



## A novel *PKP2* mutation and intrafamilial phenotypic variability in ARVC/D

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### Abstract

**Background:** Arrhythmogenic ventricular cardiomyopathy (AVC) is an inherited cardiac disorder affecting 1 in 1000 individuals worldwide. The mean diagnosed age of disease is 31 years. In this article, an Iranian family reported that they were affected by ARVC due to a novel *PKP2* mutation.

**Methods:** Clinical evaluations, 12-lead ECG, CMR, and signal-averaged ECG were performed. After DNA extraction, genetic testing was done, and PCR-sequencing was applied to find causal mutations. Segregation analysis was also performed for the family.

**Results:** ARVC criteria were documented in the patients. Genetic testing revealed a novel chain termination mutation (p.Tyr168Ter) in *PKP2* gene; this mutation was transmitted from the mother to her 23-year-old son, but only the son was affected with ARVC.

**Conclusion:** Modifier genes were indicated using interactome analysis of Plakophilin 2 protein (*PKP2*); they might have led to phenotypic variability through cellular mechanisms, such as nonsense-mediated mRNA decay. At least, 9 proteins were identified that might have affected Plakophilin 2 protein function, and consequently, rationalizing this intrafamilial phenotypic variability. This study highlighted the role of modifier genes involved in ARVC as well as the major role of *PKP2* mutation in developing the disease in our population.

**Keywords:** ARVC/D, *PKP2* gene, Phenotypic variability

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### Introduction

Arrhythmogenic ventricular cardiomyopathy (AVC or previously ARVC/D, arrhythmogenic right ventricular cardiomyopathy/dysplasia) is an inherited cardiac disease defined by abnormal structure and function of the right and possibly the left ventricle, resulting in the progressive fibrofatty replacement of myocardium (1). It has been estimated that ARVC/D frequency affects 1 in 1000 individuals, with a mean diagnosed age of 31 years (2). Clinical symptoms of the affected individuals include palpita-

tions, syncope, sudden death, atypical chest pain, and dyspnea ventricular heart failure. Based on the 2010 task force criteria (3), ARVC/D is diagnosed by major and minor diagnostic criteria of echocardiographic, arrhythmic, and tissue abnormalities. Approximately 30% to 50% of the patients show a positive family history (4). Mutations in desmosome coding genes have been reported to cause ARVC in 40% to 50% of the cases (5). Desmosomes act as anchors between myocytes and intermediate

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#### ↑What is “already known” in this topic:

ARVC/D frequency affects 1 in 1000 individuals, with a mean diagnosed age of 31 years. Heterozygous mutation in *PKP2* gene, encoding Plakophilin-2, is the common cause of ARVC/D.

#### →What this article adds:

A novel mutation is reported as well as segregation analysis of this mutation within the family. In silico analysis of the novel mutation, it was shown that its pathogenicity leads to ARVC phenotype. The role of modifier genes is indicated using interactome analysis. Intrafamilial phenotypic variability, which is an important issue in genetic counseling in ARVC/D, is presented in an Iranian family.

filaments/cells and involve in Wnt/B-Catenin signaling. Plakophilin-2 protein, which participates in intercellular coupling and interacts with voltage-gated sodium channel complex, has an essential role in heart morphogenesis (6).

Heterozygous mutation in *PKP2* gene, encoding Plakophilin-2, is the common cause of ARVC/D (7-9). More than 200 mutations in *PKP2* gene have been reported so far, which are dominantly categorized as point mutations. Here, a chain termination variant in *PKP2* gene is described for the first time that causes ARVC/D with low penetrance.

**Methods**

Clinical evaluations, ECG, 12-lead ECG, CMR, and SAECG were performed. Family history was also recorded. After obtaining the signed informed consent form, 5 mL peripheral blood was used for DNA extraction using standard methods. To date, mutations of 7 major genes including *RYR2*, *TMEM43*, *DSP*, *PKP2*, *DSG2*, *DSC2*, and *JUP* have been reported to cause ARVC/D. Genetic testing was performed. Briefly, coding regions of *PKP2* gene (NM\_001005242) (exons and intron boundaries) were amplified using PCR reactions (primers are available upon request), and direct sequencing was performed using

BigDye protocol by sequencing analyzer ABI 3500 (PE Applied BioSystems, US).

