CLINICAL RESEARCH

e-ISSN 1643-3750
© Med Sci Monit, 2015; 21: 3343-3347
DOI: 10.12659/MSM.894630

Received: 2015.05.12
Accepted: 2015.07.07
Published: 2015.11.03

TNNT2 Gene Polymorphisms are Associated with Susceptibility to Idiopathic Dilated Cardiomyopathy in Kazak and Han Chinese

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Source of support: This study was supported by the National Natural Science Foundation of China (no. 81260069)

Background: Dilated cardiomyopathy (DCM) is characterized by left ventricular enlargement, systolic dysfunction, and heart failure. Both genetic and non-genetic factors have been linked to DCM pathogenesis. Familial DCM (FDCM) accounts for 20%-50% of all DCM cases, highlighting the importance of genetics in pathogenesis. Indeed, more than 40 DCM-associated genes have been identified, including the gene encoding cardiac troponin T type-2 (TNNT2). We examined polymorphisms of the TNNT2 gene in idiopathic DCM (IDCM) patients of Kazak and Han ethnicity compared with healthy Kazak and Han controls.

Material/Methods: Peripheral blood samples were collected from 180 patients with IDCM (90 Kazak and 90 Han), and 180 healthy controls (90 Kazak and 90 Han). PCR was used to amplify 15 exons and nearby introns of the TNNT2 gene. The amplified products were sequenced and compared to the standard sequence in PubMed by BLAST and CHROMAS software, to identify mutation sites.

Results: Results from Kazak and Han IDCM patients were complied for Hardy-Weinberg equilibrium analysis. There was a significant difference in the genotype distribution (χ^2=6.67, P=0.015) and allele frequency (χ^2=5.71, P=0.017) between Kazaks with IDCM and Kazak controls of SNP rs3729547. There was also a difference in the genotype distribution (χ^2=6.62, P=0.036) and allele frequency (χ^2=4.91, P=0.018) between Han with IDCM and Han controls. The TNNT2 gene polymorphism *loci* rs3729547 may be associated with the IDCM onset in Kazak and Han patients (OR=2.5, 95% CI: 1.233~5.068).

Conclusions: The TNNT2 polymorphisms might play an important role in susceptibility to DCM in Xinjiang Kazak and Han patients.

MeSH Keywords: **Cardiomyopathy, Dilated • DNA Mutational Analysis • Polymorphism, Single Nucleotide**

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/894630

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Background

Dilated cardiomyopathy (DCM) is a common primary myocardial disease characterized by systolic dysfunction of the left or both ventricles, which frequently results in heart failure [1], arrhythmia, thromboembolism, and sudden death. In contrast to hypertrophic cardiomyopathy (HCM), which is strongly linked to mutations in sarcomeric proteins, DCM is associated with dysfunction in more diverse pathways, including Z-disk, nuclear lamina proteins, intermediate filaments, and the dystrophin-associated glycoprotein complex [2]. Idiopathic DCM (IDCM) is diagnosed when detectable causes of DCM (e.g., hypothyroidism, chemotherapeutic agents, alcoholism, ischemia, and known causative mutations) are excluded [3]. Research on DCM found that 20–50% of IDCM cases are caused by familial genetics [4–6], which has been linked to rare mutations in more than 40 genes [7]. In 90% of cases, inheritance is autosomal dominant, while autosomal recessive and X-linked inheritance cases are rare [8]. Most of these mutations are single-nucleotide mutations conferring changes in amino acid sequence of the encoded protein. Less frequent mutations include small in-frame insertions or deletions, but rarely large deletions [9]. Mutations in the cardiac actin gene ACTC1 were the first sarcomeric gene mutations to be associated with DCM, and the list has expanded to included 6 additional genes encoding sarcomeric proteins [10].

The TNNT2 gene encodes thin filament contractile protein that links troponin complex to tropomyosin [11]. The troponin complex is a calcium sensor regulator and an intracellular free calcium concentration, thereby strongly affecting muscle contraction. Although TNNT2 mutations often lead to HCM, surprisingly, other TNNT2 gene can also cause DCM [12].

Single-nucleotide polymorphism (SNP) is the most common type of genetic variation in the human genome, and 2 recent large-scale SNP screens in European patients with DCM revealed SNPs in several genes associated with DCM [13,14]. Based on previous studies, we speculated that a TNNT2 gene mutation could cause DCM. Therefore, we searched for mutations of the TNNT2 gene in IDCM patients and controls of both Kazak and Han Chinese ethnicity to validate this finding.

Material and Methods

The study protocol was approved by the Ethics Committee of The First Affiliated Hospital of Xinjiang Medical University, Xinjiang, China and all subjects gave written informed consent.

Subjects

Blood samples were collected from 180 cases of IDCM were also examined, 90 cases of Kazak ethnicity (37 males, 53 females) and 90 cases of Han Chinese ethnicity (43 males, 47 females), all recruited from the First Affiliated Hospital of Xinjiang Medical University clinic or hospital and 180 unrelated healthy volunteers, 90 cases of Kazak ethnicity (48 males, 42 females), and 90 cases of Han Chinese ethnicity (38 males, 52 females) served as controls.

