Association between *CDK8* gene polymorphisms and dilated cardiomyopathy in a Chinese Han population

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Background: Dilated cardiomyopathy (DCM) is one of the most common types of cardiomyopathies. Various genes have been verified to be related to DCM, but the pathogenesis remains unclear. Cyclin-dependent-kinase 8 (CDK8), encoded by the *CDK8* gene, is a transcriptional factor that regulates the phosphorylation of RNA polymerase II. It plays an important role in the transcription process and different signaling pathways. This study aimed to investigate the potential role of *CDK8* gene polymorphisms in DCM susceptibility and prognosis in a Chinese Han population.

Methods: Two single nucleotide polymorphisms (SNPs) of *CDK8*, rs17083838 (A/G) and rs7992670 (A/G), were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 341 DCM patients and 381 healthy controls. Survival analysis was performed using Kaplan–Meier curves and Cox regression analysis.

Results: The frequencies of allele A of both SNPs rs17083838 and rs7992670 were increased in DCM patients compared to healthy controls (P<0.05). Genotypic frequencies of rs17083838 and rs7992670 were associated with the susceptibility to DCM in the codominant, and recessive models (P<0.05), and AA/AG genotypes of rs17083838 were also related to DCM susceptibility in the dominant model. AA/AG genotypes of rs17083838 and the AA genotype of rs7992670 in the dominant and recessive genetic models presented a correlation with the poor prognosis of DCM patients in both univariate (P<0.05) and multivariate analyses (P<0.05) after adjusting for age, gender, left ventricular end-diastolic diameter (LVEDD), and left ventricular ejection fraction (LVEF).

Conclusions: This research is the first to reveal that *CDK8* gene polymorphisms might be related to DCM susceptibility and prognosis in the Chinese Han population.

Keywords: Dilated cardiomyopathy (DCM); single nucleotide polymorphism (SNP); *CDK8*; survival analysis; polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

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Introduction

Dilated cardiomyopathy (DCM) is a primary myocardial disease defined by dilation of the left or both ventricles and contractile dysfunction, with decreased ejection fraction (1). DCM is also the most common type of cardiomyopathy, with a prevalence rate of 1/2,500 (2). The manifestations of DCM include progressive heart failure, ventricular arrhythmia, and sudden cardiac death (3). DCM is one of the leading causes of death in Western developed countries (4). The etiology of DCM can be divided into non-genetic and genetic (5). In previous research, thousands of mutations in more than 50 genes have been reported to be related to the pathogenesis of familial DCM, such as *ACTC1*, *TTN*, *LAMA4*, and *SCN5A*, among others (3). Most of these genes define heart structures and cardiac functions, but they can only explain 40% of the pathogenesis of DCM, since DCM is genetically heterogeneous (6). At present, the mechanism of gene mutation in the pathogenesis of DCM remains partially unclear. Accumulating studies have demonstrated that some transcription factors (TFs) are significantly associated with the dysfunction of cardiac muscles, particularly under pathological stress. Additionally, some studies have raised the possibility of cyclin-dependent kinase 8 (*CDK8*) variants as potential risk factors of some cardiomyopathies (7,8).

The *CDK8* gene encodes a major isoform of 464 amino acids and is an evolutionarily conserved serine/threonine kinase component of the Module (8). The Module is a 4-subunit complex that binds to the Mediator and regulates its activity. The Mediator complex is a global regulator of gene expression through the phosphorylation of RNA polymerase II and is considered as a general TF. Thus, CDK8 has a vital role in regulating transcription processes. Meanwhile, CDK8 activity is important in the expression of numerous components of the glycolytic cascade (9) and as a key regulator of several additional signaling pathways, such as the WNT, TGFβ/BMP, STAT1, SHH, and NOTCH pathways (10,11). Some researchers recently reported that *CDK8*-related disorders present characteristic facial features, congenital heart disorders, and intellectual disability in humans (7,8). Overexpression of *CDK8* causes progressive heart failure, DCM, and premature lethality (12). The basic mechanisms of *CDK8*-related disorders have yet to be delineated.

Moreover, single nucleotide polymorphisms (SNPs) of *CDK8* have not yet been revealed as predictors or risk factors of DCM prognosis and susceptibility. This study firstly hypothesized that SNPs of the *CDK8* gene might affect the susceptibility and prognosis of DCM. A pilot study was designed to investigate the role of *CDK8* polymorphisms among DCM patients in a Chinese Han population. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/cdt-21-323).

