



Review

The genetic background of arrhythmogenic right ventricular cardiomyopathy

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ABSTRACT

Arrhythmogenic right ventricular cardiomyopathy (ARVC) is characterized by degeneration of the right ventricle and ventricular tachycardia originating from the right ventricle. Additionally, the disease is an inherited cardiomyopathy that mainly follows the autosomal dominant pattern. More than 10 genes have been reported as causative genes for ARVC, and more than half of ARVC patients carry mutations in desmosome related genes. The desmosome is one of the structures involved in cell adhesion and its disruption leads to various diseases, including a skin disease called pemphigus. Among desmosome genes, mutations in PKP2 are most frequently identified in ARVC patients. Although the genotype–phenotype correlations remain to be fully studied, many studies have reported clinical manifestations of, prognosis for, and appropriate therapies for ARVC from the perspective of gene mutations. A collective review of these reports would enhance the understanding of ARVC pathogenesis and clinical manifestation. This review discusses the clinical issues of ARVC from the genetic background.

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1. Introduction

Arrhythmogenic right ventricular cardiomyopathy (ARVC), previously called arrhythmogenic right ventricular dysplasia (ARVD), is an inherited disease characterized by right ventricular degeneration and ventricular arrhythmias. ARVC is one of the important causes of sudden cardiac deaths in young people, and especially in young athletes [1]. The disease seems to be reported at the end of the 19th century as “cor adipose” [2]. In 1982, Marcus et al. first summarized 22 cases of adult ARVC patients, including their clinical characteristics such as male predominance, onset at around 40 years of age, T wave inversion in precordial leads, and fibro-fatty replacement of the myocardium [3]. These clinical characteristics are still applied in the latest diagnostic task force criteria [4].

Familial cases of ARVC have been reported since the early 1980s. In 1985, 3 out of 5 siblings from a family were diagnosed with ARVC, and the authors hypothesized incomplete autosomal dominant inheritance mode with low penetrance [5]. Thus, ARVC was suspected to be an inherited disease from the beginning, and many physicians and researchers started to explore the genes responsible for ARVC. Studies related to the identification of causative genes are summarized in the next section.

The first diagnostic criteria for ARVC published in 1994 included family history as one of the criteria [6]. Familial disease confirmed at necropsy or surgery was classified as a major criterion. A familial history of premature sudden death (< 35 years of age) due to suspected right ventricular dysplasia or a familial history based on clinically diagnosed disease as per the criteria were classified as minor criteria.

To understand the pathogenesis of ARVC, many researchers have studied the genetic background of the disease, and many causative genes have been identified in the last decade. Among these, the identification of involvement of desmosomal genes in ARVC patients was a significant discovery [7].

Presently, genetic mutations are identified in more than 60% of ARVC patients, and familial cascade screening is useful to diagnose the disease before its onset in young family members.

In this review, I have described the causative genes, the characteristics of the genotype, and the future perspectives for ARVC from the viewpoint of genetics.

2. History of desmosome genes as the cause of ARVC

Desmosomes are a complex formed by proteins and function to bind the myocardial cells to each other. In the heart, desmosomes are composed of five proteins that is, junctional plakoglobin encoded by JUP, plakophilin-2 by PKP2, desmoplakin by DSP, desmoglein-2 by DSG2, and desmocollin-2 by DSC2 (Fig. 1). Desmosomes are indispensable for electrical conduction and mechanical contraction in myocardial cells.

In 1986, the Greek cardiologist Protonotarios and his colleagues reported cardiac abnormalities in 9 patients with familial palmo-plantar keratosis and wooly hair [8]. All patients originated from families on the Greek island of Naxos, and therefore the disease was named as Naxos disease. The island of Naxos has nearly 200 unrelated families. Medical doctors and scientists in Naxos and

London collaborated and recruited all families living on the island of Naxos for the study. They identified 9 affected families and performed linkage analysis for 38 members, including the 14 affected members. Using this analysis, they reported a homozygous genotype on 17q21 in 1998 [9].

In 2000, a homozygous deletion mutation in JUP was finally identified in 19 patients with Naxos disease [7].

After the discovery of JUP as a causative gene for ARVC in the recessive form, many researchers started genetic analysis for other desmosome genes in ARVC patients. DSP was confirmed as a causative gene in 2002 [10]. PKP2 mutations in ARVC patients were reported in 2004 [11], while DSG2 and DSC2 mutations were reported in 2006 [12,13].

