



## Significance of sarcomere gene mutation in patients with dilated cardiomyopathy

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Genet. Mol. Res. 14 (3): 11200-11210 (2015)

Received January 22, 2015

Accepted May 8, 2015

Published September 22, 2015

DOI <http://dx.doi.org/10.4238/2015.September.22.14>

**ABSTRACT.** Dilated cardiomyopathy (DCM) is a myocardial disease with a high mortality rate. Approximately 40 genes have been found to be associated with DCM to date. Non-familial DCM can also be caused by gene mutations, suggesting that genetic factors were involved in the pathogenesis of DCM; therefore genetic testing is beneficial for the early diagnosis of DCM, which can facilitate the implementation of preventive measures by and within patient's families. Here, we investigated the underlying genetic mutations involved in the cause of patients with DCM. This prospective study included 240 patients with idiopathic DCM and 240 healthy volunteers. Subject clinical data were collected and polymerase chain reaction amplification was carried out on subject DNA for three candidate genes tropomyosin (*TPM1*), cardiac troponin T type-2 (*TNNT2*), and nuclear lamina protein A/C.

Single nucleotide polymorphism (SNP) loci were detected in the *TPMI* (rs1071646) and *TNNT2* (rs3729547) genes, respectively. The genotype distributions and allele frequencies were found to satisfy Hardy-Weinberg equilibrium, which indicated that the group was representative. Statistically significant differences were found between the variant frequencies in the two SNP loci between the Kazakh patients with idiopathic DCM (IDCM) and healthy volunteers. A significant difference in the genotype distributions ( $P=0.000$ ) and allele frequencies ( $P = 0.000$ ) of SNP rs1071646, and another significant difference in the genotype distributions ( $P = 0.000$ ) and allele frequencies ( $P = 0.039$ ) of SNP rs3729547 between Kazakhs with IDCM and Kazakh controls. These results suggest that the *TPMI* (rs1071646) and *TNNT2* (rs3729547) gene variants might represent risk factors for patients with DCM in the Kazakh population.

**Key words:** Dilated cardiomyopathy; Cardiac troponin T type-2 gene; Gene mutation; Single nucleotide polymorphism; Tropomyosin gene; Nuclear lamina protein gene

## INTRODUCTION

Dilated cardiomyopathy (DCM) is a primary myocardial disease with high morbidity and mortality rates in children and adults. Its primary clinical features include the left ventricle or ventricular systolic and diastolic dysfunction, which might result in complications such as chronic heart failure, arrhythmia, sudden death, and cardiovascular diseases. The incident rate of DCM is approximately 30-40 per one lakh persons. Furthermore, the incidence is higher in males than in female, and is 2.5 times higher in blacks than in the white population (Burkett and Hershberger, 2005). Idiopathic DCM (IDCM) is diagnosed if the incidence of DCM is isolated with only a single member of a family involved, and if the cause of the disease is unclear. If at least two closely related family members are diagnosed with sporadic DCM or a young family member were reported to have died of sudden cardiac death, then familial DCM (FDCM) is indicated (Burkett and Hershberger, 2005). To date, 40 genes have been reported to be associated with DCM (Fatkin et al., 2010). Sometimes variety of genetic mutations together leads to the occurrence of DCM. At the same time, the study found that DCM in patients with familial transmissibility is up to 30-50%, and the risk of disease is known to be increased in the families of patients with DCM; therefore early genetic screening should be performed in these families. Furthermore, genetic factors play an important role in the pathogenesis of DCM (Hershberger et al., 2009) as well as in the risk. Therefore, understanding the genetic mechanism and genetic testing are not only beneficial for the early diagnosis of DCM, but also can prevent disease in the patient families through implementation of prophylactic measure.

