



Original Article

Clinical profile in arrhythmogenic cardiomyopathy and a recessive plakophilin-2 gene mutation



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ABSTRACT

Objective: Arrhythmogenic cardiomyopathy (ACM) is not an uncommon cause of cardiac morbidity in Kashmir valley. This study was designed to document various clinical features and to sequence exons 11 and 12 of plakophilin 2 (*PKP2*) gene in these patients.

Methods: ACM patients who attended cardiology outpatient department of our institute from January 2014 to April 2015 were included in the study. Their records were reviewed. Controls were randomly selected, who had no history or family history of cardiac illness and had a normal cardiac examination. A blood sample was also taken from both the groups for sequencing of exon 11 and 12 of *PKP2* gene. ACM patients were followed up until July 2016.

Results: Eleven ACM patients and seven controls were included in the study. Most common mode of presentation was ventricular tachycardia (VT). Two patients had left ventricular (LV) systolic dysfunction. One patient had a splice site mutation in exon 12 of *PKP2* gene and one patient died during follow-up. One of the controls had an intronic variation that has no pathogenic significance vis-à-vis ACM.

Conclusion: Our study describes various clinical parameters in ACM patients and a recessive plakophilin 2 mutation after a limited *PKP2* gene sequencing.

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1. Introduction

Arrhythmogenic cardiomyopathy (ACM) is a genetic and structural heart disease characterized by gradual replacement of cardiac myocytes by adipocytes and fibrosis, predominantly of the right ventricle.¹ The left ventricle is involved in up to 50% of cases.² The most common gene involved in ACM is plakophilin-2 gene (*PKP2*) that accounts for approximately 20% of the cases.³ This study was designed to document various clinical features of ACM and to sequence exons 11 and 12 of *PKP2* gene in these patients.

2. Methods

Patients of ACM (diagnosed on the basis of Revised Task Force Criteria of ACM),⁴ who were under cardiology outpatient department follow-up of our Institute, and attended the hospital from

January 2014 to April 2015 were included in the study. Their previous records were reviewed, and a blood sample was taken for sequencing, after written informed consent.

Controls were randomly selected from cardiology outpatient department who had no history or family history of cardiac illness and had a normal cardiac examination. A blood sample was also taken from them for sequencing after written informed consent.

The study was cleared by our institute's ethical committee.

Peripheral blood samples were used as the source of DNA. DNA was extracted by DNA extraction kit (Qiagen, US). The quality of the DNA obtained from the blood samples was analyzed on 1% agarose gel. Polymerase chain reaction (PCR) amplification was done using the primers (Table 1) which were designed by using PrimerQuest[®] program, IDT, Coralville, USA. PCR amplified products were checked on 2% agarose gel and sent for sequencing to Macrogen Ltd, Seoul for sequencing of exons 11 and 12. The results were compared with *PKP2* gene reference sequence (ENST00000070846.10).⁵

Exons 11 and 12 were selected based on the number of reports that have reported pathogenic variations of these two exons and because of financial constraints. Exons 11 and 12, when taken as

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Table 1
PKP2 primers.

Gene	Primers
PKP-Exon 11-F	5'- GCCTGAATGACAGAGAGAC-3'
PKP-Exon 11-R	5- CCGGTTTATCACCTACTCCTTAC-3'
PKP Exon 12-F	5'-ACTCTCCCTGATTGGTTTCC-3'
PKP Exon 12-R	5'-GACTCCTGCTTGCCTACAT-3'

combined, have the maximum number of reported pathogenic variations than any other two contiguous exons of *PKP2* gene.⁶

3. Results

Eleven patients presented to our outpatient department from January 2014 to April 2015. All of them were unrelated. Their records were reviewed.

Table 2
Baseline characteristics of ACM patients.

