Sudden death associated with a novel H401Q PRKAG2 mutation

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Introduction

PRKAG2 cardiomyopathy is an autosomal dominant inherited disorder resulting from mutations in the gene encoding the γ2 regulatory subunit of AMP-activated protein kinase.1 Clinical manifestations include cardiac hypertrophy, ventricular pre-excitation, atrioventricular conduction abnormalities, and atrial flutter and fibrillation.1 Its precise prevalence is challenging to define, in part due to phenotypic overlap on imaging with sarcomeric hypertrophic cardiomyopathy. Eighteen mutations have been reported2 to this date, most commonly Arg302Gln and Asn488Ile, constituting ~57% and ~21% of ~270 reported cases, respectively.3

Greater clinical awareness of PRKAG2 cardiomyopathy is important given that both prognosis and management are distinct from familial hypertrophic cardiomyopathy.4 For patients bearing the most frequently mutation (Arg302Gln), clinical presentation is usually during the second decade of life. Sudden cardiac death may occur before 30 years, caused by atrial fibrillation conducting with rapid ventricular rate, or later, caused by atrioventricular block.2 We report a new missense PRKAG2 mutation (His401Gln) in a family where two young adult sisters had sudden cardiac death as the initial clinical manifestation.

Methods

Patient population

Five members of three generations of one family (Figure 1).

This study complies with the Declaration of Helsinki. Locally appointed ethics committee has approved the research protocol and informed consent has been obtained from the subjects.

Endomyocardial biopsy

Tissue samples were obtained by a myocardial biopsy forceps (CookVR, USA) from the right ventricular (RV) septum. Fragments were stained with H&E, Mallory trichrome, and Periodic acid–Schiff.

Transmission electron microscopy (TEM): biopsy specimens were stained with Reynolds lead citrate. TEM was performed using a FEI Tecnai Spirit.

Standard transthoracic echocardiogram

Two- and three-dimensional studies were performed using Vivid E9 ultrasound system (GE, Norway).

Cardiac magnetic resonance

Cardiac magnetic resonance (CMR) was performed with 1.5-T scanner (Siemens, Germany). Ten minutes after injection of a contrast agent, late gadolinium enhancement (LGE) images were acquired.

Electrophysiologic study

Programmed stimulation (CardioTek-BV, The Netherlands) included atrial and ventricular pacing at increasing rates and extra-stimuli during atrial and ventricular pacing. The ventricular stimulation protocol with up to three extra-stimuli was repeated during isoproterenol infusion.

Genetic analysis

Proband DNA underwent sequencing of 52 genes linked to cardiomyopathy. A genomic library was constructed using an AmpliSeq custom panel and sequenced with a PGM Ion-Torrent system. Sequences were aligned with the hg19 reference genome build and nucleotide variants were filtered for potential candidate variants. Family members then underwent mutation-specific screening by Sanger sequencing (ABI3500XL, Thermo Fisher, USA).
Three-dimensional modelling of the PRKAG2 gene

To date no crystal structure of the AMPK complex containing the γ2 subunit is available, therefore, the AMPK α2β1γ1 structure was used to predict the effect of the His401Gln mutation. The γ isoforms are highly conserved across cystathionine-β synthase (CBS) repeats, with γ2 having a large N-terminal extension of unknown structure. Images of the AMPK structure were generated in the PyMol Molecular Graphics System programme (Schrödinger, LLC) using the structure of AMPK α2β1γ1, PDB database entry 4CFE. An arbitrary selection of
potential glutamine side chain rotamers were chosen to illustrate the potential disruption to nucleotide binding in the γ2 His401Gln mutant protein.

**Results**

**Clinical manifestation**
The proband (III.3) had paroxysmal atrial fibrillation at the age of 11 years. She is now aged 18 years and has developed exertional dyspnoea, in New York Heart Association (NYHA) functional class II, likely due to diastolic dysfunction. She is under amiodarone 200 mg daily and had no recurrence of atrial fibrillation.

Case III.1 is a 17-year-old woman who has only occasional short-lived bouts of atrial tachycardia (Figure 1). She is asymptomatic and had recently delivered a healthy baby.