Most genes associated with the onset of DCM exhibit standard patterns of autosomal dominant, autosomal recessive, and X-linked inheritance (Mestroni et al., 1999). These genes are involved in the encoding of myocardial skeleton, nuclear membrane, cardiac muscle, sarcomere, and mitochondrial proteins (Mogensen et al., 2004). This spectrum of genes is different from that found to underlie hypertrophic cardiomyopathy (HCM) (Richard et al., 2003);

However in 2000, Kamisago et al. (2000) found that the genes, encoding the sarcomere and cytoskeleton were not only associated with HCM, but also with the onset of DCM. Accordingly, genes encoding of tropomyosin (TPM1), myocardial actin, troponin T (TNNT2), troponin I and C, myosin-binding protein, and connectin have subsequently been demonstrated to be associated with IDCM (Møller et al., 2009). In addition, lamin A/C (LMNA) and X-linked muscular dystrophy proteins were also found to be involved in DCM occurrence and their disruption was shown to lead to heart blockage and bone atrophy (Schönberger and Seidman, 2001).

However, the role of these genes in IDCM remains unclear and further study is needed to clarify this issue. In this study, we investigated potential mutational changes of three of the known candidate genes: *TPM1*, *TNNT2*, and *LMNA* along with their associations with patients with IDCM of Kazakh and Han descent in Xinjiang, China.

## MATERIAL AND METHODS

### Ethical approval

This study was reviewed and approved by the Ethical Committee of the First Affiliated Hospital to Xinjiang Medical University, and all participants provided signed written informed consent before study enrollment.

### Subjects

This prospective study was conducted at the Pacing and Electrophysiology Department, First Affiliated Hospital to Xinjiang Medical University, Urumqi, Xinjiang, China. Patients with IDCM enrolled in this study included: 120 Kazakh (48 men and 72 women) and 120 Han (62 men and 58 women) ethnic individuals. The control group consisted of 240 healthy volunteers including 120 Kazakh (56 men and 64 women) and 120 Han (67 men and 53 women) individuals.

The study inclusion criteria were as follows: 1) Patient left ventricular end diastolic dimension (LVEDD) greater than 50 and 55 mm for women and men, respectively; 2) left ventricular ejection fraction (LVEF) and left ventricular decurtation rate less than 45 and 25%, respectively; and 3) expanded cardiac development revealed by X-ray and cardiothoracic ratio results greater than 50%. Clinically, the diagnosis was primary based on echocardiography, X-ray chest radiography, and cardiac isotope, and cardiac computed tomography. Magnetic resonance imaging examination was of diagnostic significance for patients with cardiac localized hypertrophy. The study exclusion criteria included myocardial damage caused by other diseases such as hypertension, coronary, pulmonary, valvular, and congenital heart diseases, alcoholism and tachycardiomyopathy, pericardial, systemic, and neuromuscular diseases as per the "Guidelines for the diagnosis and management of FDCM" (Fatkin and members of the CSANZ, 2011).

### Clinical information

Clinical data included the following: date of birth, gender, nationality, clinical diagnosis, age at diagnosis, family history of cardiovascular disease (myocardial infarction, hypertension, and myocarditis); toxin/drug exposure duration as well as cardiac structure and function

including maximum left ventricular wall thickness, ejection fraction, and area (Lakdawala et al., 2012). All subjects had detailed clinical information including general information, medical history, 12-lead synchronous electrocardiogram, echocardiography, and six-minute walk test, etc.

## Genetic analysis

A 2-mL sample of peripheral blood was collected and DNA was extracted from each subject. Polymerase chain reaction (PCR, China Shanghai Meiji Biological Testing Company) amplification was carried out to determine the exonic sequences of *LMNA* (NM\_001257374.2), *TPMI* (NM\_000366.5), and *TNNT2* (NM\_000364.3), and the amplification products were purified. All gene exon sequences underwent sequencing in both the positive and negative directions followed by analysis using the DNAMAN software, and the results were compared with the standard template sequences using NCBI BLAST and the CHROMAS software to identify the gene mutation loci (amplification and sequencing were accomplished by China Shanghai Meiji Biological Testing Company). Later, the mutation loci were examined with reverse sequencing and re-sequencing for confirmation (Tables 1-3).

