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A novel variant in *RyR2* causes familiar Catecholaminergic Polymorphic Ventricular Tachycardia

Short title

Sudden cardiac death due to RyR2 mutation

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Highlights

- Catecholaminergic polymorphic ventricular tachycardia is a cause of sudden death
- Molecular autopsy should be performed in sudden death cases showing normal autopsy
- Next Generation Sequencing technology allows a comprehensive genetic analysis
- Familial assessment is crucial to identify relatives at risk of sudden death

Abstract

Catecholaminergic polymorphic ventricular tachycardia is a rare familial arrhythmogenic disease. It usually occurs in juvenile patients with a structurally normal heart and causes exercise-emotion triggered syncope and sudden cardiac death. The main gene associated with catecholaminergic polymorphic ventricular tachycardia is *RyR2,* encoding the cardiac ryanodine receptor protein which is involved in calcium homeostasis. After the identification of a 16 year-old man presenting with exercise-induced sudden cardiac death, clinically diagnosed as catecholaminergic polymorphic ventricular tachycardia, we collected the family information and performed a comprehensive genetic analysis using Next Generation Sequencing technology. The initial electrocardiogram in the emergency department revealed ventricular fibrillation. On electrocardiogram monitoring, sinus tachycardia degenerated into bidirectional ventricular and into ventricular fibrillation. Catecholaminergic polymorphic ventricular tachycardia was clinically diagnosed in 5 of the 14 family members evaluated. There were no additional reports of seizures, pregnancy loss, neonatal death, or sudden cardiac death in family members. Genetic analysis of the index case identified only one rare novel variant p.Ile11Ser (c.32T>G) in the *RyR2* gene. Subsequent familial analysis identified segregation of the genetic variant with the disease. All current evidence supports that novel p.Ile11Ser variant in the *RyR2* gene is a potential disease-causing variant in catecholaminergic polymorphic ventricular tachycardia. To our knowledge, there has been no previous case report of catecholaminergic polymorphic ventricular tachycardia associated to this missense variant.

Keywords

Sudden Cardiac Death; Catecholaminergic Polymorphic Ventricular Tachycardia; Genetics; RyR2

1. Introduction

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare potentially fatal disease responsible for syncope or sudden cardiac death (SCD). CPVT is usually diagnosed in children and young adults. It usually presents in the context of exercise, stress and emotion. The disease usually causes bidirectional ventricular tachyarrhythmias in the absence of either structural heart disease or prolonged QT interval [1]. A family history of syncope or SCD is present in about one-third of cases. Mortality rates in untreated patients are estimated at 30–50% by age 40. The actual prevalence is probably underestimated as it can be easily misdiagnosed as idiopathic VT or as a noncardiogenic entity (such as epilepsy or vasovagal syncope). Some studies describe a prevalence estimated at 1:10,000 in population although it is difficult to predict because the absence of structural heart disease makes postmortem diagnosis speculative. For this reason, postmortem genetic testing has been suggested as a potential diagnostic tool in cases of sudden unexplained death [2]. To date, CPVT is a genetic disease associated with pathogenic variants in 6 genes. Inheritance may be autosomal dominant (*RyR2*, *KCNJ2, CALM1,* and *CALM2*) or recessive (*CASQ2* and *TRDN*). In recent years, other genetic alterations named Copy Number Variations (CNV) have been associated with CPVT in any of above mentioned genes [3]. The main gene responsible for up to 50% of CPVT cases is *RyR2*, which encodes for the cardiac isoform of the ryanodine receptor. *RyR2* mediates the release of calcium from the sarcoplasmic reticulum, required for sarcomere contraction. Genetic alterations in the *RyR2* gene increases calcium release and can trigger life-threatening ventricular arrhythmias under catecholaminergic stimulation [4]. The main therapy in CPVT patients is beta-blockers. Implantable cardiac defibrillator is indicated in current guidelines for patients with aborted SCD or CPVT during exercise. It is also recommended in adolescents with incomplete control of arrhythmias despite a high dose of medications [5]. In this study we report the identification of a novel missense

variant in the *RyR2* gene in a family showing severe cardiac arrhythmias. With the identification of the pathogenic variant responsible for the disease, we also detected genetic carriers in the family who despite being asymptomatic can be at risk of SCD.

2. Material and Methods

This study was approved by the Ethics Committee of our Hospital and conforms to the principles outlined in the Declaration of Helsinki. Alive individuals signed a written informed consent to participate, in accordance with international review board guidelines of our insitution. All samples were anonymized. Detailed family history was obtained including age of presentation, initial symptoms, and 12-lead electrocardiogram (ECG).

