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Research Article

Targeted Next-Generation Sequencing Reveals Hot Spots and Doubly Heterozygous Mutations in Chinese Patients with Familial Cardiomyopathy

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As a common cardiac disease mainly caused by gene mutations in sarcomeric cytoskeletal, calcium-handling, nuclear envelope, desmosomal, and transcription factor genes, inherited cardiomyopathy is becoming one of the major etiological factors of sudden cardiac death (SCD) and heart failure (HF). This disease is characterized by remarkable genetic heterogeneity, which makes it difficult to screen for pathogenic mutations using Sanger sequencing. In the present study, three probands, one with familial hypertrophic cardiomyopathy (FHCM) and two with familial dilated cardiomyopathy (FDCM), were recruited together with their respective family members. Using next-generation sequencing technology (NGS), 24 genes frequently known to be related to inherited cardiomyopathy were screened. Two hot spots (TNNI3-p.Arg145Gly, and LMNA-p.Arg190Trp) and double (LMNA-p.Arg190Trp plus MYH7-p.Arg1045His) heterozygous mutations were found to be highly correlated with familial cardiomyopathy. FDCM patients with doubly heterozygous mutations show a notably severe phenotype as we could confirm in our study; this indicates that the double mutations had a dose effect. In addition, it is proposed that genetic testing using NGS technology can be used as a cost-effective screening tool and help guide the treatment of patients with familial cardiomyopathy particularly regarding the risk of family members who are clinically asymptomatic.

1. Introduction

Inherited cardiomyopathy is a chronic myocardial disorder affecting all ethnic groups, resulting in a large cost burden to social health systems. The World Health Organization (WHO) has divided cardiomyopathy into four categories: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), and restrictive cardiomyopathy (RCM) [1]. Among these disorders, HCM and DCM occur most frequently in the Chinese population.

HCM is a frequent genetic heart disease with autosomal dominant inheritance and is one of the most common causes of sudden cardiac death (SCD), especially in adolescents and young athletes [2, 3]. Clinically, HCM is characterized by unexplained left ventricular hypertrophy, myocyte disarray, and myocardial fibrosis [4]. The incidence of HCM was estimated to be at least 1/500 individuals [5]; thus, approximately 2 million patients in China are affected by HCM. The first gene mutation identified to be causing heart disease was reported in 1993 [6]; since then, more than 1400 mutations have been detected in at least 20 genes [7] that encode sarcomere proteins, calcium-handling proteins, Z-disc proteins, and so forth. In particular, MYH7 and MYBPC3 sarcomere proteins mutations are most commonly related to HCM [8].

Another common inherited cardiomyopathy, DCM, is clinically characterized by left ventricular dilation with systolic dysfunction and affects at least 1/2500 of the general

population worldwide [9]. Pathogenic gene mutations play an important role in DCM pathogenesis and mostly follow Mendelian autosomal dominant patterns; however, recessive as well as X-linked inheritance may also be involved [10]. About 20~48% of DCM patients have familial forms of the disease, called familial DCM (FDCM). FDCM is often characterized by genetic heterogeneity and shows high variability even between family members; the age of onset and disease progression also differ considerably between individuals with FDCM. To date, mutations in more than 50 genes have been reported to cause FDCM, including mutations in genes encoding sarcomere proteins, cytoskeletal proteins, and nuclear envelope proteins [10].

Genetic testing may provide the genetic information required for clinical diagnosis of inherited cardiomyopathy. Once a disease-related mutation is identified in a proband, testing for this mutation should be conducted on other family members to predict the disease risk and guide preventative measures and treatment [11, 12]. In this study, comprehensive genetic analysis of 24 genes frequently known to be related to inherited cardiomyopathy (*ABCC9*, *CAV3*, *DES*, *MYBPC3*, *MYL2*, *PRKAG2*, *PSEN1*, *PSEN2*, *SGCD*, *TNNC1*, *TPM1*, *MYH6*, *MYH7*, *TNNT2*, *SCN5A*, *TNNI3*, *MYL3*, *MYPN*, *LAMA4*, *RBM20*, *VCL*, *LDB3*, *ACTN2*, and *LMNA*) was performed in FHCM and FDCM patients by using next-generation DNA sequencing (NGS) technologies to identify their potential pathogenic mutations, as well as to determine their value in clinical diagnosis and in guiding family therapy management.

2. Materials and Methods

2.1. Patient Data. In this study, the patient from Family A was diagnosed with FHCM based on the American College of Cardiology Foundation/American Heart Association (ACCF/AHA) criteria [4], using the following inclusion criterion: wall thickness >15 mm on echocardiography, with wall thickness of 13-14 mm being considered borderline. The two patients in Family B had been previously diagnosed with FDCM in accordance with European guidelines [13]. The patients resided in Yu Xi, in the central region of the Yunnan province, China. Their demographic and clinical information, including family history, clinical symptoms, echocardiography results, and 12-lead electrocardiography (ECG) records, were collected. Genetic testing was performed on the patients and their family members. This investigation was approved by the Institutional Ethics Committee of the First People's Hospital of Yunnan Province (Affiliated Hospital of Kunming University of Science and Technology), China.

