# Multiple gene mutations, not the type of mutation, are the modifier of left ventricle hypertrophy in patients with hypertrophic cardiomyopathy

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**Abstract** Genotype-phenotype correlation of hypertrophic cardiomyopathy (HCM) has been challenging because of the genetic and clinical heterogeneity. To determine the mutation profile of Chinese patients with HCM and to correlate genotypes with phenotypes, we performed a systematic mutation screening of the eight most commonly mutated genes encoding sarcomere proteins in 200 unrelated Chinese adult patients using direct DNA sequencing. A total of 98 mutations were identified in 102 mutation carriers. The frequency of mutations in *MYH7*, *MYBPC3*, *TNNT2* and *TNNI3* was 26.0, 18.0, 4.0 and 3.5 % respectively. Among the 200 genotyped HCM patients, 83

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Surgical ICU, State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Disease, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100037, People's Republic of China harbored a single mutation, and 19 (9.5%) harbored multiple mutations. The number of mutations was positively correlated with the maximum wall thickness. We found that neither particular gene nor specific mutation was correlated to clinical phenotype. In summary, the frequency of multiple mutations was greater in Chinese HCM patients than in the Caucasian population. Multiple mutations in sarcomere protein may be a risk factor for left ventricular wall thickness.

**Keywords** Hypertrophic cardiomyopathy · Multiple gene mutations · Left ventricular hypertrophy

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# Introduction

Hypertrophic cardiomyopathy (HCM) is a disease marked by left ventricular hypertrophy with predominant involvement of the interventricular septum in the absence of other causes of hypertrophy. HCM is the most prevalent, heritable cardiovascular disease and the most common cause of sudden cardiac death in young athletes [1]. We have previously reported that the prevalence of HCM is 83/100,000 in the adult Chinese population, indicating that there are approximately one million HCM patients in China [2].

Over 1400 mutations in at least 11 genes encoding sarcomere proteins have been reported to cause HCM [3, 4]. Currently, 3-5 % of patients with HCM have been estimated to carry more than one mutation in the same gene or different genes. These patients are thought to have severer clinical manifestations than did one mutation carrier. These studies were mostly performed in Caucasians and little is known about the genetic basis on multiple mutations in Chinese patients with HCM. To assess the mutation profile and the genotype-phenotype correlations, systematic mutation screening of the eight most common HCM-disease genes encoding the sarcomere proteins was carried out in 200 unrelated index Chinese patients with HCM. These HCMcausing genes encode beta-myosin heavy chain (MYH7), cardiac myosin binding protein C (MYBPC3), the regulatory and essential myosin light chains (MYL2, MYL3), alphatropomyosin (TPM1), cardiac troponin I (TNNI3), cardiac troponin T (TNNT2) and alpha-actin (ACTC1).

## Materials and methods

## Subjects

Two hundred unrelated index patients with HCM and 120 age-and sex-matched healthy controls were recruited consecutively from Beijing Fuwai Hospital, Chinese Academy of Medical Sciences, from 2002 to 2008. This study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association [5], and has been approved by the Ethics Committee of Fuwai Hospital. Written informed consent was provided by all participants. The diagnosis of HCM was ascertained in adults by a left ventricular maximal wall thickness (MWT) of greater than 15 mm on echocardiography [6]. The greatest wall thickness measured in diastole at any site in the LV wall was regarded as the maximal thickness, independent of correction for body surface area, gender or age. Subjects with extracardiac disease or secondary causes of cardiac hypertrophy were excluded. None of the control subjects had a history of serious systemic diseases. Three cardiologists independently reviewed all clinical data.

#### Mutation detection

Genomic DNA was prepared from peripheral blood leukocytes. Mutation screening was performed on the entire coding sequences and flanking regions in MYH7, MYBPC3, MYL2, MYL3, TPM1, TNN13, TNNT2 and ACTC1 with polymerase chain reaction (PCR) amplification and direct DNA sequencing on an ABI Prism 3730 XL DNA Sequencer (Applied Biosystems, Foster City, CA, USA). All variants were confirmed by sequencing in sense and anti-sense directions. Non-pathogenic polymorphisms, defined as more than 1 % frequency in 120 unrelated control subjects, were excluded. The pathological variants were defined as mutation-HCM cosegregation in all affected members in the investigated pedigrees and the gene has been reported as a HCM-causing gene in literature; the mutation is localized in a very conservative sequence region across species; and the mutation causes a significant structural or functional change by bioinformatic prediction [7, 8]. To confirm the pathogenic role for a mutation, the following programs were applied. The programs of amino acid substitution prediction (SIFT, http:// sift.bii.a-star.edu.sg/www/SIFT BLink submit.html; Polyphen -HCM, http://genetics.bwh.harvard.edu/pph/ or Polyphen-2 when prediction with Polyphen-HCM unavailable, http:// genetics.bwh.harvard.edu/pph2/; Mutpred, http://mutpred. mutdb.org/; SNPs3D, http://www.snps3d.org/; Pmut, http:// mmb2.pcb.ub.es:8080/PMut/). A missense mutation was assumed to be possibly disease-causing if at least two independent programs indicated a damaging effect. The program Human Splicing Finder (HSF) was used to determine whether any of the detected mutations destroys an existing or creates a novel splice site (HSF, http://www.umd.be/HSF/).

## Statistical analysis

Statistical analysis was performed with SPSS for Windows, release 18.0 (SPSS, Chicago, IL). Continuous variables were expressed as mean  $\pm$ S.D. ANOVA analysis was used to calculate the difference between groups. Qualitative parameters were compared between groups by Chi squared test or Fisher's exact test. A two-tailed *P* value of <0.05 was considered significant. To rule out the influence of gender or age, the General Linear Model was introduced to adjust the differences in clinical characteristics among the groups.

#### Results

## Genotype analysis

Among the 200 patients with HCM, 102 (51 %) were identified with the disease-causing mutations in the genotyped 8 genes. Mutations were detected in 61 % (58/95) of the familial probands and 41.9 % (44/105) of the sporadic cases. Seventy (47.9 %) male and 32 (59.3 %) female patients were found to harbor mutations, respectively.

A total of 98 mutations were identified in 102 mutation carriers (Table 1), 58 % (57/98) of these mutations were novel mutation. The novel mutation was defined as not reported in the previous publications or the mutation databases (http://www.cardiogenomics.org and http://www.hgmd.org). The mutation was distributed mostly in *MYH7* (26.0 %, 52/200) and in *MYBPC3* (18.0 %, 36/200), less in *TNNT2* (4.0 %, 8/200) and in *TNNI3* (3.5 %, 7/200). Mutations no more than 1.5 % was found in each of the following genes, including *MYL2* (1 %, 2/200), *MYL3* (1.5 %, 3/200), *TPM1* (1.5 %, 3/200) and *ACTC1* (1.5 %, 3/200), as shown in the Fig. 1. Various types of mutations were detected in *MYBPC3*, including missense, splicesite, nonsense and frame shift mutation. In contrast, missense mutations were predominantly identified in the genes other than *MYBPC3*.

Among the 102 HCM mutation carriers, 83 had a single mutation identified, 18 (9 %) had two mutations, and 1 had three mutations, respectively (Table 2). Eight of the double mutations were located within a single gene (*MYH7* or *MYBPC3*), whereas the other double mutations and the triple mutations presented in distinct genes.

