Clinical Features and Natural History of PRKAG2 Variant Cardiac Glycogenosis

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This work was supported by grants from Instituto de Salud Carlos III (PI17/ 01941, AC16/0014, PI17/01690, PI18/01582 and PT17/0015/0043); ERA-CVD Joint Transnational Call 2016 (GENPROVIC) to Dr. Garcia-Pavia; the DETECTIN-HF project (ERA-CVD framework) to Dr. Bilinska; the Wellcome Trust (107469/Z/15/); the National Institute for Health Research (NIHR) Royal Brompton Cardiovascular Biomedical Research Unit; the NIHR Imperial Biomedical Research Centre; a Health Innovation Challenge Fund award from the Wellcome Trust and the Department of Health, United Kingdom (HICF-R6-373); the British Heart Foundation (SP/10/10/28431); Obra Social La Caixa Foundation (ID 100010434); and Fundacio Privada Daniel Bravo Andreu. Grants from Instituto de Salud Carlos III and the Spanish Ministry of Economy and Competitiveness are supported by Plan Estatal de I.D.I. 2013–2016, European Regional Development Fund (“A Way of Making Europe”). Dr. Lopes is a recipient of a Medical Research Council Clinical Academic Research Partnership Award. Dr. Asselbergs is supported by the UCL Hospitals NIHR Biomedical Research Centre. Dr. Pezzoli is supported by Fondazione per la Ricerca Ospedale Maggiore. Dr. Kaski is supported by the NIHR Great Ormond Street Hospital Biomedical Research Centre. The views expressed in this publication are those of the authors and not necessarily those of the National Health Service, the NIHR, or the Department of Health. Hospital Universitario Puerta de Hierro, Hospital Universitario Virgen de la Arrixaca, Hospital Sant Joan de Deu, the Great Ormond Street Hospital, and St. Bartholomew’s Hospital are members of the European Reference Network for Rare and Low Prevalence Complex Diseases of the Heart (ERN GUARD-Heart). The authors have reported that they have no relationships relevant to the contents of this paper to disclose.

ABSTRACT

BACKGROUND PRKAG2 gene variants cause a syndrome characterized by cardiomyopathy, conduction disease, and ventricular pre-excitation. Only a small number of cases have been reported to date, and the natural history of the disease is poorly understood.

OBJECTIVES The aim of this study was to describe phenotype and natural history of PRKAG2 variants in a large multicenter European cohort.

METHODS Clinical, electrocardiographic, and echocardiographic data from 90 subjects with PRKAG2 variants (53% men; median age 33 years; interquartile range [IQR]: 15 to 50 years) recruited from 27 centers were retrospectively studied.

RESULTS At first evaluation, 93% of patients were in New York Heart Association functional class I or II. Maximum left ventricular wall thickness was 18 ± 8 mm, and left ventricular ejection fraction was 61 ± 12%. Left ventricular hypertrophy (LVH) was present in 60 subjects (67%) at baseline. Thirty patients (33%) had ventricular pre-excitation or had undergone accessory pathway ablation; 17 (19%) had pacemakers (median age at implantation 36 years; IQR: 27 to 46 years), and 16 (18%) had atrial fibrillation (median age 43 years; IQR: 31 to 54 years). After a median follow-up period of 6 years (IQR: 2.3 to 13.9 years), 71% of subjects had LVH, 29% had AF, 21% required de novo pacemakers (median age at implantation 37 years; IQR: 29 to 48 years), 14% required admission for heart failure, 8% experienced sudden cardiac death or equivalent, 4% required heart transplantation, and 13% died.

CONCLUSIONS PRKAG2 syndrome is a progressive cardiomyopathy characterized by high rates of atrial fibrillation, conduction disease, advanced heart failure, and life-threatening arrhythmias. Classical features of pre-excitation and severe LVH are not uniformly present, and

diagnosis should be considered in patients with LVH who develop atrial fibrillation or require permanent pacemakers at a young age.

ABBREVIATIONS AND ACRONYMS

ACMG = American College of Medical Genetics and Genomics

AF = atrial fibrillation

HCM = hypertrophic cardiomyopathy

HF = heart failure

HTx = heart transplantation

ICD = implantable cardioverter-defibrillator

IQR = interquartile range LV = left ventricular

LVEF = left ventricular ejection fraction

LVH = left ventricular hypertrophy

MACE = major adverse cardiac event(s)

MWT = maximal wall thickness

PPrV = probable pathogenic rare variant

PS = PRKAG2 syndrome SCD = sudden cardiac death

VUS = variant(s) of unknown significance

Hypertrophic cardiomyopathy (HCM) is a predominantly autosomal-dominant disorder associated with increased morbidity and mortality (1). It is characterized by increased cardiac mass, as a result of cardiomyocyte hypertrophy and fibrosis, and is caused mainly by genetic variants in genes encoding sarcomeric proteins. However, 5% to 10% of adult cases of HCM are caused by rare, non-sarcomere-related genetic defects, including inherited neuromuscular and metabolic diseases such as PRKAG2 syndrome (PS) (2). PS is caused by genetic variants in the PRKAG2 gene that encodes the adenosine monophosphate– activated protein kinase gamma 2 regulatory subunit (3). In the heart, PRKAG2 variants result in glycogen accumulation within cardiomyocytes and are classically associated with the triad of severe ventricular hypertrophy, electrocardiographic pre-excitation, and conduction system disease (4). Because of the complex electrophysiological impact of the disease, an incidence of premature (<40 years) sudden cardiac death (SCD) as high as 20% has been suggested (5).

The true prevalence of PS is unknown, and data regarding clinical characteristics and outcomes of patients with PS are scarce, as only a small number of patients have been reported to date (5–8). The aim of this study was to describe the clinical characteristics and natural history of PS by analyzing a large cohort of patients recruited from an international multicenter cardiomyopathy collaboration.

METHODS

STUDY DESIGN AND COHORT COMPOSITION

This was a multicenter, retrospective, longitudinal cohort study consisting of probands and relatives with PRKAG2 genetic variants recruited from 27 European cardiomyopathy centers (Supplemental Table 1). Baseline and follow-up clinical data were collected at each participating center.

The study conformed to the principles of the Declaration of Helsinki. A.L.-S., F.D., and P.G.-P. had access to all data and had final responsibility for submission of the manuscript. The

authors from each participating center guarantee the integrity of data from their institution and had approval from a local ethics committee or internal review board. All investigators have agreed to the manuscript as written.

