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Polymorphisms of *CD247* gene is associated with dilated cardiomyopathy in Chinese Han population

Chunmei Li[1], XiaoChuan Xie[1], Kun Li[2] and Li Rao[1*]

Abstract

Background Dilated cardiomyopathy (DCM) is a major cause of heart failure and heart transplantation. Recently, some studies have reported that the autoimmune response in myocardial cells might be related to the pathogenesis of DCM. The *CD247* gene has been previously found to be involved in autoimmune disease. Therefore, our study aimed to clarify the hypothesis that there is a certain linkage between polymorphisms of the *CD247* gene and the triggering of DCM risk.

Methods In the present study, two single nucleotide polymorphisms (SNPs) of the *CD247* gene, rs12141731 and rs858543, were genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) in 355 DCM patients and 404 age- and sex-matched controls.

Results Pearson's chi-squared test for the *CD247* gene revealed that SNP rs858543 ($p=0.001$, OR = 0.72, 95% CI = (0.588–0.882), but not SNP rs12141731, was associated with DCM in the Chinese Han population. Haplotype analysis revealed that the CC haplotype was associated with increased DCM susceptibility, while CT was a protective haplotype. Cox multivariate survival analysis indicated that the rs858543 TT genotype (HR: 0.608, 95% CI = 0.402–0.921, $p=0.019$) was an independent multivariate predictor for longer overall survival in DCM patients. *CD247* mRNA expression levels were significantly decreased in DCM patients ($p=0.02$).

Conclusions Our study suggested that a polymorphism in the *CD247* gene may be a risk factor for DCM in the Chinese Han population.

Trial registration ChiCTR2000029701.

Keywords *CD247*, Genetic polymorphisms, Dilated cardiomyopathy

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Introduction

Dilated cardiomyopathy (DCM) is characterized by the enlargement of one or both ventricles and systolic dysfunction with reduced ejection fraction [1, 2]. In the late stages of DCM, the major clinical manifestations include progressive congestive heart failure, arrhythmias, thromboembolism, and sudden death [3, 4]. The morbidity and mortality of DCM are very high [5]. The main goal of treatment measures, which include beta-blockers, ACE inhibitors, angiotensin-II receptor antagonists, diuretics, aldosterone antagonists, digitalis and resynchronization therapy, is to alleviate symptoms and myocardial damage in DCM patients. Many patients may eventually need to undergo heart transplantation to sustain life [3]. Therefore, the study of etiologies is very important for the diagnosis and treatment of DCM. However, the etiology of DCM, despite years of study, remains unclear [6]. Previous researchers have reported that genetic factors, uncontrolled apoptosis, toxicity and metabolism may play important roles in the pathogenesis of DCM [5, 7]. Recently, several studies have shown that immune abnormalities are becoming more obvious in the triggers of DCM by the following points [5, 7–11]. First, previous studies have shown that some pathologically related cardiac-specific autoantibodies, such as cardiac troponin I, the β1-adrenoceptor and α-myosin, are related to DCM [12–14]. Second, myocarditis caused by viral infection may be linked to DCM, and 67% of DCM have been related to viral genomes in large samples of endomyocardial biopsy [15]. Third, more than 150 individuals with DCM who underwent EMB had interstitial and endothelial immune activation [8]. Furthermore, infiltration of CD4+and CD8+T cells has also been detected in patients with DCM by endomyocardial biopsy. In addition, DCM mice exhibit myocardial fibrosis and an increase in the left ventricular end-diastolic dimension via the transfer of lymphocytes [8–10]. This evidence strengthens the view that immune abnormalities may play an important role in the pathogenesis of DCM.

Currently, related studies have reported that the *CD247* gene is involved in many autoimmune-related disorders, such as systemic lupus erythematous (SLE) [16], rheumatoid arthritis [17], and immunodeficiency virus infection [18], via the CD4+T-cell-mediated immune pathway. Previous researchers have shown that CD4+T cells are significantly increased in DCM patients during inflammation, and the proportion of CD4+T cells is obviously linked to systolic dysfunction and increased end-diastolic volume, which is similar to the changes in DCM patient hearts [10, 19–21]. A previous study also showed that the *CD247* gene may be involved in cardiovascular disease, such as hypertension, by altering T lymphocyte infiltration [22]. In addition, the *CD247* gene encodes CD3ζ protein. Research suggests that genetic variation in the *CD247* gene is closely related to the regulation of disease cell apoptosis, which may regulate the cellular inflammatory response and apoptosis through the expression of the ZAP-70 protein and/or related cytokines and may participate in the occurrence and development of diseases [23]. Therefore, we speculate that the *CD247* gene is related to the regulation of cell apoptosis and that its encoded protein may regulate cell proliferation, differentiation, apoptosis, oxidative stress, inflammation, etc., through ZAP-70 and downstream MAPK and/or PI3K/Akt pathways, participating in the occurrence and development of DCM.

