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REVIEW

Clinical interpretation of genetic variants in arrhythmogenic right ventricular cardiomyopathy

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Abstract Arrhythmogenic right ventricular cardiomyopathy is an inherited cardiac entity characterized by right ventricular, or biventricular, fibrofatty replacement of myocardium. Structural alterations may lead to sudden cardiac death, mainly in young males during exercise. Autosomal dominant pattern of inheritance is reported in most parts of pathogenic genetic variations identified. Currently, 13 genes have been associated with the disease but nearly 40 % of clinically diagnosed cases remain without a genetic diagnosis. New genetic technologies allow further genetic analysis, generating a significant amount of genetic data in novel genes, which is often classified as of ambiguous significance. We focus on genetic advances of arrhythmogenic right ventricular cardiomyopathy, helping clinicians to interpret and translate genetic data into clinical practice.

Keywords Sudden cardiac death · Arrhythmogenic right ventricular cardiomyopathy/dysplasia · Genetics · Pathogenicity

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Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC), also called arrhythmogenic right ventricular dysplasia (ARVD), arrhythmogenic cardiomyopathy (AC), or arrhythmogenic dysplasia (AD), is a rare cardiac disease characterized by a progressive myocardial fibrofatty replacement of right ventricle (RV), although up to 50 % of cases also show a left ventricular (LV) involvement [1]. The presence of focal inflammatory infiltrates in the myocardium is commonly found in autopsies and biopsies of ARVC [2], as well as, viral particles and markers for apoptosis [3].

These structural alterations are responsible for electrical abnormalities, which may cause subsequent ventricular arrhythmias, syncope, and sudden cardiac death (SCD) [4]. Unfortunately, sudden death is often the first symptom of the pathology, mainly in young males during exercise. Prevalence of ARVC in general population is 1:2,500–5,000, depending on gender (3.1 in men) [5]. ARVC especially affects young athletes, being responsible for up to 15 % of SCD in this group [6]. The diagnosis of the disease is established using a set of criteria as proposed by an international Task Force in 1994, and revised in 2010 including genetic as a criterion [7].

Clinical diagnosis

Four clinical-pathologic stages can be considered in ARVC: the early "silent phase" that can manifest as sudden death because arrhythmias occur without evident structural abnormalities; the "overt electrical disorder" in which right ventricular arrhythmias are associated with structural abnormalities; the "phase of right ventricular failure" with extension of the fibrofatty substitution that leads to RV dysfunction; and finally the phase of "biventricular failure" which is often indistinguishable from dilated cardiomyopathy.

Although imperfect, the diagnosis of ARVC is still based on the Revised Task Force Report Criteria [7], which includes structural, histological, electrocardiographic, and familial factors. The diagnosis is confirmed if two major, one major, and two minor, or four minor criteria from different categories are present. A borderline diagnosis is considered with one major and one minor or three minor criterion from different categories and a possible diagnosis with only one major or two minor criterion. Based on this report, a comprehensive approach for the assessment of a patient suspected of suffering ARVC includes:

- Electrocardiographic evaluation (12-lead ECG and signal-averaged ECG): the ECG can be normal in up to 40 % of patients in the early phases, but as the disease progress abnormalities systematically occur within the first 3–6 years after diagnosis [8, 9]. These include repolarization abnormalities manifested by T-wave inversion in V_1 to V_3 (in 55–85 % of patients) and depolarization abnormalities such as complete or incomplete right bundle-branch block (RBBB) (6–18 %), the pathognomonic epsilon waves (10–37 %), and prolongation of the QRS duration $\geq$110 ms in V_1 to V_3 (26–75 %) [10–16]. Late potentials identified by signal-averaged ECG (SAECG) can also be observed in 50–80 % of patients [10] (Fig. 1).
- Structural evaluation (echocardiography, magnetic resonance, RV angiography): the echocardiogram represents the first-line imaging technique and it may show RV enlargement and regional contraction abnormalities [17]. However, it may be of limited use in the early stage of ARVC. For this reason, cardiac magnetic resonance imaging with late gadolinium enhancement has emerged as a major diagnostic tool for the morphological and functional assessment of the RV, including tissue characterization by distinguishing muscle from fat [18–20]. However, healthy individuals can also show fatty infiltration [21], and therefore, minor abnormalities must be carefully considered in order not to misdiagnose ARVC [22]. RV angiography has traditionally been considered the gold standard for the diagnosis of ARVC with a high specificity (90 %) in case of evidence of akinetic/dyskinetic bulging in the regions of the triangle of dysplasia [23], but it is often reserved for doubtful cases (Fig. 2).
- Evaluation of arrhythmias (12-lead ECG, Holter monitoring, exercise testing, electrophysiological study): the presence of >500 premature ventricular contractions in 24 h or the documentation of ventricular tachycardia of left bundle-branch block morphology (particularly if a superior axis is present) support the diagnosis of ARVC [7].
- Histological evaluation (endomyocardial biopsy): a myocyte loss with fibrosis and fatty infiltration suggests the diagnosis [7, 24]. Due to the patchy nature of the disease this technique is most sensitive when sampling the triangle of dysplasia [25]. The use of electroanatomic mapping has shown to improve the diagnostic accuracy of myocardial biopsy as low-amplitude areas are associated with myocyte loss and fibrofatty replacement [26–29]. Immunohistochemical analysis of conventional biopsy samples may also increase the sensitivity and specificity of this diagnostic tool [30] (Fig. 3).
- Genetic evaluation: according to the Rhythm Society/European Heart Rhythm Association Consensus Statement, a comprehensive or targeted ARVC genetic testing may be useful for patients satisfying task force diagnostic criteria [31]. However, it must be underlined that genetic testing may only support a clinical diagnosis, and a negative test cannot rule out ARVC. On the other hand, once the ARVC causative mutation has been identified in the index case, genetic testing is recommended for family screening [31].