2.2. Candidate Gene Sequencing. For each sample, genomic DNA was extracted from anticoagulated whole blood by using a commercial genomic DNA midiprep kit (AxyPrep, Corning, CA, USA). The PCR primer panel was designed to amplify the coding exons and partial introns of a number of genes frequently known to be related to inherited cardiomyopathy: *ABCC9*, *CAV3*, *DES*, *MYBPC3*, *MYL2*, *PRKAG2*, *PSEN1*, *PSEN2*, *SGCD*, *TNNC1*, *TPM1*, *MYH6*, *MYH7*, *TNNT2*, *SCN5A*, *TNNI3*, *MYL3*, *MYPN*, *LAMA4*, *RBM20*, *VCL*, *LDB3*, *ACTN2*, and *LMNA*. The amplicons were submitted to Agilent Technology (CA, USA) for construction of a multitarget gene library and then sequenced using a Genome Analyzer IIx (Illumina, CA, USA).

2.3. Molecular Genetic Analysis. The sequencing results were aligned to the National Center for Biotechnology Information (NCBI) human reference genome assembly (GRCh37/hg19), and PCR duplicates were removed using the SAM tools software package (version 0.1.16) [14]. To identify nucleotide mutations, including both unknown mutations and those that have been reported in the dbSNP137 (http://www.ncbi.nlm.nih.gov/projects/SNP) and 1000 Genome Project (http://www.1000genomes.org), the genomic variations in the sequences were annotated with the ANNOVAR software [15]. Synonymous and noncoding region mutations were excluded in this step, and the remaining coding region variations were considered putative pathogenic mutations. Putative pathogenic mutations were analysed computationally using the PolyPhen-2, SIFT, and MutationTaster [16–18] algorithms. These algorithms can distinguish mutations with functional effects from other neutral mutations.

2.4. Mutation Validation. The putatively pathogenic mutations were verified using custom-designed Sanger sequencing methods. Exon 7 of *TNNI3*, exon 3 of *LMNA*, and exon 25 of *MYH7* were amplified using primers covering exon 7 of the mutated *TNNI3*-c.433C>G, primers covering exon 3 of the mutated *LMNA*-c.568C>T, and primers covering exon 25 of the mutated *MYH7*-c.3134G>A, respectively (Table 2). Genomic sequences of the family members of the patients were also confirmed using the Sanger sequencing platform after PCR amplification of the corresponding exons to confirm the results.

3. Results

3.1. Familial Phenotypes of HCM and DCM. Families A and B were diagnosed with HCM and DCM, respectively; data regarding their clinical symptoms and the demographic information were collected during interviews. In family A, the proband II: 3 was a 44-year-old woman with clinical symptoms of dyspnoea and chest tightness after exercise. Her father (I: 1), sister (II: 1), and brother (II: 2) died of SCD around the age of 40 years. Her daughter (III: 1, 22 years of age) presented as clinically unaffected at the time of the interview (Figure 2(a)). No clinical data were available for III: 1. According to the clinical records of this proband (II: 3), her heart rate was 68 beats/min, and the ECGs showed changes in T-waves, which is common in patients with HCM. Additionally, the Q-wave was abnormal in the sidewalls, while the QRS (86 ms) duration was within the normal range (Figure 1(a)). Moreover, echocardiography showed an increase in the interventricular septum thickness (IVST, 14.8 mm), as well as in the thickening of the left atrium (LA, 32 mm).

In family B, the proband II: 3 was a 43-year-old man with severe DCM symptoms. His elder brother (II: 2) had died