Genomic DNA was extracted from whole blood (PerkinElmer Inc). DNA was fragmented and library preparation was performed according to the manufacturer's instructions (SureSelect XT Target Enrichment System for Illumina Paired-End Sequencing Library protocol, Custom 1-499 Kb library, Agilent Technologies Inc). Sequencing process was developed on MiSeq System (Illumina). We analyzed the most prevalent 76 genes involved in SCD-associated pathologies (*ABCC9, ACTC1, ACTN2, AKAP9, ANK2, BAG3, CACNA1C, CACNA2D1, CACNB2, CASQ2, CAV3, CRYAB, CSRP3, DES, DMD, DSC2, DSG2, DSP, EMD, FKTN, GLA, GPD1L, HCN4, JPH2, JUP, KCND3, KCNE1, KCNE2, KCNE3, KCNE5, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, LAMP2, LDB3, LMNA, MYBPC3, MYH6, MYH7, MYL2, MYL3, MYOZ2, MYPN, NEBL, NEXN, NOS1AP, PDLIM3, PKP2, PLN, PRKAG2, RANGRF, RBM20, RyR2, SCN1B, SCN2B, SCN4B, SCN5A, SGCD, SLMAP, SNTA1, TAZ, TCAP, TGFB3, TMEM43, TMPO, TNNC1, TNNI3, TNNT2, TP63, TPM1, TRDN, TRPM4, TTN*, and *VCL*). Variants were annotated with dbSNP human Build 142 IDs (http://www.ncbi.nlm.nih.gov/SNP/); NHLBI Exome Sequencing Project (ESP) ESP6500SI-V2 data release (http://evs.gs.washington.edu/EVS/); the 1000 Genomes browser Phase 3 data release (http://www.1000genomes.org/); the Exome Aggregation Consortium (ExAC) v.0.3 data release (http://exac.broadinstitute.org/); the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php); the ClinVar archive

(http://www.ncbi.nlm.nih.gov/clinvar/); the Ensembl information (http://www.ensembl.org/index.html), and in-home database. Variants were annotated and allelic frequency was consulted using the same databases. *In silico* pathogenicity of novel genetic variations were assessed using CONDEL, PROVEAN, and Mutation Taster. Alignment among species was also performed using the Universal Protein Resource database. Non-common (Minor Allele Frequency – MAF- < 1%) genetic variants were confirmed by Sanger method. Familial cosegregation of rare genetic variants was also performed using Sanger technology. All coding exons and flanking intronic sequences of *CALM1* and *CALM2* were also sequenced using Sanger technology. Regions were amplified by PCR, purified and directly sequenced in both directions (3130XI Applied Biosystems). SeqScape Software v2.5 (Life Technologies) was used to compare results with the reference sequence from Hg19. CNV analysis was performed using an algorithm capable to identify this type of genetic alterations from custom enrichment gene panel designs [6].

3. Results

3.1. Phenotype

A 16 year-old man presented with exercise-induced syncope. He was referred to hospital and the initial ECG in the emergency department revealed ventricular fibrillation. On ECG monitoring, bidirectional ventricular tachycardia with degeneration into ventricular fibrillation was detected. The diagnosis was CPVT (Figure 1). Unfortunately, a new arrhythmogenic episode occurred in the hospital and the young-boy died. Complete autopsy did not identify any histological abnormality responsible for the death. Family members were clinically assessed (basal ECG, exercise testing) and CPVT was diagnosed in 5 of the 14 family members after evaluation (Figure 2 and 3). Family history was negative for seizures, pregnancy loss, neonatal death, or SCD.

3.2. Genotype

Next Generation Sequencing analysis revealed a call rate of 99.8% at 30X (18 exons failed but they were sequenced by Sanger technology). Mean coverage was 461, and the percentile 25 and 75 was

340 and 580, respectively. We identified only one rare variant never reported so far (novel), which was later confirmed by conventional Sanger sequencing (p.11I>S_*RyR2)*. The amino acid isoleucine (Ile, I) change to serine (Ser, S) at position 11 (nucleotide 32, where T changes to G, c.32T>G) (Figure 4). The position is highly conserved between species, and predicted *in silico* as deleterious/pathogenic (Figure 5). In addition, the variant is localized at the beginning of the *RyR2* gene, a zone already described as one of the three hot spots for pathogenic variants in *RyR2*. No CNVs were identified in any of genes analyzed.