Cases II.1 and II.5 both died suddenly and unexpectedly in their early 20s. Both had a diagnosis of hypertrophic cardiomyopathy and were in NYHA Class I. The clinical diagnosis was made early because of heightened awareness, as their mother (Case I.1) already knew about the familial aspect of her cardiac disease. Case II.5 had sporadic palpitations. She died at the age of 23 years while pregnant (35 weeks). She experienced a sudden onset of chest pain and palpitations and died within 30 min before receiving medical attention. Her sister, Case II.1 died unexpectedly at home (21 years), 1 year after delivering her first daughter. There was no record of symptoms either during pregnancy or after delivery.

Case I.1, a 56-year-old woman, received a pacemaker due to persistent symptomatic sinus bradycardia at age 46 years. She had a myocar-
dial infarction at 44 years, evolving into Killip II requiring a 30 days hospital stay. Coronary angiography did not disclose abnormalities. Currently, she is in NYHA functional Class II.

**Electrocardiogram**
All three carriers exhibit ventricular pre-excitation (Figure 2).

**Echocardiogram**
Case I.1 exhibits significant left atrial (48 mm) and left ventricular (LV) dilatation [LV end-diastolic diameter (LVEDD) 62 mm], together with severe systolic impairment [left ventricular ejection fraction (LVEF) 31% by 3D]. Akinesia with thinning of the basal septal segment and part of the mid-anterosepetum and inferoseptum were evident with concentric hypertrophy elsewhere. Doppler revealed significant diastolic dysfunction with evidence of increased LV filling pressure. Pulmonary artery systolic pressure was estimated as 50 mmHg.

The proband and her cousin (III.1) exhibited moderate to severe LV hypertrophy without resting outflow tract obstruction: estimated myocardial mass index was 246 and 218 g/m² (Penn), interventricular septal and LV posterior wall thickness was 22/22 and 19/17 mm, respectively. Left atrial dimensions and LVEF were within normal limits. Global longitudinal strain determined by automated function imaging measured 13.4% and 12.6%, respectively. Myocardial longitudinal deformation depicted as a bull’s eye plot showed different deformation levels in a ‘striped’ pattern.

**Cardiac magnetic resonance**
The proband (III.3) and her sister (III.1) had diffuse and severe left ventricular hypertrophy (LVH), more pronounced in the interventricular septum, and mid-inferior LV wall. Myocardial mass index was 254 and 222 g/m², respectively. Interventricular septal wall thickness varied significantly along the septum: measurements performed at the basal, mid, and apical septum were 17, 15, and 20 mm in III.3, and 15, 14, and 18 mm in III.1. The left ventricular posterior wall measured 13, 11, and 10 mm in III.3, and 12, 11, 11 in III.1, at the basal, mid, and apical regions. The LV mid-inferior wall was severely hypertrophied at 31 (III.3) and 26 mm (III.1). Left ventricular end-diastolic diameter was 54 (III.3) and 52 mm (III.1). Notably, however, none had LGE.

**Electrophysiologic study (Cases III.1 and III.3)**
This revealed ventricular pre-excitation due to a fasciculoventricular pathway (fixed HV interval of 28 and 32 ms, despite atrial pacing at increasing rate, and unchanged QRS morphology during induced atrial fibrillation with a minimum RR interval of 260 and 280 ms). No re-
entrant tachycardia was induced. Atrioventricular block occurred with atrial pacing at a cycle length of 280 and 310 ms, respectively. No ventricular tachycardia was inducible.

**Endomyocardial biopsy**
Histological evaluation of endomyocardial biopsy samples of proband III.3 and individual III.1 demonstrated vacuolization in most of the fibres due to cytosolic glycogen storage (Figure 3). No interstitial fibrosis or inflammation was observed. On ultrastructural examination, large amounts of glycogen were evident in cytosolic pools, mostly in the perinuclear region (Figure 3).