Methods

Study subjects

The case group contained a total of 341 unrelated patients diagnosed with DCM (mean age: 49.37±14.432; gender: male, 226; female, 115) recruited from West China Hospital of Sichuan University from July 2005 to August 2019. These patients were diagnosed with DCM according to the criteria established by the World Health Organization/ International Society and Federation of Cardiology Task Force on the Classification of Cardiomyopathies in 1995 (before 2006) (13), the Scientific Statement on the Classification of Cardiomyopathies proposed by the American Heart Association in 2006 (2006–2008) and the Classification from the European Society of Cardiology in 2008 (after 2008) (14). Cardiac function was graded by the New York Heart Association (NYHA) classification, and 72 patients were NYHA II, 207 patients were NYHA III, and 62 patients were NYHA IV. All patients were treated under the guidance of clinical guidelines in this study. Echocardiography was used to assess heart function. A total of 381 healthy unrelated individuals (mean age: 48.98±16.772; gender: male, 234; female, 147) from routine health surveys were recruited as the control group, with NYHA functional classes ranging from I to II. Age and gender in the control group were matched with the case group. Individuals with a history of coronary heart disease, cardiac valve disease, hypertension, tachyarrhythmia, acute viral myocarditis, familial DCM history, heavy alcohol intake, diabetes, obesity, insulin resistance, systemic diseases of putative autoimmune origin, skeletal myopathies, Parkinson's disease, or Alzheimer's diseases were excluded intentionally. Clinical data were collected from the hospital record system. We did not detect whether these patients had the most frequent monogenic causes of DCM, and all subjects were from the Chinese Han population of Sichuan Province in southwestern China. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This research was approved by the Hospital Ethics Committee (No. 81670346) and written informed consents

Table 1 Information on PCR-restriction fragment length polymorphisms in enrolled subjects

SNP ID	Primer sequence	Annealing temperature (℃)	Restriction enzyme	Allele (bp)
rs17083838	F: 5'-TCTGTTGCACTTGCCATATCA-3'	58	Mspl	A [194]
	R: 5'-TCCAAAGACTTTCAAAGACACTCA-3'			G [194=68+126]
rs7992670	F: 5'-TTTCATGCAGATACCACGTCA-3'	58	Mboll	A [151]
	R: 5'-TGGATTCACGGGTTCGTTAT-3'			G [151=113+38]

bp, base pair; SNP, single nucleotide polymorphism.

from all subjects were obtained.

SNP selection and genotyping

EDTA-anticoagulated peripheral blood samples (5 mL) were drawn from each participant. A DNA isolation kit (BioTeke, Peking, China) was used according to the manufacturer's protocol to extract genomic DNA from 200 µL whole blood samples (15). These DNA products were stably stored in a –80 ℃ freezer until assayed.

Tag SNPs were selected based on the data of the Chinese Han in Beijing China population sample from the HapMap Project (Data Release 24/Phase II, NCBI build 36 assembly, dpSNPb126). Two tag SNPs of *CDK8*, rs17083838 (A/G) and rs7992670 (A/G) were selected by the National Institute of Environmental Health Sciences (NIEHS) online software. PCR primers were designed using the Primer 3 software (https://primer3.ut.ee/) (16).

CDK8 gene polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR reactions were performed in a 10 µL reaction system containing 50 ng genomic DNA, 0.15 µL forward and reverse primers, 5 µL 2× Power Taq PCR Master Mix (Biosharp, Peking, China), and 10 µL ddH$_2$O. The PCR conditions were an initial denaturation step at 94 ℃ for 2 min, 34 cycles at 94 ℃ for 30 s, annealing at 58 ℃ for 30 s (rs17083838 and rs7992670), extension at 72 ℃ for 30 s, and final elongation at 72 ℃ for 10 min. PCR products were digested stably by the specific restriction enzymes MspI for 45 min (rs17083838) and Mboll for 30 min (rs7992670) at 37 ℃ according to the manufacturer's instructions (New England Biolabs, Peking, China) (*Table 1*). Ultimately, the digested products were electrophoresed and visualized by 6% polyacrylamide gels with silver staining. A double-blind method was applied during the genotyping of the enrolled subjects. Although DNA sequencing is the most accurate choice for SNPs genotyping, PCR and agarose gel electrophoresis were still used in our study as they are

convenient and fast. To verify the accuracy of the genotyping results, ~10% PCR-amplified DNA samples randomly underwent repeated visualization, and all results were 100% consistent.

