Mutations in the Cardiac Ryanodine Receptor Gene (hRyR2) Underlie Catecholaminergic Polymorphic Ventricular Tachycardia

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**Background**—Catecholaminergic polymorphic ventricular tachycardia is a genetic arrhythmogenic disorder characterized by stress-induced, bidirectional ventricular tachycardia that may degenerate into cardiac arrest and cause sudden death. The electrocardiographic pattern of this ventricular tachycardia closely resembles the arrhythmias associated with calcium overload and the delayed afterdepolarizations observed during digitalis toxicity. We speculated that a genetically determined abnormality of intracellular calcium handling might be the substrate of the disease; therefore, we considered the human cardiac ryanodine receptor gene (hRyR2) a likely candidate for this genetically transmitted arrhythmic disorder.

**Methods and Results**—Twelve patients presenting with typical catecholaminergic polymorphic ventricular tachycardia in the absence of structural heart abnormalities were identified. DNA was extracted from peripheral blood lymphocytes, and single-strand conformation polymorphism analysis was performed on polymerase chain reaction–amplified exons of the hRyR2 gene. Four single nucleotide substitutions leading to missense mutations were identified in 4 probands affected by the disease. Genetic analysis of the asymptomatic parents revealed that 3 probands carried de novo mutations. In 1 case, the identical twin of the proband died suddenly after having suffered syncopal episodes. The fourth mutation was identified in the proband, in 4 clinically affected family members, and in none of 3 nonaffected family members in a kindred with 2 sudden deaths that occurred at 16 and 14 years, respectively, in the sisters of the proband.

**Conclusions**—We demonstrated that, in agreement with our hypothesis, hRyR2 is a gene responsible for catecholaminergic polymorphic ventricular tachycardia. (Circulation. 2001;103:196-200.)

**Key Words:** arrhythmia ■ genetics ■ tachycardia ■ ryanodine receptor calcium release channel

Catecholaminergic ventricular tachycardia (VT) occurring in the structurally intact heart was described by Coumel et al in 1978 and by Leenhardt et al in 1995 as a distinct clinical entity of unknown origin with manifestations in childhood and adolescence. Affected individuals present with syncopal events and with a distinctive pattern of highly reproducible, stress-related, bidirectional VT in the absence of both structural heart disease and a prolonged QT interval. A family history of juvenile sudden death and stress-induced syncope is present in approximately one third of cases. Recently, Swan et al showed a linkage of the disease to chromosome 1q42-q43 in 2 affected families.

On the basis of both the typical electrocardiographic pattern and on the hypothesis that delayed afterdepolarizations underlie arrhythmias in this disease, we hypothesized that mutations of the human cardiac ryanodine receptor gene (hRyR2) mapped to 1q42-q43 may be associated with catecholaminergic polymorphic VT. We detected 4 missense mutations cosegregating with the clinical phenotype. These data suggest that hRyR2 is a gene for catecholaminergic VT.

**Methods**

**Study Population**

The study included 12 probands presenting with bidirectional VT that was reproducibly induced by exercise stress testing and/or isoproterenol infusion in the absence of structural heart abnormalities. All probands had a clinical diagnosis of catecholaminergic polymorphic VT. A familial history of syncope and sudden death was present in 5 of the 12 patients (41%). All patients had a normal ECG at enrollment, normal atrioventricular conduction, and a normal QT interval.

All patients gave informed consent to the clinical and genetic study; the study was approved by the internal ethics committee of Fondazione Salvatore Maugeri.
Mutation Screening

Mutation screening was performed on genomic DNA samples that were extracted from peripheral blood lymphocytes using standard methods, and the genomic structure of the \textit{hRyR2} gene was obtained (by N.T. and G.A.D.). Intronic primers that amplify 90 of 103 exons, including those corresponding to the protein domain involved in interactions with FKBP12.6, were used for polymerase chain reaction amplifications. Polymerase chain reaction products ranging from 120 to 300 bp were analyzed by single-strand conformation polymorphisms (SSCP) on nondenaturing polyacrylamide gels. All abnormal conformers were sequenced using an ABI310 genetic analyzer. A control group of 400 healthy and unrelated subjects (800 alleles) was used to exclude the possibility of the detected mutations being DNA polymorphisms. Additionally, for all patients included in the present study, the entire coding sequences of the \textit{KCNQ1}, \textit{KCNH2}, \textit{SCN5A}, \textit{KCNE1}, and \textit{KCNE2} genes were screened using previously described primer pairs to exclude the possibility of atypical variants of long-QT syndrome.