GENETIC TESTING. Genetic testing in probands was undertaken at participating institutions or at a regional accredited genetics laboratory. Pathogenicity of variants was established according to the current American College of Medical Genetics and Genomics (ACMG) guidelines (9). Variants not fulfilling ACMG criteria for pathogenic or likely pathogenic were classified as probable pathogenic rare variants (PPrVs) and considered causal of PS if they were associated with classical phenotypic expression of the disease and/or typical histological findings on endomyocardial biopsy (Figure 1) and exhibited a minor allele frequency of $<1 \times 10^{-4}$ in the ExAC database (10). The complete list of genetic variants with interpretations and genetic classification is available in Supplemental Table 2.

CLINICAL EVALUATION AND FOLLOW-UP. Clinical data and cardiac test results were extracted from available hospital records. Patients were considered clinically affected by PS if they had 1 or more of the following: otherwise unexplained left ventricular hypertrophy (LVH) (maximal left ventricular [LV] thickness $13 mm), LV ejection fraction (LVEF) <50%, advanced conduction disorders, sustained ventricular tachycardia, supraventricular arrhythmias (atrial fibrillation [AF] or atrial flutter or supraventricular tachycardia), electrocardiographic abnormalities (including pre-excitation, conduction disease, and repolarization abnormalities), or skeletal myopathy. Carriers of PRKAG2 variants with none of these findings were considered nonaffected.

Standard 12-lead electrocardiographic recordings at baseline and follow-up were examined. Ventricular pre-excitation on electrocardiography was diagnosed on the basis of a short PR interval (.120 ms) with a widened QRS complex (110 ms) or with an abnormal delta wave; Wolff-Parkinson-White syndrome was defined by the presence of pre-excitation associated with

supraventricular arrhythmia. Sokolov-Lyon index criteria were used to evaluate LVH on electrocardiography.

Creatine kinase and N-terminal pro–brain natriuretic peptide levels at the baseline visit were recorded when available.

Details of clinical events prior to first clinical contact and during follow-up (including the timing of events) were collected. Events were characterized as follows: new-onset AF, de novo pacemaker implantation, LV assist device implantation, heart transplantation (HTx), sustained ventricular tachycardia, successfully resuscitated ventricular fibrillation, appropriate implantable cardioverter-defibrillator (ICD) shock, SCD, and cardiac and all-cause mortality. SCD was defined as an unexpected death due to cardiac causes occurring within 1 h of the onset of symptoms. Hospitalizations for heart failure (HF) were also registered. Major adverse cardiac events (MACE) were defined as a composite of appropriate ICD shock, aborted SCD, SCD, HTx, LV assist device implantation, and pacemaker implantation.

STATISTICAL ANALYSIS. Results are presented as mean ± SD for continuous variables with normal distributions, as median (interquartile range [IQR]) for continuous variables without normal distributions, and as number (percentage) for categorical data. For statistical analysis, Student's t-test and the Mann-Whitney U nonparametric test were used in 2-group comparisons. The chi-square test or Fisher exact test was used for categorical variables. The cumulative probability of the occurrence of clinical events was estimated by using the Kaplan-Meier method, and factors were compared using the log-rank (Mantel-Cox) method. Statistical analyses were performed using SPSS Statistics version 20.0 (IBM, Armonk, New York).

RESULTS

The study cohort comprised 90 subjects (53% men; median age at first evaluation 33 years; IQR: 15 to 50 years) from 47 families (median subjects per family 3). Forty-seven patients (52.2%) were probands and 43 (47.8%) were relatives. All but 2 patients were Caucasians of European ancestry. Most subjects (98%) carried missense variants (Supplemental Table 2). After a comprehensive analysis of the main genes currently associated with HCM, no additional rare variants were identified in any of the probands included in our study. The 2 non-Caucasian patients were men from Pakistan and India, respectively. Neither of them showed pre-excitation on electrocardiography, and maximal wall thickness (MWT) was 15 and 18 mm, respectively. The patient from Pakistan had a pacemaker implanted at 38 years of age because of advanced atrioventricular block and carried a PPrV (p.His401Asp) not described in ExAC or ClinVar. The other patient carried a frameshift variant classified as pathogenic (p.Leu352Lysfs*6).

CLINICAL CHARACTERISTICS. At first evaluation, 71% of the 90 subjects (n 1/4 64; 56% men; median age 37 years; IQR: 18 to 50 years) had evidence consistent with the PS and were considered clinically affected; the remaining 26 were considered nonaffected carriers (Table 1). Probands (n 1/4 47) had a median age of 40 years (IQR: 19 to 54 years), and 60% were men. Their mean MWT was 20 ± 8 mm, and 32% had pre-excitation.

Patients with mild (MWT <15 mm) or normal phenotype (n 1/4 33) carried PPrVs in 36% of cases, compared with 28% of individuals with more severe expression of disease (p 1/4 0.50). This group of patients with a milder phenotype were mostly relatives (n 1/4 24 [73%]) and had median age of 33 years (IQR: 13 to 52 years) (compared with a median age of 41 years [IQR: 23 to 54 years] in the group with a more severe phenotype; p 1/4 0.38).

Almost 40% of patients in the entire cohort (n 1/4 90) reported a family history of SCD in a first-degree relative, 18% had either a history of or current AF, and 4% had had strokes (Table 1).

Most subjects (n = 74 [82%]) were in sinus rhythm on their first available electrocardiogram. Mean PR interval duration was 120 ± 49 ms, and 35 patients (39%) had PR intervals shorter than 120 ms. Only 30 patients (33%) showed pre-excitation patterns, and 7 (8%) had undergone accessory pathway ablation previously; 13% showed first-degree atrioventricular block. Mean QRS interval duration was mildly prolonged (126 ± 36 ms), and left or right bundle branch block was common (19% and 13%, respectively). At first evaluation, 17 patients (19%) already had permanent pacemakers implanted, and 3 subjects had ICDs (including 1 for secondary prevention).

Symptoms of skeletal muscular involvement such as proximal muscle weakness or myalgia were seldom reported (2% of patients); 19 subjects (21%) had increased creatine kinase levels (>90 U/l).

Most of the 64 affected subjects (93%) were in New York Heart Association functional class I or II, and only 4 (6%) had LVEFs <50%. Overall, 60 of the 64 affected individuals at baseline evaluation (94%; 55% men; median age 37 years; IQR: 17 to 50 years) had LVH, with mean MWT $13 mm, including 50 (83%; 60% men; median age 36 years; IQR: 19 to 50 years) with LVH that was in the range of HCM (MWT $15 mm, mean 20 ± 8 mm). Mean LVEF in these 50 subjects was 60 ± 12%, and mean left atrial diameter was 41 ± 10 mm. None had LV outflow tract obstruction >30 mm Hg or evidence of systolic anterior movement of the mitral valve.

NATURAL HISTORY AND CLINICAL EVENTS. During a median follow-up period of 6 years (IQR: 2.3 to 13.9 years), 4 of 26 initially unaffected subjects (15%) developed LVH and AF; 2 had LVEFs in the lower range of normality (50%) on their last echocardiograms.

