Prevalence and significance of rare *RYR2* variants in arrhythmogenic right ventricular cardiomyopathy/dysplasia: Results of a systematic screening

Nathalie Roux-Buisson, MD, PhD,[1,2,3,*] Estelle Gandjbakhch, MD, PhD,[4,5,*] Erwan Donal, MD, PhD,[6] Vincent Probst, MD, PhD,[7] Jean-Claude Deharo, MD,[8] Philippe Chevalier, MD, PhD,[9] Didier Klug, MD,[10] Nicolas Mansencal, MD, PhD,[11] Etienne Delacretaz, MD, PhD,[12] Pierre Cosnay, MD,[13] Patrice Scanu, MD,[14] Fabrice Extramiana, MD, PhD,[15] Dagmar Keller, MD,[16] Françoise Hidden-Lucet, MD,[4,5] Jonathan Trapani,[2] Pierre Fouret, MD, PhD,[4,17] Robert Frank, MD,[5] Veronique Fressart, MD, PhD,[5,18] Julien Fauré, PhD,[1,2,3] Joel Lunardi, PharmD, PhD,[1,2,3] Philippe Charron, MD, PhD[4,5,11]

From the [1]INSERM U836, Grenoble Institut des Neurosciences, Equipe Muscle et Pathologies, Grenoble, France, [2]CHRU de Grenoble, Hopital Michallon, Biochimie et Génétique Moléculaire, Grenoble, France, [3]Université Joseph Fourier, Grenoble, France, [4]UPMC Université Paris-6, INSERM UMRS-1166, Paris, France, [5]Département de Cardiologie et Centre de Référence pour les Maladies Cardiaques Héréditaires; APHP, Hôpital Universitaire Pitié-Salpêtrière, Paris, France, [6]Service de Cardiologie, Hôpital Pontchaillou, Rennes, France, [7]Service de Cardiologie, Institut du thorax, CHU de Nantes, Nantes, France, [8]Service de Cardiologie, Hôpital La Timone, Marseille, France, [9]Service de Cardiologie, Hôpital Est, Lyon, France, [10]Service de Cardiologie, Hôpital Cardiologique, Lille, France, [11]Service de Cardiologie, Université de Versailles-Saint Quentin, Hôpital Ambroise Paré, AP-HP, Boulogne, France, [12]Service de Cardiologie, Hôpital de l'Ile, Berne, Switzerland, [13]Service de Cardiologie B, Hôpital Universitaire de Tours, Tours, France, [14]Service de Cardiologie, Hôpital Côte de Nacre, Caen, France, [15]Service de Cardiologie, Hôpital Lariboisière, AP-HP, Université Paris 7, Paris, France, [16]Service de Cardiologie, Hôpital Universitaire de Bâle, Bale, Switzerland, [17]Service d'Anatomie Pathologique, APHP, Hôpital Universitaire Pitié-Salpêtrière, Paris, France, and [18]Service de Biochimie, Unité de Cardiogénétique et Myogénétique, AP-HP, Hôpital Universitaire Pitié-Salpêtrière, Paris, France.

BACKGROUND Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a genetic disease predominantly caused by desmosomal gene mutations that account for only ~50% of cases. Ryanodine receptor 2 (*RYR2*) gene mutations usually cause catecholaminergic polymorphic ventricular tachycardia but have been associated with a peculiar phenotype named ARVC2.

OBJECTIVE We aimed to determine the prevalence and phenotype associated with *RYR2* mutations in a large ARVC/D population.

METHODS We analyzed the whole *RYR2* coding sequence by Sanger sequencing in 64 ARVC/D probands without desmosomal gene mutations.

RESULTS We have identified 6 rare missense variants: p.P1583S, p.A2213S, p.G2367R, p.Y2932H, p.V3219M, and p.L4670V. It corresponds to a 9% prevalence of rare *RYR2* variants in the ARVC/D population (6 of 64 probands), which is significantly higher than the estimated frequency of rare *RYR2* variants in controls (Fisher exact test, $P = .03$). Phenotypes associated with *RYR2* variants were similar to desmosome-related ARVC/D, associating typical electrocardiographic abnormalities at rest, frequent monomorphic ventricular tachycardia, right ventricular dilatation, wall motion abnormalities, and fibrofatty replacement when histopathological examination was available.

CONCLUSION In this first systematic screening of the whole coding region of the *RYR2* gene in a large ARVC/D cohort without mutation in desmosomal genes, we show that putative *RYR2* mutations are frequent (9% of ARVC/D probands) and are associated with a conventional phenotype of ARVC/D, which is in contrast with previous findings. The results support the role of the *RYR2* gene in conventional ARVC/D.