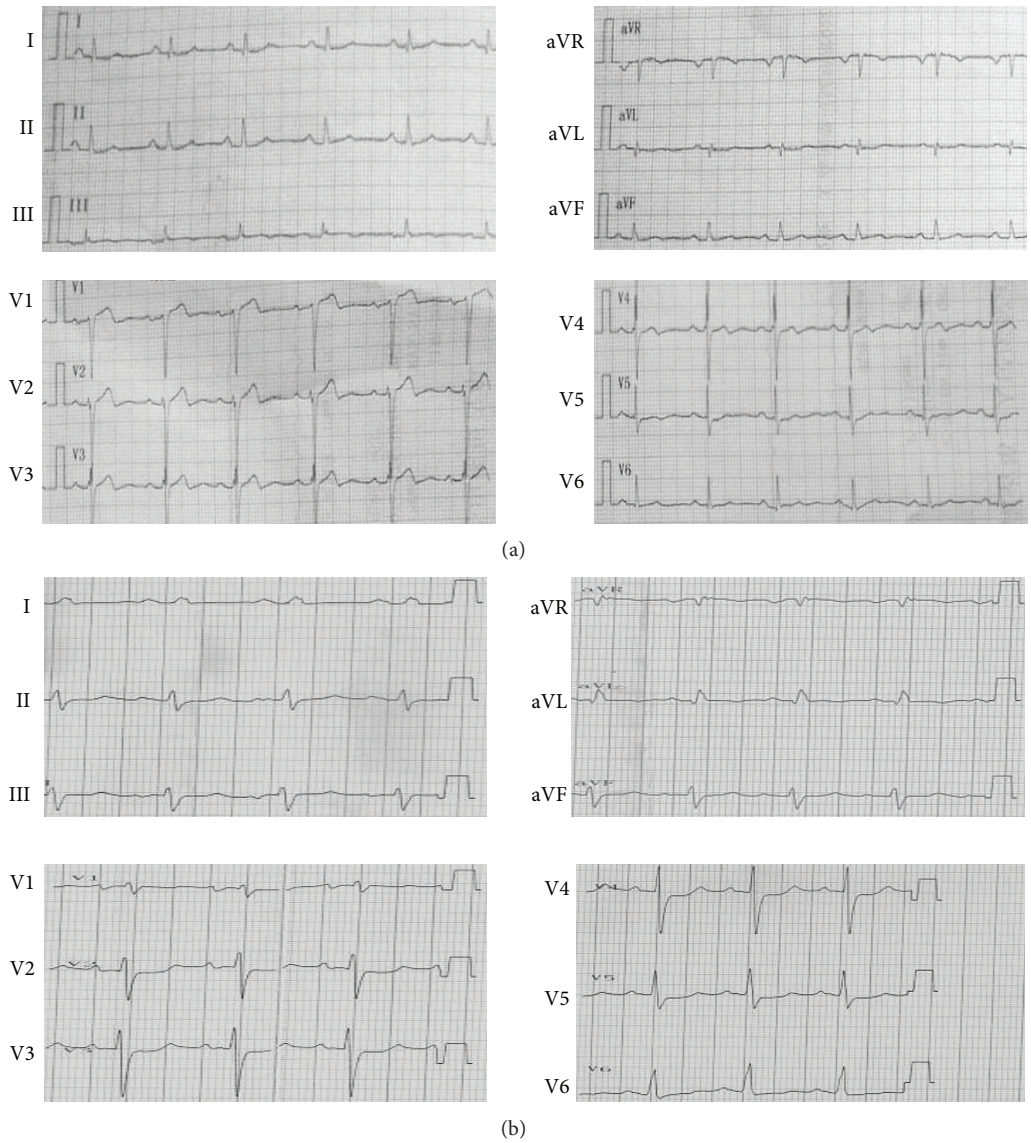

Figure 1: The electrocardiogram of probands with familial hypertrophic cardiomyopathy and familial dilated cardiomyopathy. (a) shows the electrocardiogram of the proband with HCM from family A (II: 3); the Q-wave was abnormal in sidewalls and the T-wave is changed; (b) shows the electrocardiogram of the proband with DCM from family B (II: 3); it reveals a significant ST-segment depression.

of SCD, and his sister (II: 1) had been diagnosed with DCM before that, while his daughter (III: 1) and father (I: 1) showed no clinical symptoms. No data were available for the individuals Family B III: 1 and B I: 1 (Figure 2(b)). Echocardiographic tests for II: 3 showed severe cardiac enlargement and increased left ventricular end-diastolic diameter (LVEDD, 77.8 mm), while the left ventricular end-systolic diameter (LVESD, 69.8 mm) and left ventricular ejection fraction (LVEF, 21% < 50%) were lesser than those observed in the general population. His heart rate was 78 beats/min, and ECG revealed significant ST-segment depression in II, III, aVF, V2, V3, and V4, which is indicative of ischemia, a predisposing condition seen in patients with FDCM [19]. Notably, a widened QRS complex (120 ms > 110 ms) (Figure 1(b)) may indicate a bundle branch block.

3.2. Summary of Targeted Sequencing for the Patients and Their Families. We analysed the number of reads that covered the variations in 24 genes frequently known to be related to inherited cardiomyopathy; we considered that a read depth ≥30 reads (30x, Q30 = 99.9% chance of the correct base being called) for each targeted variation indicated that it was correctly covered [20]. A summary of the nonsynonymous mutations and coverage of three patients (Family A, II: 3; Family B, II: 1 and II: 3), after exclusion of synonymous and noncoding region mutations, is shown in Table 1. Using bioinformatics analysis of the sequencing results, we identified the pathogenic mutations TNNI3-p.Arg145Gly in Family A and LMNA-p.Arg190Trp plus MYH7-p.Arg1045His in Family B. The functional impact of amino acid changes was predicted using three computational programs (PolyPhen-2, SIFT,