In the entire cohort (n = 90), arrhythmic complications were common. Of note, 14% of subjects who were in sinus rhythm at baseline evaluation developed AF, and the total prevalence of AF at the end of follow-up in the entire cohort was 29% (n = 26). Age at AF

onset was very young, with an average of 43 ± 16 years. Interestingly, 32% presented the first episode before 35 years of age (median age 43 years, IQR: 31 to 54 years).

Fifteen patients (21%) without conduction disease at baseline required permanent pacemakers during follow-up (median age at implantation 37 years; IQR: 29 to 48 years). The main indication was advanced atrioventricular block in 8 subjects (53%). Notably, 2 subjects required pacemakers after accessory pathway ablation. The total proportion of subjects with pacemaker at the end of follow-up in the entire cohort was 36% (32 of 90 subjects), with a mean age at implantation of 37 ± 16 years (median 37 years; IQR: 28 to 48 years). Finally, a total of 19 patients (21%) received ICDs during follow-up, including 4 for secondary prevention following SCD events.

Ten of 68 affected patients at last follow-up (15%) had LVEFs <50%; 13 (19% of affected) were admitted with HF (median age at first admission 49 years; IQR: 33 to 73 years), and 4 patients (6%) required HTx (mean age 37 ± 17 years).

Twelve subjects in the entire cohort (13%) died during follow-up (median age 52 years; IQR: 35 to 60 years). Causes of death in the affected patients included SCD in 3 (25%), end-stage HF in 2, and stroke in 2. A total of 5 nonaffected subjects died (1 because of sepsis and respiratory failure, unknown causes in the other 4) (Figure 2).

Table 2 shows the clinical, electrocardiographic, and echocardiographic parameters at last evaluation in the entire cohort and in affected and unaffected subjects. Event rates at the end of follow-up in the entire cohort and in affected subjects are shown in Figure 3. Median age free of MACE and death was 64 years (95% confidence interval: 53 to 75 years), and median age free of MACE, death, and AF was 52 years (95% confidence interval: 42.5 to 61.5 years) (Figure 4, Central Illustration).

We did not find differences in baseline characteristics and events according to sex, except that women exhibited shorter PR intervals (136 ± 40 ms vs. 115 ± 30 ms; p = 0.002) (Supplemental

Table 3). However, mean MWT at diagnosis was significantly increased and LVEF at baseline significantly decreased in subjects with MACE during follow-up (20 ± 9 mm vs. 16 ± 7 mm [p = 0.04] and 55 ± 16% vs. 64 ± 8% [p = 0.01], respectively). Pre-excitation was not associated with MACE in our cohort, with similar baseline PR intervals in patients with and without events (131 ± 63 mm vs. 115 ± 43 mm; p = 0.30).

Because 2 of the rare genetic variants (p.Arg302Gln and p.Asn488Ile) were present in 44% of the patients included in the cohort, we compared subjects with these genetic variants with those with other rare genetic variants (Table 3). The 32 subjects who carried the p.Arg302Gln variant belong to 10 different families from 6 different countries (Spain, Italy, Israel, Denmark, Portugal, and the United Kingdom), with a median of 3 subjects per family (IQR: 1 to 4). The 7 subjects who carried the p.Asn488Ile variant come from 2 different families in the United Kingdom (1 with 1 subject and the other with 6 subjects).

Patients with these 2 genetic variants exhibited pre-excitation more frequently and had a lower prevalence of syncope but otherwise showed a very similar clinical profile. There were no differences in cardiovascular event rates during follow-up, with the exception of AF, which was more common in patients with the most common 2 variants (Table 3).

At the end of follow-up, 76% of patients (68 of 90) had signs and symptoms of PS, but penetrance of PS was only 31% at 40 years of age or less.

DISCUSSION

This study shows that patients with PRKAG2 genetic variants have a poor prognosis with a high rate of complications including juvenile onset of conduction disease, advanced HF, and potentially lethal arrhythmias (Central Illustration). The detailed phenotypic characterization of our cohort reveals that the classical features of PS, such as pre-excitation and severe LVH, are not uniformly present in affected patients, while AF is particularly common and presents almost a decade earlier than in sarcomeric HCM; unaffected subjects may develop a clinical

phenotype relatively late in life, although mean age at onset of PS manifestations in affected subjects generally occurs between the third and fifth decade of life.

PS is a rare disease that is mostly identified as a phenocopy of HCM. Patients with clinical features of PS were initially described in the second half of the 20th century, but it was not until 2001 that the responsible gene was identified (3). Since then, several case series and small patient cohorts have been reported (5–8). Most underline the classical triad of severe cardiac hypertrophy, electrocardiographic pre-excitation, and conduction system disease. However, our findings show that the PS phenotype is quite heterogeneous, ranging from severe presentation in infancy to cases of late-onset mild LVH (Figure 5). Similarly, although PS is classically associated with severe LVH, fewer than one-half of the affected subjects in our cohort had LVH $20 mm.

The prognosis of HCM phenocopies associated with defects in glycogen metabolism is generally worse than that of disease caused by sarcomeric protein gene variants (5,11). Danon disease is an X-linked disease in which hemizygous men do not have any unaltered copy of the LAMP2 gene and have a worse prognosis than women, who are heterozygotes for the genetic defect (11). The prognosis of patients with PRKAG2 genetic variants in our cohort was better than in Danon disease, particularly for men (PS is an autosomal-dominant disease, and no differences in phenotype were observed related to sex), but still poor compared with sarcomeric HCM (11,12).

Notably, patients with PS are burdened with a high incidence of HF and sudden death. SCD occurred in 3 subjects, and 4 additional subjects received ICDs for resuscitated SCD, for a total prevalence of SCD in the entire cohort of 8% (9% if the patient with an ICD for secondary prevention at baseline evaluation is considered). Clinical characteristics of these patients are shown in Supplemental Table 4.

The cause of SCD in patients with PS is likely to be multifactorial, with advanced heart block (13) and ventricular fibrillation due to rapid conduction through accessory pathways

(5) as possible triggers. It has been speculated that in younger patients, SCD might be secondary to the degeneration of rapid supraventricular arrhythmias (14) and, in patients older than 30 years, due to cardiac conduction system disease and asystole (15). Because of the lack of adequate patient cohorts with sufficient follow-up data, risk stratification for SCD remains challenging in PS, and the decision to implant an ICD in primary prevention should be made on an individual basis. In our cohort, the decision for prophylactic ICD implantation at participating centers was made taking into account phenotype and family history of SCD, particularly in symptomatic patients with unexplained syncope.

