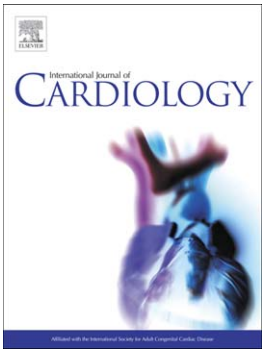

Brugada Syndrome and *PKP2*: Evidences and uncertainties

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PII: S0167-5273(16)30642-8
DOI: doi: 10.1016/j.ijcard.2016.03.194
Reference: IJCA 22323

To appear in: *International Journal of Cardiology*

Received date: 24 March 2016
Accepted date: 27 March 2016

Please cite this article as: Campuzano Oscar, Fernández-Falgueras Anna, Iglesias Anna, Brugada Ramon, Brugada Syndrome and *PKP2*: Evidences and uncertainties, *International Journal of Cardiology* (2016), doi: 10.1016/j.ijcard.2016.03.194

Brugada Syndrome and *PKP2*: evidences and uncertainties

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Disclosure

The authors declare no conflicts of interest to disclose

Acknowledgement

None

Key Words

Sudden Cardiac Death, Brugada Syndrome, Desmosome, PKP2

Abstract

Common electrocardiographic patterns in Brugada Syndrome and Arrhythmogenic Cardiomyopathy have been reported despite phenotypic alterations during its clinical course. Recently, potentially pathogenic variants in the *PKP2* gene, the most prevalent gene associated with Arrhythmogenic Cardiomyopathy, has been associated with Brugada Syndrome. In addition, *in vitro* studies demonstrated the interaction between plakophilin-2 and sodium channel, the most prevalent gene associated with Brugada Syndrome. All these facts reinforce the suggested overlapping between both entities but little is known about the pathophysiological mechanisms. We have performed a comprehensive genetic revision of all *PKP2* genetic variants currently associated with Brugada Syndrome. In all variants we identified a lack of solid evidences in order to establish a definite genotype-phenotype association. Hence, despite we believe that *PKP2* analysis should be considered as a part of molecular genetic testing in Brugada Syndrome patients, comprehensive clinical and molecular studies should be performed before establish pathogenic association. Therefore, *PKP2* variants in Brugada Syndrome cases should be interpreted carefully and additional studies including family segregation should be performed before translation into clinical practice.

In 1992, Brugada Syndrome (BrS) was described as a new cardiac entity characterized by an electrocardiographic (ECG) pattern of right bundle branch block and persistent ST elevation in leads V1-V3, and association with sudden cardiac death (SCD) without structural heart alterations [1]. Currently, it is classified as an inherited rare channelopathy, which typically causes SCD at rest, mainly in men in the third-fourth decade of life [2]. Several studies established that around 25% of clinically diagnosed BrS cases carry at least one pathogenic variant in the *SCN5A* gene. This gene encodes the sodium voltage-gated channel alpha subunit 5 (Nav1.5), and it is the most prevalent gene associated with BrS. Additional pathogenic variants located in other 17 genes have also been associated with the disease, despite representing all together only nearly 10% of clinically diagnosed cases [2]. One of the last genes associated with BrS is *PKP2* (ENSG00000057294) which encodes the protein plakophilin-2 (PKP2, ENSP00000070846). It is one of the main components of the armadillo complex located in the outer dense plaque of cardiac desmosomes that interacts with multiple other cell adhesion proteins. Therefore, absence and/or alteration of plakophilin-2 structure in the cardiac desmosomes impair myocytes interactions among myocytes, inducing myocardium disruption, particularly in response to mechanical stress [3]. To date, more than 200 pathogenic variants have been reported in this gene as cause of arrhythmogenic cardiomyopathy (AC), an inherited rare disease characterized by fibro-fatty substitution of myocardium leading to malignant arrhythmias and SCD [4]. The most prevalent variations in the *PKP2* gene correspond to small *insertion/deletion* with frameshift pattern (40%), followed by *nonsense* variants in 25% of cases, *missense* variants in 20%, and pathogenic splice site variations in nearly 15% of cases [5].

Currently, phenotype similarities are on debate and differential diagnosis is a current matter of argue on the basis of common predominant RV involvement with abnormalities in right precordial ECG repolarization, RV outflow tract conduction, and RV arrhythmias leading to ventricular fibrillation (VF) [6, 7]. Several cases showing overlapping of entities have been reported but characteristic clinical features remain still unclear [8]. Hitherto, the main evidence that AC may present with phenotypic features characteristically observed in BrS was published by Corrado et al in the largest study on SCD victims with an ECG pattern of right precordial ST-segment elevation [9]. Recently, structural and/or ECG abnormalities commonly considered characteristics of AC have been observed in BrS patients [10-12]. In some cases, initial BrS ecg features can be observed

in the course of developing AC [13]. Therefore, a connection between AC and BrS has been postulated as possible, at least, since the *PKP2* gene has been associated with BrS [13]. Concerning this fact, in 2013, Cerrone et al suggested for the first time that "*PKP2 mutations may be a molecular substrate leading to the diagnosis of Brugada Syndrome*" [14]. Previously, in 2012, it was reported that plakophilin-2 not only participates in intercellular coupling, but it also interacts, directly or indirectly, with the voltage-gated sodium channel complex [15]. Also in 2012, another study reported that a mouse model of haploinsufficiency for plakophilin-2 showed sodium current deficit, leading to flecainide-induced ventricular arrhythmias and SCD [16]. In addition, a study performed in human heart reported that sodium channel signal was decreased in nearly 65% of patients diagnosed of AC [17, 18]. Therefore, integrity of plakophilin-2 is essential for microtubule anchoring and safe delivery of sodium channel to the intercalated disc [19], suggesting the fact that AC and BrS are not two separate entities [7].

