Research Article

**TNNT2 Gene Polymorphisms Are Associated with Susceptibility to Idiopathic Dilated Cardiomyopathy in the Han Chinese Population**

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Received 27 September 2012; Accepted 11 January 2013

Academic Editor: Yasemin Alanay

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**Background.** Idiopathic dilated cardiomyopathy (DCM) is characterized by ventricular chamber enlargement and systolic dysfunction. The pathogenesis of DCM remains uncertain, and the **TNNT2** gene is potentially associated with DCM. To assess the role of **TNNT2** in DCM, we examined 10 tagging single nucleotide polymorphisms (SNPs) in the patients.

**Methods.** A total of 97 DCM patients and 189 control subjects were included in the study, and all SNPs were genotyped by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

**Results.** In the **TNNT2** gene, there was a significant association between DCM and genotype for the tagging SNPs rs3729547 ($\chi^2 = 6.63, P = 0.036, OR = 0.650$, and $95\% \text{ CI} = 0.453–0.934$) and rs3729843 ($\chi^2 = 9.787, P = 0.008, OR = 1.912$, and $95\% \text{ CI} = 1.265–2.890$) in the Chinese Han population. Linkage disequilibrium (LD) analysis showed that the SNPs rs7521796, rs2275862, rs3729547, rs10800775, and rs1892028, which are approximately 6kb apart, were in high LD ($D^2 > 0.80$) in the DCM patients.

**Conclusion.** These results suggest that the **TNNT2** polymorphisms might play an important role in susceptibility to DCM in the Chinese Han population.

1. **Introduction**

Idiopathic dilated cardiomyopathy (DCM) is a cardiac muscle disease of unknown origin that is characterized by ventricular chamber enlargement and systolic dysfunction with thinning of the left ventricular wall. DCM leads to progressive heart failure and a decline in left ventricular contractile function, conduction system abnormalities, thromboembolism, and sudden or heart failure-related death; only 50% of DCM patients survive more than 5 years beyond their initial diagnosis [1, 2]. Coronary artery disease, viral myocarditis, thyroid disease, immunologic processes, and toxins are known causes of DCM; however, the underlying pathology is not known in most cases [3–5]. In a population-based study, the prevalence of DCM was estimated to be 36.5 cases per 100,000, and 20–50% of these cases are familial [6–8]. Candidate gene analysis revealed that the cardiac actin encoding gene **ACTC1** mutations were the first sarcomeric gene mutations that caused DCM [9]. To date, mutations have been found in at least six genes encoding sarcomeric proteins: $\beta$-myosin heavy chain, cardiac myosin binding protein C, titin, cardiac actin, $\alpha$-tropomyosin, cTnI, and cTnC [9–15].

The **TNNT2** gene (OMIM number *191045) encodes the protein cardiac TnI, which contains 15 exons and spans 25 kb.
on chromosome 1q32 [16]. Mutations in the TNNT2 gene can cause three phenotypically distinct cardiomyopathies: hypertrophic, restrictive, and dilated [10, 17–19]. TNNT2 mutations are responsible for approximately 15% of all cases of familial hypertrophic cardiomyopathy (HCM) [20–22]. Recent data indicated that TNNT2 mutations are also associated with DCM, and the overall frequency of TNNT2 mutations in familial DCM is approximately 3–6% [23, 24].

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation in the human genome, and two recent large-scale SNP screens in European patients with DCM showed that SNPs in several genes were associated with DCM [25, 26]. Based on the above findings, we hypothesized that some cases of DCM are associated with specific polymorphisms in the TNNT2 gene. To test this hypothesis and further understand the pathogenesis of DCM, we investigated 10 tagging SNPs in the TNNT2 gene in DCM patients and normal control subjects from a Chinese Han population using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) techniques. Our results indicated that the SNPs rs3729547 and rs3729843 in the TNNT2 gene were associated with DCM in the Chinese population, suggesting that the TNNT2 polymorphisms may play an important role in susceptibility to DCM in the Chinese population.

2. Materials and Methods

2.1. Subjects and Selection of Tagging SNPs. This case-control study enrolled 97 unrelated DCM patients from the Fuwai Hospital. The clinical diagnosis was made in accordance with the revised criteria [1]. A total of 189 healthy unrelated individuals from a routine health survey were enrolled as controls. Patients with a history of hypertension, coronary heart disease, cardiac valve disease, diabetes, acute viral myocarditis, systemic diseases of putative autoimmune origin, and family history of DCM were intentionally excluded. This study was approved by the Ethics Committee of our hospital; the subjects involved were all of Han nation in the North of China and were informed of the study aims and provided written informed consent prior to participating.