A total of 4 patients required HTx and 2 died of end-stage HF in our study (7% of the whole cohort, 8% of affected subjects), and 9 (13% of the affected patients) were in New York Heart Association functional class III or IV (Supplemental Table 4). Few studies have reported data on HTx or advanced HF in patients with PS (3,12,16), but comparing our data with those reported in HCM series (12) (1.6% and 2.5% of HTx and HF death, respectively), it appears that advanced HF complication rates are worse in patients with PS than in other patients with HCM.

Considering global MACE, MWT and LVEF at baseline proved to be prognostic markers. Both features are also related to worse outcomes in patients with HCM (17), but it is interesting to note that the mean LVEF of patients with events was 55%, suggesting that values at the lower limit of the normal range could already have clinical implications.

Compared with previously published series (5,6,13), our cohort displayed considerable genetic heterogeneity, with a total of 26 rare unique genetic variants, most of which were missense. A number of the rare genetic variants included in our study do not fulfil ACMG criteria for pathogenic or likely pathogenic but were included on the basis of a classical PS phenotype or typical histological findings and very low minor allele frequency. Although still the standard for genetic interpretation, the validity of ACMG criteria in classifying some rare gene variants in specific cardiovascular-related genes has been questioned (18). ACMG

or any other criteria for calling the pathogenicity of gene variants should always be considered in combination with expert review and clinical judgment. Most of the patients with PPrVs in our study had rare variants that had not been previously reported and that cosegregated with phenotype in the families. The ACMG criteria for variant classification provide a framework for genetic variant interpretation but might not be as useful in patients with prominent characteristics linked to certain syndromes, as happens in PS. The ACMG criteria would probably need an adaptation for PS, as has occurred already with FBN1 variants in Marfan's syndrome (19) or more recently with MYH7 in dilated cardiomyopathy (20).

In our opinion, the diagnosis of PS should always be made on the basis of genetic findings, but specific clinical characteristics and positive cosegregation in the family should have a strong role in interpreting variants of unknown significance (VUS) found in the PRKAG2 gene. Moreover, when interpreting a PRKAG2 VUS, it is important to consider the patient's clinical context. Incidental findings of VUS in unaffected patients without family histories of LVH, conduction disease, or atrial arrhythmia and VUS in patients with other phenotypes should not be considered disease-causing variants. In any case, longitudinal follow-up is highly recommended in these patients in order to monitor the phenotypic expression of PS and enable possible variant reclassification.

In this regard, we are confident that the variants classified as PPrVs in our study represent PS-causing variants. In fact, clinical characteristics and event rates between patients with pathogenic or likely pathogenic rare genetic variants and those with PPrVs did not differ (Table 4).

PS might be a suitable candidate disease for enzymatic replacement therapy or for a gene therapy approach, as in other lysosomal storage HCM phenocopies also caused by enzymatic defects. Fabry disease and Pompe disease already have approved enzyme

replacement therapies (21), and gene therapy clinical trials are currently being conducted in Danon disease (NCT03882437) and Fabry disease (NCT04040049). In this regard, our study would be useful in designing appropriate clinical trials for PS in the future.

Although there is no specific treatment for PS yet, this study shows that lifelong follow-up of genetic carriers is necessary, considering the high incidence rate of cardiovascular events. In our study, 15% of subjects who were unaffected at baseline went on to develop signs of the disease during a median follow-up period of just 2.8 years. Some developed substantial cardiac involvement and all had AF, highlighting the need for regular surveillance with ambulatory electrocardiography. Furthermore, frequent ambulatory electrocardiography should also be recommended in affected patients given the high rate of atrial arrhythmia and conduction disorders found in this study.

STUDY LIMITATIONS. The study was not designed to evaluate treatment effects. Causes of death were not available in all nonaffected genetic carriers. The study was subject to selection and referral bias, as the participating centers are all specialized cardiomyopathy centers. Furthermore, even though this cohort is the largest PS cohort published to date, given the rarity of this disease, the reduced number of subjects included limits the possibility of identifying prognostic factors.

CONCLUSIONS

PS is a severe progressive cardiac disease characterized by a high rate of complications, including atrial arrhythmias, conduction disease, advanced HF, and SCD at a young age. Affected patients should be closely monitored to facilitate early detection of arrhythmia and conduction problems. PS should be considered in patients with LVH who develop AF or require permanent pacemakers at a young age. Early recognition is important to allow prompt identification and appropriate management of genetic carriers.

COMPETENCY IN MEDICAL KNOWLEDGE: Variants of the PRKAG2 gene cause a syndrome of cardiac glycogenosis that is characterized by progressive cardiomyopathy associated with a high incidence of heart failure, AF, conduction system disease, and life-threatening arrhythmias. The clinical characteristics are heterogeneous and the classical findings of pre-excitation and severe LVH are not uniformly present, but the diagnosis should be considered in young patients with LVH who develop AF or require pacemaker implantation.

TRANSLATIONAL OUTLOOK: Because penetrance is incomplete and expressivity variable, further studies are needed to identify the factors causing some individuals with PRKAG2 syndrome to experience cardiac events, specifically arrhythmic complications. In the future, enzyme replacement therapy may become feasible, but efficacy and safety would require validation in clinical trials.

	Entire Cohort (N = 90)	Affected (n = 64)	Nonaffected (n = 26)
Male	48 (53)	36 (56)	12 (46)
Age, yrs	33 (15–50)	37 (18–50)	18 (9-39)
Family history of SCD	35 (39)	24 (38)	11 (46)
Stroke	4 (5)	4 (6)	0 (0)
Myopathy	2 (2)	2 (3)	0 (0)
Syncope	28 (32)	24 (38)	4 (18)
Chest pain	15 (17)	10 (16)	5 (22)
Palpitations	41 (49)	31 (50)	10 (46)
CK, U/l	79 (56–117)	106 (2–365)	66 (2-130)
NYHA functional class III	6 (7)	5 (8)	1 (5)*
NT-proBNP, pg/ml	120 (21–1200)	170 (37–2168)	47 (10-224)
Pre-excitation	30 (33)	30 (44)	0 (0)
QRS, ms	126 ± 36	131 ± 37	108 ± 26
Atrial fibrillation	16 (18)	16 (25)	0 (0)
LVH on ECG	43 (49)	37 (64)	6 (38)
LV MWT, mm	18 ± 8	20 ± 8	10 ± 2
LA diameter, mm	39 ± 8	41 ± 8	33 ± 5
LVEF, %	61 ± 12	60 ± 13	66 ± 8
PPrV	28 (31)	20 (31)	8 (31)

TABLE 1 Clinical characteristics at baseline evaluation in patients with PRKAG2 variants.