No.	Year of diagnosis	Age at presentation	Sex	Mode of presentation	Reduced LVEF
1	2008	43	M	Syncope(VT)	No
2	2012	18	F	Syncope(VT)	No
3	2013	14	M	Syncope(VT)	Yes
4	2013	18	M	Syncope(VT)	Yes
5	2010	45	M	Palpitations(VT)	No
6	2012	38	M	Syncope(VT)	No
7	2012	22	M	Palpitations(VT)	No
8	2010	50	M	Palpitations(VT)	No
9	2010	40	M	Palpitations(VT)	No
10	2012	22	M	Palpitations(VT)	No
11	2007	37	M	Palpitations(VPCs)	No

Table 3
Revised Task Force criteria and other findings in each case.

No.	Major criteria present	Minor criteria present	Other Findings
1	1. ECG: T wave inversion in leads V1–V4 2. Echo: Thinned out RV apex and free wall and PSAX RVOT diameter of 37 mm 3. LBBB morphology VT with superior axis		
2.	1. ECG: T wave inversion in leads V1–V6 2. Echo: Grossly dilated RA & RV, PSAX RVOT diameter of 38 mm 3. Cardiac MRI: RV free wall hypokinesia and RV ejection fraction of 26.3% 4. LBBB morphology VT with superior axis	ECG: More than 1000 VPCs on 24-h Holter monitoring	Echo: Severe Low-Pressure TR
3.	1. LBBB morphology VT with superior axis 2. Cardiac MRI: Grossly dilated and hypokinetic RV; RVEF of 17%	1. Echo: Dilated and hypokinetic RV and PSAX RVOT diameter of 35 mm	Echo: LV systolic dysfunction
4.	1. ECG: T wave inversion in leads V1–V6 2. Echo: Grossly dilated RA & RV, RV free wall hypokinetic and PSAX RVOT diameter of 39 mm 3. LBBB morphology VT with superior axis 4. Cardiac MRI: Thinned out RV free wall and markedly decreased RV systolic function		Echo: Severe low-pressure TR, LV systolic dysfunction
5.	1. ECG: T wave inversion in leads V1–V4 2. Echo: Grossly dilated RA & RV, PSAX RVOT 40 mm, RV systolic dysfunction 3. Cardiac MRI: Thinning of RV free wall and RVEF OF 21.9%	2. LBBB morphology VT with inferior axis	
6.	1. ECG: T wave inversion in leads V1–V4 2. LBBB morphology VT with superior axis		Echo: Mildly dilated RA & RV, PSAX RVOT = 31 mm, mild TR Cardiac MR: Fatty infiltration of RV free wall, mild hypokinesia of RV free wall, RVEF 41% ^{††}
7.	1. ECG: T wave inversion in leads V1–V5 2. ECG: Epsilon waves 3. Echo: Thinning of RV apex and PSAX RVOT of 40 mm	1. LBBB morphology VT with inferior axis	
8.	1. ECG: T wave inversion in leads V1–V5 2. ECG: Epsilon waves 3. LBBB morphology VT with superior axis	1. RV Apical thinning and PSAX RVOT dia of 35 mm	
9.	1. Echo: Thinned out RV lateral wall and PSAX RVOT of 40 mm 2. CMRI: Severe RV lateral wall thinning and RVEF of 19%	1. History of ACM in brother [†] 2. LBBB morphology VT with inferior axis	
10.	1. ECG: T wave inversion in leads V1–V4 2. Echo: RV free wall hypokinesia and PSAX RVOT diameter Of 41.5 mm 3. LBBB morphology VT with superior axis		
11.	1. Echo: RV lateral wall thinning and PSAX RVOT diameter of 49 mm 2. Cardiac MRI: Thinned out anterior RV wall and RVEF of 27%		

ECG: electrocardiogram; LBBB: left bundle branch block; VT: ventricular tachycardia; RVOT: right ventricular outflow tract; RV: right ventricular; RVEF: right ventricular ejection fraction; RA: right atrium; ACM arrhythmogenic cardiomyopathy; MRI: magnetic resonance imaging; LV: left ventricular; TR: tricuspid regurgitation; VPC: ventricular premature contractions.

[†] In whom it was not possible to determine whether he met current Task Force criteria.

^{††} These finding do not fulfil any major or minor criterion.

