Clinical Features and Natural History of cardiac glycogenosis due to PRKAG2 variants

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ABSTRACT:

Background:

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

Objectives:

To describe phenotype and natural history of *PRKAG2* variants in a large multicenter European cohort.

Methods:

We retrospectively studied clinical, electrocardiographic and echocardiographic data from 90 individuals with *PRKAG2* variants (53% males, median 33 years (IQR: 15-50) recruited from 27 centers.

Results:

At first evaluation, 93% of patients were in NYHA functional class I or II. Maximum left ventricular (LV) wall thickness was 18±8 mm and LV ejection fraction was 61±12%. LV hypertrophy (LVH) was present in 60 (67%) subjects at baseline. Thirty patients (33%) had ventricular preexcitation or had undergone an accessory pathway ablation; 17 (19%) had a pacemaker (median age at implantation 36 years (IQR: 27-46)) and 16 (18%) had atrial fibrillation (AF) (median age 43 years (IQR: 31-54)).

After a median follow-up of 6 years (IQR:2.3-13.9), 71% of individuals had LVH, 29% had AF, 21% a de novo pacemaker (median age at implantation 37 years (IQR: 29-48)), 14% required admission for heart failure (HF), 8% experienced sudden cardiac death or equivalent, 4% required a heart transplant and 13% died.

Conclusions:

PRKAG2 syndrome is a progressive cardiomyopathy characterized by high rates of AF, conduction disease, advanced HF and life-threatening arrhythmias. Classical features of preexcitation and severe LVH are not uniformly present and diagnosis should be considered in patients with LVH who develop AF or require a PPM at a young age.

CONDENSED ABSTRACT

PRKAG2 gene variants cause a syndrome characterised by cardiomyopathy, conduction disease and ventricular preexcitation. We describe phenotypes and outcomes in a cohort of 90 individuals with a *PRKAG2* variants (53% males, median age 33 years (IQR: 15-50) followed at 27 European centers. After a median follow-up of 6 years (IQR: 2.3-13.9), 51 individuals (57%) had developed cardiovascular complications including conduction disease, atrial fibrillation, heart failure, SCD and heart transplant (36%, 29%, 14%, 8% and 4%, respectively). Classical features described in patients with this condition such as preexcitation and LVH were not uniformly present (41% and 67% at baseline, respectively).

KEY WORDS:

Glycogen-storage disease, Heart failure, hypertrophic cardiomyopathy, left ventricular hypertrophy, pre-excitation, PRKAG2, sudden cardiac death, pacemaker.

ABBREVIATIONS LIST

AF: Atrial fibrillation
HCM: Hypertrophic Cardiomyopathy
HT: Heart transplant
LVH: Left ventricular hypertrophy
LVEF: Left ventricular ejection fraction
MWT: Maximal wall thickness

NYHA: New York Heart Association
PS: PRKAG2 syndrome.
SCD: Sudden Cardiac Death
SVT: Sustained ventricular tachycardia

INTRODUCTION

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 AMP-activated protein kinase (AMPK) 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, ECG pre-excitation and conduction system disease (4). Due to the complex electrophysiologic 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 PS patients are scarce, as only a small number of individuals have been reported to date (5–8). This study sought 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 is a multicenter, retrospective longitudinal cohort study consisting of probands and relatives with *PRKAG2* genetic variants recruited from 27 European cardiomyopathy centers (Online Table 1). Baseline and follow-up clinical data were collected at each participating center.

The study conforms to the principles of the Helsinki declaration. ALS, FD and PGP had access to all the 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 / 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 Pathogenic/Likely pathogenic ACMG criteria, were classified as Probable pathogenic rare variants (PPrV) 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 (MAF) of $<1x10^{-4}$ in the ExAC database (10). The complete list of genetic variants with interpretations and genetic classification is available in online supplementary Appendix Table 2.