Values are n (%), median (interquartile range), or mean ± SD. *Cardiogenic shock in a pre-term newborn due to sepsis.

CK = creatine kinase; ECG = electrocardiography; LA = left atrial; LV = left ventricular; LVEF = left ventricular ejection fraction; LVH = left ventricular hypertrophy; MWT = maximal wall thickness; NT-proBNP = N-terminal pro–brain natriuretic peptide; NYHA = New York Heart Association; PPrV = probably pathogenic rare variant; SCD = sudden cardiac death.

	Entire Cohort (N 1/4 90)	Affected (n 1/4 68)	Nonaffected (n 1/4 22)
Male	48 (53)	38 (56)	10 (50)
Age, yrs	42 (25–58)	43 (31–59)	28 (14–44)
NYHA functional class III or IV	10 (11)	9 (13)	1 (5)
Atrial fibrillation	26 (29)	26 (39)	0 (0)
LV MWT, mm	17 ± 7	19 ± 7	10 ± 3
LA diameter, mm	39 ± 10	42 ± 9	31 ± 7
LVEF, %	59 ± 13	57 ± 13	68 ± 7
LVEF <50%	10 (11)	10 (15)	0 (0)

TABLE 2 Clinical, electrocardiographic, and echocardiographic parameters at last evaluation in the entire cohort and in affected and unaffected individuals. Values are n (%), median (interquartile range), or mean + SD. Abbreviations as in Table 1.

	p.Arg302Gln p.Asn488Ile (n 1/4 39)	Other PRKAG2 Variants (n 1/4 51)	p Value
Baseline characteristics			
Males	22 (56)	26 (51)	NS
Age, yrs	32 (15–44)	36 (14–50)	NS
Family history of SCD	14/38 (36)	21/49 (43)	NS
Stroke	1/38 (3)	3/49 (6)	NS
Syncope	10/38 (20)	18/49 (47)	0.008
Chest pain	7/38 (18)	8/49 (16)	NS
Palpitations	20/38 (53)	21/49 (46)	NS
Affected	28 (72)	36 (71)	NS
Myopathy	2 /39 (5)	0 (0)	NS
CK level, U/l (range)	56 (13–81)	98 (76–134)	NS
Pre-excitation	17/34 (50)	13/47 (28)	0.002
LVH on ECG	19/33 (58)	24/43 (56)	NS
PR interval, ms	103 ± 52	131 ± 45	NS
LV MWT, mm	19 ± 10	17 ± 7	NS
LVEF, %	57 ± 14	62 ± 11	NS
LVEF <50%	5/37 (13)	5/44 (10)	NS
Follow-up			
Pacemaker implantation	15 (38)	17 (33)	NS
Sudden cardiac death	3 (8)	0 (0)	NS
Heart transplantation	1 (3)	3 (6)	NS
Death	3 (8)	9 (18)	NS
Heart failure hospitalization	4 (11)	9 (18)	NS
Atrial fibrillation	17 (46)	9 (18)	0.009

TABLE 3 Clinical characteristics and events according to underlying genetic cause. Values are n (%), median (interquartile range), n/N (%), or mean + SD. Abbreviations as in Table 1.

	Pathogenic Likely Variants (n 1/4 62)	Pathogenic or Probably Pathogenic Variants (n 1/4 28)	p Value
Male	34 (55)	15 (53)	NS
Age at first evaluation, yrs	32 (14–49)	40 (17–53)	NS
Family history of SCD	22 (35)	13 (46)	NS
Stroke	4 (6)	0 (0)	NS
Myopathy	2 (5)	0 (0)	NS
Syncope	20 (32)	8 (29)	NS
Chest pain	13 (21)	2 (32)	NS
Palpitations	32 (52)	9 (58)	NS
Pre-excitation	21 (34)	9 (32)	NS
Atrial fibrillation	11 (18)	5 (18)	NS
LVH in ECG	30 (48)	13 (46)	NS
PR interval, ms	111 ± 47	139 ± 51	NS
LVEF, %	62 ± 11	57 ± 14	NS
LV MWT, mm	18 ± 9	16 ± 6	NS
Follow-up			
Pacemaker implantation	23 (37)	9 (32)	NS
Sudden cardiac death	2 (3)	1 (4)	NS
Heart transplantation	3 (5)	1 (4)	NS
Death	8 (13)	4 (14)	NS
Heart failure hospitalization	8 (13)	5 (18)	NS
Atrial fibrillation	20 (32)	6 (21)	NS

TABLE 4 Clinical characteristics and events during follow-up in patients with pathogenic or likely pathogenic variants according to ACMG criteria and in those with probably pathogenic PRKAG2 rare genetic variants. Values are n (%), median (interquartile range), or mean + SD. Abbreviations as in Table 1.

FIGURE 1 Typical histological findings in patients with PRKAG2 syndrome. (A,B) Hematoxylin-eosin staining displaying hypertrophied myocytes. Magnification 200x and 400x. (C,D) Periodic acid Schiff (PAS) staining positive for glycogen accumulation in cardiomyocyte vacuoles. Magnification 200x and 400x. Yellow arrowheads indicate $PAS^{þ}$ deposits, corresponding to glycogen. Images courtesy of Dr. Clara Salas, Department of Pathology, Hospital Universitario Puerta de Hierro Majadahonda.

