

Mutations of the Cardiac Ryanodine Receptor (RyR2) Gene in Familial Polymorphic Ventricular Tachycardia

Päivi J. Laitinen, BSc; Kevin M. Brown, BA; Kirsi Piippo, MSc; Heikki Swan, MD; Joe M. Devaney, PhD; Bhoomi Brahmhatt, BSc; Elizabeth A. Donarum, BSc; Michael Marino, PhD; Natascia Tiso, PhD; Matti Viitasalo, MD; Lauri Toivonen, MD; Dietrich A. Stephan, PhD; Kimmo Kontula, MD, PhD

Background—Familial polymorphic ventricular tachycardia is an autosomal-dominant, inherited disease with a relatively early onset and a mortality rate of $\approx 30\%$ by the age of 30 years. Phenotypically, it is characterized by salvos of bidirectional and polymorphic ventricular tachycardias in response to vigorous exercise, with no structural evidence of myocardial disease. We previously mapped the causative gene to chromosome 1q42-q43. In the present study, we demonstrate that patients with familial polymorphic ventricular tachycardia have missense mutations in the cardiac sarcoplasmic reticulum calcium release channel (ryanodine receptor type 2 [RyR2]).

Methods and Results—In 3 large families studied, 3 different RyR2 mutations (P2328S, Q4201R, V4653F) were detected and shown to fully cosegregate with the characteristic arrhythmic phenotype. These mutations were absent in the nonaffected family members and in 100 healthy controls. In addition to identifying 3 causative mutations, we identified a number of single nucleotide polymorphisms that span the genomic structure of RyR2 and will be useful for candidate-based association studies for other arrhythmic disorders.

Conclusions—Our data illustrate that mutations of the RyR2 gene cause at least one variety of inherited polymorphic tachycardia. These findings define a new entity of disorders of myocardial calcium signaling. (*Circulation*. 2001;103:485-490.)

Key Words: ryanodine receptor calcium release channel ■ sarcoplasmic reticulum ■ tachycardia ■ genetics

Inherited cardiac disorders associated with a propensity to malignant ventricular tachyarrhythmias constitute an important cause of sudden death in both young and adult individuals.¹ The identification of defective genes that cause the clinical phenotype has the potential to allow molecular diagnostics to identify benign arrhythmias from those that should be treated. In addition, knowledge of the defective protein and its cellular function will allow the development of targeted therapies. Defective genes that cause several of these types of arrhythmic disorders have been identified to date and, thus far, they primarily code for various ion channels in the cardiomyocyte plasma membrane.

Long-QT syndrome, which is characterized by a delayed repolarization phase of the cardiac action potential and a risk of life-threatening tachyarrhythmias such as torsade de pointes, was recently shown to be caused by inactivating mutations of the cardiac potassium channels KCNQ1, HERG,

minK, or MiRP or activating mutations of the sodium channel SCN5A.^{2,3} Activating mutations of SCN5A may cause Brugada's syndrome, a rare dominantly inherited electrophysiological disorder with right bundle branch block on ECG and a propensity to ventricular fibrillation.⁴ Arrhythmogenic right ventricular dysplasia (ARVD) is characterized by fatty infiltration and fibrosis of the myocardium, resulting in electric instability and risk of fatal ventricular arrhythmias. At least 6 chromosomal loci for the autosomal-dominant form of ARVD have been mapped,⁵⁻¹⁰ and a deletion of the plakoglobin gene was recently identified in patients with an autosomal-recessive form of ARVD.¹¹

Familial polymorphic ventricular tachycardia (FPVT) is an autosomal-dominant, inherited syndrome characterized by the occurrence of episodes of bidirectional and polymorphic ventricular tachycardias, typically in relation to adrenergic stimulation or physical exercise, in the absence of any

Received December 4, 2000; revision received December 20, 2000; accepted December 20, 2000.

From the Department of Medicine, University of Helsinki, Helsinki, Finland (P.J.L., K.P., H.S., M.V., L.T., K.K.); the Research Center for Genetic Medicine, Children's National Medical Center, Washington, DC (K.M.B., B.B., E.A.D., D.A.S.); the National Human Genome Research Institute, National Institutes of Health, Bethesda, MD (K.M.B., D.A.S.); Transgenomic, Inc, Gaithersburg, MD (J.M.D., M.M.); and the Department of Biology, University of Padova, Padova, Italy (N.T.).

