Dual LQT1 and HCM phenotypes associated with tetrad heterozygous mutations in KCNQ1, MYH7, MYLK2, and TMEM70 genes in a three-generation Chinese family

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Aims
Hypertrophic cardiomyopathy (HCM) mainly results from autosomal-dominant inherited single heterozygous mutations in cardiac sarcomere genes. Contributions of multiple gene mutations to disease heterogeneity in a three-generation family were investigated.

Methods
Clinical, electrocardiographic (ECG), and echocardiographic examination in members of a three-generation Chinese family was followed by exon and boarding intron analysis of 96 genes in the proband using second-generation sequencing. The identified mutations were confirmed by bi-directional Sanger sequencing in all family members and 300 healthy controls.

Results
Four missense mutations were detected in the family. These were two novel MYH7-H1717Q and MYLK2-K324E mutations accompanied by the KCNQ1-R190W and TMEM70-I147T mutations. The proband carried all four mutations and showed overlapping HCM and LQT1 phenotypes. Five family members each carried two mutations. Subject II-2 only carried TMEM70-I147T. MYH7-H1717Q and TMEM70-I147T came from the paternal side, whereas KCNQ1-R190W and MYLK2-K324E came from the maternal side. Left ventricle mass indices in MYH7-H1717Q carriers were significantly higher than in non-H1717Q carriers (90.05 ± 7.33 g/m², 63.20 ± 4.53 g/m², respectively, P < 0.01). Four KCNQ1-R190W carriers showed QTc intervals that were significantly more prolonged than those in non-R190W carriers (472.25 ± 16.18 and 408.50 ± 7.66 ms, respectively, P < 0.05). All MYLK2-K324E carriers showed inverted ECG T waves. The subject with only a TMEM70-I147T mutation showed normal ECG and echocardiographs, suggesting that this had less pathological effects at least in this family.

Conclusions
We demonstrate dual LQT1 and HCM phenotypes in this multiple LQT1- and HCM-related gene mutation carrier family for the first time and suggest that LQT-related gene mutations associate with QT interval prolongation and/or arrhythmia in HCM patients.

Keywords
Hypertrophic cardiomyopathy • Overlap phenotypes • Second-generation sequencing • Multiple mutations

Introduction
Hypertrophic cardiomyopathy (HCM) is a common disorder affecting 1 in 500 people. It is characterized by cardiac hypertrophy, myocyte disarray, and fibrosis. It is most frequently caused by single heterozygous mutations in a range of cardiac sarcomere genes showing an autosomal-dominant inheritance pattern. Genetic testing of these genes in HCM patients has provided valuable information on diagnosis...
and early identification of individuals at risk, especially among family members. It has been recommended in the latest diagnostic guidelines in several countries. Ventricular arrhythmias such as ventricular tachycardia and ventricular fibrillation is one of the main causes of sudden death especially in young people with HCM. The underlying mechanism of this still remains unclear. As with other inherited cardiomyopathies, HCM shows marked phenotypic variability, even within families. Multiple gene mutations have recently been reported from genetic screening in HCM which may further contribute to the disease heterogeneity, although most cases of HCM are heterozygous disease-causing mutations. We report the KCNQ1-R190W mutation and three novel MYH7-H1717Q, MYLK2-K324E, and TMEM70-I147T mutations and their association with overlapping LQT1 and HCM phenotypes through three generations of a Chinese family.

What’s new?
- Ninety-six genes were screened using next-generation sequencing through three generations of a Chinese family.
- Two novel HCM disease-causing, MYH7-H1717Q and MYLK2-K324E, mutations were detected.
- We demonstrate dual symptomatic LQT1 and HCM phenotypes in this multiple LQT1- and HCM-related gene mutation carrier family for the first time and suggest that LQT-related genes mutations associate with QT interval prolongation and/or arrhythmia in HCM patients.

Methods

Patients and clinical investigation
All investigations conformed to principles defined in the Helsinki Declaration and approved by the ethics committee of Xijing Hospital, Fourth Military Medical University, PRC. Eight members of a three-generation Chinese family were investigated (Figure 1) with informed written consent having been obtained from each member. For the children <16 years old, written informed consents were obtained from parents in accordance with the guidelines of the Ethics Committee of Xijing Hospital, Fourth Military Medical University. Clinical investigations included a complete medical history, physical examination, and at least two 12-lead electrocardiographic (ECG) recordings obtained at different times. Electrocardiographic traces and transthoracic echocardiogram were read by two independent, experienced physicians. QT intervals corrected for heart rate (QTc) using Bazett’s formula were measured in limb lead II. QTc intervals were averaged from five consecutive beats of at least two 12-lead ECG recordings at different time points.

