
Clinical, Genetic, and Epidemiological Characteristics of Arrhythmogenic Cardiomyopathy Associated With the Pathogenic Founder Variant NP_00454563.2 (p.Glu259Glyfs*77) in Gene PKP2

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Article

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Abstract: Introduction and objectives: arrhythmogenic cardiomyopathy (ACM) is a hereditary heart disease defined by the progressive replacement of the ventricular myocardium with fibroadipose tissue, which can act as a substrate for arrhythmias, sudden death, or even give rise to heart failure (HF). Sudden death is frequently the first manifestation of the disease, particularly among young patients. The aim of this study is to describe a new pathogenic variant in the PKP2 gene. **Methods:** A descriptive observational study that included 8 initially non-interrelated families with a diagnosis of ACM undergoing follow-up at our HF and Familial Cardiomyopathies Unit, who were carriers of the NM_004572.3:c.775_776insG; p. (Glu259Glyfs*77) variant in PKP2 gene. The genetic testing employed next-generation sequencing for the index cases, and the Sanger method for the targeted study with family members. We compiled personal and family histories, demographic and clinical characteristics, data from the additional tests at the time of diagnosis, and arrhythmic events at diagnosis and during follow-up. **Results:** We included 47 subjects, of whom 8 were index cases (17%). Among the evaluated family members, 16 (34%) were carriers of the genetic variant, 3 of whom also had a diagnosis of ACM. The majority were women (26 patients; 55.3%), with a mean age on diagnosis of 48.9 ± 18.6 years, and a median follow-up of 39 [24-59] months. Worthy of note are the high incidence of arrhythmic events as the form of presentation and in follow-up (21.5% and 20.9% respectively), and the onset of HF in 25% of the sample. The most frequent ventricular involvements were right (4 patients 16.7%) and biventricular (4 patients 16.7%); we found no statistical differences in any of the variables analysed. **Conclusions:** This variant is a pathogenic variant of gene PKP2 that has not previously been described and is not present in the control groups associated with ACM. It has incomplete penetrance, a highly variable phenotypic expressivity, and was identified in 8 families of our geographical area in Malaga (Andalusia, Spain), where we can establish a founder effect and describe the clinical and risk characteristics.

Keywords: arrhythmogenic cardiomyopathy; PKP2; founder pathogenic variant; arrhythmic events; heart failure

INTRODUCTION

Arrhythmogenic cardiomyopathy (ACM) is a heart disease with a genetic basis, defined by the progressive replacement of the ventricular myocardium with fibroadipose tissue, which can act as a

substrate for arrhythmias, sudden death (SD), or even give rise to heart failure (HF). Estimates place the prevalence of ACM among the general population at 1:3.000-5.000 inhabitants. It generally presents between the ages of 20 and 40 years, and usually manifests in the form of palpitations, syncope or presyncope, with SD often the first manifestation of the disease, particularly in the case of young patients^{1,2}.

Despite classically having been described as arrhythmogenic right ventricular dysplasia (ARVD), frequent biventricular or predominant left ventricle involvement have led to adoption of the term ACM³. Both the biventricular and the predominant left ventricular forms can be difficult to differentiate from dilated cardiomyopathy (DCM)^{3,4}.

Complex molecular mechanisms exist in the physiopathology of ACM that differ according to the genotype^{5,6}. Classically, ACM has been considered a disease of autosomal dominant inheritance with reduced penetrance and variable clinical expressivity depending on age, caused by mutation in the genes that encode desmosomal proteins^{7,8}. There are five genes that encode these proteins and are involved in the pathogenesis of ACM: plakophilin-2 (PKP2), desmoglein-2 (DSG2), desmoplakin (DSP), desmocollin-2 (DSC2) and junction plakoglobin (JUP). The literature also describes certain, much less frequent, forms with autosomal recessive transmission, which are usually syndromic⁹. Approximately 40-60% of cases with a definitive ACM diagnosis show at least one genetic variant that could explain the disease¹⁰, for the most part in a desmosomal gene, although in a low percentage of cases it is possible to identify causal variants in non-desmosomal genes (DES, PLN, TMEM43, LMNA, TTN, SCN5A, CTNNA3, CDH2, TJP1, ANK2, TP63)¹¹. Recent years have seen numerous cases of ACM described as oligogenic, or even multifactorial, with genomic and atmospheric factors playing a role in pathogenesis^{10,11}.