**Table 1.** Primer sequences for *TPMI* gene amplification.

Exon	Forward primer (5'-3')	Reverse primer (5'-3')	Amplicon size (bp)	Annealing temperature (°C)
1	ATC TAC GAA TCC ACG GTC	CCC AGC TCA TTG AGT TCA C	1639	53
2	TAT TCG CAG GAG TCT GGT CTG A	AAA CGG CAA AGG TCA CCC	1419	55
3	GGA ATA GGT GGA CGG AAC	GGT AAT AAG GGT CGA CGA	1674	53
4	AGA ACC GTC ATA CTT TGG	CAC CGA TAG GAC TTT GTC	1016	50
5, 6	ACG ATG ACT AAC CGA AGG A	AAG GAA ACC GAA CAG AGG	1805	53
7, 8	ACG ATG ACT AAC CGA AGG A	GAT GTA GTG TCT TCC CTA A	1628	50
9	CTG AGG TTG TCG TTG TAA	GTA GGT GTT CTA TGG TCG AT	813	53

**Table 2.** Primer sequences for *TNNT2* gene amplification.

Exon	Forward primer (5'-3')	Reverse primer (5'-3')	Amplicon size (bp)	Annealing temperature (°C)
1, 2, 3	GTT GAA GCA AGG AAC GGG	TTT TGG TAT TGG TGT TAC GGT A	2001	52
4, 5, 6	AAA CTG ACA GCC GAT GGA	CCC TAT CCC TCG TAA AGG	2068	53
7, 8	GCC AAA GTA ACA AAC CGA	ATG ACC TAC GAG GAA CTF AC	1518	52
9, 10	TGT TGA CCC TCT GTA GTC C	GAG TTG ACC GTG AGT ATA AC	1746	53
11, 12, 13	AGA TTT CGT AGT TCC GT	GGG TGT ATG TGG GTT CCA	1990	51
14, 15	GAA ACT CGT CAT CGT AGG G	GAA CTG GGA GGT AAG GAAA	1699	53

**Table 3.** Primer sequences for *LMNA* gene amplification.

Exon	Forward primer (5'-3')	Reverse primer (5'-3')	Amplicon size (bp)	Annealing temperature (°C)
1	GGG AAAG TCA GGG AAA GAT GA	GCT ATT GAA ACG GTA TCA GAG	1536	53
2	ATG AGT GAA GGG AAA CCG	GAA TAG GAT TAA ACC GAC G	739	50
3, 4, 5	CTC CTA CAA ACT CGT CCC	CTC TTC CCA GCG TCC TAC	1695	52
6, 7	GGT GTA GGA CGC TGG GAA	TCG TTC TAT GTG GGT TCT CG	1883	52
8, 9, 10	CCA CAT AGA ACG AGT TTC CG	GAC CAT TCC TTC CCT CAC C	1111	55
11, 12, 13	ACC AAA CGG ATG ACC CAC	CTA CCC TTA CTC CAC CCT	2424	55

## Statistical analysis

The SPSS 17.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis. Clinical data are reported as means  $\pm$  standard deviation. A Hardy-Weinberg equilibrium test was used to test the population representativeness of the sample. A direct counting method was applied to calculate the genotype distributions and allele frequencies. Chi-square ( $\chi^2$ ) test was used to analyze the differences of genotype distributions and allele frequencies between groups.

## RESULTS

### Clinical information

Statistical analyses on the clinical data of the 240 patients with IDCM and the 240 healthy volunteers were conducted. The distributions of gender and age between the patients with IDCM and the control groups showed no statistical differences. The differences of LVEDD and LVEF between the two groups were statistically significant (Table 4). A Hardy-Weinberg equilibrium test was used to assess the *TPM1* and *TNNT2* gene variants, which indicated that the group was representative of the population (Tables 5 and 6).

**Table 4.** Baseline information.

Ethnicity	Data	Patients	Controls	P
Kazakh	Gender (M/F)	48/72	56/64	0.297*
	Age (years)	46.5 $\pm$ 11.89	48.7 $\pm$ 6.65	0.616
	LVEF (%)	37.2 $\pm$ 4.04	50.8 $\pm$ 4.75	0.000
	LVEDD (mm)	56.4 $\pm$ 6.41	47.9 $\pm$ 6.69	0.001
Han	Gender (M/F)	62/58	67/53	0.517*
	Age (years)	51.6 $\pm$ 9.17	59.1 $\pm$ 7.41	0.060
	LVEF (%)	39.1 $\pm$ 2.84	45.2 $\pm$ 3.04	0.000
	LVEDD (mm)	59.9 $\pm$ 6.80	50.5 $\pm$ 3.24	0.001

