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Case Report

# TNNI3 and KCNQ1 co-inherited variants in a family with hypertrophic cardiomyopathy and long QT phenotypes: A case report

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ABSTRACT

QTc prolongation is reported in patients with hypertrophic cardiomyopathy (HCM). However, the causes of the QTc interval increase remain unclear. The main contribution to QTc prolongation in HCM is attributed to the myocardial hypertrophy and related structural damage. In a 24-year-old male proband, affected by HCM and long QTc, we identified by Next Generation Sequencing a pathogenic variant in gene *TNNI3* co-inherited with a damaging variant in *KCNQ1* gene. This evidence suggests the possibility that QTc interval prolongation and its dispersion in HCM could be associated not only to the severity of left ventricular hypertrophy but also to the co-inheritance of pathogenic variants related to both long QT Syndrome (LQTS) and HCM. Although the simultaneous presence of pathogenic variants in genes related to different heart diseases is extremely rare, counseling and genetic testing appear crucial for the clinical diagnosis. Screening of LQTS genes should be considered in HCM patients to clarify the origin of long QTc, to provide more information about the clinical presentation and to evaluate the incidence of the co-existence of LQTS/HCM gene variants that could occur more frequently than so far reported.

## 1. Introduction

Hypertrophic cardiomyopathy (HCM) is the most common cardiovascular genetic disease, inherited with an autosomal dominant pattern, incomplete penetrance and variable expressivity, affecting 1/500 individuals in the general population [1]. Family studies in large pedigrees associate strongly HCM with pathogenic variants in sarcomere genes, such as *MYH7*, *MYBPC3*, *TNNT2*, *TNNI3*, *TPM1*, *MYL2*, *MYL3*, *ACTC1* [2]. In addition, non sarcomere genes have been implicated in some cases, although without robust evidence. HCM diagnosis is based on the idiopathic presence of left ventricular hypertrophy with wall thickness $\geq$ 15 mm. A large proportion of the patients are asymptomatic, and the sudden cardiac death can be the first presentation of the disease, particularly in young patients. HCM is usually associated with electrocardiographic (ECG) abnormalities including QTc prolongation (QTc>480 ms) and its dispersion as markers of electrophysiological instability. Of note, long QTc interval at baseline ECG is an established pro-arrhythmic factor in familial long-QT syndrome (LQTS), an inherited heart disease with an incidence of about 1/2500 live births characterized by high risk of arrhythmias and possibly death. The main genes involved in LQTS are: *KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2*, *CACNA1c*, *CAV3*, *SCN5A*, *SCN4B* [3]. The most prominent variant of LQTS (LQTS1, LQTS type 1) is due to mutations in the *KCNQ1* gene, present in about half of the genotyped patients [4]. Almost all of the involved genes are associated with $K^+$ or $Na^+$ channels that determine the lengthening of the action potential, identifiable electrocardiographically as

*Abbreviations:* HCM, Hypertrophic cardiomyopathy; ECG, electrocardiographic; LQTS, long-QT syndrome; LGE, late gadolinium enhancement, late gadolinium enhancement; NGS, Next Generation Sequencing; ACMG, American College of Medical Genetics and Genomics; ICD, Implantable Cardioverter-Defibrillator.

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lengthening of the QTc. Although loss of function mutations of the two cardiac potassium channels and gain of function mutations of the cardiac sodium channel are responsible of most monogenic LQTS cases (LQTS1, 2 and 3), pathogenic variants of multiple ion channels or channel-interacting proteins (LQTS 4–15) account for an additional 5% [4]. Some patients also show additional cardiac or extra-cardiac features beyond QTc prolongation, resulting in unique cardiac phenotypes.

The mechanisms underpinning the link between prolongation of the QTc interval and HCM have not been clarified yet. In some cases, patients with HCM without left ventricular hypertrophy may have a prolonged QTc duration [5]. Therefore, mechanisms other than myocardial hypertrophy could be involved in QTc prolongation. The analysis of other genes, apart from those associated with HCM, should be considered to fully understand the etiopathogenesis of long QTc in HCM. We describe a patient with the simultaneous presence of HCM and long QTc, carrying a pathogenic variant in *TNNI3* and a damaging variant in *KCNQ1*, two genes related to the distinct clinical phenotypes of HCM and LQTS.