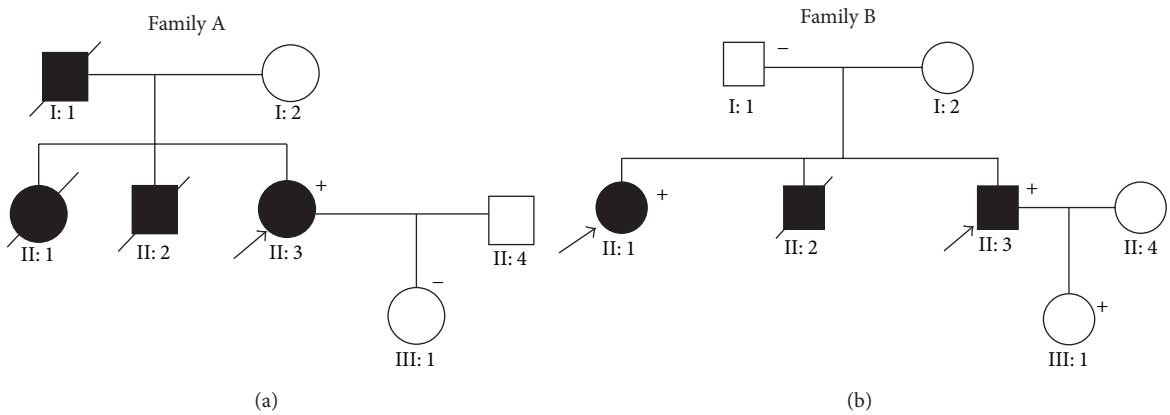

Figure 2: The pedigrees of the families with hypertrophic and dilated cardiomyopathy. Male family members are indicated by squares; female family members are indicated by circles, deceased individuals are indicated by symbols with a strikethrough, the unaffected individuals are represented by open symbols, and the solid symbols represent affected individuals. In addition, the probands are marked with a black arrow. The presence of a mutation was indicated by a "+" sign and the absence of mutations was indicated by a "–" sign. Family A: II: 3 is the proband, I: 1 and II: 2 died of sudden cardiac death, and III: 1 is clinically unaffected; the other clinical data were unavailable; Family B: II: 1 and II: 3 are the probands, II: 2 died of sudden cardiac death, and III: 1 mutation is present, but individual is clinically unaffected.

and MutationTaster). The mutations TNNI3-p.Arg145Gly, LMNA-p.Arg190Trp, and MYH7-p.Arg1045His change the amino acid sequence and may have some impact on the function of coding proteins.

3.3. Mutations in TNNI3, LMNA, and MYH7. In family A, the proband (II: 3) was diagnosed with typical HCM. In the sequenced genomic regions, we identified the known C>T pathogenic heterozygotic mutation located at nucleotide position c.433 (Transcript name: NM_000363.4) in *TNNI3* exon 7 (Table 1 and Figure 3(a)). This mutation results in the replacement of arginine at the 145th amino acid position by glycine (p.Arg145Gly). Using Sanger sequencing, this mutation was verified; it was not found in the daughter (Figure 3(a)). Further analysis showed that the amino acid Arg145 is highly conserved in many species and is localized in the first actin binding domain of the TNNI3 protein.

Molecular genetics analysis for family B showed that the probands II: 1 and II: 3 have the pathogenic doubly heterozygous mutations c.568C>T within exon 3 of *LMNA* and c.3134G>A within exon 25 of *MYH7* (Table 1 and Figures 3(b) and 3(c)). These mutations are predicted to cause substitution of a nonsynonymous charged arginine with a tryptophan at codon 190 (Transcript name: NM_005572.3, p.Arg190Trp) and an arginine with a histidine at codon 1045 (Transcript name: NM_000257, p.Arg1045His). Although the father (I: 1) and daughter (III: 1) of the proband II: 3 did not have clinical symptoms (e.g., tightness, dyspnoea, and dizziness), the same pathogenic mutation LMNA-p.Arg190Trp was also identified in the daughter (III: 1) by Sanger sequencing. In contrast, this mutation (LMNA-p.Arg190Trp) was not found in the father (I: 1) (Figure 3(b)). The identified mutated site of LMNA is also highly conserved among species and is localized in the Coil1b region of the LMNA protein. Interestingly, we also found a second pathogenic mutation (MYH7-p.Arg1045His) in family B in the probands II: 1 and II: 3, in addition to the LMNA-p.Arg190Trp mutation. We found that the amino acid MYH7-p.Arg1045 is highly conserved in many species.

4. Discussion

In this study, candidate genes were sequenced in patients with familial cardiomyopathy by using NGS technologies. Many nonsynonymous variations were detected in the familial cardiomyopathy patients and per nonsynonymous variation in coverage of more than 30x (Table 1), including three pathogenic heterozygotic mutations (*TNNI3*, c.433C>G, p.Arg145Gly; *LMNA*, c.568C>T, p.Arg190Trp; and *MYH7*, c.3134G>A, p.Arg1045His). Further, PCR and Sanger sequencing were used to verify those three mutations.

The *TNNI3* gene is located on chromosome 19q13.4 and encodes cardiac troponin I type 3. Together with the TNNT2 and TNNC1 subunits, TNNI3 forms the heterotrimeric troponin complex in the thin filaments of the cardiac striated muscle, and it is a key regulatory protein of the thin filament [21, 22]. TNNI3 contains five main functional regions: a region containing phosphorylation sites, a TNNC1 binding domain, a TNNT2 binding domain, the first actin binding domain, and the second actin binding domain (Table 3). It is the main inhibitory subunit of the troponin complex, and after calcium binding to troponin C, it blocks interaction between myosin and actin via tropomyosin and indirectly inhibits actomyosin ATPase activity. Mutation of the *TNNI3* gene and the subsequent alterations in the protein may interfere with its binding to the other subunits and disrupt the function of the entire troponin complex. Furthermore, the relevant evidence indicates that the mutated TNNI3 protein (p.Arg145Gly) increased activity of actomyosin ATPase activity via increasing the sensitivity of Ca^{2+} [23]. This causes increased Ca^{2+} sensitivity in cardiac myofilaments, which results in increased activity of the sarcomere proteins, eventually leading to the occurrence of HCM. The prevalence

Table 1: Potential of the nonsynonymous variations in patients with FHCM and FDCM.

