Research Commentary

Does p.Q247X in TRIM63 Cause Human Hypertrophic Cardiomyopathy?

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Rationale: Variants in TRIM63, including a nonsense mutation (p.Q247X), have been suggested recently to cause hypertrophic cardiomyopathy.

Objective: To verify pathogenicity of TRIM63 p.Q247X detected by whole-exome sequencing in a symptomless professional sports player seeking medical advice because of a prolonged QT interval found during a routine check-up.

Methods and Results: Clinical studies were performed in the proband and his mother, who also carried TRIM63 p.Q247X. No evidence of hypertrophic cardiomyopathy was found in either person.

Conclusions: The p.Q247X variant in TRIM63 is not likely to be a highly penetrant variant causing hypertrophic cardiomyopathy. (Circ Res. 2014;114:e2-e5.)

Key Words: cardiomyopathy, hypertrophic • TRIM63 protein, human

In a recent issue of Circulation Research, Chen et al. published an interesting article proposing that defects in the TRIM63 gene are a novel cause of human hypertrophic cardiomyopathy (HCM). TRIM63 encodes the RING finger protein 1 (MuRF1) that is expressed selectively in cardiac and skeletal muscle. The authors sequenced TRIM63 in 302 HCM probands (including 260 white individuals) and 339 control subjects (including 262 white individuals) and found 2 missense variants and a nonsense variant (p.Q247X) only among patients with HCM. Lack of these variants in the controls was confirmed by TaqMan assays in another group of 751 subjects. The pathogenicity of the TRIM63 variants was supported by functional studies, with particularly convincing results obtained for p.Q247X. In addition, TRIM63 mutation carriers were screened for mutations in MYH7, MYBPC3, TNNT2, TPM1, and ACTC1 by direct sequencing and were found to be free of putative causal variants in these genes that are relatively commonly mutated in HCM.

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We identified the TRIM63 nonsense variant p.Q247X by whole-exome sequencing (WES) (Figure 1) in 2 related subjects (son and mother) in a family who were free from HCM. The first subject was a 22-year-old Polish professional soccer player searching for medical advice with regard to his eligibility for competitive sports in Schweinfurt, Germany and in Warsaw, Poland. The reason for cardiological examination was a suspicion of long QT syndrome raised during a routine check-up by the team physician in January 2013. At that time he was referred to a cardiologist who diagnosed a QTc prolongation of 470 ms in a resting recording during bradycardia 55 bpm and an episode of nonsustained ventricular tachycardia during sleep on 24-hour ECG monitoring (asymptomatic, irregular, 8 cycles, medium cycle length of 400 ms). He was advised against continuing competitive sports until further studies in April 2013. The patient negated any symptoms; his medical history included surgery of a throat cyst at age 9 years and insertion tendinothropy of the right knee. His family history included a brother who died during a car accident, but there were no sudden cardiac or unexplained deaths in the family. His physical examination was normal, with resting blood pressure of 110/80 mmHg. On standard 12-lead ECG sinus rhythm, 72/min, normal axis, T-notching in V2–V5, U waves, and QTc 440 ms were found (Online Figure 1). Transthoracic 2-dimensional echocardiography showed normal-size ventricles with normal systolic and diastolic function, maximal wall thickness of 10 mm, left ventricle internal diastolic diameter of 53 mm (26 mm/m²), left ventricular mass of 101 g/m², and normal systolic function with left ventricular ejection fraction 60% (Simpson method; Online Figure I and Online Movie I).
On cardiopulmonary test, maximal oxygen consumption was 42.8 mL/kg per minute; QTc was 420 ms at rest and did not increase during exercise. There was no arrhythmia during exercise or any other pathological findings. With the diagnosis of low probability of long QT syndrome, it was decided to perform cardiac MRI (to exclude arrhythmogenic right ventricular cardiomyopathy and myocarditis) to examine first-degree relatives and to perform genetic tests.

Cardiac MRI in a bradycardiac patient in the detraining period showed mildly dilated ventricles (left ventricular end-diastolic volume, 120 mL/m^2; right ventricular end-diastolic volume, 122 mL/m^2) with low normal left ventricular ejection fraction of 55% and right ventricular ejection fraction of 50%. Maximal left ventricle wall thickness was 11 mm without any asymmetry (Online Figure II and Online Movie II). On gadolinium-enhanced myocardial imaging, the presence of one small intramyocardial focus of fibrosis at the junction point of the right and left ventricles was suspected. Although the patient had increased right ventricular end-diastolic volume (>110 mL/m^2), he did not have any regional right ventricular wall motion abnormalities and he did not fulfill any of the criteria for diagnosis of arrhythmogenic right ventricular dysplasia.3

Clinical and noninvasive tests were performed in the proband’s father (aged 50 years) and mother (aged 47 years), revealing asymptomatic and normotensive individuals. On standard ECG with sinus rhythm, QTc was 400 ms in the father and 429 ms in the mother (Figure 2A), with no other abnormalities. On standard echocardiogram, there were no features of any cardiomyopathy. In particular, in the proband’s mother left ventricular posterior wall thickness was 7.4 mm and intraventricular septum was 9 mm. Left ventricular end-diastolic dimension was 46.5 mm (30.2 mm/m^2) and left ventricular ejection fraction in apical 4-chamber view by Simpson method was 62.5% (Figure 2B and 2C; Online Movies III and IV).