Clinical evaluation and follow-up

Clinical data and cardiac tests results were extracted from available hospital records. Individuals were considered clinically affected by PS if they had one or more of the following: otherwise unexplained left ventricular hypertrophy (LVH) (maximal LV thickness ≥13 mm), left ventricular ejection fraction (LVEF)<50%, advanced conduction disorders, sustained ventricular tachycardia, supraventricular arrhythmias (atrial fibrillation/flutter or supraventricular tachycardia), ECG abnormalities (including preexcitation, conduction disease or repolarization abnormalities) or skeletal myopathy. carriers of *PRKAG2* variants with none of these findings were considered as non-affected.

Standard 12-lead electrocardiographic recordings at baseline and follow-up were examined. Ventricular pre-excitation on ECG 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 preexcitation associated with supraventricular arrhythmia. Sokolov-Lyon index criteria were used to evaluate LVH on ECG.

Creatine kinase (CK) and NT-proBNP 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 atrial fibrillation (AF), de novo pacemaker implantation, left ventricular assist device (LVAD) implantation, heart transplantation (HT), sustained ventricular tachycardia (SVT), successfully resuscitated ventricular fibrillation (VF), 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 hour from the onset of symptoms. Hospitalizations due to heart failure (HF) were also registered. Major adverse cardiac events (MACE) was defined as a composite of ICD appropriate shock, aborted SCD, SCD, HT, LVAD implantation and pacemaker implantation.

Statistical analysis

Results are presented as mean and standard deviations for continuous variables with normal distribution, median and interquartile ranges for continuous variables without normal distribution, and number and percentages for categorical data. For statistical analysis, Student's t-test and Mann-Whitney nonparametric test were used in two-group comparisons. Chi-square test or Fisher's exact test were 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 individuals (53% males, median age at first evaluation 33 years; IQR: 15-50) from 47 families (median subjects per family 3). 47 (52.2%) were probands and 43 (47.8%) relatives. All individuals except two were Caucasians of European ancestry. Most subjects (98%) carried missense variants (Online Table 2). After a comprehensive analysis of main genes currently associated with HCM, no additional rare variants were identified in any of the probands included in our study. The two non-Caucasians patients were males from Pakistan and India, respectively. None of them showed preexcitation in the ECG and MWT was 15 mm and 18 mm, respectively. The patient from Pakistan had a pacemaker implanted at 38 years of age due to advanced AV block and carried a PPrV (p.His401Asp) not described in ExAC and ClinVar. The other patient carried a frameshift variant classified as pathogenic (p.Leu352Lysfs*6).

Clinical characteristics

At first evaluation 71% (n=64) of the 90 individuals (56% males, median age 37 years; IQR: 18-50) had evidence consistent with the PRKAG2 syndrome and were considered clinically affected; the remaining 26 were considered non-affected carriers (Table 1). Probands (n=47) had a median age of 40 years (IQR: 19-54) and 60% were males. Their mean maximal wall thickness (MWT) was 20 ±8 mm and 32% presented preexcitation.

Patients with mild (MWT <15 mm) or normal phenotype (n=33) carried a PPrV in 36% of cases, compared to 28% of individuals with a more severe expression of disease (p=0.5). This group of patients with a milder phenotype were mostly relatives (n=24, 73%) and had a median age of 33 years (IQR:13-52) (vs median age of 41years (IQR: 23-54) in the group with a more severe phenotype, p=0.38).

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

Most individuals (n=74, 82%) were in sinus rhythm on their first available ECG. Mean PR interval duration was 120±49 ms and 35 patients (39%) had a PR interval shorter than 120 ms. Only 30 individuals (33%) showed a preexcitation pattern and 7 (8%) had undergone an accessory pathway ablation previously; 13% showed a first degree AVB. Mean QRS interval duration was mildly prolonged (126±36 ms) and left or right bundle branch block were common (19% and 13%, respectively). At first evaluation, 17 (19%) patients already had a permanent pacemaker implanted and 3 subjects had an ICD (including one for secondary prevention).