Proband	Gene name	Transcript name	Coverage	Zygosity	Nucleic acid change	Amino acid change	rs ID
Family A II: 3	*LAMA4*	NM_002290	184	Het	c.1471T>C	p.Tyr491His	rs1050348
	TNNI3	NM_000363	111	Het	c.433C>G	p.Arg145Gly	rs104894724
	MYPN	NM_032578	169	Het	c.2072G>C	p.Ser691Ile	rs10997975
	RBM20	NM_001134363	123	Hom	c.2303G>C	p.Trp768Ser	rs1417635
	VCL	NM_014000	172	Het	c.2801C>T	p.Ala934Val	rs16931179
	SCN5A	NM_198056	150	Het	c.1673A>G	p.His558Arg	rs1805124
	LAMA4	NM_002290	235	Hom	c.3328G>A	p.Gly1110Ser	rs2032567
	LDB3	NM_007078	192	Het	c.163G>A	p.Val55Ile	rs3740343
	MYPN	NM_032578	119	Het	c.2409C>G	p.Ser803Arg	rs3814182
	ACTN2	NM_001103	186	Het	c.1423G>A	p.Asp475Asn	rs80257412
	RBM20	NM_001134363	153	Het	c.3667G>C	p.Glu1223Gln	rs942077
	MYL3	NM_000258	201	Het	c.92G>A	p.Arg31His	rs199639940
Family B II: 1	*LAMA4*	NM_002290	191	Het	c.1471T>C	p.Tyr491His	rs1050348
	RBM20	NM_001134363	116	Hom	c.2303G>C	p.Trp768Ser	rs1417635
	MYH7	NM_000257	176	Het	c.3134G>A	p.Arg1045His	NA
	VCL	NM_014000	143	Het	c.2801C>T	p.Ala934Val	rs16931179
	SCN5A	NM_198056	133	Het	c.1673A>G	p.His558Arg	rs1805124
	LAMA4	NM_002290	225	Hom	c.3328G>A	p.Gly1110Ser	rs2032567
	MYH6	NM_002471	107	Het	c.3388G>A	p.Ala1130Thr	rs28730771
	MYH6	NM_002471	158	Het	c.3302T>C	p.Val1101Ala	rs365990
	TNNT2	NM_001001432	154	Het	c.740A>G	p.Lys247Arg	rs3730238
	LMNA	NM_170708	130	Het	c.568C>T	p.Arg190Trp	rs59026483
	RBM20	NM_001134363	147	Het	c.3667G>C	p.Glu1223Gln	rs942077
Family B II: 3	*LAMA4*	NM_002290	225	Het	c.1471T>C	p.Tyr491His	rs1050348
	MYPN	NM_032578	177	Het	c.1471T>C	p.Tyr491His	rs10823148
	MYPN	NM_032578	179	Het	c.2072G>A	p.Ser691Ile	rs10997975
	RBM20	NM_001134363	125	Hom	c.2303G>C	p.Trp768Ser	rs1417635
	SCN5A	NM_198056	140	Het	c.1673A>G	p.His558Arg	rs1805124
	LAMA4	NM_002290	286	Hom	c.3328G>A	p.Gly1110Ser	rs2032567
	MYH6	NM_002471	121	Het	c.3388G>A	p.Ala1130Thr	rs28730771
	MYH6	NM_002471	143	Het	c.3302T>C	p.Val1101Ala	rs365990
	MYPN	NM_032578	198	Het	c.2409C>G	p.Ser803Arg	rs3814182
	LMNA	NM_170708	114	Het	c.568C>T	p.Arg190Trp	rs59026483
	MYPN	NM_032578	199	Het	c.3403C>A	p.Pro1135Thr	rs7079481
	MYPN	NM_032578	186	Het	c.2120G>A	p.Ser707Asn	rs7916821
	RBM20	NM_001134363	173	Hom	c.3667G>C	p.Glu1223Gln	rs942077
	MYH7	NM_000257	169	Het	c.3134G>A	p.Arg1045His	NA
	RBM20	NM_001134363	166	Het	c.3170G>A	p.Arg1057Gln	rs188054898

Het: heterozygotes; Hom: homozygous; NA: not applicable.

Table 2: PCR primers for amplification of the mutation sites.