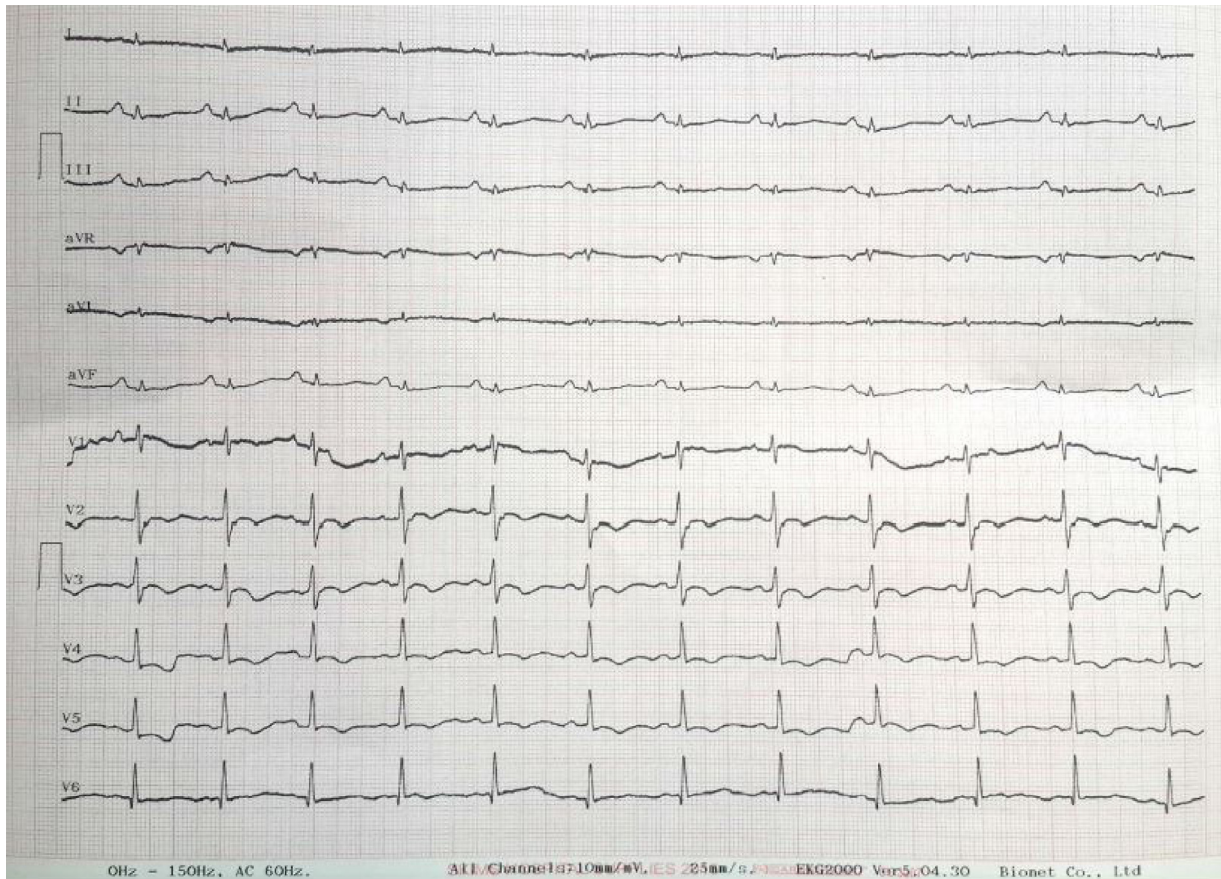


Fig. 1. Twelve lead ECG of case no. Seven.