Although most ARVC patients show the autosomal dominant inheritance, two recessive inheritance modes have been reported in syndromic ARVC. One of these is the Naxos disease caused by homozygous mutations in JUP [7], and another is the Carvajal syndrome caused by homozygous DSP mutations [14]. At first, Carvajal syndrome was reported as a syndromic dilated

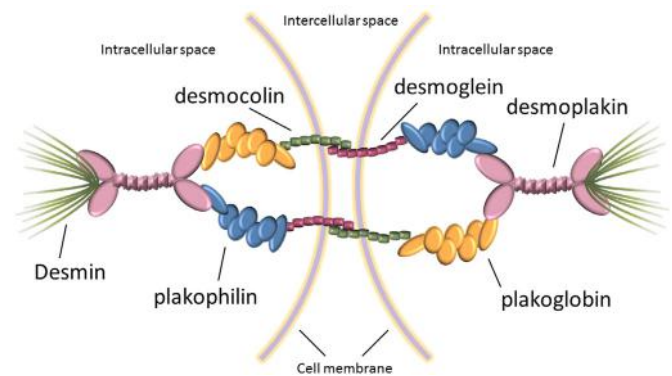


Fig. 1. A schematic diagram of the desmosome. Desmoglein and desmocollin located in the transmembrane region connect with the corresponding molecules on the neighboring cell and are linked to desmoplakin by plakophilin and plakoglobin.

Table 1
Genotypes, gene names, and locations related to ARVC.

Genotype	Gene	Location	Recessive form	Reference
ARVC1	<i>TGFB3</i>	14q24.3		[24]
ARVC2	<i>RYR2</i>	1q43		[27]
ARVC3	Unknown	14q12-q22		[54]
ARVC4	<i>TTN</i>	2q32.1-q32.3		[29]
ARVC5	<i>TMEM43</i>	3p25.1		[33]
ARVC6	Unknown	10p14-p12		[55]
ARVC7	<i>DES</i>	2q35		[37]
ARVC8	<i>DSP</i>	6p24.3	Carvajal syndrome	[10]
ARVC9	<i>PKP2</i>	12p11		[11]
ARVC10	<i>DSG2</i>	18q12.1		[12]
ARVC11	<i>DSC2</i>	18q12.1		[13]
ARVC12	<i>JUP</i>	17q21.2	Naxos disease	[7]
Others	<i>PLN</i>	6q22.1		[40]
	<i>LMNA</i>	1q22		[41]
	<i>SCN5A</i>	3p21		[44]
	<i>CTNNA3</i>	10q 22.2		[45]

cardiomyopathy with woolly hair and keratoderma caused due to a homozygous mutation in DSP [14]. In 2003, another homozygous mutation in DSP was identified in an ARVC family showing hair and skin abnormalities [15].

Causative genes for ARVC and their respective characteristics are summarized in Table 1.

3. Desmosome genes for ARVC

3.1. PKP2

PKP2 is the most major causative gene for ARVC. PKP2 encodes plakophilin-2, a protein with 881 amino acids with armadillo repeat domain (Fig. 1) and has a structure similar to that of plakoglobin, which is encoded by JUP. In 2004, PKP2 mutations were identified in 32 out of 120 unrelated individuals with ARVC [11]. Later studies demonstrated the low penetrance of PKP2 mutations in ARVC [16,17]. Among the carriers of mutations in desmosome genes, more than 70% patients in western countries carry PKP2 mutations [18]. In Asian countries, we have previously reported the results of genetic analysis in 35 ARVC probands [19]. Among 35 probands, we identified 19 carriers with desmosome mutations, with 10 showing mutations in PKP2 (52.6%). In China, Bao et al. identified 57 mutation carriers from 90 ARVC patients (63.3%); of these, 40 were PKP2 mutation carriers (70.2%) [20]. These reports indicate that genetic screening for PKP2 in ARVC patients is indispensable to understand their genetic backgrounds.

3.2. JUP

JUP was the first gene identified as causative for ARVC among the desmosome genes [7], and the structure of plakoglobin, which is encoded by JUP, is similar to that of plakophilin (Fig. 1). However, the mutation frequency of JUP in ARVC patients is low as compared to that of other desmosome genes. The number of JUP mutations identified in ARVC patients is less, but plakoglobin encoded by JUP has a remarkable effect in ARVC pathogenesis. In an experiment where desmoplakin expression in atrial myocyte cell lines was suppressed by siRNA, nuclear translocation of plakoglobin and reduction of Wnt/beta-catenin signaling were reported [21]. In 2009, translocated plakoglobin to nuclear was shown to bind the Tcf712 transcription factor, resulting in increased expression of adipogenic factors like Wnt5b and BMP7, which are normally inhibited by canonical Wnt signaling [22]. Therefore, JUP-encoded plakoglobin could be a key factor for dissolving the pathogenesis of ARVC.