3.3. Genotype-Phenotype

DNA samples from 14 family members were analyzed for the rare variant identified in index case (Figure 1). The novel variant p.I11S_*RyR2* was detected in the index case, a 16-year-old boy (III:4), and 6 relatives -in his 48 year-old father (II:3), in his 18 year-old brother (III:3), in his 46 year-old uncle (II:1), in his other 42 year-old uncle (II:5), and in two of his cousins of 2 and 6 year-old (III:2 and III:7 respectively). All family members who carried the novel variant in *RyR2* showed clinical diagnosis of CPVT except one – the 2 year-old cousin (III.2)-. It is important to remark that this young boy showed a normal basal ECG but no exercise test was performed due to young age due to negative of parents despite accepting his genetic analysis. None of negative carriers were diagnosed with CPVT.

4. Discussion

CPVT is one of the most lethal cardiac channelopathies and it is mainly expressed in young patients. The disease shows a normal structural heart and carries a mortality rate of 30%–50% [7]. In our study we report a family in which some relatives are clinically affected by CPVT, including young patients. Genetic analysis identified a novel genetic variant in the *RyR2* gene as highly probable cause of the disease. All diagnosed cases carried the novel variant except one relative who carried the genetic variant but remained clinically asymptomatic. This fact could be explained by the young age of the patient (2 years-old). Moreover, it is also well-known that nearly 85% of CPVT families show a variable expressivity and incomplete penetrance [8]. Additional analysis supported a

deleterious/pathogenic role of the variant, and the altered aminoacid is situated nearly a hot-spot area associated with CPVT [9], but no pathogenic variants have been reported in any of the previous aminoacids of this gene, so far. One of the limitations of our study is the lack of functional analysis; despite these studies may also add supportive evidence of pathogenicity, *in vitro* evidence of channel dysfunction associated with specific variants may not necessarily directly translate into a clinical phenotype in the complex biological environment of the human cardiovascular system [10]. Therefore, the variant identified in our family should be classified as pathogenic following recent ACMG guidelines [11]; in addition, the family segregation is the crucial point in pathogenic interpretation. Taking all data into account, we consider this variant as highly probably pathogenic, at least in our family.

Preventive and therapeutic strategies in this family are based on close follow-up, extreme sport avoidance and pharmacological treatment with beta-blockers. This has also been recommended in the young boy without the phenotype but carrier of the genetic variant (III.2). This approach is based on our experience with a large family with more than 170 carriers of a pathogenic variant in *RyR2* [12]. Current guidelines recommend early genetic testing for clinical management and therapeutic decisions involving family members at any age because CPVT may be presented as SCD as the first manifestation of the disease (even in infants) [5]. Hence, molecular autopsy in cases with a no conclusive cause of death after complete autopsy should be performed, as also recommended by current guidelines. Despite genetic analysis is an important result in current diagnosis of CPVT families, a complete genetic analysis identify the alteration associated with CPVT in nearly 65% of families, remaining 35% of families diagnosed with CPVT without a genetic alteration identified. Therefore, genetic analysis not should be the main result in clinical diagnosis and adoption of therapeutic measures; currently, only clinical tests allow physicians diagnose a CPVT case. In 2011, Sy et al reported two different peaks of the CPVT symptom onset suggesting that the clinical manifestations of late-onset CPVT (32–48 years old) differ from those of young-onset CPVT (10–20 years old) [13]. In addition, the authors also suggested that patients with late-onset CPVT are more

likely to be female, and at lower risk of SCD. In agreement with this report, also in 2011, Sumitomo emphasized a new classification of CPVT according to age of onset: (1) juvenile type, before 20 years of age; and (2) adult type, after 20 years of age [14]. Despite these facts, no conclusive genotype-based risk stratification or differential therapeutic approaches have been suggested based on CPVT-positive variants, so far.

We believe that the lack of solid risk stratification data based on genetics is due to the unclear clinical significance of additional rare genetic variants. It is likely that in the future, with larger amount of data and cohorts, we will be able to unravel the role of these variants in the phenotype. The use of NGS technology allows performing a cost-effective genetic analysis of a large number of genes in a reduced time but most genetic variants remain of unknown clinical significance. It is therefore of paramount importance that a multidisciplinary team of cardiologists, pediatricians, geneticists and genetic counselors discuss the clinical interpretation of each novel genetic variant, being very cautious before translation of the results into clinical practice. Despite this fact, we believe that the identification of asymptomatic genetic carriers is crucial to perform a preventive treatment and close clinical follow-up of families suffering of CPVT.