Furthermore, it has been reported that SNPs rs12141731 and rs858543 in the *CD247* gene are significantly linked to SLE [24, 25]. These results indicate that *CD247* gene polymorphisms may be genetic factors that affect susceptibility to some cardiovascular diseases, such as DCM, caused by CD4+T-cell-mediated abnormalities in immune pathways.

Based on the above findings, we hypothesized that there is a certain linkage between polymorphisms of the *CD247* gene and DCM risk. To test this hypothesis, we investigated two single nucleotide polymorphisms (SNPs) (rs12141731 and rs858543) that were identified as SNPs in the International HapMap project (http://hapmap.ncbi.nlm.nih.gov/) in the *CD247* gene in patients with DCM by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) techniques. We also discuss the associations of established clinical prognostic factors with survival in DCM patients in the present study.

Subjects and methods

Subjects

This study included 355 unrelated DCM patients from the West China Hospital of Sichuan University between 2006 and 2022. The clinical diagnosis of DCM was determined by using the revised criteria established by the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Classification of Cardiomyopathy (DCM group) [26] from 2003 to 2007; the clinical diagnosis of DCM was determined by using the 2006 American Heart Association (AHA) criteria after 2007 [27]. Patients with hypertension, tachyarrhythmia, coronary heart disease, valvular heart disease, acute viral myocarditis, heavy alcohol intake, Emery–Dreifuss muscular dystrophy, skeletal myopathies, Parkinson's disease, Alzheimer's disease, or systemic diseases of putative autoimmune origin were intentionally excluded. The control group also included four hundred and four healthy unrelated individuals according to a routine health survey. This study used PASS15.0 software to evaluate the expected sample size and calculated the expected sample size to be 312 patients based

on the expected population standard deviation (σ=0.9) and allowable error (δ=0.1). The case group (n=355) and control group (n=404) included in this study met the expected sample size requirements.

All subjects were Han individuals who lived in the Sichuan Province of southwestern China. The study was supported by the hospital Ethics Committee. Written informed consent was obtained from all participants. The trial has been registered on the Chinese Clinical Trial Registry. (Clinical Trial Number: ChiCTR2000029701)

Patient follow-up

A total of 175 patients who left a telephone number were scheduled for follow-up every three months. The clinical follow-up was performed in a blinded manner without respect to the patient's genetic status. The end point during follow-up was cardiac death, which included death due to sudden cardiac or death pump failure. Conventional echocardiography was conducted in all patients using Philips Sonos7500 and iE33 echocardiography systems (Philips Medical Systems, Bothell, WA, USA). The LVEF (left ventricular ejection fraction) was measured using the biplane Simpson method using images acquired in the apical four-chamber and apical two-chamber views. Serum brain natriuretic peptide (BNP) levels were measured by enzyme-linked immunosorbent assay (ELISA) kits in the laboratory department of West China Hospital, Sichuan University.

DNA isolation and genotyping

Genomic DNA was extracted from 200 μl of EDTA-anticoagulated peripheral blood samples by using a DNA isolation kit from Bioteke (Peking, China). The primers and restriction enzymes used in the genotyping analysis are displayed in Table 1. The sequence of the rs12141731 locus was as follows: upstream primer, 5'-CCACCCGA CTGGAGTCAA-3'; downstream primer, 5'-GAAGAC CCGCCTTCTTTCTT-3'. The sequence of the rs858543 locus was as follows: upstream primer, 5'-CAGATGGG CAGGATAGGAAG-3'; downstream primer, 5'-TTTCTC CCTGGTTCCTGCTA-3'. PCR-RFLP was performed as follows: 50 ng of genomic DNA was amplified in a 25 μl reaction mixture containing 75 mM Tris-HCl (pH 9), 1.5 mM MgCl2, 150 mM KCl, 2 mM (NH4)SO4, 200 pmol dNTPs, 10 pmol of each primer and 1.5 U of Taq (Biomede, China). The PCR conditions were as follows: 94 °C for 5 min; 36 cycles of 94 °C for 30 s, 62.2 °C for 45 s, and 72 °C for 55 s; and a final extension at 72 °C for 10 min (Eppendorf, Germany). The PCR products were digested with the corresponding restriction enzymes (HincII and MboI, New England Biolabs, MA, USA) for 2 h at 37 °C and analyzed on 6% polyacrylamide gels via silver staining. Approximately 20% of the samples were randomly selected for the repeated assays. The C allele of the rs12141731 SNP locus can be cleaved by HincII restriction endonuclease, while the T allele cannot be cleaved. Therefore, the CC genotype shows two fragments (including 145 bp and 16 bp), while the CT heterozygous genotype has three fragments (including 161 bp, 145 bp, and 16 bp), while the TT genotype only has one fragment of 161 bp (Fig. 1a). The T allele of the rs858543 SNP locus can be cleaved by MboI restriction endonuclease, while the C allele cannot be cleaved. Therefore, two fragments (including 126 bp and 34 bp) can be seen in the TT genotype, three fragments (including 160 bp, 126 bp, and 34 bp) in the CT genotype, and only one fragment of 160 bp in the CC genotype (Fig. 1b).