Results

Clinical Findings

Family A

The proband for family A (III-2 in Figure 1) is an 8-year-old boy who was referred (to S.G.P.) for clinical evaluation of recurrent syncopal events that had occurred since he was 3 years old. Events were invariably induced by exercise, and they never required resuscitation. No family history of sudden death or of syncopal episodes was present, and the parents had normal hearts and no exercise-induced arrhythmias. The resting ECG of the proband showed normal sinus rhythm, normal atrioventricular conduction, and normal QT duration. Ventricular arrhythmias (isolated premature ventricular beats, couplets, and runs of bidirectional VT) could be reproducibly induced during exercise testing and progressively worsened as the workload increased. Echocardiograms and right ventricular angiography demonstrated an entirely normal heart.

No repetitive arrhythmias were induced during programmed electrical stimulation (maximum of 3 extrastimuli on driving cycle lengths from 600 ms to 350 ms), but isoproterenol infusion (2 \( \mu \)g/min) induced the spontaneous onset of bidirectional VT. The arrhythmia had a cycle length of 300 ms and a morphology resembling that of the clinically observed VT, and it spontaneously terminated after 2 minutes when isoproterenol infusion was discontinued. The patient was treated with nadolol (2 \( mg \cdot kg^{-1} \cdot d^{-1} \)), and an implantable cardiac defibrillator (ICD) was implanted. No further syncopal episodes have occurred during 2 years of follow up, but short runs of nonsustained polymorphic VT were recorded by the ICD.

Family B

The proband of family B is an 8-year-old boy (II-1 in Figure 1) who was referred (to G.V.) because of the occurrence of repeated syncopal episodes. The identical twin of the index case had a history of repeated syncopal events and died suddenly at 7 years of age; autopsy failed to demonstrate abnormal findings, and death was attributed to cardiac arrest. The parents of the twins are asymptomatic, with normal hearts and no exercise-induced arrhythmias. Nonsustained, bidirectional VT was reproducibly induced during exercise stress testing. No structural and functional abnormalities were detected with MRI or during right and left ventriculography. The patient was treated with atenolol (2 \( mg \cdot kg^{-1} \cdot d^{-1} \)), and adequate control of the arrhythmias was achieved with no recurrences of syncope after 6 years of follow-up.

Family C

The proband of family C is a 14-year-old boy (II-1 in Figure 1) who was referred (to G.V.) because of frequent episodes of...
loss of consciousness during exercise, which began at 7 years of age. No family history of sudden cardiac death and/or syncopal episodes was present. The parents are asymptomatic, with normal hearts and no exercise-induced arrhythmias. Baseline ECG of the proband was normal, and nonsustained, bidirectional VT was reproducibly elicited by exercise stress testing. No cardiac abnormalities were identified with echocardiography, MRI, or right and left ventriculography. The patient was treated with atenolol (2 mg·kg⁻¹·d⁻¹), which prevented recurrences of syncope and the onset of repetitive exercise-induced ventricular arrhythmias during 9 years of follow-up.

**Family D**

The proband of family D (III-3 in Figure 2) underwent cardiac evaluation at 30 years when she experienced palpitations. Her family history included the sudden cardiac death of 2 sisters, which occurred when they were 14 and 16 years old, respectively. One of the 2 girls died while being tested at school and the second while climbing stairs. The Holter recording demonstrated nonsustained, bidirectional, polymorphic VT (maximum of 15 beats). Because of the family history of cardiac arrest, an ICD was implanted. The family was then referred (to S.G.P.) for clinical and genetic evaluation.

Clinical evaluation of family members across 4 generations demonstrated that the proband (III-3 in Figure 2), her mother (II-1 in Figure 2; age, 59 years), 2 of her sisters (III-2 and III-4 in Figure 2; ages, 28 and 36 years), and her brother (III-1 in Figure 2; age, 23 years) developed bidirectional VT during exercise stress testing. None of them had structural abnormalities of the heart. Six additional family members (II-2, II-3, III-5, III-6, IV-1, and IV-2 in Figure 2) underwent clinical evaluation, and none developed ventricular arrhythmias during exercise stress testing. The 92-year-old grandfather of the proband (I-1 in Figure 2) did not carry the genetic defect. He was asymptomatic for syncopal events and had no arrhythmias at Holter recording, but he could not perform exercise stress testing because of severe arthritis.