Genotype data on the TNNT2 gene from the Han Chinese in Beijing (CHB) population were downloaded from the phase 2 HapMap SNP database (available at http://www.hapmap.org/), and tagging SNPs were selected in the Haploview software (available at http://www.broadinstitute.org/haploview) using a minor allele frequency (MAF) cutoff of 0.05 and a correlation coefficient ($r^2$) threshold of 0.8.

2.2. Isolation of DNA and Genotyping by MALDI-TOF-MS. Blood samples were collected from patients using tubes containing ethylenediaminetetraacetic acid. Genomic DNA was isolated from whole blood with a QIAGen DNA Blood Mini Kit (Qiagen, Germany). Genotyping was performed by MALDI-TOF-MS as described previously [27]. SNP genotyping was performed using a MassARRAY system (Sequenom, San Diego, CA, USA) based on the MALDI-TOF-MS method, according to the manufacturer’s instructions. Completed genotyping reactions were spotted onto spectroCHIP (Sequenom) using a MassARRAY Nanodispenser (Sequenom), and the genotype was determined by MALDI-TOF-MS. Genotype calling was performed in real time with MassARRAY RT software version 3.1 (Sequenom) and analyzed using MassARRAY Typer software version 4.0 (Sequenom) (Table 1).

2.3. Statistical Analyses. Differences in the distributions of selected variables and TNNT2 genotypes between the cases and controls were evaluated using the $\chi^2$ test. The correlations between the TNNT2 genotype and the risk of DCM were estimated by computing the odds ratios (ORs) and the 95% confidence intervals (CIs) using logistic regression analysis. The $\chi^2$ test was used to test for the Hardy-Weinberg equilibrium to compare the observed and expected genotype frequencies among the control subjects. All statistical analyses were performed with SPSS 13.0. All tests were two-tailed, and the significance was set at $P < 0.05$.

3. Results

The gender and age distributions of the DCM patients and the control subjects were compared with the Pearson’s chi-square test and Student’s t-test, respectively, and no significant differences were detected (control: $n = 189, 54.0 \pm 3.6$ years, male/female = 150/39; DCM: $n = 97, 51.6 \pm 12.0$ years, male/female = 75/22, $P > 0.05$).

The observed and expected genotype frequencies of each SNP were compared with the chi-squared test in DCM patients and the control subjects separately, and no significance was detected in either group. These results indicate that the samples fit the assumption of the Hardy-Weinberg equilibrium. The DNA variants and the Hardy-Weinberg equilibrium test of the 10 tagging SNPs in the DCM patients and control subjects were shown in Table 2.

Using the chi-squared test, we compared the genotype and allele frequencies in the TNNT2 gene between the DCM patients and control subjects. Our results showed that the allele frequencies of the tagging SNPs rs3729547 ($\chi^2 = 5.474, P = 0.019$), rs1892028 ($\chi^2 = 5.855, P = 0.016$), rs3729843 ($\chi^2 = 9.620, P = 0.002$), rs12564445 ($\chi^2 = 4.351, P = 0.037$), and rs10800775 ($\chi^2 = 4.252, P = 0.039$) were significantly correlated with DCM. However, among the genotypes, only those of the tagging SNPs rs3729547 ($\chi^2 = 6.63, P = 0.036$, OR = 0.650, 95% CI = 0.453–0.934) and rs3729843 ($\chi^2 = 9.787, P = 0.008$, OR = 1.912, 95% CI = 1.265–2.890) had a significant correlation with DCM in the Chinese population. The allele and genotype frequencies of the 10 tagging SNPs in the DCM patients and control subjects and the statistical analysis results were shown in Table 3 and Figure 1.

Because the great majority of DCM patients are male [1–5], we compared the frequencies of the genotypes of SNPs rs3729547, rs3729843, and rs10927875 in TNNT2 between the DCM patients and control subjects stratified by gender. In
Table 1: Sequences of the PCR primers used to genotype SNPs in the DCM patients and control subjects.