3.3. DSP

DSP is the largest among the desmosome genes and encodes the 2872 amino acid protein, desmoplakin. The N-terminus of desmoplakin is required for localization to the desmosome and interaction with plakophilin and plakoglobin. The C-terminus of desmoplakin binds to the intermediate filaments (desmin) (Fig. 1). A DSP mutation was first identified in a homozygous manner as the cause of Carvajal disease, which shows dilated cardiomyopathy with woolly hair and keratoedema [14]. In 2002, a DSP mutation, S229R, was identified in a 18 year-old male who suffered cardiac arrest and was diagnosed with ARVC [10]. An extended clinical and genetic analysis of his family members from 4 generation confirmed that the mutation was the cause of ARVC. The residue 229 located in the N-terminus of desmoplakin is involved in binding with plakoglobin or plakophilin, and the mutation S229R would disrupt the normal binding with those proteins.

After the first report, other DSP mutations have been identified in ARVC patients. Although DSP is the largest among the desmosome genes, the number of reported mutations is small compared to other genes. Only 12 mutations have been reported in recent study with 439 families [18].

3.4. DSG2 and DSC2

The products of DSG2 and DSC2 are cadherin-like transmembrane glycoproteins that are major components of the desmosome (Fig. 1). Mutations in both genes have been reported as the cause of ARVC.

Mutations in DSG2 as a causative gene for ARVC were first reported in 2006 [12]. Among 54 ARVC probands who were negative for mutations in DSP, PKP2, and TGFβ3, 9 heterozygous DSG2 mutations were found.

DSC2 was the last one among the desmosome genes to be reported as the causative gene for ARVC. Among 77 probands who were negative for PKP2, JUP, DSP and DSG2, two frameshift mutations that resulted in the formation of a premature termination codon in DSC2 were identified from 4 families [13].

After these reports, many mutations in DSG2 and DSC2 have been reported. However, in European countries, the frequency of mutations in these genes is low compare to PKP2. In a recent report, 17 DSG2 (4%) and 5 DSC2 (1%) mutation carriers were identified from 276 genotype positive probands [18].

In Asian countries, the frequency of DSG2 mutations is rather higher than in Caucasians. We reported 3 DSG2 (15.8%) mutation carriers from 19 genotype positive patients [19] and Bao et al. reported 8 DSG2 (14%) mutation carriers from 57 patients [20]. Surprisingly, the latter reported that 3 of the 8 DSG2 mutation carriers had homozygous mutations.

DSC2 mutations in ARVC patients are also rare in Asian countries. We identified only one DSC2 mutation carrier from 19 genotype positive patients; the patient carried three DSC2 mutations; R132C, N194K, and R203C [19]. In China, 3 DSC2 mutations were identified from 57 patients, and two of these mutations were identified as a single mutation [20].

4. Other causative genes for ARVC

Although more than half of ARVC patients carry mutations in desmosome genes, other genes have also been reported as causative genes for ARVC (Table 1). In the most of these genes, their loci on chromosomes were first confirmed by linkage analysis, and then the specific genes were identified in the target families. Other than desmosome genes, 7 loci have been detected in linkage analysis and 5 causative genes for ARVC have been identified. However, the causative genes of ARVC3 in chromosome 14q12-q22 and ARVC6 in chromosome 10q14-p12 have not been identified yet.

4.1. ARVC1-TGFB3

The ARVC1 locus on chromosome 14q23-q24 was first identified in 1994 as the causative locus for ARVC on performing linkage analysis of two families, including a large family with 4 generations of ARVC patients [23]. However, identification of the causative gene, TGFB3, took a lot of time. In 2005, two nucleotide substitutions in the 5'UTR and 3'UTR of TGFB3 were identified in ARVC patients [24]. To confirm the effect of UTR mutations in TGFB3 gene expression, a luciferase reporter assay was performed, and both UTR mutations were found to increase the luciferase reporter activity. Thus, the authors explained that increased TGFB3 expression induced myocardial fibrosis in accordance with previous studies [25].