Clinical management

There is limited data on risk stratification in ARVC emerging from retrospective studies that have identified several markers of adverse prognosis: young age, competitive sport activity, history of syncope, or familial SD, QRS dispersion $\geq$50 ms, precordial T-wave inversion beyond V_1, QT dispersion >65 ms, severe RV dysfunction, LV involvement, inducible VT, and antiarrhythmic drug failure during serial electrophysiological studies [32–40] (Fig. 1).

Unfortunately, there are no clear-cut guidelines to select the best management approach in patients with ARVC, so treatment should be individualized. The different options include pharmacological treatment, the placement of an implantable cardioverter defibrillator (ICD), radiofrequency ablation, and transplantation.

In the absence of randomized trials, sotalol may be effective in the treatment of ventricular arrhythmias,

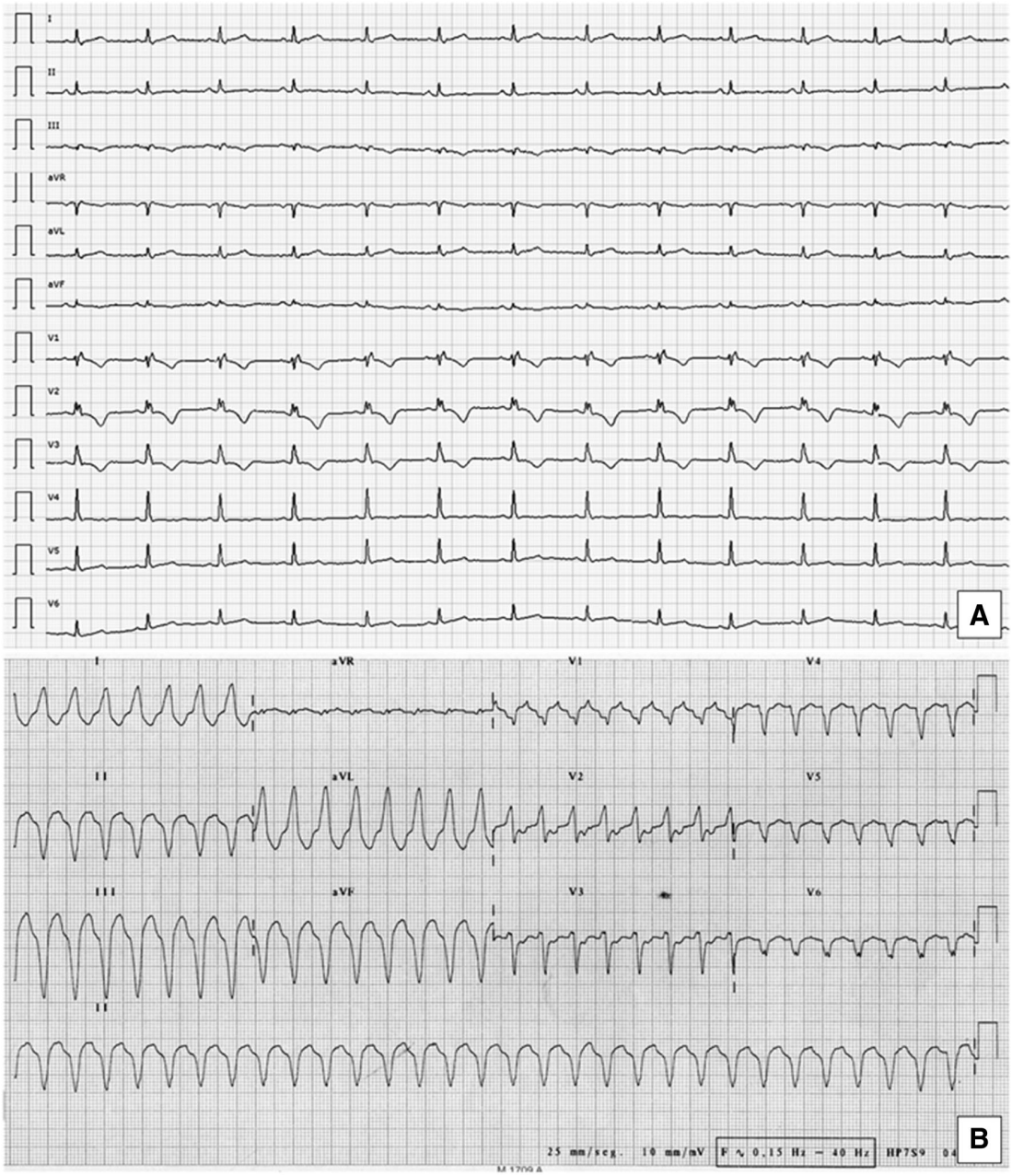

Fig. 1 12-Lead ECG during SR and sustained monomorphic VT a patient diagnosed of ARVD. **a** ECG during SR showing precordial T-wave inversion and epsilon waves in right precordial leads. **b** ECG during VT with LBBB morphology and superior axis

whereas class I drugs have not shown to be useful in this patient population [41, 42]. Amiodarone might also be of use although patients unresponsive to sotalol are unlikely to respond to amiodarone according to some authors [41, 43], whereas others have suggested superior efficacy of this drug compared to beta-blockers or sotalol [44]. The ability of drugs to prevent SD remains to be proven and data obtained from studies in patients with ICD suggest otherwise [37, 39].