**Results**

A 23-year-old male presented with an earlier history of syncope was referred to the hospital. Electrocardiogram (ECG) records showed a broad QRS tachycardia with left bundle branch block (LBBB) morphology and superior axis, with a rate of 260 beats/minute terminated by DC-shock (Fig. 1a). Medical history showed repeated episodes of sudden onset palpitation without syncope. A history of sudden cardiac arrest in the patient’s aunt was recorded.

The resting 12-lead ECG was consistent with T-wave inversion in leads V1-V5, with no bundle branch block patterns (Fig. 1b). Laboratory tests were within the normal ranges, from mild to moderate LV systolic dysfunction, with an estimated ejection fraction of 40% to 45%. The right ventricle (RV) was also severely enlarged, with moderate to severe dysfunction. Cardiac magnetic resonance imaging (CMR) of the patient revealed diffuse evidence of localized dyskinesia and microaneurismal formation in the anterior RV free wall, inferior RV wall, and the right ventricular outflow tract (RVOT) region in addition to localized LV myocardial thinning, and a few mi-



Fig. 1. (a) 12-Lead ECG of the patient showing VT with LBBB morphology and superior axis. (b) Resting 12-Lead ECG after admission to our hospital illustrating sinus rhythm and T-wave inversion in V1-V5.

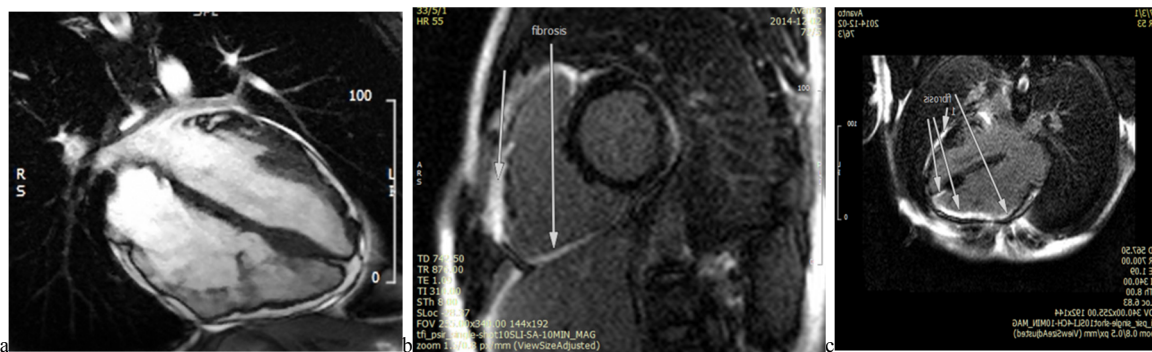


Fig. 2. CMR images depicting RV dilation and aneurysm formation (a) and diffuse fibrosis in RV and parts of LV (b and c) (videos are available upon request).

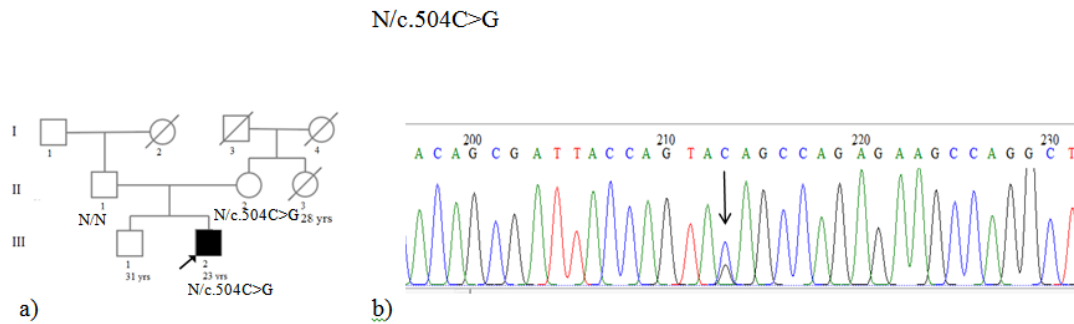


Fig. 3. (a) Pedigree of the family. Individual III2 and II2 had heterozygous c.504C>G. (b) Electropherograms of the patient showed a heterozygous stop codon mutation in Exon 3, c.504C>G, TAC>TAG, and p.Tyr168Ter.

