLE 2 | General features comparison between cases and controls |
|-----------------------------------------------|
| Indicator                                      | Case (n = 100) | Control (n = 91) | P       |
| Age                                           | 59.4 ± 7.33    | 60.3 ± 6.79      | P > .05 |
| Sex (male/female)                             | 58/42          | 53/38            | P > .05 |
| Smoking                                       | 39 (59)        | 35 (38)          | P < .05 |
| Body mass index (kg/m²)                       | 25.4 ± 3.3     | 24.7 ± 3.1       | P > .05 |
| Hypertension                                  | 71 (71)        | 19 (21)          | P < .05 |
| Diabetes                                      | 28 (28)        | 11 (12)          | P < .05 |
| Glucose (mmol/L)                              | 6.05 ± 2.79    | 5.03 ± 0.03      | P < .05 |
| Total cholesterol (mmol/L)                    | 4.78 ± 1.18    | 4.31 ± 0.96      | P < .05 |
| Triglyceride (mmol/L)                         | 2.23 ± 2.17    | 1.17 ± 0.45      | P < .05 |
| High density lipoprotein cholesterol (mmol/L) | 1.16 ± 0.52    | 1.21 ± 0.58      | P > .05 |
| Low density lipoprotein cholesterol (mmol/L)  | 2.87 ± 0.96    | 2.78 ± 0.86      | P > .05 |

4 | DISCUSSION

Coronary heart disease is a complex disease. Multiple gene mutations and risk factors lead to the occurrence and development of such disease. So far, more than 200 risk factors of CHD have been reported, including sex, hypertension, hyperlipidaemia, hyperglycaemia, smoking, obesity and other traditional risk factors. Hypertension is one of the leading risk factor for CHD. Studies suggest that patients with hypertension (HTN) had 3-4 times higher risk of suffering from CHD. In the meanwhile, hypertension is independent of other risk factors to have a continuous and rising relationship with the onset of CHD. Patients with diabetes have high incidence of CVD, and their risk of developing CHD is 2-4 times as high as that of non-diabetic patients. Likewise, smoking is an independent risk factor for CHD. Smokers have been certified to have 1.5-4 times higher risk of being afflicted with CHD than non-smokers. Furthermore, people who begin smoking at an early age are at a higher risk of CHD.

With the deepening of the researches and development of biological technology, a growing body of evidence has confirmed the genetic susceptibility for CHD, and multiple genes have been found to be closely associated with the susceptibility for CHD. Hinohara et al firstly ascertained the correlation of MKL-1 -184C/T polymorphism with the onset of CHD in 2009, and the association was replicated in both Japanese (OR = 1.25, 95%CI = 1.04-1.49) and Korean (OR = 1.26, 95% CI = 1.01-1.58) populations. Other reports have demonstrated that the high expression of MKL-1 might play important roles in the occurrence and development of atherosclerosis. The present study discovered that -184C/T SNP of MKL-1 gene was closely related to the onset of CHD, and TT genotype increased the onset risk of such disease. T allele frequency was significantly higher in case group than that in control group, and it was suggested to be a risk factor for CHD. The distributions of TT genotype and T allele in case and control groups still had statically significant differences after logistic regression analysis, which attested the correlation with the onset of CHD. This is consistent with a previous study, and they also found that the -184C > T polymorphism of MKL1 is an important risk factor for CHD in Han nationality of Henan Province. The homozygosity of T allele is related to the risk of CHD and the severity of stenosis.

Many researches on the relationship between ICAM-1 polymorphisms and inflammatory diseases have been reported. Correlation
analyses on ICAM-1 K469E polymorphism and CHD have been reported as well both in domestic and abroad, \(^{28-30}\) but the results are inconclusive and contradictory. Liu et al conducted meta-analysis and found that rs5498 polymorphism was related to the reduction of CAD risk in Caucasians, but not Asians, which may be caused by different races.\(^ {31}\) The results of our study illustrated that after being assessed by logistic regression analysis, ICAM-1 K469E polymorphism was found to have no obvious association with CHD in the Chinese population.