The first two authors contributed equally to this work.

This article originally appeared Online on January 8, 2001. (*Circulation*. 2001;103:r7-r12.)

Correspondence to Kimmo Kontula, MD, PhD, Department of Medicine, University of Helsinki, 00290 Helsinki, Finland. E-mail: kimmo.kontula@hus.fi or to Dietrich A. Stephan, PhD, Research Center for Genetic Medicine, Children's National Medical Center, 111 Michigan Avenue, NW, Washington, DC 20010. E-mail dstephan@childrens-research.org

© 2001 American Heart Association, Inc.

Circulation is available at <http://www.circulationaha.org>

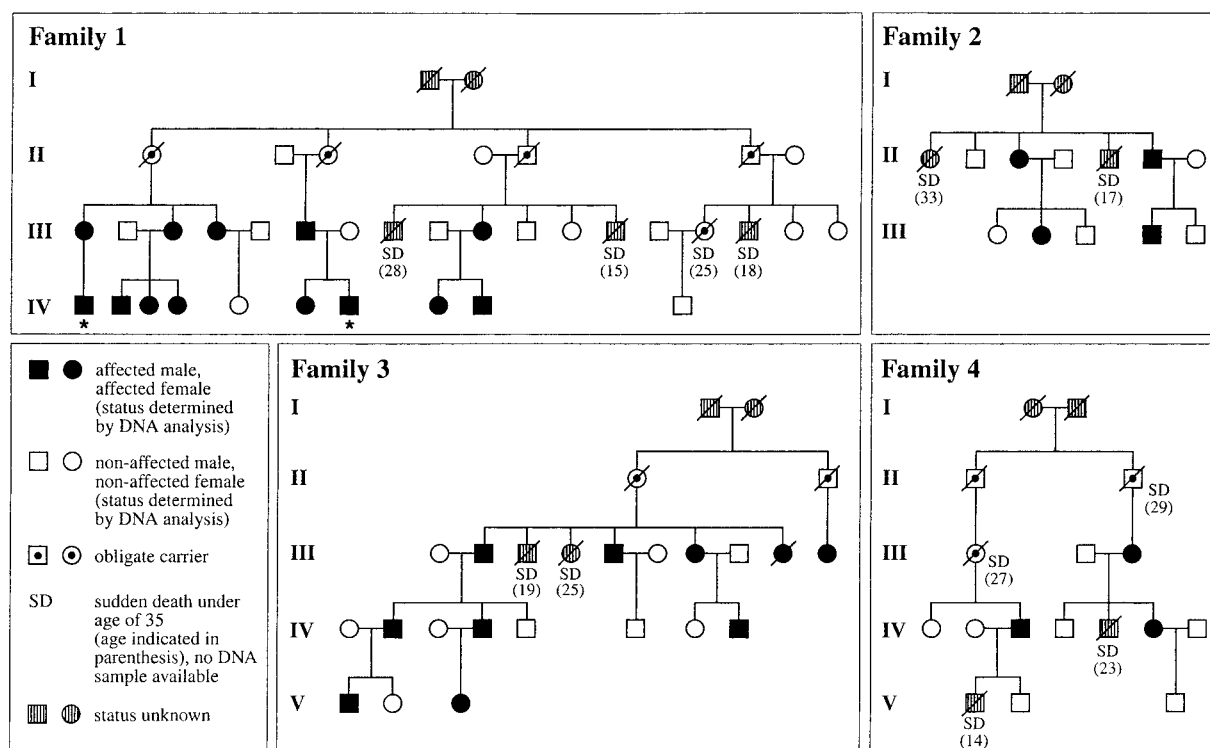


Figure 1. The pedigrees of the Finnish FPVT families. Deceased individuals are indicated with a slash, and mutation carriers without clinical symptoms with an asterisk.

evidence of structural myocardial disease.^{12–14} FPVT shows a highly malignant course, with estimates of mortality ranging from 30% to 50% by the age of 20 to 30 years.^{12,14} Recently, we described the clinical characteristics of FPVT in 2 Finnish families and assigned the disease locus to chromosome 1q42–q43.¹⁴ Now we report the identification of mutations in the cardiac calcium release channel of the sarcoplasmic reticulum (RyR2) as the cause of FPVT.