4.2. ARVC2-RYR2

RYR2 encodes the cardiac ryanodine receptor, which locates to the sarcoplasmic reticulum, and is indispensable for cardiac contraction by controlling the calcium ions. The RYR2 mutations were reported in various other cardiac diseases, including dilated cardiomyopathy (DCM) and catecholaminergic polymorphic ventricular tachycardia (CPVT). The locus, 1q42–q43 was confirmed in an ARVC family in 1995 [26] and the causative gene was identified as RYR2 in 2001 [27]. The RYR2 mutation carriers in this family showed effort-induced polymorphic ventricular tachycardia, and there were four patients of juvenile sudden death in the family. Post mortem examination of two subjects showed normal right ventricular size, but large areas of fatty-fibrous replacement localized in the sub-epicardial layer of the right ventricle were detected at the histological level [26]. In 2001, four RYR2 mutations (R176Q, L433P, N2386I, and T2504M) from three ARVC families were identified [27]. Although the affected family members fulfilled the diagnostic criteria of ARVC at that time [6], this ARVC family with RYR2 mutations might actually be a variant of CPVT.

4.3. ARVC4-TTN

ARVC4 was reported in three families with left ventricular involvement in 1997 [28], and TTN was confirmed as the causative gene for ARVC4 in 2011 [29]. TTN consists of 363 exons and encodes the protein, titin, which is the largest protein in mammals, with its name originating from the giant men in Greek mythology. TTN mutations have been reported in other cardiomyopathies such as hypertrophic cardiomyopathy (HCM) [30] and dilated cardiomyopathy (DCM) [31]. ARVC4 patients with TTN mutations displayed various phenotypes, including biventricular dysfunction and conduction block. Therefore, overlapping phenotypes with DCM are suspected in ARVC4 patients.

4.4. ARVC5-TMEM43

The locus of ARVC5 (chromosome 3p23) was detected in a large ARVC family from the island of Newfoundland including more than 200 members spanning eight generations [32]. In 2008, the causative gene of ARVC5 was confirmed as TMEM43 encoding the transmembrane protein 43, which functions as a nuclear membrane organizer [33]. The mutation identified in the large family was S358L located in the transmembrane domain of the protein. Mutations in TMEM43 were also identified in 2 of 41 unrelated individuals with Emery–Dreifuss muscular dystrophy (EDMD) [34]. As the cause of ARVC, TMEM43 mutations were regarded as rare compared to desmosome mutations. However, recent advances in genetic analysis have made it easy to identify TMEM43 mutations in ARVC patients. Recently, we identified double TMEM43 mutations in a Japanese family with RV aneurysm [35].

Although ARVC patients with TMEM43 mutations fulfill the ARVC task force criteria, the phenotype of ARVC due to TMEM43 mutations might be different from that due to desmosome mutations. One of these differences includes the early onset of ARVC. In general, ARVC onset occurs at around 30 years of age, but in our patient with TMEM43 mutation, RV aneurysm and ventricular arrhythmia were identified before birth by fetal ultrasound [35]. In addition, the penetrance of TMEM43 related ARVC is very high. In an analysis of 137 TMEM43 mutation carriers, all the patients showed ARVC specific phenotype by 63 years in males and 76 years in females [33].

4.5. ARVC7-DES

The locus of ARVC7 was first detected on chromosome 10q [36], but in 2010, ARVC7 was determined to be caused by DES located on chromosome 2q35 [37]. DES encodes desmin, which is one of the types of intermediate fibers in myocytes (Fig. 1) and is related to myofibrillar myopathies. The patients discussed in the first report of ARVC7 had myopathy and cardiomyopathy [36]. In 2010, an ARVC7 patient without myopathy was reported [37]. The mutation frequency of DES is not high, but in the screening of 91 ARVC probands, 2 DES mutation carriers were identified [38]. One of the patients carried a PKP2 mutation in addition to a rare variant of DES, whereas the other patient carried a frameshift DES mutation. Therefore, the mutation frequency of DES seems to be more than 1%.

5. Other causative genes for ARVC

By using candidate gene sequence methods instead of linkage analysis, other causative genes for ARVC have been reported.

5.1. PLN

PLN encodes phospholamban, which is indispensable for calcium handling in cardiac contractions [39] and was known as the causative gene for DCM. In 2012, van der Zwaag et al. identified PLN-R14del in 12 out of 97 ARVC and 39 out of 257 DCM patients in the Netherlands [40]. However, no PLN mutations except for R14del have been reported in ARVC patients.