#### HCM phenotype

Nineteen patients were found to carry multiple mutations. The effect of the number of mutations on the phenotype was further analyzed. Patients were classified into three groups on the basis of mutation number: patients with no mutation (98 patients), single mutation (83 patients), and multiple mutations ( $\geq 2$  mutations) (19 patients). MWT was proportionally related to the mutation numbers (Table 3), even after adjusting for age and gender. MWT was increased with the numbers of mutations harbored (non-mutation vs. 1 vs. 2 or more,  $19.7 \pm 5.1$  mm vs.  $20.5 \pm 4.8$  mm vs. 23.6 $\pm$  5.7 mm, P < 0.01) and earlier age at diagnosis (non-mutation vs. 1 vs. 2 or more,  $44.9 \pm 13.5$  years vs.  $38.4 \pm 12.7$  years vs.  $34.5 \pm 12.9$  years, P < 0.01). The left atrial internal diameter (LA) was greater in patients with mutations than in those without mutations (LA, non-mutation vs. 1 vs. 2 or more,  $38.4 \pm 6.3$  mm vs.  $41.4 \pm 8.3$  mm vs. 42.5  $\pm$  7.9 mm, P < 0.01). No significant differences in left ventricular end-diastolic diameter (LVEDD) or left ventricular ejection fraction (LVEF) were observed among the three groups. Of the 19 patients with multiple mutations, 11 carried at least one established risk markers of SCD and 1 was treated with implantable cardioverter defibrillator (ICD).

#### Discussion

We report the mutation profiling of eight genes encoding sarcomere proteins in an adult Chinese HCM cohort. As most reports, the gene mutations can be found in more than half of the HCM patients. The *MYH7* (26.0 %) and *MYBPC3* (18.0 %) were the predominant HCM-causing genes in Chinese population as well, followed by *TNNT2* (4.0 %) and *TNNI3* (3.5 %). The other four screened genes accounted for less than 1.5 % each. In addition to missense mutation, splice site, nonsense and frame shift mutations could also be identified in *MYBPC3*, but not in the other screened genes.

The gene mutations, such as R403Q in MYH7, have been found as malignant mutation in Caucasian HCM [9, 10], but were not detected in Chinese. To date, more than 1,400 mutations in at least 11 HCM-causing sarcomere genes have been identified in patients with HCM. Genotype-phenotype correlations have been challenging because of the genetic and clinical heterogeneity of HCM, such as that most pedigrees carried their own private mutation. Multiple mutations were found in Caucasians, the frequency of multiple mutations of two or more in the same gene or in different genes has been reported to be only 3-6 and 0.8 %, respectively [11-16]. Only 2.7 % (3/ 112) familial HCM patients were found harbor compound gene mutations in the selected eight genes in Japanese [17]. In contrast, we screened 8 known HCM-causing genes in 200 HCM patients, 83 had a single mutation, 18 (9 %) had two mutations, and 1 had three mutations. The frequency of multiple mutations was much higher in this study than in previous reports [11-16].

The frequency of mutation in adult proband (61.0 %, 58/95) and sporadic patients (41.9 %, 44/105) were similar to that of previously reported in HCM children (64 % in familial and 49 % in sporadic, respectively) [18]. Both familial and sporadic patients of HCM shared common gene mutations, indicating identical feasibility and necessity of genetic analyses for both types of patients, and in both children and adults.

No genetic mutation was identified in 49.0 % (98/200) of HCM patients. Three conceivable reasons maybe responsible: mutations were present in genes that were not screened; mutations were present in the non-coding (intron or promoter) regions of the genes screened; or there were technical limitations in the method of direct DNA sequencing. For example, the sequencing method used cannot detect copy number variants.

Given the heterogeneity in genetic etiologies and clinical manifestations of HCM, from asymptomatic to heart failure and sudden cardiac death, and even intra-family patients carrying the same gene mutation show impressively the wide spectrum of phenotypic presentation and

Table 1 Mutations associated with HCM in 102 unrelated index patients

Gene	Nucleotide change	Protein change	Status	Number of affected patients	Amino acid substitution prediction	Human splicing finder
MYH7	NM_000257.2 NP_	000248.2				
c.136	6T>A	F46I	Novel	1	+	
c.346	6A>T	T116S	Novel	1	+	
c.428	3G>A	R143Q	Known	2	+	
c.655	5C>G	Q219E	Novel	1	+	
c.746	6G>A	R249Q	Known	2	+	
c.923	3A>G	Y308C	Novel	1	+	
c.106	53G>A	A355T	Known	1	+	
c.117	72A>C	N391T	Novel	1	+	
c.127	73G>A	G425R	Known	1	+	
c.130	)9A>G	N437D	Novel	1	+	
c.135	57C>T	R453C	Known	2	+	
c.175	50G>A	G584S	Known	1	+	
c.181	16G>A	V606 M	Known	1	+	
c.198	37C>T	R663C	Known	1	+	
c.198	38G>A	R663H	Known	4	+	
c.210	)4A>G	I702 V	Novel	1	+	
c.214	46G>A	G716R	Known	1	+	
c.219	91C>T	P731S	Novel	1	+	
c.222	21G>T	G741 W	Known	1	+	
c.234	46C>G	S782R	Known	1	+	
c.238	39G>A	A797T	Known	1	+	
c.246	65T>C	M822T	Known	1	+	
c.246	58G>A	G823E	Known	1	+	
c.253	39_254 1delAAG	K847del	Known	1	+	
c.260	)9G>A	R870H	Known	1	+	
c.267	74C>A	Q892 K	Novel	1	+	
c.277	79G>A	E927 K	Known	1	+	
c.278	35_278 7delGAG	E929del	Novel	1	+	
c.278	38G>A	E930 K	Known	1	+	
c.326	58C>G	L1090 V	Novel	1	+	
c.350	)4G>T	E1168D	Novel	1	+	
c.383	30G>A	R1277Q	Novel	2	+	
c.406	66G>A	E1356 K	Known	3	+	
c.413	35G>A	A1379T	Known	1	+	
c.414	14C>T	R1382 W	Known	1	+	
c.414	45G>A	R1382Q	Novel	1	+	
c.425	58C>T	R1420 W	Known	1	+	
c.491	2G>A	E1638 K	Novel	1	+	
c.494	41G>C	Q1647H	Novel	1	+	
c.534	46–534 8delAAG	M1782del	Novel	1	+	
c.554	I3A>C	K1848T	Novel	2	+	
c.556	51C>T	T1854 M	Known	3	+	
MYBP	<i>C3</i> NM_000256.3 N	P_000247.2				
c.5C	>G	P2R	Novel	1	+	
c.156	6C>A	S52R	Novel	1	+	
c.478	3C>T	R160 W	Known	3	+	