All echocardiographic studies were performed using an iE33 ultrasound system (Philips Medical Systems, Bothell, WA, USA) with a 1.0–5.0 MHz transducer. The subjects were placed in the left lateral recumbent position, and the electrocardiogram was recorded simultaneously. All subjects underwent a routine two-dimensional echocardiographic study with a 55–1 probe. Left ventricular (LV) end-diastolic mass (LVED mass), and LV ejection fraction (EF) were

Figure 1 Pedigree of the family with phenotypic and genotypic information. Squares denote males; circles denote females. A cross placed inside the symbol denotes unavailable family members. The arrow indicates the proband. The genotype for each individual is noted below the symbol, where available. A: KCNQ1-R190W; B: MYH7-H1717Q; C: MYLK2-K324E; D: TMEM70-I147T.
thickness is defined as a wall thickness

Echocardiography. In the case of children, a wall thickness of 13–14 mm considered borderline based on echocardiography. Hypertrophic cardiomyopathy is recognized by a maximal LV wall thickness (MLVWT) of ≥ 15 mm, with a wall thickness of 13–14 mm considered borderline based on echocardiography. In the case of children < 18 years old, increased LV wall thickness is defined as a wall thickness ≥ 2 SD above the mean (z-score ≥ 2) for age, gender, or body size.

Genetic analysis

Genomic DNA was extracted from 10 mL of peripheral blood leukocytes using standard protocols (D2492 Blood DNA Maxi Kit, Omega Bio-Tek, Inc., Norcross, GA, USA). Each DNA sample was quantified by agarose gel electrophoresis and Nanodrop (Thermo Fisher NanoDrop, Norcross, GA, USA). Each DNA sample was quantified by agarose gel electrophoresis and Nanodrop (Thermo Fisher NanoDrop, Norcross, GA, USA). Genomic DNA was extracted from 10 mL of peripheral blood leukocytes using standard protocols (D2492 Blood DNA Maxi Kit, Omega Bio-Tek, Inc., Norcross, GA, USA). Libraries were prepared using the Illumina standard protocol. The amplified DNA was captured with a cardio-disease-related Gene Panel using biotinylated oligo-probes (MyGenostics GenCap Enrichment Technologies). The probes were designed to tile along all the exons of 96 cardio-disease-related genes (MyGenostics GenCap Enrichment Technologies). The probes were designed to tile along all the exons of 96 cardio-disease-related genes.

After the polymerase chain reaction, duplicates were removed using the Picard software, the single-nucleotide polymorphisms (SNPs) were firstly identified by the SOAP SNP program (http://soap.genomics.org.cn/soapSNP.html). Subsequently, we realigned the readings to the reference genome (hg19) using the Burrows–Wheeler Aligner and detected the insertions or deletions (InDels) using the GATK program (http://www.broadinstitute.org/gsa/wiki/index.php/Home_Page). After annotation of the identified SNPs and InDels with the Exome-assistant program (http://122.228.158.106/exomeassistant), the short read alignment, candidate SNPs, and InDels were viewed by Magic Viewer. All mutations were further confirmed by bi-directional Sanger sequencing performed by an ABI-automated cycle sequencer using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The identified mutation was screened among the remaining family members by direct

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Symptoms</th>
<th>Genotype</th>
<th>ECG</th>
<th>Heart rhythm</th>
<th>HR (bpm)</th>
<th>P (ms)</th>
<th>PR (ms)</th>
<th>QRS (ms)</th>
<th>QTc (ms)</th>
<th>Inverted T wave</th>
<th>EF (%)</th>
<th>LVMI (g/m2)</th>
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<td>60</td>
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<td>68</td>
<td>100</td>
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<td>88</td>
<td>24</td>
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<td>III-5</td>
<td>M</td>
<td>7</td>
<td>A, C</td>
<td></td>
<td></td>
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<td>80</td>
<td>140</td>
<td>98</td>
<td>9</td>
<td>No</td>
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</tbody>
</table>

Note: A: KCNQ1-R190W, B: MYLK2-K324E, C: MYH6, D: TMEM70-I147T; SR, sinus rhythm; MIVS, maximum intraventricular septum; MLVWT, maximum left ventricular wall thickness; LVMI, left ventricle mass index.

