Multiple mutations in desmosomal proteins encoding genes in arrhythmogenic right ventricular cardiomyopathy/dysplasia

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BACKGROUND Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a progressive cardiomyopathy showing a wide clinical spectrum in terms of clinical expressions and prognoses.

OBJECTIVE This study sought to estimate the occurrence of compound and double heterozygotes for mutations in desmosomal proteins encoding genes in a cohort of ARVC/D Italian index cases, and to assess the clinical phenotype of mutations carriers.

METHODS Fourty-two consecutive ARVC/D index cases who fulfilled the International Task Force diagnostic criteria were screened for mutations in PKP2, DSP, DSG2, DSC2, and JUP genes by denaturing high-performance liquid chromatography (DHPLC) and direct sequencing.

RESULTS Three probands (7.1%) showing a family history of sudden death carried multiple mutations. Family screening identified an additional 7 multiple-mutation carriers. Among the 7 double heterozygotes for mutations in different genes, 2 were clinically unaffected, 2 were affected, and 3 showed some clinical signs of ARVC/D even if they did not fulfill the diagnostic criteria. Two compound heterozygotes for mutations in the same gene and 1 subject carrying 3 different mutations showed a severe form of the disease with heart failure onset at a young age. Moreover, multiple-mutation carriers showed a higher prevalence of left ventricular involvement ($P = .025$) than single-mutation carriers.

CONCLUSION Occurrence of compound and double heterozygotes in ARVC/D index cases is particularly relevant to mutation screening strategy and to genetic counseling. Even if multiple-mutation carriers show a wide variability in clinical expression, the extent of the disease is higher compared to that in single-mutation carriers.

KEYWORDS Arrhythmia; Arrhythmogenic cardiomyopathy; Sudden death; Cell adhesion molecules; Gene mutations; Compound genotypes

ABBREVIATIONS ARVC/D = arrhythmogenic right ventricular cardiomyopathy/dysplasia; **ECG** = electrocardiogram; **DHPLC** = denaturing high-performance liquid chromatography; **ICD** = implantable cardioverter-defibrillator; **RPD** = repeat domains; **RV** = right ventricular; **VT** = ventricular tachycardia.

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Introduction

Arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) is a heart muscle disease characterized by a progressive myocardial atrophy followed by fibrofatty replacement, involving mostly the right ventricular (RV) myocardium.[1–3] The pathologic changes offer the substrate for the onset of ventricular arrhythmias that in some cases can lead to sudden death.[4] Moreover, the disease is clinically heterogeneous, with interfamilial and intrafamilial variability, ranging from benign to malignant forms with a high risk of sudden cardiac death. The mode of inheritance is mostly autosomal dominant with incomplete penetrance.

Twelve genetic loci have been discovered so far, and mutations were documented in 8 different genes. Different mutations have been detected in genes encoding desmosomal proteins: desmoplakin[5,6] (DSP, ARVD8), plakophilin-2[7] (PKP2, ARVD9), desmoglein-2[8,9] (DSG2, ARVD10), desmocollin-2[10–12] (DSC2, ARVD11), and plakoglobin[13] (JUP, ARVD12). Mutations of JUP and DSP have also been shown to cause recessively inherited syndromic forms of ARVC manifesting with palmoplantar keratoderma and woolly hair, named Naxos

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and Carvajal syndrome, respectively.[14–16] Moreover, mutations in TGFβ3 and TMEM43 are involved in ARVD1[17] and ARVD5,[18] respectively. The cardiac ryanodine receptor gene RyR2 causes a distinct clinical entity, ARVD2, characterized by juvenile sudden cardiac death and effort-induced polymorphic ventricular tachycardia (VT).[19] Whether this is a variant of ARVC/D is a controversial issue.

We report here on genetic and clinical data of 3 ARVC/D families in which mutation screening of desmosomal ARVC/D genes detected the simultaneous presence of different mutations in 1 or 2 different genes.