c43G>TR21SCNovelI+c77G>AE28 KKaownI+c97G>AE28 KKaownI+c97G>AE28 KNovelI+c97G>AE36 KNovelI+c100K1>AH34 KKaownA+c107BA>TK360XNovelI+c118G>AW39XNovelI+c127G>CA33PNovelI+c127G>CA43PNovelI+c137GdCP459 KNovelI+c1387C>TQ463XNovelI+c1387C>TQ463XNovelI+c1387C>TQ463XNovelI+c1387C>TQ463XNovelI+c1387C>TQ463XNovelI+c1387C>TQ463XNovelI+c1387C>TQ463XNovelI+c1387C>TQ463XNovelI+c1387C>TQ463XNovelI+c1387C>CSpliceNovelI+c1387C>CSpliceNovelI+c2304+IC>CSpliceNovelI+c2304+IC>CSpliceNovelI+c2304+IC>CSpliceNovelI+c2304+IC>CSpliceNovelI+c2304+IC>CSpliceNovelI+c2304-IC>CSplice<	Gene	Nucleotide change	Protein change	Status	Number of affected patients	Amino acid substitution prediction	Human splicing finder
c.7870>AE28 KKnownI+c.7870>AG263 RNovelI+c.903de0G203 KNovelI+c.1008>AE334 KKnown3+c.1078A>TK30CNovelI+c.1187G>AW396XNovelI+c.125A>TK40CNovelI+c.137ACP495 NNovelI+c.1387C>TQ403XNovelI+c.1387C>TQ405XNovelI+c.1387C>TQ405XNovelI+c.1387C>TQ405XNovelI+c.1387C>TQ405XNovelI+c.1387C>TQ405XNovelI+c.1387C>TQ405XNovelI+c.1387C>TQ405XNovelI+c.1387C>TQ405XNovelI+c.1387C>TQ405XNovelI+c.1387C>TR405XNovelI+c.2387C>GSpliceNovelI+c.2397C>CN93XNovelI+c.2397C>CN93XNovelI+c.3397A=QN13DNovelI+c.3397A=QN13DNovelI+c.3397A=QN13DNovelI+c.3397A=QN13DNovelI+c.3397A=QN13DNovelI+c.3397A=Q <t< td=""><td>c.643</td><td>SC&gt;T</td><td>R215C</td><td>Novel</td><td>1</td><td>+</td><td></td></t<>	c.643	SC>T	R215C	Novel	1	+	
c 7370×AC 263RKawaI+c.903deGL301 KNovelI+c.1000C>AH343 KNovelI+c.1078A>TK360XNovelI+c.1377ACK430XNovelI+c.1377ACH442 MNovelI+c.1377ACH442 MNovelI+c.1377ACH645XNovelI+c.1377ACH645XNovelI+c.1387ASH611 KNovelI+c.1381G>AH611 KNovelI+c.1381G>AH611 KNovelI+c.1381G>AH611 KNovelI+c.1381G>AH611 KNovelI+c.2481S+GCSpliceNovelI+c.2401>AH307 NNovelI+c.2401>AH307 NNovelI+c.24201>AH307 NNovelI+c.2542_250AblinGGCN552,M854delmSNovelI+c.2543_250AblinGGCN504NovelI+c.2373ACSpliceNovelI++c.3374CN163NovelI++c.3374CN163NovelI++c.3374CN163NovelI++c.3374CN164NovelI++c.3374CN164NovelI++c.337	c.772	2G>A	E258 K	Known	1	+	
e030dEGL301 fs.NorelI+c.1000c>AE334 KNorelI+c.1078A>TK30XNorelI+c.1187C>AW396XNorelI+c.1325A>TK442 MNorelI+c.1325A>TK442 MNorelI+c.1337C>TP450 fs.NorelI+c.1387C>TR463 NNorelI+c.1387C>TR463 NNorelI+c.1387C>TR450 NNorelI+c.1387C>TR450 NNorelI+c.1387C>TR450 NNorelI+c.1387C>TR550 NNorelI+c.2308+162 NSpliceNorelI+c.2413+346GSpliceNorelI+c.2542 C50 delimsTR361 NorelI+c.2542 C50 delimsGCNorelNorelI+c.2382 AR964NorelI+c.2382 AR964NorelI+c.2382 AR964NorelI+c.2382 AR964NorelI+c.2382 AR964NorelI+c.2382 AR964NorelI+c.2382 AR964NorelI+c.2382 AR964NorelI+c.2397 AR165NorelI+c.3397 A>GR165NorelI+ <t< td=""><td>c.787</td><td>G&gt;A</td><td>G263R</td><td>Known</td><td>1</td><td>+</td><td></td></t<>	c.787	G>A	G263R	Known	1	+	
c1008c>AE334 KKnown3+c.1078c>CK360XNovel1+c.1376cAW396XNovel1+c.1325c>TK442 MNovel3+c.1325c>TQ463XNovel1+c.1387c>TQ463XNovel1+c.1381C>ABelin KKnown1+c.1381C>AE611 KKnown1+c.1381C>ABelin KNovel1+c.1381C>ASpliceNovel1+c.2308+1C>CSpliceNovel1+c.2308+1C>CSpliceNovel1+c.2413-AddGSpliceNovel1+c.2413-AddGSpliceNovel1+c.24207>AIRONNovel1+c.24207>AIRONNovel1+c.24207>AIRONNovel1+c.24207>AIRONNovel1+c.24207>AIRONNovel1+c.24207>AIRONNovel1+c.242826/delins/CTNationalNovel1+c.2432.5SpliceNovel1+c.2442.5MS444Novel1+c.2442.5SpliceNovel1+c.2442.5Novel1+MT site brokenc.2442.5Novel1+-c.2442.5Novel1+- </td <td>c.903</td> <td>delG</td> <td>L301 fs</td> <td>Novel</td> <td>1</td> <td>+</td> <td></td>	c.903	delG	L301 fs	Novel	1	+	
c1078ATK300XNovel1+c.1187G>AW396XNovel1+c.1297G>CA13PNovel1+c.135ATK42 MNovel1+c.137TabCM59 NNovel1+c.1387C>TQ463XNovel1+c.1387C>TQ463XNovel1+c.1387C>TQ463XNovel1+c.1387C>TR95 WKnown1+c.1387C>TR95 WNovel1+c.1387C>TR95 WNovel1+c.1387C>TR95 WNovel1+c.2308+1G>CSpliceNovel1+c.2308+1G>CSpliceNovel1+c.2420T>A1807 NNovel1+c.2420T>ASpliceNovel1+c.2542_2505 delinsTR851MNovel1+c.2542_2505 delinsTR851MNovel1+c.2542_2505 delinsTR051MNovel1+c.2542_2505 delinsTR051MNovel1+c.313734DN165CNovel1+c.31374DN165CNovel1+c.31374DN165CNovel1+c.31374DN113DNovel1+c.3373-357AV1192DNovel1+c.3404CN192WNovel1+c.3404CN192WNovel <td>c.100</td> <td>00G&gt;A</td> <td>E334 K</td> <td>Known</td> <td>3</td> <td>+</td> <td></td>	c.100	00G>A	E334 K	Known	3	+	
c.1187G>AW396XNovel1+c.1297G>CA433PNovel1+c.1325A>TK42 ANNovel3+c.137G>CQ463XNovel3+c.1387C>TQ463XNovel1+c.1387C>TQ463XNovel1+c.1838C>AE611 KKnown1+c.1838C>ASpliceNovel1+c.1898-1G>ASpliceNovel1+c.2304F1G>CSpliceNovel1+c.2314F3defGSpliceNovel1+c.2420T>AI807 NNovel1+c.2420T>AI807 NNovel1+c.2420T>AI807 NNovel1+c.2420T>AI807 NNovel1+c.2548_2560deinsGCGN50,M854deinsGVNovel1+c.2538_2A>CSpliceNovel1+c.31045G>ASpliceNovel1+c.31045G>ASpliceNovel1+c.31045G>ANilizeNovel1+c.3373-387delV1125_L1129delNovel1+c.3374-387delV1120Novel1+c.3374-387delV1120Novel1+c.3374-387delV1120Novel1+c.3374-387delN104NNovel1+c.3374-387delN102NNovel1+c.	c.107	/8A>T	K360X	Novel	1	+	
c.1373cbA133PNovel1+c.1325AsTK442 MNovel3+c.1373cbP49 fsNovel3+c.1387cbQ463XNovel1+c.1483CsTR495 WKnovel1+c.1831GsAE611 KKnovel1+c.1397dbCSpliceNovel1+c.1397dbCSpliceNovel1+c.230841GsCSpliceNovel1+c.2413+3deGSpliceNovel1+c.2413+3deGSpliceNovel1+c.2420TsAR87TNovel1+c.2542_505 delinsTR850, M854delinsGNovel1+c.2542_505 delinsTR850, M854delinsGNovel1+c.2382_5CASpliceNovel1+WT site brokenc.2382_5CAR943QNovel1+WT site brokenc.2387_ACSpliceNovel1+WT site brokenc.2387_ACSpliceNovel1+WT site brokenc.3397_ASN1125_L1129dNovel1+WT site brokenc.3397_ASN1125_L1129dNovel1+WT site brokenc.3397_ASN1125_L1129dNovel1+WT site brokenc.3397_ASN1125_L1129dNovel1+WT site brokenc.3397_ASN1125_L1129dNovel1++c.3304_C1 <t< td=""><td>c.118</td><td>37G&gt;A</td><td>W396X</td><td>Novel</td><td>1</td><td>+</td><td></td></t<>	c.118	37G>A	W396X	Novel	1	+	