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

Most (93%) of the 64 affected individuals were in NYHA functional class I-II and only 4 (6%) showed LVEF <50%. Overall, 60 of the 64 affected individuals at baseline evaluation (94%; 55% males, median age 37 years; IQR: 17-50) had LVH with mean maximal wall thickness (MWT) ≥13 mm, including 50 (83%; 60% males, median age 36 years, IQR: 19-50) with LVH that was in the range of HCM (MWT ≥15 mm, mean 20±8 mm). Mean LVEF in these 50 individuals was 60±12% and mean left atria diameter was 41±10 mm. None had left ventricle outflow tract obstruction >30 mmHg or evidence of systolic anterior movement of the mitral valve.

Natural history and clinical events

During a median follow-up of 6 years (IQR: 2.3-13.9), 4 out of 26 (15%) initially unaffected individuals developed LVH and AF; 2 had an LVEF in the lower range of normality (50%) on their last echocardiogram.

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 below 35 years of age (median age of 43 years, IQR: 31-54).

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

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

Twelve individuals in the entire cohort (13%) died during follow-up (median age 52 years, IQR: 35-60). 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 non-affected subjects died (1 because of sepsis and respiratory failure, unknown cause 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 individuals. Event rates at

the end of follow-up in the entire cohort and in affected individuals are shown in Figure 3. Median age free of MACE and death was 64 years (95%CI:53-75) and free of MACE, death and AF was 52 years (95%CI:42.5-61.5) (Figure 4 and Central Illustration).

We did not find differences in baseline characteristics and events based on gender, except that women exhibited a shorter PR interval (136±40 vs 115±30 ms; p=0.002) (Online table 3). However, mean MWT at diagnosis was significantly increased and LVEF at baseline significantly decreased in subjects whith MACE during follow up (20±9 mm vs. 16±7 mm; p=0.04; and 55±16 vs 64±8 %; p=0.01, respectively). Preexcitation 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=0.3).

As two of the rare genetic variants (p.Arg302Gln and p.Asn488Ile) were present in 44% of the cases included in the cohort, we compared individuals 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 UK) with a median of 3 subjects per family (IQR: 1-4). The 7 subjects who carried the p.Asn488Ile variant come from two different families in the UK (one with one subject and the other with 6).

Patients with these two genetic variants exhibited preexcitation 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 commonest two variants (Table 3).

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

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 preexcitation and severe LVH are not uniformly present in affected patients, while atrial fibrillation is particularly common and presents almost a decade earlier than in sarcomeric HCM; unaffected individuals may develop a clinical phenotype relatively late in life, although mean age at onset of PS manifestations in affected individuals generally occurs between the third and fifth decade of life.

PRKAG2 syndrome 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 twentieth 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, ECG 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, while PS is classically associated with severe LVH, less than half of the affected individuals 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 where hemizygous males do not have any unaltered copy of the *LAMP2* gene and have worse prognosis than females, who are heterozygotes for the genetic defect (11). Prognosis of individuals with PRKAG2 genetic variants in our cohort was better than in Danon disease, particularly for males (PS is an autosomal dominant disease and no differences in phenotype were observed related to gender), but still poor compared with sarcomeric HCM (11,12).

Notably, PS is burdened with a high incidence of HF and sudden death. SCD occurred in 3 subjects and 4 additional subjects had an ICD for a resuscitated SCD, giving 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 Online Table 4.

The cause of SCD in 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 (15), and in patients older than age 30, due to cardiac conduction system disease and asystole (16). Due to 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 in 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 a heart transplant and 2 died due to end-stage HF in our study (7% of the whole cohort, 8% of affected subjects), and 9 (13% of the affected patients) showed NYHA functional class III-IV (Online Table 4). Few studies have reported data on HT or advanced HF in PS (3,12,14), but comparing our data with those reported in HCM series (12) (1.6% and 2.5% of HT and HF death, respectively), it appears that advanced HF complication rates are worse in 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 HCM (17), but it is interesting to remark that the mean LVEF of patients with events was 55%, suggesting that values in the lower limit of the normal range could already have clinical implications.