The clinical diagnosis of ACM is not an easy task, given that it shares characteristics with many other diseases such as DCM, myocarditis, sarcoidosis and some variations of normality such as athletic heart syndrome¹². Genetic diagnosis is also complex, due to the high predominance of variants that are missense-type or of uncertain significance in desmosomal genes, particularly in PKP2¹³, which means that interpretation of the genetic study must be undertaken in conjunction with clinical data and a familial study to establish the presence or not of cosegregation. Thus, the 2010 revised diagnostic criteria for ACM included genetic criteria¹⁴.

The aim of our study is to describe the clinical, genetic, and epidemiological characteristics of eight initially non-interrelated families who have a diagnosis of ACM associated with the NM_004572.3:c.775_776insG; p. (Glu259Glyfs*77) variant in the PKP2 gene. This variant has not previously been described, and we can confirm its pathogenicity, establishing a founder effect in our region in Malaga (Andalusia, Spain) that allows us to evaluate the genotype-phenotype correlation and describe the clinical and risk characteristics of both index cases and family members in follow-up at our HF and Familial Cardiomyopathies Unit.

METHODS

Study design and population

This is a descriptive observational study that included 8 families with a diagnosis of ACM related with the genetic variant NM_004572.3:c.775_776insG; p. (Glu259Glyfs*77) in gene PKP2 who underwent follow-up at our HF and Familial Cardiomyopathies Unit. We evaluated 8 probands, or index cases, who had undergone genetic testing as part of the habitual study of their heart disease. We also included 39 family members of these index cases, performing cascade testing. We broadened the initial evaluation to include further tests in cases of suspicion or indications of disease. The informed consent of each subject was obtained, and the study protocol met the ethical criteria of the 1975 Declaration of Helsinki.

The definitive diagnosis of ACM followed the 2010 modified Task Force criteria¹⁴. We compiled personal and family histories, demographic and clinical characteristics, additional tests at the time of diagnosis, and arrhythmic events both at diagnosis and during follow-up.

Genetic analysis

The study of the index cases of each family was performed by Next Generation Sequencing (NGS) using a library that included 121 genes related to ACM and DCM as a part of the habitual clinical evaluation. The genes included in this test were selected based on clinical criteria, taking into account their association with a given phenotype (among them, we highlight ACTA1, BAG3, BRAF, DES, DMD, DSC2, DSG2, DSP, EMD, FHOD3, FLNC, GLA, JUP, KCNJ2, LMNA, MYBPC3, MYH6, MYH7, PKP2, PLN, RBM20, RYR2, SCN5A, TMEM43, TTN, TTR). In addition, the method is combined with the Sanger technique for those regions with suboptimal coverage and/or quality levels, which are re-sequenced using this technique. By this method it is possible to identify point substitutions and small insertions/deletions of up to 20 nucleotides with a sensitivity and specificity of the method higher than 99%.

For the study of the PKP2 variant in family members, we used the Sanger method, sequencing only the variant under study. We obtained the blood samples used for the genetic testing after receiving signed informed consent from each subject. The pathogenicity of the genetic variants was classified according to the American College of Medical Genetics and Genomics¹⁵.

Clinical evaluation and follow-up

For all index cases identified, we exhaustively researched the family history, and prepared a family tree covering at least 3 generations, which allowed us to carry out the cascade familial screening of all accessible family members. All subjects received a clinical evaluation that included a personal and family history, a physical examination, a 12-lead ECG, and a transthoracic echocardiogram. The 24-hour Holter monitoring and cardiac magnetic resonance imaging (CMRI) were only undertaken for the index cases and for family members who were affected or had suspected incipient disease, provided no contraindications existed for the study (claustrophobia or wearers of implantable cardiac defibrillator -ICD- or other devices). The clinical information was retrospectively recorded for patients who had a previous diagnosis, and prospectively for new cases or evaluated family members.

Thus, we identified, treated and established follow-up for all carrier subjects of the NM_004572.3:c.775_776insG; p. (Glu259Glyfs*77) variant, in accordance with the latest evidence available for ACM and DCM^{16,17}.