Gene symbol	Nucleotide change	Exon	Primer (5′-3′)	Tm (°C)	Fragment size (bp)
TNNI3	c.433C>G	7	Sense: GCCTAAGCCGGGAAGAGACTGGTA Antisense: GAGGACCCCTTACTAGCTGCTTCT	55	437
LMNA	c.568C>T	3	Sense: GAGTAGCTGGGACTACAGGCGTGT Antisense: ATCTGACTCCACATCCTGCGACC	57	1338
MYH7	c.3134G>A	25	Sense: GGCAATCTCACAGTCCCCTAATAA Antisense: TTTTTGCCAGGGAGGACCATCTAA	55	508

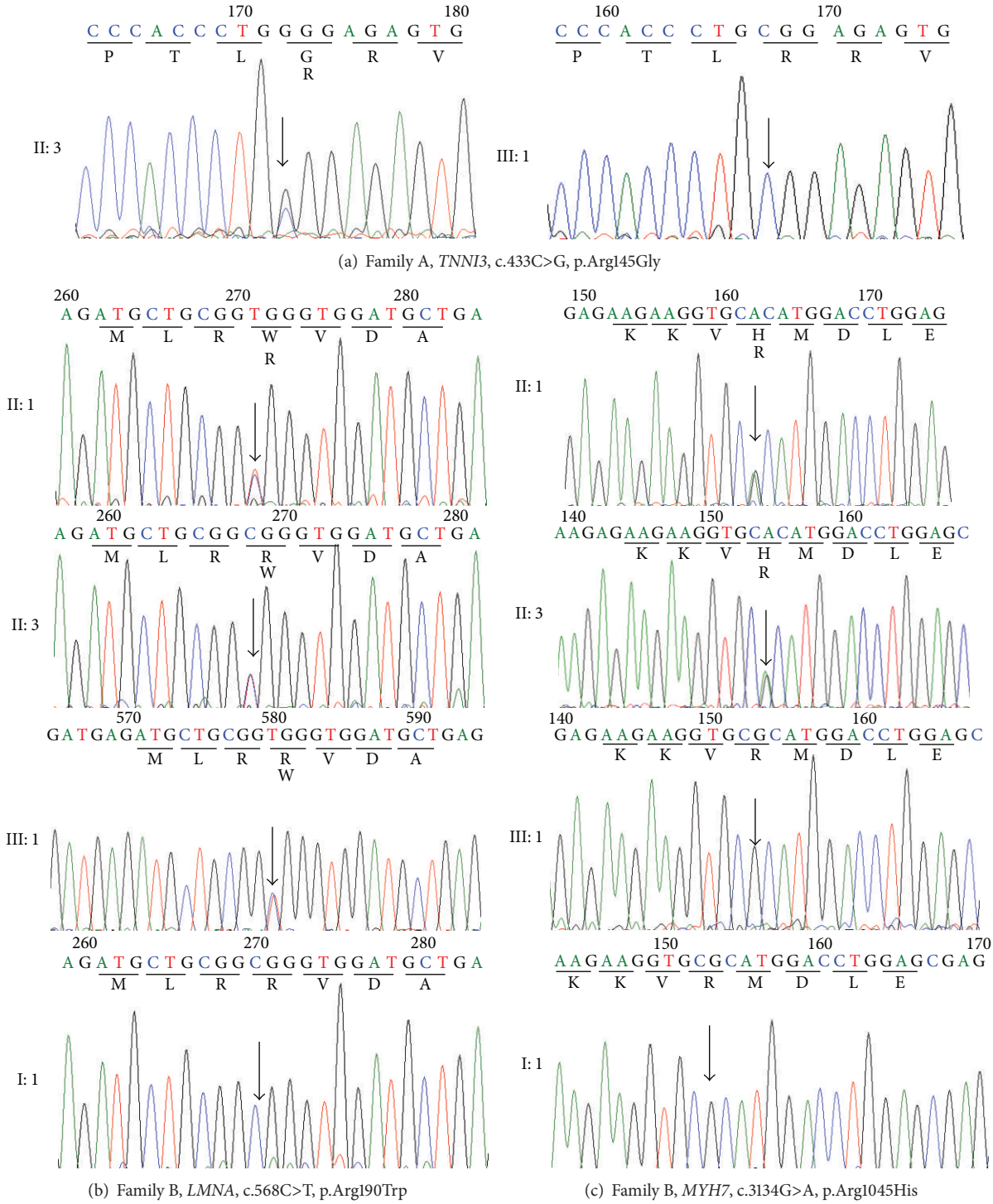

FIGURE 3: Results of the Sanger sequencing analysis. (a) shows the results for the TNNI3-p.Arg145Gly mutation in Family A; the results were positive in II: 3 and negative in III: 1; (b) shows the results for the LMNA-p.Arg190Trp mutation in Family B; the results were positive in II: 1, II: 3, and III: 1 and negative in I: 1; (c) shows the results for the MYH7-p.Arg1045His mutation in Family B; family members II: 1 and II: 3 tested positive, and III: 1 and I: 1 tested negative.

of *TNNI3* mutations is approximately 5% in HCM. To date, approximately 30 mutations have been identified to be associated with HCM (Table 3), and the most frequent *TNNI3* mutations have been reported in exons 7 and 8, which encode the domains interacting with cardiac troponin C (TNNC1) and cardiac actin (ACTC1) (Table 3). The TNNI3-p.Arg145Gly mutation identified here has been previously described in American and Korean patients (Table 3).