Table 1 Polymorphic SNPs markers, PCR primers, restriction enzymes and corresponding alleles

Marker	Primer sequence (5'- -3')	Product (bp)	Annealing temperature(^{0}C)	Restriction enzyme	Allele(bp)
rs12141731	CCACCCGACTGGAGTCAA	161	37	HincII	T (161)
	GAAGACCCGCCTTCTTTCTT				C (145, 16)
rs858543	CAGATGGGCAGGATAGGAAG	160	37	MboI	C (160)
	TTTCTCCCTGGTTCCTGCTA				T(126, 34)

CD247 mRNA determination

CD247 mRNA expression data were available for analysis for 51 patients and 55 controls. Total RNA was extracted and purified from blood samples using TRIzol® Reagent (Life Technologies, USA) according to the manufacturer's protocol. Reverse transcription-PCR (RT–PCR) was performed with a one-step RT–PCR kit according to the manufacturer's instructions (Bioneer, South Korea). Quantitative real-time PCR was carried out using SYBR Green PCR Master Mix (Roche, Switzerland), and the samples were amplified in a thermocycler as follows: 95 °C for 10 min (1 cycle), 95 °C for 15 s, 60 °C for 30 s (60 cycles), 72 °C for 45 s, 60 °C for 20 min and 95 °C for 15 s (1 cycle). The sequences of the primer pairs for *CD247* and their predicted sizes were as follows: sense, A TGGGCTCCCTCTCATCAGT; antisense, GCTTGGTG GTTTGCTACGAC (106 bp). The data were normalized for beta-actin (β-actin) expression using the comparative threshold cycle method. Triplicate Ct values were

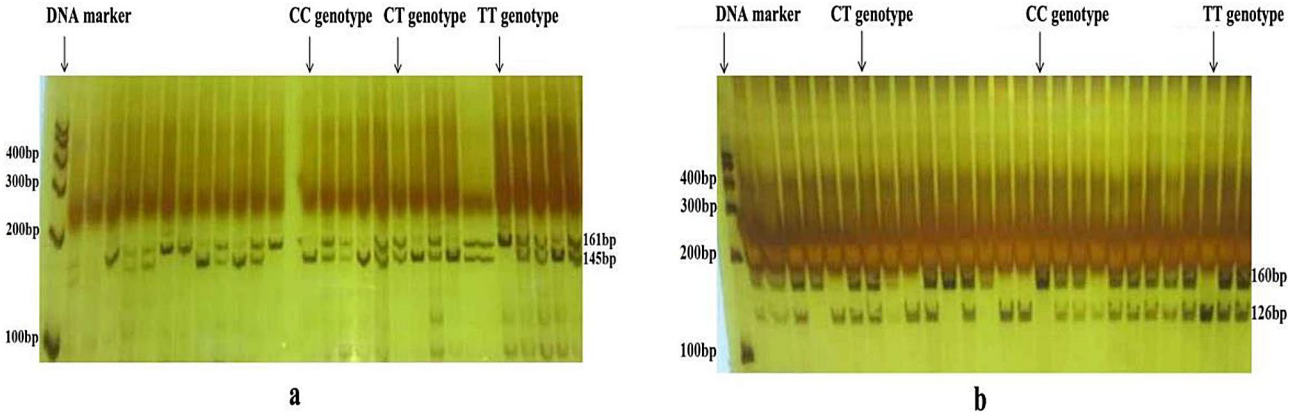

Fig. 1 **a** Genotyping of *CD247* gene rs12141731 SNP site after HincII digestion. (The 16 bp fragment has run out of the swimming lane due to its too small.) **b** Genotyping of *CD247* gene rs858543 SNP site after MboI digestion. (The 34 bp fragment has run out of the swimming lane due to its too small)

averaged, and the relative expression levels were determined as $2^{-\Delta\Delta Ct}$.

