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ORIGINAL ARTICLE

Association between MMP2 gene polymorphisms and dilated cardiomyopathy in a Chinese Han population

Shiyang Li*, Xiaobin Zeng, Shihai Wang, Xiaoshuang Xie and Jianjun Lan*

Division of Cardiology, Panzhihua Central Hospital, Panzhihua, China

Abstract

Aims Dilated cardiomyopathy (DCM) belongs to the common types of cardiomyopathies. The pathogenesis remains unclear despite the fact that various genes have been found associated with DCM. MMP2 is a zinc-dependent and calcium-containing secreted endoproteinases, which could cleave a broad spectrum of substrates including extracellular matrix components and cytokines. It has proved to play an important role in the cardiovascular diseases. This study aimed to investigate the potential role of *MMP2* gene polymorphisms in DCM susceptibility and prognosis in a Chinese Han population.

Methods and results A total of 600 idiopathic DCM patients and 700 healthy controls were enrolled. Patients with contact information were followed up for a median period of 28 months. Three tagged single nucleotide polymorphisms (rs243865, rs2285052, and rs2285053) in the promoter of *MMP2* gene were genotyped. A series of function analysis were conducted to illuminate the underlying mechanism. The frequency of rs243865-C allele was increased in DCM patients when compared with healthy controls ($P = 0.001$). Genotypic frequencies of rs243865 were associated with the susceptibility of DCM in the codominant, dominant, and overdominant models ($P < 0.05$). Besides, rs243865-C allele presented a correlation with the poor prognosis of DCM patients in both dominant (HR = 2.0, 95% confidence interval [CI] = 1.14–3.57, $P = 0.017$) and additive (HR = 1.85, 95% CI = 1.09–3.13, $P = 0.02$) model. The statistical significance remained after adjustment for sex, age, hypertension, diabetes, hyperlipidaemia, and smoking status. There were significant differences in left ventricular end-diastolic diameter and left ventricular ejection fraction between rs243865-CC and CT genotypes. Functional analysis indicated that rs243865-C allele increased luciferase activity and the mRNA expression level of MMP2 by facilitating ZNF354C binding.

Conclusions Our study suggested that *MMP2* gene polymorphisms were associated with DCM susceptibility and prognosis in the Chinese Han population.

Keywords Dilated cardiomyopathy; MMP2; Polymorphisms; Susceptibility; Prognosis

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**Correspondence to: Jianjun Lan and Shiyang Li, Division of Cardiology, Panzhihua Central Hospital, Panzhihua 34# Yi kang Ave., Panzhihua 617000, China. Email: pzhzxyyxnkljj@sina.com; lishiyangzyy@sina.com*

Introduction

Dilated cardiomyopathy (DCM), which is a major cause of heart failure, is characterized by left ventricular dilation and contractile dysfunction absence of secondary causes, such as coronary artery disease, hypertension, valvular disease, and congenital heart disease.[1] The prevalence of DCM was estimated ranged from 1:2700 to 1:250 individuals.[2] DCM is an multifactorial disease, which could be divided into non-genetic and genetic.[3] More than 100 genes have been identified to be associated with the phenotype of DCM, most of which encoded sarcomere, cytoskeleton, sarcolemma, nucleus, and the nuclear lamina.[1] But only 40% of pathogenesis of DCM could be explained by above genes.[3] At present, it remained partially unclear about the mechanism of gene variant in the pathogenesis of DCM.

The *MMP2* gene, which located on human chromosome 16q12.2, encodes the 72 kDa type IV collagenase (MMP2). MMP2 was identified as a zinc-dependent and calcium-containing secreted endoproteinases. A series of substrates including extracellular matrix (ECM) components, soluble metabolic mediators, secreted and ECM-anchored growth factors, and cytokines could be cleaved by MMP2.[4] MMP2 is widely expressed in many tissues and participates in many diseases including progression of chemoresistant lung cancer cell lines,[5] asthma susceptibility[6] gastric cancer,[7] and arterial

wall rejuvenation.[8] Importantly, a substantial of studies have demonstrated the vital role of MMP2 in myocardial infarction (MI),[9] hypertensive heart disease,[10] and atherosclerosis.[11] The expression of MMP2 was reported elevated in these pathologies. Furthermore, MMP2 has been identified as an independent predictor of all-cause mortality post-acute coronary syndrome[12] and correlated positively with total and low-density lipoprotein cholesterol.[13]

Notably, single nucleotide polymorphisms (SNPs) in *MMP2* gene have been widely investigated and associated with many diseases including oesophageal squamous cell carcinoma,[14] multiple sclerosis,[15] colorectal cancer,[16] chronic obstructive pulmonary disease,[17] and ischaemic stroke.[18] However, no study has discussed the effect of variants in *MMP2* on DCM. Considering the important role of MMP2 in cardiovascular diseases, we assumed that SNPs of this gene might be correlated with the susceptibility and prognosis of DCM. Accordingly, the association between the three tagged SNPs (rs243865, rs2285052, and rs2285053) in *MMP2* gene and the morbidity and mortality of DCM was investigated in Chinese Han Population. Further functional analysis was performed to illuminate the underlying mechanism.