Up to now, of more than 200 pathogenic or potentially pathogenic variants located in the *PKP2* gene, only 6 genetic variants have been potentially associated with BrS (p.R101H –CM1511405-, p.S183N –CM142721-, p.M365V –CM142723-, p.T526A -CM142722-, p.R635Q -CM1310619-, and p.K835R -CM1511404-) (Table 1) [14, 20]. All these variants are very rare in the population (MAF<0,05%) according to Exome Aggregation Consortium (ExAC) and Exome Sequencing Project (ESP) –Exome Variant Server, EVS- databases. In addition, these variants are *missense*, and were identified in heterozygous status in index cases with clinical diagnosis of BrS. *In silico* analysis predicted all them as benign/neutral/polymorphism except p.R635Q_*PKP2* which shows a discrepancy between *in silico* predictors. *In vitro* studies of 4 of these 6 genetic variants (p.S183N, p.M365V, p.T526A, and p.R635Q) have shown sodium current alteration. No *in vi*vo studies have been performed in any of them. Regarding variant p.T526A, it is relevant to remark that another change in the same aminoacid (p.T526M) has been reported associated with AC (CM113820) [21, 22] thus resulting in 2 variants associated with 2 different pathologies in the same aminoacid. In addition, the genetic variant p.M365V_*PKP2* has been identified in one case clinically diagnosed of Hypertrophic Cardiomyopathy (HCM) in our own sample cohort. To our knowledge more than one rare alteration has been reported in the same aminoacid of several genes, but always associated with the same cardiac pathology. According to ACMG Standards and

Guidelines for the interpretation of sequence variants [23], family segregation of 3 generations is the minimum required to accept the association variant-disease. Though, familial studies have been possible only in 2 of the variants (p.R635Q and p.K835R). In variant p.R635Q_*PKP2* family segregation was performed in 2 generations and showed complete penetrance. Again, it is relevant to notice that another rare genetic variant was reported in the same aminoacid (p.R635W) but associated with AC (CM0910839) [24]. Therefore, as it occurs in aminoacid p.T526, there exist 2 variants associated with 2 different pathologies in the same aminoacid. Finally, in variant p.K835R_*PKP2*, family segregation in two generations was performed and showed incomplete penetrance, a characteristic treat of all cardiac inherited diseases.

In conclusion, none of the six variants can be fully considered causative in BrS, and their role as phenotype modifier cannot be discarded. However, and taking all data into account, desmosomal gene analysis should be considered as a part of molecular genetic testing in BrS patients, but further clinical and molecular studies should be performed to clarify their pathogenic role in BrS. Genetic data concerning the *PKP2* gene in BrS cases should be interpreted carefully and additional studies including family segregation should be performed before translation into clinical practice.

Aminoacid	Nucleotide	HGMD	dbSNP	ExAC %	EVS % EA/AA/All	Family Segregation	*In Vitro* Studies	*In Silico* PP2/MT/MA	ClinVar	In Home Database
p.R101H (p.Arg101His)	c.302G>A	CM1511405	rs149542398	0.011 (14/121374)	0.04/0.02/0.03	No	No	B/N/P	VUS	-
p.S183N (p.Ser183Asn)	c.548G>A	CM142721	rs373222905	0.0098 (12/121370)	-	No	Yes	B/N/P	Probably Pathogenic	-
p.M365V (p.Met365Val)	c.1093A>G	CM142723	rs143900944	0.035 (43/121402)	0.02/0.01/0.01	No	Yes	B/N/P	VUS	1 sample HCM
p.T526A (p.Thr526Ala)	c.1576A>G	CM142722	rs397516999	0.014 (18/121218)	-	No	Yes	B/N/P	VUS	-
p.R635Q (p.Arg635Gln)	c.1904G>A	CM1310619	-	0.00082 (1/121356)	-	Yes -CP- (2 generations)	Yes	PD/N/P	VUS	-
p.K835R (p.Lys835Arg)	c.2504A>G	CM1511404	rs372729739	0.00082 (1/121394)	0.01/0.001/0.007	Yes -IP- (2 generations)	No	B/N/P	VUS	-

Table 1. Variants in the PKP2 gene associated with Brugada Syndrome. EA: European American, AA: African American, All: all populations, CP: Complete Penetrance, IP: Incomplete Penetrance, PP2: Polyphen2, MT: Mutation Taster, MA: Mutation Assessor, B: Benign, N: Neutral, P: Polymorphism, PD: Possibly Damaging, VUS: Variant Uncertain Significance, HCM: Hypertrophic Cardiomyopathy.

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