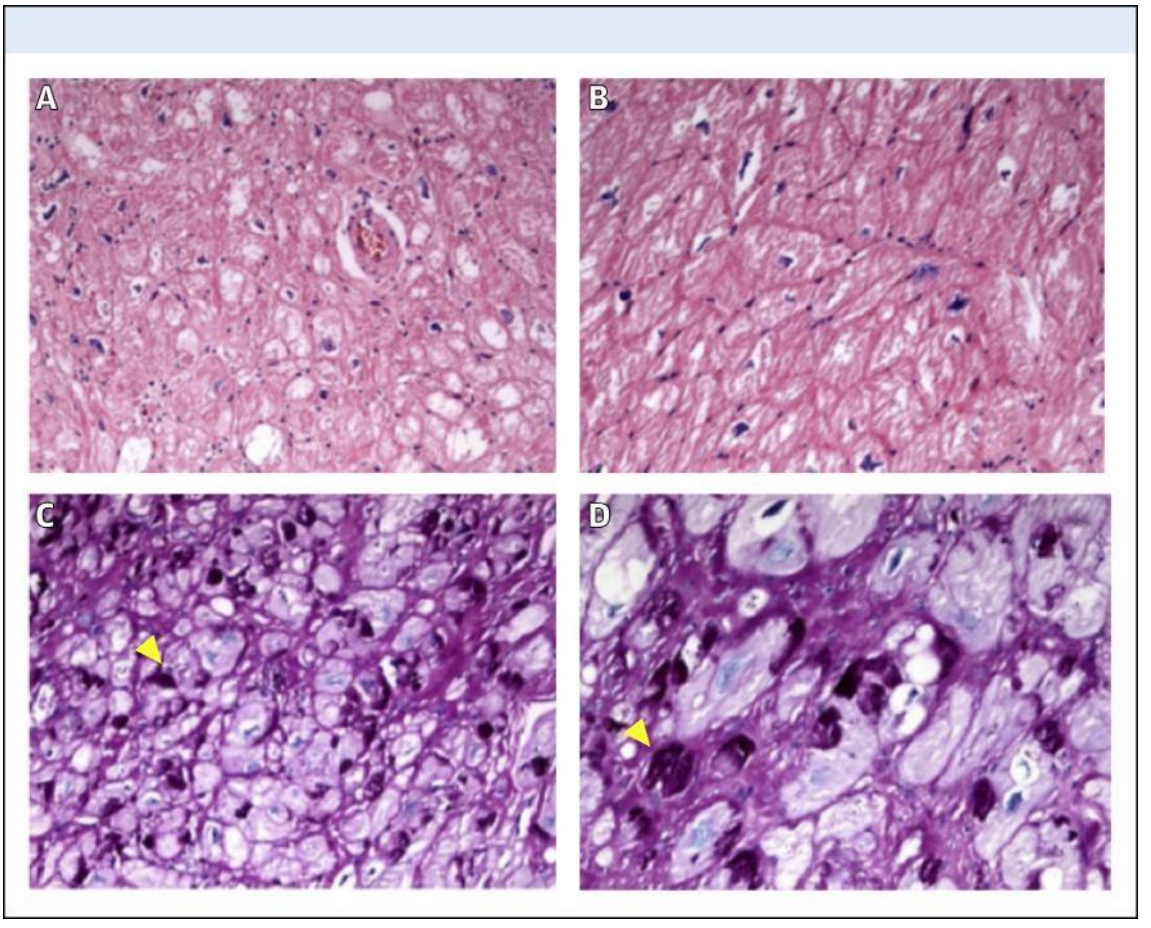
FIGURE 2 Flowchart of the subjects included in the study. Clinical events and phenotype of subjects during the study. Affected patients were subjects with 1 or more of the following: unexplained left ventricular hypertrophy, left ventricular ejection fraction <50%, advanced conduction disorders, sustained ventricular tachycardia, supraventricular arrhythmias, electrocardiographic abnormalities, or skeletal myopathy. AF 1/4 atrial fibrillation; HF 1/4 heart failure; HTx 1/4 heart transplantation; PM 1/4 pacemaker; SCD 1/4 sudden cardiac death.

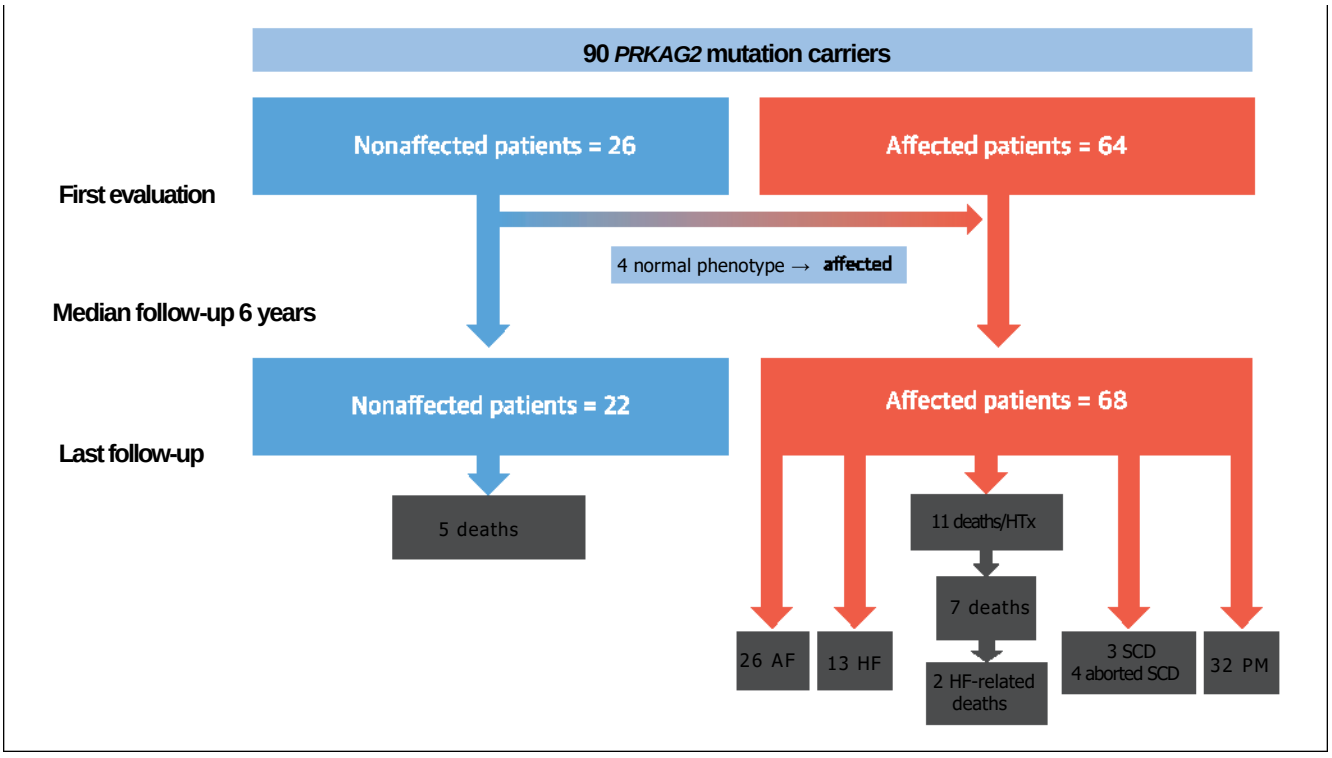
FIGURE 3 Prevalence of different complications in 90 subjects with PRKAG2 variants after a median follow-Up period of 6 years. Affected patients were subjects with 1 or more of the following: unexplained left ventricular hypertrophy, left ventricular ejection fraction <50%, advanced conduction disorders, sustained ventricular tachycardia, supraventricular arrhythmias, electrocardiographic abnormalities, or skeletal myopathy. Abbreviations as in Figure 2.