Table 3: The documented TNNI3 mutations in HCM.

Exon	Amino acid change	Local structure	Reported times	Population report group
3	p.Arg21Cys	TNNC binding domain	1	American [37] and Norwegian [35]
3	p.Arg13Cys	TNNC binding domain	1	Chinese [38]
4	p.Lys36Gln	TNNC binding domain	1	English [39]
5	p.Pro82Ser	TNNT2 binding domain	1	American [40]
7	p.Arg141Gln	First actin binding domain	2	American [41] and French [27]
7	p.Arg145Gly	First actin binding domain	8	American [2], Korean [42, 43], and Chinese [**this study**]
7	p.Arg145Gln	First actin binding domain	1	Japanese [44]
7	p.Asn185Lys	TNNC binding domain	1	English [39]
7	p.Ala157Val	TNNC binding domain	3	French [27], Norwegian [35], and Dutch [45]
7	p.Arg162Pro	TNNC binding domain	1	French [27]
7	p.Arg162Trp	TNNC binding domain	1	Japanese [44]
7	p.Arg162Gln	TNNC binding domain	2	American [41] and English [46]
7	p.Ser166Phe	TNNC binding domain	5	American [41] and Dutch [45]
7	p.Arg170Gln	TNNC binding domain	1	English [47]
7	p.Ser166Phe	TNNC binding domain	1	German [48]
7	p.Lys164Thr	TNNC binding domain	1	Dutch [49]
7	p.Asp180Gly	TNNC binding domain	1	Dutch [49]
7	p.Lys178del	TNNC binding domain	1	Dutch [49]
8	p.Arg186Gln	TNNC binding domain	1	French [27] and English [46]
8	p.Asp196Asn	Second actin binding domain	3	French [27], American [50], and Norwegian [35]
8	p.Gly203Ser	Second actin binding domain	1	Japanese [44]
8	p.Met201Thr	Second actin binding domain	1	Dutch [49]
8	p.Arg204Cys	Second actin binding domain	1	American [51]
8	p.Lys206Gln	Second actin binding domain	1	Japanese [44]
8	p.Glu209Ala	Second actin binding domain	5	Dutch [49]
8	p.Ile195Met	Second actin binding domain	1	American [51]

However, to our knowledge, our study is the first report of the TNNI3-p.Arg145Gly mutation in a Chinese patient with FHCM. It is worth noting that the p.Arg145Gly mutation is a hot spot of TNNI3 and is highly correlated with HCM in American, Korean, and Chinese populations (Table 3).

The clinical diagnosis of HCM is based on the criterion of maximal wall thickness greater than or equal to 15 mm; our patient (Family A, II: 3) had wall thickness (14.8 mm) close to 15 mm. However, studies on genotype-phenotype correlations have verified that essentially any wall thickness (including normal wall thickness) is compatible with the presence of a HCM mutant gene but also have a high incidence of sudden death [24, 25]. Our interpretation is that mutation (p.Arg145Gly) in the gene encoding TNNI3 is delayed or subclinical cardiac hypertrophy until middle age or old age; this result indicates that genetic testing has an important diagnostic value, especially for clinically equivocal patient.

The *LMNA* gene is located on chromosome 1q22 and encodes two major isoforms of lamin A/C, which is the main nuclear protein component in mammals and acts as a meshwork structure. The LMNA protein contains four major coiled-coil domains (Coil1a, Coil1b, Coil2a, and Coil2b) and three insertion regions (L1, L2, and L12) [26]. Generally, the *LMNA* gene is highly conserved throughout evolution. However, approximately 40 disease-causing mutations have been described in different populations (Table 4). *LMNA* may be one of the most frequent disease-associated genes for FDCM and has been shown to be associated with a severe clinical phenotype; the prevalence of mutations in *LMNA* is approximately 10% in DCM patients [27]. The documented data (Table 4) suggest that mutations in DCM may occur almost anywhere in *LMNA*; however, the Coil1b region, which is important for lamin A/C dimerization and lamin B interaction, seems to be affected most frequently. The LMNA-p.Arg190Trp mutation has also been documented in previous studies [28–34] (Table 4), but, to our knowledge, this is the first report of the LMNA-p.Arg190Trp mutation in a case of Chinese familial DCM. This finding further illustrates that the LMNA-p.Arg190Trp mutation is a hot spot in familial DCM in Chinese patients as well as in the general population. Interestingly, this pathogenic mutation (LMNA-p.Arg190Trp) was also identified in the proband II: 3's daughter (III: 1) who showed no clinical symptoms; it illustrates that III: 1 has an onset of the disease that appears delayed. Based on these findings, regular clinical cardiovascular testing and further genetic screening are recommended for her and all first-degree relatives of family B for future clinical management of this case of familial cardiomyopathy.