ICD therapy is the cornerstone for ARVC patients at moderate to high risk for SCD and can prolong survival in this population [37] and it should be considered in patients presenting with cardiac arrest or life-threatening ventricular arrhythmias in which drug treatment is ineffective or is associated with severe secondary effects, and in case of syncope or sustained VT in the presence of risk factors.

Finally, radiofrequency ablation of ventricular arrhythmias has traditionally only been used in medically

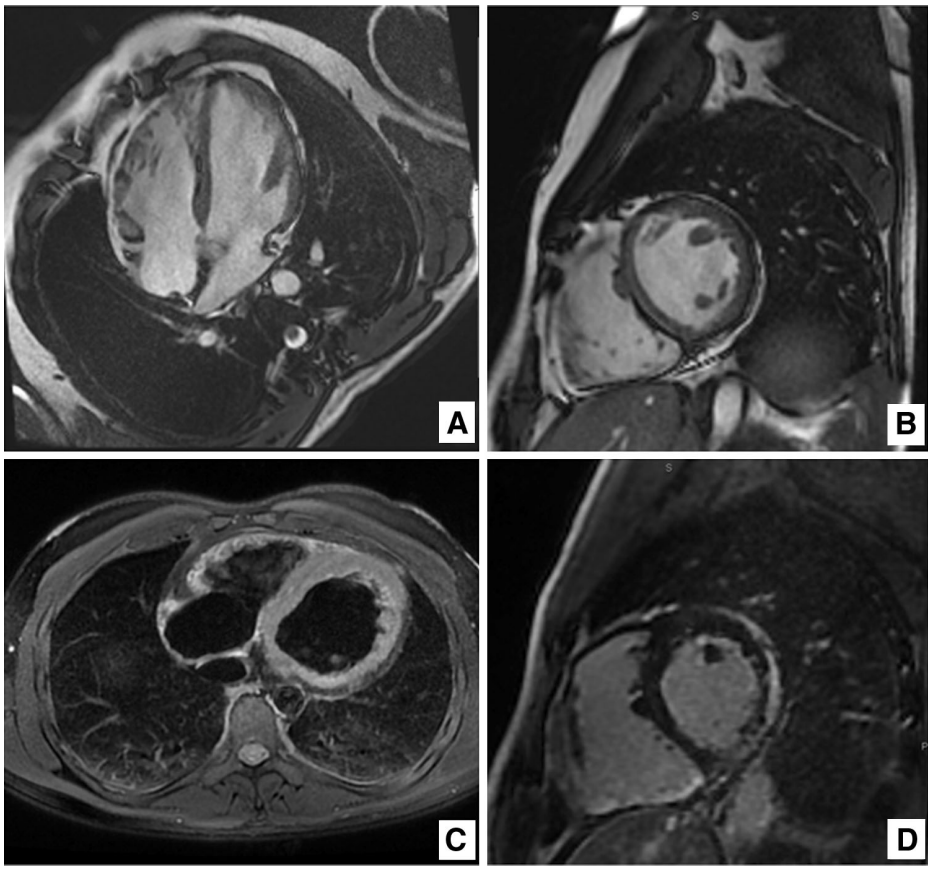

Fig. 2 High resolution contrast-enhanced cardiac MR. **a**, **b** Diastolic 4-chamber and short axis view of the RV showing dilatation and trabeculation and a thinned diaphragmatic wall. **c** Axial view in T1 showing fibrofatty infiltration of the RV wall. **d** Short axis showing late gadolinium enhancement of the RV diaphragmatic wall and the lateral wall of the LV

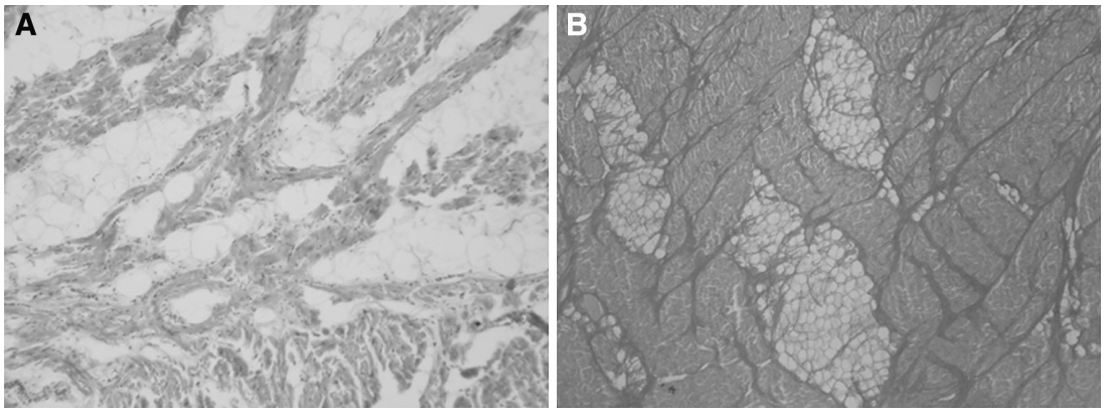

Fig. 3 Representative microscopic images of ARVC. **a** Hematoxylin–eosin staining showing fibrofatty replacement (scale: ×100). **b** Picro-sirius red staining showing fibrofatty replacement (scale: ×40)

refractory cases. However, it is a therapeutic option in patients unresponsive or intolerant to antiarrhythmic drugs, and it may also be considered a first-line approach in patients presenting with recurrent monomorphic VT as antiarrhythmic drugs have not clearly proven to be effective and ablation with the currently available techniques is successful in a significant proportion of patients [45]. A combined endocardial and epicardial mapping and ablation approach appears to improve results [46, 47].

