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TITLE PAGE

Title: Long QT syndrome and left ventricular noncompaction in 4 family members across 2 generations with *KCNQ1* mutation

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Running title: KCNQ1 mutation LVNC and LQT

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Abstract

The association of long QT syndrome and left ventricular noncompaction is uncommon, with only a handful of previous reports, and only one reported case in association with a mutation in *KCNQ1*. Here we present genetic and phenotypic data for 4 family members across 2 generations who all have evidence of prolonged QT interval and left ventricular noncompaction in association with a pathogenic mutation in *KCNQ1,* and discuss the potential mechanisms of this association. In conclusion, we suggest that it may be helpful to consider looking for mutations in *KCNQ1* in similar patients.

Introduction

Long QT syndrome (LQTS) encompasses a group of disorders characterised by mutations affecting ion channels in the myocardium, leading to prolongation of the QT interval and risk of torsades de pointes arrhythmia and ventricular fibrillation. 15 subtypes (LQT1-15) have been delineated, with *KCNQ1* (LQT1), *KCNH2* (LQT2) and *SCN5A* (LQT3) being the most commonly identified. Although arrhythmic syndromes are classically considered to be genetically distinct to hereditary cardiomyopathies, some phenotypic overlap has been reported previously. For example, mutations in *SCN5A*, which is classically associated with LQT3 and Brugada syndrome, have been found in patients with arrhythmic DCM. (1) A recent study looking at echocardiographic findings in patients with LQT gene mutations demonstrated that in 20%, subclinical cardiomyopathic changes were found, and this correlated with prolonged QT interval on ECG and history of arrhythmic events. (2) A further study has also demonstrated subtly reduced systolic and diastolic function in patients with LQTS compared to controls, again suggesting that LQTS may not purely be an electrical disease. (3) There have also been a handful of case reports of patients with both long QT syndrome and left ventricular noncompaction; this appears to be a rare association. Left ventricular noncompaction (LVNC) is defined as a congenital cardiomyopathy with increased trabeculation along with deep intertrabecular recesses of the myocardium, and can be associated with arrhythmia, cardiac failure and thromboembolism. Diagnosis is confirmed by imaging, usually cardiac MRI, looking for a specific ratio of noncompacted to compacted myocardium (>2.3). (4, 5, 6, 7) Diagnosis can be complicated by the fact that trabeculation can occur as a

physiological trait. For example, this can be seen in as a normal variant in athletes, and is most likely due to increased left ventricle preload and afterload. (8) LVNC can be sporadic or familial, and appears to be genetically heterogeneous. Mutations in tafazzin have been detected in children with LVNC (9); other genes, which have been linked to LVNC, include *SCN5A* (10)*, LMNA, MYH7*, *MYBPC3* (11), *KCNQ1* (12)*, KCNH2* (13) and *HCN4* (14, 15), but more work is needed to confirm the significance of these. It can be isolated, or occur with other features, such as congenital heart defects or neuromuscular disorders, such as myotubular myopathy. It can also occur as part of a syndrome, examples being Turner syndrome, 1p36 deletion syndrome and 22q11.2 deletion syndrome. (8) Here, we describe a pedigree in which 3 siblings have been found to have long QT syndrome and evidence of LVNC with a LQT1-associated *KCNQ1* mutation.

Materials and Methods

PCR amplification of *KCNQ1, KCNH2, KCNE1, KCNE2* and *SCN5A* was followed by PCR clean up using Agencourt AMPure XP magnetic beads on a on a Biomek NXp lab automation workstation as per manufacturer's instructions. 1µl of the cleaned up PCR product was used with ABI BigDye Terminator v3.1 sequencing kit. The sequencing reactions products were cleaned up using Agencourt CleanSEQ magnetic beads on a on a Biomek NXp lab automation workstation as per manufacturer's instructions and analysed on an ABI 3730 genetic analyser. The sequencing results were analysed using Mutation Surveyor software (current version 5.0.0) from SoftGenetics.

The complete coding regions and flanking intronic sequences of the MYBPC3 (NM_000256.3), MYH7 (NM_000257.2), TNNT2 (NM_000364.2), TNNI3 (NM_000363.4), TPM1 (NM_001018005.1), MYL2 (NM_000432.3) genes were amplified by PCR using 10x ReddyMix PCR buffer (Thermo Scientific). PCR products were cleaned up using Agencourt AMPure XP (Beckman Coulter). Products were sequenced bidirectionally using fluorescent Big Dye v3.1 (Life Technologies) and cleaned up using CleanSEQ (Beckman Coulter). Sequencing products were run on the ABI 3130 and sequencing data analysed using Mutation Surveyor software v4.0.8 (Softgenetics).