Statistical analysis

All the statistical analyses were performed using SPSS ver. 22.0 (SPSS, IBM). Pearson's chi-squared test and Student's t test were applied to compare categorical variables (gender) and continuous variables (age, systolic blood pressure, diastolic blood pressure, heart rate, left ventricular end-diastolic diameter, left ventricular end-diastolic volume, left ventricular ejection fraction, and brain natriuretic peptide). Genotype frequencies of these two SNPs were obtained by directed counting, and the allele frequencies were calculated by dividing the total allele counts by the quantity of objects. Hardy–Weinberg equilibrium was established by the chi-square test. The Pearson χ2 method was used to test whether the genotype frequency distribution of each SNP locus conformed to Hardy–Weinberg equilibrium. The Pearson chi-squared test was also applied for comparison of the genotype frequencies and the allele frequencies between the DCM and control groups. Accordingly, odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to assess the effects of any difference between genotypes or alleles. Genotypic association tests were performed by SNPstatsin, a case–control pattern presuming codominant, dominant, or log-additive genetic models [28]. The linkage disequilibrium (LD) between polymorphisms was quantified using SHESIS software. (Available online: http://analysis.biox.cn/myAnalysis.php). Survival rates were calculated using the Kaplan–Meier method, with differences assessed by the log rank test. Univariate and multivariate Cox proportional hazards regression analyses were performed to evaluate the associations of genetic and clinical variables with the end point of cardiac death. *CD247* mRNA expression levels were compared between patients and controls with Mann–Whitney nonparametric tests. Statistical comparisons of the relative expression of mRNA between the different genotypes of the two SNPs in the gene were performed with the Kruskal–Wallis and Mann–Whitney nonparametric tests in DCM samples. Continuous data are presented as the means ± SDs. In all analyses, $p<0.05$ was considered to indicate statistical significance.

Table 2 Baseline characteristics of controls and dilated cardiomyopathy (DCM) patients

Variable	Patients($n=355$)	Control($n=404$)
Gender (male/female)	217/138	249/155
Age (years)	51.89 ± 14.92	54.85 ± 13.41
NYHA	II: 43; III: 229; IV: 83	I: 345; II:59
SBP (mmHg)	103.27 ± 13.76*	123.23 ± 8.76
DBP (mmHg)	68.19 ± 10.16*	83.78 ± 4.82
Heart rate, beats/min	79.33 ± 9.27*	71.53 ± 10.29
LVEDV(mL)	216.48 ± 81.84*	97.35 ± 17.23
LVEF (%)	34.12 ± 12.51*	67.62 ± 5.99
BNP(pg/mL)	4411.59 ± 2495.31*	101.62 ± 55.96

NYHA, New York Heart Association; SBP, systolic blood pressure; DBP, diastolic blood pressure; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; BNP, brain natriuretic peptide

* Patients vs. Controls

$p<0.05$. Data are presented as the mean ± SD

Results

Baseline clinical and echocardiographic characteristics

The baseline clinical and echocardiographic characteristics of all participants are displayed in Table 2. There was no significant difference between DCM patients and controls in sex or age ($p>0.05$). A significantly increased heart rate left ventricular end-diastolic volume (LVEDV), and BNP and decreased LVEF, systolic blood pressure (SBP), diastolic blood pressure (DBP) ($p<0.05$) and a more severe NYHA functional class were detected in DCM patients.

Distribution of allele frequencies and genotypes in patients with DCM

The genotype distributions of these two SNPs of *CD247* in the DCM and control groups met the assumption of the Hardy–Weinberg equilibrium ($p>0.05$), indicating that the included study subjects were representative of the population. We analyzed the allele frequencies of the two SNPs by using the chi-squared test in the DCM and control groups. The distributions of the allele frequencies and genotypes of the two SNPs are summarized in Table 3. Compared with those in the control group, the frequency of the T allele decreased in the DCM group (0.5 vs. 0.58, OR=0.72, 95% CI=0.588–0.882, $p=0.001$) at SNP rs858543. However, there was no significant difference between the two groups for the allele frequencies of the SNP rs12141731 (OR=0.977, 95% CI=0.799–1.196, $p=0.824$). The results of genotypic association tests performed by SNPstatsin are also summarized in Table 3. These results indicated that rs858543 in *CD247* is related to DCM in a dominant genetic model ($p=0.01$, OR=0.66, 95% CI=0.48–0.91), while rs12141731 in CD247 is not associated with DCM in the Chinese Han population.

Linkage disequilibrium and haplotype analysis

LD was conducted for the two variants by using SHESIS software (available online: http://analysis.biox.cn/myAnalysis.php). The SNPs rs12141731 and rs858543 were not in linkage disequilibrium (D′=0.072, $r^2=0.004$). We further analyzed four haplotype combinations. We found a significant association in the distribution of two haplotype frequencies (CT and CC) between cases and controls ($p<0.05$) (Table 4). The CC haplotype was significantly associated with increased DCM susceptibility, while CT was a protective haplotype.