Genetics

Currently, nearly 60 % of clinically diagnosed cases show at least one pathogenic genetic variation responsible for the

Table 1 Genes associated with ARVC. AD: autosomal dominant

Gene	Locus	Protein	Inheritance	Prevalence (%)
PKP2	12p11	Plakophilin-2	AD/AR	30–40
DSP	6p24	Desmoplakin	AD/AR	10–15
DSG2	18q12.1	Desmoglein-2	AD/AR	3–8
DSC2	18q21	Desmocollin-2	AD/AR	1–5
JUP	17q21	Junction plakoglobin	AD/AR	<1
TTN	2q31	Titin	AD	<1
TMEM43	3p25.1	Transmembrane protein 43	AD	<1
TGFB3	14q24	Transforming growth factor beta-3	AD	<1
PLN	6q22.1	Phospholamban	AD	<1
LMNA	1q22	Lamin A/C	AD	<1
DES	2q35	Desmin	AD	<1
CTNNA3	10q22.3	Alpha-T-catenin	AD	–
SCN5A	3p22.2	Sodium channel, voltage-gated, type V, alpha subunit	AD	–

AR autosomal recessive

disease after comprehensive genetic screening of 13 genes associated with ARVC to date. These genes encode desmosomal proteins: plakophilin-2 (*PKP2*), desmoplakin (*DSP*), desmocolin-2 (*DSC2*), desmoglein-2 (*DSG2*), and plakoglobin—PG—encoded by the *JUP* gene; and non-desmosomal proteins: transmembrane protein 43 (*TMEM43*), transforming growth factor beta-3 (*TGFB3*), Catenin alpha-3 (*CTNNA3*), desmin (*DES*), lamin A/C (*LMNA*), titin (*TTN*), phospholamban (*PLN*) [1, 48], and recently described sodium channel, voltage-gated, type V, alpha subunit (*SCN5A*) [49] (Table 1; Fig. 4). Most pathogenic genetic variants reported have an autosomal dominant pattern of inheritance but, in some cases, a recessive pattern has also been described [50, 51]. Recently, even compound and/or digenic pathogenic genetic variants have been reported in ARVC [52, 53]. The majority of the pathogenic genetic variants associated with ARVC have been identified in genes encoding proteins of the desmosome, which are cellular structures for cell membrane, crucial to provide structural and functional integrity to adjacent cells in myocardium. However, the specific molecular defects caused by these pathogenic genetic variants are not completely understood.

The origin of adipocytes in the myocardium of ARVC has been an enigma for years. Traditionally, two different mechanisms have been proposed to be involved in the fibrofatty replacement: apoptosis and inflammation. DNA fragmentation and the expression of CPP-32, both markers of apoptosis, are present in the myocardium of 75 % ARVC cases [3]. Inflammation has also been association in ARVC since a high percentage of ARVC cases (up to 60 %) showed inflammatory infiltrates and viral particles in the myocardium, suggesting that ARVC could also have an infectious component [2]. The role of inflammation and apoptosis in ARVC is still unknown. In 2009, the

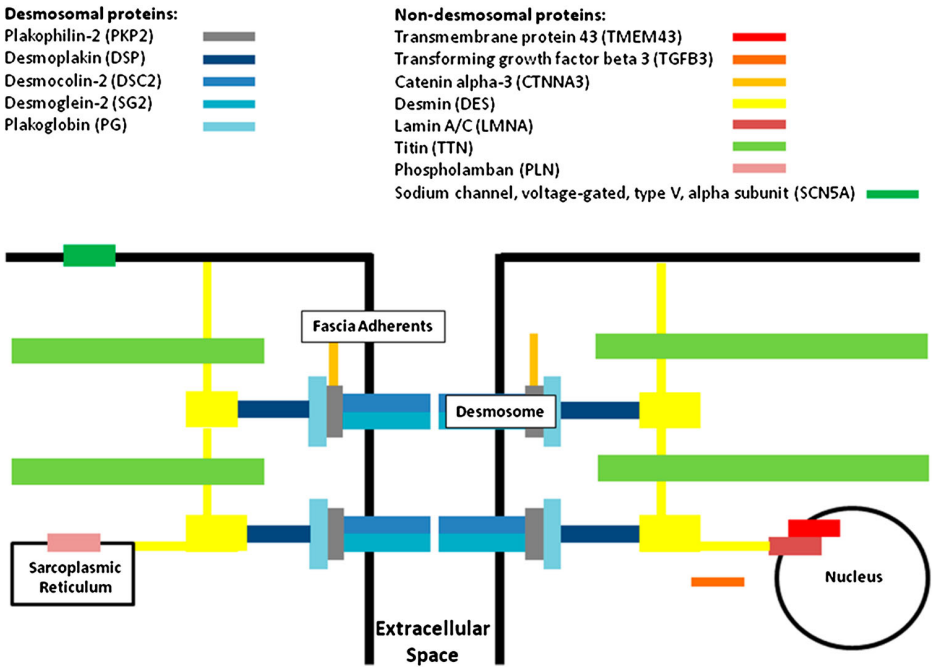

Fig. 4 Desmosomal and non-desmosomal proteins associated with ARVC. Plakophilin-2, desmoplakin, desmoglein-2, desmocollin-2, junction plakoglobin, titin, transmembrane protein 43, transforming growth factor beta-3, phospholamban, lamin A/C, desmin, alpha-T-catenin, sodium channel, voltage-gated, type V, alpha subunit