Clinical follow-up

A total of 153 patients with contact information were scheduled for follow-up every 3 months. The basic clinical characteristics (gender, age, blood pressures and heart rate) of patients and echocardiographic indicators [left ventricular end-diastolic diameter (LVEDD) and left ventricular ejection fraction (LVEF)] were obtained from medical records, and echocardiography was performed at the time of diagnosis using an S5-1 broadband phased-array transducer (1–5 MHz) (Philips Medical Systems, Bothell, WA, USA). The end points were cardiac death or loss to follow-up. During follow-up, all patients received continuous medication treatment, and no patients underwent heart transplantation. Follow-up was performed in a double-blind manner, and the genotype of patients remained unknown.

Statistical analysis

Data analysis was performed using SPSS version 26.0 software (SPSS Inc., Chicago, IL, USA). Power and Sample Size Calculation software (version 3.0.43) was used to calculate sample power (17). Differences between two independent samples of continuous data were analyzed using the Mann-Whitney U test, while categorical variables were analyzed with Pearson's chi-square test. Shapiro-Wilk's test was used to determine normality. Genotypic frequencies of SNPs were obtained by counting directly. To compare the allelic and genotypic distribution between the DCM and healthy control groups, data were evaluated on the SNPStats website in the codominant, dominant, recessive, and overdominant models (18). The chi-square test was used to evaluate the Hardy-Weinberg equilibrium (HWE).

Table 2 Baseline characteristics of DCM patients and controls

Variables	Cases (n=341)	Controls (n=381)
Age (years)	49.37±14.432	48.98±16.772
Gender (male/female)	226/115	234/147
NYHA	II: 72; III: 207; IV: 62	I: 306; II: 75
SBP (mmHg)	108.62±17.681*	114.17±12.672
DBP (mmHg)	61.62±9.453*	74.44±8.410
HR (beats/min)	91.71±12.197*	80.62±11.425
LVEDD (mm)	67.79±8.987*	47.11±5.198
LVEF (%)	32.07±11.023*	62.98±7.188

Data are presented as the mean ± SD or number. *, patients *vs.* controls P<0.05. DCM, dilated cardiomyopathy; DBP, diastolic blood pressure; HR, heart rate; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure; NYHA, New York Heart Association.

Linkage disequilibrium tests and haplotype analysis were carried out using SHEsis software (19,20). The odds ratio (OR) and its 95% confidence interval (CI) were calculated to assess the effects of any differences in the distribution of genotypes and alleles. For survival analyses, Kaplan-Meier curve analysis and Cox regression were performed. A P value <0.05 was considered statistically significant in all the statistical analyses. Bonferroni corrections were carried out for multiple testing to determine statistical significance by the number of analyzed SNPs (for 2 SNPs, a Bonferroni correction coefficient of 2 was applied to the P values).

Results

Baseline characteristics of the enrolled subjects

The baseline clinical and echocardiographic characteristics of all participants are summarized in *Table 2*. The DCM group had a lower systolic blood pressure, diastolic blood pressure and LVEF than the control group. The HR and LVEDD of DCM patients were higher than those of control subjects (both P<0.001). Age and gender were matched between the two groups (P=0.737 and P=0.175 respectively). All samples had >80% power, which revealed that the sample size was adequate in the present study.

Associations between CDK8 SNPs and the susceptibility to DCM

Two SNPs of the *CDK8* gene, rs17083838 (A/G) and

rs7992670 (A/G), were previously identified by the International HapMap project (https://www.genome.gov/10001688/international-hapmap-project). The allelic and genotypic distribution of these 2 SNPs were consistent with the expected HWE (P=0.81 for rs17083838; P=0.55 for rs7992670), suggesting that all the samples met the assumption of the HWE (P>0.05). To identify the association between susceptibility to DCM and the two SNPs, a chi-square test was performed by SNPStats to analyze the genotype and allele frequencies in the DCM and control groups. Significant differences concerning both SNPs are summarized in *Table 3*.