A multiplex PCR primer panel for the major cardiac muscle isoform (NM_003319.4) of the Titin (TTN) gene was created using the Ion AmpliseqTM Designer tool (www.ampliseq.com). This panel (IAD25063) predicts coverage of >99.7% of the coding and flanking intronic regions (+2, -2) of the TTN gene. Target enrichment (library construction) for TTN was performed using the Titin custom design primers and Ion Ampliseq library kit v2.0 (Life Technologies). Emulsion PCR and enrichment was subsequently carried out using the Ion OneTouch 2 instrument (200bp template kit) and Ion ES module. Sequencing was carried out on an Ion Torrent Personal Genome Machine (Ion PGM Sequencing 200 kit v2). All stages of the workflow were carried out according to the manufacturer's instructions. >95% of the coding sequence and flanking intronic sequences of major cardiac muscle isoform (N2-B) of TTN were covered to a minimum depth of 20 reads. Sequence data was mapped and variants identified using Variant Caller (Torrent Suite Software) and NextGENe (Softgenetics) with hg19 (GRCh37) human genome as the reference.

Case Report

The proband, III6, was a 48-year-old gentleman from a Caucasian, non-consanguineous family (Figure 1). He initially presented with atypical chest pain. He had no history of any medical problems, apart from a possible hole in the heart as a child. The resting ECG was found to be abnormal, showing sinus rhythm with inferolateral T wave inversion as well as a long QT interval (QTc 471ms) (Figure 2). Echocardiography showed appearances suggestive of a healed perimembraneous ventricular septal defect and hypertrabeculation at the apex associated with hypokinesia, an ejection fraction of 45-47% and reduced longitudinal function (Figure 3). Subsequent MRI confirmed the noncompaction. Due to the combination of abnormal findings on the ECG and echocardiogram, he was thought to have LVNC. He was advised to contact his first-degree relatives so that they could be screened clinically.

His sister, III4, was then reviewed. She was found to have a prolonged QT interval (QTc 506ms) (Figure 4) and cardiac MRI showed an area of hypertrabeculation of the apical inferolateral wall, although this did not meet the diagnostic criteria for LVNC. (Figure 5) She also had mitral valve prolapse. DNA samples were taken, with sarcomere gene testing (*MYBPC3, MYH7, TNNT2, TNNI3, TPM1* and *MYL2*) carried out initially, which did not detect any mutations. *TTN* was also tested, with no mutations detected. In view of the prolonged QT interval, channelopathy genes were screened as well (*KCNQ1, KCNH2, KCNE1, KCNE2* and *SCN5A).* A c.817C>T (p.L273F) mutation in *KCNQ1* was detected, which has been previously reported in

LQT1 (16). Her 2 adult sons have been tested and have not inherited this gene change; they both have normal echocardiograms and ECGs.

Another sibling, III1, was also reviewed. She was found to have a prolonged QT interval (QTc 507ms) (Figure 6) and cardiac MRI showed apical hypertrabeculation consistent with LVNC (Figure 7). She was subsequently tested to see if she had the same mutation in *KCNQ1* as her sister, which was the case. The proband was then tested and also found to have the *KCNQ1* mutation. His children have also been assessed, one of whom (IV8) has a prolonged QT interval (QTc 520ms). Echocardiogram showed trabeculation of the apex of the left ventricle consistent with LVNC in the context of excellent ventricular function, and she has subsequently been found to have the familial *KCNQ1* mutation. No evidence of skeletal myopathy was found in any members of the family, and none of them are known to regularly participate in sporting activity except IV8, who dances regularly. The clinical and genetic findings in each of these patients are summarised in Table 1.

Discussion

LVNC is a relatively recently established cardiomyopathy. It was first described in 1984 and is frequently associated with arrhythmia. A study by Shan et al looking at the frequency of *SCN5A* mutations in patients with LVNC showed that those with an associated arrhythmia were more likely to have a *SCN5A* mutation (50% in arrhythmia group compared to 7% in control group, p= 0.0003). These patients had a variety of arrhythmias, with 2/17 having long QT syndrome. (10)

As mentioned, there are a number of conditions and genes associated with LVNC, suggesting that it may be a consequence of a variety of disease processes. It may well be that abnormal ionic current flow seen in LQTS is one of these processes, although the association of LVNC and LQTS appears to be uncommon. This may be due to the fact that it is not necessarily looked for by imaging in LQT cases, or that it truly is a rare association. Several cases have been reported in association with mutations in ion channel genes. Along with the 2 cases reported by Shan et al, there have also been 2 cases reported by Ogawa et al with LVNC and long QT syndrome, who both have different mutations in *KCNH2*. (13) Recent reports have associated LVNC and bradycardia with *HCN4* mutations in multiple families. (14, 15)