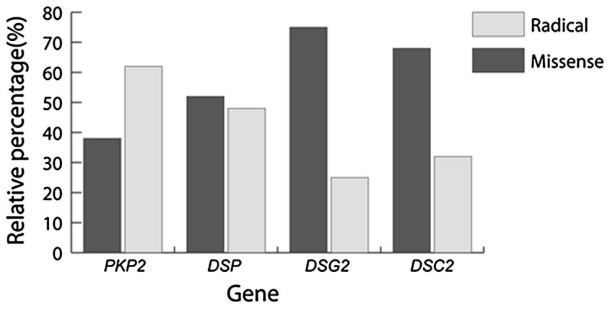

Fig. 5 Relative percentage of "radical" and *missense* pathogenic variations in each desmosomal gene associated with ARVC

adipocytes origin was elucidated when it was developed a mice model with conditionally suppressed expression of DSP in cardiac myocytes lineage. The investigators showed that nuclear localization of PG suppresses the canonical *Wnt* signaling and leads to differentiation of a subset of second heart field progenitor cells to adipocytes [54]. Thus, this mechanism would be triggered by pathogenic genetic variants in desmosomal genes, which promote nuclear localization of PG.

Desmosomal genes

As previously mentioned, ARVC has been widely linked to pathogenic genetic variants in desmosomal genes. Hence, ARVC is mainly caused, at least by one pathogenic genetic variant in one of the genes encoding desmosomal proteins: plakophilin-2 (*PKP2*), desmoplakin (*DSP*), desmocolin-2 (*DSC2*), desmoglein-2 (*DSG2*), and plakoglobin (PG), encoded by the *JUP* gene. Genetic series have reported that 30–40 % carry a pathogenic genetic variant in the *PKP2* gene, followed by *DSP* (10–15 %) [55], *DSG2* (3–8 %) [56], and *DSC2* (1–5 %) [57]. Recently, other genetic alterations such as copy number variations (CNV) in *PKP2* gene have been associated with the disease but they are very rare [58, 59]. Therefore, after a comprehensive genetic analysis, nearly 40 % of clinically diagnosed cases remain without identified genetic cause. Consequently, pathogenic genetic variants located in unknown genes and/or other genetic defects could explain, at least, a part of the ARVC cases currently without a genetic diagnosis.

Plakophilin-2

The most prevalent pathogenic genetic variants associated with ARVC are radical mutations (*nonsense* and *frame-shift* variations) in *PKP2*, followed by *missense* mutations also in this gene (Fig. 5). To date, more than 120 pathogenic genetic variants has been associated with ARVC in the *PKP2* gene [60]. The inheritance in *PKP2* is commonly through autosomal dominant pattern, although a recessive form was also described [61], who identified a novel homozygous small deletion in *PKP2*. It is also important to remark the recent role of CNV in ARVC. Hence, the first study was published in 2011 [48], which identified three *PKP2* exon deletions among 149 individuals affected by ARVC. After that, in the last 3 years [58], Mura et al. [59] reported cases of CNV in this pathology. Consequently, we strongly recommend that genetic testing in ARVC includes CNV screening when conventional sequencing analysis does not identify any pathogenic genetic variants in ARVC associated genes.

Cadherins

The desmosomal cadherins, DSG2, and DSC2 are transmembrane molecules that mediate adhesion through their extracellular domains and serve as a scaffold for assembly of the desmosomal plaque through their cytoplasmic domains. The cadherin tails associate with armadillo family members, PG and PKP2, which in turn associate with DSP. DSP completes the link with intermediate filaments (IFs) through its C-terminus [62–64]. The association between the *DSC2* gene and ARVC was reported for the first time in 2006 [65]. Recently, the *DSC2* gene was associated with a recessive pattern of inheritance with no cutaneous alterations [66]. In 2006, *DSG2* was also associated in ARVC cases [51] with both dominant and recessive pattern of inheritance. To date, about 50 pathogenic genetic variants in both *DSC2* and *DSG2* genes have been described, including four splice site pathogenic genetic variants in ARVC cases [60].

Desmoplakin

The first ARVC report of pathogenic genetic variants in the *DSP* gene was published in 2002 [67]. Up to now, more than 100 pathogenic genetic variants have been described in *DSP* (Fig. 5) [60]. Alterations of the DSP protein were also implicated in Carvajal syndrome, an autosomal recessive cardiocutaneous form of ARVC that was published as a variant of Naxos syndrome with more severe LV involvement, palmoplantar keratoma, and wooly hair [68]. Later, a recessive pathogenic genetic variant in the *DSP* gene was identified in a large family causing Naxos disease [69]. Since 2010, some studies have shown heterozygous pathogenic genetic variants in this gene causing combined phenotypes resembling Naxos–Carvajal disease [70, 71]. Interestingly, all these pathogenic genetic variants are localized in the same region of DSP (564–597), suggesting that this may be a key region in PG or PKP2 interaction.

Plakoglobin

In 2000, [72] an autosomal recessive form of ARVC caused by a pathogenic genetic variant in the *JUP* gene was reported. The phenotype showed, beside ARVC, palmoplantar keratoderma and woolly hair, characteristic of Naxos disease [72]. To date, less than 20 pathogenic genetic variants have been identified in this gene (Fig. 5) [60].