## 2. Case Presentation

A 24-year-old male with a family history of long QT (mother and maternal grandmother) received a diagnosis of long QT at the age of 16 based on the ECG evidence of a long QTc (498 msec), with abnormal T wave morphology in DI and aVL (Fig. 1a). One year later the diagnosis of long QTc, the patient was found to carry left ventricular hypertrophy and, for this reason, was referred to our centre of "Diagnosis and Treatment of Cardiomyopathies and Hereditary Arrhythmogenic Diseases", School of Medicine and Psychology, "Sapienza" University of Rome. He did not refer syncope. A 24 h ECG monitoring showed the presence of a sinus rhythm (average heart rate 61 bpm), normal atrium-ventricular and intraventricular conductions, and lengthening of the QTc interval, particularly at increased heart rate. At the same time, the cardiopulmonary test showed regular stress tolerance (VO2 max reached 40 ml/min/kg, equal to 85% of the theoretical maximum value), in the absence of signs of cardiogenic deficit, and lack of adaptation of QTc to the exercise. Besides the ECG evidence of LQTS, the echocardiogram showed hypertrophy of the lateral wall of the left ventricle, with a maximum thickness of 19 mm, normal filling and systolic function indices, normal left atrium and right sections, no significant valve morphological and functional alterations. The exam was compatible with a diagnosis of non-obstructive HCM (Fig. 1b). The patient also performed a cardiac magnetic resonance imaging, with evidence of asymmetric hypertrophy of the left ventricle (basal anterior wall