Methods

Families With FPVT

The clinical characteristics of families 1 and 2 were reported previously.¹⁴ Since then, we identified 2 additional families (families 3 and 4) with similar phenotypic characteristics (Figure 1). All families had a history of sudden unexplained death at an early age, and the index case of each family presented with frequent ventricular premature complexes and/or polymorphic ventricular tachycardia. On the basis of these exercise-induced ventricular arrhythmias or obligate carrier status, the total number of affected individuals in the 4 families was 30.

The control DNA samples from apparently healthy Finnish blood donors were provided by the Finnish Red Cross Blood Service. Informed consent was obtained from all patients, and the study was approved by the Ethical Review Committee of the department and performed in accordance with the Helsinki Declaration.

Refining the Area of Linkage on Chromosome 1q42–q43

To narrow the region of linkage in our previously described families and to test the hypothesis of 1q42–q43 linkage in the 2 new families, genotyping with a dense panel of highly polymorphic markers in the region was performed in the 4 families. Individuals were genotyped with the markers D1S179, D1S2712, D1S446, D1S2649, D1S235, ACTN2, AFM093XG5, D1S2680, D1S2850, D1S2678, and

D1S2670. Primer sequences were obtained from the Genome Database (<http://www.gdb.org/>), and pairwise linkage analyses were performed as previously described.¹⁴

Mutation Screening of the Candidate Genes CHRM3, GNG4, and RyR2

DNA samples from the probands of each family (Figure 1) and 2 healthy control individuals were screened for mutations in 3 candidate genes. The genes encoding the type-3 muscarinic receptor (CHRM3; Genbank #000740), the G-protein γ_4 subunit (GNG4; #004485), and the cardiac ryanodine receptor (RYR2; #001035) are located at 1q42–q43 according to GeneMap 98 (<http://www.ncbi.nlm.nih.gov>). The Sanger Center Blast server was used to localize these genes more accurately to the region of interest. The genomic organization of CHRM3 (1 coding exon) and GNG4 (2 coding exons) were deduced using the Sanger Center unfinished sequence contigs. After polymerase chain reaction (PCR), both genes were directly sequenced using the ABI Prism 377 DNA Sequencer (PE Biosystems).

The gene encoding RyR2 was localized to the Sanger Center physical map using the Sanger Center BLAST server. The exon-intron boundaries were determined from the National Center for Biotechnology Information's (NCBI) High Throughput Genomic Sequence database (http://www.ncbi.nlm.nih.gov/BLAST/blast_databases.html) by aligning the cDNA sequence against the genomic sequence. Primers to amplify the RyR2 exons (by N.T.) are available by contacting the authors. The RyR2 exons of 4 probands (1 from each family) were screened for mutations by 2 methods, denaturing high-performance liquid chromatography (dHPLC) analysis and direct sequencing of PCR-amplified DNA fragments, and compared with those from unaffected individuals. dHPLC analysis was performed for each PCR fragment on the Wave DNA Fragment Analysis System (Transgenomic) using a DNasep column (Transgenomic). Column temperatures were calculated using WAVEmaker software (Transgenomic). Affected samples were assumed to form heteroduplexes due to the dominant inheritance of the disease. The exons showing heteroduplexes by dHPLC analysis were directly

Clinical Characteristics of the Affected Individuals in the 3 Finnish FPVT Families With Established Missense Mutations of the RyR2 Gene

Variable	Family 1	Family 3	Family 4	All Families
Mutation	RyR2 P2328S	RyR2 V4653F	RyR2 Q4201R	
No. of males/females	5/8	6/4	1/2	12/14
Age, y	29±14 (11–53)	45±20 (13–67)	56±16 (46–74)	38±19 (11–74)
Heart rate, bpm	71±12 (53–90)	78±16 (59–112)	57	73±14 (53–112)
QT, ms	400±30 (340–450)	390±50 (340–510)	460	400±40 (340–510)
QTc, ms	430±20 (400–470)	440±50 (390–560)	448	430±40 (390–560)

Only individuals without drug treatment that could possibly affect heart rate and QT interval were included in this table.