Table 2 Clinical and genetic characteristics of the family pedigree

<table>
<thead>
<tr>
<th>Family Pedigree</th>
<th>Patient ID</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Symptoms</th>
<th>Genotype</th>
<th>ECG</th>
<th>Heart rhythm</th>
<th>HR (bpm)</th>
<th>P (ms)</th>
<th>PR (ms)</th>
<th>QRS (ms)</th>
<th>QTc (ms)</th>
<th>Inverted T wave</th>
<th>EF (%)</th>
<th>LVMI (g/m2)</th>
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</tbody>
</table>

Table 1 Full list of 96 cardiac-related genes screened in a three-generation Chinese family

<table>
<thead>
<tr>
<th>Cardiac-related genes</th>
<th></th>
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</thead>
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<td>AARS2, ABCC1, ABCC9, ACTC1, ACTN2, AKAP9, ANK2, ANKR1D1, BAG3, BRAF, CACNA1C, CACNB2, CASQ2, CAV3, CBL, CRYAB, CSRP3, DES, DMD, DSC2, DSG2, DSG3, DSP, DTNA, EMD, EYA4, FHL2, PHOD3, FKTN, GATA4D1, GLA, GD1D1L, HCN4, ILK, JPH2, JUP, KCNE1, KCNE2, KCNE3, KCNH2, KCNJ2, KCNJ5, KCNQ1, Kras, LAMP2, LDB3, LMNA, MARCKS1, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYLK2, MYOZ2, MYPTN, NEXN, NRAS, OBSCN, PDLIM3, PKP2, PLB1, PLN, PKRAG2, PSEN1, PSEN2, PTPN11, RAF1, RBM20, RYR2, SCN1B, SCN3B, SCN4B, SCN5A, SCO2, SCD, SHOC2, SLC25A, SNTA1, SOS1, TAZ, TCFAP2, TGFBI, TMEM43, TMEM70, TNPO1, TNRC1, TNRC2, TNRC3, TNNT2, TNNT3, TPM1, TRPC6, TTN, TTR, and VCL</td>
<td></td>
</tr>
</tbody>
</table>
DNA sequencing. Three hundred unrelated control individuals were randomly selected from a group of Chinese healthy volunteers with normal 12-lead ECG, echocardiography, and without reported cardiovascular history.

**Statistical analysis**

Continuous variances were expressed as means ± standard errors of the mean. Differences were analysed using Student’s significant difference tests. A P-value of <0.05 was taken as the criterion for significance. Categorical variables were compared using Fisher’s exact tests, with P < 0.05 considered significant.

**Results**

**Clinical features in the family pedigree**

Figure 1 shows the pedigree of the three-generation Chinese family with eight members available for investigation. Clinical characteristics of the family pedigree are summarized in Table 2. The proband (II-9) was a 38-year-old man. He presented with two short-lived and self-terminating episodes of syncope in the month before he visited a doctor. His ECG showed heart rate 62 bpm, PR interval 172 ms, QRS complex duration 88 ms, and diffuse repolarization changes with a late-onset inverted T wave pattern. QTc was prolonged to 469 ms (Figure 2A). Transthoracic echocardiograms showed left ventricle hypertrophy (Figure 2B and C) with an LVMI 109.55 g/m², MLVWT 24 mm and EF 70%. The proband’s father (I-1) and mother (I-2) had histories of mild episodes of dizziness. There were no cardiovascular symptoms, syncope, or sudden death reported in the remaining family members. Clinical examinations were normal without cardiac murmurs.

**Genotypes in the family with four mutations in the KCNQ1, MYH7, MKLY2, and TMEM70 genes**

Four heterozygous missense mutations were identified in this family (Table 3). Mutations include (i) c.568C>T within exon 3 of KCNQ1 resulting in an arginine to tryptophan substitution p.R190W in the Kv7.1, voltage-gated potassium channel, denoted KCNQ1-R190W(A) (Figure 3A); (ii) c.5151T>A within exon 35 of MYH7 resulting in a
Table 3  Summary of mutations in the family pedigree

<table>
<thead>
<tr>
<th>Gene</th>
<th>Encoded protein</th>
<th>Exon</th>
<th>DNA change</th>
<th>Protein change</th>
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</thead>
<tbody>
<tr>
<td>KCNQ1</td>
<td>Kv7.1, voltage-gated potassium channel</td>
<td>3</td>
<td>c.568C&gt;T</td>
<td>p.R190W</td>
</tr>
<tr>
<td>MYH7</td>
<td>MHC-β isoform, cardiac muscle</td>
<td>35</td>
<td>c.5151T&gt;A</td>
<td>p.H1717Q</td>
</tr>
<tr>
<td>MYLK2</td>
<td>Myosin light chain kinase 2</td>
<td>6</td>
<td>c.970A&gt;G</td>
<td>p.K324E</td>
</tr>
<tr>
<td>TMEM70</td>
<td>Transmembrane protein 70</td>
<td>3</td>
<td>c.440T&gt;C</td>
<td>p.I147T</td>
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</tbody>
</table>