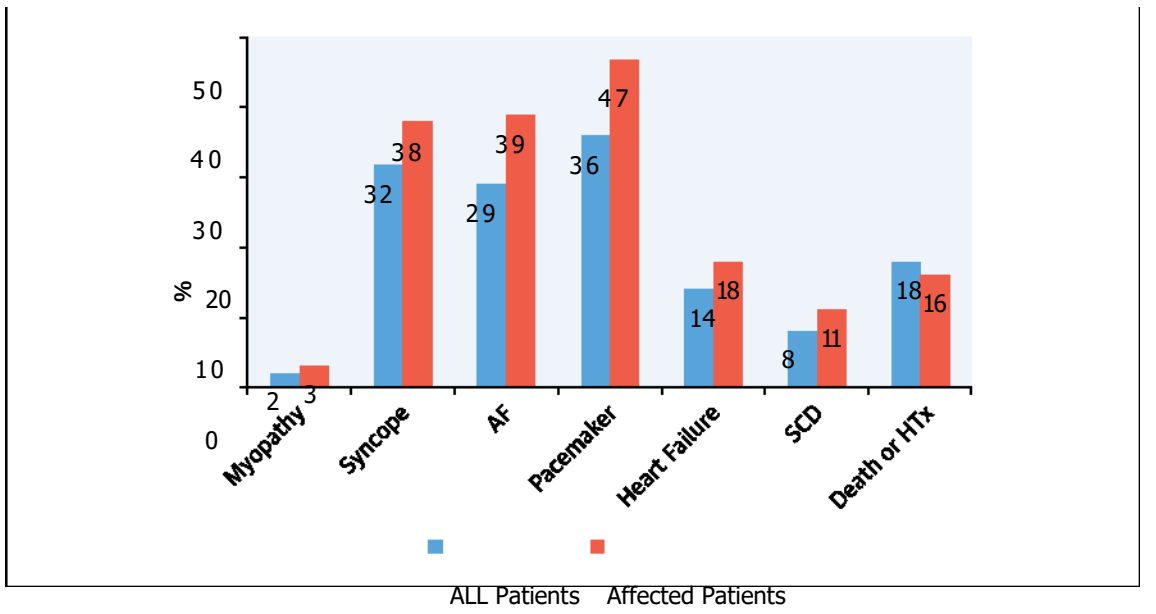
FIGURE 4 Survival curves in 90 subjects with PRKAG2 variants. Blue line indicates freedom of major adverse cardiac events (MACE) and death; red line indicates freedom of MACE, death, and atrial fibrillation (AF). MACE include sudden cardiac death (SCD), aborted SCD, appropriate implantable cardioverter-defibrillator discharge, heart failure hospitalization, heart transplantation, and pacemaker implantation.

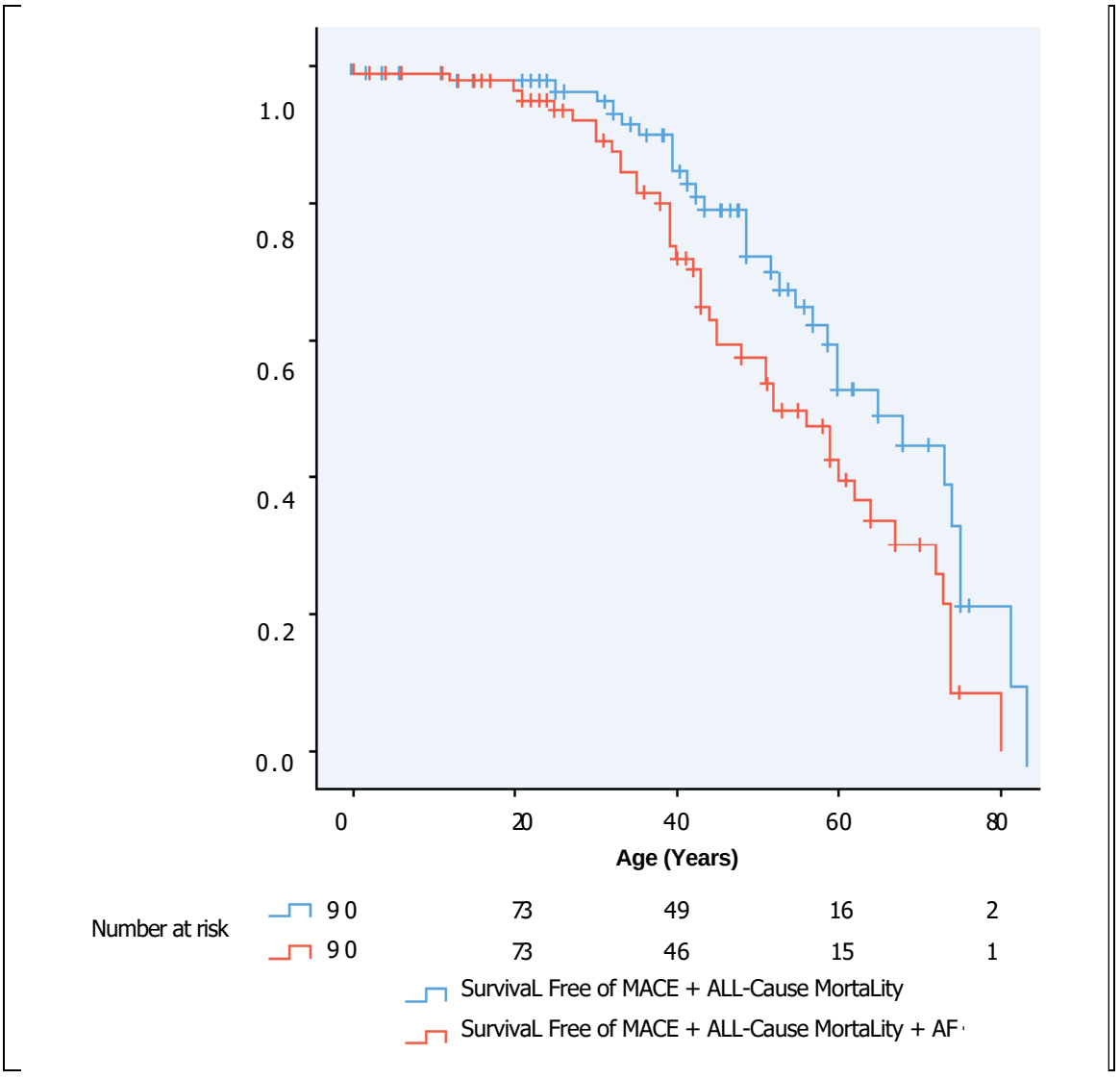
FIGURE 5 Clinical diversity of PRKAG2 syndrome. (A,B) Electrocardiogram and 2-dimensional echocardiogram of a 51-year-old patient with a PRKAG2 p.Glu342Gln variant showing pre-excitation and mild left ventricular hypertrophy. (C) Parasternal short-axis view of a 22-year-old man with the p.Arg302Gln PRKAG2 variant and a more severe phenotype (septal thickness 35 mm). (D) Late gadolinium enhanced cardiac magnetic resonance image of the patient in C 11 years after first assessment showing severe fibrosis in the intraventricular septum.