During follow-up, we collected information on arrhythmic events, including sustained ventricular tachycardia (SVT) and non-sustained ventricular tachycardia (NSVT), appropriate ICD therapies (with anti-tachycardia therapy or shock) and aborted SD. We also considered the development of heart failure (HF), the need for heart transplant, and death.

Statistical analysis

We used SPSS (version 22) to analyse the data. Quantitative variables are expressed as a mean \pm standard deviation or as a median and interquartile range according to whether they followed a normal distribution or not. Categorical variables are expressed as an absolute value and a percentage. For comparison of continuous variables, we used Student's t-test or the Mann-Whitney U test for normal or non-normal distribution respectively. Categorical variables are compared by means of contingency tables and the χ^2 test or Fisher's exact test. Two-tailed values of $p < 0.05$ were considered statistically significant.

RESULTS

Baseline characteristics of the sample

Of the 47 subjects studied, 8 were index cases or probands (17% of the sample) who met the diagnostic criteria for ACM and were carriers of the NM_004572.3:c.775_776insG; p. (Glu259Glyfs*77) variant in gene PKP2. The 39 family members evaluated underwent a targeted genetic study for the variant, and 16 (34%) were found to be carriers, 3 of whom (6.4%) were also affected by heart disease:

one subject with incipient involvement, whose study it was not possible to complete due to exitus for gastric cancer; another patient with a phenotype overlapped with hypertrophic cardiomyopathy (HCM) and with a complex genotype, who was additionally a carrier of pathogenic variants in sarcomeric genes; and another patient with incipient DCM.

Most of the subjects evaluated were women (26 patients: 55.3%), with a mean age on diagnosis of 48.9 ± 18.6 years. The left ventricular ejection fraction (LVEF) was 64% [60-65] and the follow-up period was 39 [24-59] months. Table 1A describes the baseline characteristics of the sample, and table 1B illustrates the main clinical characteristics of the subjects who are carriers of the genetic variants and were diagnosed of ACM (11 subjects, 17.2% of the sample). Among this group, in most cases, presentation of the disease was for arrhythmic events: 4 (36.3%) patients with VT, 1 (9.1%) with aborted SD and another (9.1%) for palpitations caused by high-density ventricular ectopic beats. In addition, 3 (27.2%) of the diagnoses were made for symptoms of HF and another 2 (18.2%) on conducting the familial studies. Right ventricular involvement was the most common (4 patients, 36.3%), alongside biventricular involvement (4 patients, 36.3%), and only 3 patients (27.2%) had exclusive left involvement. None of the subject with right involvement had a right ventricular dysfunction that was greater than mild, and right involvement was considered for segmental hypokinesia.

Table 1. Baseline and follow-up characteristics

A.	Carriers	Non-carriers	p	Total
Sex			0.38	
Women	15 (31.9%)	11 (23.4%)		26 (55.3%)
Men	9 (19.1%)	12 (25.5%)		21 (44.7%)
Age (years)	54 ± 16.7	43.6 ± 19.3	0.06	48.9 ± 18.6
Follow-up time (months)	48 [27-59]	27 [23-59]	0.13	39 [24-59]
LVEF (%)	63 [45-65]	64 [62-65]	0.43	64 [60-65]
B.	Men	Women	p	Total
Diagnostic ACM/DCM	6 (54.5%)	5 (45.5%)	0.50	11 (100%)
Probands	4 (50%)	4 (50%)		8 (72.7%)
Family members	2 (66.7%)	1 (33.3%)		3 (27.2%)
Age at diagnosis (years)	63.2 ± 11.2	48.4 ± 17.3	0.03	54 ± 16.7
Form of presentation			0.26	
Family screening	2 (18.2%)	0 (0%)		2 (18.2%)
Dyspnea	1 (9.1%)	2 (18.2%)		3 (27.2%)
Palpitations	0 (0%)	1 (9.1%)		1 (9.1%)
Arrhythmic events	3 (27.2%)	1 (9.1%)		4 (36.3%)
Sudden death	0 (0%)	1 (9.1%)		1 (9.1%)
Affectation			0.32	
Right	3 (27.2%)	1 (9.1%)		4 (36.3%)
Left	2 (18.2%)	1 (9.1%)		3 (27.2%)
Biventricular	1 (9.1%)	3 (27.1%)		4 (36.3%)
ECG			0.47	
RBBB	3 (27.2%)	3 (27.2%)		6 (54.4%)
T negative wave	1 (9.1%)	2 (18.2%)		3 (27.2%)
Epsilon wave	1 (9.1%)	0 (0%)		1 (9.1%)
LVEF (%)	63 [40-65]	64 [60-65]	0.68	63 [45-65]