The results revealed that the frequency of the A allele in SNP rs17083838 among DCM patients was notably elevated compared with the control group (22.0% *vs.* 16.0%; P=0.004; OR: 0.677; 95% CI: 0.520–0.883). In the codominant model, the frequencies of the GG, AG, and AA genotypes of rs17083838 were, respectively, 61.6%, 32.4%, and 6.2% in DCM cases, and 61.9%, 29.3%, and 1.6% in controls. The differences among genotype frequencies were statistically significant (P=0.0021; OR: 3.96; 95% CI: 1.05–1.94). Associations between s17083838 and DCM susceptibility were also found in the dominant model, where A carriers (AA/AG genotypes) had a higher DCM risk than the GG genotype (P=0.035; OR: 1.39; 95% CI: 1.027–1.90), and in the recessive model, where the AA genotype had a higher DCM risk than the GG/AG genotypes (P=0.0011; OR: 4.04; 95% CI: 1.61–10.12). These results remained significant even after the Bonferroni correction (P<0.025).

For the allele frequencies of SNP rs7992670, the DCM group had a significantly greater allele frequency of A than the healthy control group (50% *vs.* 43%, P=0.016, OR: 0.774, 95% CI: 0.628–0.954). AA genotypes had higher DCM risk than AG/GG genotypes in the codominant model (P=0.047; OR: 1.71; 95% CI: 1.11–2.62). In the recessive model, compared with the GG/AG genotypes, an increased risk of DCM was associated with the AA genotype (P=0.028; OR: 1.50; 95% CI: 1.04–2.16). The association was still significant after the Bonferroni correction (P<0.025). However, no significant differences were observed in the dominant and overdominant models.

There were no obvious differences between DCM patients and healthy controls in the overdominant model for both rs17083838 (P=0.4, OR: 1.15, 95% CI: 0.83–1.58) and rs7992670 (P=0.84 OR: 0.97, 95% CI: 0.72–1.30). Hence, stratified analyses were conducted for this model among 341 DCM patients. *Table 4* summarizes the results after adjusting for age, LVEF, and LVEDD. The frequency of the GG/AA genotype was higher in individuals with a

Table 3 Distributions of *CDK8* SNPs among DCM patients and controls and their associations with dilated cardiomyopathy susceptibility

Model	Genotype	Cases, n (%)	Controls, n (%)	OR (95% CI)	P value
rs17083838					
Codominant	GG	210 (61.6)	259 (61.9)	1.00	–
	AG	110 (32.4)	110 (29.3)	1.23 (0.89–1.70)	–
	AA	21 (6.2)	6 (1.6)	3.96 (1.05–1.94)*	0.0021*
Dominant	GG	210 (61.6)	259 (69.1)	1.00	–
	AG-AA	131 (38.4)	116 (30.9)	1.39 (1.02–1.90)*	0.035*
Recessive	GG-AG	320 (93.8)	369 (98.4)	1.00	–
	AA	21 (6.2)	6 (1.6)	4.04 (1.61–10.12)*	0.0011*
Overdominant	GG-AA	231 (67.7)	265 (70.7)	1.00	–
	AG	110 (32.3)	110 (29.3)	1.15 (0.83–1.58)	0.4
Allele	G	530 (78.0)	628 (84.0)	1.00	–
	A	152 (22.0)	122 (16.0)	0.677 (0.520–0.883)	0.004*
rs7992670					
Codominant	GG	85 (24.9)	114 (30.9)	1.00	–
	AG	172 (50.4)	189 (51.2)	1.22 (0.86–1.73)	–
	AA	84 (24.6)	66 (17.9)	1.71 (1.11–2.62)*	0.047*
Dominant	GG	85 (24.9)	114 (30.9)	1.00	–
	AG-AA	256 (75.1)	255 (69.1)	1.35 (0.97–1.87)	0.076
Recessive	GG-AG	257 (75.4)	303 (82.1)	1.00	–
	AA	84 (24.6)	66 (17.9)	1.50 (1.04–2.16)*	0.028*
Overdominant	GG-AA	169 (49.6)	180 (48.8)	1.00	–
	AG	172 (50.4)	189 (51.2)	0.97 (0.72–1.30)	0.84
Allele	G	342 (50.0)	417 (57.0)	1.00	–
	A	340 (50.0)	321 (43.0)	0.774 (0.628–0.954)	0.016*

P<0.05 was considered to be statistically significant; *, values indicate statistical significance. SNP, single nucleotide polymorphism; DCM, dilated cardiomyopathy; CI, confidence interval; OR, odds ratio.

larger left ventricle (LV ≥67 mm) than that in patients with LVEDD less than 67 mm for rs7992670 (57.4% *vs.* 45.1%, OR: 0.608, 95% CI: 0.396–0.935, P=0.023). No correlation was found between LVEDD and rs17083838, and there were no significant differences between the two SNPs (rs17083838 and rs7992670) and the other indicators (age, gender, and LVEF).