Figure 3  Four detected mutations in the family. (A) KCNQ1-R190W, (B) MYH7-H1717Q, (C) MYLK2-K324E, (D) TMEM70-I147T. (a) DNA change in sequencing. (b) Partial deduced amino acid sequence in wild type. (c) Amino acid sequence in mutant. (d) Schematic representation of the relevant mutation in the corresponding protein.

Table 3 summarizes the mutation details. The proband carried all four mutations. Subjects I-1, I-2, II-6, II-8, and III-5 carried two mutations in the form of B–D, A–C, C–D, A–B, and A–C, respectively. Subject II-2 carried mutation D only, whereas subject III-4 was the only member who did not carry any of the above-detected mutations (Figure 3 and Table 2).

Genotype–phenotype correlation
To confirm the genotype–phenotype correlations as well as mutation pathogenesis in the family, ECG and echocardiograph details...
were further analysed. QTc > 440 ms for men and children, QTc > 460 ms for women were used as criteria for clinical LQT diagnosis. Hypertrophic cardiomyopathy is recognized by an MLVWT ≥ 15 mm, with a wall thickness of 13–14 mm considered borderline based on echocardiography. In the case of children <18 years old, increased LV wall thickness is defined as a wall thickness ≥ 2 SD above the mean (z-score ≥ 2) for age, sex, or body size.

First, in the family, four subjects (I-2, II-8, II-9, and III-5) carried KCNQ1-R190W which previously was reported as being associated with unexpected sudden cardiac death.10,11 Four KCNQ1-R190W mutation carriers showed T waves with a delayed onset and that 16.18 ms vs. 408.5 + with a thick nes s i sd e f i n d a saw a l lt i c n e s s echocardiography. In the case of children with a wall thickness of 13–14 mm considered borderline based on echocardiography, T waves were upright (Figure 2A for II-9). Their QTc was significantly prolonged relative to that of non-KCNQ1-R190W mutation carriers (472.25 ± 16.18 ms vs. 408.5 ± 7.67 ms, respectively, P < 0.05; Figure 4Ao). QTc was indistinguishable between MYH7-H1717Q, MYLK2-K324E, TMEM70-I147T mutation carriers, and non-corresponding mutation carriers (Figure 4Ba, Ca, Da, P > 0.05, respectively). The other ECG parameters and echocardiograph parameters showed no statistically significant differences. The results suggest that KCNQ1-R190W is associated with an LQT1 phenotype in this family. Using a QTc > 440 ms for men and children, QTc > 460 ms for women as criteria for clinical LQT diagnosis. 10,11 Four subjects (I-2, II-6, II-9, and III-5) in this family carried MYH7-H1717Q mutations. MYH7 is the most common genetic pathological gene of HCM.1 Figure 4B illustrates LVMI comparisons between mutation carriers and non-corresponding mutation subjects. In MYH7-H1717Q, mutation carriers LVMI were significantly higher than in non-MYH7-H1717Q mutation subjects (96.05 ± 7.33 g/m² vs. 63.20 ± 4.52 g/m², respectively, P < 0.05). Four multiple chest leads (Figure 2 for subject II-9, Figure 5A for subject II-6) except subject III-5 who is a 7-year-old boy with an inverted T wave only in lead V1. In contrast, in four non-MYLK2-K324E mutation subjects, T waves were upright (Figure 5B for subject I-1, P < 0.05 by Fisher’s exact test). Subjects I-1 and II-6 carried the same mutation.

![Figure 4](https://academic.oup.com/europace/article-abstract/18/4/602/2466812) QTc and LVMI comparisons between mutation carriers and non-mutation subjects in the family. (A) KCNQ1-R190W, (B) MYH7-H1717Q, (C) MYLK2-K324E, (D) TMEM70-I147T. (a) QTc and (b) LVMI; *P < 0.05; NS, no significant difference.
Subject I-1 carrying mutation MYH7-H1717Q showed Q waves in lead II, III, and aVF. In contrast, subject II-6 carrying mutation MYLK2-K324E showed inverted T waves in chest leads V1–V3 without Q waves in any leads. The results suggest that the MYLK2-K324E mutation might be involved in the abnormal repolarization consequences in HCM.