Follow-up time (months)	59 [33-75]	42 [24-58]	0.07	48 [27-59]
ICD implant			0.25	
Primary prevention	1 (9.1%)	3 (27.2%)		4 (36.3%)
Secondary prevention	2 (18.2%)	2 (18.2%)		4 (36.3%)
Events in the follow-up			0.45	
SVT	2 (18.2%)	2 (18.2%)		4 (36.3%)
NSVT	0 (0%)	1 (9.1%)		1 (9.1%)
VT Ablation	1 (9.1%)	1 (9.1%)		2 (27.2%)
ICD therapies	1 (9.1%)	1 (9.1%)		2 (27.2%)
HF	3 (27.2%)	3 (27.2%)		6 (54.5%)
Heart transplantation	0 (0%)	1 (9.1%)		1 (9.1%)

A. Baseline characteristics of the sample. B. Clinical characteristics of subjects carrying the variant NM_004572.3:c.775_776insG; p. (Glu259Glyfs*77) in the PKP2 gene and diagnosed of ACM. Values expressed as n (%) or median [interquartile range]. ECG: electrocardiogram; ICD: implantable cardioverter-defibrillator; LVEF: left ventricular ejection fraction; HF: heart failure; NSVT: non-sustained ventricular tachycardia; RBBB: right bundle branch block; SVT: sustained ventricular tachycardia; VT: ventricular tachycardia.

We also detected variants in genes other than PKP2 in 9 patients. In the first 5 families (Figure 1) no other variants in other genes have been identified. In family 6 (Figure 2) we identified a variant in DSG2 gene that has been described to be associated with the development of ACM in homozygosis or compound heterozygosis, being highly probable that it contributes to the development of ACM in the presence of another pathogenic variant as is our case in PKP2 and more specifically in the index case of this family. In addition, we found another variant in DSP gene considered to be of uncertain pathogenicity, and a more extensive cosegregation study is necessary. In family 7 (Figure 2) 2 variants in the gene that encodes myosin binding protein C3 (MYBPC3) have been found to be associated with the development of HCM, unrelated to DCM and/or ACM. In this family, the HCM phenotype is only evident in patients carrying these variants in MYBPC3. In Family 8 (Figure 2) we have found variants of unknown pathogenicity in the inward-rectifier potassium channel gene (KCNJ2) and titin gene (TTN); and another variant in the ryanodine receptor 2 gene (RYR2) which, although also considered of unknown pathogenicity, could contribute to the ventricular arrhythmia phenotype in the presence of other disease-causing variants such as the PKP2 variant. Table 2 describes the genetic and clinical characteristics of the subjects diagnosed with ACM.

It should be highlighted that of the 47 patients studied, only 10 (21.3%) underwent CMRI, and only one person had positive late gadolinium enhancement. In most cases, the reason that CMRI was not possible was claustrophobia.