Cox regression analysis of cardiac death in DCM patients

Survival analysis of patients with the two SNPs of the *CD247* gene revealed that 175 DCM patients were followed for a mean period of 43.45±21.54 months. During follow-up, all patients received continuous medication treatment, and no patients underwent heart transplantation. One hundred thirty-two patients (75.4%) ,died 35 because of sudden cardiac death and 97 because of pump failure. Univariate analysis demonstrated that patients with the rs858543 TT genotype had longer overall

Table 3 Allele frequencies and genotype frequencies of SNPs in *CD247* gene among DCM patients and controls and their association with DCM risk

SNP		Patients *n*=355(46.77%)	Controls *n*=404(53.23%)	OR (95%CI)	*p* value
rs12141731	**Allele**				
	C	374(0.53)	421(0.52)	0.977(0.799–1.196)	0.824
	T	336(0.47)	387(0.48)		
	Genotype				
	C/C	94 (26.5%)	105 (26%)	1.00	
Codominant	C/T	186 (52.4%)	211 (52.2%)	1.02 (0.72–1.43)	0.97
	T/T	75 (21.1%)	88 (21.8%)	1.05 (0.69–1.59)	
Dominant	C/C	94 (26.5%)	105 (26%)	1.00	
	C/T-T/T	261 (73.5%)	299 (74%)	1.03 (0.74–1.42)	0.88
Recessive	C/C-C/T	280 (78.9%)	316 (78.2%)	1.00	
	T/T	75 (21.1%)	88 (21.8%)	1.04 (0.73–1.47)	0.83
Overdominant	C/C-T/T	169 (47.6%)	193 (47.8%)	1.00	
	C/T	186 (52.4%)	211 (52.2%)	0.99 (0.75–1.32)	0.9
rs858543	**Allele**				
	T	357(0.5)	472(0.58)	**0.72(0.588–0.882)**	**0.001**
	C	353(0.5)	336(0.42)		
	Genotype				
	T/T	87 (24.5%)	133 (32.9%)	1.00	
Codominant	C/T	183 (51.5%)	206 (51%)	**0.74 (0.53–1.03)**	**0.0051**
	C/C	85 (23.9%)	65 (16.1%)	0.50 (0.33–0.76)	
Dominant	T/T	87 (24.5%)	133 (32.9%)	1.00	
	C/T-C/C	268 (75.5%)	271 (67.1%)	**0.66 (0.48–0.91)**	**0.01**
Recessive	T/T-C/T	270 (76.1%)	339 (83.9%)	1.00	
	C/C	85 (23.9%)	65 (16.1%)	**0.61 (0.42–0.87)**	**0.0067**
Overdominant	T/T-C/C	172 (48.5%)	198 (49%)	1.00	
	C/T	183 (51.5%)	206 (51%)	0.98 (0.74–1.30)	0.88

Significant *p* values after multiple testing adjustment ($p<0.025$) are shown in italic bold; SNP, single nucleotide polymorphism; DCM, dilated cardiomyopathy; OR, odd ratio; CI, confidence interval

Table 4 Haplotypefrequencies of the *CD247* gene in the patients with DCM and in controls

CD247 gene (rs12141731/ rs858543) haplotypes	Frequency	OR (95% CI)		*p* Value
CT	173.50(0.244)	237.23(0.294)	0.778(0.619–0.978)	0.031
CC	200.50(0.282)	183.77(0.227)	1.337(1.060–1.685)	0.014
TT	183.50(0.258)	234.77(0.291)	0.851(0.678–1.067)	0.162
TC	152.50(0.215)	152.23(0.188)	1.178(0.917–1.515)	0.200

Haplotypes with frequency less than 3.0% were not analyzed; significant *p* value after multiple testing adjustment ($p<0.05$) is shown in italic bold; OR, odd ratio; CI, confidence interval

survival than did those with the CT+CC genotype (HR: 0.632, 95% CI=0.423–0.943; *p=0.025*) (Fig. 2a). Additionally, female sex (HR: 1.235, 95% CI=0.833–1.832, $p=0.294$), age (HR: 1.011, 95% CI=0.997–1.025, $p=0.113$), SBP (HR: 0.997, 95% CI=0.982–1.012, $p=0.692$), DBP (HR: 0.992, 95% CI=0.975–1.010, $p=0.401$), heart rate (HR: 1.014, 95% CI=0.992–1.036, $p=0.223$) were not significant predictors of survival in patients with DCM, while NYHA class (HR: 0.585, 95% CI=0.406–0.843, *p=0.004*), LVEDV (HR: 1.003, 95% CI=1.000-1.005, *p=0.003*) and decreased LVEF (HR: 0.976, 95% CI=0.960–0.992, *p=0.003*), BNP (HR: 0.658, 95% CI=0.451–0.961, *p=0* No statistically significant association between the rs12141731 polymorphism and overall survival time was found via univariate survival analysis (Fig. 2b). Variables including SNP rs858543, NYHA functional class, LVEDV, LVEF, and BNP were analyzed in the subsequent multivariate Cox model. Cox multivariate analysis indicated that the SNP rs858543 TT genotype (HR: 0.608, 95% CI=0.402–0.921, *p=0.019*) was an independent multivariate predictor for longer overall survival in DCM patients. However, the NYHA class (HR: 0.621, 95% CI=0.409–0.944, *p=0.026*) together with decreased LVEF (HR: 0.975, 95% CI=0.960–0.991, *p=0.002*) and BNP (HR: 0.605, 95% CI=0.408–0.897, *p=0.012*) were independent multivariate predictors for shorter overall survival in DCM patients (Table 5).