Fourthly, four subjects (I-1, II-2, II-6, and II-9) carried the TMEM70-I147T mutation. Both QTc and LVED mass remained statistically undistinguishable between TMEM70-I147T mutation carriers and non-TMEM70-I147T subjects. Subject II-2 carried TMEM70-I147T only. Her ECG and echocardiographic parameters showed neither QTc prolongation nor HCM. Taken together, multiple HCM and LQT1 genotype-phenotypes are closely correlated in this family with an autosomal-dominant trait.

Discussion

The present study identified four mutations, MYH7-H1717Q, MYLK2-K324E, KCNQ1-R190W, and TMEM70-I147T through a single three-generation Chinese family with multiple LQT1 and HCM phenotypes for the first time. Genotype–phenotype correlations associated KCNQ1-R190W with LQT1. MYH7-H1717Q and MYLK2-K324E were associated with HCM, reflected in LV thicknesses and inverted T wave ECG features. Both HCM and LQT1 are autosomal-dominant variants with phenotypes showing cumulative mutation-related effects.

KCNQ1 encodes the potassium channel alpha (Kv7.1) subunit conducting $I_{Ks}$, a major determinant of phase 3 of the cardiac action potential. Loss-of-function KCNQ1 mutations are associated with LQT1, which accounts for around half the genotyped LQT patients. Gain-of-function KCNQ1 mutations are associated with familial atrial fibrillation and short QT syndrome type 2. The R190 residue in KCNQ1 has previously been associated with R190W, R190Q, and R190L variants in LQTS patients and unexpected sudden cardiac death. In vitro studies on the R190Q mutation demonstrate a total loss of channel function. This strongly implicates R190W in the LQT phenotype. Four subjects in the family carried KCNQ1-R190W and showed prolonged QTc relative to non-carriers.
consistent with 75% penetrance in this family. However, they showed no SCD or arrhythmias suggesting a mild LQT1 phenotype.

**MYH7** encodes the MHC-β isoform mainly expressed in cardiac muscle. It is the commonest gene associated with HCM.\(^6\) **MYH7** mutations account for about 25–40% of gene testing in positive HCM patients and are associated with robust disease phenotypes.\(^1\) **MYH7-H1717Q** is a novel missense mutation and results in a histidine-to-glutamine substitution p.H1717Q in MHC-β. In our family, basal anteroseptal thickness and LVMI in **MYH7-H1717Q** mutation carriers were significantly higher than in non-corresponding mutation subjects. The **MYH7-H1717Q** mutation also resulted in diastolic dysfunction in the mutation carriers suggesting that diastolic dysfunction preceded the systolic dysfunction which occurs at late stages of HCM. Thus, **MYH7-H1717Q** had HCM pathogenic effects and is therefore proposed to be a disease-causing mutation in this family.

**MYL2** encodes myosin light chain kinase 2, a calcium/calmodulin-dependent enzyme. It is mainly expressed in skeletal muscle. **MYL2** mutations are linked to midventricular HCM.\(^7,18\) Four subjects carrying **MYL2-K324E** mutations showed inverted ECG T waves. This implicates **MYL2-K324E** in the development of ventricular hypertrophy and cardiac repolarization abnormalities in this family.

**TMEM70** encodes transmembrane protein 70. Previous reports implicate **TMEM70** mutations in an isolated ATP synthase deficiency and neonatal mitochondrial encephalohardiomyopathy.\(^19,20\) with an autosomal recessive trait. In the present family, all **TMEM70-I147T** mutation carriers carried a single mutant allele. Subject II-2 carried the **TMEM70-I147T** mutation only. Both her ECG and echocardiogram were normal, indicating that **TMEM70-I147T** is less pathogenic in this family.

### Conclusions

Our findings together suggest that LQT-related gene mutations might be associated with QT interval prolongation and/or arrhythmia in HCM patients leading to arrhythmia. Five double mutation carriers either showed mild hypertrophy or QTc prolongation without obvious symptoms. In contrast, the proband carried all four detected mutations. He was the only subject with the most severe symptomatology. In contrast, the proband carried all four detected mutations. He was the only subject with the most severe symptomatology. In contrast, the proband carried all four detected mutations. He was the only subject with the most severe symptomatology. In contrast, the proband carried all four detected mutations. He was the only subject with the most severe symptomatology. In contrast, the proband carried all four detected mutations. He was the only subject with the most severe symptomatology. In contrast, the proband carried all four detected mutations. He was the only subject with the most severe symptomatology. In contrast, the proband carried all four detected mutations. He was the only subject with the most severe symptomatolog...