Values are mean±SD (range) unless otherwise indicated.

sequenced as above. The entire coding region of the RyR2 gene was eventually sequenced in all 4 probands, even after a causative mutation was identified, to ensure that there were no other amino acid changes cosegregating with the phenotype. The promoter region of the RyR2 gene was not analyzed.

On detection of a RyR2 amino acid change in a proband, all of his or her family members, the 3 other probands, and at least 100 DNA samples from apparently healthy Finnish blood donors were screened for the presence of the change. Restriction fragment length polymorphism assays for specific mutations were based on the use of the following restriction enzymes (the allele possessing the cleavage site is indicated in parentheses): P2328S, HaeIII (wild type); Q4201R, PvuII (mutant); V4653F, NcoI (wild type, an artificial site generated by a mismatch primer); intron 91 A to C nucleotide change (13682+67 C>T), NlaIII (allele C); and polymorphism Q2958R, BsrGI (allele Q, an artificial site generated by a mismatch primer). Alleles were identified by electrophoresis through 12.5% polyacrylamide gel of the cleavage products.

Results

Identification of 2 New Affected Families With FPVT and Refinement of the Area of Linkage

The phenotypic characteristics of families 1 and 2 were reported in our previous study.¹⁴ Some clinical characteristics of the 3 families that were subsequently shown to have RyR2 missense mutations are summarized in the Table. In Families 3 and 4, the number of unexplained deaths on sudden mental stress at the age of 16 to 25 years were 5 and 1, respectively, thus confirming the high mortality rate (30% by the age of 30 years) reported earlier in families 1 and 2.¹⁴ Symptoms never occurred before the age of 10 years in any of the families studied.

The electrocardiographic appearance of arrhythmias in families 3 and 4 was similar to that reported in families 1 and 2.¹⁴ On exercise testing, when heart rate exceeded an individual threshold, isolated unifocal and multifocal ventricular premature complexes and salvos of multifocal ventricular complexes appeared. The left and right ventricular dimensions and systolic and diastolic function were normal in all affected individuals in the 4 families, with the exception of 2 cases (aged 46 and 47 years) in family 4, in whom a minor local bulging of the right ventricular outflow tract was observed on right ventricular cineangiography.

Results from haplotype analyses are summarized in Figure 2. For families 1 and 2, haplotype data were reported in our previous article¹⁴ and supplemented with additional markers in the present work. In family 3, a haplotype reaching from

D1S179 to D1S2670 was cosegregating with the disease. In family 4, a haplotype cosegregating with the disease was detected, but no informative recombinant individuals were observed. All 4 families had their own distinct haplotypes, consistent with the subsequent observation that all carried private mutations. Informative meiosis in pedigrees 1 and 3 localized the disease gene between markers D1S235 and D1S2670, an intermarker distance of ≈ 1.5 cM (Figure 2).

Family 3 had a maximum pairwise lod score of 2.43 at $\theta=0$ with the marker D1S2670. In family 4, a lod score of 1.06 with the same marker was found at $\theta=0$. The combined lod score value of all 4 families was 8.23 at this marker.

Identification of 3 Missense Mutations in the RyR2 Gene in 3 Different Families

Direct DNA sequencing of all protein-coding areas on the candidate genes CHMR3 and GNG4 failed to reveal any mutations in any of the 4 probands (data not shown). In contrast, the dHPLC and sequence analysis of the RyR2 gene revealed 3 missense mutations, each private to a distinct family (Figure 3). In family 1, a mutation substituting serine for proline at amino acid position 2328 (P2328S) was detected. Glutamine 4201 was mutated to arginine (Q4201R) in family 4. A valine to phenylalanine substitution at position 4653 (V4653F) was found in family 3. All these changes were shown to be mutations in evolutionarily conserved regions of the protein. The P2328S mutation is located in the large, footlike cytoplasmic domain of RyR2, and the latter 2 mutations occur in the carboxy-terminal part of the receptor, which contains several membrane-spanning, presumably critical regions of RyR2.¹⁵ All 3 amino acid alterations cosegregated with the clinical phenotype in the 3 pedigrees and were absent in at least 100 healthy control individuals (200 alleles).