croaneurismal formation, especially in mid and apico-lateral LV segments. Diffuse subepicardial to transmural fibrosis of RV inferior and anterior wall extended close to RVOT, and RV apex was determined in late gadolinium enhancement (LGE) images; moreover, LV inferoapical segment and a subepicardial fibrosis of mid lateral LV segment were found (Fig. 2).

Signal-averaged ECG (SAECG) indicated the presence of late potentials. Taken together, the definite clinical diagnosis was made according to 2010 Task Force major and minor criteria for diagnosis of ARVC/D (3).

Sequencing of the *PKP2* gene showed a new heterozygous stop codon mutation in Exon 3, c.504C>G (TAC>TAG), and p.Tyr168Ter (Fig. 3). This variant has not yet been reported in the literature. However, since the mutation leads to formation of a stop codon terminating the translation, the resulting truncated protein (p.Tyr168Ter) causes pathogenic effect.

Cascade screening of first-degree family members of this individual for the new variant showed that his mother is heterozygote, but the mutation was not found in his

brother and his father. His mother underwent a comprehensive cardiological work-up and ARVC was not diagnosed in this individual.

Protein-protein interaction analysis was performed using STRING10 to find whether the proteins interact with Plakophilin 2.

### Discussion

A 23-year-old male (III2) was presented with diagnosed ARVC due to a novel chain termination mutation in the *PKP2* gene. ARVC was diagnosed according to the 2010 Task Force criteria. Genetic testing was performed for his family, which showed mutation in his mother (II2), following an autosomal-dominant inheritance.

Genetic testing can be used for possible ARVC patients (1 major or 2 minor criteria of the Heart Rhythm Society/European Heart Rhythm Association's Expert Consensus Statement). Mutation identification would be useful for screening other family members, so that effective life style and predictive management could be used.

*PKP2* mutations are the most common cause of ARVC in some populations; mutation frequency has been reported to range from 10% to 78% in ARVC patients (9, 10). To date, nearly 200 mutations have been described to be pathogenic in *PKP2* gene (<http://www.hgmd.cf.ac.uk/>). c.504C>G (p.Tyr168Ter) in *PKP2* gene, which is a nonsense mutation creating a stop codon at 168 position of the protein. Of 68 reported substitution mutations, 24 are nonsense (<http://www.hgmd.cf.ac.uk/>). In general, nonsense mutations are responsible for nearly 11% of all mutations leading to inherited disease and approximately 20% of disease-associated point mutations within coding regions (11).

As mentioned, p.Tyr168Ter in *PKP2* was observed in both the son and his mother, but the mother's condition was apparently normal. The patient underwent successful ICD implantation for secondary prophylaxis of sudden cardiac death and was discharged from the hospital in a good condition after 2 days. To find out why his mother had no diseased phenotype, we considered several hypotheses; however, she might have shown very small degrees of the phenotype, so she was underdiagnosed. On the other hand, this mutation may have a reduced penetrance. There are different factors affecting the penetrance of a mutation, such as lifestyle, genetic variations, sport