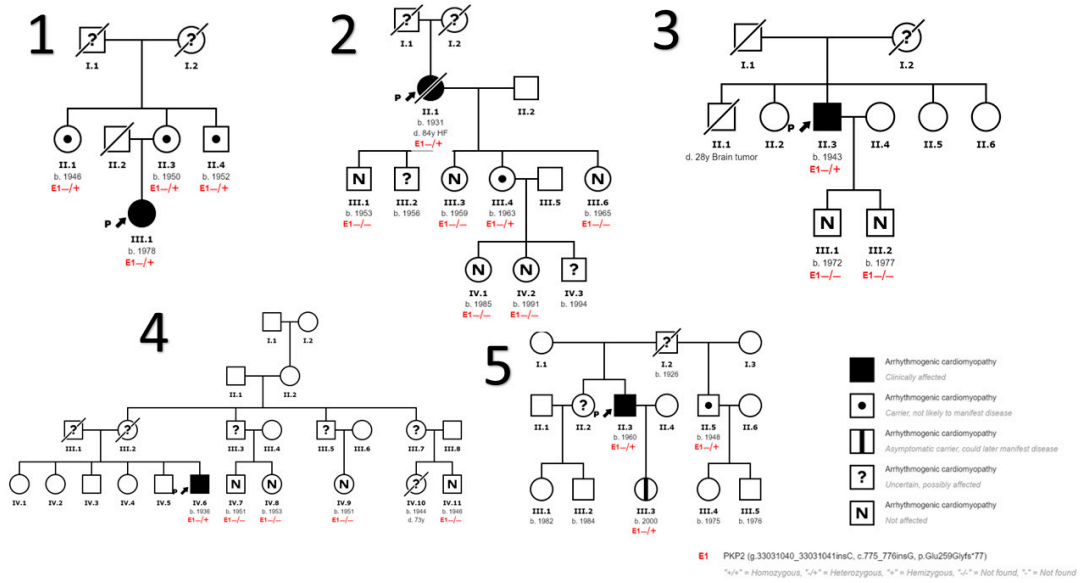


Figure 1.

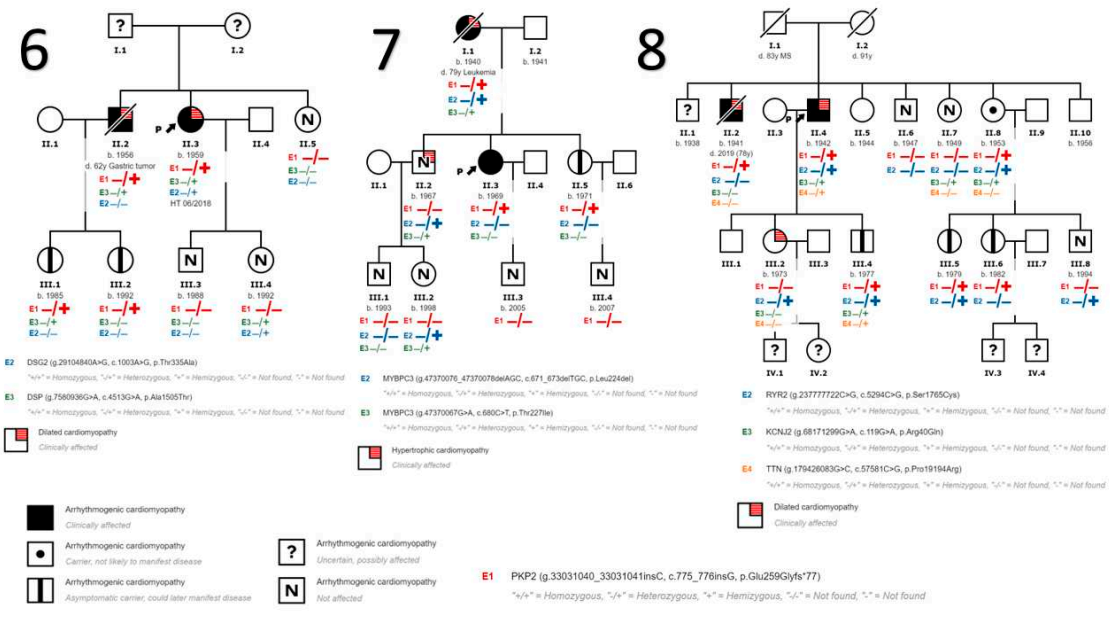


Figure 2.