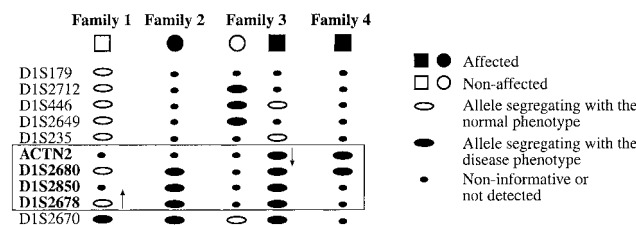


Figure 2. A summary of haplotypes delineating the disease locus (boxed) in chromosome 1q42-q43. Families do not share a common haplotype.

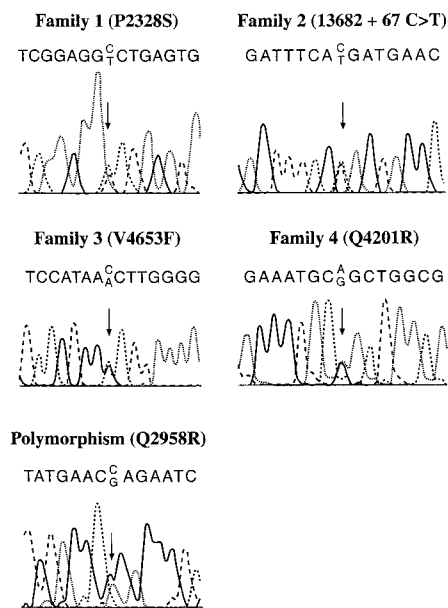


Figure 3. Sequence analysis of RyR2 mutations in 4 FPVT families and identification of a common Q2958R polymorphism. With V4653F mutation, reverse strand sequence is illustrated.

Two mutation carriers in family 1 had no clinical manifestations of the disease (aged 21 and 35 years).

In Family 2, no amino acid alterations were found in RyR2, but an intronic base change (A to C) located 67 bases downstream of the exon-intron boundary at cDNA bp 13682 was detected (Figure 3). This DNA alteration fully cosegregated with FPVT in the family, but it was also detected in 1 of 100 healthy control individuals.

An amino acid polymorphism substituting arginine for glutamine (Q2958R) was detected in all pedigrees (Figure 3). The variant Q2958R allele was present in a homozygous state in both affected and nonaffected individuals within the 4 families (data not shown), and it was also detected in healthy control individuals with allele frequencies of 0.74 for Q and 0.26 for R. While screening the RyR2 gene, the following silent nucleotide polymorphisms were also detected (the nucleotide numbering starts from initiation codon): 1359 C/T, 1776 A/T, 1863 C/T, 4160+50 A/G, 6688+72 G/T, 7221 to 56 A/G, 7808 C/T, 13 476+16 A/G, 13 783 to 6 A/G, 13 783 to 21 A/G, 13 914+8 A/C, 14 299 to 25 A/G, and 14 299 to 62 G/T (in case of an intronic alteration, positive numbers start from GT splice donor site and negative numbers from AG splice acceptor site). None of these nucleotide alterations resulted in amino acid change, and most of them were also present in control individuals.

Discussion

Stress- or emotion-induced polymorphic ventricular tachycardias occasionally cluster in families.^{12,14} Although this syndrome may represent a panel of etiologically heterogeneous disorders, the fact that life-threatening ventricular arrhythmias arise in hearts showing no apparent structural alterations favors the assumption that the underlying cause may affect the mechanisms regulating cardiac depolarization, repolarization, or excitation-contraction coupling, rather than structural

elements of the myocardium. This hypothesis proved to be correct: we were able to pinpoint the RyR2 gene as the apparent causative gene in Finnish FPVT patients.

Ryanodine receptors are the largest ion channels known to exist. Three different isoforms of ryanodine receptors, each encoded by different genes, have been characterized. The RyR1 gene is principally expressed in skeletal muscle,¹⁶ whereas RyR3 expression is virtually limited to the brain.¹⁷ The human RyR2 gene encodes a product containing 4967 amino acids¹⁵ and is abundantly expressed in myocardium and, to some extent, in brain and gestational myometrium.¹⁸ The calcium-release channel RyR2 forms a homotetrameric membrane-spanning calcium channel on the sarcoplasmic reticulum. Stimulation of voltage-sensitive L-type calcium channels (dihydropyridine) on the outer myocardial cell membrane permits the cellular entrance of minute amounts of calcium ions, which in turn activate RyR2 channels, allowing larger amounts of calcium ions to pass from the lumen of the sarcoplasmic reticulum into the cytoplasm. This is sufficient to initiate myocardial contraction. Thus, the RyR2 channels serve to couple the excitation of myocardial cells to their actin/myosin contractile apparatus by a mechanism involving a calcium-induced calcium release.^{19,20}