During 1 year of follow-up after ICD implantation, the proband experienced 2 appropriate shocks that successfully terminated ventricular fibrillation. On both occasions, emotional stress preceded the events: the first episode occurred while the patient was being fired by her boss; the second while she was acting in a play at the local school.

**Identification of** hRyR2 **Mutations**

Four different single nucleotide substitutions leading to hRyR2 missense mutations (nonconservative amino acid changes) were identified in the 4 probands with catecholaminergic VT. A Ser to Leu substitution at position 2246 (exon 44) was identified in the proband of family A (Figure 1), an Arg to Ser substitution at position 2474 (exon 49) in the proband of family B (Figure 1), an Asn to Lys substitution at position 4104 (exon 90) in the proband of family C (Figure 1), and a Arg to Cys substitution at position 4497 (exon 93) in the proband of family D (Figure 2).

The primer pairs used to amplify the mutated exons were as follows:

Exon 44: 44F, GTTACAGCACGATCCAGGTT; 44R, GAGAAAACCGTGAAAAAGCA
Exon 49: 49F, ACAGGCATTGACACCAAAAT; 49R, AGAGAGGGAGGAAGTCCATCG
Exon 90: 90bF, GAGCCATAAGCACTACACGC; 90bR, ATAGACCCCTCTCGATGCCT
Exon 93: 93F, AGGTTTCAAGCTGTTGATTC; 93R, GCCTAGGCACAGTATTTCA

In 3 cases, the mutations were de novo and they were not inherited from the parents (biological paternity was confirmed by DNA analysis). The last hRyR2 mutation was
present in all 5 individuals with the clinical phenotype (II-1, III-1, III-2, III-3, and III-4) and in none of the 6 with a negative clinical phenotype (100% concordance between clinical and genetic diagnosis; 100% penetrance). None of the mutations was present in the DNA obtained from 400 normal subjects (800 alleles).

**Discussion**

We demonstrated that mutations in \( hRyR2 \) cause catecholaminergic VT. Several lines of evidence support this conclusion. First, catecholaminergic VT has been linked with chromosome 1q42-q43,\(^3 \) where \( RyR2 \) maps. Second, \( RyR2 \) is responsible for calcium release from the sarcoplasmic reticulum in response to calcium entry from the dihydropyridine receptor\(^12 \) (voltage-dependent calcium channel). A dysfunction in this protein, which is pivotal in controlling calcium homeostasis, is compatible with the presumed mechanisms of bidirectional VT, which likely involve calcium overload-mediated, delayed afterdepolarizations.\(^13,14 \) Third, intragenic mutations leading to nonconservative amino acid substitutions have been identified in highly conserved regions of the gene and were cosegregated with the clinical phenotype. Finally, \( hRyR2 \) mutations are located in functionally important regions of the gene, in locations where mutations were identified in the \( RyR1 \) gene (the skeletal muscle homologue of \( hRyR2 \)) and are associated with malignant hyperthermia (MH) and central core disease (CCD).\(^15 \) Finally, the evidence that in 3 probands \( hRyR2 \) mutations occurred de novo support the concept that these missense mutations are sufficient to cause the disease.

**Background**

In 1995, Leenhardt et al\(^2 \) described a series of patients with a remarkably uniform pattern of stress-induced, bidirectional, polymorphic VT in the absence of structural heart disease. Approximately one third of the cases had a family history of juvenile sudden death and/or stress-related syncope. Of interest, most of the patients were the only affected individual in their family, thus suggesting either the existence of incomplete penetrance of the genetic defect or the occurrence of de novo mutations. The presence of bidirectional VT and the reproducible pattern of adrenergic-induced ventricular ectopic activity are very important distinguishing features of this disease and its pathogenesis. In contrast with patients who have long-QT syndrome, the patients described by Coumel et al\(^1 \) and Leenhardt et al\(^2 \) have a normal QT interval, they do not develop VT with a pattern of torsade des pointes, and their VT is easily inducible during exercise stress testing. In their study, Leenhardt et al\(^2 \) stated that the bidirectional arrhythmias observed in their patients had an “ECG pattern most commonly described in digitalis toxicity.”\(^16 \) This observation points to delayed afterdepolarizations as a likely arrhythmogenic mechanism in these patients.\(^13,14 \) Delayed afterdepolarizations are caused by intracellular calcium overload, as occurs with digitalis toxicity,\(^17 \) and abnormal calcium release from the sarcoplasmic reticulum; they are enhanced by adrenergic stimulation\(^13,14,15 \) and are blocked by the in vitro administration of ryanodine.\(^13 \) Thus, the human cardiac ryanodine receptor gene seemed to be a good candidate for this form of idiopathic ventricular arrhythmia.