\*Does not meet chi-square test conditions; continuity correction was used instead. LVEF = left ventricular ejection fraction; LVEDD = left ventricular end diastolic dimension. This table presents baseline information, including gender, age, LVEF, and LVEDD. The distributions of gender and age between the patients with IDCM and the control groups were not statistically significant. The differences of LVEDD and LVEF between the two groups were statistically significant. Gender, LVEF and LVEDD were represented by the mean and standard deviation, detected by analysis of variance. Age detected by the chi square test.

**Table 5.** Test of Hardy-Weinberg equilibrium for *TPM1* genotypes.

Group	rs1071646 genotype			$\chi^2$	Prob exact test
	C/C	C/A	A/A		
Patients (K)	0 (0)	4 (0.03)	116 (0.97)	0.034	0.852
Controls (K)	0 (0)	24 (0.20)	96 (0.80)	1.481	0.223
Patients (H)	36 (0.3)	56 (0.47)	28 (0.23)	0.468	0.493
Controls (H)	20 (0.17)	48 (0.40)	52 (0.43)	2.310	0.128

P > 0.05 indicates a representative group; K = Kazakh; H = Han; Prob = probability. The parentheses represented the percentage of genotype.

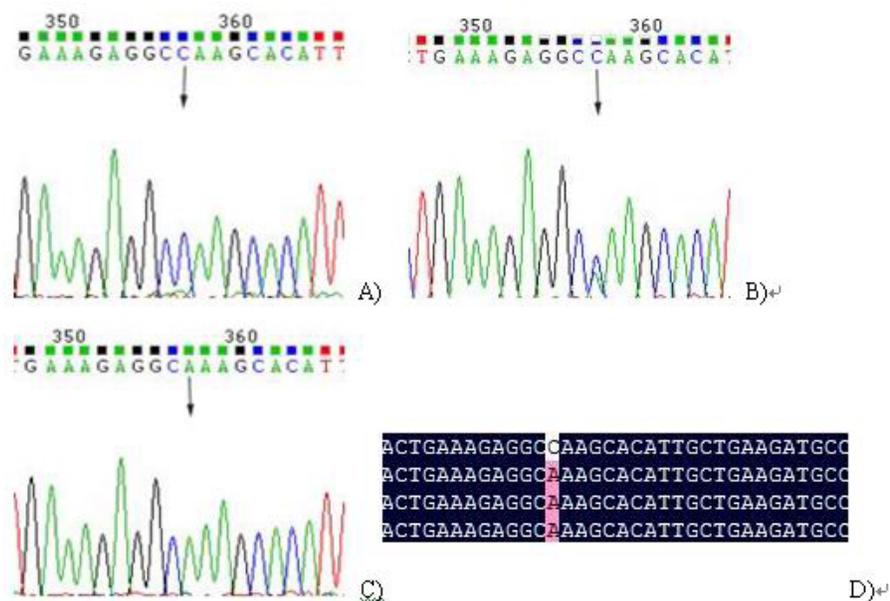
**Table 6.** Test of Hardy-Weinberg equilibrium for *TNNT2* genotypes.

Group	rs3729547 genotype			$\chi^2$	Prob Exact test
	C/C	C/T	T/T		
Patients (K)	0 (0)	12 (0.05)	108 (0.95)	0.332	0.564
Controls (K)	0 (0)	25 (0.21)	95 (0.79)	1.622	0.202
Patients (H)	24 (0.20)	52 (0.43)	44 (0.37)	1.414	0.234
Controls (H)	26 (0.22)	65 (0.54)	29 (0.24)	0.840	0.357