c.1323ATK442 MNovelI+c.1370ACP459 fsNovelI+c.137CATP463XNovelI+c.1483CATR495 WKnownI+c.1898.1GAF611 KKnownI+c.1898.1GASpliceNovelI+c.2304.1GACSpliceNovelI+c.2304.1GACSpliceNovelI+c.2401.34 MeGSpliceNovelI+c.2401.2505 delinsTR835,LNovelI+c.2562.5GY842.XNovelI+c.2542.2506 delinsGCN850.M854 delinsGVNovelI+c.2542.2506 delinsGCSN850.M854 delinsGVNovelI+c.23173.2A>CSpliceNovelI+WT site brokenc.2382.85AR940NovelI+WT site brokenc.2397A>GN102NovelI+WT site brokenc.3397A3GUV1125_L1129dLNovelI+WT site brokenc.3397A>GN1133DNovelI+WT site brokenc.3397A>GN1132DNovelI+WT site brokenc.3397A>GN1132DNovelI+WT site brokenc.3397A>GN1132DNovelI+c.3397A>GN1125_L1129dLNovelI+c.3397A>GN1128_LNovelI+c.3404C>T	c.129	97G>C	A433P	Novel	1	+	
c.1370cbCP49 95Novel3+c.1387cbTQ403XNovel1+c.1483cbTR49 5WKnown1+c.1831cbAE611 KKnown1+c.1831cbASpliceNovel1+c.1937bCISp 9MNovel1+c.2308+1CsCSpliceNovel1+c.2413-3daGSpliceNovel1+c.2413-3daGSpliceNovel1+c.2420bAM37 NNovel1+c.2420bAStabLownNovel1+c.2542_550delinsGTNSUM854delinsGVNovel1+c.2548_256ASpliceNovel1+c.2548_256ASpliceNovel1+c.2548_256ASpliceNovel1+c.3137deICT1046 fsNovel1+c.3137deICN103 WNovel1+c.3137deICN1132DNovel1+c.3373-387deIN1132DNovel1+c.3440C5PipiceNovel1+c.3440C7R102 MNovel1+c.340c5TR102 MNovel1+c.3404C7R102 MNovel1+c.3404C7R102 MNovel1+c.3404C5R168GNovel1+c.3404C5R168GNovel1+c.3404C5R164G	c.132	25A>T	K442 M	Novel	1	+	
c1831C>TQ463XNovel1+c1433C>TR495 WKnow1+c1831G>AE011 KKnow1+c1898-1G>ASpliceNovel1+c1977L>G1659 MNovel1+c2308+1G>CSpliceNovel1+c2413+3deIGSpliceNovel1+c2420T>ABN07Novel1+c250C-200R850, MS54Novel1+c2542-2505 delinsTR851, Movel1+c25242-2505 delinsTR851, Movel1+c25242-2505 delinsTR850, Movel1+c25242-2505 delinsTR804, Movel1+c25242-2505 delinsTR1033 WNovel1+c31373-3137dCT1046 fsNovel1+c31373-3137dCT1046 fsNovel1+c31373-3387delV1122_U1129delNovel1+c3373-3387delV1122_U1129delNovel1+c3373-3387delV1122_U1129delNovel1+c3374-152V1122_U1129delNovel1+c3374-152V1122_U1129delNovel1+c3374-152V1122_U1129delNovel1+c3374-152V1122_U1129delNovel1+c3374-152V1122_U1129delNovel1+c3374-152V1122_U1129delNovel1+c3304-152V12	c.137	7delC	P459 fs	Novel	3	+	
c183CTR495 WKnowI+c183G>AE611 KKnowI+c1898-1G>ASpliceNovelI+c2084-1G>CSpliceNovelI+c2413+3deGSpliceNovelI+c2413+3deGSpliceNovelI+c2420T>A1807 NNovelI+c2542-2505 delinsTR35LNovelI+c2542-2505 delinsGCNs00NovelI+c2542-2505 delinsGCNs00NovelI+c2542-2505 delinsGCNs00NovelI+c2542-2505 delinsGCNs00NovelI+c2542-2505 delinsGCNs00NovelI+c2542-2505 delinsGCNs00NovelI+c2542-2505 delinsGCNs00NovelI+c2542-2505 delinsGCNs00NovelI+c3543-25Ns00NovelI+c3373-354V1025NovelI+c3371-3537V1025NovelI+c3404CV1025NovelI+c300C>GI100 MNovelI+c300C>GI100 MNovelI+c300C>GI100 MNovelI+c300C>GI100 MNovelI+c300C>GI100 MNovelI+c412C>AR16CNovelI+c412C>A </td <td>c.138</td> <td>37C&gt;T</td> <td>Q463X</td> <td>Novel</td> <td>1</td> <td>+</td> <td></td>	c.138	37C>T	Q463X	Novel	1	+	
c.1831G>AEf11 KNowlI+c.1893-IG>ASpliceNovelI+WTsite brokenc.1977.F>GI659 MNovelI+WT site brokenc.2308+1G>CSpliceNovelI+WT site brokenc.24107-XI807 NNovelI+WT site brokenc.24107-XI807 NNovelI+WT site brokenc.2504_2050 delinsTR851NovelI+WT site brokenc.2528_250-CY842XKnownI+WT site brokenc.2548_2560delinsGCGN850_M854delinsOFNovelI+WT site brokenc.2528_2562-SSpliceNovelI+WT site brokenc.2528_2562-SSpliceNovelI+WT site brokenc.2528_2562-SSpliceNovelI+WT site brokenc.2528_2562-SSpliceNovelI+WT site brokenc.2528_2562-SSpliceNovelI+WT site brokenc.3137delSpliceNovelI+WT site brokenc.3137delVI125_L1129delNovelI+WT site brokenc.3397-SN1133DNovelI+WT site brokenc.3375T>AVI125_L1129delNovelI+c.300C>GII00 MNovelI+c.300C>GII00 MNovelI+c.300C>GII00 MNovelI	c.148	33C>T	R495 W	Known	1	+	
c.1898-1G>ASpliceNovelI+WT site brokenc.1977-GIG59 MNovelI+c.2308+1G>CSpliceNovelI+c.2413+3deGSpliceNovelI+c.2413+3deGSpliceNovelI+c.24207-AI807 NNovelI+c.2504_2505 delinsTTR835LKnovelI+c.2526CY842XKnowI+c.25248_2560delinsGGCN850_M854delinsGVNovelI+c.25288_2ASpliceNovelI+c.25286_2AR943QNovelI+c.307C>TR1033 WNovelI+c.3137delCT1046 fsNovelI+c.3137delCT1045 fsKnownI+c.3373-3387dV1125_L1129delNovelI+c.3373-3387dV1125_L1129delNovelI+c.3373-3375/AV1120_L129delNovelI+c.3424bC1P1020K fsKnownI+c.307C>CI100 MNovelI+c.300C>GI100 MNovelI+c.300C>GI100 MNovelI+c.301C>TI100 MNovelI+c.301C>TI100 MNovelI+c.301C>TI100 MNovelI+c.301C>TI100 MNovelI+c.301C>TI108 KNo	c.183	31G>A	E611 K	Known	1	+	
c.1977)>GI659 MNovelI+WT site brokenc.2413+3deGSpliceNovelI+WT site brokenc.2413+3deGSpliceNovelI+WT site brokenc.2420T>AI807 NNovelI+c.2540_2505 delinsTR85_MS43delinsONovelI+c.2548_2560delinsGCØSk9_MS43delinsONovelI+c.2548_2560delinsGCØSk9_MS43delinsONovelI+c.2548_2560delinsGCØSk9_MS43delinsONovelI+c.2548_2560delinsGCØNovelI+WT site brokenc.2548_2560delinsGCØNovelI+WT site brokenc.3097C>TR103 WNovelI+WT site brokenc.3097L>TN103 WNovelI+WT site brokenc.337A5N113DNovelI+WT site brokenc.337A5N113DNovelI+WT site brokenc.364delCN102NNovelI+WT site brokenc.364delCN102NNovelI++c.304C>TR102WNovelI++c.304C>TR102WNovelI++c.304C>TR102WNovelI++c.304C>TR102WNovelI++c.304C>TR102WNovelI++c.304C>TR16SGNovelI++ <t< td=""><td>c.189</td><td>08-1G&gt;A</td><td>Splice</td><td>Novel</td><td>1</td><td>+</td><td>WT site broken</td></t<>	c.189	08-1G>A	Splice	Novel	1	+	WT site broken