Some limitations of our research cannot be ignored. First of all, in the case-control study, our sample is relatively small, it is necessary to expand the sample size to further verify our results. Secondly, our research objects are all Han nationality, the possibility of different genetic background and surroundings caused by ethnic and regional differences might affect experimental results. Therefore, a lager or different population should be taken into account to confirm the results.

In summary, the present study suggested the correlation between MKL-1 –184C/T polymorphism and CHD, and T allele might be a susceptible factor for the disease. Meanwhile, the coexistence of TT genotype with smoking status, hypertension, diabetes or high TG level would significantly increase the morbidity of CHD.

**CONFLICT OF INTEREST**

None.

**AUTHOR CONTRIBUTIONS**

Cungang Wu: Conceptualization (equal); Data curation (equal); Formal analysis (equal); Writing-review & editing (equal). Chao Huang: Methodology (equal); Resources (equal); Software (equal); Writing–original draft (equal).

**ETHICAL APPROVAL**

With the approval of The First Affiliated Hospital of Jinzhou Medical University Ethics Committee, written informed consent was obtaining from every subject.

**DATA AVAILABILITY STATEMENT**

All data generated or analysed during this study are included in this article.

**ORCID**

Chao Huang https://orcid.org/0000-0003-0418-0777

**REFERENCES**

1. den Dekker WK, Cheng C, Pasterkamp G, Duckers HJ. Toll-like receptor 4 in atherosclerosis and plaque destabilization. Atherosclerosis. 2010;209(2):314-320.
2. Libby P. Inflammation in atherosclerosis. Nature. 2002;420(6917):868-874.
3. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med. 1999;340(2):115-126.
4. Negi S, Anand A. Atherosclerotic coronary heart disease-epidemiology, classification and management. Cardiovasc Hematol Drug Targets. 2010;10(4):257-261.
5. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352(16):1685-1695.
6. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: Part II: variations in cardiovascular risk in specific ethnic groups and geographic regions and prevention strategies. Circulation. 2001;104(23):2855-2864.
7. Bowron A, Scott J, Stansbie D. The influence of genetic and environmental factors on plasma homocysteine concentrations in a population at high risk for coronary artery disease. Ann Clin Biochem. 2005;42(Pt 6):459-462.
8. del Zoppo GZ, Wijnholds S, Hallenbeck JM, Iadecola C, Wang X, Feuerstein GZ. Inflammation and stroke: putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. Brain Pathol. 2000;10(1):95-112.
9. Ikata J, Wakatsuki T, Oishi Y, Oki T, Ito S. Leukocyte counts and concentrations of soluble adhesion molecules as predictors of coronary atherosclerosis. Coron Artery Dis. 2000;11(6):445-449.
10. Ridker PM, Hulbert-Shearon R, Stampfer MJ, Allen J. Plasma concentration of soluble intercellular adhesion molecule 1 and risks of future myocardial infarction in apparently healthy men. Lancet. 1998;351(9096):88-92.
11. Luc G, Arveiller D, Evans A, et al. Circulating soluble adhesion molecules ICAM-1 and VCAM-1 and incident coronary heart disease: the PRIME Study. Atherosclerosis. 2003;170(1):169-176.
12. O'Malley T, Ludlam CA, Rimmer RA, Fox KA. Early increase in levels of soluble inter-cellular adhesion molecule-1 (sICAM-1); potential risk factor for the acute coronary syndromes. Eur Heart J. 2001;22(14):1226-1234.
13. Cen B, Selvaraj A, Prywes R. Myocardin/MKL family of SRF coactivators: key regulators of immediate early and muscle specific gene expression. J Cell Biochem. 2004;93(1):74-82.