Methods

Study subjects

In this study, a total of 600 idiopathic DCM patients and 700 healthy controls were recruited from the Cardiology Division of Panzhihua central hospital in Sichuan between March 2014 and June 2017. The diagnostic criteria of DCM refer to the modified version of standardized diagnostic criteria for DCM.[19] In brief, patients with a history of cardiac valve disease, coronary heart disease, hypertension, tachyarrhythmia, congenital heart disease, pericardial disease, acute viral myocarditis, heavy alcohol intake, skeletal myopathies, systemic diseases of a putative autoimmune origin, diabetes, and nutrition disorders were excluded from our study. Participants of healthy controls are free of cardiac disease and cardiac dysfunction. Echocardiography was conducted for all participants to assess their heart function. This study conformed with the principles outlined in the Declaration of Helsinki and was approved by Review Board of Panzhihua central hospital. All patients have signed the informed consents.

DNA extraction, single nucleotide polymorphism selection, and genotyping

We extracted genomic DNA from peripheral blood leukocytes using Tiangen commercially available kit (Tiangen, Beijing, China). Detailed procedures of DNA extraction have been described previously.[20]

Referring to the Chinese data of the 1000 Genomes, we selected three tagged genetic variants with minor allele frequency (MAF) $>$ 0.05 in the promoter region of *MMP2* for further genotyping. The probes for genotyping came from Applied Biosystems (ABI) with the following assay ID: rs243865 (C___3225943_10, 4351379), rs2285052 (C___3225944_10, 4351379), and rs2285053 (C__26734093_20, 4351379). The genotyping procedure was conducted according to the TaqMan assay on the TaqMan 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) with the following conditions: 10 min at 95°C (enzyme activation) followed by 45 cycles at 95°C for 15 s and 60°C for 1 min (annealing/extension). An endpoint read was performed for allelic discrimination after amplification. The details of the procedure for amplification and the quality control of genotyping were mentioned in our previous study.[21]

Clinical follow-up

A total of 534 patients with contact information have finished the follow-up every 3 months. The primary endpoints were defined as cardiovascular deaths or cardiac transplantation. The clinical characteristics and echocardiographic indicators of DCM patients were obtained from medical records, and echocardiographic parameters were assessed at the time of enrolment. A double-blind method was conducted during the follow-up process.

Plasmids construction, cell culture, transient transfection, and luciferase activity assays

To determine the causal variant, we constructed the reporter plasmids including their wild and mutant type using PGL3-basic. Meanwhile, the predicted transcriptional factor ZNF354C was cloned into pcDNA3.1 using human cDNA. Related primer and restriction enzyme cutting sites were shown below: forward primer with MluI 5′ cgACGCGTAATGCTGTTCAGGCTGGATTTAG 3′, reverse primer with HindIII 5′ CCCAAGCTTCTGAGGAAGTCTGGATGCAGC 3′ for reporter plasmids construction, forward primer with KpnI 5′ GGGGTACCATGGCTGTGGATCTGCTGTCT 3′, reverse primer with NotI 5′ TTGCGGCCGCCTAAACCTCATAGGCTTTATCTCCTTT 3′ for ZNF354C expression vector construction. Cell culture and transient transfection procedures have been described previously.[20] Cells were harvested 48 h after transfection using the Passive Lysis Buffer (SIRIUS, Pforzheim, Germany). The data of luciferase expression levels were adjusted with reference to Renilla luciferase activity and relative to the average values of mutant-type for corresponding variants.

Each reporter was performed six independent experiments to avoid potential experimental errors.

Transcription assays of the MMP2 gene

A total of 201 samples of peripheral blood lymphocytes from participants undergoing coronary angiography were collected for mRNA assessment. Details about RNA isolation, mRNA transcription, and PCR performed in this study were described previously.[21] MMP2 and actin beta (ACTB) mRNA were measured using absolute quantification methods with each sample in triplicate. Related primer sequences are listed in Table S1. Detailed characteristics of individuals are shown in Table S2. The expression of MMP2 relative to ACTB was compared between individuals with CC genotype and with CT combined TT genotype.

Statistical analysis

Statistical analyses were performed with SPSS version 13.0 (SPSS, Inc, Chicago, Illinois) for Windows (Microsoft Corp, Redmond, Wash). Data were presented as mean ± standard deviation (SD) for continuous variables and numbers (percentages) for categorical variables. Linkage disequilibrium was calculated using Haploview version 4.1. The polymorphisms were tested for Hardy–Weinberg equilibrium (HWE) among the DCM patients and the controls using χ^2 test. Logistic regression analyses and Cox proportional hazards regression model were used for association analyses of variants in *MMP2* gene with the risk and prognosis of DCM, respectively. We quantify the effects of variants in *MMP2* on risk of DCM with odds ratio (OR) and its 95% confidence interval (CI). Statistical significance was calculated by either unpaired or paired, two-tailed Student's *t*-test, or one-way ANOVA followed by Bonferroni's *post hoc* test, where appropriate. $P < 0.05$ was considered as significant.