**Genetic analysis**
Proband targeted panel next-generation sequencing identified a missense heterozygous variant, p.His401Gln (Figure 1), which was not found in the gnomAD or ExAC databases. The histidine 401 residue is situated in a highly conserved region of the AMPK γ2 subunit protein, with
in silico pathogenicity prediction tools classifying the variant as possibly deleterious. Mutation-specific cascade screening identified the variant allele in individuals III.1 and I.1, confirming segregation of the variant allele with the cardiac phenotype.

Three-dimensional modelling of the mutant PRKAG2 gene

Although the AMPK \( \gamma \)2 subunit has four CBS repeats, in mammalian AMPK only three have the correct sequence to form nucleotide binding sites, by convention named AMP-1, AMP-3, and AMP-4 and is illustrated by the structure of the human AMPK \( \alpha_{2}\beta_{1}\gamma_{1} \) complex\(^5\) (Figure 4A). The three sites bind nucleotide with different affinity, which allows the protein to sense the availability of ATP compared with AMP in the cell. In addition to a pocket which binds the nucleotide adenine moiety, each binding site incorporates residues that form a series of hydrogen bonds to the phosphate moiety. The \( \gamma \)2 His401 residue side chain (equivalent to His169 in \( \gamma \)1) does not directly make hydrogen bonds with the nucleotide phosphate, but its proximity contributes to the environment that promotes the hydrogen bonds made by His151 and His298, to form the AMP-4 non-exchangeable site (Figure 4B). Substitution of the basic side chain with an amide changes the chemical environment of this binding site, but also depending on which rotamer is adopted, the glutamine side chain could sterically impede the existing hydrogen bond network and form new interactions (Figure 4B). This will lead to destabilization of the AMP binding environment and dysregulation of the allosteric nucleotide sensing mechanism and, by extension, allosteric regulation. Due to its proximity to the binding site this is potentially more disrupting than the previously characterized Thr400Asn mutation.\(^1\)

Discussion

The major clinical findings in the three family members heterozygous for a novel H401Q PRKAG2 variant were: early-onset severe LV hypertrophy, ventricular pre-excitation, symptomatic sinus bradycardia, myocardial infarction in individual I.1, absence of myocardial fibrosis, and sudden death in two individuals early in their twenties.

Early-onset of left ventricular hypertrophy

Left ventricular hypertrophy represents the most common clinical feature in patients with PRKAG2 mutations.\(^6\) A greater propensity for carriers of the Asn488Ile mutation to express LVH has been noted than for those with the Arg302Gln variant (70% vs. 42%, respectively).\(^3\) Cardiac hypertrophy is usually not associated with outflow tract obstruction, may be concentric or phenocopy that of hypertrophic cardiomyopathy to preferentially involve the septum. Infantile presentation of LVH has been reported.\(^1,4\) The Arg531Gln variant has been

Figure 2 Electrocardiogram of three His401Gln carriers. A (III.1) and B (III.3, proband) show sinus rhythm and ventricular pre-excitation. B also shows sinus bradycardia. C depicts an atrial paced rhythm and ventricular pre-excitation from individual I.1.
Figure 3  Cardiac histopathological findings: individual III.1 (A, C, E, G) and the proband III.3 (B, D, F, H). (A and B) Pronounced vacuolization of myofibres (asterisks), H&E staining. (C and D) Abundant gross granular inclusions consistent with glycogen (arrows) within vacuoles, PAS staining. (E and F) Absence of fibrosis, Mallory trichrome staining. (G and H) Transmission electron microscopy shows normal appearing mitochondria (Mit) interspersed between large amounts of glycogen (Gly). Mf, myofibrils.
reported to induce massive hypertrophy and a fatal outcome in neonates. In our current series, LVH associated with the His401Gln mutation was also present in childhood, with predominant interventricular septal hypertrophy. Given the LV macroscopic imaging findings, this suggests that in the context of PRKAG2 cardiomyopathy, RV biopsy samples can be representative of morphological changes in the contralateral ventricle.

**Ventricular pre-excitation**

It is reported in 77% of a large series of patients. Some patients have fast conducting accessory pathway, but most have a fasciculoventricular pathway, or more rarely nodo-ventricular pathway. Both patients with the His401Gln variant had a fasciculoventricular pathway.