Comparisons of the relative expression of mRNAs between the different genotypes of the two SNPs in *the CD247* gene

CD247 mRNA *relative* expression was significantly lower in the DCM group ($p=0.02$) than in the control group (Fig. 3a). However, there was no relationship between *CD247* mRNA expression and rs12141731 or rs858543 polymorphisms ($p=0.701$ and $p=0.242$, respectively) (Fig. 3b and c).

Discussion

As reported in previous studies, autoimmune abnormalities may be related to the pathogenesis of DCM [8, 9]. The immune system mainly includes cellular immunity, which involves mainly T cells involved in the immune response, and humoral immunity, which involves mainly T cells and B cells involved in the immune response. Normally, both the cellular and humoral immune components of the immune system kill foreign viruses, bacteria, and other materials to protect the body through immune activation. However, an increasing number of studies have confirmed that overactivation of the immune system may trigger a large amount of immune cell activation and ultimately lead to diseases such as systemic lupus erythematous16, rheumatoid arthritis [17], myocarditis and DCM

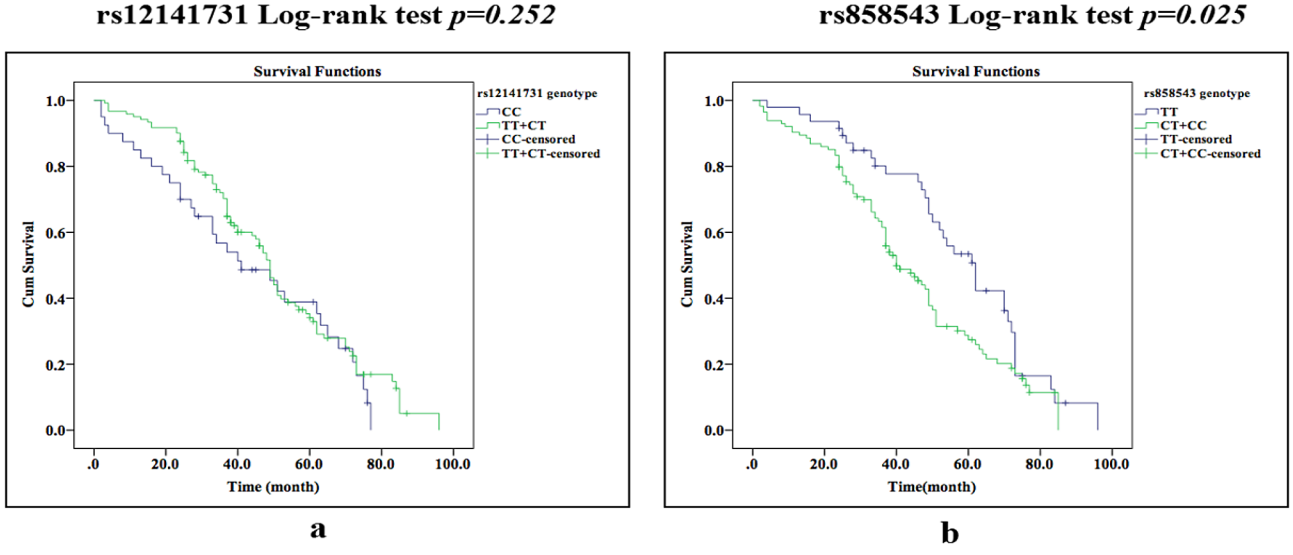

Fig. 2 Kaplan–Meier survival curves free of cardiac death for 175 DCM patients based on rs12141731 and rs858543

Table 5 Cox regression analysis of cardiac death in patients with DCM

Characteristics		Overall Survival					
		Univariate Survival Analysis			Multivariate Survival Analysis		
		HR	95%CI	*p* Value	HR	95%CI	*p* Value
SNP rs731							
Dominant	C/C	1					
	C/T-T/T	0.788	0.523–1.185	0.252			
SNP rs543							
Dominate	T/T	1					
	C/T-C/C	0.632	0.423–0.943	**0.025**	0.608	0.402–0.921	**0.019**
SEX(FEMALE)		1.235	0.833–1.832	0.294			
Age		1.011	0.997–1.025	0.113			
NYHA		0.585	0.406–0.843	**0.004**	0.621	0.409–0.944	**0.026**
SBP (mmHg)		0.997	0.982–1.012	0.692			
DBP (mmHg)		0.992	0.975–1.010	0.401			
HR(beats/min)		1.014	0.992–1.036	0.223			
LVEDV(ml)		1.003	1.000-1.005	**0.003**	1.002	0.999–1.004	0.208
LVEF (%)		0.976	0.960–0.992	**0.003**	0.975	0.960–0.991	**0.002**
BNP * >7897 pg/m		0.658	0.451–0.961	**0.030**	0.605	0.408–0.897	**0.012**