Assessing pathogenicity of desmosomal genetic alterations

The evaluation of the DNA variant has to be extremely accurate in ARVC cases because disease-associated genes have high background genetic variation rate. A recent study showed 16 % of controls carry rare genetic variant in one of those genes versus 58 % in ARVC cases [73]. However, the difference in percentage is not that clear for *missense* variations since 16 % of controls carry a genetic variant in a desmosomal gene, while 21 % of ARVC cases host a *missense* mutation. In this sense, recent studies have proved that previous ARVC-associated pathogenic genetic variants, after further comprehensive genetic studies, are present in the general population. Specifically, they observed that 18 % of previously reported ARVC pathogenic genetic variants were recently identified in the Exome Sequencing Project (ESP) population (one *nonsense* and 37 *missense*) [74]. It is remarkable that only one of these 38 pathogenic genetic variants had convincing family co-segregation, and only three variants had functional characterization with significant difference between mutant and wild-type. On the other hand, in the same mentioned study, radical mutations were hosted by only 0.5 % individuals versus 43 % of ARVC cases [73]. This fact supports the idea that radical mutations are very likely to be associated with ARVC. However, the genotype–phenotype severity correlation in radical mutations is still unclear. A recent study in Japanese population with 35 ARVC cases showed that carriers with PKP2 premature stop codon developed the disease at a significantly younger age than other mutations carriers [75], while one recent study of our group performed in European population suggests later onset in carriers with PKP2 premature stop codon [76].

So far, 155 radical mutations have been associated with ARVC, reported in Human Gene Mutation Database (HGMD). These variants are distributed as follows: 75 in *PKP2*, 52 in *DSP*, 16 in *DSG2*, and 12 in *DSC2*. However, despite being reported in the HGMD database, not all pathogenic genetic variants mentioned above denote a pathogenic role in clinical practice, especially if no family segregation has been reported. Hence, the most particularly vulnerable to misclassification are mutations identified in large cohorts of individual unrelated probands (without data on familial segregation). For this reason, it is highly recommendable to perform accurate in silico analysis, in vitro assays, and, as key point, co-segregation studies in families to assess the pathogenicity on ARVC.

In addition, 190 pathogenic *missense* genetic variants have been associated with ARVC. These variants are distributed as follows: 56 in *DSP*, 49 in *DSG2*, 48 in *PKP2*, and 24 in *DSC2*. From all *missense* pathogenic genetic variants associated with ARVC reported so far, only 62 % predicted to be deleterious by in silico platforms, using PROVEAN (Protein Variation Effect Analyzer) (http://provean.jcvi.org/index.php). Moreover, 57 % are predicted to be deleterious using Condel (CONsensus DELeteriousness score of non-synonymous single nucleotide variants) (http://bg.upf.edu/condel/analysis), which integrates five different platforms (Polyphen-2, SIFT, MAPP, LogR Pfam *E*-value, and Mutation Assessor) different in silico platforms.

Non-desmosomal genes

Although ARVC has been considered as a desmosomal disease, other non-desmosomal genes have also been identified with this pathology: transmembrane protein 43 (*TMEM43*), transforming growth factor beta-3 (*TGFB3*), Catenin alpha-3 (*CTNNA3*), desmin (*DES*), lamin A/C (*LMNA*), titin (*TTN*), phospholamban (*PLN*), and recently reported sodium channel, voltage-gated, type V, alpha subunit (*SCN5A*). All they together have a lower incidence (<5 %) [77] in ARVC clinically diagnosed cases.

Desmin

Desmin is the main intermediate filament in heart muscle cells. It forms a scaffold around Z-disk and links whole contractile structure with subsarcolemmal cytoskeleton, intercalated disk, nucleus, and other components of the cytoplasm. The *DES* gene was first associated with ARVC in 2009 [78], although it had already been related to other heterogeneous group of myopathies, including cardiomyopathies. Up to now, there are ten reported pathogenic *missense* mutations in ARVC [79]. In all cases, patients showed myopathy disorder. Thus, the probability of ARVC-related mutations in desmin with no skeletal muscle disorders is very low [80].

Transforming growth factor beta-3

TGFb3 is a member of TGF family, pleiotropic, and multifunctional peptides with diverse effects on different cell types. TGFb3 is a cytokine that stimulates fibrosis and modulates cell adhesion and expression of desmosomal genes [81]. The *TGFB3* gene was first reported in ARVC in

2005, they identified two pathogenic genetic variants in untranslated region (UTR) in two different families [82]. This study also suggested that changes in expression levels in TGFb3 are associated with the pathogenesis in ARVC. It is interesting to say that until now, only these regulatory two pathogenic genetic variants have been reported in ARVC. For this reason, more evidence in this sense is needed to eventually corroborate this mechanism.

Transmembrane protein 43

Although the function of this protein remains unclear, it has been suggested to have a role in adipogenic pathway because of their peroxisome proliferator response element (PPAR). This gene was first reported in ARVC in 2008 [83]. The authors suggest that alterations in adipogenic pathway may explain myocardial fibrofatty replacement in ARVC patient carrying pathogenic genetic variants in the *TMEM43* gene [83]. So far, only three pathogenic genetic variants have been reported in ARVC [60]. It is interesting to note the high prevalence of p.S358L_*TMEM43* in six from 195 unrelated ARVC cases.