Compared to previously published series (5,6,13), our cohort displays 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 Pathogenic/Likely Pathogenic ACMG criteria but were included on the basis of a classical PS phenotype or typical histological findings and very low MAF. While 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 pathogenicity of gene variants should always be considered in combination with expert review and clinical judgment. Most of the patients with PPrV in our study had rare variants that had not been previously reported and which co-segregated with phenotype in the families. ACMG criteria for variant classification provides a framework for genetic variant interpretation but might not be as useful in individuals with prominent characteristics linked with certain syndromes as it happens in PS. ACMG criteria would probably need an adaptation in PS as has occur already with *FBN1* variants in Marfan's syndrome (19) or more recently with MYH7 in DCM (20).

In our opinion, diagnosis of PS should always be made based on genetic findings but specific clinical characteristics and positive cosegregation in the family should have a strong role in interpreting VUS variants found in 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 history 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 PPrV in our study represent PS-causing variants. In fact, clinical characteristics and event rates between patients with Pathogenic/Likely pathogenic rare genetic variants and those with PPrV did not differ (Table 4).

PS might be a suitable candidate disease for an enzymatic replacement therapy (ERT) or for a gene therapy approach as in other lysosomal storage HCM phenocopies also caused by enzymatic defects. Fabry or Pompe disease already have ERT approved treatments (21) and gene therapy clinical trials are currently being conducted in Danon (22) and Fabry disease (23). In this regard, our study would be useful to design appropriate clinical trials for PS in the future.

While 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 subject who were unaffected at baseline went on to develop signs of the disease during a median follow-up of just 2.8 years. Some developed substantial cardiac involvement and all had AF, highlighting the need for regular surveillance with ambulatory ECG. Furthermore, frequent ambulatory ECG should also be recommended in affected patients given the high rate of atrial arrhythmia and conduction disorders found in this study.

Limitations

The study was not designed to evaluate treatments effects. Causes of death were not available in all non-affected genetic carriers. The study is subject to selection and referral bias as participant 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

PRKAG2 syndrome is a severe progressive cardiac disease characterized by high rate of complications including atrial arrhythmias, conduction disease, advanced HF and SCD at a young age. Affected patients should be closely monitored in order to facilitate early detection of arrhythmia and conduction problems. PRKAG2 syndrome should be considered in patients with LVH who develop AF or require a PPM at a young age. Early recognition is important to allow prompt identification and appropriate management of genetic carriers.

Clinical Perspectives

Clinical Competencies: Progressive conduction disease, atrial arrhythmias, SCD and advance heart failure, are frequent complications of PRKAG2 syndrome. Clinical characteristics of PRKAG2 syndrome are heterogeneous and classical findings such as preexcitation and severe LVH are not uniformly present.

Translational Outlook: PRKAG2 syndrome is a candidate disease for potential enzyme replacement therapy in the future. Our data will help in designing future clinical trials for this disease.

Because disease penetrance is incomplete and expressivity is variable, further studies are needed to identify the factors leading some affected individuals to suffer cardiac events and in particular to develop arrhythmic complications.