Only 1 other case associated with *KCNQ1* mutation has been reported so far. Nakashima et al reported the case of a 5-year-old girl who suffered a cardiac arrest and was found to have LVNC and long QT syndrome. She was found to have a previously reported pathogenic mutation in *KCNQ1* (c.1831G>T, D611Y), in the C terminus region. Several members of her family were found to carry this mutation, but none of them had ECG or echocardiographic abnormalities detected. (12)

The mutation in our pedigree lies in a distinct region of the KCNQ1 channel protein, the S5 transmembrane domain. The L273F mutation is causally linked to LQT1. (16) Functional cardiac "I_{Ks}" (slow delayed rectifier) potassium channels are formed by coassembly of KCNQ1 with KCNE1; KCNQ1+KCNE1 channels lack the marked current inactivation that is present for KCNQ1 alone. For the L273F mutant KCNQ1, KCNE1 coexpression is no longer able to eliminate KCNQ1 inactivation, and this is expected to limit repolarizing current flow through native I_{Ks} channels. (17)

Work has been undertaken to look at therapeutic options that could activate I_{Ks} channels and so overcome KCNQ1 inactivation, although there is no such agonist currently available for clinical use. For example, zinc pyrithione (ZnPy) has been found to augment current and reduce inactivation in experiments on recombinant KCNQ1 channels, though it does not augment recombinant KCNQ1+KCNE1 or native I_{Ks} (18) A small molecule activator of I_{Ks} called ML277 may have more promise. Using patient specific stem-cell derived myocytes, ML277 has been shown to increase I_{Ks} in cells with reduced I_{Ks} from LQT1 patients. (19) Thus, this compound or future derivatives may have potential for some LQT1 patients. Gene therapy would involve replacement of defective KCNQ1 with KCNQ1 lacking the L273F mutation. Were such treatment possible in an adult, this would likely correct the LQT phenotype, but whether or not it could alter established LVNC is open to question, due to the stage of development at which this occurs.

Looking at embryonic mouse models, expression of KCNQ1 mRNA and protein has been found to commence at embryonic day 9.5 (E9.5). It appears to be evenly expressed within the atrial and ventricular myocardium, including the trabeculated layer, from E9.5 to E18.5. After this time, it appears to be preferentially expressed in the conducting system. (20) Formation of trabeculae occurs at E9.5-10.5, followed by elongation and then compaction of the trabecular myocardium at E14.5-E18.5. (21) This suggests that, in mice, KCNQ1 channels are expressed at the same time as formation and compaction of the trabeculae occurs, so the channels could conceivably play a role in this process, but more work is needed to characterise the possible underlying mechanism.

Since all four affected family members with both long QT syndrome and evidence of LVNC have the *KCNQ1* mutation and the two relatives who tested negative have neither long QT nor LVNC, this suggests that as well as causing the long QT syndrome, the mutation may also be important in the development of LVNC. However, it is possible there could actually be 2 separate conditions which are co-segregating in this family. There is a 1/32 chance that this could have happened by chance. Alternatively, there may be a second genetic alteration in the family that is linked due to its proximity to *KCNQ1* on the chromosome.

As IV8 has been found to be clinically affected and carries the familial KCNQ1 mutation, this suggests autosomal dominant inheritance in the family. A possible mechanism in view of this is that there may be a genetic modifier acting in conjunction with the *KCNQ1* mutation to cause LVNC along with long QT syndrome. For example, there could be a further variant in *KCNQ1* in trans or a concurrent mutation of another gene. More work needs to be done to understand how changes in *KCNQ1* could lead to LVNC. It is notable that a recent report has linked a *KCNQ1* mutation (p.R397Q) with arrhythmogenic cardiomyopathy, although the underlying mechanism remains to be established (22). This report highlights 4 familial cases of long QT syndrome and LVNC across 2 generations, all of which have a pathogenic *KCNQ1* mutation, and illustrates the importance of considering testing for *KCNQ1* mutations in these patients, especially in the context of long QT or arrhythmia.

Conflicts of interest: none

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Legend 1

Pedigree diagram. Members with LQT and LVNC are shaded in black, and mutation status is demonstrated by +/- annotation.

Legend 2

Echocardiographic images of patient III6 demonstrating hypertrabeculation.

Legend 3

ECG of patient III6 demonstrating prolonged QT interval (512ms).

Legend 4

MRI images of patient III4 demonstrating hypertrabeculation.

Legend 5

ECG of patient III4 demonstrating prolonged QT interval (506ms).