Alpha-T-catenin

The *CTNNA3* gene encodes for α-T-catenin, which is a cytoplasmic molecule necessary for dynamic maintenance of tissue morphogenesis by integrating in cadherin–catenin complex [84]. This gene was recently reported in ARVC. The mechanism underlying ARVC in this gene would involve alteration of PKP2 and Cx43, as was shown in animal model. To date, only two pathogenic genetic variants have been reported in ARVC [60].

Lamin A/C

The gene *LMNA* encodes for lamin A and lamin C by alternative splicing. Both are proteins from intermediate filaments that take part in the constitution of the nuclear lamina, a protein complex in the inner part of the nuclear lamina [85]. Genetic alterations in *LMNA* are associated with a group of disorders, named laminopathies, which include cardiac disorders [86]. In 2012, it was described *LMNA* gene in ARVC, in a cohort of more than 100 affected patients [87]. Later, other studies showed in ARVC cases, supporting the idea that LMNA is a gene associated with this pathology [88].

Titin

The gene *TTN* encodes for titin, a giant protein located in the cardiac sarcomere, interacting with actin-based thin and myosin-based thick filaments. This gene was first associated with ARVC in 2011 [89]. However, until now, there are only these eight *missense* mutations reported.

Phospholamban

In 2012, *PLN* was first associated with ARVC. They identified a mutation in a Dutch family affected by this pathology [90]. The gene *PLN* encodes for phospholamban, a phosphoprotein associated with the cardiac sarcoplasmic reticulum. It is a regulator of the sarcoplasmic reticulum Ca^{2+} (SERCA) pump in a cardiac muscle [91]. Thus, PLN is one of the key proteins in cardiac contractility and relaxation [92].

Sodium channel, voltage-gated, type V, alpha subunit

The gene *SCN5A* encodes for the cardiac sodium channel, voltage-gated, type V, alpha subunit and it is the main gene in Brugada syndrome. In 2008, *SCN5A* was first associated with ARVC. They described a case of a 58-year-old man with ARVC and a mutation in *SCN5A* [49]. Recently, a second case with ARVC and inducible Brugada ECG pattern has been described [93], and also *SCN5A* mutations in a cohort of Chinese patients of ARVC [93]. Until now, only two *SCN5A* mutations are described in ARVC.

Assessing pathogenicity of non-desmosomal genetic alterations

As described above, non-desmosomal genes have been associated with ARVC very recently, in the last 4 years, and all together represent a low percentage of ARVC. Non-desmosomal genes associated with ARVC are a heterogeneous group of genes with different functions. The mechanisms underlying ARVC in non-desmosomal genes are still to clarify. It is interesting that most of them are cytoskeleton-associated proteins such as, DES, LMNA, CTNNA3, and TTN. In all cases, they had been previously described in other cardiomyopathies, especially with dilated cardiomyopathy (DCM) [94]. Therefore, recent studies support the concept of 'arrhythmogenic cardiomyopathy' as an entity encompassing DCM an ARVC [95]. Importantly, although non-desmosomal genes are commonly associated with these two diseases, desmosomal genes have not yet been associated with any other cardiomyopathy. Mechanisms for these differences are not yet understood.

Consequently, assessing pathogenicity in non-desmosomal genes is difficult, because of the overlapping symptoms with other cardiomyopathies. Familial co-segregation is strongly recommended to finally associate genetic variation in these genes with the phenotype, as well as, performing in vitro and in vivo assays to elucidate the role of non-desmosomal mutations in this pathology.

Current classification of cardiomyopathies is limited due to the overlapping clinical features. Distinction of hypertrophic cardiomyopathy (HCM), DCM, and ARVC has crucial implications in clinical practice, guiding both diagnostics and treatments [96–98]. As mentioned above, this overlapping also occurs in genetics, not only with DCM, but also with HCM. For example, pathogenic genetic variants in *PLN* and *TTN* have been identified in HCM cases [99, 100]. Thus, the solely identification of a genetic variant cannot guide the clinical diagnosis. Clinical characterization is currently the main approach to diagnose patients.

Clinical implications of genetics

Currently, the role of genetics in ARVC diagnosis is limited. Genetic information in ARVC helps to provide a potential cause of the pathology, but clinical findings are the main basis for diagnosis and treatment of patients. Thus, the identification of a potential pathogenic genetic variant cannot override clinical judgment regarding ARVC diagnosis. In addition, lack of pathogenic genetic variant in the setting of convincing clinical evidence neither should call the diagnosis into question nor rule out the disease [73]. All ARVC diagnosed patients or suspicious of suffering ARVC should be genetically analyzed, and for all genes associated with ARVC due to reported digenic/compound cases [52, 101]. Identification of the pathogenic genetic variant may help to clarify the cause of the disease, and posterior familial genetic analysis may identify genetic carriers that could remain asymptomatic but at risk of SCD. Genetic analysis should be one more tool in clinical diagnosis, mainly due to lack of clear pathogenic interpretation. Determining the pathogenicity of a genetic variant is the main genetic challenge in current clinical practice. Establishing solid potential therapeutic and prognostic implications of gene variations associated with ARVC is not available due to lack of clinical-genetic correlations. Despite being accepted that "radical" variants (nonsense, frame-shift) should be considered more dangerous than *missense* variants due to truncation of proteins associated with ARVC [73], genetic results should be interpreted with extreme caution and by teams including cardiologist, geneticists, and genetic counselors.