5.2. LMNA

LMNA encodes lamin A/C, which functions in the lining of the nuclear membrane and is indispensable for stabilization of cells. LMNA mutations were reported in various systemic diseases, including Emery–Dreifuss muscle dystrophy and premature aging syndrome. In the cardiovascular field, LMNA mutations were identified in DCM patients, especially those with sinus bradycardia and conduction disturbance. In 2011, four LMNA mutations in ARVC patients were reported [41]. Two of the four patients died suddenly at ages 54 and 67, and one died from severe heart failure at the age of 48 years. The histological characteristics of one of the patients were compatible with those of ARVC, including fibro-fatty replacement. Recently, we reported two ARVC families with LMNA mutations [42]. ARVC probands in both families showed bradycardia and were implanted with pacemakers.

5.3. SCN5A

SCN5A encodes the cardiac sodium channel, and mutations in SCN5A cause various cardiac diseases, including long QT syndrome type 3, Brugada syndrome, progressive cardiac conduction disease, and DCM [43]. In 2008, an ARVC patient with a SCN5A splice variant, c.3840+1g>a, was reported, and frequent VT was recorded in his ICD [44].

5.4. CTNNA3

CTNNA3 is the newest candidate gene for ARVC [45]. Alpha-T-catenin encoded by CTNNA3 binds with plakophilins and functions in the cell–cell adhesion in cardiomyocytes. Two CTNNA3 mutations, c.281t>a (p.V94D) and c.2293_2295delTTG (p.dI765L) were identified in 2 out of 76 ARVC patients without any mutations in desmosome genes. In functional analysis, CTNNA3-V94D showed disabled interaction with β -catenin, and CTNNA3-del765L showed

much stronger dimerization potential. These functional changes suggest a causal relationship between CTNNA3 mutations and ARVC pathology.

6. Genotype–phenotype correlations

In certain genetic diseases with multiple genotypes, genotype–phenotype correlations are well studied and used for diagnosis and treatment. For example in long QT syndrome, phenotypes predict the genotypes, and genotyping is useful to predict prognosis of the patients and to decide treatment. However, in ARVC, genotype–phenotype correlations have not been fully examined as of yet. One of the reasons being that most of the mutations are identified in PKP2 [18]. Another reason for the lack of study is the low penetrance of the mutations [17].

Recently, a study that analyzed the phenotypes and gene mutations related to ARVC was reported. In the probands, mutation positive status did not affect clinical characteristics and outcomes. In contrast, family members with mutations were more likely to meet Task Force Criteria for ARVC [4] (40% vs 18%), experience sustained ventricular arrhythmias (11% vs 1%), and die from a cardiac cause (2% vs 0%) than family members without mutations [18]. Among 116 desmosome gene mutation carriers in another study, the event rate was higher among patients with definite ARVC than among borderline or phenotype negative patients [46].

Correlations between mutation type and phenotype have been reported. We compared the disease onset of probands with 12 missense and 7 non-missense mutation carriers. All non-missense mutations were found in PKP2 [19]. In our study, the disease onsets were significantly younger in the patients with non-missense mutations than in those with missense mutations (29.4 ± 12.4 vs 45.8 ± 14.2 years, $P=0.027$). In contrast, Alcalde et al. reported that the disease onsets in patients with stop gain mutations were later than those in patients with missense mutations (27 vs 39 years old, $P < 0.05$). Although, it is difficult to clarify the reason of the discrepancy, the definition of onset was different between studies. It would be difficult to define disease onsets in the patients whose first symptoms were ventricular arrhythmias, because cardiologists would never recognize that the patients experienced ventricular tachycardia if the patients did not complain of their symptoms or had syncope.

7. Progress pertaining to genetic analysis and genetic noise

At present, the causative genes for ARVC are known, and we can screen all the genes in patients who are diagnosed with ARVC. The gold standard of sequencing for genetic screening is the Sanger method, which needs two times of PCR for target exons. All the target exons can be screened in genes related to ARVC. However, Sanger methods need a long time and are expensive if we screen all the exons in those genes. The total number of exons in desmosome genes is nearly 90. In addition, TTN, a causative gene for ARVC4, has 363 exons, and RYR2, a causative gene for ARVC2, has 105 exons. The screening of all ARVC-related genes by Sanger methods therefore seems to be a difficult task.