Linkage disequilibrium and haplotype analysis

Linkage disequilibrium was observed within the two

variants using SHEsis software (http://analysis.bio-x.cn/myAnalysis.php). The results showed that SNP rs17083838 and rs7992670 were not in linkage disequilibrium (D'=0.148, r^2=0.004), which meant both of the SNPs were representative. We found a significant association in the distribution of the three haplotype frequencies (AA, AG, and GG) between DCM cases and healthy controls (P<0.05) (*Table 5*) in the further analysis of four haplotype combinations. The haplotype AA was significantly associated with increased DCM susceptibility, while GG was a protective haplotype.

Table 4 Distributions of *CDK8* SNPs in patients' characteristics and their associations with DCM risk

Characteristics	rs17083838				rs7992670			
	Genotype		OR (95% CI)	P	Genotype		OR (95% CI)	P
	GG-AA	AG			GG-AA	AG		
Age (year), n (%)								
<50	111 (68.1)	52 (31.9)	–	–	90 (55.2)	73 (44.8)	–	–
≥50	120 (67.4)	58 (32.6)	1.032 (0.655–1.626)	0.893	87 (48.9)	91 (51.1)	1.290 (0.842–1.975)	0.242
Gender, n (%)								
Male	150 (66.4)	76 (33.6)	–	–	116 (51.3)	110 (48.7)	–	–
Female	81 (70.4)	34 (29.6)	0.828 (0.509–1.347)	0.448	61 (53.0)	54 (47.0)	0.934 (0.595–1.464)	0.764
LVEDD (mm), n (%)								
<67	97 (63.4)	56 (36.6)	–	–	69 (45.1)	84 (54.9)	–	–
≥67	134 (71.3)	54 (28.7)	0.698 (0.442–1.101)	0.122	108 (57.4)	80 (42.6)	0.608 (0.396–0.935)*	0.023*
LVEF, n (%)								
<30	110 (70.5)	46 (29.4)	–	–	82 (52.6)	74 (47.4)	–	–
≥30	121 (65.4)	64 (34.6)	1.265 (0.800–2.001)	0.315	95 (51.4)	90 (48.6)	1.050 (0.685–1.608)	0.823

Adjusted for age, gender, LVEDD, and LVEF. *, values indicate statistical significance. SNP, single nucleotide polymorphism; DCM, dilated cardiomyopathy; OR, odds ratio.

Table 5 Haplotype frequencies of the *CDK8* gene in the patients with DCM and in controls

CDK8 gene (rs17083838/ rs7992670) haplotypes	Frequency		OR (95% CI)	P value
	Cases	Controls		
A A	61.59 (0.090)	43.03 (0.059)	1.576 (1.052–2.361)	0.026518*
A G	90.41 (0.133)	70.97 (0.098)	1.411 (1.014–1.962)	0.040427*
G A	278.41 (0.408)	271.97 (0.375)	1.152 (0.930–1.427)	0.196346
G G	251.59 (0.369)	340.03 (0.468)	0.663 (0.536–0.821)	0.000159*

Haplotypes with frequency less than 3.0% were not analyzed; *, significant P value after multiple testing adjustments (P<0.05). DCM, dilated cardiomyopathy; OR, odd ratio; CI, confidence interval.

Survival analysis

Survival analysis was performed to reveal the correlation between DCM prognosis and the two SNPs (rs17083838 and rs7992670) of the *CDK8* gene. In this study, we continuously tracked a total of 153 DCM patients with contact information (males/females, 106/46; mean age, 52.35±12.269 years) for a mean period of 55.36±23.170 months. All included DCM patients received continuous medication treatment during the follow-up period, and none of them underwent heart transplantation. Eighteen (11.8%) DCM patients were withdrawn due to loss to follow-up, 89 (58.2%) DCM

patients died, and 46 (30.1%) were alive at the end of our follow-up.