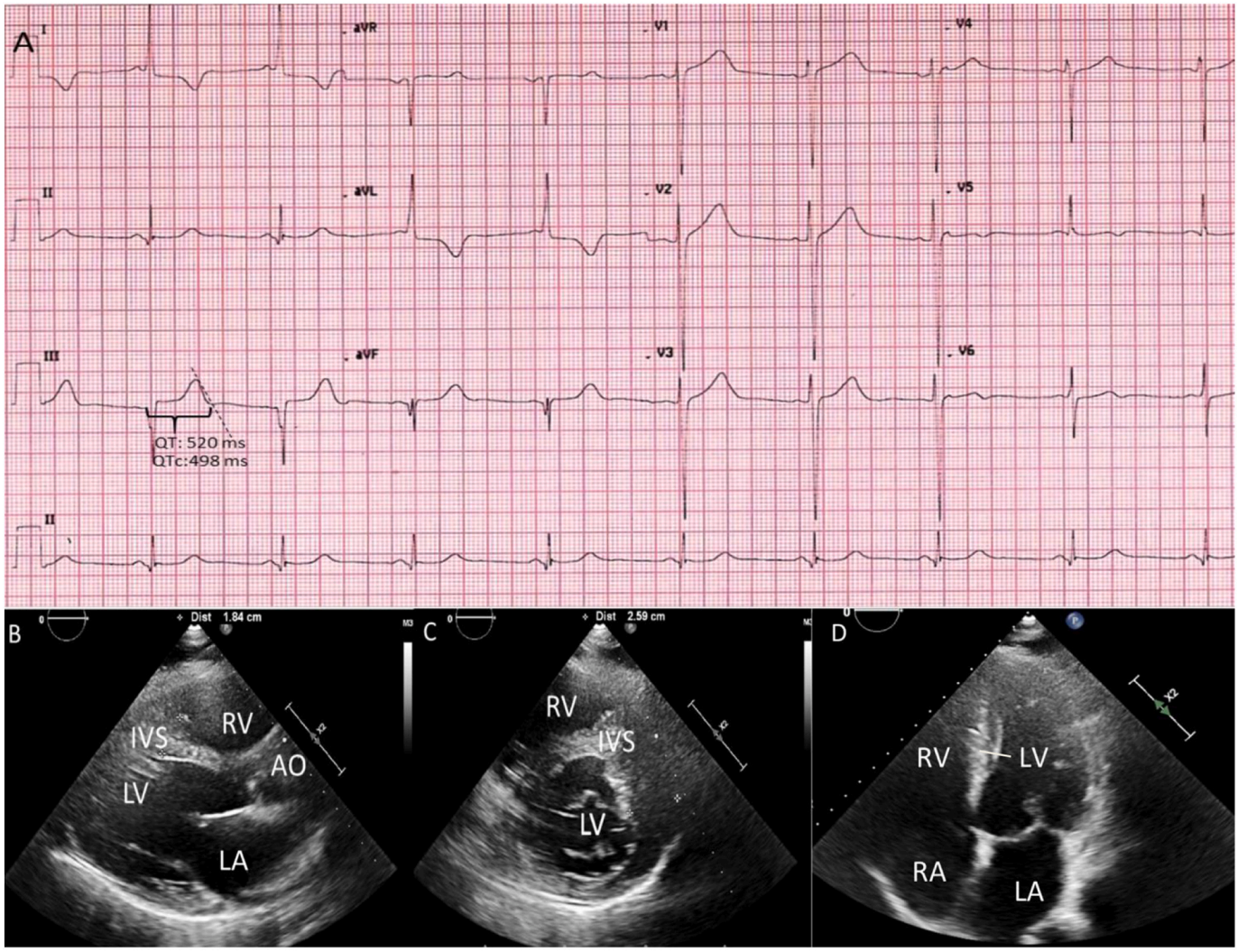


**Fig. 1.** Electrocardiographic and echocardiographic images of proband (III-3). (A) Electrocardiogram shows QT prolongation (QTc 498 ms) and inverted T waves on leads DI, aVL (calibrations: 25 mm/s, 5 mm/mV). Echocardiographic parasternal long axis (B) and short axis (C) views showing asymmetric thickening of the LV wall (19 mm anterior septum in long axis view and 26 mm lateral wall in short axis view). Apical four chamber view (D) showing asymmetric thickening of the posterior septum and left atrial enlargement. LV, left ventricle; RV, right ventricle; LA, left atrium; RA right atrium; AO, aorta; IVS, interventricular septum.

thickness of 26 mm) with shaded late gadolinium enhancement (LGE) in the anterior mid-basal septal site. Currently, the clinical condition of the patient is stable, being asymptomatic for chest pain, dyspnea, palpitations and syncope. The current echocardiogram shows the presence of non-obstructive HCM with a maximum thickness of 24–26 mm at the level of the mid anterolateral wall, normal systolic function indices, hypokinesia of the lower basal wall, which appears thinned, pseudo-normal filling, dilated left atrium (29-cm$^2$ area). The current ECG shows a sinus rhythm with prolonged QTc (470 msec). The patient is currently under therapy with bisoprolol 5 mg/day.

Based on the clinical features, the subject underwent a genetic counseling to collect family history. The proband's mother was diagnosed with LQTS1 at the age 36 and referred to be carrier of a KCNQ1 pathogenic variant identified at another center. Unfortunately, a detailed report of the identified maternal variant was unavailable. The 53-year-old father of the proband was diagnosed with HCM at the age 35 (Fig. 2a) and never underwent HCM molecular testing before our analysis. Due to the double diagnosis of LQTS1 and HCM, the proband was simultaneously screened for the possible HCM causing variants, likely inherited from the father, and for the maternal KCNQ1 pathogenic mutation, using NGS (Next Generation Sequencing) technologies. All subjects gave their informed consent for inclusion before they participated into the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of S. Andrea Hospital (approval identification number: 42 of 28 September 2007). Molecular analysis was performed on Ion PGM Platform (Life Technologies), using a custom multi-gene panel, covering the coding exons and exon–intron boundaries of 26 genes, including HCM and LQTS1 related genes (ACTC1, CSRP3, GLA, LAMP2, MYBPC3, MYH7, MYL2, MYL3, PLN, PRKAG2, TNNC1, TNNI3, TNNT2, TPM1, TTR, DSG2, DSP, KCNE1, KCNE2, KCNH2, KCNJ2, KCNQ1, PKP2, RYR2, SCN4B, SCN5A; Table S1). Pathogenic/likely pathogenic variants were validated by Sanger sequencing and reported using the Human Genome Variation Society nomenclature guidelines (https://varnomen.hgvs.org/). The clinical classification of the variants was carried out according to the American College of Medical Genetics and Genomics (ACMG) criteria [6].

## 3. Discussion and Conclusions

The genetic testing allowed to confirm in our proband the presence of the c.1781G>A on *KCNQ1* gene, very likely inherited from *KCNQ1* mutant mother, and the presence of the c.592C>G pathogenic variant on the *TNNI3* gene (Fig. 2b). The TNNI3 c.592C>G substitution was then investigated in the father by Sanger sequencing, confirming the paternal origin of the variant.