Legend 6

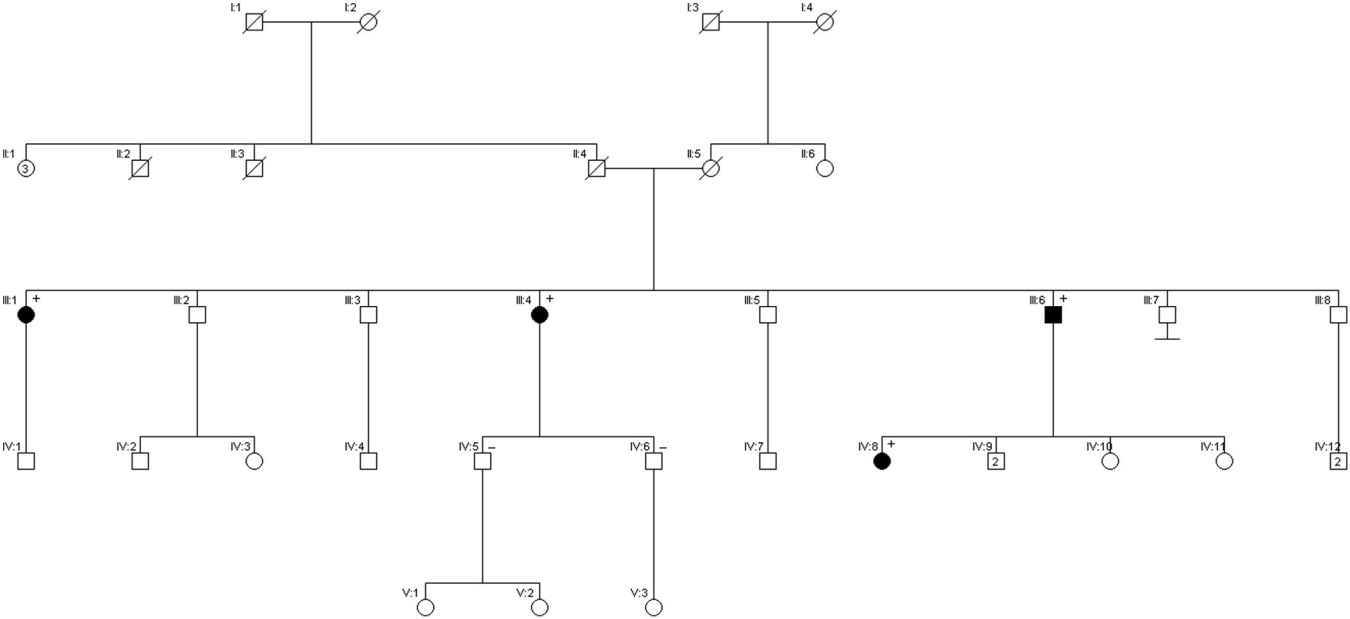
MRI images of patient III1 demonstrating left ventricular noncompaction.

Legend 7

ECG of patient III1 demonstrating prolonged QT interval (507ms).