5. Conclusions

In conclusion, CPVT is an inherited cardiac arrhythmic disorder showing a highly malignant clinical course in the absence of morphological heart alterations. We identified a new pathogenic variant (p.I11S_*RyR2*) in a family diagnosed of CPVT and with a history of SCD in a young relative. Early diagnosis and genetic identification of family members at risk may help to avoid a new case of syncope adopting preventive measures.

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Conflict of interest

The authors declare that they have no conflict of interest

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Figure legends

Figure 1.- Family pedigree. The generations are indicated in the left column and all individuals are identified with a pedigree number. The proband is indicated by arrow (III-4), and clinical assessment was performed before death while genetic analysis was performed post-mortem. In all other family members, clinical and genetic analysis was also performed except in I.1, I.2 and III.9, deceased several years before. Black round/square means clinically diagnosed patients. White round/square means negative on clinically assessment. Plus signs indicate genetic carriers of p.I11S_*RyR2*. Minus signs indicate non-genetic carriers. Slashed line indicates deceased individuals.

Figure 2.- Electrocardiogram showing CPVT in **II.1**

Figure 3.- Electrocardiogram showing CPVT in **II.5.**

Figure 4.- Electropherogram showing the novel p.I11S (c.32T>G) genetic variant in the *RyR2* gene. Arrow indicates the heterozygous change in exon 1 of the *RyR2* gene. The nucleotide 32 changes from T (Thymine) to G (Guanine), and cause a modification of the aminoacid 11, from I (Ile, Isoleucine) to S (Ser, Serine).

Figure 5.- Multiple sequence alignment and tree of species. The aminoacid 11 is indicated with an asterisk. The conservation of the aminoacid is preserved between species.

Figures

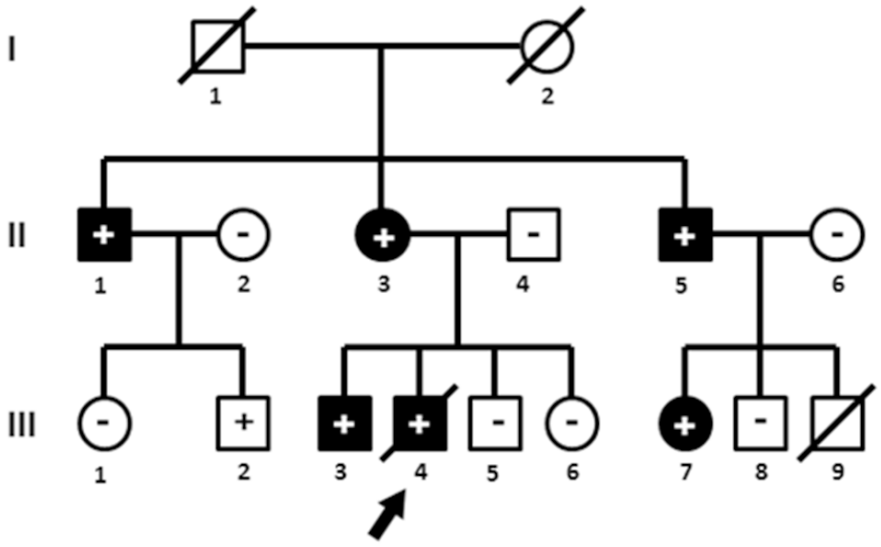

Figure 1.