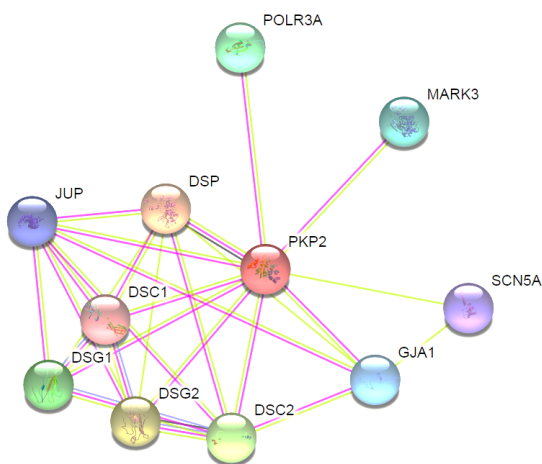


Fig. 4. Protein-protein interaction network of Plakophilin 2 with STRING10. Plakophilin 2 is one of several proteins that make up desmosomes that provide strength to the myocardium and are involved in signaling between neighboring cells. Predicted functional partners are as follow: DSP: desmoplakin; DSG2: desmoglein 2; DSC2: desmocollin 2; DSG1: desmoglein 1; DSC1: desmocollin 1; POLR3A: polymerase (RNA) III, (DNA directed) polypeptide A; GJA1: gap junction protein, alpha 1; MARK3A: MAP/microtubule affinity-regulating kinase 3; SCN5A: sodium channel, voltage-gated, type V, alpha subunit.



activity, and gender (12-16). However, interaction analysis of *PKP2* protein by STRING database Version 10.0 (17) was performed to check the probable modifier genes. STRING is a database of known and predicted proteins and showed that *PKP2* gene is related to desmoplakin [DSP], Desmoglein 2 [DSG2], Desmocollin 2 [DSC2], Desmoglein 1 [DSG1], gap junction protein, Alpha 1 (GJA1), polymerase (RNA) III [POLR3A], MAP/microtubule affinity-regulating kinase 3 [MARK3A], sodium channel, voltage-gated, type V, alpha subunit (SCN5A), and Desmocollin 1 (DSC1) (Fig. 4). DSP is a major high molecular weight protein of desmosomes that plays a role in assembling the desmosomal cadherin-plakoglobin complexes to discrete transmembrane domains and anchor desmosomal intermediate filaments. DSG2, DSC2, DSG1, and DSC1 are components of intercellular desmosome junctions. DSG1 and DSC1 may also contribute to epidermal cell positioning by mediating differential adhesiveness between cells that express different isoforms. Therefore, variants of these genes leading to different isoforms of a protein may influence and affect the penetrance and expression of this mutation. Hence, other related factors, eg, various isoforms of the protein involved in desmosome junctions, could influence or compensate for Plakophilin 2 deficiency caused by this nonsense variant; in other words, although Plakophilin 2 had a defective function in the mother, other related proteins could compensate this deficiency (of Plakophilin 2, having Tyr168Ter mutation), while no such phenomenon was found in her son. However, this hypothesis should be investigated in this family. Finding this gene is an interesting idea because it may have a significant impact on unravelling the involved factors in NMD process in this individual and can open a new window to the treatment of the disease. Further studies are needed to find the prevalence of *PKP2* mutations and modifier genes.

Alternatively, phenotypic variability may be due to effects of modifying factors including environmental and genetic agents interacting with deficient Plakophilin 2. Modifier factors may usually influence the expression, age at onset, and progression of the disease. It is likely that the existence of an unknown modifier effect, such as delE120 mutation in *GJB2* gene, may modulate this scenario (14). Nonsense mutations result in a premature termination codon and most often lead to complete loss of protein and reduce mRNA levels because of the nonsense-mediated mRNA decay (NMD). NMD, as an mRNA quality-control mechanism, starts selective degradation of mutant transcripts and improves cellular surveillance (18). In the studied family, NMD may decrease the severity of disease in his mother.

## Conclusion

This study, as a first report of *PKP2* mutation in an Iranian family, highlights the role of modifier genes involved in ARVC. Finding the cause of phenotypic variability due to this variant within the family may shed light on the road of treatment approach for ARVC. Also, *PKP2* mutation may play a major role in the development of disease in our population.

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## Conflict of Interests

The authors declare that they have no competing interests.

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