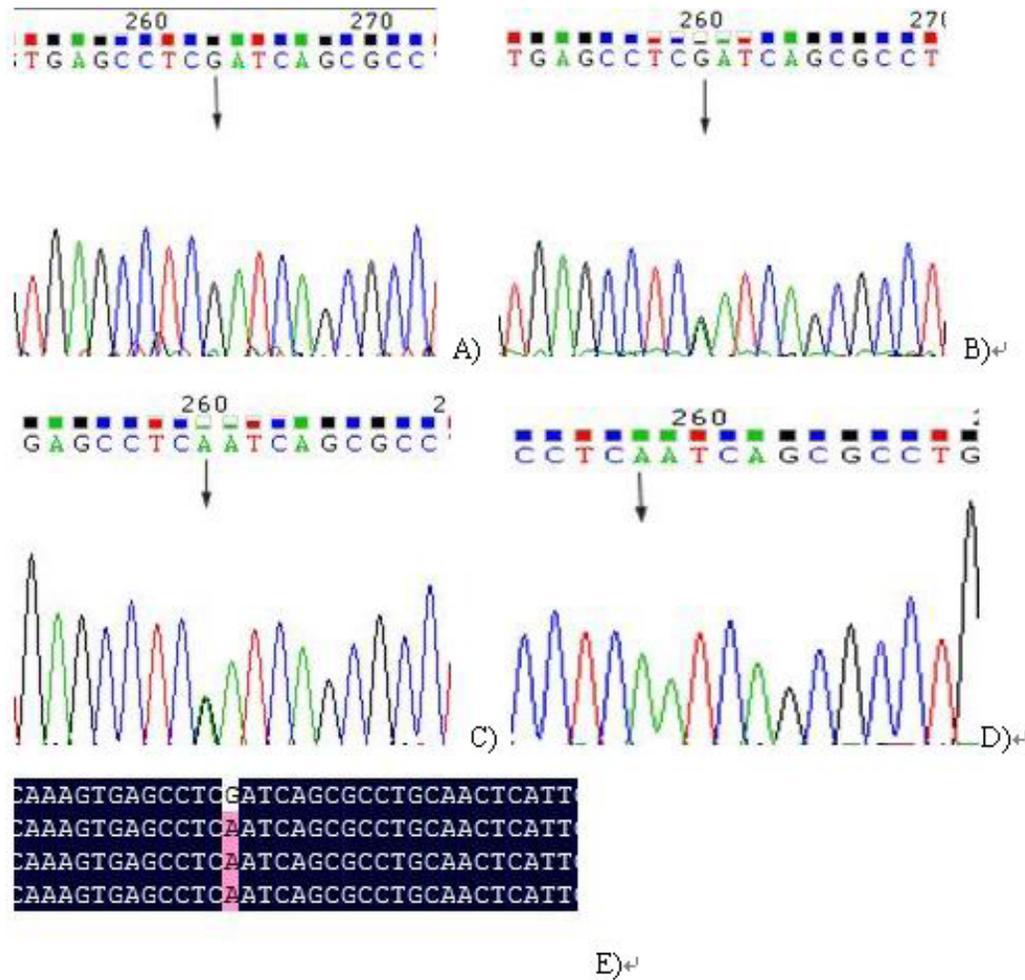
P > 0.05 indicates a representative group; K = Kazakh; H = Han; Prob = probability. The parentheses represented the percentage of genotype.

### Gene sequencing results

PCR amplification was carried on three candidate genes, and the amplification products were sequenced and a total of two mutation loci were identified: The two identified mutations, *TPM1* (rs1071646, Ala151Ala) and *TNNT2* (rs3729547, Ile116Ile), led to base replacement and were therefore considered pathological, their potential to lead to DCM was clarified using Pubmed. The genotype distributions and allele frequencies at the *TPM1* and *TNNT2* mutation loci between patients with IDCM of Chinese Xinjiang Kazakh ethnicity and the healthy population showed statistically significant differences. Figure 1 shows the rs1071646 locus in the *TPM1* gene. Figure 2 shows the rs3729547 locus in the *TNNT2* gene. In the Han Chinese population in Xinjiang, there were no statistical differences for the two candidate genes between the two groups. No mutation loci were identified for *LMNA* between the patients with IDCM and the healthy population (Tables 7 and 8).



**Figure 1.** *TPM1* exon 4 C>A Ala151Ala mutation. **A.** Wild-type homozygote C/C. **B.** Mutant heterozygote C/A. **C.** Wild-type homozygote A/A. **D.** Comparison of results using DNAMAN. Here, the top sequence represents the wild-type sequence.