However, we can now approach the new sequencing methods, next generation sequencing (NGS) methods [47]. These methods are useful not only for whole genome or exome sequencing but also for target gene sequencing [48]. Especially for cardiomyopathies, multiple panels for genetic analysis are available on the market. If these panels are used, mutations in ARVC patients can easily be detected in a week.

In the NGS era, many rare variants can be detected in genetic analysis, some of which are related with the disease. Therefore, the true mutation(s) causative for the disease need to be distinguished from other genetic noise. In 2011, Kapplinger et al. reported background genetic noise in ARVC [49]. They performed a genetic analysis of PKP2, DSP, DSG2, DSC2, and TMEM43 for 92 probands diagnosed with ARVC and 427 controls. Radical mutations resulting in stop codons were identified in 43% of probands and in 0.5% of controls. In contrast, missense mutations were identified in 21% of the probands and 16% of controls; thus, the frequency of missense mutations was similar between probands and controls. They further analyzed the ARVC related genes and locations of missense mutations that were highly detected in controls and found that missense mutations in DSP and DSG2, especially at the C-terminals of both genes, were highly detected in controls [49]. To avoid misunderstanding of the detected variants in the ARVC related genes, a comparison of the detected mutations with data for ethnically matched controls is needed, for example, the 1000 genome study [50] and the ExAC Browser (<http://exac.broad-institute.org/>).

In silico prediction software, SIFT [51], PolyPhen2 [52], CADD [53], and other prediction systems are useful to evaluate a specific mutation which is detected in a patient. However, even if the in silico software predicts a mutation as pathogenic, we need to examine whether the genetic result is compatible with the phenotype.

8. Conclusion

In the last decade, there has been remarkable progress in determining the genetic background of ARVC and new technologies for genetic analysis have been developed. The next step is to utilize this genetic progress for the treatment of ARVC, including the prevention of sudden cardiac death in young people.

Conflict of interest

All authors declare no conflicts of interest related to this study.

References

- [1] Basso C, Corrado D, Marcus FI, et al. Arrhythmogenic right ventricular cardiomyopathy. *Lancet* 2009;373:1289–300.
- [2] William O. Fatty heart. In: *The principles and practice of medicine*. New York: D.Appleton and Company; 1892. p. 642–3.
- [3] Marcus FI, Fontaine GH, Guiraudon G, et al. Right ventricular dysplasia: a report of 24 adult cases. *Circulation* 1982;65:384–98.
- [4] Marcus FI, McKenna WJ, Sherrill D, et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: proposed modification of the task force criteria. *Circulation* 2010;121:1533–41.
- [5] Ruder MA, Winston SA, Davis JC, et al. Arrhythmogenic right ventricular dysplasia in a family. *Am J Cardiol* 1985;56:799–800.
- [6] McKenna WJ, Thiene G, Nava A, et al. Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Task Force of the Working Group Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology. *Br Heart J* 1994;71:215–8.
- [7] McKoy G, Protonotarios N, Crosby A, et al. Identification of a deletion in plakoglobin in arrhythmogenic right ventricular cardiomyopathy with palmoplantar keratoderma and woolly hair (Naxos disease). *Lancet* 2000;355:2119–24.
- [8] Protonotarios N, Tsatsopoulou A, Patsourakos P, et al. Cardiac abnormalities in familial palmoplantar keratosis. *Br Heart J* 1986;56:321–6.
- [9] Coonar AS, Protonotarios N, Tsatsopoulou A, et al. Gene for arrhythmogenic right ventricular cardiomyopathy with diffuse nonepidermolytic palmoplantar keratoderma and woolly hair (Naxos disease) maps to 17q21. *Circulation* 1998;97:2049–58.