Table 1

Summary of ECG and imaging findings in all family members tested for the familial *KCNQ1* mutation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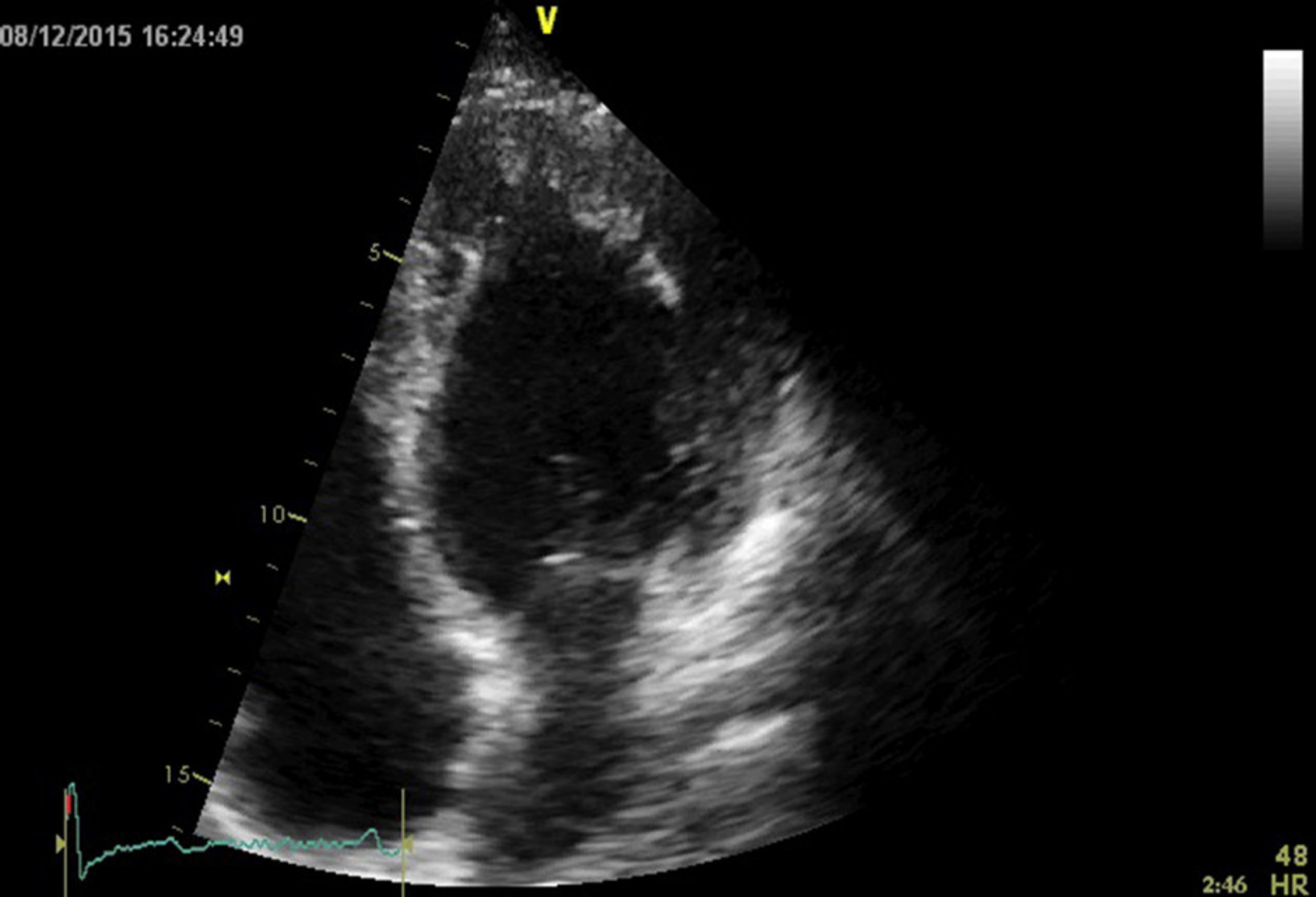
