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Experimental research

Role of novel *DSP*_p.Q986X genetic variation in arrhythmogenic right ventricular cardiomyopathy

Oscar Campuzano [a,1], Mireia Alcalde [a,1], Paola Berne [b], Esther Zorio [c], Anna Iglesias [a], Josep Navarro-Manchón [c], Josep Brugada [b], Ramon Brugada [a,*]

[a] Cardiovascular Genetics Center, UdG-IdIBGi, Girona, Spain
[b] Arrhythmias Unit, Hospital Clínic of Barcelona, Barcelona, Spain
[c] Sudden Death Risk Unit, Cardiology Department, Hospital Universitario y Politécnico La Fe, Valencia, Spain

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ABSTRACT

Introduction: Arrhythmogenic right ventricular cardiomyopathy is an inherited disease characterized by a progressive myocardium fibrofatty replacement. This abnormality disrupts electrical transmission causing ventricular arrhythmias and sudden cardiac death. This genetic disease is transmitted mainly with an autosomal dominant pattern. Our aim was to identify the genetic defect responsible for the pathology in a Spanish family, and to perform its phenotype connotations.
Material and methods: A total of 15 individuals in a three-generation Spanish family were screened after the sudden cardiac death of one family member. All they underwent a complete physical examination, 12-lead electrocardiogram, 2-dimensional echocardiography, magnetic resonance imaging, exercise stress test, 24-h Holter and genetic testing.
Results: Autopsy revealed the presence of biventricular arrhythmogenic dysplasia in deceased member. Six family members showed clinical symptoms but only three of them fulfilled definite diagnostic criteria of the disease. Genetic analysis showed a novel *nonsense* genetic variation in nine family members. All family members with clinical symptoms carried the genetic variation.
Conclusions: Genetic testing in families affected by arrhythmogenic right ventricular cardiomyopathy helps to identify the genetic cause responsible for the disease. The incomplete penetrance and variable phenotypic expression highlights the need of comprehensive genetic analysis and further phenotype implications of genetics to clarify the pathophysiology of the disease.

1. Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC, MIM 107970) is characterized by a progressive replacement of myocytes by fibro-adipose tissue. The pathological findings are usually present in the right ventricle (RV), however up to 50% of cases may also show a left ventricular affectation (biventricular dysplasia) [1]. The presence of fibrofatty tissue in the myocardium impairs cell-to-cell electrical signalling, cause ventricular arrhythmias and even sudden cardiac death (SCD) [2]. The National Center for Biotechnology Information (NCBI) establishes its prevalence between 1:1000 and 1:5000, being more common in young men (3:1) [3]. Unfortunately, sudden death may be the first symptom in ARVC cases, being responsible for 5% of all SCD cases every year [4].

The diagnostic criteria for ARVC were recently improved, increasing the sensitivity for diagnosis in relatives of affected patients [5]. The disease has an autosomal dominant inheritance, with incomplete penetrance and a wide variety of clinical manifestations. Genetic testing can help ensuring a proper diagnosis, not only for the index case but also for family members. Up to 60% of ARVC patients are carriers of mutations, mainly in genes encoding for desmosomal proteins. Mutations in plakophilin (*PKP2*) gene are found in nearly 30–35% of ARVC patients, followed by desmoplakin (*DSP*) (10–15%), desmoglein (*DSG2*) (10%) and desmocollin (*DSC2*) (2–5%) genes [6]. In addition, non-desmosomal genes have been also identified responsible for the ARVC (*TMEM43*, *TGFβ3*, *TP63* and *DES*) but with a lower incidence (<1%). Recently, mutations in titin protein (*TTN*) [7] and lamin A/C protein (*LMNA*) [8] have been described in ARVC patients although further studies are required to confirm the gene-disease relationship. Therefore, around 35% of

* Corresponding author. Dean School of Medicine, Director Cardiovascular Genetics Center, C/Pic de Peguera 11, 17003 Girona, Spain. Tel.: +34 972 183366; fax: +34 972 183367.
E-mail address: ramon@brugada.org (R. Brugada).
[1] Both authors equally contributed.

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ARVC patients remain without a genetic diagnosis [9]. In the present study we report ARVC patients of a three-generation Spanish family who carry a novel *nonsense* genetic variation in *DSP* gene.

2. Material

The present study was approved by the ethics committee of the Hospital Josep Trueta (Girona, Spain), and informed consent was obtained from all participants. All individuals included in our study were assessed clinically at the Arrhythmia Unit, Hospital Clinic of Barcelona (Spain), and at Sudden Death Risk Unit Hospital La Fe of Valencia (Spain). Patients were all Caucasians and natives of Spain.

The index case (III.4) was identified after suffering an episode of SCD, with a positive autopsy for biventricular arrhythmogenic dysplasia. Family members were clinically diagnosed based on the revised Task Force Criteria (TFC) of the European Society of Cardiology/International Society and Federation of Cardiology [5]. Clinical data and pedigree from the family are shown in Table 1 and Fig. 1. All family members underwent clinical evaluation, consisting of electrocardiogram (ECG), echocardiogram (ECHO), cardiac magnetic resonance (MR), exercise test (ET) and Holter (HO) (except I.1 due to old age and I.2, dead 20 years before).