- [10] Rampazzo A, Nava A, Malacrida S, et al. Mutation in human desmoplakin domain binding to plakoglobin causes a dominant form of arrhythmogenic right ventricular cardiomyopathy. *Am J Hum Genet* 2002;71:1200–6.
- [11] Gerull B, Heuser A, Wichter T, et al. Mutations in the desmosomal protein plakophilin-2 are common in arrhythmogenic right ventricular cardiomyopathy. *Nat Genet* 2004;36:1162–4.
- [12] Pilichou K, Nava A, Basso C, et al. Mutations in desmoglein-2 gene are associated with arrhythmogenic right ventricular cardiomyopathy. *Circulation* 2006;113:1171–9.
- [13] Syrris P, Ward D, Evans A, et al. Arrhythmogenic right ventricular dysplasia/cardiomyopathy associated with mutations in the desmosomal gene desmocollin-2. *Am J Hum Genet* 2006;79:978–84.
- [14] Norgett EE, Hatsell SJ, Carvajal-Huerta L, et al. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. *Hum Mol Genet* 2000;9:2761–6.
- [15] Alcalai R, Metzger S, Rosenheck S, et al. A recessive mutation in desmoplakin causes arrhythmogenic right ventricular dysplasia, skin disorder, and woolly hair. *J Am Coll Cardiol* 2003;42:319–27.
- [16] Syrris P, Ward D, Asimaki A, et al. Clinical expression of plakophilin-2 mutations in familial arrhythmogenic right ventricular cardiomyopathy. *Circulation* 2006;113:356–64.
- [17] Dalal D, James C, Devanagondi R, et al. Penetrance of mutations in plakophilin-2 among families with arrhythmogenic right ventricular dysplasia/cardiomyopathy. *J Am Coll Cardiol* 2006;48:1416–24.
- [18] Groeneweg JA, Bhonsale A, James CA, et al. Clinical presentation, long-term follow-up, and outcomes of 1001 arrhythmogenic right ventricular dysplasia/cardiomyopathy patients and family members. *Circ Cardiovasc Genet* 2015;8:437–46.
- [19] Ohno S, Nagaoka I, Fukuyama M, et al. Age-dependent clinical and genetic characteristics in Japanese patients with arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Circ J* 2013;77:1534–42.
- [20] Bao J, Wang J, Yao Y, et al. Correlation of ventricular arrhythmias with genotype in arrhythmogenic right ventricular cardiomyopathy. *Circ Cardiovasc Genet* 2013;6:552–6.
- [21] Garcia-Gras E, Lombardi R, Giocondo MJ, et al. Suppression of canonical Wnt/beta-catenin signaling by nuclear plakoglobin recapitulates phenotype of arrhythmogenic right ventricular cardiomyopathy. *J Clin Invest* 2006;116:2012–21.
- [22] Lombardi R, Dong J, Rodriguez G, et al. Genetic fate mapping identifies second heart field progenitor cells as a source of adipocytes in arrhythmogenic right ventricular cardiomyopathy. *Circ Res* 2009;104:1076–84.
- [23] Rampazzo A, Nava A, Danieli GA, et al. The gene for arrhythmogenic right ventricular cardiomyopathy maps to chromosome 14q23-q24. *Hum Mol Genet* 1994;3:959–62.
- [24] Beggina G, Occhi G, Nava A, et al. Regulatory mutations in transforming growth factor-beta3 gene cause arrhythmogenic right ventricular cardiomyopathy type 1. *Cardiovasc Res* 2005;65:366–73.
- [25] Leask A, Abraham DJ. TGF-beta signaling and the fibrotic response. *FASEB J* 2004;18:816–27.
- [26] Rampazzo A, Nava A, Erne P, et al. A new locus for arrhythmogenic right ventricular cardiomyopathy (ARVD2) maps to chromosome 1q42-q43. *Hum Mol Genet* 1995;4:2151–4.
- [27] Tiso N, Stephan DA, Nava A, et al. Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). *Hum Mol Genet* 2001;10:189–94.
- [28] Rampazzo A, Nava A, Miorin M, et al. ARVD4, a new locus for arrhythmogenic right ventricular cardiomyopathy, maps to chromosome 2 long arm. *Genomics* 1997;45:259–63.
- [29] Taylor M, Graw S, Sinagra G, et al. Genetic variation in titin in arrhythmogenic right ventricular cardiomyopathy-overlap syndromes. *Circulation* 2011;124:876–85.
- [30] Satoh M, Takahashi M, Sakamoto T, et al. Structural analysis of the titin gene in hypertrophic cardiomyopathy: identification of a novel disease gene. *Biochem Biophys Res Commun* 1999;262:411–7.
- [31] Itoh-Satoh M, Hayashi T, Nishi H, et al. Titin mutations as the molecular basis for dilated cardiomyopathy. *Biochem Biophys Res Commun* 2002;291:385–93.