c.2308+1G>C   Splice   Novel   1   +   WT site broken     c.2414)+340G   Splice   Novel   1   +   WT site broken     c.2420T>A   1807 N   Novel   1   +   WT site broken     c.2504_2505 delinsTT   R35L   Novel   1   +   -     c.2526_CCG   Y842_MS   Novel   1   +   -     c.2528_2CSG   N850_MS54delinsGV   Novel   1   +   -     c.2528_2CSG   Splice   Novel   1   +   WT site broken     c.2828CA   R943Q   Novel   1   +   WT site broken     c.3097C>T   R1033 W   Novel   1   +   WT site broken     c.3190+5C>A   Splice   Novel   1   +   WT site broken     c.3373-3387del   V1125_L1129del   Novel   1   +   WT site broken     c.3404CA   Plice   Novel   1   +   -   -     c.3404CA   Ploz   Novel   1   +   -   -   -     c.300C>C   I1	c.197	77T>G	I659 M	Novel	1	+	
c2413+3deG   Splice   Novel   1   +   WT site broken     c.240T>A   1807 N   Novel   1   +     c.2504   T835L   Novel   1   +     c.25265   N42X   Novel   1   +     c.25262   N50_M854delinsGV   Novel   1   +     c.25282-A   Splice   Novel   1   +     c.27382-A>C   Splice   Novel   1   +     c.307C>T   R1033 W   Novel   1   +     c.3137delC   T1046 fs   Novel   1   +     c.3377AS   N1132D   Novel   1   +   WT site broken     c.3377AS   N1192D   Novel   1   +   WT site broken     c.3377AS   V1192D   Novel   1   +   WT site broken     c.337ASC   N1132D   Novel   1   +   WT site broken     c.337ASCA   V1192D   Novel   1   +	c.230	08+1G>C	Splice	Novel	1	+	WT site broken
c.2420T>A     I807 N     Novel     I     +       c.25402505 delinsT     R850.     Novel     I     +       c.2526C>G     Y842X     Novel     I     +       c.2548_2560delinsGCG     N850_MS84delinsO     Novel     I     +       c.2548_2560delinsGCG     N850_MS84delinsO     Novel     I     +       c.2548_2560delinsGCG     N890_MS40     Novel     I     +       c.3070C>T     R1033 W     Novel     I     +       c.3137delC     I1046 fs     Novel     I     +       c.3373-3387del     V1125_L1129del     Novel     I     +       c.33791-SA     N113D     Novel     I     +       c.33797A>G     N113D     Novel     I     +       c.3401-IS>A     Splice     Novel     I     +       c.3404-DSA     P102M     Novel     I     +       c.3404-DSA     P102M     Novel     I     +       c.300C>G     I100 M     Novel     I     + <td>c.241</td> <td>3+3delG</td> <td>Splice</td> <td>Novel</td> <td>1</td> <td>+</td> <td>WT site broken</td>	c.241	3+3delG	Splice	Novel	1	+	WT site broken
c.2504_2505 delinsTT     R35L     Novel     I     +       c.2526     Y842X     Known     I     +       c.2548_2560delinsGGC0     N850_M854delinsO     Novel     I     +       c.2738_2A     Splice     Novel     I     +     WT site broken       c.2828G>A     R943Q     Novel     I     +     WT site broken       c.3097C>T     R1033 W     Novel     I     +     WT site broken       c.3137_3317delC     T104 fs     Novel     I     +     WT site broken       c.3373_3387dal     V1125_L1129del     Novel     I     +     WT site broken       c.3373_3387dal     V1125_L1129del     Novel     I     +     WT site broken       c.3373_3387dal     V1125_L1129del     Novel     I     +        c.3373_3387dal     V1125_L1129del     Novel     I     +        c.3373_302     V1125_L     Novel     I     +        c.3376_MCON364_L     P120k     Novel     I     + <td< td=""><td>c.242</td><td>20T&gt;A</td><td>I807 N</td><td>Novel</td><td>1</td><td>+</td><td></td></td<>	c.242	20T>A	I807 N	Novel	1	+	
c.2526C>G   Y842X   Known   1   +     c.2548_2560delinsGCG   N50_M854delinsGV   Novel   1   +     c.25382A>C   Splice   Novel   1   +     c.2828G>A   R943Q   Novel   1   +     c.3097C>T   R103 3W   Novel   1   +     c.3137delC   T1046 fs   Novel   1   +     c.3190+5G>A   Splice   Novel   1   +     c.3397ASG   N1133D   Novel   1   +     c.3397A>G   N1133D   Novel   1   +     c.3401-1G>A   Splice   Novel   1   +     c.3402-1G>A   If00 M   Novel   1   +     c.300C>G   If00 M   Novel   1   +     c.300C>G   R168G   <	c.250	4_2505 delinsTT	R835L	Novel	1	+	
c.2548_2560delinsGGCG   Nsb0_M854delinsGV   Novel   1   +     c.2738-2A>C   Splice   Novel   1   +   WT site broken     c.2828>A   R943Q   Novel   1   +   WT site broken     c.3097C>T   R1033 W   Novel   1   +   WT site broken     c.3137delC   T1046 fs   Novel   1   +   WT site broken     c.3190+5G>A   Splice   Novel   1   +   WT site broken     c.3373-3387del   V1125_L1129del   Novel   1   +   WT site broken     c.3491-1G>A   Splice   Novel   1   +   WT site broken     c.3575T>A   V1192D   Novel   1   +      c.3624delC   P1208 fs   Know   3   +      c.300C>G   1100 M   Novel   1   +       c.300C>G   100 M   Novel   1   +         c.314C>T   R140C   Novel   1   + </td <td>c.252</td> <td>26C&gt;G</td> <td>Y842X</td> <td>Known</td> <td>1</td> <td>+</td> <td></td>	c.252	26C>G	Y842X	Known	1	+	
c.2738-2A>C     Splice     Novel     I     +     WT site broken       c.2828G>A     R943Q     Novel     I     +       c.3007C>T     R1033 W     Novel     I     +       c.3137delC     T1046 fs     Novel     I     +       c.3137delC     T1046 fs     Novel     I     +       c.31373a87del     V1125_L1129del     Novel     I     +     WT site broken       c.33373-3387del     V1125_L1129del     Novel     I     +     WT site broken       c.3397A>G     N1133D     Novel     I     +     WT site broken       c.35575A     V1192D     Novel     I     +     WT site broken       c.3624delC     P1208 fs     Novel     I     +     WT site broken       c.3502A     P100 M     Novel     I     +        c.300C>G     I100 M     Novel     I     +        c.412G>A     R168G     Novel     I     +        c.502C>G     R168G	c.254	8_2560delinsGGCG	N850_M854delinsGV	Novel	1	+	