Methods

Clinical evaluation

A cohort of 42 consecutive index cases with a typical form of ARVC/D was investigated at the Department of Cardiothoracic and Vascular Sciences of the University of Padua, Italy. All of these patients gave informed consent to be evaluated and to give blood samples for DNA study. The study protocol[20] included: physical examination, family history, 12-lead electrocardiogram (ECG), signal-averaged ECG, 24-hour Holter ECG, 2-dimensional echocardiogram. As far as ECG evaluation, the term RV conduction delay was used to define both the presence of a QRS duration in V1 to V3 of 110 to 120 ms and of incomplete bundle branch block (QRS $<$120 ms, presence of a secondary R wave in V1, or S wave notched with a prolonged duration in V1 or an S1 to S2 to S3 aspect with a secondary R wave in lead aVR).[21,22] Measurements of the QRS voltages in the precordial leads were calculated summing the amplitude of the different QRS components in each of the precordial leads.[22] Moreover, premature ventricular complexes were defined as abnormal when more than 1,000/24 h were present. Diagnosis was made according to Task Force criteria.[23] Moreover, family members of these 42 subjects were also analyzed using the same clinical protocol. The clinical and instrumental findings of multiple mutations were compared with those of single-mutation carriers. All continuous variables were expressed as the mean value $\pm$ SD. An unpaired Student *t*-test was used for comparison of normally distributed data. To compare noncontinuous variables, the Fisher exact test was performed to contingency tables.

Mutation screening

Forty-two consecutive index case patients were screened for mutations in PKP2, DSP, DSG2, DSC2, JUP, and TGFβ3 genes by denaturing high-performance liquid chromatography (DHPLC) and direct sequencing. The analysis was performed using the Wave Nucleic Acid Fragment Analysis System 3500HT with DNASep HT cartridge technology (Transgenomic Inc, Omaha, USA). The temperatures for sample analysis were predicted using the Wave Navigator software. Samples showing a change in DHPLC pattern were directly sequenced using the Big Dye dideoxy-terminator chemistry (Perkin Elmer, Waltham, USA) on an ABI 377 DNA sequencer (PE Applied Biosystems, Foster City, USA). Chromas 1.5 software (Technelysium) and Lasergene package computer programs (DNASTAR) were used to edit, assemble, and translate sequences. DSP sequences were compared with reference sequence NM_004415, PKP2 sequences to NM_001005242, DSG2 sequences to NM_001943, DSC2 sequences to NM_024422, JUP sequences to NM_002230, and TGFβ3 to NM_003239.

A control group of 250 healthy and unrelated subjects (500 alleles) from the Italian population was used to exclude that the detected mutations were DNA polymorphisms. Identified mutations altering restriction sites were confirmed by restriction digest according to the manufacturer's protocol (New England Biolabs, Ipswich, USA). Mutation screening was performed in all living family members of index cases in whom a mutation was detected.

Results

Among 42 unrelated ARVC/D index cases, 7 (16.6%) carried a PKP2 mutation, 5 (11.9%) a DSP mutation, 4 (9.5%) a DSG2 mutation, 2 (4.8%) a DSC2 mutation, and 2 (4.8%) a TGFβ3 mutation. No one carried JUP mutations. Overall, 3 index cases (7.1%) carried multiple mutations in the same gene or in different genes.

Family #137

Genetic findings

Index case (IV,5) showed 2 different mutations in the DSP gene (V30M and R2541K) (Figure 1A). Substitution c.88G$>$A, leading to a replacement of a valine by methionine (V30M), was previously reported.[24] Methionine in position 30 of DSP does not show phylogenetic conservation in vertebrates (Figure 1B). On the contrary, strong conservation is present in the upstream residues. Mutation R2541K (c.7622G$>$A) occurred in a highly conserved residue (Figure 1B), placed in a region linking repeat domains (RPD) of DSP C-terminal. None of such nucleotide changes were detected in 250 control subjects (500 chromosomes) from the same population. Restriction digestion with BstEII was used as an independent genotyping method to validate sequence analysis results for DSP V30M. No additional mutations were detected in PKP2, DSG2, DSC2, and JUP genes.

Analysis of family members available to the study showed that the index case inherited V30M from her mother (III,3) and R2541K from the paternal side. V30M was detected as well in subjects IV,2; V,1; V,3; V,4; V,5; and V6, whereas R2541K was detected in subjects IV,7; V,7; V,8; and V,9 (Figure 2).

Clinical findings

Among the 28 family members, 7 died before the time of the study. The index case (IV,5) required cardiac evaluation because of palpitations at the age of 36, and she was diagnosed as carrying a biventricular form of ARVC/D (left ventricular [LV] end diastolic volume = 75 ml/m^2, LV ejection fraction = 50%, RV end diastolic area = 32 cm^2, RV fraction shortening = 30%). At the age of 40, the patient showed symptoms and signs of heart failure, and at