KEYWORDS Arrhythmogenic right ventricular dysplasia/cardiomyopathy; *RYR2* gene; Mutation

ABBREVIATIONS ARVC/D = arrhythmogenic right ventricular cardiomyopathy/dysplasia; **CPVT** = catecholaminergic polymorphic ventricular tachycardia; **ECG** = electrocardiogram/electrocardiography;

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EP = electrophysiology; **EVS** = Exome Variant Server database; **LBBB** = left bundle branch block; **LV** = left ventricular; **MRI** = magnetic resonance imaging; **NSVT** = nonsustained ventricular tachycardia; **RV** = right ventricle/ventricular; ***RYR2*** = ryanodine receptor 2; **SVT** = sustained ventricular tachycardia; **TFC** = Task Force criteria; **TWI** = T-wave inversion; **WMA** = wall motion abnormality

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Introduction

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a rare cardiac muscle disorder characterized by progressive fibrofatty replacement of the myocardium. The right ventricle (RV) is predominantly affected, but left ventricular (LV) involvement is also present in more than half of the cases.[1] These structural alterations can lead to ventricular arrhythmias and heart failure. ARVC/D is a frequent cause of sudden death in young people and athletes. The diagnosis of ARVC/D is currently based on the presence of major and minor standardized Task Force criteria (TFC) that consider ventricular arrhythmias episodes, electrocardiographic abnormalities, RV function and morphology, histopathology, family history, and genetic status.[2]

ARVC/D is usually inherited as an autosomal dominant disease with reduced penetrance and variable expression. So far, the major genes involved in ARVC/D encode components of the cardiac desmosome: plakophilin-2 (*PKP2*),[3] desmoglein-2 (*DSG2*),[4,5] plakoglobin (*JUP*),[6] desmoplakin (*DSP*),[7] and desmocollin-2 (*DSC2*).[8,9] Comprehensive mutation screening of the 5 main desmosomal ARVC/D genes can detect genetic abnormalities in at least 40%–50% of probands.[10] Nondesmosomal genes have also been associated with ARVC/D phenotypes, including the cardiac ryanodine receptor 2 gene (*RYR2*),[11] the transforming growth factor beta 3 gene (*TGFB3*),[12] the Transmembrane protein 43 (*TMEM43*) gene,[13] and more recently the lamin A/C (*LMNA*),[14] the titin (*TTN*),[15] the desmin (*DES*),[16] and the phospholamban (*PLN*)[17] genes. Mutations in the *RYR2* gene are usually associated with catecholaminergic polymorphic ventricular tachycardia (CPVT), a rare and severe inherited arrhythmia without structural cardiac abnormality[18,19] (http://www.fsm.it/cardmoc/).

RYR2 is one of the largest human genes (105 exons) encoding an mRNA of 16,365 bp (NM_021991.2). In 1988, Nava et al[20] reported a family with autosomal dominant form of RV cardiomyopathy (supported by histological data) associated with polymorphic ventricular tachycardia induced by exercise stress testing and juvenile sudden death. Then, Rampazzo et al[11,21] mapped the locus to chromosome 1q42-q43 and identified *RYR2* mutations in 4 independent families with the same clinical presentation of ARVC/D (named ARVC/D2). This clinical presentation differs from desmosome-related forms of ARVC/D and is rather close to CPVT because of the presence of exercise-induced ventricular arrhythmias, its high penetrance, and a 1:1 sex ratio. The association between a typical form of ARVC/D and *RYR2* mutations remains unclear, and the prevalence of *RYR2* mutations in the ARVC/D population remains unknown since few mutations have been associated with ARVC/D.[11,22–24]

In this study, we aimed to determine the prevalence of *RYR2* mutations in a large cohort of 64 well clinically characterized ARVC/D probands for whom mutations in *PKP2*, *DSG2*, *DSP*, *DSC2*, and *JUP* were previously excluded. Sequencing of the entire coding region of *RYR2* in these ARVC/D probands led to the identification of 6 putative missense mutations in 6 unrelated probands. The pathogenic role of the variations is discussed along with the consequences for clinical practice.

Methods

Patients

This multicenter prospective study included a cohort of unrelated probands with a diagnosis of ARVC/D recruited in France and Switzerland according to the TFC used at the time of enrollment[25] and then focused on 64 probands for whom no mutation was identified in *PKP2*, *DSG2*, *DSP*, *JUP*, and *DSC2* genes.[10]

Clinical evaluation of all probands was performed as described previously[10] and included evaluation of personal and familial history, physical examination, 12-lead standard electrocardiography (ECG), standard echocardiography, cardiac magnetic resonance imaging (MRI) or RV angiography, 24-hour ambulatory ECG, and signal-averaged ECG.[10] The baseline exercise test was performed in all probands, except in particular situations requiring urgent therapeutic management (such as implantable cardioverter defibrillator or antiarrhythmic drugs). The electrophysiology (EP) study was performed, when considered clinically relevant by the physician, according to the following protocol: 2 different sites, 2 rates, with up to 3 extrastimuli, at baseline, and with infusion of isoproterenol. In the case of cardiac transplant, pathological analysis of the explanted heart was performed. Clinical evaluation of relatives was performed when available. This study was approved by the Pitié-Salpêtrière Hospital ethics committee, and written informed consent was obtained from all individuals.

Genetic analysis

For each proband, 105 exons and intron-exon junctions of the *RYR2* gene were amplified from genomic DNA (OMIM 180902, transcript: NM_021991.2, protein: 4967 amino acid, Q92736-1, primer sequences available upon request). The analysis of the entire coding sequence of *RYR2* was performed by direct sequencing on an ABI 3130 DNA sequencer (PE Applied Biosystems, Foster City, CA).

When unreported variants were detected, they were searched among 400 chromosomes from ethnically matched and healthy control subjects (Caucasian: n = 400 or