Table 2. Clinical and genetic characteristics of patients diagnosed with ACM

Family/ Patient	Sex	Age 1st visit (years)	Index case	Other genetic variants	Presentation	Affectation	LVEF (%)	ICD	Arrhythmias under follow- up	HF	Death
1/III.1	Woman	34	Yes	No	Palpitations (VPB)	Right	65	Primary Prevention	SVT with focus ablation	No	No
2/II.1	Woman	76	Yes	No	VT	Biventricular	38	Secondary Prevention	SVT	Yes	Yes (HF)
3/II.3	Man	69	Yes	No	VT	Right	65	Secondary Prevention	SVT	No	No
4/IV.6	Man	73	Yes	No	VT	Right	65	No	No	No	No
5/II.3	Man	54	Yes	No	VT	Right	65	Secondary Prevention	SVT with focus ablation	No	No
6/II.3	Woman	56	Yes	DSP p.Ala1505Thr (+?) DSG2 p.Thr335Al (+++)	Dyspnea	Left	30	Primary Prevention	NSVT	Yes (CT)	No
6/II.2	Man	63	No	DSP p.Ala1505Thr (+?)	Family Study	Left	40	No	No	Yes	Yes (gastric Ca)
7/II.3	Woman	48	Yes	No	SD	Biventricular	65	Secondary Prevention	No	No	No
7/I.1	Woman	65	No	MYBPC3 p.Leu224del (++)* MYBPC3 p.Thr227Ile (++)*	Dyspnea	Biventricular	38	Primary Prevention	No	Yes	Yes (Leuke mia)
8/II.4	Man	63	Yes	RYR2 p.Ser1765Cys(+?) KCNJ2 p.Arg40Gln (-?)	Dyspnea	Left	40	No	No	Yes	No

				TTN p.Pro19194Arg (-?)							
8/II.2	Man	74	No	RYR2 p.Ser1765Cys(+?) KCNJ2 p.Arg40Gln (-?) TTN p.Pro19194Arg (-?)	Family Study	Biventricular	30	Primary Prevention	No	Yes	Yes (older age)

The genetic and clinical characteristics of the patients carrying the variant under study and diagnosed with ACM are shown. The 8 probands and the 3 patients diagnosed at the time of the family study are included. CT: Cardiac transplant; HF: Heart failure; ICD: automatic implantable defibrillator; DSG2: desmoglein 2; DSP, desmoplakin; LVEF, left ventricular ejection fraction; KCNJ2: potassium inward rectifier channel; SD: sudden death; MYBPC2: sudden death; SD: sudden death; MYBPC3: cardiac myosin-binding protein C; TTN: titin; VPB: entricular premature beats; VT: ventricular tachycardia, NSVT: non-sustained ventricular tachycardia; SVT: sustained ventricular tachycardia; RYR2: ryanodine receptor. (-?) Possibly NOT associated with the disease. (+?) Possibly associated with disease; (++) Very Possibly associated with disease; (+++) Pathogenic or associated with disease. * Genetic variants related to HCM, diagnosis also made in this family.

Follow-up

During the mean follow-up period of 48 [27-59] months for the patients who were carriers of the described genetic variant, 5 patients (20.9%) suffered arrhythmic events, 4 (16.7%) as SVT and 1 (4.2%) as NSVT. Only 2 patients (8.3%) underwent an ablation procedure for VT; and 8 patients (33.3%) had an ICD implant, 4 (16.7%) as primary prevention and the other 4 (16.7%) as secondary prevention (2 subjects with right-involvement and the other two with biventricular involvement). Regarding ICD therapies, 2 patients (8.3%) received appropriate therapies for SVT during follow-up.

The onset of HF occurred in 6 patients (25%), one of whom eventually required a heart transplant with a good subsequent progression.

We did not find any statistically significant differences between the sexes regarding events during follow-up (Table 1B). Neither were there any differences for LVEF between the sexes (63% [40-65] among men and 64% [60-65] in women, $p=0.68$), although we did find a statistical significance for age on diagnosis, which was lower for women (48.4 ± 17.3 years, $p=0.03$).

During follow-up, 4 patients died, one of them due to HF and three others not directly related to ACM: in 2 cases it was due to oncological processes (gastric cancer and leukaemia) and the third was multifactorial in an elderly patient.

DISCUSSION

PKP2 encodes plakophilin-2, the protein that localizes to desmosomes and nuclei, and is involved in regulation of cellular signalling and in cell-cell junctions through intermediate filaments in the cytoskeleton. It is expressed in various tissues, including the myocardium, and high levels of this protein are detected in the RV. The intention of this study is to describe our carrier population of the NM_004572.3:c.775_776insG; p. (Glu259Glyfs*77) variant in PKP2, which has been identified among 8 families of our geographical area in Malaga (Andalusia, Spain) who were initially non-interrelated but for whom we can establish a founder effect. This variant has not previously been described or reported in the databases used as population control (Exome Sequencing Project, 1000 Genomes Project, ExAC, etc.). It is also a radical variant provoking truncation in the protein, which causes functional deficit and/or premature degradation. Radical variants (nonsense, frameshift and splicing) are considered clearly pathogenic¹⁵ as they cause a premature stop codon and are most frequently associated with the pathogenesis of ACM, although deletions and duplications have also been identified as the cause of the phenotype.