The RyR2 channel is composed of a carboxy-terminal domain, approximately one-fifth of its total size, that is anchored to the sarcoplasmic reticulum by 4 to 10 membrane-spanning hydrophobic motifs and a very large, foot-like cytoplasmic domain that is in close connection with the outer cell membrane L-type calcium channels.²¹ Amino acids 4822 to 4829 seem to constitute part of the pore-forming segment.²² A potential ATP-binding site is situated between residues 2618 and 2653; calmodulin-binding sites are present between residues 2774 to 2806, 2876 to 2897, and 2997 to 3015; and residues for other potent modulators may be present in these same areas between residues 2618 to 3015.^{21,22} One of the 3 RyR2 mutations we identified (P2328S) is present in the amino-terminal domain, and the 2 other mutations (Q4201R and V4653F) are located in the carboxy-terminal region containing the transmembrane segments.

There are several lines of evidence that implicate these mutations as causes of FPVT in our families. First, all 3 mutations affect amino acid residues that are fully conserved in both the human¹⁵ and rabbit RyR2,²³ human RyR1¹⁶ and RyR3,¹⁷ and pig RyR1.²⁴ Second, each mutation cosegregates with the trait in the corresponding family. Third, the 3 amino acid changes were not seen in 200 chromosomes from phenotypically unaffected individuals. Finally, it is unlikely that these mutations would represent innocent linkage markers of putative causative RyR2 mutations because the entire coding area was sequenced in each proband, and no other amino acid changes specific to the affected individuals were found. Whether these mutations affect calcium-induced activation, calcium permeation, modulator binding, or some other function of RyR2 awaits *in vitro* expression studies using mutant RyR2 cDNA constructs.

In contrast, we cannot judge whether the intronic A>C nucleotide change plays any pathogenic role. Because this change was also present in 1 of the 100 controls tested, it may

represent an innocent polymorphism. Thus, we cannot fully exclude the possibility that in family 2, a putative causal mutation escaped detection by the 2 techniques used or that there is a different causal gene in this family. The common amino acid polymorphism Q2958R is localized within the area of interaction with the RyR2 modulator¹⁵ and has remained highly conserved during the evolution of the ryanodine receptors.^{15–17,23,24} Therefore, it will be of interest to study the functional consequences of this variation in vitro.

The critical role of RyR2 is substantiated by the demonstration that mutant mice lacking RyR2 die during embryonic life with morphological abnormalities in the developing heart tube.²⁵ RyR2 is downregulated in experimental heart failure in dogs,^{25,26} and the RyR2 protein and mRNA levels are also subnormal in failing human myocardium.²⁷

Our data suggest that FPVT is a disease showing close kinship to malignant hyperthermia and central core disease, which are rare, dominantly inherited disorders of calcium signaling in striated muscle resulting from mutations of the RyR1 gene.^{28,29} Malignant hyperthermia is characterized by skeletal muscle rigidity and contractures, malignant cardiac arrhythmias, hypermetabolism, and eventually fever, which is typically triggered by the administration of halothane or depolarizing skeletal muscle relaxants. In contrast, patients with central core disease show muscular hypotonia and weakness but are at risk of hyperthermia during the administration of inhalational anesthetics. In both cases, mutations of the RyR1 gene seem to cluster in 3 regions: between residues 1 to 614, 2162 to 2458, and 4800 to 4900.²⁹

A specific concealed form of ARVD (ARVD2) that has effort-induced polymorphic tachycardias but no changes in cardiac size or standard ECG has also been shown to map to chromosome 1q42-q43 in specific Italian families.⁵ The clinical picture of ARVD2 shows striking similarities with that of Finnish patients with FPVT, but differences were reported on histopathological examination: large areas of fatty-fibrous replacement were detected in the right ventricular wall of the Italian patients^{5,30} but were absent in the Finnish patients.¹⁴ These 2 diseases may be allelic,³¹ thus forming another pair of ryanodine receptor syndromes in addition to that composed of malignant hyperthermia and central core disease and showing subtle phenotypic variations that originate from similar molecular defects. Interestingly, mutations of the cardiac sodium channel SCN5A may result in type LQT3 of the long-QT syndrome,³² Brugada's syndrome,⁴ or a progressive cardiac conduction defect,³³ adding even more complexity to the phenotypic sequelae stemming from defects of single cardiac ion channels.