BIBLIOGRAPHY

1. Geske JB, Ommen SR, Gersh BJ. Hypertrophic Cardiomyopathy: Clinical Update. JACC Hear Fail 2018;6:364–75.
2. Arad M, Maron BJ, Gorham JM, et al. Glycogen storage diseases presenting as hypertrophic cardiomyopathy. N Engl J Med 2005;352:362–72.
3. Gollob MH, Green MS, Tang AS, et al. Identification of a gene responsible for familial Wolff-Parkinson-White syndrome. N Engl J Med 2001;344:1823–31.
4. Gollob MH., Green MS, Tang AS, Roberts R. PRKAG2 cardiac syndrome: familial ventricular preexcitation, conduction system disease, and cardiac hypertrophy. Curr Opin Cardiol 2002;17:229–34.
5. Thevenon J, Laurent G, Ader F, et al. High prevalence of arrhythmic and myocardial complications in patients with cardiac glycogenosis due to PRKAG2 mutations. Europace 2017;19:651-659
6. Murphy RT, Mogensen J, McGarry K., et al. Adenosine monophosphate-activated protein kinase disease mimicks hypertrophic cardiomyopathy and Wolff-Parkinson-White syndrome: natural history. J Am Coll Cardiol 2005;45:922–30.
7. Arad M, Seidman JG, Seidman CE, et al. Constitutively active AMP kinase mutations cause glycogen storage disease mimicking hypertrophic cardiomyopathy. J Clin Invest 2002;109:357–62.
8. Sternick EB, Oliva A, Gerken LM, et al. Clinical, electrocardiographic, and electrophysiologic characteristics of patients with a fasciculoventricular pathway: The role of PRKAG2 mutation. Hear Rhythm 2011;8:58-64
9. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet

Med 2015;17:405–24.

10. ExAC Browser. Available at: http://exac.broadinstitute.org/. Accessed August 28, 2019.

11. López-Sainz Á, Salazar-Mendiguchía J, García-Álvarez A, et al. Clinical Findings and Prognosis of Danon Disease. An Analysis of the Spanish Multicenter Danon Registry. Rev Esp Cardiol 2019;72:479-86

12. Lorenzini M, Anastasiou Z, O'Mahony C, et al. Excess mortality and sex differences in outcome in hypertrophic cardiomyopathy: an European multicentre study. JAMA Cardiol 2019 Nov 27. doi: 10.1001/jamacardio.2019.4534. [Epub ahead of print]

13. Porto AG, Brun F, Severini GM, et al. Clinical Spectrum of PRKAG2 Syndrome. Circ Arrhythm Electrophysiol. 2016 ;9:e003121 14.

14. Blair E, Redwood C, Ashrafian H, et al. Mutations in the gamma(2) subunit of AMP-activated protein kinase cause familial hypertrophic cardiomyopathy: Evidence for the central role of energy compromise in disease pathogenesis. Hum Mol Genet 2001;10:1215–20.

15. Gollob MH, Seger JJ, Gollob TN, et al. Novel PRKAG2 mutation responsible for the genetic syndrome of ventricular preexcitation and conduction system disease with childhood onset and absence of cardiac hypertrophy. Circulation 2001;104:3030–3.

16. Sternick EB, Oliva A, Magalhaes LP, et al. Familial pseudo-Wolff-Parkinson-White syndrome. J Cardiovasc Electrophysiol 2006;17:724–32.

17. Dominguez F, Sanz Sanchez J, Garcia-Pavia P, Zorio E. Follow-up and prognosis of HCM. Glob Cardiol Sci Pract. 2018; 12: 33

18. Kelly MA, Caleshu C, Morales A, et al. Adaptation and validation of the ACMG/AMP variant classification framework for MYH7-associated inherited cardiomyopathies: Recommendations by ClinGen's Inherited Cardiomyopathy Expert Panel. Genet Med

2018; 20: 351-359

19. Muiño-Mosquera L, Steijns F, Audenaert T, et al. Tailoring the American College of Medical Genetics and Genomics and the Association for Molecular Pathology Guidelines for the Interpretation of Sequenced Variants in the FBN1 Gene for Marfan Syndrome: Proposal for a Disease- and Gene-Specific Guideline. Circ Genom Precis Med 2018;11:e002039.
20. Morales A, Kinnamon DD, Jordan E, et al. Variant Interpretation for Dilated Cardiomyopathy (DCM): Refinement of the ACMG/ClinGen Guidelines for the DCM Precision Medicine Study. Circ Genom Precis Med 2020. doi: 10.1161/CIRCGEN.119.002480
21. Nair V, Belanger EC, Veinot JP. Lysosomal storage disorders affecting the heart: a review. Cardiovasc Pathol 2019;39:12-24.
22. NCT03882437. Available at https://clinicaltrials.gov/ct2/show/NCT03882437 Accessed December 28, 2019.
23. NCT04040049. Available at https://clinicaltrials.gov/ct2/show/NCT04040049 Accessed December 28, 2019.