| Markers | Forward primer (5'-3') | Reverse primer (5'-3') | Amplicon size (bp) | Temp. (°C) | GC (%) |
|---------|------------------------|------------------------|-------------------|-----------|--------|
| rs7521796 | TGCCACAGAGAGGTGTCTTC | CTTGAGGCTCAAGCTAATTG | 93                | 46.5      | 56.3   |
| rs2275862 | AAATGGGTGAGGCCGCTCAT | TATTACGCAAGCGTGTAAC | 99                | 48.4      | 52.9   |
| rs3729547 | GAAAGGAGTGAAGTTGGGC | AGAAACGACTCTCCTTCTCT | 99                | 50.2      | 60.0   |
| rs10800775 | AATCCCTTCCCAAGTCTTCT | TCAACTACAGCTCTCTGCC | 98                | 50.7      | 47.4   |
| rs1892028 | AGAGGGGACCATTGTCCAG | TCTAGGAGCTTATGATTTG | 100               | 48        | 62.5   |
| rs3729843 | TCAAGGTCTCTGTTCTGAGC | TCTTGCTAGGCTTATGCTG | 99                | 47.1      | 44.4   |
| rs3729842 | TCAAAGGTGGATTGGGAG | AGAACAGGCTTTCCCATGTG | 99                | 46.7      | 31.8   |
| rs12563114 | TGGAAGGGCAGAGTAGGAGA | AATTCTGAGGAGCTGC | 100               | 45.2      | 44.4   |
| rs12564445 | AACTCGGAGACTGTTTCTAC | CTCTCTGACTTCAGTTAACC | 95                | 47.7      | 47.1   |
| rs4915232 | CAATCTCGCTATTCTCTGC | AGAAGAGTTTGAGGACTGG | 95                | 48.6      | 62.5   |

Table 2: Identified DNA variants and the Hardy-Weinberg equilibrium of 10 SNPs in the TNNT2 gene in the DCM patients and control subjects.

| Markers | Location of nucleotide change | Amino acid change | Note | Obs HET | Expt HET | HWE (P) | MAF |
|---------|-------------------------------|------------------|------|---------|----------|---------|-----|
| rs7521796 | Intron 201330019 A>G | Non-coding | Novel noncoding SNP | 0.115 | 0.115 | 1 | 0.061 |
| rs2275862 | Intron 201330366 C>G | Non-coding | Novel non-coding SNP | 0.329 | 0.319 | 0.7683 | 0.199 |
| rs3729547 | Intron 201334382 T>C | Non-coding | Novel non-coding SNP | 0.474 | 0.448 | 0.771 | 0.339 |
| rs10800775 | Intron 201336386 C>T | Non-coding | Novel non-coding SNP | 0.489 | 0.498 | 0.7396 | 0.47 |
| rs1892028 | Intron 201336641 A>G | Non-coding | Novel non-coding SNP | 0.285 | 0.331 | 0.5949 | 0.21 |
| rs3729843 | Intron 201336984 G>A | Non-coding | Reported non-coding SNP | 0.225 | 0.241 | 0.3594 | 0.14 |
| rs3729842 | Intron 201337170 C>T | Non-coding | Reported non-coding SNP | 0.081 | 0.084 | 0.5638 | 0.044 |
| rs12563114 | Intron 201344908 C>T | Non-coding | Novel non-coding SNP | 0.435 | 0.427 | 0.7891 | 0.309 |
| rs12564445 | Intron 201345487 G>A | Non-coding | Novel non-coding SNP | 0.535 | 0.499 | 0.7407 | 0.482 |

Note: Obs HET: observed heterozygosity, Expt HET: expected heterozygosity, HWE (P): P value from the Hardy-Weinberg equilibrium test, and MAF: minor allele frequency.

males, the distributions of the SNP rs3729547 genotypes were not significantly different between the DCM patients and control subjects, but the distributions of the SNP rs3729843 genotypes were significantly different in the DCM patients and control subjects ($\chi^2 = 8.102, P = 0.017$). In females, the distributions of the genotypes of rs3729843 and rs3729547 were not significantly different in the DCM patients and control subjects.

The nonrandom associations between polymorphic variants at different loci on the TNNT2 gene were then measured by the degree of linkage disequilibrium (LD). LD analysis showed that the SNPs rs7521796, rs2275862, rs3729547, rs10800775, and rs1892028 in the TNNT2 gene, which are approximately 6 kb apart (block 3, Figure 2), were in high LD in the DCM patients (Figure 2, $D^' > 0.80$). The haplotype analysis showed that ACTCA ($\chi^2 = 6.66, P = 0.0099$) and AGCTG ($\chi^2 = 4.003, P = 0.0454$) in block 3 and AG ($\chi^2 = 3.988, P = 0.0458$) in block 4 (rs12564445 and rs4915232) of the TNNT2 gene correlated significantly with DCM (Figure 2, Table 4).