The present findings have a major impact on the understanding of both normal and pathological excitation-contraction coupling mechanisms of the human myocardium, as well as on the molecular diagnosis of severe ventricular arrhythmias. If RyR2 mutations result in increased sensitivity of calcium-induced activation of the calcium-release channel complex, it will be important to determine whether the induction of arrhythmias in the FPVT patients can be dampened by blocking the L-type calcium channels.

In conclusion, mutations in the cardiac calcium-release channel type 2 are at least 1 cause of inherited polymorphic

ventricular tachycardia, a dominantly inherited syndrome with a poor prognosis and hitherto unknown pathogenetic background. These findings should foster careful in vivo and in vitro studies on calcium signaling in the affected patients and should lead to a much better understanding of myocardial excitation-contraction coupling pathways.

Acknowledgments

This study was supported by grants from the Council of Health Sciences of the Finnish Academy, the Sigrid Juselius Foundation, the Finnish Foundation for Cardiovascular Research, and Biomedicum Helsinki Foundation. D.A.S. and K.M.B. are supported by American Heart Association grant 0030118N. B.B. is supported by a fellowship through the Society for Experimental Biology, and E.A.D. is supported through an Intramural Research Training Award from the National Institutes of Health. The authors thank Sirpa Stick for expert technical assistance and Christiane Robbins at NHGRI/NIH for sequencing assistance. We also thank Dr Tom Krusius of the Finnish Red Cross Blood Service for providing blood samples from healthy blood donors.

References

1. Zipes DP, Wellens HJ. Sudden cardiac death. *Circulation*. 1998;98:2334–2351.
2. Ackerman MJ. The long QT syndrome: ion channel diseases of the heart. *Mayo Clin Proc*. 1998;73:250–269.
3. Chiang CE, Roden DM. The long QT syndromes: genetic basis and clinical implications. *J Am Coll Cardiol*. 2000;36:1–12.
4. Chen Q, Kirsch GE, Zhang D, et al. Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. *Nature*. 1998;392:293–296.
5. Rampazzo A, Nava A, Danieli GA, et al. The gene for arrhythmogenic right ventricular cardiomyopathy maps to chromosome 14q23-q24. *Hum Mol Genet*. 1994;3:959–962.
6. Rampazzo A, Nava A, Erne P, et al. A new locus for arrhythmogenic right ventricular cardiomyopathy (ARVD2) maps to chromosome 1q42-q43. *Hum Mol Genet*. 1995;4:2151–2154.
7. Rampazzo A, Nava A, Miorin M, et al. ARVD4, a new locus for arrhythmogenic right ventricular cardiomyopathy, maps to chromosome 2 long arm. *Genomics*. 1997;45:259–263.
8. Severini GM, Krajcinovic M, Pinamonti B, et al. A new locus for arrhythmogenic right ventricular dysplasia on the long arm of chromosome 14. *Genomics*. 1996;31:193–200.
9. Ahmad F, Li D, Karibe A, et al. Localization of a gene responsible for arrhythmogenic right ventricular dysplasia to chromosome 3p23. *Circulation*. 1998;98:2791–2795.
10. Li D, Ahmad F, Gardner MJ, et al. The locus of a novel gene responsible for arrhythmogenic right-ventricular dysplasia characterized by early onset and high penetrance maps to chromosome 10p12-p14. *Am J Hum Genet*. 2000;66:148–156.
11. McKoy G, Protonotarios N, Crosby A, et al. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). *Lancet*. 2000;355:2119–2124.
12. Leenhardt A, Glaser E, Burguera M, et al. Short-coupled variant of torsade de pointes: a new electrocardiographic entity in the spectrum of idiopathic ventricular tachyarrhythmias. *Circulation*. 1994;89:206–215.
13. Fisher JD, Krikler D, Hallidie-Smith KA. Familial polymorphic ventricular arrhythmias: a quarter century of successful medical treatment based on serial exercise-pharmacologic testing. *J Am Coll Cardiol*. 1999;34:2015–2022.
14. Swan H, Piippo K, Viitasalo M, et al. Arrhythmic disorder mapped to chromosome 1q42-q43 causes malignant polymorphic ventricular tachycardia in structurally normal hearts. *J Am Coll Cardiol*. 1999;34:2035–2042.
15. Tunwell RE, Wickenden C, Bertrand BM, et al. The human cardiac muscle ryanodine receptor-calcium release channel: identification, primary structure and topological analysis. *Biochem J*. 1996;318:477–487.
16. Zorzato F, Fujii J, Otsu K, et al. Molecular cloning of cDNA encoding human and rabbit forms of the Ca²⁺ release channel (ryanodine receptor)