FIGURE LEGENDS

Figure 1. **Typical histological findings in patients with PRKAG2 syndrome.** A & B: Hematoxylin-eosin staining displaying hypertrophied myocytes. Magnification x200 and x400. C & D: PAS (Periodic Acid Schiff) staining positive for glycogen accumulation in cardiomyocyte vacuoles. Magnification x200 and x400. Yellow arrowheads point PAS+ deposits, corresponding to glycogen.

Figure 2. Flowchart of the individuals included in the study. Clinical events and phenotype of individuals during the study. Affected patients: subjects with one or more of the following: unexplained left ventricular hypertrophy, LVEF<50%, advanced conduction disorders, sustained ventricular tachycardia, supraventricular arrhythmias, ECG abnormalities or skeletal myopathy.

Figure 3. Prevalence of different complications in 90 individuals with *PRKAG2* variants after a median follow-up of 6 years. Affected patients: subjects with one or more of the following: unexplained left ventricular hypertrophy, LVEF<50%, advanced conduction disorders, sustained ventricular tachycardia, supraventricular arrhythmias, ECG abnormalities or skeletal myopathy.

Figure 4. Survival curves in 90 individuals with *PRKAG2* variants. Blue line: Freedom of major cardiovascular events (MACE) and death; **Green line**: Freedom of MACE, death and atrial fibrillation. MACE includes SCD, aborted SCD, appropriate ICD discharge, heart failure hospitalization, heart transplantation and pacemaker implantation.

Figure 5. Clinical diversity of PRKAG2 syndrome. A and **B:** ECG 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 male with the p.Arg302Gln *PRKAG2* variant and a more severe phenotype (septal thickness 33 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 MACE and death and outcomes of 90 individuals with variants in *PRKAG2* gene.

MACE: composite of ICD appropriate shock, aborted SCD, SCD, HT, LVAD implantation and pacemaker implantation. Affected patients: subjects with one or more of the following: unexplained left ventricular hypertrophy, LVEF<50%, advanced conduction disorders, sustained ventricular tachycardia, supraventricular arrhythmias, ECG abnormalities or skeletal myopathy.

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

	ENTIRE COHORT (n=90)	AFFECTED (n=64)	NON-AFFECTED (n=26)
Male Gender, n (%)	48 (53)	36 (56)	12 (46)
Age, years (IQR)	33 (15-50)	37 (18-50)	18 (9-39)
Family History of SCD, n (%)	35 (39)	24 (38)	11 (46)
Stroke, n (%)	4(5)	4(6)	0
Myopathy, n (%)	2 (2)	2 (3)	0
Syncope, n (%)	28 (32)	24 (38)	4 (18)
Chest pain, n (%)	15 (17)	10 (16)	5 (22)
Palpitations, n (%)	41 (49)	31 (50)	10 (46)
CK, U/L (IQR)	79 (56-117)	106 (2-365)	66(2-130)
NYHA III-IV, n (%)	6(7)	5 (8)	1 (5)*
NT-proBNP, pg/ml (median,IQR)	120 (21-1200)	170 (37-2168)	47 (10-224)
Pre-excitation, n (%)	30 (33)	30 (44)	0
QRS, ms	126±36	131±37	108±26
Atrial Fibrillation, n (%)	16 (18)	16 (25)	0
LVH in ECG, n (%)	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, n (%)	28(31)	20 (31)	8 (31)

CK: creatine kinase; IQR: Interquartile range, LA: left atrium; LVEF: left ventricular ejection fraction; LVH: left ventricular hypertrophy; LV MWT: left ventricular maximal wall thickness; PPrV: Probably pathogenic rare variant; SCD: sudden cardiac death.

*Cardiogenic shock in preterm new born due to sepsis

Table 2. Clinical, electrocardiographic and echocardiographic parameters at last evaluation in the entire cohort.