The proband and his family were well aware of diagnostic possibilities offered by genomic testing. Given their considerable anxiety and the possibility of congenital long QT syndrome, we performed WES in the patient with follow-up of suspicious variants by Sanger sequencing. WES was performed on HiSeq 1500 using TruSeq Exome Enrichment Kit (Illumina). After standard 2×100 Illumina run, the data were processed by the CASAVA. Generated reads were aligned to the hg19 reference genome with Burrows-Wheeler Alignment Tool4 and processed further by Genome Analysis Toolkit.5 Base quality score recalibration, indel realignment, duplicate removal, and the variant calling were performed as described.6 The detected variants were annotated using Annovar2 and converted to MS Access format for final manual analyses. Alignments were viewed with Integrative Genomics Viewer.7 Sanger sequencing was performed on ABI PRISM 3500XL capillary sequencer. With WES we found 2 heterozygous variants of note: the p.Q247X in TRIM63 (rs148395034) (Figure 1) and p.V392I in DSG2 (rs193922639). These variants were confirmed by direct sequencing in 2 blood samples taken on separate days. Both variants, p.Q247X in TRIM63 and p.V392I in DSG2, were found in the proband’s asymptomatic mother.

The p. Q247X variant described by Chen et al1 occurred in 2 unrelated families. As the authors commented,1 because all carriers of the p. Q247X shared the same haplotype for 5 STR markers and 21 single nucleotide polymorphisms at the TRIM63 locus (1p34-1p33), an independent origin of the variant in these 2 families could not be established. Of interest, clinical characteristics of the 3 affected subjects (Online Table IV) showed late onset of the disease, namely the diagnosis of severe obstructive HCM was made at ages 45, 53, and 57 years, respectively. The identification of the p.Q247X variant in our proband’s asymptomatic mother with a structurally normal heart at age 47 years argues against the possibility of preclinical HCM in the young proband.

Figure 1. Integrative Genomics Viewer view of TRIM63 c.C739T (p.Q247X) mutation found in the proband by whole-exome sequencing (left). Chromatograms from direct sequencing by the Sanger method showing the p.Q247X mutation in the proband (right, top), his mother who also carries the variant (right, middle), and his father (wild-type; right, bottom).
The lack of disease expression in carriers of TRIM63 p.Q247X reported by us might be because of some unknown protective alleles in the family. However, whereas p.Q247X in TRIM63 could be a pathological variant with incomplete penetrance, it should also be considered that TRIM63 (or perhaps Q247X in this gene) has been incorrectly assigned as causative for HCM.

Another variant of note in the family was V392I in the DSG2 gene, reported first as pathogenic with phenotype of arrhythmogenic right ventricular cardiomyopathy by Syrris et al.19 by Bauce et al.9 as arrhythmogenic right ventricular cardiomyopathy in the context of double heterozygosity (DSG2 V392I and PKP2 T50SfsX60), and by Garcia-Pavia,11 who placed it among pathogenic mutations with phenotype of dilated cardiomyopathy leading to heart transplantation. Of interest, Gaertner et al.13 did not show any functional differences comparing the DSG2 V392I protein with the wild-type variant. In silico prediction classifies the V392I in the DSG2 as benign and in the arrhythmogenic right ventricular cardiomyopathy database (http://www.arvcdatabase.info); this variant is currently classified as a variant of unknown significance.15

According to the National Center for Biotechnology Information dbSNP database, the p.Q247X TRIM63 variant has been described as SNP rs148395034, with 4 heterozygous subjects among 662 participants of European descent from the ClinSeq (http://genome.gov/20519355) project who have undergone WES (http://www.ncbi.nlm.nih.gov/projects/SNP/). Furthermore, in the same population, another SNP (rs199956613, p.C173X) introducing a stop codon even earlier than Q247X was found in a single individual, suggesting that the prevalence of damaging variants in TRIM63 is currently classified as a variant of unknown significance.15

The prevalence of null alleles in TRIM63 reported by National Heart, Lung, and Blood Institute Exome Sequencing Project (≈7500 subjects)4 was 0.0036 in the whole cohort and 0.0045 among subjects of European descent. In the European cohort, the most prevalent (n≈28/8254) variant was c.481_482del2, predicted to cause a frameshift (p.S161Cfs*8), whereas p.Q247X (rs148395034) was observed 6 times among 8600 alleles. Furthermore, low-frequency truncating alleles of damaging TRIM63 are relatively high in the studied population (1:132). ClinSeq is a population-based study recruiting adults aged 45 to 65 years with special, but not exclusive, interest in atherosclerosis (http://www.genome.gov/25521305).

V147Lfs*23C159+1 G>A were reported with prevalences of 1 in 8254 and 2 in 8600, respectively. The prevalence of V392I in the DSG2 gene (rs193922639) in the National Heart, Lung, and Blood Institute Exome Sequencing Project was 17 in 8220 (ie, 0.0021) among subjects of European descent. Thus, in the European population, the carriage rates of null variants in TRIM63 and the V392I variant in DSG2 are relatively high (≈1/113 and ≈1/242, respectively).

Taken together, the p.Q247X variant in TRIM63 was an unexpected finding discovered by chance in 2 generations of a family free from HCM. This observation together with an apparently high population prevalence of damaging variants in TRIM63 argue against the proposition that the p.Q247X mutation in this gene causes on its own human HCM with high penetrance.1 This calls for a judicious interpretation of novel findings during assessments of single or several genes in the diseased population, even in cases supported with excellent functional studies.

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Disclosures

None.

References

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