- of skeletal muscle sarcoplasmic reticulum. *J Biol Chem*. 1990;265:2244–2256.
17. Nakashima Y, Nishimura S, Maeda A, et al. Molecular cloning and characterization of a human brain ryanodine receptor. *FEBS Lett*. 1997;417:157–162.
 18. Awad SS, Lamb HK, Morgan JM, et al. Differential expression of ryanodine receptor RyR2 mRNA in the non-pregnant and pregnant human myometrium. *Biochem J*. 1997;322:777–783.
 19. Stokes DL, Wagenknecht T. Calcium transport across the sarcoplasmic reticulum - Structure and function of Ca²⁺-ATPase and the ryanodine receptor. *Eur J Biochem*. 2000;267:5274–5279.
 20. Missiaen L, Robberecht W, Van Den Bosch L, et al. Abnormal intracellular Ca²⁺ homeostasis and disease. *Cell Calcium*. 2000;28:1–21.
 21. Coronado R, Morrissette J, Sukhareva M, et al. Structure and function of ryanodine receptors. *Am J Physiol*. 1994;266:C1485–C1504.
 22. Zhao M, Li P, Li X, et al. Molecular identification of the ryanodine receptor pore-forming segment. *J Biol Chem*. 1999;274:25971–25974.
 23. Otsu K, Willard HF, Khanna VK, et al. Molecular cloning of cDNA encoding the Ca²⁺ release channel (ryanodine receptor) of rabbit cardiac muscle sarcoplasmic reticulum. *J Biol Chem*. 1990;265:13472–13483.
 24. Fujii J, Otsu K, Zorzato F, et al. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. *Science*. 1991;253:448–451.
 25. Hittinger L, Ghaleh B, Chen J, et al. Reduced subendocardial ryanodine receptors and consequent effects on cardiac function in conscious dogs with left ventricular hypertrophy. *Circ Res*. 1999;84:999–1006.
 26. Vatner DE, Sato N, Kiuchi K, et al. Decrease in myocardial ryanodine receptors and altered excitation-contraction coupling early in the development of heart failure. *Circulation*. 1994;90:1423–1430.
 27. Brillantes AM, Allen P, Takahashi T, et al. Differences in cardiac calcium release channel (ryanodine receptor) expression in myocardium from patients with end-stage heart failure caused by ischemic versus dilated cardiomyopathy. *Circ Res*. 1992;71:18–26.
 28. Zucchi R, Ronca-Testoni S. The sarcoplasmic reticulum Ca²⁺ channel/ryanodine receptor: modulation by endogenous effectors, drugs and disease states. *Pharmacol Rev*. 1997;49:1–51.
 29. MacLennan DH. Ca²⁺ signalling and muscle disease. *Eur J Biochem*. 2000;267:5291–5297.
 30. Baucé B, Nava A, Rampazzo A, et al. Familial effort polymorphic ventricular arrhythmias in arrhythmogenic right ventricular cardiomyopathy map to chromosome 1q42–43. *Am J Cardiol*. 2000;85:573–579.
 31. Tiso N, Stephan DA, Nava A, et al. Identification of mutations in the cardiac ryanodine gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). *Hum Mol Genet*. In press.
 32. Wang Q, Shen J, Splawski I, et al. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell*. 1995;80:805–811.
 33. Schott JJ, Alshinawi C, Kyndt F, et al. Cardiac conduction defects associate with mutations in SCN5A. *Nat Genet*. 1999;23:20–21.