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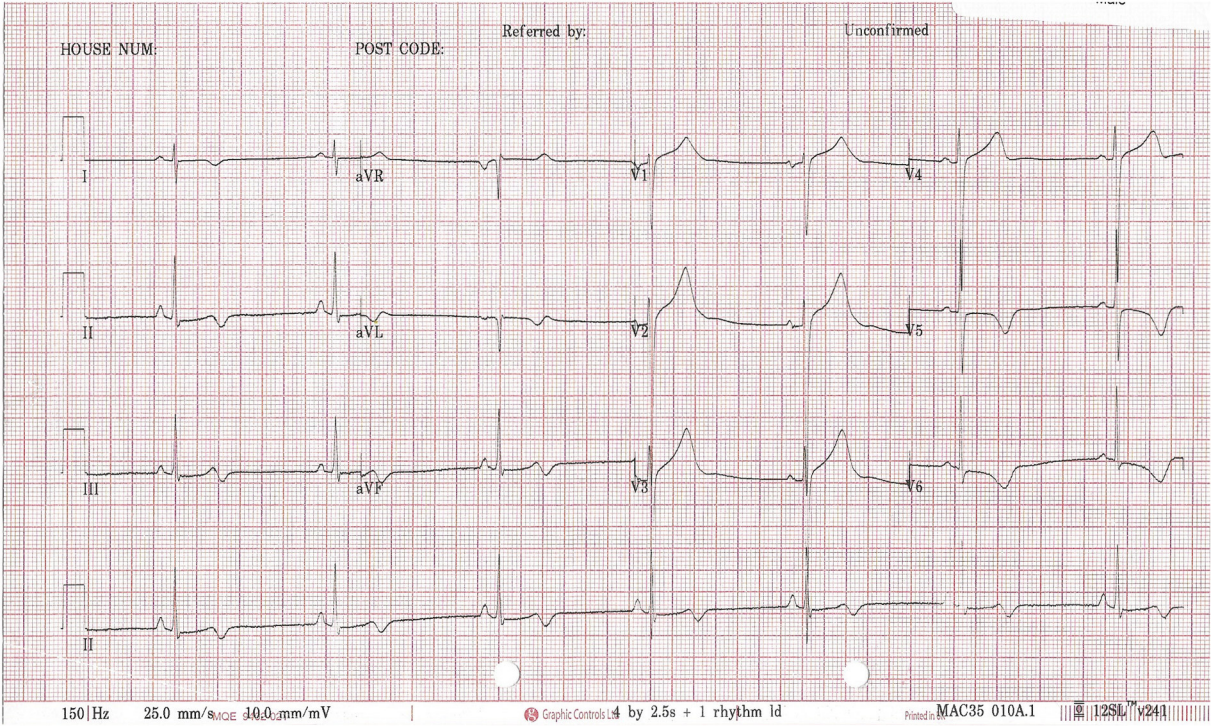
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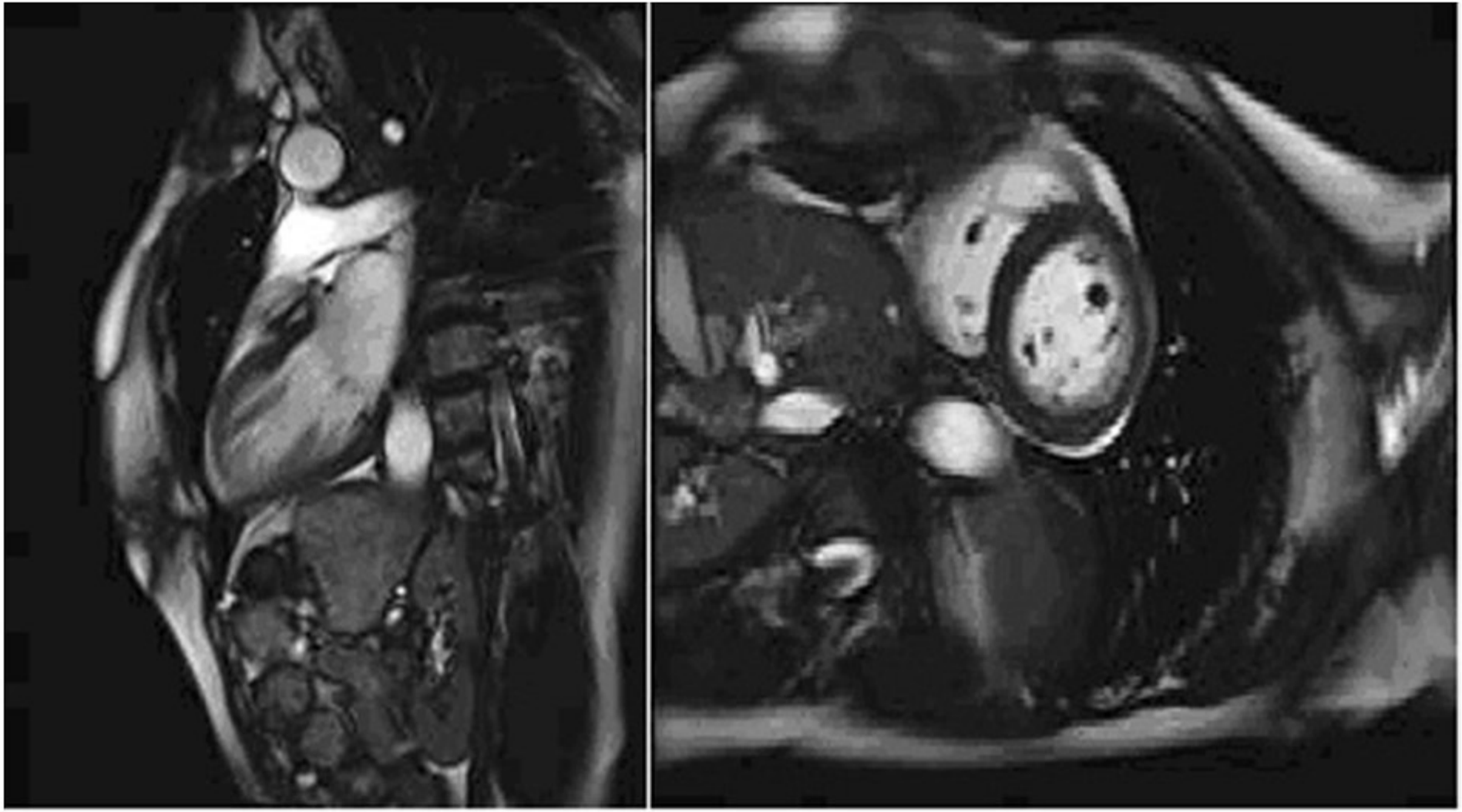