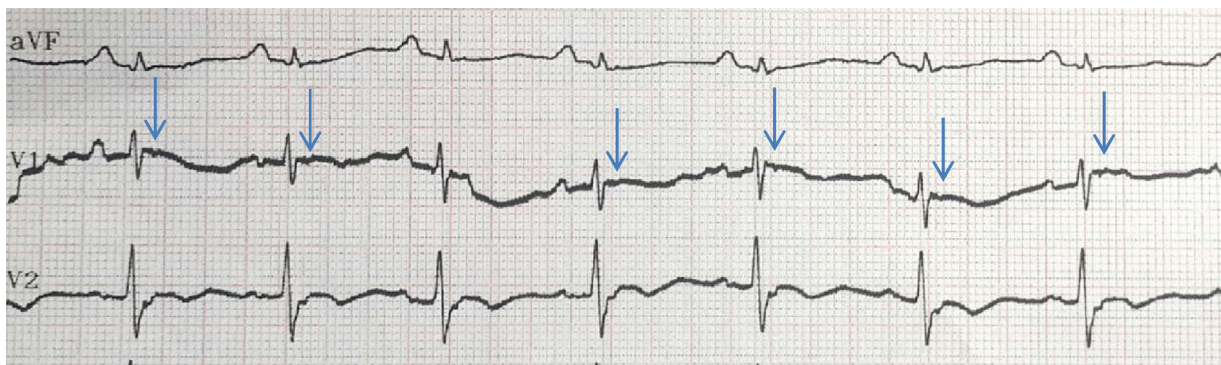


Fig. 2. Magnified view of Lead V1 in Fig. 1 showing Epsilon waves.

Mean age at diagnosis was 31 years. One (9%) of the 11 patients was female. Two patients (case no. 2 and 3) were products of consanguineous marriage. Two patients (18%) had a positive family history. Case no. 2 had a second-degree relative who had died suddenly and case no. 9 had a first-degree relative with ACM who had been implanted an implantable cardioverter defibrillator (ICD) and died suddenly in 2010. Baseline characteristics are mentioned in Table 2.

The major and minor Revised Task Force criteria for the diagnosis of ACM in each case are mentioned in Table 3.

The most common presenting symptom was palpitations that were present in six patients (54%). The second most common presenting symptom was syncope that was present in five patients.

All of the patients were in sinus rhythm at the time of the last follow-up. The most common electrocardiographic abnormality

was T wave inversions in precordial leads that were present in eight of the eleven patients (72.7%). Case no. 2 and 11 had frequent ventricular premature complexes. Epsilon waves were present in two patients (case no. 7 and 8). (Figs. 1 and 2) Ten patients had documented ventricular tachycardia. ICD was implanted in nine of them. All documented ventricular tachycardia (VT) were of left bundle branch block (LBBB) morphology with case no. 3 having both right bundle branch block (RBBB) and LBBB morphology tachycardia.

Case no. 2 had multiple appropriate and inappropriate ICD shocks and was admitted twice during the study period.

Case no. 4 was also admitted four times for recurrent ICD shocks. All but one of these shocks was inappropriate and anti-shock therapy was turned off after detailed discussion with the patient and his parents.

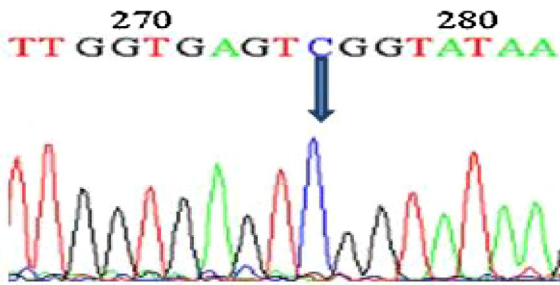


Fig. 3. Partial electropherogram of exon 11 showing intronic variation c.2299 + 7C > T (Case no. 2).

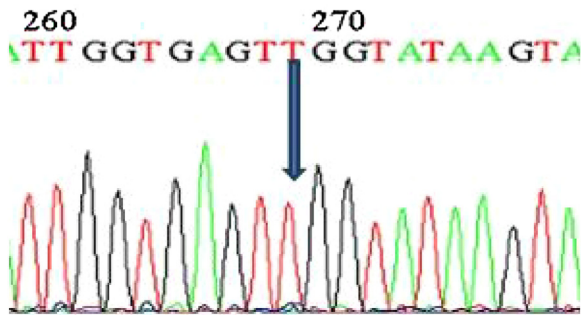


Fig. 4. Partial electropherogram of exon 12 showing intronic variation c.2489 + 72G > A (Case no. 2).