Kaplan-Meier curves indicated that the AA genotype of *CDK8* rs17083838 was associated with a worse prognosis for DCM patients in the dominant model (Log- rank: P=0.020, *Figure 1*). For rs7992670, the AA genotype was associated with a worse prognosis of DCM patients in the recessive model (log-rank: P<0.001, *Figure 2*). In the univariate Cox regression analysis, a positive correlation was found between the genotypic frequencies of rs17083838 and the overall survival (OS) of DCM patients in the dominant model (HR: 1.623, 95% CI: 1.069–2.464, P=0.023), with the A carriers
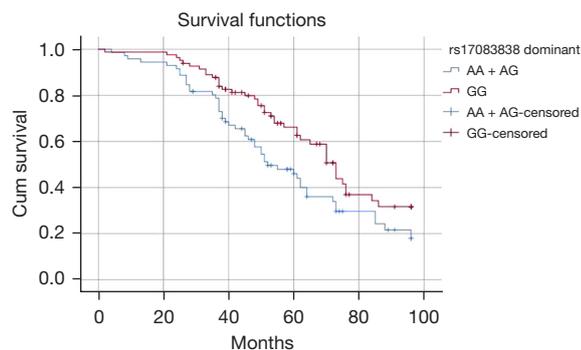
Figure 1 Relation of AA genotype of rs17083838 and the worse prognosis of DCM patients in the dominant model (log-rank: P=0.02). DCM, dilated cardiomyopathy.
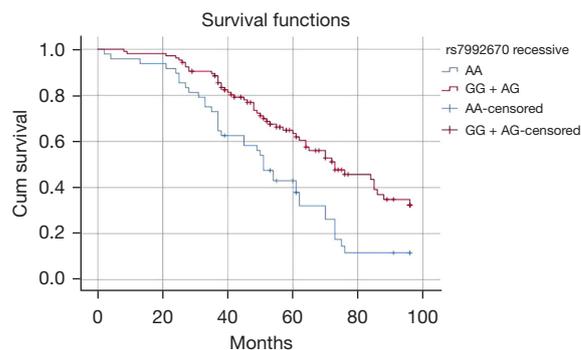

Figure 2 Relation of AA genotype of rs7992670 and the worse prognosis of DCM patients in the recessive model (log-rank: P<0.001).

Table 6 Association between *CDK8* SNPs and overall survival in DCM patients

Characteristic model	Genotype	Overall survival					
		Univariate survival analysis			Multivariate survival analysis*		
		HR	95% CI	P value	HR	95% CI	P value
rs17083838							
Dominant	GG	1.00	–	–	1.00	–	–
	AA/AG	1.623	1.069–2.464[#]	0.023[#]	1.539	1.013–2.340[#]	0.043[#]
rs7992670							
Recessive	GG/AG	1.00	–	–	1.00	–	–
	AA	2.054	1.347–3.133[#]	0.001[#]	2.146	1.405–3.280[#]	<0.001[#]

[#], values indicate statistical significance; *, adjusted for gender, age, LVEDD, and LVEF. SNP, single nucleotide polymorphism; DCM, dilated cardiomyopathy; HR, hazard ratio; CI, confidence interval.

(AA/AG) associated with a worse prognosis. Meanwhile, the AA genotype of rs7992670 in the recessive model had a worse prognosis among DCM patients (P=0.001; HR: 2.054; 95% CI: 1.347–3.133). These results were identical to those of the Kaplan-Meier analysis. The results remained statistically significant in the multivariate Cox analysis after adjusting for age, gender, LVEDD and LVEF (*Table 6*). Additionally, the Bonferroni correction was applied for multiplicity comparison. All the above data remained significant after the Bonferroni correction (P<0.025).

Discussion

The pathogenic mechanism of DCM remains unclear and is a research hotspot in cardiology. Numerous genes have been reported to be related to the susceptibility and prognosis of DCM. Determining the exact gene responsible

for DCM is necessary on clinical grounds, since various pathological mechanisms lead to DCM (3). Recently, several TFs that respond to developmental, mechanical, metabolic, and extracellular cues have been identified to be associated with regulating cardiac remodeling.

The *CDK8* gene is composed of 13 exons and is located at chromosomal region 13q12.13. It encodes 464 amino acids to form a 53-kDa major isoform, which is an evolutionarily conserved serine/threonine kinase (8). CDK family members are known to play critical roles in different biological processes, such as metabolism, angiogenesis, spermatogenesis, neuronal differentiation, hematopoiesis, stem cell self-renewal, and carcinogenesis (21). As a special subtype of the CDK family, CDK8 has been suggested to act as both a positive and negative regulator of transcription (22).