**Figure 2.** *TNNT2* exon 10 complementary sequence G > A Ile116Ile. **A.** Wild-type homozygote G/G. **B.** and **C.** Mutant heterozygote G/A. **D.** Mutant homozygous A/A. **E.** Comparison of results using DNAMAN. Here, the top sequence represents the wide-type sequence.

**Table 7.** Chi-square tests for the *TPM1* gene polymorphism.

Group	rs1071646 genotype					rs1071646 allele				OR (95%CI)
	C/C	C/A	A/A	$\chi^2$	P	C	A	$\chi^2$	P	
Patients (K)	0 (0)	4 (0.03)	116 (0.97)		0.000	4 (0.01)	236 (0.99)		0.000	0.15 (0.052-0.447)
Controls (K)	0 (0)	24 (0.20)	96 (0.80)			24 (0.10)	216 (0.90)			
Patients (H)	36 (0.3)	56 (0.47)	28 (0.23)	1.61	0.446	128 (0.53)	112 (0.47)	1.63	0.201	1.26 (0.883-1.808)
Controls (H)	20 (0.17)	48 (0.40)	52 (0.43)			88 (0.37)	152 (0.63)			

P < 0.05 indicates Statistical significance; K = Kazakh; H = Han. The parentheses represented the percentage of genotype and allele.

**Table 8.** Chi-square test for the *TNNT2* gene polymorphism.

Group	rs3729547 genotype					rs3729547 allele				OR (95%CI)
	C/C	C/T	T/T	$\chi^2$	P	C	T	$\chi^2$	P	
Patients (K)	0 (0)	12 (0.05)	108 (0.95)		0.000	12 (0.05)	228 (0.95)	4.94	0.039	0.45 (0.222-0.923)
Controls (K)	0 (0)	25 (0.21)	95 (0.79)			25 (0.17)	215 (0.82)			
Patients (H)	24 (0.20)	52 (0.43)	44 (0.37)	4.60	0.101	100 (0.42)	140 (0.58)	2.43	0.142	0.75 (0.524-1.077)
Controls (H)	26 (0.22)	65 (0.54)	29 (0.24)			117 (0.48)	123 (0.52)			

P < 0.05 indicates Statistical significance; K = Kazakh; H = Han. The parentheses represented the percentage of genotype and allele.

## DISCUSSION

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation in the human genome, and many SNPs have been identified in patients with DCM. Two consecutive SNP screenings conducted in Europe showed that SNPs have close relationships with DCM (Kimura, 2011). *TPM1* and *TNNT2* gene mutation loci were detected in all Kazakh patients with IDCM. Such high mutation frequency was also common in the Han population. But after statistical analysis, the incidence of the Han nationality makes no sense. This is because Xinjiang is a remote area in China, and the diet, living habit, environment, and marital status of the Kazakh ethnic population are different from those of the Han ethnic population. This mutation might be transmitted to the next generation, furthermore, both genotype distributions and gene frequencies showed statistically significant differences in patients with DCM compared with the healthy Kazakh volunteers. The detection of SNP loci in the *TPM1* and *TNNT2* genes in both the Xinjiang Kazakh and Han population indicated that genetic factors are involved in the pathogenesis of disease (Table 4).

Previous studies have shown that the same genetic mutations can lead to HCM as well as causing DCM (Nanni et al., 2003), although the mechanisms remain unclear. Subsequent studies have pointed out that the same genetic mutations can lead to different appearances of HCM and DCM, which might be due to different functions of specific gene expression in these disorders (Debold et al., 2007; Robinson et al., 2007). For example, *TNNT2* gene mutation can result in both decline and enhancement of myocardial contraction force in patients with DCM and HCM, respectively (Kamisago et al., 2000; Mirza et al., 2005). However, variability in clinical features within DCM induced by the same mutation loci apart from that which can be explained by genetic, environmental, and other factors has also been observed.

In our study, the gene mutation detection rates in Xinjiang Han patients with IDCM were 76% (*TPM1*) and 80% (*TNNT2*). However, their genotype distributions and gene frequencies were not statistically significantly different compared with those of healthy Han volunteers, suggesting that although genetic factors play a major role in this disorder, external factors were also involved in its occurrence.