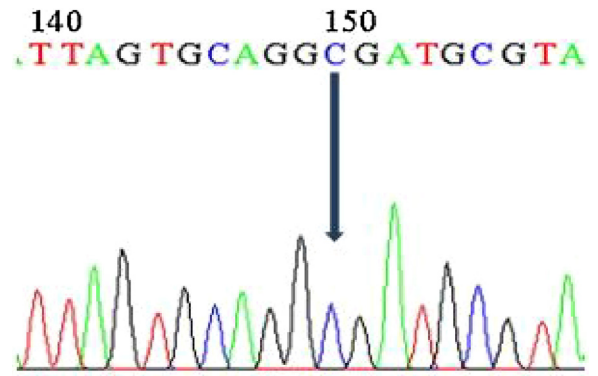
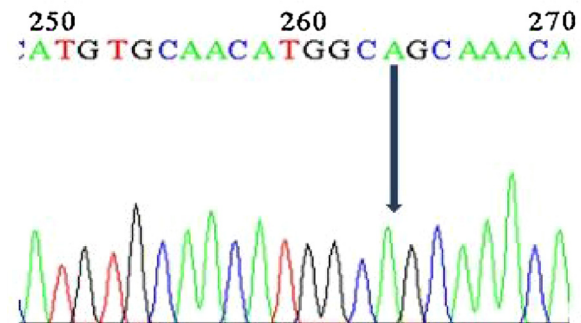
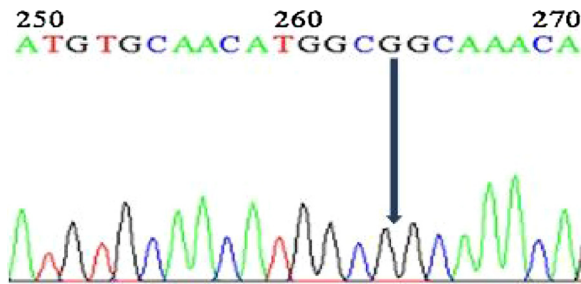
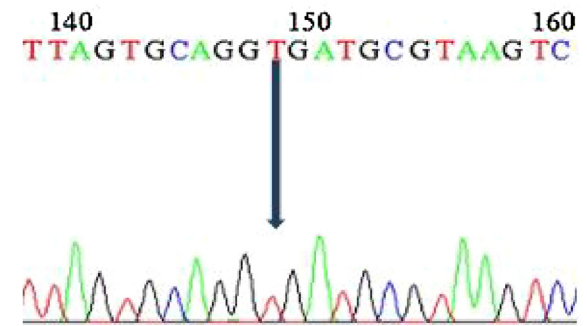


Fig. 5. Partial electropherogram of exon 12 showing c.2484C > T variation (Case no. 2).



Case no. 8 had a VT that was not reverted by ICD shocks. He developed acute kidney injury on the background of an established chronic kidney disease and died subsequently of multi-organ dysfunction. That was the only mortality of the cohort during the study period.

Other patients had an uneventful follow-up.

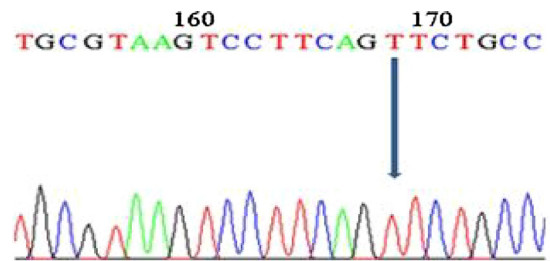
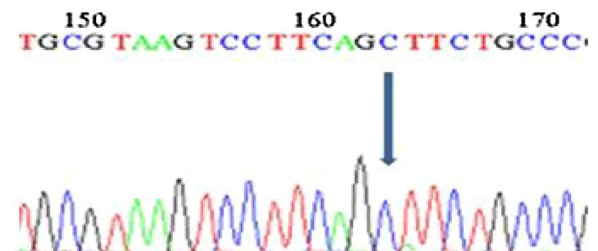


Fig. 6. Partial electropherogram showing cytosine insertion c.2489 + 14insC (Control).