Inclusion criteria were: (1) left ventricular end diastolic diameter (LVEDd) >50 mm in females and 55 mm in males, (2) left ventricular ejection fraction (LVEF) <45%, and (3) heart shadow widening on cardiac X-ray with cardiothoracic ratio >50%. Patients with myocardial damage caused by other diseases, such as hypertension, coronary heart disease, valvular heart disease, congenital heart disease, alcoholic cardiomyopathy, tachycardia, and pericardial disease, as well as patients with systemic, lung, and neuromuscular diseases, were excluded. All diagnosis were based on "Guidelines for the diagnosis and management of familial dilated cardiomyopathy" and were based on clinical echocardiography, with X-ray, heart isotopes, cardiac computed tomography, and MRI when required.

Clinical data

Demographic and clinical information were collected included date of birth, gender, race/ethnicity, clinical diagnosis, age at diagnosis, family history, cardiovascular history (myocardial infarction, hypertension, myocarditis, toxin/drug exposure), and cardiac structure and function, including maximal LV (left ventricular) wall thickness, LV ejection fraction, and LV dimensions [15]. All researchers had access to detailed clinical information, including general information, medical history, 12-lead synchronous ECG, echocardiography, and 6-min walk test results.

Genomic DNA extraction, PCR, and sequencing

We collected 2 ml of peripheral blood and extracted DNA from each subject. Polymerase chain reaction (PCR) was carried out for gene exon sequences of TNNT2 (NM_000364.3), and the amplification products were purified. All gene exon sequences underwent sequencing of the positive and negative directions with DNAMAN software, and the results were compared with the standard template sequences of the BLAST software on the PUBMED and CHROMAS software to identify the gene mutation *loci*. Later, the mutation *loci* were analyzed with reverse sequencing and re-sequencing for confirmation (Table 1).

Statistical analysis

Clinical data are presented as mean ± standard error of the mean (SEM). All sample groups were tested for Hardy-Weinberg equilibrium. The direct counting method was used to calculate genotype distributions and allele frequencies. Between-group differences in genotype distribution and allele frequencies were

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Table 1. Primer sequences.

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 GAA A	1699	53

Table 2. Clinical and demographic data.

Race	Data	IDCM	Controls	P
Kazak	Gender (M/F)	37/53	48/42	0.101
	Age (years)	45.83±7.62	56.83±9.98	0.505
	LVEDD (mm)	65.33±3.93	48.50±1.87	0.026
	LVEF%	38.34±2.50	60.00±6.13	0.034
Han	Gender (M/F)	43/47	38/52	0.454
	Age (years)	49.84±4.62	56.83±9.98	0.172
	LVEDD (mm)	64.85±2.31	45.83±0.75	0.029
	LVEF%	37.80±2.69	56.84±4.79	0.016

LVEDD – left ventricular end diastolic diameter, LVEF – left ventricular ejection fraction; P<0.05 statistically difference.

compared by chi-square (χ^2) test. All statistical analyses were conducted using SPSS17.0 software.

Results

Clinical data

Clinical characteristics and demographic data on the 180 IDCM patients and 180 controls are summarized in Table 2. There were no significant differences in sex ratio or mean age between IDCM patients and controls for either Kazak or Han ethnicity groups. Both LVEDD and LVEF differed significantly between IDCM patients and respective controls groups. All sample groups were complied for Hardy-Weinberg equilibrium (Table 3).

IDCM

A SNP rs3729547 (c.348C>T, rs3729547, Ile116Ile) in exon 10 was found in Kazak and Han IDCM. The genotype distribution (χ^2=6.67, P=0.015) and allele frequency (χ^2=5.71, P=0.017) between Kazak IDCM and Kazak controls of rs3729547 had typical differences. The genotype distribution (χ^2=6.62, P=0.036) and allele frequency (χ^2=4.91, P=0.018) between Han IDCM and Han controls of rs3729547 also had typical differences. TNNT2 gene polymorphism *loci* rs3729547 may be associated with the onset of IDCM from Kazak and Han (OR=2.5, 95% CI: 1.233~5.068). The TNNT2 gene SNP rs3729547 may be an important susceptibility gene for DCM in the both Kazaks and Han in Table 4.

Discussion

With an incidence of about 1 in 2500, DCM is a relatively rare myocardial disease. Both genetic and non-genetic factors have been linked to DCM pathogenesis. Studies have confirmed a multitude of genes linked to disease susceptibility. The cardiac histological features are myocardial necrosis and myocardial fibrosis, which induce compensatory hypertrophy of myocardial cells and result in a disorganized, enlarging heart chamber, thinning ventricular walls, weaker myocardial contraction, and reduced ejection fraction. These changes may eventually lead to chronic heart failure and sudden death. The Heart Rhythm Society/European Heart Rhythm Association expert consensus statement recommends genetic testing for patients with DCM and cardiac conduction disease [3].