Table 1 Baseline characteristics of population

Variable	DCM (n = 600)	Control (n = 700)	P
Age	56.2 ± 13.8	56.1 ± 13.5	0.15
Gender (male/female)	396/204	455/245	0.71
SBP (mmHg)	107.7 ± 25.2	112.1 ± 24.7	0.002
DBP (mmHg)	61.0 ± 18.2	70.6 ± 13.6	<0.001
HR (b.p.m.)	90.2 ± 19.6	77.3 ± 11.5	<0.001
IVSD (mm), mean ± SD	9.6 ± 1.7	10.9 ± 3.4	<0.001
LVEDD (mm), mean ± SD	65.1 ± 8.5	46.5 ± 4.3	<0.001
LVPWD (mm), mean ± SD	9.6 ± 1.5	10.0 ± 1.7	<0.001
LVEF (%)	34.3 ± 12.4	62.3 ± 6.6	<0.001

Abbreviations: DBP, diastolic blood pressure; HR, heart rate; IVSD, end-diastolic interventricular septal diameter; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVPWD, end-diastolic left ventricular posterior wall diameter; SBP, systolic blood pressure; SD, standard deviation.

Results

Baseline characteristic of population

As shown in *Table 1*, 600 DCM patients and 700 controls were matched by gender (66% male vs. 65% male, P = 0.71) and age (56.2 ± 13.8 vs. 56.1 ± 13.5, P = 0.15). The DCM patients presented lower systolic blood pressure, diastolic blood pressure, end-diastolic interventricular septal diameter, end-diastolic left ventricular posterior wall diameter, and left ventricular ejection fraction (LVEF) than the healthy controls. On the contrary, the heart rate and left ventricular end-diastolic diameter (LVEDD) of DCM group were higher than those of controls (both $P < 0.001$).

Associations of *MMP2* single nucleotide polymorphisms with the susceptibility to dilated cardiomyopathy

Referring to the Chinese data of the 1000 Genomes, we found eight common variants with MAF > 0.05 in the promoter region of *MMP2* gene. As shown in *Table 2*, total

Table 2 Characteristics of MMP2 variants identified by the Chinese data of the 1000 Genomes

Haploblock structure	Gene position[a]	dbSNP ID[b]	Gene region	Maj > Min[c]	MAF
Haploblock 1	chr16:55510822	rs12599478	Promoter	C > T	0.466
	chr16:55510853	rs12599483	Promoter	C > G	0.464
	chr16:55512157	rs2285052	Promoter	A > C	0.469
Haploblock 2	chr16:55511537	rs243866	Promoter	G > A	0.101
	chr16:55511806	rs243865	Promoter	C > T	0.101
	chr16:55512322	rs243864	Promoter	T > G	0.101
Haploblock 3	chr16:55512053	rs17859821	Promoter	G > A	0.245
	chr16:55512377	rs2285053	Promoter	C > T	0.24

Abbreviation: MAF, Minor allele frequency.
[a]Base pair position is based on NCBI GRCH37.
[b]Polymorphism are numbered relative to transcription start site.
[c]With major allele given first, followed by minor allele.

of three haploblock structures were identified through linkage disequilibrium analyses. Finally, we selected three tagged SNPs (rs243865, rs2285052, and rs2285053) for further genotyping.

The distribution of *MMP2* rs243865, rs2285052, and rs2285053 genotype frequencies conformed to HWE ($P > 0.05$). We compared the frequencies of genotypic and allelic of rs243865, rs2285052, and rs2285053 between DCM patients and controls to explore the association of the three SNPs with susceptibility to DCM. As shown in *Table 3*, the frequency of rs243865-T allele in DCM group is notably lower than that in healthy controls (7.7% vs. 11.6%, OR = 0.63; 95% CI = 0.48–0.82, $P = 0.001$). In codominant model, individuals carrying CT genotype displayed reduced risk of DCM when compared with those carrying CC genotype (OR = 0.64; 95% CI = 0.47–0.86, $P = 0.003$). Furthermore, rs243865-T allele carriers (CT + TT) also showed significant association with reduced DCM risk in the dominant genetic model (OR = 0.62; 95% CI = 0.46–0.83, $P = 0.001$). Besides, the CT frequency of rs243862 was lower in DCM group than that in healthy controls (14% vs. 20.1%, OR = 0.65; 95% CI = 0.48–0.87, $P = 0.004$). However, no statistical significance was detected for the association between rs2285052, rs2285053, and risk of DCM (*Table 4*).