c.2828G>A   P.943Q   Novel   1   +     c.3097C>T   R1033 W   Novel   1   +     c.3137AclC   T1046 fs   Novel   1   +     c.3190+5G>A   Splice   Novel   1   +     c.3373-3387del   V1125_L1129del   Novel   1   +     c.3397A>G   N1133D   Novel   1   +     c.3491-1G>A   Splice   Novel   1   +     c.35757>A   V1192D   Novel   1   +     c.362delC   P1208 fs   Novel   1   +     c.300C>G   1100 M   Novel   1   +     c.304C>T   R102 W   Novel   1   +     c.412G>A   E138 K   Novel   1   +     c.502C>G   R168G   Novel   1   +     c.504C>T   R184C   Novel   1   +     c.504C>C   R2SP   Novel   1   +     c.504C>C   R2SP   Novel   1   +     c.504C>C   R2SP   Novel   1 <td>c.273</td> <td>8-2A&gt;C</td> <td>Splice</td> <td>Novel</td> <td>1</td> <td>+</td> <td>WT site broken</td>	c.273	8-2A>C	Splice	Novel	1	+	WT site broken
c.3097C>T     R1033 W     Novel     I     +       c.3137delC     T1046 fs     Novel     I     +       c.3197delC     T1046 fs     Novel     I     +       c.3197delC     Splice     Known     I     +     WT site broken       c.3397A>G     N113D     Novel     I     +     WT site broken       c.3397A>G     N113D     Novel     I     +     WT site broken       c.3491-IG>A     Splice     Novel     I     +     WT site broken       c.3624delC     P1208 fs     Known     3     +      WT site broken       c.3624delC     P1208 fs     Known     3     +           c.300C>G     I100 M     Novel     I     +            c.412G>A     E138 K     Novel     I     +                   <	c.282	28G>A	R943Q	Novel	1	+	
c.3137delC   T1046 fs   Novel   1   +     c.3190+5G>A   Splice   Known   1   +   WT site broken     c.3373-3387del   V1125_L1129del   Novel   1   +   WT site broken     c.3397A>G   N1133D   Novel   1   +   WT site broken     c.3397A>G   N1133D   Novel   1   +   WT site broken     c.3397A>G   N1133D   Novel   1   +   WT site broken     c.3491-IG>A   Splice   Novel   1   +   WT site broken     c.3575T>A   V1192D   Novel   1   +   WT site broken     c.362delC   P1208 fs   Novel   3   +      r.MV72 NM_000364.2 NP_UOUS5L   V   Novel   1   +      c.300C>G   I100 M   Novel   1   +       c.412G>A   E138 K   Novel   1   +       c.418C>T   R140C   Novel   1   +        c.472C>A   R168G   Novel	c.309	7C>T	R1033 W	Novel	1	+	
c.3190+5G>A     Splice     Known     1     +     WT site broken       c.3373-3387del     V1125_L1129del     Novel     1     +       c.3397A>G     N1133D     Novel     1     +       c.3491-1G>A     Splice     Novel     1     +     WT site broken       c.3491-1G>A     V1192D     Novel     1     +     WT site broken       c.3562delC     P1208 fs     Known     3     +        c.300C>G     100 M     Novel     1     +        c.412G>A     E138 K     Novel     2     +        c.418C>T     R140C     Novel     1     +        c.470C>T     R13C     Novel     1     +        c.432G>A	c.313	7delC	T1046 fs	Novel	1	+	
c.3373-3387del   V1125_L1129del   Novel   1   +     c.3397A>G   N1133D   Novel   1   +     c.3397A>G   Splice   Novel   1   +     c.3491-IG>A   Splice   Novel   1   +     c.3575T>A   V1192D   Novel   1   +     c.3624delC   P1208 fs   Known   3   +     T/NYT2 NM_000364.2 NP_UUU355.2   -   -   -     c.300C>G   I100 M   Novel   1   +     c.300C>G   I100 M   Novel   1   +     c.304C>T   R102 W   Novel   1   +     c.412G>A   E138 K   Novel   2   +     c.418C>T   R140C   Novel   1   +     c.436C>C   R285P   Novel   1   +     c.37C>T   R13C   Novel   1   +     c.434G>A   R141Q   Known   2   +     c.434G>A   R145Q   Known   2   +     c.434G>A   R145Q   Known   1 <td< td=""><td>c.319</td><td>00+5G&gt;A</td><td>Splice</td><td>Known</td><td>1</td><td>+</td><td>WT site broken</td></td<>	c.319	00+5G>A	Splice	Known	1	+	WT site broken
c.3397A>G   N1133D   Novel   1   +     c.3491-1G>A   Splice   Novel   1   +   WT site broken     c.3575T>A   V1192D   Novel   1   +   WT site broken     c.3575T>A   V1192D   Novel   1   +   WT site broken     c.3624delC   P1208 fs   Known   3   +      TNVT2 NM_000364.2 NP_000355.2    +       c.290T>A   P97Y   Novel   1   +      c.300C>G   1100 M   Novel   1   +      c.304C>T   R102 W   Novel   1   +      c.412G>A   E138 K   Novel   2   +      c.412G>A   E138 K   Novel   1   +      c.412G>A   R168G   Novel   1   +      c.854G>C   R285P   Novel   1   +      c.432G>A   R141Q   Known   1   +      c.434G>A   R145Q   Known   2   + <td>c.337</td> <td>/3–3387del</td> <td>V1125_L1129del</td> <td>Novel</td> <td>1</td> <td>+</td> <td></td>	c.337	/3–3387del	V1125_L1129del	Novel	1	+	
c.3491-1G>A   Splice   Novel   1   +   WT site broken     c.3575T>A   V1192D   Novel   1   +     c.3624delC   P1208 fs   Known   3   +     TNNT2 NM_000364.2 NP_000355.2    +   +     c.390T>A   F97Y   Novel   1   +     c.300C>G   1100 M   Novel   1   +     c.304C>T   R102 W   Novel   1   +     c.412G>A   E138 K   Novel   2   +     c.418C>T   R140C   Novel   1   +     c.502C>G   R168G   Novel   1   +     c.502C>G   R168G   Novel   1   +     c.502C>G   R168G   Novel   1   +     c.432G>A   R132C   Novel   1   +     c.434G>A   R141Q   Known   1   +     c.434G>A   R145Q   Known   2   +     c.434G>A   R145Q   Known   2   +     c.470C>T   A157 V   Known   2	c.339	07A>G	N1133D	Novel	1	+	
c.3575T>A   V1192D   Novel   1   +     c.3624delC   P1208 fs   Known   3   +     TNNT2 NM_000364.2 NP_000355.2	c.349	01-1G>A	Splice	Novel	1	+	WT site broken
c.3624delC   P1208 fs   Known   3   +     TNNT2 NM_000364.2 NP_000355.2     c.290T>A   F97Y   Novel   1   +     c.300C>G   1100 M   Novel   1   +     c.300C>G   1100 M   Novel   1   +     c.304C>T   R102 W   Novel   1   +     c.412G>A   E138 K   Novel   2   +     c.418C>T   R140C   Novel   1   +     c.502C>G   R168G   Novel   1   +     c.502C>G   R168G   Novel   1   +     c.432G>A   R132C   Novel   1   +     c.432G>A   R141Q   Known   1   +     c.432G>A   R141Q   Known   1   +     c.433C>T   R145 W   Known   2   +     c.434G>A   R145Q   Known   1   +     c.470C>T   A157 V   Known   2   +     c.173G>A   R58Q   Known   1   +	c.357	/5T>A	V1192D	Novel	1	+	