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Table 3. Test of Hardy-Weinberg equilibrium in IDCM groups.

Group	rs3729547 Genotype				
	C/C	C/T	T/T	χ^2	*Prob Exact*
IDCM (K)	0 (0)	30 (0.33)	60 (0.67)	3.60	0.057
Controls (K)	0 (0)	15 (0.16)	75 (0.84)	0.74	0.388
IDCM (H)	8 (0.10)	26 (0.28)	56 (0.62)	0.08	0.773
Controls (H)	10 (0.11)	41 (0.45)	39 (0.44)	0.02	0.87

P>0.05 meet Hardy-Weinberg balance of verification.

Table 4. rs3729547 Genotype and Allele frequency in IDCM and controls.

Group	rs3729547 genotype					rs3729547 allele frequency			
	C/C	C/T	T/T	χ^2	P	C	T	χ^2	P
IDCM(K)	0 (0)	30 (0.33)	60 (0.67)	6.67	0.015	30 (0.17)	150 (0.83)	5.71	0.017
Controls (K)	0 (0)	15 (0.16)	75 (0.84)			15 (0.09)	165 (0.91)		
IDCM(H)	8 (0.10)	26 (0.28)	56 (0.62)	6.62	0.036	42 (0.23)	138 (0.76)	4.91	0.018
Controls (H)	10 (0.11)	41 (0.45)	39 (0.44)			61 (0.33)	119 (0.67)		

P<0.05 statistically difference.

In this study, the TNNT2 gene rs3729574 CC mutation was more frequent and TT genotype much less frequent in Kazak patients with IDCM and FDCM compared to Kazak controls. In contrast, SNP rs3729574 CC mutation also can be found in Han IDCM and controls, but the mutation rate is less than in Kazaks. This result suggests this SNP rs3729574 increases the incidence rate in Kazaks and may play an important role in disease pathogenesis. In this study, we found a significant feature in the Kazakh IDCM patients – all of IDCM and FDCM were detected rs3729547C SNP, according to the haplotype analysis, and this SNP may come from a common ancestor [16]. It may be due to remote areas, diet, living habits, living environment, Kazak population intermarried, and ethnicity differences, indicating a serious genetic factor involved in the pathogenesis of the disease. Studies have found that the same genes can cause HCM and DCM, because the function of the gene expression is different [17,18], However, clinical symptoms of the same mutation are also different, suggesting that environmental factors and others can also cause DCM, implying that the genetic factor play a major role, but common external factors are also involved in the occurrence of the disease. This also explains why TNNT2 was a susceptibility gene but incidence in Hans is low.

Cardiac troponin (cardiac TnT), composed of troponin C, troponin I, and troponin T, regulates myocardial contraction and relaxation by controlling the Ca^{2+} concentration-dependent interaction of actin [13]. The TNNT2 gene mutation may affect complex stability and/or the interaction between tropomyosin and troponin T, which could then alter the actin interaction [19]. Studies have shown that TNNT2 gene mutations can reduce the sensitivity of the complex to Ca^{2+}, thereby reducing the contractile force of the myocardium. In contrast, mutations in genes encoding sarcomere proteins associated with HCM can increase Ca^{2+} sensitivity, thereby increasing cardiac contractility [13,19]. Thus, mutations in different proteins may be associated with a common cardiac disease phenotype (DCM or HCM) depending on the ultimate effect of the mutation on cardiac myocardial contractile strength.

Although environmental factors, sex, age, and lifestyle can impact the occurrence of a disease and facilitate or impede disease progression, susceptibility is conferred by allelic variants, of which the most common are single-point mutations (SNPs) [13,20]. It is usually difficult to confirm DCM as familial (FDCM) because most pathogenic mutations are rare, requiring complete sequencing of the candidate genes. However, detection of these variants is important for early detection and treatment. There is evidence indicating that prophylactic treatment of asymptomatic DCM patients with depressed cardiac function (as evidenced by lower LVEF) is beneficial [15], but by this time the disease is already relatively advanced. In contrast, genetic testing may detect elevated DCM risk in young children (<10 years old), allowing for initiation of preventative treatment long before irreversible cardiac damage [21]. Moreover, genetic testing of saved tissue samples from deceased patients

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may help reveal the causes of heart failure, sudden death, and atrioventricular block lesions, shedding further light on DCM pathogenesis.

Conclusions

The TNNT2 polymorphisms might play an important role in susceptibility to DCM in Xinjiang Kazak and Han ethnicity populations.

Acknowledgments

We thank the Kazak families, patients, volunteers, referring physicians for their participation in the Familial Dilated Cardiomyopathy Research Program, without which these studies would not have been possible.

Study limitations

We will continue to collect and expand the sample sizes to confirm and identify new candidate causative genes for genome-wide and functional studies of DCM.

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