Associations between *MMP2* SNPs and the prognosis of dilated cardiomyopathy

In the study, we have finished follow-up of 534 DCM patients with contact information, which received optimal medical treatment (*Table 5*). The median follow-up time reached up to 28 months, during which 151 cardiovascular deaths or cardiac transplantation occurred. Survival analyses were performed to determine the association between three SNPs (rs243865, rs2285052 and rs2285053) in *MMP2* and prognosis of DCM. The results revealed that rs243865 was significantly associated with the mortality risk of DCM in dominant and additive model, both with or without adjustment for conventional risk factors (*Table 6* and *Figure 1*). The cardiovascular deaths or cardiac transplantation had occurred in 138 patients (30.3%) in CC genotype group, 12 patients (16.2%) in CT genotype group and 1 (25%) in TT genotype group for rs243865. The baseline clinical characteristics based on MMP genotype were shown in *Table 7*. The statistical significance in multivariate analysis remained after adjustments for sex, age, hypertension, diabetes, hyperlipidaemia, smoking state (HR = 0.56, 95% CI = 0.38–0.82, $P = 0.003$ in dominant model, HR = 0.42, 95% CI = 0.24–0.74, $P = 0.002$ in additive model) (*Table 6*). We identified no statistically significant difference between rs2285052, rs2285053 and the prognosis of DCM (*Table 6*).

Correlation between *MMP2* single nucleotide polymorphisms and clinical characteristics

Furthermore, we conducted stratified analysis to detect the association between three tagged SNPs and clinical characteristics. As shown in *Figure 2*, patients carrying rs243865-CT genotype displayed reduced LVEDD and increased LVEF when compared with rs243865-CC ($P = 0.001$ for LVEDD and $P = 0.039$ for LVEF) genotype. Owing to the limited number of individuals carrying TT genotype, we observed no difference for LVEDD and LVEF between patients with CC and TT genotype. The differences for end-diastolic interventricular septal diameter and end-diastolic left ventricular posterior wall diameter between different genotypes of rs243865 were also statistically insignificant (*Figure 2*). Subsequently, we compared the clinical characteristics of individuals carrying different genotype of rs2285052 and rs2285053, and no difference were detected (*Figure 3*).

Table 3 Distributions of rs243865 among dilated cardiomyopathy patients and controls and their associations with dilated cardiomyopathy susceptibility

				Logistic regression	
Genetic model	Genotype	Case, *n* (%)	Control, *n* (%)	OR (95% CI)	*P*
Codominant	CC	512 (85.3)	548 (78.3)	Reference	Reference
	CT	84 (14)	141 (20.1)	0.64 (0.47–0.86)	0.003*
	TT	4 (0.7)	11 (1.6)	0.39 (0.12–1.23)	0.11
Dominant	CC	512 (85.3)	548 (78.3)	Reference	Reference
	CT + TT	88 (14.7)	152 (21.7)	0.62 (0.46–0.83)	0.001*
Recessive	CC + CT	596 (99.3)	689 (98.4)	Reference	Reference
	TT	4 (0.7)	11 (1.6)	0.42 (0.13–1.33)	0.14
Overdominant	CC + TT	516 (86)	559 (79.9)	Reference	Reference
	CT	84 (14)	141 (20.1)	0.65 (0.48–0.87)	0.004*
Allele	C	1108 (92.3)	1237 (88.4)	Reference	Reference
	T	92 (7.7)	163 (11.6)	0.63 (0.48–0.82)	0.001*

Abbreviations: CI, confidence interval; DCM, dilated cardiomyopathy; OR, odds ratio; SNP, single nucleotide polymorphism.
*$P < 0.05$.

Table 4 Distributions of MMP2 SNPs among DCM patients and controls and their associations with dilated cardiomyopathy susceptibility

Genetic model	Genotype	Case, *n* (%)	Control, *n* (%)	Logistic regression	
				OR (95% CI)	*P*
rs2285052					
Codominant	AA	169 (28.2)	200 (28.6)	Reference	Reference
	AC	299 (49.8)	351 (50.1)	1.01 (0.78–1.3)	0.95
	CC	132 (22)	149 (21.3)	1.05 (0.77–1.43)	0.77
Dominant	AA	169 (28.2)	200 (28.6)	Reference	Reference
	AC + CC	431 (71.8)	500 (71.4)	1.02 (0.80–1.3)	0.87
Recessive	AA + AC	468 (78)	551 (78.7)	Reference	Reference
	CC	132 (22)	149 (21.3)	1.04 (0.8–1.36)	0.76
Overdominant	AA + CC	301 (50.2)	349 (49.9)	Reference	Reference
	AC	299 (49.8)	351 (50.1)	1.0 (0.79–1.23)	0.91
Allele	A	637 (53.1)	751 (53.6)	Reference	Reference
	C	563 (46.9)	649 (46.4)	1.02 (0.88–1.2)	0.78
rs2285053					
Codominant	CC	346 (57.7)	421 (60.2)	Reference	Reference
	CT	219 (36.5)	234 (33.4)	1.14 (0.9–1.44)	0.27
	TT	35 (5.8)	45 (6.4)	0.95 (0.6–1.51)	0.82
Dominant	CC	346 (57.7)	421 (60.2)	Reference	Reference
	CT + TT	254 (42.3)	279 (39.8)	1.11 (0.89–1.38)	0.37
Recessive	CC + CT	565 (94.2)	655 (93.6)	Reference	Reference
	TT	35 (5.8)	45 (6.4)	0.9 (0.57–1.42)	0.66
Overdominant	CC + TT	381 (63.5)	466 (66.6)	Reference	Reference
	CT	219 (36.5)	234 (33.4)	1.15 (0.91–1.44)	0.25
Allele	C	911 (75.9)	1076 (76.9)	Reference	Reference
	T	289 (24.1)	324 (23.1)	1.05 (0.88–1.26)	0.57

Abbreviations: CI, confidence interval; DCM, dilated cardiomyopathy; OR, odds ratio; SNP, single nucleotide polymorphism.
$^{*}P < 0.05$.