The variables analyzed in the multivariate Cox model included SNP rs7986131, gender, age, NYHA functional class, SBP, DBP, HR, LVEDD, LVEDV and LVEF, BNP; $p<0.05$ was considered to be statistically significant and the values were given in italic bold font; SNP, single nucleotide polymorphism; NYHA, New York Heart Association; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, hazard ratio; CI, confidence interval; LVEDD, left ventricular end-diastolic diameter; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; BNP, brain natriuretic peptide

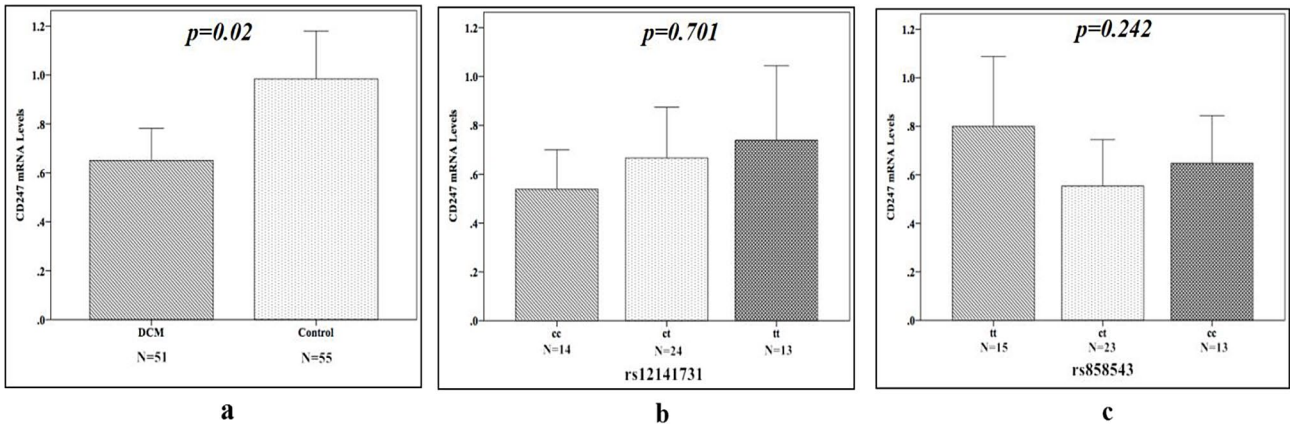

Fig. 3 **a***CD247* mRNA expression was significantly decreased in DCM blood samples ($p=0.02$). **b** No significant relationship was found between *CD247* mRNA expression and polymorphism of rs12141731 in DCM samples($p=0.701$). **c** No significant relationship was found between *CD247* mRNA expression and polymorphism of rs858543 in DCM samples($p=0.242$). *p* value was calculated by Mann–Whitney and Kruskal–Wallis test on log-transformed values

[5, 7]. Previous studies have reported that T cells are vital initiators and mediators in the course of autoimmune myocarditis disease [19, 20]. In addition, infiltration of CD4+T and CD8+T cells has been detected in patients with myocarditis and DCM via endomyocardial biopsies [9, 10, 29]. The role of CD4+T cells, which are considered initiators and markers of myocarditis progression, is much greater than that of CD8+T cells in the progression of autoimmune myocarditis according to immunohistochemistry [9, 10, 30]. An increased proportion of CD4+T cells was obviously related to the deterioration of systolic function and increased end-diastolic volumes in DCM patients [19–21]. Furthermore, previous studies have shown that IL-2 levels are a marker of CD4+T-cell activation [21]. CD4+T-cell clone expansion is accompanied by increased IL-2 levels in patients with DCM [21]. Unregulated IL-2 levels are significantly increased and are considered an independent clinical predictor for the progression of DCM [31]. Therefore, the above findings suggested that the CD4+T-IL-2 pathway may be involved in the pathogenesis of dilated cardiomyopathy through immune responses.

Previous researchers have reported that the *CD247* gene plays an important role in many autoimmune abnormality diseases by regulating human CD4+T-cell differentiation and activation via the CD3 zeta chain [16–18, 32]. The *CD247* gene is mapped to the chromosome at lq24.2 and encodes the T-cell receptor zeta chain

(CD3ζ), which is a component of the T-cell receptor (TCR) signaling machinery and is important for effector CD4+T-cell activation [32]. Many studies have reported that deficient expression of the CD3ζ-chain is linked to many autoimmune abnormality diseases through its involvement in effector CD4+T-cell activation and generation [16–18, 32]. After the loss of CD3ζ protein expression, the expression and phosphorylation levels of related protein tyrosine kinases (ZAP-70 proteins), as well as the expression levels of cytokines such as IL-2, IL-4, IL-10, IL-17 A, IFN-γ, and TNF-α, significantly changed. Cell inflammation and apoptosis are increased, while cell proliferation and differentiation are decreased [33]. When ZAP-70 undergoes phosphorylation and activation, activated ZAP-70 activates downstream related proteins, thereby activating the MAPK and PI3K/Akt signaling pathways [23] and regulating cell proliferation, differentiation, apoptosis, oxidative stress, and inflammation. It is closely related to the regulation of cell proliferation, differentiation, apoptosis, oxidative stress, inflammation, and other factors.