Post-mortem cases

It is well known that SCD may be the first symptom of ARVC. Most of these cases are diagnosed in the autopsy setting due to cardiac structural modifications after a thorough macroscopic and microscopic analysis [102]. The need to adopt strict criteria for diagnosis is important for the forensic and general pathology. These criteria are also vital for both epidemiologic purposes and for clinical implications, since the identification of a genetic disease as a cause of death represents the starting point for familial screening and sudden death prevention [103]. At present, considerable importance is given to the finding of fatty infiltration of the myocardium, since cardiac magnetic resonance imaging has the ability to identify adipose tissue in vivo with consequent diagnostic and therapeutic implications. It is still a matter of debate whether fatty infiltration of the right ventricle per se should be considered a morphologic hallmark of ARVC [104].

We need to remember that the original distinction in two histologic variants, i.e., fatty and fibrofatty, has been a source of confusion, since it has not been sufficiently appreciated that even in the fatty variant a certain amount of replacement-type fibrosis and myocyte abnormalities should be found to label it as ARVC [33]. Fatty accumulation is reported in several large series of patients, particularly at older age, and since they were not convinced of the clinical or pathological significance of isolated fat infiltration, they concluded that this entity is different from ARVC [21, 105–108]. In particular, in autopsy hearts of people dying suddenly with pure fatty infiltration, the right ventricular myocardium was of normal or increased thickness, the left ventricle was mostly normal, and there was no inflammation or myocyte atrophy [21]. Moreover, the authors pointed out that while up to 15 % fatty replacement is distinctly abnormal in the right ventricular outflow tract or posterior wall, it is probably normal in the anterior wall near the apex [108]. It was reported that 85 % of hearts from people who died of noncardiac causes contained at least some intramyocardial fatty tissue, in the absence of fibrosis or inflammation, with significantly more fat replacement noted in the right ventricle of older subjects and in females than in males. Massive fatty infiltration of the right ventricle, without any evidence of fibrosis and myocyte degeneration, represents a questionable cause of sudden death and does not have a familial tendency [106]. In this condition, the myocytes seem to be pushed apart rather than replaced and they appear structurally normal. In contrast to fatty infiltration of the right ventricle, both the so-called fatty and the fibrofatty ARVC variants consistently show degenerative changes of the myocytes and nuclei, often resembling those observed in DCM.

Thus, in addition to fat, two additional histologic features are essential in order to provide an unequivocal diagnosis of ARVC: (a) replacement-type fibrosis; and/or (b) degenerative changes of the myocytes. Newly available cardiac imaging techniques—such as cardiac magnetic resonance with gadolinium enhancement—which are able

to detect not only fatty tissue deposition but also fibrous tissue could play a key role in differential diagnosis [104]. A corollary is that the finding of fatty infiltration of the right ventricular myocardium in the absence of wall motion abnormalities should be interpreted with caution. In the case of a patient with sudden death, where extensive right ventricular fatty infiltration is observed in the heart, it would be preferable to state this finding without implying a cause and effect relationship. In other words, this would not necessarily indicate that death was due to ARVC. If pathologists would ascribe sudden death as due to ARVC based on simple observation of adipose infiltration, there will be a huge increase in its frequency as a cause of sudden death which in most instances will be totally spurious [109]

Notably, an extensive forensic autopsy investigation reported an extremely high incidence (more than 10 %) of ARVC in people aged 1–65 years who died suddenly in France: in the majority of cases, there was no any evidence of fibrous tissue replacement [110]. Nowadays, in order to reach an accurate diagnosis, disease-causing genes are available for mutational analysis at post-mortem sudden death cases. In these cases, post-mortem genetic investigation could help to identify the pathogenic genetic variants responsible fordeath, identifying even asymptomatic relatives, who may be at risk of SCD. The DNA quality from post-mortem formalin-fixed and paraffin-embedded (FFPE) tissue represents a main limitation to identify potential pathogenic genetic variants underlying the disease [111]. However, high-throughput genetic technology has emerged as a useful tool to solve the difficulty of analyzing degraded DNA. Sequenom Mass ARRAY System (Sequenom, Inc) has demonstrated its capability in analyzing low DNA quality samples. This technology has already been used to identify pathogenic genetic variants in ARVC [112] and it had been also used in other diseases such as long QT syndrome (LQTS) and HCM [113]. It is interesting to remark that most part of Sudden Unexpected Death victims with Negative Autopsy (SUDNA) remain without genetic testing, and recent studies in post-mortem series identified ARVC pathogenic genetic variants in up to 25 % of cases [114, 115]. We believe that molecular autopsy and post-mortem mRNA expression analysis of heart tissue should be implemented in forensic field, especially in cases with a no-conclusive cause of death after comprehensive autopsy investigation [116, 117]

Conclusions

Arrhythmogenic right ventricular cardiomyopathy is a rare inherited cardiomyopathy leading to SCD. The clinical diagnosis of ARVC is complex and incorporates multiple components including criteria derived from clinical, genetic, electrical, and imaging characteristics. VT ablation in ARVC is conditioned by the patchy and progressive nature of the myocardial replacement of the RV by fibro-fatty tissue. In spite of the variable long-term success rates reported, ablation is a useful tool for the management of recurrent ventricular arrhythmias in this group of patients. Hundreds of pathogenic genetic variants have been identified in 13 genes encoding mainly desmosomal proteins. Genetic testing has been progressively incorporated into clinical diagnosis. Hence, nearly 35 % of clinically ARVC-diagnosed cases remain without identified genetic cause. In recent years, massive genetic sequencing has been incorporated progressively into ARVC filed. The main challenge is the clinical interpretation of the amount of data generated by NGS technology, since only about 60 % of ARVC-related variants are predicted to be pathogenic by in silico platforms.

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Conflict of interest None.

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