An additional mutation (MYH7-p.Arg1045His) was found in family B in patients II: 1 and II: 3; the proband (family B, II: 3) with the doubly heterozygous mutations displayed a more malignant clinical phenotype of cardiomyopathy

Table 4: The documented LMNA mutations in DCM.

Exon	Amino acid change	Local structure	Reported times	Population report group
1	p.Lys97Glu	Coil1b	1	Italian [30]
1	p.Arg101Pro	Coil1b	1	American [31]
1	p.Glu111*	Coil1b	1	Italian [30]
1	p.Arg89Leu	Coil1b	2	American [31, 52]
1	p.Leu85Arg	Coil1b	1	American [53]
1	p.Arg60Gly	Coil1b	1	American [53]
1	p.Arg89Leu	Coil1b	1	American [52]
1	p.Glu82Lys	Coil1b	1	Chinese [54]
2	p.Arg166Pro	Coil1b	1	American [31]
2	p.Glu161Lys	Coil1b	1	German [26]
3	p.Arg190Trp	Coil1b	7	Spanish [28], Italian [30], American [31], German [32], English [33], Finland [34], Korea [28], and Chinese [**this study**]
3	p.Arg189Trp	Coil1b	1	Italy [12]
3	p.Glu203Val	Coil1b	1	German [26]
3	p.Arg190Gln	Coil1b	2	German [26] and American [31]
3	p.Glu203Lys	Coil1b	1	American [31]
3	p.Ile210Ser	Coil1b	1	American [31]
3	p.Asn195Lys	Coil1b	1	Dutch [55]
3	p.Glu203Gly	Coil1b	1	American [53]
3	p.Asn195Lys	Coil1b	1	American [53]
3	p.Asp192Gly	Coil1b	1	English [33]
4	p.Gly232Val	L12	1	Chinese (Taipei) [56]
4	p.Lys219Thr	L12	1	German [26]
4	p.Leu215Pro	L12	1	American [31]
4	p.Lys219Thr	L12	1	Italy [57]
4	p.His222Pro	L12	1	French [45]
4	p.Arg225*	L12	2	American [31] and Dutch [55]
4	p.Gln234*	L12	1	American [31]
6	p.Arg349Leu	Coil2b	1	Spanish [28]
6	p.Glu317Lys	Coil2b	1	Italian [30]
6	p.Ala318Thr	Coil2b	1	American [31]
7	p.Arg388His	Tail	1	American [31]
7	p.Arg377His	Tail	1	American [52]
8	p.Arg471His	Tail	1	American [31]
8	p.Tyr481*	Tail	1	English [33]
10	p.Ser573Leu	Tail	1	American [52]
10	p.Arg541Ser	Tail	1	English [33]
11	p.Arg644Cys	Tail	2	German [26] and Chinese [58]
11	p.Arg654*	Tail	1	American [31]

*Stop codon.

(Figures 2(b) and 3(c)). This described for the first time the double heterozygous mutations of LMNA-p.Arg190Trp plus MYH7-p.Arg1045His in FDCM patients. The *MYH7* gene, which encodes the myosin heavy chain, is one of the most important causative genes in inherited cardiomyopathy. Previous studies have reported a relationship between the MYH7-p.Arg1045His mutation and HCM [35, 36]; however, our study is the first to discover the association of the MYH7-p.Arg1045His mutation with FDCM. We believe that these results indicate that double mutations (LMNA-p.Arg190Trp plus MYH7-p.Arg1045His) had a dose effect. As a result, it led to the patients (LMNA-p.Arg190Trp plus

MYH7-p.Arg1045His carriers) showing a clinical malignant phenotype. This result is an additional indication of the genetic heterogeneity of inherited cardiomyopathy.

5. Study Limitations

In this study, the number of recruited participants was limited, and the obtained clinical data were not consistent for all subjects. In addition, we used the accepted normal range of each clinical parameter in this study. A larger study on Chinese familial cardiomyopathies will be essential to confirm further the mutations identified in this study.

6. Conclusions

To our knowledge, this is the first study to discover the mutations TNNI3-p.Arg145Gly and LMNA-p.Arg190Trp in Chinese patients with familial HCM and DCM, respectively. A double mutation (LMNA-p.Arg190Trp plus MYH7-p.Arg1045His) was first discovered in familial DCM. These mutations were identified using NGS technologies and were confirmed by Sanger sequencing. Considering both previous data and our new findings, it is proposed that TNNI3-p.Arg145Gly and LMNA-p.Arg190Trp are hot spots of FHCM and FDCM, respectively. The discovery of the mutational status with respect to the double mutations (LMNA-p.Arg190Trp plus MYH7-p.Arg1045His) may be useful in family B for assessment of individuals at risk for familial DCM; the results further indicate the genetic heterogeneity of inherited cardiomyopathy. Genetic testing may provide more predictive information for inherited cardiomyopathy diagnosis, particularly regarding the risk to family members who are clinically asymptomatic. In addition, genetic testing of candidate genes by using NGS technologies is becoming increasingly viable and economical. In the future, NGS could be used as a complementary approach for the clinical diagnosis of FHCM and FDCM.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Yue Zhao and Yue Feng contributed equally to this work.

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