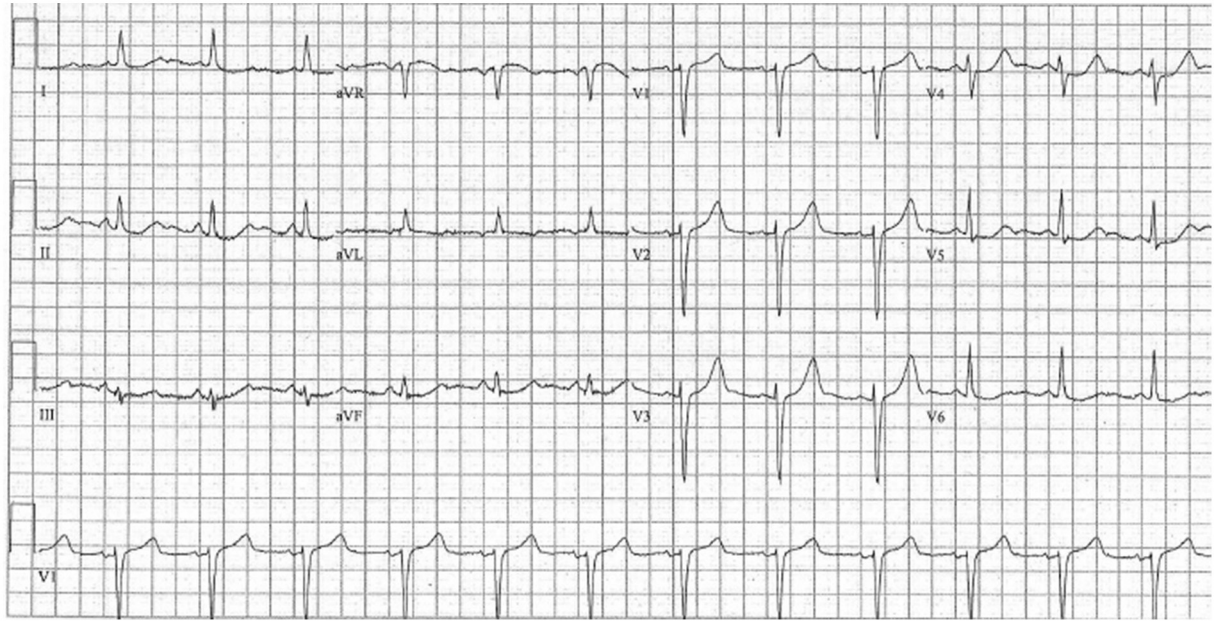
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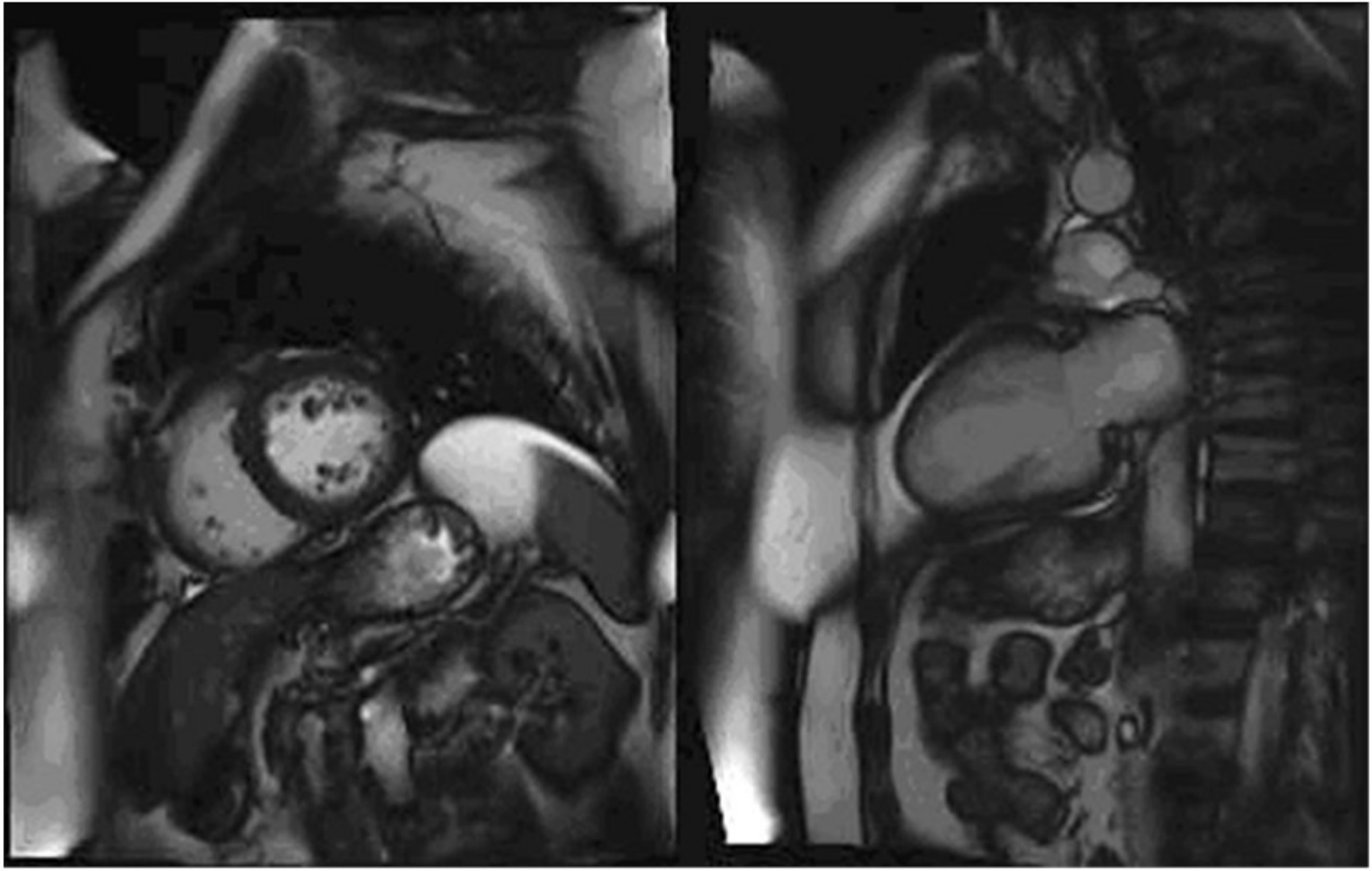