Abnormalities in cytoskeletons connecting the sarcomere and sarcolemma are mainly responsible for the pathogenesis of DCM. The fracture of this cytoskeleton can cause heart cavity expansion and decreased heart pump function. *TPM1* encodes the sarcomere myofilament, is located on human chromosome 15q22.2, and contains 9 exons. The *TPM1* gene product consists of two parallel polypeptide chains twisted into a spiral molecule and its function is to interact, strengthen, and stabilize the actin filament (Lakdawala et al., 2010). *TPM1* combines

with actin filament and troponin T, their binding loci are composed of troponin complexes, which are sensitive to ionized calcium ( $\text{Ca}^{2+}$ ). Therefore, increasing the  $\text{Ca}^{2+}$  levels promotes the movement of the tropomyosin-troponin complexes thereby prompting muscle contraction.

Three elements of the troponin complexes such as troponin T, joint troponin C, and I comprise myocardial troponin, which can combine with myosin and adjust myocardial contraction and relaxation, and can coordinate with  $\text{Ca}^{2+}$  concentration-dependent adenine triphosphatase activity in the muscle fiber (Richard et al., 2003). Recent studies have shown that cardiac troponin not only maintains the structural integrity of the troponin complexes, but also affects the contraction force of the sarcomere components and myocardium (Sehnert et al., 2002). *TNNT2* gene mutation not only affects the combination of the tropomyosin with troponin T, and actin, but also changes the combination of the troponins C, I, and T (Mirza et al., 2005).

LMNA is the most commonly identified pathogenic gene in DCM. It is located on chromosome 1q21.2-q21.3, and has a total genomic sequence length of 56.7 kb including a coding of approximately 24 kb, which contains 12 exons (Wydner et al., 1996). *LMNA* gene-encoded protein products include lamin A and C, which are important components of the nucleus fiber layer and are the main cytoskeleton protein for maintaining normal nuclear membrane morphology. It has been confirmed that the first symptom of DCM induced by *LMNA* gene mutation is severe arrhythmia, whereas decreased heart function often appears late but progresses quickly in these patients (Bécane et al., 2000). However, in this study, most patients with IDCM were diagnosed with decreased ventricular function and heart ultrasonic LVEF was considered as an evaluation criterion. As these characteristics are inconsistent with the presentation following LMNA mutation, no LMNA gene mutations were detected.

The LVEDD and LVEF differences between the patients with IDCM and the healthy volunteers were statistically significant, which indicated that the genetic mutations might influence the expression of sarcomere proteins and the connection of tropomyosin with actin and troponin thus impacting myocardial cytoskeleton structure and inducing decreased myocardial systolic ability, ejection fraction, and systolic function leading to expanded ventricular cavity that ultimately results in the onset of DCM. As gene sequencing can speed up and simplify the diagnosis of disease and can inform medical treatment and health care in patients at the genome level, the risk of DCM in FDCM family members should be evaluated. Studies have shown that the genetic testing is beneficial for preventing the occurrence of patients with decreased cardiac function (evaluated by echocardiogram LVEF) (The SOLVD Investigators, 1992). Therefore, early diagnosis could prevent disease in patients with asymptomatic IDCM as well.

The *TPMI* and *TNNT2* genes might be susceptibility loci for patients with IDCM of Kazakh descent in Xinjiang. The results of this study results have suggested that the sarcomere gene mutation frequency in patients with IDCM was significantly higher than previously reported (Robinson et al., 2007). Familial transmission suggests that at least one kind of genetic mutation affects the onset of DCM. Therefore, it is necessary to carry out comprehensive gene sequencing of the patient, which might promote early diagnosis of the disease and enable timely treatment and prevention.

## ACKNOWLEDGMENTS

We thank the Kazakh families, patients, volunteers, and the referring physicians for their participation in the Familial Dilated Cardiomyopathy Research Program, without whom

these studies would not have been possible. Research supported by the National Natural Science Foundation of China (#81260069).

### Conflicts of interest

The authors declare no conflict of interest.

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