4. Discussion
To our knowledge, this is the first study to show an association between DCM and SNPs in the TNNT2 gene in the Chinese population. DCM is regarded as a heterogeneous disease. The present study shows that, in at least a subgroup of DCM patients, the SNPs in the TNNT2 (rs3729547 and rs3729843) gene may be involved in the pathogenesis of DCM.
Table 3: Genotype and allele frequencies of the SNPs from the TNNT2 gene in the DCM patients and control subjects.

| Marker | Genotype | $\chi^2$, P value | Allele | $\chi^2$, P value | OR (95% CI) |
|--------|----------|------------------|--------|------------------|-------------|
| rs7521796 | A/A | 0.50 | G/G | 1.303 (0.613–2.772) |
| rs2275862 | C/C | 1.562 (0.987–2.471) |
| rs3729547 | A/G | 1.578 (1.090–2.291) |
| rs10800775 | C/C | 1.486 (1.019–2.169) |
| rs1892028 | A/G | 1.912 (1.265–2.890) |
| rs3729842 | C/C | 1.158 (0.697–1.925) |
| rs12563114 | A/G | 0.899 (0.390–2.07) |
| rs4915232 | A/G | 1.155 (0.505–2.45) |

DCM represents the third most frequent cause of heart failure and the most frequent cause of heart transplantation. Among patients with the so-called idiopathic DCM, 20–50% of cases are of genetic origin [6, 7]. Over the past decade, de novo mutations have been found in more than 30 genes encoding essential sarcomeric, cytoskeletal, and nuclear proteins in DCM patients [28], and mutations in the TNNT2 gene have been found to be associated with familial HCM and DCM [10, 17–19, 23, 24]. Recent studies have suggested that cardiac TnT is essential not only for the structural integrity of the troponin complex but also for sarcomere assembly and cardiac contractility [22]. The troponin complex is a calcium sensor that regulates the contraction of striated muscle, and TnT is important in
mediating the interaction between tropomyosin and actin and the rest of the troponin complex, which appears to modulate the activation of actomyosin ATPase activity and force [29]. Countless studies in reconstituted systems have provided valuable information on the functional effects of disease-associated mutations in TnT. The most extensively studied DCM-associated TnT mutation to date is ΔK210; functional studies of the ΔK210 mutation showed that the mutated protein reduced the Ca$^{2+}$ sensitivity of actomyosin ATPase activity, which resulted in a decreased maximum speed of muscle contraction [30, 31]. Thus, DCM mutations in the troponin complex may induce a profound reduction in force generation, leading to impaired systolic function and cardiac dilation.

In this study, we assessed whether polymorphism within the TNNT2 gene might affect DCM susceptibility by comparing ten tagging SNP loci in DCM patients and normal control subjects. The representative SNP in a region of the genome with high linkage disequilibrium is called a tagging SNP. Among the ten tagging SNPs in the TNNT2 gene, we found a significant association between the genotypes of rs3729547 (synonymous variant) and rs3729843 (noncoding SNP) and DCM. Although the allele frequencies of five tagging SNPs (rs3729547, rs3729843, rs1892028, rs1256445, and rs10800775) were significantly associated with DCM, the genotypes of rs1892028, rs1256445, and rs10800775 were not significantly associated with DCM, possibly because of the limited number of patients enrolled in the present study. LD analysis of the polymorphic SNPs observed in our study revealed a group of five SNPs, rs7521796, rs2275862, rs3729547, rs10800775, and rs1892028, located 6 kb apart; these alleles were in high LD and associated with DCM risk. As the majority of SNPs are likely to be allelic variants that do not affect expression or function of a protein, such SNPs are commonly used as genetic markers to localize nearby disease-causing variations in linkage and association analyses. SNPs that directly influence phenotype may be located within coding or regulatory regions of genes. SNPs within regulatory regions tend to have more quantitative effects, for example, by altering the expression level of a receptor or signaling protein, and result in a more subtle variation in the associated phenotype [32]. Recently, study showed that polymorphism in intron 3 of TNNT2 significantly affected the mRNA expression pattern by skipping exon 4 during splicing in cardiomyopathy patients [33]. Missing exon 4 in cardiac troponin T is corresponding to isoforms cTnT2 and cTnT4, and the two isoforms increase might be related to hemodynamic stress [34]. These results in our study suggest that TNNT2 gene polymorphism, as like genetic markers to localize nearby disease-causing variations in linkage and association analyses, may play an important role in DCM susceptibility in the Chinese Han population. However, further functional analyses are needed to confirm the role of these polymorphisms in the pathogenesis of DCM.

In the present study, we have provided the evidence that shows that SNPs in the TNNT2 gene may be implicated in the pathogenesis of DCM in a Chinese population. However, because the frequencies of genetic polymorphisms vary greatly among ethnic populations, further studies in other populations are needed to exclude a population-oriented association. In addition, the outcomes of the present study may be influenced by the limited sample size; larger studies are therefore required to investigate the potential associations between the SNPs in the TNNT2 gene and the DCM susceptibility.

**Authors’ Contribution**

X. Li and H. Wang contributed equally to this work.

**Acknowledgments**

This study was supported by Grants from the National Natural Science Foundation of China (no. 81000104 and no. 81160141) and the Postdoctoral Fellows Foundation of Chinese Academy of Medical Sciences (2011-XH-9).

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