Except cases no. 2 and 11, all of them were on amiodarone. Case no. 2 was on sotalol and case no. 11 was not on any antiarrhythmic therapy.

The most common echocardiographic finding was dilated right atrium (RA)/right ventricle (RV) that was present in all patients along with various degrees of tricuspid regurgitation. Severe regurgitation was present in two patients (case no. 2 and 4). Regional RV akinesia was present in all patients except case number six. Left ventricular systolic dysfunction was present in two cases (case no. 3 and 4). Case no. 7 had thinned out left ventricular apex with normal overall contractility of the left ventricle.

Seven patients had undergone cardiac MR imaging. All seven had dilated RA/RV. All seven had decreased RV systolic function except case no. 6. RV free wall fatty infiltration was found in three cases (case no. 6, 9 and 11). Patchy enhancement of RV free wall was present in two cases (case no. 2 and 5), and RV trabecular disarray was found in case no. 3 and 9. Left ventricular systolic dysfunction was present in two cases (case no 3 and 4).

On sequencing exon 11 and 12 of the patients, one patient had a *PKP2* alteration. Case no. 2 had two intronic variations, c.2299 + 7C > T (exon 11) as shown in Fig. 3 and c.2489 + 72G > A (exon 12) as illustrated in Fig. 4. The same patient had variation in exon number 12, c.2484C > T in which thymine replaced cytosine base in GGC codon as depicted in Fig. 5. All these variants were in homozygous state.

No other case had any variation in exon 11 and exon 12.

Controls consisted of seven subjects, six males and one female with mean age of 63.7 years. All of them were on cardiology outpatient department follow-up for hypertension. They had no history and evidence of cardiac disease. Exon 11 and 12 of *PKP2*

gene were also sequenced in them. One of the controls, a 63-year-old male had an intronic variation of cytosine insertion c.2489 + 14 insC (exon 12) as shown in Fig. 6.

4. Discussion

In this study, we assessed the clinical features of ACM and did a limited *PKP2* gene sequencing. Age of presentation in our patients ranged from fourteen to fifty years.

The reported prevalence of familial involvement is around 50%,⁷ but in our study, only one patient had a confirmed family history of ACM.

Although ACM accounts for 11%–22% of cases of sudden cardiac death in the young athlete patient population,⁸ none of our patients were involved in athletic activity.

Unpublished data from our Institute suggest that ACM is an underlying cause of VT in 14% of the patients presenting with VT (personal communication). In our study, all but one patient presented with VT.

On sequencing of exon 11 and exon 12, we found changes in one patient.

Case number two had two intronic variations, c.2299 + 7C > T (C nucleotide is replaced by T nucleotide, exon 11) and c.2489 + 72G > A (G nucleotide is replaced by A nucleotide, exon 12). Both of these variations have no known pathogenic role in ACM. The former variation was found at a frequency of >1% in general population.⁹ and there are no reports of any relationship with the last variation with ACM pathogenesis.⁶

The same patient had an exonic variation in exon 12 i.e. c.2484C > T where thymine replaced cytosine. The codons are GGC and GGT respectively. This change should not lead to any change in