CDK8 is a component of the Mediator complex, which is a large multi-subunit complex that plays a central role

in the transcription regulation of eukaryotes. CDK8 and its regulatory subunits, MED12, MED13, and cyclin C, constitute the Mediator transcriptional regulatory complex. The Module is a 4-subunit complex that reversibly binds to the Mediator and regulates its activity by changing the structure and function of the core Mediator. The Mediator serves as a bridge in both transcriptional activation and repression by phosphorylation of the carboxy-terminal domain of the largest subunit of RNA polymerase II. The phosphorylation targets of CDK8 include the core Mediator-RNA-polymerase-II complex and many other transcriptional regulators, such as tissue-specific and general TFs, chromatin-remodeling factors, pause or release factors, and histone H3 (23).

Most previous research has focused on the role of human *CDK8* in carcinogenesis as an oncogene, and its function to be a therapeutic target in many cancers (24). It has been demonstrated that the *CDK8* gene is frequently amplified in colorectal cancer (CRC) (25,26), and overexpression of *CDK8* has also been demonstrated in the pathogenesis of breast cancer, ovarian cancer (27), melanoma (28), acute myeloid leukemia (29), and prostate cancer (30). CDK8 modulates β-catenin-mediated transcription directly or indirectly. Knockdown of *CDK8* induces aberrant activation of the Wnt/β-catenin signaling pathway in CRC. However, the role of *CDK8* in cancer is context-dependent. For example, in contrast to the findings above, deletion of *CDK8* in a murine intestinal tumor model induced increased tumor size and growth rate, which resulted in reduced survival (24). Although many studies have reported the successful development of effective CDK8 inhibitors, many of them have shown unacceptable toxicity (31). The toxicity of these CDK8 inhibitors might provide insights into the essential functions of CDK8 in the process of normal development (8). Several *in vitro* and *in vivo* experiments have demonstrated that deficiency of CDK8 causes developmental arrest of embryos through transcriptional deregulation of developmentally critical genes, resulting in embryonic lethality (32).

A recent study reported by Calpena *et al.* (8) firstly assessed 12 patients with 8 heterozygous nonsynonymous variants of *CDK8*. All predicted substitutions localized at the ATP-binding pocket of the kinase domain, which led to a decrease in the phosphorylation level of the CDK8 substrate, STAT1-Ser727. Six of the 12 subjects suffered congenital heart defects (CHDs), classified as left ventricular obstruction and conotruncal heart anomaly. They pointed out that the developmental disorder caused by missense mutations in

CDK8 has overlapping phenotypes with symptoms related to mutations of other subunits of the Mediator kinase module, thus indicating that there might be overlap in pathogenic mechanisms. Uehara *et al.* (7) reported 2 cases of *CDK8*-associated disorder involving two *de novo* missense mutations also within the kinase domain of *CDK8*, which suggested that craniofacial and cardiac defects are critical components of the *CDK8*-related disorder. A kinase assay of human CDK8 showed decreased kinase activity. A zebra fish model was used for functional analysis, which showed that non-synonymous variants in the kinase domain of *CDK8* act as hypomorphic alleles that can cause human craniofacial and heart defects. These data demonstrate the essential role of *CDK8* in prenatal development and cardiovascular disease. Hall *et al.* (12) found that CDK8 protein expression levels increased in human failing heart explants and identified that the same heart failure phenotype was present in cardiac-specific *CDK8*-expressing mice. Myofilament gene isoforms not typically expressed in adult cardiomyocytes were observed to be enriched, while oxidative phosphorylation and fatty acid biosynthesis genes were decreased. Interestingly, the expression levels of candidate upstream TFs and MAPK signaling pathways are considered to regulate cardiomyocyte size remained relatively unaffected. Their research revealed that manipulating cardiac gene expression through increased CDK8 levels is detrimental to the heart by novel differential regulation of a transcriptional profile enriched in sarcomeric and metabolic gene expression pathways, which induces pathological remodeling and eccentric hypertrophy in heart failure. Moreover, the tension-based model related to *CDK8* may be able to predict DCM since *CDK8* transgenic cardiomyocytes can re-express fetal myofilament genes, compromise the sarcomeric structure, and introduce cardiac dilation.