Furthermore, CD3ζ-chain (*CD247* gene) expression is associated with IL-2 leakage in chronic inflammatory disease [34, 35]. In an AngII-induced hypertension mouse model, a previous study revealed that *CD247* gene knockout is involved in the regulation of blood pressure by altering T lymphocyte function [22]. Taken together, the above findings suggest that the *CD247* gene—CD4+T-cell—IL-2-mediated pathway axis may play a vital role in the pathogenesis of some cardiovascular conditions, such as DCM.

However, whether SNPs in the *CD247* gene influence susceptibility to DCM has not been confirmed. This study is the first to show the relationship between DCM and SNPs in the *CD247* gene. In the present study, we evaluated whether the polymorphism of the *CD247* gene is a factor influencing susceptibility to DCM by comparing two SNP loci in DCM patients and normal controls. Therefore, we selected two SNPs (rs858543 and rs12141731) in the *CD247* gene. The two SNP locus are in the 5' region (intron 1) of *CD247*, suggesting a possible role in the regulation of the expression of this gene [21]. However, our results showed that rs858543 was significantly linked to DCM. At this locus, the frequency of the T allele was obviously lower in DCM patients than in normal controls, which suggested that the T allele may be a protective factor against DCM. One possible reason is that mutation of the rs858543 locus is highly important for regulating gene function. However, there was no obvious association between rs12141731 and DCM. One possible reason is that the rs12141731 locus does not affect protein function. Another possible reason is that our study included a small sample size.

Our results showed that CC was significantly associated with increased DCM susceptibility, while CT was a protective haplotype. The results demonstrated that haplotype analysis of the two SNPs might be vital for predicting DCM susceptibility.

Interestingly, patients with the rs858543 TT genotype had longer overall DCM survival, which was also related to decreased DCM risk in the present study. Previous studies have reported that deficient expression of the CD3ζ-chain is linked to autoimmune abnormality diseases such as SLE [16], rheumatoid arthritis [17], some chronic infection diseases [18], hypertension [22] and renal carcinoma [32] via effector CD4+T-cell activation and generation. Studies of the CD4+T-cell pathway in the heart of DCM patients have shown that CD4+T cells are significantly increased in DCM patients during inflammation and that the proportion of CD4+T cells is strongly linked to systolic dysfunction and increased end-diastolic volumes [10, 19–21]. Accordingly, the *CD247* gene-CD4+T-cell-mediated pathway is involved in DCM progression. This result suggested the distinct genetic contributions of the rs858543 TT genotype in controlling the onset and outcome of DCM.

Our results showed that the expression of *CD247* mRNA in the DCM group was significantly lower than that in the control group, which suggested that the *CD247* gene is involved in the triggering of DCM and is consistent with previous findings from other researchers [16–18, 32]. However, we discovered that there was a lack of association between the two SNP genotypes and *CD247* mRNA expression. Two reasons may explain these results. (1) Studies with small sample sizes were included. (2) Other SNPs in the *CD247* gene may participate in the regulation of *CD247* transcription.

Conclusion

In conclusion, the above results suggested that the *CD247* gene may play an important role in triggering DCM in the Chinese Han population. This study is the first to display the relationship between DCM and SNPs in the *CD247* gene. Several issues remain to be resolved in the future: (1) whether *CD247* gene SNPs are linked to the amount of CD4+T cells and IL-2 expressed in DCM; (2) studies should be conducted in other populations to exclude population-oriented association diseases; and (3) studies should be conducted to investigate the molecular mechanisms of the *CD247* gene that are correlated with susceptibility to DCM.

Abbreviations

DCM	Dilated Cardiomyopathy
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
OR	Odds Ratio
CI	Confidence Intervals

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Author contributions

CM.L. wrote the main manuscript text and prepared Tables 2 and 5; Fig. 1. XC.X.prepared Fig. 2; Table 1 . K.L.prepared Tables 3 and 4. L.R. prepared Fig. 3 . All authors reviewed the manuscript.

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Data availability

The datasets generated and/or analysed during the current study are available in the dbSNP repository, the International HapMap project (http://hapmap.ncbi.nlm.nih.gov/). The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. The datasets used and analyzed in the current study are included within the article.

Declarations

Ethics approval and consent to participate

The present study was approved by the hospital Ethics Committee. Written informed consent was obtained from all participants.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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