**Sinus bradycardia**

Progressive and severe sinus bradycardia likely reflects activation of γ2 AMPK complexes in the sinoatrial node and down-regulation of key sinoatrial cellular pacemaker mechanisms. However, the incidence of sinus node dysfunction may be underestimated given the large proportion of carriers who develop concomitant atrioventricular septal hypertrophy and receive a pacemaker before the bradycardia typically becomes symptomatic, like in individual I.1, who received a pacemaker or symptomatic sinus bradycardia.

**Myocardial infarction**

Individual I.1 experienced a myocardial infarction. Two other cases of myocardial infarction have been reported, both with significant septal LV hypertrophy and with the R302Q gene mutation, all without obstructive coronary artery disease.

**Sudden death at a young age**

Sudden death occurs in 32% of individuals, with a mean age of 44 years. In light of the high rate of pacemaker implantation (43%), frequently in the age range of 30–40 years, it is likely that timely pacemaker implantation prevents sudden death in many mutation carriers. In younger

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**Figure 4** (A) Representation of the structure (PDB code 4CFE) of the AMPK complex focused on the γ1 subunit showing the three nucleotide binding sites. The CBS repeats are shown in shades of cyan. The α- and β-subunits are coloured green and orange, respectively. (B) Close up of the AMP-4 binding site showing the environment and interactions of the His169 (γ2-His401 equivalent) side chain. (C) A selection of glutamine side chain rotamers (lemon) superposed on the His169 position shown to illustrate the potential disruption of the nucleotide binding site due to the His401Gln mutation. CBS, cystathionine-β synthase.
This is the first report of a His401Gln mutation in a single family. Evaluation of a larger number of mutation carriers from different families as well as linkage studies with large number of non-carriers will be required to add to the evidence for pathogenicity and confirm the high risk of sudden death associated with this specific variant.

Study limitations

While runs of non-sustained ventricular tachycardia have been reported in a small proportion of PRKAG2 mutation carriers with severe LVH,9 there are no published data regarding aborted episodes of sudden cardiac death in patients who have received an implantable defibrillator. In our own experience, those who have received a defibrillator were initially misdiagnosed with hypertrophic cardiomyopathy and underwent implantation for primary prevention. Three of our PRKAG2-R302Q patients with an implantable cardioverter-defibrillator had inappropriate therapies caused by atrial fibrillation.9 Notably, in a series of patients carrying the Arg302Gln mutation who underwent electrophysiologic study, none had inducible sustained ventricular tachycardia,16 an observation mirrored during study of transgenic mice expressing the same mutation.15 Similarly, we were unable to induce ventricular tachycardia in either of our two patients carrying the His401Gln variant. These findings may in part reflect the lower degree of myocardial fibrosis as substrate for re-entrant VT encountered in PRKAG2 cardiomyopathy compared with familial hypertrophic cardiomyopathy (Figure 3). A recent CMR study identified LGE, a marker of focal fibrosis, in only two of six mutation carriers analysed who had severe LVH.16 The molecular substrate for this relative paucity of fibrosis is uncertain, but recent findings from human iPS cell-derived cardiomyocyte models bearing the Asn488Ile mutation suggest that this may involve attenuation of pro-fibrotic transforming growth factor-β signalling through mutation-associated AMPK activation.17

His401Gln PRKAG2 variant

Supporting the pathogenicity of this newly described variant are: its occurrence in a residue highly conserved across species; its absence from large population-based cohorts (gnomAD); the high likelihood of pathogenicity based on in silico prediction tools (Figures 1 and 4, Table 1); the proximity of the amino acid residue to the AMP-4 non-exchangeable site—immediately adjacent to an established PRKAG2 mutation (c.1199C>G, p.Thr400Asn)—with the potential to destabilize the AMP binding environment and thereby dysregulate allosteric nucleotide sensing; and its co-segregation with disease in multiple affected individuals with a cardiac phenotype consistent with PRKAG2 cardiomyopathy.

Conflict of interest

We report a family with a novel PRKAG2 gene mutation (His401Gln) characterized by an early-onset of LVH and a high incidence of sudden death at a young age.

Conflict of interest: none declared.

References