- [32] Ahmad F, Li D, Karibe A, et al. Localization of a gene responsible for arrhythmogenic right ventricular dysplasia to chromosome 3p23. *Circulation* 1998;98:2791–5.
- [33] Merner ND, Hodgkinson KA, Haywood AF, et al. Arrhythmogenic right ventricular cardiomyopathy type 5 is a fully penetrant, lethal arrhythmic disorder caused by a missense mutation in the TMEM43 gene. *Am J Hum Genet* 2008;82:809–21.
- [34] Liang WC, Mitsuhashi H, Keduka E, et al. TMEM43 mutations in Emery-Dreifuss muscular dystrophy-related myopathy. *Ann Neurol* 2011;69:1005–13.
- [35] Honda T, Kanai Y, Ohno S, et al. A fetal case of arrhythmogenic right ventricular cardiomyopathy with double mutations in TMEM43. *Pediatr Int* 2015 <http://dx.doi.org/10.1111/ped.12832>, in press.
- [36] Melberg A, Oldfors A, Blomstrom-Lundqvist C, et al. Autosomal dominant myofibrillar myopathy with arrhythmogenic right ventricular cardiomyopathy linked to chromosome 10q. *Ann Neurol* 1999;46:684–92.
- [37] Klauke B, Kossmann S, Gaertner A, et al. De novo desmin-mutation N116S is associated with arrhythmogenic right ventricular cardiomyopathy. *Hum Mol Genet* 2010;19:4595–607.
- [38] Lorenzon A, Beggina G, Bauce B, et al. Desmin mutations and arrhythmogenic right ventricular cardiomyopathy. *Am J Cardiol* 2012;111:400–5.
- [39] MacLennan DH, Kranias EG. Phospholamban: a crucial regulator of cardiac contractility. *Nat Rev Mol Cell Biol* 2003;4:566–77.
- [40] van der Zwaag PA, van Rijsingen IA, Asimaki A, et al. Phospholamban R14del mutation in patients diagnosed with dilated cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy: evidence supporting the concept of arrhythmogenic cardiomyopathy. *Eur J Heart Fail* 2012;14:1199–207.
- [41] Quarta G, Syrris P, Ashworth M, et al. Mutations in the Lamin A/C gene mimic arrhythmogenic right ventricular cardiomyopathy. *Eur Heart J* 2012;33:1128–36.
- [42] Kato K, Takahashi N, Fujii Y, et al. LMNA cardiomyopathy detected in Japanese arrhythmogenic right ventricular cardiomyopathy cohort. *J Cardiol* 2015 <http://dx.doi.org/10.1016/j.jcc.2015.10.013>, in press.
- [43] Remme CA. Cardiac sodium channelopathy associated with SCN5A mutations: electrophysiological, molecular and genetic aspects. *J Physiol* 2013;591:4099–116.
- [44] Erkapic D, Neumann T, Schmitt J, et al. Electrical storm in a patient with arrhythmogenic right ventricular cardiomyopathy and SCN5A mutation. *Europace* 2008;10:884–7.
- [45] van Hengel J, Calore M, Bauce B, et al. Mutations in the area composita protein alphaT-catenin are associated with arrhythmogenic right ventricular cardiomyopathy. *Eur Heart J* 2013;34:201–10.
- [46] Zorzi A, Rigato I, Pilichou K, et al. Phenotypic expression is a prerequisite for malignant arrhythmic events and sudden cardiac death in arrhythmogenic right ventricular cardiomyopathy. *Europace* 2015 <http://dx.doi.org/10.1093/europace/euv205>, in press.
- [47] Metzker ML. Sequencing technologies—the next generation. *Nat Rev Genet* 2010;11:31–46.
- [48] Rehm HL. Disease-targeted sequencing: a cornerstone in the clinic. *Nat Rev Genet* 2013;14:295–300.
- [49] Kapplinger JD, Landstrom AP, Salisbury BA, et al. Distinguishing arrhythmogenic right ventricular cardiomyopathy/dysplasia-associated mutations from background genetic noise. *J Am Coll Cardiol* 2011;57:2317–27.
- [50] Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. *Nature* 2015;526:68–74.
- [51] Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res* 2003;31:3812–4.
- [52] Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7:248–9.
- [53] Kircher M, Witten DM, Jain P, et al. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet* 2014;46:310–5.
- [54] Severini GM, Krajcinovic M, Pinamonti B, et al. A new locus for arrhythmogenic right ventricular dysplasia on the long arm of chromosome 14. *Genomics* 1996;31:193–200.
- [55] Li D, Ahmad F, Gardner MJ, et al. The locus of a novel gene responsible for arrhythmogenic right-ventricular dysplasia characterized by early onset and high penetrance maps to chromosome 10p12–p14. *Am J Hum Genet* 2000;66:148–56.