**Mutations in the \( hRyR2 \) Gene in Patients With Catecholaminergic VT**

We identified mutations in the \( hRyR2 \) gene in 4 of 12 probands. Two of the 4 genotyped probands had a family history of juvenile sudden cardiac death. In one case (family B), the identical twin of the proband, who had history of stress-related syncopal events since he was 3 years old, died suddenly (negative autopsy) at 7 years of age while running on the beach. In the second case (family D), 2 sisters of the proband (both with negative autopsy) died suddenly at 14 years (while being tested at school) and 16 years (while climbing stairs). A clinical evaluation of the family members of the proband in family D demonstrated that the 5 gene carriers presented with bidirectional VT when performing even a moderate degree of exercise. The pattern of arrhythmias was identical in the proband and the other 4 carriers (Figure 3). The mutation identified in this 4-generation family clearly cosegregates with the clinical phenotype. In this family, cardiac arrest occurred during adolescence in the 2 sisters who died suddenly but several years later (30 years) in the proband, suggesting a variable age-related manifestation of the disease.
On the basis of the published amino acid sequence of hRyR2, all 4 mutations involve highly conserved residues among the hRyR homologues and RyR proteins from other animal species, thus reinforcing the hypothesis that these mutations are associated with functional changes in the hRyR2 gene product. Some interesting observations stem from the comparison of the mutations identified here and those found in the RYR1 gene (skeletal muscle homologue of RyR2) that are associated with 2 human diseases affecting skeletal muscle, MH and CCD. MH is a pharmacogenetic disorder of skeletal muscle triggered by common anesthetics and depolarizing muscle relaxants and, in some susceptible individuals, by severe exercise. CCD is a nonprogressive myopathy characterized by hypotonia and proximal muscle weakness. CCD is usually closely associated with MH.15

Mutations in MH and CCD patients are clustered in 3 regions of the RYR1 gene: region 1 corresponds to the first 614 amino acids (aa); region 2 corresponds to a region between aa and 2162 to 2458; and region 3 corresponds to the C-terminal region of the RYR1-encoded protein, where mutations have been found in the pore-forming region of the encoded channel between aa and 4800 to 4900.15 An alignment of the mutations Ser 2246 Leu and Arg 2474 Ser, which was found in families A and B, with the corresponding aa sequence in the RYR1 protein locates these 2 mutations: one is in the center of the second cluster of MH/CCD mutations and the second is very close to the same region. However, Asn 4104 Lys is very close to and Arg 4497 Cys lies within the “D1” region (divergent region 1),18–20 which is one of the most investigated region of the RYR1 protein because it appears to contain important sites for regulating channel functions.15,18–21

Marks et al22 recently reported that adrenergic stimulation of cardiomyocytes results in the hyperphosphorylation of RyR2 through PKA, which results in FKBP12.6 displacement and altered channel activity and, thus, cardiac dysfunction. Mutations found in MH/CCD patients increase the sensitivity of the mutant RYR1 channels to activating concentrations of Ca2+.15,18,19 In analogy, it is reasonable to envision that patients with catecholaminergic VT, as a consequence of the observed mutations in the hRyR2 gene, have an increased sensitivity to Ca2+; therefore, intense adrenergic stimulation due to emotional stress and/or increased physical activity may lead to calcium overload and precipitate severe tachyarrhythmias.

We identified hRyR2 mutations in 4 of 12 patients with catecholaminergic VT. This may be due to the fact that we have not yet completed the screening of this very large gene or to incomplete sensitivity of the screening techniques. We favor the hypothesis that, in analogy with other arrhythmogenic disorders, genetic heterogeneity underlies catecholaminergic VT. The finding of hRyR2 mutations in ARVD2,8 mapped to 1q42-q43,23 raises the question of the possible allelism of ARVD2 and catecholaminergic VT.

In summary, we present evidence suggesting that mutations in RyR2 are likely to cause catecholaminergic VT. This finding suggests cardiac intracellular calcium release channels may have a role in the genesis of human arrhythmogenic disorders.

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