Table 5 Details about the treatment in DCM patients

Characteristics	Sequencing population ($N = 534$)
ACEI[a]	426 (80%)
ARB[a]	38 (7%)
Spironolactone[a]	433 (81%)
Beta-blocker use[a]	464 (87%)

Note: Data are expressed as percentages.
Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers.
[a]Listed as number (%).

Functional analysis of the causal single nucleotide polymorphism

Considering the fact that rs243866, rs243865, and rs243864 were in linkage disequilibrium with each other, we performed fluorescent reporter gene assay to identify the causal variant in AC16 cell. As shown in *Figure 4A*, the luciferase activity of rs243865-C was significantly higher than that of rs243865-T allele. However, we did not observe any effect on luciferase activity in rs243866 and rs243864 luciferase assays in AC16 cells. Subsequently, we conducted the bioinformatics analysis using Jaspar (http://jaspar.genereg.net/) and found rs243865-T allele destroyed that binding site of ZNF354C in the promoter of MMP2. We then experimentally assessed the function of rs243865 on ZNF354C-mediated gene transcription using luciferase activity assays. As shown in *Figure 4B*, transfection of ZNF354C expression plasmids increased the transcription activity for the rs243865-C but not T MMP2 promoter construct. This indicated that the rs243865-T allele could disrupt the binding site of ZNF354C in the MMP2 promoter and reduced the transcriptional activity of the gene. Meanwhile, we compared MMP2 mRNA relative expression in lymphocytes including 201 samples (161 CC genotype and 40 CT + TT genotype) and found that samples with CC genotype displayed significantly higher MMP2 mRNA level than samples with CT + TT genotype (*Figure 4C*).

Discussion

DCM is a multifactorial disease and characterized by reduced left ventricular systolic function.[22] Numerous genes have been demonstrated associated with the susceptibility and prognosis of DCM. However, the pathogenic mechanism and genetic background hiding behind DCM remained unclear and deserved deeply investigation.[3] In this study, we continuously recruited 600 DCM patients and 700 healthy controls. Association analysis were conducted to explore the relationship between three tagged SNPs in *MMP2* and the susceptibility and prognosis of DCM. We found that rs243865 was associated with both the risk and prognosis of DCM, with the C allele increasing the susceptibility and mortality risk of DCM. Besides, the C allele of rs243865 was found related to enlarged left ventricle and reduced cardiac function. A

Table 6 Association of the MMP2 polymorphisms with cardiac mortality or transplantation in DCM patients

	Dominant model		Resessive model		Additive model	
SNPs	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
rs243865						
Unadjusted	0.5 (0.28–0.88)	0.017*	1.17 (0.16–8.39)	0.87	0.54 (0.32–0.92)	0.02*
Adjusted	0.56 (0.38–0.82)	0.003*	1.04 (0.14–7.94)	0.97	0.42 (0.24–0.74)	0.002*
rs2285052						
Unadjusted	0.78 (0.56–1.10)	0.16	0.87 (0.59–1.3)	0.5	0.86 (0.69–1.08)	0.2
Adjusted	0.79 (0.56–1.11)	0.17	0.86 (0.58–1.27)	0.44	0.86 (0.68–1.08)	0.19
rs2285053						
Unadjusted	0.98 (0.71–1.36)	0.91	1.36 (0.71–2.58)	0.35	1.03 (0.79–1.35)	0.81
Adjusted	1.01 (0.73–1.40)	0.96	1.00 (0.52–1.94)	1	1.06 (0.81–1.38)	0.69

Note: Hazard ratio (HR) and 95% confidence intervals (95% CI) were obtained by Cox regression, without and with adjustment for sex, age, hypertension, diabetes, hyperlipidaemia and smoking status.
Abbreviation: SNP, single nucleotide polymorphism.
*$P < 0.05$.

Figure 1 Effects of rs243865 on the prognosis of DCM patients. (A) Prognosis analysis in additive model showed that rs243865 is associated with cardiovascular deaths or cardiac transplantation (unadjusted HR = 0.54, 95% CI = 0.32–0.92; *P* = 0.02). (B) In dominant model, patients carrying rs243865-TT and TC genotype showed reduced risk of cardiovascular deaths or cardiac transplantation when compared with patients carrying CC genotype (unadjusted HR = 0.5, 95% CI = 0.28–0.88; *P* = 0.017).