3. Methods

3.1. Genetic studies

Blood samples were processed and genomic DNA extracted using commercial protocols (PUREGENE DNA, QIAGEN). Subsequently amplification of each gene of interest was done by polymerase chain reaction (PCR), and purified by ExoSAP-IT (ISOGEN). The analysis of the exonic and intron–exon regions was performed by direct sequencing (Genetic Analyzer 3130XL, Applied Biosystems). The mother (II.4) of our index case (III.4) underwent genetic studies of *PKP2*, *DSP*, *DSC2*, *DSG2*, *TGFβ3* and *LMNA*. Plakoglobin protein (*JUP* gene) was not studied because none patient in our cohort had phenotype of Naxos disease [10]. Each sequence was compared with the reference sequence (Ensembl genome browser): *PKP2*-ENST00000070846, *DSP*-ENST00000379802, *DSC2*-ENST00000379802, *DSG2*-ENST00000261590, *TGFβ3*-ENST00000238682 and *LMNA*-ENST00000368300.

Table 1
Clinical data. Results from clinical findings in all family included in the study. Major and Minor criteria indicate clinical findings based on the revised Task Force Criteria (TFC) of the European Society of Cardiology/International Society and Federation of Cardiology. Data from III.4 patient was obtained after autopsy examination. Abbreviations: ECG: electrocardiogram. ECHO: echocardiogram. MR: magnetic resonance. F: female. M: male. NE: no evaluated.

Family member	Age	Gender	Genetic variation	ECG	ECHO	MR
I.1	82	M	p.Q986X	NE	NE	NE
I.2 -Died-	53	F	NE	NE	NE	NE
II.2	53	F	p.Q986X	Major	Minor	Normal
II.3	52	F	No	Normal	Normal	Normal
II.4	50	F	p.Q986X	Normal	Major	Normal
II.6	49	M	No	Normal	Normal	Normal
II.7	46	M	p.Q986X	Normal	Normal	Normal
II.9	42	F	p.Q986X	Normal	Normal	Normal
III.1	30	M	No	Normal	Normal	Normal
III.2	25	F	No	Normal	Normal	Normal
III.3	17	F	p.Q986X	Normal	Normal	Normal
III.4 -Proband, Died-	18	M	NE	NE	NE	NE
III.5	15	F	p.Q986X	Minor	Major	Normal
III.6	11	F	No	Normal	Normal	Normal
III.7	18	M	p.Q986X	Normal	Normal	Normal
III.8	12	F	p.Q986X	Normal	Normal	Normal

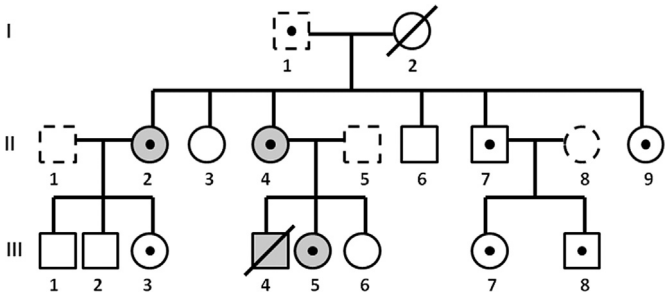

Fig. 1. Familial pedigree. Squares represent men, circles represent woman, and slashes represent deceased family members. III.4 is the proband. Clinical affected individuals are shown as grey filled circle/squares. White filled circles/squares are no clinical affected individuals. White filled circles/squares with discontinuous line represent no clinically evaluated individuals. Black dots within the circle/square are p.Q986X carriers in *DSP*. Black dots within white circle/square represent family members carry genetic variation without clinical symptom. Note that III.4 was diagnosed after autopsy. In addition, I.1 was not evaluated due to old age.

For this novel genetic variation detected, genetic analysis was realized in 206 Spanish control subjects (412 control alleles) (individuals not related to any patient and of all them with Spanish ancestors). This novel genetic variation was not previously identified as shown in consulted genetic variants database as 100° genomes (http://browser.1000genomes.org/) and Exome sequencing project (http://evs.gs.washington.edu/EVS/). In addition, alignment between species was also performed using UniProt database (http://www.uniprot.org/).

4. Results

4.1. Clinical

The proband (index case), a 18 year-old male (III.4), was diagnosed post-mortem after suffering an episode of SCD immediately after exercise (handball training). He was asymptomatic before the SCD event. The autopsy revealed anatomopathological changes (biventricular fibro-fatty substitution), consistent with the diagnosis of biventricular arrhythmogenic dysplasia. Other possible causes of death were ruled out, including coronary disease. The event of SCD in this young individual led to the clinical investigation of first degree family members: parents (II.4 and II.5) and two sisters (III.5 and III.6) (Table 1; Fig. 1).

The mother (II.4) of our index case, a 53-year-old female, suffered several syncopes not related to physical activity. Her ECG only showed low QRS voltages in DII, DIII and aVF leads, but typical ARVC ECG abnormalities were absent. An ECHO showed normal biventricular ejection fraction and aneurisms in apex and basal segments of RV plus parasternal long-axis view of the right ventricular outflow tract (RVOT) of 33 mm, and parasternal short-axis view RVOT of 39 mm. As a result, II.4 was diagnosed with ARVC fulfilling 2 major diagnostic criteria, namely regional dysfunction and structural alterations, and family history of SCD in first-degree relative with ARVC (confirmed after autopsy). Therefore, an implantable cardiac defibrillator (ICD) was implanted because of her symptoms and received sotalol as pharmacological treatment. The index case's father (II.5) wasn't clinically evaluated because he was apparently healthy and without previous family history of ARVC or SCD. The index case had two sisters (III.5 and III.6). The older sister (III.5), a 15 year-old girl who practiced basketball on a regular basis, showed altered ECG: low QRS voltages in limb leads, flat T-waves in DIII and aVF, inverted T-waves from V4 to V6.