14. Du KL, Chen M, Li J, Lepore JJ, Mericko P, Parmacek MS. Megakaryoblastic leukemia factor-1 transduces cytoskeletal signals and induces smooth muscle cell differentiation from undifferentiated embryonic stem cells. J Biol Chem. 2004;279(17):17578-17586.
15. Parmacek MS. Myocardin-related transcription factors: critical coactivators regulating cardiovascular development and adaptation. Circ Res. 2007;100(5):633-644.
16. Elberg G, Chen L, Elberg D, Chan MD, Logan CJ, Turman MA. MKL1 mediates TGF-beta1-induced alpha-smooth muscle actin expression in human renal epithelial cells. Am J Physiol Renal Physiol. 2008;294(5):F1116-F1128.
17. Cambien F, Poirier O, Lecerf L, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature. 1992;359(6396):641-644.
18. Tanne D, Haim M, Boyko V, et al. Soluble intercellular adhesion molecule-1 and risk of future ischemic stroke: a nested case-control study from the Bezafibrate Infarction Prevention (BIP) study cohort. Stroke. 2002;33(9):2182-2186.
19. Aghor-EEtang BB, Setaro JF. Management of hypertension in patients with ischemic heart disease. Curr Cardiol Rep. 2015;17(12):119.
20. Haffner SM, Miettinen H. Insulin resistance implications for type II diabetes mellitus and coronary heart disease. Am J Med. 1997;103(2):152-162.
21. Ziegler D. Type 2 diabetes as an inflammatory cardiovascular disorder. Curr Mol Med. 2005;5(3):309-322.
22. Vogiatzis I, Tsikrika E, Sachpekidis V, Pittas S, Kotsiani A. Factors affecting smoking resumption after acute coronary syndromes. Hellenic J Cardiol. 2010;51(4):294-300.
23. Hinohara K, Nakajima T, Yasunami M, et al. Megakaryoblastic leukemia factor-1 gene in the susceptibility to coronary artery disease. Hum Genet. 2009;126(4):539-547.
24. Sata M, Saito S, Kuniyoshi T, et al. Hematopoietic stem cells differentiate into vascular cells that participate in the pathogenesis of atherosclerosis. Nat Med. 2002;8(4):403-409.
25. Xu Y, Li B, Bao YZ, et al. MKL1-184C>T gene polymorphism is associated with coronary artery disease in the Chinese Han population. Genet Mol Res. 2014;13:590-597.
26. Amoli MM, Mattey DL, Calvino MC, et al. Polymorphism at codon 469 of the intercellular adhesion molecule-1 locus is associated with protection against severe gastrointestinal complications in Henoch-Schonlein purpura. J Rheumatol. 2001;28(5):1014-1018.
27. Boiard L, Salvarani C, Casali B, et al. Intercellular adhesion molecule-1 gene polymorphisms in Behcet’s disease. J Rheumatol. 2001;28(6):1283-1287.
28. Aminian B, Abdi Ardekani AR, Arandi N. ICAM-1 polymorphisms (G241R, K469E), in coronary artery disease and myocardial infarction. Int J Immunol. 2007;4(4):227-235.
29. Jiang H, Klein RM, Niederacher D, et al. C/T polymorphism of the intercellular adhesion molecule-1 gene (exon 6, codon 469). A risk factor for coronary heart disease and myocardial infarction. Int J Cardiol. 2002;84(2-3):171-177.
30. McGlinchey PG, Spence MS, Patterson CC, et al. The intercellular adhesion molecule-1 (ICAM-1) gene K469E polymorphism is not associated with ischaemic heart disease: an investigation using family-based tests of association. Eur J Immunogenet. 2004;31(5):201-206.
31. Liu A, Wan A, Feng A, Rui R, Zhou B. ICAM-1 gene rs5498 polymorphism decreases the risk of coronary artery disease. Medicine. 2018;97:e12523.

How to cite this article: Wu C, Huang C. ICAM-1 and MKL-1 polymorphisms impose considerable impacts on coronary heart disease occurrence. J Cell Mol Med. 2020;24:10338–10342. [https://doi.org/10.1111/jcmm.15645](https://doi.org/10.1111/jcmm.15645)