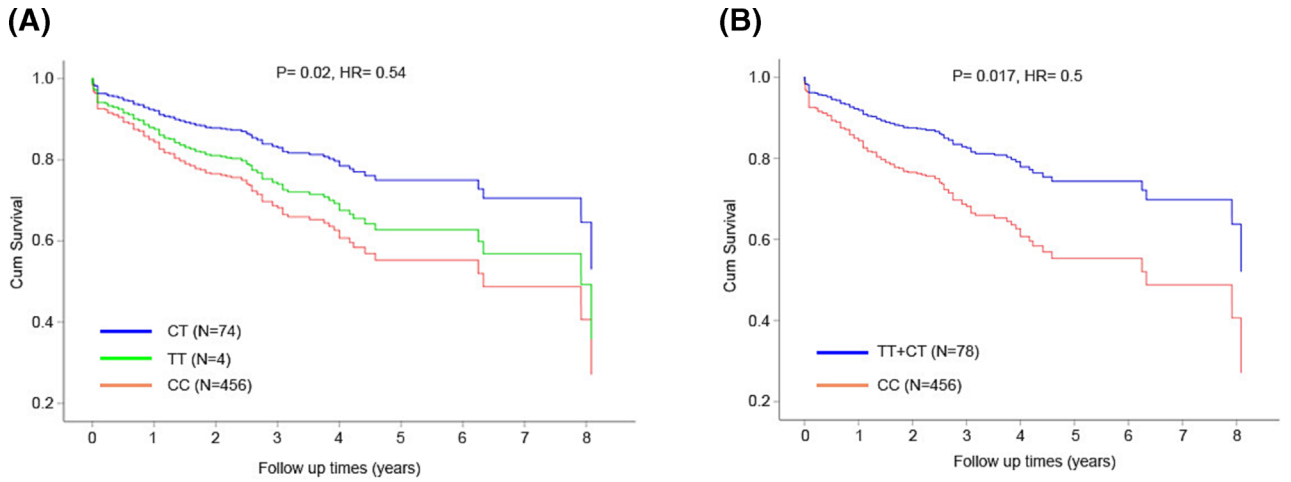


Table 7 Baseline clinical characteristics of DCM patients grouped by rs243865 genotype

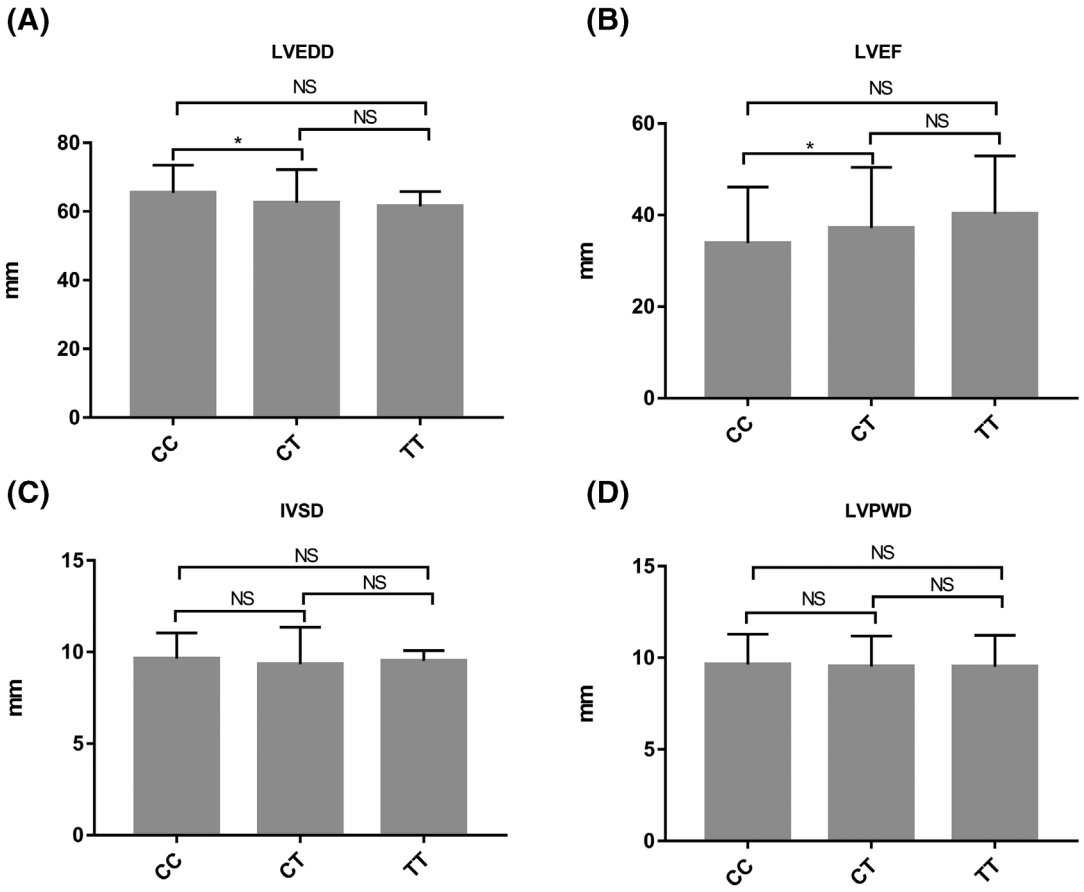
	Genotype		
Characteristics	CC (*N* = 456)	CT + TT (*N* = 78)	*P*
Age (years)	54.87 ± 13.74	62.87 ± 12.28	<0.001
Male, %	64	67	0.654
HBP, %	44	36	0.189
Diabetes, %	18	17	0.779
Hyperlipidaemia, %	5	7	0.617
Current smoker, %	39	36	0.701

Note: All continuous variables are expressed as mean ± SD or percentages.
Abbreviation: HBP, high blood pressure.

series of functional analysis also demonstrated that rs243865-C allele increased the transcriptional activity of MMP2 by facilitating ZNF354C binding.