The c.1781G>A variant within the *KCNQ1* gene replaces the amino acid arginine with glutamine in position 594, p.(Arg594Gln), in the K$^+$ Kv7.1 channel protein. This variant, that is rare in the general population (frequency of $1.1 \times 10^{-5}$ in gnomAD database), has been reported in the heterozygous condition in multiple individuals and families with long QT [3,7,8] and in compound heterozygous state in patients with Jervelle and Lange Syndrome [9]. Functional studies have shown that p.(Arg594Gln) results in a complete loss of *KCNQ1* channel function due to a trafficking defect of the mutant channel in the plasma membrane [9,10]. Missense variants associated with LQTS are reported in HMGD (http://www.hgmd.cf.ac.uk/docs/login.html) at the same codon and on adjacent codons, suggesting the functional importance of the amino-acidic residues of this region. Based on these data and according to the ACMG criteria, the *KCNQ1* c.1781G>A variant is classified as pathogenic.

The c.592C>G change identified in *TNNI3* gene replaces leucine with valine at codon 198 of the corresponding coding protein, p.(Leu198Val). This variant is reported in the literature in several unrelated HCM patients and segregates with the disease in different families [11–14]. The prediction tools used to evaluate the impact of the variant on the protein

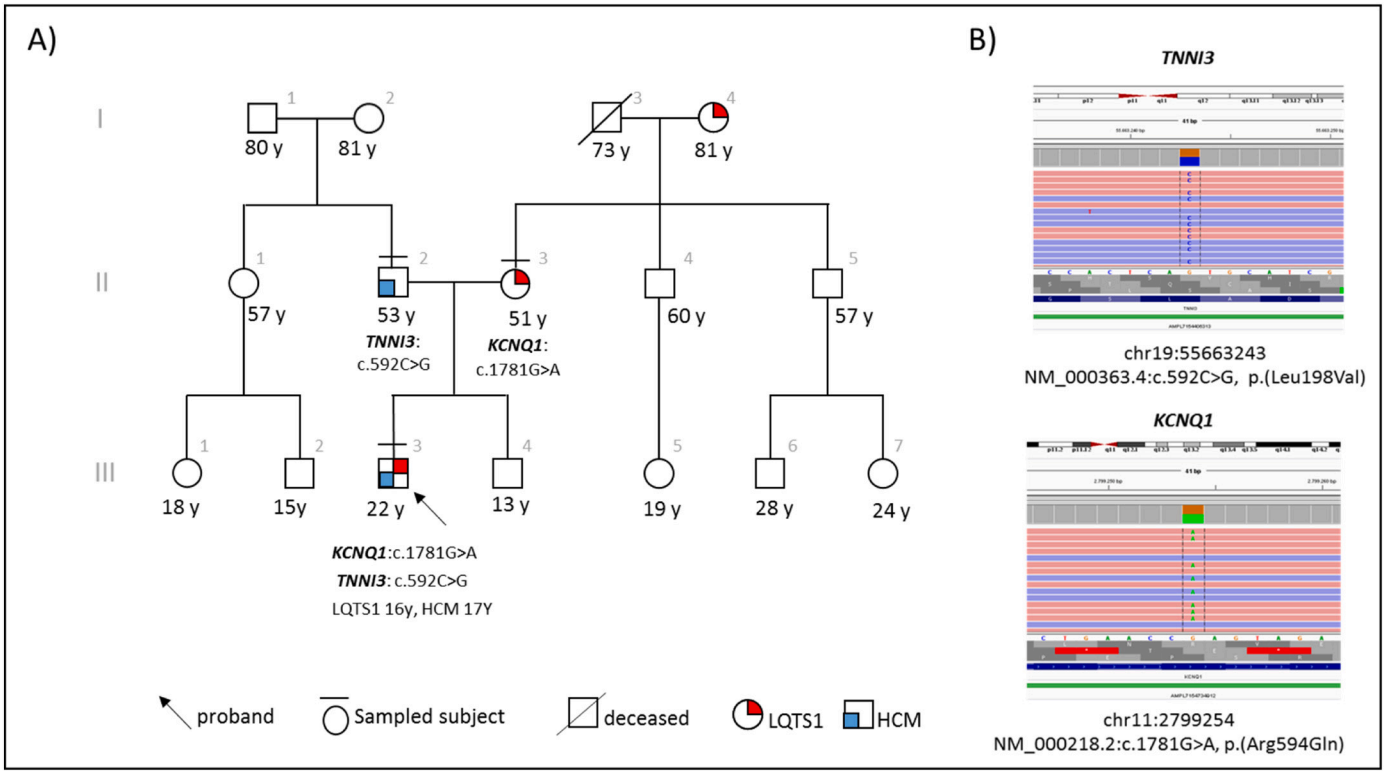


**Fig. 2.** (A) Pedigree of Family with co-inheritance of TNNI3 and KCNQ1 variants. Male is represented by a square; female is represented by a circle. LQTS1, Long QT Syndrome; HCM, Hypertrophic Cardiomyopathy, Y, years. (B) Multi-gene panel analysis. The identified pathogenic variants in TNNI3 and KCNQ1 genes are visualized by Integrative Genome Viewer (IGV) software. Ref Seq (Reference sequencing) used for variants annotation: NM_000363.3 and NM_000218.2.

gave conflicting opinions: SIFT: deleterious, PolyPhen: benign, Align GVGD: C0. Other missense variants have been reported at the same codon, independently classified as pathogenic or likely pathogenic, in patients with HCM [15]. In Atlas of Cardiac Genetic Variations (https://www.cardiodb.org/acgv/) the c.592C>G is classified as VUS favor pathogenic. According to the ACMG criteria, the c.592C>G can be classified as likely pathogenic.

These results demonstrate that the clinical expression of long QT and HCM in our proband is due to two independent genetic events. Of note, the co-inheritance of two monogenic diseases involving LQT1 was also shown by combination of LQT1 pathogenic variants with metabolic disease [16].

Of note, our observations indicate that a possible increased risk of arrhythmias, due to the effect of the two pathogenic variants, should be considered for the subsequent follow-up of the patient. In fact, in this case the length of the QTc interval becomes a classic risk factor, as per LQT1.