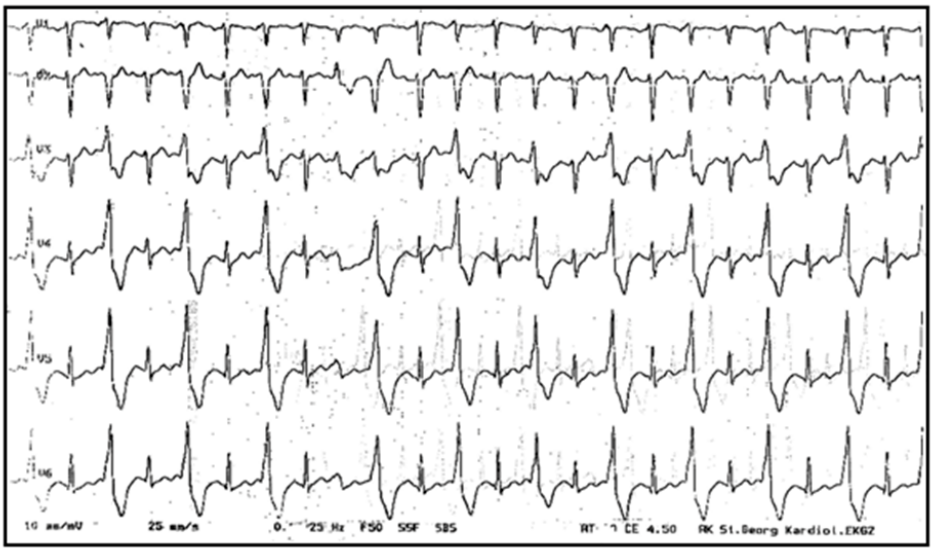

Figure 2.

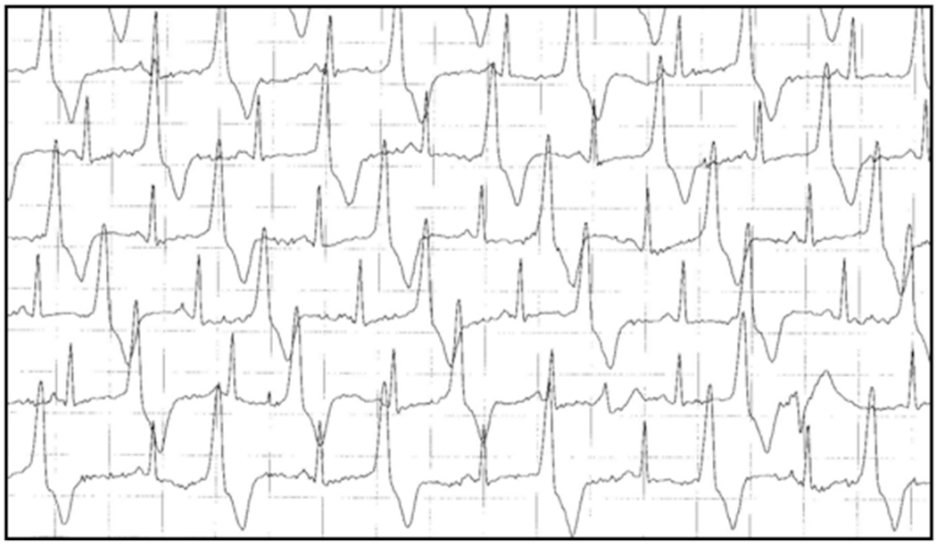

Figure 3.

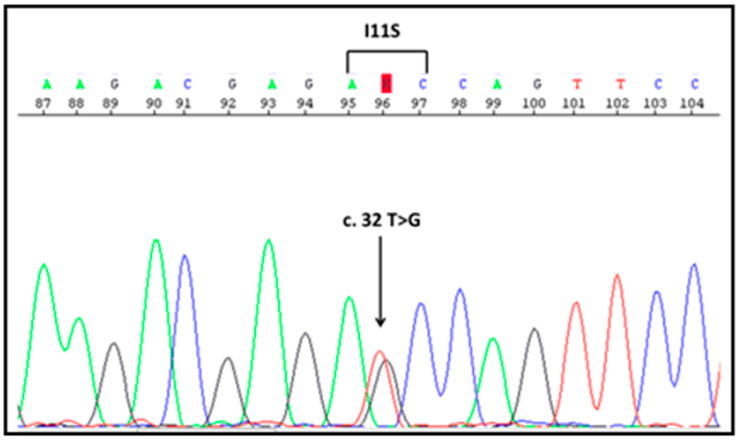

Figure 4.

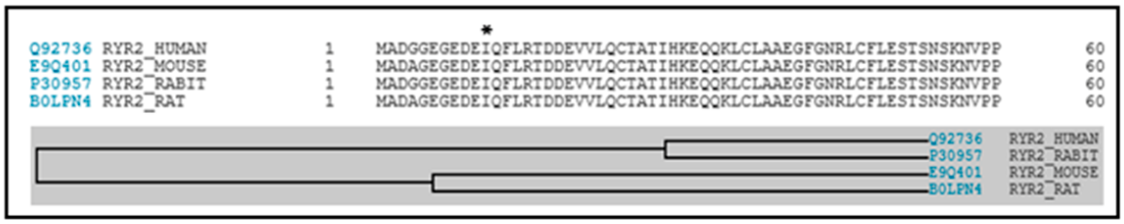

Figure 5.