Under normal condition, MMP2 is constitutively expressed and synthesized by cardiomyocytes and endothelial cells.[23] As a zinc-dependent and calcium-containing secreted endoproteinases, MMP2 has been reported involved in a substantial of pathophysiological process. Zhao et al. revealed that MMP2 implicated in the initiation and progression of gastric cancer.[24] Carotid artery stiffening was also demonstrated associated with MMP2.[8] Besides, increased MMP2 expression is related to osteosarcoma metastasis.[25] Importantly, the role of MMP2 in cardiovascular diseases has gained widely attention recent years. In the mouse model of acute MI, inhibition of MMP2 activity could improve the survival rate by preventing cardiac rupture and delaying post-MI remodelling through reduction in macrophage infiltration.[9] Besides, Hidenori et al. also demonstrated the beneficial role of MMP2-deletion in pressure

Figure 2 Comparison of clinical characteristics including LVEDD (A), LVEF (B), IVSD (C) and LVPWD (D) among DCM with different genotypes of rs243865. IVSD, end-diastolic interventricular septal diameter; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVPWD, end-diastolic left ventricular posterior wall diameter.



overload-induced left ventricular hypertrophy and dysfunction.[10] Pharmacological inhibition of MMP2 was demonstrated to produce cardioprotection in both normal and hyperlipidaemic rats.[26] MMP2 was also shown to contribute to the development of atherosclerosis in apoE$^{-/-}$mice.[11] A series of clinical research have also supported the deleterious role of MMP2 in heart diseases. In patients with acute coronary syndrome, the level of MMP2 in plasma was first proved to be an independent prediction of death.[12] Immediate administration of atorvastatin could significantly reduce the plasma level of MMP2 and improve the prognosis of acute heart failure.[27] Besides, higher serum levels of the active form of MMP2 were a predictor of remodelling 6 months after MI.[28]

Furthermore, genetic research has been widely conducted for *MMP2* using human population. The *MMP-2* C1306T (rs243865) was found to be associated with a wide range of diseases, including the risk of cervical cancer,[29] colorectal cancer,[16] and coronary artery disease,[30] susceptibility to salivary gland cancer[31] and oesophageal squamous cell carcinoma,[14] chronic obstructive pulmonary disease severity,[17] intracranial aneurysm,[32] and oral cancer.[33] In the female group, the association between *MMP-2* C-735T and risk of multiple sclerosis was statistically significant.[15] Besides, rs1132896-C allele and rs243849-T allele were significantly related to reduce stroke risk in a Hainan population.[18] However, the association between genetic variants in *MMP2* and DCM remained unexplored. In this study, we identified rs243865 in the promoter region of *MMP2* as the risk variant for the susceptibility and prognosis of DCM, with the C-allele increased the susceptibility and mortality risk of DCM. Maladaptive ECM remodelling represented the common feature of ventricular remodelling for DCM.[34] Matrix metalloproteinases (MMPs), which is responsible for ECM degradation, is closely related to the development of DCM.[35] Besides, many reports have revealed that patients carrying rs243865-CC genotype displayed higher MMP2 level and activity in serum and cervical tissue when compared with patients carrying CT or TT genotype,[17,29] which is consistent with our results that rs243865-C allele increased luciferase activity of MMP2 promoter region and the mRNA expression level of MMP2 by facilitating ZNF354C binding. Considering the deleterious

Figure 3 Comparison of clinical characteristics including LVEDD, LVEF, IVSD, and LVPWD among DCM with different genotypes of rs2285052 (A, B, C, D) and rs2285053 (E, F, G, H). IVSD, end-diastolic interventricular septal diameter; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; LVPWD, end-diastolic left ventricular posterior wall diameter.