Pathogenic variants in PKP2 are one of the most frequent causes of arrhythmogenic cardiomyopathy, and research suggests that such variants are responsible for between 30-50% of all ACM cases, although there is disparity between the different studies, probably due to geographical distribution^{18,19}. Some series have found mutations of this gene in up to 70% of patients with a family history, above all in the Netherlands¹⁸ with proportions that are higher than other registries for other parts of the world²⁰.

As in our sample, the incidence of desmosomal mutations described in the different published series varies between 30% to 60%^{10,21,22}. The work of Ruiz-Salas et al.²³ indicates a higher incidence, most likely due to the selection of the sample, all of whom had received a definitive diagnosis of ACM. Likewise, the Medeiros-Domingo et al.²⁴ paper detected a higher percentage of mutation in desmosomal genes among patients with definitive RVACM compared to borderline or possible RVACM, who additionally had other variants in genes associated with ACM, the supposition was that this could consist of overlapped ACM and DCM or phenocopies.

Our research reports a previously undescribed genetic variant that also has a founder effect in our geographical area. The description of new mutations in ACM registries is not always specified, and as many as 25%-70% of all of the pathogenic variants reported in the main series have not been previously described^{25,26}. As we have already mentioned, there is wide geographic variability in the distribution of PKP2 mutations¹⁸ with multiple founder mutations described in areas such as the Netherlands²⁷. These data support the suspicion that the genetic spectrum of ACM may have

singularities in each geographical zone and that large-scale registries and studies are necessary to establish the genotype-phenotype relationship.

Our series seems to suggest incomplete penetrance, with most of the affected carriers over the age of 55 years. Although this variant is sufficient to cause presentation of the disease, our cases with a phenotype at an earlier age are usually women or cases in which a second impact exists, such as the presence of another pathogenic genetic variant or participation in sports activities. Thus, our data concur with others described in the literature, with a variable expressivity of the disease among patients with PKP2 mutations, with some very severe forms and others that are milder within the same family, with incomplete penetrance, depending on age, but differing in terms of sex, as these earlier and more severe cases have normally been described in males²⁸.

In most cases of ACM associated with PKP2 mutations, right involvement predominates, and predominantly left forms, or those diagnosed as DCM, are rarer, although some reports suggest that the possibility of left involvement may be as high as 60% of carriers of pathogenic mutations in PKP2²⁹. The previously cited Ruiz-Salas et al. study²³ found an association between PKP2 mutations and cases of exclusively right ventricle involvement, whereas mutations in DSP correlated with predominantly left ventricle involvement. Different series, such as that of Bhonsale et al.³⁰, have also observed the same results; likewise our series found the most frequent ventricular involvement to be either right or biventricular (16.7% in both cases).

The phenotype of these patients is characterised by presentation with ventricular arrhythmia, generally from youth³¹, and several studies report an association between the presence of these mutations with a higher likelihood of arrhythmic events and presentation of the disease at an earlier age in comparison to patients with a negative genetic study, as occurred in the den Haan et al. series^{24,25}, which detected more VT events and an earlier age of diagnosis for carrier patients (73% versus 44%, and 33 versus 41 years). In our research, we found a high rate of arrhythmic events as the form of presentation (25.1%) and in follow-up (20.9%), in addition to appropriate ICD therapies and the need for ablation for VT (8.3% respectively), taking into account that this is a very select group, as occurred in the Ruiz-Salas et al. series²², where the most frequent presentation of the disease was with arrhythmic events; 86% of the sample presented an arrhythmic episode throughout their lives. However, Bhonsale et al.²⁹, who published the largest series of RVACM genetic mutation carrier patients, did not find a worse arrhythmic profile or worse prognosis than for non-carriers, which could be attributable to the different profile of their sample with only 60% definitive RVACM. Among our series, the high rate of HF during follow-up (25%) is notable, despite the fact that this phenotype has more frequently been described in cases of ACM with mutations in genes DSG2 and DSP, with predominantly left involvement³⁰.