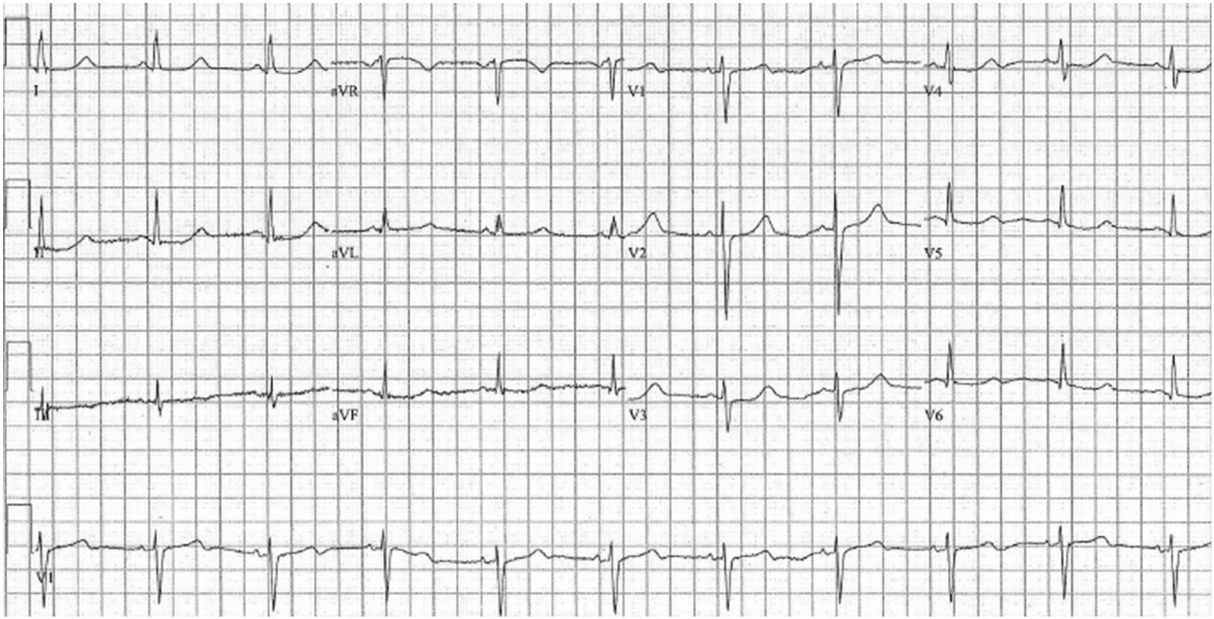
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III
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V3
V6
V1

Family member	ECG findings	Imaging findings	KCNQ1 mutation
III1	Prolonged QT interval – QTc 507ms	Apical hypertrabeculation	+
III4	Prolonged QT interval – QTc 506ms	Hypertrabeculation apical inferolateral wall	+
III6	Prolonged QT interval – QTc 471ms	Apical hypertrabeculation	+
IV8	Prolonged QT interval – 520ms	Apical hypertrabeculation	+
IV5	Normal	Normal	-
IV6	Normal	Normal	-