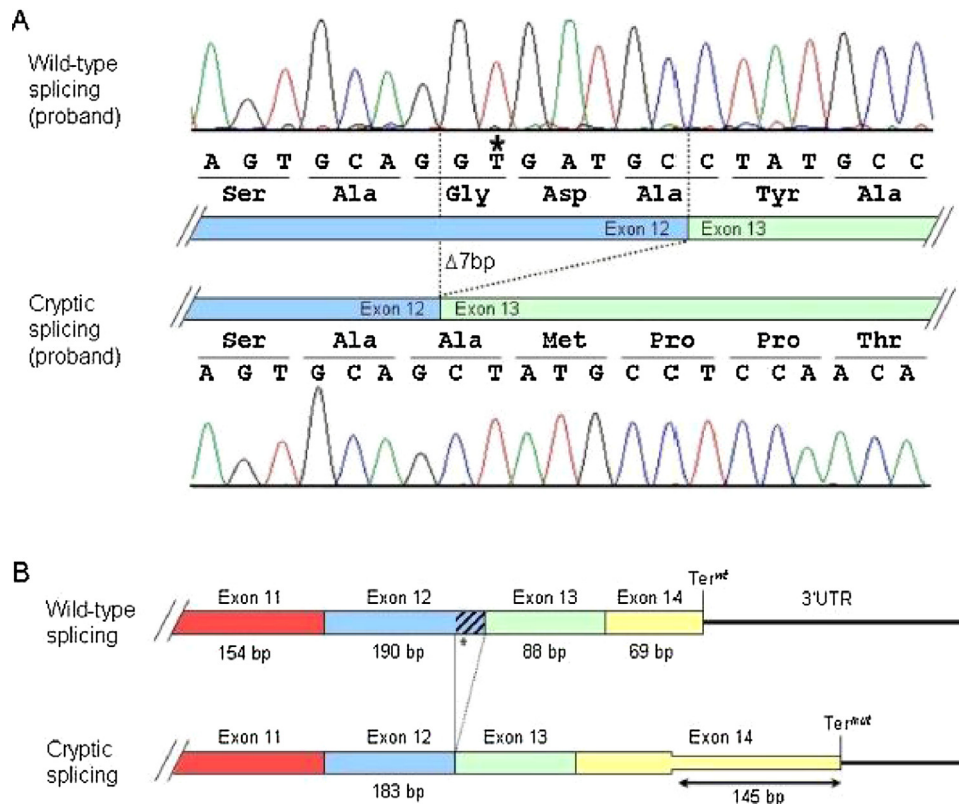


Fig. 7. Cryptic splicing of the *PKP2* transcript caused by the c.2484C > T mutation.

A: Chromatograms of cloned RT-PCR products. Top panel: wild-type splicing in the proband. Bottom panel: mutant splicing results in deletion of the last seven nucleotides in exon 12 and causes a subsequent frameshift. B: Diagram illustrating consequences of cryptic splicing on translation. Boxes indicate exons, and the 3'UTR is drawn as a line. Ter^{wt} and Ter^{mut} indicate the wild-type and mutant termination codons, respectively. (Copyright © John Wiley and Sons, used with permission)

the protein as both the codons represent glycine {p. (G828G)}. However, in the first case report of such mutation, Awad et al described the same mutation in a patient suffering from ACM. They showed that this change leads to a cryptic splice mutation with a 7-nucleotide deletion in exon 12 as illustrated in Fig. 7. The ensuing frame-shift disrupts the last 54 amino acids of *PKP2* and extends the open reading frame by 145 nucleotides into the 3' untranslated region as shown in Fig. 5.¹⁰ This is also the first report which described a recessive mutation of *PKP2* causing ACM. Since then there have been many other reports of the same mutation in other ACM patients.¹¹

The patient described by them was a 44 year old female who was diagnosed in her late teens and was of Caucasian European ancestry. She had greater than 1000 VPCs in 24-h Holter monitoring, had no cutaneous abnormalities and had normal left ventricular systolic function. Our case no. 2 was diagnosed at 18 years of age. She also had more than 1000 VPCs on 24h Holter monitoring and had no cutaneous findings. In their study, den Haan AD et al have mentioned a 23 old male with the same variation who had one major structural criterion and three minor criteria, the details of which they haven't mentioned.¹¹

The variation found in a control subject (c.2489 + 14insC) is a common finding in general population.⁶

5. Conclusion

Our study describes various clinical parameters in ACM patients from the Kashmir valley and a recessive mutation in *PKP2* gene responsible for ACM after a limited *PKP2* gene sequencing. More ACM patients are being diagnosed in our institute. So we suggest a detailed study of the disease. Setting up of an ACM Registry will be a baby step for the same.

6. Limitations

The limitations of our study are:

- a A small number of patients.
- b Inability to sequence the entire *PKP2* gene. A study that includes sequencing of the majority of the genes, not only *PKP2*, involved in ACM will provide more comprehensive results.
- c Inability to verify the cryptic splice mutation that results from c.2484C > T variation.

d We did not study the family members of the cohort.

Conflict of interest

We have no conflict of interest to declare.

Source of funding

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