Central Illustration. Manifestations, survival curve free of major adverse cardiac events and death, and outcomes in 90 subjects with variants in the PRKAG2 gene. Major adverse cardiac events were a composite of appropriate implantable cardioverter-defibrillator shock, aborted sudden cardiac death (SCD), SCD, heart transplantation, left ventricular assist device implantation, and pacemaker implantation. Affected patients were subjects with 1 or more of the following: unexplained left ventricular hypertrophy, left ventricular ejection fraction <50%, advanced conduction disorders, sustained ventricular tachycardia, supraventricular arrhythmias, electrocardiographic abnormalities, or skeletal myopathy.


A
B
C
D


90 PRKAG2 mutation carriers
First evaluation
Nonaffected patients = 26
Affected patients = 64
4 normal phenotype → affected
Median follow-up 6 years
Last follow-up
Nonaffected patients = 22
Affected patients = 68
5 deaths
11 deaths/HTx
7 deaths
2 HF-related deaths
26 AF
13 HF
3 SCD
4 aborted SCD
32 PM

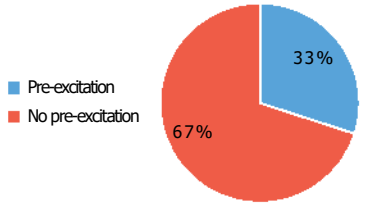

50
40
30
20
10
0
%
2
3
32
38
29
39
36
47
14
18
8
11
18
16
Myopathy
Syncope
AF
Pacemaker
Heart Failure
SCD
Death or HTx
ALL Patients
Affected Patients


1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
Age (Years)
Number at risk
90 73 49 16 2
90 73 46 15 1
SurvivaL Free of MACE + ALL-Cause MortaLity
SurvivaL Free of MACE + ALL-Cause MortaLity + AF

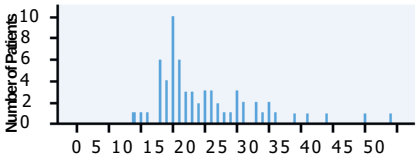
PRKAG2 SYNDROME

CLASSICAL FEATURES

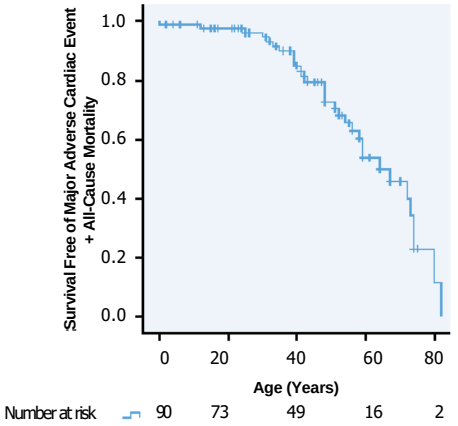
Pre-excitation in Electrocardiography



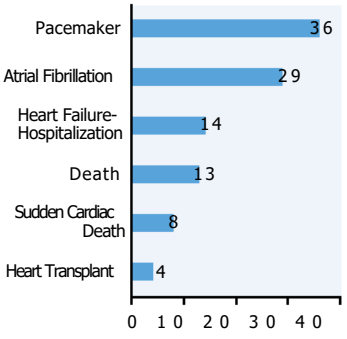
Maximal Left Ventricular Wall Thickness in Affected Patients (mm)

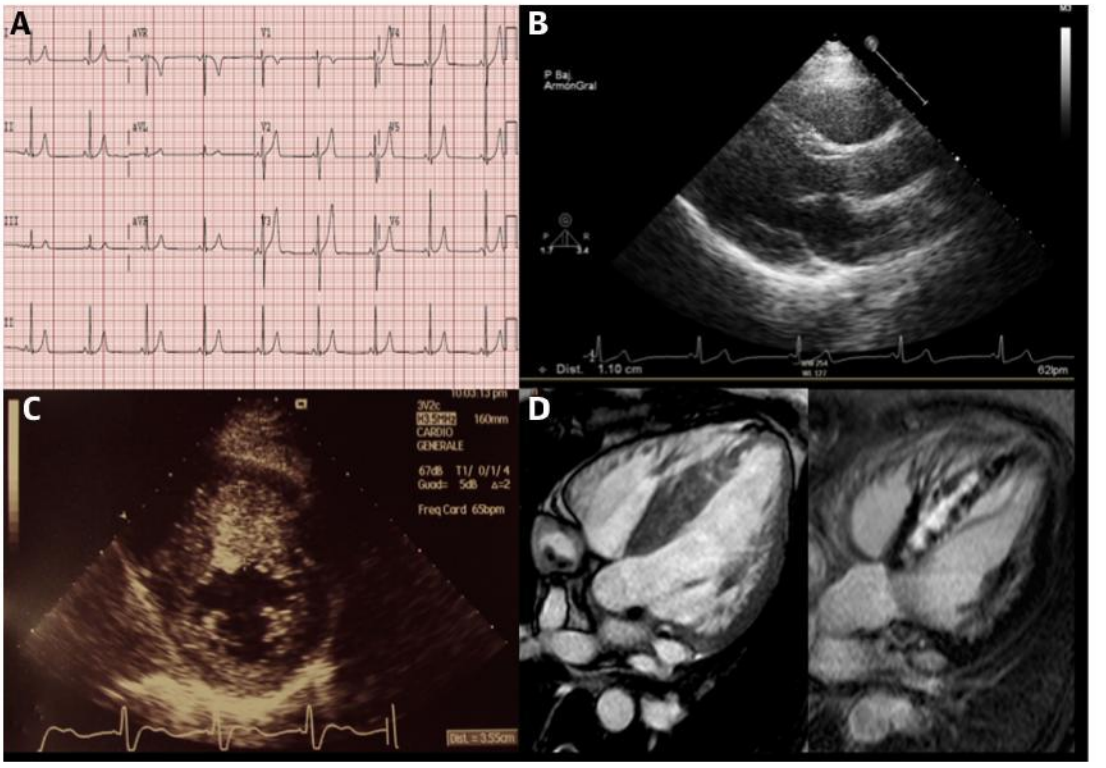


✓ 63% of patients with left ventricular wall thickness <20 mm



EVENTS AT END OF FOLLOW-UP (%)




A
B
P Baj
ArmónGral
Dist. 1.10 cm
62bpm
C
3V2c
160mm
CARDIO
GENERALE
67dB T1/ 0/1/4
Guad= 5dB A=2
Freq Card 65bpm
Dist. = 3.55cm
D

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