One of the challenges currently facing cardiology remains the prognosis and risk stratification for sudden death in ACM¹⁶, as there is a considerable lack of coherence between the factors associated with a greater risk of SD and/or ventricular arrhythmias in the studies and series of published cases. What has been generally reported is a lower incidence of ventricular arrhythmia among family members diagnosed during the familial screening in comparison with the index cases, as also occurs in our series, although recent studies appear to cast doubt on this³².

Our research also seeks to highlight the importance of genetics in the study of cardiomyopathies and in particular of ACM, since not only does it allow us to confirm diagnosis, and indeed is one of the diagnostic criteria of the Task Force¹⁴, but also to perform a familial screening for early detection of the disease and to establish recommendations for physical activities and work, thus avoiding unnecessary diagnostic tests or follow-up among patients with negative genetic testing results. In many cases, it also represents a guide for prognosis, with higher or lower risk of arrhythmias or of HF in some of the genetic mutations identified, as we have already indicated³⁰. Nevertheless, we should not forget that genetic testing requires suitable interpretation in conjunction with clinical data by specialist personnel, since recent awareness of hereditary diseases and the new sequencing technology available mean that genetic testing is increasingly requested and consequently the number of described variants associated with ACM is higher.

Limitations

This is a descriptive single-centre study, with a small number of patients and no control group, which does not allow extrapolation of the data to all patients who are carriers of PKP2 variants. Moreover, as most of the data used are retrospective, confounders exist that may affect the results.

Another limitation has been that some family members rejected a clinical or genetic study; and for the cases that underwent the study, the request for imaging tests indicated "familial screening for ACM" which may have introduced bias.

In our study, we also refer to penetrance, but many of the subjects diagnosed were asymptomatic and diagnosis was by means of familial screening.

WHAT IS KNOWN?

-ACM is a heart disease defined by the progressive replacement of the right ventricle myocardium with fibroadipose tissue, and may cause arrhythmias, sudden death, and heart failure. Currently, 40–60% of patients have at least one genetic mutation related with the disease.

WHAT IS NEW?

-This study presents a PKP2 variant not previously described or present in the control population and establishes a probable founder effect in our region.

-This variant has incomplete penetrance and a highly variable phenotypic expressivity, is strongly influenced by age, sex, and the presence of other genetic or environmental factors such as sport, characteristics that the majority of desmosomal gene mutations share.

CONCLUSIONS

Our study describes the p.Glu259Glyfs*77 variant in PKP2 gene, previously undescribed and not present among the control groups, which we identified in 8 families of our geographical area in Malaga (Andalusia, Spain) who were initially non-interrelated, and for whom we were able to establish a founder effect in our region. This allowed us to evaluate the genotype-phenotype correlation and describe the clinical and risk characteristics of both the index cases and the family members under follow-up in our HF and ICD Unit.

This variation is a pathogenic mutation associated with ACM; it has incomplete penetrance until advanced ages, and with some male sex-dependent tendency; phenotypic expressivity is extremely variable and heavily influenced by the presence of other genetic or environmental factors, characteristics shared by most mutations in desmosomal genes.

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Abbreviations

ACM: arrhythmogenic cardiomyopathy

ARVD: arrhythmogenic right ventricular dysplasia

CMRI: cardiac magnetic resonance imaging

DCM: dilated cardiomyopathy

DSC2: desmocollin-2

DSG2: desmoglein-2

DSP: desmoplakin

HCM: hypertrophic cardiomyopathy

HF: heart failure

ICD: implantable cardiac defibrillator
JUP: junction plakoglobin
KCNJ2: inward-rectifier potassium
LVEF: Left Ventricular Ejection Fraction
MYBPC3: myosin binding protein C3
NGS: next-generation sequencing
NSVT: non-sustained ventricular tachycardia
PKP2: plakophilin-2
RYR2: ryanodine receptor 2
SD: sudden death
SVT: sustained ventricular tachycardia
TTN: titin

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