	ENTIRE COHORT (n=90)	AFFECTED (n=68)	NON-AFFECTED (n=22)
Male Gender, n (%)	48 (53)	38 (56)	10 (50)
Age, years (IQR)	42 (25-58)	43 (31-59)	28 (14-44)
NYHA III-IV, n (%)	10 (11)	9 (13)	1 (5)
Atrial Fibrillation, n (%)	26 (29)	26(39)	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%, n (%)	10 (11)	10 (15)	0

IQR: Interquartile range, LA: left atrium; LVEF: left ventricular ejection fraction; LVH: left ventricular hypertrophy; LV MWT: left ventricular maximal wall thickness

Table 3. Clinical characteristics and events according to underlying genetic cause.

	p.Arg302Gln and p.Asn488Ile n=39	Other PRKAG2 variants n=51	p
Baseline characteristics			
Males, n (%)	22 (56)	26 (51)	NS
Age, years (IQR)	32 (15-44)	36 (14-50)	NS
Family History of SCD, n (%)	14/38(36)	21/49(43)	NS
Stroke, n (%)	1/38(3)	3/49(6)	NS
Syncope, n (%)	10/38 (20)	18/49 (47)	0.008
Chest pain, n (%)	7/38 (18)	8/49(16)	NS
Palpitations, n (%)	20/38 (53)	21/49 (46)	NS
Affected, n (%)	28 (72)	36 (71)	NS
Myopathy, n (%)	2 /39(5)	0	NS
CK levels, U/L (range)	56 (13-81)	98 (76-134)	NS
Pre-excitation, n (%)	17/34(50)	13/47(28)	0.002
LVH in ECG, n (%)	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%, n (%)	5/37 (13)	5/44 (10)	NS
Follow-up			
Pacemaker implantation, n (%)	15 (38)	17(33)	NS
Sudden cardiac death, n (%)	3 (8)	0	NS
Heart transplantation, n (%)	1(3)	3(6)	NS
Death, n (%)	3 (8)	9 (18)	NS
Heart failure hospitalization, n (%)	4 (11)	9 (18)	NS
Atrial fibrillation, n (%)	17 (46)	9 (18)	0.009

IQR: Interquartile range, LVEF: left ventricular ejection fraction; LVH: left ventricular hypertrophy; LV MWT: left ventricular maximal wall thickness; SCD: sudden cardiac death.

Table 4. Clinical characteristics and events during follow-up in patients with pathogenic/likely pathogenic variants according to ACMG criteria and in those with probably pathogenic *PRKAG2* rare genetic variants.

	Pathogenic/likely pathogenic variants (n=62)	Probably pathogenic rare variants (n=28)	p
Male Gender, n (%)	34 (55)	15 (53)	NS
Age at first evaluation (IQR)	32 (14-49)	40 (17-53)	NS
Family History of SCD, n (%)	22 (35)	13 (46)	NS
Stroke, n (%)	4 (6)	0	NS
Myopathy, n (%)	2 (5)	0	NS
Syncope, n (%)	20 (32)	8 (29)	NS
Chest pain, n (%)	13 (21)	2 (32)	NS
Palpitations, n (%)	32 (52)	9 (58)	NS
Pre-excitation, n (%)	21 (34)	9 (32)	NS
Atrial Fibrillation, n (%)	11 (18)	5 (18)	NS
LVH in ECG, n (%)	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, n (%)	23 (37)	9 (32)	NS
Sudden cardiac death, n (%)	2 (3)	1 (4)	NS
Heart Transplantation, n (%)	3 (5)	1 (4)	NS
Death, n (%)	8 (13)	4 (14)	NS
Heart failure hospitalization, n (%)	8 (13)	5 (18)	NS
Atrial fibrillation, n (%)	20 (32)	6 (21)	NS

LVEF: left ventricular ejection fraction; LVH: left ventricular hypertrophy; LV MWT: left ventricular maximal wall thickness; SCD: sudden cardiac death