The presence of long QTc is found in about 12% of HCM patients [17] and it is considered a predictor for the need of ICD (Implantable Cardioverter-Defibrillator) implantation. The QTc length risk in HCM was also a feature noted by other groups [18,19]. Concerning the cause of QTc abnormalities in HCM, either a structural or a genetic origin could be hypothesized. The first hypothesis suggests that QTc abnormalities derive mainly from the repolarization dispersion due to the myocardial hypertrophy. The second hypothesis proposes the independent genetic origin of the ECG alterations. In favor of the second hypothesis, the literature reports that the QTc length in HCM can be influenced by polymorphisms in LQT genes/NOS1AP [20]. Interestingly, the existence of a Chinese family carrying pathogenic variants related to both HCM and LQTS phenotypes has been previously described [21].

As our clinical report demonstrates, the co-existence of pathogenic variants of *KCNQ1* and *TNNI3* correlates with the clinical expression of both LQTS and HCM, suggesting the possibility of complex genotypes in HCM patients with prolonged QTc interval.

Our findings support the hypothesis that the clinical expression of long QTc could be due to the co-inheritance of causative gene variants related to LQTS, rather than to the degree of cardiac hypertrophy, at least in some HCM patients. The simultaneous analysis of HCM and LQTS related genes in patients with HCM could be helpful in establishing the hereditary nature of the disease and to identify those cases with dual heterozygous damaging mutations in genes related to HCM and LQTS, respectively. This achievement will ultimately allow a better clinical management of the family probands and proper segregation studies among their relatives.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2021.100743.

## Ethics approval

This is an observational retrospective patient report that did not involve any research-based intervention. All interventions were intended to diagnose and treat the patient. No aspect of the case report is in contradiction with the Helsinki Declaration of 1975, as revised in 2000.

## Submission declaration and verification

This report has not been published previously and is not under consideration for publication elsewhere. Publication of this report is approved by all authors.

## Patient consent

Written informed consent for the present study was obtained from the patient's parents.

## Author contributions

FC, EC, SF, MLM, SB, MBM, CA: data curation and investigation; CS, AG: methodology and analysis; SR, MP: supervision and writing; MRT: supervision. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. All authors confirm the absence of previous similar or simultaneous publications.

## Declaration of Competing Interest

None.

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