TNNT2 NM_000364.2 NP_000355.2     c.290T>A   F97Y   Novel   1   +     c.300C>G   I100 M   Novel   1   +     c.304C>T   R102 W   Novel   1   +     c.412G>A   E138 K   Novel   2   +     c.418C>T   R140C   Novel   1   +     c.502C>G   R168G   Novel   1   +     c.854G>C   R285P   Novel   1   +     c.37C>T   R13C   Novel   1   +     c.433C>T   R145 W   Known   2   +     c.434G>A   R140   Known   1   +     c.433C>T   R145Q   Known   1   +     c.434G>A   R145Q   Known   2   +     c.434G>A   R145Q   Known   2   +     c.470C>T   A 157 V   Known   2   +     MYL2 NM_000432.3 NP_000423.2   V   V   +     c.173G>A   R58Q   Known   1   +	c.362	24delC	P1208 fs	Known	3	+	
c.290T>A   F97Y   Novel   1   +     c.300C>G   I100 M   Novel   1   +     c.304C>T   R102 W   Novel   1   +     c.412G>A   E138 K   Novel   2   +     c.418C>T   R140C   Novel   1   +     c.502C>G   R168G   Novel   1   +     c.737C>T   R13C   Novel   1   +     c.432G>A   R141Q   Known   1   +     c.433C>T   R145W   Known   2   +     c.434G>A   R145Q   Known   1   +     c.434G>A   R145Q   Known   2   +     MYL2 NM_000432.3 NP_000423.2   V   Novel   1   +	TNNT2	2 NM_000364.2 NP_0	000355.2				
c.300C>G   I100 M   Novel 1   +     c.304C>T   R102 W   Novel 1   +     c.412G>A   E138 K   Novel 2   +     c.412G>A   E138 K   Novel 1   +     c.412G>A   E138 K   Novel 1   +     c.412G>A   E138 K   Novel 1   +     c.418C>T   R140C   Novel 1   +     c.502C>G   R168G   Novel 1   +     c.854G>C   R285P   Novel 1   +     r.37C>T   R13C   Novel 1   +     c.432G>A   R141Q   Known 1   +     c.433C>T   R145 W   Known 2   +     c.434G>A   R145Q   Known 1   +     c.434G>A   R145Q   Known 2   +     MYL2 NM_000432.3 NP_000423.2   -   +     c.173G>A   R58Q   Known 1   +	c.290	)T>A	F97Y	Novel	1	+	
c.304C>T   R102 W   Novel 1   +     c.412G>A   E138 K   Novel 2   +     c.418C>T   R140C   Novel 1   +     c.502C>G   R168G   Novel 1   +     c.854G>C   R285P   Novel 1   +     TNNI3 NM_000363.4 NP_000354.4   -   +     c.37C>T   R13C   Novel 1   +     c.433C>T   R145 W   Known 1   +     c.434G>A   R145Q   Known 1   +     c.434G>A   R145Q   Known 2   +     c.470C>T   A157 V   Known 2   +     MYL2 NM_000432.3 NP_000423.2   -   +     c.173G>A   R58Q   Known 1   +	c.300	)C>G	I100 M	Novel	1	+	
c.412G>A   E138 K   Novel 2   +     c.418C>T   R140C   Novel 1   +     c.502C>G   R168G   Novel 1   +     c.854G>C   R285P   Novel 1   +     TNNI3 NM_000363.4 NP_000354.4   +   +     c.37C>T   R13C   Novel 1   +     c.422G>A   R141Q   Known 1   +     c.433C>T   R145 W   Known 2   +     c.434G>A   R145Q   Known 1   +     c.470C>T   A157 V   Known 2   +     MYL2 NM_000432.3 NP_000423.2   -   +     c.173G>A   R58Q   Known 1   +	c.304	C>T	R102 W	Novel	1	+	
c.418C>T   R140C   Novel 1   +     c.502C>G   R168G   Novel 1   +     c.854G>C   R285P   Novel 1   +     TNNI3 <nm_000363.4 np_000354.4<="" td="">   +   +     c.37C&gt;T   R13C   Novel 1   +     c.422G&gt;A   R141Q   Known 1   +     c.433C&gt;T   R145 W   Known 2   +     c.434G&gt;A   R145Q   Known 1   +     c.470C&gt;T   A157 V   Known 2   +     MYL2 NM_000432.3 NP_000423.2   -   +     c.173G&gt;A   R58Q   Known 1   +</nm_000363.4>	c.412	2G>A	E138 K	Novel	2	+	
c.502C>G   R168G   Novel 1   +     c.854G>C   R285P   Novel 1   +     TNNI3 NM_000363.4 NP_000354.4   -   -     c.37C>T   R13C   Novel 1   +     c.422G>A   R141Q   Known 1   +     c.433C>T   R145 W   Known 2   +     c.434G>A   R145Q   Known 1   +     c.470C>T   A157 V   Known 2   +     MYL2 NM_000432.3 NP_000423.2   -   -   +     c.173G>A   R58Q   Known 1   +	c.418	3C>T	R140C	Novel	1	+	
c.854G>C   R285P   Novel 1   +     TNNI3 NM_000363.4 NP_000354.4   -   -     c.37C>T   R13C   Novel 1   +     c.422G>A   R141Q   Known 1   +     c.433C>T   R145 W   Known 2   +     c.434G>A   R145Q   Known 1   +     c.470C>T   A157 V   Known 2   +     MYL2 NM_000432.3 NP_000423.2   -   -   +     c.173G>A   R58Q   Known 1   +	c.502	C>G	R168G	Novel	1	+	
TNNI3 NM_000363.4 NP_000354.4     c.37C>T   R13C   Novel 1   +     c.422G>A   R141Q   Known 1   +     c.433C>T   R145 W   Known 2   +     c.434G>A   R145Q   Known 1   +     c.470C>T   A157 V   Known 2   +     MYL2 NM_000432.3 NP_000423.2   -   -   +     c.173G>A   R58Q   Known 1   +	c.854	G>C	R285P	Novel	1	+	
c.37C>T   R13C   Novel 1   +     c.422G>A   R141Q   Known 1   +     c.433C>T   R145 W   Known 2   +     c.434G>A   R145Q   Known 1   +     c.470C>T   A157 V   Known 2   +     MYL2 NM_000432.3 NP_000423.2   -   -   +     c.173G>A   R58Q   Known 1   +	TNNI3	NM_000363.4 NP_0	00354.4				
c.422G>A   R141Q   Known 1   +     c.433C>T   R145 W   Known 2   +     c.434G>A   R145Q   Known 1   +     c.470C>T   A157 V   Known 2   +     MYL2 NM_000432.3 NP_000423.2   E   E   E     c.173G>A   R58Q   Known 1   +	c.370	C>T	R13C	Novel	1	+	
c.433C>T   R145 W   Known 2   +     c.434G>A   R145Q   Known 1   +     c.470C>T   A157 V   Known 2   +     MYL2 NM_000432.3 NP_000423.2   -   -   -     c.173G>A   R58Q   Known 1   +	c.422	2G>A	R141Q	Known	1	+	
c.434G>A   R145Q   Known 1   +     c.470C>T   A157 V   Known 2   +     MYL2 NM_000432.3 NP_000423.2   -   -     c.173G>A   R58Q   Known 1   +	c.433	C>T	R145 W	Known	2	+	
c.470C>T A157 V Known 2 + MYL2 NM_000432.3 NP_000423.2 c.173G>A R58Q Known 1 +	c.434	G>A	R145Q	Known	1	+	
MYL2 NM_000432.3 NP_000423.2     Known 1     +       c.173G>A     R58Q     Known 1     +	c.470	C>T	A157 V	Known	2	+	
c.173G>A R58Q Known 1 +	MYL2	NM_000432.3 NP_00	00423.2				
	c.173	G>A	R58Q	Known	1	+	