Furthermore, several previous studies have demonstrated that CDK8 plays a significant role as a regulator in several signaling pathways, including WNT, TGFβ/BMP, STAT1, SHH, and NOTCH (10,11), most of which have been reported to be associated with the pathogenesis of DCM, especially TGFβ/BMP. The TGFβ family of cytokines are important regulators of cell cycle, differentiation, apoptosis, and inflammation (33). CDK8 enhances the transactivation potential and turnover of receptor-regulated Smad proteins in the TGFβ/BMP pathways (34). The abnormal expression of TGFβ may mediate myocardial injury through immunizing inflammation, and can cause the occurrence and development of DCM by signal pathway dysfunction. The apoptosis of cardiomyocytes plays a key role in the

progression of DCM-induced heart failure, while the AP-1/ Smad signaling pathway has been identified as the final common pathway of cardiac myocyte apoptosis. Moreover, Euler-Taimor and Heger (35) revealed that myocardial fibrosis and myocardial cell apoptosis are the consequences of negative regulation of inhibitory SMADs proteins, which are activated by the TGFβ family. CDKs have been proven to be vital regulators of cell cycle progression and RNA transcription. It has been shown that several genetic and epigenetic events cause universal overactivity of cell CDKs, and their inhibition can lead to both cell cycle arrest and apoptosis (36). Therefore, once the biological equilibrium of CDK8 activity has been deregulated, there might be modification of these developmental signaling networks related to the pathogenesis of DCM.

The A genotype of both rs17083838 and rs7992670 were found to be risk factors of DCM in our study. The rs17083838 polymorphism has been linked with pituitary adenoma (37), but the clinical significance of rs7992670 has not yet been examined. Although the 2 SNPs are located in the intron regions of *CDK8*, they may affect the RNA secondary structure, which may cause abnormal gene regulation and phenotypes (38). It has been confirmed that introns and noncoding RNAs serve as endogenous network control molecules, which allow direct communication between genes and multitasking in eukaryotic genomes (39). In addition, CDK8 plays a vital role in the process of RNA transcription. Taken together, the two SNPs may cause DCM through the deregulation of gene-gene communication rather than changing the encoded protein. According to the results of our survival analysis, the two SNPs found within the *CDK8* gene might be related to the poor prognosis of DCM.

Nevertheless, the prognosis of DCM is associated with an LVEF <35%, right ventricular involvement and NYHA functional class III or IV. Furthermore, various factors can worsen the prognosis of DCM, such as functional mitral regurgitation, ventricular arrhythmia, myocardial fibrosis, dyssynchronous ventricular contraction, and enlargement of other chambers. Exogenous factors, such as enteroviruses, have also been related to poor prognosis (40). The treatments for DCM mainly focus on reducing symptoms of heart failure and improving cardiac function. Management can also affect the prognosis. All the patients in this study were treated under the guidance of clinical guidelines. The current survival analysis showed that the two SNPs were related to a worse prognosis. This result reveals that genotypic variations might also be prognostic factors of DCM.

Consequently, *CDK8* is a possible biological candidate gene that plays a vital role in the pathogenesis of some cardiovascular diseases, including DCM. However, up to now, no study or analysis has mentioned which SNPs of *CDK8* are associated with susceptibility to DCM. We firstly defined the correlation between *CDK8* SNPs (rs17083838 and rs7992670) and DCM in a Chinese Han population. However, due to the small sample size, regional differences, and ethnic differences, this study still has some limitations. The main results were obtained based on a regression model that established a relationship between SNPs of *CDK8* and DCM. In the absence of identifying specific pathophysiological links between genotypes and phenomena, this model cannot be recognized as adequate. Furthermore, various factors can affect the prognosis of DCM. Hence, the positive results do not necessarily mean that the pathogenic SNPs have been found. Further studies of the pathogenic mechanisms with stricter screening, larger samples sizes, and more races are indispensable.

Conclusions

Taken together, we firstly revealed that SNPs rs17083838 and rs7992670 of the *CDK8* gene might be associated with the higher DCM risk and worse prognosis in the Chinese Han population. In China, the Han population is the largest ethnic group, which is representative. The *CDK8* gene is currently a research hotspot. *CDK8* gene polymorphisms might be candidate risk factors for DCM in Chinese. Therefore, larger sample sizes of *CDK8* SNPs in multiple populations would be required to confirm our findings. More studies are also necessary to investigate the specific mechanistic link between *CDK8* and DCM, which may potentially promote the development of novel genetic therapies that involving *CDK8*.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the West China Hospital of Sichuan University ethics committee (No. 81670346) and individual informed consents for this retrospective analysis were obtained.

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