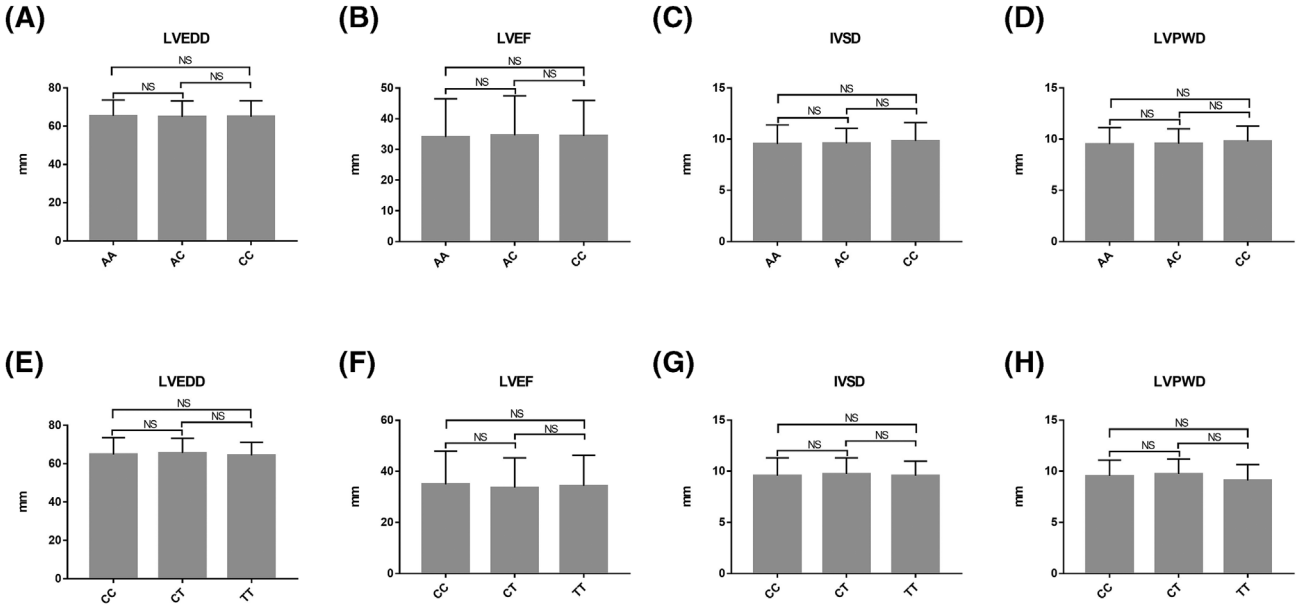
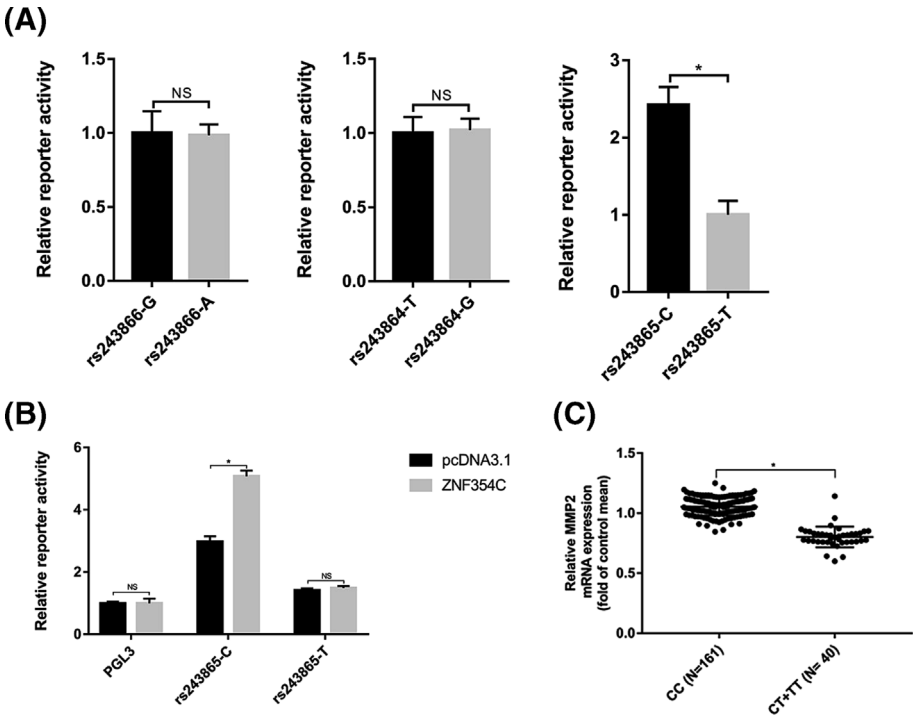


Figure 4 Functional analysis of rs243865. (A) Firefly luciferase assays were performed in AC16 and rs243865-C allele displayed higher luciferase activity than T allele. (B) ZNF354C could significantly increases the transcriptional activity of rs243865-C allele but not T allele. (C) Compared with CT + TT genotypes, samples carrying CC genotype showed higher mRNA level of MMP2. *$P < 0.05$, NS, not significant.



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role of MMP2 in heart and higher expression activity of rs243865-CC genotype, it was reasonable to assume that rs243865-C allele contributed to high susceptibility and mortality risk of DCM through increasing the expression of MMP2, which could accelerate ECM remodelling and eventually lead to left ventricular dilation. Importantly, patients with rs243865-CC genotype displayed increased LVEDD and reduced LVEF when compared with rs243865-CT genotype, which could also be attributable to increased MMP2 expression.

In conclusion, our study suggested that rs243865 in the promoter of *MMP2* are associated with the susceptibility as well as prognosis of DCM in a Chinese Han population. MMP2 might play an important role in pathogenesis of DCM. Further studies and large samples are still indispensable to confirm our findings and to explore a more distinct mechanism of MMP2 in DCM.

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Conflict of interest

None declared.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Sequences of primers used for quantitative RT-PCR.
Table S2. Baseline characteristics of 201 individuals undergoing coronary angiography.

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