Table 1 continued

Gene	Nucleotide change	Protein change	Status	Number of affected patients	Amino acid substitution prediction	Human splicing finder
c.26	0G>A	G87E	Novel	1	+	
MYL3	NM_000258.2 NP	_000249.1				
c.28	1G>A	R94H	Known	1	+	
c.44	6T>C	M149T	Novel	1	+	
c.46	0C>T	R154C	Novel	1	+	
TPM1	NM_001018005.1	NP_001018005.	.1			
c.38	0T>A	M127 K	Novel	1	+	
c.52	3G>A	D175 N	Known	1	+	
c.62	9A>G	Q210R	Novel			
ACTC	1 NM_005159.4 N	P_005150.1				
c.14	5A>C	M49L	Novel	2	+	
c.94	0C>T	R314C	Novel	1	+	

To confirm the causative role of a mutation, the following programs were applied: the programs for amino acid substitution prediction (SIFT, http://sift.bii.a-star.edu.sg/www/SIFT\_BLink\_submit.html; Polyphen-HCM, http://genetics.bwh.harvard.edu/pph/ or Polyphen-2, http:// genetics.bwh.harvard.edu/pph//; Mutpred, http://mutpred.mutdb.org/; SNPs3D, http://www.snps3d.org/; Pmut, http://mmb2.pcb.ub.es:8080/PMut/). A missense mutation was assumed to be possibly disease-causing if at least two independent programs indicated a damaging effect. The program HSF was used to determine whether any of the detected mutations destroys an existing or creates a novel splice site (HSF, http:// www.umd.be/HSF/)

+ means the variant is predicted to be deleterious; WT site broken means wild type splice site was broken



Fig. 1 Mutation distribution of the 8 disease-causing genes in studied HCM patients

outcome, some researchers questioned the appropriateness of attempts at genotype-phenotype correlation analyses [19]. Will the type of gene mutation really predict a certain clinical phenotype and the age of onset and prognosis of individual patients with HCM? Our study as well as the clues from human and animal model studies support that it is the number of mutation, not the type of mutation, predict the prognosis and disease course in HCM [12–16, 20].

At present, the risk markers of HCM has been established, including prior personal history of ventricular fibrillation, or sudden cardiac death, or sustained ventricular tachycardia, family history of sudden cardiac death, unexplained syncope episodes, non-sustained ventricular tachycardia, maximum left ventricular wall thickness  $\geq$  30 mm, abnormal blood pressure response during exercise. ICD placement is recommended for HCM patients with one of above-mentioned factors. However, most such factors have a low positive predictive value and the absence of risk factors does not convey absolute immunity to sudden cardiac death. The presence of >1 HCM-associated sarcomere mutation is associated with greater severity of disease, which provides an opportunity to predict clinical outcomes of HCM by using genetic information.

In our study, a positive correlation between the number of mutations and left ventricular hypertrophy and chamber enlargement of HCM was observed, supporting that the multiple gene mutations may be used to predict some clinical manifestations and prognosis of HCM. To some extent, MWT is not an ideal marker to predict clinical outcome. By following up, the correlation of mutations with the cardiac events can be better verified in young asymptomatic HCM patients.

We determined that about one-fifth of mutation carrier harbored two or more mutations in merely eight sarcomere genes. The proportion of multiple gene mutation carriers is expected to be even higher when additional HCM-related genes are screened. Our story suggested that new mutation searching efforts should not be suspended until a comprehensive genotype completed.

Table 2 Genotypes and risk markers of SCD in patients with multiple mutations

Patient	Mutation numbers	Genotype	Risk markers	Invasive therapy
1	2	<i>MYH7</i> : M822T+R1420W	No	/
2	2	<i>MYBPC3</i> :R215C+ <i>TNNT2</i> : R140C	mLVWT	PTSMA
3	2	<i>MYBPC3</i> : c.2308+1G>C+R160W	mLVWT	PTSMA
4	2	MYBPC3: R160W+T1046fs	No	/
5	2	MYBPC3: P1208fs+TNNI3:R145Q	No	/
6	2	MYH7: R663C+Q892K	Family history of SCD	/
7	2	MYBPC3: N850_M854delinsGV+P2R	Family history of SCD	PM
8	2	<i>MYH7</i> :R1277Q+ <i>MYBPC3</i> : Q463X	Family history of SCD	/
9	2	<i>MYBPC3</i> : I659M+R943Q	Unexplained syncopal episodes	/
10	2	MYH7:A1379T+TNNT2: E138K	No	PM
11	2	MYH7:M1782del+TNNT2: E138K	No	/
12	2	<i>MYBPC3</i> : V1192D+ <i>TPM1</i> : D175N	No	/
13	2	<i>MYBPC3</i> : A433P+E258K	No	/
14	2	<i>MYH7</i> :R663H+ <i>MYBPC3</i> : K442M	mLVWT	PTSMA
15	2	<i>MYH7</i> :L1090V+ <i>MYL2</i> : G87E	Family history of SCD	/
16	2	<i>MYBPC3</i> : E334K+R1033W	No	PM
17	2	MYH7:K1848T+TNNI3: R145W	Prior personal history of sustained VT	ICD
18	2	<i>MYH7</i> :R453C+ACTC1: M49L	Family history of SCD	/
19	3	MYBPC3:R160W+MYL3:R94H+TNNI3: R13C	mLVWT	PTSMA

SCD sudden cardiac death, VT ventricular tachycardia, mLVMT maximum left ventricular wall thickness  $\geq$ 30 mm, PTSMA percutaneous transluminal septal myocardial ablation, ICD implantable cardioverter defibrillator, PM pace maker

Table 3	The effect	of multiple	mutations on	phenotype	of HCM

Number of	Number of cases	Demographic Features			Age of onset	Echocardiography			
mutations		Age (years.)	Height (cm)	Weight (Kg)	(years.)	MWT (mm)	LA (mm)	LVEDD (mm)	LVEF (%)
0	98	52.0 ± 15.3	$168.7 \pm 7.6$	$74.5 \pm 14.1$	44.9 ± 13.5	19.7 ± 5.1	38.4 ± 6.3	45.7 ± 5.7	$66.3 \pm 8.2$
1	83	$47.6 \pm 13.5$	$168.1\pm7.7$	$70.7\pm12.8$	$38.4\pm12.7^*$	$20.5\pm4.8$	$41.4\pm8.3^*$	$45.2\pm8.2$	$65.3 \pm 10.0$
≥2	19	$42.7 \pm 12.9$	$162.2\pm7.9$	$69.8\pm7.7$	$34.5\pm12.9^*$	$23.6\pm5.7^{*\dagger}$	$42.5\pm7.9^*$	$47.1\pm8.6$	$65.3 \pm 11.3$

P < 0.05, compared with no mutation group; P < 0.05, compared with one mutation group

#### Conclusion

Our results once again proved that multiple mutations may be more practical and useful for prediction of HCM prognosis. Multiple mutations are much more frequent than that in literature reported by using more advanced sequencing technology and screening more HCM-related genes. Our result support that it is the number of mutation, not the type of mutation (such as R403Q mutation in MHY7), is the prognosis predictor.

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